Survey of microplastics in a freshwater and saltwater ecosystem

G.Lau, M.Fadaie, B.Durrani
University of British Columbia Department of Zoology

Abstract:

The objective of our study was to quantify the abundance of microplastics across a salmon migration gradient, using a freshwater and seawater site as proxies for the habitats that salmon encounter along their migration route. Three 190L water samples were filtered using a plankton net at two different sites in the city of Vancouver: the mouth of Salish Creek, and the waters off Jericho Beach. Samples were treated using Proteinase-K digestion and microplastics were visually identified using Zeiss Axiostar compound microscopes and a microplastic identification key. An unpaired two-tailed t-test returned a t-value of 2.4010 and a p-value of 0.0743. Although the results are not statistically significant (p-value < 0.05), we did find a trend showing that the ocean samples contained a larger quantity of microplastic fragments. The presence of microplastic fragments in both the freshwater and ocean water samples is alarming since microplastics have been known to inflict negative effects on fish physiology and behaviour. This is especially alarming for the restoration of habitats for Pacific salmonids as microplastics in the freshwater streams may harm juveniles and returning spawners.

Introduction:

Plastic production and consumption has increased over the past 50 years, with almost 300 million tons of plastic produced in 2013 (Gourmelon, 2015). Plastics have become a common material in every facet of life, and synthetic polymers have, up until recently, replaced other resources such as wood and metal in manufactured goods (Anderson, Park, & Palace, 2016; Gourmelon, 2015). Increased production and consumption has resulted in accumulation of plastic litter across the globe, and plastics now account for the largest amount of anthropogenic litter in aquatic environments (Barnes, Galgani, Thompson, & Barlaz, 2009; Eriksen et al., 2013). Common plastics include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) (Andrady, 2011). Their lightweight, oxygen and moisture barrier qualities along with their cheap production costs have resulted in their use across a wide array of industries such as cosmetics, packaging, healthcare,

fishery, and motor vehicles (Andrady, 2011; Gourmelon, 2015; Napper, Bakir, Rowland, & Thompson, 2015).

Increased plastic use leads to increased amounts of plastic ending up in landfills instead of being recycled. It is estimated that approximately 80% of marine plastic debris has a land-based origin, with the other 20% coming from an aquatic use-based origin, such as fishing (Li, Tse, & Fok, 2016). The exact number of plastics that end up in the ocean each year is estimated to be between 10-20 million tons (Gourmelon, 2015). As they travel in the aquatic environment, they become brittle and break up, and if given enough time and corrosion, can break up into microplastics (Cole, Lindeque, Halsband, & Galloway, 2011; Li, et al. 2016). Although the exact definition of a microplastic varies, most agree that microplastics are defined as pieces of plastic that are <5mm in diameter (Anderson et al., 2016; Dikavera & Simon, 2019). Due to differing densities, microplastics can be found in surface water, in the water column, and denser plastics, such as PVC, can be found in the sediment (Anderson at al., 2016).

Microplastics that reside in the water column limits the ability to move through ocean currents (Andrady, 2011; Barnes & Milner, 2004). This is particularly a concern for marine life, such as Pacifc Salmon (Desforges, Galbraith, & Ross, 2015). A study by Lu et al. in 2016 on the Zebrafish (*Danio rerio*) found microplastic accumulation in the gills, liver, and guts of the fish. The same study also found increased levels of necrosis and lipid accumulation in hepatocytes (Lu et al., 2016). Studies in juvenile fish have also found wide ranging effects on fish behaviour. A study on juveniles of the Common Goby (*Pomatoschistus microps*) found that exposure to microplastic particles resulted in reduced predatory efficiency and performance, which may have negative consequences for fitness (de Sa, Luis, & Guilhermino, 2015). Ingestion of microplastics have also been found to structurally alter the distal intestines of fish, with effects including widening of the lamina propria, shortening and swelling of villi, and increase of goblet cells at the top of the villi (Peda et al., 2016). Salmon are not only essential to marine life but also to human populations. Salmon are considered to be of sentimental value for the indigenous people as they depend on it for cultural and material uses but unfortunately, salmon returns, and salmon spawning are decreasing in many sites around the world. A study conducted by Ween & Colombi (2013) analyzed two rivers, the Tana River in Northern Norway and the Columbia

River on the northwest coast of the United States, both of which host indigenous communities. Salmon from both rivers are considered as essential to indigenous diets as well as human-salmon relations which enact gender, kinship, and identity (Ween & Colombi, 2013). The amount of salmon in the present day in both rivers reflect the chance of there being salmon extinction which may negatively impact the indigenous populations that depend upon them. In the Tana River in 2009, it was seen that there was a significant (50%) decrease in salmon catching from the year before and the weight of the average fish had also decreased dramatically. The Columbia River was also once the site of high salmon production but in the current state has portrayed a significant decline in salmon spawning and return. Both rivers are now being managed to conserve and protect the quantity and quality of salmon present and returning to accommodate the necessities of the indigenous communities relying on them (Ween & Colombi, 2013).

Zooplankton, which are the primary source at the base of the food chain, are one of the main food supplies for many marine biota including salmon. Desforges et al. (2015) conducted a study on two zooplankton species, calanoid copepod (*Neocalanus cristatus)* and the euphausiid (*Euphausia pacifia).* By means of this study, they found that zooplankton ingest microplastic fibers and fragments as they mistake it for food at a rate of 1 particle/ every 34 copepods and 1 particle/ every euphausiids (Desforges et al, 2015). Since these species are the primary food sources for salmon, it is estimated that microplastic containing zooplankton will be encountered by juvenile salmon at a rate of 2-7 microplastic particles/day and ≤ 91 microplastics particles/day in returning adults in coastal British Columbia. The researchers also mention that microplastic particle ingestion may contribute more towards physical defects as microplastics fibers/fragments may entangle feeding appendages or block internal organs which may result in injury, reduced feeding, and even death (Desforges et al, 2015).

Mattson et al conducted a study on three different marine organisms, green algae (*Scenedesmus* sp.), zooplankton (*Daphnia magna*) and Crucian Carp (*Carassius carassius*), using a freshwater trophic system to discover the effects of polystyrene nanoparticles on their behaviour, physiology, and metabolism. Through filming the behaviour of fish, the researchers compared the differences on the 25_{th} and 62_{nd} day between the nanoparticle ingested fish and control fish (nanoparticle free). The results achieved depicts that the fish that consumed polystyrene

nanoparticles had slower movement, less activity, and took twice as long to feed compared to unaffected fish, which led to a smaller fish size. Also, the fish that fed on polystyrene nanoparticles had larger, swollen brain tissue due to higher water content. The metabolism of fish that consumed the nanoparticles was also lower compared to the ones that were the control (nanoparticle free).

As freshwater streams are typically exposed to less human activity than the open ocean, we predict that Jericho Beach will be the site that contains the higher number of microplastic particles due to a higher probability of human activity and domestic runoff. Our null and alternative hypotheses were as follows:

H_0 = There is no difference in the average number of microplastic particles between the stream and the ocean site.

H_a = There is a difference in the average number of microplastic particles between the stream and the ocean site.

Methods & Materials:

Study Sites

We collected samples from two sites in the city of Vancouver, a coastal city in southwestern Canada that borders the Pacific Ocean. We selected the mouth of Salish Creek as our freshwater site and sampled three random spots in the creek. This creek was recently restored and is currently undergoing efforts to rehabilitate the creek for salmon habitat. We selected the waters off Jericho Beach as our ocean water sample and sampled three random nearshore areas. The beach borders the Burrard Inlet which connects to the Pacific Ocean and is known to contain adult salmon.

Sample collection and treatment

Samples were collected on the same day at both sites in October 2019. At each site, we used 3 50 micrometer plankton nets to filter 190 liters of water. At the stream site, we used 4-liter jugs to pour water through the net as the water was too shallow to let the nets drift in the stream. At the ocean site, we towed the nets at the surface of the water to collect 190 liters of water as

calculated using the volume of the nets. We used distilled water to rinse the debris in the net into a falcon tube attached at the end of the net, which we emptied into a collecting jar. The samples were labelled and sent back to the laboratory where they were stored in the refrigerator for five days. A negative control of distilled water was also run through the plankton nets and stored in a collecting jar.

To isolate microplastic samples, we employed a similar digestion protocol to the one used by Cole et al. (2014) where they used optimized Proteinase-K digestion to isolate microplastics from plankton in seawater. We refiltered the samples through the same nets to concentrate the debris onto a smaller surface area so that we could use less distilled water to wash the debris into one autoclaved 250mL glass beaker per sample. We then placed the beakers in a drying oven set to 60 degrees Celsius and let them evaporate for 20 hours. After achieving the beakers from the oven, we scraped out the dried material into a weigh boat on a scale to determine the dry weight of our sample in order to calculate the amount of Proteinase-K required for digestion. We used the same ratio as the one in the study by Cole et al. (2014), 500micrograms of Proteinase-K/mL for every 0.2 grams of dry sample. In order to wash each sample into a 2mL microcentrifuge tube, we calculated how much additional buffer we would need, in addition to the amount of Proteinase-K, to make 2mL of liquid. The volume of the dry sample was negligible. We then used the calculated amount of buffer to wash the dry samples from the weigh boat into the 2mL microcentrifuge tube. We incubated the samples in a 50 degree Celsius water bath for 2 hours for digestion of organic material. After, we incubated the samples in a 95 degree Celsius water bath for 15 minutes in order to stop enzyme activity. Samples were stored in the refrigerator for another five days before we could identify the plastics using visual identification.

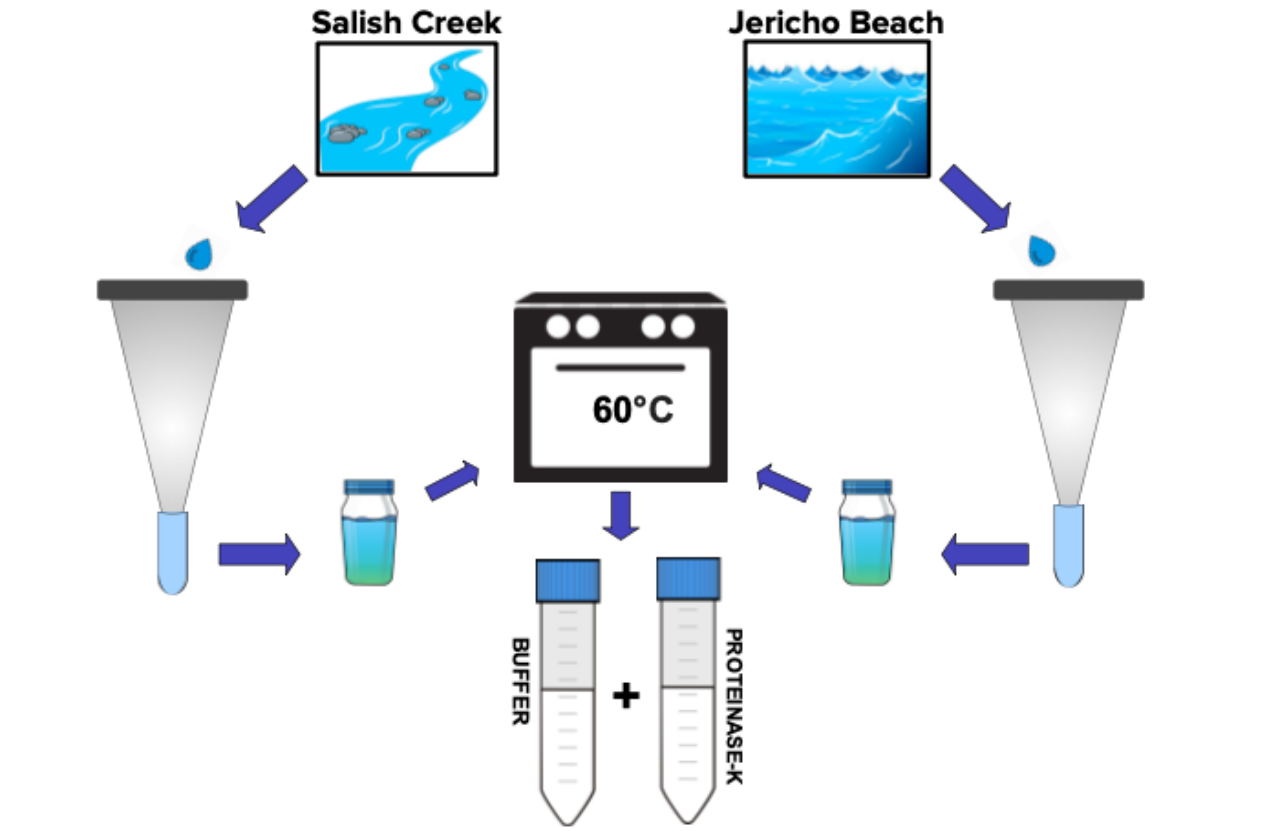

Figure 1. General summary of experimental process. Water samples from each site were filtered through plankton tow nets, followed by evaporation of water from dry sample in a 60°C drying oven. The dry samples were reconstituted in buffer and a pre-calculated amount of proteinase-K was added to digest organic material within each sample.

Visual identification

For the visual identification of microplastics and quantification, each lab group member was tasked with analyzing one stream sample and one ocean sample. In order to resuspend any debris left at the bottom of the microcentrifuge tube, we vortexed each sample for 20 seconds before using a micropipette to draw 50 microliters of sample from the microcentrifuge tube. We then prepared a wet mount slide and used a compound microscope to thoroughly analyze the entire slide. This step was repeated three times for each sample and the negative control. Because we could not use any infrared identification to determine whether or not the item in question was truly plastic in origin, we only quantified items that were abnormal in colour, or abnormal in shape.

Results:

The total number of microplastics found in all of the stream samples was 15 (n=3). The average was 5 pieces/250 microliters. The standard deviation of the mean was 5.291503 pieces and the 95% confidence interval of the mean was from -8.144821 to 18.144821. The total number of microplastic pieces found in all of the ocean samples was 43 (n=3). The mean was 14.3333 pieces/250 microliters. The standard deviation of the mean was 4.163332 pieces and the 95% confidence interval of the mean was from 3.991043 to 24.675623.

An unpaired two-tailed t test on the means of the number of pieces of microplastics/250 microliters at each site returned the following statistics. The difference between the means of the stream sample and the ocean sample is 9.33 and the p-value was calculated to be 0.0743. Since the calculated p-value is greater than alpha, conventionally set to alpha = 0.05, we fail to reject the null hypothesis. The t-value calculated was 2.4010, with 4 degrees of freedom.

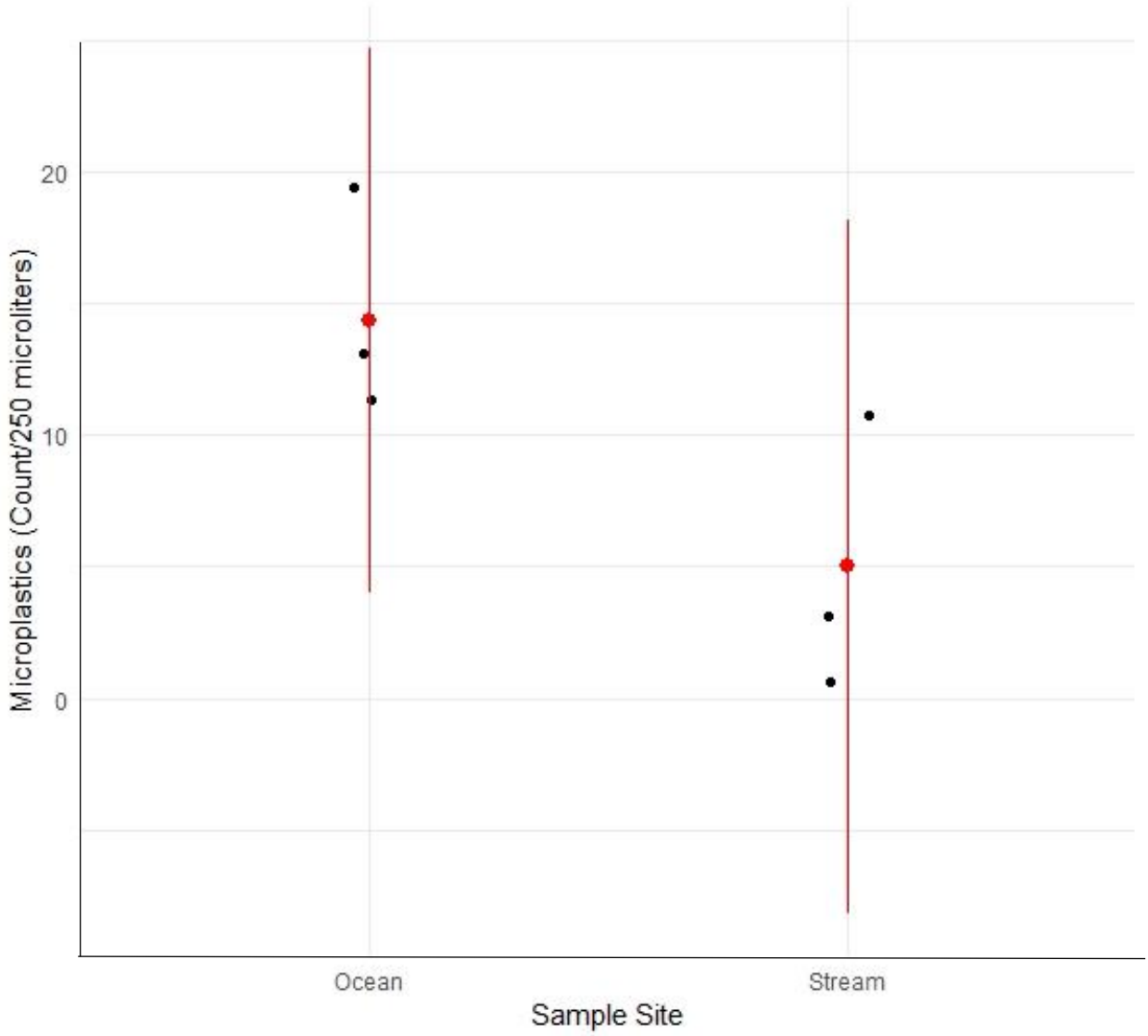

Figure 2. Microplastic counts per 250 microliters in samples from the ocean (n=3) and stream (n=3) sites. The red dots indicate the mean at each site; 14.33333 pieces/250 microliters at the ocean site and 5 pieces/250 microliters for the stream site. Data were analyzed using an unpaired two-tailed t-test. *P*=0.0743, t=2.4010, df=4.

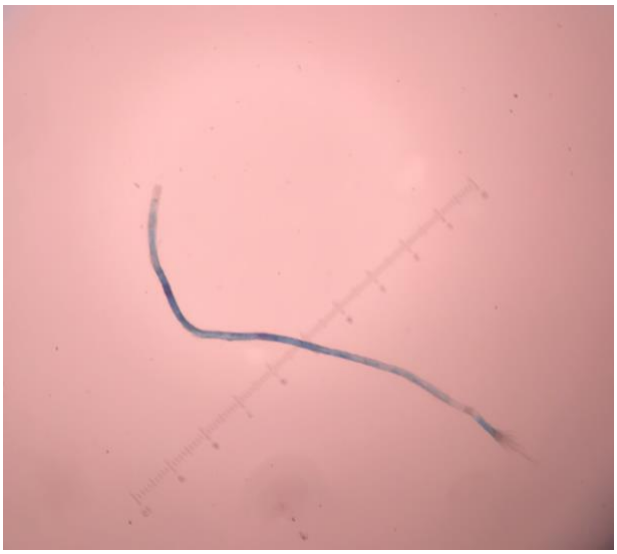

Figure 3. Microplastic identified from ocean sample collected from Jericho Beach visualized under a Zeiss Axiostar compound microscope at 400X magnification.

Discussion:

The purpose of this experiment was to determine if the quantity of microplastics in the water differed between the mouth of Salish Creek and Jericho Beach. Based on the data analysis from the two-sample t-test, we obtained a p-value of 0.0743. Thus, we fail to reject the null hypothesis; the difference in the quantity of microplastics from the two sites was not statistically significant. Since the number of microplastics between the two sites do not differ, we cannot infer a difference in microplastic accumulation within different stages of the salmon life cycle (Lu et al., 2016). That is, the salmon that spend their life cycle in different bodies of water (stream and ocean) will not have a statistically significant difference of exposure to microplastics.

The formidable presence of microplastics in the water further leads to detrimental effects on trophic systems and indirect negative effects on salmon species. This occurs because smaller invertebrates will feed on the microplastics found in the water, which are trophically passed onto the salmon species in the ecosystem. Mattsson et al. (2015) conducted a study to understand how incorporating microplastics within the trophic system can eventually get passed on and lead to negative effects on salmon that ingested microplastics indirectly. Consequently, ingestion of microplastics damages the physiological features of salmon and leads to overall structural damage (Peda et al., 2016). Damaging the physiological features of salmon will decrease their

lifespan and eventually decrease the population of salmon in various ecosystems. One of the direct examples of microplastics being passed on in the different trophic levels can be achieved from the study conducted by Desforges et al (2015). In this study, the researchers considered two types of zooplankton, calanoid copepod *(Neocalanus cristatus)* and the euphausiid (*Euphausia pacifia).* Upon examining these primary food sources of salmon, the researchers concluded that both zooplankton species ingest microplastics at different rates and have a strong chance of being ingested by juvenile and adult salmon as well. Furthermore, since the salmon play a significant role as a keystone species in British Columbia, we can conclude that damaging the lifestyle of salmon will bring stress to the salmon species and the ecosystem as a whole.

The presence of microplastics indicate harmful anthropogenic effects on the ecosystem of both Salish Creek and Jericho Beach waters. The local effects contribute to the increase in global amounts of litter in aquatic environments (Barnes, Galgani, Thompson, & Barlaz, 2009; Eriksen et al., 2013). With the tremendous growth of plastic-producing industries around the world, the discharge of microplastics within the environment are difficult to contain (Andrady, 2011; Gourmelon, 2015; Napper, Bakir, Rowland, & Thompson, 2015). Not only does this damage the direct and indirect services that the environment provides for humans, but it also leads to destruction of the diversity and aesthetic beauty of nature. In order to prevent the large magnitude of deterioration on the ecosystem, sanctioning of plastic usage and conservation efforts should be enforced and followed consistently.

There are also some design limitations and sources of error that are important to consider for this experiment. When micropipetting the ocean samples throughout the procedure, microplastics may have been left within the micropipette tip. This is a design limitation as it does not fully allow us to determine the accurate quantity of microplastics from our field samples. It is very important to obtain an accurate representation of data in order to determine if the difference in microplastic quantities between the two sites are statistically significant. Furthermore, throughout the identification process of microplastics, we utilized our own discretion to differentiate between pieces of microplastic and other materials visualized under the microscope. Since only three individuals were part of this project, the identification was solely based on our

own discretion and may have introduced a bias to our results. For further research, we should obtain more replicates from the field sites in order to minimize possible errors in our results and have more individuals confirming microplastic presence under the microscope.

Conclusion:

We found evidence of microplastics at both the freshwater and saltwater ecosystems, with a higher average obtained from the saltwater site. The difference between the average number of microplastics identified was not statistically significant. In future conducted studies, a larger sample size will likely be necessary in order to achieve significant results. This will help us understand which ecosystem contains more microplastics and has larger detrimental effects on salmon populations. Since salmon are considered a keystone species in British Columbia, this result may offer vital evidence as to why the conservation of salmon populations is an urgent predicament that must be acknowledged and attended to.

Acknowledgements:

We would like to express our gratitude to Jordan Hamden and Tessa Blanchard for their input and continuous support throughout our research. We would also like to thank Mindy Chow and Chanelle Chow for continually supporting our experiment and assisting our setup. Lastly, we would like to thank the University of British Columbia and Metro Vancouver for allowing us to conduct this research.

Reference List:

Anderson, J. C., Park, B. J., & Palace, V. P. (2016). Microplastics in aquatic environments: Implications for Canadian ecosystems. *Environmental Pollution, 218*, 269-280.

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin, 62*(8), 1596-1605.

Barnes, D. K. A., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society Biological Sciences, 364*, 1985-1998.

Barnes, D. K. A., & Milner, P. (2004). Drifting plastic and its consequences for sessile organism dispersal in the Atlantic Ocean. *Marine Biology, 146*(4), 815-825.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin, 62*(12), 2588-2597.

Cole, M., Webb, H., Lindeque, P. K., Fileman, E. S., Halsband, C., & Galloway, T. S. (2014). Isolation of microplastics in biota-rish seawater samples and marine organisms. *Scientific Reports, 4*, 1-8.

Desforges, Jean-pierre W, Galbraith, Moira, Ross, & Peter S. (2015). Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. *Archives of Environmental Contamination and Toxicology, 69(3),* 320-330.

Dikavera, N., & Simon, K. S. (2019). Microplastic pollution in streams spanning an urbanisation gradient. *Environmental Pollution, 250*, 292-299.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., … Amato, S. (2013). Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Marine Pollution Bulletin, 77*(1-2), 177-182.

Gourmelon, G. (2015). Global plastic production rises, recycling lags. *Vital Signs,* 1-7.

Li, W. C., Tse, H. F., & Fok, L. (2016). Plastic waste in the marine environment: A review of sources, occurrence and effects. *Science of the Total Environment, 566-567,* 333-349.

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., … Ren, H. (2016). Uptake and accumulation of polystyrene microplastics in Zebrafish (*Danio rerio*) and toxic effects in liver. *Environmental Science & Technology, 50*(7), 4054-4060.

Mattsson, K., Ekvall, M. T., Hansson, L., Linse, S., Malmendal, A., & Cedervall, T. (2015). Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. *Environmental Science & Technology, 49*(1), 553-561.

Napper, I. E., Bakir, A., Rowland, S. J., & Thompson, R. C. (2015). Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Marine Pollution Bulletin, 99*(1-2), 178-185.

Peda, C., Caccamo, L., Fossi, M. C., Gai, F., Andaloro, F., Genovese, L., … Maricchiolo, G. (2016). Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: Preliminary results. *Environmental Pollution, 212,* 251-256.

de Sa, L. C., Luis, L. G., & Guilhermino, L. (2015). Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. *Environmental Pollution, 196,* 359-362.

Ween, G. B., & Colombi, B. J. (2013). Two rivers: The politics of wild salmon, indigenous rights and natural resource management. *Sustainability, 5*(2), 478-495.

Appendix:

Table 1.

Sample name	Weigh boat + Dry wgt. (g)	Weigh boat wgt. (g)	Dry wgt. (g)	Buffer required (mL)	Proteinase-K required (mL)
Stream A	n/a	n/a	0.015	1.925	0.075
Stream B	0.496	0.491	0.005	1.975	0.025
Stream C	0.477	0.475	0.002	1.99	0.01
Ocean A	0.5	0.492	0.008	1.96	0.04
Ocean B	0.498	0.490	0.008	1.96	0.04
Ocean C	0.497	0.496	0.001	1.995	0.005
Negative control	0.483	0.483	0	1.995	0.005*

*Negative control received the same amount of Proteinase-K as the smallest measured sample- Ocean C (0.005mL).

Formula for Proteinase-K requirement:

$$\frac{mL}{0.2g} \times Dry\ weight\ (g) = Proteinase\ K\ (mL)$$

Table 2. Quantity of microplastics identified from three freshwater samples collected at Salish Creek and the calculated average.

Stream Site	A	B	C
# of microplastics/250 microliters	11	3	1
Average of all stream sites	5		

Table 3. Quantity of microplastics identified from three saltwater samples collected at Jericho Beach and the calculated average.

Ocean Site	**A**	**B**	**C**
# of microplastics/250 microliters	13	19	11
Average of all ocean sites	14.3		

Table 4. Quantity of microplastics identified from negative control sample.

	Negative Control
# of microplastics/250 microliters	0