

Macro and micro plastics in an urbanized and non-urbanized fjord
estuary in the Northeast Pacific Ocean

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Abstract

Plastics, synthetic polymers which are durable and long lasting, have been appearing as marine debris for many decades. Macro-plastics (larger than 5 mm) and their effects on marine life have been well studied, documenting effects such as strangulation and digestive blockage in animals. Micro-plastics (0.3 mm to 5 mm in size), a result of either plastic produced in small pieces or fragmentation, have only recently begun being researched. This study characterizes macro and micro plastics in two NE Pacific estuaries, Puget Sound, WA and Nootka Sound, Canada. Macro-plastic concentrations were zero at all stations in both estuaries. Average micro-plastic concentrations at the surface ranged from 0 pieces/m³ to 102 pieces/m³, 5 m depth concentrations were 0 pieces/m³ to 44 pieces/m³ and 10 m depth concentrations were 0 pieces/m³ to 5300 pieces/m³. Fibers of plastic, assumed to be primarily marine in origin, had a higher concentration at most stations than those in pellet form, assumed to be terrestrial in origin. Understanding plastics' distribution, origins, and effects on estuaries will require significant spatial and temporal sampling, beginning at the source.

Introduction

The study of plastics on the marine environment has primarily focused on surface macro-plastic concentrations within the five oceanic gyres. Persistent global surface currents are primarily responsible for the distribution and accumulation of plastics throughout the ocean. The effects of macro-plastics on marine biota has been studied fairly extensively. Strangulation, intestinal blockages and sequestration of plastics within tissues are some of the known effects of macro-plastics on fish, birds, marine mammals and other marine organisms (Eriksen et al. 2014). Neglected in most of these studies are the direct observations and sampling of smaller, coastal regions. This research looked at plastics in an urbanized and non-urbanized fjord estuary in the Northeast Pacific, Puget Sound, WA and Nootka Sound, Canada.

Plastics, which are a synthetic polymer created by humans, are lightweight and highly durable and therefore widely present in many consumer products, many of which end up being disposed. Significant marine plastic waste comes from run-off or dumping. Additionally, plastics are widely used in fishing material such as nets and rope, further contributing to the concentration of plastics in marine environments. Plastics' slow degradation process in combination with increased plastic production and waste have caused concentrations of plastics in the ocean to increase (Carolina and Gimenez 2015). Due to marine processes, such as surface circulation and mixing, large pieces of plastic (visible by the naked eye) are constantly broken down into smaller pieces rather than completely degrading over time (Moore 2008). Because of the durability of these materials, many plastics persist in the ocean for decades and can release toxic chemicals, although very little is known about the biological consequences of these toxins (Moore 2008). Plastics tend to converge in the oceanic gyres, closed bays and seas (Eriksen et al.

2014). Quantifying the concentrations of plastics in the ocean has been a difficult process due to inconsistencies in methodology of collection and analysis (Hidalgo-ruz et al. 2012).

The primary categories of plastics present in the oceans are polyethylene (PE) primarily used for containers such as bags and milk jugs, polystyrene (PS) used for Styrofoam and food containers, and polypropylene (PP) use for ropes, bottle caps and fishing gear (Carolina and Gimenez 2015). Plastics are then generally separated into two size categories, macro-plastics which are typically items larger than 5 mm and micro-plastics, items smaller than 5 mm (Law and Thompson 2014).

Direct effects of plastics on marine organisms has primarily focused on animals large enough to ingest plastics, such as sea birds, turtles and marine mammals (Sigler 2014). Consumed macro-plastics have been found to block excretory pathways, cause internal injuries, depress growth and reduce stomach capacity. Additionally, marine animals can get tangled in plastics, which can cause drowning and strangulation (Wright et al. 2013). Fish have also been shown to consume plastics, but studies are lacking on the associated biological effects (Sigler 2014). The effects of consumption of micro-plastics by fish, birds and other marine animals have not been studied, but in the lab, it has been shown that consuming micro-plastics can cause the plastics to release toxins which can be contained in the flesh of the animal (Law and Thompson 2014).

Plastics in the ocean aren't detrimental to all organisms. Floating particles of plastics have provided an additional substrate within the water column that organisms which are typically benthic and sessile can now attach to and move around the ocean (Zettler et al. 2013). The implications of this movement are not well understood, although, it is clear that this provides a new vector for invasive species proliferation (Reisser et al. 2014). Other considerations are the

effects on organisms which encounter their normal prey attached to plastic and consume both the plastic and the prey, what type of organisms live on what types of plastics and the effects of toxins released by the plastic on the organisms living on plastic, and the effects on the local ecology when plastics are introduced.

Fjord estuaries are bodies of water whose basins were carved by glaciers, leaving deep basins with shallow sills, and receiving fresh water input from rivers. Water in fjords can be strongly stratified with incoming cold dense seawater near the bottom and outgoing less dense fresh water at the top. The combination of deep basins, shallow sills and fresh water create slow circulation which results in long residence times for the water (Khangaonkar et al. 2012). Plastics may enter these regions from terrestrial sources, such as rivers draining into the basin, or marine sources, from the many boats and ships traveling through the region and lost fishing gear. Additional circulation complications such as variations in basin shape can create convergence zones, where plastics can reside in high concentration.

The Puget Sound, a highly urbanized fjord estuary located in northwestern Washington State, has very active recreational and commercial fisheries and aquaculture, all of which are of great economic importance to the region (Washington Department of Fish and Wildlife 2010). Basins within the Puget Sound vary in terms of currents, circulation and residence times (Khangaonkar et al. 2012). Plastic pollution entering these basins can change water properties such as the depth of the photic zone, toxins present and the introduction of invasive species. Little is understood about plastic distribution and concentration and how these factors impact fisheries and aquaculture in the Puget Sound.

Nootka Sound is also a fjord estuary located in the Northeast Pacific (Canada), with an active fishery and aquaculture economy. However, in comparison to the Puget Sound, this

estuary has remained virtually non-urbanized. Anthropogenic inputs to this estuary primarily consist of mining upriver, a few small towns and an active fishery. The impact of plastics on the environment and ecology is important for managing healthy fisheries and aquaculture.

Due to the complicated physical processes which move plastics through the ocean and differences in sample collection and analysis, quantifying plastic concentrations in the ocean has been difficult. This research looks to characterize plastic concentrations in two fjord estuaries in the Northeast Pacific, one which has been highly urbanized (Puget Sound, WA) and one which has been urbanized very little (Nootka Sound, Canada), through the collection and analysis of plastics within the fjords. Additionally, convergence zones within the estuaries will be examined where plastics may collect and reside for long periods of time and terrestrial vs. oceanic plastic contributions will be compared.

Methods

Field sampling took place aboard the R/V Thomas G. Thompson from 10 December 2015 through 20 December 2015, travelling from the Puget Sound in Seattle, WA to Nootka Sound, Canada. Stations sampled in and around the Puget Sound include Shilshole Bay, Admiralty Inlet, Fort Casey, and the Straits of Juan de Fuca. Victoria, Canada, and the open ocean between Victoria and Nootka Sound (Figure 1). Stations sampled in Nootka Sound, Canada, near Esperanza Inlet, in both the inlet and the mouth, and at the entry points of Port Eliza, Espinosa Inlet and Zeballos Inlet/Hecate Channel (Figure 2). Tahsis Inlet was also sampled, near the town of Tahsis and at the Tahsis Narrows (within Tahsis Inlet). Finally, in areas near Muchalat Bay, sample sites were Eliza Passage, Zuciarte Channel, Gold River in Muchalat Inlet, Matchlee Bay and the entry point between Muchalat Bay, Williams/Kings Passages (Figure 3).

Surface samples were collected using a manta net with 0.335 mm mesh and cod end filter, with the net towed about 30 m behind the vessel to avoid artificial mixing. Surface tows were performed as the ship was travelling between stations and was towed for about 15 minutes each at a speed of about 2 knots. Beginning and ending latitudes and longitudes were collected from the ship's log along with the exact speed travelled to calculate the volume of water being filtered. Contents of the net were then examined, with large pieces of plastic removed by hand for analysis and the remaining sample rinsed into the cod end. The remaining sample was then wet sieved through a 5.6 mm sieve, after which the larger pieces were collected and stored for further analysis. The last of the sample was wet sieved with a 0.3 mm sieve, rinsed with deionized water, and stored for further analysis.

Sub-surface samples were collected at depths of 5 m and 10 m. Samples collected at 5 m depths were collected using the vessel's salt water flow-through system. The flow-through system has a filter size of 3 mm, preventing large objects from entering the system. The water was then run through a 3 μm filter for about 15 minutes per station, after which the contents were rinsed and placed in a jar for on-shore analysis. Flow rates were determined by filling a 500 mL graduated cylinder with water from the system and tracking the time to fill. This data was then multiplied by the time the flow system was running, giving the volume of water filtered. Additionally, one hand deployed 3 L Niskin bottle was deployed to collect samples at 10 m depths at each sample site. This water was then filtered through a 3 μm filter and the contents rinsed and placed in a jar for on-shore analysis.

The following separation and identification procedures are slightly modified procedures taken from the National Oceanic and Atmospheric Administration's "Laboratory Methods for the Analysis of Microplastics in the Marine Environment" (Masura et al. 2015) and were performed

onshore. The collected samples were wet sieved through stacked stainless steel mesh sieves of sizes 5.6 mm and 0.3 mm. After rinsing, the material retrieved from the sieves were placed in a pre-weighed 600 mL beaker, then into a drying oven set a 90° C for 24 hours while the sample dried. Once dry, the beaker and sample were weighed and the mass of the sample was determined. Removal of biological material was performed by adding 20 mL of a 30% hydrogen peroxide solution for oxidizing the material and 20 mL of aqueous 0.05 M Fe(II) solution to catalyze the reaction with the dry solids, under a fume hood. After letting the mixture sit for 5 minutes, a stir bar was added and the beaker was covered with a watch-glass while the mixture was heated to 75° C on a hotplate. Once boiling, the beaker was removed from the hot plate until the boiling subsided, then was heated on the hotplate for an additional 30 minutes at 70° C. An additional 20 mL of the 30% hydrogen peroxide solution was added if biological material was visible. Next, 6 g of sodium chloride was added for every 20 mL of sample, increasing the density of the aqueous solution. The mixture was then heated to 75° C until the salt was dissolved. This solution was then transferred to a density separator made with funnel placed in a ring stand and fitted with latex tubing at the stem and a pinch clamp to control liquid flow. The density separator was then covered and the solids left to settle overnight. Once settled, the solid iron was drained and inspected for any plastic material. The remaining solution was vacuum filtered through a 1 or 3 μm filter (1 μm filters were used when 3 μm filters were unavailable). The solids and the filters were then placed in a petri dish and allowed to dry overnight. Once dried, a 1% solution of rose Bengal dye was added to the filter, staining the filter and any remaining biological material and leaving synthetic material unstained. Filters were then placed under a dissecting microscope at 30X magnification and plastic materials were counted and

categorized as either fibers/filaments with a marine origin or pellets/indeterminate shape, with a terrestrial source.

Determination of plastic concentrations were made using the volume of water filtered and the number of plastic particles/volume of water. Concentrations are in pieces of plastic/m³.

Results

Macro-plastics were not found at any station or at any depth. No further analysis regarding macro-plastics will be discussed.

Micro-plastics were found at the surface at most stations. Concentrations at the surface ranged from 0 pieces/m³ at the head of Tahsis to 102 pieces/m³ at Victoria, Canada and a mean concentration of 21 pieces/m³ (Figure 4). All stations showed a higher contribution of marine originated plastics, with Eliza, Victoria and the open ocean stations as exceptions. Both Victoria and the open ocean showed equal numbers of plastics with marine and terrestrial origins while the Eliza sample contained only plastics assumed to originate terrestrially (Figure 5). The average concentration of micro-plastics for the Puget Sound was 38 pieces/m³ and for Nootka Sound the average concentration was 4 pieces/m³.

Subsurface (5 m depth) samples contained micro-plastic concentrations ranging from 0 pieces/m³ at Fort Casey to 60 pieces/m³ at Eliza, Admiralty Inlet as well as the Straits of Juan de Fuca (Figure 4). Five meter subsurface micro-plastics with marine origins were present in higher numbers than at the surface, with the Gold River and Matchlee Bay stations having equal numbers of marine and terrestrial micro-plastics and Tahsis Narrows having higher terrestrial micro-plastics (Figure 6). Average 5 m depth concentrations for the Puget Sound was 3 pieces/m³ and for Nootka Sound the average was 25 pieces/m³.

Micro-plastic concentrations at 10 m depths ranged in concentrations from 0 pieces/m³ at the Straits of Juan de Fuca, Victoria and Zebellos to 5300 pieces/m³ at Gold River (Figure 7). All stations had higher concentrations of marine micro-plastics with Fort Casey being the only exception (Figure 8). Average concentrations at 10 m depth in the Puget Sound was 533 pieces/m³ and in Nootka Sound the average concentration was 1190 pieces/m³.

Discussion

Macro-plastics were not present in either Puget Sound or Nootka Sound. A study performed in Puget Sound during December 2014 found macro-plastics to be present at stations in the Puget Sound (Swaffield 2015), contributing 2.68% of the total pieces of plastics found. The difference from 2014 to 2015 in macro-plastic concentrations demonstrates the temporal variation in plastic concentrations and the difficulty in using short term studies to characterize conditions which can change significantly over short periods of time. This research also supports the hypothesis posed by Barnes et al. that plastic input to the ocean has decreased, but the micro-plastics in the ocean is increasing through further fragmentation of existing macro-plastics (Barnes et al. 2009).

Concentrations of plastics at 5 m depths were much higher in Nootka Sound than in the Puget Sound. This was an unexpected result and could be a function of vertical mixing in high velocity wind in Nootka Sound (Table 1), although a strong correlation with wind speed and plastic concentrations was not found (Figure 10). Higher shipping traffic in Puget Sound likely had an additional effect on surface currents, potentially effecting the vertical mixing (by down-mixing surface plastics) and the distribution of plastics (by moving plastics to just outside the shipping lanes).

High concentrations of plastics at 10 m depths were likely a result of the small sample size, especially when compared with the surface and 5 m depth sample sizes. With the small sample size, a single piece of plastic can result in a very high concentration. This data set demonstrates that plastics can occur within the water column and not necessarily be restricted to the surface or sediment. Further research is needed to understand the effects of plastics throughout the water column as this research demonstrates that plastics can be present from the surface to varying depths. High concentrations of plastics in the water column could have profound effects on a wide range of pelagic marine fauna, through the release of toxins, changes in the depth of the photic zone or the introduction of invasive species.

No evidence of convergence zones was found. Plastic concentrations from the 2014 study were not similar to concentrations found in this study. Additionally, the relative concentrations varied between studies. For the 2014 study, the highest concentration was at Victoria and the lowest concentration in the Straits of Juan de Fuca while for this study, the highest concentration was at Admiralty Inlet and the lowest in the Straits of Juan de Fuca.

Conclusion

This research will help establish a baseline for future studies. It will allow researchers to be able to understand if and what type of changes in plastic composition and concentrations are occurring within Puget Sound and Nootka Sound. Additional research is needed to fully understand the sources, fate, and temporal changes associated with plastics. Assuming that all fiber and strand plastics are marine in origin and that deformed or pellet plastics are terrestrial in origin helps us to understand how plastics enter the marine environment and could be used to establish programs focused on reducing plastic pollution from entering Puget Sound and Nootka

Sound, although there is a great deal of uncertainty involved. To fully gauge the sources of plastic pollution, plastics would need to be logged and tracked at the source on a very consistent basis and over a longer period of time, which was out of the scope of this study.

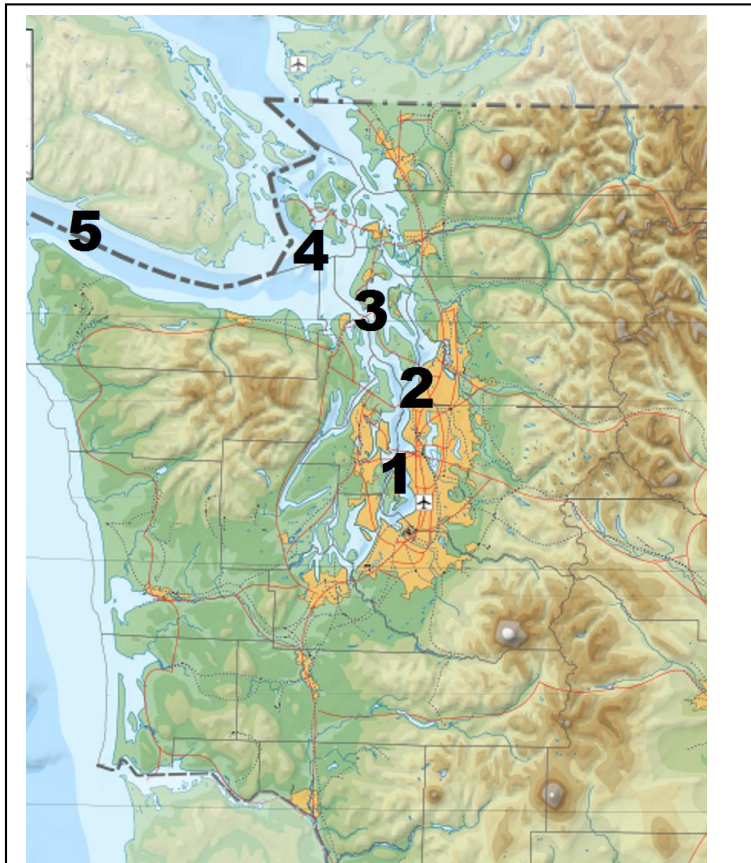


Figure 1. Sampling sites in Puget Sound
Sampling sites in and around the Puget Sound. 1) Shilshole Bay, 2) Admiralty Inlet, 3) Fort Casey, 4) Straits of Juan de Fuca, 5) Victoria and the 6) Open Ocean (not pictured).



Figure 2. Sampling sites in Nootka Sound
 Sampling sites in Nootka Sound, Tahsis Basin. 7) Port Eliza, 8) Zeballos Inlet/Hecate Channel, 9) Tahsis, and 10) Tahsis Narrows.

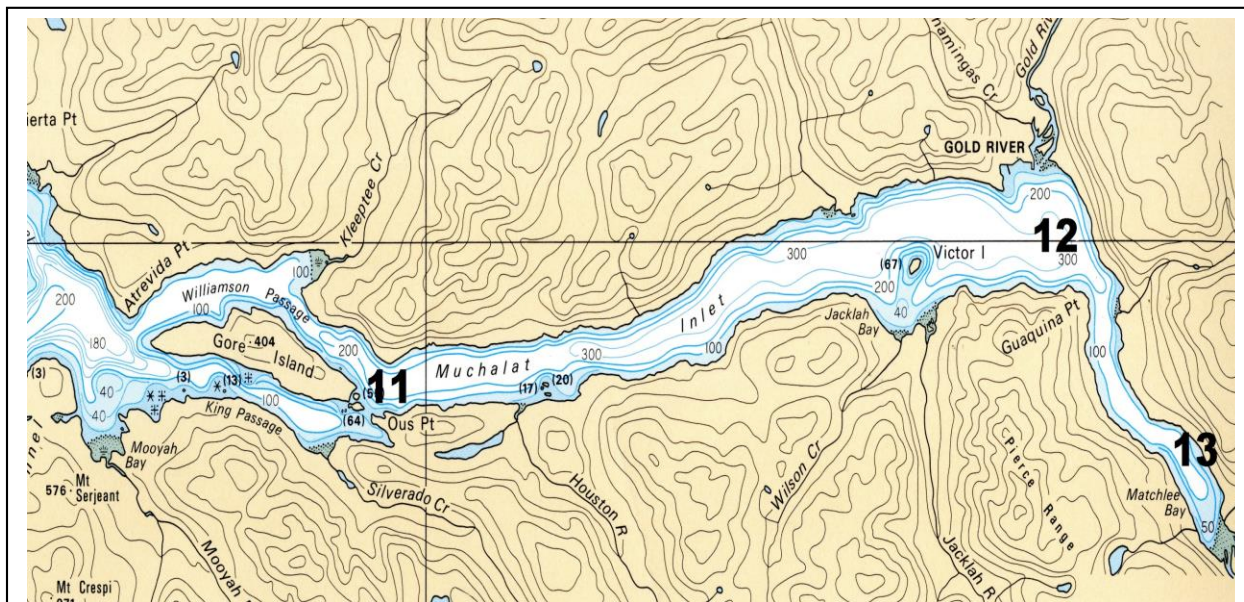


Figure 3. Sampling sites in Nootka Sound
 Sampling sites in Nootka Sound, Muchalat Basin. 11) Williams/Kings Passages, 12) Gold River, and 13) Matchlee Bay.

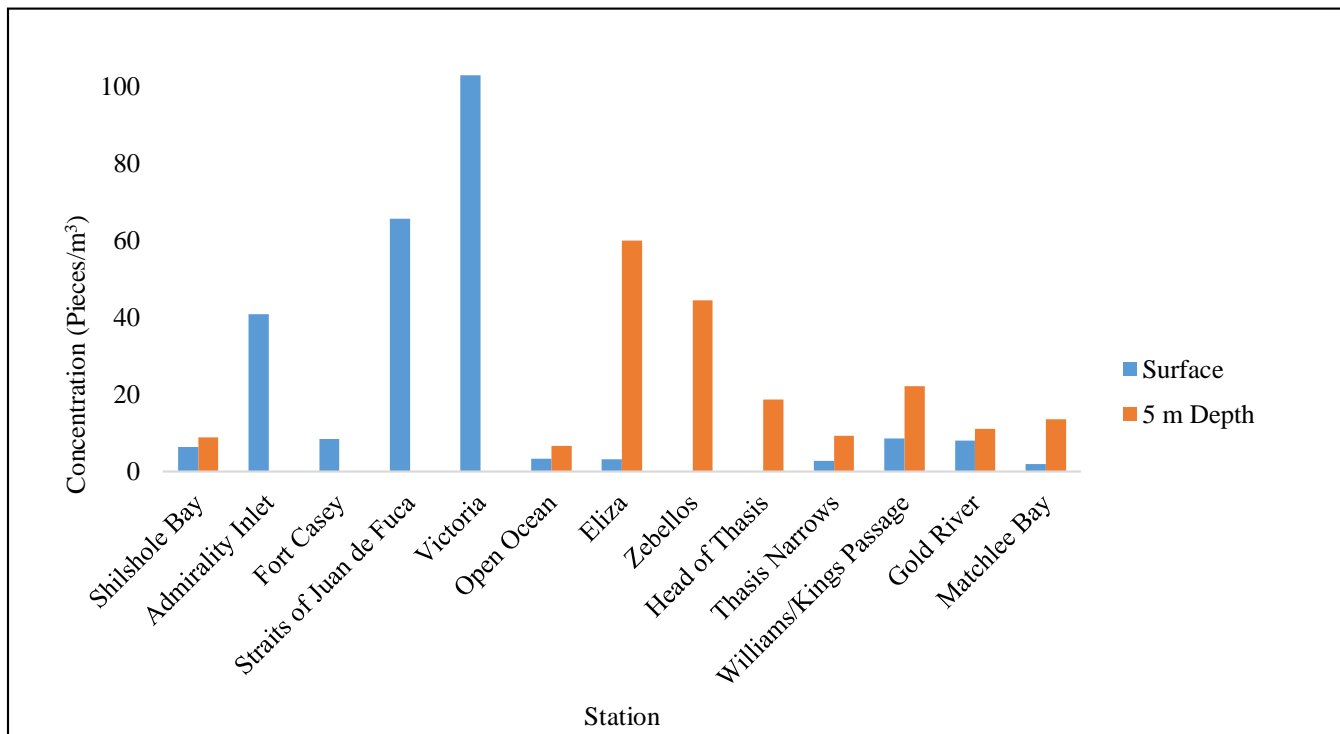


Figure 4. Surface and 5 m Depth Plastic Concentrations (pieces/m³) by Station
 Surface plastic concentrations (pieces/m³) in blue and subsurface (5 m) concentrations (pieces/m³) in orange at each station. Puget Sound stations are listed from Shilshole Bay to Victoria.

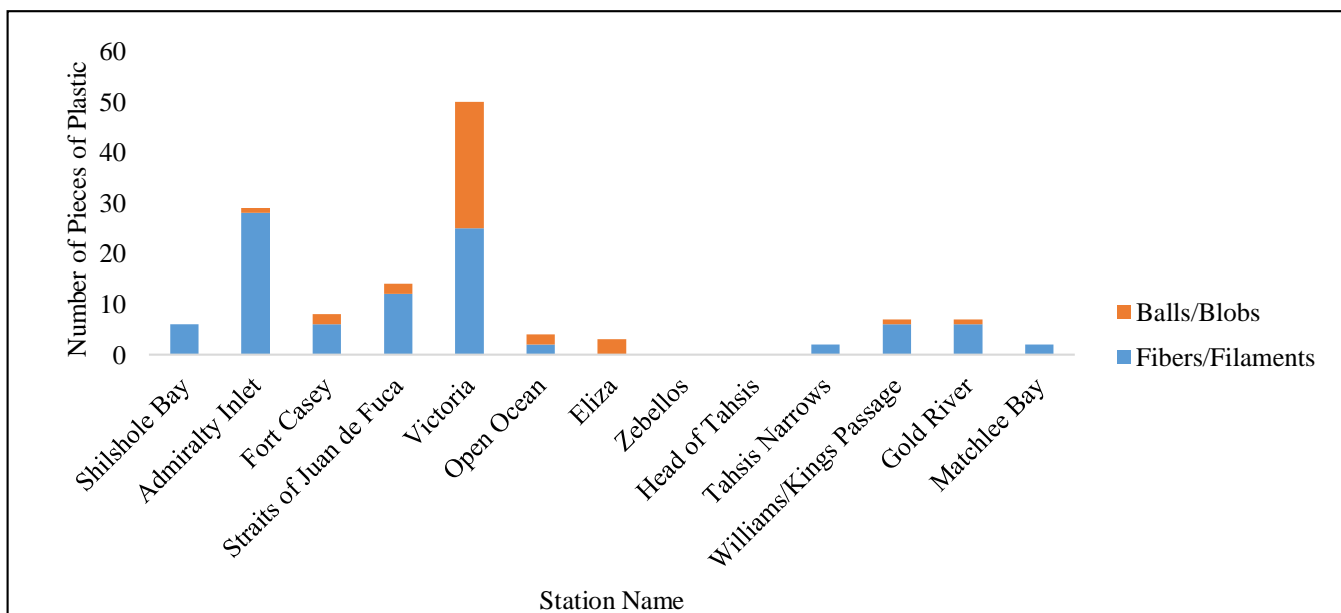
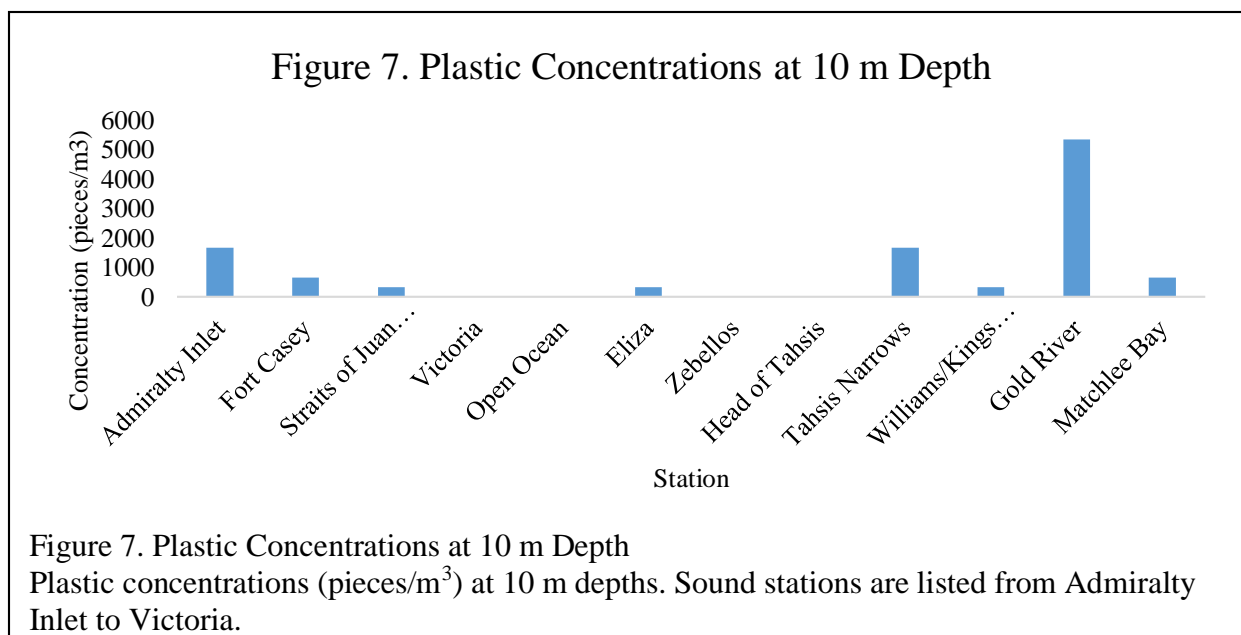
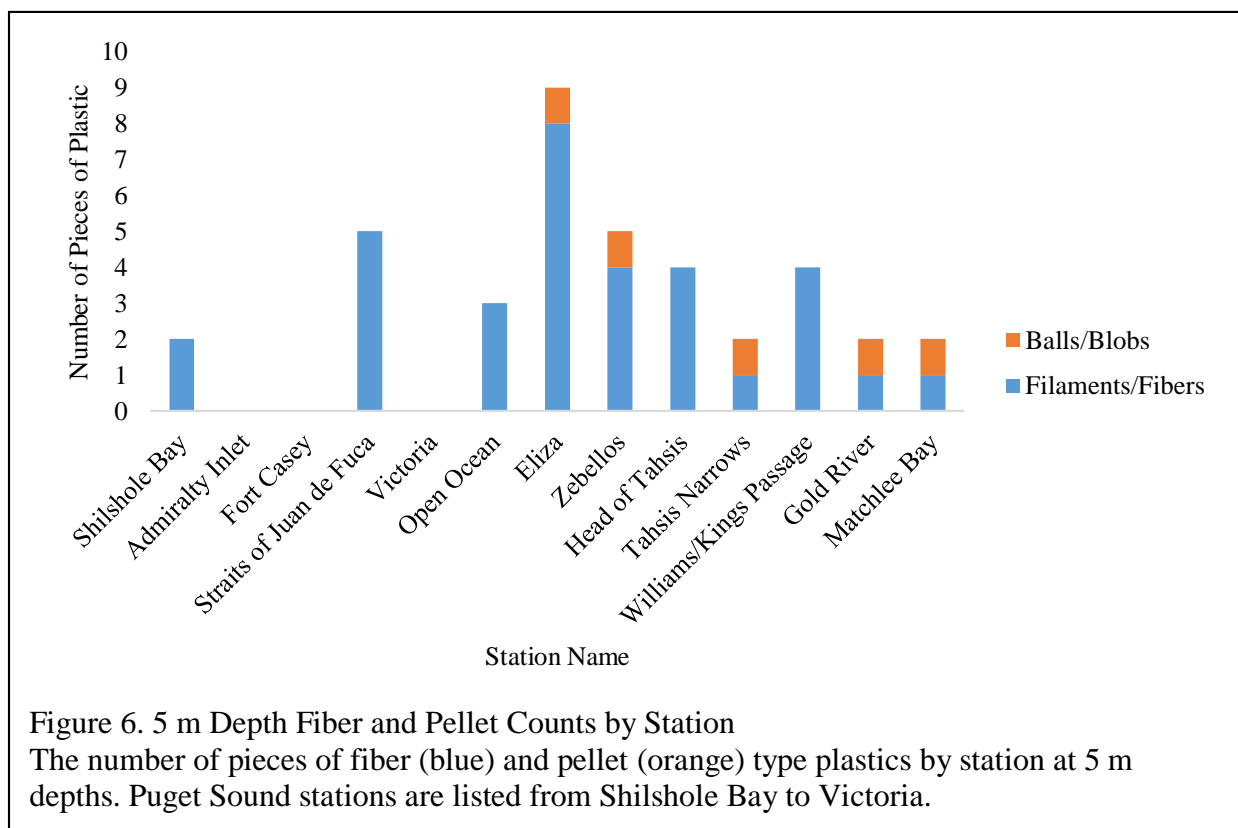


Figure 5. Surface Fiber and Pellet Counts by Station
 The number of pieces of fiber (blue) and pellet (orange) type plastics by station. Puget Sound stations are listed from Shilshole Bay to Victoria.



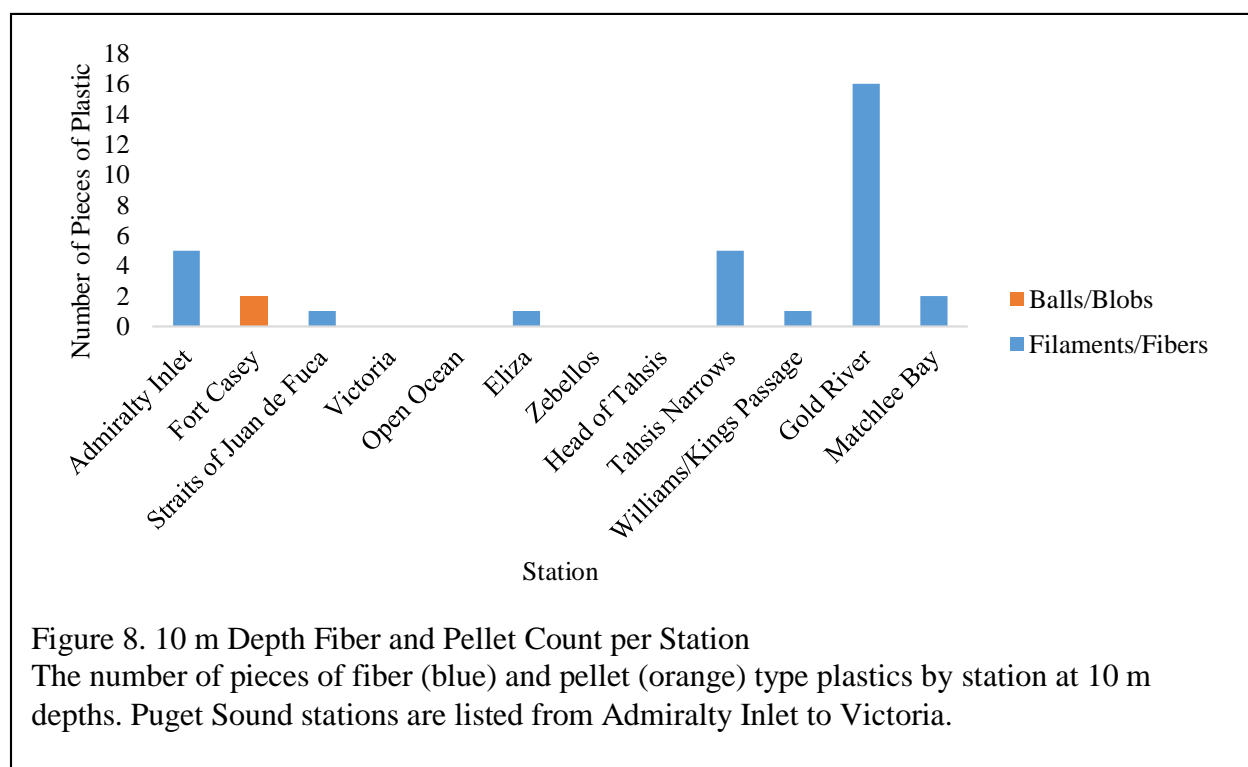
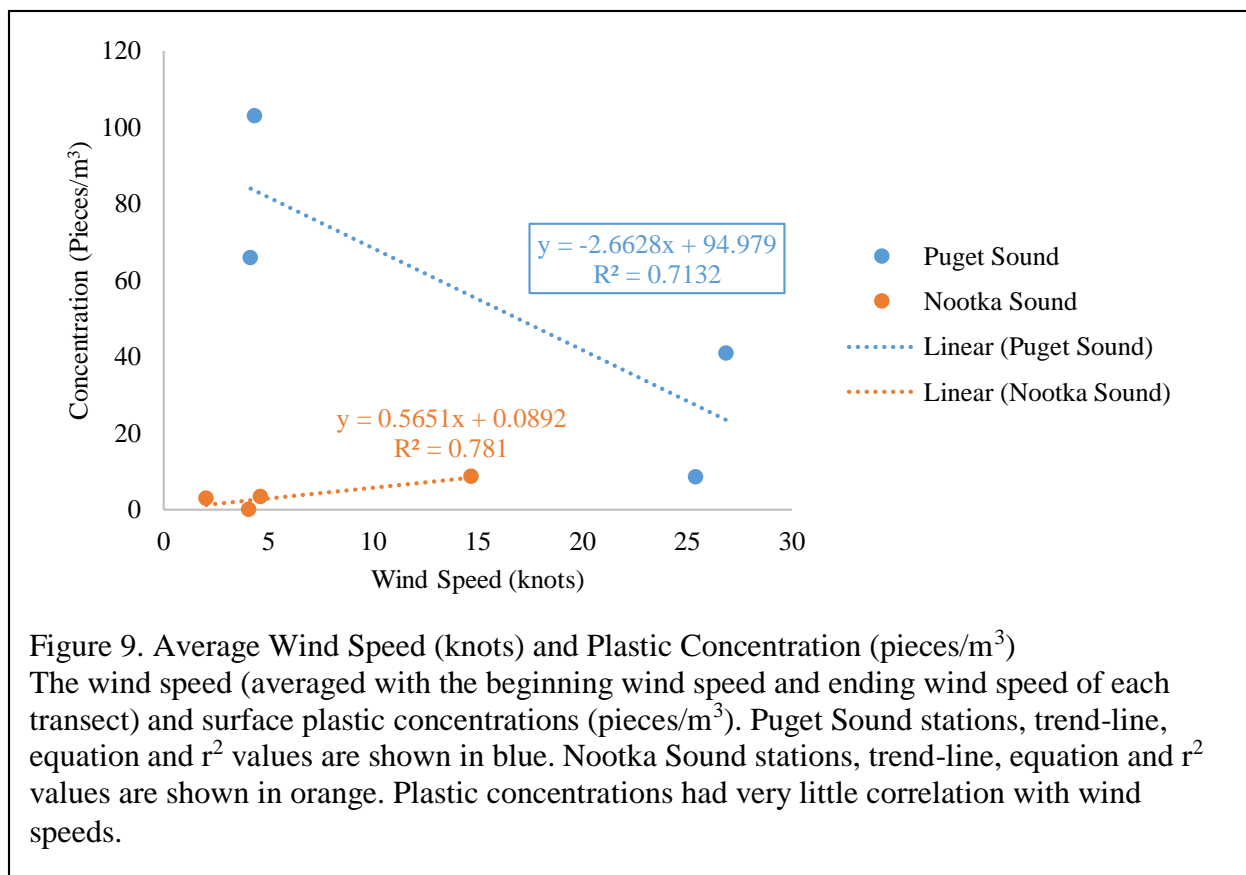


Table 1. Average Wind Speed (knots) and Plastic Concentration (pieces/m³)

Station	Average Wind Speed (kts)	Concentration (pieces/cubic m)
Shilshole Bay	25	6.341011844
Admiralty Inlet	26.875	40.86429855
Fort Casey	25.425	8.454682459
Straits of Juan de Fuca	4.15	65.75864134
Victoria	4.35	102.938504
Open Ocean	15.015	3.302610335
Eliza	4.64	3.235210124
Zebellos		
Head of Thasis	4.08	0
Thasis Narrows	2.035	2.830808859
Williams/Kings		
Passage	14.69	8.66935213
Gold River	2.545	7.997672596
Matchlee Bay	1.89	1.981566201

The wind speed (averaged using the speed at the start and end of each transect) and surface plastic concentrations (pieces/m³).



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