Monitoring the abundance of microplastics in mussels and marine ecosystems may indicate human health risks.

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This Master’s Project

Monitoring the abundance of microplastics in mussels and marine ecosystems may indicate human health risks.

By

Puja Janda

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Your Name       Date         Allison Luengen, Ph.D.   Date
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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT3</td>
<td>l-free triiodothyronine</td>
</tr>
<tr>
<td>FT4</td>
<td>l-free thyroxine</td>
</tr>
<tr>
<td>HeLa</td>
<td>Human Epithelial Cells</td>
</tr>
<tr>
<td>MFCA</td>
<td>Minimum Frequency of Assessment &amp; Collection Program</td>
</tr>
<tr>
<td>Micro FTIR Spectra</td>
<td>Micro Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>OPC SAT</td>
<td>Ocean Protection Council Advisory Team</td>
</tr>
<tr>
<td>SWRCB</td>
<td>State Water Resources Control Board</td>
</tr>
<tr>
<td>T3</td>
<td>l-triiodothyronine</td>
</tr>
<tr>
<td>T98G</td>
<td>Cerebral Cell Lines</td>
</tr>
<tr>
<td>TMDLs</td>
<td>Total Maximum Daily Loads</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nations Environmental Programme</td>
</tr>
</tbody>
</table>
Abstract

The numerous scientific methodologies and procedures used in marine research on microplastic pollution restrict our present understanding of this severe environmental issue threatening marine organisms and human health. The abundance and bioaccumulation of microplastic in the food web is causing adverse implications for marine wildlife and potentially human health via seafood consumption. The absence of standardized methods for quantifying and characterizing microplastics is a major limiting factor in comparing current research. To evaluate the issue of monitoring microplastics in the marine environment, I conducted a Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis on mussels as a biomonitoring tool. Because mussels are resilient, abundant, accessible worldwide, and commonly used as bioindicators in the marine environment, they retain higher microplastic particles and are significant biomarkers for microplastic particles. Furthermore, I conducted a comparative analysis of five different trash capture systems for fighting microplastic pollution. Among the five trash capturing methods, end of pipe nets and bioretention rain gardens were cost-effective and efficient in the long run. I suggested the implementation of a Total Maximum Daily Loads for trash (TMDLs) and the trash capture system to aid in a framework for achieving the zero waste goals by 2030 in California. Recommendations for microplastic management include a two-track strategy: (1) implementing preventative measures, clean-up pathways, and regulatory initiatives; (2) advancing scientific research through risk assessments and thresholds to improve existing solutions by adopting the standardized protocol.
1. Introduction

Microplastic is gaining attention as an emerging environmental pollutant. Without preventative methods, the abundance of plastic pollution will exceed the number of fish in the oceans by 2050 (Everaert et al., 2020). Over the last decade, the production and use of plastic have significantly increased due to its cost-effectiveness, resulting in a high discharge of non-recyclable synthetic polymers in the marine environment. Moreover, the abundance of microplastics is creating a global ecological issue. Today, we are considered the "plastic era," where plastic has become widely used in multiple products (i.e., bags, packaging, bottles, etc.).

In 1907, Dr. Leo Bakeland created Bakelite, the first fully synthetic plastic, which originates from fossil fuels using coal by-products (formaldehyde and phenol) (Plastic Industry Association, 2022). Bakelite's appearance and inexpensive cost increased its popularity among artists in the 1800s who used natural resources such as elephant ivory for piano keys. In addition, industrial manufacturers also worked with plastic materials to design war machinery for World War II. This marked the beginning of the modern plastic industry, which started in the 1920s and significantly grew since the 1940s (Fig. 1) (Plastic Industry Association, 2022).

![Historical timeline of plastic from 1907 to 2022](https://example.com/timeline.png)

**Fig. 1.** Historical timeline of plastic from 1907 to 2022. (Information pulled from (Plastic Industry Association, 2022).)
Studies have shown a significant increase in plastic pollution in marine environments. The increase in plastic pollution results from an increase in plastic products worldwide, which was recorded to be ~359 million tonnes in 2018 (Fig. 2), and in 2017 demand was ~348 million tonnes (Wang et al., 2021). Despite increased recognition of plastic pollution and attempts to decrease it, annual plastic output continues to rise. An estimated 6300 million tons of plastic waste were generated from 1950 through 2015, where 79% of the plastic entered landfills or the natural environment (Wang et al., 2021).

Fig. 2 (from Plastic Europe, 2019) Distribution of global plastic pollution in 2018 reached 359 million tonnes.

Furthermore, microplastics are formed when marine plastic litter degrades into smaller fragments by environmental matrixes (i.e., UV radiation). Microplastics are described as plastic particles that are less than 5 millimeters (mm) (0.2 inches) in diameter (Smith et al., 2018; Webb et al., 2019; Wu et al., 2018). Although, research studies conducted on surface waters identify microplastic particles to range between 0.355mm and 5mm (Carbery et al., 2018; Wu et al., 2018) (Table 1). In addition, smaller particles less than 100 nanometers are known as nanoplastics (Wu et al., 2018) but are beyond the scope of this paper (Table 1).
Table 1. (from Carbery et al., 2018) Main Plastic-type, size, and definition are abundantly found in the marine environment, consisting of microplastics and nanoplastics.

<table>
<thead>
<tr>
<th>Plastic Type</th>
<th>Size</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-plastic</td>
<td>0.355mm – 5mm</td>
<td>Tiny plastic particles less than 5 millimeters.</td>
</tr>
<tr>
<td>Nano-plastic</td>
<td>&lt;100nm</td>
<td>Polymer material that is less than 100 nanometers.</td>
</tr>
</tbody>
</table>

Despite the efforts to reduce plastic use, our dependence and continuous use of plastics have led us to a worldwide plastic pollution disaster. Every piece of plastic ever manufactured still circulates in the environment today in some way or form. Eventually, plastic waste degrades into primary and secondary microplastics that never wholly degrade in the marine environment (Stevens 2020). Instead, plastic waste will endure indefinitely as microplastic and continuously affect the marine ecosystems.

In 2015, 60-90 metric tons of microplastic waste were measured in the marine environment (Lebreton and Andrady, 2019). As the number of microplastic is doubling and tripling each year and entering the marine environment, we are seeing a surge in research studies (Fig. 3) over the last decade to identify the short and long-term impacts of microplastic in the environment. However, most research studies have not monitored the relationship between microplastic bioaccumulation in marine organisms and human consumption of contaminated microplastic seafood.
While the toxic effects of massive plastic pollution have been a problem for wildlife for decades, growing scientific data suggests that microplastic pollution is just as damaging, if not more, to marine species and human health (Beyer et al., 2017a). Furthermore, microplastic contamination has affected marine organisms at all trophic levels, including humans. The ingestion of microplastic pollution by marine organisms can affect the biochemical and physical features of a variety of species, such as mussels and fish (Beyer et al., 2017). However, the specific negative consequences for health risks remain unknown because of the different species' exposure lengths, varying shapes, plastic composition, and microplastic size. Researchers have conducted various microplastic exposure studies on mussels and fish to close the knowledge gap between microplastics in wildlife and human health. As a result, I anticipate that my research proposal will pique the interest of more researchers to close the knowledge gap on the effects of microplastics on humans and marine organisms.
1.1 Research Question
This research aims to investigate the number of microplastics in mussels as a biomonitoring tool, including marine wildlife, which may indicate higher risks for human health, necessitating improved preventative strategies and TMDLs regulatory measures. The first portion looks at how the bioaccumulation of microplastics in marine species can help future studies on potential human health risks from consuming contaminated seafood. Section two explores possible biomonitoring standards for the bioaccumulation of microplastic in mussels. Section three compares California prevention methods and TMDLs regulatory policies using feasible full trash capture systems. Lastly, section four focuses on recommendations on management strategies to reduce microplastic in the marine environment based on the research's literature review and analysis examined for this paper.

2. Methodology
My first research objective was to conduct a literature review on the bioaccumulation of microplastics that threatens marine wildlife and may indicate the potential impact on human health through the consumption of contaminated seafood. I used Scimago, a ranking database, to pull out Q1 and Q2 journals from Scopus, PubMed, and Academic Search Complete databases. After analyzing data from 2010 to 2022, I picked two highly cited journals for three highly studied marine organisms (fish and mussels). Two highly cited journals for each potential human health (nervous system, digestive system, placental barrier, endocrine disruptors, and reproductive toxicity) were conducted in-vitro and ex-vitro experiments. By doing so, I was able to present urgency to why the consumption of microplastic contaminated seafood future research is needed to close the knowledge gap for analyzing the potential toxic effect of microplastic on human health and determine long-term exposure risks.

My second objective is to conduct a qualitative Strength, Weakness, Opportunities, and Threats (SWOT) analysis on mussels as a biomonitoring tool for microplastics. A wide range of literature review studies was the primary method to develop this analysis from Q1 and Q2 sources from Scopus and PubMed databases. However, additional information was pulled from grey literature and governmental agencies, including the Mussel Watch Program and the Regional Monitoring Program for San Francisco Estuary. This analysis will evaluate if monitoring mussels are good bioindicators for microplastic pollution.
Afterward, I plan to conduct a comparative analysis on five (i.e., catch basin inserts, full structural vortex separation system, end of pipe nets, bioretention rain gardens, and wastewater treatment – reverse osmosis) preventive strategies for microplastic pollution, which includes trash TMDLs, and other regulatory processes. The primary methods used to develop this analysis will come from governmental agencies consisting of the U.S Environmental Protection Agency (prevention strategies), California Water Boards (trash TMDLs), and California State Legislative Information (environmental policies) for California. By doing so, I will be able to report recommended microplastic strategies in the marine environment and the urgency for future framework collaboration to tackle microplastic pollution in the environment.

3. Background

3.1 Sources & Types of Microplastics

Microplastics contaminating the marine environment are classified into primary and secondary microplastics. Primary microplastic is a synthetic polymer intended for commercial use (Boucher and Friot, 2017; European Food Information Council, 2021). The leading sources of primary microplastics in the marine environment mainly originate from land-dwelling activities that include microbeads in cosmetics (e.g., facial scrubs microplastic size < 2 mm) and in health care products (microplastic toothpaste size < 5mm), which contain polystyrene, polypropylene, polyethylene beads (Boucher and Friot 2017). Additional primary microplastic can also be found in car tires, plastic fibers in synthetic textiles (e.g., nylon), and plastic pellets usage in industrial manufacturers.

They contain minuscule plastic pieces that are intentionally manufactured and are discharged directly (~19 to 31%) into the marine environment as microplastics through various pathways (Fig. 4) (European Food Information Council, 2021). For instance, personal care products that contain microplastic are being discharged into wastewater systems through household drains; leaks during manufacturing or transportation that result in unintentional microplastic waste; or roughness caused by washing clothes created by synthetic textiles fibers.
Secondary microplastics are generated from the degradation of plastic bottles, bags, and fishing nets and account for 69-81% of microplastic in the ocean (Fig. 4) (European Food Information Council, 2021). The primary sources of secondary microplastic in the marine environment mainly originate from larger pieces of plastic. Over time, the long-term exposure to chemical, physical, and biological, including sunlight radiation and ocean waves, contributes to the degradation of plastic waste to the point that it is not visible to the human eye. Studies show the combined influence of various environmental factors. The breakdown process for synthetic polymers from larger plastic materials begins when deposited in the oceans (Costa et al., 2018). This process of degrading larger plastic pieces into microplastics is also known as fragmentation. The polymers' decomposition rate is slow, making them highly persistent in the marine environment; they endure for hundreds of years, causing wildlife implications (Costa et al. 2018).

### 3.2 Physical and Chemical Properties of Microplastics?

Microplastic is a pollutant with a wide range of chemical and physical properties. The breakdown of these polymers is composed of long chains consisting of hydrogen, oxygen, carbon, chloride, and silicon, obtained from coal by-products, oil, and natural gas (Roland et al., 2017). A wide range of synthetic polymers generates the term plastic. The most predominant plastic synthetic polymer in the marine environment is polyethylene, polystyrene, polypropylene,
low-density polyethylene, high-density polyethylene, polyvinyl chloride, and polyethylene terephthalate.

As shown in Table 2, the seven groups of synthetic polymers account for 90% of the earth’s plastic pollution (Roland et al., 2017). Many plastic polymers have high levels of chemical additives, which are used to strengthen the functionalities of the plastic (i.e., flame retardants and plasticizers). Although, plastic additives and polymers are chemical components that contribute to microplastic contamination in the marine environment (Roland et al., 2017).

Table 2: Predominate Microplastic Pollution in Marine Environment (Information pulled from Roland et al. (2017))

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Structure</th>
<th>Primary Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene (PE)</td>
<td><img src="image" alt="Polyethylene Structure" /></td>
<td>Packaging industry (shampoo bottles, milk jugs, water bottles, and plastic bags)</td>
</tr>
<tr>
<td>Polystyrene (PS)</td>
<td><img src="image" alt="Polystyrene Structure" /></td>
<td>Beverage/foam cups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Toys</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Window in envelopes</td>
</tr>
<tr>
<td>Polypropylene (PP)</td>
<td><img src="image" alt="Polypropylene Structure" /></td>
<td>Heavy-duty microwavable containers</td>
</tr>
<tr>
<td>Low-Density Polyethylene (LDPE)</td>
<td><img src="image" alt="Low-Density Polyethylene Structure" /></td>
<td>Plastic bags</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plastic wraps</td>
</tr>
</tbody>
</table>
The physical components of microplastic particles are available in a wide variety of shapes and sizes that cannot be seen by the naked eye, especially in the marine environment. With the aid of microscopes, particles are generally classified into five different categories based on the particle shape and chemical make-up: fragments, fibers, pellet, and film and foam (Table 3) (Wu et al., 2018). The distinctions between microplastic shape, size, and chemical make-up can impact the way particles travel through the marine environment and possibly alter the potential for toxicity (Wu et al., 2018).

Table 3: Microplastic is classified into five categories based on shape and size: fragments, fibers, pellets, film, and foam. Information pulled (from Wu et al., 2018)

<table>
<thead>
<tr>
<th>Plastic Categories</th>
<th>Characteristics</th>
<th>Picture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fragment</strong></td>
<td>rough, rugged, jagged particle</td>
<td></td>
</tr>
<tr>
<td><strong>Fiber</strong></td>
<td>thin, fibrous line; straight plastic</td>
<td></td>
</tr>
</tbody>
</table>
Pellet | hard and rounded particle
---|---
Film | thin, flimsy plastic
Foam | lightweight, soft, porous plastic

### 3.3 Plastic Lifecycle, Fate, & Transportation

The initial phase of the microplastic lifecycle starts with creating macroplastic through mining or drilling methane gas and crude oil (Brown, 2021). Land-based anthropogenic activities are the primary route of ocean pollution that leads to microplastic fragments. The potential lifecycle of microplastic in the marine environment goes through various processes of (1) weathering, (2) chemical leakage from plastic, and (3) human and wildlife ingestion (Fig. 5) (Sutton et al., 2016). Polymers are highly challenging to break down due to their chemical structure that never entirely degrades. Instead, plastic waste is broken down into microplastics in the marine environment by a slow weathering process of photo-oxidative degradation. The polymer reacts with oxygen and sunlight in the marine environment (Fig. 5).
Fig. 5: (from Ocean Garbage Patch, 2022) Potential lifecycle of microplastic in the marine environment. It starts with plastic litter in the ocean that weathers from photo-oxidation. The microplastic is degraded, and depending on the material, it either sinks or floats. Over time chemicals leach into the ocean from the plastic and disperses, while other hazardous toxins attach to the microplastics. Eventually, microplastic is spread through the food web.

Thus, microplastics will either float or sink based on the polymer material. For instance, microplastic composed of polypropylene and polyethylene are lighter in weight and are positively buoyant, will cause plastic particles to float on surface waters, and distribute to a larger area of waterways (Sutton et al., 2016). The large oceanic (gyres) current will further break down microplastic and accumulate the floating particles. In contrast, plastic particles composed of polyvinyl chloride, polyester, and polystyrene have a higher density than surface water. They are negatively buoyant, causing microplastic to sink to the bottom of the seabed and accumulate in the hadal zone (the most profound part of the sea and possibly the earth's most significant microplastic sink) (Sutton et al., 2016).

As plastics are weathered and degraded into microplastics, plasticizers and additive chemicals associated with plastic waste leach into the ocean and disperse in the waterways (Costa et al.,
Studies have analyzed that microplastics dispersed in the marine environment can acquire persistent organic contaminants (i.e., PCBs, PAHs, DDT, & HCB). The persistent organic pollutants have a higher binding affinity for plastics than water, and since microplastic is abundant in the surrounding water, it increases the risks of adverse ecological impacts (Costa et al. 2018). Microplastic's distinct chemical properties, size, and long lifespan make marine organisms mistake plastic particles for food. Furthermore, microplastics can be reintroduced to marine organisms through digestion, passing, decomposition, and eventually transported along with the food web (Prata et al., 2021). Thus, making the release and binding of the small plastic to toxic chemicals easily assessable to a large spectrum of marine organisms, including humans. The following section below is a literature review on the potential human health implication of microplastic contaminated marine organisms.

4. Literature Review on the Bioaccumulation of Microplastic that Threatens Marine Wildlife and Potential Human Health Risks

4.1 Microplastic Exposure to Marine Wildlife

The main pathway of human exposure to microplastics is seafood ingestion (Rochman et al., 2015; Smith et al., 2018). This section will focus on the bioaccumulation of microplastics in marine wildlife that threaten human exposure to microplastic through contaminated seafood consumption. Each study analyzed the adverse health implications resulting from the bioaccumulation of microplastic in marine tissues for fish and mussel species. One of the common ways to digest microplastics from marine organisms includes soft tissue (wet weight), a digester (hydrogen peroxide), and catalyst (Fe (II) combined in a beaker for a 24-to-48-hour period.

Furthermore, to assess human health implications in the body, the studies reviewed the adverse implications in human cell lines in cerebral cells; human gastric adenocarcinoma epithelial cells; placental perfusion models that mimic biological behaviors of the placental tissue cells; and adult rats because of their genetic and biological similarities to humans. A standard protocol used for quantifying and characterizing microplastics in the studies reviewed utilized the Fourier
Transform Infrared Spectroscopy (micro-FTIR spectra). A micro-FTIR spectrum is an essential tool to identify unknown materials because it offers precise data about chemical interactions and molecule structures; the FTIR is valuable for analyzing organic and inorganic microplastic materials. In addition, the absorption of infrared radiation at various wavelengths is used to identify microplastic compounds generating an infrared spectrum. Compared to standardized microplastic identification recorded in libraries, the spectrum is employed as a DNA fingerprint.

Microplastics cause obstruction, bioaccumulation, and inflammation in multiple marine organisms (Rochman et al., 2015). The distribution and abundance of ingested microplastics across the trophic levels of the marine food web are determined by each organism's feeding methods. Microplastics bioaccumulate in the gastrointestinal tract, gills, and dorsal muscle tissues in marine wildlife, which can cause detrimental cellular alterations (Stepniak et al., 2018). Bioaccumulated microplastics cause neurotoxicity, oxidative stress, reproductive stress impairment, and physical and chemical toxicity (Barboza et al., 2020). This section examines the adverse implications of ingesting microplastics in primary food sources from the marine wildlife that pose potential risks to human health through the following: 1) fish and 2) mussels.

4.1.1 Fish

The abundance of microplastic in marine ecosystems increases the risks of bioaccumulation in fish tissue. However, their feeding habits regulate the amount of microplastic consumed by fish species. Various marine organisms such as anchovies have selective diets preventing bioaccumulation of microplastics. However, other fish species like European bass (Dicentrachus labrax), Atlantic horse mackerel (Trachurus trachurus), and Atlantic chub mackerel (Scomber colias) hunt other prey, making them more vulnerable to ingesting microplastics (Barboza et al. 2020). Microplastics taken up by the gills are retained during the water filtration process, highly dependent on the microplastics' size, shape, and morphology (Schirinzi et al., 2017). In addition, the presence of microplastics in the dorsal muscle specifies the ingestion of microplastics has been rooted in the tissue; fish can also bioaccumulate microplastics through the skin by lesions and skin alteration (Fig. 6).
Fig. 6 (Barboza et al., 2020) Microplastics are being ingested, retained, and internalized by fish species in this conceptual model.

Barboza et al. (2020) detected the presence of microplastics found in the gill, muscle, and gastrointestinal tract tissues in three commercially sold fish: European bass, Atlantic horse mackerel, and Atlantic chub mackerel in the Northeast Atlantic Ocean. Microplastics were identified in 49% of the fish (73 fish) of the 150 fish tested (50 per species), where 35% (52 fish) were found in the gastrointestinal tract, 36% (54 fish) were found in the gills, and 32% (48 fish) in the dorsal muscle (Fig. 6) (Barboza et al., 2020). Furthermore, the 150 species yielded 368 microplastic particles, where 175 came from the gastrointestinal tract, 112 microplastics were identified in the gills, and 81 microplastics from the dorsal muscle.

A wide variety of effects have been found in fish that contained significant amounts of microplastics in the gastrointestinal tract (175 microplastic) and dorsal muscles (81 microplastic) concentration than gills (112), which promotes neurotoxicity. Therefore, acetylcholinesterase
inhibition negatively impacts nerves and muscles' functionality because the bioaccumulated microplastic in fish promotes lipid oxidation, a process in which free radicals steal electrons from the lipid cell membranes, producing cell damage. Ingestion of microplastics impairs the activity of energy-related enzymes (lactate dehydrogenase and isocitrate dehydrogenase) in the brain, muscle, and tissues resulting in cell damage (Barboza et al., 2020).

The frequency rate of microplastic in farmed Nile tilapia (*Oreochromis niloticus*) and wild freshwater fish from Colombia (*Prochilodus magdalenae*) were measured (Garcia et al., 2021). The methods used to extract microplastics from fish tissues used 200 milliliters of 30% hydrogen peroxide, along with 5 grams of tissues taken from the gut, gill, and flesh. Each tissue sample was put into separate beakers for 24 hours. The results showed no significant difference in microplastics found within the gut, gill, and flesh tissues from farmed and wild fish, as shown in Fig. 7. The main differences showed higher microplastic in gill and gut tissues than in flesh tissues for farmed and wild fish analyzed. The micro-FTIR spectra and PerkinElmer Spectrum Spotlight (16 gold-wired detectors) were used to quantify the amount of microplastic.

With the help of these two methods, Garcia et al. (2021) identified 161 microplastics that also surpassed the acceptable threshold for a polymer identity match rate of 70%. The 161 particles were speculated from the filter that contained fish tissues, and 53% corresponded to cellulose. Only 44% of farmed fish had microplastics, and about 77% were identified in wild fish (Garcia et al., 2021).
Fig. 7 (from Garcia et al., 2021) Box plot demonstrates the abundance of plastic particles per gram of tissues in gut, gill, and flesh from farmed and natural fish sources. The geometric dots that are shown above and below the whiskers that are show outliers in the data.

The prevalent microplastics in all fish were polyethylene terephthalates, polyesters, and polyethylene (Garcia et al., 2021). The farmed fish had a more comprehensive range of polymer types. The edible flesh in fish exhibited a lower prevalence of microplastics than gill and gut with a higher frequency and diversity (Fig. 7). In this preliminary study, the occurrence and form of microplastics are different in farmed versus wild fish and tissue types; significantly fewer microplastics are detected in edible flesh than in the gill and stomach (Garcia et al., 2021). Fish mistake microplastics for food, which bioaccumulate in their gastrointestinal tract, gills, and flesh, whether wild or farmed. Although fish are not the only marine organisms affected by microplastics, mussels are also at risk, as humans consume microplastic-polluted seafood. Mussels are primary consumers and the first to be exposed to microplastic particles. Although, compared to fish and other seafood, mussels bioaccumulate the most microplastic particles because of their unique natural filtering system.
4.1.2 Mussels

Green-lipped mussels, a popular delicacy sold in New Zealand and Thailand markets, have a 100% microplastic detection rate in one study by Bouwmeester et al. (2015). Mussels are valuable natural filter feeders in a microplastic-polluted marine environment. Depending on the size and abundance of microplastics, mussels can either filter the particles out or retain them (Bouwmeester et al., 2015). Although, the microplastic exposure in marine fish is most likely caused by the ingestion of plastic particles from surface water, seabed, or prey already bioaccumulated microplastic.

A study conducted in New Zealand coastal waters by the green-lipped mussel (*Perna canaliculus*) was examined to have abundant microplastic in the range of 0 to 1.5 microplastic particles per mussel with a concentration of 0 to 0.48 particle per gram of tissue in wet weight in six out of the nine regions (n = 12 per location site) (Webb et al., 2019). Following the results, another study by (Imasha and Babel, 2021) detected microplastic in 90 green mussels, analyzed in three different markets in Thailand (Khlong Dang, Talad Thai, and Talad Siummang).
The identification of microplastics in mussel tissues using the micro-FTIR picture and FTIR spectra for microplastics in Thailand markets Khlong Dang, Talad Thai, and Talad Siummang. The figure shows the most common polymers and percent match rate using the micro-FTIR spectroscopy found within the mussel tissues: 90.15% match ethylene/propylene copolymer, 92.22% match polypropylene, 92.14% match polyethylene, and 83.41% match of polyethylene terephthalate polymer material.
A standard method used to extract microplastics includes soft mussel tissue (0.05-millimeter precision), a digester (300 milliliters of 30% of hydrogen peroxide), and a catalyst (0.1 molar Fe (II) were added to a beaker for a 24-to-48-hour period. The green-lipped mussels detected an average microplastic bioaccumulated of 7.32 to 8.33 items per mussel gram and 1.53 to 2.04 items per gram in wet weight (Imasha and Babel, 2021). Moreover, fragments were predominated in the mussel tissues by 75.4%, followed by 24.6% fibers.

The micro-FTIR spectra and the Nile Red staining method were used to quantify and characterize microplastic shapes to identify microplastic particles in the soft mussel tissues. The imagining taken from the micro-FTIR spectra was taken to identify microplastic materials that included rubbers, fillers, paints, resin, adhesives, and coatings. However, the predominant microplastic found within the soft mussel's tissues matched with the FTIR spectra with the polymer library database (Hummel Polymer Sample Library). An 80% polymer identification for microplastic is considered acceptable, and a 90% or greater is a confirmation for identifying the characteristics of the microplastic. In the study, identification match rate percentages of ethylene/propylene copolymer (90.15% match rate), polypropylene (92.22% match rate), polyethylene (92.14% match rate), and polyethylene terephthalate (83.41% match rate) were identified in the mussel's soft tissues (Fig. 8) (Imasha and Babel, 2021).

These results suggest that eating green-lipped mussels as a dish may increase microplastic exposure to people. Moreover, the cultivation strategy for the green-lipped mussel affects the form and polymer composition of microplastic. Smaller-sized microplastic (<5μm) was analyzed to be more predominant within the green-lipped mussels and are likely to have higher health impacts (Imasha and Babel, 2021).

Webb et al. (2020) conducted another green-lipped mussel study. However, this study exposed the soft mussel tissues to microplastic and triclosan (a chlorinated organic compound with antifungal and antibacterial properties) simultaneously and individually. The triclosan was mainly used as a disinfectant in households and is seen abundantly in the marine environment through wastewater treatment plants and stormwater. The study tested soft mussel tissue and followed the same microplastic extraction methods. The microplastic particles and triclosan were
tested separately (Webb et al., 2020). Following this test, another test was set up to combine microplastic particles and triclosan with the soft mussel tissue. The separate and combined tests of microplastic and triclosan indicated decreased oxygen uptake. The combined test resulted in a triclosan spike onto the microplastic, increasing mussel superoxide dismutase and lipid peroxidation activity (Webb et al., 2020). Superoxide dismutase is in all living cells, which increases chemical reactions within the body. The evidence of increased superoxide enzyme indicates an increased breakdown of harmful oxygens of the superoxide radical into oxygen and hydrogen.

Although superoxide is a by-product of oxygen, the increased superoxide dismutase in mussels may cause cell damage if not regulated. In addition, lipid oxidation elevations in mussels indicate a free radical chain reaction, when free radicals steal electrons from lipids containing carbon-carbon double bonds (primary polyunsaturated fatty acids) from the cell membrane in cell damage (Webb et al., 2020). The negative consequences of superoxide in cells caused by hydrophobic contaminants and microplastics raise concerns about bioaccumulation and trophic transfer that affects human health by consuming contaminated microplastic fish and mussels. In addition, microplastic mussel consumption, on average, retains as high as 8,407 microplastic particles annually per person for Canadians (Bouwmeester et al., 2015).

4.1.3 Estimated Human Consumption of MPs

There is no standard method for assessing microplastic consumption from seafood. Various methods for determining and identifying microplastics have, nevertheless, been developed. Plastic particle extraction and characterization are the first steps in evaluating the presence of microplastic in marine species. The extraction and characterization of microplastics from biological tissues involves several methods. Basic, acidic, or oxidative conditions can degrade pH-sensitive microplastic polymers and are among the several digesting methods for removing microplastic from marine species tissues (Mercogliano et al., 2020). As a result, analyzing microplastics becomes challenging. Although enzymatic digestion looks practical, it is not a feasible tool for broad microplastic detection due to its high cost.

The European Food Safety Authority estimated that ingestion of contaminated fish in 1-year-old children averages 112 microplastic items per year (Elizalde and Gómez, 2021). In addition,
children two- to six years of 140 microplastic items/per year, six years and older 562 microplastic items/year, and the general population (adults) ingested 842 microplastic items per year, as shown in Table 4 (Elizalde and Gómez, 2021). Thus, indicating microplastic is prevalent in all age groups.

Table 4: (from Elizalde and Gómez, 2021) Estimated human ingestion of microplastics found in *Dicentrarchus labrax*, *Trachurus trachurus*, and *Scomber colias*. Following the EFSA recommendations, ingestion of MPs contaminated fish per week in children (varying ages), adults, and the general population

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>Adults or the general population</th>
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<tbody>
<tr>
<td></td>
<td>(1 y)</td>
<td>(2–6 y)</td>
</tr>
<tr>
<td>g fish muscle/week</td>
<td>40 g</td>
<td>50 g</td>
</tr>
<tr>
<td>MP items/week</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>g fish muscle/year</td>
<td>2080 g</td>
<td>2600 g</td>
</tr>
<tr>
<td>MP items/year</td>
<td>112</td>
<td>140</td>
</tr>
</tbody>
</table>

Nicole (2021) conducted a literature review from 50 primary peer-reviewed research studies that analyzed the microplastic in the crustacean, mollusks, and fish (sardines and anchovies) by a novel risk of bias quality assessment to examine the experimental design and execution (Nicole, 2021). Each marine organism was weighted by microplastics per gram (wet weight). The finding suggests a range of 0-10.5 microplastic/g mollusks, 0.14-8.6 microplastic/g in crustaceans, and 0.35-2.3 microplastic/g in fish with multiple data gaps in the study; the annual estimated maximum human ingestion is ~53,864 microplastic particles (Nicole, 2021). The calculation was based on the global consumption of 15.21 kg/ per individual for fish, 2.65 kg/ per individual for mollusks, and 2.06 kg/ per individual (Nicole 2021).

Thus, it indicates that human consumption of marine organisms contaminated by microplastic is evident. However, given the volume of research in this field, comparing available data becomes challenging because of the wide variety of research methods and quantifying units used. It is
essential to analyze microplastic transport through trophic transfer to narrow the knowledge gap. A standardized method of measuring is necessary to assess the potential health effect on humans.

4.2 Potential Toxic Effects of Microplastic on Human Health

The bioaccumulation of microplastic in contaminated seafood may potentially result in adverse human health implications through consumption. Several research studies have claimed that substantial concentrations of microplastic may generate hazardous effects for living organisms in multiple research studies conducted in-vitro (Velázquez and Gómez, 2021; Forte et al., 2016; Schinrinzi et al., 2017); ex-vitro (Barboza et al., 2018; Grafmueller et al., 2015), and rat experiments (Amereh et al., 2019; Amereh et al., 2020). Evidence shows that synthetic microplastic smaller than 150μm can absorb into the mammalian gastrointestinal epithelium and plays a crucial role in food digestion, absorbing nutrients, and protecting the human body from pathogenic infections. Although only 0.3% of these microplastic particles are absorbable, even microplastic between 5μm to 10μm (0.1%) can reach cell membranes and organs (Barboza et al. 2018).

However, there is no documented literature stating the harmful effects of microplastics on the human body because there are no epidemiology studies that have been done on humans. This section will examine the toxicological consequences in human cell lines that microplastic have at various levels through the ingestion of contaminated seafood through the following: 1) nervous system, 2) digestive system, 3) placental barrier, 4) endocrine disruptors, and reproductive toxicity (Fig. 9).
### Nervous System

Exposure to MPs increased cerebral human cells (T98G) & how oxidative stress is a vital mechanism of cytotoxicity cellular level (Schirinzi et al. 2017).

### Digestive System

- MPs effected gene expression in gastric adenocarcinoma cell, which caused inflammation, changes in cell morphology & cell viability (Forte et al. 2016)

### Placental Barrier

Exposure to MPs suggest syncytiotrophoblast plays a crucial role to transferring MPs to the placenta (Barboza et al. 2018); (Grafmueller et al. 2016).

### Endocrine Disruptor & Reproductive Toxicity

- MPs exposure hinder spermatogenesis process and causes endocrine disturbance by histological lesion (Amereh et al, 2020)
- MPs significantly increased thyroid stimulating hormone and the suppressed serum levels and the T3 hormone (Amereh et al. 2019)

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**Fig. 9.** An overview of the potential toxicity of MPs on diverse human cells in peer-reviewed literature studies. (Author)

#### 4.2.1 Nervous System

Schirinzi et al. (2017) conducted a study on the cerebral cells (T98G) and epithelial (HeLa) human cells to better comprehend cell viability and cell oxidative stress (analyzing reactive oxygen species effect) levels. To further evaluate the cytotoxicity of metal oxides (ZrO$_2$, CeO$_2$, TiO$_2$, and Al$_2$O$_3$), carbon nanomaterial (C$_{60}$ fullerene and graphene), polyethylene, and polystyrene consequence of microplastics in the human cells, they were exposed to microplastics that included metal oxides, carbon nanomaterial, polyethylene, and polystyrene in various
concentrations of 50μg/mL to 10 mg/L in vitro. According to their findings, the microplastic exposure showed no significant signs of reduced cell viability. Thus, cytolysis of the T98G and HeLa did not occur (Schirinzi et al., 2017).

Although, the synergistic and antagonist interactions between organic pollutants such as organophosphate insecticides, surfactants, and plasticizers bound to microplastics were evaluated. This study suggests that oxidative stress is a vital mechanism of cytotoxicity at the cellular level that was analyzed in both cell lines, which are concurrent with current studies of the adverse implication of microplastic that are bound to organic pollutants (Schirinzi et al., 2017).

4.2.2 Digestive System

Microplastic exposure may present toxicity within the digestive system through the ingestion of marine contaminated seafood—the internalization of various molecules, including microplastic entering cells uptake in various pathways (Bodei et al., 2014). For instance, clathrin-mediated endocytosis is a pathway that needs the help of the clathrin protein to form circular, coated vesicles around the microplastic. The coating of the microplastics occurs in the cytoplasm for trafficking the plastic particle inside the cells with additional help from the AP-2 complex proteins (Fig. 10). Although, clathrin can coat particles between 60 to 200nm. Particles more significant than that cannot be completely coated. Particles less than 60nm cannot absorb clathrin (Bodei et al., 2014).

In addition, Forte et al. (2016) conducted an in-vitro toxicological research study on the human gastric adenocarcinoma epithelial cells. In clathrin-mediated endocytosis, they evaluated 44nm and 100nm uptake kinetics of unmodified polystyrene microplastic. Both 44nm and 100nm polystyrene microplastic showed a dependent absorption mode and a clathrin-mediated endocytosis route, as shown in Fig. 10, where cells absorb proteins, hormones, and in some cases, microbes (Forte et al., 2016).
Thus, the internalization of the microplastic fuses to the early endosomes transporting it to the lysosome cycle, which leads to further degradation of microplastic due to the acidic environment in the early endosomes. The degraded microplastic particle is then open for cellular use, which can cause multiple implications in the human cell lines, including degraded adenocarcinoma cell viability and distorted cell morphology in epithelial cell lines.

In this case, Fort et al. (2016) report that the absorption of polystyrene microplastic with a concentration of 10µg/L of 44 nanometers strongly up-regulated the IL-6 (protein-coding) and IL-8 (mediator in inflammatory responses) genes; the most significant cytokines attribute in gastric pathologies studies. As a result, the absorption of polystyrene microplastic negatively impacted cell morphology, cell viability, and inflammatory gene expression and negatively impacted human health (Forte et al., 2016). Moreover, recent studies have also measured adverse effects in microplastic exposure studies on human cell lines.
A recent study by, Velázquez et al. (2021) conducted an in-vitro incubation study on positively charged polystyrene microplastic in cell lines with a concentration of 10g/L, 50 g/L, & 100g/L. The incubation resulted in a concentration reduction in adenocarcinoma cell viability, HT29 (a cell line with epithelial morphology), and Caco2 (epithelial cell from colon tissue), which was confirmed by an optical microscopy demonstrating completely distorted cell morphology from the interaction of microplastic (Velazquez and Olivan 2021).

Table 5: The function of caspase enzymes in apoptosis in human cell lines.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-3</td>
<td>It catalyzes the selective breakdown of several critical biological proteins.</td>
<td>Velázquez et al. (2021)</td>
</tr>
<tr>
<td>Caspase-7</td>
<td>Building and helps cell detachment in apoptosis.</td>
<td>Velázquez et al. (2021)</td>
</tr>
<tr>
<td>Caspase-9</td>
<td>Regulates both normal cell death and degenerative tissue</td>
<td>Velázquez et al. (2021)</td>
</tr>
</tbody>
</table>

The results reveal that high polystyrene concentrations affect the normal cell death processes in apoptosis (the function of regular and controlled death of cells). In apoptosis, all caspase enzymes play a critical role in regulating cell death and inflammation, which increases cytotoxicity (Forte et al., 2016). Caspases 3, 7, and 9 displayed changed functions with polystyrene interactions (Table 5). Thus, generating high rates of abnormal cell death during the apoptotic process in human cell lines with microplastic polystyrene interactions may adversely influence human health through microplastic ingestion, including transplacental transfer in pregnant women.

4.2.3 Placental Barrier

The unknown exposure to microplastics from contaminated seafood during pregnancy raises the possibility of intrusion into the human placenta barrier (Muoth et al., 2016). In both ex-vivo experiments examined, a human placental perfusion model was employed to investigate the effects of microplastic circulation in the blood vessels between fetal and maternal tissues over the placental barrier. A human placental perfusion model is a non-invasive and practical way to investigate microplastic and environmental chemical transplacental transit in humans.
As a result, the syncytiotrophoblast made up of epithelial tissue cells of the human placenta acts as a barrier between the mother and fetal blood. It is examined that the syncytiotrophoblast plays a crucial role in transferring microplastic into the human placenta (Grafmueller et al., 2015; Wick et al., 2010). The placental barrier allows nutrient and gas exchange. In addition, the translocation of polystyrene microplastic beads in the placenta model may be a transport pathway that is energy-dependent and not passive. Studies indicate that polystyrene beads up to 240 nanometers can cross the placenta barriers with the aid of the syncytiotrophoblast (Wick et al. 2010).

Following these results, Grafmueller et al. (2015) examine whether microplastics can intrude through the placental barrier and impact the fetus and determine if this process is relevant to the microplastic particle size with carboxylate modified polystyrene (50-300nm). The main findings indicate that polystyrene beads crossed the placental barrier accumulated in the syncytiotrophoblast of the placenta tissue. Both studies provide evidence that translocation of microplastic with the aid of syncytiotrophoblast in the placenta is relevant to size (Grafmueller et al., 2015; Wick et al., 2010). Thus, reducing the knowledge gap highlights the necessity for more microplastic toxicological research on this vital organ system (Barboza et al., 2018; Wick et al., 2020).

4.2.4 Endocrine Disruptor & Reproductive Toxicity

Microplastic contains and leaches dangerous endocrine-disrupting chemicals (e.g., BPAs), damaging the reproductive and hormonal systems. The endocrine disruption and reproductive toxicity linked to polystyrene and microplastic ingestion were evaluated in male rats in two studies (Amereh et al., 2019; Amereh et al., 2020). Laboratory rats are commonly used in research experiments as a model because they are genetically, physiologically, and anatomically like humans. In addition, rats have significant benefits, including a small body, easy management, relatively short lifespan, and abundant genetic resources (Amereh et al., 2019; Amereh et al., 2020). Thus, making them great species to examine possible human exposure to microplastic poses as they pose a substantial danger to endocrine disruption and reproductive toxicity, where the interactions with cells and tissues are primarily unknown. Thus, to better understand the implications of microplastic exposure in adult rats, they were orally fed
polystyrene particles at doses of 1, 3, 6 and 10 mg/kg-day in both studies reviewed (Amereh et al., 2019; Amereh et al., 2020).

The rats exposed to polystyrene microplastics (average 38.92 nanometers) were analyzed for changes in the hormonal milieu, endocrine-disrupting signatures, and semen quality (Amereh, F. et al., 2020). The finding suggests that tissue, cell impairment, DNA damage, and alternation in the sperm morphology were noticed with a dose-response pattern. In addition, the RT-qPCR data showed a significant down-regulation of gene expression (Table 6) in rat testis (Amereh et al., 2020). In addition, microplastic exposed rats hinder the spermatogenesis process, and the endocrine disturbance was caused by microplastic bioaccumulation and histological lesion (Amereh et al., 2020).

Table 6: Function of the down-regulated gene expression in rat's testis conducted in two studies. The rat has expressed disturbance in the spermatogenesis and endocrine disturbances caused by microplastic accumulation and histological lesion.

<table>
<thead>
<tr>
<th>Gene Expression</th>
<th>Function</th>
<th>Negative Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>The promyelocytic Leukemia Zine Finger (PLZF)</td>
<td>A protein that aids in renewing and maintaining the early progenitor cell and stem cells.</td>
<td>Decreases the development and growth of cells.</td>
<td>Amereh et al. (2020)</td>
</tr>
<tr>
<td>Deleted in Azoospermia-Like (DAZL)</td>
<td>A protein that binds to RNA and is required for gamete formation.</td>
<td>Decreases spermatogenesis.</td>
<td>Amereh et al. (2020)</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone (FSH)</td>
<td>A hormone essential for sexual development and functioning.</td>
<td>Decreases spermatogenesis and sexual development.</td>
<td>Amereh et al. (2020)</td>
</tr>
<tr>
<td>1-thyroxine (T4)</td>
<td>Treatment for hypothyroidism, enlarged thyroid gland, and thyroid cancer.</td>
<td>Thyroid treatment.</td>
<td>Amereh et al. (2019)</td>
</tr>
<tr>
<td>1-free thyroxine (FT4)</td>
<td>Measurement is used to examine if the thyroid gland is working.</td>
<td>Biomarker to examine thyroid gland.</td>
<td>Amereh et al. (2019)</td>
</tr>
<tr>
<td>1-triiodothyronine (T3)</td>
<td>The regulation of hormone and cellular growth rate; supports the brain, heart, digestion, bone, and metabolic rate.</td>
<td>Decreases hormonal and cellular functionalities.</td>
<td>Amereh et al. (2019)</td>
</tr>
</tbody>
</table>
## 1-free triiodothyronine (FT3)

A measurement used to examine thyroid function; is elevated in hyperthyroidism and low in hypothyroidism. Biomarker to check thyroid functions. Amereh et al. (2019)

## Luteinizing Hormone (LH)


A previous study conducted by Amereh et al. (2019) detected the effects of thyroid endocrine status and biochemical stress within adult male rats through exposure to polystyrene microplastic (exact dosage of 1,3,6 and 10 mg/kg-day). The study compared the microplastic exposure in the rats to five thyroid hormones, I-thyroxine, I-triiodothyronine (T3), I-free triiodothyronine (FT3), and I-free thyroxine (FT4), along with the thyroid-stimulating hormone serum levels (Table 6) (Amereh et al. 2019).

Findings showed that exposure to microplastic significantly increased thyroid-stimulating hormone and the suppressed serum levels and the T3 hormone. However, there was a slight increase in FT3 and FT4 thyroid hormones. Furthermore, the exposure to microplastics indicated signs of kidney impairment and nephrotoxicity in exposed rats, which may result from significantly increased creatinine levels (Amereh et al., 2019). Thus, microplastic consumption indicates early warning signs of reproductive damage and provides evidence of endocrine disruption and metabolic deficit in exposed rats, potentially impacting human health.

### 5. SWOT Analysis: Monitoring Method for Microplastics Using Mussels

Despite the uncertainties of microplastic and their impact on human health and the environment, scientific research on mussels has revealed enough evidence to conclude that microplastics are hazardous. There are no regulations or standards to monitor the widespread presence of microplastics and their high bioavailability, which poses unknown risks to human health and marine wildlife. As a result, it is critical to have an accurate and uniform biomonitoring tool to evaluate methods for microplastic pollution.
To explore the biotic effects of microplastic exposure in mussels, it is essential that the following conditions must be met to have a reliable bioindicator for monitoring the marine environment, which include:

- A broad distribution range,
- A vital role in the ecosystem
- A well-studied biology
- Immobility – for site-specific data
- A uniform response to microplastic pollution
- Distinguishable toxic effects from the varied levels of microplastic exposure

Although mussels have been utilized as bioindicators for marine microplastic contamination in many studies, there is still a lack of a solid and apparent explanation for their usage as an indicator species. This section reviews scientific literature studies on microplastics in mussels based on field observations and laboratory tests. With the accumulated data, I conducted a SWOT analysis on mussels as a biomonitoring tool for microplastic contamination in the marine environment by looking at their (1) strengths, (2) weaknesses, (3) opportunities, and (4) threats (Fig.12).

5.1 Strengths

The mussel *Mytilus* *spp.* is the most researched species for recognizing microplastics, which has also successfully been used as a biomonitoring tool for contaminants in the aquatic environment (Gunther et al., 1999). *Mytilus* genus comprises seven subspecies that can crossbreed and are found worldwide (Fig. 11). Increased demand for farming mussels worldwide resulted in long-term transportation far from native water to regions around the globe, resulting in the vast geographical spread. As a result, some mussel species, such as *M. galloprovincialis*, have become invasive in South Africa, California, Japan, Australia, and New Zealand (Beyer et al., 2017b). Despite being an invasive species, the mussel's vast geographical dispersion and high environmental tolerances (Li et al., 2019) make them an ideal biomonitoring model for studying the fate of microplastics in humans and marine wildlife (Huang et al., 2021).
With data dating back to the 1960s, more than 50 countries have used mussels as an environmental biomonitoring species for trace elements and organic pollutants (Cantillo, 1998). To further access current and emerging contaminants, management programs like the California Mussel Watch Program and the Regional Monitoring Program were established to monitor the abundance of toxic substances. Thus, transplanted mussels in the San Francisco Estuary were used to monitor trace elements (Gunther et al., 1999).

Gunther et al. (1999) published results from 15 years (the 1980s to 1996) of biomonitoring for trace substances in the mussel (Mytilus californianus, Crassostrea gigas, and Corbicula funeina) in the San Francisco Estuary using data from the California Mussel Watch Program and Regional Monitoring Program. As a result, the mussels portrayed significant declines (p < 0.01) in PCBs, dieldrin, Ag, and cis chlordane, although results indicated a significant increase in chromium (p < 0.05). These findings suggest that mussels can provide site-specific data on microplastic contamination due to their immobility (Gunther et al., 1999). Also, when undertaken as part of a long-term program, biomonitoring with transplanted mussels can offer considerable information on the spatially and temporally estimated prevalence of specific contaminants in marine environments.

In addition, mussels are also known for their substantial filter feeding traits, which directly exposes them to microplastic pollution in the marine environment. During active feeding, the mussel's ciliated gills (positioned in the epithelium surface) aid in the pumping and filtration of seawater. They can pump about 50 milliliters of seawater per minute and 3 liters per hour, which cause mussels to ingest more particles over time (Li et al., 2019). Due to their filter-feeding traits, mussels can easily absorb chemical contaminants and particles from the ocean, which validate them as an ideal biomonitoring tool that can efficiently measure the concentration and bioavailability of microplastic pollution.

As primary consumers, mussels act as a transport system for anthropogenically generated microplastics, including human consumption, into the marine food chain. Because mussels are consumed entirely by humans, they are among the largest microplastic contributors. Based on a significant number of exposure studies (Huang et al., 2021; Li et al., 2019; Qu et al., 2018) globally, mussels have been verified as an ideal biomonitoring tool for better understanding the
uptake, bioaccumulation, and toxicity of microplastic in marine species and potentially human health. Thus, from a practical aspect, research highlights the feasibility and benefits of mussels as bioindicators for assessing microplastics.

5.2 Weaknesses

There are potential disadvantages to employing mussels as a biomonitoring tool for microplastics. Due to mussels' unique ecological and biological characteristics, scientists have been particularly interested in *Mytilus* spp. as an environmental biomonitoring tool for analyzing microplastic pollution in coastal water bodies. Although, because of their unique properties of high tolerance for various environmental factors, including salinity, oxygen levels, food supply, and water temperature, microplastic uptake may vary with their unique properties.

For example, Gunther et al. (1999) investigated the survival and condition rate of transplanted mussels in the San Francisco Estuary, indicating that environmental matrices in some places cause physical stress in the mussels. Suppose the mussels exhibit severe physiological stress or die. In that case, they are considered compromised and cannot be used as a biomonitoring tool because the uptake for contaminants will be influenced. In addition, the absorption rate for mussels will be stagnant and will not be able to maintain equilibrium with the marine environment (Gunther et al., 1999).

As a result, *Mytilus californianus*, commonly found on the west coast of North America, has a 50 to 150 percent saline tolerance level and has exhibited reasonable survival rates in Alameda, Yerba Buena Island, and Horseshoe Bay because of their salinity concentration (Gunther et al., 1999). However, during the winter seasons, an increase in freshwater caused a reduction in salinity in the aquatic environment. Thus, according to the Mussel Watch Program and Regional Monitoring Program monitoring stations in San Francisco Bay (Alameda, Yerba Buena Island, and Horseshoe Bay), the data showed low survival rates for transplanted mussels (Gunther et al., 1999).

Furthermore, mussels do not absorb all environmental contaminants in the same way. For instance, the accumulation factor (contamination pre-deployment vs. deployment) for trace metal contamination from deployed mussels from 1993 to 1996 differed from the examined species
(Mytilus californianus, Crassostrea gigas, and Corbicula fuminea) (Gunther et al., 1999). Such distinctions affect both species' accumulating capability and variability in the polluted spatial and temporal distribution in the Estuary. For instance, mussel species illustrated a low accumulation factor for mercury concentration (with the highest being 1.3) in the San Francisco Estuary; it also has a public health advisory for seafood consumption (Gunther et al., 1999). These findings suggest that mussels may not be an effective biomonitoring tool for evaluating microplastic pollution in the marine environment and human health concerns.

Measuring microplastic exposure in mussels is difficult worldwide due to significant inconsistencies in research findings due to their survival and condition rates. Even though several reviews have extensively analyzed the current microplastic identification methods and extraction techniques, there are still significant limitations, and discussions regarding the selection of transplanted mussels are a suitable methodology. Additionally, recommendations for standardizing the efficiency and validity of ecotoxicological investigations on microplastics are required, which should entail the characterization of physical qualities, chemical qualities, and quantification. Moreover, this essential information will aid future experimental exposure studies on mussels and help better understand the correlation between the implication of microplastics and adverse consequences on marine wildlife and human health.

5.3 Opportunities

Based on the literature analysis above, the suggested usage of mussels as a biomonitoring tool for microplastic pollution in the marine environment would be an essential water quality parameter. First, it is essential to promote the governmental agency to form a global collaboration group to investigate the implications of microplastic in mussels, including the underlying physical and psychological behavioral responses. The collaborative working group should be part of an international institution, such as the United Nations Environmental Programme (UNEP). It is necessary to organize a global collaboration group so that academics from other fields can share and debate monitoring strategies, including future goals to educate the public on microplastic reduction strategies. A possible way to promote this topic to the state members is through the Ad Hoc Open-Ended Expert Group, a group of experts established
during the third meeting of the UN Environmental Assembly that examines alternatives to reduce marine plastic litter, including microplastics from all sources.

Mussels are already being proposed as a biomonitoring technique for microplastic in several geographical locations. In Belgium, the bioaccumulation of microplastic particles in mussels has been classified as a criterion of marine health status. The levels of microplastic contamination in mussels have been observed in European databases and are identified as a growing concern for human consumption of seafood. Moreover, a workshop held in 2017 by the IOC Sub-Commission for the Western Pacific on the fate, source, distribution, and implications of microplastic pollution in the Pacific and Asian marine environments proposed that mussels be used as a biomonitoring species due to their worldwide abundance (Fig 11). Using mussels as a biomonitoring tool will allow repeated sampling and accurately reflect water quality parameters.

Fig. 11 (from Gaitán et al., 2016) The global geographical distribution of seven subspecies of *Mytilus* marine mussels.

Because mussels have been used in numerous worldwide studies, Norway supports them as a biomonitoring tool for microplastic in the marine environment (Gaitán et al., 2016). According to Lusher et al. (2017), mussels are an excellent biomonitoring species for microplastics smaller than 1 mm in waterbodies. Microplastic abundance in mussels is highly related to anthropogenic activities (Li et al., 2019). He reported a total number of microplastics ranging between 0.9 and
4.6 items/g and between 1.5 and 7.6 items/g in farmed and wild mussels. In wild groups, the microplastics of M. edulis (2.7 items/g) were higher than that of farming (1.6 items/g). The areas where human activities were intensively involved and significantly higher than (1.6 items/g) with less human activities showed the abundance of microplastics was 3.3 items/g. The abundance of mussels in contact with microplastic can aid in researching a standardizing method for measuring microplastic in mussels (Lusher et al., 2017).

Moreover, mussels can provide a comparable biomonitoring base, which is essential for establishing and implementing a standardized protocol. Using mussels as a standardized protocol can evaluate regional and temporal comparisons and investigate the presence of microplastics worldwide. Lusher et al. (2017) have provided a precise method for measuring microplastics in mussels and a possible benchmark standard. As a result, it is critical that inter-calibration across equipment and dataset exercises aid in confirming and coordinating methodologies utilized by different researchers.

Overall, standardized microplastic monitoring in mussels should be practiced globally. There is a scarcity of comparative data regarding microplastic pollution in mussels worldwide. Thus, it is beneficial to encourage researchers to utilize a combined standardized method for monitoring microplastics in the marine environment using mussels in the current monitoring studies. This will allow research studies to be comparable and focus on future opportunities to improve the implications of microplastic pollution in the marine environment. However, numerous problems remain unanswered, and additional criteria should be considered when developing an efficient and cost-effective method applicable for future mussel monitoring programs.

5.4 Threats

The threats of using mussels as a microplastic biomonitoring method include inefficient assessments induced by physiological stressors generated by microplastics. Recent research on mussel interaction with microplastics has discovered that mussels can actively eject and absorb 70% of plastic particles (Woods et al., 2018). Despite its favorable implications, microplastic consumption and expulsion resulted in a 50% reduction in algal intake (mussel's primary food source). Over time, this decreased feeding capability progresses into nutritional deficiencies and a weakened state (Woods et al., 2018). The poor nutritional state may impair mussels' growth
development and the ability to grip surfaces, causing them to drift and collapse from strong tidal waves. A recent study conducted by Green et al. (2019) reported that exposure to microplastic reduces blue mussel species' attachment strength and hemolymph proteome (blood plasma-circulates oxygen, nutrients, and proteins). The blue mussels were exposed to polyethylene for 52 days where their ability to attach to hard surfaces was reduced to 50%, which was caused by decreased production of byssal threads that aid in an attachment (Green et al., 2019).

Mussels under physiological stress cannot provide accurate data for measuring the abundance and effects of microplastic in the environment. Therefore, hindering the process of being a biomonitoring tool. As a result, microplastic exposure may impair the mussel's ability to function as a biomonitoring instrument for assessing microplastics in the marine environment because of their physiological stressors and survival conditions.

Moreover, the National Oceanic and Atmospheric Administration conducted a research study using the Mussel Watch Program in the Milwaukee Estuary in Wisconsin. The study assessed (1) the concentration and size of microplastics and (2) the correlation of microplastics between three classes of chemical contaminants (alkylphenols, polyaromatic hydrocarbons, and petroleum biomarkers) in *dreissenid* mussels near an outfall of a wastewater treatment plant (Hoellein et al., 2021). Mussels were deployed for 30-day and 60-days and categorized in size, class, and body burdens from chemical contaminants. As a result, microplastic was identified in larger mussels during the 30-day deployment, but in smaller mussels, there were no distinctions between the sites for 30-day deployment or 60-days. In addition, there were no correlations between the chemical contaminants and microplastic particles in the mussels (Hoellein et al., 2021).

The microplastic concentration discovered in mussel tissues varied substantially across different sizes of dreissenid mussels and between different sample collection periods. These differences were more significant than or equivalent to the variability between sites (Hoellein et al., 2021). Because microplastic concentrations and chemicals were different across mussels and sites, comparing microplastic between mussels was impossible. As a result, the dreissenid was not an effective biomonitoring instrument for obtaining site-specific data on microplastics or chemical pollutants in mussel tissues.
Overall, I recommend using mussels as a biomonitoring method to assess microplastic pollution and its impact on marine animals and human health. Because of their wide geographical distribution, filter-feeding and site-specific data will be an excellent worldwide biomonitoring tool for applying standardized microplastic pollution measures. With the help of the Mussel Watch Program and Regional Monitoring Program (created in the 1970s), the Mytilus species can be employed in various long-term biomonitoring investigations to identify microplastic pollution as they have done for toxic substances in the San Francisco Estuary. In addition, mussels will aid in establishing a risk assessment and threshold for microplastic pollution. Moreover, to address the limitations and risks of utilizing mussels as a biomonitoring tool, researchers can examine the pre-deployment environmental circumstances of the transplanted mussels to understand their physiological stressors (Fig. 12).

![SWOT analysis on mussels as a biomonitoring tool for microplastic pollution in the marine environment.](Author)

6.1 California’s Prevention Strategies

California is a global leader leading in advanced scientific research on microplastic pollution that has been actively fighting against plastic pollution from adversely impacting marine wildlife and human health. California developed the 2018 *California Ocean Litter Prevention Strategy to prevent microplastic pollution: Addressing Marine Debris from Source to Sea*. The plan was a collaboration with the Ocean Protection Council (OPC) and the National Oceanic and Atmospheric Administration’s Marine Debris Program (NOAA MDP) (2018) to provide a framework in 6 years (2018-2024) to reduce plastic pollution in the ocean. In this preventative strategy, the focus of the OPC and NOAA MDP priorities are listed as three goals:

- **Goal 1 – Land Based Ocean Litter:** Encourage legislation prohibiting plastic garbage from entering the ocean to protect marine habitats and the species that rely on them.

- **Goal 2 – Microplastic and Microfibers:** Conduct advanced scientific research to better comprehend microplastics and microfibers in the marine environment and create and implement a practical framework to reduce plastic particle pollution.

- **Goal 3 – Fishing and Aquaculture Gear:** Reduce microplastic debris in the ocean from fishing-related activities.

In addition, to the three goals, a wide range of stakeholders’ objectives is to address land-based and ocean-based litter. The objectives were established by scientists, wastewater treatment managers, fishermen, frontline workers, and the plastic industry. The stakeholders prioritized six objectives:

- **Objective 1:** Reduce the number of common plastic products detected in the ocean through incentives and legislation aimed at companies and public organizations.
➢ **Objective 2:** Reduce the number of common plastic products detected in the ocean by changing the plastic product’s design, production, and management.

➢ **Objective 3:** Improve waste management strategies before litter enters the ocean.

➢ **Objective 4:** Conduct new research studies by using existing studies as a guide to develop feasible frameworks to manage macro and microplastic pollution in the ocean.

➢ **Objective 5:** Provide education on how ocean litter negatively impacts the ocean's ecosystem and wildlife.

➢ **Objective 6:** Reduce plastic ocean litter debris sources by conducting trash capture mechanisms effective for microplastic debris.

The two phases will support California’s efforts to reduce and manage plastic pollution in the marine environment, eventually achieving zero-plastic waste by 2030, including the economic recovery of recycled plastic. Despite ongoing attempts to reduce plastic pollution in the marine environment, California has an omitted feasible trash TMDLs for impaired water bodies in their strategies.

6.2 *Regulatory Implementation of TMDLS: Trash Capture Systems*

The benefits of implementing TMDLs for water bodies impaired by plastic polluted trash is such recreational and ecological uses (i.e., fishing, swimming, estuarine habitat, marine habitat, wildlife habitat, wetland habitat, fish migration, spawning, reproduction, shellfish harvesting, and endangered species) can be protected. Water bodies' recreational and ecological uses are impaired by an accumulation of plastic trash, which (i.e., suspended solids and microplastic debris) negatively harms the environment and prevents organisms from performing their ecological duties to the ecosystem. Common products that the Water Regional Boards staff has identified various types of plastic material, including plastic bottles, balls, toys, plastic containers, plastic straws, styrofoam cups and take-out containers, beverage cans, and plastic bags transported through the river system into the marine environment.
The purpose of using TMDLs for trash-impaired watersheds is to examine plastic pollution as a reduction and management approach for preventing microplastics from entering the marine ecosystem. In the Clean Water Act (CWA), each state must develop a TMDLs process for impaired water bodies listed in Section 303(d) that do not meet the water quality standards listed in a priority ranking system. TMDLs are based on a calculation (USEPA 2021):

\[
\text{TMDLs} = \Sigma \text{WLA} + \Sigma \text{LA} + \text{MOS}
\]

\[\text{TMDLs} = \text{Waste Load Allocation (point sources)} + \text{Load Allocation (nonpoint sources and background)} + \text{Margin of Safety}\]

The TMDL calculation measures the maximum concentration of the pollutant and is determined by a point source or nonpoint source pollution. TMDLs are used as a preventative technique to maintain water quality standards by establishing a framework for load reduction targets for the pollutant sources to protect marine wildlife, drinking water, and human health. In California, 73 impaired water bodies are listed for trash or debris, but only two water bodies (Los Angeles River Watershed and Ventura River Watershed) have implemented trash TMDLs (USEPA 2020).

6.2.1 TMDLs for Trash in Los Angeles River Watershed

Due to growing concerns about plastic trash in waterways, the Los Angeles (LA) Regional Water Quality Control Board enacted the nation’s first trash TMDLs for the LA River watershed to regulate trash as a pollutant; 80 percent of the river was damaged by plastic trash in the waterway. The plastic trash TMDLs went into effect in 2008, intending to achieve zero waste removal for nine years ending in 2016, because it impaired beneficial uses such as wildlife and recreational (Table 7) (California Regional Water Quality Boards, 2015)
The Los Angeles (LA) Regional Water Quality Control Board enacted a 9-year trash TMDLs implementation plan for the LA River watershed. The plastic trash TMDLs were implemented as non-point and point sources, with urban stormwater runoff being the primary source of pollution. Therefore, municipal storm drains, and Caltrans permittees became the compliance point for the plastic trash TMDLs process is the focus of achieving their standards. The baseline waste load allocation for municipal stormwater permittees and Caltrans for the watershed was reduced by 10% every implementation year. The compliance strategies used to reduce trash pollution for the TMDLs included full capture and partial capture devices (California State Water Board, 2015).
Fig. 13 (from California Regional Water Quality Boards, 2015) Graphs (a) and (b) represent the Los Angeles River Watershed trash TMDLS implementation plan in the year 2013 to 2014. The blue line reports the estimated trash reduction, and the red is the required reduction from the allocated baseline waste load. Graph (a) illustrates trash discharged in percentage (1% increase of trash reduction). Graph (b) illustrates 50 tons reduction in trash discharge.

In Fig. 13, we can analyze that the trash discharged reduced in both percentage and tons from the TMDLs implementation year of 2013 to 2014 for LA River Watershed significantly improved. Both graphs are based on full capture systems. Thus, estimates may be lower but still portray trash reduction by the end of 2014, where we can assess a 1% increase in trash discharge and a reduction of 50 tons of trash being discharged. Thus, implementing the trash TMDLs process was a successful attempt (California Regional Water Quality Boards, 2015)
As a result, yearly compliance requirements according to the plan were not met, but the water quality outcomes reported significant reductions in the trash. By 2015, all required measures and compliance points of sustaining 50% were met for the plastic trash TMDLs, including the structural controls for the stormwater drains systems, completed in 2013. Therefore, suggesting trash TMDLs should be implemented for waterbodies that are listed as impaired for reducing macro- and microplastic pollution in the marine environment (California Regional Water Quality Boards, 2015)

6.2.2 TMDLs for Trash in Ventura River Watershed

Following the LA River Watershed reductions of the plastic trash TMDLs, the Los Angeles (LA) Regional Water Quality Control Board asked for a water quality improvement TMDLs strategy for the Ventura River Watershed (Fig. 14). Implementing the plastic trash TMDLs was also a 9-year plan to achieve a 100% trash reduction plan. The primary pollutant sources came from urban stormwater runoff and non-point source runoff from adjacent lands, established by the Minimum Frequency of Assessment and Collection Program (MFAC). It is a program that was created by the California Water Boards to count collected trash from the full trash capture devices (California Regional Water Quality Boards, 2018).
The plastic trash TMDLs process included various compliance strategies such as full capture devices and MFAC procedures to reduce trash waste in the watershed to achieve their waste allocation goals. According to graph (a) the trash reduction in the watershed from 2010 to 2013, the MFAC protocols required each piece of plastic trash to be counted. Because the graph does not show a consistent decreasing trendline and counting each piece of trash was time-consuming, this method was ruled out, and full trash capture was adopted (California Regional Water Quality Boards, 2018).

![Graph (a) represents the trash reduction in Ventura River Estuary where pieces collected trash from 2010 to 2013; the trendline did not portray a decreasing reduction. Counting pieces of trash was resource-intensive in 2014. So, they changed the trash collection to weight (tons). Graph (b) represents the waste load allocation by installing a full capture device. The installation research was 100% in 2014, two years earlier than required.](image)
In 2014, MFAC revised its methods to full trash capture devices, which later became the primary approach, as shown in graph (b) (Fig. 15). In addition, they were able to achieve 100% installation two years earlier than the requirement because each full trash capture device was installed in each basin draining into the Ventura Estuary. From 2014 to 2017 started to show a decreasing trendline with the revised strategy where trash collected was collected in weight (tons). At the same time, the point sources will continue to use the full trash capture devices as an efficient reduction strategy for the plastic trash TMDLS for the watershed (California Regional Water Quality Boards, 2018).

6.2.3 Synthesis

Establishing plastic pollution reduction initiatives and viable plastic trash TMDLs method will aid in estimating the maximum concentration of trash pollution that can enter the waterbody. Thus, I evaluated the Los Angeles River Watershed and the Ventura River Watershed, the only two watersheds that have successfully developed trash TMDLs out of 73 trash-impaired water bodies in California. Both watersheds used full trash capture systems, including catch basins inserts (Fig.16) and a full capture vortex separation system (Fig.17).

The waste allocation target in the Los Angeles River Watershed was not met by 2016, although, as shown in graphs (a) and (b) in Fig. 13, garbage reduction in the watershed was improving, but not quickly enough. On the other hand, Ventura River Watershed used the same trash capture systems and achieved a 100% waste reduction by 2017 while deploying all trash capture systems by 2014 after transitioning to a functional matrix from pieces to weight to count the amount of trash gathered. Furthermore, the implementation of both watersheds took nine years, which is a lengthy procedure, but with the addition of a new cost-effective and efficient trash capture system, the TMDLs' requirements for L. A River Watershed is achievable.

Furthermore, a feasible TMDLs process can be utilized as a measuring instrument to maintain the pollutant's water quality guidelines for minimizing marine wildlife and human health concerns worldwide for microplastic ingestion degraded from plastic trash. Implementing a plastic trash TMDLs approach will aid in the determination of pollutant reduction goals and the development of microplastic pollution control techniques in the marine environment. Without an appropriate regulating framework, such as a feasible trash TMDLs for plastic pollution, there
would undoubtedly be irreversible environmental effects on marine wildlife and human health due to the abundance of microplastic. Still, it is also essential to evaluate the economic cost of implementing trash capture systems for TMDLs.

6.3 Comparative Analysis: Implementing Trash Capture Systems

The goal of conducting a comparative analysis is to offer practical, cost-effective, and efficient data that can be utilized to help design future frameworks for implementing trash TMDLs in impaired watersheds to minimize microplastic contamination in the marine environment. As a result, I evaluated the financial cost and feasibility of five different trash collection methods to determine which option is the most effective in reducing plastic trash from discharging in stormwater systems and wastewater treatment plants. The methods utilized for implementing trash TMDLs to achieve the baseline waste load allocations are (1) catch basin inserts, (2) full structural vortex separation system, (3) end of pipe nets, (4) bioretention rain gardens, and (5) wastewater treatment – reverse osmosis.

6.3.1 Catch Basin Inserts

A catch basin is a system that collects plastic trash and suspended debris from stormwater runoff before it empties into the ocean, river, or stream from drains or storm sewers. Installing a catch basin might cost up to $800 per insert; they have a 5mm mesh great for reducing microplastic debris (California Regional Water Quality Boards, 2007) (Fig. 16).
The catch basin inserts are characterized as a full capture device capable of removing large plastic waste and 5mm microplastic debris carried in stormwater runoff.

The California Regional Water Board report for L.A River Watershed in 2007 assessed that it was the least expensive of the three approaches for reducing trash in the short term. Because catch basins only capture a portion of the waste, they must be swept and monitored regularly. The cost of implementing the basins on a yearly basis would be $51.3 million (Table 8). The overall capital cost for retrofitting catchment basins for the whole LA River watershed (574 square miles) would be $120, including operation and maintenance (California Regional Water Quality Boards, 2007).

Table 8: (from California Regional Water Quality Boards, 2007) Catch basin inserts yearly costs (in millions) for capital, operation, maintenance, and services for the LA River Watershed to implement trash TMDLs.

<table>
<thead>
<tr>
<th>Number of years into the program</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capital Cost (yearly)</td>
<td>$12</td>
<td>$12</td>
<td>$12</td>
<td>$12</td>
<td>$12</td>
<td>$12</td>
<td>$12</td>
<td>$12</td>
<td>$12</td>
<td>$12</td>
<td>$00</td>
<td>$00</td>
</tr>
<tr>
<td>Operation &amp; Maintenance Costs (yearly)</td>
<td>$5.1</td>
<td>$10.2</td>
<td>$15.4</td>
<td>$20.5</td>
<td>$25.6</td>
<td>$30.1</td>
<td>$35.9</td>
<td>$41.0</td>
<td>$46.2</td>
<td>$51.3</td>
<td>$51.3</td>
<td>$51.3</td>
</tr>
<tr>
<td>Costs per year (servicing + capital costs)</td>
<td>$17.1</td>
<td>$22.2</td>
<td>$27.4</td>
<td>$32.5</td>
<td>$37.6</td>
<td>$42.1</td>
<td>$47.9</td>
<td>$53</td>
<td>$58.2</td>
<td>$51.3</td>
<td>$51.3</td>
<td>$51.3</td>
</tr>
</tbody>
</table>
6.3.2 Full Capture Vortex Separation System

A full capture vortex separation system is a permanent structural device; it is considered the best alternative for removing plastic pollution, including microplastic debris from stormwater runoff. The device separates trash and pollutants from the inflowing stormwater into a containment chamber through a screen (Fig. 17). The suspended solids in the containment chamber at the bottom of the device are known as the sediment storage sump. The continuous motion keeps the treated clean water flowing. Studies have reported that the system can remove almost all trash, including oil separation.

Fig. 17 (from Hydro International, 2020) The full capture vortex separation system depicted above is a specialized separator that uses vortex technology to remove 5mm trash and suspended solids, as the California Water Board requires.

Although the cost to implement and install a full capture vortex separation system is high, the yearly costs for servicing each unit are $2000. In addition, depending on the location, unit size capacity and cost may differ. For instance, the Ventura River Estuary trash TMDLs implemented the full capture system for a 4,000 acres point source (California Regional Water Quality Boards, 2008). Depending on the capacity, acres, and devices needed for the estuary, they could access the capital cost and yearly servicing fees.
Table 9: (from California Regional Water Quality Boards, 2008) Full capture vortex separation system yearly cost for implementing the devices as a trash TMDLs for Ventura River Estuary.

<table>
<thead>
<tr>
<th>Capacity</th>
<th>Acres (average)</th>
<th>Unit Capitol Costs</th>
<th># Of devices needed on Urban Portion of Watershed</th>
<th>Capital Costs</th>
<th>Yearly Costs for servicing all devices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 2 cfs</td>
<td>5</td>
<td>$12,800</td>
<td>788</td>
<td>$10,086,400</td>
<td>$1,576,000</td>
</tr>
<tr>
<td>6 – 8 cfs</td>
<td>30</td>
<td>$45,000</td>
<td>131</td>
<td>$5,895,000</td>
<td>$262,000</td>
</tr>
<tr>
<td>19 – 24 cfs</td>
<td>100</td>
<td>$90,000</td>
<td>39</td>
<td>$3,510,000</td>
<td>$78,000</td>
</tr>
</tbody>
</table>

In Table 9, the low-capacity system (1-2 cfs) reports that the watershed requires a higher number of devices (788) compared to the high-capacity system (19-24 cfs). Thus, retrofitting high-capacity systems is more beneficial due to fewer numbers of devices (39) required, and the capital costs ($3,510,000) and yearly servicing ($78,000) are also less compared to low-capacity systems (California Regional Water Quality Boards, 2008).

6.3.3 End of Pipe Nets

End of pipe nets is an economical technique for monitoring plastic pollution trash loads from stormwater drainage systems. The installment of the plastic mesh varies depending on the size.

In Table 10, the estimated costs for a release net can range from $10,000 to $80,000 (Table 10) (California Regional Water Quality Boards, 2008). Although the plastic mesh size is either ½ inch or 1 inch, it cannot prevent microplastic debris, as shown in Fig. 18. Because of their net size, this method is considered a partial trash capture system. The nets are placed at the end of the drainage pipe, and

Fig. 18 (from Storm Water Systems, 2022) The picture illustration of an end of pipe nets. Mesh size is either ½ inch or 1 inch and are engineered to capture plastic pollution from stormwater runoff.
the water will go through the mesh capturing all debris larger than 1 inch (Fig. 18). Thus, the end of pipe nets is only used as a monitoring or capturing system.

Table 10: (from California Regional Water Quality Boards, 2008) The end of pipe net sizes and the cost estimation of release nets.

<table>
<thead>
<tr>
<th>Pipe Size</th>
<th>Release Nets (Cost Estimates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>End of 3 ft pipe</td>
<td>$10,000</td>
</tr>
<tr>
<td>End of 4 ft pipe</td>
<td>$15,000</td>
</tr>
<tr>
<td>End of 5 ft pipe</td>
<td>$20,000</td>
</tr>
<tr>
<td>In 3 ft pipe network</td>
<td>$40,000</td>
</tr>
<tr>
<td>In 4 ft pipe network</td>
<td>$60,000</td>
</tr>
<tr>
<td>In 5 ft pipe network</td>
<td>$80,000</td>
</tr>
</tbody>
</table>

6.3.4 Bioretention Rain Gardens

Bioretention rain gardens are cost-effective and environmentally friendly, capable of preventing pathogenic microorganisms, hazardous pollutants, and microplastic debris from entering the marine environment from stormwater runoff. A bioretention rain garden, as depicted in Fig. 19, incorporates layers of porous media such as mulch, bioretention soil, gravel bed, and existing soil as a filtration system and resilient native plants. Although different soils have variable infiltration rates, the cost may vary.

The cost of installing a bioretention rain garden is determined by the type of soil present. As a result, if the soil is permeable and has a higher value of 0.2 inches of runoff per hour, the cost to build is $1.50 to $3.00 per foot square because the water may infiltrate the groundwater table, but if the soil is not permeable and has a value of fewer than 0.2 inches of runoff per hour. Then the cost per square foot is $4.00 to $6.00 because the stormwater runoff will migrate to surface water into pipes after the treatment from the bioretention rain garden. Also, bioretention rain gardens operate as a pre-treatment and prevent stormwater runoff from infiltrating combined sewer systems, reducing high-cost treatment for microplastic debris and saving residents money over time.
The bioretention cell model depicts a pre-treatment for urban stormwater runoff and how rainfall catches and filters microplastic particles via (1) native plants, (2) mulch, (3) bioretention soil, (4) gravel bed, and (5) existing soils. The stormwater runoff finally finds its way to the nearest body of water.

6.3.5 Wastewater Treatment: Reverse Osmosis

To prevent microplastics from entering the marine environment, wastewater treatment plants must modernize their equipment to include reverse osmosis. Reverse osmosis consists of a membrane with a pore size of 0.1μm and is a well-studied treatment procedure in the tertiary treatment process that is beneficial in minimizing microplastic contamination (Fig. 20). However, it is not a feasible method due to its high cost.
Fig. 20 (from Open PR, 2022). A diagram showing how a reverse osmosis membrane works as a tertiary treatment in a wastewater treatment plant. During the backflush, unclean water reaches the membranes (0.1μm), which clean and remove microplastic particles and bacteria. As a result, pure water is generated.

The cost to implement reverse osmosis varies by water flow rate and levels of treatment necessary. Implementing reverse osmosis in a simple system of 5 to 10 gallons per minute can cost up to $60,000. A large (~300 gallon per minute) system, complex treatment systems can cost $2 to 4 million dollars (Marshall 2018).

Fig. 21 (from Sun et al., 2019) A wastewater treatment plant portraying the removal of microplastic in the preliminary 41-65%), primary (2-50%), secondary, and final (0.1-2%) treatment stages.
Although, we cannot deny that wastewater treatment plants are a known source of microplastic pollution due to the presence of microfibers in receiving waters. One study examined microplastics at several phases of the wastewater treatment plant and found significant microplastic discharge in primary treatment (2% -50%) and secondary treatment (Fig. 21) (0.2 % -14%). Tertiary advanced treatment methods (0.1% -2%) detected relatively little microplastic in treated effluent (Sun et al., 2019). Incorporating tertiary treatment at treatment facilities in highly polluted areas will help reduce microplastic pollution from discharging directly into the oceans. However, an effective and efficient method is needed.

6.3.6 Synthesis

Establishing TMDLs for waste has been problematic because of the lengthy implementation processes that can take up to 9 years, as shown in Table 7 for L.A River Watershed. Therefore, to speed up the implementation of TMDLs in impaired water bodies, including a combination of trash capture systems to reduce plastic pollution from degrading into microplastic debris. In addition, the cost of establishing a TMDL for trash-impaired waterbodies is put in place to decrease the impact of microplastics on the marine environment, and human health should be considered a framework as a preventative strategic plan for global leaders such as California.

In section 6.3, for instance, the comparative analysis evaluates the annual costs and efficiency of five trash capture devices in removing plastic trash and microplastic debris. Catch basins cost $53.1 billion each year in the Los Angeles River Watershed and can reduce significant plastic pollution in watersheds to prevent plastic breakdown (short-term) from reaching the marine environment. However, catch basins are inefficient and incompetent at reducing microplastic pollution (California Regional Water Quality Boards, 2007).

On the contrary, reverse osmosis is a tertiary treatment in a wastewater plant that can 100% remove pollutants, including microplastic debris. Although, running this treatment is highly inefficient and unfeasible in conventional systems worldwide. It can cost up to $60,000 for simple systems (measured in gallons per minute) and $2 to 4 million for complex systems (Marshall 2018). Similarly, the full capture vortex separation system removes 100 percent of
waste, and costs vary depending on capacity size and the number of units required (Table 9). Annual expenditures for larger units might range from $78,000 to $1,576,000 for smaller ones (California Regional Water Quality Boards, 2008). However, suppose larger full capture vortex units are used instead of smaller units. This may be a viable solution in the long run because larger systems require fewer units and less maintenance.

Among the five trash capture systems, the end of pipe net and bioretention rain gardens are full capture systems that can remove debris as small as 5mm, a requirement from the California Water Boards. In addition, the end of pipe nets and rain gardens/bioretention cells are easily operatable and need minimal maintenance annually. The end of the pipe nets an annual range of $10,000 to $80,000, (Table 10) depending on the pipe size (California Regional Water Quality Boards, 2008). The rain garden/bioretention cells cost $1.50 to $6.00 per garden (Hirsch, 2019). Both trash capture systems are cost-efficient and effective for implementing faster trash TMDLs, including a feasible alternative for adopting a management plan for watersheds substantially polluted by microplastic debris.


There is no one-stop solution for preventing microplastic pollution from entering the aquatic ecosystem. Global action and national cooperation are essential to avoid further microplastic pollution from negatively impacting marine wildlife and human health. Our global focus should emphasize the importance of (1) reducing non-reusable or recyclable plastic; (2) developing innovative designs that use biodegradable materials; (3) establishing policies to reduce toxic waste generation; (4) reducing the number of plastic wastes dumped into the oceans and (5) capturing waste in wastewater and stormwater operations before it flows into the ocean.

The goal of this section will provide the management strategies mentioned above in a two-track approach with the attention to providing an efficient preventive action to reduce the amount of microplastic pollution worldwide. The first approach consists of multi-benefit solutions that can be applied via policy/regulatory measures, such as plastic pollution prevention, clean-up interventions, and public education. The second approach involves developing a feasible research strategy that allows science to improve future microplastic monitoring and risk assessment actions.
When the combination of the two-track method is implemented, they should be able to strengthen and support one another (Table 11). The global urgency of immediate changes is necessary, along with the development of scientific knowledge to provide a framework to implement. The two methods applied together can provide data on the non-point and point sources and the various detrimental impacts of microplastic waste. These research strategies will narrow and fill a significant knowledge gap and provide future management recommendations for microplastic pollution in the marine environment.

Table 11: A two-track approach to addressing microplastic contamination in the marine environment, consisting of (1) implementing practical actions and (2) discovering and adopting innovative solutions based on advanced scientific understanding.

<table>
<thead>
<tr>
<th>Track #1: Policy/Regulatory Solutions that can be implemented as scientific knowledge continues to develop.</th>
<th>Track #2: Developing a feasible research strategy that allows science to improve future solutions to microplastic pollution.</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Pollution Prevention</td>
<td>• Monitoring</td>
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<tr>
<td>To begin eradicating plastic waste, products</td>
<td>To track worldwide microplastic contamination trends while</td>
</tr>
<tr>
<td>and materials must be banned.</td>
<td>collecting data to develop standardized identification,</td>
</tr>
<tr>
<td>• Clean-up Intervention</td>
<td>classification, and quantification techniques.</td>
</tr>
<tr>
<td>To intervene in urban stormwater runoff and</td>
<td>• Risk Assessment &amp; Threshold</td>
</tr>
<tr>
<td>wastewater treatment plants that transfer</td>
<td>To acquire a better scientific understanding of the critical</td>
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<td>microplastic pollution into the ocean.</td>
<td>thresholds at which microplastic exposure affects marine</td>
</tr>
<tr>
<td>• Public Outreach &amp; Education</td>
<td>wildlife and potentially causes risks to human health</td>
</tr>
<tr>
<td>Increasing public awareness of the sources,</td>
<td></td>
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<tr>
<td>consequences, and preventative actions of</td>
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<td>microplastic pollution on marine wildlife and</td>
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<td>human health through outreach programs and</td>
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<td>educational campaigns would help to minimize</td>
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<td>pollution.</td>
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7.1 Track #1 Approach: Policy/Regulatory Solutions

7.1.1 Recommendation #1: Pollution Prevention

Disincentivizing the use of single-use plastic products (AB-1276) and the sale of plastic pellets in personal care (AB-888) products may aid in preventing the further spread of microplastic pollution in the marine environment. As a result, I advocate for continuing the ban on AB-1276
and AB-888 on specific single-use plastics that are not easily recyclable. For example, AB1276 and AB-888 are statewide regulations prohibiting expanded polystyrene in take-out boxes and plastic bags. The regulatory bans were implemented as a preventative measure to keep plastics from entering stormwater collecting systems (Storm Water Systems 2022), wastewater treatment facilities (Marshall 2018), and the aquatic environment (Sutton et al., 2019).

In addition, I propose that other toxic plastic items, such as tobacco filters and products, should be prohibited because they are a significant source of microplastics in the marine environment (Sutton et al., 2019). The prohibition of tobacco products, which also include hazardous substances, will aid in the prevention of microplastic pollution in bodies of water. Furthermore, various microplastics are purposefully employed in cosmetics, detergents, and disinfectants, which should be severely outlawed globally.

7.1.2 Recommendation #2: Clean-Up Intervention

Precipitation events wash microplastic particles into stormwater collection systems and are abundantly present in treating wastewater effluent in cities worldwide. A research study conducted in the San Francisco Bay Area (SF Bay) also measured significantly high amounts of microplastic (estimated annual flow of 7 trillion) in stormwater (Sutton et al., 2019). The high amounts of microplastics were also consistent with the limited available scientific investigations published in international locations worldwide (Moran et al., 2021). As a result, the number of microplastics observed in SF Bay was caused by urban stormwater runoff and is projected to be 300 times more than all wastewater treatment plants that discharge into SF Bay (Sutton et al., 2019). This data should be enough evidence to determine runoff treated effluent as dominant pathways for plastic pollution. However, to address these challenges in a viable manner, it is necessary to enhance stormwater and wastewater management to avoid microplastic from municipalities from entering the marine environment.

As a reduction strategy for microplastic pollution in trash-impacted watersheds, I advocate a realistic and faster process for implementing TMDLs while adding full trash capture devices such as the end of pipe nets and bioretention rain garden to capture plastic pollution.
The addition of full trash capture devices for stormwater and wastewater management should be applied worldwide. It is a cost-effective and efficient management strategy for removing plastic litter as small as 5mm from flowing into the receiving waters. End of pipe nets and bioretention rain gardens need minimal maintenance. For instance, the end of pipe nets is easily removed when filled and does not take long to install. Moreover, the trash capture devices portrayed a 100% reduction of trash in the Ventura River Watershed and mitigated microplastic pollution by preventing the fragmentation of large plastic particles.

Similarly, bioretention rain gardens are being implemented in metropolitan places worldwide as a preventative technique for urban stormwater runoff for microplastic pollution in the marine environment. Rain gardens are a low-impact development strategy that contains landscape depressions that aid in capturing and filtering urban runoff from impermeable surfaces through porous engineering media that include plants and mulch (Fig. 19). Studies show that bioretention gardens effectively retain suspended solids from physical filtrations, and because microplastic are also suspended solids, they should also be effectively removed. According to Spraakman et al. (2021), a bioretention cell can remove up to 80% of microplastic from urban stormwater runoff. The benefits of using a low-impact development strategy like bioretention rain gardens will aid in capturing large plastic litter and microplastic pollution before they end up in receiving waters, including treated effluent. Moreover, the trash capture devices portrayed a 100% reduction of trash in the Ventura River Watershed and mitigated microplastic pollution by preventing the fragmentation of large plastic particles.

7.1.3 Recommendation #3: Public Outreach and Education

Developing outreach initiatives and educating the public are critical preventive measures for preventing large plastic and microplastic pollution from entering the marine environment. Spreading public awareness on the harmful effects of microplastic pollution on marine wildlife and potential human health concerns is critical to facilitating environmental changes through policymakers and public behavior, which can only be accomplished through public involvement and education.

Public outreach programs and involvement are critical for assessing the effects of microplastic exposure and assuring that specific initiatives and pollution prevention techniques are motivated...
by societal needs. For instance, California already has obligations and agreements to execute efficient internal government processes with the California Native American Tribes to protect ancestral lands and waters used for cultural resources from plastic pollution. This government-to-government cooperation allows improved communications for plastic pollution data sharing and data access to create effective policies and regulations that may affect tribal communities.

In addition, I also recommend raising public awareness of the implications of microplastic in the marine environment through the aid of educational campaigns. Economic growth for plastic replacements or repurposing can be promoted because educational campaigns reduce plastic items' appeal, availability, and usage. Furthermore, enhancing public awareness contributes to a better understanding of the sources and effects of microplastics on human health and marine wildlife. Thus, developing and combining public awareness and educational campaigns is necessary to develop goals and policies.

For instance, campaigns may involve collaborating with global organizations such as the National Oceanic and Atmospheric Administration Marine Microplastic to raise public awareness about accumulated global data on how microplastic pollution. In addition, the collaboration aids in spreading vital data (National Oceanic and Atmospheric Administration, 2022) on the impacts of marine water quality, particularly coastal habitats (such as salt marshes and mangrove forests) that aid in nutrient cycling and serve as breeding grounds for coastal marine wildlife (i.e., mussels and oysters). Another global authority is the United Nations Environmental Programme, which oversees environmental issues such as nature, climate, and pollution, including microplastics. It promotes safe consumer behaviors and regulations to protect the marine environment from plastic pollution. In addition, I propose that cooperation with the Department of Education should be prioritized so that instructors may educate K-12 students about the causes and impact of microplastics on the environment and human health.

7.2 Track #2 Approach: Science to Improve Future Solutions

7.2.1 Recommendation #4: Monitoring

To provide effective management strategies for microplastic pollution. It is essential to have a standardized monitoring protocol, which can be developed from previous monitoring studies
conducted on stormwater, surface waters, wastewater treatment effluent, and exposure studies on mussels. A reliable monitoring approach for microplastics in the marine environment is critical since it provides information on identification, classification, and polymer particle sizes. This data will enable researchers to expand their scientific expertise for future solutions on preventative management measures for microplastic pollution. The monitoring data will also reveal which organisms are bioaccumulating microplastics and are thus at risk.

As mentioned in the SWOT analysis, I recommend using mussels as a biomonitoring tool for monitoring microplastic pollution in the marine environment. Mussels have been used in multiple microplastic exposure studies, but because of the lack of standardized methods, the studies were incomparable to one another. However, there is consensus that mussels make an excellent biomonitoring tool due to their extensive filter-feeding attributes, resilience in various environmental matrices, and a wide geographical range. In addition, they are abundant worldwide and come directly in contact with plastic particles, which adds to why they should be studied more extensively for a standardized method as an indicator species for microplastic pollution.

7.2.2 Recommendation #5: Risk Assessment and Thresholds

Microplastics are prevalent in the oceans worldwide, making it critical to apply management measures that involve a risk assessment evaluation to understand better the extent and multitude of human and wildlife effects. In the U.S, Senate Bill 1236 was passed in 2018 to conduct a risk assessment on microplastic from vehicle tires and the implications on marine organisms. The State Water Resources Control Board organized an expert workshop called The Ocean Protection Council Advisory Team (OPC SAT) to research critical thresholds for microplastic exposure on marine organisms. The OPC SAT built a framework for measuring microplastic tire particles based on existing research studies; hence, advances in scientific studies may strengthen future risk assessments on microplastics, and other preventative microplastic measures should be evaluated.

As a result, I urge that prior studies be used to investigate primary and secondary microplastic sources in the marine environment to update and reinforce precise thresholds through risk assessment evaluations. Furthermore, accurate thresholds will give enhanced management tactics
across various environmental matrices. The findings will aid in determining which marine habitats and species are most harmed by microplastic pollution, as well as potential human exposure, and which management actions are required as a preventative approach. Microplastic risk assessment should consider the possibility of exposure, which includes the frequency, severity, duration, and negative consequences of microplastic contamination in the marine environment and potentially human health.

Prioritizing in advance research on assessing microplastic pollution in the marine environment in highly contaminated locations worldwide will guide worldwide future management strategies. It is also vital to focus increasing scientific studies on urban runoff, treated effluent, and its relationship to microplastics that have yet to be addressed globally. Thus, investing in the research priorities will provide feasible risk thresholds, including identifying management strategies on a microplastic source to adhere to water quality parameters to protect marine wildlife and human health.

8. Conclusion

This document addresses the abundance and ambiguity of microplastics in the marine environment. My first research objective was to review the bioaccumulation of microplastic in marine wildlife and assess the implications of contaminated microplastic seafood that may pose human health risks. My second research objective was to construct a SWOT analysis for using mussels as a biomonitoring tool for microplastic pollution in the marine environment. My third objective was to conduct a comparative analysis between five different trash capture systems for cost-effectiveness and efficiency in removing microplastic pollution and better understand existing policies for microplastic pollution that should be implemented nationwide.

An upsurge in plastic pollution has been detected in the last decade, and research studies have reported abundant bioaccumulation of microplastic in marine organisms. For instance, mussels and fish species have identified significantly high microplastics, which bioaccumulates gill muscle and gastrointestinal tract tissues. In addition, exposure to microplastics in fish promotes lipid oxidation and neurotoxicity, including cell damage in the brain, muscle, and tissues. In mussels, microplastic promotes superoxide dismutase (the oxygen breakdown into hydrogen and
oxygen) and lipid oxidation that can transfer up in the food web and potentially cause human health concerns. However, further research is necessary to confirm this transfer.

Furthermore, in-vitro (Velázquez and Gómez, 2021; Forte et al., 2016; Schinrinzi et al., 2017), ex-vitro (Barboza et al., 2018; Grafmueller et al., 2015), and in-vivo experiment reported significant microplastic implication in human cell lines which included nervous system, digestive system, placental barrier, endocrine disruptors, and reproductive toxicity. Microplastic exposure increases human cerebral cells, and how the indication of oxidative stress is an essential mechanism for cytotoxicity on the cellular level (Schinrinzi et al., 2017). Microplastic affected gene expression that distorted the cell morphology and cell viability in the gastric adenocarcinoma cell in the digestive system.

Research conducted with the human placental perfusion model reported that the syncytiotrophoblast plays an essential role in transferring particles to the placenta. Also, two studies (Amereh et al. 2019; Amereh et al. 2020)) conducted a microplastic exposure study on rats and measured significant disturbances in the spermatogenesis by microplastic histological lesions. In addition, the rat’s thyroid reported significant increases in the thyroid-stimulating hormone, along with a suppressed serum level and T3 hormone. Even though we have several existing research studies on the implications of microplastics in marine organisms and human cell lines, there is a lack of incomparable standardizing protocols.

As a result, I did a SWOT analysis on mussels as an indicator species for microplastic pollution as a biomonitoring tool. Mussels would make an excellent biomonitoring tool for monitoring microplastic pollution because of their unique attributes in location-specific data, filter-feeding, resilient nature, and wide distribution. In addition, several past scientific data from the Mussel Watch Program and Regional Monitoring Program have shown mussels as a great biomonitoring tool for trace metals. However, the lack of standardization and multiple intercalibrated methods in retrieving microplastic from mussels’ tissues make the studies incomparable. Therefore, creating a nationwide or statewide standardization for classifying, identifying, and quantifying microplastic in mussel’s tissues will decrease the knowledge gaps on the implications of microplastic in the marine environment and potential human health concerns.
Mussels are a common species to test the water quality parameters (i.e., trace metals), including microplastic, which may cause overharvesting or fragmentation in the mussel bed. Furthermore, mussel bed fragmentation encourages invasive species to take over, negatively impacting biodiversity by disrupting biogenic reefs generated by mussels, which feed nutrients to over 88 species. Thus, it is crucial for leading countries like the U.S to join and learn from one another to understand better the implications of microplastic in the marine environment by enforcing regulatory policies as a preventative method to reduce plastic pollution.

California's leadership in microplastic research studies has instilled preventive methods, regulatory measures, and trash capture systems to reduce plastic pollution. As a result, bioretention rain gardens and end of pipe nets are full trash capture systems analyzed to be cost-effective and efficient in being a preventative method for microplastic pollution. Developing a feasible and quicker TMDLs process with the addition of full capture systems and full trash capture devices will aid impaired waterbodies to prevent plastic pollution and reduce microplastic degradation from entering the marine environment.

Thus, I recommended a two-track solution for reducing microplastic pollution. (1) The first track would be immediate action to reduce unnecessary plastic (i.e., straws and plastic bottles) and ban it for commercial and industrial use. In addition, it is essential to establish policies, identify clean-up interventions, incorporate green infrastructure (i.e., bioretention rain gardens), and provide outreach programs and educational campaigns to reduce microplastic pollution. (2) The second track aid in advancing scientific knowledge to improve existing solutions by developing and adopting a standardized protocol for mussels as a global bioindicator for microplastics and conducting a risk assessment to identify the thresholds for microplastic. Researchers can develop a data pool for comparable research studies to further close knowledge gaps on microplastic pollution in the marine environment.
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