Microfiber Content in Pacific Oysters (*Crassostrea gigas*) from Morro Bay, California

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ABSTRACT

Plastics are a major source of marine pollution. One form of plastic pollution is microfibers, which are synthetic fibers five micrometers or smaller that are shed by artificial clothing. The size of microfibers enables them to easily be ingested by a number of marine organisms, including oysters. Oysters are filter feeders and a major aquaculture asset, which presents a concern for the effects of microfiber ingestion on human health. Very few studies have been conducted quantifying microfibers using Pacific oysters (*Crassostrea gigas*) sourced from California. This study quantifies microfiber content in the Pacific oyster farmed for human consumption in Morro Bay, California. Microfibers were quantified after being isolated from oyster samples. An average of 9.12 microfibers were recovered per oyster sample. Although some of the smaller oysters contained more microfibers compared to larger oysters, this difference was not significant. There also was no significant difference between the quantities of black and blue microfibers. However, there was a significant increase in quantities of black microfibers compared to green or red microfibers. The results of this study indicate that a large amount of microfibers are present in commercial oysters, but more research needs to be conducted to determine how this will impact human health.

KEYWORDS

Marine Pollution; Microplastic; Microfiber; Trophic Transfer; Keystone Species; Aquaculture; Oyster; Human Health

INTRODUCTION

Plastics are synthetic, non-biodegradable, persistent environmental pollutants that are found in a variety of environments. Plastic pollution has been increasing dramatically in recent years, doubling from the year 2000 to 2019 from 230 million tons to 460 million tons.[1] In 2019, 1.7 million tons of plastic waste entered the ocean, which contributed to the estimation of over 30 million tons of plastic in the ocean.[1] Plastic pollution is a growing concern due to its longevity, as well as the variable forms it can take on in the environment. Persistence of both bulk and microplastic forms can exceed 20 years (on the basis of plastic bag decomposition), and even approach 600 years for fishing line.[2] This study aims to raise awareness and offer evidence of the growing problem of plastic pollution by providing additional data from the Central Coast of California, USA.

Microplastics are fragments of plastics that are five μm or less in size. Due to their small size and low density, microplastics are ubiquitous to the marine environment.[3–6] Not only does the presence of plastics and microplastics pose an environmental issue, but so do the chemicals they contain. Additive chemicals that help maintain their structural integrity, such as plasticizers, pigments, and flame retardants leach into the environment and tissues of organisms that ingest them.[7] Microfibers are a category of microplastics that are polymer-based fibers that originate from synthetic fabrics including acrylic, spandex, nylon, and polyester that get sloughed off in washing machines. A single load of laundry can release up to several thousand microfibers.[8] Due to their small size, wastewater treatment plants are unable to isolate microfibers, enabling them to enter the ocean. Another source of microfibers is the inappropriate disposal of clothing or fabrics, such as illegal dumping, which directly contributes to pollution.[9]

Once in the ocean, microplastics, including microfibers, absorb persistent organic pollutants (POPs), also known as forever chemicals, which are toxic chemicals made of organic compounds.[7] The ingestion of plastic and the chemicals they contain presents a health issue for marine species that includes blockage of the gastrointestinal (GI) tract, GI and muscle irritation, and endocrine dysfunction.[10]

In addition to microplastics and microfibers being found in various water columns due to their size,[11] they are also transferred through the ecosystem via trophic transfer.

Trophic transfer refers to smaller prey organisms ingesting microfibers and then being eaten by larger predatory organisms. Trophic transfer enables microfibers, along with the chemicals they contain (POPs), to travel through the food web and reach even the major predators at the top.[12] Fish that ingested microplastics showed signs of abnormal feeding, reproduction, and movement behaviors.[13] Additionally, microplastics were transferred from mother to offspring, and exposure in the larval state of the offspring resulted in abnormal development.[13] Not only do microfibers negatively affect fish, but they also impact bivalves. Bivalves ingest food by filtering the water around them, a process during which they also remove organic particles that nourish algae. Accumulation of microfibers in the gills of bivalves can inhibit filtration. After 39 days of exposure to microfibers, bivalve filtration has been found to decrease by 21.3%.[14] The decrease in biofiltration can have negative effects on an ecosystem by contributing to ocean eutrophication. With the decrease in filtration, organic particles remain in the water and allow algal blooms to flourish. Such algal blooms use up the oxygen in the water, resulting in the deaths of surrounding plant and animal life.

One species of bivalves that is affected by plastic pollution is oysters. Oysters are filter feeders that extract plankton or algae from the water by filtering it through their gills. They are a keystone species to the marine environment because they are an essential source of food, habitat, and biofiltration.[14–17] The close connection between the ecosystem and oysters also makes them useful as an indicator species, meaning the health of the oysters signifies the overall health of the ecosystem. Therefore, oysters can be used to indicate the amount of microfibers present in their environment. Varying amounts of microfibers have been recovered in oysters across global locations with 4–57 microplastics being quantified in Shanghai, China and 104–140 microfibers being found in Chesapeake Bay, U.S.A.[3, 6]

Oysters are a major aquaculture product. Therefore, when humans eat oysters and other seafood, they are at risk of ingesting microfibers and other microplastics. Europeans who consume shellfish may ingest up to 11,000 microplastics annually.[18] It has also been estimated that humans who eat seafood can ingest up to 54,864 microplastics in a year with mollusk consumption contributing approximately 2.65 kg of microplastics per person.[19] When ingested, microfibers can interfere with the digestive tract and leach chemicals, resulting in localized particle toxicity and irritation.[7] While little research has been conducted to evaluate the full extent of the health effects of consuming microfibers, continuous ingestion of microfibers may contribute to infections, stomach and brain damage, reproductive issues, cardiovascular issues, and cancer.[9, 20]

In this study, Pacific oysters (*Crassostrea gigas*) farmed for human consumption from Morro Bay, California were measured, weighed, and the quantity of microfibers content was recorded. The aim of this study was to contribute to evidence of microfiber pollution in the marine environment and determine if there is a correlation between oyster size and number of microfibers. The data collected was used to test the following hypotheses: 1) oysters with greater visceral wet weight would have fewer microfibers due to the allometric relationship between mass and filtration.[21] As filter feeders, oysters obtain food from the environment by siphoning water over their gills and collecting food particles that are then ingested. This filtration rate has been found to be affected by the relation between oyster size and metabolic rate.[21] Metabolic rates are allometrically scaled in relation to mass in a variety of organisms (law of allometric scaling of metabolism), including oysters.[21] Therefore as oyster mass increases, metabolic rates decrease, and filtration rates decrease. 2) Oysters with larger shell lengths will have fewer microfibers. A positive correlation has been found between shell length and weight.[22] Therefore, if oyster mass and filtration rate are allometrically related,[21] and shell length and weight are positively correlated, it is expected that shell length and filtration rate will also be allometrically related. Understanding whether size is correlated to microfiber quantity can enable consumers to predict the number of microfibers they may ingest depending on the size of the oyster they consume. Furthermore, identifying how many microfibers are being consumed can help determine the range of microfibers that needs to be ingested in order to result in aversive health effects.

METHODS AND PROCEDURES

Sample collection

Oyster samples used in the study were Pacific oysters (*Crassostrea gigas*) purchased from Grassy Bar Oyster Company based in Morro Bay, California (**Figure 1**). The oysters were purchased during the summer (July/August) and winter February/March) months with each shipment containing between 100–120 oysters. Although the details of exact harvest days are not available, oysters are frozen upon harvest, then bundled for freezing and shipment. The samples were shipped frozen and were stored in plastic bags at –20 °C until use. Before processing for microfibers, the oysters (n = 197) were measured, dissected and weighed.

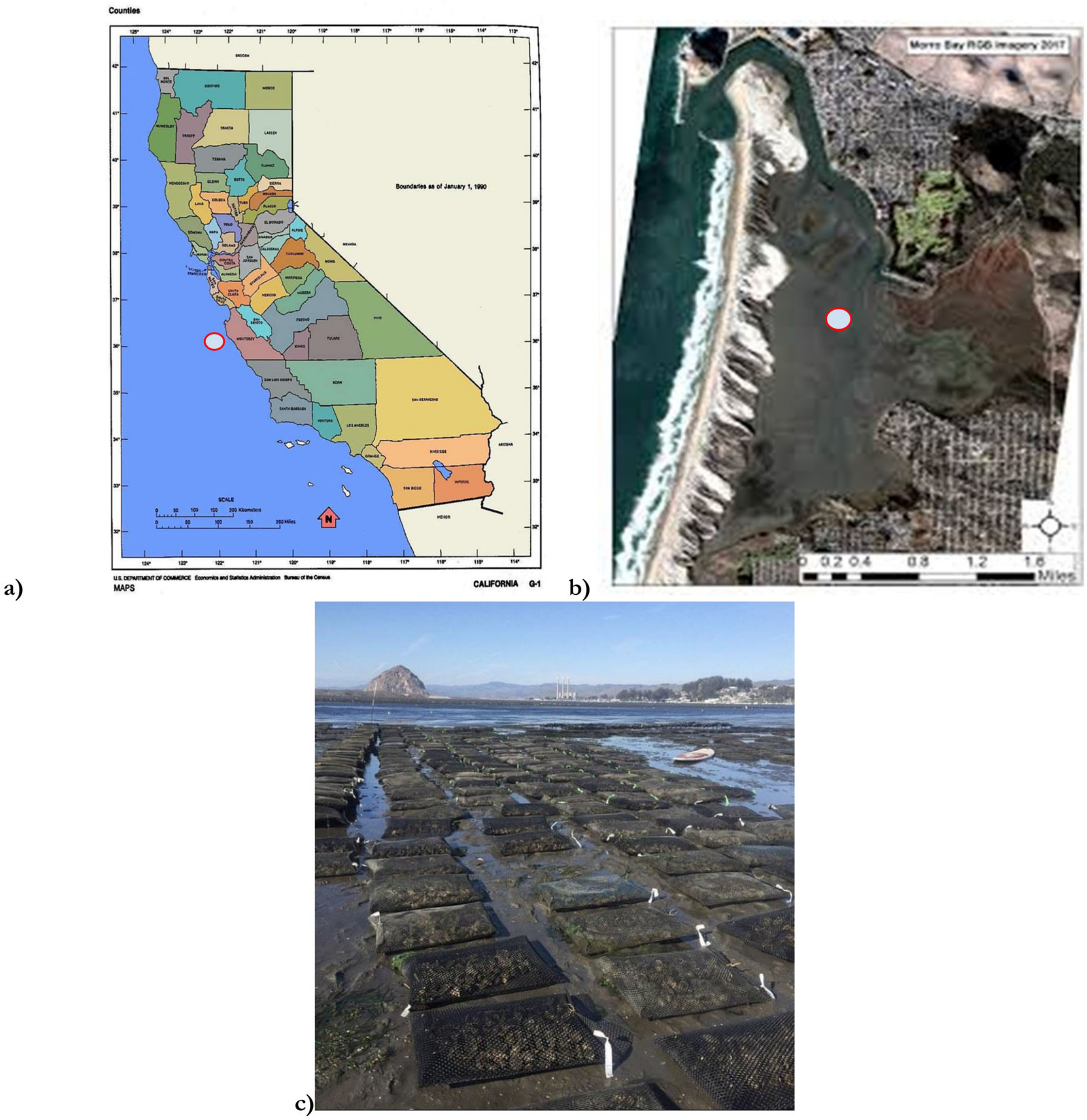


Figure 1. a) The white circle indicates the location of Morro Bay, California. **b)** The white circle indicates the Grassy Bar Oyster farm. **c)** Photograph of the Grassy Bar Oyster Company harvest site obtained from the Grassy Bar Oyster Company *Instagram* account.[23]

Oyster preparation

The shell length, width, and height of each oyster were measured using a caliper, and the soft tissue, or visceral mass, was removed and weighed for wet weight. The tissue was then cut into smaller pieces using dissection scissors and ground using a mortar and pestle. The sample was added to a 500 mL beaker (beaker one) and 30 parts per thousand artificial seawater was added to bring the solution to 100 mL. All artificial seawater is made in a closed system and tested for microfiber contamination (see *Controlling for contamination* below). Due to their light weight and small size, a number of microfibers in the sample will float to the top of the beaker. To extract the floating microfibers, a wash was performed. The top layer of the sample solution was poured into a 200 mL beaker (beaker two) and more artificial sea water was added to bring both beakers to 100 mL. In order to account for as many microfibers as possible in the animal tissue, the tissue was dissolved using 30% hydrogen peroxide. For each 100 mL beaker, 100 mL of 30% hydrogen peroxide was added. Both beakers were placed on an Innova 2000 Platform Shaker set for 100 rpm for 24–48 hours. The samples were filtered using 11 µm Whatman filter paper and a Buchner funnel system with a 500 mL side arm flask.

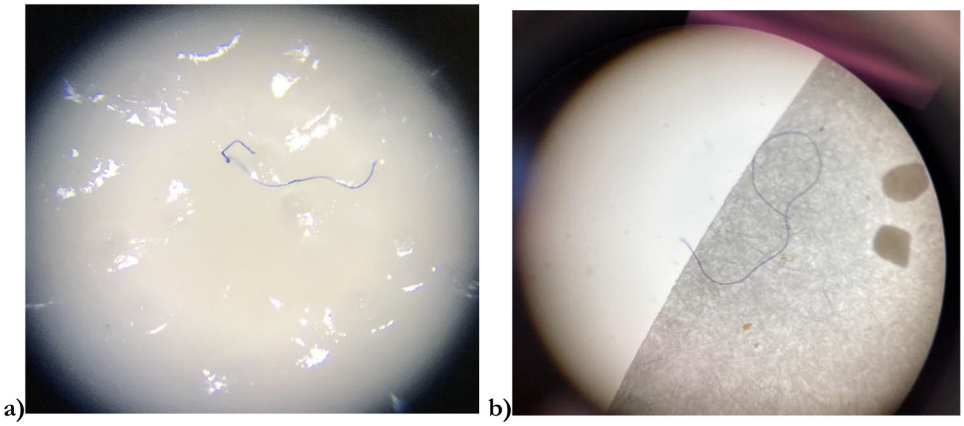
Microfiber quantification
All microfibers on the filter paper were counted and categorized based on color as blue, black, red, or green. The filter paper containing microfibers was divided into a grid of 16 sections and examined using a Stemi 305 Stereo microscope. The microfibers were visible under the microscope when viewed at 10–40x magnification. Microfibers were identified by physical characteristics including size (< 5.0mm), color, and structure.[24] When a single oyster sample was filtered on more than one filter paper, the total number of microfibers for each filter paper was summed to obtain the total number of microfibers in that individual oyster.

Controlling for contamination
Clothing with synthetic fibers is constantly shedding microfibers, even when they are not being washed, with between 1,200 and 7,000 microfibers having been found in the air in various indoor environments.[25] In order to understand the level of contamination in the laboratory, when the samples were being prepared and processed, everyone in the lab area was required to wear a 100% cotton white lab coat. Since the lab coats were made of natural fibers, they did not shed any microfibers that could contaminate the oyster samples. In addition, all samples were covered with aluminum foil after being added to the beakers, when sitting, and when being filtered. 15 saltwater blanks were filtered to control for microfiber contamination that may occur during the preparation procedure. When observed for microfiber content, an average of 0.80 microfibers per blank saltwater sample were recovered.

Statistical analysis
Statistical analysis of microfiber quantities and oyster sizes was performed using Microsoft Excel 2008 Version 16.0. The sum and average microfiber quantity were calculated. The average oyster length and wet weight was also calculated. Linear regression was used to determine the correlation between oyster wet weight and microfiber quantity as well as shell length and microfiber quantity. A coefficient of determination (r^2) value of 0.7 was determined as indicative of a significant correlation. A one-way analysis of variance (ANOVA) was performed to compare the quantities of different colors of microfibers. Black microfibers have been found to be an abundant color of microfiber,[25] so the number of black microfibers recovered in this study were compared with blue, red, and green microfibers quantities to determine whether there were significantly more ($p < 0.05$) black microfibers than other colors.

RESULTS

The results of this study determine the quantity of microfibers in commercial oysters farmed from Morro Bay, CA. A total of 197 oysters were processed, and examined for microfibers with a total of 1,797 microfibers being positively quantified. An average of 9.12 ± 7.09 microfibers/oyster were recovered (**Figure 2**) and an average of 0.79 ± 0.90 microfibers/g wet weight (ww) was found. In order to determine if there is a correlation between oyster size and number of microfibers, both wet weight and shell length were studied. The oysters had an average visceral mass wet weight of 13.27 g (range of 5.30–24.50 g) and an average shell length of 66.46 mm (range of 50.0–85.0 mm) (**Figure 3**). The standard deviations for mean microfibers show diverse degrees of variation because the number of microfibers quantified in each sample ranged from 0–39 microfibers/sample.



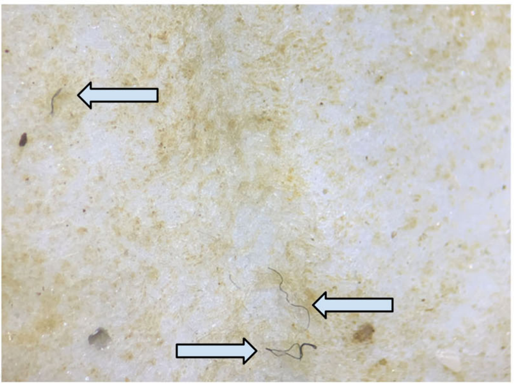


Figure 2. Representative image of **a, b)** blue and **c)** black microfibers observed on filter paper.

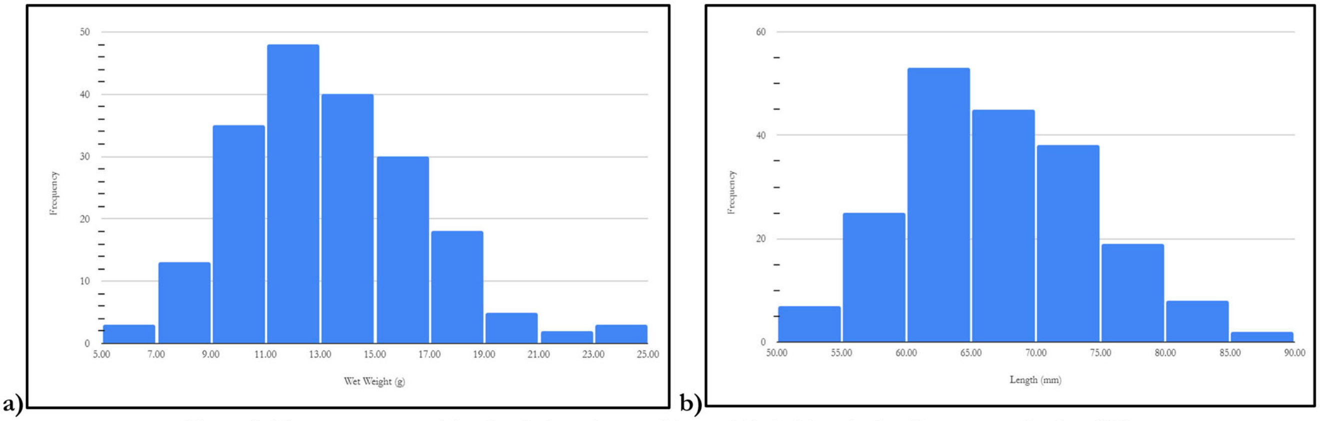


Figure 3. Histograms summarizing the **a)** visceral wet weights and **b)** shell lengths for all oyster samples (n = 197).

Oyster wet weight varied with a difference of 19.2 g between the lowest recorded weight (5.3 g) and the highest (24.5 g). The quantity of microfibers also varied over the range in wet weight (**Figure 4**). A linear regression was performed using the number of microfibers quantified and the visceral mass wet weight of the oysters to determine if weight may be indicative of the levels of microfiber pollution (**Figure 5**). While some smaller oysters were found to contain more microfibers, the coefficient of determination indicates that this correlation is not significant ($r^2 = 0.04$). The equation obtained from the line of best fit is: Microfibers/Sample = –0.406 * (wet weight) + 14.5.

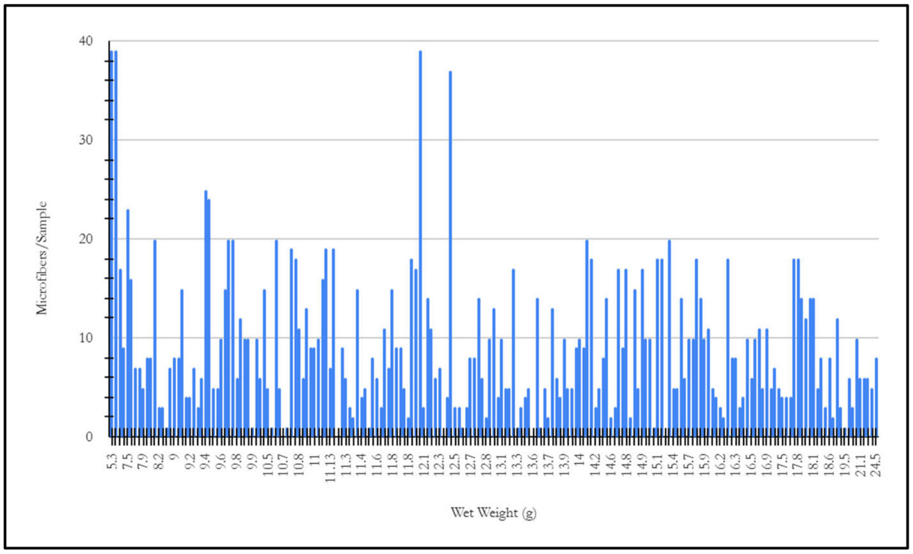


Figure 4. Graphical representation of the variation in wet weight and corresponding microfiber quantity.

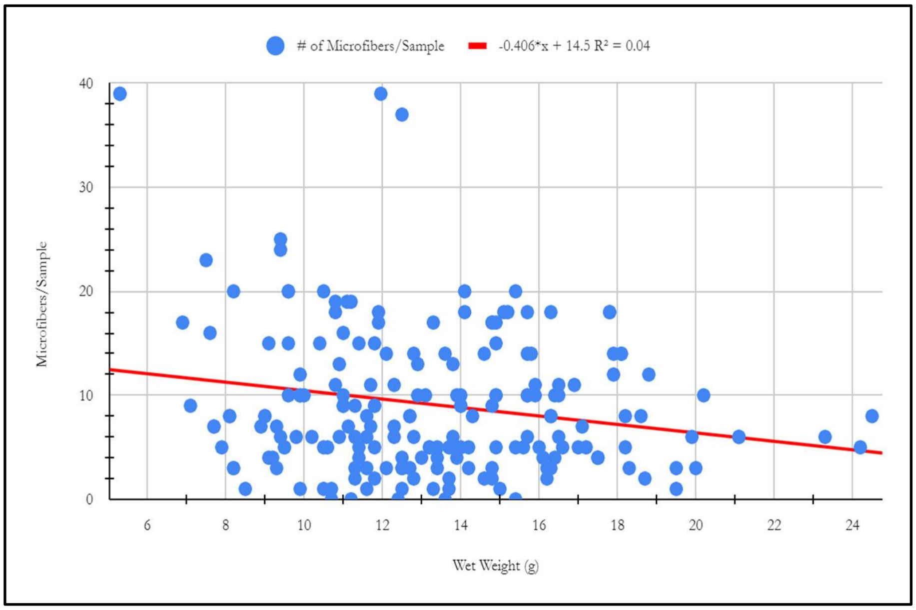


Figure 5. Linear regression comparing wet weight (g) and microfibers/sample ($r^2 = 0.04$). The red line represents the line of best fit (Microfibers/Sample = –0.406 * (wet weight) + 14.5).

Oyster shell length varied with a difference of 35.0 mm between the lowest recorded length (50.0 mm) and the highest (85.0 mm). The quantity of microfibers also varied over the range in shell length (**Figure 6**).

A linear regression was performed using the number of microfibers recovered and the shell length of the oyster samples to determine if length may be indicative of the levels of microfiber pollution (**Figure 7**). There was no correlation found between shell length and microfiber quantity ($r^2 = 0.00$). The equation obtained from the line of best fit is: Microfibers/Sample = 1.26E–3 * (shell length) + 9.04.

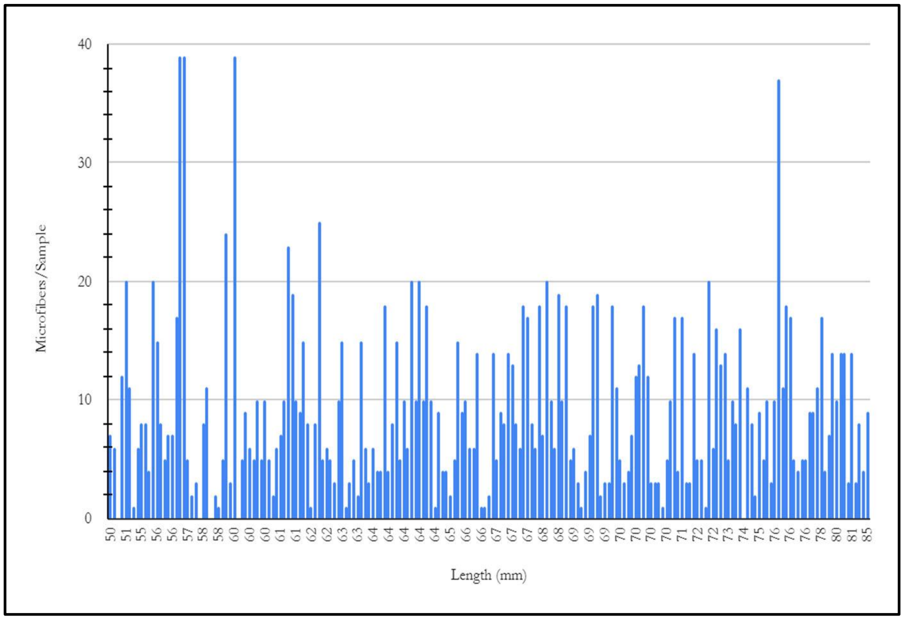


Figure 6. Graphical representation of the variation in shell length and corresponding microfiber quantity.

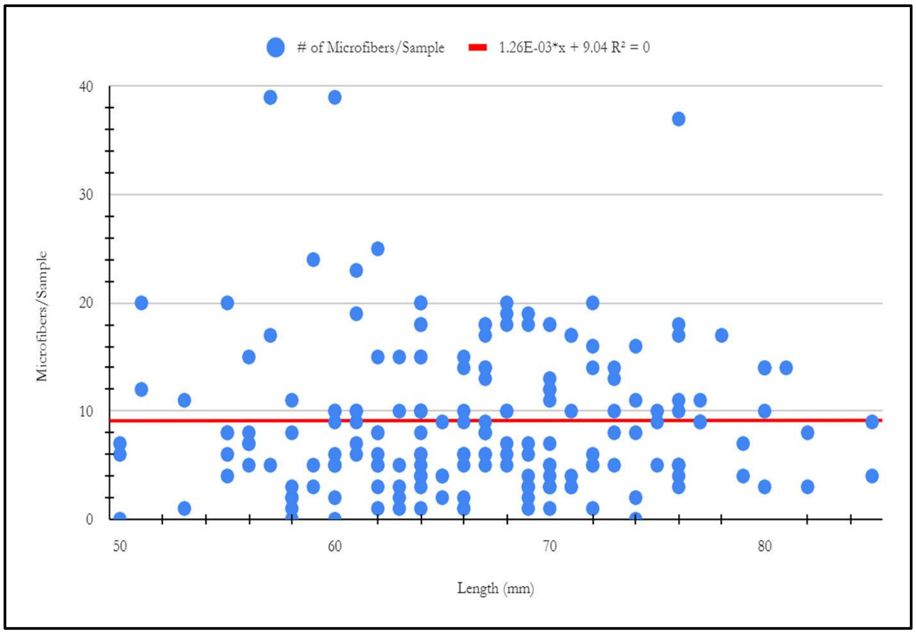


Figure 7. Linear regression comparing shell length (mm) and Microfibers/sample ($r^2 = 0.00$). The red line represents the line of best fit (Microfibers/Sample = 1.26E–3 * (shell length) + 9.04).

The colors of microfibers were compared to determine what color was most prevalent (**Figure 8**). Black microfibers were expected to be most prevalent, so the quantity of black microfibers was compared to the other colors.

The colors of the microfibers were vibrant, and all clearly stood out in contrast to the white filter paper. The only colors observed were black, blue, red, and green, all of which have been included in the data analysis. There were significantly more black microfibers than green ($p = 2.68E–11$) and red ($p = 5.60E–24$), but not blue ($p = 0.22$). 50.1% of the microfibers quantified were black ($n = 900$), 37.5% were blue ($n = 673$), 8.1% were green ($n = 146$), and 4.3% were red ($n = 78$) (**Figure 9**).

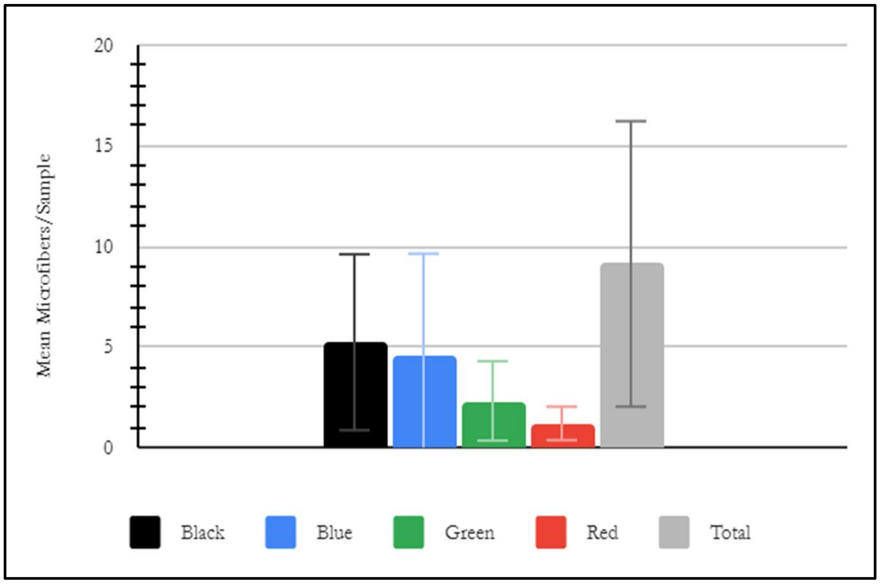


Figure 8. Mean number of black ($\bar{x} = 5.23 \pm 4.37$ microfibers/sample), blue ($\bar{x} = 4.58 \pm 5.05$ microfibers/sample), green ($\bar{x} = 2.32 \pm 1.97$ microfibers/sample), red ($\bar{x} = 1.20 \pm 0.83$ microfibers/sample), and total ($\bar{x} = 9.12 \pm 7.09$ microfibers/sample) microfibers recovered per oyster ($n = 197$). Error bars represent the standard deviation.

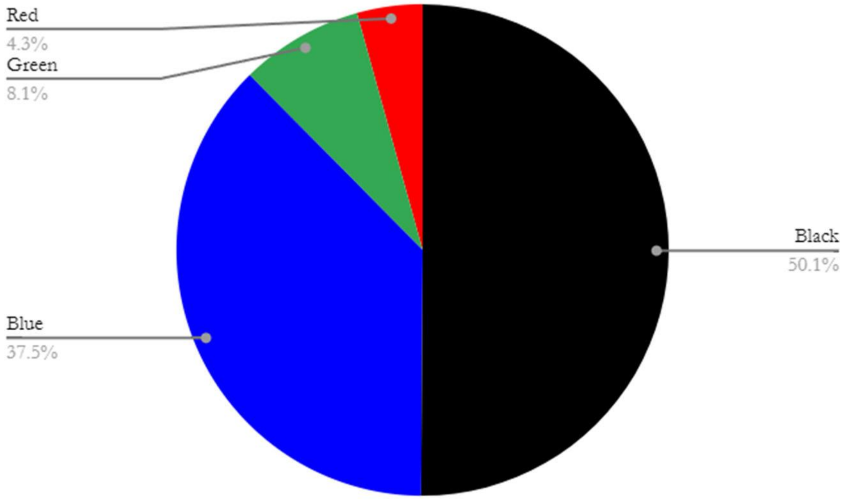


Figure 9. The percentage of black, blue, green, and red microfibers out of the total amount of microfibers (n = 1,797) recovered from all sampled oysters (n = 197).

DISCUSSION

The purpose of this study was to quantify microfiber pollution in Pacific oysters farmed for human consumption and determine if wet weight and shell length were indicative of microfiber quantity. Very few studies have been conducted quantifying microfibers using Pacific oysters sourced from Morro Bay, California. This study recovered a total of 1,797 microfibers with an average of 9.12 microfibers per oyster sample and an average of 0.79 microfibers/g ww. While oysters with less wet were found to contain more microfibers/sample compared to larger oysters, the relationship was not significant. There was no correlation between shell length and microfiber quantity.

There were significantly more black microfibers observed compared to green or red microfibers. There was no significant difference between black and blue microfibers.

Other studies along the West Coast of the United States using oysters and other bivalves have reported varied quantities of average microfibers compared to this study. While quantities were varied, lower average quantities of microfibers/sample were recovered at other locations including in commercial bivalves harvested from the coast of Baja California, Mexico ($\bar{x}$ = 0.14–0.36 microfibers/sample),[26] bivalves from San Francisco, California ($\bar{x}$ = 0.87–1.4 microfibers/sample),[27] and oysters from Washington State, USA ($\bar{x}$ = 0.77 microfibers/sample).[28] These studies were all conducted during the years 2018 and 2019, while the present study was conducted between 2021 and 2022. Due to the accumulation of microfibers over time,[12] the current study being conducted years after the mentioned West Coast studies may contribute to the greater microfiber quantities found in the present study. The variation between bivalves along the Pacific coast indicates that sample location may be a contributing factor to microfiber quantities and should be a consideration when consuming seafood. While there is variation in the amount of microfibers in seafood, filter feeders have been found to contain more microfibers than fish,[29] which increases health-related risks for communities that rely on seafood, and in particular filter feeders.

Although few studies have focused on the effects of microfiber ingestion in humans, persistent organic pollutants (POPs) that have been found in microfibers have presented a public health concern due to their contribution to metabolic diseases including type 2 diabetes as well as birth defects and dysfunctions in the endocrine and immune systems.[7, 30, 31] The limited data regarding the effect of microfibers and the chemicals they leach may give rise to uncertainty about food safety.

This study found no correlation between oyster shell length and levels of microfiber content. Oysters harvested from the coast of Oregon, U.S.A. were also found to bear no significant correlation between oyster length or weight.[32] This study found no significant correlation between microfiber quantity and wet weight, although a negative trend with smaller oysters containing more microfibers was noted. A similar result was noted in bivalves harvested from Italy with smaller oysters (4.00–5.75 g, $\bar{x}$ = 1.69 microplastics/g ww) containing more microplastics/g ww compared to large oysters ($\bar{x}$ = 0.78 microplastics/g ww).[4]

The average quantity of microfibers/g ww varies depending on the global location. The present study recovered an average of 0.79 microfibers/g ww. However, bivalves harvested from the French-Belgian-Dutch coastline found an average of 0.2 microplastics/g ww,[33] while commercial bivalves collected from the Tyrrhenian Sea found an average of 1.33 microplastics/g ww,[4] and an average of 0.04–0.07 microplastics/g ww were found in bivalves from Baja California, Mexico.[26] The variation in microplastics/g in both commercial and wild bivalves also supports the implication that the location of oyster harvest contributes to levels of microfiber pollution.

Oyster ingestion of microfibers being a factor of location rather than shell length or wet weight has ramifications for human consumption. In order to limit microfiber intake, consumers should consider the location of oyster growth and harvest. Bivalves harvested in Asia have been found to contain increased levels of microplastic contamination.[19] Three times more anthropogenic debris have been recovered in fish and bivalves from Indonesia compared to California.[5] An average of 13.3 microplastics/oyster was found in oysters collected in the Philippines and an average of 57.2 microplastics/oyster was quantified in bivalves from China.[34, 3] Contributing factors for higher levels of microfiber contamination in Asia include plastic mismanagement, population size, and major rivers that flow into the ocean.[35, 36] This bears implications for our study in Morro Bay and other locations in contact with the Pacific Ocean due to ocean currents.

Microplastics are distributed across the ocean with accumulation sites where ocean currents converge.[37, 38] The connection between continents via ocean currents makes microplastics contamination a global issue rather than a regional issue and illustrates the need for a standardized method of wastewater treatment and plastic disposal.[39]
There was no significant difference between the quantities of black and blue microfibers. However, there were significantly more black microfibers than green and red microfibers, which is consistent with initial expectations. Black and blue microfibers have been found to be more than 50% more common than other colors of microfibers in oysters from the Salish Sea, Washington,[28] Baja California, Mexico,[26] and Florida.[40] Black and blue microfibers are also more common than other colors year-round.[25]

The dominance of blue and black clothing preferences during summer and winter may contribute to why black and blue microfibers were consistently more common overall.[22] The present study was performed over the course of a year, and black and blue microfibers were consistently found to be the most common regardless of when the oysters were harvested (one harvest occurred during summer and another during mid-winter). The results indicate that synthetic black and blue fabric is a prominent contributor to microfiber pollution. To help reduce such pollution, initial efforts can be taken by fabric producers to use natural fibers for black and blue clothing. Not only are natural fibers such as cotton, hemp, linen, and jute more eco-friendly in terms of biodegradability, but also are more sustainable, renewable, and lower-cost.[41] Spreading awareness of such properties can incentivize fabric producers and other industrial companies to increase their usage of natural plant fibers.

CONCLUSIONS

The ubiquity of plastic pollution makes it both an environmental and a human health issue. Quantifying plastic pollution is imperative to drawing awareness. For this reason, it is important that microfibers be quantified. While there have been studies of microfibers quantities across the world,[3–6, 26, 33] few studies have focused on oysters found on the coast of California. The findings of this study provide a clearer number of how many microfibers a consumer may ingest. A total of 1,797 microfibers were recovered in 197 sample oysters with an average of 9.12 microfibers/oyster. Additionally, 0.79 microfibers/g ww were quantified with oysters with less wet weight containing more microfibers. Microfiber quantity has been found to vary depending on location, wet weight, and shell length with a wide range of microfibers in oysters harvested from the Pacific coast of the United States as well as from various global locations including Mexico,[26] China,[3] and France.[33]

While there is a limited number of studies that have identified precisely the effects of microfibers on humans, there is evidence of a multitude of consequences including metabolic, reproductive, and cardiovascular difficulties stemming from microfiber ingestion.[30, 31] Future research related to microfiber consumption and human health should study the range at which microfiber quantities become hazardous to human health.

Microfiber pollution can be decreased by strengthening wastewater treatment infrastructure, which will increase the amount of microfibers isolated from water that is later drained into the ocean. Furthermore, a shift towards including natural plant fibers in textiles will decrease the amount of microfibers being shed from clothing and other fabrics. Shifting from synthetic fibers to natural fibers can be initiated by drawing awareness to the benefits of natural fibers including low density, versatility, and strength.[41]

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PRESS SUMMARY

One form of plastic pollution is microfibers, which are synthetic fibers five µm or smaller that are shed by synthetic-based clothing. Once in the ocean, microfibers are ingested by a variety of marine organisms including oysters. Oysters are filter feeders and a major aquaculture asset, which presents a concern for the effects of microfiber ingestion on human health. This study quantifies microfiber pollution in Pacific oysters farmed for human consumption from Morro Bay, California.

An average of 9.12 microfibers were recovered per oyster sample with no significant correlation between microfiber content and oyster visceral wet weight or shell length. Black and blue microfibers were recovered in higher quantities than red and green microfibers. The results of this study indicate that a large number of microfibers are present in commercial oysters, but more research needs to be conducted to determine how this will impact human health.