

# Increasing watershed responsibility through a local inventory of microplastics and educational community outreach

Final Report prepared for Pensacola and Perdido Bay Estuary Program

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## Introduction

Microscopic plastics (microplastics) are ubiquitous in aquatic environments and have become a serious concern as plastic production and use increases. Microplastics, defined as plastic particles smaller than 5 mm, can be those that are manufactured at a microscopic size for use in facial cleansers and cosmetics. Alternatively, microplastics can be secondary, in that they are the result of the physical breakdown of larger plastic debris as a result of UV solar radiation, thermal, and chemical degradation (Arthur et al. 2009, Costa et al. 2010, Andrady 2011). Microplastics are washed down drains, into rivers and eventually collect in the ocean, where they are persistent. One estimate of microplastics in the ocean is over 5 trillion floating pieces, or 250,000 tons (Eriksen et al. 2013), covering 88% of the ocean's surface (Cozar et al. 2014). Importantly, recent studies indicate that microplastic pollution in small freshwater bodies can be more severe than in estuaries and coastal waters (Luo et al. 2019); thus, monitoring of microplastics is recommended within entire river networks.

Escambia and Santa Rosa Counties have a combined population of 450,000 and environmental issues have long raised concern in this region. Specifically, a Grand Jury on Air and Water Quality impaneled by the First Judicial Court of the State of Florida (Grand Jury Report, 1999) addressed the deterioration of environmental health in Northwest Florida due to pollution from point sources (industrial, military, and Superfund sites) and nonpoint sources (storm water runoff, septic tanks, lead contaminated homes, contaminated aquifers, and other diffused sources of pollution). The Pensacola Bay watershed is not exempt from the microplastics problem, however information on microplastics pollutants for this region is limited. The report from the Environmental Quality of Pensacola and Bay System (2016) indicated the lack of knowledge on microplastics concentrations as a limitation for environmental management, conservation, and restoration of Pensacola Bay. Thus, demonstrating the need for microplastics studies in the Pensacola Bay watershed.

In addition to a need for inventory of microplastics for Pensacola Bay watershed, it is also important to help create responsible environmental stewards within the local community. It has been documented that engagement of public participation in scientific research promotes ownership and appreciation for nature while increasing scientific knowledge. Dixon School of Arts & Sciences serves at-risk youth (less likely to transition successfully into adulthood) students living in Escambia County, providing an ideal platform to develop research projects in partnership with the University of West Florida. In particular, these two institutions worked collaboratively to establish a baseline of microplastic pollution for our watershed, while working to promote responsible environmental stewards in our local community.

The objectives of this project were to increase public awareness of microplastic pollution in our local watershed through microplastic sampling and educational outreach. Specifically, the following objectives were carried out:

1. Quantification of microplastics using water collected from throughout the Pensacola Bay watershed to establish a baseline of plastic pollution

2. Help promote responsible engagement of environmental stewardship of local students from Dixon School of the Arts and Sciences with the following activities:

- a. Microplastics education unit
- b. High-impact educational outreach field trip
- c. Active hands-on learning: students will collect water samples from a local water body near where they live for microplastics quantification

# **Material and Methods**

The objectives for this project consisted of both data collection and educational outreach. This project was conducted by A.M. Janosik, associate professor of marine biology, and V. E. Bogantes, postdoctoral researcher, from the University of West Florida in collaboration with E.D. Eubanks, science specialist, at Dixon School of the Arts and Sciences. Undergraduate students J. M. Kleinschmidt and H. E. Koch each devoted 10 hours a week to working on this project.

To carry out Objective 1: **Quantification of microplastics using water collected from throughout the Pensacola Bay watershed,** the following methods were used. Surface water samples (1 liter) were collected from across the Pensacola Bay Watershed from 40 sites by A.M. Janosik, V.E. Bogantes on 01/22/2021, 02/24/2021, 02/26/21, 3/10/21, and 3/25/2021 (map of collection sites: **Figure 1**; list of collection sites: **Table 1**).

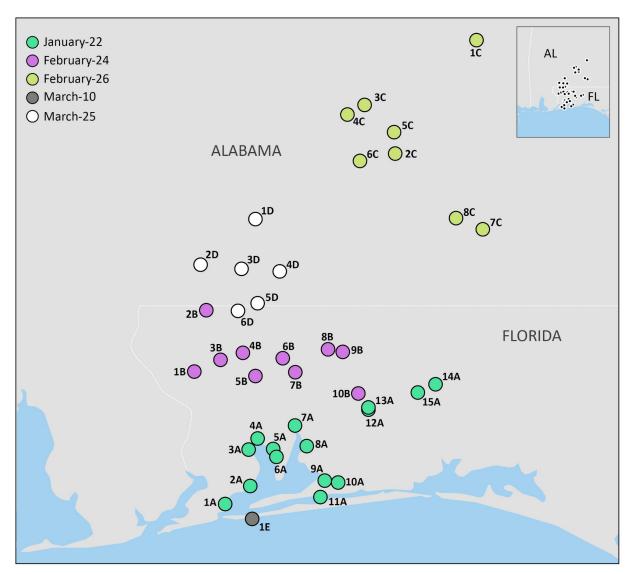


Figure 1. Map of collection sites from across Pensacola Bay Watershed.

Site No.	Date	Latitude	Longitude	Fibers	Fragments	Beads	Total
1A	1/22/21	30.375	-87.281	26	0	1	27
2A	1/22/21	30.432	-87.189	30	1	0	31
3A	1/22/21	30.547	-87.195	60	1	1	62
4A	1/22/21	30.582	-87.162	11	0	0	11
5A	1/22/21	30.549	-87.105	20	0	0	20
6A	1/22/21	30.524	-87.093	1	0	1	2
7A	1/22/21	30.623	-87.025	18	0	0	18
8A	1/22/21	30.558	-86.982	8	0	0	8
9A	1/22/21	30.449	-86.916	6	0	2	8
10A	1/22/21	30.443	-86.867	10	0	1	11
11A	1/22/21	30.397	-86.932	142	0	2	144
12A	1/22/21	30.673	-86.756	13	0	1	14
13A	1/22/21	30.68	-86.756	10	0	0	10
14A	1/22/21	30.753	-86.51	11	1	0	12
15A	1/22/21	30.727	-86.575	3	0	0	3
1B	2/24/21	30.793	-87.394	8	1	0	9
2B	2/24/21	30.986	-87.35	7	0	1	8
3B	2/24/21	30.83	-87.298	8	0	1	9
4B	2/24/21	30.852	-87.216	6	0	0	6
5B	2/24/21	30.779	-87.17	2	0	0	2
6B	2/24/21	30.835	-87.07	7	0	0	7
7B	2/24/21	30.791	-87.024	1	0	0	1
8B	2/24/21	30.863	-86.904	7	0	0	7

**Table 1.** List of collection sites of water samples across Pensacola Bay Watershed.Microplastics are categorized as fibers, fragments, and beads identified for each site.Abundance is expressed as microplastics/liter.

9B	2/24/21	30.855	-86.85	6	0	0	6
10B	2/24/21	30.724	-86.793	1	0	0	1
1C	2/26/21	31.831	-86.36	11	1	0	12
2C	2/26/21	31.477	-86.658	1	0	0	1
3C	2/26/21	31.629	-86.77	9	0	0	9
4C	2/26/21	31.599	-86.833	2	0	0	2
5C	2/26/21	31.544	-86.662	16	1	1	18
6C	2/26/21	31.454	-86.787	17	0	0	17
7C	2/26/21	31.24	-86.337	19	3	0	22
8C	2/26/21	31.275	-86.435	6	0	0	6
1E	3/10/21	30.328	-87.182	13	1	0	14
1D	3/25/21	31.272	-87.17	3	0	0	3
2D	3/25/21	31.129	-87.371	7	0	0	7
3D	3/25/21	31.116	-87.221	14	0	0	14
4D	3/25/21	31.108	-87.081	4	1	0	5
5D	3/25/21	31.008	-87.162	10	0	0	10
6D	3/25/21	30.984	-87.234	2	1	0	3
TOTAL				556	12	12	580

Water samples were collected in sterile and rinsed 1L Nalgene bottles. Nalgene bottles were immediately sealed in order to prevent contamination. Water samples were filtered using a vacuum hand pump through a 0.45µ gridded cellulose filter (Whatman). To avoid contamination the filtering apparatus, a magnetic 500 mL filter cup and magnetic filter base were flushed using milliQ water in between each sample and were covered during filtration to help prevent contamination. Each filter was then stored in a sterile Petrislide<sup>™</sup> (MilleporeSigma<sup>™</sup>) for drying and quantification. A one-liter sample of milliQ water was also filtered to control for microplastics contamination during the seawater filtration process.

Each filter was quantified by direct examination of each square of gridded filtered using a compound microscope (4x and 10x magnification) by both H.E. Koch and J.M.

Kleinschmidt. Visual identification was performed to accurately differentiate plastics from other natural organic debris such as algae, sediment, invertebrates, and plant material. To avoid misidentification and inaccurate estimation of micro-plastics, visual criteria, including examination for cellular and organic structures, equal thickness throughout the length of fibers, and homogeneous particle color, similar to Hidalgo-Ruz et al. (2012) was used. To distinguish between plastic and natural organic material, fibers were tested with two procedures. Specifically, a metal probe was heated and placed next to the putative microplastic. Hendrickson et al. (2018) found that using the "melt test," all plastic fibers melted, but cotton and wool fibers burned. Therefore, in this study, if the item melted it was considered a microplastic (Hidalgo-Ruz et al., 2012). Microplastics were characterized and quantified according to type: microbeads, microfibers, microfragments, microfoams.

Measurements were taken using ImageJ v 1.52a bundled with Java 1.8.0\_172 for Windows. Prior to the melt test, photographs of each filter were taken with a Nikon DS-Fi2 microscope and corresponding software, NIS-Elements, was used to burn the set scale of 100  $\mu$ m to every photo when capturing each image. Using the straight-line tool in ImageJ, the scale line was traced and set before beginning measurements. All fibers and fragments were traced using the freehand tool to accommodate twisting and bending.

To carry out Objective 2: Help promote responsible engagement of environmental stewardship of local students from Dixon School of Arts and Sciences, the following steps were undertaken. Students from Dixon School of the Arts and Sciences were given four lessons on microplastics and watershed responsibility. Before the first lesson and after the final lesson, students completed a survey on plastic pollution and watershed responsibility. Students were asked the following questions in both surveys: What grade are you in?; What is your favorite animal?; What do you want to be when you grow up?; Do you think the ocean is important and valuable to humans? Please describe why or why not.; I think conservation is important.; I am confident in my ability to do science.; I think protecting our watershed is important.; Do you recycle?; What are microplastics?; Do you think marine animals are in danger and need our help?; I believe I can make a difference for marine organisms and conservation.; Do you identify as underrepresented? For purposes of this survey UR is defined (from National Institute of Health) as: someone whose racial or ethnic make-up is from one of the following: African American, Black, Asian (Filipino, Hmong, or Vietnamese only), Hispanic (Latinnix) Native American (including Alaska Native). Native Hawaiian (or other Pacific Islander). Two or more races when one is from this list. Students were asked the following questions in the post survey: Where do microplastics come from?; What action will you take to reduce plastic pollution?.

The first microplastics lesson plan focused on an overview of aquatic pollution and organism impacts was held on February 3, 2021. At the first workshop, students were encouraged to submit a design that represents challenges with pollution and watershed. The winning design was selected, printed on a canvas bag and students were each given a reusable canvas bag for encouraged use instead of one-time use plastic bags. A second microplastics lesson plan was held on February 17th and focused on watershed responsibility. Additionally, at this lesson plan, students were provided with collection materials and encouraged to collect a one-liter water sample from a water source near their residence. These water samples were filtered and photographed for student quantification. Collection locations of student water samples can be seen in Figure 2. A student field trip was held on March 10, 2021 at Escambia County Bay Park West, near Fort Pickens. A.M. Janosik, V.E. Bogantes, and E.D. Eubanks led a field trip for Dixon middle school students to examine and learn about intertidal habitats and organisms. The third microplastics lesson was held on April 7, 2021 and focused on microplastics pollution and identification and quantification of microplastics. Students examined and quantified microplastics from the filters of their water samples. A fourth and final microplastics lesson plan was held on May 5, 2021 to discuss microplastics data collected in the Pensacola Bay Watershed and the student data results.

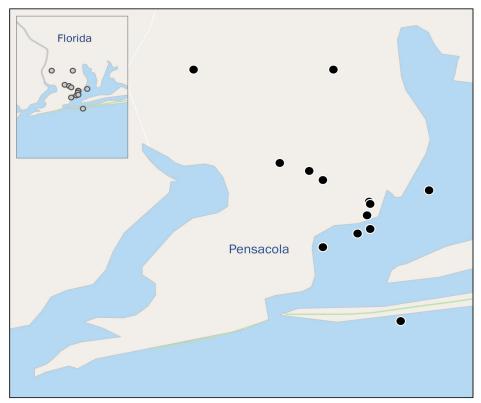
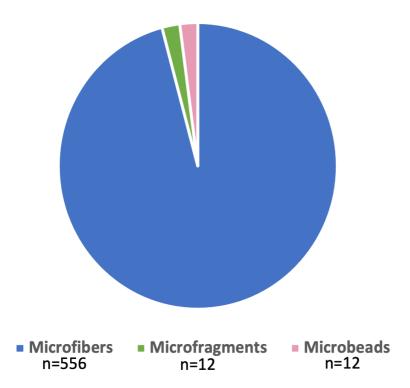


Figure 2. Map of water samples collected by Dixon middle school students.

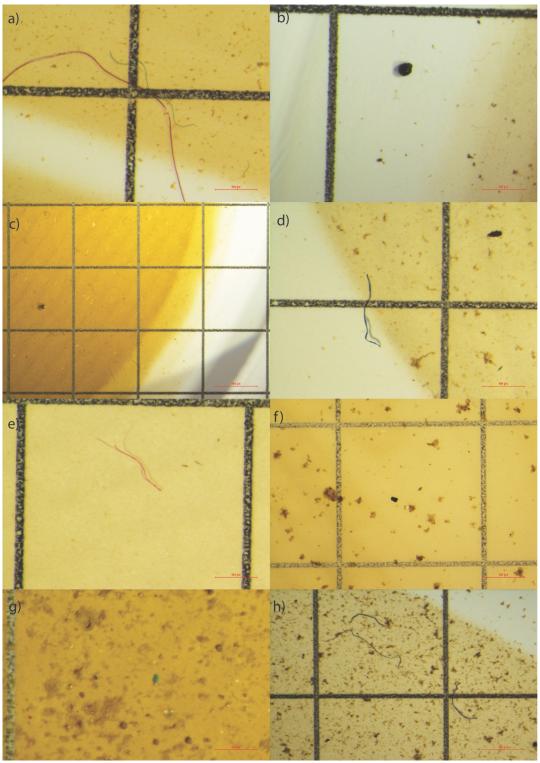
## Results

For Objective 1, a total of 580 microplastics were collected from 40 liters of water throughout the Pensacola Bay Watershed (mean 14.12 microplastics/L). Specifically, 556 microfibers were collected, 12 microfragments, and 12 microbeads (**Figure 3**). No microfoams were recovered.

Specific numbers of microfibers, microfragments and microbeads from each sampled site can be seen in **Table 1**. Microfibers were collected at all 40 sites. Microfragments were collected at 9 of 40 sites, and microbeads were collected at 10 of 40 sites. Representative microplastics can be seen in **Figure 4**.



**Figure 3.** Total microplastics according to type. Microfibers are depicted in blue (n=556), microfragments are depicted in green (n=12), and microbeads are depicted in pink (n=12).



**Figure 4.** Representative examples of microplastics from water filtration samples. a.) from site 6; b.) from site 10; c.) from site 11; d.) from site 9; e.) from site 8; f.) from site 6, g) from site 5; h) from site 2.

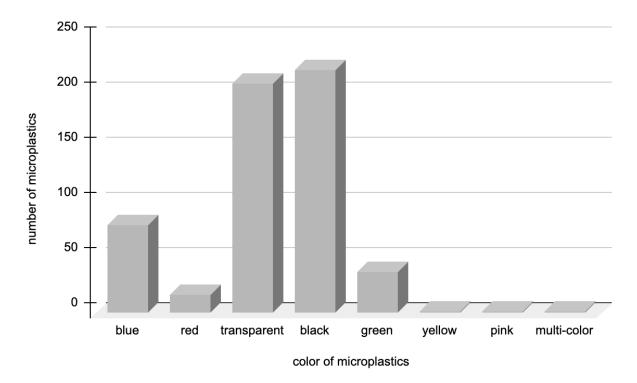
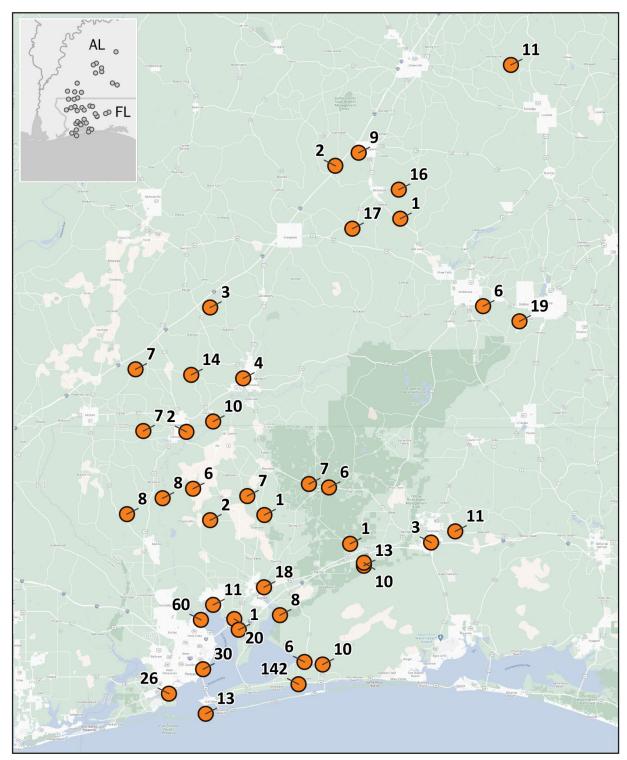


Figure 5. Microplastics counts from Pensacola Bay Watershed by color.

Black (n=220) and transparent (n=208) were the most commonly recovered colors of microplastics. Blue (n=80), green (n=37), red (n=16), yellow (n=1), pink (n=1), and multi-color (transparent/blue n=1) microplastics were also recovered (**Figure 5**). Site 11A on Santa Rosa Sound, near Navarre, Florida, had the most microplastics, with 144 total (142 microfibers, 2 microbeads). Average length of microfibers was 110.38  $\mu$ m. Average surface area of microfragments was 58.72  $\mu$ m<sup>2</sup>. Average surface area of microfragments was 58.72  $\mu$ m<sup>2</sup>.

Site 3A at Smith's Fish Camp on the Escambia River had the second highest number of microplastics, with 62 total (60 microfibers, 1 microfragment, 1 microbead). Sites where microfibers were collected, along with counts of microfibers can be seen in **Figure 6**. Sites were microfragments were collected can be seen in **Figure 7**. Sites were microbeads were collected can be seen in **Figure 8**.



**Figure 6.** Map of water collection sites with microfiber counts per site. Counts are microfibers per 1 liter of water.

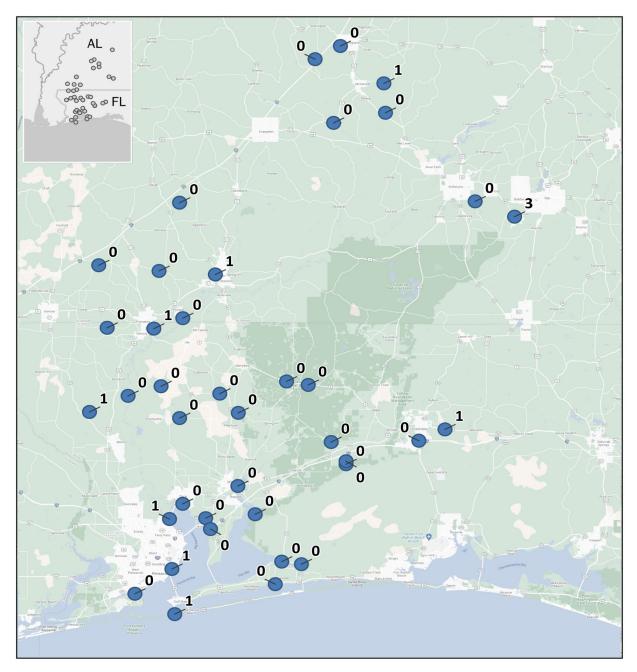


Figure 7. Map of water collection sites with microfragments counts per site. Counts are microfragments per 1 liter of water.

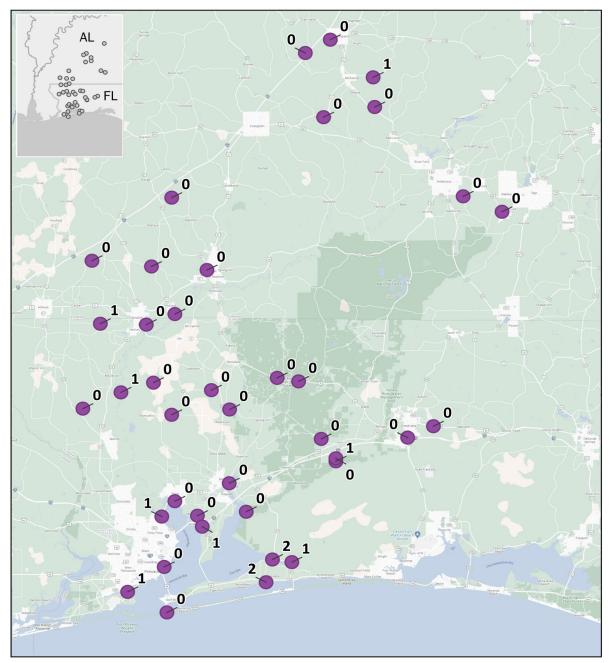


Figure 8. Map of water collection sites with microbead counts per site. Counts are microbeads per 1 liter of water.

For Objective 2, a total of 45 microplastics were collected from 13 liters of water that was collected by students from Dixon School of the Arts and Sciences (mean 3.46 microplastics/L). Specifically, 43 microplastics were microfibers, two were microfragments, and zero microbeads were collected (**Table 2**).

**Table 2.** List of collection sites of water samples collected by Dixon School of the Arts and Sciences students. Microplastics are categorized as fibers, fragments, and beads identified for each site. Abundance is expressed as microplastics/liter.

Student Name	Latitude	Longitude	Fibers	Fragments	Beads	Total
Jaleel	30.466	-87.296	2	0	0	2
Courtney	30.548	-87.378	3	0	0	3
Elivia	30.327	-87.181	6	0	0	6
Nashawn	30.432	-87.211	4	0	0	4
Rylee A.	30.392	-87.255	8	0	0	8
Meya D.	30.459	-87.268	2	0	0	2
Angel.	30.242	-87.154	0	1	0	1
Aiyanna	30.404	-87.222	4	1	0	5
Alisha	30.43	-87.21	3	0	0	3
DeAzyzia	30.451	-87.255	4	0	0	4
Elizabeth	30.42	-87.213	1	0	0	1
Kadence	30.408	-87.21	4	0	0	4
Laila	30.548	-87.245	2	0	0	2
			43	2	0	45

In total, 15 students from Dixon School of the Arts and Sciences participated in the presurvey. Specifically, there were five 8th graders, three 7th graders, and seven 6th graders. Two of the 15 students listed a marine species as a favorite organism, while the remaining listed terrestrial species. Six of 15 students reported a preference for a STEM career. All students indicated that the ocean is valuable to humans. Ten of 15 students indicated "strongly agree" for the prompt "conservation is important". Five of 15

students indicated "strongly agree" for the prompt, "I am confident in my ability to do science". Eight of 15 students indicated "strongly agree" for the prompt, "I think protecting our watershed is important". Ten of 15 students indicated that they recycle. Ten of 15 students indicated "strongly agree" for the prompt, "Do you think marine animals are in danger and need our help?". Thirteen of 15 selected "yes" for the question, "Do you identify as underrepresented?".

In total, 17 students from Dixon School of the Arts and Sciences participated in the postsurvey. Specifically, five students were 8th graders, three students were 7th graders, and nine students were 6th graders. Three of 17 students listed a marine species of their favorite organism, while the remaining listed a terrestrial species. Six of 17 students reported a preference for a STEM career. All students indicated that the ocean is valuable to humans. Ten of 17 students indicated "strongly agree" or "agree" for the prompt "conservation is important". Ten of 17 students indicated "strongly agree" or "agree" for the prompt, "I am confident in my ability to do science". Fourteen of 17 students indicated "strongly agree" or "agree" for the prompt, "I think protecting our watershed is important". Thirteen of 17 students indicated that they recycle. Eleven of 17 students indicated "strongly agree" and need our help?". Additionally, for the post-survey, students were asked, "Where do microplastics come from? And "What action will you take to reduce plastic pollution?" Student responses can be seen below in **Table 3**.

Where do microplastics come from?	What action will you take to reduce plastic pollution?
Bigger chunks of plastics or for the fibers cloth	not use plastic straws.
It can come from plastic water bottles, plates bags, etc	You can recycle
Bigger pieces of plastic.	Recycle and join a beach clean up.
It comes from plastic.	Start recycling.
Microplastics come from clothes and any type of form of plastic.	I will stop using so much plastic and start non- biodegradable products.
Microplastics are very small plastic particles that can originate from a variety of sources.	refusing disposable plastic whenever you can
plastic trash, clothes	stop buying things with so much plastic
It comes from pollution that humans make.	l will recycle plastic.

**Table 3.** Student responses from final two post-survey questions.

microplastics come from big pieces of plastic and it gets broken down into the ocean to become microplastic	don't use plastic things
They come from fabrics, beauty products and water.	I would put a filter in the ocean that swallows plastics and other things to keep sea creatures safe.
other plastics	make art
They come from old plastics bottles or something else like that	To keep the ocean safe for the sea creates
Microplastics comes from rubber and things like that.	Reuse things
Broken down pieces of bigger plastic.	recycle and use non-plastic products.
Microplastics are very small generally less than 5 millimeters in size plastic particles that can originate from a variety of sources, such as ingredients in cigarette filters, textile fibers and cleaning or personal care products, and dust from car and truck tires, as well as from larger plastic products broken down	Wean yourself off disposable plastics. Ninety percent of the plastic items in our daily lives are used once and then chucked: grocery bags, plastic wrap, disposable cutlery, straws, coffee-cup lids. Take note of how often you rely on these products and replace them with reusable versions.
Plastic and litter	No plastic water bottles
Old plastic bottles	By getting it out the ocean

## Discussion

Water sampling of surface waters and filtration revealed microplastics contamination in the Pensacola Bay Watershed (mean 14.12 microplastics/L). The most common type of microplastics were microfibers. Often, microfibers are the dominant type of microplastics recovered (e.g.McEachern et al. 2019; Whitaker et al. 2019). Sites in the Pensacola Bay Watershed with the highest counts of microfibers were more southern in the watershed. Of particular notice, sites on Pensacola Bay (XX, XX, XX) had the highest counts of microplastics from sites sampled throughout the watershed. This may be indicative that fibers are being washed down from higher in the watershed and are more concentrated toward the lower end of the watershed. Further, the streams and rivers throughout the Pensacola Bay Watershed are likely acting as a source and means of transport to the ocean (Gulf of Mexico). Site XX near Navarre on Santa Rosa Sound had the highest concentration of microplastics per liter (n=142.) This site is surrounded by high human

urbanization and use. Fibers in the Pensacola Bay Watershed may arise from a variety of sources such as from wastewater of laundry of clothing with synthetic fibers, cigarette buts, ropes, nets, fishing activity, and atmospheric deposition (Wright et al. 2013; Dris et al. 2016; Wang et al. 2018). Microfragments were the second most common microplastic recovered in this study. Fragments are secondary microplastics that result from the breakdown of larger plastic debris.

Impacts on marine and freshwater organisms have not been thoroughly investigated, but microplastics are considered to be bioavailable to all aquatic organisms in the food web. Microplastics may act as vectors for transferring novel bacterial assemblages (Barnes 2002, Gregory 2009) and may contain adsorbed chemical pollutants (Carpenter et al. 1972). Additionally, microplastics are ubiquitous in terrestrial environments potentially leading to increased contamination in aquatic environments. A report from the World Health Organization (2019) suggested that human exposure of microplastics is of great concern to human health. Even though it is not clear yet how microplastics cause negative effects on organism growth, reproduction, and lead to weakened immune systems. Humans rely heavily on aquatic biodiversity and ecosystems, both of which are greatly impacted by microplastics.

Our goal for including students in this project is to engage students in local sciences and conservation issues, working to promote responsible environmental stewards in our local community, while at the same time building their confidence and capacity for science. Students of Dixon School of the Arts and Sciences also filtered water samples, many from household water sources such as bathroom faucets, hoses, or kitchen faucets. Overall the count of microplastics from student water samples was lower (mean 3.46 microplastics/L), but several plastics were nonetheless recovered. From the results of the pre- and post-surveys, student confidence in their ability to do science increased after completion of the microplastics educational program. Specifically, pre-survey results indicate that 33% of students were "confident in their ability to do science", while post-survey results indicate 59% of students were "confident in their ability to do science". Time and again, studies show that confidence in scientific abilities is a predictor of student success. Additionally, results from the pre- and post-surveys indicate that more students think "protecting our watershed is important" after the completion of the microplastics educational program. Specifically, in the pre-survey, 53% of students indicated "protection of our watershed is important", while in the postsurvey, 82% of students indicated "protection of our watershed is important". Even a greater percentage of students indicated that they would recycle after the completion of the program (67% to 76%).

Overall, this study demonstrated that microplastics were widely and in many cases abundantly distributed in the surface waters of the Pensacola Bay Watershed. This work also demonstrated that microplastics research serves as a strong educational tool for aiding in building student confidence in science and engagement in conservation of resources. This work also further solidified the need to combine educational outreach and scientific research. Despite the difficulty of undertaking, local and regional management initiatives need to be developed and carried out to manage microplastic pollution in our watershed. Removing microplastics from the water remains an almost impossible task. The solution, rather, lies within preventing plastic pollution from entering the watershed and increasing community awareness and involvement for protection of our watershed.

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