Regional Studies in Marine Science 77 (2024) 103668

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Spatial and temporal trends in microplastic pollution in the Eastern Oyster, *Crassotrea virginica*, in Tampa Bay, Florida

A. Murray [a], I.C. Romero [b], M. Riedinger-Whitmore [a], P. Schwing [c], H. Judkins [a,*]

[a] Integrative Biology Department, University of South Florida St. Petersburg, St. Petersburg, FL, USA
[b] College of Marine Science, University of South Florida, St. Petersburg, FL, USA
[c] Eckerd College, Marine Science, St. Petersburg, FL, USA

ARTICLE INFO

Keywords:
Plastic pollution
Watershed
Estuary
Plastic ingestion

ABSTRACT

Microplastic pollution is an increasingly alarming concern with widespread global distribution in aquatic environments. Spatial and temporal differences in microplastic abundance were evaluated in the Eastern Oyster, *Crassostrea virginica*, at six sites within Tampa Bay. Oyster tissue was digested using 30 % hydrogen peroxide (H_2O_2) and microplastics were quantified using Nile Red stain and fluorescent particle excitement. A total of 3025 microplastics were found throughout six study sites over two seasons (winter 2021 and summer 2022) with varying site types. Microfragments (n=2867) made up the majority of microplastics, as compared to microfibers (n=158). Significant differences were observed among the sites studied, site type, and their location in the bay. Outflow and marina areas had significantly higher ($p<0.01$) amounts of microplastics compared to preserve areas, and the east bay had significantly higher ($p<0.05$) amounts than the west bay. Findings suggest microplastic contamination is associated with higher urbanization, proximity to drainage basins, and recreation.

1. Introduction

Microplastics have been classified as a heterogenous mixture of particles with various composition and shapes, ranging from several microns to less than five mm diameter (Li et al., 2015; Lusher et al., 2015; Chubarenko et al., 2016). They can be produced directly from large factories or by being broken down to smaller pieces via chemical, physical, and biological processes in the environment (Ding et al., 2018; Waite et al., 2018). More than 335 million tons of plastic are produced globally every year with roughly 10 % transported into the oceans through watershed environments and carried by maritime vessels and ocean currents (Lusher et al., 2015; Ding et al., 2018; McEachern et al., 2019). Over 75 % of aquatic plastic comes from terrestrial sources (Thushari and Senevirathna, 2020). Once in the environment, microplastics are exposed to multiple processes such as wave action, sand grinding, photodegradation, thermal degradation, and biodegradation (Lusher et al., 2015; Waite et al., 2018; Amelia et al., 2021). While less prevalent, excretion from other organisms may also serve as a vector for microplastic dispersion (Hoang and Felix-Kim, 2020; Bourdages et al., 2021). Microplastics are globally ubiquitous, having been found in every ocean basin, deep-sea sediments, coastal sand, and estuarine environments (McEachern et al., 2019). While their universal distribution is widely accepted, their abundance in the Gulf of Mexico is still understudied (Wessel et al., 2016; Di Mauro et al., 2017; Plafcan et al), in review).

Microplastics are a marine pollutant of growing concern, as a majority of mismanaged plastics (i.e. plastics that uncontrollably enter the environment) find their way into aquatic ecosystems (Andrady, 2011), Amelia et al., 2019, (Ford et al., 2022), (Wootton et al., 2022). Microplastics ingestion has been recorded in over 180 marine species, including filter feeding organisms such as bivalves, which lack the capacity to select particles during ingestion (Wang et al., 2016; Waite et al., 2018). Also, once ingested, microplastics cannot be digested or absorbed because marine organisms do not have the capacity to breakdown their synthetic polymers, affecting their reproduction and energy uptake (Andrady, 2011; Guzzetti et al., 2018; Sussarellu et al., 2016; Carpenter et al., 2019; Provencher et al., 2019). Moreover, microplastic ingestion by oysters and other bivalves can lead to biomagnification of marine debris throughout food webs, up to marine predators and humans causing exposure both directly and indirectly (Waite et al., 2018; Cho et al., 2021). It is estimated that 85 % of oyster reefs have been globally lost and most are functionally extinct due to declining water conditions (Beck et al., 2011). There is still very little known regarding microplastic exposure to human health, but the risk

* Corresponding author.
E-mail address: Judkins@usf.edu (H. Judkins).

https://doi.org/10.1016/j.rsma.2024.103668
Received 26 January 2024; Received in revised form 23 June 2024; Accepted 1 July 2024
Available online 10 July 2024

factors outside of inflammation and lodging are being explored (Waite et al., 2018; Campanale et al., 2020; Pironti et al., 2021). Human consumption effects may vary with particle size, chemical properties, and microbial growth (Campanale et al., 2020) which may be problematic for individuals such as European shellfish consumers who may ingest up to 11,000 microplastics a year (Van Cauwenberghe and Janssen, 2014).

Tampa Bay is Florida's largest open water estuary located on the west-central coast of Florida and the second largest estuary in the Gulf of Mexico (Sherwood and Greening, 2013, (Beck et al., 2019). Estuaries have shown to be microplastic sinks due to directional wind patterns and tidal gradients (Browne et al., 2010). Tampa Bay's average depth is approximately four meters, and it has an area of 1000 km^2 which includes a watershed system almost five times the size of the bay itself (McEachern et al., 2019). Four major river systems contribute to the Bay: the Alafia, Manatee, Little Manatee, and Hillsborough Rivers (Xian et al., 2007). The Tampa Bay watershed has become a highly developed, urbanized area with more than 42 % of land developed and a population nearing four million people (Sherwood and Greening, 2014; Trottet et al., 2021). It is among the most highly developed regions in Florida, with more than 60 % urban land use within 15 km of the shoreline, making it more susceptible to microplastic contamination as microplastics are more prevalent in highly industrialized areas (Beck et al., 2019; Cho et al., 2021). Previous research has shown that microplastic concentrations are higher in Tampa Bay compared to open ocean systems (McEachern et al., 2019). Recent studies report there are approximately four billion microplastics in Tampa Bay (McEachern et al., 2019; Pariatamby et al., 2020). The Tampa Bay area is densely populated, supports high levels of tourism, fishing, and boating; all of which may contribute to higher concentrations of microplastics (Fibbe et al., 2023).

Demand for plastic production has reached a pivotal point, due to its versatility, low cost, and durability. Plastic manufacturing has become widespread across numerous industries, from food packing to medical and technological fields (Frias and Nash, 2019). Continued monitoring of microplastic contamination is vital as plastic pollution causes ecological, economic, biological, and social stress (Chubarenko et al., 2016). This study investigated the spatial and temporal occurrence of microplastics fragments and fibers in the Eastern Oyster, *Crassostrea virginica*, in Tampa Bay, Florida. Bivalves serve as keystone species, providing habitat and settling areas for other organisms (Waite et al., 2018). They are also a natural source for aquatic health, cleaning waterways through high filtration rates. They are economically important,

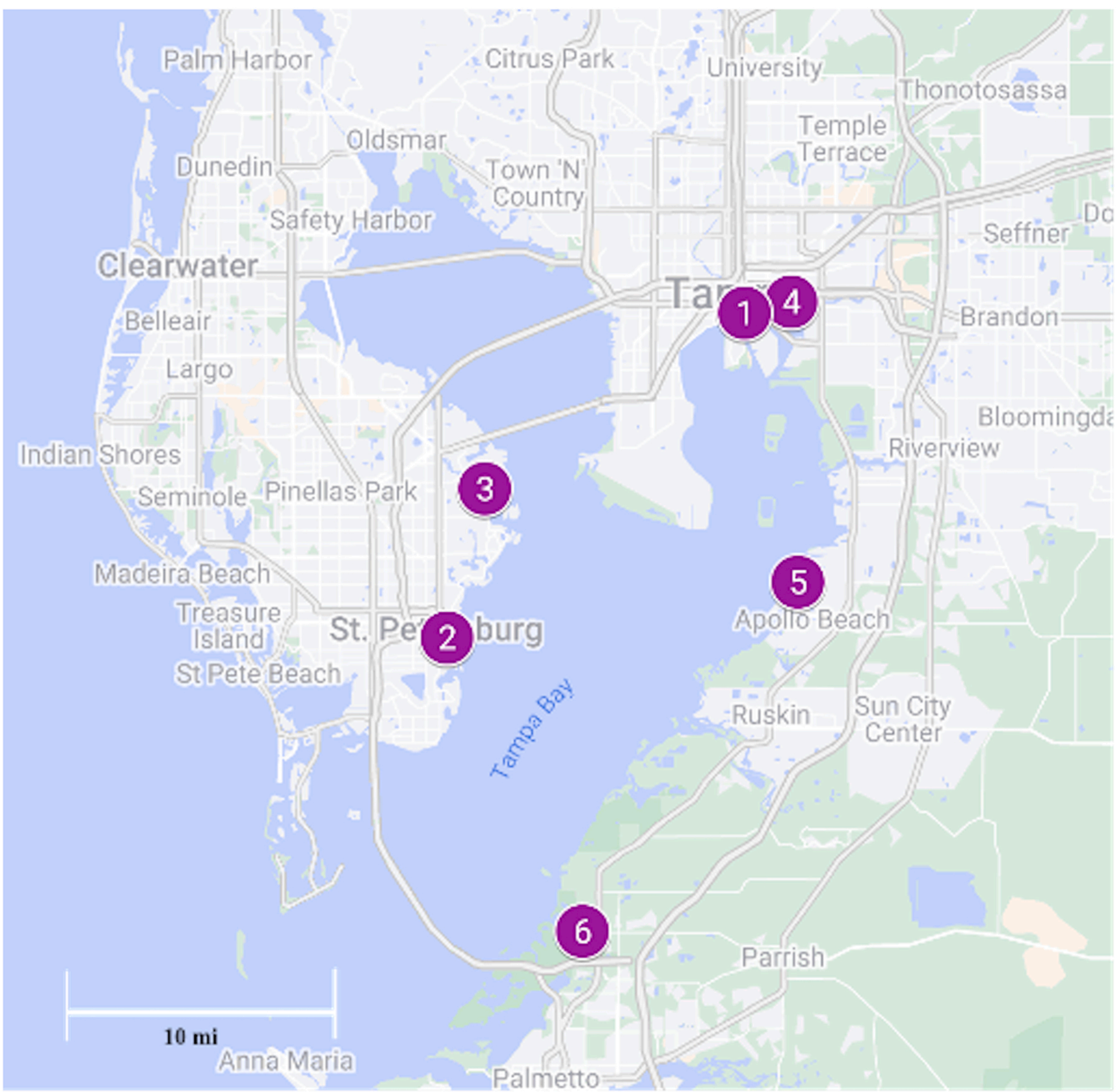

Fig. 1. Sampling locations in Tampa Bay, Florida by site categories: Marinas (1): Garrison Channel (2): Bayboro Harbor, preserves (3): Riviera Bay (4): McKay Bay, outflows (5): Big Bend Channel (6): Bishop Harbor. Map was created using Google My Maps.

serving as a widespread food source making them a species of particular concern (Dowarah et al., 2020). Microplastics may also travel through various trophic levels through bioaccumulation, leading to biomagnification of metals, additives, toxins, and other harmful organic compounds associated with microplastics (Waite et al., 2018). Examining spatial trends will provide a better understanding for microplastic vectors while temporal sampling will account for potential changes in microplastic abundance during varying seasons. Overall, this study provides a better understanding of general trends in microplastic pollution and enhances our knowledge regarding microplastic uptake in *C. virginica*.

2. Methods

2.1. Study sites

Tampa Bay has a subtropical and temperate climate (Sherwood and Greening, 2014) with a rainy season from June to mid-October and a mild dry season from November through May (Radabaugh et al., 2018). In general, seasons appear to influence estuary oyster microplastic abundance due to periods of increased rainfall which transports particles into coastal environments during the summer months (Baechler et al., 2019). Six sites were selected throughout Tampa Bay (Fig. 1, Table 1). These sites were selected to cover broad regions with varying degrees of human activities. Sites were categorized in three groups: marina, preserve, and outflow. Marinas were Garrison Channel (1) and Bayboro Harbor (2). Garrison Channel houses a large marina located in downtown Tampa, outside of Port Tampa, the largest port in Florida. Bayboro Harbor is located near the University of South Florida St. Petersburg campus and contains the Harborage Marina. It is also adjacent to Albert Whitted Airport (SPG). Preserve area sites were Riviera Bay (3) and McKay Bay (4), and outflow areas were Big Bend Channel (5) and Bishop Harbor (6). Preserves are areas where animals and plants are protected and there are few buildings on site. The sampled outflow sites act as vectors to discharge potentially hazardous contaminants to surrounding aquatic areas. Wastewater has been associated as a vector for microplastic contamination (Waite et al., 2018; Baechler et al., 2019). In spring 2021, 814 million liters of phosphate mining wastewater and marine dredge were released into lower Tampa Bay from Piney Point near Bishops Harbor (Beck et al., 2022). The TECO Power Plant discharges cooling water, utilized for machinery into discharge conduits, leading directly into the Bay near Big Bend Channel (McConnell et al., 2003).

Sites were also categorized regionally: north bay (Garrison Channel, McKay Bay), east bay (Big Bend Channel, Bishop Harbor), and west bay (Riviera Bay, Bayboro Harbor). The east Bay is located next to two large drainage basins, the Little Manatee and Alafia Rivers (Xian et al., 2007). The Hillsborough River basin drains into north bay while west bay is farther away from the Bay's watershed drainage (Xian et al., 2007). The size of the sampling areas is shown in Table 1. Areas were estimated using Google Earth.

2.2. Collection

Collections of *Crassostrea virginica* samples were timed during low tide in inshore environments. Sampling was conducted in December 2021 and repeated in July-August 2022 to compare possible changes in seasonality (i.e. winter and summer). A 20 m transect was used at all collection sites with samples taken from random spots one meter from either side of the transect. Twenty oysters were taken from each site and were kept in 5-gallon buckets with site specific water during transport to the laboratory, where morphological measurements were taken before storing oysters at −20 °C. Oysters smaller than 50 mm were not used for this study. Morphological measurements were taken with a digital caliper which included shell length, width, and across the midline of each shell. For the purpose of this study, the midline is defined as the start of the curvature of the shell or midway from the anterior to posterior if no curvature was present. Water quality measurements were conducted onsite with a few exceptions (e.g. nitrite) which was measured with greater certainty in the laboratory. These measurements were taken within roughly an hour of sampling. Nitrite readings were void for three out of six test sites in December. Salinity was measured using a Milwaukee MR Refractometer. All other parameters utilized color changing reagents and a gradient scale. Dissolved oxygen was measured using CHEMets Kit K-7512, and all other measurements utilized Hanna HI water quality instruments.

2.3. Extraction and enumeration of microplastics

Oysters were thawed to room temperature and opened using an oyster shucker and hammer. All organs except the mantle tissue were weighed and placed in aluminum foil and frozen at −20 °C until ready for extraction. Erlenmeyer flasks (250 mL) were filled with 150 mL of Fischer Scientific (certified ACS) 30 % hydrogen peroxide (H_2O_2) and the sample. Samples were covered with aluminum foil, placed in an incubator for 24 hours at 65°C, then placed on a shaking table inside a hood for an additional 24 hours at 80 rotations per minute (rpm). The temperature fluctuated from 61 to 75°C with an average of 64 °C during the digestion period. After these steps, the digested tissues were filtered using Sterlitech polycarbonate PCTE filters (5μm, diameter 47 mm black) in a fume hood.

Methanol-based Nile Red liquid stain was used to enhance fluorescence of hydrophobic particles and distinguish plastic versus organic matter with previous literature suggesting Nile Red stain is comparable to spectroscopy techniques, such as FTIR, for microplastic quantification (Maes et al., 2017; Dowarah et al., 2020; Nalbone et al., 2021). Each filter was flushed with stain while still in vacuum filtration. Filters were placed in Fisherbrand sterile petri dishes (triple rinsed with deionized water), covered, and left to dry in a dark cabinet. Samples were analyzed in a dark room using a SF-2TRA dissecting scope with NIGHTSEA fluorescent attachments including two cyan excitation lights (490–515 nm), barrier filter (550 nm long pass), and viewing shield. Images were taken through an AmScope MU1803 digital microscope camera. Microplastics were counted and categorized into fragments or fibers. FTIR was not part of this study. This work is paired with a subsequent publication utilizing the visual analysis of microplastic counts from this study and

Table 1
Coordinates and site types of each sampling area.

ID	Site	Site Type	Region	Latitude	Longitude	Size
1	Garrison Channel	Marina	North	27.94033	-82.45118	0.16 km^2
2	Bayboro Harbor	Marina	West	27.762185	-82.634928	0.34 km^2
3	Riviera Bay	Preserve	West	27.844176	-82.611166	3.53 km^2
4	McKay Bay	Preserve	North	27.945764	-82.422315	3.68 km^2
				27.94676	-82.428184	
5	Big Bend Channel	Outflow	East	27.79337	-82.41828	20.11 km^2
6	Bishop Harbor	Outflow	East	27.60177	-82.55194	1.07 km^2

Table 2
Water quality measurements at all sampling sites.

Date	Location	pH	Temp °C	Phosphate	Nitrate	Nitrite	DO	Salinity
12/10/21	Riviera Bay	8.0	23.0	1.0 mg/L	<10 mg/L	NA	5.0 mg/L	23 ppt
7/18/22	Riviera Bay	7.6	31.5	<1.0 mg/L	<10 mg/L	<0.2 mg/L	5.0 mg/L	21 ppt
12/13/21	Bayboro Harbor	7.7	25.5	<1.0 mg/L	<10 mg/L	<0.2 mg/L	5.0 mg/L	22 ppt
7/19/22	Bayboro Harbor	7.4	30.3	5.0 mg/L	<10 mg/L	<0.2 mg/L	5.0 mg/L	10 ppt
12/15/21	Garrison Channel	7.9	21.8	<1.0 mg/L	<10 mg/L	NA	4.0 mg/L	25 ppt
7/28/22	Garrison Channel	7.5	30.0	4.0 mg/L	< 10 mg/L	0.2 mg/L	5.6 mg/L	14 ppt
12/22/21	McKay Bay	7.9	17.7	<1.0 mg/L	<10 mg/L	NA	4.0 mg/L	26 ppt
8/1/22	McKay Bay	7.8	32.7	2.0 mg/L	<10 mg/L	<0.2 mg/L	5.5 mg/L	23 ppt
12/24/21	Big Bend Cannel	8.1	22.2	2.0 mg/L	<10 mg/L	<0.2 mg/L	6.0 mg/L	26 ppt
7/29/22	Big Bend Cannel	7.8	32.9	1.0 mg/L	<10 mg/L	<0.2 mg/L	5.0 mg/L	25 ppt
12/30/21	Bishop Harbor	7.7	23.6	2.0 mg/L	<10 mg/L	<0.2 mg/L	5.0 mg/L	29 ppt
7/25/22	Bishop Harbor	7.8	30.4	5.0 mg/L	<10 mg/L	<0.2 mg/L	6.0 mg/L	30 ppt

analyzing chemical compounds found within oyster tissue, with an emphasis on phthalate contamination and other emerging contaminants of concern.

2.4. Quality control

To prevent contamination, a 100 % cotton lab coat was worn during dissection periods as well as nitrile gloves (Wang et al., 2021). All glassware, dishes, and other tools were rinsed three times with deionized water (Li et al., 2015). During dissections, one blank filter was left exposed in an uncovered petri dish per site to account for possible air borne contamination (Li et al., 2015). When left uncovered for roughly two hours, blanks had on average three airborne microplastics (range 0–10). For digestion, deionized water and H_2O_2 blanks were used to account for potential contamination in either media. Blanks were subjected to the same flask size, foil, incubation, and shaking periods. All deionized water and H_2O_2 blanks showed insignificant contamination (an average of less than one microplastic per blank). Samples were double counted under a dissecting scope and a subset of at least five samples were verified by an additional technician.

2.5. Data analysis

One-Way ANOVA (α=0.05) analysis was run to compare variation across sampling sites. Tukey-Kramer HSD multiple comparison tests were performed to determine significance between microplastic counts among individual sites. Nested ANOVA (α=0.05) analyzes were conducted for comparisons between microplastic counts among site types, regions, and to measure variation between sites. Results reported did not undergo Satterthwaite approximation corrections as variation within sample sets did not significantly alter results. Least fit models were used to determine significance between morphological differences in oyster size and microplastic accumulation. Data that did not follow a normal distribution was normalized by square root transformations for least fit analyses. All analyses were done using JMP Pro 16 statistical software. JMP®, Version 16. SAS Institute Inc., Cary, NC, 1989–2023.

3. Results

3.1. Morphological measurements

All morphological measurements (mean ± SD) across all sites are in Table 3. On average shell length was 70.4 mm ± 11.5 ranging from 50.6 mm to 111.6 mm. Body weight also varied among sites averaging 27.8 g ± 11.7 and ranged from 9.9 g to 70.0 g. Least fit models showed there were no overall significant trends with microplastic contamination and oyster size. Shell length (mm) and shell midline (mm) were the only significant ($p<0.05$) results when compared to microplastics (MP).

3.2. Quantifying microplastics

Microplastics were found in all sample sites over the two seasons sampled. Photos from this methodology (Fig. 2) demonstrates the Nile Red stain's ability to discern plastic and organic material. A total of 3025 MP were found, with fragments making up 94.8 % of MP and fibers making up the remaining 5.2 %. Average MP counts for *C. virginica* was 13.8 ± 17.1 MP per individual. Average (mean ± SD) MP per site are shown in Table 4. Mean microfragments per individual and mean microfibers per individual are shown in Fig. 3. Microplastic concentrations were calculated by dividing the total MP/individual by sample tissue wet weight (g). Average concentration was 5.2 ± 6.6 MP/gram sample tissue (mean ± SD).

Microplastic accumulation significantly (F_{5213}=8.2068, $p<0.0001$) varied between individual sites. Specific p-values within each site are

Table 3
Average oyster morphological measurements (mean ± SD) per site.

Site	Digested Tissue (g)			Shell Weight (g)			Shell Length (mm)			Shell Width (mm)			Shell Midline (mm)		
Bishop Harbor	3.4	±	1.8	19.9	±	9.0	62.0	±	7.1	17.8	±	4.8	30.6	±	5.2
Big Bend Channel	2.7	±	1.1	22.1	±	8.6	73.9	±	10.0	17.6	±	4.2	31.0	±	4.1
Garrison Channel	3.2	±	1.2	28.9	±	11.7	75.9	±	14.4	19.1	±	4.2	35.8	±	5.0
Bayboro Harbor	2.8	±	1.3	27.4	±	10.6	70.2	±	10.3	20.5	±	5.4	33.9	±	6.8
McKay Bay	1.9	±	1.0	22.0	±	9.1	68.7	±	12.5	17.8	±	3.2	27.9	±	4.1
Riviera Bay	3.8	±	1.7	28.2	±	11.7	70.7	±	8.5	19.5	±	4.5	37.5	±	6.2

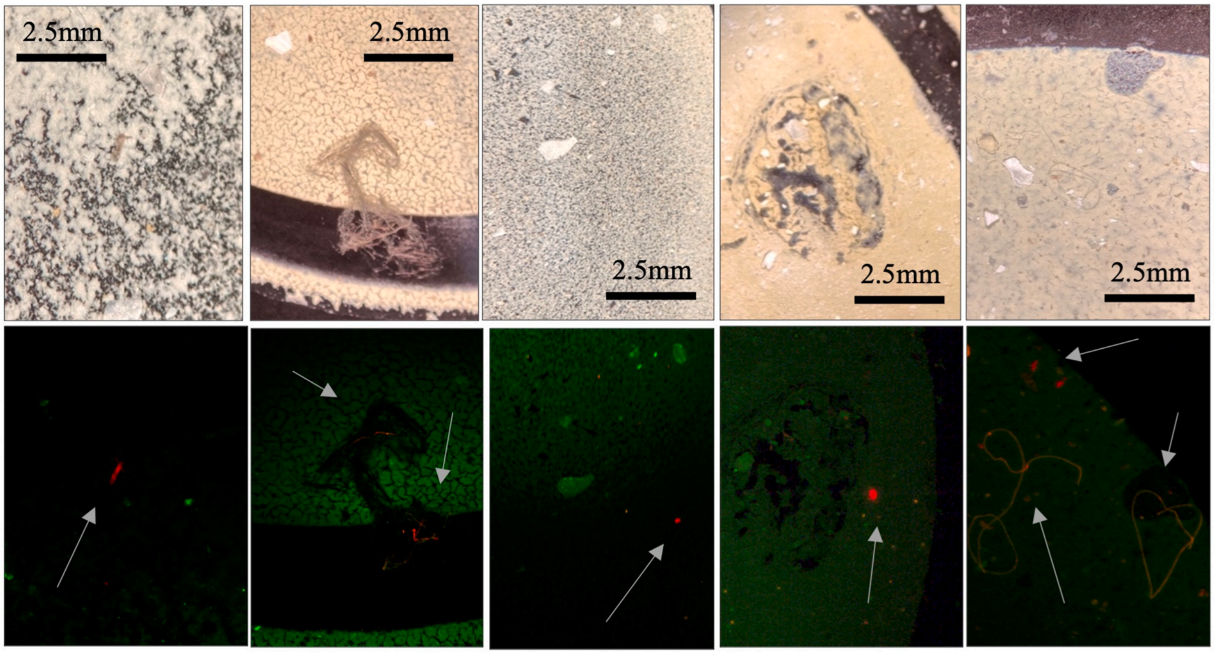

Fig. 2. Examples of dyed samples under a dissecting scope in ambient light (top row), bottom row shows same sample under fluorescent light.

Table 4
Microplastic particles per individual (mean ± SD) at each site.

Site	Mean Fragments (MP/individual)			Mean Fibers (MP/ individual)			Total Mean (MP/ individual)		
Bishop Harbor	7.3	±	12.6	0.6	±	0.9	7.9	±	12.5
Big Bend Channel	25.6	±	23.4	0.4	±	0.6	26.0	±	23.3
Garrison Channel	18.1	±	12.4	0.6	±	1.0	18.7	±	12.7
Bayboro Harbor	9.4	±	13.4	1.2	±	1.4	10.6	±	14.1
McKay Bay	6.1	±	6.2	0.8	±	1.5	6.9	±	6.4
Riviera Bay	10.1	±	19.4	0.7	±	1.5	10.8	±	19.3

shown in Table 5. Differences between site types (marina, preserve, outflow) were not significant ($F_{2,3}$=0.4836, p=0.6576) (Fig. 4). However, there was significant variation (F_{3213}=9.9853, p<0.0001) in microplastic counts among sites within categorized site type. Big Bend Channel had 26.0 ± 23.3 MP/individual (mean ± SD) while the other outflow sample site, Bishop Harbor, had only 7.9 ± 12.5 MP/individual (mean ± SD). Similar variation was found among marinas with Garrison Channel reporting 18.7 ± 12.7 MP/individual (mean ± SD) and Bayboro Harbor reporting 10.6 ± 14.1 MP/individual (mean ± SD). Oysters sampled from Big Bend Channel and Garrison Channel had the highest average counts out of the dataset. Average MP counts were higher in marina sites (Bayboro Harbor, Garrison Channel) and outflow areas (Big Bend Channel, Bishop Harbor) compared to preserves (Riviera Bay, McKay Bay) (Fig. 4). Outflow areas had the highest mean MP count (17.7 ± 21.1 MP/individual) while preserves had the lowest (8.9 ± 14.5 MP/individual).

Differences among regions ($F_{2,3}$=0.2575, p=0.7885) were not significant, but there was significant (F_{3213}=11.2471, p<0.0001) variation among sites within each region. Mean counts for east bay were 17.6 ± 21.1 MP/individual compared to west bay which was 10.7 ± 16.6 MP/individual (Fig. 4). Overall, east Tampa (Big Bend Channel, Bishop Harbor) had the highest MP mean counts compared to west Tampa (Bayboro Harbor, Riviera Bay) and north Tampa (Garrison Channel, McKay Bay). Winter and summer counts did not drastically differ, though four out of the six sites did show an increase in MP/individual. Water quality measurements are shown in Table 2. Bayboro Harbor's salinity in the winter more than doubled compared to summer months but other parameters were showed less variability comparatively.

4. Discussion

The aim of this research was to study the spatial and temporal trends of microplastic pollutants in the Eastern Oyster, *Crassostrea virginica*, throughout Tampa Bay. Samples were collected during two different seasons to account for possible differences in microplastic contamination in the rainy versus dry season at six locations throughout the Bay. Microplastics were found in oyster tissue at all six sites with most samples having at least some contamination. Total microplastic counts did not vary with seasonality. These results differ from previous studies that found higher microplastic counts during periods of heavy rain (Baechler et al., 2019; McEachern et al., 2019). Looking at water quality parameters (Table 2), the low salinity for Bayboro Harbor during summer sampling confirms that rainfall alone did not increase microplastic abundance since microplastic counts during seasonal water inflow were not significantly higher. This suggests microplastic contamination may be more closely related to recreational activities versus freshwater influx at Bayboro Harbor. Further investigation is needed to justify a definitive pathway and confirm this finding. Winter and summer replicates should be continued to get a better understanding of any seasonal trends happening in the Bay.

Microplastic fragments were more abundant than fibers in this study across all sites during both sampling seasons. Other studies showed higher abundance of microfibers in bivalves and shallow aquatic systems (Waite et al., 2018; Baechler et al., 2019; Lozano-Hernandez et al., 2021). However, Woods et al. (Woods et al., 2018) found that when exposed to microfibers, the blue mussel, *Mytilus edulis*, was able to expel 71 % of fibers as "pseudofeces." This contradiction may be due to studies suggesting microfragment accumulation in bivalves is higher due to smaller particle size, making them easier to transfer and remain in the digestive tract (Woods et al., 2018). The ability of bivalves and other filter feeding invertebrates to reject undesirable particles acts as an alternative mechanism to maintain the organism's health, suggesting that fibers could be easier to expel compared to fragments (Woods et al., 2018). This may explain the stark difference in fragment to fiber findings compared to previous studies looking at microplastics in Tampa Bay (McEachern et al., 2019).

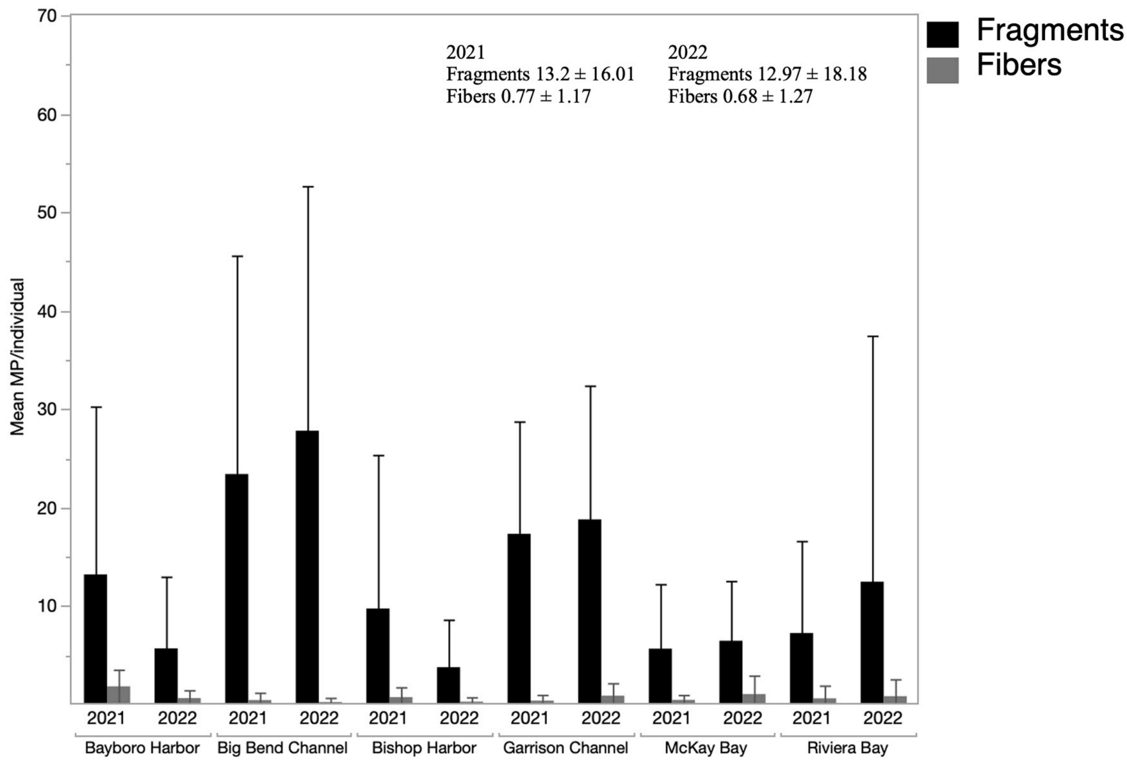

Fig. 3. Bar graph depicting mean (mean ± SD) number of microfragments and microfibers per site in December 2021 and July-August 2022. Black bars represent fragments and gray bars represents fibers. Error bar was constructed using one standard deviation from the mean.

Table 5
Mean MP differences between sites with significance and SD. Data set analyzed through One-Way ANOVA (F_{5213}=8.2068, p<0.0001, α=0.05). Tukey-Kramer HSD was used for multiple comparison to calculate significance among sites.

Level		Difference	Std Err Dif	p-value
Site				
Big Bend Channel	McKay Bay	19.04	3.70	<0.0001
Big Bend Channel	Bishop Harbor	18.10	3.70	<0.0001
Big Bend Channel	Bayboro Harbor	15.36	3.57	0.0004
Big Bend Channel	Riviera Bay	15.12	3.67	0.0008
Garrison Channel	McKay Bay	11.82	3.77	0.0237
Garrison Channel	Bishop Harbor	10.88	3.77	0.0487
Garrison Channel	Bayboro Harbor	8.14	3.64	>0.05
Garrison Channel	Riviera Bay	7.90	3.74	>0.05
Big Bend Channel	Garrison Channel	7.22	3.62	>0.05
Riviera Bay	McKay Bay	3.92	3.82	>0.05
Bayboro Harbor	McKay Bay	3.68	3.72	>0.05
Riviera Bay	Bishop Harbor	2.98	3.82	>0.05
Bayboro Harbor	Bishop Harbor	2.74	3.72	>0.05
Bishop Harbor	McKay Bay	0.94	3.85	>0.05
Riviera Bay	Bayboro Harbor	0.24	3.69	>0.05

The average MP count per individual and each MP's concentration are similar to previous studies in estuary and coastal environments. A study completed in Mosquito Lagoon, an eastern Florida estuary, found 16.5 MP/individual of *C. virginica* (Waite et al., 2018) while another study along the Oregon coast detected 11.0 MP/individual of *C. gigas* (Baechler et al., 2019). These studies align with findings here which indicate that microplastics are prevalent in many estuaries around the United States. Microplastic estimates vary internationally (Table 6) but counts appear to be higher in the United States compared to other global studies.

Among individual *C. virginica*, size variation did not have a significant impact on microplastic levels which coincides with previous literature that there is not a significant trend with organism size and microplastic burden (Beachler et al., 2019, (Joshy et al., 2022). Shell size is a better indicator for growth stage compared to body weight because soft tissue is easily influenced by metabolic status (Wu et al., 2022). Counting higher numbers of microplastic particles in larger oysters is not surprising given mature organisms are typically associated with higher clearance rates (Sylvester et al., 2005). Therefore, any influence with microplastic uptake should be associated with shell size rather than body weight. However, there is not enough evidence in this study to support an overall trend with oyster size and microplastics.

The total number of microplastics did not significantly differ between marina, outflow, and preserve areas. Both marinas and outflow areas were expected to have higher quantities compared to preserves which are areas of managed land designated to maintain their natural ecosystems as areas with higher urbanization gradients are associated with microplastic distribution and abundance (Browne et al., 2011; Li et al., 2023). Recreational activities and boating are linked to higher microplastic abundance (Waite et al., 2018). Microplastics can accumulate near marinas through both boating equipment and boat-wake driven water patterns (Waite et al., 2018). Outflow areas act as vectors for contaminant capture.

Differences in microplastic distribution were also not significant between regions, though microplastic counts were higher in east bay compared to west bay. This difference may be due to the east bay's proximity to the Little Manatee and Alafia drainage basins. Recent studies have shown a widespread number of microplastics in freshwater

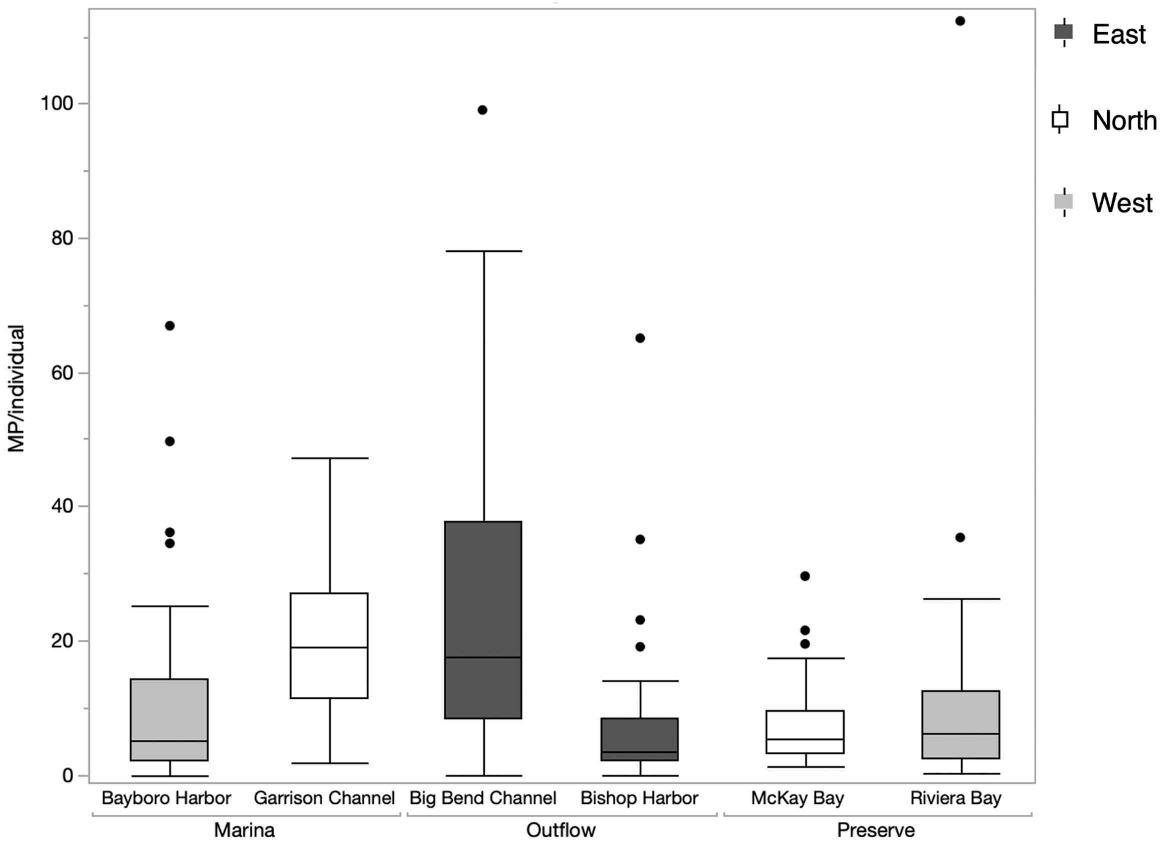

Fig. 4. Box plot showing total microplastics (n=3025) across all six sites in Tampa Bay: Bayboro Harbor, Big Bend Channel, Bishop Harbor, Garrison Channel, McKay Bay, Riviera Bay. Sites are split among site types and color coordinated per region.

Table 6
Comparison of Mean MP/individual and Mean MP/g wet weight of oyster tissue (wet weight) from this study to other global literature.

Location	Species	Mean MP/ individual	Mean MP/g	Reference
South Korea	*Crassostrea gigas*	1.21	0.33	Cho et al., (Cho et al., 2021)
China	*Saccostrea cucullata*	N/A	1.84	Wang et al., (Wang et al., 2021)
Australia	*Crassostrea gigas*	0.83	0.09	Wootton et al., (Wootton et al., 2022)
India	*Magallana bilineata*	6.90	1.23	Patterson et al., (Patterson et al., 2019)
Vietnam	*Crassostrea gigas*	18.54	3.84	Do et al., (Do et al., 2022)
United States	*Crassostrea virginica*	16.50	0.35	Waite et al., (Waite et al., 2018)
United States	*Crassostrea gigas*	10.69	0.35	Baechler et al., (Baechler et al., 2019)
United States	*Crassostrea virginica*	12.80	5.20	This Study

basins, and research is shifting toward looking at drainage inputs (McEachern et al., 2019; Li et al., 2023). Results showed microplastic counts were significantly different when comparing individual sites while microplastic counts comparing spatial differences showed little variation. Future studies should focus on increasing sampling sites within targeted categories to broaden the dataset and create a more accurate depiction of accumulation trends. Although water quality measurements fluctuated slightly, there was no significant trend between total microplastics and measured water quality (Table 2).

5. Conclusions

It is evident that microplastics are present in oysters throughout Tampa Bay even in protected preserve environments. This work showed the presence of microplastics in all areas, with little to no differences in water quality among sites. Microplastic abundance was higher at sites associated with higher human activity although there were no significant trends between grouped sampling sites. There was strong variation with microplastic accumulation throughout the Bay, thus calling for stronger research initiatives to have a better understanding of spatial trends. Tampa Bay's intricate hydrology also serves as an effective pollutant distribution mechanism. With continued development of the Tampa Bay area, it is imperative to continue monitoring microplastic contamination as this research has shown microplastic accumulation is more reliant on location versus oyster size. Without continued implementation of management practices, it is reasonable to expect an increase in contamination.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant No. 1930451. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Funds for sample processing were provided by a Duke Energy Conservation Biology Graduate Student Award to support graduate student research. This project could not have been completed without materials and space provided by Eckerd College.

CRediT authorship contribution statement

A. Murray: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **I.C.Romero:** Writing – review & editing, Methodology, Conceptualization. **M.Riedinger-Whitmore:** Writing – review & editing. **P.Schwing:** Writing – review & editing. H.Judkins: Writing – review & editing, Supervision, Conceptualization.

References

Amelia, T.S.M., Khalik, W.M.A.W.M., Ong, M.C., Shao, Y.T., Pan, H.-J., Bhubalan, K., 2021. Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. Prog. Earth Planet. Sci. *8* (1) https://doi.org/10.1186/s40645-020-00405-4.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Baechler, B.R., Granek, E.F., Hunter, M.V., Conn, K.E., 2019. Microplastic concentrations in two Oregon bivalve species: spatial, temporal, and species variability. Limnol. Oceanogr. Lett. 5 (1), 54–65. https://doi.org/10.1002/lol2.10124.

Beck, M.W., Sherwood, E.T., Henkel, J.R., Dorans, K., Ireland, K., Varela, P., 2019. Assessment of the cumulative effects of restoration activities on water quality in Tampa Bay, Florida. Estuaries Coasts 42 (7), 1774–1791. Retrieved from. 〈https://www.ncbi.nlm.nih.gov/pubmed/31853233〉.

Beck, M. W., Altieri, A., Angelini, C., Burke, M. C., Chen, J., Chin, D. W., Gardiner, J., Hu, C., Hubbard, K.A., Liu, Y., Lopez, C., Medina, M., Morrison, E., Phlips, E.J., Raulerson, G.E., Scolaro, S., Sherwood, E.T., Tomasko, D., Weisberg, R.H., & Whalen, J. 2022 Initial estuarine response to inorganic nutrient inputs from a legacy mining facility adjacent to Tampa Bay, Florida. Mar Pollut Bull 178:113598. doi: 10.1016/j.marpolbul.2022.113598.

Bourdages, M.P.T., Provencher, J.F., Baak, J.E., Mallory, M.L., Vermaire, J.C., 2021. Breeding seabirds as vectors of microplastics from sea to land: Evidence from colonies in Arctic Canada. Sci. Total Environ. *764*, 142808 https://doi.org/10.1016/j.scitotenv.2020.142808.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45 (21), 9175–9179. https://doi.org/10.1021/es201811s.

Browne, M.A., Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris along Estuarine shorelines. Environ. Sci. Technol. 44 (9), 3404–3409. https://doi.org/10.1021/es903784e.

Campanale, C., Massarelli, C., Savino, I., Locaputo, V., Uricchio, V.F., 2020. A Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human Health. Int J. Environ. Res Public Health *17* (4). https://doi.org/10.3390/ijerph17041212.

Carpenter, J.S., B, Masonjones, H., 2019. The Impact of Microplastics on *Crassostrea virginica* Filtration Efficiency. Acta Spartae 4 (1), 13–16. https://doi.org/10.48497/xp2c-fq23.

Cho, Y., Shim, W.J., Jang, M., Han, G.M., Hong, S.H., 2021. Nationwide monitoring of microplastics in bivalves from the coastal environment of Korea. Environ. Pollut. 270, 116175 https://doi.org/10.1016/j.envpol.2020.116175.

Chubarenko, I.B., Zobkov, A., M, Esiukova, E., 2016. On some physical and dynamical properties of microplastic particles in marine environment. Mar. Pollut. Bull. 108, 105–112. https://doi.org/10.1016/j.marpolbul.2016.04.048. Retrieved from.

Di Mauro, R., Kupchik, M.J., Benfield, M.C., 2017. Abundant plankton-sized microplastic particles in shelf waters of the northern Gulf of Mexico. Environ. Pollut. 230, 798–809. https://doi.org/10.1016/j.envpol.2017.07.030.

Ding, J.L., Sun, J., He, C., Jiang, C., Gao, F., Zheng, F.L., 2018. Separation and Identification of Microplastics in Digestive System of Bivalves. Chin. J. Anal. Chem. 46 (5), 690–697. https://doi.org/10.1016/S1872-2040(18)61086-2 (Retrieved from).

Do, V.M., Dang, T.T., Le, X.T.T., Nguyen, D.T., Phung, T.V., Vu, D.N., Pham, H.V., 2022. Abundance of microplastics in cultured oysters (*Crassostrea gigas*) from Danang Bay of Vietnam. Mar. Pollut. Bull. 180, 113800 https://doi.org/10.1016/j.marpolbul.2022.113800 (Retrieved from).

Dowarah, K.P., Thirunavukkarasu, A., Jayakumar, C., Devipriya, S., S, 2020. Quantification of microplastics using Nile Red in two bivalve species *Perna T viridis* and *Meretrix meretrix* from three estuaries in Pondicherry, India and microplastic uptake by local communities through bivalve diet. Mar. Pollut. Bull. 153 https://doi.org/10.1016/j.marpolbul.2020.110982 (Retrieved from).

Fibbe, M.C., Carroll, D., Gowans, S., Siuda, A.N.S., 2023. Ingestion of microplastics by copepods in Tampa Bay Estuary, FL. Front. Ecol. Evol. *11* https://doi.org/10.3389/fevo.2023.1143377.

Ford, H.V., Jones, N.H., Davies, A.J., Godley, B.J., Jambeck, J.R., Napper, I.E., Suckling, C.C., Williams, L.C., Woodall, L.C., Koldewey, H.J., 2022. The fundamental links between climate change and marine plastic pollution. Sci. Total Environ. *806* (Pt 1), 150392 https://doi.org/10.1016/j.scitotenv.2021.150392.

Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar Pollut Bull 138, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022.

Guzzetti, E., Sureda, A., Tejada, S., Faggio, C., 2018. Microplastic in marine organism: Environmental and toxicological effects. Environ. Toxicol. Pharm. 64, 164–171. https://doi.org/10.1016/j.etap.2018.10.009.

Hoang, T.C., Felix-Kim, M., 2020. Microplastic consumption and excretion by fathead minnows (*Pimephales promelas*): Influence of particles size and body shape of fish. Sci. Total Environ. *704*, 135433 https://doi.org/10.1016/j.scitotenv.2019.135433.

Joshy, A., Krupesha Sharma, S.R., Mini, K.G., 2022. Microplastic contamination in commercially important bivalves from the southwest coast of India. Environ. Pollut. *305*, 119250 https://doi.org/10.1016/j.envpol.2022.119250.

Li, W., Li, X., Tong, J., Xiong, W., Zhu, Z., Gao, X., Li, S., Jia, M., Yang, Z., Liang, J., 2023. Effects of environmental and anthropogenic factors on the distribution and abundance of microplastics in freshwater ecosystems. Sci. Total Environ. 856 (Pt 2)), 159030 https://doi.org/10.1016/j.scitotenv.2022.159030.

Li, J., Yang, D., Li, L., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. Environ. Pollut. 207, 190–195. https://doi.org/10.1016/j.envpol.2015.09.018.

Lozano-Hernandez, E.A., Ramirez-Alvarez, N., Rios Mendoza, L.M., Macias-Zamora, J.V., Sanchez-Osorio, J.L., Hernandez-Guzman, F.A., 2021. Microplastic concentrations in cultured oysters in two seasons from two bays of Baja California, Mexico. Environ. Pollut. *290*, 118031 https://doi.org/10.1016/j.envpol.2021.118031.

Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. Sci. Rep. 5, 14947 https://doi.org/10.1038/srep14947.

Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci. Rep. 7, 44501 https://doi.org/10.1038/srep44501.

McConnell, R., Robison, D., Janicki, T., 2003. Tampa Bay Water's Hydrobiological Monitoring Programs. In: SESSION, *1*. FISH & WILDLIFE, 67.

McEachern, K., Alegria, H., Kalagher, A.L., Hansen, C., Morrison, S., Hastings, D., 2019. Microplastics in Tampa Bay, Florida: Abundance and variability in estuarine waters and sediments. Mar. Pollut. Bull. 148, 97–106. https://doi.org/10.1016/j.marpolbul.2019.07.068.

Nalbone, L., Panebianco, A., Giarratana, F., Russell, M., 2021. Nile Red staining for detecting microplastics in biota: Preliminary evidence. Mar. Pollut. Bull. 172, 112888 https://doi.org/10.1016/j.marpolbul.2021.112888.

Pariatamby, A., Hamid, F.S., Bhatti, M.S., Anuar, N., Anaur, N., 2020. Status of Microplastic Pollution in Aquatic Ecosystem with a Case Study on Cherating River, Malaysia. J. Eng. Technol. Sci. *52* (2), 222–241. https://doi.org/10.5614/j.eng.technol.sci.2020.52.2.7.

Patterson, J., Jeyasanta, K.I., Sathish, N., Booth, A.M., Edward, J.K.P., 2019. Profiling microplastics in the Indian edible oyster, Magallana bilineata collected from the Tuticorin coast, Gulf of Mannar, Southeastern India. Sci. Total Environ. *691*, 727–735. https://doi.org/10.1016/j.scitotenv.2019.07.063.

Pironti, C., Ricciardi, M., Motta, O., Miele, Y., Proto, A., Montano, L., 2021. Microplastics in the Environment: Intake through the Food Web, Human Exposure and Toxicological Effects. Toxics *9* (9). https://doi.org/10.3390/toxics9090224.

Plafcan, Martina M. and Schwing, Patrick T. and Romero, Isabel C. and Brooks, Gregg R. and Larson, Rebekka A. and O'Malley, Bryan J. and Stallings, Christopher D., Benthic Foraminifera in Gulf of Mexico Show Temporal and Spatial Dynamics of Microplastics (In Review). Benthic foraminifera in Gulf of Mexico show temporal and spatial dynamics of microplastics. Mar Pollut Bull.

Provencher, J.F., Ammendolia, J., Rochman, C.M., Mallory, M.L., 2019. Assessing plastic debris in aquatic food webs: what we know and don't know about uptake and trophic transfer. Environ. Rev. 27 (3), 304–317. https://doi.org/10.1139/er-2018-0079.

Radabaugh, K.R., Moyer, R.P., Chappel, A.R., Powell, C.E., Bociu, I., Clark, B.C., Smoak, J.M., 2018. Coastal Blue Carbon Assessment of Mangroves, Salt Marshes, and Salt Barrens in Tampa Bay, Florida, USA. Estuaries Coasts 41 (5), 1496–1510. https://doi.org/10.1007/s12237-017-0362-7.

Sherwood, E.T., Greening, H.S., 2014. Potential impacts and management implications of climate change on Tampa Bay estuary critical coastal habitats. Environ. Manag. 53 (2), 401–415. https://doi.org/10.1007/s00267-013-0179-5.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E., Le Goic, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. USA 113 (9), 2430–2435. https://doi.org/10.1073/pnas.1519019113.

Sylvester, F., Dorado, J., Boltovskoy, D., Juarez, A., Cataldo, D., 2005. Filtration rates of the invasive pest bivalve *Limnoperna fortunei* as a function of Size and Temperature. Hydrobiologia 534 (1-3), 71–80. https://doi.org/10.1007/s10750-004-1322-3.

Thushari, G.G.N., Senevirathna, J.D.M., 2020. Plastic pollution in the marine environment. Heliyon *6* (8), e04709. https://doi.org/10.1016/j.heliyon.2020.e04709.

Trottet, A., George, C., Drillet, G., Lauro, F.M., 2021. Aquaculture in coastal urbanized areas: A comparative review of the challenges posed by Harmful Algal Blooms. Crit.

Rev. Environ. Sci. Technol. *52* (16), 2888–2929. https://doi.org/10.1080/10643389.2021.1897372.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010.

Waite, H.R., Donnelly, M.J., Walters, L.J., 2018. Quantity and types of microplastics in the organic tissues of the eastern oyster *Crassostrea virginica* and Atlantic mud crab *Panopeus herbstii* from a Florida estuary. Mar. Pollut. Bull. 129 (1), 179–185. https://doi.org/10.1016/j.marpolbul.2018.02.026.

Wang, D., Su, L., Ruan, H.D., Chen, J., Lu, J., Lee, C.H., Jiang, S.Y., 2021. Quantitative and qualitative determination of microplastics in oyster, seawater and sediment from the coastal areas in Zhuhai, China. Mar. Pollut. Bull. 164, 112000 https://doi.org/10.1016/j.marpolbul.2021.112000.

Wang, J., Tan, Z., Peng, J., Qiu, Q., Li, M., 2016. The behaviors of microplastics in the marine environment. Mar. Environ. Res 113, 7–17. https://doi.org/10.1016/j.marenvres.2015.10.014.

Wessel, C.C., Lockridge, G.R., Battiste, D., Cebrian, J., 2016. Abundance and characteristics of microplastics in beach sediments: Insights into microplastic accumulation in northern Gulf of Mexico estuaries. Mar. Pollut. Bull. 109 (1), 178–183. https://doi.org/10.1016/j.marpolbul.2016.06.002.

Woods, M.N., Stack, M.E., Fields, D.M., Shaw, S.D., Matrai, P.A., 2018. Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel (*Mytilus edulis*). Mar. Pollut. Bull. 137, 638–645. https://doi.org/10.1016/j.marpolbul.2018.10.061.

Wootton, N., Sarakinis, K., Varea, R., Reis-Santos, P., Gillanders, B.M., 2022. Microplastic in oysters: A review of global trends and comparison to southern Australia. Chemosphere *307* (Pt 4)), 136065. https://doi.org/10.1016/j.chemosphere.2022.136065.

Wu, Y., Yang, J., Li, Z., He, H., Wang, Y., Wu, H., Xie, L., Chen, D., Wang, L., 2022. How does bivalve size influence microplastics accumulation? Environ. Res *214* (Pt 1)), 113847. https://doi.org/10.1016/j.envres.2022.113847.

Xian, G., Crane, M., Su, J., 2007. An analysis of urban development and its environmental impact on the Tampa Bay watershed. J. Environ. Manag. 85 (4), 965–976. https://doi.org/10.1016/j.jenvman.2006.11.012.