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Microplastics in Tampa Bay, Florida: Abundance and variability in estuarine waters and sediments

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ABSTRACT

This study provides the first measurement of microplastic abundance and distribution in surface waters and sediments in Tampa Bay, FL. Microplastic concentrations in discrete water samples ranged from 0.25 to 7.0 particles/L with an average of 0.94 (\pm 0.52) particles/L. Samples taken with a 330 μ m plankton net had 1.2–18.1 particles/m³ with an average of 4.5 (\pm 2.3) particles/m³. Discrete samples were 200 times higher than net samples, suggesting substantial losses or undersampling with the net. For both discrete and plankton tow samples, there were no significant differences in concentrations between stations or regions. Intense rainfall events in the summer always preceded samples with substantially higher counts. Most (> 75%) microplastics were fibers. Using an average value of 1 particle/L, Tampa Bay contains ~4 billion microplastic particles. Surface sediments had an average of 280 (\pm 290) particles/kg, ranging from 30 to 790 particles/kg. Highest concentrations of microplastics were found in sediments close to industrial sources; lowest values in Middle and Lower Tampa Bay are consistent with shorter residence times.

1. Introduction

Plastic pollution is pervasive in the marine environment and poses a threat to marine ecosystems (Law, 2017). The global production of plastic now reaches over 335 million metric tons per year (PlasticsEurope, 2017) with a cumulative production estimated at 8300 million metric tons (Geyer et al., 2017), expected to increase to 33 billion tons by 2050 (Zalasiewicz et al., 2016). While plastics are remarkably useful and versatile, they have also become a problematic and pervasive pollutant. The attractive characteristics of plastic, lightweight, durable, inexpensive, and long lasting, also contribute to serious environmental concerns. The scope and range of plastic pollution has become increasingly apparent and is now regarded as a major and growing contaminant. Estimates are that 4.8 to 12.7 million metric tons of plastic per year enter the marine environment, equivalent to 5 grocery bags full of plastic per foot of coastline around the globe (Jambeck et al., 2015).

Plastic pollution in the marine environment has been a concern for decades, in large part due to its high visibility, aesthetic concerns and negative interaction with animals (Law, 2017). More recently, microplastics, commonly defined as < 5 mm (GESAMP, 2015), have been

recognized as an important subset of plastic pollution and are pervasive in the marine environment. Microplastics have been identified in surface waters of every ocean basin (Cózar et al., 2014; Lusher et al., 2015), the deep sea (Chiba et al., 2018), coastal sediments, beach and deep-sea sand (Mohamed Nor and Obbard, 2014; Van Cauwenberghe et al., 2013), and estuaries (Gray et al., 2018). There are numerous sources of microplastics including stormwater runoff, wastewater treatment plant effluent, tire wear, and atmospheric deposition (e.g. Auta et al., 2017; Barrows et al., 2018; Browne et al., 2011; Dris et al., 2017; Kole et al., 2017). Because of their small size, microplastics can be ingested by a wide range of marine organisms, including numerous marine invertebrates (e.g. Cole et al., 2011), a wide range of fish (Lusher et al., 2013) and amphipods in the deepest ocean trenches (Jamieson et al., 2019).

While plastics are considered chemically inert, persistent organic pollutants (POPs) and heavy metals adsorb to microplastic particles due to the hydrophobic nature of their surfaces. While the toxicological impacts and biological effects of ingestion of microplastic particles are not fully understood, emerging evidence indicates toxicity to marine organisms when microplastic particles are ingested (Cole et al., 2015; Kühn et al., 2015; Rochman, 2015; Rochman et al., 2016).

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Microplastic Sampling Stations in Tampa Bay

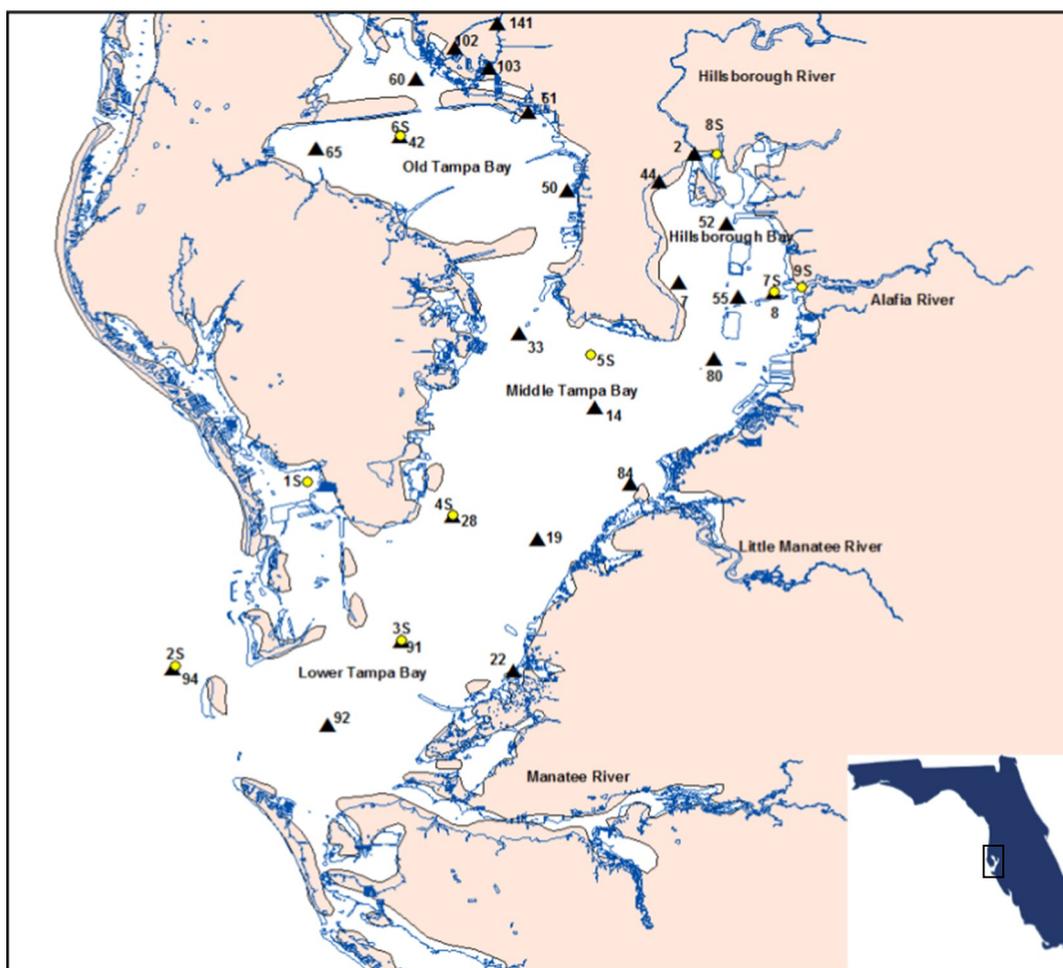


Fig. 1. Sample locations in Tampa Bay, FL. Discrete water and plankton tow samples indicated by black triangle, sediment samples indicated by yellow circle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Most microplastic studies published to date have been one-time efforts that do not fully consider spatial or temporal patterns. This study examines microplastic concentrations at 24 stations over 14 months in order to constrain temporal variations over an annual cycle. We measure microplastic abundance at sites throughout Tampa Bay including at the mouth of major rivers, small tributaries, close to wastewater treatment facilities, near industrial facilities and near relatively pristine coastal mangroves.

Tampa Bay, Florida's largest open-water estuary, is located on the west coast of central Florida and has an average depth of about 4 m and an area of 1000 km² (Fig. 1). It is a highly urbanized watershed, and supports one of the most active shipping ports in the U.S. The watershed is nearly five times as large as the bay, almost half of which is urban/suburban land use. Input of freshwater includes the Hillsborough, Alafia, Manatee and Little Manatee Rivers. The residence time of water in Tampa Bay depends upon location, fresh water discharge, precipitation, tides and winds. Model derived estimates of the residence time are 36 and 156 days (Meyers and Luther, 2008), or between 30 days at the bay entrance to 90 days in Old Tampa Bay (Zhu et al., 2015).

After decades of rapid urbanization and associated water pollution in the late 20th century, Tampa Bay is widely recognized as a national environmental success story due to the reduction of nutrient loads, increased water clarity, and dramatic recovery of seagrasses (Lewis III

et al., 1999; Yates et al., 2011). The Tampa Bay estuary encompasses freshwater, salt marsh, mangrove and sea grass habitats, as well as spawning and juvenile fish habitat.

Our objective is to present the first reliable estimates of microplastics in Tampa Bay, Florida's largest estuary, including spatial and temporal variations and microplastic values in the water column and surficial sediments. We also compare the more commonly used sampling method of a towed plankton net with discrete or grab water sampling.

2. Materials and methods

2.1. Site description

Twenty-four stations were chosen from the Environmental Protection Commission of Hillsborough County (EPC-HC) monthly sampling locations to collect discrete and plankton tow samples. Five sites were located in Old Tampa Bay (OTB: 65, 42, 60, 61, and 50); six sites in Hillsborough Bay (HB: 44, 52, 7, 8, 55, and 80); five sites in Middle Tampa Bay (MTB: 84, 28, 19, 33, and 14); and four sites in Lower Tampa Bay (LTB: 91, 22, 92, and 94). Four sites were in rivers that flow into Tampa Bay: stations 141, 102, and 103 were in tributaries to Old Tampa Bay; station 2 was at the mouth of the Hillsborough River. Sediment samples were collected at nine stations. Different parts of the

Table 1
Sample locations: discrete and plankton tow.

Bay region	Station #	Sample type	Latitude	Longitude
OTB	50	D, P	27.9185	−82.5379
OTB	61	D, P	27.9687	−82.5621
OTB	60	D	27.9899	−82.6316
OTB	65	D	27.9456	−82.6943
OTB	42	D, P	27.9528	−82.6416
HB	44	D	27.9237	−82.4807
HB	7	D	27.8589	−82.4686
HB	80	D	27.8096	−82.4460
HB	8	D, P	27.8524	−82.4093
HB	52	D	27.8970	−82.4382
HB	55	P	27.8493	−82.4314
MTB	14	D	27.7780	−82.5203
MTB	84	D	27.7290	−82.4987
MTB	19	D	27.6934	−82.5559
MTB	33	D	27.8261	−82.5675
MTB	28	D, P	27.7084	−82.6092
LTB	22	D	27.6081	−82.5712
LTB	92	D	27.5737	−82.6868
LTB	94	D, P	27.6100	−82.7832
LTB	91	D	27.6279	−82.6415
OTB Trib	141	D	28.0261	−82.5812
OTB Trib	102	D	28.0106	−82.6078
OTB Trib	103	D	27.9976	−82.5863
HR Trib	2	D	27.9418	−82.4585

OTB = Old Tampa Bay, HB = Hillsborough Bay, MTB = Middle Tampa Bay, LTB = Lower Tampa Bay, OTB Trib = Old Tampa Bay Tributaries, HR Trib = Hillsborough River Tributary; D = discrete sample; P = plankton tow sample.

Tampa Bay system, population densities, industrial and wastewater outputs, as well as proximity to relatively pristine bodies of water and healthy mangrove, oyster, and seagrass ecosystems were all represented by the sample sites chosen (Fig. 1 and Table 1).

2.2. Sample collection

Microplastic particles suspended in the water column were collected using two different sampling methods: discrete water samples and plankton tows. The discrete samples were captured using a Van Dorn sampler that was triple-rinsed in seawater then lowered 1 m below the surface to collect the sample. The sample was poured into a 1 L HDPE collection bottle that was cleaned in the laboratory with filtered deionized water (Milli-Q®) prior to field sampling and triple-rinsed in situ with sea water. Plankton tow samples were taken using a 330 µm plankton net with a 50 cm diameter at stations in Old Tampa Bay (50 and 42); Hillsborough Bay (55 and 8); Middle Tampa Bay (28); and Lower Tampa Bay (94). The net and collection bottle were triple-rinsed with seawater at the station before being deployed, towed at ~2 knots (1 m/s) one to two meters below the sea surface for 3 min outside the research vessel's wake. After detaching the cod-end collection bottle, the net was thoroughly rinsed with seawater to collect microplastic particles. Samples were frozen until processed. Sediment samples were collected using a Shipek grab sampler on March 21–23, 2017.

2.3. Sample processing

2.3.1. Discrete samples

In the laboratory, discrete water samples were vacuum-filtered through a 1.2 µm pore size, 47 mm diameter, gridded cellulose nitrate (CN) filter paper. Microplastic particles were counted using a Nikon® dissecting microscope at 33× magnification. Characteristics including shape, color, and type of each plastic piece were recorded. Great care was taken to avoid airborne or waterborne contamination of microplastics during processing. Cotton (100%) lab coats were always worn and clothing that shed microplastic fibers was not worn. Laboratory

benches were wiped down before each use; filter parts and glassware were thoroughly rinsed before each use with filtered deionized water. Furthermore, a watch glass was placed over the top of the filter during use. Blanks of 0.2 µm filtered deionized water (Milli-Q®) were taken to establish values for contamination in the lab.

2.3.2. Plankton tow samples

Plankton tow samples contained a large amount of organic matter that needed to be removed in order to accurately count microplastic particles. The larger pieces, including sea grass, were first manually separated from the sample. Smaller pieces, mainly plankton, were removed via an enzymatic digestion process developed by Cole et al. (2014) and described in detail elsewhere (McEachern, 2018). Samples were split five times with a Folsom plankton splitter to reduce sample volume, passed through a 212 µm wire mesh sieve, rinsed with DI water, then filtered onto a 1.2 µm cellulose nitrate filter. The material retained on the wire mesh was desiccated at 65–80 °C in glass vials, then 15 mL of homogenizing solution (15.77 g Tris HCl, 4.38 g EDTA, 1.53 g NaCl, and 1.26 g SDS in 250 mL deionized water) was added to each vial. Samples were vortexed for 30 s and homogenized with an 18-gauge needle and syringe, then heated at 50 °C for 20 min in a hot water bath. Finally, 375 µL of Proteinase K (500 µg/mL) was added and samples were incubated for 2–4 h at 50 °C. After incubation, 5 mL of 5 M NaClO₄ was added to each vial and vortexed for 30 s. Digested samples were filtered onto a 1.2 µm, 47 mm diameter gridded, cellulose nitrate filter paper. Microplastic particles were counted under a dissecting microscope. As with the discrete samples, considerable efforts were made to avoid contamination from ambient microplastic particles.

2.3.3. Sediment samples

Our method for quantifying microplastics in sediments used volume reduction of the sample by elutriation followed by a density separation using a high density (6.7 M; 1.6 g/cm³) NaI solution. Using NaI in lieu of the conventional, and lower density saturated NaCl solution extracts all types of microplastics present in environmental samples, including high-density plastics and results in a high (98%) extraction efficiency (Claessens et al., 2013).

The first step was to concentrate less dense microplastic particles from the bulk of the sediment using an elutriation column (Claessens et al., 2013). The sediment sample (~150 g) was put into the top of the column in which flowing water (3 L/min) and small air bubbles forced the less dense particles up the column where they spilled over into a 53 µm sieve. The sample was then transferred to a 50 mL centrifuge tube with 6.7 M NaI and centrifuged for 15 min at 3350 rpm. Supernatant was filtered to collect and count the microplastic particles. Careful attention was paid to prevent airborne contamination by covering all open containers with Al foil and keeping the elutriation column and collecting sieve capped. Overlying water collected with the grab sampler, which includes microplastic particles associated with the benthic boundary layer, was included in sample processing. Dry weight was determined by drying a 10–15 g sample of homogenized wet sediment at 90 °C to a constant weight.

2.4. Blank values

Blank samples of 0.2 µm filtered Milli-Q® deionized water were processed in the laboratory to assess contamination in processing both discrete water sampling and plankton tow samples. As described earlier, great care was taken to avoid contamination by airborne or waterborne microplastic particles. In each case, for both types of samples, no microplastic particles were found in these blank samples, suggesting that contamination was not a significant concern (see Table 2).

2.5. Microplastic identification

Microplastics were identified as pieces ≤5 mm that possess no

Table 2
Microplastic laboratory process blank.

Sampler type	Date	Microplastics
Discrete	8/29/16	0
Discrete	9/13/16	0
Plankton tow	10/4/16	0
Plankton tow	10/6/16	0

cellular structures, are equally thick throughout their entire length, and either clear or homogeneous in color throughout (Hidalgo-Ruz et al., 2012). Any pieces believed to be plastic were probed with a hot dissecting needle. If the material quickly melted or disfigured, the sample was classified as plastic. Biological materials do not melt - they burn and react less to heat (Hidalgo-Ruz et al., 2012). Microplastics were categorized as one of six shapes: fiber (thread-like), bead, fragment, film, flake, or foam.

2.6. Statistical methods

To assess the data for normality, histograms, Q-Q plots, and the Shapiro Wilk test of normality were utilized on the discrete and plankton tow concentrations. The discrete microplastic data did not follow a normal distribution. After log transformation of the data set, the data did not follow a normal distribution; therefore, it was analyzed with non-parametric statistical methods. The plankton tow microplastic data was log transformed with a $\log_{10}(x + 1)$ transformation; data then fit a normal distribution so parametric tests were used.

3. Results

3.1. Type of microplastic

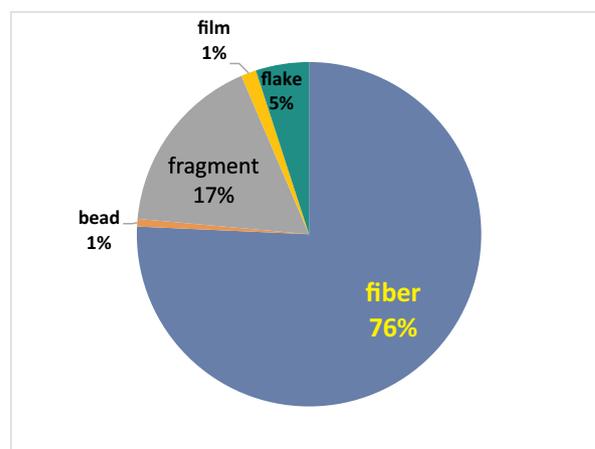
For both discrete and plankton tow samples, the most common type of microplastic particle was classified as fiber, or thread-like, with 76% and 88% fibers in discrete and plankton tow samples, respectively (Fig. 2 a, b). The second and third most common microplastics identified in both types of sampling efforts were fragments (discrete: 17%; tow: 5%) and flakes (discrete: 5%; tow: 3%). Foam microplastics, made of expanded polystyrene (EPS), were scarce: Middle and Lower Tampa Bay plankton tow samples had the only foam microplastics we found, with < 0.5% foam pieces out of almost 500 total particles counted.

3.2. Abundance

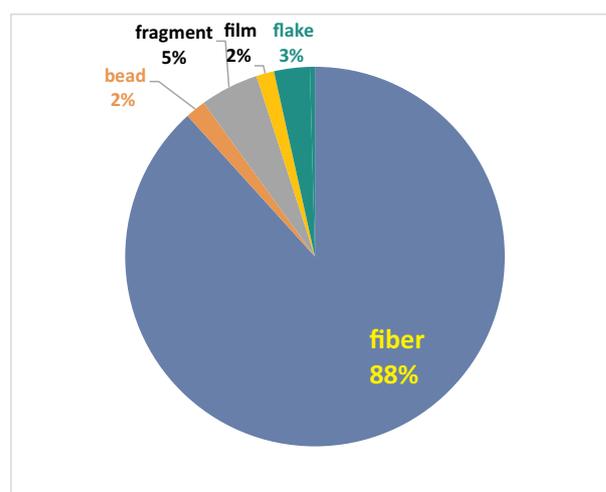
The average microplastic concentration for all discrete water samples collected in Tampa Bay (not including tributaries) over the entire sampling period was 0.94 (± 0.52) particles/L (Table 3). The average concentration over the sampling period in discrete water samples was Hillsborough Bay (0.68 \pm 0.93), Lower Tampa Bay (0.92 \pm 1.05), Old Tampa Bay (1.03 \pm 1.63), and Middle Tampa Bay (1.14 \pm 1.64) particles/L. The average value for the tributaries flowing into Old Tampa Bay and the Hillsborough River was 1.3 (± 1.2) particles/L. The individual concentrations for the two tributaries were 1.67 (± 1.4) and 0.25 (± 0.50) particles/L, respectively.

The average concentration of microplastic particles in all plankton tow samples over the entire sampling period was 4.5 (± 2.3) particles/ m^3 (Table 4). Regional average microplastic values for plankton tow samples in Old Tampa Bay, Hillsborough Bay, Middle Tampa Bay, and Lower Tampa Bay were 3.8 (± 1.5), 4.6 (± 2.0), 4.4 (± 2.6), and 5.8 (± 7.1) particles/ m^3 , respectively (Table 4). Complete results of discrete and plankton tow samples for each sampling effort are provided in Tables 3 and 4.

There was no statistically significant difference in discrete concentrations per station or bay region. With p values > alpha at 0.05



a: Type of microplastics: Discrete samples.



b: Type of microplastics: Plankton tow samples

Fig. 2. Type of microplastics: a) Discrete samples; b) Plankton tow samples.

there is not enough evidence to reject the null hypothesis that the sum of the ranks of the discrete microplastic concentrations of the different stations or bay regions are all equal. Since the result from the Kruskal-Wallis test was not significant, there was no justification to perform a set of uncorrected Mann-Whitney *U* tests to determine which of the stations or bay regions may be significantly different from one other. There was no statistically significant difference in plankton tow concentration per station or bay region. Because p values > alpha, the results of the Welch's ANOVA test was not significant and there was no need to conduct the Tukey post hoc test of multiple comparisons of plankton tow concentration data.

Sediment samples had a bay-wide average of 280 (± 290) microplastic particles/kg dry weight, ranging from 30 to 790 particles/kg (Table 5). The overlying water collected in the grab sample with the sediment contained, on average, 40% of the total microplastic particles.

4. Discussion

4.1. Particle type

Both discrete and plankton tow sampling methods revealed that the most commonly found type was fibers, which are thin, long, thread-like plastics. These may be from laundering of clothing with synthetic fabrics; the fibers are transported through and released from wastewater

Table 3
Microplastic concentrations: discrete samples (particles/L).

Region	Sta #	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Nov-16	Apr-17	May-17	Jun-17	Jul-17	Avg	Std dev
OTB		6/8		8/1		10/3	11/1	4/10		6/5	7/5		
	65	0		0		0	2	0		0	2	0.57	0.98
	42	0		0		1	4	0		0	5	1.43	2.15
	60	0		0		0	0	1		5	1	1.00	1.83
	61	0		0		0	0	4		1	3	1.14	1.68
	50	4		2		0	1	0		0	0	1.00	1.53
	Mean											1.03	1.63
HB		6/14	7/11	8/9	9/12	10/11	11/7	4/11	5/8	6/13	7/10		
	44	0	2	0	1	2		0	0	1	4	1.11	1.36
	52	0	0	0	0	1	2	0	1	1	1	0.60	0.70
	7	0	2	0	0	0	1	0	0	0	1	0.40	0.70
	8	0	2	0	0	0	0	1	1	0	2	0.60	0.84
	80	2	0	1	0	3	0	0	0	1	0	0.70	1.06
	Mean											0.68	0.93
MTB		6/8, 6/14, 6/20	7/5, 7/11, 7/25	8/1 8/9, 8/15	9/6, 9/12, 9/20	10/3 10/11, 10/24	11/1, 11/7, 11/15	4/10, 4/11, 4/17	5/2, 5/8, 5/16	6/5/, 6/13, 6/19	7/10, 7/18, 7/24		
	84	2	0	0	0	0	0	0	1	7	1	1.10	2.18
	28	4	0	0	1	0	2	0	0	0	1	0.80	1.32
	19	0	0	0	0	2	2	0	0	4	0	0.80	1.40
	14	0	2	0	1	2	0	1	0	3	1	1.00	1.05
	33	6		2		1	1	0		0	4	2.00	2.24
	Mean											1.14	1.64
LTB		6/20	7/25	8/15	9/20	10/24	11/15	4/17	5/16	6/19	7/18, 7/24		
	91	0	0	1	0	5	3	0	0	2	2	1.30	1.70
	22	2	0	0	0	1	2	1	0	0	1	0.70	0.82
	92	2	2	1	0	0	0	1	1	1	1	0.90	0.74
	94	2	0	0	0.67	0	2	1	0	0	2	0.77	0.92
	Mean											0.92	1.05
Average												0.94	0.52
OTB Trib		6/13		8/2									
	141	4		0								2.00	2.83
	102	2		2								2.00	0.00
	103	0		2								1.00	1.41
	Mean											1.67	1.41
HR Trib		6/9	7/13	8/23	9/14								
	2	0	0	0	1							0.25	0.50
	Mean											0.25	0.50

OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay, OTB Trib: Old Tampa Bay Tributaries, HR Trib: Hillsborough River Tributary.

treatment facilities. These threads might also be derived from the breakdown of synthetic fishing lines, nets, and ropes. A slightly higher fraction of particles found in the plankton net was fibers, which could be due to the fact that the longer, thread-like particles are easily

entangled in the net and are selectively collected in net samples.

Fragments and flakes are secondary microplastics, most likely derived from the breakdown of larger plastics. The paucity of foam particles (expanded polystyrene or EPS) is likely due to the high buoyancy

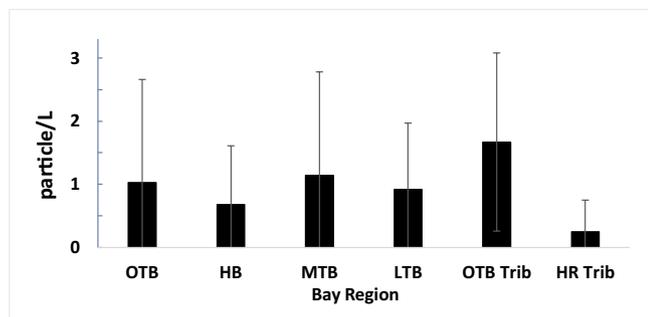
Table 4
Microplastic concentrations: plankton tow samples (particles/m³).

Region	Sta #	Oct-16	Nov-16	Dec-16	Apr-17	May-17	Jun-17	Jul-17	Avg	Std dev
OTB		10/3/16	11/1/16		4/10/17		6/5/17	7/5/17		
	50	4.1	2.6		5.4		8.2	2.6	4.6	2.3
	42	2.3	3.9		3.1		2.4	3.5	3.1	0.7
	Mean								3.8	1.5
HB		10/11/16	11/7/16	12/13/16	4/11/17	5/8/17	6/13/17	7/10/17		
	55	10.3	1.8	5.8	1.9	5.2	6.5	2.9	4.9	3.0
	8	4.9	4.4	5.2	4.2	2.2	4.0	4.9	4.3	1.0
	Mean								4.6	2.0
MTB				12/19/16	4/17/17	5/16/17		7/24/17		
	28			6.6	3.4	6.3		1.2	4.4	2.6
	Mean								4.4	2.6
LTB				12/19/16	4/17/17	5/16/17	6/19/17	7/18/17		
	94			1.9	2.0	1.6	18.1	5.4	5.8	7.1
	Mean								5.8	7.1
Average									4.5	2.3

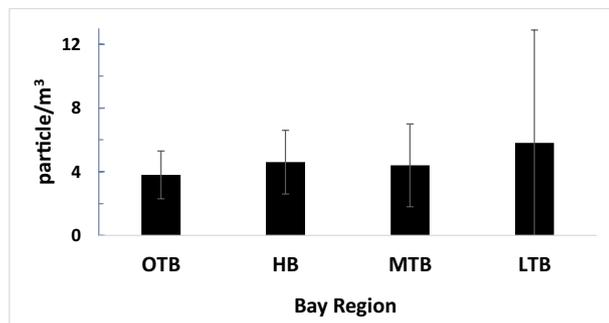
OTB: Old Tampa Bay, HB: Hillsborough Bay, MTB: Middle Tampa Bay, LTB: Lower Tampa Bay, OTB Trib: Old Tampa Bay Tributaries, HR Trib: Hillsborough River Tributary.

Table 5
Microplastic concentrations: sediment samples (particles/100 g dry weight).

Site	Location	Latitude	Longitude	Depth (m)	Particles/kg
1S	Boca Ciega Bay	27° 43.800' N	082° 42.000'W	2.6	380
2S	Egmont Key	27° 36.66' N	082° 46.920'W	3.6	200
3S	Lower Tampa Bay	27° 37.674'N	082° 38.490'W	8.4	110
4S	Mid Tampa Bay	27° 42.504'N	082° 36.552'W	5.3	40
5S	Mid Tampa Bay	27° 48.708'N	082° 31.392'W	4.0	30
6S	Old Tampa Bay	27° 57.168'N	082° 38.496'W	3.4	180
7S	Hillsborough Bay	27° 51.144'N	082° 24.558'W	8.9	60
8S	Ybor Channel	27° 56.457' N	082° 26.698'W	10.5	790
9S	Alafia R. mouth	27° 51.334' N	082° 23.536'W	11.0	720
	Average				280



a: Average regional microplastic concentrations: Discrete samples (particles/L)



b: Average regional microplastic concentrations: Plankton tow samples (particles/m³)

Fig. 3. Average microplastic concentrations: a) Discrete samples; b) Plankton tow samples.

of the material, the density of which is much lower than water, ranging from 0.1 to 0.3 g/cm³. EPS particles would be expected to float at the water surface, while we sampled 1 m and 1–2 m below the surface for discrete and plankton tow samples, respectively.

4.2. Sampling methodology

Microplastic concentrations collected by discrete sampling were, on average, 230 times higher than those collected by plankton tow. Only 1 L was collected in discrete sampling whereas tens of thousands of liters were sampled with the tow. The discrete sampling collected a lower absolute particle count for each sample, but substantially more microplastics per volume of water were found compared to plankton tows. What factors lead to the large difference in microplastic concentrations for the different sampling approaches? Process controls in the laboratory indicate that contamination is not a significant or substantial contributor (Table 2). The plankton tow method resulted in a lower value since plastic particles smaller than the mesh size of 330 μm will pass through the net. Fibers are typically thinner than this mesh size

and would likely pass through the net while the discrete water sampler collects all particles irrespective of size or shape. It is well known that a large fraction of phytoplankton is small enough to pass through plankton nets; the same is true of microplastic particles.

Grab sampling collects all the microplastic particles, while the plankton net does not. Song et al. (2014) found four times more microplastic particles in bulk samples than in tows using 330 μm nets. Barrows et al. (2017) found that grab samples collected three orders of magnitude more particles per volume of water than 335 μm neuston tows. Both results are consistent with ours and confirm that samples taken with a plankton or neuston net result in an underestimation of microplastic concentrations. Our inability to identify and recognize microplastic particles smaller than 50 or 100 μm also results in underestimation of the abundance.

It is possible that the difference between the two sampling methods is due to missed identification of microplastic particles in the net samples. There is a considerable amount of other material in the net including sea grass, algae, and larger plankton which may obscure identification of smaller microplastics. Another consideration is the lower efficiency of collection if the net clogs with plankton, which reduces the total volume of water that passes through the net. It is well known that the volume of water passing through the net is hard to measure exactly, and it is difficult to get an accurate flow meter reading. Reduced efficiency of the net, when water that flows around the net rather than through the net, is not considered; our estimate of the water passing through the plankton net could be different from the actual value.

While we did not detect any contamination in our process blank samples, contamination in the discrete samples would alter the final value more than plankton tow samples, since relatively few particles (0–7) were counted in the discrete samples compared to plankton tow samples.

4.3. Spatial differences

It is tempting to interpret differences in microplastic concentrations between the different geographic locations or the different regions in Tampa Bay. However, the large standard deviations for both average discrete and plankton tow sample concentrations (Fig. 3a,b) indicate that variability between stations is relatively high and suggests the different values are not significantly different from one another. A non-parametric Kruskal-Wallis H test showed no statistically significant difference in discrete microplastic concentrations between the stations ($\chi^2 = 12.19$, $p = 0.96$) or between bay regions ($\chi^2 = 4.74$, $p = 0.58$). With p values $>$ alpha at 0.05, there is not enough evidence to reject the null hypothesis that the sum of the ranks of the microplastic concentrations of the different stations or bay regions are all equal. The parametric Welch's ANOVA test, which allows for variances to be unequal, was used to test for differences in plankton tow concentrations among stations and bay regions. The results for the stations indicated an F ratio of 1.48 and a p value of 0.27, while the results for the bay

regions gave an F ratio of 0.34 and a p value of 0.80. With p values > alpha at 0.05, there is not enough evidence to reject the null hypothesis that the means of the microplastic concentrations of the different stations or bay regions are equal.

4.4. Temporal differences: discrete samples

Previous research reveals that rainfall increased microplastic abundance in a Brazilian estuary (Lima et al., 2014). Microplastics in the Chesapeake Bay were also influenced by major rain events as well as proximity to population density and urban/suburban development (Yonkos et al., 2014). Does rainfall also influence microplastic concentrations in Tampa Bay?

Precipitation in Tampa Bay is highly seasonal, with 60 to 75% of the annual precipitation occurring during the four-month period from June to September (Duever et al., 1994; Florida Climate Center, 2015). Intense convective rains, erratic tropical storms and hurricanes, which occur primarily in summer and early fall, contribute substantially to precipitation. Our hypothesis is that heavy rainfall leads to increased microplastic content in Tampa Bay, likely due to increased discharge from rivers and storm water runoff. To test this hypothesis, we identified sampling efforts where more than double the average number of microplastics on a given sampling date were found. We wanted to know if unusually heavy rainfall preceded these sampling efforts. Daily precipitation data (NOAA National Centers for Environmental Information, 2019) from three weather stations around Tampa Bay (Tampa International Airport, St. Petersburg Albert Whitted Airport, and Bradenton Airport) were used to obtain a geographic average for the bay and were binned into 7-day averages. For reference, the median value of rainfall for Tampa Bay is 0.4 in./week.

Six discrete water samples fit the criterion of having twice the average number of microplastics. In Old Tampa Bay (OTB), the highest observed value in discrete sampling was 2.2 particles/L on 7/5/17, more than double the average of 1.0 particles/L. This high value for microplastic particles followed a period of intense precipitation, an average of 2.2 in./week for the 5 weeks preceding the sampling date. In Hillsborough Bay (HB), the highest discrete value recorded (1.6 particles/L) was more than double the average (0.7 particles/L) and also occurred in early July (7/10/17) following more than a month of heavy rain (average > 2.2 in./week). The other high value of 1.2 particles/L on 10/11/16 was recorded following a week of high rainfall during which 2.4 in. of rain fell on Tampa Bay.

In Middle Tampa Bay (MTB), there was a high average value of 2.4 (± 2.6) particles/L for the month of June 2016 on three sampling dates. The highest value (6 particles/L) was recorded at station 33 on 6/8/16, following a week of intense rain (6.8 in.). The second highest value (4 particles/L) was recorded on 6/20/16, following 2.4 in. of rain the preceding week. High microplastic values were recorded on 6/19/17 at stations 84 and 19 with 7 and 4 particles/L, respectively. It rained 3.3 in. the week prior to collection. Relatively high values (3 particles/L) were also recorded at station 14 on 6/13/17 which were collected during a week of heavy rain (3.3 in.) with another week of heavy rain (3.7 in.) preceding the collection. Relatively high counts (4 particles/L) were found in the OTB tributary at station 141 on 6/13/16, following an exceptionally heavy week of rain (6.3 in.).

In Lower Tampa Bay, samples with higher microplastic counts did not appear to be related to heavy rainfall prior to sampling. Relatively high values were observed in October and November 2016, 1.5 and 1.8 particles/L, respectively, when dry conditions prevailed, with 0.1 in. or less each week from 10/13/16 to 12/1/16. This is consistent with the fact that Lower Tampa Bay is influenced mostly by Gulf of Mexico waters, and is less influenced by rain-driven runoff.

In summary, the discrete water samples with more than double the number of microplastic particles were sampled following periods of heavy rain. The only exception was Lower Tampa Bay, which is influenced more by Gulf of Mexico waters than by river discharge or

stormwater runoff.

4.5. Temporal differences: plankton tow samples

To determine if high microplastic values found in plankton tow samples were also associated with periods of high rainfall, we identified those samples with microplastic concentrations substantially higher than the average for that site - at least 75% higher than the average plankton tow sample. Three samples fit this criterion: HB on 10/11/16, OTB on 6/5/17, and LTB on 6/19/17. The same precipitation data used with the discrete samples were used to obtain a geographic average for the bay. We found that each of the three samples with high microplastic concentrations were associated with a period of relatively heavy and protracted rainfall preceding the sampling event by 5–14 days.

The first unusually high value on 10/11/16 in HB (station 55) had over twice the average microplastic concentration for this station and followed two weeks of high precipitation; 2.5 in. of rain fell the week before sampling. The other high concentrations both occurred in June 2017. The first rainy period followed a prolonged period of abnormally low rainfall since October 2016, considered one of the worst droughts in recent history. The second unusually high microplastic value sampled via plankton net was almost twice the average value for station 50 in OTB. Over two inches of rain fell the week prior to the date of collection, 6/5/17. The other high microplastic sample was collected at station 94 in LTB on 6/19/17, following two weeks of heavy rainfall (3.3 and 3.7 in./week, respectively). As with the discrete water samples, plankton tow samples with substantially greater microplastic concentrations were sampled following periods of heavy rain. Intense rainfall events influence the abundance of microplastic particles using both sampling methods.

4.6. Wastewater treatment plant discharge

While wastewater treatment plants (WWTPs) remove the vast majority (> 99%) of microplastics during treatment (Magnusson and Norén, 2014), nonetheless, they are known sources of microplastics in the freshwater and marine environment given their incomplete removal during wastewater treatment (e.g. Estahbanati and Fahrenfeld, 2016). Our objective was to determine if WWTPs are a significant source of microplastics to Tampa Bay.

We identified WWTPs that discharge > 1 million gallons per day (mgd) into Tampa Bay, and classified them into the four different regions of the bay (McEachern, 2018). Using Spearman's Rank correlation test, we found no significant correlation between microplastic concentration in either discrete or plankton tow samples and regions with different wastewater treatment effluent (McEachern, 2018). This suggests that the impact of wastewater effluent on microplastic concentrations is not regionally specific in Tampa Bay. More detailed investigation into the point (discharge effluent) and non-point (reclaimed water) sources of microplastics from wastewater treatment plants, as well as a better understanding of the exact discharge location and surface circulation, may be necessary to determine a relationship between WWTPs effluent and microplastics in Tampa Bay. While some studies reveal a clear relationship between WWTPs and an increase in downstream microplastic concentration in rivers (e.g. Estahbanati and Fahrenfeld, 2016), point-source pollution from WWTPs is more difficult to identify due to more complex physical circulation in Tampa Bay and possible higher removal rates at these WWTPs.

4.7. Comparison with other studies

The average plankton tow concentration of 4.5 particles/m³ in Tampa Bay is similar to the range in microplastic concentrations measured by similar methods in other estuarine studies, including the Goiana Estuary in NE Brazil (3.1 particles/m³, Lima et al., 2014) and the Great Lake Tributaries (median value of 1.9 particles/m³, Baldwin

et al., 2016). The average discrete concentration in this study, 0.94 particles/L (940 particles/m³), is orders of magnitude higher than surface water samples collected by plankton net. This discrepancy is due to collecting more microplastic particles via discrete sampling than plankton nets. Our microplastic data reveals significantly higher concentrations in Tampa Bay compared to open ocean values, e.g. 0.28 particles/L (Desforges et al., 2014), 1.1 particles/m³ (Kanhai et al., 2017), and 0.13/particles/m³ (Mu et al., 2019). Tampa Bay microplastic concentrations were substantially lower than sea surface concentrations in Charleston Harbor and Winyah Bay, South Carolina with average values of 6.6 (± 1.3) particles/L and 30.8 (± 12.1) particles/L, respectively (Gray et al., 2018). These higher values reflect sampling the sea surface microlayer, where microplastics concentrations are higher than in the water column below due to the relatively low density, and high buoyancy of many plastics. The choice of sampling method clearly has a large influence on the concentration.

4.8. Sediments

While many plastics are buoyant in seawater due to their lower density, biofouling can contribute towards the settling and subsequent burial of previously buoyant plastic (Andrady, 2011; Zettler et al., 2013). Thus, sediments are likely a sink for microplastics. A wide range of microplastic concentrations were found in sediment samples, with the lowest value of 30–40 particles/kg at sites 4S and 5S in Middle Tampa Bay. Estuarine water in this region has a short residence time compared to other parts of the bay (Meyers and Luther, 2008; Meyers et al., 2007), and is quickly flushed, which is consistent with the lowest microplastic values. These sites are also relatively distant from land-based sources of microplastics. Other relatively low microplastic values were found in Lower Tampa Bay (3S) and at the entrance of Tampa Bay near Egmont Key (2S), resulting in 110 and 200 particles/kg, respectively, that also have short residence times (Meyers and Luther, 2008). The highest value (790 particles/kg) is in a highly industrialized area in Ybor Channel (8S) near a shipyard and asphalt terminal. This location is expected to have a low residence time with little flushing. The other high value (720 particles/kg) is at the mouth of the Alafia River (site 9S), close to a processing plant for the Mosaic phosphate company. It is not possible to distinguish whether the high concentration of microplastics at this site is due to discharge from the Alafia River, the processing plant, or both.

Moderate numbers of microplastic particles (180 particles/kg) are found in Old Tampa Bay (6S), which has the longest residence time (~150 days) in Tampa Bay (Meyers and Luther, 2008; Meyers et al., 2007). Given the sluggish circulation, Boca Ciega Bay (1S) would also be expected to have a relatively long residence time and sediments there have moderate concentrations of microplastics (380 particles/kg). Spatial differences of microplastics in Tampa Bay sediments appear to be related to proximity (river, industry) and residence time in the bay. Since sediments accumulate slowly over time, they integrate relatively short-term temporal changes (less than a year) and provide good indications of large sources.

How do our observed values in Tampa Bay compare with other microplastic measurements in sediments? Comparisons are problematic since a wide range of sampling techniques is used and because values are reported using different units. Estuarine sediments near Plymouth, UK had 35 microplastic particles/kg (Thompson et al., 2004), similar to our lowest values in the middle of Tampa Bay. We converted their values with units of particles/mL to particles/kg using an average sediment density of 1.6 g/cm³ and an average wet/dry sediment ratio of 1.25. The average value in sediments of three Belgian coastal harbors was 167 particles/kg (Claessens et al., 2011), about half of our average value of 280 particles/kg. The highest value in these Belgian harbors was 391 particles/kg, about half of our highest measured value in Tampa Bay. Using these two studies as comparisons, the abundance of microplastic particles in Tampa Bay is high relative to other estuarine

and coastal sediments. Concentration of microplastics in deep sea sediments is substantially less than Tampa Bay based on one study (Van Cauwenberghe et al., 2013). However, only when consistent methodologies are developed and established will such comparisons between studies be conclusive and accurate.

5. Conclusion

This study demonstrates that microplastic particles are widely dispersed and abundant in surface water and sediments in Tampa Bay, FL. The average concentration of microplastics in discrete water samples was 0.94 (± 0.52) particles/L; plankton net samples yielded an average of 4.5 (± 2.3) particles/m³. Assuming an average value of 1 particle/L, consistent with our discrete water sample, an average bay depth of 4 m and area of 1000 km² (Kunneke, 1984), Tampa Bay contains approximately 4 billion microplastic particles. Tampa Bay sediments contain, on average, 290 microplastic particles/kg or 130 particles per pound of dry sediment. Highest concentrations were found close to industry and at the mouth of a major river while lower sediment values were consistent with shorter residence times. Our sediment values are substantially higher than other coastal and estuarine environments that have been sampled. Given the abundance and ubiquity of these microplastics, local and regional management initiatives need to be developed and implemented to manage them, despite the clear difficulty of this undertaking.

Differences of two orders of magnitude between measured values in discrete water samples and plankton tow samples indicate that sampling with plankton net results in substantial loss of microplastics. Given the abundance of microplastics in virtually every environment, and the low total particle counts in samples, it is critically important to prevent contamination in the field and in the laboratory.

While identification techniques used in this study provided reliable data, our laboratory protocols have shifted to staining samples with Nile Red, a lipophilic fluorescent dye, combined with image analysis to identify microplastics (Erni-Cassola et al., 2017; Maes et al., 2017). Our collection methods have evolved; we now collect larger discrete water samples (10–20 L) to increase sample counts and to reduce potential impacts of contamination. We are also shifting to micro-Raman and Raman spectroscopy/microscopy on select particles, which can identify the structural properties of micro-scale particles (Anger et al., 2018).

Our research reveals that unusually high microplastic concentrations in both net and discrete samples were preceded by intense and/or prolonged rainfall in Tampa Bay. These events result in significant surface runoff, and are likely to increase transport of microplastic particles into Tampa Bay and the Gulf of Mexico. Establishing a baseline of microplastic contamination in Tampa Bay is important in order to measure changes as the occurrence of these extreme rainfall events increases as predicted by climate models (Easterling et al., 2017; Janssen et al., 2016).

This study affirms that microplastic contamination is ubiquitous including Tampa Bay. Although it is tempting to clean up the mess, it is impractical, if not impossible, to remove the numerous and tiny microplastic particles from the water column or separate microplastic contamination from sediments. Only by removing the sources of plastics and microplastic particles can we successfully decrease the potential risks of plastics in the marine environment.

CRedit authorship contribution statement

Henry Alegria: Writing - review & editing. **Cypress Hansen:** Writing - review & editing. **Samantha Morrison:** Writing - review & editing. **David Hastings:** Funding acquisition, Writing - original draft, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

None.

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