Pervasive occurrence of microplastics in Hudson-Raritan estuary zooplankton

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ABSTRACT

Microplastics (MP) are considered emerging contaminants in the water environment, and there is an interest in understanding their entry into the food web. As a growing body of literature demonstrates the ingestion of MP by zooplankton in controlled laboratory studies, few data are available demonstrating *in situ* observations of MP in zooplankton. A field survey was performed to collect zooplankton in the highly urbanized Hudson-Raritan estuary. Following washing, sorting by species, and enumeration, three dominant species of copepods (*Acartia tonsa*, *Paracalanus crassirostris* and *Centropages typicus*) were digested. MP were filter concentrated and characterized by size, morphology, and color via microscopy and polymer type by micro-FTIR imaging and/or Raman spectroscopy. MP were observed in all extracts performed on the three copepod species with averages ranging from 0.30 to 0.82 MP individual^{-1}. Polyethylene and polypropylene were the dominant polymer types observed and fragments and beads the most commonly observed morphologies for MP. These data were used to estimate the flux of MP through zooplankton based on gut turnover times, which we compare to estimates of MP entering this environment though the local waterways. The estimated fluxes were sufficiently large, indicating that ingestion by zooplankton is a major sink of MP in the size range subject to zooplankton feeding in surface estuarine waters.

Keywords: plastics, copepods, polymer, Raman micro-spectroscopy

1. Introduction

Plastic pollution in aquatic environments is an increasingly important concern. The human population produces an average of about 1.5 megatons of plastic waste every year (Boucher and Friot, 2017). Plastic waste not recycled, combusted for energy recovery, or properly landfilled (representing an estimated 8.7%, 15.8%, and 75.6% of US plastics generated in 2018, respectively) can enter the land and water environment where most of this plastic will not break down completely (United States Environmental Protection Agency, 2021), but rather will be subject to mechanical or photo oxidative degradation processes that will lead to the fragmentation of the macroscopic plastics into microscopic plastic particles (Andrady, 2011). These particles, categorized as microplastics (hereafter, MP), are defined as plastic fragments that are 5 mm or less in diameter. The tendency for discarded plastic products to ultimately end up in waterways is primarily responsible for the ubiquity of MP in lakes (Dusaucy et al., 2021; Iannilli et al., 2020; Pastorino et al., 2021), rivers (Nel et al., 2018; Ravit et al., 2017), estuaries and coasts (Bailey et al., 2021; Frias et al. 2014; Rodrigues et al., 2019; Zhao et al. 2014), the open ocean (Cózar et al. 2014; Moore et al. 2001), and deep-sea sediments (Kanhai et al. 2019; Woodall et al. 2014) from tropical to polar ecosystems (Alfaro-Núñez et al., 2021; Burns and Boxall, 2018; Waller et al. 2017). Regions identified as most at risk from MP pollution, estuaries and the coastal ocean, are those exposed a high number of MP sources (Cole et al., 2011). MP concentrations up to 2.75 microplastic/m^3 for 500-2000µm and 4.71 microplastic/m^3 for 250-500µm were recently reported from the mouth of the Raritan River out to the coastal ocean (Bailey et al., 2021). Generally, concentrations of macro and microplastics in lakes, rivers, and oceans have been reported between 10^{-3}-10^3 microplastic/m^3 (Alimi et al., 2018), the variation being a function not only of study location but also methods, with higher concentrations observed when smaller particles and more morphologies were included in analyses.

MP that pollute the aquatic environment may enter the food chain through consumption by organisms that inhabit terrestrial, water column (pelagic), and benthic environments such as semiterrestrial amphipods, zooplankton, fish, crabs, and shellfish (Farrell and Nelson, 2013; Iannilli et al., 2020; Savoca et al., 2021; Setälä et al., 2014; Van Colen et al., 2020). Zooplankton are particularly susceptible to MP ingestion due to similarity in size and density (i.e., buoyancy) of their natural prey sources (Costa et al., 2020; Rodrigues et al., 2021; Zheng et al., 2020), and the presence of MP has been detected in 28 taxonomic orders encompassing nearly 40 species, including several different copepods (Zheng et al., 2020). Furthermore, biofilm formation on the surface of aged MP has been reported to increase the attractiveness of particles as food for zooplankton (Vroom et al., 2017), but can also serve to change the buoyancy of MP particles and therefore impact their fate in aquatic environments.

MP can pose many threats to marine organisms (Avio et al., 2016; Botterell et al., 2019; Derraik, 2002; Foley et al., 2018; Wright et al., 2013). In zooplankton, MP ingestion has been associated with decreases in survival (Lee et al., 2013; Svetlichny et al., 2021; Yu et al., 2020; Zhang et al., 2019), development and growth (Cole et al., 2019; Jeong et al., 2017), fecundity (Jeong et al., 2017; Zhang et al., 2019), and egg hatching success (Cole et al., 2015). Furthermore, plastic additives or monomers can be hazardous, impact mobility, development, and reproduction of zooplankton (Botterell et al., 2019; Cole et al., 2011; Lee et al., 2013).

Although an increasing number of studies have focused on the relationship between MP and zooplankton, most published results are from laboratory settings rather than field collection involving the digestion of whole zooplankton to quantify all MP ingested (Rodrigues et al., 2021). Of those field studies, research in the open ocean predominates and thus is not representative of MP-zooplankton relationships in highly populated, biologically productive coastal systems. The discrepancy between the number of laboratory versus field studies is likely a result of the methodological challenges of extracting and analyzing environmental MP from environmental organisms. Laboratory studies typically use colored or fluorescent MP beads or fragments that can be visually inspected in organism guts or stomachs once ingested. Visual identification of these colored or fluorescently labeled plastics is possible. However, the detection and analysis of small MP ingested by zooplankton in natural systems. requires chemical digestions of collected organisms, ideally optimized to reduce non-target debris from the organism without altering the polymers targeted. A second challenge is analysis of the extracted particles, which even with optimized protocols still contain non-anthropogenic debris, and for the size range relevant to ingestion by zooplankton, use analytical techniques that are more challenging than for large particles. Chemical analysis of MP can be performed by FTIR and Raman spectroscopy, techniques that are non-destructive and require minimal sample preparation after particles have been extracted from the environmental matrix. For particles smaller than 500 µm, a microscope is commonly coupled to the spectrometer. Raman spectroscopy has a lower diffraction limit; hence, smaller particles (< 15 µm) can be accurately identified.

Interactions between MP and marine organisms is facilitated in coastal waters because of enhanced MP pollution and high organism abundance (Clark et al., 2016; Sun et al.,2018a). The few studies that have examined MP ingested by zooplankton in natural seawater highlighted the ubiquity of occurrence, but also demonstrated high variability in ingestion incidence and MP characteristics in terms of size, morphology, and polymer type (Desforges et al., 2015; Kosore et al., 2018; Sun et al., 2018a, b; Taha et al., 2021; Zheng et al, 2020). Additionally, there have been no published studies reporting *in situ* ingestion of MP by zooplankton in the Hudson-Raritan estuary (Fig. 1), the location of interest in the present study. We note that in addition to being highly urbanized, this system is of historical significance because General Bakelite, the first company in the world to produce synthetic plastic opened up at the mouth of the Raritan in Perth Amboy in 1909 (Crespy et al., 2008). Finally, ingestion of MP by zooplankton may represent a major sink of MP in the marine environment (Kvale et al., 2020), but to our knowledge there are no system-scale estimates of the fraction of MP discharged into an estuary or coastal system that are ingested by zooplankton.

Here we present the first comprehensive characterization of MP ingested by planktonic copepods in the highly urbanized Hudson-Raritan Estuary (Fig. 1) using micro-FTIR imaging and/or Raman spectroscopy. We predicted that the MP ingestion incidence by zooplankton would be high. Therefore, the objective of this study was to determine MP ingestion incidence and characterize MP ingested by multiple species of zooplankton by size, morphology, color and polymer type. The field campaign included a single day field effort in July 2018 to test and develop protocols followed by a two-day effort in April of 2019. Sampling was performed along a salinity gradient on these three dates that also exhibited different flow conditions. This strategy allowed us to test the potential effects of these parameters on MP ingestion. Comparisons were also made to water column MP concentration and polymer profile observations previously reported (Bailey et al., 2020). These data were used to estimate the flux of MP through zooplankton based on gut turnover times, which we compared to estimates of MP entering this environment though the local waterways.

2. Materials and methods

2.1. Sampling area

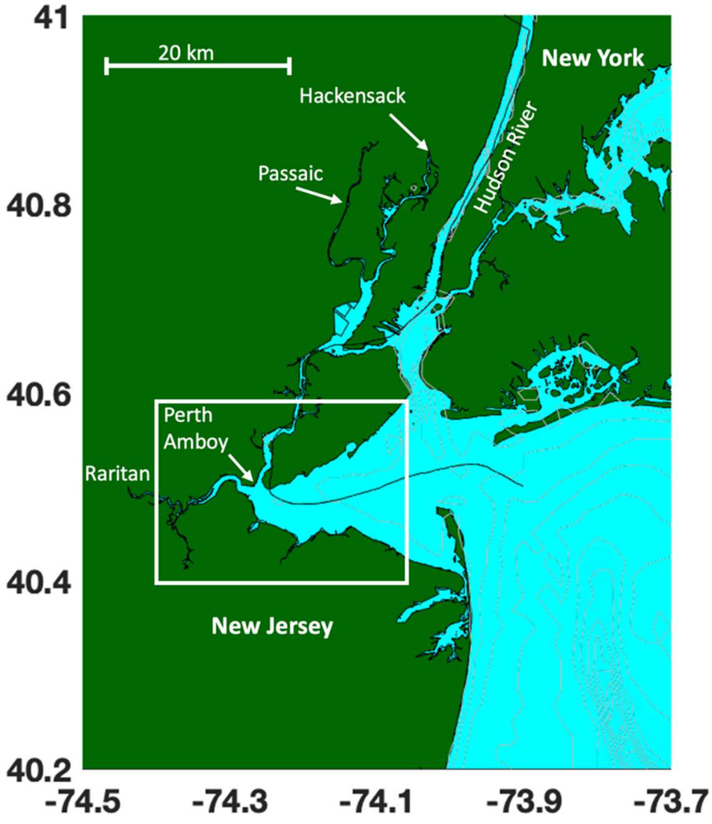

Fig 1. Map of the Hudson-Raritan Estuary. Latitude in decimal degrees North, Longitude in degrees West. White box represents sampling area depicted in Figure 2. Solid black line designates state boundary between New Jersey and New York.

The present study was performed in a highly urbanized estuary where MP pollution may be significant due to the proximity to high-population areas. The Hudson-Raritan watershed is home to nearly five million people and hundreds of various aquatic species, making the environmental impact of MP of particular importance (New York State Office of the Attorney General, 2015).

2.2. Sample collection

Paired samples to determine and characterized MP in water and in zooplankton were collected aboard the R/V Rutgers on one date in July 2018 and two sampling dates in April 2019 (Fig. 2). Sampling dates were selected to capture different flow conditions: low flow (July 2018), moderate flow (April 11, 2019), and high flow (April 16, 2019) (Bailey et al. 2021). Sampling was performed along the salinity gradient, based on real time salinity data from a flow through CTD aboard the ship, at sites in Raritan River (4/11/19 Site 6), at the river mouth (4/11/19 Site 5; 4/16/19 Site 3), and at frontal locations within the estuary (7/26/18 Site 2; 4/11/19 Site 4; 4/16/19 Sites 1 and 2). The characterization of MP in surface water, from samples collected using nets, for these locations has been previously reported (Bailey et al., 2021). Briefly, duplicate 20.3 cm diameter ring nets (mesh size 80 or 150 µm, Science First, Yulee, FL) were used to collect buoyant particles at the water surface at each sampling site. Collected samples were wet sieved, and particles were subjected to wet peroxide oxidation followed by density separation with sodium chloride (NaCl; Masura et al., 2015), buoyant particles were filtered on to 63 µm stainless steel wire mesh (TWP, Berkeley, CA) and analyzed via FTIR and/or Raman spectroscopy.

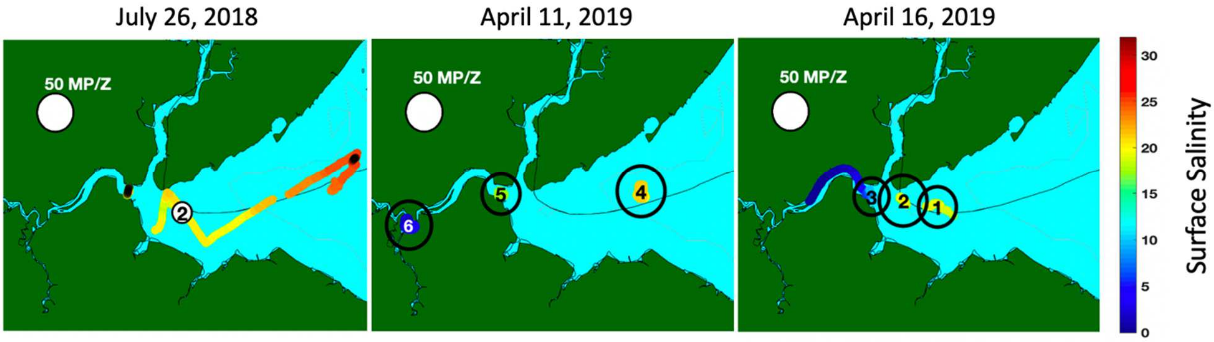

Fig. 2. Bubble plots displaying the average number of MP extracted per 100 zooplankton (MP/Z) across the sampling sites. The number inside of each bubble indicates the sampling site number, and the color corresponds to surface salinity at each site. Average MP values were calculated based on two replicates from 4/16 Site 2 and three replicates of all other samples. Solid black line designates state boundary between New Jersey and New York.

Duplicate surface tows for zooplankton were conducted at each site using 0.5 m ring net with 200 µm mesh and fitted with flowmeters (General Oceanics, Model 2030R) at the net openings and filtering cod-ends. Nets were towed for approximately 5 minutes at a speed of 1-2 knots. The contents of the cod-ends were then rinsed with filtered seawater from the cod-ends into glass collection jars and preserved in a 10% buffered formalin solution until analysis.

2.3. Extraction of MP from zooplankton

Subsets of zooplankton were removed from preserved sample jars and concentrated on a 200 µm sieve while rinsing with 0.2 µm MilliQ water (MilliporeSigma). Processing small sample aliquots at a time, zooplankton were then rinsed into glass petri dishes and examined under a dissecting microscope. Copepods were sorted and morphologically identified by species. The dominant species observed in each sample, determined via microscopic analysis using the preserved sample from the duplicate tow (see Section 3.1), were targeted for MP digestion and analysis. These included *Acartia tonsa*, *Centropages typicus*, and *Paracalanus crassirostris.* Individual copepods were rinsed copiously with 0.2 µm filtered MilliQ water and inspected microscopically for any MP attached to their appendages or exoskeleton. If detected, external particles were removed using steel forceps. After cleaning and inspection, copepods were placed in 7 ml glass scintillation vials with PTFE-lined caps in sets of 100 individuals of the same species per vial, or sample. Triplicate samples (each with 100 copepods) for each sampling date and study site, with the exception of duplicate samples for April 16 Site 2, were prepared. Each sample was digested in 3 mL of concentrated (70%) nitric acid at 80° C for two hours (Desforges et al., 2015). Samples were then diluted with 0.2 µm MilliQ water in a 1:1 ratio and filtered onto 0.2 µm pore size 25 mm Anodisc membranes (Whatman) under low vacuum. Filters were rinsed with additional MilliQ water and then placed into glass petri dishes with glass lids. Procedural blanks (7 mL vials filled only with 3 mL of the nitric acid digesting agent) and a matrix blank spiked with 15 um polystyrene beads were performed alongside each digestion. The matrix blank was prepared by diluting a white coloured polystyrene microbead stock solution (Sigma #74964) with 0.2 µm MilliQ water such that the final concentration of beads in each matrix blank sample (N=2) was calculated to be approximately 50 beads. These microbeads were selected because they were available in a comparable size to the environmental particles we expected to be extracted from the copepods and are easily quantifiable.

Due to the high particle counts, random subsampling (25% - 90% of total filter area analyzed) of each filter was performed. Particles were observed to be uniformly distributed across the filters, and MP totals were determined by scaling up the numbers of MP detected in each subsection to represent 100% of the filters. Subsampled MP were enumerated, measured for size, and characterized for color, morphology, and composition (polymer type) through visual (described in section 2.4) and chemical (described in section 2.5) analyses. MP ingestion incidence, reported here as MP individual^{-1}, was calculated by dividing MP counts on each filter by 100 individuals. Average and standard deviation (SD) of ingestion incidence were calculated from the replicate samples processed from each sampling date and study site.

2.4. Visual analysis

Visual characteristics, such as color and morphology, as well as the size of each particle were documented prior to spectral acquisition. Particle morphologies were classified as either beads, fragments, or films. The few fibers observed were omitted from this study due to the possibility of aerial contamination (Wesch et al., 2017; Woodall et al., 2015). The size of each particle was measured on the Raman microscope using the distance and profile measurement tool in Horiba's LabSpec software (Version 6.5). All sizes were reported as the length of the longest axis of the particle.

2.5. Chemical analysis & spectral interpretation

Recalcitrant particles remaining following the digestion were analyzed for MP content using micro-FTIR imaging and/or Raman microscopy. An effort was made to collect both IR and Raman spectra for all samples. However, IR was not successful on all MP and therefore some samples were limited to the collection of Raman spectra only.

Each sample was analyzed directly on the Anodisc membrane, an appropriate substrate for both spectroscopic techniques that were utilized. Micro-FTIR imaging was performed using a Bruker Hyperion 3000 FTIR microscope (Bruker Optics, Billerica, MA) equipped with a 64x64 element focal plane array (FPA) detector and a 15x IR microscope objective. All spectra were collected in transmission mode in the wavenumber region of 4000 - 1250 cm^{-1} due to absorbance features from the filters below 1250 cm^{-1} that would interfere with sample spectra. Open air was used as a background, and all spectra were acquired with 32 background scans and 32 sample scans at a spectral resolution of 8 cm^{-1}. False-color images were then generated by integration of the 3000 - 2800 cm^{-1} (aliphatic C-H stretching) spectral region in order to identify probable organic particles. Positions of these particles relative to the center of the filter were noted, and subsequent Raman spectroscopic analysis was performed to confirm potential MP.

Raman analysis was conducted using a Horiba XploRA PLUS confocal Raman microscope equipped with 532, 638, and 785 nm excitation wavelengths and 10x [numerical aperture (N.A.) = 0.25], 50x LWD (N.A. = 0.50) and 100x (N.A. = 0.90) microscope objectives. Measurement parameters were adjusted for each sample to optimize the signal-to-noise ratio and maximize the quality of the spectra. Raman spectra were evaluated through a combination of manual interpretation (Socrates, 2004) and spectral searching programs OpenSpecy (Cowger et al., 2021) and BioRad KnowItAll (Academic Edition). When an exact determination of polymer type could not be made, MP were classified broadly (e.g., polyester or epoxy resin) according to the functional groups and linkages present in the sample.

2.6. Data analysis

Statistical analysis was performed using R (www.rproject.org). ANOVA was used to compare the total MP per copepod as a function of sampling date and species with a post-hoc Tukey test. A Shapiro test was used to confirm the normality of MP counts per copepod. The distributions of polymer types found in surface seawater and in copepods were square root transformed, a Bray-Curtis dissimilarity matrix was calculated, and results are presented via non-metric multidimensional scaling (nDMS). ANOSIM was performed to test for differences in the polymer profiles using a nested approach for matrix (surface seawater vs. in copepod) and sampling date. ANOSIM was also performed to compare the MP particle size profiles observed in the copepod samples between site and date. Spearman rank correlations were tested between MP abundance per 100 copepods and MP concentrations previously reported in the water column in the 250-500 µm and 500-2000 µm size range.

To quantify the fraction of MP entering the Hudson-Raritan system that are ingested by zooplankton, we estimated the volume of MP discharged into the system based on prior studies and the flux of MP through the zooplankton community. To estimate the flux of MP into the system, we used data from Meijer et al. (2021) who estimated the mean USA loadings of MP to the ocean to be 7.4 Tons of MP million people^{-1} and scaled that to the population in the Hudson-

Raritan watershed. To estimate flux of MP through zooplankton, we first estimated the mean volume of plastics per zooplankton, V_p, as

$$V_p = \frac{\alpha \sum_{i=1}^{n} N_i L_i^3}{N_z} \tag{1}$$

where α is a shape factor, defined as the ratio of the longest dimension of MP to the shortest dimension, and is taken from the literature (Cózar et al., 2014), N_i is the number of plastics reported in each of n=5 size classes, L_i is the size class, and N_z =2000 is the total number of zooplankton sampled. For L_i, we chose the mid-point for i=2 to 4 (i.e., 17.5µm, 37.5µm, and 75µm) and the minimum (i.e., 10 µm) and maximum (i.e., 100 µm) for i=1 and 5, respectively. The flux of MP through zooplankton is the ratio of our estimate of V_p to gut retention time, and this is discussed in more detail in the results and discussion.

3. Results

3.1. Zooplankton abundance and community composition

A total of 28 zooplankton taxa were identified in net tows conducted in the Hudson-Raritan study location. Total zooplankton present in the study location ranged from 58-5771 individuals m^{-3}, and were highly variable between sampling date and site (Table 1). Copepods comprised 70-98% (mean ± SD = 89 ± 10%) of the total zooplankton present in the collected samples in the study area. These abundance values are within range of those reported in the study location previously (Jeffries, 1964; Rothenberger et al., 2014; Stepien et al., 1981). Although the highest abundance of copepods occurred at the highest measured salinity, there was no significant linear correlation between salinity and abundance ($p = 0.28$). Among copepods, two species/genera were present in all samples processed (*Acartia tonsa* and *Paracalanus* spp.), and *Centropages typicus* was present in all but two samples. When present, these three species/genera were typically the most abundant. A few exceptions occurred. For instance, *Eurytemora* spp. were most abundant at one sampling date and site (4/16/2019 Site 4); however, they were only present in three of the processed samples. Within genus *Paracalanus*, dominance fluctuated between *P. crassirostris* and *P. parvus*; however, we selected only *P. crassirostris* for the MP analysis for consistency as this was the species that dominated in the samples that were processed first. *A. tonsa*, *C. typicus*, and *P. crassirostris* were therefore the three copepod species targeted for MP analyses in the present study.

Table 1. Abundance of zooplankton, and specifically copepods, in the Hudson-Raritan study location. Abundance of total copepods includes the younger copepodite life stages. The abundance of select copepods includes only the three copepod species that were persistent and typically the dominant adult stages in the processed tow samples and therefore used in the MP analyses (*Acartia tonsa*, *Centropages typicus*, and *Paracalanus crassirostris*).

Sampling Date and Site		Total Zooplankton (individuals m^{-3})	Total Copepods (individuals m^{-3})	Select Copepods (individuals m^{-3})
7/26/2018	Site 2	58	41	32
4/11/2019	Site 4	95	86	57
	Site 5	426	416	288
	Site 6	1462	1386	885
4/16/2019	Site 1	5771	5305	3381
	Site 2	503	485	335
	Site 3	1308	1089	100

3.2. Total MP content in copepods

Three species of copepods (*A. tonsa*, *P. crassirostris* and *C. typicus*) were targeted, and MP were detected in all 20 samples analyzed (Table 2 and Fig. 3; Each 'sample' represents 100 individuals). Average ingestion incidence (MP individual^{-1}) in the study area ranged from 0.30-0.82 (Table 2). No significant differences were observed in total MP extracted from the copepods between species (ANOVA, all $p > 0.35$, Table 2) or between the two April sampling dates ($p = 0.65$), but total MP extracted from copepods was significantly lower in the July 2018 samples compared to samples from the two April 2019 dates (ANOVA, both $p < 0.009$). Furthermore, no significant correlation between site-specific copepod abundance and ingestion incidence was observed, suggesting that the amount of MP found within zooplankton was not dependent upon zooplankton abundance.

Table 2. MP ingestion incidence of target copepod species in the Hudson-Raritan study location. Dominant zooplankton species were targeted for MP analysis at each study site (*Acartia tonsa*, *Centropages typicus*, and *Paracalanus crassirostris*). Ingestion Incidence (MP individual^{-1}) was calculated from number of MP per 100 copepods and reported here as an average ± standard deviation (SD) at each study site. Averages and SDs were calculated based on two replicates from 4/16 Site 2 and three replicates of all other samples.

Sampling Date and Site	Zooplankton Species	Ingestion Incidence (MP individual^{-1}) Average ± SD
7/26/2018 Site 2	*A. tonsa*	0.30 ± 0.07
4/11/2019 Site 4	*A. tonsa*	0.73 ± 0.09
Site 5	*P. crassirostris*	0.60 ± 0.08
Site 6	*P. crassirostris*	0.74 ± 0.14
4/16/2019 Site 1	*A. tonsa*	0.69 ± 0.13
Site 2	*C. typicus*	0.82 ± 0.48
Site 3	*A. tonsa*	0.51 ± 0.14

3.3. MP characterization and size-structure in copepods

Polyethylene and polypropylene were the most commonly observed polymer types across all copepod samples, followed by polystyrene. Polyesters, such as polyethylene terephthalate (PET), as well as polydimethylsiloxane (PDMS) rubber and other polymers, including epoxy resins and vinyl copolymers were also observed (Fig. 3). No differences in polymer profiles were observed between the sampling sites or dates (ANOSIM, all $p \geq 0.10$) with replicates clustering with 51.7% (Site 2, July 26 and April 16) to 80.1% (Site 6, April 11) similarity.

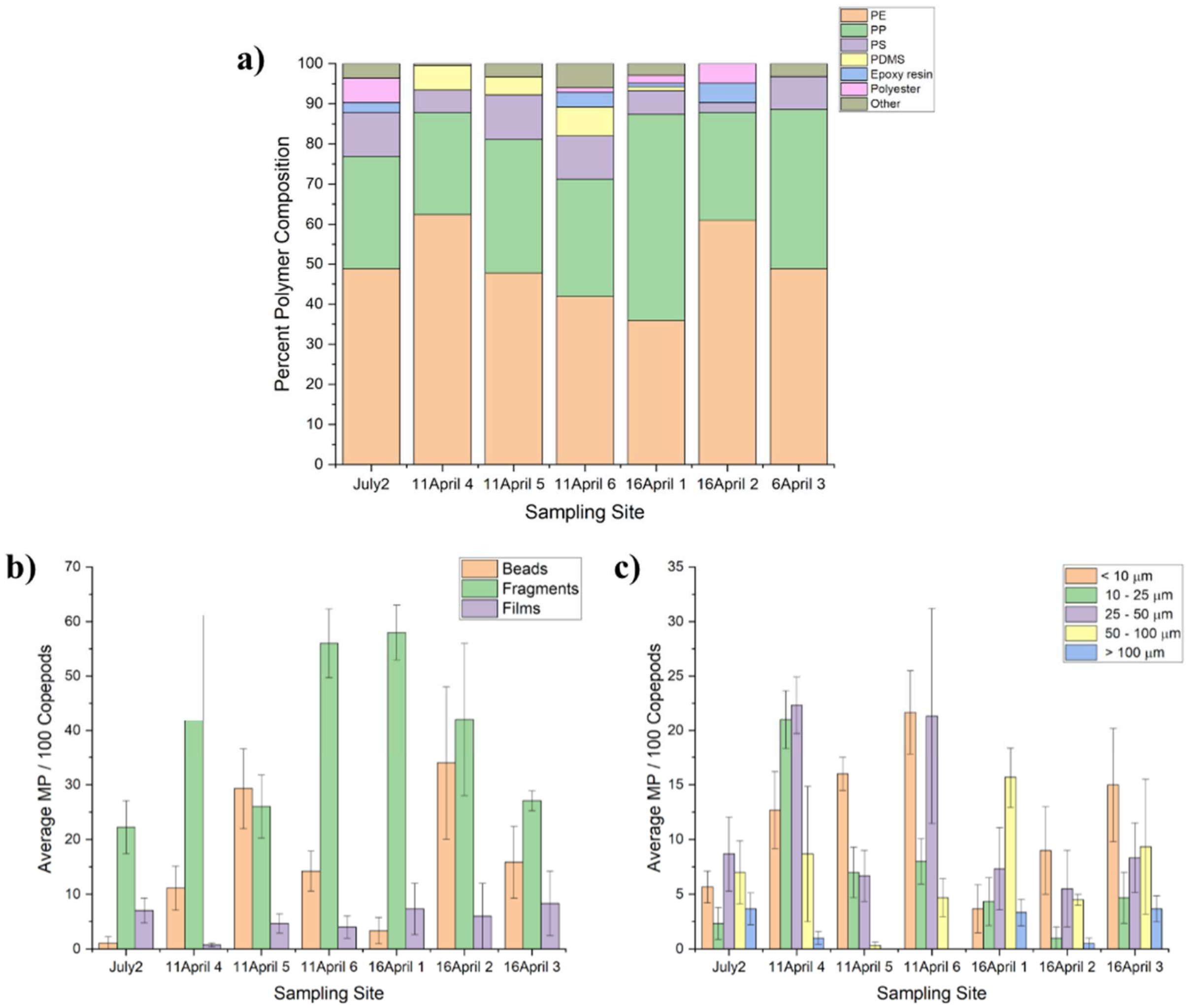

Fig 3. Characterization and size of MP found in copepods collected from the Hudson-Raritan study site. (a) Percentage of polymer types in the total MP, (b) average MP per 100 copepods by morphology, and (c) average MP per 100 copepods for different size classes extracted from copepods collected from each sampling site. Sampling site names are listed by Day Month Site. Average values are reported for N=2-3 replicates per site.

Raman spectra of common polymers, such as polyethylene (Fig. 4a) and polystyrene (Fig. 4d), could typically be evaluated on sight. The Raman spectra of most polypropylene MP indicated extensive polymer oxidation (Fig. 4c), as evidenced by the introduction of bands at approximately 1300 cm^{-1} and 1050 – 1000 cm^{-1}, which can be correlated with oxygen-containing functional groups. PDMS MP (Fig. 4e) were identified by key bands at 1440 cm^{-1} (CH_3 deformation), 1050 cm^{-1} (Si-O-Si symmetric stretch), 810 cm^{-1} (Si-C stretch) and 575 cm^{-1} (Si-O-Si asymmetric stretch). Similarly, epoxy resins (Fig. 4f) were identified by key bands at 1500 cm^{-1} (CH_2 deformation), 1250 cm^{-1} (epoxide C-O stretch), 810 cm^{-1} (ring vibration) and 750 cm^{-1} (ring vibration). Pigmented polyethylene and polypropylene MP were observed in a variety of colors, including red (Fig. 4), green, blue, purple and orange. All epoxy resin particles were blue (Fig. 4). Overall, colorless or gray/brown MP were most abundant (> 75% of all MP observed). Six out of seven procedural blanks were confirmed to be free of MP, with the exception of fibers greater than 400 μm in size. Two 15 μm polystyrene beads were found on one of the blanks. Accordingly, 15 μm polystyrene beads found on any subsequent samples were omitted from average MP counts. A recovery of 54% was reported for the matrix spike.

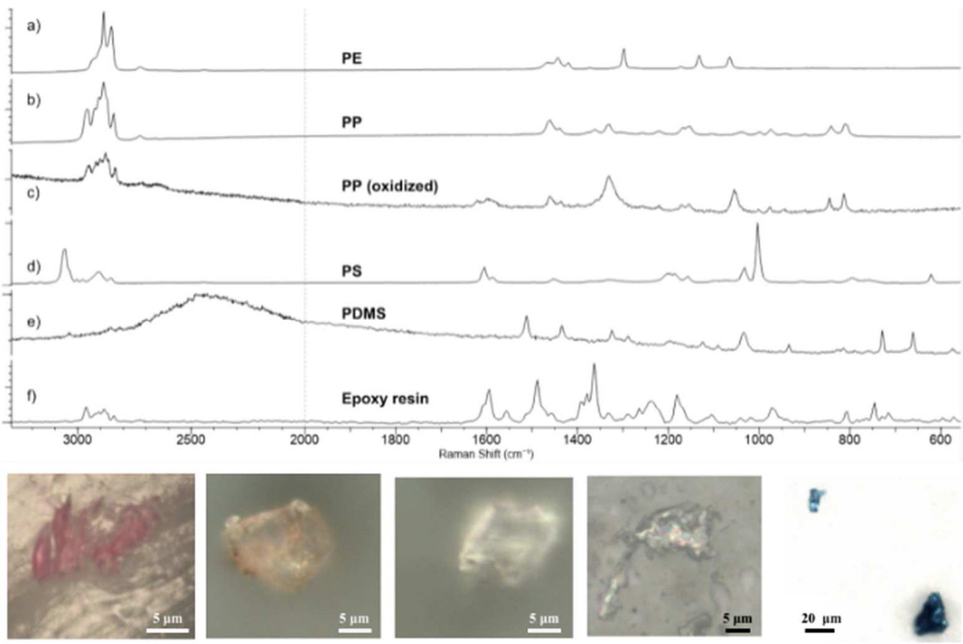

Fig. 4. Representative Raman spectra and images of MP observed in Hudson-Raritan estuary copepods. Top: Representative Raman spectra of (a) polyethylene, (b) polypropylene, (c) oxidized polypropylene, (d) polystyrene, (e) polydimethylsiloxane and (f) epoxy resin MP. Bottom: Example MP images, from left to right, are polyethylene, polypropylene, polystyrene, polydimethylsiloxane, and epoxy resin (both blue particles). All images were captured using a 100x microscope objective.

Fragments were the most commonly observed morphology found in the digested copepod samples in all but one site (Fig. 3b) (4/11/2019 Site 5). Beads were the dominant morphology found in copepods collected from the mouth of the Raritan River on 4/11/2019 (Site 5) and were also found in relatively high amounts in copepods collected near this location on 4/16/2019 (Sites 2 and 3). It is noteworthy that, although fibers were intentionally excluded from MP analysis, no fibers within the expected size range of particles ingested by the copepods analyzed were observed.

All beads were measured to be 5 µm in diameter and spectroscopically determined to be polyethylene. Films ranged in size from 7-60 µm, with approximately 75% of all films observed measuring less than 25 µm. Fragments were more varied in size, ranging from approximately 3-165 µm. Over half (57%) of all fragments fell within the size range of 10-50 µm (Fig. 5).

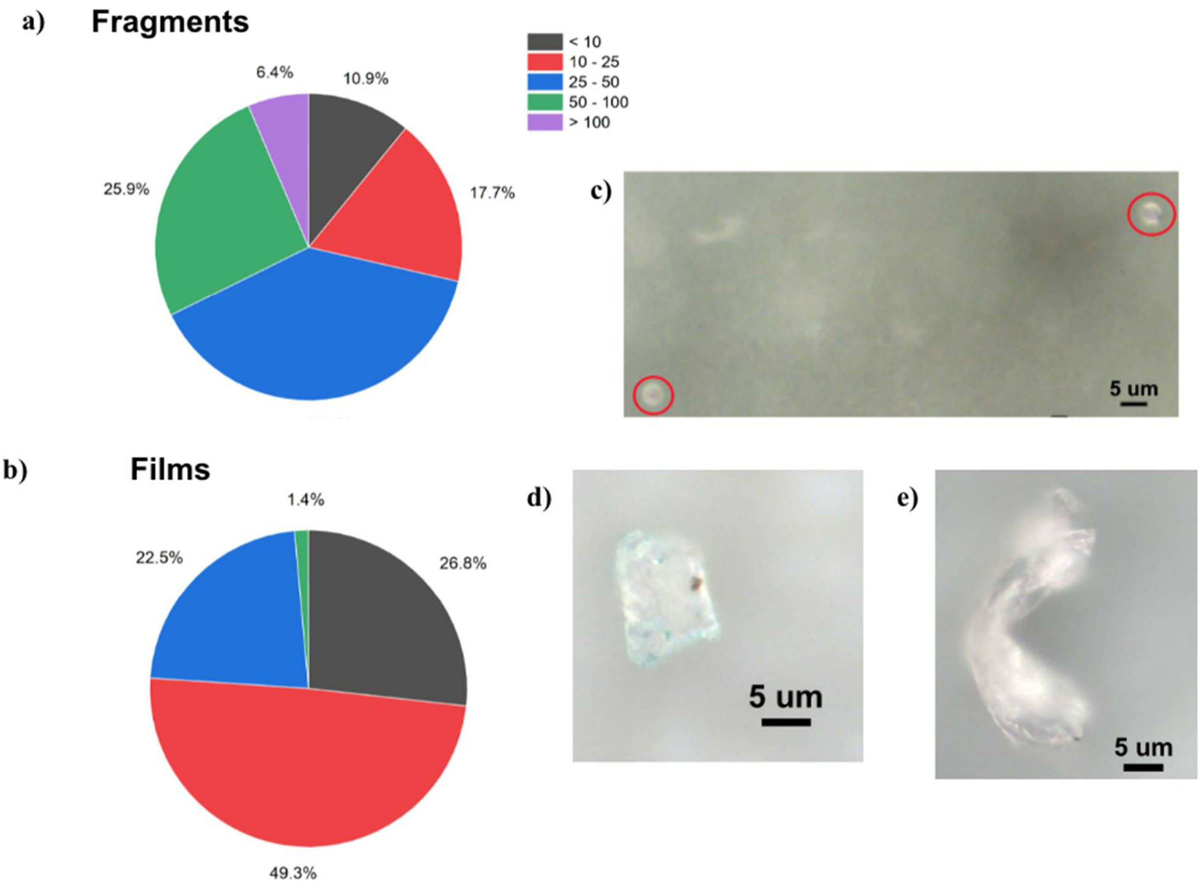

Fig. 5. Size distributions of (a) fragments and (b) films, as well as example images of various MP morphologies observed: (c) beads, (d) fragments and (e) films. Size distributions represent all fragments and films observed across copepod samples from each sampling site.

The particle size distributions observed in the copepods were significantly different by sampling site and date (ANOSIM, $p = 0.001$). As can be seen in Fig. 3c, the size distribution of MP per 100 copepods varied with date and sampling site. The smallest size class (<10 µm) predominates at Sites 5 on April 11 and at Sites 1 and 3 on April 16 and are observed in high concentrations at Site 6 on April 11. The next largest size class, 10-25 µm, can be noted at Site 4 on April 11, while the 25-50 µm size MP were found in the highest amount at Sites 4 and 6 on April 11. The

50-100 µm size class was predominant at Site 1 on April 16. Across all sites, MP of the largest size class (> 100 µm) were the least frequently observed.

3.4. Comparison of microplastics in copepods and water

The MP abundances observed in copepods were compared to MP concentrations we previously reported in the water column (250-500 µm and 500-2000 µm) for paired samples (Bailey et al., 2021), understanding these particles were larger than those bioavailable to the copepods. Smaller MP were not analyzed in water column samples in our previous study because the nets used for sampling had aperatures of 80-153 µm to prevent clogging. No correlation was observed between paired MP concentration for either size class studied in water and MP abundance in zooplankton (both $p > 0.40$, Spearman Rank, Fig. A.1). nMDS demonstrated clustering by matrix between polymer profiles observed in MP ingested by zooplankton and in small size class (250-500 µm) of MP in water samples but not by sampling site (Fig. A.2, ANOSIM by matrix across sites $p = 0.034$, by site $p = 0.23$).

3.5. MP budgets in the system

Here we make an estimate of the volume of MP in the guts of zooplankton in Raritan Bay and use this to discuss a MP budget by contrasting estimates of the loading of MP to the Hudson-Raritan system to the flux of MP though the zooplankton community. We note that this is a crude order of magnitude estimate due to large uncertainties for select parameters. A first uncertainty is estimating the volume of MP based on the reported size of MP in this paper, because the size reflects the largest dimension, L, of each MP. Cozar et al. (2014) report a shape factor, α=0.1, to relate the volume of a single MP, $V_{mp}=\alpha L^3$. The reported size range, proportional to L^3, for particles larger than 5 mm was consistent with a constant shape factor across particle size indicating that MP shapes are self-similar. With particles less than 1-2 mm the volume begins to deviate from L^3, but this was assumed to be due to loss of the smaller MP rather than a change in the shape factor. The mean volume of plastics per zooplankton, was estimated using (Eq. 1; Section 2.6) which yielded an estimate $V_p=8.6 \times 10^{-15}$ m^3.

A second uncertainty is the well-recognized spatial heterogeneity, or patchiness, of zooplankton in marine systems (Folt and Burns, 1999). Such heterogeneity is apparent in Table 1 showing total zooplankton and copepod abundances spanning two orders of magnitude and ranging from 58-5571 ind. m^{-3}. Over 90% of the zooplankton collected were copepods, with a mean concentration from the sampling dates from our study of 1258 ind. m^{-3}, with more than half of these (725 ind. m^{-3}) consisting of one of the three "select copepods (Table 1). The Bay's surface area is approximately 200×10^6 m^2 with a mean depth of 5m and thus corresponds to an estimated volume of 10^9 m^3. Using the mean copepod abundance, we calculate that this corresponds to 1.2×10^{12} copepods and 7.25×10^{11} of the select copepods in the Bay, respectively. Thus, if our estimates of MP present in the gut are representative of all the copepods in the Bay the total volume of ingested plastics in copepods would be 0.011 m^3, while for the select alone copepods it would be 0.006 m^3.

We estimated the volume of MP released annually into the Hudson-Raritan system to be 86.56 MT yr^{-1}. This estimate is based on estimate of US loadings to the marine systems (Meijer et al.,

2021) and human population residing in the Raritan (8.88M), Passaic (2.5M) and Hudson (8M) rivers watersheds. Assuming that MP have a density close to 1000 kg m^{-3} we convert this loading to 86.56 m^3 yr^{-1}. We note that for the Raritan and Passaic Rivers alone, this method yields an estimate of 8.88 and 18.5 m^3 yr^{-1}, respectively, which is close to an estimate reported in Ravit et al. (2017) of 12.6 and 26 m^3 yr^{-1} from these systems. If we apply a gut retention time of natural food in zooplankton of 1 hour (ranges ~20-120 minutes for *Acartia* spp.; Kiørboe and Tiselius, 1987; Tirelli and Mayzaud, 2005), the above estimate of volume of MP ingested in zooplankton corresponds to a flux of over 95 m^3 yr^{-1} of MP through the guts of copepod and 54 m^3 yr^{-1} through the guts of the select copepods alone.

4. Discussion

The results from the present study, which is the first to examine MP ingestion by dominant zooplankton species in the highly urbanized Hudson-Raritan estuary, highlight the ubiquitous nature of MP ingested by the lower levels of the food chain. MP were observed in every sample analyzed. Although the presence of MP was consistently observed in every copepod sample processed, the total number, size, morphology and polymer type of MP ingested by copepods, and the relationship between ingestion incidence and copepod abundances, were highly variable between sampling dates and site locations as well as between and among species. This is likely a function of: 1) the generalist feeding nature of these species of copepods; and 2) highly variable MP distributions, concentrations, polymer types, and sizes *in situ* (Bailey et al., 2021).

4.1 MP ingested by copepods

Ingestion incidence reported for copepods in the present study were higher than those reported previously in other highly urbanized environments including copepods in the Yellow Sea (0.13 MP individual^{-1}; Sun et al., 2018a), copepods in the Terengganu Estuary and offshore waters of Malaysia (<0.05 MP individual^{-1}; Taha et al., 2021), other zooplankton taxa from the Yellow Sea and East China Sea (0.13-0.35 MP individual^{-1} for amphipods, chaetognaths, and euphausiids; Sun et al., 2018a, b), and amphipods, chaetognaths, fish larvae, and medusae in the Bohai Sea (0.01-0.12 MP individual^{-1}; Zheng et al., 2020). Ingestion incidence reported for copepods in the present study were also higher, for the exception of July 2018, than that found for copepods off the coast of Kenya (0.33 MP individual^{-1}; Kosore et al., 2018). Higher ingestion incidences have been observed, however, in marine stomapods (1.17 MP individual^{-1}; Sun et al., 2018b), marine ichthyoplankton (1-27 MP individual^{-1}; Rodrigues et al., 2019; Steer et al., 2017), and semiterrestrial amphipods in inland volcanic lakes (1.8-5 MP individual^{-1}; Iannilli et al., 2020).

The composition and morphology of MP ingested by zooplankton between the present study and those conducted previously were highly variable. Polyethylene and polypropylene, the most commonly ingested polymer types, were also the most dominant polymer types in surface water samples (250-500 µm size class) analyzed in Bailey et al. (2021). The predominant polymer types observed have densities less than (i.e., PE, PP, PDMS all $\rho < 0.97$ g cm^{-3}) or near (i.e., PS with $\rho = 0.96$-1.05 g cm^{-3}) to 1 g cm^{-3}; therefore, no relationship was observed between the polymer buoyancy and ingestion by sampling site/salinity. And fragments (or beads in one study site) were the most common MP morphology ingested in the present study, while fibers were not observed. However, fibers (Sun et al., 2018a; Taha et al., 2021; Zheng et al., 2020) or filaments

(Kosore et al., 2018) dominated MP ingested by zooplankton in other studies. Furthermore, MP consisting of cellophane dominated MP ingested by zooplankton in Zheng et al. (2020), while in Sun et al. (2018a), organic oxidation polymers and poly-octenes accounted for nearly 50% of the MP in zooplankton. This suggests the type of MP ingested is likely a function of the composition of MP in surrounding seawater.

Size ranges of MP ingested by each copepod species was highly variable, particularly for the larger copepods *A. tonsa* (adults = 800-1000 µm) and *C. typicus* (adults - 1000-2000 µm). These two species are omnivorous and have been observed to feed on a large range of prey type and size (*A. tonsa*: 2-250 µm, Berggreen et al., 1988; *C. typicus*: 3-300 µm, sometimes sizes up to 3600 µm, Calbet et al., 2007). MP ingested by the smallest species analyzed in the present study, *P. crassirostris* (adults = 350-450 µm), mostly consisted of size ranges <50 µm. This species is mainly herbivorous, grazing on nanophytoplankton 2-20 µm (Calbet et al., 2000), but has been observed to feed on protozoans greater than 200 µm (Sant'Anna, 2013). In lab-based feeding studies, when introduced to a range of sizes of MP polystyrene beads (2-17.9 µm), *P. crassirostris* fed most efficiently on beads 7.0-7.9 µm (Ma et al., 2021). Therefore, the copepods were likely not preferably selecting any one size class as prey but were instead feeding on the sizes of particles (prey and MP), and MP type mentioned in the above paragraph, that were present in the water column at the time. In the future, paired water and zooplankton sampling for MP, specifically focused on the same MP size classes, should be conducted to better inform whether copepods are more preferential or opportunistic in MP ingestion.

The particle sizes observed in copepod samples underscore the importance of using Raman microscopy for MP analysis for this matrix rather than FTIR. MP smaller than 25 µm comprised between 23-77% of all MP observed across all species and sampling sites studied (Fig. 3c). This size class is near the diffraction limit of FTIR microscopy. Particles smaller than 10 µm are below the diffraction limit of FTIR and can only be effectively studied using Raman microscopy.

It should be noted that concentrated nitric acid, the digestion agent used to isolate MP ingested by copepods in the present study, has been documented to depolymerize or solubilize particular polymer types (e.g., polyurethanes, polyethers and diene polymers/rubbers) and cause particle fragmentation of polymers such as polyesters (e.g., PET) and polyamides (e.g., Nylon 66) (Enders et al., 2017; Thiele et al., 2019). We initially attempted an enzymatic digestion using proteinase-K according to Cole et al. (2014); However, the digested samples contained large amounts of residual exoskeleton (chitin) that made visual identifications of MP difficult compared to those digested using nitric acid (Sipps and Arbuckle-Keil, 2021). Thus, the values presented here may be underestimates of total MP ingested due to the breakdown of certain polymers during the acid digestion. Sizes of polyester and polyamide MP may also be skewed toward smaller size classes and higher particle counts may have been observed due to fragmentation of large particles into multiple smaller particles during digestion.

4.2 MP budgets in the system

Based on the calculated fluxes of MP through the guts of copepods, one could conclude then that the copepod community alone could process the annual loadings of MP to this system, although we note that there will be considerable temporal variability to this based on zooplankton phenology. Notably, in addition to those described above, an additional uncertainty in this calculation is the gut retention time of MP in zooplankton. Cole et al. (2013) found variable gut retention time of MP for copepods with some gut retention time similar to natural foods (hours) while others retained within guts for weeks and that irregularly-shaped microplastics may become entangled within the intestinal track and increase gut retention time. Indeed, numerous studies (referenced in Cole et al., 2013) found long or even 'near-indefinite' gut retention times in marine wildlife and that prolonged gut-retention times. Thus, as gut retention time increases the fraction of MP loadings that passes through the guts of zooplankton decreases. In the case of a short gut retention time, we suggest that large fraction of MP discharged into this system would be pass through the guts of zooplankton and be incorporated into sinking fecal pellets and retained in the system due to the strong tendency for estuarine systems to trap settling particles (Burchard et al., 2018). In contrast, if gut residence time is long, most of the positively buoyant MP would be discharged into the coastal ocean. Based on Cole et al. (2013) indicating variable gut residence of MP, we suggest that reality lies between these two extremes. Yet, while more research is needed to better quantify the impact of zooplankton on the fate and transport of MP, the mere possibility that zooplankton feeding could constrain the transport of MP between land and sea is remarkable.

5. Broader Significance

Zooplankton are not only key players in the ocean food web, transferring energy from primary producers to higher trophic levels, but they also play a critical role in the recycling and export of nutrients (Steinberg and Saba, 2008; Mitra et al., 2014; Turner, 2015). As such, MP ingestion by zooplankton can have important implications on MP fate and transport. Ordinarily buoyant MP particles may be repackaged in fecal pellets excreted by the zooplankton, altering the bioavailability of the MP to organisms throughout the water column (Cole et al., 2016). Furthermore, the incorporation of MP in fecal pellets can alter the pellets' densities and sinking rates, disrupting the vertical transport of organic matter and nutrients in the water column that is an integral part of the biological pump (Cole et al., 2016; Coppock et al., 2019; Shore et al., 2021) The "sink" of MP through the food web is one possible mechanism for the large mis-match between the total loadings of plastics to the marine environment and the vastly smaller global inventory of plastics at the ocean's surface (Cózar et al., 2014).

Expanded studies investigating the potential for other zooplankton species to ingest MP, along with MP ingestion occurrence and transit times of MP in zooplankton guts would be highly valuable in determining, on a community level, the comprehensive role of zooplankton in MP bioaccumulation through the food web and transport and fate in aquatic systems.

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Dataset

MP data collected during the present study are available in the Rutgers University CORE data repository ([dataset] Arbuckle-Keil, 2021).

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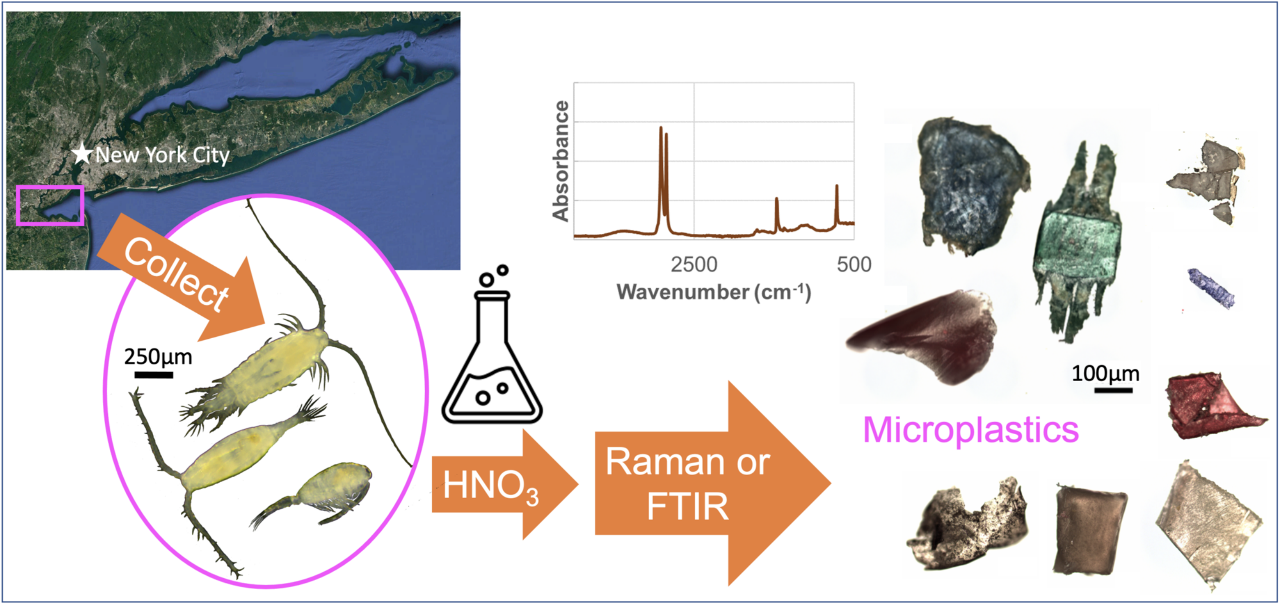
New York City
Collect
250µm
Absorbance
2500
500
Wavenumber (cm-1)
HNO3
Raman or FTIR
100µm
Microplastics