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Note

Microplastics and microfibers in surface waters of Monterey Bay National Marine Sanctuary, California

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ABSTRACT

Despite a recent report of high concentrations of microplastics and microfibers in the mesopelagic waters of Monterey Bay National Marine Sanctuary (MBNMS), little is known about these particles in surface waters. From 2017 to 2019, we sampled two nearshore and two offshore locations within MBNMS using a manta trawl and analyzed these samples for microplastics and microfibers. We found an average concentration of 1.32 ± 0.70 (SE) particles per m^3. We found the highest concentration of particles closest to shore, and the lowest concentration above the remote Davidson Seamount. Fiber-like debris was more common in offshore, as compared to nearshore, sites. Overall, particles in our samples were primarily buoyant synthetic polymers, including polypropylene and polyethylene. Our results provide baseline data on the degree of microplastic and microfiber pollution in MBNMS surface waters and confirm that this pollution can be found in waters from the surface to at least 1000 m depth.

1. Introduction

Microplastics and microfibers (i.e., anthropogenic 'particles' <5 mm in length) are the most pervasive marine debris. Surface seawater (the top meter) has consistently been the focus of microplastic and microfiber quantification efforts due to its ecological relevance and accessibility to sampling (Cózar et al., 2014; Eriksen et al., 2014). Surface water-concentrations span ten orders of magnitude from 10^{-5} particles per m^3 in the eastern equatorial Pacific (Spear et al., 1995) to 10^5 particles per m^3 off of Geoje Island, Korea (Song et al., 2014). Globally, the mean concentration in surface waters, given net mesh sizes ranging from 280 to 350 μm, is 0.96 ± 2.05 particles per m^3 (Shim et al., 2018). The ocean's sunlit surface is highly productive; diel migratory plankton and fish ascend en masse to the sea surface at night to feed. This is one route through which surface microplastics may enter the pelagic food web (Setälä et al., 2014).

These particles are concerning in part because they concentrate hydrophobic contaminants from surrounding seawater and release additives into the environment (Rochman et al., 2019). This diverse contaminant suite includes a variety of persistent organic pollutants (POPs) (e.g. polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane (DDTs)), heavy metals, and chemical additives (e.g. phthalate plasticizers). Microplastics and microfibers are consumed by hundreds of marine organisms, and while the effects on wild organisms, ecological communities, and marine ecosystems are largely unknown, there is burgeoning resource management interest (Bucci et al., 2020). Microplastics and microfibers have also been widely reported in seafood sold for human consumption (Baechler et al., 2019; Karami et al., 2018; Pellini et al., 2018; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014). POPs associated with anthropogenic debris may bioaccumulate through the food web with the potential to harm organisms that never ingest debris, thus understanding the distribution of these particles is vital to inform conservation efforts and assess ecological health.

Monterey Bay National Marine Sanctuary (MBNMS) is the largest marine sanctuary on the west coast of the United States and provides numerous commercial and recreational fisheries including the California market squid (*Doryteusthis apalescens* and *Loligo opalescens*), the northern

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anchovy (*Engraulis mordax*), and the Dungeness crab (*Metacari magister*). In addition, the region is home to a robust ecotourism industry. Despite the region's reliance on healthy marine ecosystems, there has been little research to assess the microplastic pollution of the sanctuary's waters. The only assessment of microplastic concentrations in MBNMS surface waters was from sampling efforts at four locations in 2006–2007, which reported concentrations of ≤0.08 particles per m^3 (Doyle et al., 2011). This is low in comparison to other coastal regions, and we were interested in how these concentrations may have changed in the intervening decade.

A more recent study uncovered concentrations of microplastics and microfibers as high as 15 particles m^3 in the sanctuary's epi- and mesopelagic waters (5-1000 m) (Choy et al., 2019). These particles were primarily polyethylene terephthalate (PET), commonly used in disposable water bottles and food packaging, and dominated Choy et al.'s samples possibly due to the negative buoyancy of PET (1.38 g/m^3) in seawater. However, more buoyant polymers are typically found in surface waters. For example, in the highly polluted Mediterranean, high- and low-density polyethylene (HPDE and LDPE; 0.94 and 0.92 g/m^3, respectively) made up 52% of sampled particles while PET made up less than 1% (Suaria et al., 2016). Similar to MBNMS, the Pelagos Sanctuary for Mediterranean Marine Mammals off the northwest coast of Italy is critical habitat for marine megafauna, and has a high degree of human impacts (Fossi et al., 2014, 2017). While plastic debris is an established stressor in Mediterranean marine ecosystems such as the Pelagos Sanctuary, the concentration and effects of plastic debris in MBNMS is less well known.

In the present study, we were interested in how the composition of microplastics on the surface of MBNMS differed from those found at depth by analyzing a subset of the particles we isolated via Fourier-transform infrared spectroscopy (FTIR). We hypothesized that buoyant plastics (e.g., LDPE, HDPE) would be most common. Finally, we expected to find higher concentrations of particles nearshore, despite Choy et al. (2019) reporting higher concentrations offshore than nearshore at depth in MBNMS. We based our prediction on a recent study of surface seawater in the nearby San Francisco Bay region, where higher particle concentrations were reported closer to shore (Box, 2019).

2. Methods

2.1. Sample collection

We collected surface seawater samples during the summers of 2017–2019 at two nearshore (Santa Cruz Boardwalk and Marina Outfall) and two offshore (Sur Ridge and Davidson Seamount) locations (Fig. 1). We sampled surface waters using a manta trawl net (355 μm mesh size; nylon) and completed transects at 1.5 knots in 30-min intervals in accordance with Manta Trawl Trawlshare protocol from 5 Gyres for collecting microplastic particles (Gyres, 2018). We collected nearshore samples from the small research vessels (<20 m), the *Sheila B* and the *John Martin*, owned and operated by Moss Landing Marine Laboratories (MLML), and offshore samples from large research vessels (>20 m), the FSV *Bell M. Shimada* – a NOAA ship – and the R/V *Western Flyer* owned and operated by Monterey Bay Aquarium Research Institute (MBARI). We attached a flowmeter to the manta trawl to calculate the distance traveled by the net. We multiplied this distance by the width (0.61 m) of the mouth of the manta, and the approximate height of the trawl that was submerged (0.095 m) while sampling to estimate the volume of water in which our samples were collected (Box, 2019). After we retrieved the net, we rinsed its contents from the detachable cod-end into a metal sieve (300 μm) and stored samples in labeled, sterile glass jars with 70% isopropyl for laboratory analysis.

2.2. Filtration and digestion

As depicted in Fig. 2, we first filtered the water samples through a

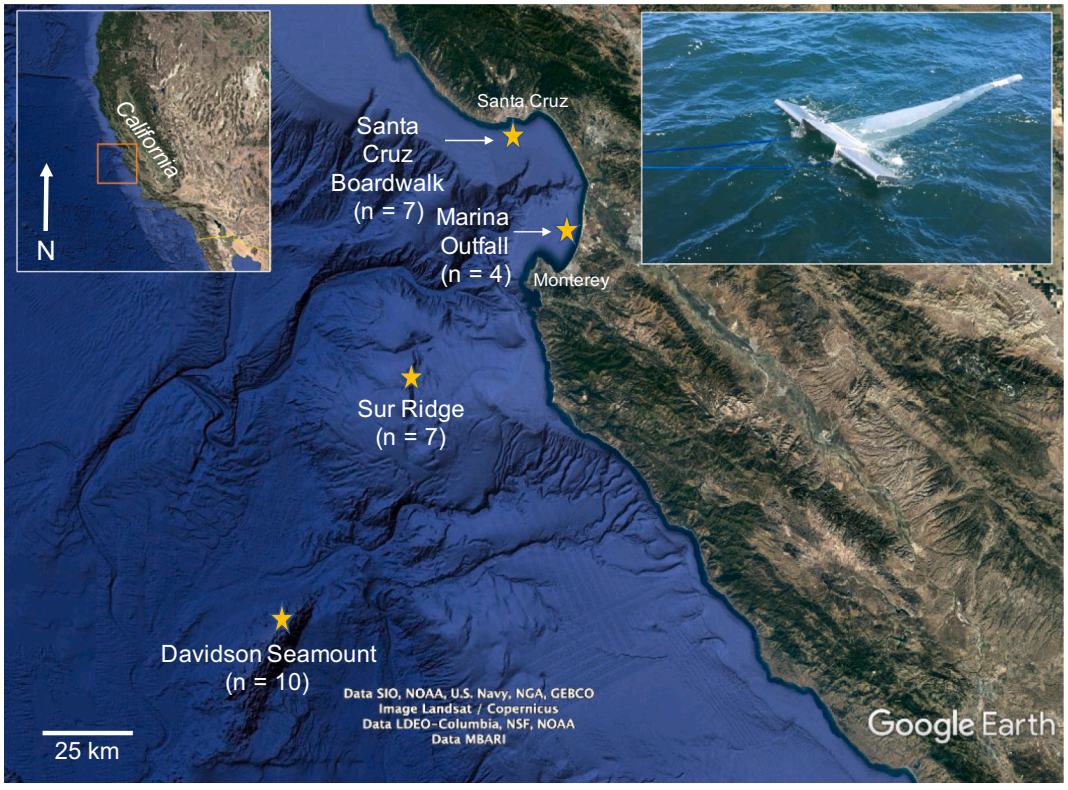

Fig. 1. Locations of two nearshore (Santa Cruz Boardwalk and Marina Outfall) and two offshore (Davidson Seamount and Sur Ridge) sample sites in MBNMS, off the central California coast (see inset, upper left). The manta net used for sampling efforts is also pictured (see inset, upper right).

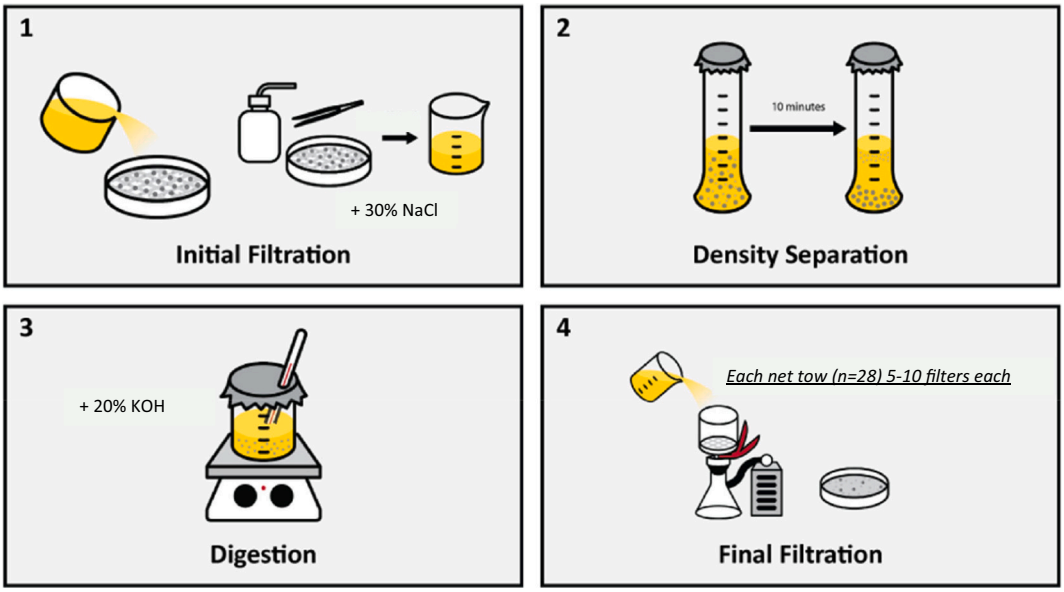

Fig. 2. Sample preparation workflow. (1) Initial filtration: sample is poured onto a mesh filter to strain excess stored 70% isopropyl & material on the mesh filter is transferred to a beaker with 30% NaCl (2) Density separation: NaCl & material solution is spun in a graduated cylinder and left to settle for at least 10 min (3) Digestion: top portion of density separated solution is poured into a new beaker with 20% KOH & digests at 60 °C for 12 h-7 days (4) Final filtration: following digestion, solution is vacuum filtered (see Methods for more details).

metal sieve (90 mm) to remove the excess 70% isopropyl in which the samples were stored. Then, we transferred the material remaining on the sieve into a glass beaker using an UltraPure water rinse and metal tweezers. Following initial filtration, we performed a density separation by adding approximately 150% of the sample volume of 30% NaCl solution and stirred on high using a magnetic stir bar for 5–7 min. Following this, we transferred each sample into 1000 mL glass graduated cylinders and allowed the sample to settle for 15–20 min. Once the solution was well separated, we poured off the top portion of the sample into a separate 1000 mL beaker and added 100% the sample volume of 20% KOH. We left the samples on hot plates at 60 °C for 12 h to 7 days depending on the amount of organic matter left in each sample. Following KOH digestion, we used a vacuum filtration system (Büchi V-500 vacuum pump) and used cellulose filter paper (Whatman grade 1, 11 μm pore size) to collect our samples; however, cellulosic filters are not ideal for particle analysis via FTIR as the filter itself creates a high background for cellulose. If feasible, fiberglass (silicon) or gold filters are a preferred alternative to minimize undesirable background interference in the infrared spectra. Due to high volumes, we used multiple (1−10) pieces of filter paper to collect single samples. We stored samples in petri dishes sealed with parafilm for future analysis. These methods were modified from (Li et al., 2015; Mathalon and Hill, 2014; Rochman et al., 2015) (see Fig. 2).

2.3. Quality control

To minimize contamination, cotton coats and clothes as well as nitrile gloves were worn at all times during lab work. All glassware was triple washed with UltraPure water, Alconox, and natural fiber brushes after use. Glassware was also rinsed with UltraPure water before use. To account for any environmental contamination, we completed laboratory procedural blanks and field blanks. Before collecting samples in the field, we rinsed seawater through the manta trawl net and into sterile glass jars. These field blank samples were run through the vacuum filtration system. In the laboratory, we used UltraPure water and completed all listed laboratory isolation and extraction techniques alongside field samples for each day of analysis. We also filtered all solutions we prepared (NaCl and KOH) through a 11 μm Whatman filter as a conservative precaution to eliminate particle contamination. We also conducted density separation and digestion steps in a chemical hood. Concentrations from procedural blanks were subtracted when quantifying particle counts in our samples.

2.4. Identification and quantification of microplastics and microfibers

After filtration, density separation, and heat-assisted chemical digestion, we assumed that the majority of particles we detected were of synthetic origin. To quantify these particles, we divided and numbered all filters, contained in petri dishes, into quadrants. We used a random number generator to determine a single quadrant per petri dish for photography, stereomicroscopy, and counting. We then used ImageJ (Collins, 2007) to aid in identifying, counting, and segregating (e.g., fiber or non-fiber) particles on our filters. First, we imported images and created stacks to edit multiple (10) photos at once. We then converted the stacks to grayscale by changing the image type to 8-bit, cropped them, and adjusted image thresholds (ranges fell between 50 and 200) to enhance the contrast between particles on the filters and the filter background itself. By using stacks, we were able to edit multiple photos at once. Cropping the images ensure we were only focused on parts of the filters in which our solutions were run over. We used grayscale to both enhance the contrast between particles and backgrounds, but also to run the image thresholds functions, the grayscale was necessary. For filters with extensive particles, we used the Analyze Particle function to retrieve particle counts. We tested this on different filters along with manual counts to ensure accuracy (±2 particles on filters with >20 particles). These methods were modified from (Erni-Cassola et al., 2017).

We sent five filters to the Thermo Fisher research and applications laboratory in San Jose, CA to identify particles present on the filters. This work was conducted on a Thermo Scientific NicoletTM iN10MX FTIR microscope equipped with a Mercury Cadmium Telluride detector. Particles were individually analyzed with a Germanium micro-ATR. All spectra were collected at a resolution of 4 cm^{-1}. To enhance particle identification, 128 scans were co-added to achieve a high signal-to-noise spectra. Moisture and carbon dioxide contribution was eliminated from spectra using the built-in atmospheric suppression feature of OMNICTM PictaTM software. The identification of spectra was achieved, using the library search feature in the software. A correlation algorithm was used

to carry out all searches. Similar methods were used for an additional subset of particles (n = 6) analyzed on a Nicolet Summit Pro FTIR-ATR (Thermo Scientific) to determine their polymer type. FTIR is a surface technique, and surface residue interferes with the ability of the instrument to accurately identify the particles of interest. Therefore, before FTIR analysis, each particle was rinsed with UltraPure water to remove surface residue that remained on the particle. The spectra generated by the FTIR and FTIR-ATR were compared to known spectra from the ThermoFisher spectral library to determine polymer type.

2.5. Statistical analysis

For analyses on location and region, our response variable was the estimated number of particles per m^3 from each net tow. To generate these numbers, we multiplied the empirical particle count on a filter quadrant by four to get an estimate of the total number of particles on the whole filter. If there was more than one filter per net tow, we added the particle counts from all filters from the same net tow to generate a total for each net tow. We blank-corrected our estimates by subtracting the mean of our procedural blanks from the total counts to account for environmental and laboratory contamination. Depending on the analysis, our predictor variable was either region (offshore or nearshore) or sampling location. We used generalized linear models (GLMs) with a negative binomial distribution implemented with the glm.nb function in the MASS package in R (Venables and Ripley, 2002) to test for differences between regions and sampling locations. To test for differences in the relative frequency of particle type (fiber or non-fiber) by region, we used a GLM with a binomial distribution in R (v. 3.6) where the sample size of the response variable (number of fibers vs. number of non-fibers per net tow) was preserved.

One net tow from the Boardwalk sampling site (B211) was a high outlier compared to all other data points. As a result, we conducted all statistical analyses with and without this outlier. Nevertheless, we believe that outlier to be a correct count, so our primary results and conclusions use all available data. All values are reported as mean ± standard error unless otherwise indicated.

3. Results

Across all net tows (n = 28), we found the mean concentration of particles to be 1.32 ± 0.70 particles per m^3 (median: 0.43 particles per m^3). We found some evidence that there were lower concentrations of particles in offshore sampling sites as compared to nearshore sites (z-value = −1.75, P = 0.08). However, after removing the outlier sample from the Boardwalk (net tow ID: B211), this trend no longer held (z-value = 0.72, P = 0.47). Particle concentrations in nearshore samples (n = 11) was 2.20 ± 1.72 particles per m^3 (median: 0.48 particles per m^3) and offshore samples (n = 17) was 0.75 ± 0.34 particles per m^3 (median: 0.22 particles per m^3). When analyzing particle concentrations by sampling location, we found a significant effect in that the nearshore Boardwalk site had the highest particle concentrations (3.21 ± 2.69 particles per m^3; z-value = 2.46, P = 0.01), and offshore Davidson Seamount had the lowest (0.26 ± 0.09 particles per m^3; z-value = −1.89, P = 0.06; Fig. 3). Even when omitting the Santa Cruz Boardwalk outlier, the Davidson Seamount still had the lowest particle concentrations (z-value = 2.12, P = 0.03).

Regarding particle type, we found more non-fibers (65%) than fibers (35%) overall; however, removing the Boardwalk outlier, this result was reversed (non-fibers = 38%; fibers = 62%). Despite this, there were significantly higher proportions of microfibers to non-fibers in offshore samples compared to nearshore samples in both the full dataset (z-value = 34.03, P < 0.0001) and the dataset with the Boardwalk outlier sample omitted (z-value = 8.85, P < 0.0001) (Fig. 4). Of the 11 particles we analyzed for via FTIR, five were polyethylene and two were polypropylene (Fig. 5). The other particles identified included a polyisoprene fragment, a rayon fiber, and a cellulose fiber (Fig. 5).

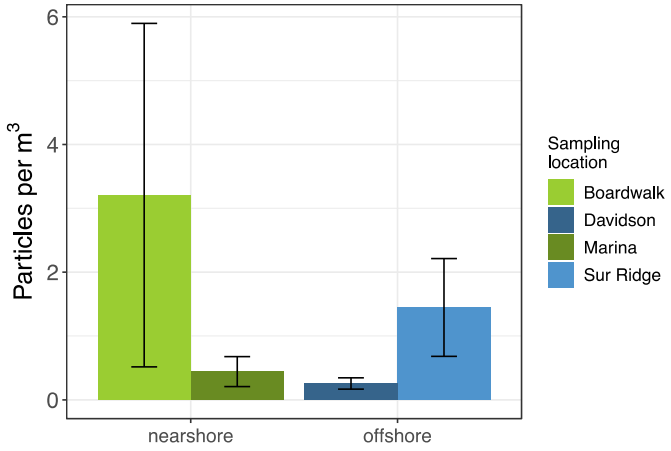

Fig. 3. Mean particle concentration (particles per m^3) at each sampling site sorted as nearshore (green) and offshore (blue). Mean nearshore concentration 2.20 ± 1.72; mean offshore concentration 0.75 ± 0.34 The nearshore boardwalk site adjacent to Santa Cruz, CA had the highest particle concentrations, and the offshore Davidson Seamount site had the lowest. Error bars represent SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

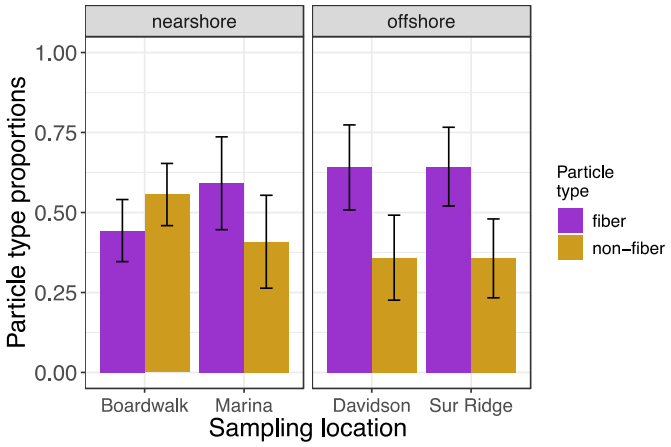

Fig. 4. Proportion microplastic particle types, fibers (purple) to non-fibers (gold), in each sampling location. A higher proportion of fibers to non-fibers particles were found at offshore sites as compared to nearshore sites. Error bars represent SEM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

This study was the first in a decade to report on microparticles in the surface waters of MBNMS. Given its designation as a National Marine Sanctuary, we did not expect to find high microplastic concentrations similar to global concentrations on comparable studies (0.96 particles per m^3) (Shim et al., 2018). However, our mean concentration across all samples, 1.32 particles per m^3, was slightly higher than the global average. This was less than what has been reported in the nearshore surface waters of the Santa Monica Bay (Lattin et al., 2004) and in a more extensive and recent study in the San Francisco Bay (Box, 2019) (Table 1). Only ~100 km to the north, the dense human population in the San Francisco Bay area may explain the differences between our findings. Focusing solely on their offshore sites – Greater Farallones NMS and Cordell Bank NMS – Box (2019) reported a median concentration of 1.12 particles per m^3, which is nearly identical to the concentrations we found in MBNMS (Table 1). Similar to the findings of Box (2019), we found lower concentrations of microparticles at offshore,

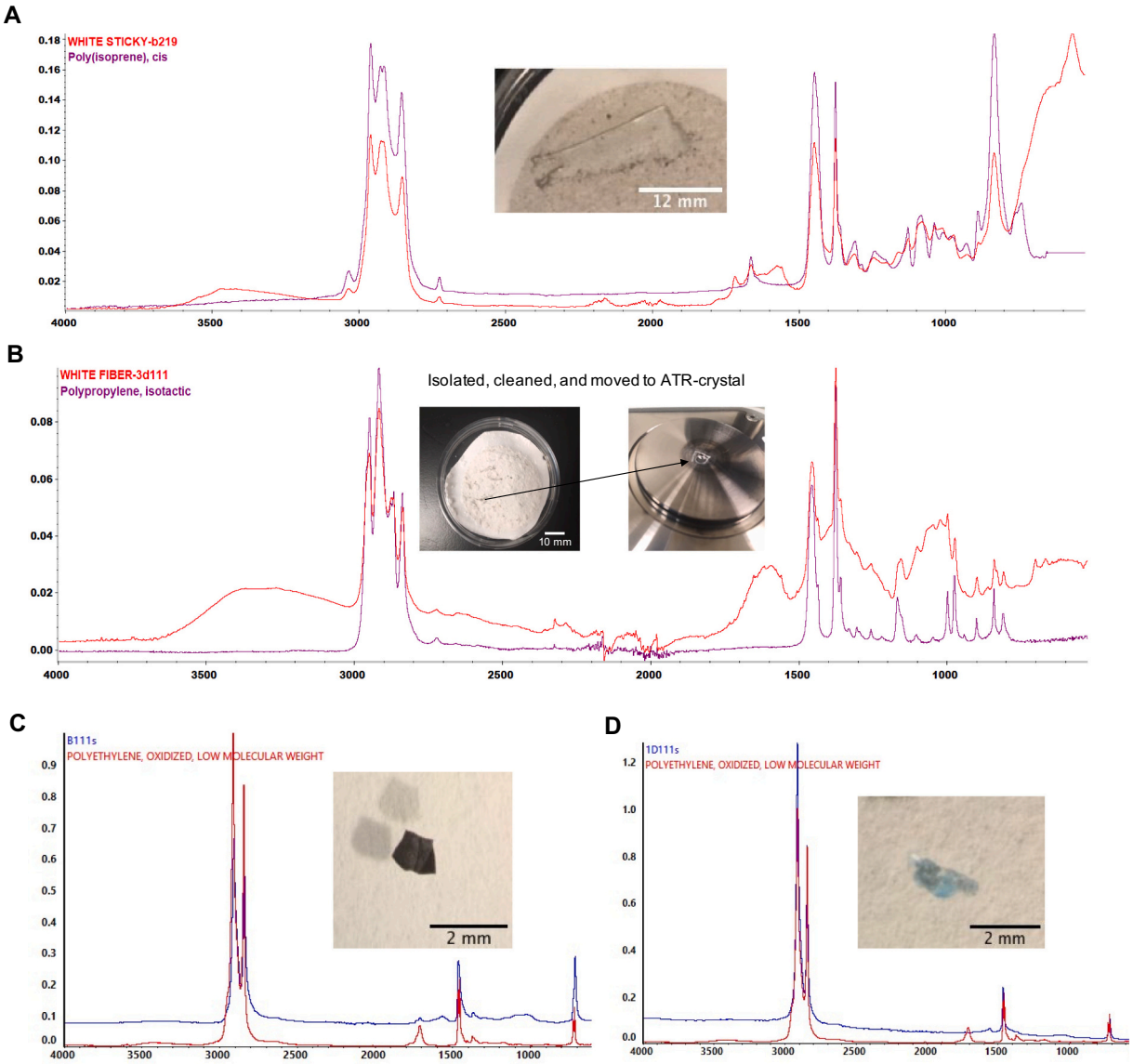

Fig. 5. Microscope images of four anthropogenic particles analyzed via FTIR. Particle spectra (red) were matched to library spectra (purple and blue). A–B) examples of two particles made of polyisoprene and polypropylene analyzed by Thermo Fisher. C–D) examples of two particles made of polyethylene analyzed by the authors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compared to nearshore, sites. Our most remote sampling location, the surface waters above the Davidson Seamount, had the lowest particle concentrations of any location we sampled (Fig. 3). Nearly half (40%) of net tows from this location recovered no particles. A similar trend – a negative relationship between particle concentrations and distance from land – was also found in the San Francisco Bay region (Box, 2019), suggesting that anthropogenic particles in the surface waters off central California likely originate from land, rather than long-range transport.

Within MBNMS, we found particle concentrations an order of magnitude higher than what had been reported a decade prior (Doyle et al., 2011) (Table 1). This may be due to variations in methodology and sample analysis; Doyle et al. (2011) used a binocular dissecting microscope to optically separate microplastic and microfiber particles from their water samples, whereas in the present study, we used density separation, chemical digestion, and vacuum filtration to isolate particles before microscopy. Therefore, we may have isolated and quantified more particles than would have been detected by optical selection alone. Another explanation is that marine microparticle concentrations have indeed increased over the past decade. The populations of Santa Cruz and Monterey Counties – the two counties that border MBNMS – have increased rapidly over the past two decades, and thus it is likely that more waste has been entering MBNMS as a result.

The highest concentrations of micro-debris in MBNMS, 5–15 particles m^3, have been found at 200-600 m depth; however, samples from 5 m depth revealed concentrations of 0–2 particles m^3 (see Table 1) (Choy et al., 2019). Concentrations reported here extend these measurements to the surface and fall within the 0.1–3 particles per m^3 range. The discrepancy between concentrations found at or near the surface compared to deeper in the water column may be due to biological and physical processes. For example, vertical migrating organisms may consume microparticles in surface waters and excrete them in deeper waters each day (Lusher et al., 2016). Additionally, the biofouling of marine plastics can affect their density and reduce their buoyancy causing them to sink (Fazey and Ryan, 2016; Kaiser et al., 2017). The processes that take microplastics deeper than the mesopelagic and down to abyssal sediments are largely unknown but may include similar biophysical mechanisms (Katija et al., 2017).

While we did not find statistically significant differences between

Table 1
Our results in context with studies that quantified microplastic and microfiber concentrations in coastal California seawater. Italic font represents data from water samples taken at depth in the water column, all other values from surface seawater. "Surface" here refers to the sampling of the top 0.5 m of the water column; all surface water studies used a manta trawl net with a 300–400 μm mesh size.

	Location	Year(s) of data collection	Sample depth (m)	Mean no. particles per m^3	Median no. particles per m^3	Source
National Marine Sanctuaries	Monterey Bay	2017–2019	Surface	1.32	0.22	This study
	Monterey Bay	2006–2007	Surface	0.00–0.07	NA	Doyle et al., 2011
	Monterey Bay	2017	5, 25, 50	NA	2.92	Choy et al., 2019
	Monterey Bay	2017	200	NA	11.00	Choy et al., 2019
	Monterey Bay	2017	400	NA	8.40	Choy et al., 2019
	Greater Farallones and Cordell Bank	2017–2018	Surface	1.12	0.86	Box, 2019
	Channel Islands	2006–2007	Surface	0.00–0.03		Doyle et al., 2011
Urbanized locations	Santa Cruz Boardwalk[a]	2017–2019	Surface	3.21	0.58	This study
	San Francisco Bay	2017–2018	Surface	4.11	2.94	Box, 2019
	Santa Monica Bay	2001	Surface	3.92	NA	Lattin et al., 2004

[a] Includes samples from the Santa Cruz Boardwalk site only (n = 7 samples total).

nearshore and offshore concentrations of anthropogenic particles in MBNMS surface waters, we did find that the sampling location closest to land (Santa Cruz Boardwalk site) had the highest concentration of debris. However, the high concentration of plastic at the Santa Cruz Boardwalk site, and large uncertainty of the overall particle concentration (Fig. 3) here, was driven by a single outlier. More sampling is needed in this region specifically to determine if these values hold. As compared to the Santa Cruz Boardwalk site, the most remote sampling location (Davidson Seamount site) had the lowest particle concentration. The collection methods (ROV) and specific sampling sites of Choy et al. (2019) differed slightly from ours and may account for our different findings. Our offshore sites were farther offshore than Choy et al.'s (2019) offshore site. In addition, our study was the first to sample the Davidson Seamount region of MBNMS for microparticles. Due to our limited sample size, we are unable to conclude that there are no significant differences in offshore or nearshore surface water particle concentrations in MBNMS; however, our results did indicate a trend for higher levels of microparticles in offshore waters that deserves further study. Though it is difficult to determine the sources and sinks of marine debris, microparticles in this region could originate from terrestrial runoff. For example, Elkhorn Slough, located at the mouth of the Salinas River in the middle of the Monterey Bay coastline, is known for high levels of contamination, attributed primarily to agricultural runoff (Rice et al., 1993). In contrast, offshore sources of anthropogenic debris may be driven by large-scale ocean currents, but more research is necessary to fully understand the sources and sinks of microplastics in this region and beyond.

Our FTIR analysis uncovered mostly buoyant particles. The majority of particles analyzed (8 of 11) were synthetic or semi-synthetic in origin, including polypropylene (PP), polyethylene, polyisoprene (PI), and rayon, while several others were natural cellulose and chitin (Fig. 5). Polyisoprene is a rubber-like material often synthesized for use in rubber bands, baby bottles, and sporting goods. Polyethylene (PE) was the most common polymer we identified (5 of 11); PE is commonly used in food packaging, industrial parts, single-use bags, and children's toys. As predicted, the synthetic polymers we identified in surface waters tended to be positively buoyant (e.g., PP, PE, PI), and notably, we did not identify any negatively buoyant plastics (e.g., polyvinyl chloride or PET). This suggests that despite physical mixing, biofouling, and biological transport processes (Galloway et al., 2017), buoyant plastics tend to remain at the surface. However, more research is needed to determine the full spectrum of polymers present in MBNMS surface waters.

Numerous field studies have found plastic additives (e.g., phthalates), and/or POPs that adsorb to plastic at sea, can transfer to organisms that consume this debris and may have deleterious physiological effects (Baini et al., 2017; Lavers et al., 2019; Rochman et al., 2014; Tanaka et al., 2013). As humans are exposed to synthetic microparticles through inhalation and ingestion (Barboza et al., 2018; Prata, 2018; Su et al., 2019), more research is necessary to understand the effects of this diverse contaminant suite on human health. Regardless, both local and global trends have shown increases in microparticle concentrations in surface seawater (Isobe et al., 2019; Thompson et al., 2004). Based on our findings and others (Box, 2019; Fossi et al., 2014), it is evident that microplastics and microfibers are pervasive even in marine sanctuaries. Continuing to monitor the presence and effects of small anthropogenic debris is imperative to maintain resource sustainability and ecosystem health.

CRediT authorship contribution statement

Lauren M. Kashiwabara: Laboratory methodology, Investigation, Writing – Original Draft **Shirel R. Kahane-Rapport:** Investigation, Fieldwork, Writing – Review & editing **Chad King:** Conceptualization, Fieldwork, Methodology, Resources **Marissa DeVogelaere:** Conceptualization, Fieldwork, Methodology, Resources, Writing – Review & Editing **Jeremy A. Goldbogen:** Supervision, Laboratory space & equipment, Project administration **Matthew S. Savoca:** Formal analysis, Data processing and statistical analyses, Writing – Review & editing, Supervision.

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.

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