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Original Article

A baseline study of macro, meso and micro litter in the Belize River basin, from catchment to coast

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The mismanagement of waste and subsequent presence of litter in the environment is an increasingly significant problem. Globally, rivers have been shown to be a major pathway for mismanaged waste. We investigated the distribution of macro, meso and micro litter along the Belize river basin. The North-East Atlantic OSPAR beach litter monitoring protocol was adapted for Belize, taking into account local issues such as mangroves and *Sargassum sp.* accumulations. On average, 77.3% of litter items consisted of plastic, and the most common items categories were unidentifiable plastic pieces (0–2.5 and 2.5–50 cm), broken glass, and metal bottle caps. The study indicated that there is an increase in the litter load as you move from catchment to coast, with both Plastic Pieces (PP) and Fishing Related (FR) items also increasing in numbers down the system. Additionally, microplastics abundance was determined in riverine sediments and in the riverine fish *Cichlasoma synspilum* ($n = 22$). All sediment samples contained microplastics, with a concentration of 200–6500 particles per kg dry sediment. Microplastics were found to be present in 36% of the riverine fish. The data from this study will provide evidence for the formation of Belizean legislation to reduce marine litter.

Keywords: beach, Belize, marine Litter, microplastics, monitoring, river, pollution, waste.

Introduction

Marine litter is "any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment" (UNEP, 2009). Marine litter has been found throughout all marine compartments: on beaches, at the sea surface, within the water column, on the seafloor and ingested by biota all over the world (Bergmann *et al.*, 2015; Galgani *et al.*, 2015). It predominantly consists of plastic items, and with plastic production rates exponentially increasing, the issue of marine litter is recognised as a growing global problem (PlasticsEurope, 2020). Synthetic polymers are inexpensive to produce, versatile and lightweight, properties which make them useful for human activities. However, these same properties make plastics a global threat when leaked into the environment (Kershaw *et al.*, 2011). Plastic items can trap and constrict many forms of life, can be ingested by animals, and transport invasive species (Rech *et al.*, 2016; Fossi *et al.*, 2018; Galgani *et al.*, 2018). Plastic persists a long time in the marine environment, and fragments through photodegradation, oxidation and mechanical abrasion.

The presence of litter in the marine environment has been noted since the 1960s (Kenyon and Kridler, 1969), and in the last two decades there has been rapid development of this research field (Galgani *et al.*, 2015). The need for scientific investigation, to understand the drivers of marine litter and inform policy decisions, is recognised. Globally, it has been estimated that 80% of marine pollution results from direct or indirect discharge of solids and liquids from land-based sources such as outfalls, waterways, agricultural runoff, and infrastructure (Jambeck *et al.*, 2015), with rivers representing the main pathways for the transport of plastics to oceans (Lebreton *et al.*, 2017; González-Fernández *et al.*, 2021; Meijer *et al.*, 2021). The remaining 20% enters the oceans through offshore industrial activities, shipping, discarded fishing gear, and the atmosphere. In the Caribbean, poor household waste collection services are among the significant reasons why plastics enter the marine environment with an estimated 322 745 tons of plastic going uncollected each year across selected Caribbean countries (Diez *et al.*, 2019). Of these, 22% of the households dispose of waste in waterways or on land where it can be washed into the waterways (Diez *et al.*, 2019). Recent studies have explored how riverine input of sediments, and wave and tidal energy regimes influence the geomorphology of the coastlines globally, and thus the dispersal and fate of plastic pollution (Harris, 2020; Harris *et al.*, 2021).

Microplastics can be defined as "synthetic water-insoluble polymers of 5 mm or less in any dimension" (ECHA, 2018) either from primary sources (e.g. accidental spillages of pre-production pellets) or resulting from the degradation process of larger materials via the action of chemical and physical processes (e.g. UV or mechanical degradation). Plastics in landfills and illegal dumpsites, as well as those lost from the waste stream into the environment, break down into microplastics which is cause for concern not only to the environment but also to human health (UNEP, 2009; Barboza *et al.*, 2018). Microplastics have been found on/in beaches, coastal zones, open-sea and deep-sea sediments worldwide (Galgani *et al.*, 2015). Similarly to larger plastic debris, the microplastics that enter the sea originate from land-based sources, such as sewage and storm water, or ocean-based sources, including discarded and lost fishing items (Li, 2018). Deep-sea sediments have been suggested as a likely final sink for microplastics in the marine environment (Woodall *et al.*, 2014), although more recent studies have shown that riverine, estuarine and coastal sediments may in-fact account for the highest loads or particles per kg of sediment (Harris, 2020). Studies on the abundance of microplastics in freshwater and estuaries are however limited, despite rivers being important pathways for the transport of microplastics from land to the marine environment (Wagner and Lambert, 2018). Settlement of microplastics in freshwaters is influenced by many factors including river morphology, rainfall events, hydrological conditions, vegetation cover, etc. (Yan *et al.*, 2021). Understanding transport of microplastics in riverine systems is however essential to assessing impacts and to guide policy actions (Whitehead *et al.*, 2021). Microplastics have also been reported for freshwater organisms including molluscs, invertebrates, fish and birds (O'Connor *et al.*, 2020). Detrimental physical effects of microplastics have been reported following ingestion (Wright *et al.*, 2013). There is also evidence that microplastics collect harmful sorbed co-contaminants (i.e. hydrophobic organic compounds, additives, pathogens) with the potential for transfer to biota following ingestion (Rochman *et al.*, 2013; Tanaka *et al.*, 2013; Bakir *et al.*, 2014). However, it has also been suggested that the transfer of sorbed co-contaminants from microplastics to biota would be negligible compared to other routes of exposure. Gaps of knowledge remain, however, about the effects of the transfer of plastic additives, often added at high concentrations during their production (Bakir *et al.*, 2016; Koelmans *et al.*, 2016; Lohmann, 2017).

This study took place in Belize, a Caribbean Small Island Developing State (SIDS) situated on the north-eastern coast of Central America. Sampling was primarily centred around the urban area of Belize City, with sampling of the Belize River and its tributaries within the Belize River basin providing rural sampling, upstream from the urban area. Regionally in the Caribbean, poor waste management systems, illegal dumping and limited recycling with weak enforcement have increased marine pollution (Clayton *et al.*, 2020). The Belizean government has recognised the social, economic and environmental impacts of plastic pollution and has responded with policies to curb single use plastics and its leakage. However, progress is difficult to follow in the absence of scientific studies on the abundance and distribution of riverine and marine litter. Clean-up and citizen science activities remove litter while educating the public and raising awareness on the issue of marine litter (Matthews and Doyle, 2012). Previous beach litter studies in the region reported on average > 75% of macrolitter was comprised of plastic, with plastic bottles being the most prevalent item (Bennett-Martin *et al.*, 2016). Prior studies looking at microplastics in Belize have focused on seagrasses and the potential for microplastics to enter the food chain (Goss *et al.*, 2018), as well as coral tissues (Oldenburg *et al.*, 2021) with, to the best of our knowledge, no previous studies reporting on the presence and abundance of microplastics in Belizean river sediments. The Belize river and its tributaries were selected because the river's source starts across the border in Guatemala. Therefore, the river crosses the central width of Belize (around 120 km), and passes through key areas of urban development, in particular Belize City, which is by far the largest population centre in the country.

The data collected in this study provided the Belizean government with an understanding of the magnitude and prevalence of macrolitter and microplastics in their waters and the capability to produce a baseline to evaluate the efficiency of planned legislative measures, such as the Belize National Marine Litter Action Plan. We investigated how the abundance and composition of microplastics and macro (> 2.5 cm) and meso (0.5–2.5 cm) litter changes along a river basin towards the marine environment. Understanding abundance, composition and spatial distribution and the sources of both macro/meso litter and microplastics allows for improved management of waste, protecting vital ecosystems from leakages. Using recognised and harmonised monitoring methodologies enables global comparison and further understanding of this transboundary issue.

Materials and Method

Site selection

The sample locations were focused along the Belize River and its tributaries, as well as around the urban centre of Belize City, the largest city in Belize with a population of just over 61 000, where the Belize river discharges into the Caribbean Sea. The Belize River is 290 km long (including the Mopan River, which originates in Guatemala) and its catchment area dominates the central region of the country, covering a total area of 9434 km^2 (approx. 60% of which is within Belize) (Esselman and Boles, 2001; Karper and Boles, 2004). The Belizean coastline is dominated by mangroves (Murray *et al.*, 2003) and access to beaches is limited due to dense jungle and lack of road infrastructure. The modelled offshore

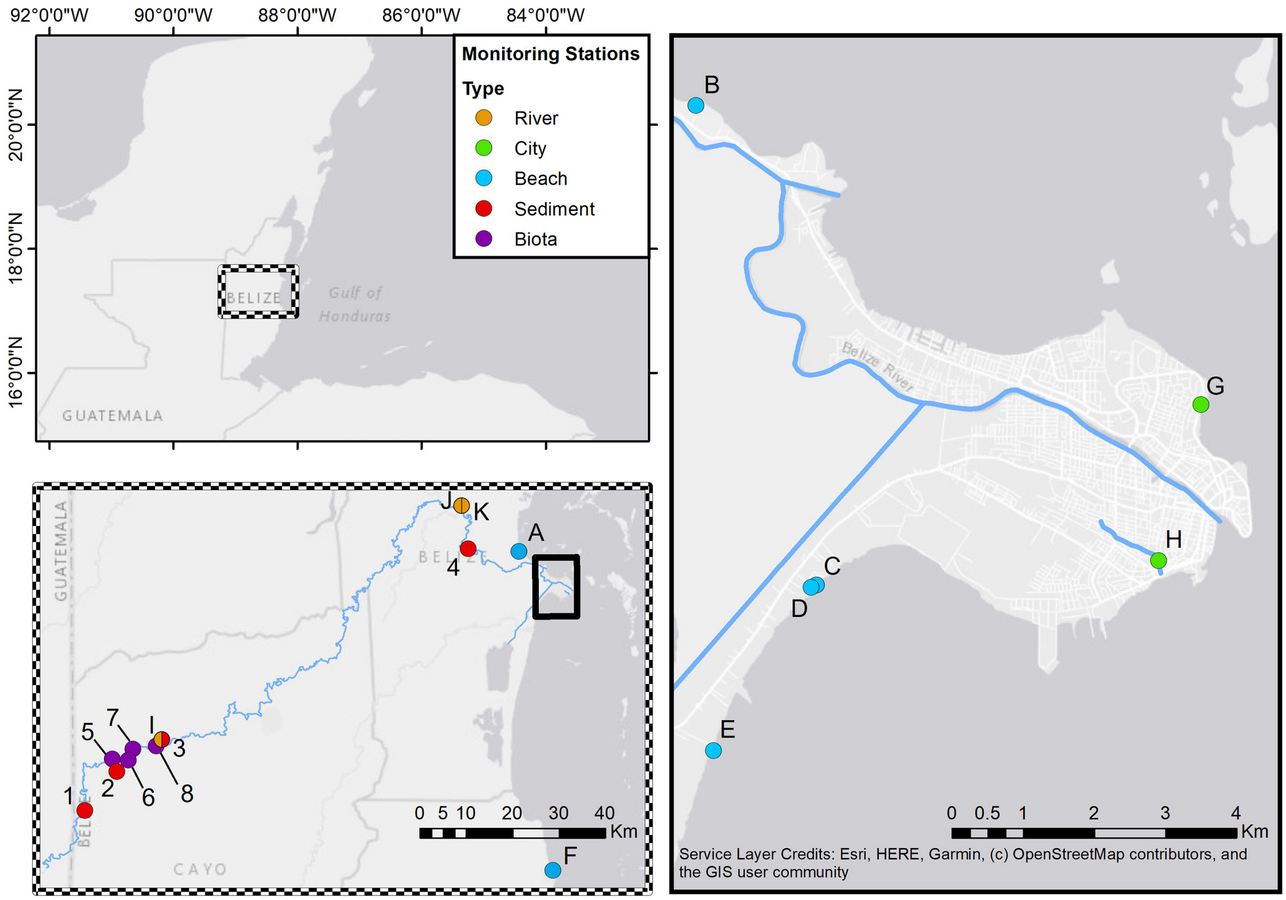

Figure 1. Map of monitoring locations across Belize for both macro/meso litter surveys (river, city, and beach) and microplastic sampling (sediment and biota).

currents of the Belizean Shelf in the Western Caribbean Sea flow in a north-westward direction, which persists all year round, although becoming weaker in the months February to November (Tang *et al.*, 2006). The selection of the sampling sites was identified following a consultation with key Belizean stakeholders: the Department of Environment (DOE); the University of Belize (UB); the Belize Coastal Zone Management Authority and Institute (CZMAI); and the Scouts Association of Belize. This was done to ensure the sampling locations and data would be representative for the entire country while allowing the determination of inputs from point sources.

An overview of the station locations can be seen in Figure 1, broken down into macro/meso litter (beach, city and river) and microplastics (sediment and biota). The purpose of the survey design was to gather macro/meso and micro litter data from multiple locations along the Belize River, waterways within Belize City and beaches to the north and south of Belize City. This provides an insight into changing quantity of macrolitter being transported downstream and into potential transport systems of litter entering the ocean around this large urban centre. The microplastics sampling locations were focused on the upstream Belize River and its tributaries to characterise riverine inputs and concentrations of microplastics.

At the three river, two city and six beach macro/meso litter site (Figure 1, A–K), a baseline survey was conducted. Additionally, subsequent surveys were conducted after the baseline survey at five of the beach, one city and one river location(s) (Table S1, Supporting Information). In total, 25 macrolitter surveys were conducted between 13th May and 20th June 2019. Sediment sampling for microplastics occurred at four locations along the Belize river and its tributaries (Figure 1, 1–4) between 22nd May and 27th May 2019. Biota samples were collected within the middle reaches of the Belize River, between Santa Familia village and Georgeville village (Figure 1, 5–8).

Macro and meso litter

Sampling protocol

Beach

The North-East Atlantic OSPAR guideline for monitoring marine litter on beaches (OSPAR, 2010), was adapted for use along Belizean shorelines. The protocol recommends at least 100 m of clear beach transect, however Belizean coasts are often dominated by mangroves (Murray *et al.*, 2003) leading to reduced sample areas and litter accumulation amongst mangrove tree roots. Where a 100 m stretch of beach could not be achieved, the survey length was noted to allow for data to be normalised to 100 m. All macro (> 2.5 cm) and meso (0.5–2.5 cm) litter items were collected from the high tide line to the back of the beach, where substantial vegetation begins, in accordance with the OSPAR guidelines. Where mangroves were the dominating feature along the shoreline (Site A), litter items were collected from the high tide line within the mangrove roots to a change in vegetation density, indicated by land-use change to aquaculture through the cutting of the mangroves. Additionally, Belizean beaches often accumulate dense mats of *Sargassum sp.* seaweed, which smother the beach and can potentially impede the collection of litter during a survey as it is more easily obstructed and tangled within the seaweed. This is a regional problem impacting both the environment, fisheries and the local tourism industry (Cabanillas-Terán *et al.*, 2019; Rodríguez-Martínez *et al.*, 2019). Where *Sargassum sp.* was present (Site E), litter items were collected from on-top of the seaweed, instead of on-top of the beach sediment. Metadata about each survey site was also collected following the OSPAR protocol and GPS coordinates (WGS84) of the start and end point of the survey transect were recorded. On all subsequent return surveys, the same area of beach was sampled again, using GPS tracking to ensure the start and end point of the survey area was identical, but using the most recent hightide line to indicate the nearshore sampling boundary.

River and city

The OSPAR guidelines (OSPAR, 2010) were additionally adapted for the riverbank and city waterway surveys. Where possible, a sandy meander on the riverbank was located and the length recorded to normalise the data to 100 m as per the standard protocol. Similarly to the beach surveys, all macro and meso litter between the high waterline and the vegetation was collected. The sites for city waterway surveys were selected in areas which are not routinely cleaned by city workers and where litter could visibly make its way into the sea. They were either a storm drain or a canal, which were surveyed similarly to the riverbanks, from the high waterline to either the nearest boundary either side or to a maximum distance of 5 m from the waterway, whichever was closest. Additionally, on subsequent surveys, the same survey areas were used, as described previously.

Macro and meso litter analysis

After collection of the litter, the items were separated and counted. The OSPAR methodology quantifies litter items in 11 material categories (plastic, rubber, textile, metal, paper, wood, glass, ceramic, sanitary, medical, and other) and 112 predefined item subcategories. However, in this study the OSPAR list was expanded to 174 predefined item subcategories, which include national items of interest, as well as subcategories added in accordance with previous studies undertaken as part of the Commonwealth Litter Programme (CLiP) (Binetti *et al.*, 2020). This adapted item category list was used across the three survey types to allow for easy cross comparison of results, as well as allowing for global comparisons with other regions where a similar methodology was also implemented. Following separation into the predefined subcategories, the total number of items within each subcategory were counted and recorded. After analysis, all the collected litter items were removed from the locations and disposed of. Data collected in accordance to this protocol can be fed into the UNEP programme as the methods are compatible, as reviewed and discussed in Caporusso and Hougee (2019). Some global efforts to harmonise marine litter monitoring lists have also been made, such as the joint list set out by the Marine Strategy Framework Directive (MSFD) in Europe (Fleet *et al.*, 2021), which takes the OSPAR category list into consideration and, through the move towards a singular list, allows for comparisons to historic data while ensuring comparability between countries and indicators.

No additional methods were utilised to collect meso litter (such as quadrants and sieves), nor was the meso litter separated out from the macro litter during quantification (except for plastic/polystyrene fragments as defined by the size category 0–2.5 cm). This methodology does not provide a reliable estimate of meso litter since the previous strandlines, which were buried prior to the time of the surveys, were not sampled (Ryan *et al.*, 2009). As such, an underestimation of meso litter is expected from this study. If future studies include meso litter, protocols such as those set out in Barnardo and Ribbink (2020) should be followed, though it is noted that these are difficult to implement in the sites covered in this study.

In order to allow for intra- and inter-survey comparison, regardless of survey area length, all data was normalised to a survey area length of 100 m. Notably, as the return surveys (after T0) were not performed at delineated time intervals daily for a sustained period of time, these results do not represent litter accumulation rates at the sites, as laid out in previously published studies on litter accumulation (Ryan *et al.*, 2014; Dunlop *et al.*, 2020; Thiel *et al.*, 2021). Therefore, in this study this is referred to as the litter load.

Microplastics

Sampling protocol

Sediments

Sediment samples (n = 4) were collected at various locations (Figure 1, Site 1–4) along the Belize River using a small handheld Van Veen grab (Duncan and Associates, UK, sampled area 0.025 m^2). Sediment grabs were subsampled for microplastic and particle size analysis (PSA) (n = 4) using a stainless-steel spoon into pre-rinsed sample jars. The jars were pre-rinsed three times with Reverse Osmosis (RO) water in the laboratory and covered with pre-rinsed aluminium foil before being capped with a plastic lid. Another pre-rinsed glass jar was exposed to the atmosphere during the time of sampling to investigate background contamination during sampling. The samples were stored frozen (−18°C) until further analysis.

Biota

Fish samples were collected within the middle reaches of the Belize River, between Santa Familia village and Georgeville village (Figure 1, Sites 5–8). Specimens of *Cichlasoma synspilum* were collected using a 1.8 m (6 ft) tall, 3.0 m (10 ft) diameter, 30 mm mesh-size cast net. Fish were collected during the dry season months of June and July 2019. A sample size of n = 22 was included in this study. After collection, fish samples were immediately anesthetized in buffered solution containing clove oil (100 mg L^{-1}) and individually wrapped in foil paper and placed inside double Ziploc plastic bags. Bags containing fish were placed in a cooler with ice and immediately transported to the Aquatic Environmental Studies laboratory of the University of Belize for processing. All sampling points were georeferenced and are shown in Figure 1.

Microplastic analysis

Sample preparation and extraction—sediment

Collected sediment samples were homogenised and dried at 50°C for a minimum of 3 days until a stable weight was achieved. In a PCR (polymerase chain reaction) workstation, three subsamples of 5 g sediment were placed into three 50 mL polypropylene centrifuge tubes. Density separation was carried out by adding a 1.2 g mL^{-1} solution of saturated sodium chloride (NaCl). The saturated sodium chloride solution was previously filtered using a 47 mm diameter regenerated cellulose filter with a 0.2 μm pore size. Each tube was shaken by hand for one minute and centrifugated at 3900 × g for 5 minutes. The supernatant of individual subsamples was transferred to a previously cleaned glass filtration unit and filtered using a 0.2 μm porosity cellulose nitrate membrane. The whole process was repeated to obtain three replicates and the supernatants combined on the respective filters. Glass beakers, funnels and filters were rinsed with 100 mL RO water. Each filter was then carefully transferred to previously RO water cleaned 100 mL glass beakers covered with glass lids, to prevent corrosion by the alkaline solution, and digested, using 30 mL of a 30% KOH: NaClO solution v: v (Enders *et al.*, 2017). The cellulose nitrate filters were digested within 10 min of the incubation process. After 72 h, digests were filtered on a 47 mm diameter regenerated cellulose filter with a 0.2 μm pore size, rinsed through with 100 mL RO water and stained with Nile Red before imaging and particle counting using as detailed in Bakir *et al.* (2020a) and Preston-Whyte *et al.* (2021). The method and the different steps are summarised in Figure S1.

Sample preparation and extraction—biota

For the fish samples, the gastrointestinal tracts (GITs) were removed in a PCR workstation with air circulation (VWR, UK) and transferred to a pre-RO rinsed 120 mL glass collecting jar. 5 mL of a 30% KOH: NaClO v:v solution was added per g of wet weight tissue collected. Samples were sonicated for 15 min and were incubated at 40°C for three days under constant agitation at 120 rpm before filtration using 47 mm diameter Whatman glass microfibre filters (GF/D) 2.7 μm porosity. Sample processing and analysis was carried out based on density-based extraction and selective fluorescent staining using Nile Red (NR), followed by quantification as detailed in Bakir *et al.* (2020a, 2020b). A summary of the method is given in Figure S2.

Contamination control

To reduce ambient contamination in the laboratory, 100% cotton lab coats were used for the duration of the study to avoid contamination from synthetic fibres. Room air recirculation was also minimised during the day to reduce contamination with dust. Manipulation of the samples was carried out under a laminar flow to reduce ambient contamination. Prior to use, all glassware was cleaned using a laboratory detergent and rinsed using reverse osmosis (RO) water. All chemical solutions used in this study were previously filtered using a 47 mm diameter 0.2 μm regenerated cellulose membrane. Contamination control was carried out by using blank filters processed in the same way as environmental samples for each batch of samples processed. The number of microplastics quantified onto blank filters were then removed from the total number of microplastics quantified in the environmental samples (i.e. sediment and biota). For time efficiency, a procedural control was prepared with every batch of samples and subjected to the same preparation steps. Additional blanks were used during field work, consisting of empty, pre-rinsed collecting jars opened during sample transfer to monitor ambient contamination (i.e. sediment).

Recovery study

Each type of filter membrane (Regenerated cellulose and GF/D) used in this study was spiked with a known number of PE particles (fragments and foams) particles (n = 10) to investigate recovery rates using both a visual and an automatic particle counting method. Recovery studies were carried out in duplicates.

Validation

Polymer identification of some selected particles was carried out using attenuated total reflection Fourier Transform infrared spectroscopy (ATR-FT-IR) using a Thermo Fisher Scientific Nicolet iS5 ATR-FTIR with OMNIC software (version 9.9.473) and by comparison of their IR spectra to a polymer library (HR Nicolet Sampler Library, HR Spectra IR Demo and Hummel Polymer Sample Library). ATR-FT-IR has been shown to be a fast and effective tool for the identification of polymers of plastic marine debris, including those ingested by marine organisms (Jung *et al.*, 2018). Particles exhibiting extended weathering or below the suitable size range for ATR-FT-IR were recommended for analysis using μ-FT-IR with microscope. Between 1 and 10% were chosen for polymer identification by spectroscopy. Due to size limitation using ATR-FT-IR, only particles above ~300 μm in size could be analysed. Spectra were collected in the range 4000–650 cm^{-1} at a resolution of 4 cm^{-1}. Polymer identification was verified based on the % match against a polymer library. Only spectra matched greater than 70% were accepted. Quality control was carried out with the analysis of a polystyrene (PS) and polyethylene (PE) reference material before each batch.

Particle size analysis (PSA)

For each sediment sample, a PSA was carried-out to relate abundance of microplastics to sediment type and fines content, this method is summarised in Figure S3 (Bakir *et al.*, 2020a; Preston-Whyte *et al.*, 2021). After collection, the samples were kept in a freezer at −18°C until ready for analysis. PSA was carried out using a modified NMBAQC protocol from Mason (2011) for fast PSA screening based on wet splitting into silt/clay (< 63 μm), sand (63 μm—4 mm) and gravel (> 4 mm) fractions (Figure S3). After settling for 24 h, excess overlying water was removed and the fraction split samples were transferred to pre-weighed trays, before being placed in an oven at 50°C until fully dry. Once dry, samples were weighed, and the proportion of each fraction was calculated.

Results

Macro and meso litter

Over the six-week sampling period, five of the beaches were surveyed multiple times to compare litter loads. The exception was Site A where, on returning to the site, it was noted that the increased accumulation of *Sargassum sp*. entangled in the mangrove roots on the shoreline prevented litter from being deposited on the beach beyond the high tide line. Therefore, no repeat surveys were performed, and no intra- site comparison could be made. Two city sites were identified, only one of which, Site G, was surveyed a total of five times during the sampling period. This was due to the unsuitability of Site H for ongoing monitoring, due to regular cleaning of the area by the municipal city cleaners, which only became apparent after the initial sampling effort. Three river sites were identified and surveyed, but only one return survey was achieved during the monitoring period at Site I. Sites J and K were unsuitable for ongoing monitoring because of the morphological nature of the beaches and the heavy use by local residents living adjacent.

In total across the 25 surveys, plastic was proportionally the largest material category, making up 73.8% by count of all items

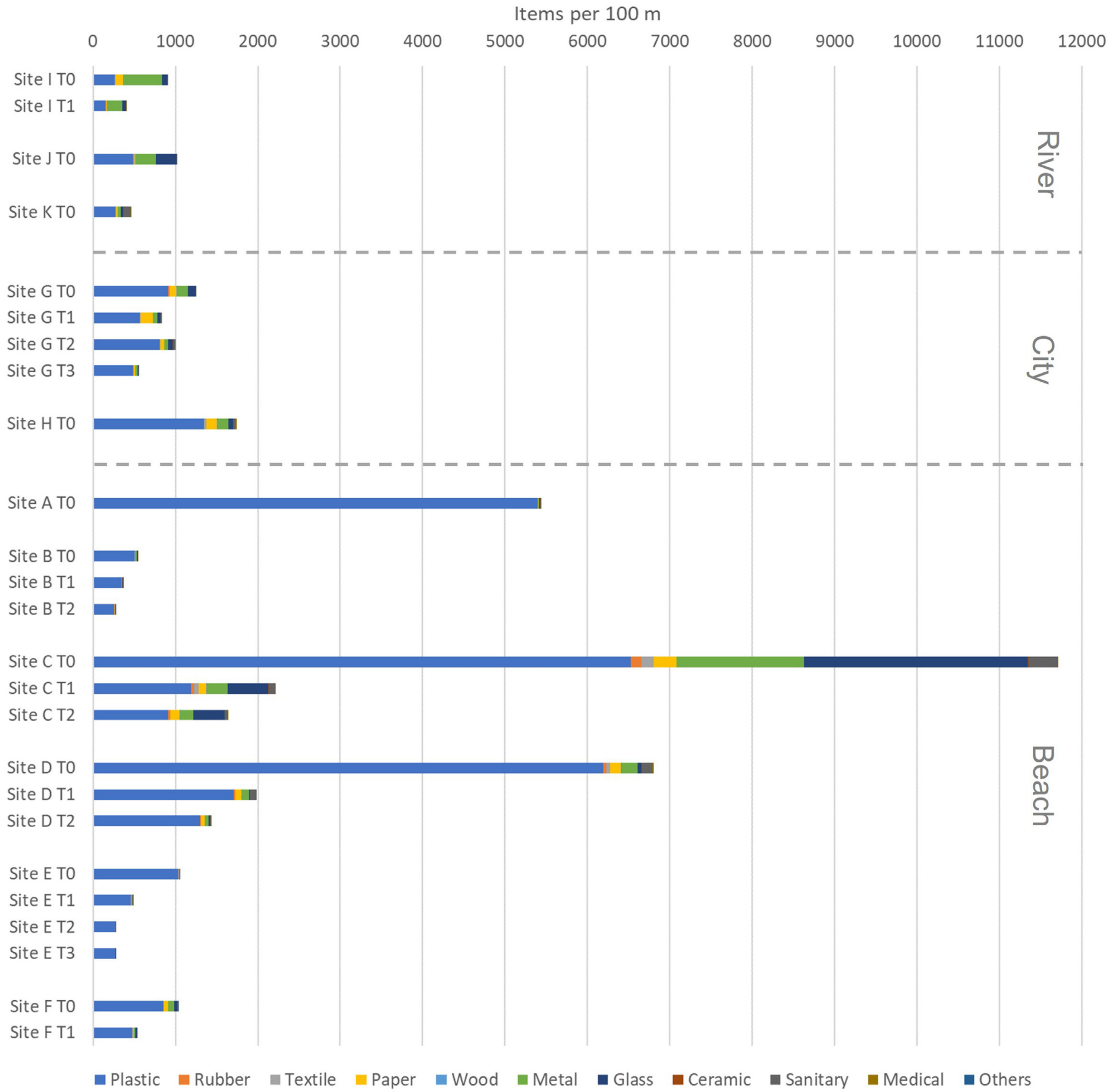

Figure 2. Abundance of macro/meso litter items per survey, normalised to 100 m survey area length, and divided by material category group.

collected, followed in descending order of percentage composition by metal (9.4%), glass (9.3%), paper (3.8%), sanitary (1.7%), textile (0.8%), rubber (0.7%), wood (0.2%), medical (0.2%), ceramic (0.1%), and others (< 0.1%).

The results of the river, city and beach surveys by litter item material category count, standardised to a 100 m survey area length, are presented in Figure 2. The number of items per 100 m varied both temporally (within a site) and spatially (between sites). At every location where repeat surveys were conducted (except Site G), the number of items per 100 m decreased with each time-point. However, the proportion of each material category present at a location did not vary greatly. The T0 surveys at the beach sites A, C and D had the greatest number of items per 100 m (5432, 11 713, and 6804, respectively), however, at sites C and D where repeat surveys were conducted, the resulting number of items per 100 m was far lower than the T0 surveys. Notably, the average number of litter items at each monitoring location by type was 702, 1075, and 2253 items per 100 m at river, city and beach respectively. This indicates that there is an overall trend of increased litter load by count as you travel from catchment to coast. The number of items per monitoring location type with average indicated and a breakdown of the litter load per repeat survey by material category can be found in Figure S4 and Table S2, respectively.

At the majority of survey locations, at every time-point, plastic was the most numerous material category. The exception was at Site I, the furthest up-stream river location, where metal made up a greater proportion of items than plastic; 475 and 189 metal items per 100 m at T0 and T1 respectively (46.0% and 52.2%

composition), compared to 261 and 152 plastic items per 100 m at T0 and T1 respectively (28.7% and 37.1% composition). At the river site, Site J, plastic was the most numerous material category at 485 items per 100 m (47.3% composition), however metal and glass combined made up a greater number of items, at 245 (23.9%) and 255 (24.9%) respectively. River site, Site K had the largest proportion of plastic items of the river surveys, which at 58% was comparable to the lowest proportion of plastic of any beach survey at Site C (53.7–55.7%). At the city sites, plastic made up between 73.0% and 87.9% while at all the remaining beach site, bar Site C, plastic consistently made up > 82.0% of the items per 100 m.

Across all surveys conducted, the 20 most frequently occurring litter item categories by count were identified and ranked (Figure 3). A breakdown of the overall top 10 most frequently occurring litter item categories at each survey type (river, city and beach) can be found in Table S3. Of the 20 items, 15 (75%) are from the plastic material category, including the two most frequent items, plastic pieces 0–2.5 cm (ranked 1st) and plastic pieces 2.5–50 cm (ranked 2nd). However, the third and fourth most frequent items, glass broken and bottle caps, are from the glass and metal material categories respectively.

At the river sites Site I and Site J, glass broken and metal bottle caps were more numerous than plastic pieces. However, at the river site Site K, plastic bags (ranked 7th) were most numerous and this site had the highest proportion of items not found on the top 20 list (51.6%) of all the survey locations and time-points.

At the city sites, a high proportion (82.0–88.2%) of the items collected were from the top 20 most frequently occurring categories by count, slightly more on average than the beach sites (73.1–93.9%). At Site G where return surveys were conducted, the most frequently occurring item varied between each survey event, where at T0 and T2 the most common item found at the site was plastic pieces 0–2.5 cm (ranked 1st), while at T1 it was other paper items (ranked 12th) and at T3 it was foam sponge (foam cups/food packs and trays) 2.5–50 cm (ranked 9th).

At the beach sites A, B, D, and F, plastic pieces 0–2.5 cm (ranked 1st) was consistently the most frequent item at each survey event, accounting for between 18.1–55.2% of all items collected. However, at Site C, the most frequent item at each survey event was glass broken (ranked 3rd), with plastic pieces 0–2.5 cm coming second. At Site E, the most frequently found item changed between each survey event, where at T0 and T3 the most common item was plastic pieces 0–2.5 cm (ranked 1st), while at T1 it was plastic pieces 2.5–50 cm (ranked 2nd) and at T2 was polystyrene pieces 0–2.5 cm (ranked 14th).

Plastic composition was explored further by grouping items into single use plastics (SUP), fisheries related (FR) and plastic pieces (PP), groupings as outlined and described in table 2 in Binetti *et al.* (2020), and split by survey location type (Figure 4). In analogy with European studies, cigarette butts were included in the SUP category, even though the OSPAR methodology lists their material category as paper. The difference was nominal, adding 1.0%, 0.9%, and 0.7% to the river, city and beach SUP percentage composition respectively. SUP composition was relatively consistent between the river and beach locations, but was highest at the city locations where SUP outnumbered PP and FR. PP percentage composition greatly increased from river to city to beach, resulting in PP occurring more frequently at beach locations than SUP or FR. There were also no FR items found at the river locations, with increasing percentage composition from city to beach. When compared with previously published data that used the same OSPAR methodology at beaches in two Pacific SIDS (Binetti *et al.*, 2020), the percentage composition values for SUP, FR, and PP at Belizean beaches are all within a comparable range However, substantially fewer FR related items were found on Belizean beaches compared to European studies, an observation similarly made by Binetti *et al.* (2020) at Pacific SIDS beaches.

Microplastics

Contamination and quality control

Results are shown in Figure S5. Atmospheric input of plastic particles was relatively low for three of the four sampling sites presented here with a mean value of 1.7 items per collecting pot during sediment sampling. However, the field control collecting pot was heavily contaminated for one site (Site 1) with 14 suspected plastic items onto the filter. This high contamination level was likely due to the collection of sediment samples near a ferry transporting passengers and vehicles. City dust and road wear have been defined as important sources of microplastics including paint polymers, fibres from clothes as well as synthetic rubber particles from car tyres (Kole *et al.*, 2017).

Recovery study

Recoveries ranged from 75 ± 7% for RC filters to 80 ± 7% for GF/D filters. Manual recoveries based on microscopic evaluation was also in agreement with the automatic counting tool applied for the automatic counting of fluorescent particles.

Occurrence of microplastics in sediments in the riverine environment

The occurrence and abundance of microplastics in sediment were investigated for a limited number of sites ($n = 4$). No macroplastics (> 2.5 cm) or mesoplastics (0.5–2.5 cm) were found in sediments, while microplastics (< 5 mm) were detected in all the sediments under investigation. Concentrations ranged from 200 to 6500 ± 1273 (mean ± SD) particles per kg dry weight sediment (Figure 5). Concentration of microplastics were significantly higher for Site 1 ($P < 0.01$) as compared to other sites and level of contamination followed the order Site 1 > Site 3 > Site 4 = Site 2.

To investigate the impact of sediment particle size on the abundance of microplastics in sediments, sediments collected alongside the grab samples were analysed for particle size analysis (PSA) (Figure S6). Scatterplots of both the number of particles per kg dry weight sediment and the standard deviation values against % gravel, % sand and % silt/clay indicated an increase in abundance with a higher percentage of silt/clay as compared to higher percentages of gravel and sand. However, a higher variability between the replicates was also observed with an increase in standard deviations (SD) for samples with a higher percentage of silt/clay (Figures S7 and S8).

Occurrence of microplastics in riverine fish

Plastic items were detected in 36% of the fish investigated ($n = 22$). No macro/meso plastics (> 5 mm) were observed. A total of 10 items were extracted from the tissues with an average of 0.7 items per fish. The most commonly found polymer was poly(ethylene: propylene: diene) (50%), followed by polyethylene (30%) and cellophane (20%). 44% of items suspected to be plastics were either

Figure 3. Top 20 most frequently occurring litter items by count, displayed as percentage composition and ranked in descending order of frequency, with the outstanding 154 categories summed as "remaining categories."

not identified due to low library match (< 70%) or were identified as natural particles (Figure S9).

Discussion

Macro and meso litter

Baseline data on the abundance and composition of macro and meso litter entering the marine environment is essential to provide evidence and support the creation and implementation of national marine litter action plans. This data provides a snapshot of the state of marine litter in Belize, which can be used as the basis for a national monitoring plan and to inform and identify baseline indicators for long term monitoring efforts, enabling future measures of the effectiveness of remediation actions. However, such studies need to be scaled-up and long-term monitoring established so that more robust inferences can be drawn and implemented legislative action can be effective.

The macro/meso litter monitoring protocol used for these surveys was developed based on the existing OSPAR beach litter monitoring method, which was designed as part of a Coordinated Environmental Monitoring Programme (CEMP) to determine standing stocks of litter on north-western European beaches, in order to show spatial and temporal trends. Transplanting this method to other regions means that adaptations had to be made. For this study, the monitoring method was also adapted for use on riverbanks and urban waterways. While this method does not provide data on the flow of litter down the river, it does provide

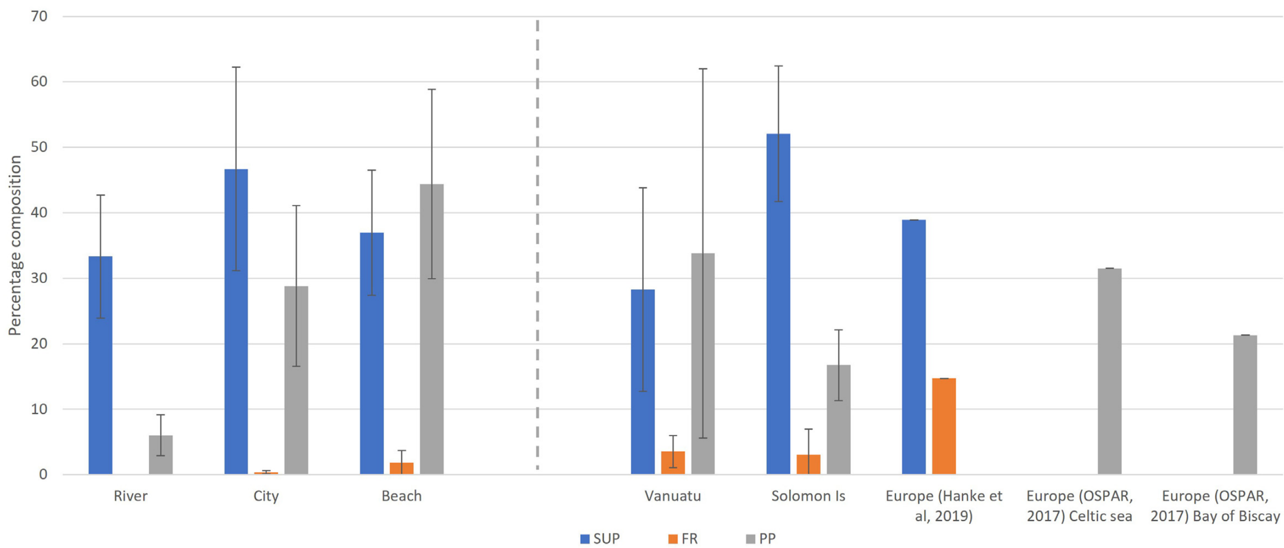

Figure 4. Percentage composition of single use plastics (SUP), including cigarette butts, fishing related items (FR) and plastic pieces (PP) from the river, city and beach locations, with standard deviation error bars. Data for beaches in Vanuatu and Solomon Islands are from Binetti *et al.* (2020), data for beaches in Europe are from OSPAR (2017) and Hanke *et al.* (2019).

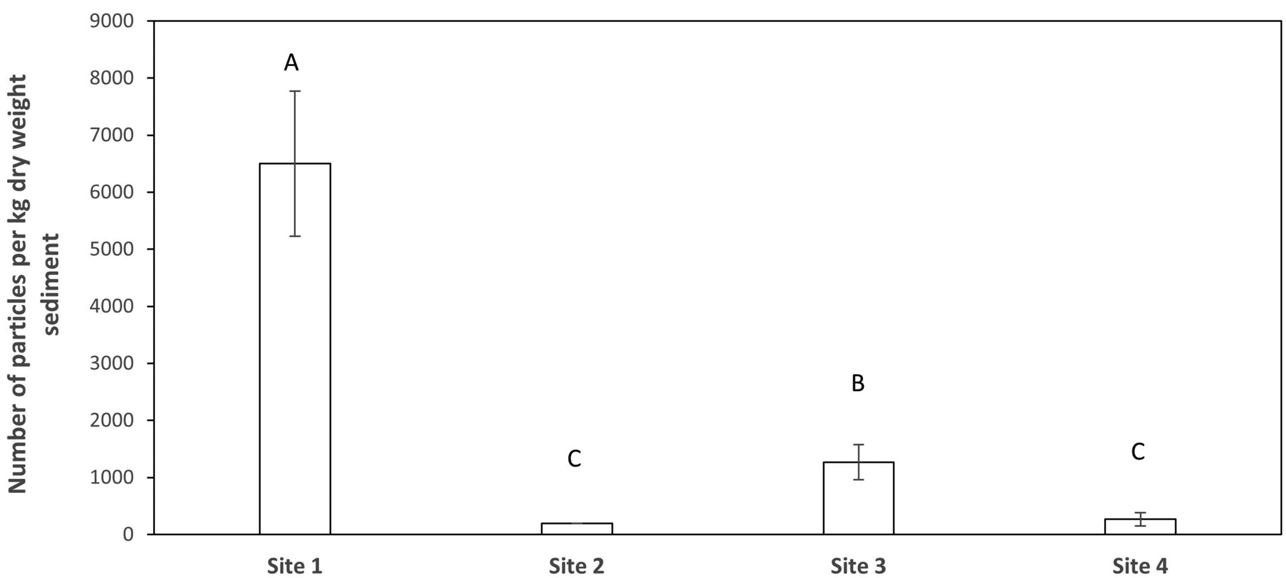

Figure 5. Number of particles per kg dry weight sediment collected for selected locations in Belize. Letters refer to grouping following a one-way ANOVA using a Tukey (HSD) post hoc test after a Box-Cox transformation of the data. Means that do not share a letter are significantly different ($P = 0.01$).

a proxy for the amount of litter in the watershed (González *et al.*, 2016). There are multiple settlements and larger urban areas along the length of the Belize river and its tributaries, stretching the width of the country and across the Guatemalan border, with the catchment area housing 130 000 Belizeans and a further 100 000 individuals beyond the Belizean border (Boles and Boles, 2016). The river is used for transport (there are multiple ferry crossings and small vessels traverse up and down the river), fishing, swimming and other recreational activities on the riverbanks. The monitoring of the waterways in Belize city used locations which were very different to the standard shoreline. Data from these surveys should therefore be taken as a preliminary survey to provide an indication of dominant litter item categories. This is especially the case for Site H, which was later found to be actively cleaned by city workers and monitoring at this location ceased. Both city locations were open waterways towards the sea, which would normally have been tidally influenced, however blockades at the seaward ends were designed to prevent litter from entering the marine environment from the city and *Sargassum sp.* from accumulating in the drainage system, thereby affecting the input of litter and the deposition on the banks. However, litter loads at the river and city sites were analogous with the litter loads at the beach locations Sites B, E and F.

In contrast with the long, sandy and clear coastlines recommended for monitoring in the OSPAR beach litter guidelines

(OSPAR, 2010), the Belizean coast consists of long stretches of mangroves. The OSPAR method recommends a standard length of 100 m of beach, but this requirement was never met in Belize with all beaches surveyed between 30 and 70 m long. The data were therefore extrapolated to 100 m to allow for inter-site comparisons, however the surrounding vegetation or geomorphology which limited the beach length may well have affected the litter accumulated on the beach. Additionally, there was a large difference in beach width between locations. The OSPAR method limits the beach width to the start of vegetation or a strong difference in beach gradient. At Site C this was beyond the area where cars would park when being visited. As there was no physical barrier, it was difficult to ascertain where the beach ended and the carpark began, so the entire width was surveyed to the start of the vegetation. This may have resulted in the particularly high number of items collected during the T0 survey at Site C of 11713.0 items per 100 m. Additionally, the results may well have been affected by the type of beach. As discussed in Harris *et al.* (2021), coastal deltas, rocky shorelines and mangrove dominated coasts all have different characteristics affecting litter turnover rates. The beach sites in this study were characteristic of a mangrove dominated habitat and were also heavily affected by the accumulation of *Sargassum sp.* seaweed. Sites A, C, and D where the highest standing stock number of items per 100 m were recorded, were flanked by mangroves or, in the case of Site A, the survey area was dominated by mangroves and the litter was collected from within the root system. These three sites had the greatest number of items per 100 m consistently across the sampling time-period, suggesting the mangroves increased the litter deposition on the beach, which is in agreement with previously reported studies (Martin *et al.*, 2019). Along the Belizean coastline, large volumes of *Sargassum sp.* accumulate, which impacted the planned sampling within this study. At Site A monitoring was ceased following the deposition of *Sargassum sp.* within the mangrove roots at this site, which acted as a barrier for litter deposition above the high tide line, a requirement of the OSPAR protocol. Additionally, *Sargassum sp.* has the potential to smother litter deposition, complicating monitoring efforts and trapping litter for later re-entry into the marine environment. Future studies into the interaction between *Sargassum sp.* and marine litter would be beneficial, both the effects of offshore trapping and transport of floating marine litter, as well as the impacts on coastal deposition and monitoring efforts.

Previous studies in the region reported that the majority of debris collected comprised of plastic (Bennett-Martin *et al.*, 2016; Blanke, 2020), which is comparable with the results presented in this study. On average, across all monitoring location types and time-points, 77.3% by count of all macro and meso litter items collected were plastic, compared to 77.6 and 68.1% reported by Bennett-Martin *et al.* (2016) and Blanke (2020), respectively. A clear difference between the river surveys and those on the coast was that the river surveys had far lower numbers of plastic items, in particular fragments, and the top items were broken glass and metal bottle caps. This is likely due to all the river sites being heavily used for recreational purposes, and therefore a lot of litter were likely brought to the site and incorrectly disposed of, rather than having been transported down the river. In Belize, there are bottle return schemes where consumers return glass bottles to vendors for a refund. However, there is no requirement to also return the bottle cap, thus this could explain the large number of metal bottle caps and broken glass, but a lack of whole glass bottles. Additionally, plastic items transported by the river have not had the chance to degrade yet, while beaches receive more weathered fragments from offshore sources which have degraded over time at sea and exposure to UV radiation. The city locations had, on average, the highest proportion of items present from the top 20 list and more items in common with the beach locations than the river location. Many of the items commonly found at the city locations were related to food and drink, particularly at Site G which was adjacent to a large number of food stalls. The fact that fewer items from the top 20 list were noted at the beach locations strongly suggests an additional source of litter outside of the Belize River catchment area is responsible for litter input. This is further supported by the positive trend observed in the litter load moving from inland to the coast. Where repeat surveys were performed, the average litter load increased from river to city to beach. A driving factor affecting beach litter loads can be related to beach use and human activities, such as recreation. A large proportion of beach litter comes from the sea, however active littering, such as was observed at Site C, can be a secondary source of litter, making it difficult to estimate the relative importance of the different inputs. Indeed, the effect of active littering at that location can be seen in the high abundance of broken glass, clearly indicating that besides wind and currents depositing litter, human activity on beaches is a defining characteristic of litter composition. This is further supported by the results from the adjacent Site D, which was not heavily used by the public and where plastic was more abundant. At the majority of the beach locations, the most frequent item was plastic pieces (either 0–2.5 cm or 2.5–50 cm), together with various single use plastic items such as food packaging, bottles, and plastic bags, a very common composition across the globe (Verlis and Wilson, 2020). Proportions of SUP, PP, and FR were also similar to results from other global studies (OSPAR, 2017; Hanke *et al.*, 2019; Binetti *et al.*, 2020), although the proportion of FR items was low. The presence in large numbers of region-specific items, such as plastic drinking pouches, which are not on the standard European OSPAR categories list, indicates the importance of tailoring monitoring to inform national and regional policy making.

Microplastics

Data on the occurrence and abundance of microplastics in sediment for Belize are limited and there is an urgent need to fill this knowledge gap with monitoring baseline data. Microplastics were detected in all the sediment samples collected while no macro/meso plastics were observed. The abundance of microplastics was significantly higher for Site 1 ($P < 0.01$) with 6500 ± 1273 particles per kg dry weight sediment, followed by Site 3 (1267 ± 306) then Sites 4 and 2 with concentrations of 267 ± 115 and 200 particles per kg dry weight sediment (Figure 5).

Investigation into the abundance of microplastics in riverine sediments highlighted different hotspots for microplastic contamination at Site 1 and Site 3 (Figure 5). Local activities at the Xuanantunich Hand Cranked River Ferry (Site 1) are varied and include tourism, recreational activities and a Ferry service transporting passengers and vehicles across the river (Figure S10). The high contamination level of the field blank at that site indicated that atmospheric input of particles was prevalent in that area. City dust and urban runoffs, including synthetic fibres, paint polymers and synthetic rubber particles from car tyres have been identified as important sources of microplastics in the environment (Dris *et al.*, 2016; Kole *et al.*, 2017). Rivers have also been identified as important pathways for the entry of microplastics in the marine environment (Rochman, 2018) and it is thus not surprising that high concentrations of microplastics have been found in a riverine system. While the abundance of microplastics was much lower for Site 3 as compared to the Site 1, the concentration of microplastics was elevated compared to the other sites (Figure 5). High concentration of microplastics in sediment could be explained by the proximity of the site to the Spanish Lookout, which is a settlement in the Cayo district of Belize with a population of 2253 in 482 households (Statistical Institute of Belize, 2017). Microplastics can also enter the environment via untreated sewage (Woodward *et al.*, 2021). About 45% of Belize's population (360 926, 2015) lives in the coastal zone and about 28% of the population (living in Belize City, San Pedro Town and Belmopan City) are connected to sewerage systems (WHO/UNICEF, 2012). The 227.9 million litres of sewage (including wastewater) from the Belmopan sewerage system are disposed of directly into the Belize River. About 248.6 million litres of sewage from San Pedro flows into a shallow pond and into the sea; and 3039.3 million litres of sewage from Belize City is disposed of directly into the sea (Silva, 2015). Concentrations recorded for Belize (200 to 6500 $\pm$ 1273 (mean $\pm$ SD) particles per kg dry weight sediment) were in the same order of magnitude for the concentration of microplastics reported for large Amazon rivers with concentrations ranging from 417 to 8178 particles per kg dry weight sediment (Gerolin *et al.*, 2020) (Table S4).

Particle size analysis (PSA) suggested a greater abundance of microplastics for sediments with higher silt/clay composition. This was in agreement with previous studies that have shown that microplastic density was directly proportional to the content of silt/clay (Kazmiruk *et al.*, 2018; Wahyuningsih *et al.*, 2018). However, data suggested that associated variations between replicates were much higher for sediments with a higher % of silt/clay probably due to these samples being more difficult to homogenise (Bakir *et al.*, 2020a; Preston-Whyte *et al.*, 2021).

The study confirmed the presence and occurrence of microplastics in the GITs of riverine fish in Belize. While several studies have reported the occurrence and abundance of microplastics for marine fish (Table S5), only a limited amount of data is available for freshwater fish with a clear knowledge gap in monitoring data for Central America. Andrade *et al.* (2019) investigated the occurrence of microplastics in 172 individuals of 16 serrasalmid species from the Xingu River, the largest clear-water tributary of the Lower Amazon River (Goulding *et al.*, 2003; Andrade *et al.*, 2019). They reported a 26.7% occurrence of microplastics in serrasalmids under investigation. This study shows the occurrence of microplastics in riverine fish from the Belize River was in the same range with microplastics being present in 36% of the fish under investigation. Andrade *et al.* (2019) also analysed the polymer type of the extracted items using ATR-FT-IR and characterised 12 polymer types with a predominance of PE items (27%), followed by PVC (13%), Polyamide (PA) (13%) and PP (13%). PE has been characterised as the most common plastic type encountered in biota globally, followed by PP, PES, PA, and PS (de Sá *et al.*, 2018), which is consistent with our study with a prevalence of poly(ethylene: propylene: diene) (50%) followed by PE (30%). Rayon, a cellulose product, was also reported in fish from the Amazon River but at only 7% occurrence (Andrade *et al.*, 2019). This was also in agreement with our findings with cellophane accounting for only 11% of the identified plastic items. Cellophane in biota has also been reported in several studies but did not represent the main prevalent polymer type (Castillo *et al.*, 2016; de Sá *et al.*, 2018). By contrast, Schmid *et al.* (2018) reported a prevalence of PA (97.4%) followed by Rayon and PE ($<$ 2%) (Schmid *et al.*, 2018). The number of items per individual (0.7 items per individual for fish) was substantially lower than other reported concentrations for riverine fish. However, the lack of data for freshwater makes comparison between studies difficult. Schmid *et al.* (2018), reported an occurrence of microplastics in 13.7% of 14 fish species from the Amazon River Estuary corresponding to a mean concentration of 1.75 (0–12.8) items per individual (Schmid *et al.*, 2018). The occurrence of microplastics in biota causes several concerns ranging from a concern for biodiversity for individuals and populations, to food safety with direct implications for human health. Although the transfer of microplastics from biota to human is still poorly understood, it is considered as negligible for larger fish as their gut is removed before consumption and larger particles cannot translocate into cells. There is however a concern for smaller seafood such as mussels, oysters, shellfish or sardines usually consumed whole (Bakir *et al.*, 2020b). Previous studies have suggested that microplastics can have a physical impact on biota following ingestion (Wright *et al.*, 2013). Other studies have also suggested that microplastics can act as vectors for the transfer of sorbed co-contaminants with potential for release to biota following ingestion (Bakir *et al.*, 2014). However, model studies have suggested that the transfer of sorbed co-contaminants from microplastics to biota is negligible compared to other pathways (i.e. contaminated prey and uptake from water) (Bakir *et al.*, 2016; Herzke *et al.*, 2016; Koelmans *et al.*, 2016). However, it is still unclear whether plastic additives, often added a high concentration, could have a significant chemical impact following ingestion. Regarding human health implications, it has been suggested by the Food and Agriculture Organization of the United Nations FAO that the transfer of sorbed co-contaminants and additives from the ingestion of plastic particles would be negligible due to the low dietary exposure to such contaminants (Lusher *et al.*, 2017).

Conclusion

The results presented here provides a baseline dataset for marine litter along the Belize River basin and beaches by using an adaptation of the OSPAR beach litter monitoring method. The study indicated that there is an increase in the litter load as you move from catchment to coast, with both Plastic Pieces (PP) and Fishing Related (FR) items also increasing in numbers down the system. Additionally, it provides an indication on the occurrence and abundance of microplastics in riverine sediments and fish for selected locations along the Belize River. These data were valuable in comparing low to high impacted sites and additional monitoring data are required to fully understand main sources and transport of microplastics from catchment to coast.

Several of the top 20 macro/meso litter items are related to single use food packaging (e.g. foam food packs, metal and plastic bottle caps, crisp/sweet packets, plastic drinking pouches), which are already being targeted in Belize by existing national bans and plastic alternatives. From a national point of view, it is important to use a protocol that can record region-specific items, although this can hinder global harmonised monitoring efforts. However, the presence of Belize specific categories in the top 20 items, such as plastic drinking pouches, shows the importance of a dynamic item category list, as this information would have otherwise been lost. This approach, allowing the identification of national or regional specific items in marine litter, provides useful data to help identify unique problem areas within a nation and further provides evidence to prioritise actions.

Different concentrations of microplastics were detected in all riverine sediment samples investigated, total concentrations were relatively low compared to other locations worldwide. Reported concentrations (200 to 6500 $\pm$ 1273 (mean $\pm$ SD) particles per kg dry weight sediment) were comparable to regional concentrations of microplastics for the Amazon river (417 to 8178 particles per kg dry weight sediment). Microplastics were also detected in fish collected from the Belize River demonstrating the occurrence of microplastics for freshwater biota. The high microplastics content in the atmospheric blanks at Xuanantunich Hand Cranked River Ferry (Site 1) also indicated the importance of atmospheric sources of microplastics for freshwater systems.

Continuing from this baseline data set, a national monitoring programme should be established to regularly monitor consistent locations in order to establish any temporal and spatial patterns in litter loads. The programme, for example, could be used to inform policy makers on the most prevalent items and materials, assess the impact of legislative bans, and be used to identify targets and risks to wildlife. The impacts of marine litter are numerous, however, a better understanding of harm marine litter causes to the environment is needed to be able to set quantitative threshold values and assessment indicators. Regular microplastic samples collected at monitoring locations over an extended period of time would enable similar monitoring for microplastics in the environment, both sediment and biota. This would not only complement national legislative action, but also feed directly into national, regional, and global efforts to inform the changing state of marine litter.

Data availability statement

All data underlying this article are available in the Cefas Data Portal, at https://doi.org/10.14466/CefasDataHub.96, https://doi.org/10.14466/CefasDataHub.99 and https://doi.org/10.14466/CefasDataHub.98.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Conflict of interest

The authors have no conflict of interests to declare.

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