Centre for Environment Fisheries & Aquaculture Science

Cefas

Commonwealth Litter Programme - Belize

Annex: CLiP Belize laboratory and training

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Executive Summary

Monitoring strategies included the monitoring of **microplastics for biota and sediments** to fill the knowledge gap in monitoring data for Belize and more widely for the Caribbean and Central America. Occurrence of microplastics was confirmed for sediments with abundances ranging from 200 to 6,500 ± 1273 particles per kg dry weight sediment with polyethylene. Occurrence of microplastics was confirmed for the economically important Queen conch as well as for the freshwater cichlid *Cichlasoma synspilum* used for human consumption by locals. Plastic items were present in 41% of the Queen conch samples and in 36% of the riverine fish samples investigated. Polypropylene and cellophane were the most prevalent polymers found in conchs while poly(ethylene:propylene:diene) and polyethylene being the most commonly found polymers in fish.

Further monitoring is however recommended for both sediments and biota due to the relatively restricted sample size investigated. Additional monitoring of microplastics in biota is recommended to investigate commercially available species and subsequent impacts on human health.

Training courses, on microplastics monitoring and analysis in environmental samples, were delivered to the Department of the Environment (DOE), the university of Belize (UB) as well as other public and private organisations including the Belize Bureau of Standards (BBS), The Belize Agricultural Health Authority (BAHA) and the Belize Coastal Zone Management Authority and Institute (CZMAI). Training courses were delivered to ensure knowledge transfer and staff building capacity to produce long-term monitoring baseline datasets.

Table of Contents

Tables

Figures

1 Introduction

1.1 Sources, occurrence and fate of microplastics in the marine environment

Plastics are polymers of either natural or synthetic organic compounds, considered to be biochemically inert due to their high molecular mass, to which additives are added to enable processing and/or to give properties that are desired in a certain application (OECD, 2009). World plastics production has increased from 322 million tonnes in 2016 to 335 million tonnes in 2018. A high proportion of marine litter comprises of plastics contributing to the increase of microplastics in the marine environment. Microplastics have been defined as plastic particles with size < 5 mm in diameter (Arthur et al, 2008), which can be divided into primary microplastics and secondary microplastics. Primary microplastics are most commonly found in industrial, domestic cleaning products and synthetic textiles, while secondary microplastics are usually fragmented from larger plastic debris via weathering, ultraviolet degradation, biodegradation or a combination of these.

Microplastics have been found on beaches, coastal zones, open-sea and deep-sea sediments worldwide. Microplastics have also been reported in high concentrations in remote locations as far as the Arctic (A. Lusher et al., 2015; Bergmann et al., 2017; Kanhai et al., 2018). Most plastic, including microplastic that enters the sea, originates from land-based sources, such as sewage and storm water, or ocean-based sources, including discarded and lost fishing items (Li, 2018). It has been estimated that land sources represent 80% of microplastic pollution in the ocean (Jambeck et al., 2015) with rivers representing the main pathways for the transport of plastics to ocean (Lebreton et al., 2017). Deep-sea sediments have been suggested as a likely final sink for microplastics in the marine environment (Woodall et al., 2014).

Field and laboratory studies have demonstrated the ingestion of microplastics by a large range of marine organisms representing various trophic levels including seabirds, marine mammals, fish and invertebrates (GESAMP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2015). Detrimental physical effects of microplastics have been reported following ingestion (Wright et al., 2013). There is also evidence that microplastics can act as carriers for 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). 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 (Bakir et al., 2016; Koelmans et al., 2016; Lohmann, 2017).

1.1.1 Monitoring of microplastics in the marine environment

1.1.1.1 Microplastic monitoring in sediments

Microplastics have been more frequently monitored in sediments than in the water column (Hidalgo-Ruz et al., 2012; Löder and Gerdts, 2015). Monitoring has been mainly focusing on beaches or subtidal sediments (Frias et al., 2010; Maes et al., 2017). Subtidal sediments can be sampled from vessels with grabs, e.g. Van Veen or Ekman grab or corers (Löder and Gerdts, 2015).

1.1.1.2 Microplastics monitoring in biota

The occurrence and fate of microplastics in biota has been reported in several studies. Organisms were sampled from a range of habitats including the sea-surface, water column, benthos, estuaries, beaches and aquacultures (Ward et al., 2009; Foekema et al., 2013; Lusher et al., 2013; Avio et al., 2015; Lusher et al., 2017; Hermsen et al., 2017). Consequently, considering the wide range of habitats targeted, a range of sampling methods have been used. Sampling techniques included the use of grabs, traps, creels and trawls (Murray and Cowie, 2011; Welden and Cowie, 2016). Some species are also collected by hand, which is often the case for annelids, bivalves and crustaceans (Van Cauwenberghe et al., 2015; Li et al., 2018). Another method is the direct collection from shellfish, fish farms and commercial fish markets, where the capture method is unknown (Li et al., 2018).

A range of methods has also been used for the isolation of microplastics in biota including dissection, depuration and digestion of tissues with chemicals or enzymes. Visualisation of suspected plastic particles is usually being carried out under microscope and □-FT-IR and ATR-FT-IR the main techniques for polymer identification.

The Caribbean region is widely recognised to be a hot spot for marine biodiversity (Birchenough., 2017). The area hosts a wide range of habitats and species of conservation and fisheries importance. The main fisheries in the area concentrates on spiny lobster and Queen conch. These species are key to sustain sources of human protein intake, livelihoods and economic trade in these areas. On the direction of the DOE and the Belize Fisheries Department, Queen conch was selected as one of the most important economic species. Only one such economically important species was chosen due to time constraints. Furthermore, specimens were already available from the Commonwealth Marine Economies (CME) programme. The project, based in Belize since 2017, entitled “Assessing the vulnerability of commercial species to ocean acidification and fisheries” has a programme of work which collects water and biological samples for both species. Some of these samples were dissected and the digestive tracks were kept for further analysis. These samples were stored and further analysed for the

presence of microplastics in support of the ongoing research in Belize under the Commonwealth Litter Programme (CLiP).

1.1.1.3 Characterisation and quantification of microplastics in environmental samples

Different methods are currently being applied for the extraction and characterisation of microplastics in environmental samples. The lack of standardisation in analytical techniques implies some difficulties in the comparison between datasets generated from various sources. Characterisation and Identification of polymer type has been mainly carried out by the following techniques: i) micro-Fourier Transformed Infrared spectroscopy (µ-FT-IR), ii) focal plane array (FPA) Fourier Transformed Infrared microscopy (FPA FT-IR), iii) Attenuated Total Reflection Fourier Transformed Infrared spectroscopy (ATR-FT-IR) iv) micro-Raman spectroscopy (µ-RAMAN), v) Pyrolysis-gas chromatography-mass spectrometry (Py-GCMS) and vi) Fluorescence tagging of polymers using Nile red. It is worth noting that □-FT-IR and ATR-FT-IR have been mainly used as it allows both the visual and chemical characterisation of polymers as compared to Py-GCMS.

1.2 Creation of a Belizean scientific hub

One of the deliverables of the project was the creation of a sustainable microplastic laboratory for the long-term monitoring of macro and microplastics, in environmental matrices including biota and sediments. The development of the laboratory facilities incorporated a training programme in analytical techniques (see section 8) as well the production of baseline data for the occurrence and abundance of macro and microplastics in biota and in coastal and offshore sediments. Ensuring staff building capacity in Belize will allow the delivery of long-term monitoring programmes with the regular production of data, which could be integrated in environmental management programmes or used as evidence to measure impacts from recently implemented national environmental policies.

1.2.1 Location of the microplastic laboratory

The laboratory facilities, also referred to as the Belizean scientific hub, was located at the University of Belize, Belmopan over the duration of the project before its relocation to a brand-new facility located at the DOE.

Figure 1.1 Location of the Belizean scientific hub at the University of Belize, Belmopan campus.

2 Aims and objectives

The main aim of this part of the project, included in the CLiP Science theme, was to create an analytical laboratory facility for the extraction, detection and quantification of macro and microplastics in environmental samples. The main objectives included i) providing essential laboratory equipment for the successful delivery of the project, ii) to enable staff building capacity from training courses and practical demonstrations, iii) to investigate the abundance, quantities and properties of macro and microplastics in biota, iv) to investigate the abundance, quantities and properties of macro and microplastics in sediment, v) to investigate impacts of some biotic and abiotic factors on the quantities of macro and microplastics in environmental samples including feeding strategies for biota and sediment particle size.

3 Materials and methods

3.1.1 Sediments

Sediments were collected by using a small Van Veen grab (Duncan and Associates, UK, sampled area 0.025 m^2) from the 22 May 2019 to the 6 June 2019. The selection of the sediment sampling sites was identified following a consultation with key monitoring stakeholders as identified by the DOE. This included, DOE, UB, Belize CZMAI and Scouts Association. Identified

sites were further validated by the Cefas monitoring team, following a site inspection, to ensure practical feasibilities according to technical and safety requirements.

Samples were collected at six locations around Belize: Succotz Ferry, San Ignacio Board Bridge, Mennonite Beach and Burrell Boom Bridge to characterise riverine inputs of microplastics and Belize River Northern Highway and Haulover Creek Pier to characterise coastal microplastic concentrations (Figure 3.1). Sediment grabs were subsampled for microplastic and Particle Size Analysis (PSA) with a rinsed 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 collecting pot 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 after returning from the site. In total six sediment grabs were sampled and due to time restraints only four sites have been processed for the quantification of microplastics (section 3.4).

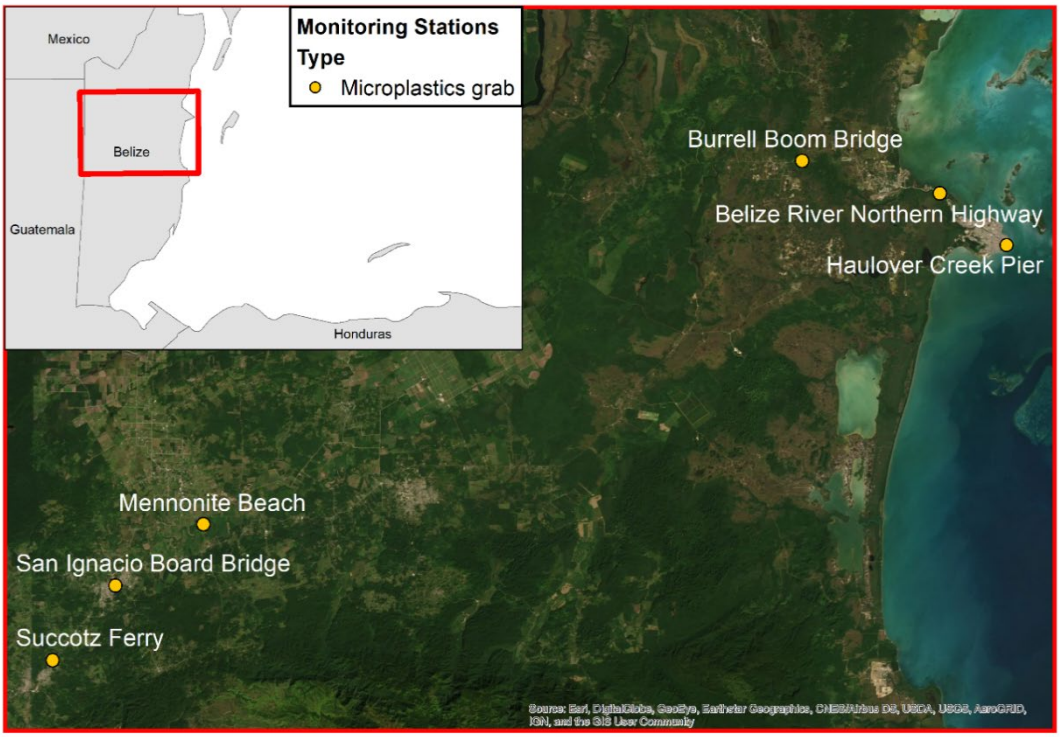

Figure 3.1 Sediment sampling sites for microplastics analysis

3.1.2 Biota

Occurrence and abundance of microplastics was carried out for the Queen conch *Lobatus gigas* and some riverine fish. The selection of the biota of interest was decided following a consultation with the DOE and the Belize Fisheries Department (BFD) to ensure selection of

commercially and ecologically important species with a direct impact on human health. Further criteria were assessed including (GESAMP, 2019):

- Regional representation (sessile or mobile species representative of a specific geographical range)
- Ethically sound (not protected or endangered)
- Abundant in the sampled area
- Species that are directly linkable to impact and effects
- Species that are globally comparable to understand global contexts

Following the selection process, species investigated included the Queen conch (*L. gigas*) and specimens of *Cichlasoma synspilum*, commonly known as redhead cichlid.

The Queen conch can be found throughout the coast of Belize and is a species of ecological and commercial importance. Twenty-two samples of *L. gigas* (Queen conch) were provided by the CME programme and were collected between the 03 to the 05 of December 2018 from fishing areas 1 and 2 Figure 3.2(a) and sampling areas are shown by the red dots in Figure 3.2(b).

Specimens of *Cichlasoma synspilum*, commonly known as redhead cichlid, were used in this study. *C. synspilum* is a native cichlid in Belize which lives in freshwater and feeds on a variety of food items including fishes, insects, snails and plant material. This fish is of importance to the local ecosystem and is also used for human consumption by a large proportion of the Belizean community. Fish samples were collected within the middle reaches of the Belize River, between Santa Familia village and Georgeville village (Figure 3.3 and Figure 3.4). Specimens of *C. synspilum* were collected using a 6 ft tall, 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 (n=50) was included in this study. After collection, fish samples were immediately anesthetized in buffered solution containing clove oil (100 ppm) 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 for later mapping of the sites sampled. Total length (L) of each fish was measured to nearest 0.1 cm using a measuring ruler and a piece of string, and body weight (W) was measured in grams using a FD6 balance scale (Ohaus Cooperation, Pine Brook, NJ USA).

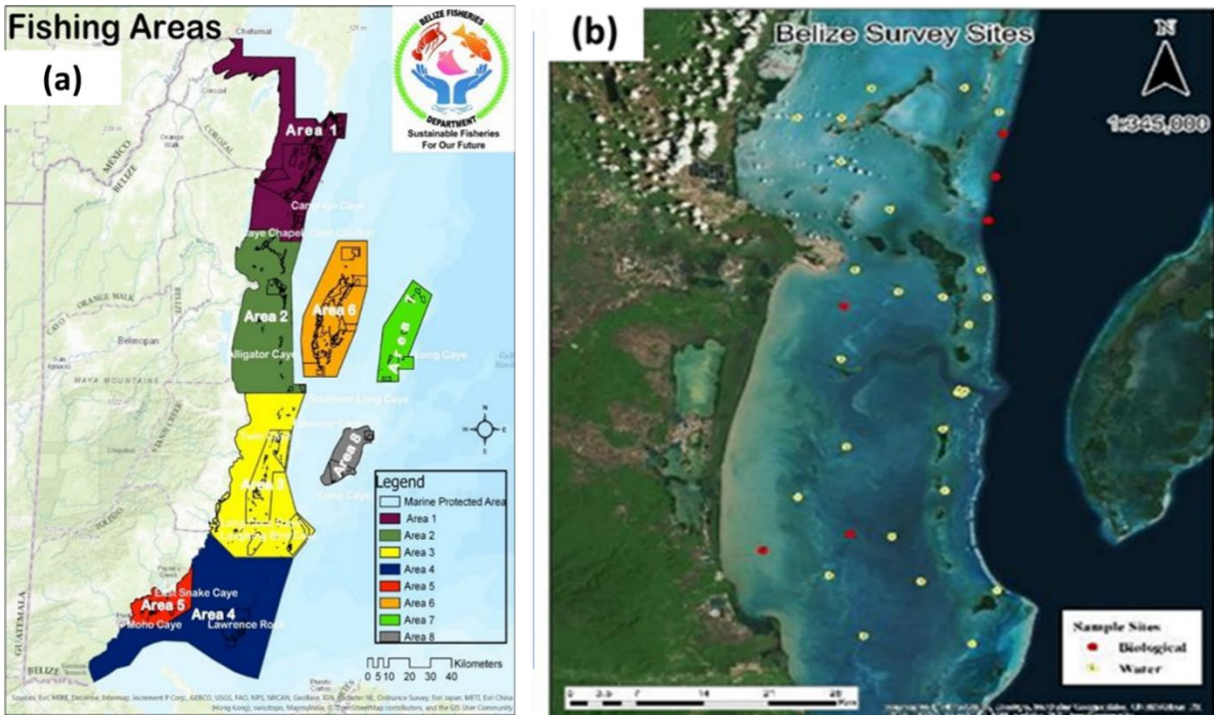

Figure 3.2 (a) Fishing areas in Belize and (b) sampling locations for (red) biological (conchs) and (yellow) water samples.

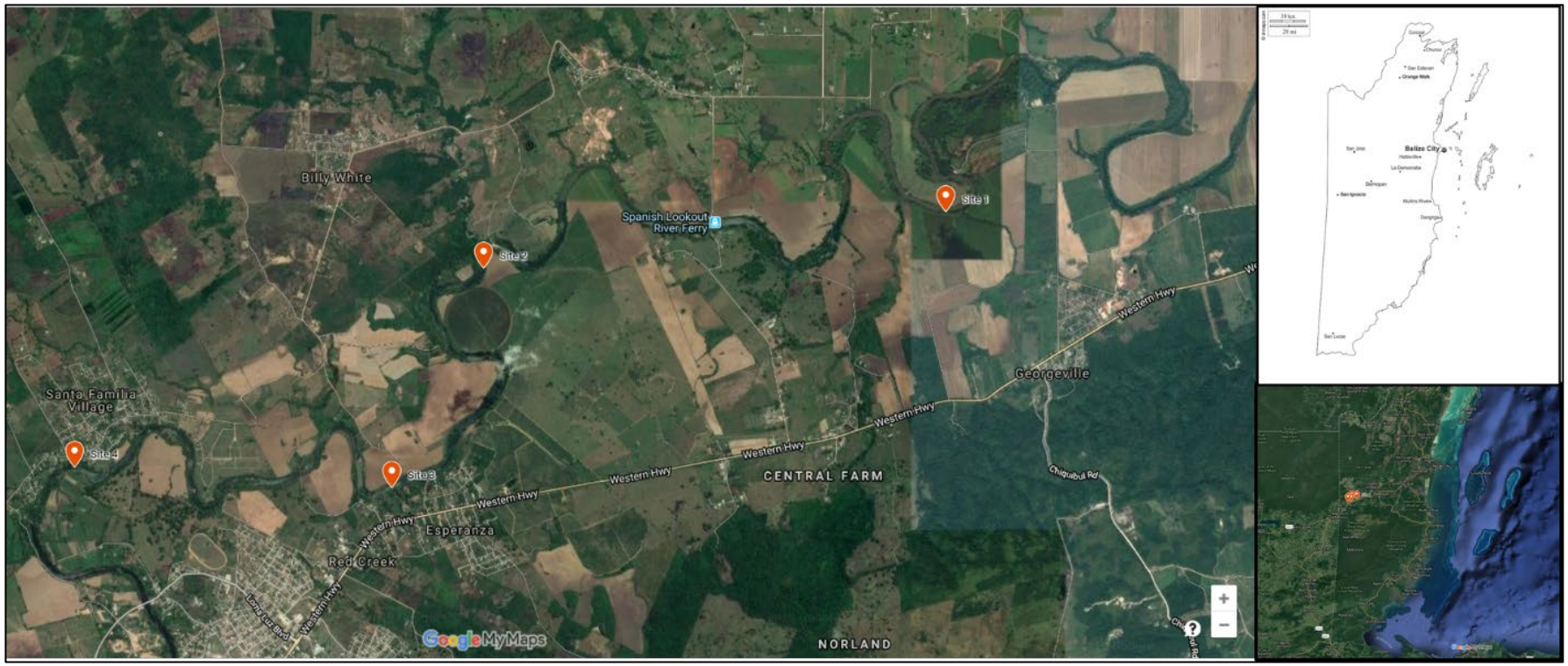

Figure 3.3 Sampling sites for the riverine fish species.

Figure 3.4 Collection of the riverine fish *Cichlasoma synspilum*. Photo by Elisabeta Waqa.

3.1.3 Chemicals

The chemicals used in this study are listed in Table 3.1 and were sourced from a local supplier (Femagra, Belize).

Table 3.1 List of chemicals, manufacturers and suppliers

Chemicals	Molecular formula	Manufacturer/Supplier	Purity (%)
Potassium hydroxide	KOH	Femagra, Belize	-
Sodium hypochlorite	NaClO	Femagra, Belize	13% active chlorine
Ethanol	C2H6O	Femagra, Belize	95% purity
Nile red	C20H18N2O2	VWR	99% purity
Sodium chloride	NaCl		Industrial grade

3.1.4 Contamination control

Microplastics are ubiquitous in the environment and it is important to have contamination control procedures in place when working in a microplastic laboratory. Sources of contamination can vary ranging from synthetic clothing to dust including plastic contamination from plasticware (Wesch et al., 2017). A series of step were taken in order to reduce ambient contamination in the laboratory but also in the field during the collection of samples. To reduce ambient contamination in the laboratory, 100% cotton lab coats were purchased and used for the entire duration of the study to reduce contamination of the samples with synthetic fibres. Room air recirculation was also minimised during the day to reduce contamination with dust.

Manipulation of the samples (sediments and biota) 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 r RO water. All chemical solutions used in this study were previously filtered using a 47 mm diameter 0.2 m 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 to compensate for background contamination. Control filters were used alongside the preparation of the sediment samples. For time efficiency, a procedural control was prepared with every batch of samples and subjected to the same preparation steps as detailed in sections 3.2.

3.1.5 Recovery study and validation

As a validation step, each type of filter membrane used in this study were spiked with a known number of plastic particles to investigate recovery rates using both a visual and an automatic particle counting method. Polymers used included LDPE in a film like state as well as a foam like state.

3.1.6 Quality control and polymer identification using Fourier Transform infrared spectroscopy (FT-IR)

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 an OMNIC software (version 9.9.473) and by comparison of their IR spectra to a polymer 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 -FT-IR with microscope. For microplastics, about only one particle was identified for 100 samples (1%). 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 reference material before each batch. Results are shown in Appendices 1 to 4.

3.2 Occurrence of microplastics in sediments in the coastal and marine environment

3.2.1 Sample preparation and extraction

The sample preparation process is summarised in Figure 3.1. The main steps include drying of the sample, density separation, alkaline digestion, fluorescence tagging and digital imaging of the samples.

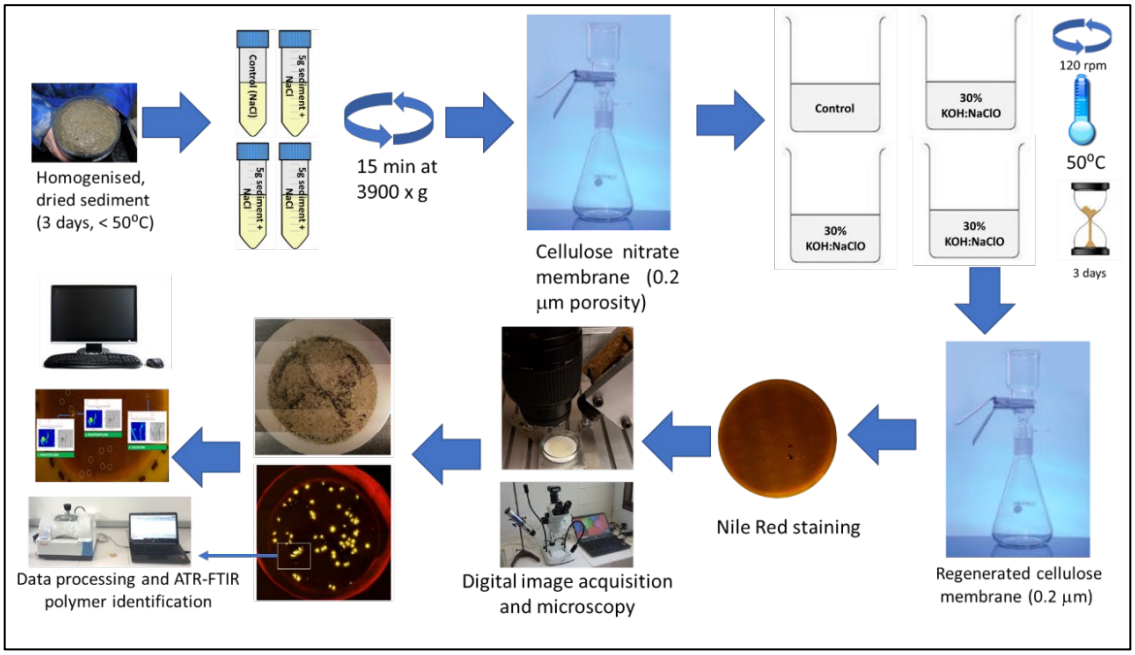

Figure 3.5 Schematic diagram of the protocol for the detection and quantification of microplastics in sediment samples.

3.2.2 Drying of the sediments

Collected sediment samples were thoroughly homogenised using a metal spatula in a fume cupboard and the lids were replaced with 15 cm Whatman 509 filter papers, held into place using small metal wires. Each sample was dried in a drying cabinet below 50°C for three days.

3.2.3 Weighing of the samples

Prior to weighing of the samples, each pot was homogenised using a rinsed cleaned metal spatula. Using two figures analytical balance, 5g of the sediment were weighed into three 50 mL polypropylene centrifuge tubes in a fume cupboard with ventilation.

3.2.4 Density separation

Density separation was carried out by using 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 with a 0.2 µm pore size. Approximately 35 mL of sodium chloride was added to each of the centrifuge tube and 37 mL was added to an empty tube as a control. The tubes were then well shaken to homogenise the samples. Each tube was centrifugated at 3900xG for five minutes. Each supernatant was transferred to a previously cleaned filtration unit and filtered using a 0.2 µm porosity Whatman cellulose nitrate membrane. The whole process was repeated two more times and the supernatants combined on the same filter. Residues of sodium chloride were rinsed with 100 mL of deionised water and particles stuck onto the funnels were rinsed using deionised water. Each filter was then carefully transferred to previously cleaned 100 mL glass beakers with a glass lid for the alkaline digest process (section 3.2.5.2).

3.2.5 Preparation of the chemical solutions

3.2.5.1 Preparation of the saturated sodium chloride solution

A saturated solution of sodium chloride (NaCl) was prepared by adding 200 g of a technical grade NaCl to about 800 mL of RO water in a clean 1 L beaker on a heating place at 30°C with a magnetic stirrer. Additional NaCl was added until saturation of the solution. The solution was filtered through a regenerated cellulose 0.2 µm pore size filter disk into a RO washed conical filter flask and was transferred to a RO ground glass stoppered bottle until ready for use. Density of the solution (min 1.2 g mL^{-1}) was confirmed by weight using an analytical balance.

3.2.5.2 Preparation of the 30% KOH:NaClO solution

For 1 L of reagent 150 ml of saturated KOH solution (1120 g L-1) and 160 mL NaClO solution (13% active chlorine) is mixed into 690 mL RO water. The solution was filtered through a regenerated cellulose 0.2 µm pore size filter disk into a RO washed conical filter flask and was transferred to a RO ground glass stoppered bottle until ready for use.

3.2.5.3 Preparation of the Nile red solution

0.01 g of Nile Red powder was weighed into a small petri dish on an analytical balance and dissolve in 1 L of ethanol using the glass coated magnetic stirrer to ensure fully dissolved. The solution was filtered through a regenerated cellulose 0.2 µm pore size filter disk into a RO washed conical filter flask to remove contaminating particles and transfer into well sealed (e.g. screw cap) amber glass bottles to prevent loss of volatile alcohol.

3.2.6 Particle Size Analysis (PSA)

PSA was carried out using wet splitting into silt/clay (< 63 µm), sand (63µm – 4 mm) and gravel (> 4mm) fractions only. For each sediment grab sample collected, a sub-sample was kept for

PSA analysis in a 120 mL collecting glass pot. The sample was kept in a freezer at -18°C until ready for analysis.

3.2.7 Occurrence of microplastics in biota in the freshwater and marine environment

The sample preparation process is summarised in Figure 3.6.

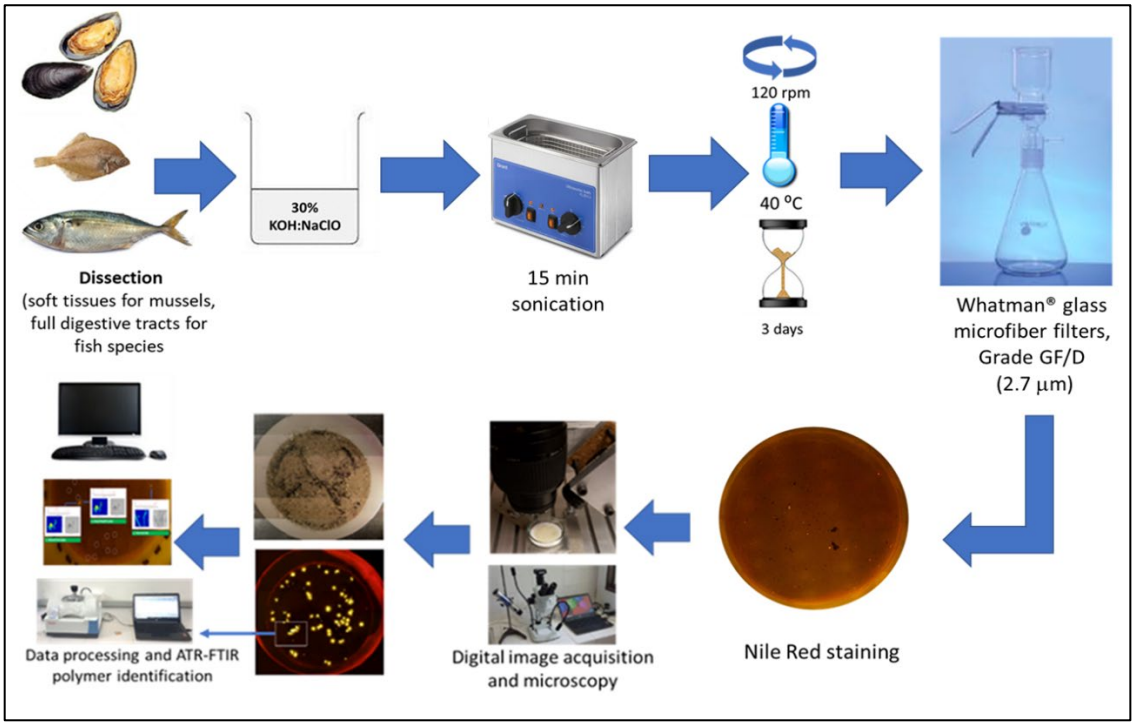

Figure 3.6 Schematic diagram of the protocol for the detection and quantification of microplastics in sediment samples.

Whole conchs (removed from their shells) (n=22) were provided to the laboratory and further dissected to remove the digestive tract using an air recirculating cabinet Figure 3.7.

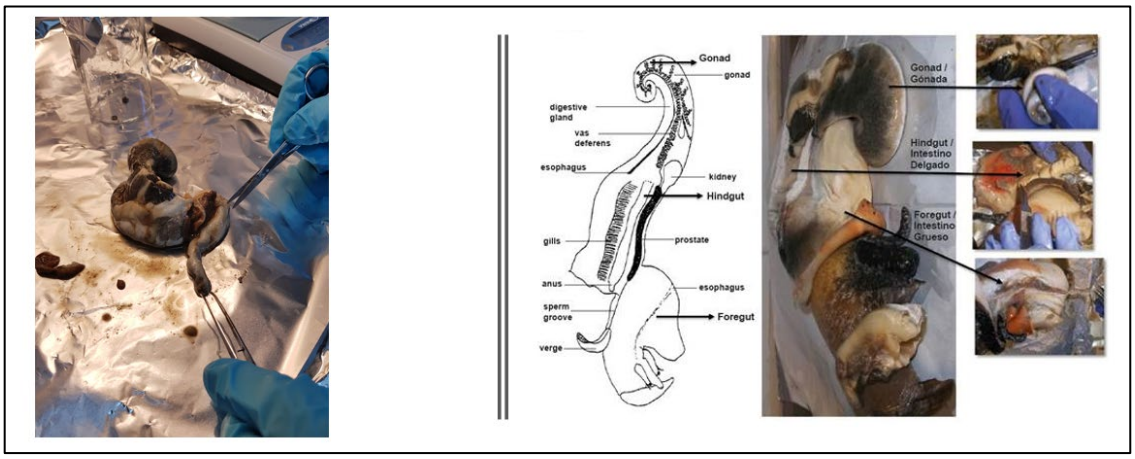

Figure 3.7 Dissection and removal of the conch's digestive tract and conch's morphology (Higuita-Valencia et al., 2018).

Each digestive tract was transferred to a 120 mL previously RO cleaned glass collecting pot and the wet weight of the tissues (including the gut content) recorded. 5 mL of a 30% KOH:NaClO solution was added per g of wet weight tissue collected. Samples were sonicated for 15 minutes 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 m porosity. Due to the large sediment content in their digestive tract, a large solid deposit was left after incubation. To avoid any blockages of the filters during the filtration process, the solid phase was carefully transferred into centrifuge tubes and potential plastic items were further extracted by flotation using the saturated solution of sodium chloride (d: 1.2 g mL-1). For time efficiency, the extraction of plastic particles from the solid phase was only carried out once. After the filtration step, about 5 mL of the Nile red solution was added to each filter and left between 30 and 60 minutes before rinsing with RO water. Imaging and counting of the fluorescence tagged particles was carried out as detailed in section 3.2.8.

For the fish samples, the digestive tract was removed following dissection in the laboratory while respecting the integrity of the gut content Figure 3.4. Each digestive tract was transferred to a 120 mL previously RO cleaned glass collecting pot and the wet weight of the tissues (including the gut content) recorded. Samples were digested and incubated as detailed above.

Figure 3.8 Collection, dissection and analysis of the fish samples by staff from UB in collaboration with the DOE and local fishermen.

3.2.8 Imaging, quantification and reporting units

Following filtration of the sediment and biota samples and staining process using Nile red, filters were investigated using a VisiScope® SZT360-6 stereo microscope (VWR, UK) equipped with a digital USB camera (VisiCam® B5) for image acquisition. A digital camera was also used (Canon EOS 800D DSLR with EF-S 18-55 mm) for the imaging of the filters using a blue light (420-470 nm) (Foster & Freeman Limited, UK) to induce fluorescence of the stained plastic particles Figure 3.9. The digital imaging process are detailed in Maes et al. (2017). Quantification of microplastics was carried by counting the number of fluorescent particles. Plastic particles for sediment samples were expressed in number of particles per kg dry weight sediment, while

reporting units for biota included number of items per individual and number of items per g wet weight.

Figure 3.9 Quantification of microplastics in the laboratory: (a) Fluorescence tagging of polymers, (b) digital imaging and (c) microscopic analysis.

3.3 Statistical analysis

To study variation among and between groups, ANOVA (analysis of variance) was used to analyse the differences among group means, followed by Tukey HSD post hoc test as the multiple comparison procedure using PAST version 3.25 (Hammer et al., 2019). Both ANOVA and Tukey's test assume independence of samples, homogeneity of variance and normality of residuals (Zar, 1999). Box-Cox transformation was used to transform non-normalised data. In addition, both ANOVA and Tukey's test assumes approximately similar population sizes, even though both tests seem to be relatively robust against deviations from the assumptions (Norwegian Environment Agency, 2018; Osborne, 2010; Zar, 1999). As an additional precautionary step for any type I errors a more conservative p value was selected ($p < 0.01$) where heterogeneous variances remained after transformation.

4 Results

4.1 Contamination and quality control

4.1.1 Contamination control

Initial concentrations of microplastics onto blank filters are shown in Figure 4.1(a). Level of ambient contamination was relatively high for the first set of blank filters with nine and eight particles per filter, respectively. Following this issue, additional contamination control procedures were implemented including cleaning of the surfaces before use, reduction of the room air recirculation system and additional dusting of the working areas. The implemented extra steps allowed a reduction of the ambient contamination with a reduction to one to two items per blank filter Figure 4.1(a).

All the collecting pots were pre-rinsed with RO water and capped with foil. Some prepared pots were analysed to determine laboratory ambient contamination. During sampling, an empty collecting pot was left uncovered to compensate for atmospheric input of plastic particles. Results are shown in Figure 4.1(b). 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. The field control collecting pot was heavily contaminated for one site (Succotz Ferry) with 14 suspected plastic items onto filter. This high contamination level was 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).

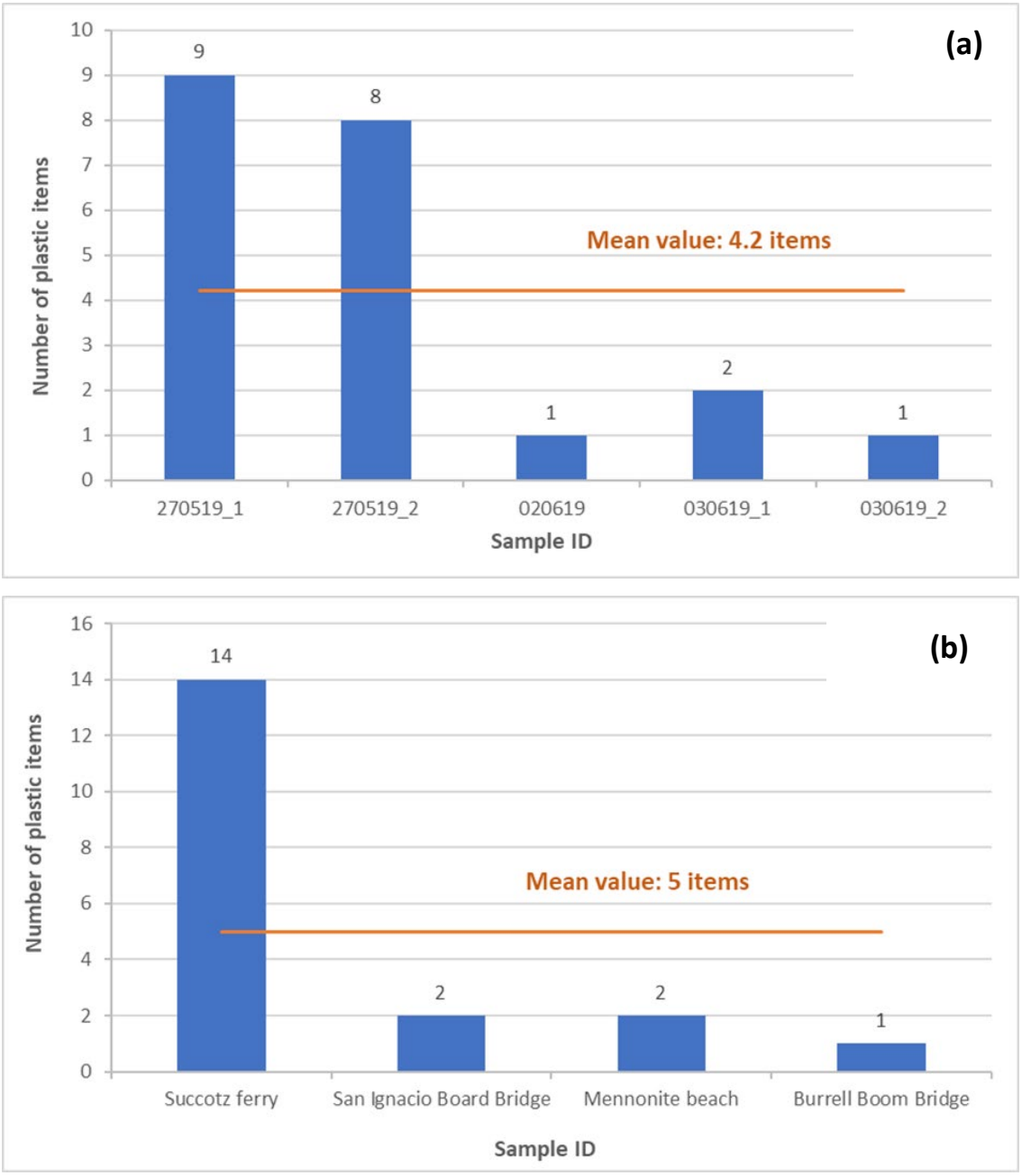

Figure 4.1 Summary of the suspected plastic items onto blank filters (regenerated cellulose, 47 mm diameter, 0.2 m porosity) for (a) laboratory blank filters and (b) field blank filters (regenerated cellulose, 47 mm diameter, 0.2 m porosity).

4.1.2 Recovery studies

Recovery studies were carried out to validate the fluorescence of plastics and to investigate the accuracy of the automatic counting method used here. The recovery study was only carried out in duplicate to highlight any deviations from the previous larger scale recovery studies carried out during CLiP South Pacific and internally at Cefas on an extended range of polymers including Nylon, unplasticised PVC (uPVC) and polyethylene. Results are presented in Figure 4.2.

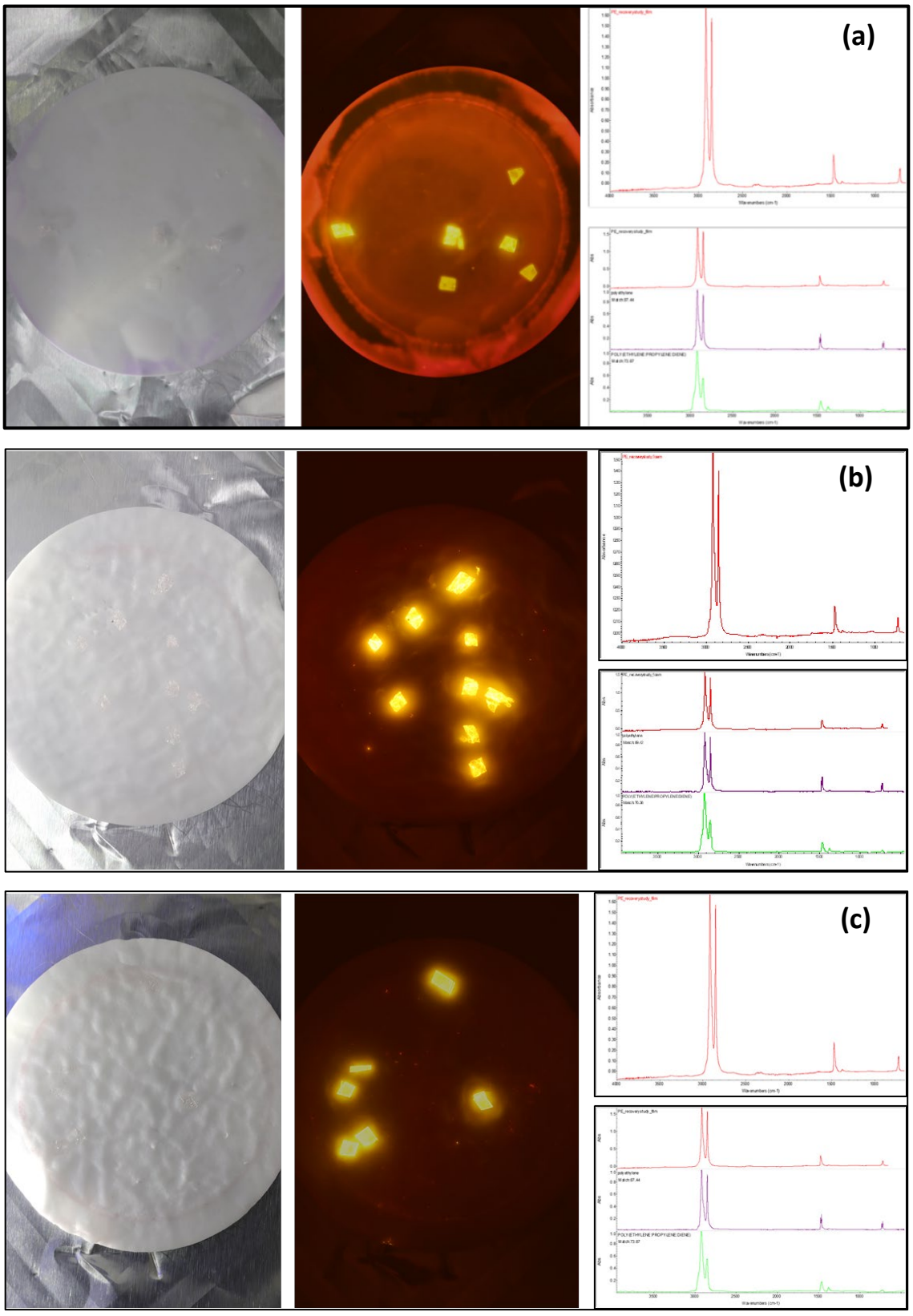

Figure 4.2 Recovery studies for (a) Regenerated cellulose filter spiked with PE items (47 mm – 0.2 m porosity), (b) GF/D filter spiked with PE foam items (47 mm – 2.7 m porosity) and (c) GF/D filter with film PE items (47 mm – 2.7 m porosity).

4.2 Occurrence of microplastics in sediments in the costal environment

4.2.1 Spatial variations

The occurrence and abundance of microplastics in sediment were investigated for a limited number of sites (n=4). No macroplastics (> 5 mm) were found in sediments while microplastics (< 5 mm) were detected in all the sediments under investigation. Concentrations ranged from 200 to 6500 ± 1273 particles per kg dry weight sediment (Figure 4.3). Concentration of microplastics were significantly higher for Succotz Ferry ($p<0.01$) as compared to other sites and level of contamination followed the order Succotz Ferry > Mennonite Beach > San Ignacio Board Bridge = Burell Boom Bridge.

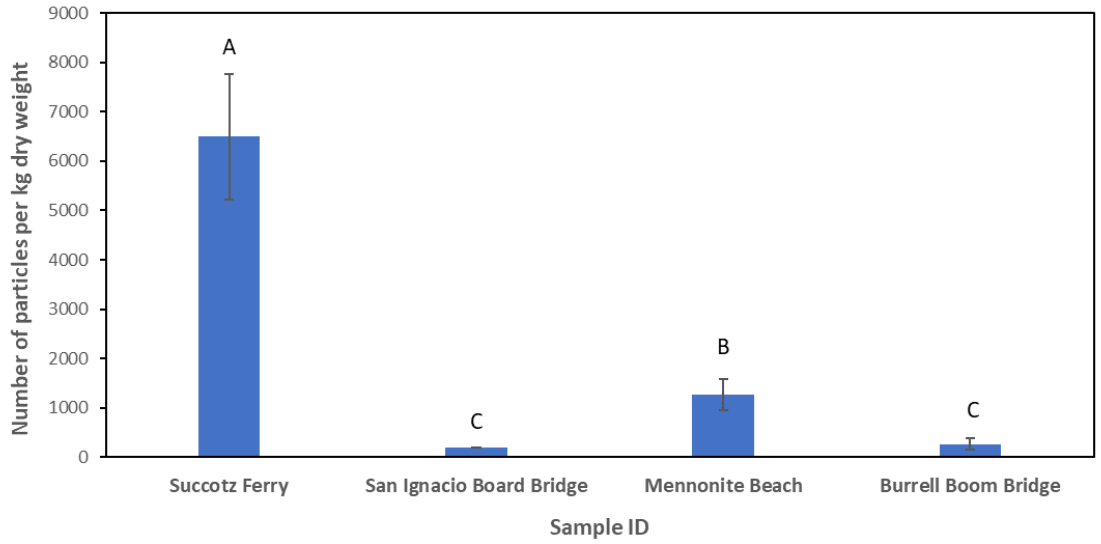

Figure 4.3 Number of particles per kg dry weight sediment collected for selected locations in Belize. Letters refer to grouping following a one-way ANOVA using a Tukey (HSD) post hoc test after a Box-Cox transformation of the data. Means that do not share a letter are significantly different (p=0.01).

4.2.2 Impact of PSA

To investigate the impact of particle size on the abundance of microplastics in sediments, sediments collected alongside the grab samples were analysed for PSA (Figure 4.4).

A scatterplot 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 (see Appendix 5).

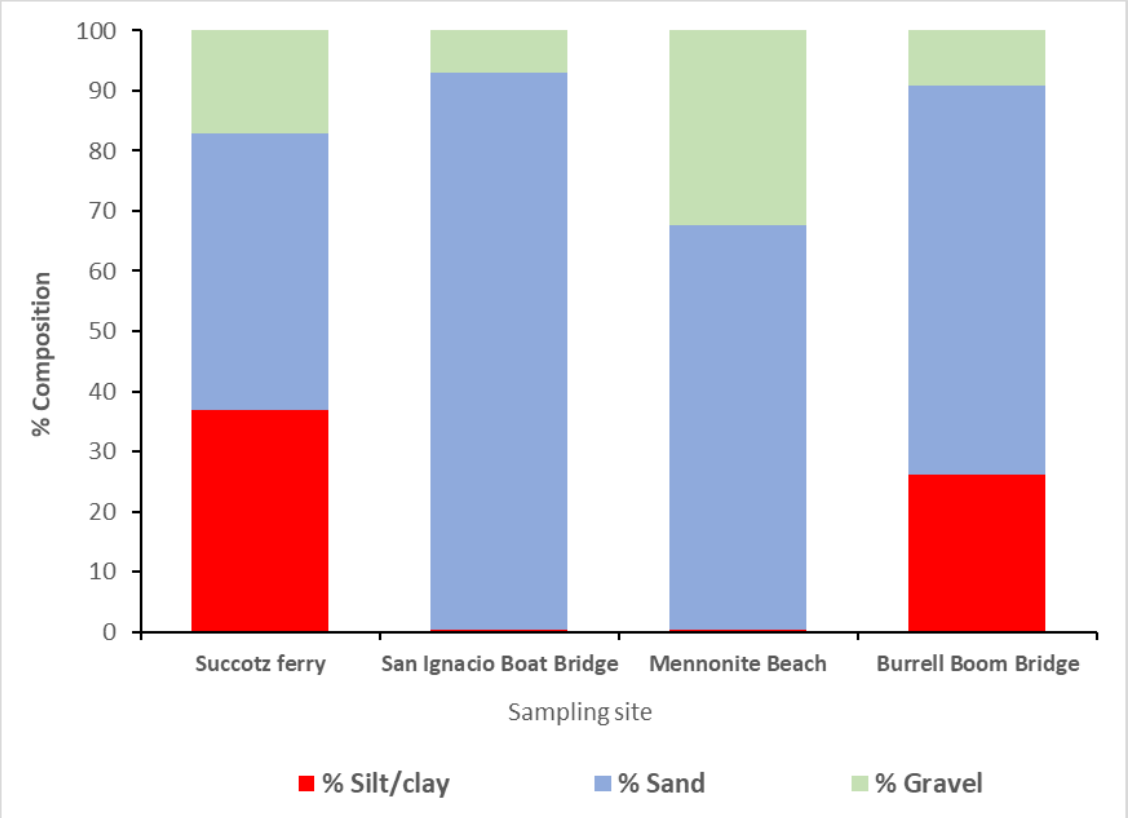

Figure 4.4 PSA for the sediments investigated for the occurrence and abundance of microplastics.

4.2.3 Uncertainties and evaluation of method

For any analytical methods, associated limitations occur. False positives can occur from the presence of some biological materials which can also sorb the dye and produce a fluorescence similar to plastic polymers including crushed shells or pieces of corals (Maes et al., 2017). While the occurrence of false positives cannot be entirely removed from the technique, some steps are being implemented and used to reduce the occurrence of false positives including microscopic analysis complementary to fluorescence counting as well as the use of ATR-FTIR for polymer identification.

4.2.4 Occurrence of microplastics in biota

4.2.5 Microplastics in Conchs

No macroplastics have been found in the digestive tract of conchs (n=22). Microplastics were present in 41% of the individuals under investigation with an average concentration of 1.3 (0 – 7) items per individual. This corresponded to an average of 0.09 (0 – 1.01) items per g wet weight tissues (Figure 4.5).

Analysis of the polymer type using ATR-FTIR indicated that PP and cellophane represented the most commonly found type of plastic in conchs with a 40% occurrence. 20% of the suspected particles analysed could not determine due to poor library match probably due to their

advanced weathering status or due to their biological origin (i.e. non-plastics) (Figure 4.6). Only library matched above 70% were considered in this study and low spectral match percentage for cellophane was considered to correspond to cellulose-based material types rather than a synthetic material. Spectral data analysis is presented in Appendix 3.

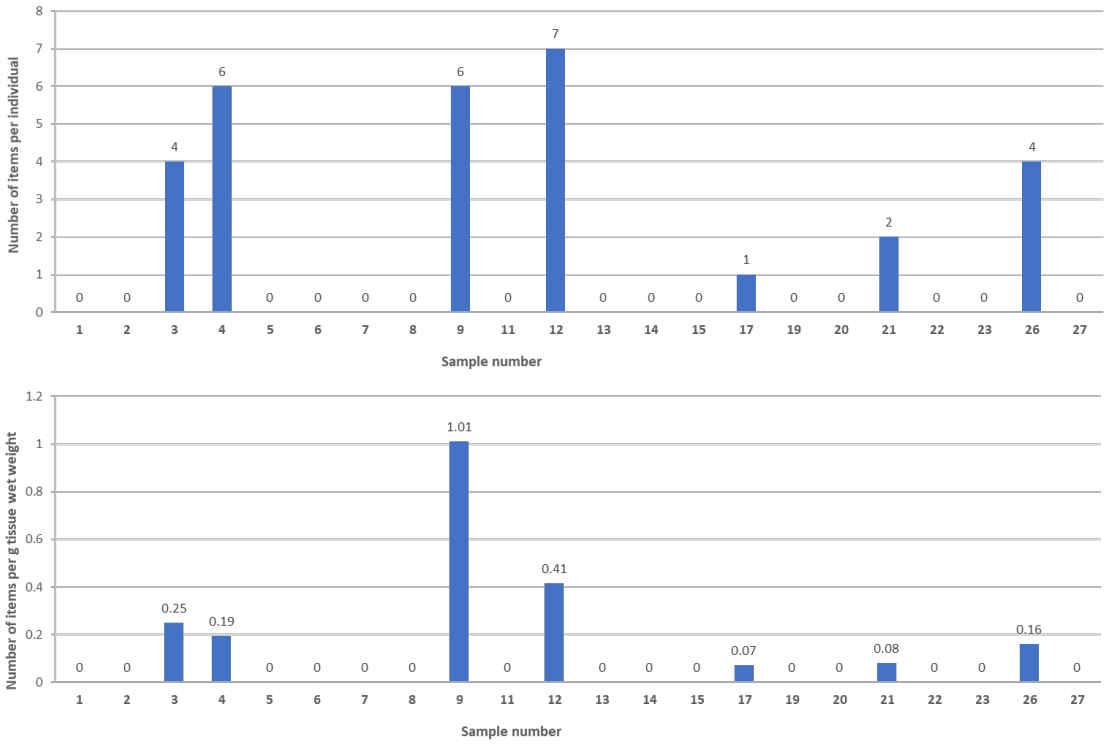

Figure 4.5 number of plastic items per individual and per g tissue wet weight (n=22).

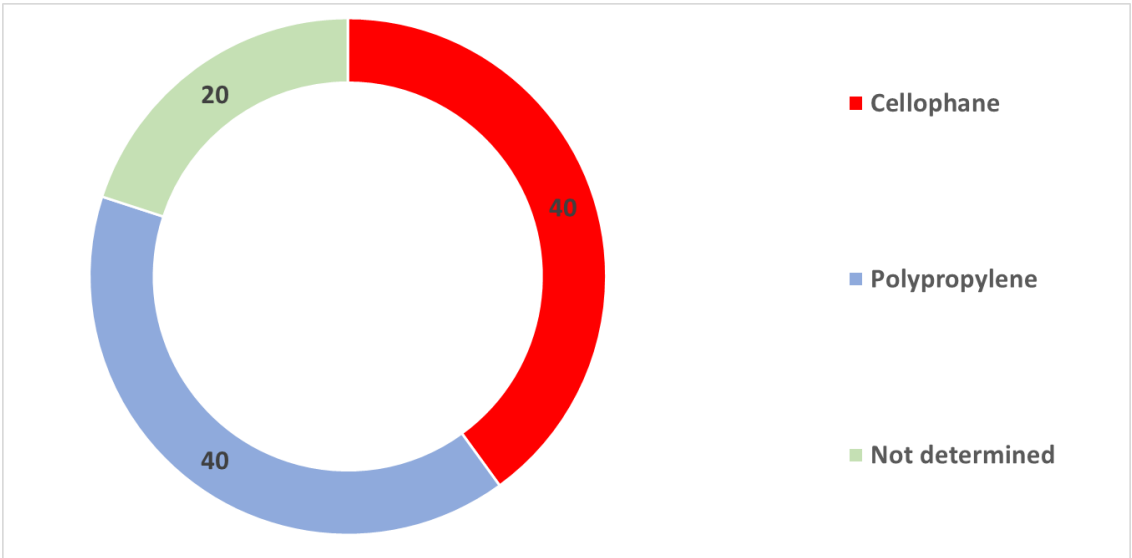

Figure 4.6 Microplastics (below 5 mm in size) extracted from conchs and analysed for QC classified according to their polymer type (n=5).

4.2.6 Microplastics in riverine and marine fish

Plastic items were detected in 36% of the fish investigated (n=22). No macroplastics (> 5mm) were observed. A total of ten items were extracted from the tissues with an average of 0.7 items per fish. 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 not identified due to low library match (< 70%) or were identified as natural particles (Figure 4.7).

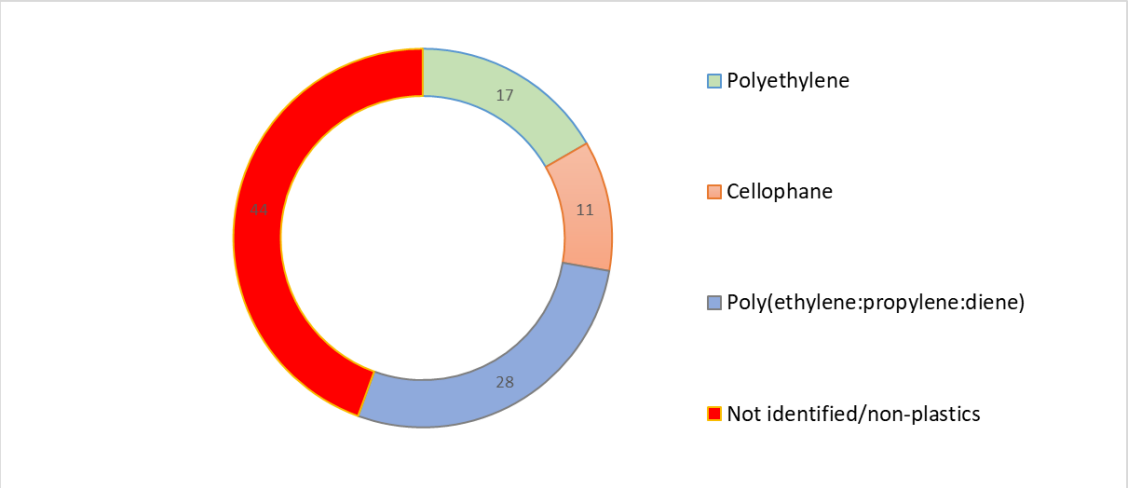

Figure 4.7 Microplastics (below 5 mm in size) extracted from fish and analysed for QC classified according to their polymer type (n=18).

5 Discussion

5.1 Occurrence and abundance of microplastics in sediment in the coastal environment

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 some monitoring baseline data. Microplastics (< 5mm) were detected in all the sediment samples collected while no macroplastics (> 5 mm) were observed. The abundance of microplastics in sediments ranged from 200 to 6500 ± 1273 particles per kg dry weight sediment. The abundance of microplastics was significantly higher for the Succotz Ferry sites ($p<0.01$) with 6500 ± 1273 particles per kg dry weight sediment followed by Mennonite Beach (1267 ± 306), Burrell Broom Bridge (267 ± 115) and San Ignacio Board Bridge with 200 particles per kg dry weight sediment.

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.

Investigation into the abundance of microplastics in sediments highlighted different "hot spots" for microplastic contamination including the Succotz Ferry site and Mennonite Beach (Figure 3.1).

- Xuanantunich Hand Cranked River Ferry (Succotz Ferry)

Succotz Ferry was characterised as a "hot spot" for microplastic contamination. Local activities at that site are varied including tourism, use of the river for recreational activities with the operation of a Ferry service transporting passengers and vehicles across the river (Figure 5.1). The high contamination level of the field blank at that site (see section 4.1.1) indicated that atmospheric input of particles was important for that area. City dust and urban runoffs, including paint polymers and synthetic rubber particles from car tyres have been identified as important sources of microplastics in the environment (Kole et al., 2017). Rivers have also been identified as important pathways for the entry of microplastics in the marine environment (Rochman et al., 2013) and it is not surprising that high concentrations of microplastics have been found for a riverine system.

Figure 5.1 Anthropogenic activities related to the Xuanantunich Hand Cranked River Ferry (Succotz Ferry site).

- Mennonite Beach

While the abundance of microplastics was much lower for Mennonite Beach as compared to the Succotz Ferry site, concentration of microplastics was elevated compared to the other sites (Figure 4.2). 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 2,253 in 482 households (Statistical Institute of Belize, 2017). Main activities in the area include agriculture and woodwork.

The number of particles varies greatly between studies and between sampling areas (Table 5.1). Concentrations recorded for Belize (200 – 6500 ± 1273 particles per kg dry weight sediment) were relatively low compared to other locations. As a comparison the smaller concentration range was reported by Crichton et al. (2017) for the Canadian shorelines with a concentration between 83 and 161.8 particles per kg sediment dry weight. Highest concentrations were reported by Manalu et al. (2017) for Jakarta Bay with a concentration ranging from 18,405 to 38,790 particles per kg sediment dry weight.

Table 5.1 Number of particles per kg dry weight sediment reported in the literature for several locations.

			Quantity in sediment	
Continent	**Sampling area**	**Characteristics**	**(numbers of particles/kg dw)**	**References**
Oceania	**South Pacific Ocean**	**Port Vila harbour**	**333 – 33,300**	**CLiP South Pacific**
	South Pacific Ocean	**Solomon Islands**	**450 – 15,167**	**CLiP South Pacific**
America	Canada	Baynes Sound and Lambert Channel, British Columbia	up to 25,000	(Kazmiruk, Kazmiruk and Bendell, 2018)
	Canada	Intertidal, Halifax Harbour, Nova Scotia	2,000 - 8,000	(Mathalon and Hill, 2014)
	Canada	Shoreline	83 - 161.8	(Crichton et al., 2017)
	Canada	Canadian Lake Ontario	20 – 27,830	(Ballent et al., 2016)

		nearshore, tributary and beach sediments		
	Belize		**200 – 6,500**	**This study**
Africa	Northern Tunisia	Lagoon-Channel of Bizerte	3,000 – 18,000	(Abidli et al., 2017)
Arctic Ocean		Deep-sea	42 - 6,595	(Bergmann et al., 2017)
Asia	Tokyo	Tokyo Bay	1,900	(Matsuguma et al., 2017)
	China	Beibu Gulf/Coastline of China Sea	5,020 – 8,720	(Qiu et al., 2015)
	Jakarta	Jakarta Bay	18,405 - 38,790	(Manalu, Hariyadi and Wardiatno, 2017)
	Jakarta	Mangrove area Pantai Indah Kapuk (PIK)	216.8 - 2,218.4	(Manalu, Hariyadi and Wardiatno, 2017)
	Eastern Asia	Gulf of Thailand	100 - 1,900	(Matsuguma et al., 2017)
Europe	Belgium	Continental Shelf	97.2 - 166.7	(Claessens et al., 2011)
	Barents Sea	Norwegian Continental Shelf	830 - 3,900	(Norwegian Environment Agency, 2018)

	Central North Sea	Norwegian Continental Shelf	180 - 31,000	(Norwegian Environment Agency, 2018)
	Norway	Reference areas in the Norwegian coastal shelf	1 - 400	(Mareano, 2017)
	The Netherlands	Subtidal	100 - 3,600	(Leslie et al., 2017)
	Italy	Venice Lagoon	672 - 2,175	(Vianello et al., 2013)
	Italy	Lido di Dante (Beach)	1512	(Lots et al., 2017)
	Sweden	Subtidal	16 - 2,590	(KIMO Sweden, 2007)
	Slovenia	Beach	170.4 - 177.8	(Laglbauer et al., 2014)
	UK	North Sea and English Channel	0 – 3,146	(Maes, Van der Meulen, et al., 2017)
	Kachelotplate Island	Beach transects	0 - 62,100	(Liebezeit and Dubaish, 2012)
	Romania	Beach	100 - 5,500	(Popa et al., 2014)
	Baltic Sea	Isle of Rügen	55.01 - 114.72	(Hengstmann et al., 2018)

5.2 Occurrence and abundance of microplastics in biota

This study confirmed the presence and occurrence of microplastics in the digestive tracts of Queen conchs and in riverine fish in Belize. Both species under investigation were of economic importance and used as a food source with a direct implication to human health. Queen conch resides in seagrass meadows and sandy substrates. Sediment represented a large proportion to

their gut content during analysis and as sediments are known to be the ultimate sink for microplastics in the marine environment (Woodall et al., 2014; Näkki, Setälä and Lehtiniemi, 2019), a high occurrence of microplastics in their gut was expected. Microplastics were present in 41% of the conch individuals investigated with a mean concentration of 1.3 items per individual (0 – 7). ATR-FT-IR identified PP and cellophane to be the prevalent polymer (40%). PP and cellophane have been reported for sediment samples globally (Shahul Hamid et al., 2018). Microplastics have been reported for a wide range of marine organisms ranging from marine worms to seabirds with varying concentrations according to the species and locations (Table 5.2). To the extent of our knowledge, no other studies have explored the occurrence of microplastics in conchs for Belize and globally. Therefore, data from Belize could not be expressed in a global context.

While several studies have reported the occurrence and abundance of microplastics for marine fish (Table 5.2), 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, Barthem and Ferreira, 2003; Andrade et al., 2019). They reported a 26.7% occurrence of microplastics in serrasalmids under investigation. Reported 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. The authors 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 a much lower extent with 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. (2019) reported a prevalence of PA (97.4%) followed by Rayon and PE (< 2%).

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 is making 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, as well as 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 entirely.

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 compare 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).

Table 5.2 Number of items per g wet weight and per individual reported in the literature for several locations. Results from this study are included in green for conch and fish samples.

	Organism	Location	Number of items/g w.w.	Number of items per ndividual	Reference
Marine worms					
	A. marina	French_Belgian_Dutch border	1.2 ± 2.8		(Van Cauwenberghe et al., 2015)
Sea snails					
	C. abbreviatus	Western Pacific Ocean	0.16	2.9	(Abbasi et al., 2018)

	Lobatus gigas	**Belize**	**0.09 (0 - 1.01)**	**1.3 (0 – 7)**	**This study**
Prawns					
	P. indicus	Western Pacific Ocean	0.59	2.3	(Abbasi et al., 2018)
Mussels					
	M. edulis	French_Belgian_Dutch border	0.2 ± 0.3		(Van Cauwenberghe et al., 2015)
	M. edulis	French Atlantic coast		0.61 ± 0.56	(Phuong et al., 2018)
	M. edulis	China	0.9 – 4.6		(Li et al., 2016)
	M. edulis	UK	0.7 – 2.9	1.1 – 6.4	(J. Li et al., 2018)
	M. modiolus	UK	0.086 ± 0.031	3.5 ± 1.29	(Catarino et al., 2017)
	M. edulis	UK	3.0 ± 0.9	3.2 ± 0.52	(Catarino et al., 2017)
	M. edulis	Norway	0 – 24.45	0 – 14.67	(A. Lusher et al., 2017)
	M. edulis	Germany	0.36 ± 0.07		(Van Cauwenberghe and Janssen, 2014)
	M. edulis	Canada		34 - 178	(Mathalon and Hill, 2014)
	Mytilus edulis, Perna viridis	Coastal waters of China	1.52 – 5.36		(Qu et al., 2018)

	Bivalves	**China**		**4.3 – 57.2**	**(Li et al., 2015)**
Oysters					
	Saccostrea cucullata	China	1.5 – 7.2	1.4 – 7.0	(H. X. Li et al., 2018)
	C. gigas	Brittany, France	0.47 ± 0.16		(Van Cauwenberghe and Janssen, 2014)
	C. gigas	French Atlantic coast		2.10 ± 1.71	(Phuong et al., 2018)
Crabs					
		Vanuatu	**0.021**	**1.71**	**CLiP South Pacific**
Fish					
Marine fish		**Vanuatu**	**0.11**	**2.9**	**CLiP South Pacific**
Redhead cichlid	*Cichlasoma synspilum*	**Belize river**		**0.7**	**This study**
Riverine fish		Amazon River Estuary in North-eastern Brazil		1.75 (0 – 12.8)	(Schmid et al., 2018)
Whiting	*S. Sihama*	Western Pacific Ocean	0.25	1.5	(Abbasi et al., 2018)
Greater lizardfish	S. tumbil	Western Pacific Ocean	0.37	2.8	(Abbasi et al., 2018)
Pelagic and demersal fish		English Channel		1.9 ± 0.10	(Lusher, McHugh and Thompson, 2013)
Adriatic fish mullet		Adriatic Sea, Italy		1 – 1.78	(Avio, Gorbi and Regoli, 2015)

Large pelagic fish	*Xiphias gladius, Thunnus; thynnus and Thunnus alalunga*	Mediterranean Sea		4 - 16	(Romeo et al., 2015)
Demersal fish		Spanish Atlantic and Mediterranean coasts		1.56 ± 0.5	(Bellas et al., 2016)
Pelagic and demersal fish		North and Baltic Sea		0.03 ± 0.18	(Rummel et al., 2016)
Sunfish bluegill and Longear	*Lepomis Macrochirus & Lepomis megalotis*	Brazos River Basin, Central Texas, USA		10.1 – 13.9	(Peters and Bratton, 2016)
Demersal & pelagic fish		Northeast Atlantic around Scotland		1.8 ± 1.7	(Murphy et al., 2017)
Flying fish	*C. rapanouiensis*	South Pacific coastal waters around Easter Island		1.0 ± 0.0	(Chagnon et al., 2018)
Commercial fish		Mondego estuary in Portugal		1.67 ± 0.27	(Bessa et al., 2018)
Sardines and Anchovy	*Sardina pilchardus and Engraulis encrasicolus*	Spanish Mediterranean coast		0 - 3	(Compa et al., 2018)
Tuna					
Yellow Fin Tuna		**South Pacific Ocean Vanuatu**	**0.02**	**4.5**	**CLiP South Pacific**
Yellow Fin Tuna	*T. albacares*	South Pacific Ocean Coastal waters of the Eastern Island		5.0	(Chagnon et al., 2018)

Whales					
		Northern Ireland		2.95	(A. L. Lusher et al., 2015)
SeaBirds					
Northern Fulmars	*Fulmarus glacialis*	Pacific and Grays Harbor counties, Washington		13.3	(Terepocki et al., 2017)
Sooty Shearwaters	*Ardenna grisea*			19.5	(Terepocki et al., 2017)

6 Conclusions

Some scientific evidence has been provided on the occurrence and abundance of microplastics in sediments and in some selected biota species (Queen conch *L. gigas* and the riverine fish *Cichlasoma synspilum)*. However, additional monitoring is required for the collection of robust baseline monitoring data and to identify the main sources contributing to these high inputs of microplastics from land to riverine sources and their subsequent transport to the marine environment.

7 Challenges and recommendations

This report compiled the very first monitoring baseline concentration for microplastics of sediment and biota for Belize. Due to the relatively small data set investigated during this phase of the project, some challenges and recommendations were formulated:

- **Identification of likely sources of contamination:**
    - Controlling/reducing sources of microplastics requires a good knowledge of transport processes and fluxes between environmental compartments.
    - Greater spatial coverage required to characterise the main inputs of microplastics in the environment for Belize.
- **Mapping of "sensitive areas" for Belize:**
    - Risk assessment approach for microplastic monitoring with sampling for more "sensitive areas"
    - Matching "hot spots" of contamination with potentially sensitive systems (e.g. marine protected areas, fishing areas with high economic impact).

- **Dose response impacts?** Relate environmental concentrations with potential ecotoxicological effects (Predicted No-Effect Concentration (PNEC) approach).
- **Funding schemes:** Ensuring long-term investment to produce long-term data sets with dedicated trained staff.

These recommendations provide further detail to their related actions as part of the Belize Marine Litter Action Plan. For example, Actions 1.

8 Training

8.1 Core training course

Staff building capacity and knowledge transfer was carried out as a priority following the installation of the laboratory facilities based on the "train the trainer" approach. Training opportunities included a core training course over a 3 days period followed by optional laboratory demonstrations over the duration of the project. The core training course was delivered at University of Belize over three days and the agenda followed as presented in Table 8.1. The training exercise included a theoretical aspect with a presentation on the issue of plastics and microplastics in the environment as well as the introduction of Health and Safety requirements when working in a laboratory environment. The remaining training included the practical demonstration of the extraction, isolation and quantification of macro and microplastics in environmental samples, including sediments and in biota. The training was delivered to 11 participants from the Department of the Environment , the University of Belize, the Belize Bureau of Standards, The Belize Agricultural Health Authority and to the Belize Coastal Zone Management Authority and Institute (Figure 8.1).

Staff building capacity was carried out by sharing Cefas Standard Operating Procedures (SOPs) on the extraction and analysis of microplastics in environmental matrices as well as related risk assessments and Control of Substances Hazardous to Health (COSHH) forms.

Training was also delivered to some students from University of Belize already involved in plastic research. Delivery was achieved by shadowing specific activities until the students felt confident enough to conduct full activities under minimum supervision. Training courses were delivered in a flexible manner to adapt for different levels of technicality and complexity and to reflect personal motivations and personal goals previously defined via informal discussions.

Quality and effectiveness of the delivery was subsequently assessed by circulating feedback questionnaires (see Appendix 6 and Figure 8.2). 40% of the participants scored the training course as extremely useful, 40% as useful and 20% as moderately useful indicating that the right stakeholders have been identified for the training exercise. 60% of the participants also

considered that the training course was delivered at an acceptable level. A certificate of attendance was also provided upon completion of the core training course (see Appendix 6). Written comments from the feedback questionnaires have also been compiled and will help to improve the delivery of training for the rest of the CLiP project.

Table 8.1 Agenda of the training activities related to the laboratory activities delivered as part of CLiP.

Date of delivery	**Activity**	**Location**
22/05/2019	Theoretical aspects of marine litter research and safety requirements when working in a laboratory	UB – Classroom
22/05/2019	Chemical handling and Health and Safety	UB – microplastic laboratory
23/05/2019	Extraction and analysis of microplastics in sediments	UB – microplastic laboratory
23/05/2019	Introduction to ATR-FTIR	UB – microplastic laboratory
24/05/2019	Extraction and analysis of microplastics in biota	UB – microplastic laboratory
24/05/2019	Introduction to ATR-FTIR	UB – microplastic laboratory

Figure 8.1 Participants registered for the training exercise.

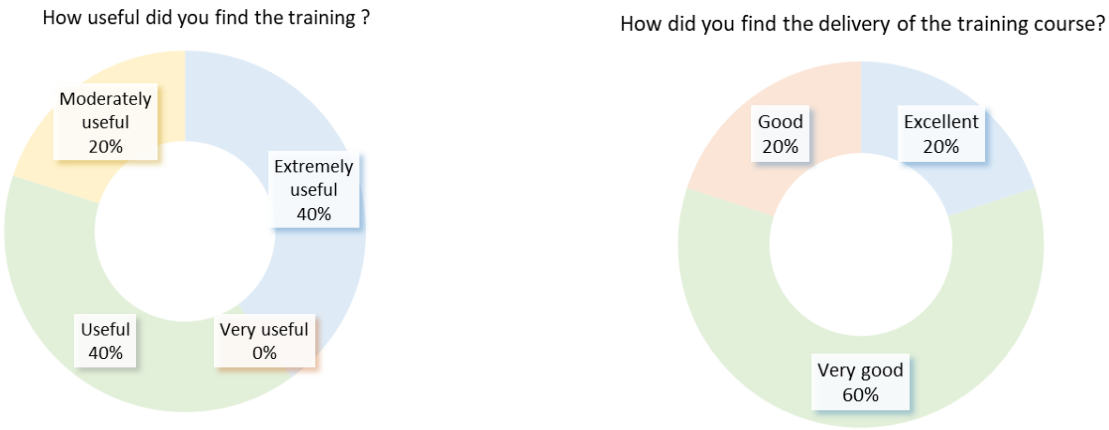

Figure 8.2 Overall ratings of the training exercise delivered for the laboratory aspect of CLiP (n=5).

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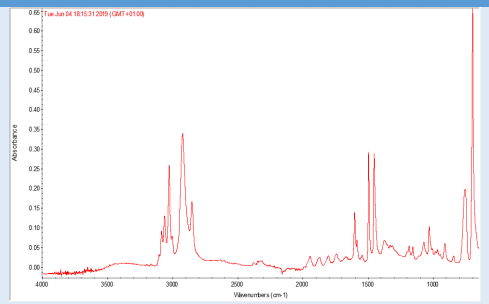
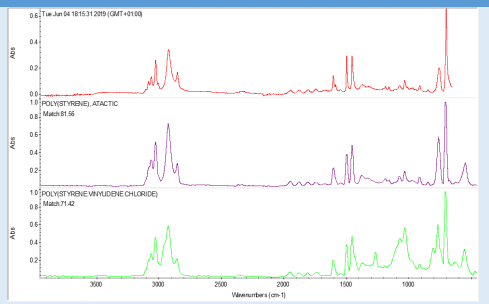
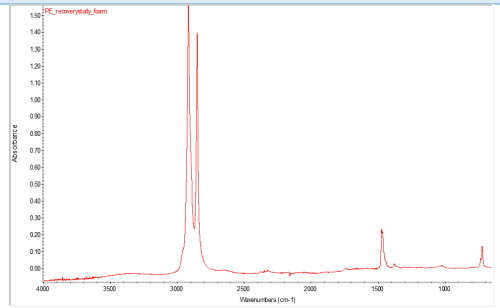
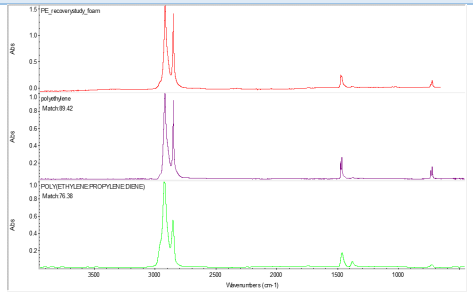
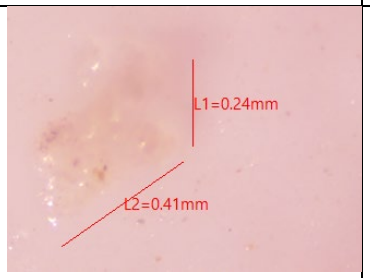
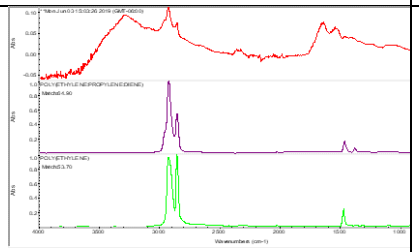
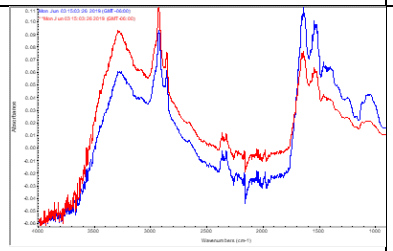
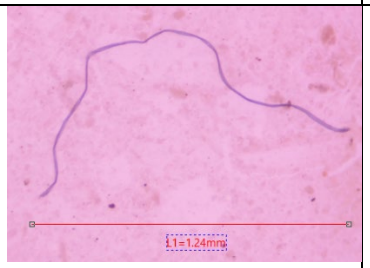
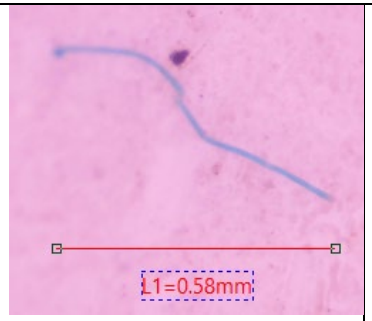
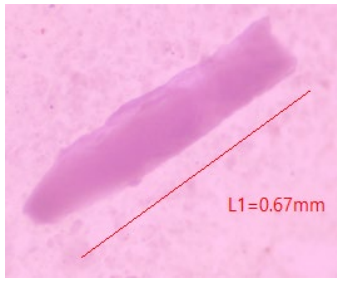
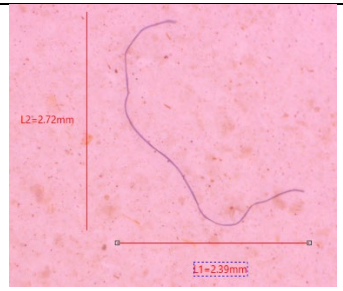
Appendix 1 QA/QC using ATR -FTIR

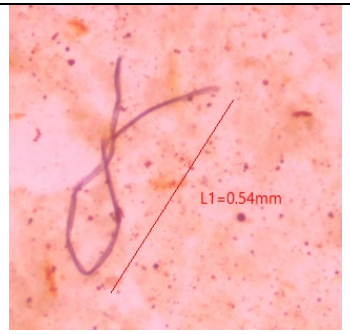
Table A1. Example of reference materials used for the quality assessment of the ATR-FTIR data.

Reference material	FTIR spectra	Library match	Polymer type	Library match (%)
Polystyrene			Polystyrene	82%
Polyethylene			Polyethylene	90%

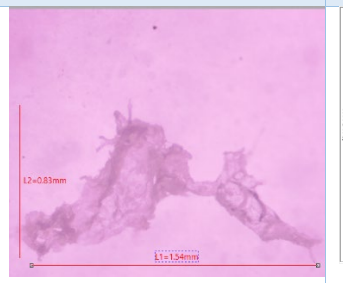
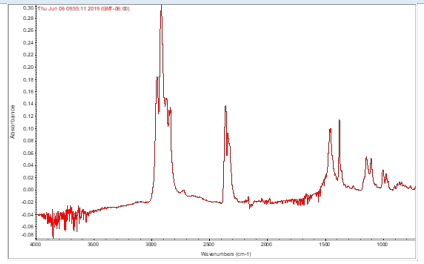
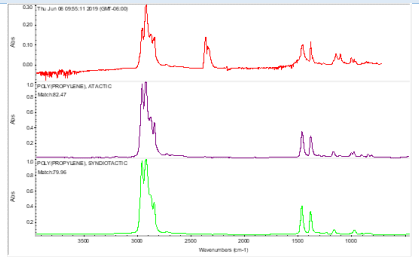
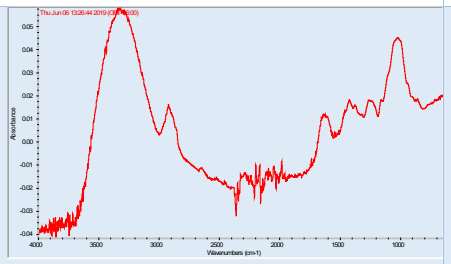
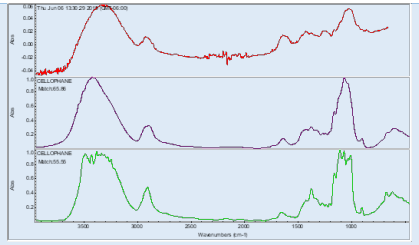
Appendix 2 Abundance, occurrence and properties of macro and microplastics in sediments. Suspected items extracted from sediment samples and ATR-FTIR data for the items selected for QA/QC.

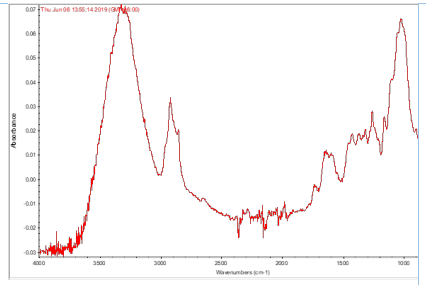
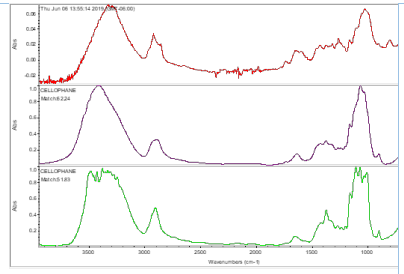
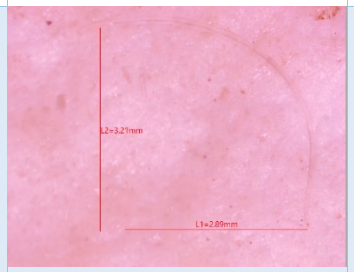
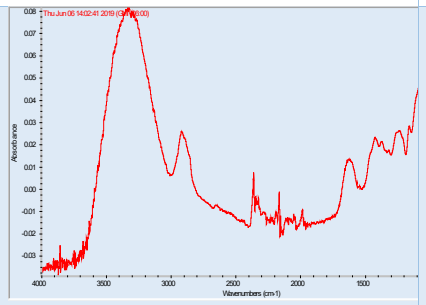
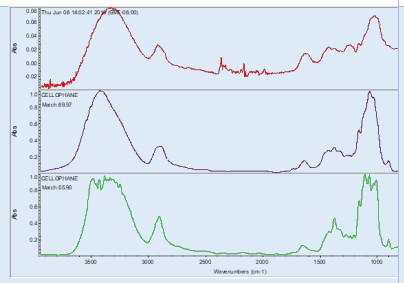
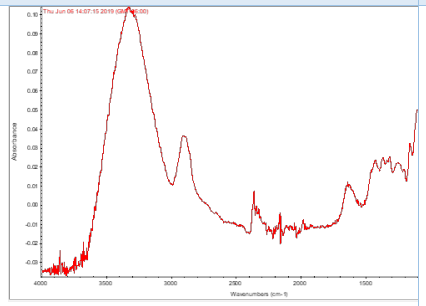
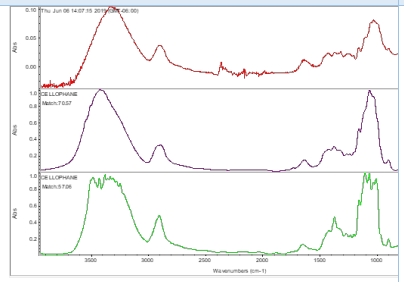
Sample ID	Image	FTIR spectra	Library search	Polymer type
BCRF1G1_Rep3				Polyethylene (65% match)
BCRD1G1_Rep1		nd	nd	

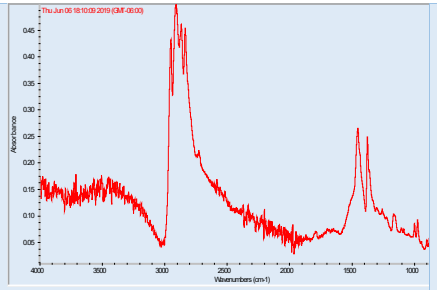
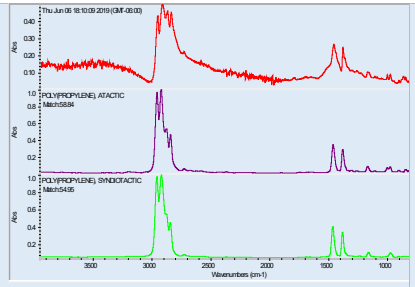
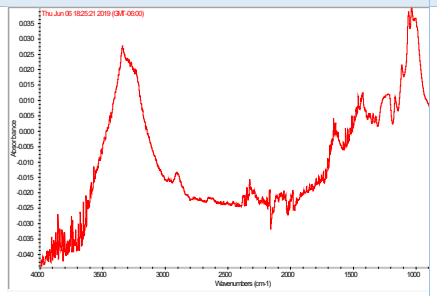
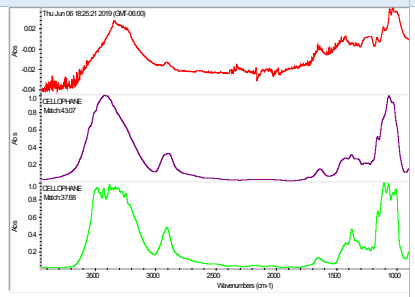
	 			
BCRH1G1_Rep1		nd	nd	

BCRH1G1_Rep 3		nd	nd	

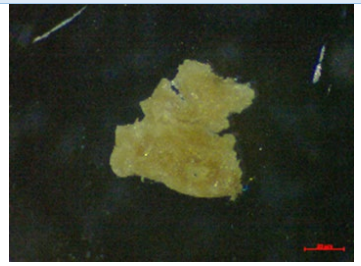
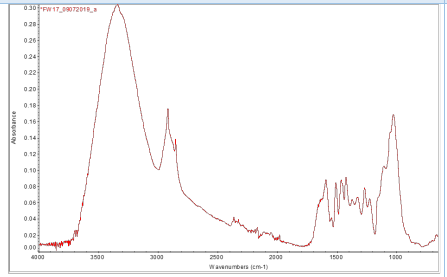
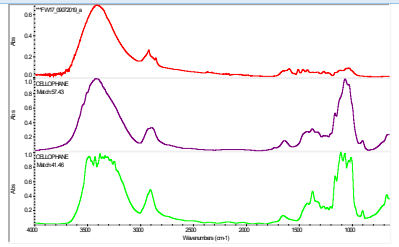
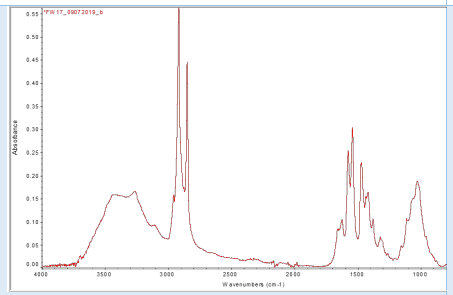
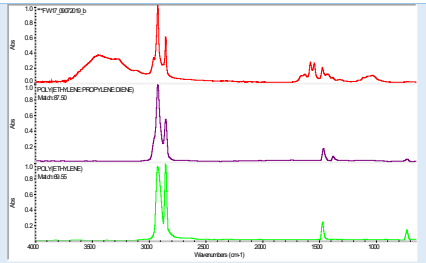
Appendix 3 Abundance, occurrence and properties of macro and microplastics in biota - Suspected items extracted from Conch samples and ATR-FTIR data for the items selected forQA/QC.

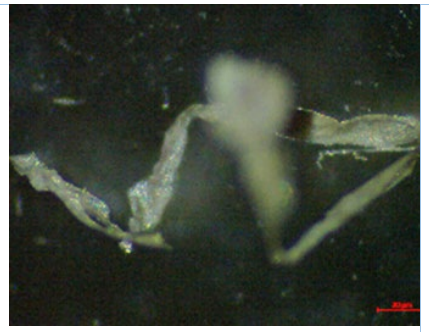
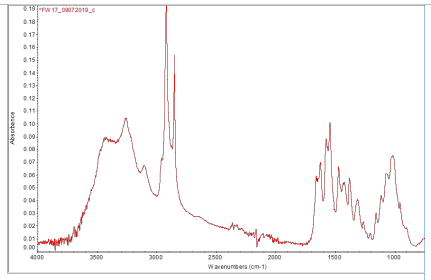
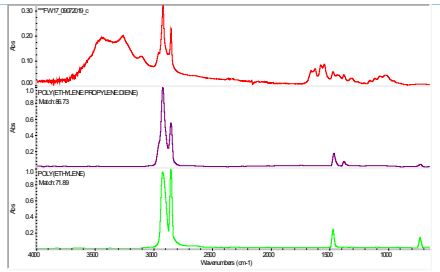
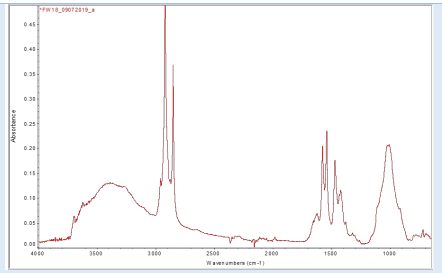
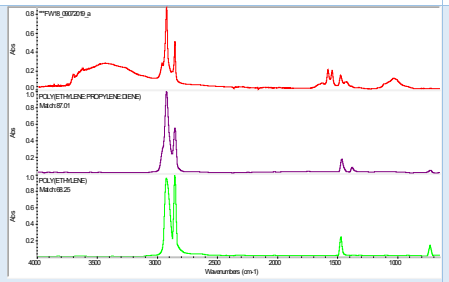
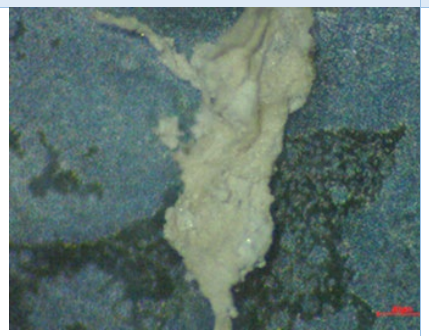
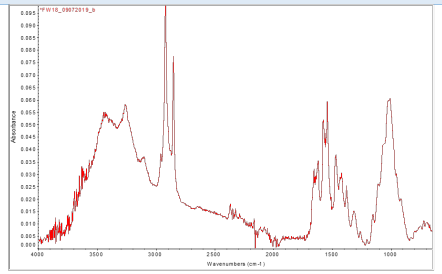
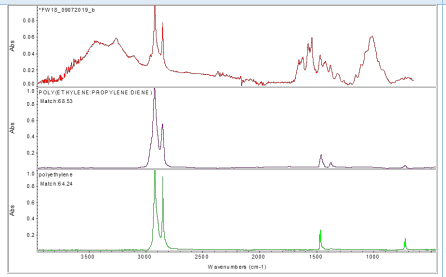
Conch sample ID	Extracted items			
	Image	FTIR spectra	Library search	Polymer type
Conch_st54_nber54_060619_Item1				Polypropylene 83%
Conch_st70_nber70_060619_5				Cellophane 65%

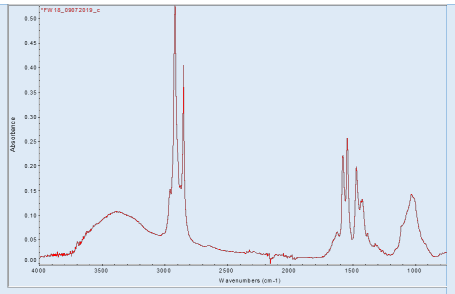
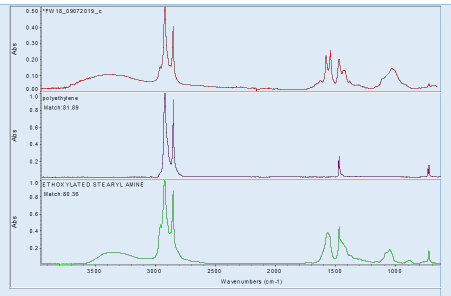
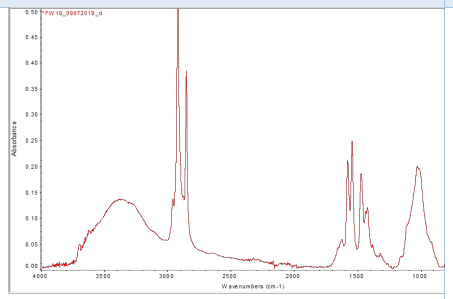
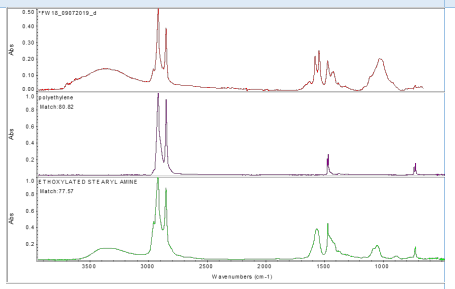
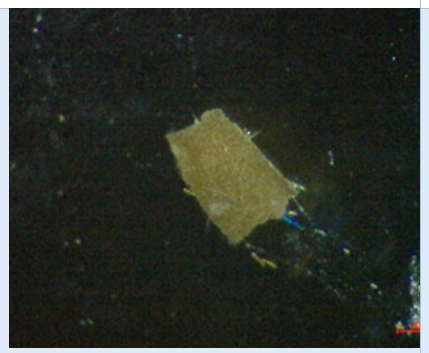
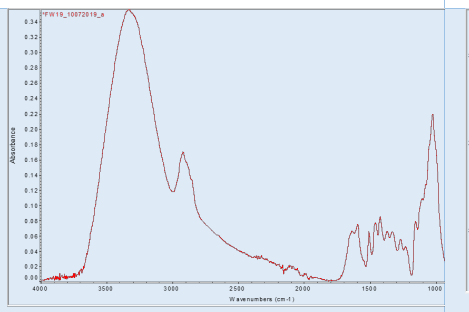
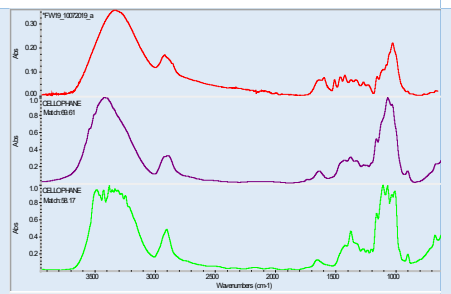
Conch_st54_nber54_060619 _Item2				Cellophane 62%
Conch_st70_nber70_060619 _Item1				Cellophane 70%
Conch_st70_nber70_060619 _Item2				Cellophane 70%

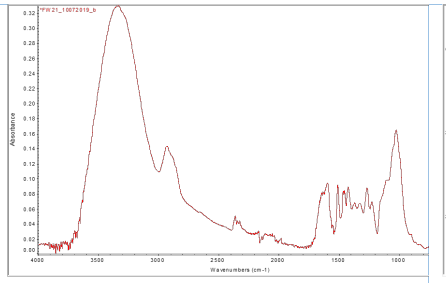
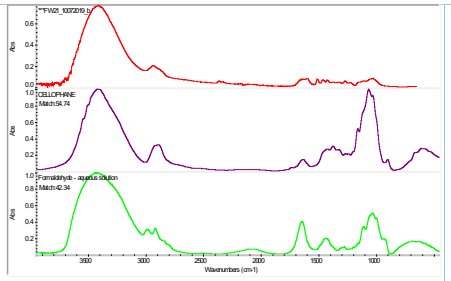
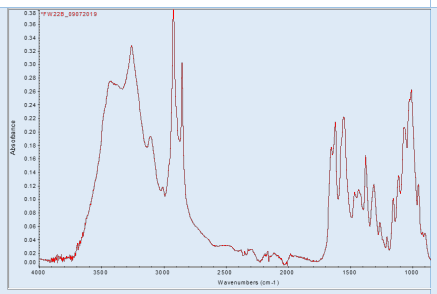
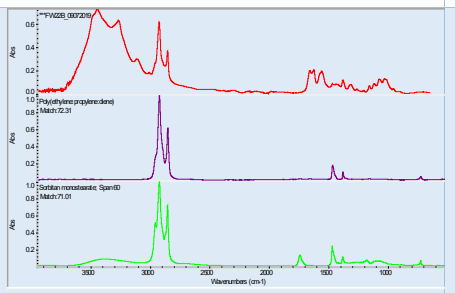
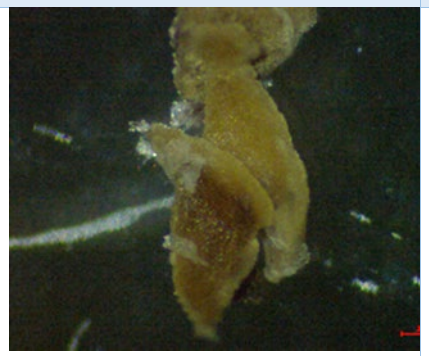
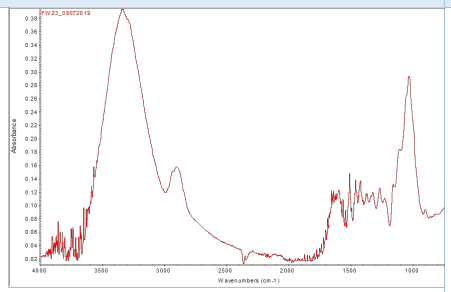
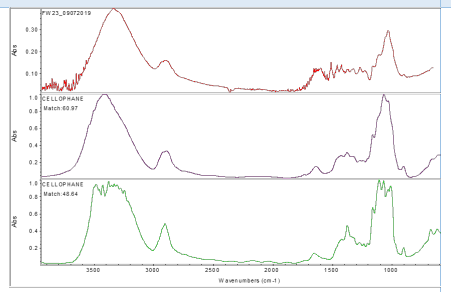
Conch_St59_nber59				Polypropylene 60%
Conch_Stn2_sample3				Cellophane 43%

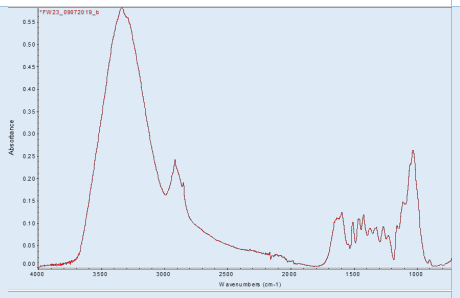
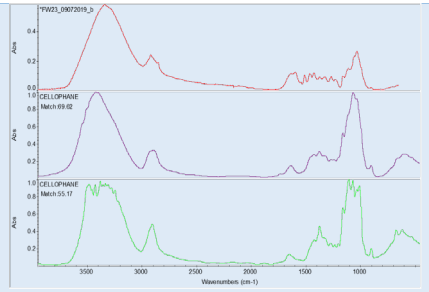
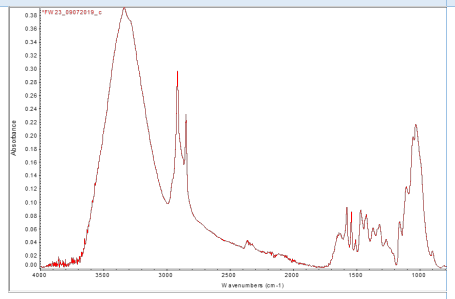
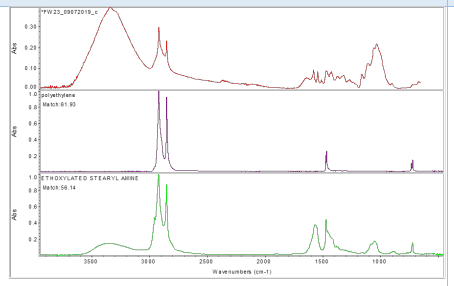
Appendix 4 Abundance, occurrence and properties of macro and microplastics in biota - Suspected items extracted from riverine fish samples and ATR-FTIR data for the items selected forQA/QC.

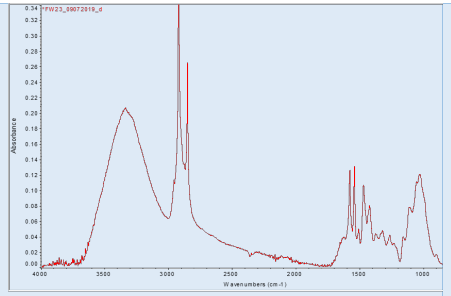
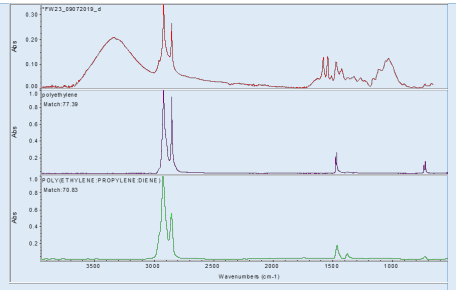
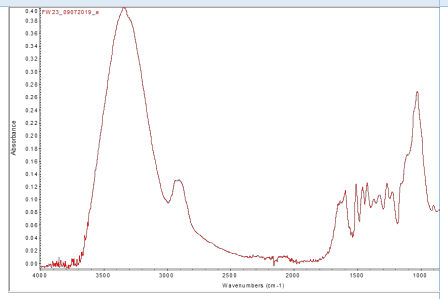
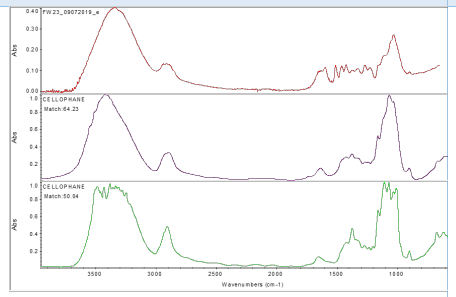
Fish sample ID	Extracted items			
	Image	FTIR spectra	Library search	Polymer type
FW17_A				Cellophane (60% library match) Not included as match <70%
FW17_B				Polyethylene (88% match)

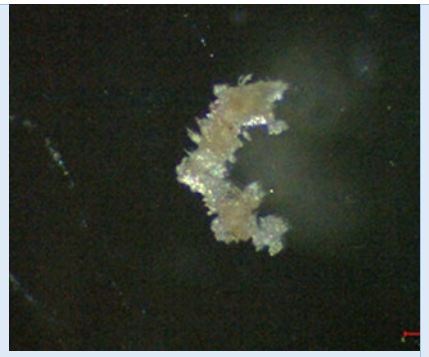
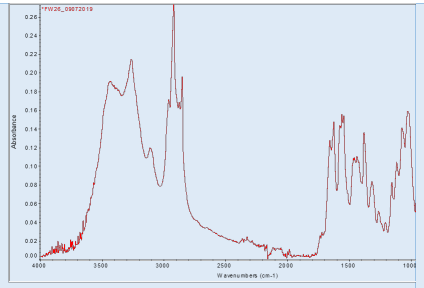
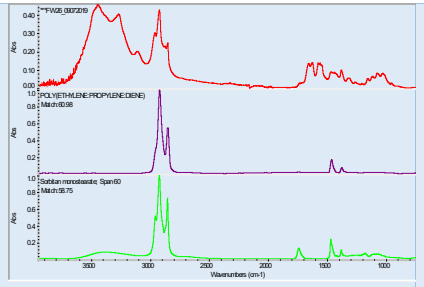
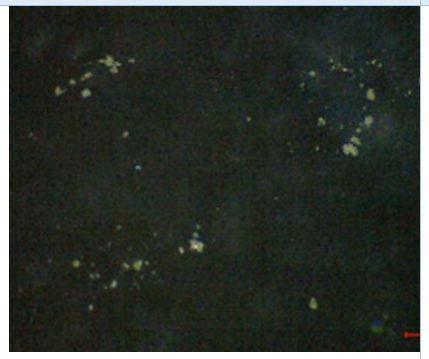
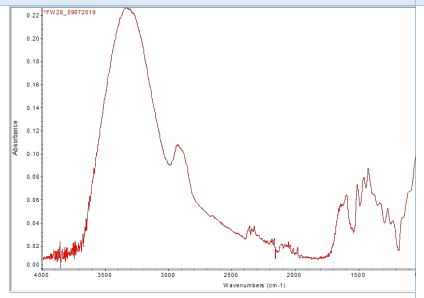
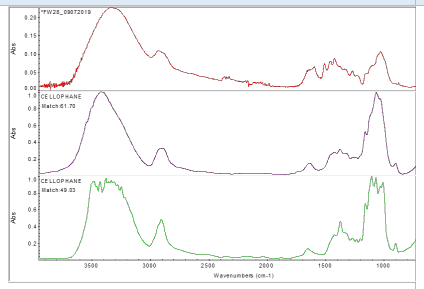
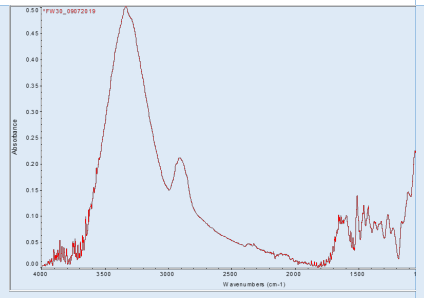
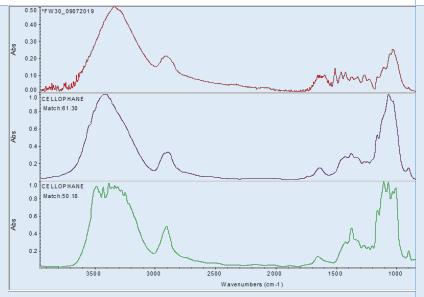
FW17_C				Poly(ethylene:propylene:diene) (87% library match)
FW18_A				Polyethylene (87% library match)
FW18_B				Poly(ethylene:propylene:diene) (80% library match)

FW18_C				Poly(ethylene:propylene:diene) (89% Library match)
FW_18D				Poly(ethylene:propylene:diene) (88% Library match)
FW19				Cellophane (70%)

FW21				Cellophane (55%) Not included as match <70%
FW22B				Poly(ethylene:propylene:diene) (72% library match)
FW23_A				Cellophane (61%) Not included as match <70%

FW23_B	Image not included			Cellophane (70%)
FW23_C	Image not included			Polyethylene (62%) Not included as match <70%

FW23_D	Image not included			Polyethylene (79%)
FW23_E	Image not included			Cellophane (64% library match) Not included as match <70%

FW26				Poly(ethylene:propylene:diene) (61% library match) Not included as match <70%
FW28				Cellophane (62% library match) Not included as match <70%
FW30				Cellophane (61% library match) Not included as match <70%

Appendix 5. Impact of particle size analysis (PSA) on the number of particles in sediment and associated variations between replicates.

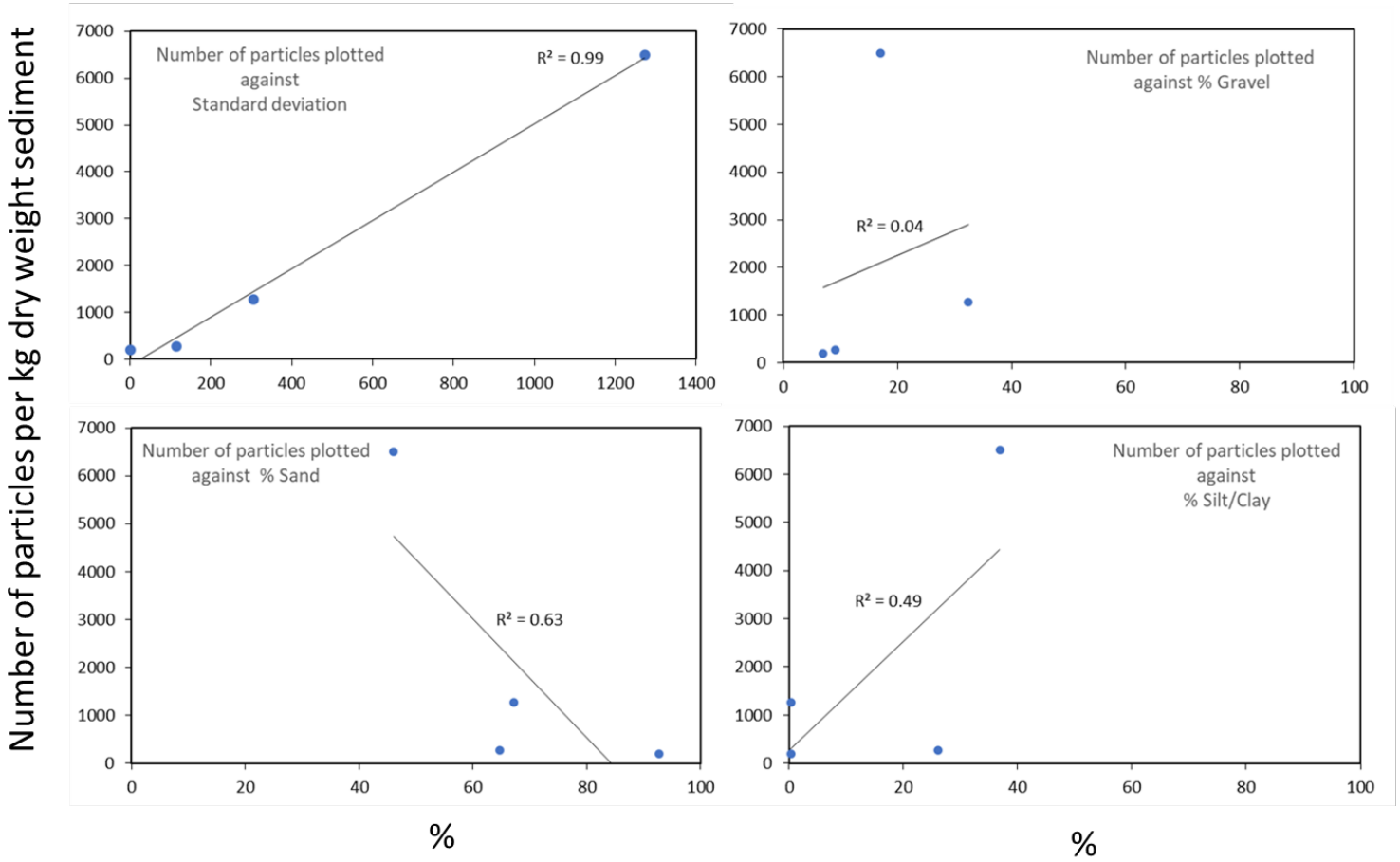


Figure A5.1 Number of particles per kg dry weight sediment plotted against % gravel, % sand and % silt/clay

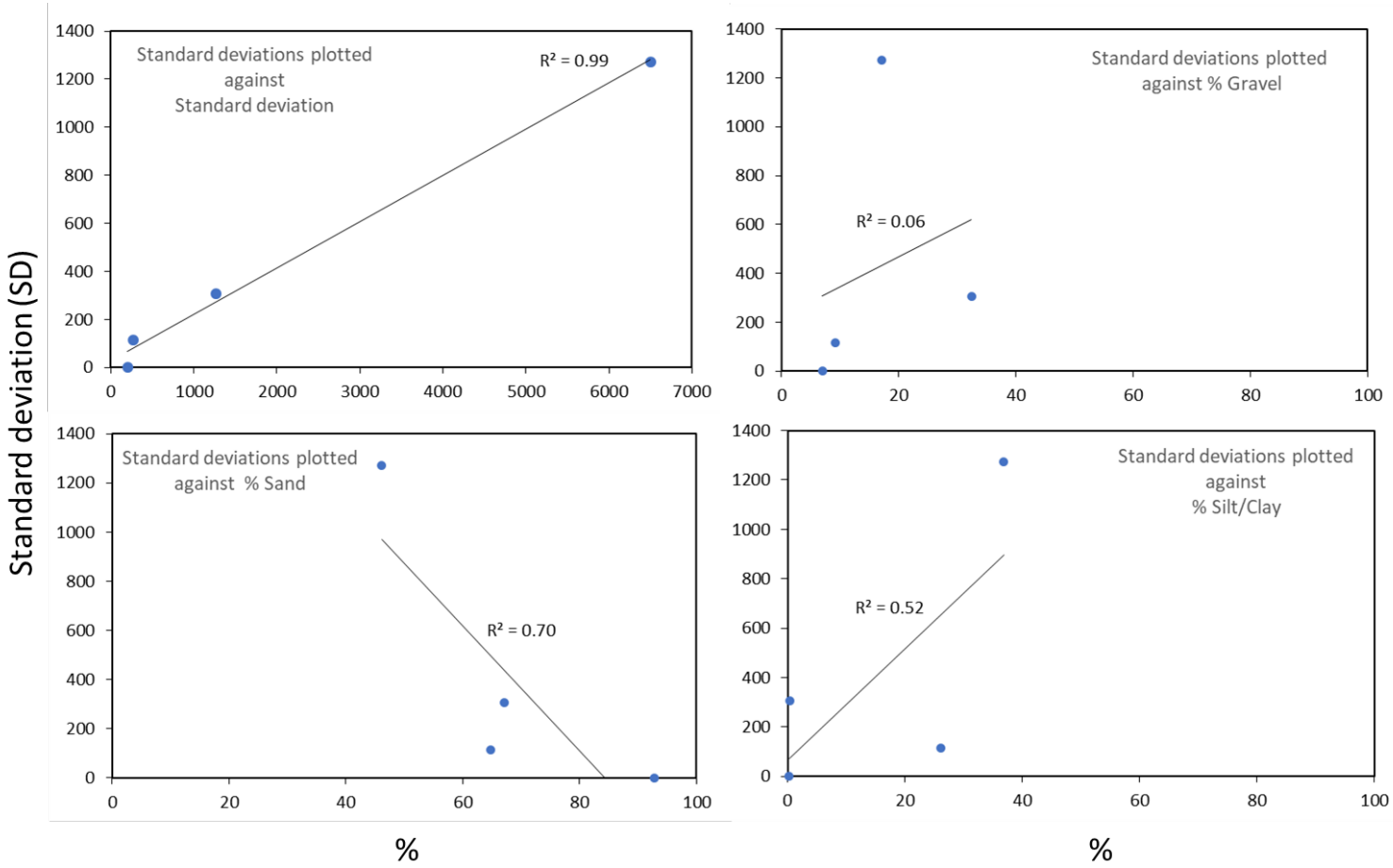


Figure A5.2 Number of particles per kg dry weight sediment plotted against % gravel, % sand and % silt/clay

Appendix 6. Certificate of participation and feedbacks questionnaires circulated to participants upon delivery of the core training.

CERTIFICATE of PARTICIPATION

THIS ACKNOWLEDGES THAT

Recipient Name

HAS SUCCESSFULLY COMPLETED THE

CLiP laboratory training course on extraction and quantification of microplastics in environmental samples between Wednesday 22nd May 2019 and Friday 24th May 2019, hosted at the University of Belize.

24 MAY 2019

SIGNED Dr Adil Bakir, Cefas

SIGNED Ms Freya Tooley, Cefas

Dear participant,

We would like to take this opportunity to thank you for attending the CLiP laboratory training course on the extraction and analysis of microplastics in environmental samples. We kindly ask that you provide some feedback following the event to help us improve our future training courses. Please return the completed questionnaire to freya.tooley@cefas.co.uk or adil.bakir@cefas.co.uk. Thank you for your participation and contributions to the training course — we hope that you enjoyed the training!

We ask that the following questions are answered on a scale from 1 — 10 [1: very poor, 5: average, 10: very positive]. Please mark the relevant box with a X. There will be room for any comments you may have.

1. How useful did you find this training?

1	2	3	4	5	6	7	8	9	10

Comments: _____

_____

2. How did you find the delivery of this training?

1	2	3	4	5	6	7	8	9	10

Comments: _____

_____

3. How did you find the catering facilities?

1	2	3	4	5	6	7	8	9	10

Comments: _____

_____

The following questions are open-ended and will be followed with space for comments. We'd appreciate any feedback you can offer.

4. What did you like most about the course?

_____
_____
_____

5. What did you like the least about the course?

_____
_____
_____

6. What can we do to improve the workshop for future events?

_____
_____
_____

Thank you for finding the time to complete this survey. We look forward to continuing our collaboration with you!

Centre for Environment Fisheries & Aquaculture Science

Cefas

About us

We are the Government's marine and freshwater science experts. We help keep our seas, ocean and rivers healthy and productive and our seafood safe and sustainable by providing data and advice to the UK Government and our overseas partners.

We are passionate about what we do because our work helps tackle the serious global problems of climate change, marine litter, over-fishing and pollution in support of the UK's commitments to a better future (for example the UN Sustainable Development Goals and Defra's 25 year Environment Plan).

We work in partnership with our colleagues in Defra and across UK government, and with international governments, business, maritime and fishing industry, non-governmental organisations, research institutes, universities, civil society and schools to collate and share knowledge.

Together we can understand and value our seas to secure a sustainable blue future for us all, and help create a greater place for living.

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Innovative, world-class science is central to our mission. Our scientists use a breadth of surveying, mapping and sampling technologies to collect and analyse data that are reliable and valuable. We use our state-of-the-art Research Vessel Cefas Endeavour, autonomous marine vehicles, remotely piloted aircraft and utilise satellites to monitor and assess the health of our waters.

In our laboratories in Lowestoft and Weymouth we:
- safeguard human and animal health
- enable food security
- support marine economies.

This is supported by monitoring risks and disease in water and seafood; using our data in advanced computer models to advise on how best to manage fish stocks and seafood farming; to reduce the environmental impact of man-made developments; and to respond to serious emergencies such as fish disease outbreaks, and to respond to oil or chemical spills, and radioactivity leaks.

Overseas, our scientists currently work in Commonwealth countries, United Kingdom Overseas Territories, South East Asia and the Middle East.

Our customer base and partnerships are broad, spanning government, public and private sectors, academia, non-governmental organisations (NGOs), at home and internationally.

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