LABPLAS

Horizon 2020
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Land-Based Solutions for Plastics in the Sea

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D2.2 First sampling campaigns and sample preparation

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PROJECT INFORMATION

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Nº	Participant name	Acronym	Country	Type
1	UNIVERSIDADE DE VIGO	UVI	SPAIN	HES
2	UNIVERSIDADE DA CORUÑA	UDC	SPAIN	HES
3	Bundesanstalt fuer Gewaesserkunde	BfG	GERMANY	RTO
4	LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA	INL	PORTUGAL	RTO
5	KATHOLIEKE UNIVERSITEIT LEUVEN	KUL	BELGIUM	HES
6	HELMHOLTZ ZENTRUM FUR OZEANFORSCHUNG KIEL	GEOMAR	GERMANY	RTO
7	NATIONAL OCEANOGRAPHY CENTRE	NOC	UNITED KINGDOM	RTO
8	SORBONNE UNIVERSITE	SU	FRANCE	HES
9	OPEN UNIVERSITEIT NEDERLAND	OUNL	NETHERLANDS	HES
10	LEIBNIZ INSTITUTE FOR BALTIC SEA RESEARCH	IOW	GERMANY	RTO
11	ASSOCIACAO PARA O DESENVOLVIMENTO DO ATLANTIC INTERNATIONAL RESEARCH CENTRE	AC	PORTUGAL	RTO
12	UNIVERSIDADE FEDERAL DE SAO PAULO	UNIFESP	BRAZIL	HES
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15	CONTACTICA S.L.	CTA	SPAIN	SME
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Executive summary:	This report corresponds to Deliverable 2.2 *First sampling campaign and sample preparation report,* resulting from Task 2.2: It covers the description of the sampling campaign of the first year including target environments, matrices, and details about sample preparation.

Version	Date	Comments
1.0	11/05/2022	Initial version
2.0	20/05/2022	GEOMAR, IOL, NOC and UDC revisions
3.0	27/05/2022	Revised, corrected and formatted version
4.0	10/02/2023	Reviewed with changes to address comments by the PO and reviewer

Disclaimer

The views and opinions expressed in this document reflect only the authors' views, and not necessarily those of the European Commission.

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ABBREVIATIONS AND ACRONYMS

Abbreviation / Acronym	Description
AI	Artificial Intelligence
Cux	Cuxhaven
d.w.	dry weight
GIT	Gastro intestinal tract
HH	Hamburg
MP	Microplastics
SDS	Sodium dodecyl sulfate
SML	Water Surface Microlayer
SMNP	Small micro and nano plastics
SOP	Standard Operating Procedure
TBD	To be determined
ULW	Underlying Water

1 INTRODUCTION

Plastic is pouring from land into our oceans at a rate of nearly 10 million tonnes a year. Once in the sea, plastics fragment into particles moving with the currents and ocean gyres before washing up on the coastline. The smaller the size the higher the risk posed by these particles to organisms and human health. Because small, micro- and nano-plastics (SMNP) cannot be removed from oceans, proactive action regarding research on plastic alternatives and strategies to prevent plastic from entering the environment should be taken promptly. The LABPLAS project is a 48-month project whose vision is to develop new techniques and models for the detection and quantification of SMNP. Specifically, LABPLAS will determine reliable identification methods for a more accurate assessment of the abundance, distribution, and toxicity determination of SMNP and associated chemicals in the environment. It will also develop practical computational tools that should facilitate the mapping of plastic-impacted hotspots and promote scientifically sound plastic governance.

This document corresponds to Deliverable 2.2 *First sampling campaign and sample preparation report*. Standard Operating Procedures (SOPs) for sample preparation are described in WP3 and WP4 and therefore these methods are only briefly explained in this document. Annex 1 shows the sampling and characterisation particle sizes throughout the project.

Winter sampling campaigns on the Elbe and the Thames rivers were successfully conducted and a delay occurred for the Mero-Barcés and the North Sea sampling sites. The delayed samplings will be reported in D2.3. To maintain the connection between the Thames, Elbe and the North Sea, the seasonal sampling campaigns in the river basins will be conducted at the same time as the two North Sea cruises in the Winter and Summer of 2023.

2 SAMPLING CAMPAIGNS

2.1 River Thames

In January 2022, the team at the National Oceanography Centre (NOC) successfully completed their first sampling campaign in the River Thames. Six sites were sampled (Fig. 1). This campaign was divided into two parts: 1) tidal and estuarine sampling (sites 4-6), and 2) freshwater sampling (sites 1-3). Part 1 was carried out together with the Environment Agency which does monthly surveys of the tidal Thames. This collaboration enabled the team to access all three designated LABPLAS sites in the tidal part of the river: the estuary, central London, and the upper tidal limit. Part 2 was carried out with a small boat to the designated sites in the freshwater Thames, sampling at three sites corresponding to urban and rural influences.

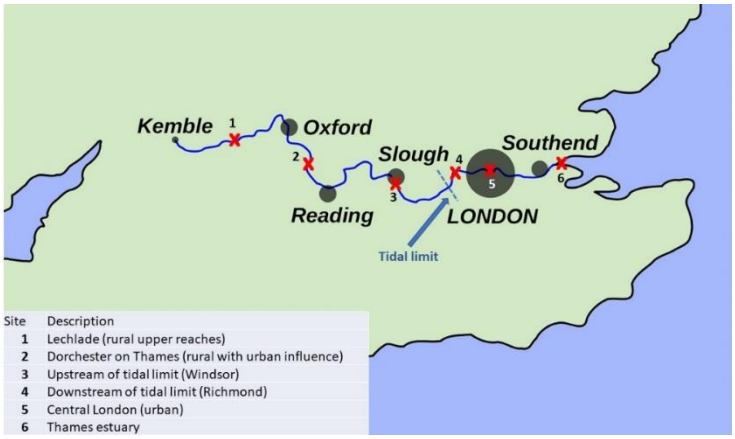


Figure 1. Map of sampling sites in the River Thames

Surface water samples were collected with a custom-built pump-filter system enabling the filtering of tens to hundreds of litres of water at each site, to collect microplastics >10 µm in size. The device consists of a fuel-powered pump (Honda WX 10 portable 1" water pump), connected to a 10 µm filter cartridge within a stainless-steel filter holder (EYS Spectrum Inox Economic Stainless Steel Cylindrical Cartridge, within SPECTRUM Inox Economic EFH Filter Housing). All hosing prior to the filter is made of silicon, to eliminate the possibility of plastic contamination of samples from the equipment itself. The sampler has been designed so that the filter unit can be isolated using a ball valve, and the pump system flushed with water between sites to prevent cross-site contamination (Fig. 2). The pump-filter unit is housed within a frame for easy transportation (Fig. 3).

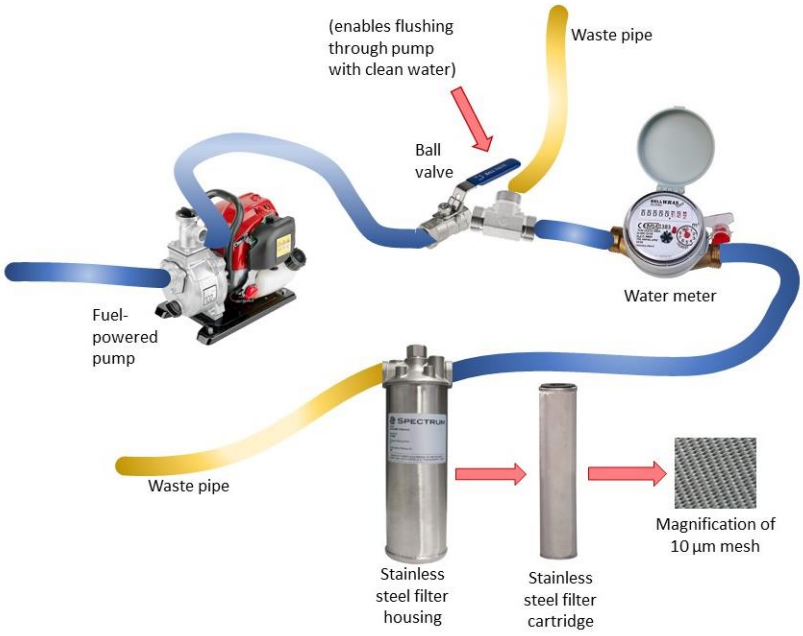


Figure 3. Diagram of pump-filter sampling equipment used for water sampling in the River Thames

Figure 2. Photo of pump-filter sampling equipment used for water sampling in the River Thames

Based on time constraints, filtering was always carried out for 20 minutes, regardless of the ultimate volume pumped. The filtered volume, therefore, ranged from 45-450 L depending on the turbidity of the water at each site. Between each site, the filter and housing were changed out to collect the new sample. At any time when the filter holders containing filter cartridges were not connected to the pump system, the inlet and outlet of the filter holders were covered with aluminium foil to prevent contamination from entering the filter.

A Manta trawl (Hydrobios Microplastic Net) was also used to collect particles in surface water greater than 335 µm in size, i.e. larger particles. This is a common method used in global surface water sampling efforts, and thus enables comparison with other studies. The Manta net was trawled for ~15-20 minutes or 17-55 m^3 sampled depending on the flow at each site.

Sediment and invertebrates were collected from the riverbed using a 15 L Day or a 5 L Van Veen grab at the tidal survey locations and in the freshwater survey locations, respectively. Samples were transferred into 1 L glass jars for transportation and storage until analysis.

The sampling campaign will be repeated in July 2022 as planned. In the meantime, protocols are being developed for the processing and analysis of different sample types between LABPLAS partners, to determine microplastic amounts within the different matrices.

2.2 Elbe River

In January 2022, the first sampling campaign of the LABPLAS along the Elbe river took place. During two weeks, researchers from the German Federal Institute of Hydrology (BfG) sampled seven sites in the river. Cooperation with the Federal Water and Shipway administration enabled the researchers to be on a vessel and to sample in the middle of the river.

The tidal part of the Elbe was sampled in the estuary (Cuxhaven), downstream of the urbanized area of Hamburg and at the tidal limit in Geesthacht. As a barrage is located at the tidal limit, samples were taken up and downstream to see how much the barrage influences the sedimentation of plastics. Freshwater samples included industrial and rural influences at Dömitz, Dessau and Wittenberg (Fig. 4).

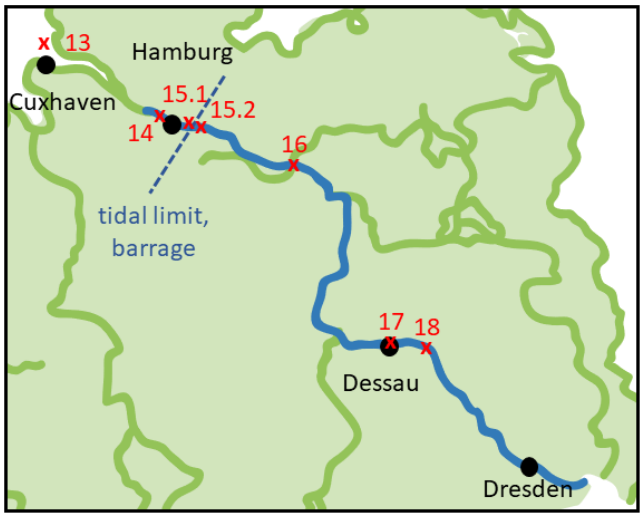


Figure 4. Map of sampling sites in the River Elbe

Small microplastics were collected with a filter cascade and a submersible pump (10 µm to 1000 µm), comparable to the one used for the Thames. At the Elbe, 10 µm and 1000 µm filter cartridges were used. A 100 µm filter cartridge was added in between to prevent fast clogging. If a cartridge clogged, it was replaced by a new one. Filtering of the water was carried out for 3-5 hours, depending on the availability of a ship with a total water volume of 0.7 to 1.9 m³.

Figure 5. Filter cascade 10 µm – 1 mm

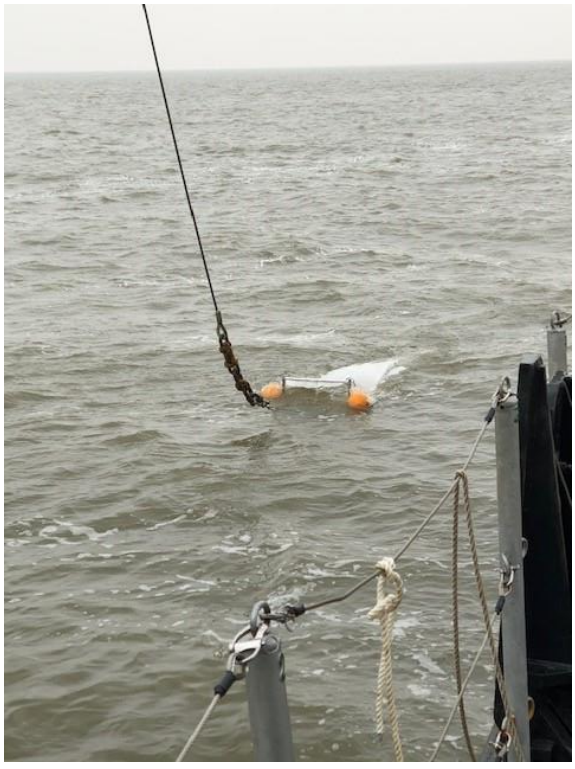


Figure 6. Manta trawl with a mesh size of 335 µm

Larger microplastics >335 µm were collected with the same Manta net (Hydrobios Microplastic Net) used for the Thames. The Manta net was attached to a crane next to the boat and hung in the water for 15-20 minutes. Only at one site, trawling was necessary as the current of the river was not strong enough. 130-250 m³ of water were sampled depending on the sampling site. As the samples were scanned for zooplankton first, they were fixed immediately with 4% buffered formalin.

Further samples for zooplankton were taken with a 200 µm manta net at two different depths (40 cm, 1.50 m) in the estuary, and for nanoplastics, at the site of Hamburg, one sample was collected via the pump and another one via the microlayer.

Sediments were taken with a Van-Veen grabber from the edge of the river where fine-grained particles accumulate.

Water surface microlayer (SML) sampling was conducted at the Elbe river on the upwind side of the vessel using a screen sampler (Garrett-Screen, stainless steel, mesh size 1.2 mm). Samples were collected in deionized water-rinsed, brown borosilicate bottles. Reference samples were taken from the underlying water (ULW).

Macroplastics were not spotted in the Elbe river. Therefore, macroplastics were collected along a beach in the city of Hamburg. Several pieces of macroplastics were sent to the University of Vigo for further analysis.

2.3 Mero-Barcés River Basin

A delay in the first sampling campaign at Mero-Barcés planned for January 2022 occurred due to problems with the custom-built pump-filter system (fig. 7). The sampler system is based on the design by ASTM Standard D8332 (2020) for the collection of water samples with suspended solids for identification and quantification of microplastic particles and fibres. The water sample is passed through a series of stacked stainless-steel sieves with the following mesh sizes: 1000 µm, 300 µm and 10 µm. The last 10 µm filter led to an overpressure in the filtering module that created leaks and damaged the filter itself. The latter issue has been solved in cooperation with the factory that made the filter sieves and ongoing adjustments are being made in the laboratory of the UDC. The Consortium has agreed to re-schedule this delayed campaign and conduct it in December 2022.

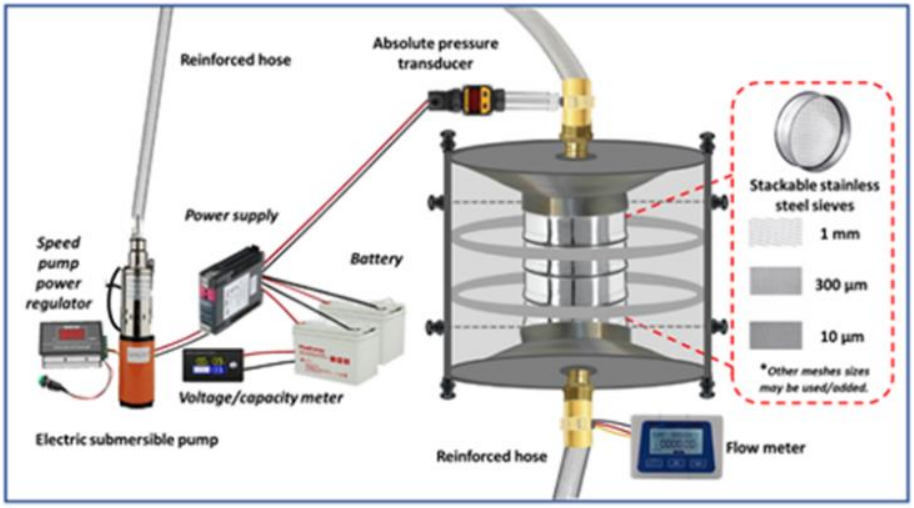


Figure 7. Diagram of the pump-filter system for water sampling 10 µm–1 mm

Meanwhile, the first summer sampling campaign is expected to be carried out successfully in July 2022, as planned, once the adjustments to the sampling system have been overcome (fig. 8).

Figure 8. Proposed sampling sites at the Mero-Barcés river basin

2.4 North Sea

The North Sea cruises planned for year 1 in LABPLAS are delayed due to scheduling challenges. A proposal for two North Sea cruises on RV ALKOR (Proposal number GPF 22-1/049, "North Sea Plastics I & II") was submitted to the Office of the Assessment Panel for Research Vessels (GPF) of the German Research Foundation (Geschäftsstelle des Begutachtungspanels Forschungsschiffe (GPF), Deutsche Forschungsgemeinschaft e.V.) in August 2021. Normally, proposals associated with awarded projects such as LABPLAS are granted ship time according solely to the appropriateness of the proposed work and requested duration. However, the current proposal was sent for full review and eventually declined because it was considered standalone and not an integral component of LABPLAS, as it should have been. This has been discussed with the GPF, and the proposal has been resubmitted (Proposal number GPF 22-2/029). We have every expectation that the next evaluation will be successful and the cruises awarded within an acceptable time for LABPLAS.

To mitigate risks associated with the delay, alternative options for sample collection in the North Sea have been identified to provide LABPLAS partners with the materials required for method development. The collection of these samples is planned for May and October 2022.

3 LABELLING OF SAMPLES

The LABPLAS partners decided to conduct a consistent sample labelling as each partner receives samples from other laboratories for sample treatment and analysis.

Example: January pumped water sample at Chapman Buoy: T6-01-PF-01-01

Sampling site:

Thames: T	North Sea: NS	Elbe: E	Mero-Barcés: MB
T1 = Lechlade	NS7 = Thames estuary	E13 = Cuxhaven	MB1 = Mero River upstream
T2 = Benson	NS8 = Southern Bight	E14 = Hamburg	MB2 = Barcés River upstream
T3 = Windsor	NS9 = Broad Fourteens	E15.1 = Elbstorf	MB3 = Reservoir
T4 = Isleworth	NS10 = West Frisian Is.	E15.2 = Geesthacht	MB4 = Reservoir
T5 = Victoria Dock	NS 11 = East Frisian Is.	E16 = Dömitz	MB5 = Mero downstream
T6 = Chapman Buoy	NS12 = Elbe estuary	E17 = Dessau	MB6 = Burgo estuary
		E18 = Wittenberg	

Sampling round:

01 = Jan 2022
02 = July 2022
03 = Oct 2022
04 = Jan 2023
05 = April 2023
06 = July 2023

For equipment codes:

PF - pump filter
MN - manta net
SG - sediment grab
BO - bottle (for nanoplastics)
SM - surface microlayer

For sample type:

01 = surface water (pumped)
02 = surface water microplastics (trawled)
03 = surface water zooplankton (for those partners who will take a separate zooplankton sample)
04 = bulk sediment
05 = sediment invertebrates
06 = nanoplastics

Sample number:

01/02/03 etc (most of these will be 01 as we are not taking replicates)
Depth (if present): in cm

Sediment cores:

Sampling site: Baltic Sea: BS

Sampled core:

GF= Gulf of Finland

EGB= East Gotland Basin

LD= Landsort Deep

For equipment codes: SC - sediment core

Sample layer in each core: 01/02/03 etc being the first sample at the top of each core

Depth: in cm

4 SAMPLE PREPARATION

4.1 Laboratory analysis of collected samples

To compare all results from field surveys, LABPLAS partners decided that each sample compartment would be analysed by one laboratory within this Consortium. Samples are at this moment in transport or already started to be analysed by each partner.

- **Water Small microplastics** (10 µm - 1 mm): preparation and analysis at the National Oceanographic Center (NOC)
- **Water Larger plastics** (> 335 µm): one part of the samples: preparation at the Sorbonne University (SU), scanned for zooplankton analysis or preparation directly at GEOMAR; plastics characterized at GEOMAR
- **Water Surface microlayer**: sample preparation at GEOMAR, analysis at UDC
- **Sediment**: preparation and analysis at the German Federal Institute of Hydrology (BfG)
- **Sediment cores**: preparation at IOW, analysis at BfG
- **Invertebrates**: at Sorbonne University (SU), TBD
- **Zooplankton**: at Sorbonne University (SU)
- **Bivalves**: preparation and analysis at BfG/UDC
- **Fish**: preparation and analysis at National Oceanographic Center (NOC)
- **Atmospheric deposition**: sample preparation and analysis at BfG/UDC (TBD)
- **Blank**: each laboratory

4.2 Sample preparation methods

The collected samples are currently prepared based on the SOPs submitted in D3.1 *SOPs for MP isolation, characterization and identification* (WP3). These SOPs will be reviewed and modified until 30.11.2022 when MS25 *Harmonized analytical methodologies for MP determination in environmental samples* is due. Following, details about sample preparation are described, which have not been included in D3.1.

4.2.1 Water and zooplankton samples

4.2.1.1 Nanoplastics

The water samples for nanoplastics analysis were collected in glass bottles protected from light and with aluminium foil in the mouth avoiding contact with plastic caps. The samples were not treated or pre-processed (neither acidification nor filtration) to avoid degradation or nanoplastics' aggregates losses. Samples were shipped and stored at 4ºC for further analysis (within 1-2 weeks since reception). Details about sample preparation for nanoplastics determination are described in D4.1. *Guideline for methods for extraction, pre-concentration and purification of SMNP*

4.2.1.2 Surface microlayer

Depending on the particulate material contained in the samples a volume of 500-1500 ml was filtered onto one filter by deploying a vacuum pump (fig. 9). The filtered water was placed in glass jars and 50 ml SDS solution was added (fig. 10). Samples were incubated at +40°C at 130 U/min for 24 hours in an incubation chamber (New Brunswick Scientific, Innova 44, fig. 11), 30% H_2O_2 was added and incubated for 48h at 130 U/min.

Figure 9. Filtration set-up

As the samples of Hamburg and Cuxhaven were still not transparent and filtration was very slow, they were again incubated with 10% KOH and 0.1% TX-100 solution. Samples were recovered by filtration onto a 10 µm stainless steel mesh using a vacuum filter apparatus. For transport, all filters with the sample extracts were placed into MilliQ-washed jars and sealed with an aluminium cover.

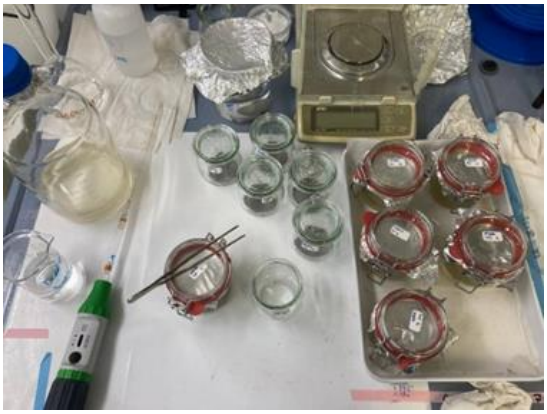


Figure 10. Aluminium-covered jars used for sample treatments

Figure 11. Incubation chamber

4.2.1.3 Small microplastics (10µm-1mm)

In a laminar flow hood, the filter cartridges (10 µm) from the sampling were put in a sample beaker and the interior was rinsed three times with MilliQ. The particles outside the filter cartridge were rinsed off with MilliQ water starting from the top to the bottom. With a silicone spatula, the particles that got stuck on the mesh were scraped and rinsed off the spatula. For ensuring that all particles were washed off, the filter cartridge was rinsed again a few more times (fig. 12).

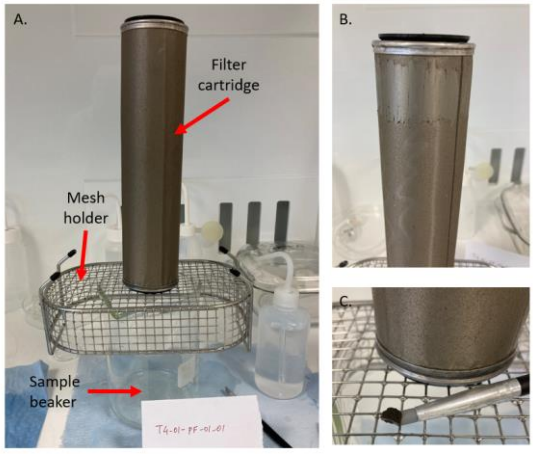


Figure 12. The set-up for rinsing particles off the 10 µm filter cartridge in the laminar flow hood. The main components of the set-up are indicated in A. The scraping of the particles with the silicone spatula is shown in B and C

4.2.1.4 Manta net and zooplankton samples

Plastics and zooplankton were collected at the surface using Manta nets and vertically using a WPII. In the laboratory, samples were gently transferred to a Petri dish and plastic particles were manually separated from other components such as wood, zooplankton and organic tissues. Each matrix (plastic and zooplankton) was enumerated by digital imaging using a ZoosCan scanner with a resolution of 2400 dpi (Gorsky et al., 2010). Post-processing of the images was performed with Zooprocess and plankton Identifier software that provides a large set of morphological parameters for each object and taxonomic identification of zooplankton. The following parameters were calculated for each particle: surface area (mm^2), length (mm), equivalent spherical diameter, and circularity. Plastics were also semi-automatically classified into six shape categories (rigid fragments, films, foam, granules, rope filaments, and microfibers) using an AI-based machine-learning process. The polymeric composition of the plastic particles was then analyzed.

4.2.2 Sediments

4.2.2.1 Sediment grab samples

Grain size analysis will be carried out for all sediment samples. The analysis provides information about the texture (percentage of clay, silt and sand particles). Samples were treated with sodium pyrophosphate ($Na_4P_2O_7$) to avoid coagulation and measured with laser diffraction (Laser Horiba LA 960; size from 0.4 to 2000 µm).

All samples were wet-sieved with stainless steel sieves and mesh sizes of 10, 20, 50, 100, 200 and 1000 µm (Retsch AS 200) (fig. 13). Plastic particles >1000 µm are picked manually from the sediment. Two fractions (e.g. 10-100/200 and 100/200-1000 µm) were density-separated with saturated potassium formate (K(HCOO)) solution to remove inorganic sediment and treated with 35% hydrogen peroxide (H_2O_2) to dissolve the organic matter (for further details see 4.2.2.2 sediment core samples). The different sizes of the fractions are necessary as the LDIR cannot analyse particles with a too-large size difference. Size classes were chosen depending on the amount of material available. After measuring, the entire sample will be represented with one result from 10-1000 µm or 10-5000 µm if available.

Figure 13. Wet sieving of sediments

4.2.2.2 Sediment core samples

The first core was successfully sampled and the purification of samples is ongoing (Figs. 14a-d). The core is EMB 262 12-2 and was retrieved in the Gulf of Finland in 2021. The sampling strategy aimed to maximize MP recovery by merging consecutive layers to have at least 1 g d.w. for analysis. For this core, 20 samples will be analysed. Interesting features of this core are its proximity to the coast, and relatively high sedimentation rates mainly around after 1950

Figure 14. a, b, c, d: EMB 262 12-2 during sampling. Contamination was minimized to guarantee excellent results

All samples are processed inside clean benches to minimise airborne contamination (fig. 14a). These benches are used exclusively for the LABPLAS project. The other 2 cores that will be sampled and analysed have already been selected, one at the Landsort deep (POS507/25-1 MUC + POS507/29-2 MUC) and one from the East Gotland basin (EMB262/6-28 MUC). These are excellent cores and are already available at the IOW to be sliced and sampled.

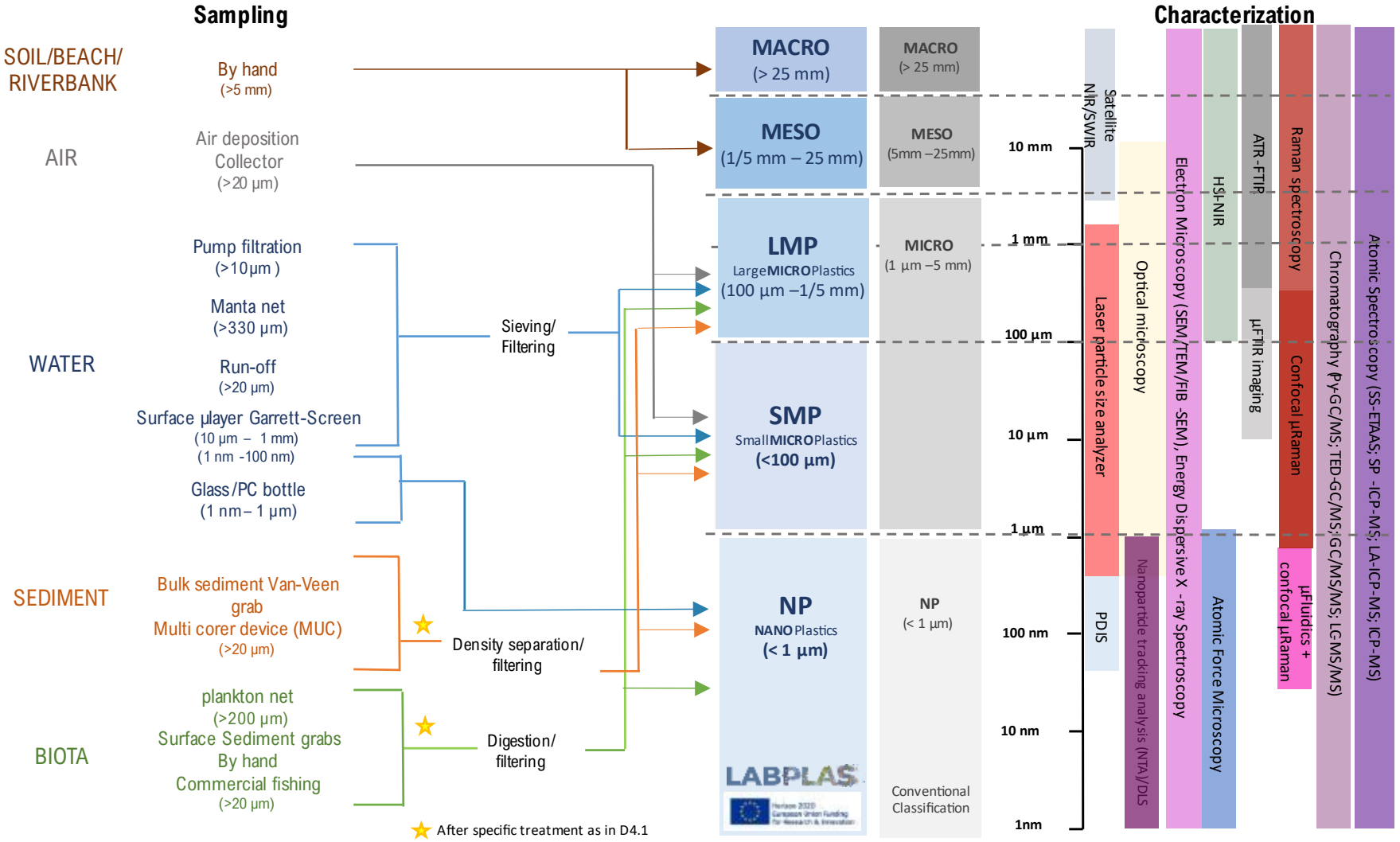
Additionally, a long core EMB262/6-30 GC has been sampled to create a contamination background for the laboratory at the IOW. Consecutive layers at the Medieval Climate Anomaly (1,200-700 years BP), when conditions are comparable to post-1950 conditions were sampled. Samples are ready for spectroscopy analysis.

4.2.3 Fish processing

Fish are weighed and measured for total length and fork length (where fork length is relevant to the species). The gastrointestinal tract (GIT) is dissected (Fig. 15), placed into a clean glass beaker and weighed. To this, 10% KOH is added, and the sample is incubated in a rotating shaker for 48h at 40 °C. After 48h, the remaining undigested particles are filtered out onto a 10 µm stainless steel mesh using a vacuum filter apparatus and resuspended in MilliQ water. To this, canola oil is added and the sample is vigorously mixed to ensure the oil is dispersed within the water. The sample is then left overnight to separate. After 24 hours, the overlying oil is poured off, and again oil is added to the remaining water. The mixing and overnight settling are then repeated twice more. After 3x flotation, all oil is filtered onto the same 10 µm stainless steel mesh, and the sample is then added to H_2O_2. The sample is again incubated in a rotating shaker for 48h at 40 °C. Following this second digestion, the sample is filtered onto the same 10 µm stainless steel mesh and suspended in Decon 90 detergent for 24h to remove any residual oil. Finally, the Decon 90 is filtered out using a clean 10 µm stainless steel mesh, the filter is flushed well with MilliQ water, and the sample is rinsed into a glass vial with 70% ethanol.

Figure 15. Dissection and removal of GIT from plaice fish

ANNEX 1: LABPLAS PROJECT SAMPLING AND CHARACTERIZATION PARTICLE SIZES



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