COLLECTION STANDARD OPERATING PROCEDURE

MICROPLASTICS

I. Sample collection

- Once at the station
- Perform a CTD cast to retrieve the vertical profile of Temperature and Salinity
- Record the GPS coordinates, the exact starting time and the flow meter reading (Fig. 1B).
- Deploy the manta net from the side of the vessel using a spinnaker boom out of the wake zone (approx. 3 – 4 m distance from the boat – Fig. 1A)

Figure 1 A) deployment of the manta net; B) recording coordinates, time and flow meter readings

- Navigate in one straight direction with a speed of approx. 2 – 3 knots for 30 min (20 min is allowed if the load is predicted to be high)
- After 30 min stop the boat and record the final GPS coordinates, the stop point of the flow meter and the average boat speed
- Rinse the manta net thoroughly with seawater from outside and from mouth to the cod end in order to collect all particles into the cod end.

Warning: Never rinse the sample through the opening of the net in order to prevent contamination.

- Perform the CTD cast at the end station
- Safely remove the cod end and pour the content into a bucket half filled with seawater (Fig. 2)

Figure 2 Emptying cod end into bucket with seawater

- After collecting the larger floating microplastics, if needed, pour the content through two metal sieves of 5000 and 300 micron size and 20 cm in diameter

Warning: Pour delicately and gradually, as the 300 micron sieve might clog faster that the larger one, especially when lots of organic particles are present (e.g. zooplankton)

- Take a photo of the two sieves (Fig. 3)

Figure 3 Picture of the two sieves after filtering

- Select representative particles of microplastics from the 300 micron sieve using previously sterilized tweezers (with alcohol) (Fig. 4).

Figure 4 Selected particles of microplastics

- Selected pieces should be representative of type, shape, color and abundance
- Dispose the microplastics in a clean glass Petri dish for further processing
- Move the content of the 300 micron sieve into a glass container previously labeled with station name and date.
- Add 20 ml of Ethanol EM grade to fix the sample
- Store in the refrigerator (4°C)

II. Microplastics processing

- Rinse the single microplastics with sterile filtered seawater
- If possible, subdivide the pieces in 3 with a sterile razor blade
- Clean the razor blade with alcohol between samples
- Place one piece in a 2-ml eppendorf filled with 500 µl lysis buffer for DNA extraction
- Make sure that the piece is immersed in the buffer (lysis buffer is kept at RT).
- Sample is stored at 4°C
- Place another piece in a 2-ml eppendorf to be later filled with 500 µl 4% paraformaldehyde for SEM analysis
- Make sure that the piece is immersed in the fixative (paraformaldehyde is kept in the refrigerator at 4°C).
- Sample is stored at 4°C

Warning: After 1-22 hours the SEM samples need to be transferred to PBS:EtOH 50:50 (v:v) and stored at -20°C

- Place the third piece in an empty 2-ml
- Sample is stored at RT

Warnings:

- If the piece is too small to be cut in 3, cut in 2 and do only the first 2 processing. In case is not possible to cut in equal pieces, dedicate the largest sample to DNA extraction
- If a piece is too small to be cut, use it for DNA only
- Make sure to mark any detail on the logsheet, especially the vial number, microplastic type, color, processing (DNA/SEM/etc)

III. List of material, equipment and supplies

- Tweezers
- Bucket
- Glass containers
- Eppendorf tubes
- Marker
- Sieves (300 um and 5 mm)
- Petri dishes
- Razor blade
- Lysis Buffer
- 4% PF
- Ethanol 70%
- Parafilm
- Tape for labelling
- Cooler
- Pipette P1000
- Tips 1000
- Milliq
- Filtered Seawater
- Ruler
- Alternative cod-end