15. MICROPLASTICS - A POTENTIAL THREAT TO THE REMOTE AND PRISTINE ECOSYSTEMS OF THE ANTARCTIC SEAS?

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Objectives

Microplastics (MP) pose an emerging threat to the global environment. Wherever one searches, MP is found, albeit in differing concentrations and constitutions. The Southern Ocean around Antarctica is thought to be an exception because it is considered beyond the reach of human impact (apart from scientific and leisure tourism). Furthermore, the major current systems of the Southern Ocean are thought to provide an effective barrier against the transfer of MP from lower latitudes to the Antarctic Ocean. However, very recent studies indicate that MP is present in the Southern Ocean (Cincinelli et al., 2017). This highlights an urgent need for investigations into the possible origin and fate of MP: concentrations and distributions in the Southern Ocean, sources originating in Antarctica, and, finally, potential transfer into Antarctic food webs. The conducted microplastic studies should furthermore provide a baseline for future research on the topic.

This project will explore MP occurrence and distribution in water and biota such as zooplankton and fish. To obtain insights into the origins and impacts of MP, we will study the microbial community composition on the plastic particles, as well as the persistent organic pollutant (POP) load of MP. To structure the research, we address these hypotheses: (1) The concentration of MP is higher in the more anthropogenically-exposed Western Antarctic Peninsula (WAP) and Scotia Sea than in the Weddell Sea (WS); (2) MP in the Southern Ocean around Antarctica originates from outside the ACC, i.e. north of the Polar front; (3) MP from local sources, i.e. research stations, and research and cruise vessels, contribute detectably to the MP load; (4) Microbial colonization of MP can inform MP origins; (5) The abiotic polymer characteristics (particle morphology, polymer type) and the POP profile of MP in the Southern Ocean around Antarctica reflect the characteristics of their sources; and, (6) Microplastic particles will enter Antarctic food webs. To reach the goals, we will sample MP in the water column, in filter-feeding zooplankton and fish of the different sites in the Southern Ocean (WS, WAP, Scotia Sea) and compare them with selected samples from subtropical southern gyres; we will characterize the MP particles with respect to their morphology, their polymer composition, the profile of adsorbed POPs and of the resident microbial communities. The microbiome work will include molecular analyses, as well as novel microscopic characterization using multiple probes combined with spectral analysis to unravel the spatial organization of the microbial communities. The polymer analysis will comprise ATR-FT–IR and microscopic FT–IR. Both approaches will contribute to assessing the importance of local sources (e.g. waste water treatment plants) and their potential origin from sites outside the ACC. The results will provide critical empirical data for ocean circulation transport models to backtrack the origin of floating MP based on probabilistic

models of surface flow in the Southern Ocean. Our ultimate goal is to answer questions on the concentration and distribution of MP in the Southern Ocean, its potential sources and its uptake into the food web.

Work at sea

Surface water was sampled for MP with a Manta Trawl (floating neuston net towed to on-board crane for trawling; *n* = 10, Figure and Table 15.1). Sub-surface water was sampled by filtering pumped seawater from beneath the vessel at approximately 11.2 m depth (*n* = 27, Figure and Table 15.2). MP collected will be analysed for polymer type, particle morphology and size, POP and Plastisphere (inhabiting microbial community characterizations). 13 sediment samples were taken with a multicorer (MUC, Table 15.3).

Suspended surface solids sampled by Manta Trawl

15 trawls were attempted during PS111, 10 were completed successfully (Fig. 15.3, Table 15.1). Two trawls were aborted due to grease ice accumulation in the mesh after 15 and 20 minutes when air temperatures surpassed –13.2° C with winds of >5.4 m s^{-1} and water temperatures below –1.7° C (MT Nr. 12); two were cancelled pre-sampling due to ice conditions and 1 was cancelled pre-sampling due to too strong winds for launching (38 knots, 8 Beaufort). The Manta Trawl (MT) (aperture: 60 cm x 18 cm) is equipped with a mechanical flowmeter and a 300 µm mesh with a removable cod end. The MT (total weight ≈ 15 kg) was deployed by an on-board crane adding an 8 kg steel weight to improve stability against dynamic forces such as wind and waves (Fig. 15.1). The steel rope was released long enough to allow for a flat sampling angle (≤ 30°) approximately 20 m behind the crane at 5–8 m away from the side of the hull (depending on wind, current and vessel course) at starboard. The tows were performed at a vessel speed of 3 knots during a target trawling duration of 30 minutes (in case the cod end filled up with plankton or ice the sampling time was reduced, see Table 15.1). This resulted in an average (± SD) of approximately 306 ± 92 m^3 of filtered seawater per successful sample and a total of 3,064 m^3. After every tow the MT was hauled from the water and the content of the removable cod and subsequently transferred into a Bogorov counting chamber for visual inspection (*n* = 7, using a stereomicroscope (Olympus SZ61) equipped with a camera (Olympus SC50) and connected to the imaging software CellSens Entry (Version 1.17.16030.0; three samples will need purification treatment at the mainland laboratory prior to visual inspection due to the high abundance of biological residue). Putative anthropogenic particles were sorted and characterized microscopically. Samples for microbial DNA-analysis were fixed in 2 mL PureGene lysis buffer, samples for microscopy via FISH and CLASI-FISH were fixed in paraformaldehyde (for less than 24 hours) then transferred to 50 % ethanol in PBS for storage at –20°C. To compare free-living microbial communities with those on MP, 2 L of seawater were drawn with

Fig. 15.1: Manta Trawl deployed in the Southern Ocean

a plastic bucket during MT trawling and subsequently filtered through a 0.2 µm Sterivex™ cartridge filter (Millipore). Cartridges were subsequently flooded with 2 mL of PureGene lysis buffer. If particles conspicuously appear to be plastic as determined by eye (e.g. based on color, texture and shape; Noren et al., 2007), these will be analysed for microbial community composition first and subsequently identified using FT–IR. 7 particles from the Trawls 1, 3, 7, 9 and 10 were saved for this purpose.

Sub-surface suspended solids sampled by on-board sea water pump

To address the MP load in the sub-surface water layer, 32 samples were taken from pumped seawater intake in the on-board wet lab. A Klaus Union Sealex Centrifugal Pump (Bochum, Germany) delivered seawater from approx. 11.2 m depth to the laboratory via stainless steel pipes (first described by Lusher et al., 2014). The water was filtered through a stack of geological sieves. 14 samples were taken using a 20 µm sieve (combined with 100 µm and 300 µm sieves) and 18 samples were taken using a 100 µm sieve as the lower mesh boundary (combined with a 300 µm sieve). Sieves were protected from airborne contamination by a dimension-tailored solid wooden construction additionally sealed off with aluminium foil (Fig. 15.2, adapted from Kanhai et al., 2016). A mean (± SD) water flow duration of 10.3 ± 9.5 hours at 0.05 ± 0.02 L s^{-1} resulted in an average of 2.35 ± 2.36 m^3 filtered seawater per sample. After every filtration process residues of all involved sieves were pooled in a pre-rinsed glass jar using pumped seawater from the stainless steel wet lab pipe system and a PTFE squirt bottle as a rinsing agent. Samples were sealed with metal lids, labelled by the lowest applied mesh size and stored in v:v 50:50 suspended sample:EtoH at 4° C.

Fig. 15.2: Stack of geological sieves (20, 100 and 300 µm, left). Covered sieve stack with wooden protection, sealed, fastened, and connected to the seawater intake system (right).

Sediment samples

13 sediment samples were taken by multicorer (MUC, Chapter 14) from the Weddell Sea at depths ranging from 332–1,418 m (Table 15. 3). For the purpose of MP investigation the top 3 cm of the kindly provided MUC cores (inner diameter: 6.2 cm) were sub-sampled using a Mili-Q rinsed steel pipe (inner diameter: 2.7 cm) to a depth of approx. 3 cm resulting in approximately 17 cm^3 each. Samples were transferred into pre-combusted (12 h at 400° C) glass vials (40 ml) and stored at 4° C. The sediment samples will be further processed by a ZnCl density separation (Imhof et al., 2012), a Fenton's reagent purification (Tagg et al., 2017) and analysed with focal plane array (FPA) micro Fourier transform infrared spectroscopy (FT–IR) (Löder et al., 2015).

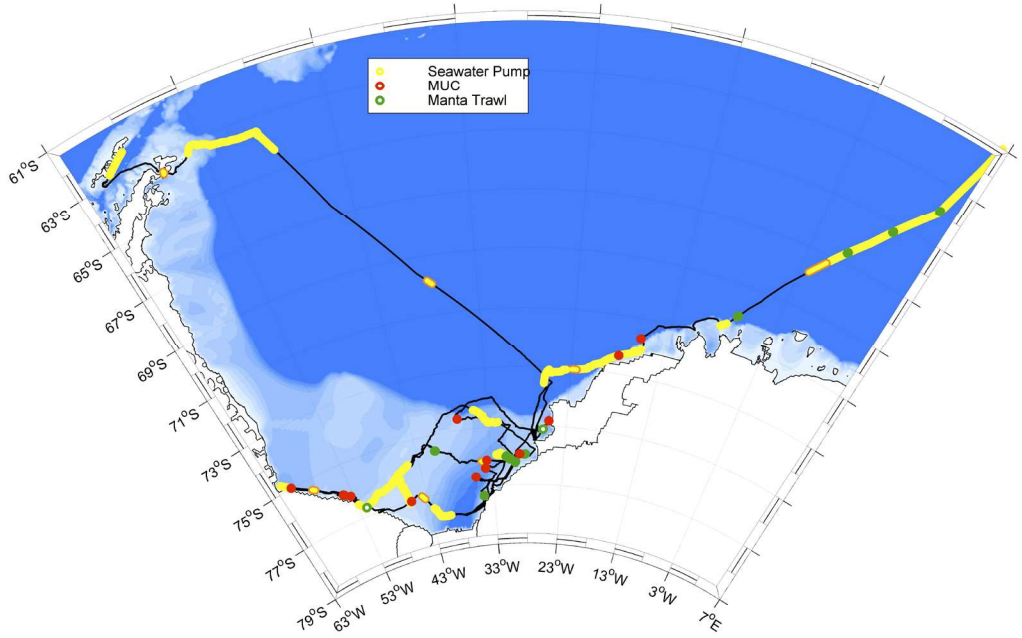

Fig. 15.3: Sampling locations. Yellow transects represent 0.1 mm mesh. Yellow transects bordered by an orange line represent 0.02 mm mesh. Red and green marks indicate multicorer and Manta Trawl samples, respectively. The black line indicates the Polarstern track on the cruise PS111.

Tab. 15.1: Summary of Manta Trawl samples for microplastics on PS111

Sample ID	Station Name	Date	Coordinates Start	Coordinates End	Transect [m]	Filtered Vol. [m3]
Manta Trawl (MT) 1	PS111_9-1	2018-01-26	-63.905159; 5.053958	-63.936306; 5.036211	3,483.9	376.3
MT 2	PS111_10-1	2018-01-26	-65.306004; 2.70799	-65.331941; 2.663314	3,551.1	383.5
MT 3	PS111_12-1	2018-01-27	-66.632579; 0.189451	-66.657449; 0.135565	3,299.1	356.3
MT 4	PS111_13-1	2018-01-28	-70.040489; -6.74798	-70.061082; -6.820045	3,561.3	384.6
MT 5	PS111_19-1	2018-02-04	-76.104748; -30.370666	-76.120101; -30.440141	2,577.6	278.4
MT 6	PS111_20-1	2018-02-04	-76.0127; -30.884445	-76.03348; -30.95367	3,104.1	335.2
MT 7	PS111_28-4	2018-02-05	-75.973637; -28.406722	-75.961511; -28.300494	3,105.3	335.4

Sample ID	Station Name	Date	Coordinates Start	Coordinates End	Transect [m]	Filtered Vol. [m3]
MT 8ab	PS111_35-1	2018-02-09	-76.707562; -51.845019	-76.711764; -51.893151	98.4	10.6
MT 9	PS111_64-1	2018-02-15	-75.548026; -40.44837	-75.544617; -40.54514	2,772.0	299.4
MT 10	PS111_74-5	2018-02-17	-76.214619; -29.683427	-76.236688; -29.720002	2,085.6	225.2
MT 11c	PS111_85-2	2018-02-19	-77.308448; -34.520721	-77.316982; -34.564203	831.0	89.7
MT 12a	PS111_138-2	2018-02-28	-75.126413; -26.038154	-75.11979; -25.960787	1,433.1	154.8

[a]Aborted due to grease ice accumulation in Manta Trawl

[b]Defect of flowmeter due to freezing

[c]Hoisting of Manta Trawl after 15 minutes due to phytoplankton accumulation in the cod end

Tab. 15.2: Summary seawater pump samples for microplastics on PS111

Sample ID	Coordinates Start	Coordinates End	Filtered Vol. [L]	Mesh Size [µm]
Seawater Pump (SP) 1	-43.310596; 14.789067	-45.873514; 13.714196	4,762.8	20
SP 2	-46.295037; 13.517873	-48.338984; 12.609975	2,829.0	100
SP 3	-48.386536; 12.587699	-51.529018; 11.144197	4,766.0	100
SP 4	-51.566922; 11.126238	-55.276444; 9.378046	5,651.0	100
SP 5	-55.325263; 9.35429	-60.391373; 6.767601	8,017.1	100
SP 6	-60.435814; 6.752008	-65.385393; 2.566424	8,099.0	100
SP 7	-65.385256; 2.567096	-67.142484; 0.856763	3,952.0	100
SP 8	-67.264469; -1.106756	-67.795751 -2.210384	297.0	20

Sample ID	Coordinates Start	Coordinates End	Filtered Vol. [L]	Mesh Size [µm]
SP 9	-70.093846; -6.85067	-70.093689; -6.850199	114.9	20
SP 10	-70.40211; -7.624916	-70.546071; -8.142461	4,363.1	100
SP 11	-72.041787; -15.520476	-72.995041; -21.430651	4,075.1	100
SP 12	-73.008257; -21.640274	-73.04826; -22.900553	217.0	20
SP 13	-73.075024; -23.532446	-73.568687; -26.071103	2,194.0	100
SP 14	-76.044146; -30.987383	-76.087175; -30.452237	4,685.9	100
SP 15	-75.975035; -28.416919	-75.963451; -28.315971	181.1	20
SP 16	-74.860296; -31.814451	-74.37882; -34.858545	2,408.1	100
SP 17	-75.788026; -44.511445	-76.505807; -52.811142	3,168.0	100
SP 18	-74.983358; -60.000768	-74.731947; -61.026099	1,164.9	100
SP 19	-75.381265; -57.707349	-75.440777; -57.341792	99.0	20
SP 20	-77.068797; -45.64848	-76.201814; -46.084503	2,283.0	100
SP 21	-76.106115; -34.6476	-76.196562; -33.885373	153.0	20
SP 22	-76.213615; -29.675775	-76.237923; -29.71535	89.0	20
SP 23	-77.308453; -34.519384	-77.335053; -34.582502	101.9	20
SP 24	-77.794927; -40.00413	-77.439638; -42.348412	3,467.0	100
SP 25	-76.943152; -43.734653	-77.070112; -43.341808	160.0	20

Sample ID	Coordinates Start	Coordinates End	Filtered Vol. [L]	Mesh Size [µm]
SP 26	-75.131051; -26.038469	-75.091618; -25.849534	269.0	20
SP 27	-69.972507; -37.418955	-69.830417; -37.864793	186.0	20
SP 28	-64.294352; -47.924158	-63.330227; -54.137903	2,792.0	100
SP 29	-63.608203; -56.437342	-63.513015; -56.394712	133.0	20
SP 30	-62.768051; -60.033051	-62.021279 -57.275862	2,505.0	100
SP 31	-59.417025; -57.03486	-59.296198; -57.209862	148.0	20
SP 32	-58.966349; -57.684771	-56.796928; -60.647233	4,244.0	100

Tab. 15.3: Summary of sediment samples for microplastics on PS111

Sample ID	Station Name	Coordinates	Depth
Sediment Core (SC) 1	PS111_15-1	-71.665894; -15.783055	1,402 m
SC 2	PS111_16-3	-72.384301; -17.816957	1,418 m
SC 3	PS111_27-1	-75.954850; -29.084043	425 m
SC 4	PS111_40-2	-76.000968; -54.239456	513 m
SC 5	PS111_42-1	-76.144926; -53.356988	493 m
SC 6	PS111_47-2	-74.984737; -60.001606	660 m
SC 7	PS111_53-3	-76.025914; -54.120589	496 m
SC 8	PS111_60-3	-77.019015; -45.400304	332 m
SC 9	PS111_70-2	-76.114507; -33.658977	796 m
SC 10	PS111_80-3	-76.642137; -35.428931	933 m
SC 11	PS111_114-3	-76.379052; -33.942022	839 m
SC 12	PS111_131-2	-74.612930; -36.931811	386 m
SC 13	PS111_139-2	-74.825079; -25.271639	665 m

Preliminary (expected) results

All of the visually inspected Manta Trawl samples (n = 7) yielded conspicuous coloured fragments (mean concentration 0.02 ± 0.02 SD m^{-3}) and fibres (0.04 ± 0.05 m^{-3}; Fig. 15.4). However, it was not yet possible to conclusively determine the origin of these particles. A first estimate of the vessel activity-related contamination potential suggests that the fragments likely constitute ship paint fragments (Figs 15.5 and 15.6) while the fibres likely stem from

textiles present in the launching and hauling area of the Manta Trawl as well as in the on-board laboratories. All Manta Trawl samples will undergo further visual and chemical investigation (FT–IR) for the final assessment of the oceanic microplastics load.

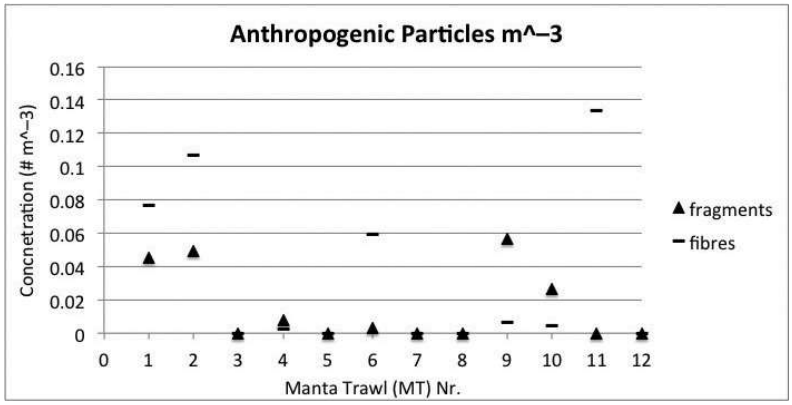

Fig. 15.4: Frequency distribution of putative anthropogenic residue m^{-3} in the Manta Trawl (MT) samples 1–12 of sampled surface seawater. MT Samples 3, 5, 7 were not yet assessed due to excess biogenic material. MT samples 8 and 12 were aborted mid-sampling due to grease ice accumulation in the mesh (Table 15.1). All putative anthropogenic particles will be analysed using Fourier transform infrared spectroscopy (FT–IR).

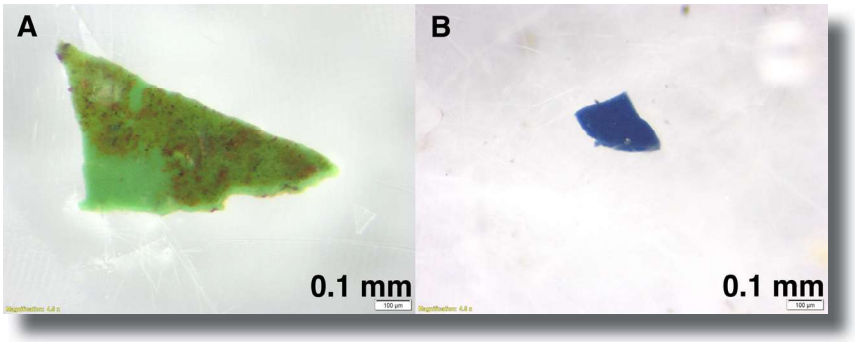

Fig. 15.5: Green and blue potentially anthropogenic fragment from MT 2 (A) and MT 1 (B) respectively. Initially assessed as putative microplastic particles due to colouring and homogenous, non-cellular texture they will now be subject to chemical analysis (FT–IR) in the mainland laboratory. Visual similarity (e.g. colour) suggests that the particles might originate from Polarstern vessel paint.

Quality control and contamination protection

The Manta Trawl (MT) aperture was sealed with a cotton cloth for transport to the launching area on the working deck (E-deck). Any rinsing activity on the working deck which could result in anthropogenic particles being washed into the surrounding waters of *Polarstern* were halted prior to MT trawling. The continuously running seawater hose on the working deck was pulled out of the drain and hung directly over the reling to avoid additional washing of particles into the surrounding waters (e.g. ship paint or plastic fragments from scientific devices such as the multicorer, etc.). The grey water outlet of the on-board waste water treatment plant (WWTP, outlet located on the opposite hull side on portside) was interrupted at least 30 minutes prior to MT trawling starting from MT Nr. 7. Whenever possible sampling was conducted with winds facing starboard to avoid lining up *Polarstern* between wind origin and MT. White lab coats

(100 % cotton) and blue nitrile gloves were worn in the laboratory when handling and inspecting samples. Glassware was used as far as possible. If the use of synthetic polymer material (e.g. pipe for seawater sampling) was necessary, items were rinsed before use thoroughly.

Procedural blanks were run in the dry lab where MT samples were inspected (*n* = 4). For this purpose two petri dishes at a time (diameter: 6 cm, first set 17 hours [assessment yielded 39 and 34 fibres, respectively], second set 26 hours) were placed on the lab bench and subsequently filtered onto glass microfiber paper (GF/C); Whatman: 47 mm, pore size: 1.2 µm, using a Buchner funnel and a vacuum flask. Filters were subsequently folded, sealed in aluminium foil and stored in glass petri dishes. Following the same filtration and storing procedure background samples were furthermore taken from the work-passage on E-deck (0.4 L glass jars, 24 hours, *n* = 2) dry lab freshwater supply (2 L, *n* = 3), lab ethanol supply for sample conservation (100 mL, *n* = 3), freshwater hose working deck for externally rinsing the MT mesh pre and post trawling (2 L, *n* = 3) and seawater for Sterivex filtration (1 L, *n* = 3).

Procedural blanks were run for the seawater pump sampling by rinsing the sieve stack upon exposure into a pre-rinsed (Mili-Q) glass jar and fixating with v:v 50% EtoH (*n* = 3).

For later (forensic) referencing, material samples from the yellow synthetic MT bridle, orange synthetic AWI work deck jackets, red synthetic AWI Tempex suits, blue synthetic fastening straps in the lab and a shaving of the multicorer (MUC) tube ware preserved and sealed seperately in colourless polypropylene (PP) tubes (cf. Fig. 7 and 8). The same was done with fragments of ship paint (green, blue, red/orange and white – the main paint colours on *Polarstern*, cf. Fig. 15.6).

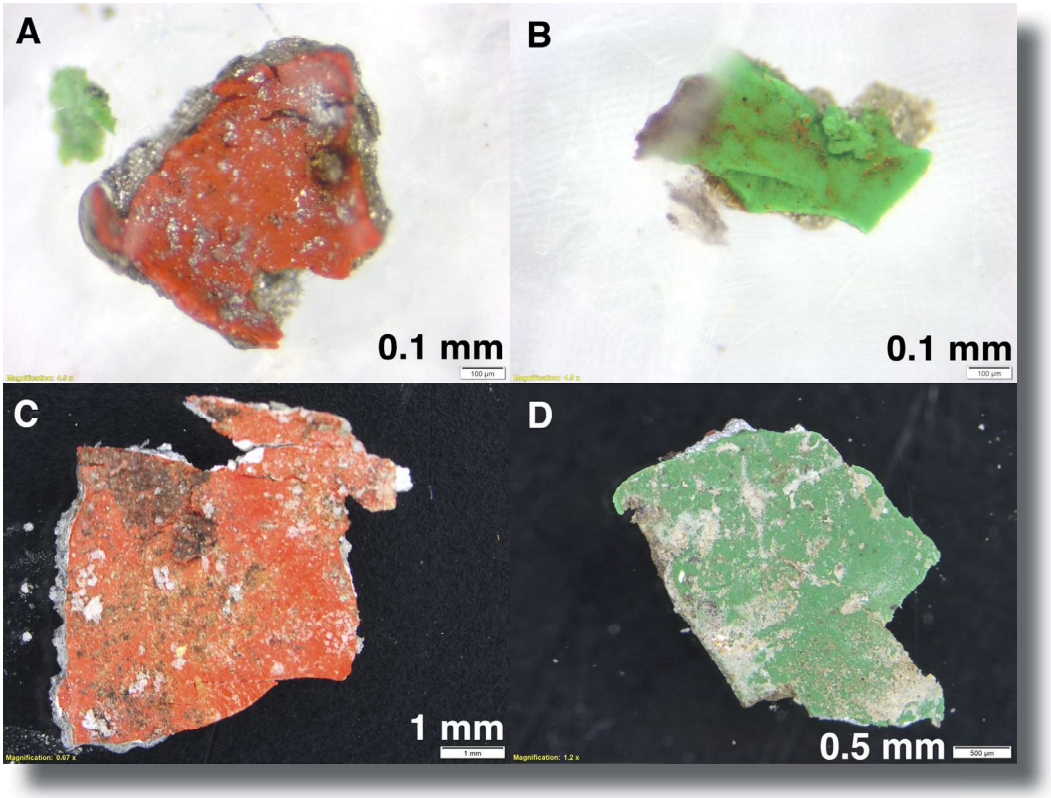

Fig. 15.6: Red/orange and green likely vessel paint fragment from MT 2 (A) and MT 1 (B) respectively. For comparison ship paint fragments deliberately collected from the working deck (E-deck) red/orange (C) and green (D).

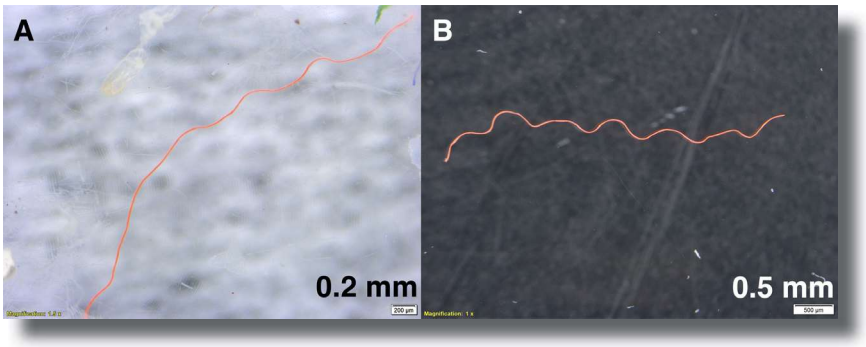

Fig. 15.7: Orange fibre from sample MT 1 (A) and reference sample taken from an orange AWI working jacket (B).

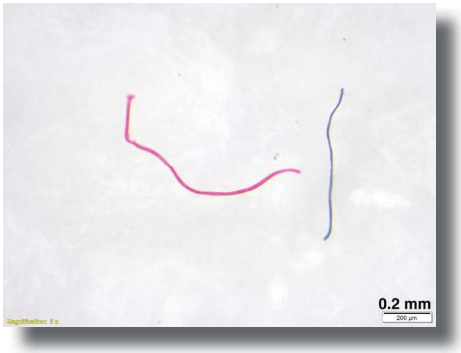

Fig. 15.8: Red and blue fibre from the lab bench procedural blank 1 (petri dish diameter 6 cm exposed to dry lab atmosphere for 17 hours, yielding 39 fibres).

Proposed developments for future microplastic research from Polarstern

Investigating an anthropogenic contaminant, such as microplastics, in a pristine and remote environment like Antarctic seas from a research vessel e.g. *Polarstern*, requires rigid measures against research activity and vessel-induced contamination potential. While sampling on the seafloor may offer a relatively undisturbed compartment (despite the synthetic MUC coring tubes), sampling immediately below the vessel hull (seawater pump) or a few metres away from the hull on the water surface (MT) constitutes a great challenge for avoiding anthropogenic contamination of samples. In an ideal scenario sampling for microplastics would be conducted independently from the vessel as much as possible. Many of the previously described precautionary measures were developed during PS111 in cooperation with fellow scientists. Some further, cooperatively established, optimisation ideas for the deployment of the MT or similar neuston net devices shall be listed here:

- Installing of shutter-mechanism which can be released immediately when device reaches the water surface
- Deployment of MT from crane in front of vessel bow
- Elastic trawling component for buffering wave and current induced tugging and “flying” of MT in rough conditions
- Installing of outward steering rudders for creating more distance to the hull and working deck (E-deck on *Polarstern*)
- Development of sampling protocol for trawling far behind the vessel (>500 m)
- Deployment of MT or similar device by helicopter or drone

Data management

Microplastic samples will either be destroyed by analysis or those not analysed will be stored at the home laboratory at University of Basel. All sequence data will be deposited in EBI's European Nucleotide Archive and will conform to the minimum information standards recommended by the Genomics Standards Consortium (http://gensc.org/projects/mixs-gscproject/). Metadata and results will be stored at data servers of the University of Basel. After a thorough quality control, processing and publication in a peer reviewed journal, the processed data will be stored in the PANGAEA data base.

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