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***MGF-Ostsee Project:
Potential effects of closure for bottom fishing in the marine protected
areas (MPAs) of the western Baltic Sea - baseline observations***

Cruise No. EMB238/Leg1+2

26.05.2020 – 09.06.2020,
Rostock (Germany) – Rostock (Germany)
MGF-Ostsee / DAM-Pilotmission - Ostsee
(MPA-DAM 2020 A)



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1 Cruise Summary

1.1 Summary in English

The cruise was a first of a series of planned cruises under the framework of the DAM MGF-Ostsee Project: Potential effects of closure for bottom fishing in the marine protected areas (MPAs) of the western Baltic Sea – baseline observations (funded by BMBF). Its major aim is the initial assessment of temporal variability and environmental state in the pre-closure condition in particular habitat of the German EEZ designated marine protected area “Fehmarnbelt”, where the effects of the planned closure for bottom fishing are expected to be most pronounced. Additionally, the control areas outside MPA (and thus outside closure areas) in similar habitat and (based on available data) under initially comparable fishing intensities was sampled. First, baseline hydroacoustic survey was done to characterize and monitor the development of the trawl marks on the seafloor. Then sampling for obtaining a comprehensive picture and estimate of variability of biological sediment communities (key players, diversity and activity in prokaryotes, protists, meiofauna, macrozoobenthos) and sediment biogeochemical composition was carried out.

Sandy sediment was expected based on existing literature sources and sediment maps from the region. However homogenous muddy sediment (sandy mud to silt) was observed in the study area.

1.2 Zusammenfassung

Die Fahrt ist Teil eines vom Bundesministerium für Bildung und Forschung (BMBF) geförderten interdisziplinärem Forschungsprojektes zur „Untersuchung der erwarteten Auswirkung des Ausschlusses mobiler, grundberührender Fischerei in marinen Schutzgebieten der Ostsee“ (Kürzel: „MGF-Ostsee“). Dies ist gleichzeitig ein Pilotprojekt der Deutschen Allianz für Meeresforschung (DAM) (DAM Pilotmission – Schutzgebiete Ostsee, FKZ: 03F0848A). In diesem Forschungsprojekt untersucht ein Konsortium von WissenschaftlerInnen wie sich die Ökosysteme der Natura 2000-Gebiete in der deutschen ausschließlichen Wirtschaftszone (AWZ) der Ostsee nach Ausschluss der mobilen grundberührenden Fischerei (MGF) entwickeln. Hauptziele sind ein besseres Verständnis der Nachhaltigkeit von Meeresbodenlebensräumen und Biota in den Natura 2000 Gebieten unter dem derzeitigen Grundschleppnetzbetrieb, eine generelle Bewertung der Auswirkungen der bodenberührenden Fischerei auf benthische Gemeinschaften und Sedimentfunktionen sowie deren Entwicklung nach Fischerei-Ausschluss. Die Fahrt ist die erste Aufnahme aller Komponenten des benthischen Nahrungsnetzes, von Prokaryonten bis Makrozoobenthos, Sedimenteigenschaften und biogeochemische Prozesse in ausgewählten Untersuchungsflächen (Weichbodensedimente) innerhalb und außerhalb der Schutzgebiet Fehmarnbelt. In der Regel erfolgte die Probenahme zwischen 6:00 – 18:00, hydrographische Messung (Multibeam echo sounder MBES & Sediment echo sounder SES); in einzelnen Nächten. Ferner wurden kurze UW-Videotransekte zur Charakterisierung des Meeresboden durchgeführt. Das Programm umfasste geologische, chemische, physikalische und biologische Ansätze.

2 Participants

2.1 Principal Investigators

Name	Institution
Gogina, Mayya Dr.	IOW Warnemünde

Piontek, Judith Dr.	IOW Warnemünde
Schönke, Mischa Dr.	IOW Warnemünde
Zeller, Mary, Dr.	IOW Warnemünde
Kallmeyer, Jens, Dr.	GFZ Potsdam
Clemens, David	GEOMAR Kiel
Hohlfeld, Manon	Uni Köln
Forster, Stefan, Dr.	Uni Rostock
Powilleit, Martin	Uni Rostock

2.2 Scientific Party

Name	Discipline	Institution
Gogina, Mayya, Dr.	Macrozoobenthos / Chief Scientist	IOW Warnemünde
Schönke, Mischa, Dr.	Sedimentology / Geophysics / Chief Scientist	IOW Warnemünde
Piontek, Judith, Dr.	Prokaryotes / radiation protection	IOW Warnemünde
Pohl, Frank	Macrozoobenthos / Technician	IOW Warnemünde
Zeller, Mary, Dr.	Geochemistry	IOW Warnemünde
Kallmeyer, Jens, Dr.	Geochemistry / radiation protection	GFZ Potsdam
Bill, Nicolas	Biogeochemistry fluxes at SWI / technician	GEOMAR Kiel
Clemens, David	Biogeochemistry fluxes at SWI	GEOMAR Kiel
Hohlfeld, Manon	Protists/Meiofauna	Uni Köln
Forster, Stefan, Dr.	Benthos/Bioturbation	UNI Rostock
Powilleit, Martin, Dr.	Benthos/Bioturbation	UNI Rostock

2.3 Participating Institutions

IOW Warnemünde	The Leibniz Institute for Baltic Sea Research, Warnemünde
GEOMAR Kiel	Helmholtz-Zentrum für Ozeanforschung Kiel
GFZ Potsdam	Das Helmholtz-Zentrum Potsdam – Deutsches GeoForschungsZentrum
Uni Köln	Die Universität zu Köln
Uni Rostock	Die Universität Rostock

3 Research Program

3.1 Description of the Work Area

The investigation area is located 17 km west of the island Fehmarn in the German EEZ and costal water of Schleswig-Holstein (Fig. 3.1) with water depth between 19 and 25 m. Additional to the area of 4.7 km² within marine protected area (MPA) site, a control area outside the MPA were sampled, in which similar habitat conditions and similar fishing intensities were expected. The control area covers an area of 3.7 km² and is located 1.4 km west of the MPA. Geologically, the

south-east of the investigation area borders on the edge of abrasion platform composed of Lag deposits, which extend west of Fehmarn with water depth between 5-15 m. The sediment composition of the Lag deposits results from wave motions removing grain sizes within the range of sand from the underlying till and leaving gravel and coarser material behind (Tauber et al., 1999). Lag deposits are commonly surrounded by areas with sandy sediment composition (Zeiler et al., 2008). Due to the prevailing west winds (Duphorn et al., 1995), most of the sandy material is remobilized and transported to the east. With the investigation area located in the west of the abrasion platform, it is sand starved and dominated by muddy sediments with a fine sand component (BSH, 2016).

Environmental variables such as temperature, salinity, sediment grain size, organic content and dissolved oxygen concentration are important for the structure of the bottom biological communities at the seafloor in the Fehmarnbelt and are all highly correlated to water depth. Stratified water column and position of the halocline determine the exposure of communities to ambient fluctuations of salinity, oxygen, and temperature (FEMA, 2013). The depth of variable boundary layer (pycnocline) between the lower-saline surface water and higher-saline bottom water lie in the 10-16 m range. Surface salinity in the study area during the cruise ranged from 11.1 to 13.3 and bottom salinity was 17.2 to 18.6. Typically, around 25 m depth salinity can be as high as 25-30 (FEMA, 2013). Near-bottom water temperature ranged between 9.6 and 10.3°C. Near-bottom oxygen concentration varied from 3.8 to 4.8 mg/l (based on the data from Leg 2 CTD casts).

3.2 Aims of the Cruise

As part of an interdisciplinary research project to investigate the effects of mobile bottom-fishing in the marine protected areas (Natura 2000) in the German Baltic Sea, general aims of the cruise included obtaining a comprehensive picture of biological sediment communities (key players, diversity and activity in prokaryotes, protists, meiofauna, macrozoobenthos) and sediment biogeochemical composition and activity, and the variability of the different components in one key MPA (sandy sediment), and sediment-water interface fluxes in Fehmarnbelt MPA. The special feature of the cruise was a combined assessment of sedimentological, micro- and microbiological and biogeochemical parameters, and provide a baseline for a series of following monitoring cruises planned in the future. It was particularly targeting the investigation (both quantitatively and mechanistically) of interactions between micro-/macrofauna and sediment biogeochemistry.

3.3 Agenda of the Cruise

Despite limitations due to COVID-19, that forced separation of the cruise into two legs, focusing integrated sampling of all components on one MPA allowed all project WPs to obtain data (thereby at least partly to fulfill obligations), optimize joint sampling and screen for gaps (scale, sufficient and feasible number of samples) to consider for future monitoring in 2021.

Project related RV Solea 777 cruise preceded EMB238, and data on location of trawling and UW transects and position of sampling stations were shared for coordination and to avoid overlapped sampling. Work during the first leg (26.05. – 03.06.2020), in the investigation areas inside MPA Fehmarnbelt and control area outside of it, included:

- Soundprobe deployment to measure the sound speed in the research area

- Hydroacoustic mapping of selected investigation areas for detailed trawl marks mapping, positioning of sampling location for sedimentological, biogeochemical and biological sampling, and in order to -and possibly for a refined area evaluation for the 2021 baseline monitoring
- CTD water column and bottom water characterization
- Collection of sediment cores with multicorer (MUC, 4 stations within each area subject to capacity) for prokaryotes, protists, meiofauna, microphytobenthos
- MUC sampling for sediment biogeochemistry, porosity, pore waters, sulfate reduction rates and interfacial element fluxes
- Two BIGO Lander (MPA and Control) and one eddy correlation (Control) incubations

After the exchange of scientific staff in Rostock Fischereihafen, the work during the second leg (04.06. – 09.06.2020) continued (in both investigation areas inside MPA and control area outside) with:

- Grab samples to obtain the sand material for Uni Rostock on the way to the study area
- CTD water column and bottom water characterization
- Sampling of macrozoobenthos with Van Veen Grab (also fine 500-1000 μm). Sampling with HAPS was not required due to muddy sediment, samples for vertical distribution of macrozoobenthos were collected from MUC cores.
- MUC cores were sampled for Chlorophyll-*a*, porosity, and gain cores for pore water analysis from missing location that remained from the first leg
- UW Video transects
- Two BIGO Lander (MPA and Control) and one eddy correlation (MPA) incubations
- Dredge samples were taken to obtain full characterization of macrofaunal biodiversity including moving, rare or large species, and to investigate the condition and damage degree of the shells of *Arctica islandica*

List of equipment used: Edgetech 4000 Sidescan System, Evo Logistig Ultra Short Baseline Acoustic System (USBL), small Sound velocity probe kleine Schallsonde, Multicorer (MUC), Frahm-Lot, 2 van-Veen Greifer, Lander BIGO with benthic chambers (GEOMAR), Eddy Correlation system (GEOMAR), Dredge, SeaViewer UW Video System, CTD, MilliQ, Titrino

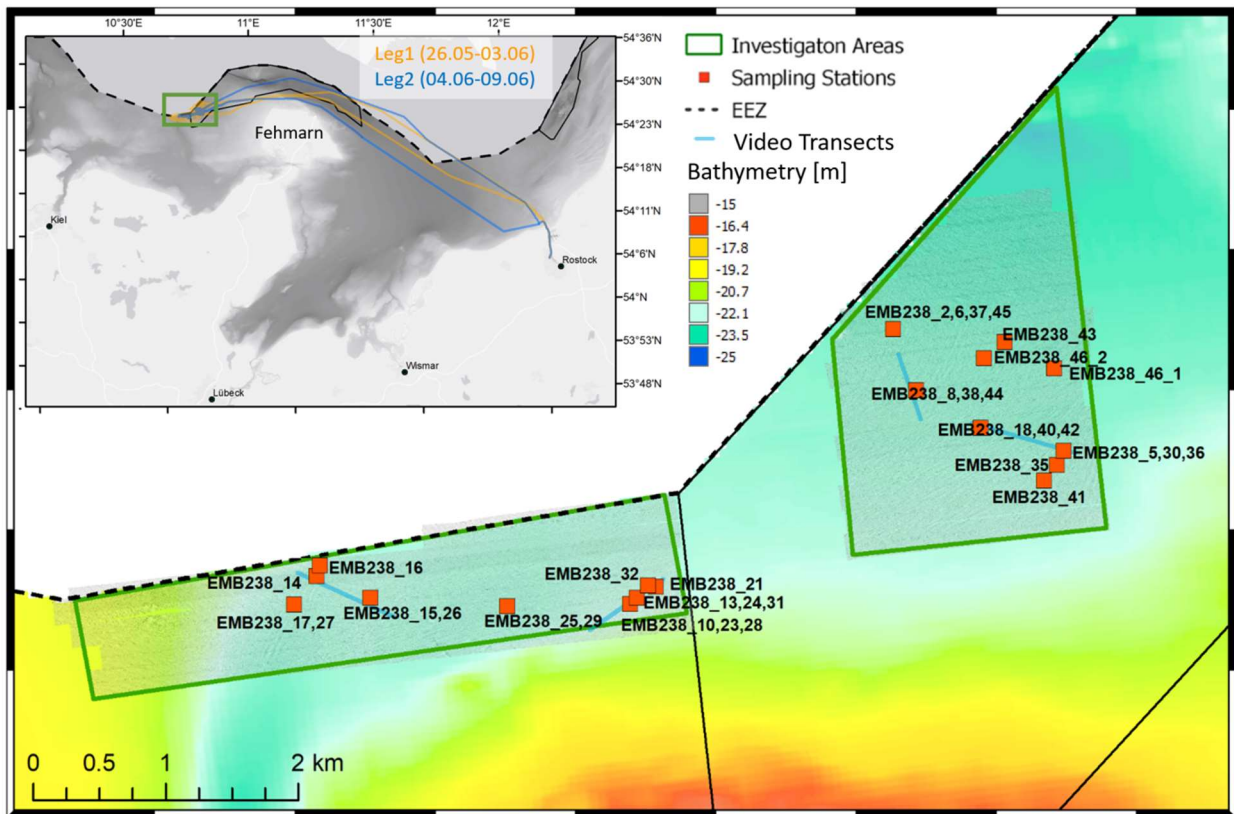


Fig. 3.1 Track chart of RV Elisabeth Mann Borgese EMB238 and location of sampling stations in the investigation area.

4 Narrative of the Cruise

Leg 1

On 26th of May, the RV Elisabeth Mann Borgese departed from Rostock at 18:30 with a delay of 6 h for transit to the investigation area located at Fehmarnbelt. The delay of 6 h was caused by the fact that packing and departure day coincided and occurring setup issues of the new USBL system could not be fixed in advance. To compensate for the delay, the MPA were mapped at Wednesday night (0:00 – 5:30 clock). A preliminary backscatter map obtained by the record SSS data revealed strong evidence for bottom fishery activities in the entire MPA. On Wednesday morning, it was decided to sample a location in the north-west of the MPA, which revealed a comparable large number of suspected trawl marks. To keep the sampling during the entire first cruise leg as comparable as possible, the following procedure was decided: First, a CTD cast to record a water column profile and to collect water samples, secondly at least four eightfold equipped MUC cast to collect ground samples. Contrary to expected sandy sediments in the investigation area, the first MUC samples revealed muddy sediment with a fine sand component, which had significantly reduced the time and effort required for coring activities. However, due to the manpower shortage of the science crew, the further processing of the recovered samples (which lasted additional 3 – 4 h) turned out to be the limiting factor. On Wednesday 27th of May after MUC operations were finished, survey profiles No. 8 in the MPA were remapped to fill data gabs caused by evade maneuvers, before leaving the MPA and start mapping the control area. The preliminary evaluation of the acoustic data suggested, that both areas (MPA and control area) were very homogeneous and similar in backscatter characteristics. Based on the results of the acoustic mapping and the

sampling experience of the previous day, it was decided to reduce the planned number of stations in the MPA and Controlarea from 5 down to 4 (to sample one station per day), so that the expected large number of MUC samples could be reconciled with the time consuming sample processing. Two stations per area should be sampled with strong and two with weak evidence for bottom fishery activities. A detailed overview of sampling activity is shown in the station list (Chapter 7).

On Thursday 28th of May a station in the south-east of the investigation area were sampled, to confirm the homogeneous sedimentation characteristics revealed by the hydroacoustic data. After the station was finished, the BIGO1-01 Lander system was deployed at the station sampled on the 27th of May for the next 36 h. After the successful deployment, the MPA area were mapped by using the MBES System. On Friday, the 29th of May we were forced to return to the Lander station to fix issues with the signal buoy. After the daily sampling program, the MPA were mapped a second time using the SSS system to close gabs to reach 100% area coverage.

On Saturday the 30th of May morning, we successfully recovered BIGO1-01. Based on the information, that military operations in the control area over Pentecost will be suspended until Tuesday 2nd of Jun morning, we decided to use the time window and stop sampling in the MPA and start our way to the control area. Within this time window, Lander deployments in the control area could not have been affected by military operation for 36 h or longer.

From the 30th of May until 3rd of Jun morning four station in the control area were successful sampled. Within this timeframe two Lander deployments were performed in areas with strong evidence for bottom fishery activities. The first the Lander BIGO1-01 was deployment from the 30th of May until Jun 2nd for about 36 h, and secondly the Eddy-Correlation Lander with a northern offset of 150 m was deployed from 31st of May until 3rd of June. Hydroacoustic mapping were done on 30th of May evening, using first the SSS to close data gabs and to reach 100% area coverage in the control area and secondly the MBES system were used until the 31st of May morning to obtain multifrequency backscatter and bathymetry maps. The shorter sampling program on 1st and 2nd of Jun was used to work of all samples before crew exchange.

After the Eddy-Correlation Lander was recovered on the 3rd of June morning, we went back to the MPA area to sample the fourth station. A comparison of the acoustic data revealed that a positioning error occurred within the first recorded profiles on the 27th of May. To close data gabs parts, several profiles of the MPA were remapped on the 3rd of Jun before leaving the research area for the scheduled scientific crew exchange in Rostock.

The weather conditions during the first Leg were good, with average temperature around 15 degrees. It was partly cloudy and mostly sunny, with hardly any swell and a breeze from N to NE with an average strength of BF 4 (Beaufort scale), with a BF range between 2 and 5.

Tuesday 26th of May

	Departure from Rostock to the investigation area Fehmarnbelt (MPA) with a
18:30 – 24:00	delay of 6h
24:00	Arriving at the research area around midnight

Wednesday 27th of May (Sampling MPA)

	SSS survey of MPA with subsequent processing of the data to determine the
00:00 – 05:15	sampling location in the MPA

9:00 – 16:30	Seafloor and water column sampling using CTD and MUC
17:00	Remapping survey profile 8 of the MPA using SSS (due to evade maneuvers)
18:30	Transit to the control area
18:40 – 23:30	Mapping of the control area using SSS
23:30	Transit back to the MPA

Thursday 28th of May (Sampling MPA)

08:00 – 15:30	Seafloor and water column sampling using CTD and MUC
16:00 – 16:30	Deployment Lander System BIGO1-01
17:00	Start Mapping MPA using MBES system

Friday 29th of May (Sampling MPA)

03:40	End mapping MPA using MBES system
08:00	Fixing Buoy (15 min) + daily sampling program
08:15 – 14:00	Seafloor and water column sampling using CTD and MUC
14:00 – 20:00	SSS Survey MAP for 100 % coverage

Saturday 30th of May (Sampling Control)

08:00 – 08:30	Recover Lander System BIGO1-01
	Transit from MPA to control area
09:00 – 13:30	Seafloor and water column sampling using CTD and MUC
14:00 – 18:00	SSS survey for 100 % coverage
19:30	Start mapping control area using MBES system

Sunday 31st of May (Sampling Control)

02:30	End mapping control area
02:30	Transit control MPA
08:00 – 09:00	Finish SSS survey (from Friday 29 th of May)
09:00	Transit MPA control
09:20 – 15:00	Seafloor and water column sampling using CTD and MUC
16:00	Deployment Lander System BIGO1-01

Monday 1st of Jun (Sampling Control)

8:30 – 13:30	Seafloor and water column sampling using CTD and MUC
16:00	Deployment Eddy Correlation Lander System

Tuesday 2nd of Jun (Sampling Control)

08:00 – 08:20	Recovery of Lander System BIGO1-01
09:00 – 14:00	Seafloor and water column sampling using CTD and MUC

Wednesday 3rd of Jun (Sampling MPA)

08:00 – 08:20	Recovery Eddy Correlation Lander
08:20 – 9:00	Transit control area MPA
9:00 – 14:15	Seafloor and water column sampling using CTD and MUC

14:50 – 20:00 SSS remapping MAP (due to positioning error from 26.05.20)
20:00 Leaving investigation area for scientific crew exchange in Rostock

Leg 2

On June 04th 2020 RV Elisabeth Mann Borgese (EMB) came to Rostock port for the exchange of scientific personnel. 4 new people came on board (with confirmed negative results of COVID-test). They were brought up to date about the measurements and sampling conducted on leg 2. After CTD maintenance (due to some issues with one of the temperature sensors during the last CTD casts of leg 1), the ship left the harbor by 10:30 UTC and headed back to investigation area. On the way, on front of Warnemünde, two Van Veen grab samples were quickly collected to obtain the sand material for the research program of Uni Rostock. The weather and sea conditions were good, partly cloudy. Equipment and laboratories were rearranged and prepared for sampling; the solution to attach the USBL also to Van Veen grab for accurate estimate of samples position on the seafloor with respect to the trawl marks was found (see Fig. XX). First BIGO deployment (21_1, planned for ca. 36 hours) and CTD/rosette sampling (22_1) was done in control area after dinner (17:00 –18:26).

On June 05th works continued in the control area and focused on characterization of macrofauna (MZB) and bioturbation (Chl-*a*). Due to relative homogeneity of trawling marks concentration and considering the intention for future synthesis with data on microbiological and biogeochemical components, it was decided to take three grab samples at each of four sampling locations selected (in control area) in leg 1 and at one additional location in-between (stations 23-27). For bioturbation measurements with Chl-*a*, vertical MZB and to obtain material for supplementary sediment characterization (grain size distribution and organic content) one MUC cast (8 cores) at each of these locations was sufficient, of which two were sampled with MUC at this day. The day working program was subject to adjustments due to time spent for optimization of fractioned sieving, attempts to reduce overfilling of Van Veen grab due to “soft” substrate, damage of one grab and its replacement, availability of only one USBL trans user for grab and MUC, and crew working (with MUC deployments requiring more people on deck). A CTD cast at the western location of the study area (27_5) was done at the end. Weather was good.

On June 06th BIGO retrieval had to be postponed due to weather conditions (wind and waves), and works continued with MUC and grab casts in both control and MPA areas (including MUC 2 cores for missing pore water analysis collected at similar location as station 5 (30_1) in MPA). BIGO was successfully retrieved at 11 o'clock (UTC), and deployed again in the new location within MPA (St. 35). One dredge (32), two underwater video transects (33, 34) were carried out in the control area, thereby completing the planned works there. After dinner a CTD cast (36) was done in MPA.

On June 07th work within MPA continued with MUC (37-40) and grab (42-45) sampling, and an Eddy correlation Lander deployment (41). CTD profile (45_4) was obtained at the most north-west location of MPA. After 9pm (UTC) a cutter has disturbed the marking of BIGO Lander, despite all the warnings from the captain. For hours the frustrated Danish fisherman tried to escape his boat from the buoy rope connected to the Lander. Appraising the height of cutter's deck, the range of vision from it, the waves and the elevation of lights from the Lander buoy, one would image that the fisherman could have overlooked the lights, suggesting the need for better (higher) marking in the future monitoring area of high fishing activity. Around midnight (UTC) fisherman

succeeded and handed over the buoy to EMB, presumably when maneuvering to come closer to the ship he managed to hit the buoy of the Eddy correlation Lander, that was seen until then, but was not seen ever after.

On June 08th in the morning using echo sounder we could detect the position of both Landers on the sea floor. USBL system also indicated that location of BIGO Lander is 40 meter away from where it was deployed. The Submaris AG diving team, Kiel was contacted to help to recover the devices to minimize damage to the equipment. They organized everything in the shortest time possible, and with their highly professional help both devices were recovered by around 4 pm (UTC). Urgency was necessary as next day EMB had to be in Rostock to prepare for the next cruise. During the day two dredge casts (46_1, 46_2), two underwater video transects (47_1, 48_1) and a CTD cast (48_2) were carried out to finalize the sampling program in MPA, and equipment and laboratories were prepared for landing. On June 09th at 6:00 (UTC) the ship entered the port of Rostock.

The weather conditions during the second Leg were generally good, with average temperature around 13 degrees, warmest (up to 20°C around noon on 04/06/20 and coldest, slightly over 9°C in the morning on 06/06/20). It was mostly cloudy to sunny with only occasional rain, most of the time wind speed did not exceed 5 to 10 m/s and southwest wind directions prevailed. Wind and waves were gusty, with scuds above 15 m/s (up to 20 m/s) at the first half of the day on 06/06/20, and a sudden short strong scud occurred on 07/06/20 around 14:00 UTC.

Table 4.1 List of numbers of EMB238 stations and major sampling gear casts at both legs located in proximity (here referred to as clusters)

Gear/leg of Station_cast	Clusters in control area outside MPA					Clusters in area within MPA				
	15,26,34	17,27	10,23,28	13,24,31	25,29	5,30,36	2,37,45	8,38,44	39,43	18,40,42
MUC leg 1	15_2,3,4,5	17_3,4,5,6	10_2,3,4,5	13_2,3,4,5	-	5_2,3,4,5	2_3,4,5,6,7	8_2,3,4,5	-	18_3,4,5,6
MUC leg 2	26_4	27_1	28_1	-	29_1	30_1, pw!	37_1	38_1	39_1	40_1,2
VV leg 2	26_1,2,3	27_2,3,4	23_3,4,5*	24_1,2,3, 31_1(sed)*	25_1,2,5	30_2,3,4	45_1,2,3	44_1,2,3	43_1,2,3	42_1,2,3
CTD leg 1	15_1	17_1,2	10_1	13_1	-	5_1	2_1	8_1	-	18_1,2
CTD leg 2	27_5	27_5	22_1	22_1	22_1 (1km)	36_1	45_4	45_4 (500m)	48_2 (650m)	48_2
UW video (close to)	34_1	34_1	33_1	33_1		48_1	47_1	47_1	48_1	48_1
Dredge			32_1	32_1					46_1,2	46_1,2
BIGO		14_1	21_1			35_1	6_1			
EDDY		16_1				41_1				

*both closer to Leg_1 MUCs 10_, then to 13_

Station numbers are always listed first, whereas numbers following after "_" symbol indicate cast numbers

5 Preliminary and Expected Results

5.1 Sedimentology and geophysics (WP 1.1)

(M. Schönke, P. Feldens)

Main task of the acoustic mapping during the cruise was to visualize the impacts of bottom fisheries on seafloor morphology and composition. In this study two different types of echo sounder system were used, which are both common in terms of seafloor mapping. The main recording device was the towed sidescan sonar system (SSS), with the advantage of a large areal

coverage in shallow water to obtain high quality, high resolution backscatter maps. The common drawback of the towed system is the difficulty to determine the device position relative to the ship positioning could be significantly improved by the usage of an ultrashort baseline (USBL) positioning system attached to the SSS. The second device was the hull mounted R2Sonic multibeam system (MBES) with the advantage to obtain seafloor bathymetry and multifrequency backscatter map simultaneously. The disadvantage compared to the SSS are the lower area coverage. Before each hydroacoustic survey a sound velocity profile (SVP) was recorded to determine the raytracing of the acoustic waves through the water column during the postprocessing.

During EMB238 the investigation area has been mapped with 100 percent coverage by hydroacoustics (Figure 5.1.1). With the MBES only 80 percent coverage could be achieved with sufficient data quality, due to a defective sound probe at the transducers impacting beam forming (defect known advance but could not be solved in time). The defect sound probe causes an overall significant loss in the MBES data quality, which cannot be corrected on a later stage during data processing.

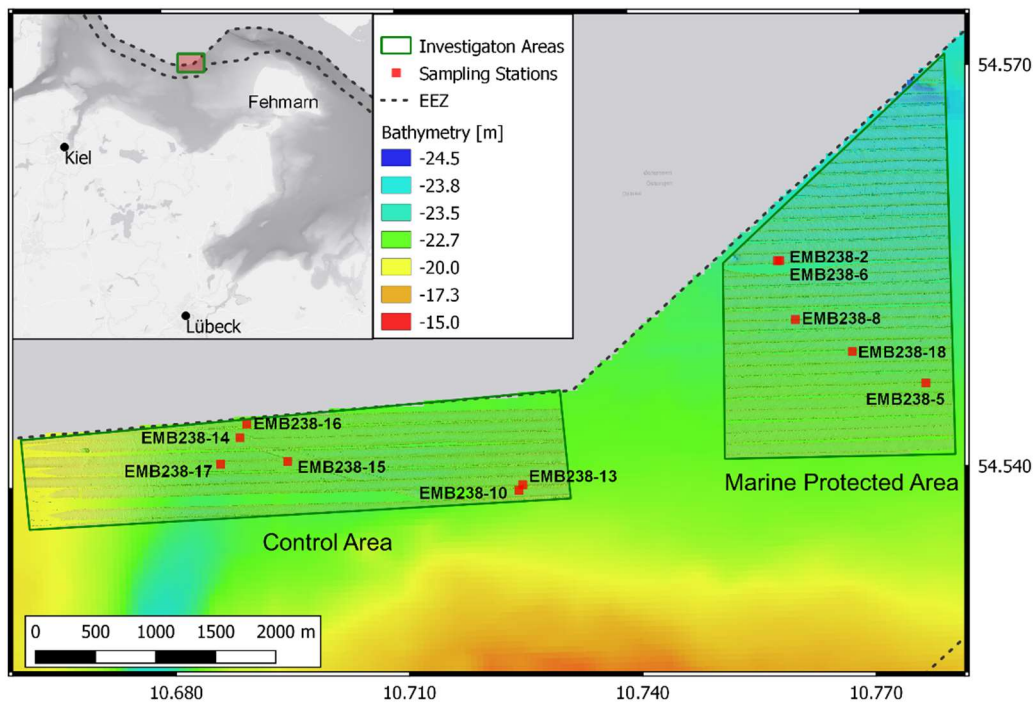


Figure 5.1.1 MBES bathymetry of the investigation area mapped during EMB238 by the R2Sonic multibeam system. As background map bathymetry data published by the BSH (2016) were used. The total size of the mapped investigation area corresponds to 8.54 km², which is subdivided into the MPA with an area of 4.72 km² and the control area with an area of 3.73 km². The stations marked with a red square are the location sampled during the first leg by CTD, MUC and Lander devices.

5.1.1 Methods

Sidescan echosounder system (SSS)

For sidescan backscatter data, the dual frequency (100/500 kHz) Klein Marine System, Series 4000 was used. Additionally, a USBL transponder (see paragraph EvoLogics S2C R for system description) was attached to the SSS to improve the positioning (Fig. 5.1.2). The system was towed 13-17 m over ground distance with a vessel speed of 4-4.5 knots resulting in a ground coverage of 120 m on each side. Water column stratification, which significantly reduces data quality was observed during data recording but could not be fixed, as it was not possible to tow the sidescan towfish below the interface. Therefore, data quality drops significantly from a distance of about 80 m to each side from the device. To fill gaps with bad data quality, each area was mapped with a profile offset of 85 m. Onboard data were processed with the program SonarWiz 7 by Chesapeake. The processing on board included slant range correction, auto time-variant-gain correction, automatic gain correction, nadir filter and layback correction. The resulting backscatter map was used to determine sampling location in both areas.



Fig. 5.1.2 Sidescan system with mounted USBL positioning system

Multibeam system (MBES) R2Sonic

Seafloor bathymetry and multifrequency backscatter data were recorded by using the hull mounted MBES R2Sonic. The MBES was operated by using a swath width of 170 deg with a corresponding ground coverage of 180 m and multi-frequency ping mode (200/300/400 kHz). Best results (based on waterfall mode observation in the recording software) were achieved with the following settings: pulse length 15 μ s, gain 7 dB, spreading 40, absorption according to frequency 30/80/110. Vessel speed during MBES data acquisition was 4.5-5 knots. During the cruise, no processing software was available for a screening of the recorded data quality during the cruise. For a later processing the software QPS Marine software solution was used (Figure 5.1.1).

EvoLogics S2C R ultrashort baseline (USBL) positioning System

The ultrashort baseline (USBL) positioning System is an underwater positioning system designed for shallow water operation to a maximum operation depth of 200 m and an operation range of 3500 m. The USBL transceiver is mounted to the vessel hull and communicates (13,9 kbps) within a frequency range of 18 -34 kHz with a transponder attached to a target device. It is possible to use the USBL system to track multiple targets at once, that on the EMB238 the SSS and the Lander system could be tracked parallel. The USBL measures the travel time between the transceiver and the transponder to determine the distance between the instruments. By using the phase-difference

method, the transceiver (consisting of multiple hydrophones) computes the angle to the transponder to calculate the relative position. By implementing the USBL SINAPS client recording software (by EvoLogics GmbH) to the AHRS and GPS ship sensor, the positions of the transponders are displayed and recorded as real-world coordinate. Unfortunately, during the installation and the setup of the USBL recording software, the position of the USBL relative to the ship was configured incorrectly. This caused a double offset effect in the recorded data by the USBL system and all coordinate positions must be corrected by -25 m in heading direction. USBL data were stored in a SQL database file format.

Sediment samples

At each sampling location a short core was taken for sedimentological and geophysical analysis. The short cores were sealed directly after recovery and stored in an upright position (Fig. 5.1.3). Some of the cores are used for x-ray imaging, with the aim to visualize sediment density changes caused by bioturbation processes. The remaining short cores will be used to test vertical core logging, with the aim to measure p-waves velocity, shear strength and grain size with a resolution of 1 cm.



Fig 5.1.3 shows all cores taken for analysis later in the IOW geophysical lab. The four cores on the left hand side (2 - 4, 5 - 4, 8 - 2, 18 - 3) were taken in the MPA, and the four cores on the right hand side (10 - 2, 13 - 2, 15 - 2, 17 - 3) were taken in the control area.

5.1.2 Expected results

A first overview shows in the entire investigation area morphological seafloor features, which are with a high probability trawl marks caused by fishing activities. The seafloor features are observed in the sidescan backscatter mosaic and the MBES obtained seafloor bathymetry. At the first view, no clear differences could be observed between the designated MPA and Control Area with regard to the impacts of bottom fisheries on seafloor surface morphologies, due to the overall high degree of seafloor morphological features. It was surprising, that morphological features corresponding to fishery activity are so well visible in the MBES bathymetry data (Fig. 5.1.4d). Further, the recorded multi-frequency backscatter data (not processed yet) by MBES will allow a multi-spectral

interpretation approach of the acoustic data, for an advanced classification and characterization of seafloor features. The combination of both acoustic system backscatter dataset including five frequencies offers a still unknown potential for interpretation.

In addition to the USBL system, a known ship wreck mapped in the north-west of the MPA and the large anchor stone of a buoy mapped in the north-west of the control area (which marks the border of the shooting area) are used as common reference points to improve the positioning comparison between the data recorded by the towed SSS system and the hull mounted MBES. The use of the USBL system is a major advantage in terms of matching real-world coordinates to ground-truthing sample position. Due to the mentioned false offset, to match the USBL position data to the samples will require additional processing but will be a major advantage in terms of interpretation. The exact positioning of the sample location within the acoustic data is essential to the data analysis and interpretation of the outer research foci.

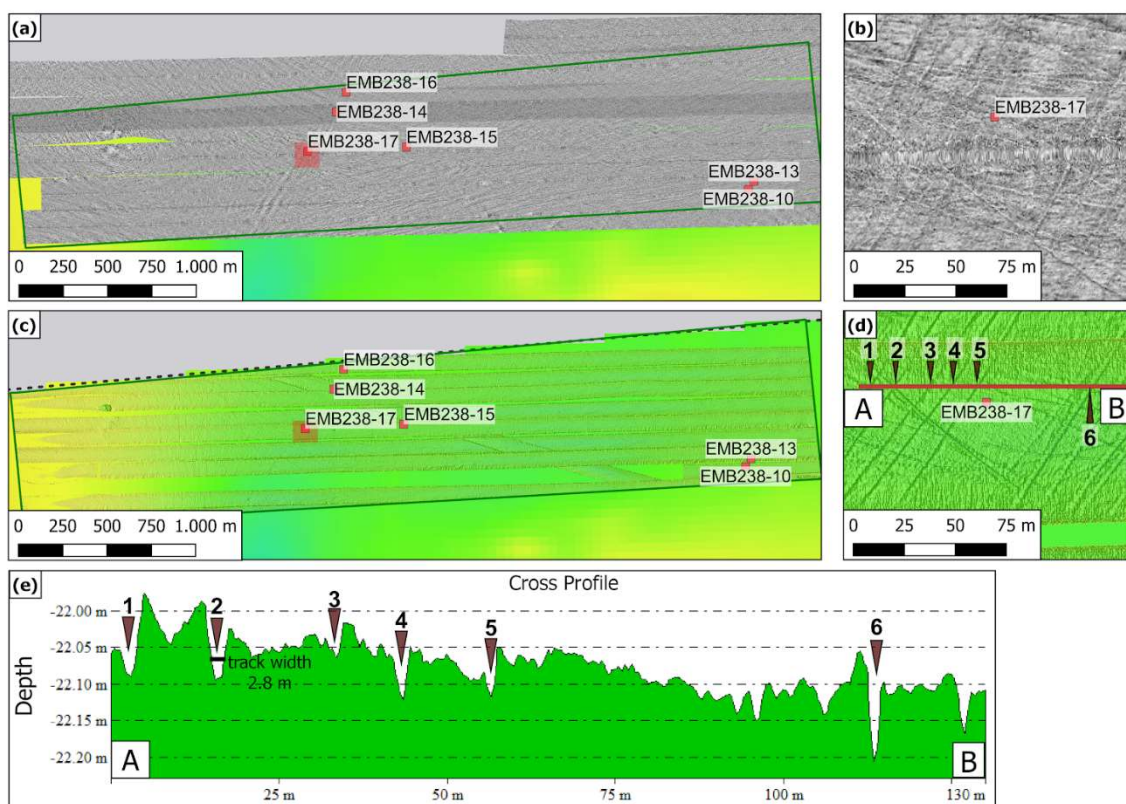


Fig. 5.1.4 (a) Recorded Backscatter mosaic of the control area by SSS, processed on board with 0.5 m resolution. (b) Close up on the backscatter of planned station 17, (Overview of the marked area in (a)), which shows seafloor structures that are the very unlikely to be geological origin. (c) Recorded Bathymetry of the control area by the MBES, processed in the IOW, with also a resolution of 0.5 m. (d) The close of Station EMB238-17, which shows the close up of the marked area in (c), shows similar seafloor structures as (b), that are also very unlikely to be geological origin. Additionally, the line drawn in (d) shows the location of the cross profile demonstrated in (e). (e) shows a cross profile of the bathymetry, to give an overview of depths of the morphological features observed in (d).

5.2 Biogeochemical processes in the surface sediments (WP 1.2) (M.A. Zeller, M.E. Böttcher)

5.2.1 Method

Sediment cores (3-4) were collected with the first MUC each morning at each site, using core liners modified with threaded holes at 1 cm intervals for rhizon sampling. The holes were initially covered with electrical tape to prevent overlying and porewater loss during collection. One core per site was collected for further sediment geochemical characterization (TOC, TIC, TN, Hg, porosity, and grain size distribution), and sectioned at 1cm intervals for the top 5 cm and at 2 cm intervals further down core. These sections were split between empty Sarstedt tubes and tubes treated with ZnOAc solution, and all sediment samples were kept frozen (-20 °C) upon collection until further analyses. Additionally, 1-3 cores were sampled with rhizons (~12 µm pore size) for porewater chemistry at 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 20, 25 cm intervals, after the removal of the overlying water via syphon. Porewater samples were collected for lab-based analyses for metals (ICP-OES: P, S, Fe, Mn, Ca, K, Mg, Na, Si, Ba, Li, Mo), sulfide (photometry), nutrients (ICP-OES, autoanalyzer), $DI^{13}C$, $DO^{13}C$ (gas mass spectrometry), total alkalinity (TA; titriprocessor), pH (on board: ion selective electrode), and water isotope (laser CRDS) analyses. In general, 1-2 cores were dedicated for metals, sulfide, nutrients, $DI^{13}C$, $DO^{13}C$ for every site, and at 5 sites an extra core was sampled for pH, TA, $DI^{13}C$, and water isotopes. For MUC 30_1, only one core was collected and it was treated somewhat differently: The porewater was collected at 0 (i.e. overlying bottom water, just above the sediment/water interface), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 30 cm with rhizons. Porewater samples from this core were collected for metals, sulfide, nutrients, pH, TA, $DO^{13}C$, and water isotope analyses.

In addition to sediment cores, bottom water samples were collected during a majority of CTD casts, as well as water column samples at one site inside and one site outside of the MPA at 4 different depths. These water samples were analyzed for metals, sulfide, nutrients, $DI^{13}C$, $DO^{13}C$, pH, TA, and water isotopes. Furthermore, a CONTROS HydroC CO₂ analyzer was incorporated on the CTD rosette and used to measure bottom water CO₂ concentration during a majority of the CTD casts.

5.2.2 Expected and preliminary results

Sample and data analysis are still at preliminary stages, as we only have results for sediment water content and porewater sulfide, metals, and pH. Selected results are displayed in Figure 5.2.1. We anticipated that we would not be able to distinguish sediment cores collected from inside of the MPA from those collected outside of the MPA for this first cruise, as at present both areas experience bottom contact trawling. This appears to be the case, although there is large variability between cores collected from different MUC deployments, especially in the sulfide data.

In general, we can see that for all cores, the water content is highest near the surface of the sediment. For some cores, which is especially obvious in MUC 5_2, 10_2, and 13_2, there is a local minima of water content around 5-10 cm or 10-15 cm, suggesting a decrease in porosity in these fractions. In the sulfide data, we see great variability between cores collected from different MUC deployments. Consider the 20 cm depth interval, where MUC 10_2 has values of ~15-30 µM while MUC 2_3 has concentrations of 1200 µM sulfide. The profiles show the strong connection between sulfur and iron quite clearly. There is very little to no sulfide present in the top 5 cm, where there are high concentrations of Fe²⁺, as the sulfide diffusing upwards precipitates as Fe(II)S compounds. Likewise, there is very little Fe below 7-10 cm, as the iron diffuses down core and meets high sulfide concentrations, again precipitating as Fe(II)S compounds. In the Mn

and Fe data, we can see that despite being produced in the top few centimeters of sediment, there is almost none of these redox sensitive metals in the bottom water (BW) or overlying water (see “0” cm in MUC 30_1), as these metals precipitate in the form of oxy-hydroxides when in contact with oxygen. In the pH profiles, we can see that in general the pH is highest in the bottom water or overlying water (again, see “0” cm in MUC 30_1), at about 7.75. For most cores, the pH decreases quite a lot in the first 1 cm fraction, and rises again from 5-15 cm, before decreasing again down core.

Focusing on the sulfide data, it becomes clear that despite the variability between MUC deployments, there is consistency within each MUC deployment. We have analyzed sulfide in duplicate for MUCs 2_3, 8_2, 10_2, 13_2, 15_2, 17_3, and 18_3. Especially true for 2_3, 10_2, 15_2, and 18_3, the sulfide profiles for these pairs follow the consistent trends. For example, the second core from 2_3 (which ends at 9 cm depth) also shows high sulfide concentrations, while both cores for 10_2 have very low sulfide concentrations. Both cores from MUC 15_2 and 18_3 have intermediate sulfide concentrations and similar profiles. Despite this consistency within a MUC deployment, there is high variability between sites, even those of very similar location. MUC 10_2 and MUC 13_2 were collected only one day apart, from very similar geographic locations, and yet show very different sulfide profiles. Similarly, MUC 5_2 and MUC 30_1 were intended to be from the same site location, and were collected one week apart, yet the core profiles are very different, with 30_1 having much higher sulfide concentrations.

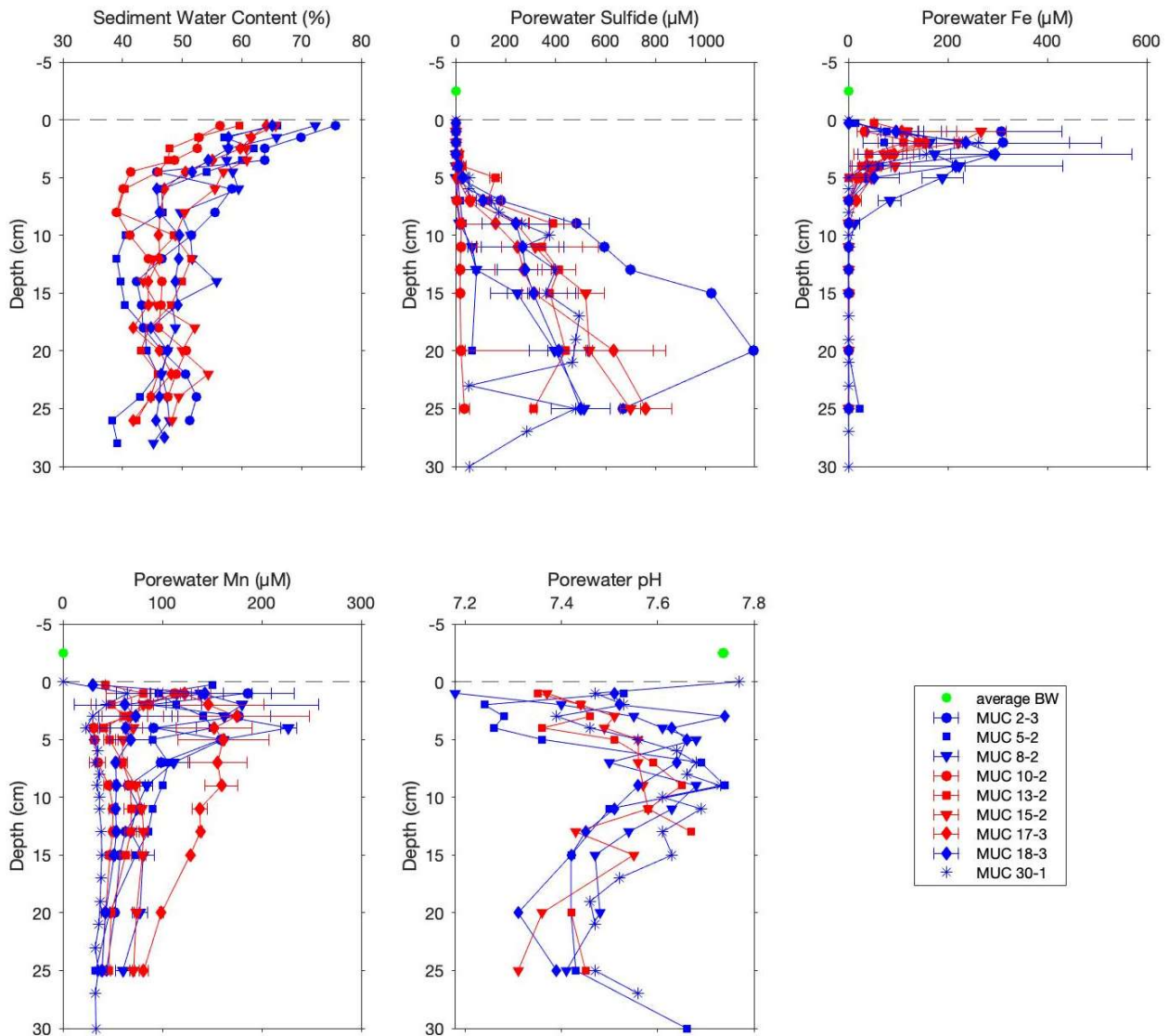


Fig. 5.2.1 Sediment profiles for water content, as well as porewater sulfide, Fe, Mn, and pH. Profiles are separated by MUC, and when averaged for duplicates the range is provided. Blue colors denote cores taken from inside the MPA, while red colors denote cores taken from outside of the MPA. The average bottom water (BW) value for each analyte is given in green. The depth for the average BW is set at 2.5 cm above the sediment/water interface for convenience, however these samples were collected with the CTD rosette from ~1 m above the sea floor.

5.3 Sulfate reduction rate measurements (WP 1.2)

(J. Kallmeyer)

5.3.1 Method

Sulfate reduction rates (SRR) were quantified using incubations of intact sediment cores with radioactive $^{35}\text{SO}_4^{2-}$ radiotracer (Jørgensen, 1978). Using a single MUC core per sampling site, three 40 cm-long acrylic tubes (30 mm OD, 24 mm ID) were pushed vertically into the sediment to retrieve mechanically undisturbed subcores. Suction was employed to avoid compression of the sediment during insertion of the tubes. Each tube has a single row of 2 mm holes drilled in 1 cm resolution drilled along its side, the holes are sealed with silicone, to avoid seepage of porewater but allow injection of radiotracer. Immediately after retrieval of the MUC, the core was subsampled and the three acrylic tubes stored in an incubator at approx. in-situ temperature (10 °C).

After termination of deck operations in the late afternoon all samples from this day were incubated. For incubation, 15 µl of radiotracer (activity ca. 200 kBq) was injected into each hole from the sediment-water interface down to 20 cm below sea floor (cmbsf). Immediately after injection of radiotracer, the core tube was put back into the incubator and incubated for 24 hrs. As all sampling sites had oxygenated bottom waters, the core tubes were left open to avoid oxygen deficiencies. Changes in salinity due to evaporation can be neglected at such temperatures.

Incubations were terminated by pushing the sediment out of the core tubes, slicing them into depth sections and transferring the sediment into 50 ml centrifuge tubes, filled with 10 ml of 20% (w/v) zinc acetate solution. The following resolution was used on all cores

0-6 cm: 1 cm

6-10 cm: 2 cm

10-20 cm: 5 cm

The vials were thoroughly shaken to break up all sedimentary structures and effectively stop all microbial activity. Due to space limitations on board the samples could not be frozen but were stored at room temperature for the remainder of the cruise. Additional samples were taken for blank measurements, usually by inserting an acrylic core tube in left over MUC tubes. Different types of blanks were taken:

Time Zero Blanks: Samples were injected with radiotracer like regular samples, but incubation was stopped within 10 minutes after injection.

Killed Controls: Sediment was first mixed with 10 ml of 20% (w/v) zinc acetate solution, tracer was added after fixation of the sample.

Tracer Blanks: 15 µl of tracer was directly dropped into a 50 ml centrifuge tube filled with 10 ml of 20% (w/v) zinc acetate solution.

All three types of blank samples are treated like regular samples.

Additional SRR samples were taken from the two chambers of the BIGO Lander. During Leg 1 two deployments brought back sufficient amounts of sediment in the incubation chambers. From each of the two chambers a triplicate of acrylic core tubes could be retrieved and was incubated like regular tubes from MUC cores. A total of 443 samples were collected.

5.3.2 Expected and preliminary results

No analyses were performed on board. Upon return to the home lab at GFZ Potsdam the biologically produced radioactive reduced sulfur species (TRIS, total reduced inorganic sulfur) is currently extracted from the sample using cold chromium distillation (Kallmeyer et al., 2004). Initial results show measurable activity in all samples that have been processed so far (about half). As we do not have access to the pore water sulfate and porosity profiles yet, it is not possible to draw any conclusion, other than the fact that sulfate reducing activity is ubiquitous in the near-surface sediment of the studied areas.

5.4 Benthic flux measurements using the BIoGeochemical Observatory (BIGO) (WP 1.3)

(D. Clemens, N. Bill)

5.4.1 Method

Using the GEOMAR BIGO Lander (Fig. 5.4.1 c), in-situ solute fluxes across the sediment-water interface are measured. For each deployment parallel incubations in the 2 benthic chambers (\varnothing 288 mm) of the BIGO are performed. The incubations last approximately 36 h during which the chamber water as well as the background bottom water is autonomously sampled 8 times whilst some parameters are measured continuously (via optodes and conductivity cells). The platform additionally allows for injections of other aqueous solutions (e.g. to determine the volume of the overlying water or to perform in-situ experiments). At the end of the incubation the chamber content (sediment & overlying water) is enclosed and is recovered together with the Lander for subsequent sediment and porewater sampling on deck.

Auxiliary sensors are attached to the Lander such as a SBE 19plus CTD, 3 Hobo pendant light loggers (M. Paar, University of Rostock) and an underwater USBL positioning system (M. Schönke, IOW).

5.4.2 Expected and preliminary results

We performed 2 BIGO deployments in both the control area as well as the MPA (4 total) and were able to recover the sediment enclosed in the chambers (Fig. 5.4.1 b). The last deployment was unfortunately cut short by a trawling fisher boat. Despite the damage done, parts of the data of that deployment will still be usable.

With the upcoming analysis of our water, porewater and solid phase samples in our home laboratory, we will be able to determine O_2 , NO_3^- , NO_2^- , PO_4^{3-} , TA, DIC fluxes as well as ions determined by IC and ICP analysis.

The Lander platform was additionally used in the following cross-subproject joint-efforts: In cooperation with S. Forster (University of Rostock) we injected bromide in the beginning of the incubation in order to being able to determine bioturbation as well as the chamber volume (see the salinity increase in Fig. 5.4.1 a).

Moreover, the sediment recovered from 1 deployment was entirely sieved for its fauna by S. Forster, M. Powilleit (Universität Rostock) and M. Gogina (IOW).

Additionally, benthic sulfate reduction rates were measured via radiotracers in triplicates in each chamber during 2 deployments by J. Kallmeyer (GFZ Potsdam).

In partnership with M. Zeller (IOW) the porewater nutrients of the recovered chamber sediments will be measured.

The already available optode and conductivity data shows that all incubations were successful, and oxygen was consumed in variable amounts (Fig. 5.4.1 a). A preliminary analysis of O_2 fluxes measured *in situ* in benthic chambers revealed a strong difference between the MPA (mean: $-38.1 \text{ mmol m}^{-2} \text{ d}^{-1}$, std.: 8.0, n: 4) and the reference site (mean: $-14.4 \text{ mmol m}^{-2} \text{ d}^{-1}$, std.: 1.3, n: 4). Whether this is related to difference in fishing activity awaits further analysis.

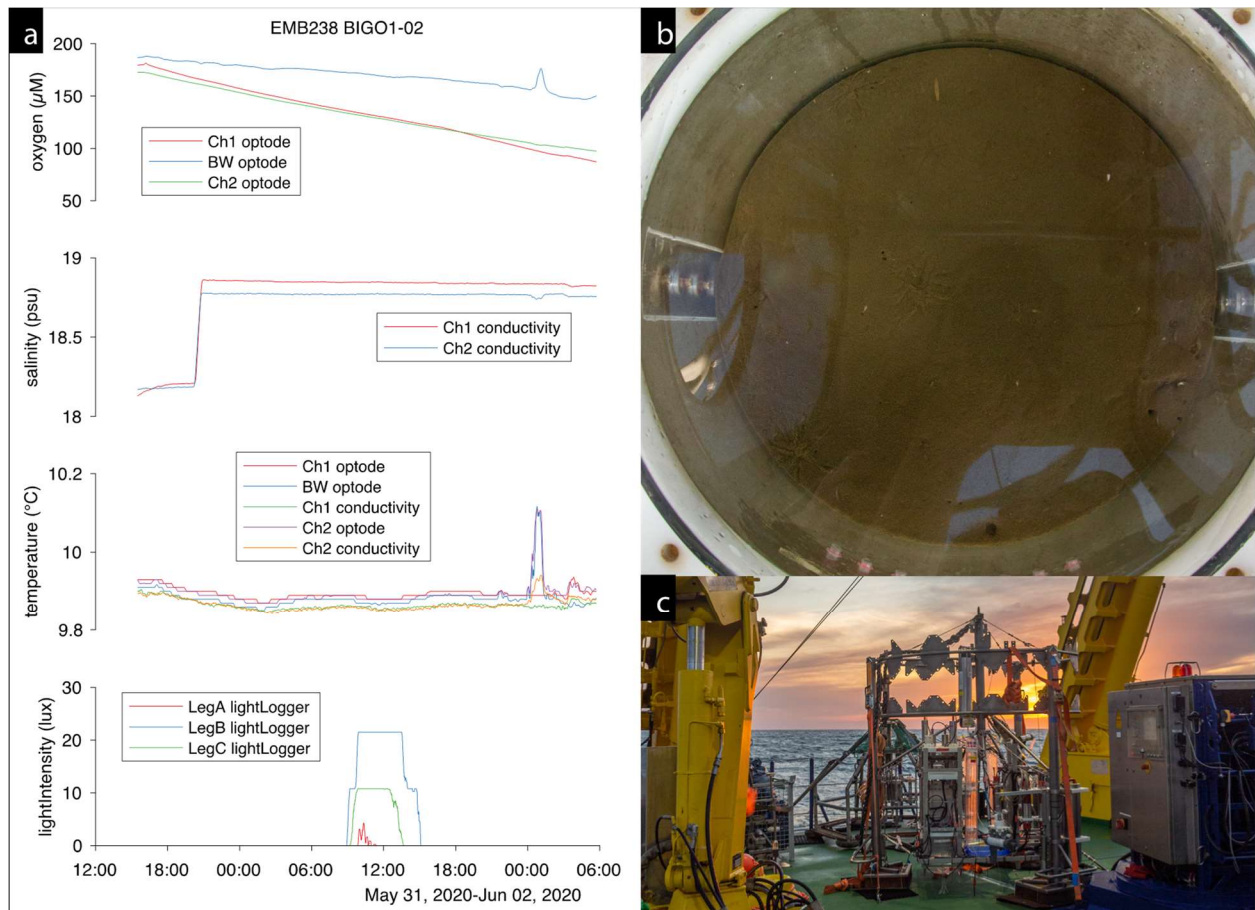


Fig. 5.4.1 Shown are (a) an example of the oxygen consumption and other environmental parameters during the BIGO incubation of BIGO1-02 in chamber 1 (Ch1) and 2 (Ch2) as well as the bottom water (BW) background, (b) the sediment recovered in chamber 2 of BIGO1-02 and (c) the BIGO Lander aboard the EMB.

5.5 Benthic flux measurements using the aquatic eddy correlation technique (WP 1.3)

(D. Clemens, N. Bill)

5.5.1 Method

Using the GEOMAR EC Lander (Fig. 5.5.1), in-situ oxygen fluxes across the sediment-water interface are measured. At the Landers core a Nortek acoustic doppler velocitometer (ADV) in combination with 2 Pyroscience Piccolo2 fiber-optic oxygen meters are used to measure turbulent fluxes of oxygen.

Auxiliary sensors are attached to the Lander such as an independent Aanderaa oxygen optode, a SBE 37-SM CTD, 1 Hobo pendant light loggers (M. Paar, University of Rostock), 3 GoPro cameras and an underwater USBL positioning system (M. Schönke, IOW).



Fig. 5.5.1 The EC Lander prior to deployment.

5.5.2 Expected results

We performed 1 EC deployment in both the control area as well as the MPA (2 total). The last deployment was unfortunately cut short by a trawling fisher boat. Despite the damage done, parts of the data of that deployment will still be usable.

With the upcoming analysis, we will be able to determine O₂ fluxes non-invasively in combination with oceanographic parameters for both the MPA and control area. Fluxes over an entire diurnal cycle in the high flow environment of the Fehmarnbelt will thus be available.

5.6 Prokaryotes (WP 2.1)

(J. Piontek)

Aims

The major goal of this work package is to investigate how the exclusion of bottom trawl fisheries from the MPAs will alter the composition and functioning of benthic prokaryotic communities. Cell numbers, phylogenetic composition, heterotrophic activity and the functional potential of communities in sediment samples of the envisaged exclusion zone will be analysed and compared with samples collected in a nearby reference area with similar fishery intensity. The composition of benthic assemblages will be analyzed by sequencing the 16S rRNA. The relative abundances of 16S rRNA sequences will be used to characterize the active key players of the communities. Heterotrophic activity of benthic prokaryotes will be explored by means of microbiological assays for hydrolytic extracellular enzymes and biomass production. Furthermore, metagenomic and -transcriptomic approaches will be used to assess the functional potential of the communities. The analysis of gene abundances and gene expression patterns will be focused on metabolic processes that are directly linked to important sediment ecosystem services. These processes include carbon remineralization, the release of inorganic nutrients and transformations within the sulfur cycle at the sediment-water interface.

Work on board

At four stations in the envisaged exclusion zone and at four stations in the reference area, respectively, sediment samples were collected by MUC hauls. For the microscopic enumeration of prokaryotic cells and for the extraction and subsequent sequencing of nucleic acids, sediment

cores were sliced into seven sections (0-1 cm, 1-2 cm, 2-4 cm, 4-6 cm, 6-10 cm, 10-15 cm, 15-20 cm). Samples were stored frozen until further analysis. Heterotrophic activity in surface sediment samples of three stations per area was analysed on board. Rates of leucine-aminopeptidase were determined using the fluorescent substrate analogue 7-amino-4-methylcoumarin. Prokaryotic biomass production was estimated from the uptake of 3H-thymidine.

5.7 Nano- and microfauna (WP 2.2)

(M. Hohlfeld, H. Arndt)

Up to our present knowledge, protists (unicellular eukaryotes) comprise the majority of all eukaryotic genotypes in the world's oceans (e.g. de Vargas et al., 2015). The nano- (protists in the size range from 1-20 μm) and microbenthos (protists in the size range from 20-200 μm) are essential parts of the benthic food web as they channel bacterial production to higher trophic levels as meiofauna and macrozoobenthos which in turn act as nutritional basis for demersal fish. The bacterial abundance and production is assumed to be regulated by the predation pressure of the nano- and microfauna. Thereby also a variety of geochemical processes determined by the oxygen consumption of bacteria should be influenced by protists. We assumed, that a disturbance of the sediment structure through trawling would significantly change the microbial food web and its functions.

We planned to use a combination of different methods to investigate the diversity, abundance and activity of the nano- and microfauna inside of the marine protected area (MPA) in the Fehmarnbelt and outside of the MPA. In order to successfully compare the benthic nano- and microfauna of the two above mentioned areas we used four different approaches since all methods have their advantages and disadvantages (Schoenle et al., 2016). To estimate abundances and investigate the diversity and activity of protists, we carried out sampling to allow for a combined analysis of live-counting, counting of fixed samples, determination of cultivable protist species and we preserved samples for metabarcoding analyses of the rDNA and rRNA (Fig. 5.7.1). For comparisons of the benthic and the pelagic protist communities, water samples were taken for abundance and diversity analyses.

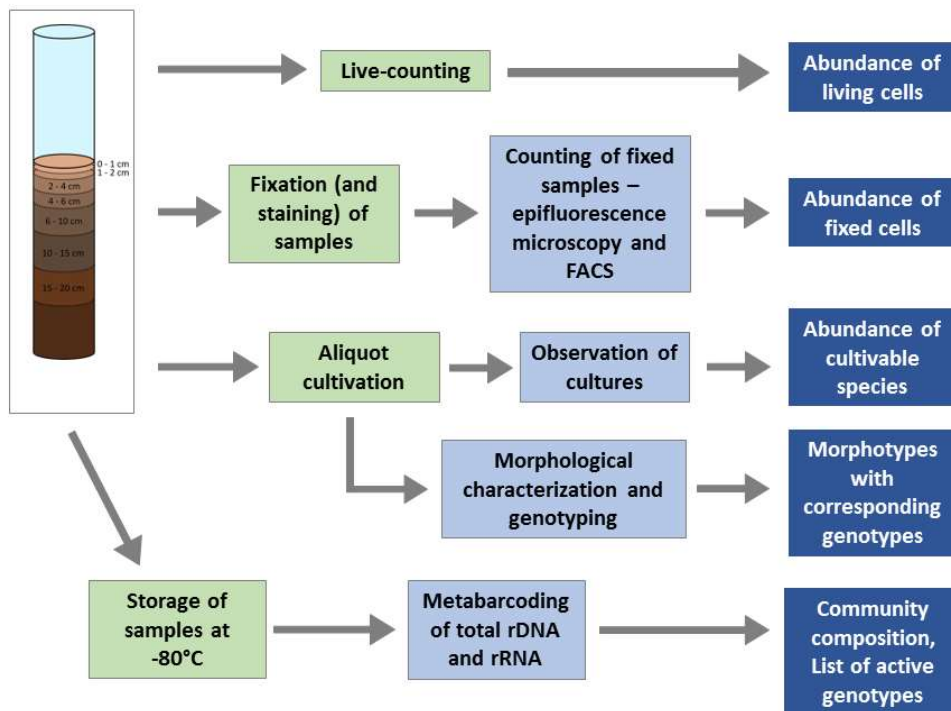


Fig. 5.7.1 Sampling protocol to estimate the diversity and abundance of nano- and microfauna. Each core was sliced into seven sediment layers. For each layer samples were deep frozen at -80°C for metabarcoding analyses. Live-counting, fixation of samples, DAPI-staining and the establishment of crude cultures were carried out on board (green boxes). Methods in light blue boxes will be carried out in the home laboratory.

Methods:

Sediment sampling

Sediment samples were taken at eight stations, four within the MPA and four outside the MPA with the multi-corer (MUC) system. Undisturbed sediment cores obtained by the MUC were used for quantitative and qualitative analyses of benthic nano- and microfauna. At stations 2-4, 5-5, 8-5 and 10-4, four sediment cores were sampled. Three cores were used for the DNA/RNA metabarcoding samples and one core was used for abundance estimations and the cultivation approach. At stations 13-6, 15-5, 17-6 and 18-6, three sediment cores were sampled of which all were used for DNA/RNA metabarcoding samples, one core was also used for abundance estimations and cultivation (Table 5.6.1). All cores were sliced into seven sediment layers (0-1 cm, 1-2 cm, 2-4 cm, 4-6 cm, 6-10 cm, 10-15 cm, 15-20 cm). Due to a closing mechanism at the top and bottom of the cores, the risk of contamination with organisms and cysts from upper water layers was reduced. However, the problem is that samples had to be processed fast after sampling, as protists are stressed by changes in e.g. temperature.

Abundance estimations of benthic nano- and microfauna

At each station one core was used for abundance estimations of all seven sediment layers. Per sediment layer, a volume of 156 μl sediment was mixed with 4 ml of Baltic sea water which had been filtered through a filter with a pore size of 0.2 μm previously. These suspensions were stored on water with a temperature of $\sim 10^{\circ}\text{C}$ and used to detect living protists under the microscope. Inspection and counting of 1-5 μl subsamples of sediment suspensions were conducted using a light microscope (40 x phase-contrast objectives) combined with video recording (Arndt et al.,

2000). Besides quantitative estimations, live-counting techniques offer the opportunity to determine several living specimens up to the morphospecies level and to verify the presence of living specimens of genotypes only known from metagenomic studies. Limitation of this method is the narrow time frame for observations of sediment suspensions on board, since several nanofauna organisms die after a few minutes due to rising temperatures and light. However, the direct counts can serve as a cultivation-independent record of species being active at the time of sampling and which cannot yet be identified from the study of data bases since reliable morphological and molecular identifications are missing.

Fixation and staining methods are advantageous due to the possibility of long-term storage and observation of samples. We used two different approaches for abundance estimations of fixed samples, DAPI staining with subsequent epifluorescence microscopy and FACS (flow cytometry). For DAPI staining, we used a volume of 156 μ l sediment and mixed it with 2 ml of filtered Baltic sea water (filter with a pore size of 0.2 μ m). This suspension was fixed with 2 ml of 4% formaldehyde (final concentration 2%). After 24 hours, samples (100 μ l) were filtered as triplicates on 0.2 μ m polycarbonate filters at a vacuum of less than 200 mbar (Morgan-Smith et al., 2011) and stained with 20 μ l DAPI (10mg/l) for 5 minutes. Filters were mounted on microscopic slides and were deep frozen and kept at -20°C until further processing in Cologne. The staining fluorochrome DAPI binds to cell components such as DNA to detect potentially eukaryotic cells (Porter and Feig, 1980; Sherr et al., 1993). Counting of fixed and DAPI stained samples will be conducted under an epifluorescent microscope in the laboratory in Cologne.

For abundance estimations by flow cytometry (FACS), a volume of 625 μ l sediment was mixed with 9 ml of filtered Baltic sea water (filter with a pore size of 0.2 μ m). The suspension was fixed with 0.8 ml of 25% glutardialdehyde (final concentration 2%) and stored at 4°C. Counting with the flow cytometer will be conducted in the home laboratory in Cologne.

Cultivation of benthic nano- and microfauna

Cultivation of protist species aims to relate the molecular identity of species to their morphology and ecology to derive an idea on the functioning of the benthic microbial food web. From each sediment layer, a volume of 156 μ l sediment was mixed with 4 ml autoclaved Baltic sea water. This suspension was added to two 50 ml tissue culture flasks filled with 30 ml autoclaved Baltic seawater. All cultures were supplied with autoclaved quinoa grains to enrich co-occurring bacteria as a food source for protists. In the home laboratory in Cologne, the liquid-aliquot method will be used to establish monoclonal cultures to analyze species' genotype, taxonomy, phylogeny and their ecology.

Environmental sequencing of benthic nano- and microfauna

The analyses of bulk DNA from marine sediments by high-throughput sequencing methods allow for a qualitative analysis of the protist community and a rough assignment to trophic functions of the nano- and microfauna (e.g. Bik et al., 2012). From all sampled sediment cores, seven sediment layers were sampled. Sediment was deep frozen in liquid nitrogen and stored at -80°C. In addition, from one core at each station, 2 ml of sediment were fixed with 10 ml RNA Later and stored at -20°C for metabarcoding analysis of RNA.

A large proportion of DNA in marine sediments is extracellular (Dell'Anno and Danovaro, 2005). Thus, it is uncertain whether protist species, detected by environmental sequencing are

actually active or if sequences originate from sedimented cells from the water column, cysts or extracellular DNA (e.g. Stoeck et al., 2007). To reduce this bias, we will use rRNA libraries to gain information on the active part of the microbial community. Additionally, the cultivated strains (see above) should serve as a valuable reference.

Table 5.7.1 List of stations and samples for benthic nano- and microfauna analyses.

Station	Gear	Core	Date	Sample type	Area
2-4	MUC	1	27.05.2020	DNA/RNA	MPA
2-4	MUC	2	27.05.2020	DNA/RNA	MPA
2-4	MUC	3	27.05.2020	DNA/RNA	MPA
2-4	MUC	4	27.05.2020	DAPI, FACS, cultivation, live-counting	MPA
5-5	MUC	1	28.05.2020	DNA/RNA	MPA
5-5	MUC	2	28.05.2020	DNA/RNA	MPA
5-5	MUC	3	28.05.2020	DNA/RNA	MPA
5-5	MUC	4	28.05.2020	DAPI, FACS, cultivation, live-counting	MPA
8-5	MUC	1	29.05.2020	DNA/RNA	MPA
8-5	MUC	2	29.05.2020	DNA/RNA	MPA
8-5	MUC	3	29.05.2020	DNA/RNA	MPA
8-5	MUC	4	29.05.2020	DAPI, FACS, cultivation, live-counting	MPA
10-4	MUC	1	30.05.2020	DNA/RNA	Control area
10-4	MUC	2	30.05.2020	DNA/RNA	Control area
10-4	MUC	3	30.05.2020	DNA/RNA	Control area
10-4	MUC	4	30.05.2020	DAPI, FACS, cultivation, live-counting	Control area
13-6	MUC	1	31.05.2020	DNA/RNA	Control area
13-6	MUC	2	31.05.2020	DNA/RNA	Control area
13-6	MUC	3	31.05.2020	DNA/RNA, DAPI, FACS, cultivation, live-counting	Control area
15-5	MUC	1	01.06.2020	DNA/RNA	Control area
15-5	MUC	2	01.06.2020	DNA/RNA	Control area
15-5	MUC	3	01.06.2020	DNA/RNA, DAPI, FACS, cultivation, live-counting	Control area
17-6	MUC	1	02.06.2020	DNA/RNA	Control area
17-6	MUC	2	02.06.2020	DNA/RNA	Control area
17-6	MUC	3	02.06.2020	DNA/RNA, DAPI, FACS, cultivation, live-counting	Control area
18-6	MUC	1	03.06.2020	DNA/RNA	MPA
18-6	MUC	2	03.06.2020	DNA/RNA	MPA
18-6	MUC	3	03.06.2020	DNA/RNA, DAPI, FACS, cultivation, live-counting	MPA

Pelagic nano- and microfauna

To estimate the abundance and diversity of nano- and microfauna in the water column, water samples were taken from 4 different depths with the CTD rosette at one station in the MPA and one station outside the MPA (Table 5.7.2). For estimations of the abundance of protists, at each depth 2 ml water were fixed with 2 ml 4% formaldehyde (final concentration 2%). Samples were stored overnight and filtered as triplicates (1 ml per filter) on 0.2 µm black polycarbonate filters at

a vacuum of less than 200 mbar. Samples were stained with 20 µl DAPI (10 mg/l) for 5 minutes. Filters were mounted on microscopic slides and stored at -20°C until further investigations. Abundances of nano- and microfauna will be determined using an epifluorescence microscope (Porter and Feig, 1980; Sherr et al., 1993).

To investigate the diversity of pelagic protist communities, 1 L seawater from each depth was filtered on a 0.4 µm glass fibre filters directly after sampling. Filters were deep frozen in liquid nitrogen and stored at -80°C for molecular analyses of protistan DNA which will be performed in Cologne. Environmental DNA will be isolated, amplified by PCR and sequenced by high-throughput sequencing techniques. The resulting dataset will be compared with the sediment dataset to gain information on which sequences in the sediment may originate from the pelagic zone.

For microscopic investigations of the diversity and identification of morphotypes, 1 L of seawater from each depth was fixed with 1 ml Lugol solution. Sample will be screened in Cologne.

Table 5.7.2 List of stations and samples for pelagic nano- and microfauna analyses.

Station	Gear	Depth [m]	Date	Sample type	Area
2-2	CTD	2.6	27.05.2020	Filter for DNA, Lugol fixed water, DAPI staining	MPA
2-2	CTD	9	27.05.2020	Filter for DNA, Lugol fixed water, DAPI staining	MPA
2-2	CTD	14	27.05.2020	Filter for DNA, Lugol fixed water, DAPI staining	MPA
2-2	CTD	21	27.05.2020	Filter for DNA, Lugol fixed water, DAPI staining	MPA
13-1	CTD	3	31.05.2020	Filter for DNA, Lugol fixed water, DAPI staining	Control area
13-1	CTD	9	31.05.2020	Filter for DNA, Lugol fixed water, DAPI staining	Control area
13-1	CTD	15	31.05.2020	Filter for DNA, Lugol fixed water, DAPI staining	Control area
13-1	CTD	22	31.05.2020	Filter for DNA, Lugol fixed water, DAPI staining	Control area

Preliminary results

Live-counting

Preliminary data of the abundance of nano- and microfauna are available only for the direct live-counts. They revealed the highest abundance of protists in the surface sediment layer (0-1 cm) ranging from 500 to 1,500 protists per cm³ as a minimum estimate of active protists. This is noticeable lower than for instance estimations from shallow waters of the Baltic Sea around Hiddensee, where abundances of $8 * 10^3$ up to $104 * 10^3$ nanoprotists per cm³ were observed (Dietrich and Arndt, 2000). Deeper sediment layers showed even lower abundances ranging from 167 to 1,000 individuals per cm³. In sediment layers deeper than 6 cm, no protists could be detected during live observations.

Besides the abundance of protists, live-counting also gives the opportunity to gain information on the relative contribution of different taxonomic groups and their major roles in the benthic food web. The majority of protists observed were heterotrophic flagellates with a bacterivorous feeding type. Bicosoecids (*Caecitellus*, *Cafeteria*), ancyromonads (*Ancyromonas*), bodonids (*Neobodo*, *Rhynchomonas*), cercozoans (*Massisteria*, *Metromonas*) and euglenids (e.g. *Petalomonas*) were observed (Fig. 5.7.2).

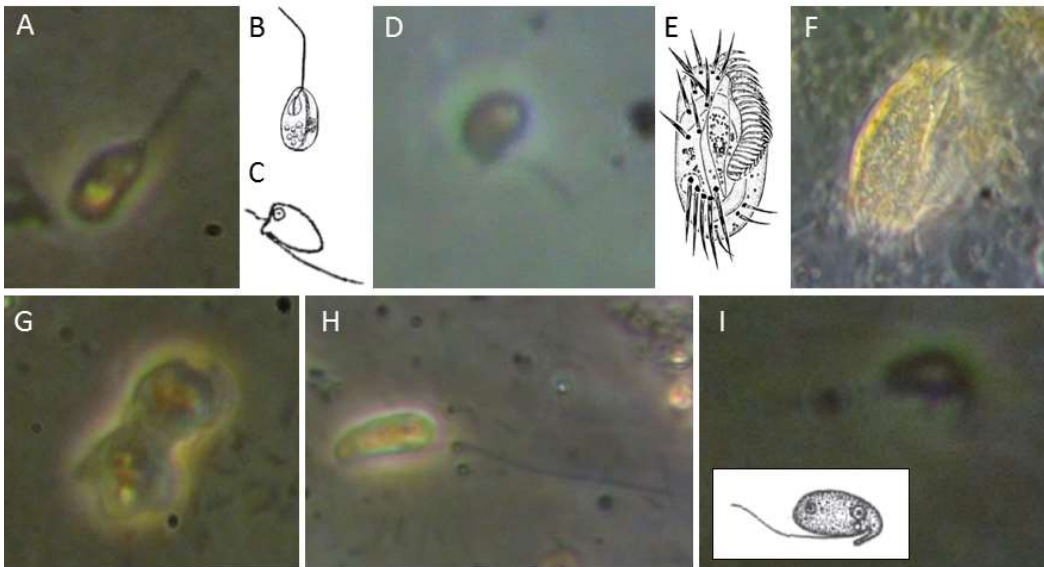


Fig. 5.7.2 Examples of live nano- and microprotists from different phyla observed during direct live-counting and in culture. A. *Petalomonas* sp. (Euglenida), B. drawing of *Petalomonas*, C. drawing of *Ancyromonas* (Ancyromonadida), D. *Ancyromonas* sp., E. drawing of *Euplotes dominicanus* (Ciliophora), F. *Euplotes* sp., G. undetermined amoeba, H. undetermined euglenid, I. *Rhynchomonas* sp. (Kinetoplastea).

Cultivation

During the cruise, raw cultures were obtained which will be further processed and isolated in Cologne, but several nano- and microprotists could already be observed and roughly identified in raw cultures (Fig. 5.7.2). Table 5.7.3 summarizes our preliminary results on the occurrence of morphotypes in cultures of the different sediment depth layers at three different stations. Most often detected in all seven sediment layers were small amoeba, ancyromonads and bicosoecids, typical r-strategists which often occur first in cultures. Less often found were bodonids (*Neobodo*, *Rhynchomonas*) and euglenids (e.g. *Petalomonas*). Only one ciliate, *Euplotes* sp., appeared in culture. Further analyses of the geno- and morphotypes are needed to determine the species and to investigate their role and function in the benthic food web.

Table 5.7.3 Preliminary list of protistan morphotypes identified in raw cultures from three different stations and seven different sediment layers.

Station	Sediment layer	Taxa
2-4	0-1cm	<i>Petalomonas</i> , different amoebas, <i>Ancyromonas</i>
2-4	1-2cm	<i>Petalomonas</i> , different amoebas, <i>Rhynchomonas</i>
2-4	2-4cm	bicosoecids, amoeba
2-4	4-6cm	<i>Neobodo</i> , bicosoecids
2-4	6-10cm	<i>Euplotes</i> sp., amoebas, bicosoecids
2-4	10-15cm	<i>Ancyromonas</i> , amoebas, bicosoecids
2-4	15-20cm	<i>Ancyromonas</i>

5-5	0-1cm	<i>Neobodo</i> , <i>Massisteria</i> , bicosoecids, amoebas, <i>Petalomonas</i> , undetermined flagellates
5-5	1-2cm	<i>Ancyromonas</i> , bicosoecids, euglenids
5-5	2-4cm	<i>Ancyromonas</i> , bicosoecids
5-5	4-6cm	<i>Ancyromonas</i> , bicosoecids, amoebas
5-5	6-10cm	amoebas
5-5	10-15cm	none
5-5	15-20cm	<i>Ancyromonas</i>
15-5	0-1cm	different amoebas, bicosoecids, <i>Rhynchomonas</i> , undetermined euglenids, <i>Ancyromonas</i>
15-5	1-2cm	<i>Petalomonas</i> , bicosoecids, amoebas, bodonids
15-5	2-4cm	amoebas, bicosoecids, undetermined flagellates
15-5	4-6cm	bodonids, amoebas, undetermined flagellates
15-5	6-10cm	bicosoecids, amoebas, undetermined flagellates
15-5	10-15cm	amoebas, bicosoecids
15-5	15-20cm	amoebas, bicosoecids

5.8 Microphytobenthos (WP 2.3)

(Ramona Kern)

Primary production of microphytobenthos of sediment cores

Sediment cores taken by S. Forster and M. Powilleit (Table 5.8.1) were incubated in the lab at 10°C and in the dark and at 5 different light intensities. The change of oxygen was measured over the time using optode modules fixed at the top of the cores. The primary production was analyzed for four cores of the exclusion area and four cores of the control area. One additional core per area was used to trace the temperature change.

After the incubation experiment the top centimeter was taken to measure water and organic content. Furthermore, the chlorophyll a content, organic carbon and nitrogen and the cell count will be analyzed.

Table 5.8.1 Sediment cores for primary production.

Core	Area	Station	Date	Time (MEZ)	Temperature (°C)	Light PAR (μE)
I	Control	27-1	5/6/2020	15:35	9.99	0
II						
III						
IV						
V						
VI	Exclusion	40-1	7/6/20	9:36	9.51	0.16
VII						
VIII						
IX						

X						
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Biodiversity of the microphytobenthos community

For the molecular identification of the algal community J. Piotnek and M. Hohlfeld took samples of the first and the second centimeter of a sediment core taken by the multi corer. The DNA and/or RNA of the samples will be isolated and corresponding marker genes will be amplified and sequenced. Per area, 4 stations were defined, whereby three cores per station were taken and slices in 2 different depths for the microphytobenthos were made (in total 48 samples).

For the microscopic determination of diatoms samples were taken by M. Hohlfeld, M. Powilleit and S. Forster (Table 5.8.2). Therefore, the top centimeters slice of a sediment core were soluted in water or mixed with Lugol's solution.

Table 5.8.2 Samples taken for determining the biodiversity of diatoms. The samples taken from M. Powilleit and S. Forster were not listed in that table because the corresponding metadata have not been shared yet. In total they provided 20 samples.

Sample	Area	Station	Core	Date
L	Exclusion	2-4	4	27/5/20
11				
M		5-5	4	28/5/20
12				
O				
13	8-5	4	29/5/20	
P	Control	10-4	4	30/5/20
14				
Q		13-6	3	31/5/20
15				
16				
R		15-5	3	1/6/20
17				
S	17-6	3	2/6/20	
S				
S	Exclusion	18-6	3	3/6/20
T				

5.9 Meibenthos (WP 2.4)

(K.H. George, P. Martínez Arbizu)

Meiofauna was sampled with a Multicorer (MUC); each core covers a sampling area of 0.00785 m² (responsible for sampling on board: Manon Hohlfeld). Altogether, 24 stations were sampled (Tab. 5.9.1). They split into 13 samples taken from the MPA region, and 11 samples from the control area outside the MPA. From each haul, from 2 MUC cores the overlaying water and the upper 5 cm of Sediment were fixed with buffered formalin (final solution ~4%) for morphological faunistics of Copepoda Harpacticoida, and 2 additional cores were preserved with DESS (20% solution of di-methyl-sulfoxide) for the metabarcoding analyses of meiofauna. Sample processing is undertaken in the laboratories of the DZMB (Senckenberg am Meer Wilhelmshaven). To extract

the meiofauna, a density centrifugation with 40% Levasil® and kaolin is performed 3 times at 5,000 rpm for 5 minutes. For the morphological faunistics of Copepoda Harpacticoida at species level, the resulting individuals are sorted by hand using a Leica M125 stereomicroscope. To date, for harpacticoid faunistics almost half of the samples have been centrifuged, and sorting of Harpacticoida has started. However, due to the enormous content of organic material even in centrifuged sample fractions, we are forced to split each sample; otherwise the sorting would be disproportionately time-consuming. For splitting the centrifuged samples, a Jensen-Splitter is used (Jensen 1982). It splits each sample into eight equal sub-samples. From these, one sub-sample will be sorted for Harpacticoida. First sorting results yielded abundance values of about 600 individuals per subsample. Moreover, sorting leads to the impression of Harpacticoida being the dominant meiobenthic taxon in the samples.

Centrifugation of the samples for metabarcoding starts soon. In the Laboratory, DNA will be extracted from the samples and the two gen fragments will be amplified using a PCR. After indexing the samples in a second PCR step, they will be pooled together and sequenced using an Illumina MiSeq sequencer (protocol explained in Rossel et al. 2019). Sequences will be demultiplexed and error corrected using the dada2 Pipeline and assigned to taxonomic groups using custom scripts. Diversity of meiofauna communities will be assessed comparing the 18S V1V2 fragment, that amplifies well in a diverse range of taxonomic groups. In addition, the COI fragment will be used for harpacticoid copepods (this fragment does not amplify well in nematodes)

Table 5.9.1 List of stations sampled with the MUC for meiobenthos. The list includes the number of cores taken per station for each harpacticoid faunistics (morphology) and metabarcoding. Furthermore, the number of already centrifuged cores is given.

No.	Date	Region	Station	MUC cores morphology		MUC cores metabarcoding	
				No. cores	Centrifuged	No. cores	Centrifuged
1	27.05.2020	MPA	2-3	2	0	2	
2			2-5	2	2	2	
3			2-6	2	0	2	
4			2-7	2	0	2	
5	28.05.2020	MPA	5-2	2	1	2	
6			5-3	1	1	1	
7			5-4	2	0	2	
8	29.05.2020	MPA	8-3	2	2	2	
9			8-4	2	2	2	
10			8-5	2	1	2	
11	30.05.2020	Control	10-3	2	1	2	
12			10-4	2	0	2	
13			10-5	2	1	2	
14	31.05.2020	Control	13-4	2	0	2	
15			13-5	2	2	2	
16	01.06.2020	Control	15-3	2	1	2	
17			15-4	2	2	2	
18			15-5	2	1	2	
19	02.06.2020	Control	17-4	2	0	2	
20			17-5	2	1	2	
21			17-6	2	1	2	
22	03.06.2020	MPA	18-4	2	2	2	
23			18-5	2	1	2	
24			18-6	2	1	2	

			Sum:	47	23	47	0
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5.10 Macrozoobenthos (WP 3.2)

(M. Gogina, S. Forster, M. Powilleit)

Biodiversity of macrofauna

The sampling of the benthic macrofauna (responsible Mayya Gogina, Stefan Forster, Martin Powilleit) was performed using a van Veen grab (75 kg, sieve lid) with a sampling area of 0.1 m² and multicorer (MUC) with sampling area of 0.00785 m². Successive fractionated sieving (1.0 mm and 0.5 mm) on 30 grab hauls (15 for each area i.e. future exclusion area within MPA and control area outside MPA). Collected samples will be sorted at Universität Rostock and IOW, including a mutual exchange, to ensure the correct determination of benthic macrofauna and completeness of macrofauna data, particularly to capture population dynamics of key species *Arctica islandica* by covering full size-spectrum of individuals down to 0.5 mm (see Fig 5.10.1). To estimate the occurrence, distribution and spatial variability of the benthic macrofaunal species and communities, and analyze the influence of MGF intensity on them, species abundance, dry and wet biomass, biological traits structure, as well as size classes distribution and condition of key species will be determined in the home laboratory.



Fig. 5.10.1 Photos illustrating sampling procedure. The two right-most photos show the fractions left on the 1.0 mm and 0.5 mm sieves.

The procedure of fractionated sieving was notably optimized: the entire grab sample was collected in the large plastic tub, sediment and water were homogenized gently by hand mixing, small portions were added and washed through the two sieves on top of each other, iterative rinsing from one 0.5 mm sieve-tub to another accelerated the washing out of fine fraction.

To record quick moving, rare or large species at each area (within and outside MPA) the Kieler Kinderwagen dredge has been used (inner opening wide - 92 cm, mesh size - 5 mm, towed with speed of up to 1 knot over the ground). The towing time due to the predominant substrate type (silt) was set to 1 minute. Semi-quantitative dredge samples were also investigated for damage of the shells of *A. islandica*. For this purpose, same dredge samples were used, and also additional (second) dredge sample was taken from the exclusion area.

At each MUC station surface sediment sample was taken from one core for later sediment granulometry and organic content analysis.

During the first part of the cruise 3 cores (remaining after other WPs had sufficient number of cores for their samples) were collected at 6 MUC stations and sieved through 1 mm sieve (also to have the possibility of comparing the area covered by samples obtained by each gear type – grab and MUC).

For all macrofauna samples, animals together with the remaining substrate were preserved with 4% formaldehyde seawater solution in sea water mixture.

Table 5.10.2 List of samples that will be analyzed in “IOW Benthos Labor”.

Station	Date	Sample No.	Number of Kautex	Comment to fixation
2-4	27.05.2020	Core 1-3	1*3	Marble gravel in Formol
10-3	30.05.2020	Core 1-3	1*3	Marble gravel in Formol
13-5	31.05.2020	Core 1-3	1*3	Marble gravel in Formol
15-3	01.06.2020	Core 1-3	1*3	Marble gravel in Formol
17-4	02.06.2020	Core 1-3	1*3	Marble gravel in Formol
18-4	03.06.2020	Core 1-3	1*3	Marble gravel in Formol
23-3, 23-4, 23-5	05.06.2020	1-3	1*3	Marble gravel + UNI Formol buffered
24-1, 24-2, 24-3	05.06.2020	1-3	1*3	Marble gravel + UNI Formol buffered
25-1, 25-2, 25-5	05.06.2020	1-3	1*3	Marble gravel + UNI Formol buffered
26-1, 26-2, 26-3	05.06.2020	1-3	1*3	Marble gravel + UNI Formol buffered
27-2, 27-3, 27-4	05.06.2020	1-3	1*3, 0.5*1 (27-4B)	Marble gravel + UNI Formol buffered
30-2, 30-3, 30-4	06.06.2020	1-3	1*3	Marble gravel + UNI Formol buffered
32-1	06.06.2020	Dredge	1	qualitativ ,control area
42-1, 42-2, 42-3	07.06.2020	1-3	1*3	Formol unbuffered + marble gravel
43-1, 43-2, 43-3	07.06.2020	1-3	1*3	Formol unbuffered + marble gravel
44-1, 44-2, 44-3	07.06.2020	1-3	1*3	Formol unbuffered + marble gravel
45-1, 45-2, 45-3	07.06.2020	1-3	1*3	Formol unbuffered + marble gravel
26-4	06.06.2020	Core 1	7	7 slices: 7 x 0.5l
28-1	06.06.2020	Core 1	7	7 slices: 7 x 0.5l
29-1	06.06.2020	Core 1	7	7 slices: 7 x 0.5l
30-1	06.06.2020	Core 1, Core 2PW, Core 3	7*3	7 slices: 7 x 0.5l
37-1	06.06.2020	Core 1	7	7 slices: 7 x 0.5l
38-1	06.06.2020	Core 1	7	7 slices: 7 x 0.5l
39-1	06.06.2020	Core 1	7	7 slices: 7 x 0.5l
40-1	06.06.2020	Core 1	2	2 slices (0-2,2-6): 2 x 0.5l
40-2	06.06.2020	Core 2	7	7 slices: 7 x 0.5l
46-1	08.06.2020	Dredge	1	qualitative, MPA area

To derive accompanying information on environmental conditions at each station near-bottom values for salinity, temperature and oxygen content were obtained from CTD. Based on the first visual estimate taxonomic composition of macrofauna was similar in exclusion and control areas (thereby justifying the choice of the latter one), and seem to represent the typical so-called Arctica community. Among the species dominating abundance and biomass, besides the name-giving ocean quahog (*Arctica islandica*), are species like *Abra alba*, polychaetes like *Nephtys ciliate* and *Terebellides stroemii*, echinoderms *Ophiura albida* and *Asterias rubens*, gastropods like *Peringia ulvae*, cumaceans *Diastylis rathkei*.

Habitat characteristics were investigated using a hand-held underwater video system (two 30 min transects per area). Multiple trawling marks were recorded, and at the edge of the largest marks, on the underwater video a kind of graveyard of ocean quahog (*Arctica islandica*) empty shells could be seen. Whether this effect is due to shells transport caused by hydrodynamics or is a result of mortality caused by fishery remains to be investigated.

Table 5.10.3 shows preliminary estimates of environmental variables relevant for macrofauna distribution. Preliminary results suggest that sediment organic content (estimated by loss on ignition) ranged from 3.5 to 7.8% and was somewhat higher in area within the MPA, but differences are not statistically significant (Kruskal-Wallis test: p-value = 0.117). Sediment grain size distribution is poorly to very poorly sorted, with median grain size within MPA $50.0 \pm 11.2 \mu\text{m}$ (MEAN \pm SD) and in the control area outside MPA $57.2 \pm 5.28 \mu\text{m}$.

Table 5.10.3 Preliminary results of sediment grain size distribution analyzed with Mastersizer 3000, sediment organic content values estimated by loss on ignition, depth and accompanying environmental variables from closest CTD casts within and outside MPA.

Area Station_cast	Control outside MPA					Within MPA				
	26_4	27_1	28_1	31_1	29_1	30_1	37_1	38_1	39_1	40_2
Median grain size [μm]	58	48	63	61	55	51	50	39	43	68
Fraction finer 63 μm [%]	52.1	58.2	50.1	50.6	53.3	55.9	56.4	64.2	60.7	48.0
Fraction coarser 2000 μm [%]	0.0	0.3	0.0	0.6	0.0	0.0	0.4	0.0	0.0	1.8
Sorting [phi]	1.9	1.9	1.9	2.0	2.0	1.9	2.0	1.8	1.9	2.3
Skewness [phi]	-0.3	0.0	-0.4	-0.1	-0.2	-0.2	0.1	0.0	-0.1	-0.1
Total organic content [%]	6.0	5.4	4.4	3.5	7.8	6.4	7.2	6.1	6.3	6.5
Depth [m]	23.8	23.8	23.9	23.7	24.1	22.9	23.8	23.4	23.7	23.4
Salinity (near bottom)	17.6	17.6	18.6	18.6	18.6	17.2	18.2	18.2	17.7	17.7
Temperature (near bottom) [$^{\circ}\text{C}$]	10.0	10.0	9.6	9.6	9.6	10.3	9.5	9.5	9.8	9.8
Oxygen (near bottom) [mg/l]	4.5	4.5	4.0	4.0	4.0	4.9	4.8	4.8	4.3	4.3

Bioturbation and permeability of sediment

Samples for the measurement of depth distribution of chlorophyll-*a* as particle tracer were obtained from 10 sediment cores on five MUC hauls in each of the areas (excl, ref). Additionally, 500 ml surface sediment (0-1 cm) was accumulated and a 10 $^{\circ}\text{C}$ chlorophyll decomposition experiment started to determine the rate constant of chlorophyll-*a* decay in this sediment.

Permeability of the sediment was checked on board using a constant head set-up. It can be concluded that this sediment is not permeable in any diagenetically relevant way ($k < 10^{-14} \text{ m}^2$).

Sodium bromide was successfully injected in three Lander experiments and subsampled from the retrieved sediment cores of both chambers. Control experiments on board determining diffusive tracer flux were also performed in the EMB cold labs using Br^- tracer. All samples will be analyzed and fluxes calculated in Rostock.

Preliminary results suggest permeability of the sediments, k , to be below $< 10^{-14} \text{ m}^2$.

6 Ship’s Meteorological Station

According to the data from ship weather station, average temperature was around 15 °C during leg 1 and 13 °C during leg 2. The general meteorological conditions during the 1 leg were characterized by continuous high pressure and moderate wind conditions, whereas it changed to low pressure after 2nd June, with predominantly southwest winds (Fig. 6.1).

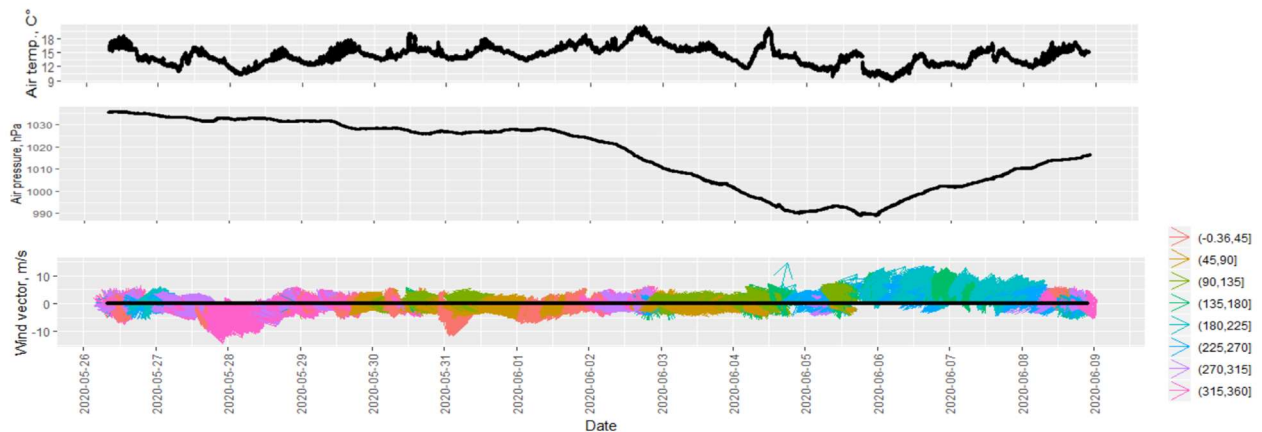


Figure 6.1. Air temperature, pressure and wind vector measured by the ship weather station of RV Elisabeth Mann Borgese (color of arrows at lower pane indicate wind direction).

7 Station List EMB238

7.1 Overall Station List

Station No.	Date	Gear	Time	Latitude	Longitude	Water Depth	Remarks/Recovery
EMB238 and MARUM	2020		[UTC]	[°N]	[°E]	[m]	
EMB238_1-1	26.05.	SSS	22:00	54°32.43'	10°46.60'	23.8	end 27.05. 05:15
EMB238_2-1	27.05.	CTD	07:37	54°33.38'	10°45.51'	24.8	
EMB238_2-2	27.05.	Niskem Bottle	08:17	54°33.38'	10°45.51'	24.8	
EMB238_2-3	27.05.	MUC	11:03	54°33.35'	10°45.53'	23.0	
EMB238_2-4	27.05.	MUC	12:11	54°33.37'	10°45.52'	23.5	
EMB238_2-5	27.05.	MUC	12:59	54°33.36'	10°45.53'	23.7	
EMB238_2-6	27.05.	MUC	13:33	54°33.36'	10°45.54'	23.6	
EMB238_2-7	27.05.	MUC	14:03	54°33.34'	10°45.53'	23.6	
EMB238_3-1	27.05.	HySo	14:32	54°33.07'	10°47'	23.6	
EMB238_3-2	27.05.	SSS	15:05	54°33.15'	10°45.46'	23.0	end 27.05. 16:25
EMB238_4-1	27.05.	SSS	16:40	54°32.31'	10°43.89'	22.0	end 27.05. 21:15

EMB238_5-1	28.05.	CTD	06:25	54°32.76'	10°46.67'	23.4	
EMB238_5-2	28.05.	MUC	06:44	54°32.8'	10°46.62'	23.4	
EMB238_5-3	28.05.	MUC	07:48	54°32.8'	10°46.62'	23.3	
EMB238_5-4	28.05.	MUC	11:02	54°32.79'	10°46.62'	23.3	
EMB238_5-5	28.05.	MUC	11:41	54°32.77'	10°46.61'	23.0	
EMB238_6-1	28.05.	Lander BIGO	13:56	54°33.33'	10°45.51'	23.6	
EMB238_6-1	30.05.	Lander BIGO	06:20	54°33.33'	10°45.51'	23.6	
EMB238_7-1	28.05.	HySo	14:57	54°32.34'	10°47.17'	22.3	
EMB238_7-2	28.05.	MBES	15:35	54°32.43'	10°46.60'	23.8	
EMB238_7-2	29.05.	MBES	01:44	54°32.43'	10°46.60'	23.8	
EMB238_8-1	29.05.	CTD	07:10	54°33.11'	10°45.67'	22.8	
EMB238_8-2	29.05.	MUC	07:30	54°33.08'	10°45.67'	23.8	
EMB238_8-3	29.05.	MUC	08:12	54°33.1'	10°45.62'	23.8	
EMB238_8-4	29.05.	MUC	11:09	54°33.09'	10°45.62'	23.9	
EMB238_8-5	29.05.	MUC	11:36	54°33.08'	10°45.63'	23.9	
EMB238_9-1	29.05.	HySo	12:05	54°32.46'	10°47.10'	22.0	
EMB238_9-2	29.05.	SSS	12:10	54°32.46'	10°46.52'	23.0	
EMB238_9-2	29.05.	SSS	18:00	54°32.46'	10°46.52'	23.0	
EMB238_10-1	30.05.	CTD	07:12	54°32.34'	10°43.48'	22.9	
EMB238_10-2	30.05.	MUC	07:32	54°32.36'	10°43.48'	22.7	
EMB238_10-3	30.05.	MUC	08:06	54°32.35'	10°43.49'	22.8	
EMB238_10-4	30.05.	MUC	11:09	54°32.36'	10°43.49'	22.8	
EMB238_10-5	30.05.	MUC	11:39	54°32.36'	10°43.49'	22.8	
EMB238_11-1	30.05.	HySo	12:05	54°32.33'	10°44.51'	22.5	
EMB238_11-2	30.05.	SSS	12:14	54°32.28'	10°43.84'	22.4	
EMB238_11-2	30.05.	SSS	16:03	54°32.28'	10°43.84'	22.4	
EMB238_12-1	30.05.	HySo	16:40	54°32.22'	10°43.9'	22.7	
EMB238_12-2	30.05.	MBES	16:55	54°32.29'	10°43.45'	21.5	
EMB238_12-2	30.05.	MBES	20:47	54°32.29'	10°43.45'	21.5	
EMB238_12-3	30.05.	MBES	21:08	54°32.58'	10°39.65'	22.2	
EMB238_12-3	31.05.	MBES	00:18	54°32.58'	10°39.65'	22.2	
EMB238_9_3	31.05.	SSS	05:50	54°33.90'	10°46.21'	24.0	
EMB238_9_3	31.05.	SSS	06:50	54°33.90'	10°46.21'	24.0	
EMB238_13-1	31.05.	CTD	07:38	54°32.36'	10°43.51'	22.8	
EMB238_13-2	31.05.	MUC	08:00	54°32.38'	10°43.52'	22.9	
EMB238_13-3	31.05.	MUC	08:37	54°32.37'	10°43.51'	23.2	
EMB238_13-4	31.05.	MUC	11:05	54°32.37'	10°43.54'	23.0	
EMB238_13-5	31.05.	MUC	11:26	54°32.39'	10°43.52'	23.0	
EMB238_13-6	31.05.	MUC	12:39	54°32.34'	10°43.55'	23.0	
EMB238_14-1	31.05.	Lander BIGO	14:10	54°32.61'	10°41.34'	23.3	
EMB238_14-1	02.06.	Lander BIGO	06:02	54°32.61'	10°41.34'	23.3	
EMB238_15-1	01.06.	CTD	06:54	54°32.51'	10°41.72'	23.0	
EMB238_15-2	01.06.	MUC	07:11	54°32.51'	10°41.72'	23.0	
EMB238_15-3	01.06.	MUC	07:46	54°32.5'	10°41.73'	23.0	
EMB238_15-4	01.06.	MUC	11:07	54°32.51'	10°41.71'	23.0	
EMB238_15-5	01.06.	MUC	11:31	54°32.51'	10°41.71'	23.2	
EMB238_16-1	01.06.	Lander EDDY	14:05	54°32.66'	10°41.36'	23.0	
EMB238_16-1	03.06.	Lander EDDY	06:35	54°32.66'	10°41.36'	23.0	
EMB238_17-1	02.06.	CTD	07:24	54°32.49'	10°41.17'	21.2	
EMB238_17-2	02.06.	CTD	08:11	54°32.48'	10°41.18'	23.6	
EMB238_17-3	02.06.	MUC	08:25	54°32.5'	10°41.16'	23.5	
EMB238_17-4	02.06.	MUC	08:53	54°32.5'	10°41.14'	23.1	
EMB238_17-5	02.06.	MUC	11:07	54°32.5'	10°41.16'	23.0	

EMB238_17-6	02.06.	MUC	11:37	54°32.5'	10°41.16'	23.0	
EMB238_18-1	03.06.	CTD	07:09	54°32.91'	10°45.95'	21.8	
EMB238_18-2	03.06.	CTD	08:02	54°32.92'	10°46.11'	22.8	
EMB238_18-3	03.06.	MUC	08:14	54°32.92'	10°46.11'	23.1	
EMB238_18-4	03.06.	MUC	08:36	54°32.92'	10°46.12'	24.5	
EMB238_18-5	03.06.	MUC	11:08	54°32.92'	10°46.11'	24.4	
EMB238_18-6	03.06.	MUC	12:06	54°32.93'	10°46.11'	24.4	
EMB238_18-7	03.06.	HySo	12:15	54°32.93'	10°46.11'	23.0	
EMB238_19-1	03.06.	SSS	12:44	54°32.45'	10°46.68'	23.0	
EMB238_19-1	03.06.	SSS	18:00	54°32.45'	10°46.68'	23.0	
EMB238_20-1	04.06.	VVG	11:56	54°11.68'	012°3.87'	10.0	
EMB238_20-2	04.06.	VVG	11:58	54°11.68'	012°3.87'	10.0	
EMB238_21-1	04.06.	Lander BIGO	17:18	54°32.39'	10°43.50'	24.1	
EMB238_21-1	06.06.	Lander BIGO	11:12	54°32.39'	10°43.50'	24.1	
EMB238_22-1	04.06.	CTD	18:09	54°32.33'	10°43.55'	23.8	
EMB238_23-1	05.06.	VVG	06:05	54°32.35'	10°43.53'	23.9	
EMB238_23-2	05.06.	VVG	06:17	54°32.36'	10°43.5'	23.7	
EMB238_23-3	05.06.	VVG	06:24	54°32.36'	10°43.5'	23.8	
EMB238_23-4	05.06.	VVG	06:32	54°32.35'	10°43.49'	23.8	
EMB238_23-5	05.06.	VVG	06:37	54°32.35'	10°43.49'	23.8	
EMB238_24-1	05.06.	VVG	08:16	54°32.33'	10°43.47'	23.7	
EMB238_24-2	05.06.	VVG	08:22	54°32.33'	10°43.47'	23.5	
EMB238_24-3	05.06.	VVG	08:28	54°32.34'	10°43.46'	23.7	
EMB238_25-1	05.06.	VVG	11:03	54°32.4'	10°42.64'	24.1	
EMB238_25-2	05.06.	VVG	11:08	54°32.4'	10°42.63'	24.1	
EMB238_25-3	05.06.	VVG	11:14	54°32.4'	10°42.63'	24.1	
EMB238_25-4	05.06.	VVG	11:18	54°32.4'	10°42.63'	24.0	
EMB238_25-5	05.06.	VVG	11:23	54°32.4'	10°42.62'	24.1	
EMB238_26-1	05.06.	VVG	12:16	54°32.49'	10°41.67'	24.0	
EMB238_26-2	05.06.	VVG	12:35	54°32.49'	10°41.67'	23.8	
EMB238_26-3	05.06.	VVG	12:41	54°32.48'	10°41.68'	23.9	
EMB238_26-4	05.06.	MUC	13:00	54°32.5'	10°41.66'	23.8	
EMB238_27-1	05.06.	MUC	13:33	54°32.52'	10°41.19'	23.8	
EMB238_27-2	05.06.	VVG	13:53	54°32.51'	10°41.14'	23.8	
EMB238_27-3	05.06.	VVG	13:58	54°32.52'	10°41.13'	23.8	
EMB238_27-4	05.06.	VVG	14:04	54°32.53'	10°41.15'	24.1	
EMB238_27-5	05.06.	CTD	16:40	54°32.47'	10°41.14'	24.1	
EMB238_28-1	06.06.	MUC	06:27	54°32.35'	10°43.49'	22.5	
EMB238_29-1	06.06.	MUC	07:16	54°32.41'	10°42.62'	22.8	
EMB238_30-1	06.06.	MUC	08:23	54°32.78'	10°46.61'	23.1	
EMB238_30-2	06.06.	VVG	08:40	54°32.77'	10°46.6'	22.9	
EMB238_30-3	06.06.	VVG	08:47	54°32.78'	10°46.62'	22.9	
EMB238_30-4	06.06.	VVG	08:52	54°32.78'	10°46.62'	22.7	
EMB238_31-1	06.06.	VVG	09:21	54°32.33'	10°43.47'	22.3	
EMB238_32-1	06.06.	DRG	11:35	54°32.4'	10°43.62'	22.3	
EMB238_33-1	06.06.	UWV	13:23	54°32.43'	10°43.72'	22.2	
EMB238_33-1	06.06.	UWV	13:55	54°32.43'	10°43.72'	22.2	
EMB238_34-1	06.06.	UWV	14:15	54°32.45'	10°41.86'	22.7	
EMB238_34-1	06.06.	UWV	15:03	54°32.45'	10°41.86'	22.7	
EMB238_35-1	06.06.	Lander BIGO	18:00	54°32.73'	10°46.56'	22.9	
EMB238_35-1	08.06.	Lander BIGO	15:53	54°32.73'	10°46.56'	22.9	
EMB238_36-1	06.06.	CTD	18:45	54°32.77'	10°46.62'	22.9	
EMB238_37-1	07.06.	MUC	06:03	54°33.34'	10°45.58'	23.8	
EMB238_38-1	07.06.	MUC	06:30	54°33.09'	10°45.61'	23.6	

EMB238_39-1	07.06.	MUC	07:00	54°33.23'	10°46.25'	23.7	
EMB238_40-1	07.06.	MUC	07:25	54°32.9'	10°46.06'	23.4	
EMB238_40-2	07.06.	MUC	07:56	54°32.92'	10°46.05'	23.4	
EMB238_41-1	07.06.	Lander EDDY	08:52	54°32.67'	10°46.45'	23.2	recovered 08.06. 16:20
EMB238_42-1	07.06.	VVG	11:08	54°32.92'	10°46.08'	23.4	
EMB238_42-2	07.06.	VVG	11:14	54°32.91'	10°46.09'	23.4	
EMB238_42-3	07.06.	VVG	11:19	54°32.91'	10°46.09'	23.4	
EMB238_43-1	07.06.	VVG	11:56	54°33.26'	10°46.3'	23.7	
EMB238_43-2	07.06.	VVG	12:00	54°33.26'	10°46.3'	23.6	
EMB238_43-3	07.06.	VVG	12:05	54°33.25'	10°46.29'	23.7	
EMB238_44-1	07.06.	VVG	13:31	54°33.1'	10°45.63'	23.4	
EMB238_44-2	07.06.	VVG	13:36	54°33.09'	10°45.65'	23.8	
EMB238_44-3	07.06.	VVG	13:45	54°33.09'	10°45.64'	23.4	
EMB238_45-1	07.06.	VVG	14:32	54°33.35'	10°45.54'	23.8	
EMB238_45-2	07.06.	VVG	14:36	54°33.35'	10°45.54'	23.7	
EMB238_45-3	07.06.	VVG	14:41	54°33.35'	10°45.54'	23.8	
EMB238_45-4	07.06.	CTD	16:26	54°33.33'	10°45.49'	23.7	
EMB238_46-1	08.06.	DRG	07:56	54°33.11'	10°46.71'	23.6	
EMB238_46-2	08.06.	DRG	08:16	54°33.18'	10°46.17'	23.5	
EMB238_47-1	08.06.	UWV	09:15	54°33.00'	10°45.64'	23.3	
EMB238_48-1	08.06.	UWV	11:03	54°32.77'	10°46.69'	23.2	
EMB238_48-2	08.06.	CTD	12:02	54°32.96'	10°46.02'	23.4	

7.2 Profile Station List

Station No.	Profile Station No.	Date	Time	Latitude	Longitude	Max. Depth	Bottom	Profile numbers
EMB238_		2020	h	[°N]	[°W]	[m]	[m]	
2-1	1	27.05.	07:05	54° 33.38'	10° 45.51'	22.5	24.8	V0001F01
5-1	1	28.05.	06:09	54° 32.79'	10° 46.67'	22.2	23.4	V0002F01
8-1	1	29.05.	06:45	54° 33.02'	10° 57.37'	22.5	22.8	V0003F01
10-1	1	30.05.	06:50	54° 32.31'	10° 43.47'	21.7	22.9	V0004F01
13-1	1	31.05.	07:20	54° 32.29'	10° 43.5'	21.7	22.8	V0005F01
17-1	1	02.06.	07:00	54° 32.49'	10° 41.17'	22	21.2	V0006F01
17-2	1	02.06.	08:03	54° 32.5'	10° 41.16'	22	23.6	V0007F01
18-1	1	03.06.	07:07	54° 32.91'	10° 45.95'	22	21.8	V0008F01
18-2	2	03.06.	07:40	54° 32.93'	10° 46.11'	22.2	22.8	V0009F01
22-1	1	04.06.	17:44	54° 32.34'	10° 43.54'	21.7	23.8	V0010F01
27-5	1	05.06.	16:25	54° 32.49'	10° 41.14'	22	24.1	V0011F01
36-1	1	06.06.	18:27	54° 32.79'	10° 46.59'	21.7	22.9	V0012F01
45-4	1	07.06.	16:13	54° 33.35'	10° 45.51'	22.7	23.7	V0013F01
48-2	1	08.06.	11:54	54° 32.96'	10° 46.01'	22.2	23.4	V0014F01

8 Data and Sample Storage and Availability

Data collected during the cruise EMB238 will be used in MGF-Ostsee project. After the scientific publication or at the latest 3 years after the end of the project, all data will be placed into the PANGAEA database for access of wider scientific public. The metadata for this cruise will be made publicly available immediately after the cruise (via MARUM). The raw and processed acoustic data will be archived on the dedicated data servers (see Table 8.1). The data collected by all sub-projects will be critically checked and made available to the project partners via an internal database within the deadlines that result from the milestones. For the data collected at the Leibniz

Institute for Baltic Sea Research Warnemünde, the metadata information system IOWMETA (<http://iowmeta.io-warnemuende.de>) is available. In addition, research data of the project from various sub-projects are archived in the PANGAEA database or DNA / RNA sequence data in the public databases Genbank, GFBio, NCBI and/or IOW database "BenthosDB" (for details see MGF-Ostsee data management plan).

Table 8.1 Overview of data availability

Type	Database	Available	Free Access	Contact
raw data CTD, ADCP, multibeam	PANGAEA	Jun 21	Jun 24	mischa.schoenke@io-warnemuende.de

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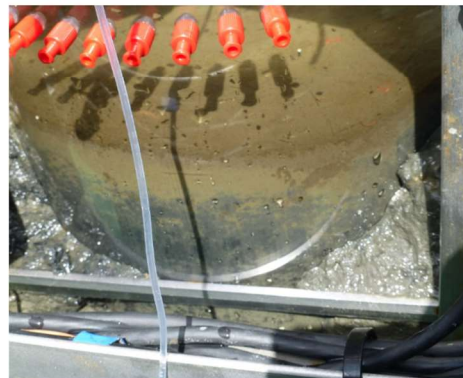
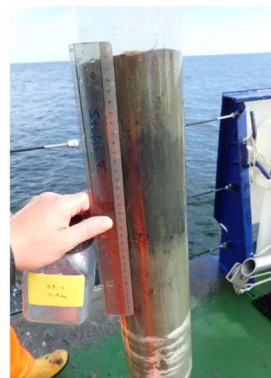
11 Abbreviations

SSS: Sidescan Sonar

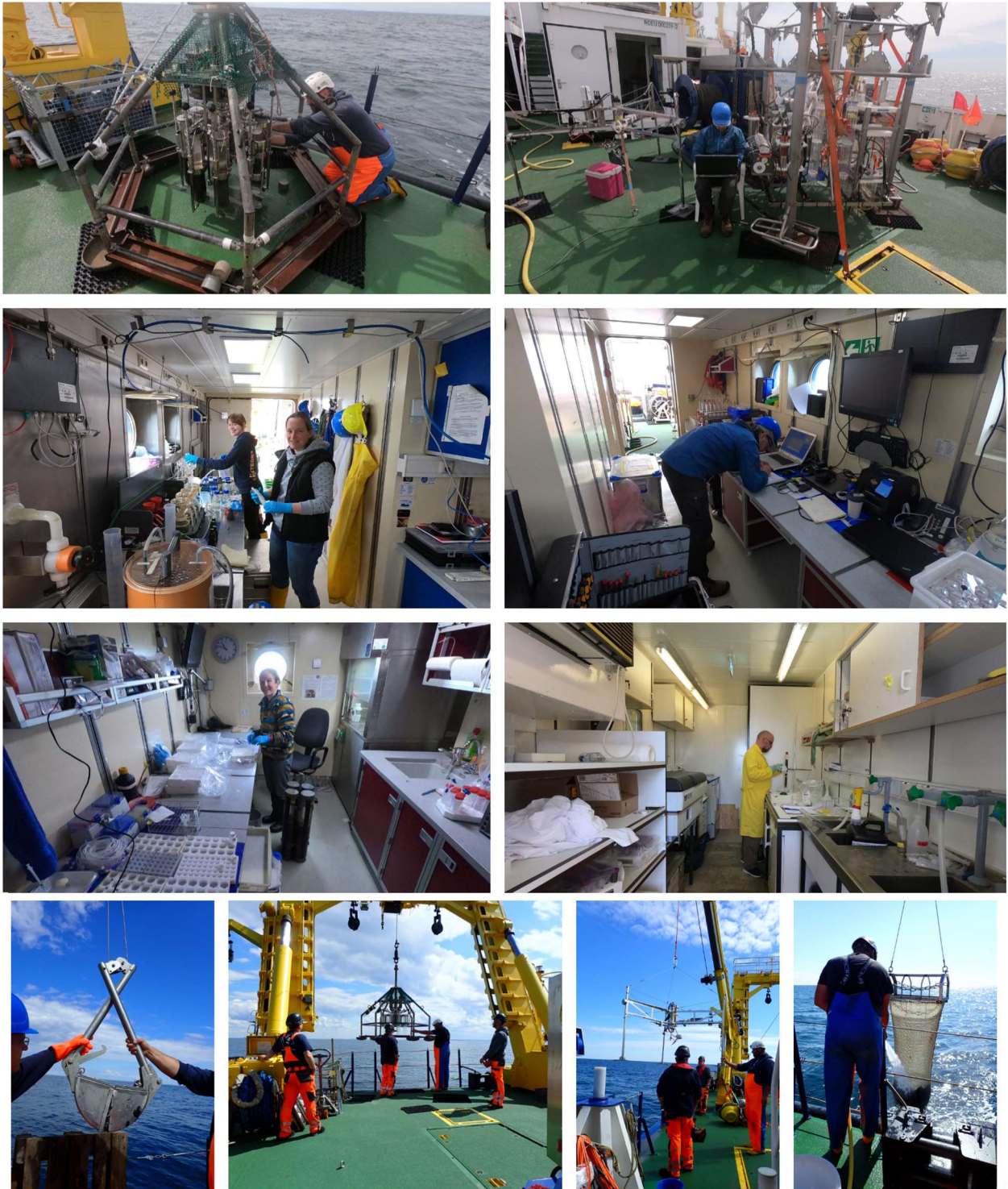
USBL	Ultra-Short Baseline
MUC:	Multi Corer
HySo:	Hydrosonde
CTD:	CTD
Lander BIGO:	Biogeochemical Observatory-Lander
MBES:	Multibeam Echosounder
Lander EDDY:	Eddy Correlation- Lander
VVG:	Van Veen Grap
DRG:	Dredge
UWV:	Underwater Video System

12 Appendices

12.1 Selected Pictures of Samples



12.2 Selected Pictures of Shipboard Operations



Three upper rows of pictures: © M. Schönke