ELISABETH MANN BORGESE-Berichte

Field-experiment to determine the short-term impact of bottom trawling on the benthic ecosystem in the German coastal Baltic Sea

Cruise No. EMB345

16.07.2024-02.08.2024
Rostock (Germany) - Rostock (Germany)
MGF-OSTSEE-II TR

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Table of contents

1. Cruise Summary 2
1.1. Summary in English 2
1.2. Zusammenfassung 2
2. Participants 3
2.1. Principal Investigators 3
2.2. Scientific Party 3
2.3. Participating Institutions 3
3. Research Program 4
3.1. Description of the Work Area 4
3.1.1. Experimental Area Kühlungsborn 4
3.1.2. Fehmarnbelt 5
3.2. Aims of the Cruise 5
3.3. Agenda of the Cruise 6
4. Narrative of the Cruise 6
5. Preliminary Results 11
5.1. AP 1.1 Hydroacousitcs & Sedimentology 11
5.1.1. Hydroacoustics 11
5.2. AP 2.1 Lower Trophic Layers (Protists & Meiofauna)) 17
5.2.1. Meiofauna 17
5.2.2. Protists - Nano- and Microfauna 21
5.3. AP 2.2 Prokaryotes & eDNA 25
5.4. AP 2.3 Makrozoobenthos 28
6. Ship’s Meteorological Station 36
7. Station List EMB345 37
7.1. Station list experimental area Kühlungsborn 37
7.2. Station list Fehmarnbelt 41
8. Data and Sample Storage and Availability 43
9. Acknowledgments 44
10. References 45
11. Abbreviations 47
12. Appendices 48

1. Cruise Summary

1.1. Summary in English

The cruise EMB345 is part of the interdisciplinary Deutsche Allianz für Meeresforschung (DAM) mission "MGF-Ostsee II", which is funded by the German Federal Ministry of Education and Research (BMBF) (FKZ 03F0936X). The project focuses on the exclusion of mobile bottom-contact fishing in marine protected areas of the German EEZ. In this context, a consortium of scientists is investigating how the ecosystems of Natura 2000 sites in the German Exclusive Economic Zone (EEZ) of the Baltic Sea develop after exclusion of mobile bottom-dwelling fisheries (MGF). The main objectives are to better understand the sustainability of seabed habitats and biota in Natura 2000 areas under current MGF operations, a general assessment of the impact of MGF on benthic communities and sediment functions, and their evolution after fisheries exclusion. As part of these objectives, the cruise EMB345 included a trawling experiment off Kühlungsborn, which was conducted in close cooperation with the Thünen-Institute, Geomar Kiel and University of Rostock, and their research vessels. The experiment consisted of the investigation of intense bottom trawling impact on the benthic community on a short term scale as well as detailed investigations along sections of a single trawl track, and sampling of resuspended sediment. Analyses focused on diversity in macro-, meso- and microfauna, microbial diversity and activity as well as transport of suspended matter due to trawling activity. Further, the cruise included sampling acquisition in Marine Protected Areas (MPA) and reference areas at Fehmarnbelt as part of the time series within the MGF-Ostsee II project.

1.2. Zusammenfassung

Die Fahrt EMB345 ist Teil der interdisziplinären Mission „MGF-Ostsee II" der Deutschen Allianz für Meeresforschung (DAM), die vom Bundesministerium für Bildung und Forschung (BMBF) (FKZ 03F0936X) gefördert wird. Das Projekt konzentriert sich auf den Ausschluss von mobilen grundberührenden Fischereien in Meeresschutzgebieten der deutschen Ausschließlichen Wirtschaftszone (EEZ). Ein Konsortium von Wissenschaftler:innen untersucht, wie sich die Ökosysteme der Natura-2000-Gebiete in der deutschen EEZ der Ostsee nach dem Ausschluss von Grundschleppfischereien entwickeln. Ziele sind ein besseres Verständnis der Nachhaltigkeit von Meeresbodenhabitaten und Organismen in Natura-2000-Gebieten, eine Bewertung der Auswirkungen dieser Fischerei auf benthische Lebensgemeinschaften und Sedimentfunktionen sowie deren Entwicklung nach dem Fischereiausschluss. Die Forschungsfahrt EMB345 umfasste ein Schleppnetzexperiment vor Kühlungsborn, welches in Zusammenarbeit mit dem Thünen-Institut, GEOMAR Kiel und der Universität Rostock durchgeführt wurde. Das Experiment beinhaltete die Untersuchung kurzzeitiger Auswirkungen von MGF auf die benthische Gemeinschaft, Untersuchungen entlang spezifischer Schleppnetzabschnitte sowie die Beprobung aufgewirbelten Sediments. Analysen fokussierten auf die Diversität der Makro-, Meso- und Mikrofauna, die mikrobielle Diversität und Aktivität sowie den Transport von suspendierten Stoffen durch den Grundschleppnetzeinsatz. Darüber hinaus beinhaltete die Fahrt Probennahmen im Meeresschutzgebiet (MPA) Fehmarnbelt und angrenzenden Referenzgebieten im Rahmen der Zeitreihenstudien des MGF-Ostsee II Projekts.

2. Participants

2.1. Principal Investigators

Name	Institution
Jürgens,Klaus, Prof. Dr.	IOW
Piontek, Judith, Dr.	IOW
Gogina, Mayya, Dr.	IOW
Arndt, Hartmut, Prof. Dr.	Uni Köln

2.2. Scientific Party

Name	Discipline	Institution
Jürgens,Klaus, Prof. Dr.	Microbiology, Chief Scientist	IOW
Piontek, Judith, Dr.	Microbiology	IOW
Gogina, Mayya, Dr.	Macrozoobenthos	IOW
Arndt, Hartmut, Prof. Dr.	Protists	Uni Köln
Bruhns, Torben	Coordination/ Microbiology	IOW
Meeske, Christian	Technician/ Microbiology	IOW
Hehl, Uwe	Technician	IOW
Gordetckaia, Olga	Student/ Microbiology	IOW
Schulze, Inken, Dr.	Hydroacousics	IOW
Schwerwass, Anja, Dr.	Protists	Uni Köln
Heene, Toralf	Technician	IOW
Ostmann, Alexandra, Dr.	Meiofauna	SaM
Hoffmann, Sven	Technician/ Meiofauna	SaM

2.3. Participating Institutions

IOW Leibniz Institute for Baltic Sea Research Warnemünde
Uni Köln University of Cologne
SaM Senckenberg am Meer - German Centre for Marine Biodiversity Research

3. Research Program

3.1. Description of the Work Area

3.1.1. Experimental Area Kühlungsborn

The research area is located offshore Kühlungsborn, near Rostock (**Fig. 3.1**) and west of the previous trawling experiment area of EMB268 (2021). Here, water depths of 22-24m (BSH-GeoSeaPortal) are located in close vicinity to the coastline (approx. 3 nm). The seafloor is composed of muddy sand, which is comparable to the sediments of the main investigation sites of the MGF-Ostsee project in the Fehmarnbelt area. The close vicinity to the coastline allows the target area to be reached by small vessels and divers for purposes of the experiment

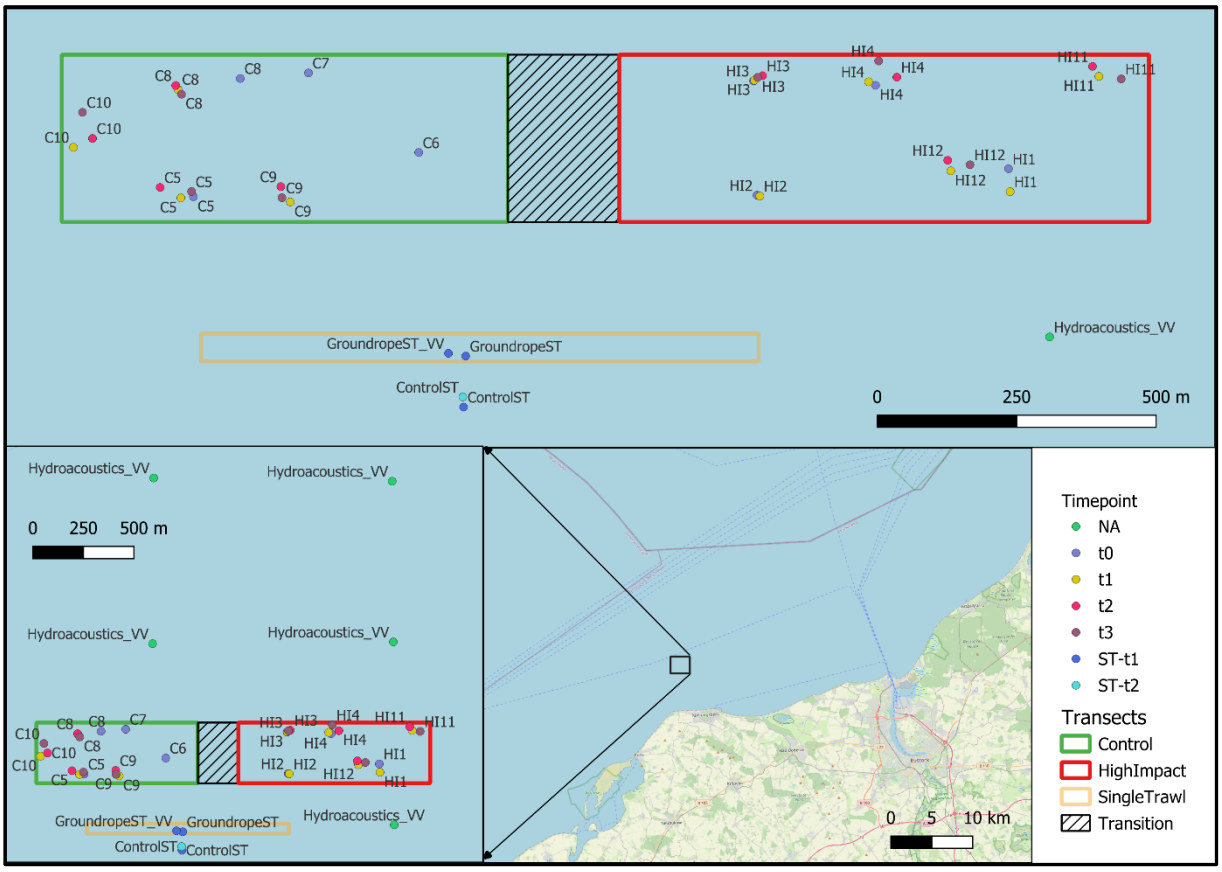

Fig. 3.1 Overview of the experimental area with sampling stations. Unless stated differently with the suffix "VV" (=Van Veen Grab), the stations indicate the location of the first successful MUC sampling. Overview map is provided by OpenStreetMap.

3.1.2. Fehmarnbelt

The investigation area is part of the time series established within the MGF-Ostsee project in 2020 and was sampled by previous cruises (EMB238, EMB320, AL570). It is located 17 km west of the island Fehmarn in the German EEZ (**Fig. 3.2**) with water depth between 22-24m (BSH-GeoSea-Portal). Additional to the area of 4.7 km^2 within marine protected area (MPA) site, a control area outside the MPA was sampled, in which similar habitat conditions and similar fishing intensities are found. The control area covers an area of 3.7 km^2 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 underlaying till and leaving gavel 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).

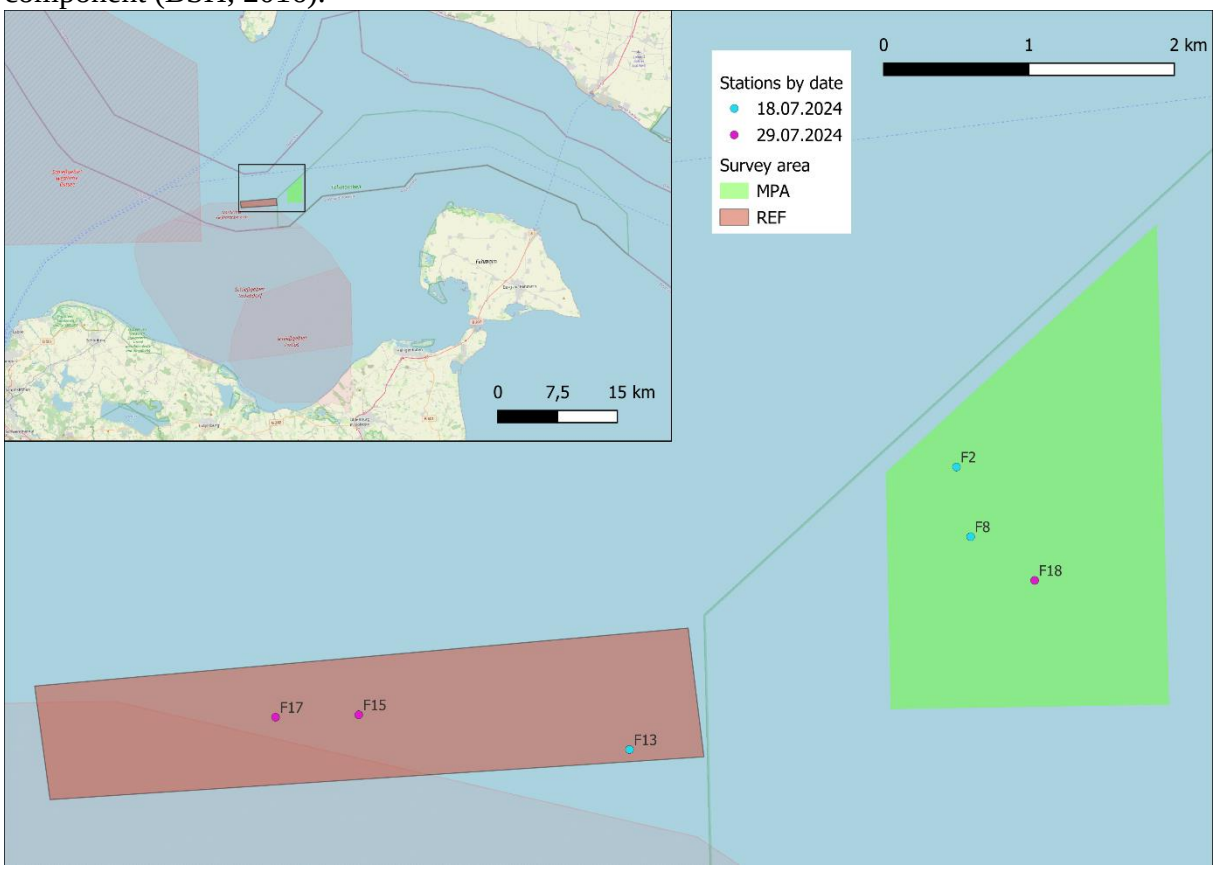

Fig. 3.2 Survey area at Fehmarnbelt. Black outline in overview (top left) indicates the location of the detailed view. Stations (circles) indicate the location of the first successful MUC sample. MPA = marine protected area, REF = reference area. Background map is provided by OpenStreetMap.

3.2. Aims of the Cruise

Based on the previous cruise EMB268, the main aim of the present cruise was to investigate the short-term effects of bottom trawling fisheries on sediment integrity and biogeochemistry, as well as on the composition and function of the benthic communities in the southwestern Baltic Sea. Within the framework of the BMBF funded DAM Pilot Mission MGF-Ostsee II, the present

experimental cruise is a counterpart to the monitoring activities carried out in designated marine protected areas within the MGF-Ostsee project (Phase I & II).

Specifically, the present experimental cruise aimed to address the following objectives:

- Assessment of sediment resuspension, including nutrients and methane caused by MGF
- Detection of immediate trawling effects on benthic communities including macrozoobenthos, meiofauna, protists and prokaryotes
- Investigation of the short-term (weeks) recovery of the previous mentioned benthic communities after a trawling event
- Fine-scale spatial analysis of sediments and benthic communities along a single otter board track

3.3. Agenda of the Cruise

The cruise took place in a framework of an experiment including 5 vessels: A fishery research Vessel (CLUPEA) from the Thünen-Institute to create a bottom trawl mark, diving base vessel LIMANDA of Rostock University, research Catamaran KLAASHAHN of IOW as well as ALKOR from GEOMAR Kiel and ELISABETH MANN BORGESE (EMB) for multi-purpose sampling and hydroacoustic surveying. The number of involved vessels and disciplines allowed a holistic sampling of a freshly created trawl mark.

The Declaration of Responsible Marine Research (emphasized in Appendix 1 of the Cruise Proposal) as defined by the DFG Senatskommission für Ozeanographie and the KDM were accepted and fully implemented. The environmental impact on the marine ecosystem was minimized by limiting the use of invasive sampling methods as well as by the reduction of fuel usage.

4. Narrative of the Cruise

Throughout the cruise, weather conditions were mostly favorable. Temperatures during the day were mostly around 18-22 (Fig. 6.1). Windspeeds mostly fluctuated between 0 and 10 m/s (Fig. 6.2) and thus, allowed an efficient workflow. The normal sampling procedure included MUC and VVG samples. For selected stations, additionally CTD and underwater video (UWV) transects were taken (**Table 7.1**). Samples gained from multicorer (MUC) and Van Veen grab (VVG) were processed between the different hauls with different sub-samples, measurements, incubations and fixations (e.g. bacterial productivity, fixation for bacterial cell counts and fixation of meiofauna samples). Sampling was conducted throughout the day until evening. Further, every two stations, MUC hauls were conducted consecutively between the stations (i.e. first station MUC haul at last, second station MUC haul at first) to allow parallel processing of the microbial samples. The total cruise track is shown in **Fig. 4.1**.

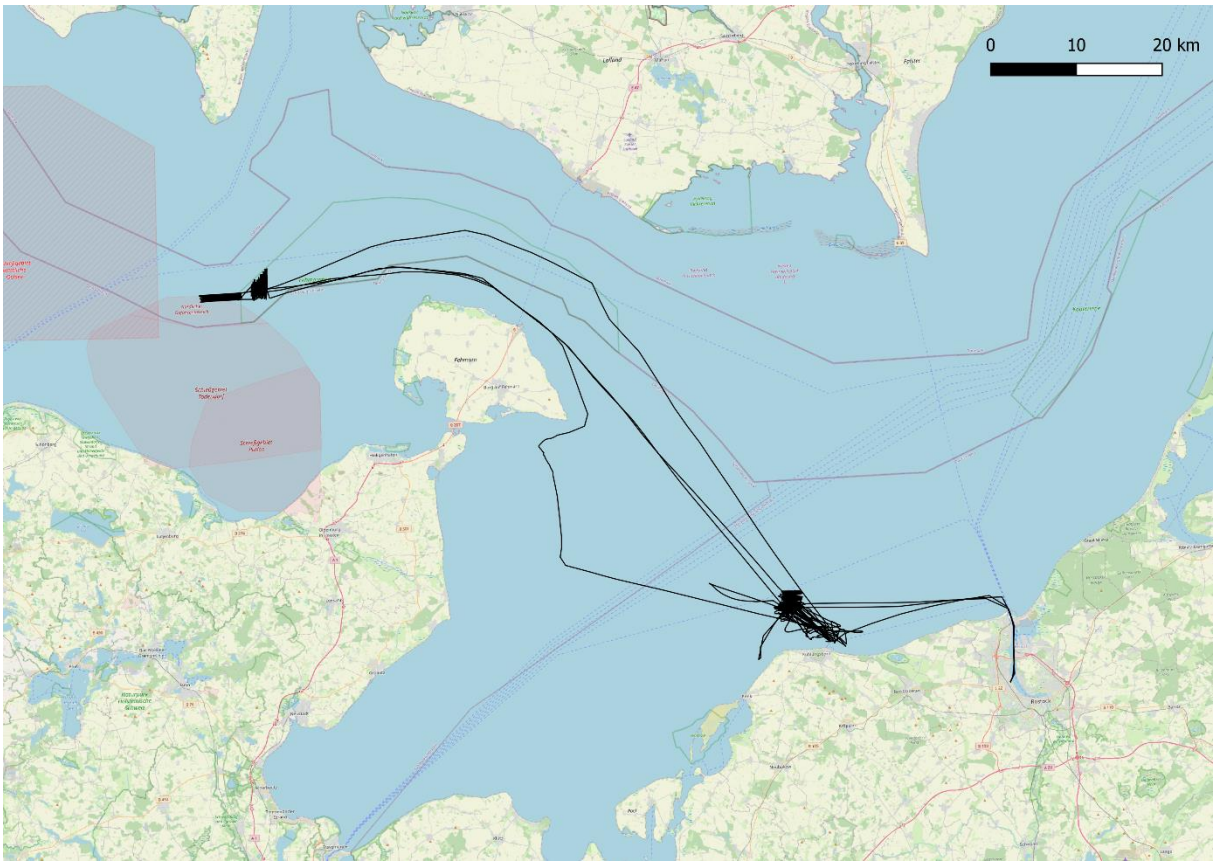

Fig. 4.1 Track of the EMB345 Cruise. Background map is provided by OpenStreeMap.

16.07.2024

Departure at 10:00 in Marienehe followed by the safety briefing. The experimental area was reached at 12:30 and T0 sampling of the experimental area started. The first day included stations HI2 and HI3 (for location of all sampling spots see **Fig. 3.1**). In the evening, CTD sampling was tested in preparation for the planned plume sampling. During the day, the ADCP measurement was constantly running and evaluated to estimate the currents for the following days. At night, the experimental area was mapped using multibeam measurement until 07:00 of the following day.

16.07.2024

In the morning, HI1 and HI4 were sampled. In addition, a UWV transect was recorded between HI4 and HI1. After lunch C6 and C7 were sampled and a video transect from C7 to C6 recorded. During station work, the ADCP measurement was constantly running and evaluated to estimate the currents for the following days. Before dinner, Kühlungsborn was approached and the ADCP technician brought on land using the ship's boat. Unless multibeam measurement was in progress, the ADCP measurement was kept running on automatic mode throughout the cruise. Afterwards, course was set to the MPA survey area at Fehmarnbelt. The area was reached at around 23:30 and multibeam mapping conducted until 07:20 the following day.

18.07.2024

At the Fehmarnbelt, three stations were sampled (F8, F2 and F13 in this order, station overview in **Fig. 3.2**). Fehmarnbelt sampling includes MUC, VVG and CTD. In addition, in south-west direction of F2, a dredge haul (~450m) was taken and a UWV transect recorded in direction west

from F13. After dinner, the multibeam mapping of the MPA area was finished until 01:00 of the following day.

19.07.2024

After multibeam mapping was finished at Fehmarnbelt, EMB returned to the experimental area off Kühlungsborn and reached at 07:00. Stations for the morning included C8 and C5. A UWV transect was recorded from C5 in eastern direction. Before lunch, the EMB approached the ALKOR for handing over MUC cores, that were taken at Fehmarnbelt, using the ship's working boat. After lunch, a technician for CTD maintenance was picked up at Kühlungsborn marina using the ship's working boat. T0 samples (=before trawling) were taken in the experimental area using the CTD. Following, several functional CTD, ADCP and camera tests were run. After diner, the CTD technician was dropped off at Kühlungsborn marina and a scientist picked up. Afterwards, the multibeam mapping of the experimental area was continued until 07:10 the following day.

20.07.2024

On the 5th day of the cruise, weather conditions were mostly stable, with occasional periods of increasing wind speeds throughout the day. These conditions were suitable for the planned trawling track. Around 08:00, the CLUPEA arrived at the experimental area and began trawling in the high-impact zone, while the EMB remained on standby south of this area. Once the CLUPEA completed its pass, EMB followed a south-to-north transect across the high-impact area. The objective was to locate the sediment plume using the multibeam, position the vessel above it, and collect samples with the CTD. Initially, the plume could not be reliably targeted, but successful sampling was achieved later during the trawling process.

Unfortunately, trawling operations were terminated prematurely by the CLUPEA due to technical issues. However, this was irrelevant for the experiment, as shortly after all available vessels were redirected to respond to a marine distress call off the coast of Meschendorf around 15:00. A diver had been reported missing, prompting a coordinated search and rescue operation, involving the vessels participating in the experiment. Fortunately, the diver had simply failed to report back to the diving base, and the search was called off.

Approximately one hour after the distress call, we resumed the scientific program, conducting T1 sampling of high-impact stations HI4 and HI3 using the MUC and VVG. Additionally, an UWV transect was recorded between stations HI3 and HI2, perpendicular to the trawling marks, completing the day's sampling efforts. The EMB then headed towards Kühlungsborn marina, where a scientific crew pickup was performed via the ship's boat. Processing of the plume samples continued into the night.

21. - 22.07.2024

Days six and seven (21&22.07) continued the T1 sampling following the normal sampling program for the stations HI1, HI2, HI11, HI12, C5, C8, C9 and C10, CTD sampling for HI1 HI11, C5 and C10 and a video transect from C8 to C9. Day six additionally included personnel deployment at Kühlungsborn before lunch.

23. - 24.07.2024

In the morning of day eight, a technician was taken on board in the course of rudder test of the ship. Due to good weather conditions and efficient progress in the sampling campaign, T0 and T1 sampling were completed. However, due to bad weather forecast, the planned diving activity of the upcoming days was shifted by two days. Therefore, after the rudder test and deployment of the technician, course was set to Marienehe harbor where the ship was moored at around 12:30. Some of the samples were transported to the IOW and URO. The rest of the day was used for further preparations but also for an exchange of information and discussion of upcoming activities with the scientists and crews of the Alkor, Limanda and CLUPEA. Day nine could be used for processing samples, preparing equipment, or for other activities.

25.07.2024

Day 10 continued with the experimental campaign and was in the focus of a single trawl track (ST) for detailed sampling. In the morning, we left Marienehe harbor with three day-guests, including two reporters from dpa. The investigation area was reached ~2h later and we started with a CTD as a control for the single ST sampling. Unfortunately, not all bottles closed, but the haul could be used for analysis. After lunch, the CLUPEA placed the ST in an area south of the HI area (Fig. 3.1). The EMB followed the trawling path, searching for the plume using the multibeam and UWV. The plume was successfully sampled, however, the divers that should sample the otter-board track, could not gather intact sediment samples. Therefore, a MUC haul on the groundrope line of ST was conducted. The day-guest left the EMB via the KLAASHAHN and were brought back to Marienehe by LIMANDA.

26.07.2024

As the diving operation of the previous day was not successful, day 11 was in the focus of a second diving attempt. This time it was successful, supported by calm weather conditions. In the morning, the control station of ST was sampled (MUC, CTD, VVG) until the diving cores arrived from the Limanda via Klashaahn. The sampling acquisition for the day was ended with a UVW from HI3 to HI2.

27.07.2024

On day 12, the Stations C5, C8, HI3 and HI4 were sampled according to the previous scheme. A UWV transect was recorded from HI3 to HI2 and C8 to C5, respectively.

28.07.2024

Day 13 was in the focus of a visit by an ARD dokumentation team, including the actor Benno Fürmann. Thus, all stations of the day (HI11, HI12, C9 and C10) were sampled before lunch according to the standard scheme. Before lunch, the ARD team arrived via speedboat and was given a tour across the ship. We explained and demonstrated the sampling equipment and methodology, introduced the background of the project and exchanged knowledge with the film crew. In the afternoon, the team was dropped off at Kühlungsborn and we headed south of Fehmarn were EMB stayed until the next morning due to bad weather.

29.07.2024

In the morning of day 14, EMB approached again the sampling area of Fehmarbelt. The sampling of the remaining stations (F 15, F17 and F18) followed the scheme of the previous Fehmarnbelt sampling. During the night, again a multibeam profile was taken.

30.07.2024

In the morning of Day 15, the experimental area at Kühlungsborn was approached. VVG samples were taken for the verification of the multibeam measurements. Afterwards, the EMB was on standby until divers samples (T2) of the ST track arrived. These samples were processed throughout the day and personnel transferred to the LIMANDA to be brought back to Rostock.

31.07. - 01.08.2024

Day 16 and 17 followed the standard scheme including the stations HI3, HI4, HI11, HI12, C5, C8, C9 and C10. On Day 16, the control station for the ST track was additionally sampled via MUC and VVG. UWV transects were recorded from HI3 to HI2, C8 to C5, HI4 to HI12 and C10 to C9, which marked the last sample acquisition. Samples were processed throughout the last day and equipment packed.

5. Preliminary Results

5.1. AP 1.1 Hydroacousitcs & Sedimentology

5.1.1. Hydroacoustics

(Inken Schulze)

MGF-Experiment

During EMB345, the hull-mounted R2Sonic 2024 multibeam echosounder (MBES) was utilized to generate detailed bathymetric and backscatter maps of the seafloor surface within the experimental area at different times. Baseline mapping prior to the trawling by RV CLUPEA documented the initial seafloor conditions, including sediment homogeneity and any pre-existing trawl tracks. Fresh trawl tracks were promptly mapped after gear deployment, with subsequent monitoring of their disintegration over the following days. The sound velocity profiles (SVP) from several CTD casts were used during post-processing, ensuring accurate depth measurements by compensating for the variability in the speed of sound within the water column.

Furthermore, hydroacoustic techniques proved to be an essential tool for detecting the underwater sediment plume created by bottom trawling within the water column, facilitating a precise and timely sampling using the CTD rosette.In this process, an EK80 echosounder installed in the moon pool of RV EMB was utilized alongside the MBES and the hull-mounted sediment echosounder INNOMAR SES2000 medium.

To ground truth the sediments in the extended experiment area, five grab samples were taken at additional stations and frozen for later grain size analysis.

MGF-Fehmarnbelt

The primary objective of the hydroacoustic mapping conducted during the divided Fehmarnbelt (FB) survey was to assess the impact of bottom fisheries on seafloor morphology and composition. This survey extends the time series, which began in 2020, by an additional year. The hull-mounted R2Sonic 2024 multibeam echosounder (MBES) was utilized to collect comprehensive seafloor bathymetry and backscatter data for two survey sites at the FB area. The sound velocity profiles (SVP) from several CTD casts at every survey site were used during post-processing, ensuring accurate depth measurements by compensating for the variability in the speed of sound within the water column.

5.1.1.1. Methods

R2Sonic 2024 multibeam echosounder (MBES)

Table 5.1 R2Sonic 2024 settings used for different parts of the cruise.

	MGF_EXP	MGF_Plume	FB_MPA	FB_REF
Frequency [kHz]	400 (200/300)	400 + water column	400	400
Swath Width [°]	120/130	130	120	120
Ping Rate [Hz]	12.5	12.6	12.6	12.6
Range [m]	40/50/70	50	50	50
Power [dB]	209	215	209	209
Pulse Width [µs]	50	20	50	50
Gain	6	6	6	6

Absorption [dB/km]	80	80	80	80
Spreading	20	20	20	20
BSM	uhd	uhd	uhd	uhd

The seafloor bathymetry and backscatter data were acquired using a hull-mounted multibeam echosounder (MBES) system manufactured by R2Sonic. MBES is a sophisticated sonar system commonly used for mapping the seafloor surface. The system was operated with a swath width of 120 or 130 degrees, which refers to the angle covered by the sonar beams. Usually, the recording frequency of the MBES was set to a frequency of 400 kHz, while some profiles were surveyed in multifrequency mode changing between 200 and 300 kHz at every ping. The frequency choice is significant as it impacts the resolution and depth penetration of the sonar signal. The following recording parameters were selected:

The vessel's speed during data acquisition was 4 to 5 knots, adjusted according to water depth and the consequential ping rate. The profile distances were 50 m or 75m at the experiment site and 50 m for both survey sites at the FB area. The MBES data was post-processed using the QPS Qimera and FMGT software. Sound velocity data from CTD measurements are available for post processing.

INNOMAR SES2000 (sediment echosounder, SES)

Although designed as a sub-bottom profiler, the sediment echo sounder was utilized to detect the sediment plume from trawling within the water column. The parametric system operated at a fixed high frequency (HF) of 100 kHz, while the lower frequency (LF) was set to 15 kHz. Nonlinear interactions of the two signals in the water result in the generation of new frequencies, one of which is the difference frequency, with a bandwidth similar to that of the primary frequency. The parametric acoustic system provides several advantages, including a narrow beam width at low frequencies, deep penetration with high-resolution imaging of sediment layers and objects, and precise depth measurements using the high-frequency signal. Ping rate was around 20 pps, gains were both set to 0, and the range covered the water depths of 5 to 30 m. The data was only used to locate the plume on the fly for sampling with the CDT rosette and only few profiles were recorded. The data is not displayed in this report.

Kongsberg Maritime EK80 echosounder

The two transducers of the EK80 echo sounder were installed in the EMBs moon pool operating at 70 kHz and 120 kHz. The range was set 0 to 25 m, Ping interval 100 ms. Unfortunately, the position and motion from the ships network were not directly saved into the files. The EK80 system was not calibrated during this cruise. The data was only used to locate the plume on the fly for sampling with the CDT rosette and only few profiles were recorded. The data is not displayed in this report.

5.1.1.2. Preliminary Results

MGF-Experiment T0

The main experimental area was surveyed three times at time T0: twice at 400 kHz frequency to improve data quality at the experimental site and once in dualfrequency mode changing between 200 kHz and 300 kHz frequency after every ping. The first MBES survey covered not only the

experimental site but was extended towards the 3 mile-limit in the north to gain a comprehensive overview of the surrounding seafloor. During this survey an area of about 6 km^2 was covered (**Fig. 5.1**).

The bathymetry maps show a decrease in water depth of 3 m from the southeast at -22.5 m to the northwest at -25.5 m. At the experimental site, variations in water depth are within 1 m and the seafloor appears widely homogeneous.

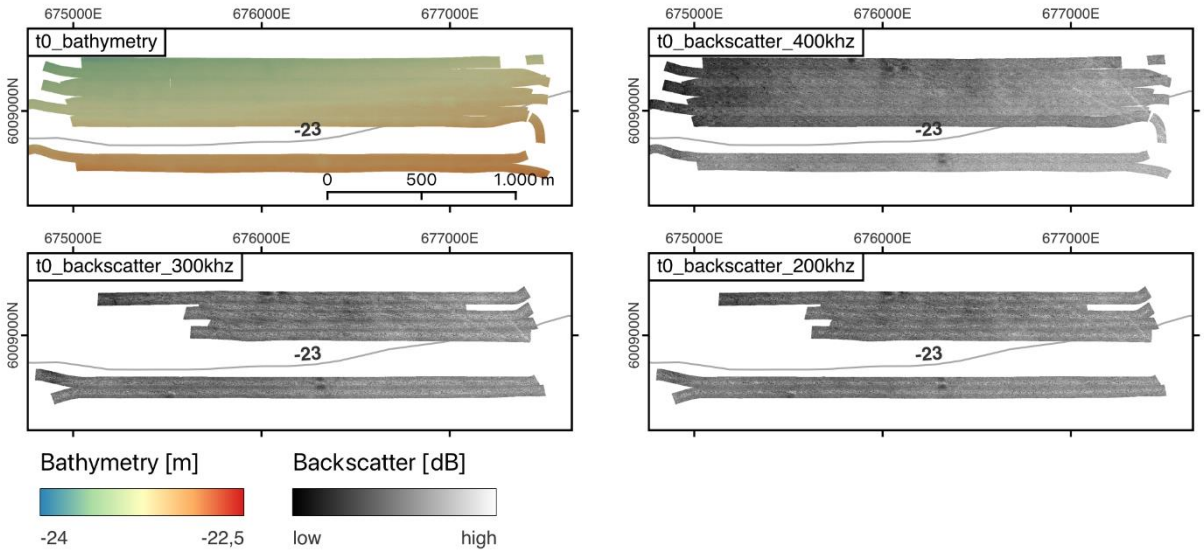

Fig. 5.1 The hydroacoustic survey was extended around the main experimental site to gain comprehensive overview of the surrounding seafloor at time T0. Both, the bathymetry and backscatter map show a homogeneous seafloor for the experimental site with only few deviations and no trawl tracks present. To the north, trawl tracks occur and their density increases. Please note: Data processing is preliminary, noise may be present.

The backscatter mosaic of the extended area shows a decrease in intensity toward the northwest. While the seafloor composition within the experimental site appears generally homogeneous, patches of low backscatter occur within an area of higher backscatter values in the northeast. Moving north, there is an increase in visible trawl tracks. Two tracks, crossing in an east-west direction, are particularly distinct, indicating a more recent origin. The increase in trawl tracks in different stages of degenerations is also reflected in the bathymetry.

MGF-Experiment T1

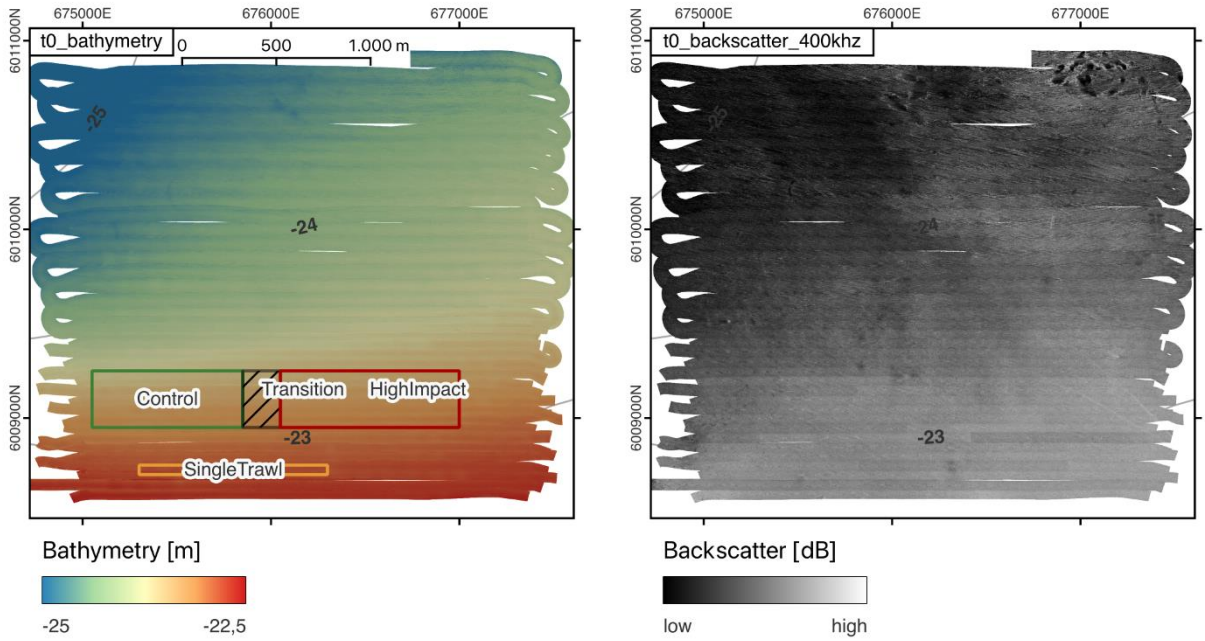

Fig. 5.3 The main experimental site was surveyed twice again at time T0 to improve data quality and to include two more frequencies. The bathymetry (at 400 kHz) shows a homogeneous flat seafloor surface. The differences in backscatter between the three frequencies utilized are small. Please note: Data processing is preliminary, noise may be present

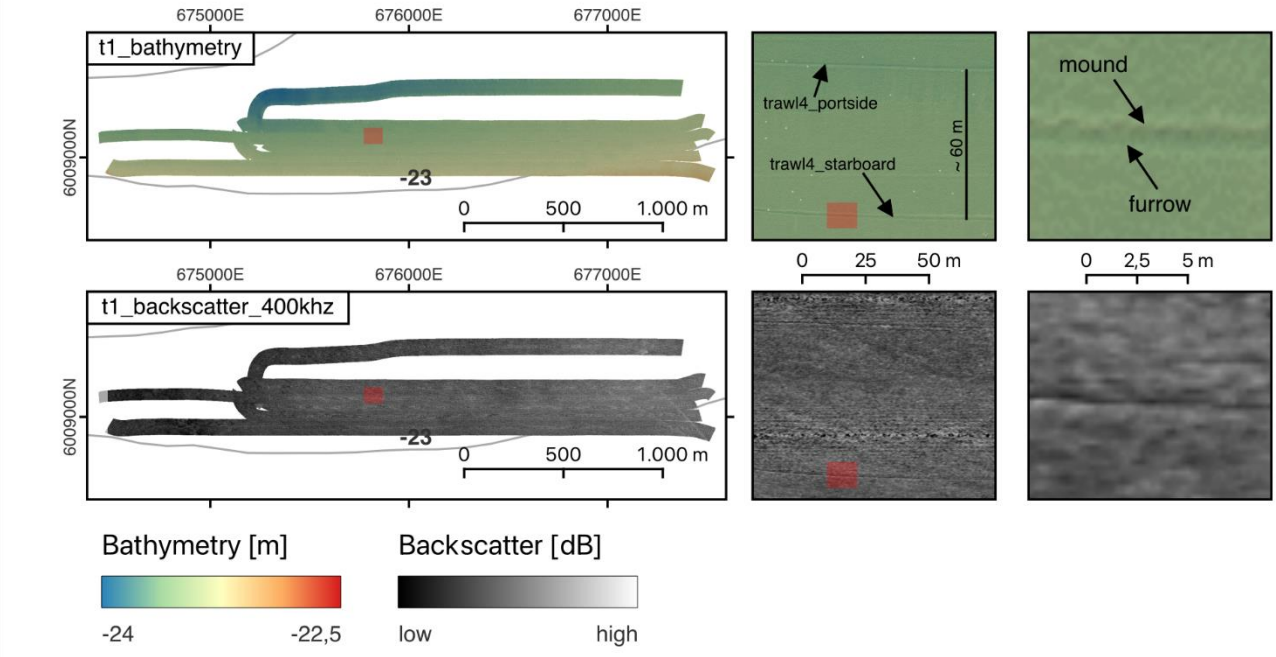

Fig. 5.2 The experiment area was surveyed at T1 to record the trawl tracks arranged by RV CLUPEA earlier the same day. The trawl tracks are visible in both, the bathymetric and backscatter maps as elongated, parallel lines. The otter boards leave behind two continuous furrows flanked by mounds formed by the overturned sediment. The furrows are represented by low backscatter values and the mounds by slightly increased values compared to the surrounding seafloor. Please note: Data processing is preliminary, noise may be present.

The bathymetric mapping during the night after the bottom trawling by RV CLUPEA allowed for the detailed localization of the trawl tracks. As four gill nets were placed within the main investigation area or close by at the time, some parts of the site could not be surveyed, resulting in data gaps (**Fig. 5.3**).

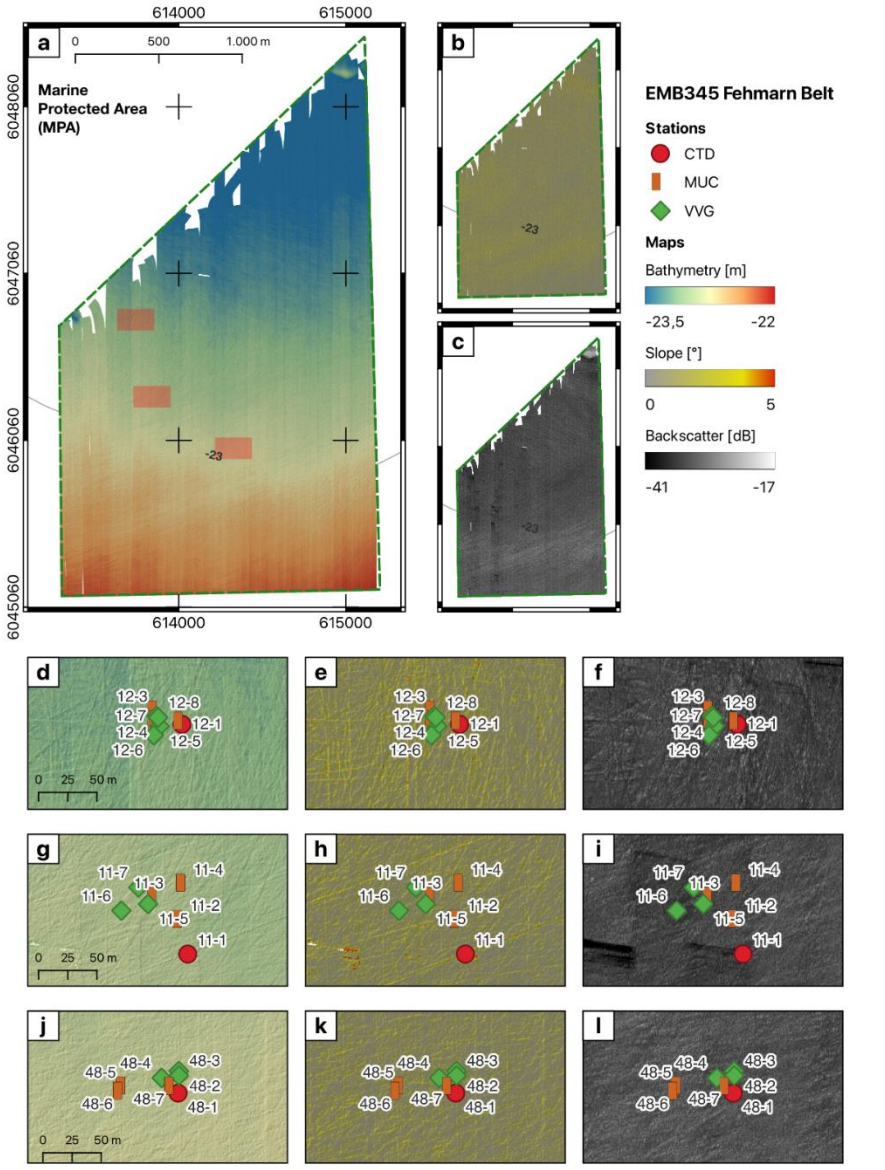

Fig. 5.4 The hydroacoustic maps of the MPA at Fehmarnbelt reveal a dense pattern of both old and recent trawl tracks at the seafloor surface. These maps include: (a) bathymetry with hillshading, (b) slope with hillshading and (c) backscatter at 400 kHz. Map sections provide a more detailed view of the seafloor around the stations EMB345_12 (d-f), EMB345_11 (g-i) and EMB345_48 (j-l). Please note: Data processing is preliminary, noise may be present.

The trawl tracks were visible in all hydroacoustic datasets gridded with a resolution of 0.25 m. In the bathymetric maps the tracks are visible as pairs of parallel, continuous furrows with depths of up to 10 cm and a width of about 1 m. The furrows are flanked by bumpy mounds to the inner side, which show elevations of up to 10 cm and are about 1 m wide.

In the backscatter maps (Fig. 5.3 and Fig. 5.3), the furrows appear as lines of low backscatter values, while the mounds show slight increase backscatter values compared to the surrounding

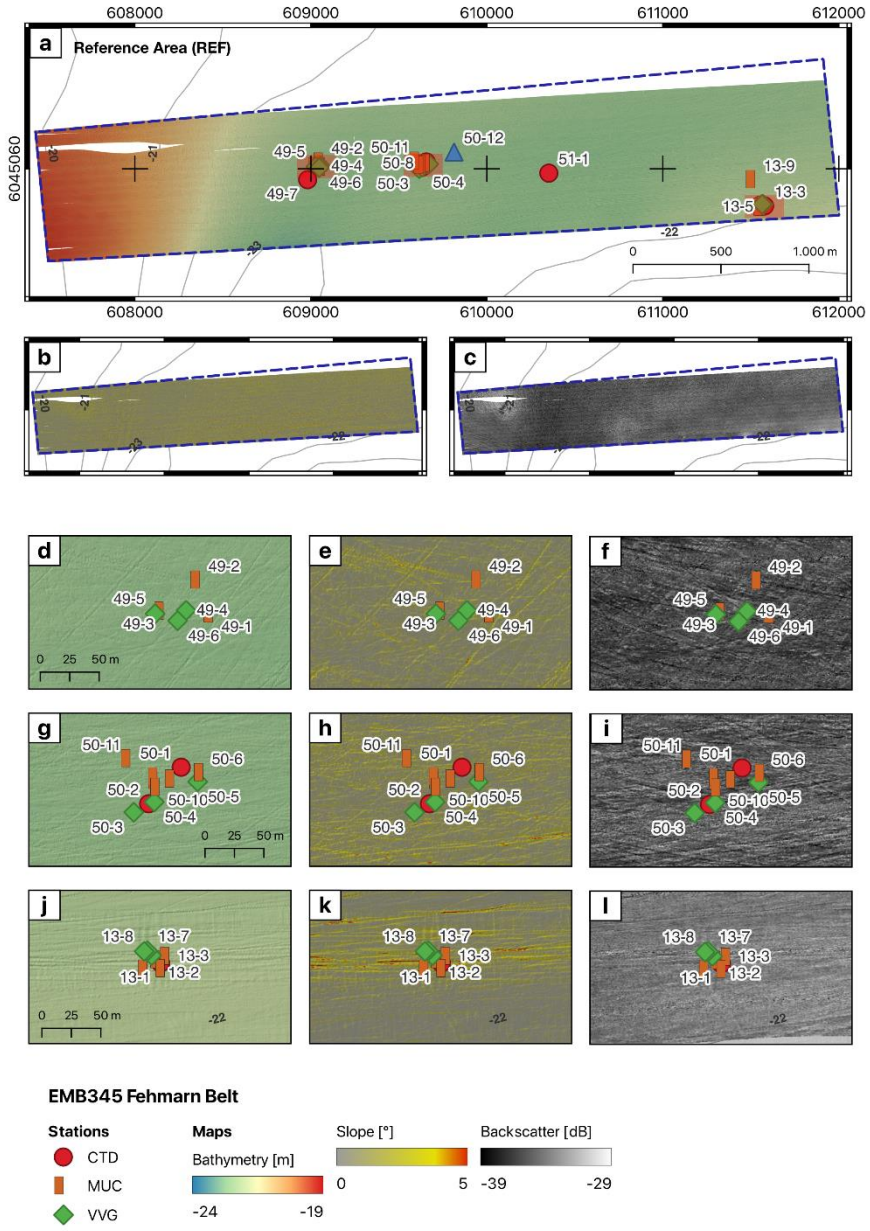

Fig. 5.5 The hydroacoustic maps of the reference area at Fehmarnbelt reveal a dense pattern of both old and recent trawl tracks at the seafloor surface. These maps include: (a) bathymetry with hillshading, (b) slope with hillshading and (c) backscatter at 400 kHz. Map sections provide a more detailed view of the seafloor around the stations EMB345_49 (d-f), EMB345_50 (g-i) and EMB345_13 (j-l). Please note: Data processing is preliminary, noise may be present

areas. However, the backscatter data is affected by noise in the nadir area, leading to variations in data quality.

Digitalizing the trawl tracks allowed for the precise identification of the exact areas that were trawled. The trawling affected not only the High Impact area (HI) and transition zone, but extended significantly into the Control area, which was intended to remain untouched.

Please note: There is a hydroacoustic dataset for the time T2 surveyed by RV LIMANDA equipped with a NORBIT iWBMSe.

MGF-Fehmarnbelt

Two distinct areas located in the Fehmarnbelt were subject to hydroacoustic surveys for the fifth time in a span of five years covering a Marine Protected Area (MPA) and a reference area. Both are characterized by a significant presence of trawl marks crossing the seafloor surface, which are clearly visible in backscatter and bathymetric data.

In the survey area of the MPA (**Fig. 5.4**), several pathways with different orientations show of distinct, likely recent trawl tracks. Most of the areas beside these are characterized by a fragmented pattern of elongated, elevated mounds, which may result from previous trawling activities. A known wreck is located at the northwestern edge of the survey area. The reference area (**Fig. 5.5**) to the west of the MPA also displays a similar pattern of pathways formed by distinct trawl tracks. The same characteristic fragmented pattern of elongated elevations as in the MPA can be observed. Additionally, there is a data gap in the northwest part of the survey area around a navigation mark around which fewer trawl tracks are observed.

5.2. AP 2.1 Lower Trophic Layers (Protists & Meiofauna))

5.2.1. Meiofauna

(Alexandra Ostmann, Sven Hoffmann)

Samples were collected in the Fehmarnbelt and in the trawling experiment area off Kühlungsborn using the multicorer (MUC) or directly by divers using push cores.

The MUC was equipped with eight cores. Each MUC core has an inner diameter of 10 cm and covers a sampling area of 78.54 cm², while the push cores are 6 cm in inner diameter and cover 28.27 cm². The top five cm of each core (push cores and MUC cores), as well as the supernatant water (sieved through 40 µm), were transferred into a Kautex vial. The samples were preserved in 96% denatured ethanol.

In the experimental area (**experiment**), 4 stations in the high-impact area (HI) and 4 stations in a control area (C) were sampled at four points in time (t0, t1, t2, t3, see **Table 5.2**). Samples were taken from one MUC deployment per station. At each station, one sediment sample was taken for morphological determination and two samples for genetic analyses. At some stations, an additional sample was taken for morphological determination. A total of 39 samples for morphological determination and 68 samples for metabarcoding were taken in the experimental area.

Table 5.2 List of stations in the experimental area sampled with the MUC for meiobenthos. The list includes the number of cores taken per station for each morphology and metabarcoding.

Cruise	Date	Region	Area	Timeslot	Station	Cast	No of cores for Morphology	No of cores for Metabarcoding	Depth
EMB345	16.07.2024	High Impact	HI2	t0	1	3	1	2	24.0
EMB345	16.07.2024	High Impact	HI3	t0	2	2	1	2	25.0
EMB345	17.07.2024	High Impact	HI1	t0	6	3	2	2	23.7
EMB345	17.07.2024	High Impact	HI4	t0	7	1	2	2	23.8
EMB345	17.07.2024	Control	C6	t0	8	3	1	2	24.0
EMB345	17.07.2024	Control	C7	t0	9	1	2	2	24.0
EMB345	19.07.2024	Control	C8	t0	15	3	1	2	24.4
EMB345	19.07.2024	Control	C5	t0	16	1	1	2	24.0

EMB345	20.07.2024	High Impact	HI4	t1	22	2	1	2	24.1
EMB345	20.07.2024	High Impact	HI3	t1	23	2	2	2	24.4
EMB345	21.07.2024	High Impact	HI1	t1	26	3	1	2	24.0
EMB345	21.07.2024	High Impact	HI2	t1	27	1	2	2	24.2
EMB345	21.07.2024	Control	C5	t1	28	3	1	2	24,0
EMB345	21.07.2024	Control	C8	t1	29	1	1	2	24.3
EMB345	22.07.2024	High Impact	HI11	t1	31	4	1	2	24.1
EMB345	22.07.2024	High Impact	HI12	t1	32	3	1	2	24.0
EMB345	22.07.2024	Control	C10	t1	33	4	1	2	24.0
EMB345	22.07.2024	Control	C9	t1	34	1	1	2	23.5
EMB345	27.07.2024	High Impact	HI4	t2	40	3	1	2	24.0
EMB345	27.07.2024	High Impact	HI3	t2	41	1	1	2	24.1
EMB345	27.07.2024	Control	C5	t2	42	3	1	2	24.0
EMB345	27.07.2024	Control	C8	t2	43	1	1	2	24.3
EMB345	28.07.2024	High Impact	HI11	t2	44	3	1	2	24.0
EMB345	28.07.2024	High Impact	HI12	t2	45	1	1	2	24.0
EMB345	28.07.2024	Control	C9	t2	46	2	1	2	24.1
EMB345	28.07.2024	Control	C10	t2	47	1	1	2	24.2
EMB345	31.07.2024	High Impact	HI4	t3	54	3	1	2	24.1
EMB345	31.07.2024	High Impact	HI3	t3	55	1	1	2	24.1
EMB345	31.07.2024	Control	C5	t3	56	3	1	2	24.0
EMB345	31.07.2024	Control	C8	t3	57	1	1	2	24.3
EMB345	01.08.2024	High Impact	HI11	t3	59	3	1	2	24.1
EMB345	01.08.2024	High Impact	HI12	t3	60	1	1	2	24.0
EMB345	01.08.2024	Control	C9	t3	61	3	1	2	24.1
EMB345	01.08.2024	Control	C10	t3	62	1	1	2	24.3

At the **single track** (**Table 5.3**), divers took three sediment samples from the furrow and three sediment samples from the mount of a trawling track at t1 and t2 using push cores (**Fig. 5.6**). Furthermore, the MUC was deployed in a control area outside the track (Control; t1 and t2) and in between the otter boards within the net area (Net, only t1).

Table 5.3 List of stations of the single track sampled with the MUC or by divers for meiobenthos. The list includes the number of cores taken per area for each morphology and metabarcoding

Cruise	Date	Region	Area	Timeslot	Station	Cast	Gear	No of cores for Morphology	No of cores for Metabarcoding	Depth
EMB345	25.07.2024	Impact	Net	t1	35	7	MUC	1	2	23.6
EMB345	26.07.2024	Control ST	Control	t1	36	3	MUC	1	2	23.4

FK Limanda	26.07. 2024	Impact	Mount	t1			Diver	1	2	
FK Limanda	26.07. 2024	Impact	Furrow	t1			Diver	1	2	
FK Limanda	30.07. 2024	Impact	Mount	t2			Diver	1	2	
FK Limanda	30.07. 2024	Impact	Furrow	t2			Diver	1	2	
EMB345	31.07. 2024	Control ST	Control	t2	58	1	MUC	1	2	23.6

For morphology one core was used, for genetic analysis two cores were used. In total, 14 sediment samples were taken for genetic analyses and seven samples for morphological determination.

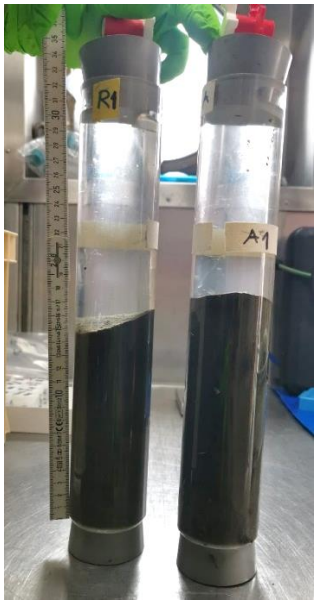

Fig. 5.6 Push cores of the single track from the furrow (R1) and the mount (A1).

Due to the time constraints during the EMB342 cruise, the Fehmarnbelt was visited during EMB345 (**Table 5.4**). In the Fehmarnbelt, three stations in the reference area (REF) and three stations in the marine protected area (MPA) were sampled with the MUC. From three casts, three cores each were used for meiofauna investigation. One core from each cast will be used for morphological determinations, and two cores will be used for metabarcoding and eDNA. The samples for eDNA analysis were taken as a subsample (~1 gr) from the sediment surface of the samples used for metabarcoding (Fig. 5.7). The eDNA samples are stored in –20 °C until further processing.

Table 5.4 List of stations sampled in the Fehmarnbelt with the MUC for meiobenthos. The list includes the number of cores taken per station for each morphology, metabarcoding and eDNA.

Cruise	Date	Region	station-replica	Station	Cast	No of samples for Morphology	No of samples for Metabarcoding	No of samples for eDNA	Depth
EMB345	18.07.2024	MPA	f8	11	2	1	2	2	23.6
EMB345	18.07.2024	MPA	f8	11	3	1	2	2	23.6
EMB345	18.07.2024	MPA	f8	11	4	1	2	2	23.6
EMB345	18.07.2024	MPA	f2	12	2	1	2	2	23.8
EMB345	18.07.2024	MPA	f2	12	3	1	2	2	23.8
EMB345	18.07.2024	MPA	f2	12	4	1	2	2	23.8
EMB345	18.07.2024	REF	f13	13	2	1	2	2	22.9
EMB345	18.07.2024	REF	f13	13	3	1	2	2	22.8
EMB345	18.07.2024	REF	f13	13	5	1	2	2	22.8
EMB345	29.07.2024	MPA	f18	48	5	1	2	2	23.6
EMB345	29.07.2024	MPA	f18	48	6	1	2	2	23.6
EMB345	29.07.2024	MPA	f18	48	7	1	2	2	23.6
EMB345	29.07.2024	REF	f17	49	1	1	2	2	23.4
EMB345	29.07.2024	REF	f17	49	2	1	2	2	23.3
EMB345	29.07.2024	REF	f17	49	3	1	2	2	23.4
EMB345	29.07.2024	REF	f15	50	9	1	2	2	23.4
EMB345	29.07.2024	REF	f15	50	10	1	2	2	23.3
EMB345	29.07.2024	REF	f15	50	11	1	2	2	23.3

A total of 18 samples were taken for morphological determination. The genetic material comprises 36 samples for metabarcoding and 36 samples for eDNA analyses.

Fig. 5.7 Taking eDNA samples of the sediment surface.

The samples are processed in the laboratories of the DZMB (German Centre for Marine Biodiversity Research, Senckenberg am Meer, Wilhelmshaven). To extract the meiofauna from the sediment, the samples are centrifuged with Levasil® and kaolin. The supernatant is washed into small Kautex vials with ethanol. These samples will be used for metabarcoding.

For metabarcoding, DNA will be extracted from the samples (**Fig. 5.8**) and two gene fragments, COI mtDNA and the hypervariable region V1V2 of the 18S rRNA, 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, error corrected 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).

DNA will be extracted from the eDNA sample (**Fig. 5.8**). Similar gene fragments as in metabarcoding will be amplified and processed.

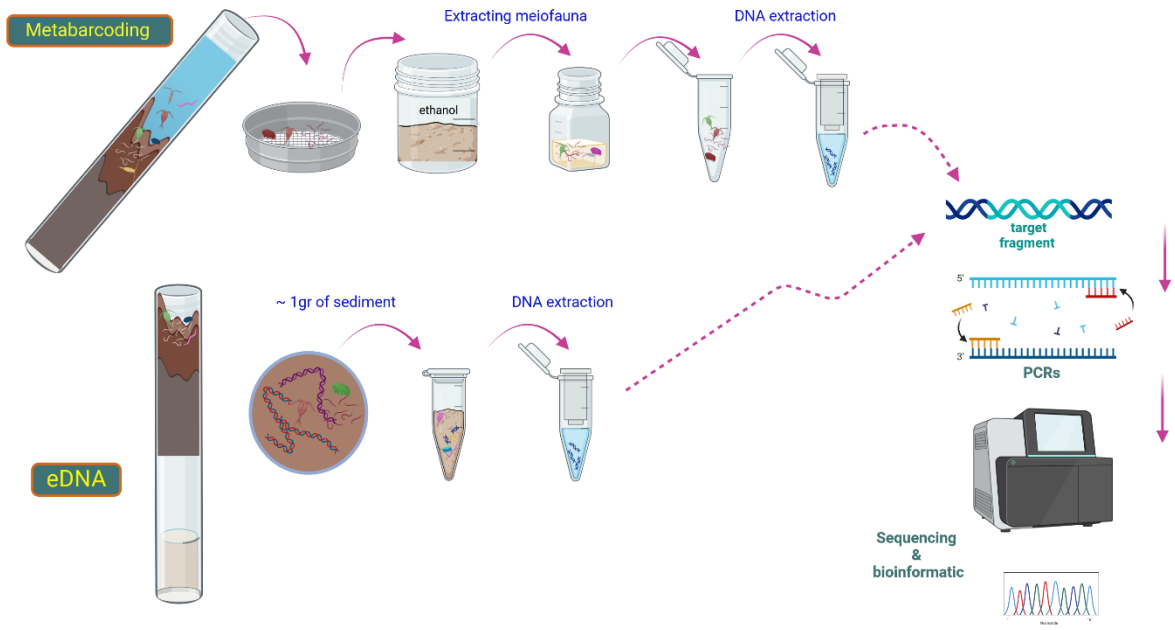

Fig. 5.8 Process of DNA extraction and amplification from the samples (created by Dr Sahar Khodami, DZMB).

Morphological determination is performed only, if DNA fragments cannot be assigned to any of the known species. For this purpose, Meiofauna would be sorted and counted manually by means of a Leica M125 stereomicroscope. Benthic copepods (Copepoda Harpacticoida) would be determined at family, genus and species level.

5.2.2. Protists - Nano- and Microfauna

(Hartmut Arndt, Aanja Scherwaß)

Unicellular eukaryotes account for the majority of all eukaryotic genotypes in the world's oceans (e.g., de Vargas et al., 2015; Gooday et al., 2020; Schoenle et al., 2021). Benthic nanofauna in the size range of 1–20 µm (mainly heterotrophic nanoflagellates and small amoebae) and microfauna in the size range of 20–200 µm (ciliates, heterotrophic dinoflagellates, amoeboid protists, etc.) are known to be important parts of the benthic food web as they channel bacterial production to higher trophic levels (e.g., meiofauna, macrozoobenthos) that provide a food source for demersal fishes. Bacterial abundance and production are thought to be regulated and controlled by microbial predators. In the process, a variety of geochemical processes determined by the oxygen

consumption of bacteria are also potentially regulated by nano- and microfauna organisms. In addition, many parasitic protists are known to populate marine sediments (Schoenle et al., 2021). In our subproject, we assume that the disturbance of sediment structures by trawling alters the microbial food web and its functions. During the present expedition EMB345, our task was to estimate the effect directly after the trawling activity. As previously described in the general introduction to the experiment, experimental trawling was carried out in a previously untouched area near Kühlungsborn in the Mecklenburg Bight. Nano- and microfauna samples (MUC cores) were examined from all stations of the affected areas as well as from the control areas without trawling (except the stations of the preliminary investigation). In addition, cores taken by divers at a freshly created impact zone of an otter board were examined. In addition, protist samples from the second visit to the Fehmarnbelt region during EMB 345 were examined. Nano- and microfauna abundance and diversity were estimated by combining different approaches (Schoenle et al., 2016, Jeuck et al., 2017). Nano- and microfauna abundance and diversity were estimated by a combined analysis of live counts on board, determination of culturable protist species in the laboratory of the University of Cologne and - in collaboration with the microbiology group (Prof. Jürgens and Dr. Piontek from the IOW) - expansion of the GenBank database for Baltic protists by sequencing the V4 and V9 region of isolated microeukaryotes for better evaluation of eDNA metabarcoding analyses.

5.2.2.1. Methods

Sediment sampling

Sediment samples were collected using an IOW multicorer (MUC, Fig. 5.9 C, D) system (see **Table 7.1**). Undisturbed sediment cores obtained from the MUC were used for quantitative and qualitative analyses of benthic nano- and microfauna. Additionally, smaller cores obtained by scuba diving (see Fig. 5.9 E, F) from the impact area of an otter board were examined. In all cases, samples were taken from the same cores, which were also analyzed for microbiological parameters and eDNA. Cores were cut into eight sediment layers (0-1 cm, 1-2 cm, 2-3 cm, 3-4 cm, 6-7 cm, 9-10 cm, 14-15 cm, 19-20 cm). With the help of 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 and samples were processed as quickly as possible and stored cooled until further processing on board. For quantitative and qualitative analyses of the benthic nano- and microfauna of protists, the analysis on board began with the analysis of the surface layer at each station, followed by the analysis of the next deeper layer. The microscopic analysis was stopped for a station if no protists were found in three parallel subsamples of a depth layer. This was generally the case at a depth of 3-4 cm.

Abundance estimations of benthic nano- and microfauna

Three times, 0.5 cc (1.5 cm^3) of sediment was collected from each 1 cm slice of a core mixed with 3 ml of filtered (<0.2 µm) ambient seawater. This suspension was used to detect live protists under the microscope (ZEISS Axiostar equipped with phase contrast objectives and video camera). Examination and counting of 5-50 µl subsamples of the sediment suspensions were performed using 10x, 20x (for microfauna) or 40x (for nanofauna) phase contrast objectives (Arndt et al., 2000; Jeuck & Arndt, 2013). In addition to quantitative estimates, live counting techniques offer the possibility to determine a certain percentage of organisms down to the morphospecies level

and to verify the presence of live specimens of genotypes known only from metagenomic studies. The limitation of this method is the short time frame available for observing sediment suspensions on board, as many nanofauna organisms die after a certain time due to rising temperatures and light exposure. However, the direct counts can serve as cultivation-independent evidence of the species that are active at the time of sampling.

Cultivation of benthic nano- and microfauna

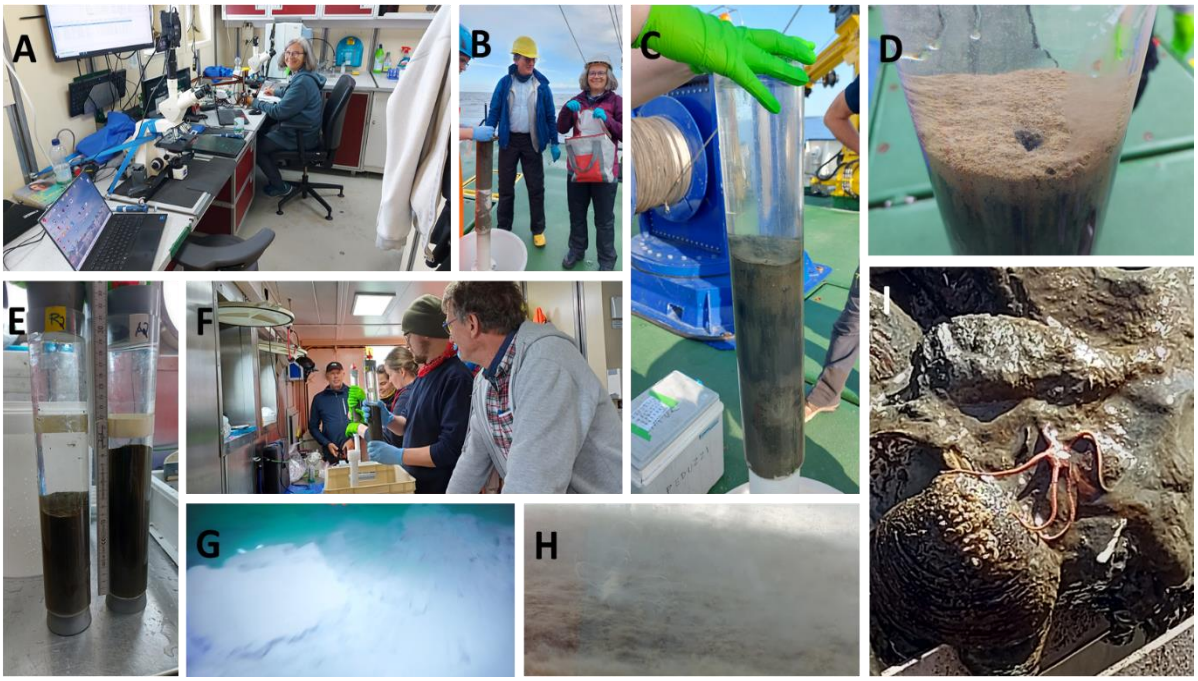

Fig. 5.9 Live-counting of protists on board. A – Microscopic investigation by video-microscopy; B - Core slicing for subsequent live-counting, and cultivation of sediment samples; C – Typical core from Kühlungsborn; D – Thick fluff layer on the sediment; E,F – Cores obtained by SCUBA diving; G – Mound resulted from by otter board action in about 20m depths; H – Small sediment resuspension by benthic macrofauna (I) in a core before slicing.

From several stations, subsamples were mixed with sterile filtered ambient seawater and supplemented with autoclaved quinoa or wheat grains to enrich the co-occurring bacteria as a food source for protists. These suspensions were used for subsequent cultivation of protist species with the aim of relating the morphological identity of the species to their genotype and ecology in order to derive an idea of the function in the benthic microbial food web. For this purpose, 50 ml tissue culture flasks were used to establish crude cultures. In addition, the liquid aliquot method was used to establish monocultures. Subsequent investigations and further isolations are carried out in the laboratory in Cologne to analyze the genotype, morphology, taxonomy, phylogeny and ecology of the successfully cultured taxa.

5.2.2.2. Preliminary results

Live counting studies on board (Fig. 5.9 A-F) typically showed highest abundances of nanofauna (up to 100 ind./cm³) and microfauna (up to 20 ind./cm³) in the upper sediment layer. Before a detailed analysis of the data, it seems that the abundances of nano- and microfauna are more related to the presence of a thick fluff layer than to whether trawling is carried out at the station or not. However, trawling seems to be a factor influencing the presence of the fluff layer, in addition to

the currents over the sediment. Statistical analyses of the abundance and biomass data have to be performed in the home laboratory. The community structure of nano- and microfauna was relatively similar to previous studies within the MGF Baltic Sea project. The abundance of nano- and microfauna in the study area off Kühlungsborn was lower than that we recorded in shallower regions such as the Oderbank or the shallow coastal waters of the island of Rügen (Dietrich & Arndt, 2000; Sachs et al., 2023), but it was comparable to the Fehmarnbelt region. The numerically dominant groups of nanofauna were kinetoplastids (neobodonids) and euglenids. In addition, colorless dinoflagellates, bicosoecids, colorless cryptomonads, ancyromonads, amastigomonads, cercomonads and small gymnamoebidae were occasionally important. Noteworthy were the relatively abundant hemimastigophorans (*Spironema*-like). Regarding microfauna, ciliates and large colorless euglenids dominated the sediment samples. In several cases, large Testaceafilosea (*Cyphoderia*-like species) and also foraminifera (e.g. *Cribroelphidium*-like, see also Frenzel et al., 2005) occurred. Among the ciliates, voracious bacterivores dominated (e.g. scuticociliates, karyiorelictid ciliates, *Cytopharynx*, *Metacystis*), but predatory forms (e.g. *Litonotus*) and anaerobic forms (e.g. *Plagiopyla*) were also detected. Some representatives present in the live counts are shown in Fig. 5.10. The vertical stratification of the distribution patterns was very obvious. In the upper first centimeter, heterotrophic flagellates dominated, which were gradually replaced by ciliates in deeper sediment layers. This vertical stratification was altered in the cores affected by otter boards.

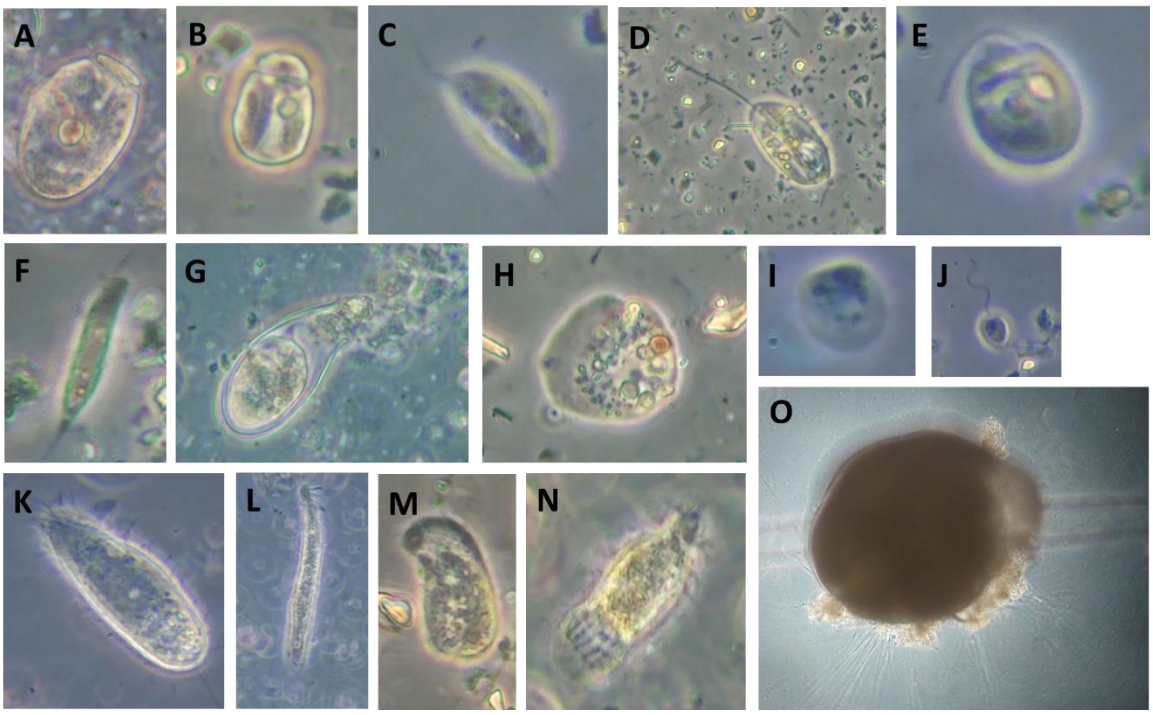

Fig. 5.10 Different heterotrophic protozoan species observed during live-counting at the Kühlungsborn sampling site. A, B - colourless benthic dinoflagellates; C – amastigomonad flagellate; D – colourless euglenid flagellate; E – goniomonad flagellate; F – Spironema sp.; G – Cyphoderia-like Testaceafilosea; H – thecamoebid amoeba; I – vannellid amoeba; J – Pteridomonas sp.; K - Scuticociliate; L - Remanellid ciliate; M - Cryptopharynx sp.; N - Metacystis sp.; O – benthic foraminiferan.

In addition to the sampling campaign immediately after the impact, the area and also the single track sampled by divers were re-examined one week later. At the current stage of the analysis, no clear response of the nano- and microfauna was evident, but we await a more detailed analysis of

the data in our home lab. On 29 July, the nano- and microfauna were surveyed at three stations in the Fehmarnbelt (**Table 7.2**) to fill the gap left by the EMB 342 sampling campaign in June 2024. The taxonomic composition and vertical stratification of the nano- and microfauna were comparable to previous campaigns in this region. Due to the pronounced vertical stratification observed for almost all nano- and microfauna, a potentially strong influence of sediment restructuring by bottom fishing must be expected.

5.3. AP 2.2 Prokaryotes & eDNA

(J. Piontek, C. Meeske, T. Bruhns, K. Jürgens)

The major goals of this work package are (1) to investigate how bottom trawling fisheries affect the composition and functioning of benthic prokaryotes and (2) to develop a non-invasive approach for the assessment of benthic biodiversity in Marine Protected Areas (MPAs) of the Baltic Sea using environmental DNA (eDNA).

5.3.1.1. Methods – Trawling Experiment

During the trawling experiment, samples for the analysis of bacterial biomass production, taxonomic community composition by 16S rRNA-amplicon sequencing and the quantification of bacterial cell numbers were collected in both the trawled area and the conrol area before bottom trawling (t0) and at three timepoints after bottom trawling (t1, t2, t3). Four to five stations per area and timepoint were sampled with the multicorer.

In addition, sediment cores with a diameter of 60 mm were taken by divers from small-scale structures of a trawl track one day after trawling (t1-single track) and four days later (t2-single track). These cores were sampled for the same parameters as described above.

Both MUC cores and diver cores were sliced on board and sampled in seven to eight discrete depth intervals from the surface to 15-20 cmbsf were taken (depth intervals MUC cores: 0-1, 1-2, 2-3, 3-4, 5-6, 9-10, 14-15, 19-20 cmbsf, depth intervals diver cores: 0-1, 1-2, 2-3, 3-4, 6-7, 9-10, 14-15 cmbsf). Sub-samples for cell counting were fixed with formaldehyde, washed with PBS and stored frozen in an ethanol : PBS mixture at -20°C. Samples for the taxonomic analysis of benthic prokaryotes by amplicon sequencing were shock-frozen in liquid nitrogen and stored at -80°C until further analysis in the lab. Prokaryotic biomass production in surface sediments was estimated from uptake rates of ^{3}H-leucine. Incubations with the radioactive tracer were conducted on board.

5.3.1.2. Preliminary Results – Trawling Experiment

Prokaryotic biomass production in both the trawling area and the untrawled control area showed substantial temporal variability over the time of the cruise but no significant difference between trawling area and control area after the trawling was conducted (t1, t2, t3) ().

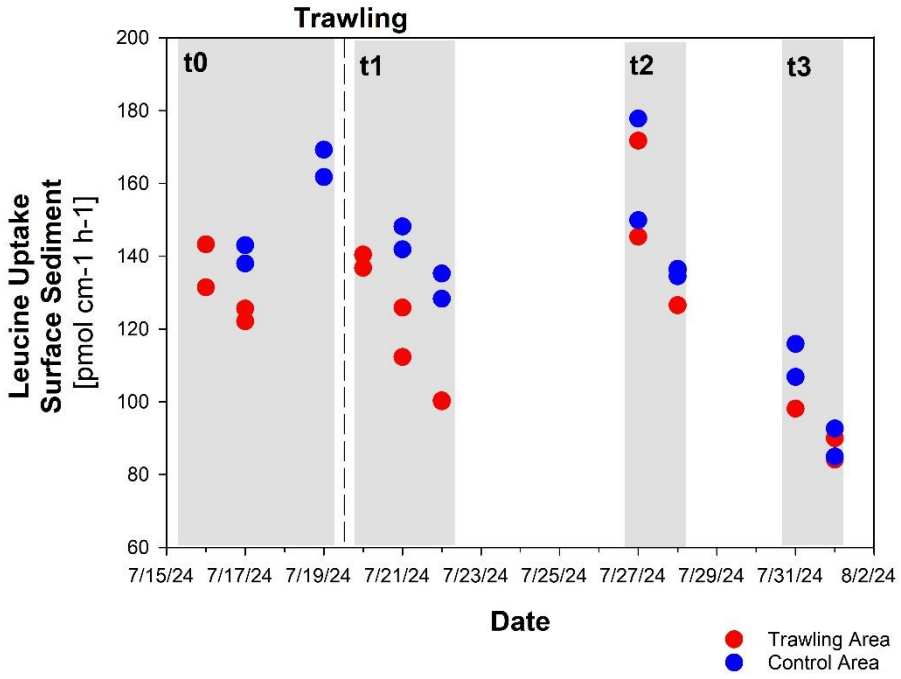

Fig. 5.11 Leucine uptake rates in surface sediments of the trawled area (red) and of the control area (blue) before (t0) and after (t1, t2, t3) trawling

Rates declined in both areas in the first three days after trawling, with a slightly stronger decrease in the trawled area. Further analyses of cell numbers and the taxonomic community composition will reveal whether this difference can be attributed to effects of bottom trawling.

Samples collected at small-scale structures of a trawl track showed a clear impact of bottom trawling on prokaryotic biomass production (**Fig. 5.12**).

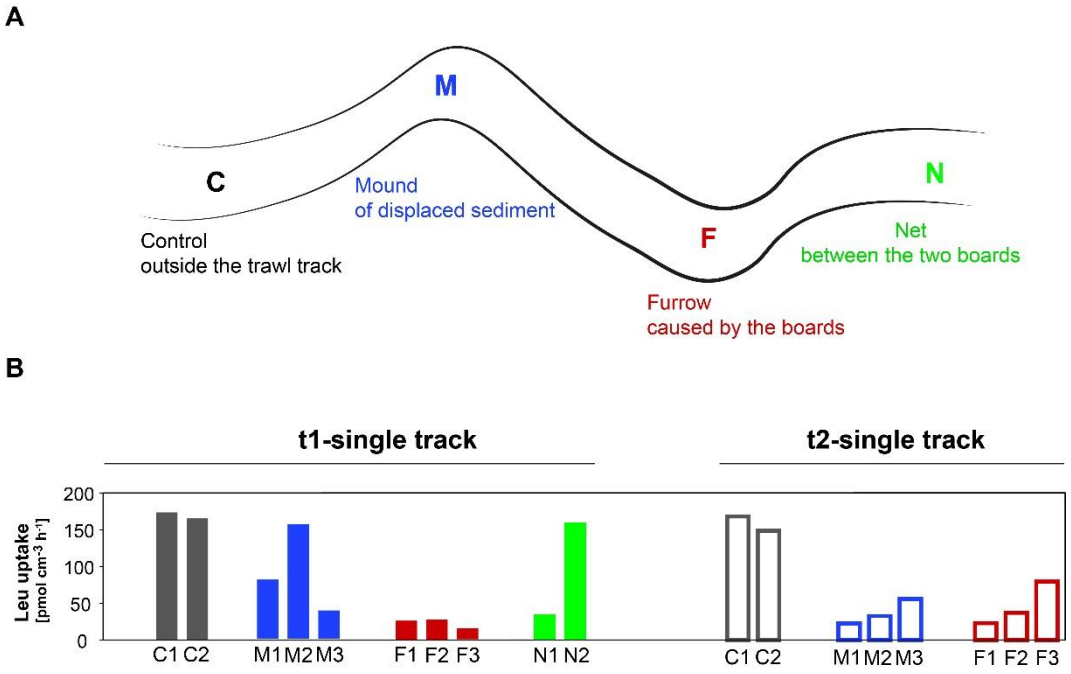

Fig. 5.12 Leucine uptake rates in surface sediment sampled at small-scale strucutres of a trawl track one day after trawling (t1-single track) and five days after trawling (t2-single track). Samples from the mound of displaced sediment (M) and from the furrow caused by the boards of the fishing gear were taken by divers. Control samples outside the trawl track (C) and from the area potentially affected by the net (N) were taken by the multicorer.

The leucine uptake rates in the furrow of the trawl track and in the adjacent mound of displaced sediment were 2 to 7 times lower than in the control samples taken outside the trawl track on both the first and fifth day after trawling. The leucine uptake rates in the two samples taken in the area where the trawl net possibly touched the bottom showed diverging results. While a reduction in the leucine uptake rate by a factor of 5 was observed in one sample, the rate in the second sample of the net area was similar to that in the control samples.

5.3.1.3. Methods – Time Series Fehmarnbelt

Sediment sampling for the analysis of prokaryotic biomass production, cell numbers and community composition continues continues the time series of (almost) annual samplings started in 2020 in the MGF project. Sediment cores collected at six stations with the multicorer were sliced on deck and sampled in seven discrete depth intervals from the surface to 20 cmbsf (depth intervals 0-1, 1-2, 3-4, 5-6, 9-10, 14-15, 19-20 cmbsf). Sampling was conducted as described above in the section "Methods – *Trawling experiment*".

In addition to the analyses of benthic prokaryotes, bottom water of the stations was sampled for the analysis of eDNA. The analysis of eDNA suspended in seawater in trace amounts, e.g. derived from skin, scales and tissues, offers the possibility of using suitable genetic markers to draw conclusions about the spatial distribution of multicellular taxa. Samples for eDNA analysis have been taken during the Fehmarnbelt time-series cruises since 2023 to test the potential of this method as a non-invasive tool for the monitoring of fishes and macrozoobenthos in the marine protected areas.

Samples for the analysis of eDNA were collected using a rosette sampler equipped with Niskin bottles. Bottles were closed 1-2 m above ground without disturbing the sediment. Subsequently, particulate matter of 1-4 litres of bottom water was collected by the use of Sterivex filter cartridges with 0.22 µm pore size that were stored frozen at -80°C. Sixteen Sterivex filters were collected at the six sampling stations at Fehmarnbelt during EMB345. In addition to the filters, macrofauna specimens for sequencing marker genes from tissue samples were collected and stored either at -20°C or in ethanol at room temperature.

5.3.2. Preliminary Results – Time Series Fehmarnbelt

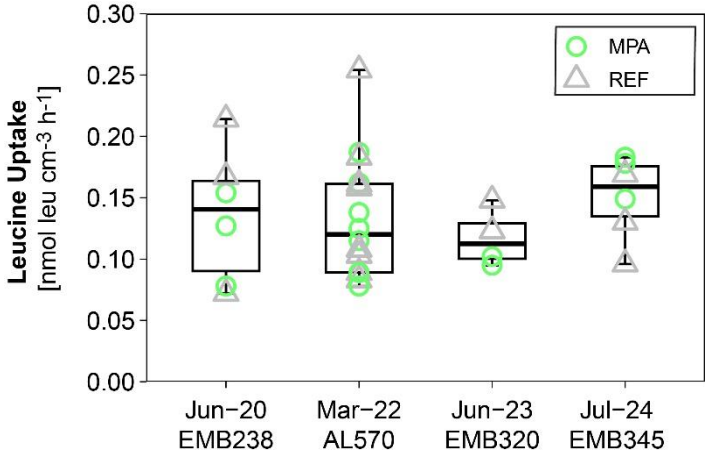

Fig. 5.13 Leucine uptake rates in surface sediments of Fehmarnbelt. Stations inside the MPA and outside (REF) were investigated.

Leucine uptake rates of 0.15±0.03 nmol cm^{-3} h^{-1} corresponded to heterotrophic prokaryotic biomass production of 0.23±0.05 µg C cm^{-3} h^{-1} in Fehmarnbelt surface sediments (**Fig. 5.13**).

The rates were similar to those determined in previous years. Like in previous years, there were no differenes between samples taken in the MPA and samples taken in an adjacent area outside the MPA (REF).

5.4. AP 2.3 Makrozoobenthos

Mayya Gogina (IOW)

To investigate the immediate and short-term effects of bottom trawl fisheries on macrobenthic communities, samples were collected in the trawling experiment area off Kühlungsborn (see **Fig. 5.14**). Additionally, two sampling days within EMB345 (between experimental sampling time points) were used to gain samples from MGF-Ostsee focus area in Fehmarnbelt (see **Fig. 5.15**) that could not be visited during MGF time-series cruise EMB342 in June, due to military restrictions.

5.4.1.1. Methods

Due to strong effect of sampling gear and size on the outcome of the study (also evidenced from the data obtained during previous experiment, in EMB268 cruise), here benthic macrofauna at each station and time point was collected by Van Veen garb (75 kg, sieve lid, sampling area 0.1 m², samples sieved on-board using 1.0 mm sieve). For vertical distribution of macrozoobenthos, expected to be impacted by trawling, core samples were obtained by multicorer (60 cm long acrylic tubes, sampling area of 0.00785 m²; sediment penetration in the area ca. 30 cm). Cores were sliced (intervals 0-2, 2-4, 4-6, 6-8, 8-10, 10-15 and >15 cm sediment depth) and sieved separately using 0.5 mm sieve (exception: deepest sediment slice >15 cm - sieved over 1 mm sieve for operational reasons, usually these sediment layers are not inhabited). After sieving, remaining material was fixed in the buffered 4% seawater-formaldehyde solution. Grab and cores samples were taken in every treatment area at each time point before and after trawling (T0 - pre-survey targets the state of community before disturbance and addresses natural variability of biodiversity, T1 – immediately or a day or two after trawling, T2 – about a week after, T3 – over 10 days after the disturbance). In the laboratory of the Leibniz-Institute for Baltic Sea Research in Warnemünde organisms are later identified morphologically to the lowest taxonomic level possible (mainly to species level), counted and weighted. Biological traits of taxa are used to describe ecological functioning.

For sedimentology at each sampling event additional surface sediment sample (from ca. top 2 cm) were conducted from a separate grab or MUC core. Corresponding environmental parameters (near-bottom water temperature, salinity and oxygen concentrations) were obtained from a CTD cast.

To examine habitat properties and characteristics, large mobile species and epifauna, as well as visual effects of disturbance non-invasive underwater video transects were carried out with a hand-held SeaViewer HD underwater video systems with mounted GoPro camera (to cameras are intended to capture the seafloor at different angles).

Kieler Kinderwagen dredge was used only in the Fehmarnbelt (one short haul per MPA and REF area) to better cover the biodiversity and capture qualitatively quick moving, rare or large

species at each area (gear specification: inner opening wide - 92 cm, mesh size - 5 mm, towed for 1 min with a speed of up to 1 knot over the ground). Additionally, few individuals of polychaetes and other groups from Fehmarnbelt were fixed in ethanol for identification and later DNA sequencing, but unfortunately there was no capacity for more intensive collection.

5.4.1.2. Preliminary Results

In total of 34 grab and 34 sliced MUC core samples were collected for macrozoobenthos in the control and high impact areas before and after trawling (4 per treatment with the exception of 6 sampling locations for T1 in HI area). Additionally 3 grab samples and 4 cores were taken in the single track area (for capacity reasons samples from scuba divers from furrow and mount areas of the single trawling track were not available and not planned for macrozoobenthos during this experiment, and only net/rope area and close control location were sampled). In the Fehmarnbelt 3 grab replicates and 3 sliced cores were collected at 3 locations in MPA and in REF, each (see **Table 5.5**). Taxonomical analysis is currently in progress in the Laboratory of WG "Ecology of benthic organisms" at IOW.

Coordinates correction for the positions of sediment sampling gears at the seafloor (for Van Veen grab and multicorer), to adjust for the offset of GPS antenna and the position of crane and winch off the starboard, was done using records from DSHIP for latitude and longitude (transformed to projected coordinate system WGS 1984 UTM Zone 32N, in m) and Gyro_GPS.GPHDT.Heading (converted to radians, refered to as HDG.rad). For adjustment the following equations for the delta_x and delta_y for the offset from ship GPS antenna were used:

delta_x = (-20) * sin(HDG.rad) + 6 * cos(HDG.rad)

delta_y = (-20) * cos(HDG.rad) - 6 * sin(HDG.rad)

Coordinates correction for video imaging was done in the same way adjusting to the position of the camera operator on the port-stern corner:

delta_x = (-33.2) * sin(HDG.rad) - 5 *cos(HDG.rad)

delta_y = (-33.2) * cos(HDG.rad) + 5 *sin(HDG.rad)

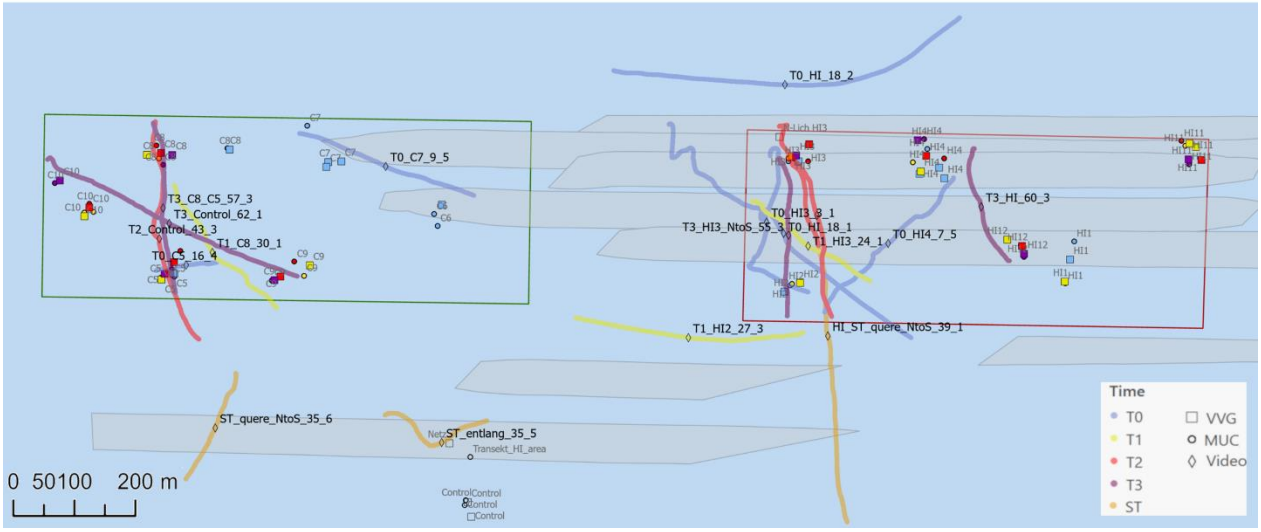

Fig. 5.14 Map showing positions of grab samples (squares), MUC samples (circles) and underwater video transects (showed by colored lines, based on time stamps and video protocols, not yet filtered for acceptable seafloor visibility) in the experimental trawling area (corrected coordinates). Intended control area is outlined by green rectangle, and HI area is outlined by red rectangle. Section Grey

polygons show trawling tracks based on hydroacoustic data (collected and processed by I. Schulze; see section 5.2. for more details).

Table 5.5 Full list of samples collected during the cruise for the analysis of benthic macrofauna.

Cast_Timeslot_Stat	D	Samples		Cast_Timeslot_Sta	D	MUC
Experimental control and high impact area						
EMB345_1_T0_HI	16	1	grab	EMB345_1_T0_HI	16	Kern 1
EMB345_2_T0_HI	16	1	grab	EMB345_2_T0_HI	16	Kern 1
EMB345_6_T0_HI	17	1	grab	EMB345_6_T0_HI	17	Kern 1
EMB345_7_T0_HI	17	1	grab	EMB345_7_T0_HI	17	Kern 1
EMB345_8_T0_C6	17	1	grab	EMB345_8_T0_C6	17	Kern 1
EMB345_9_T0_C7	17	1	grab	EMB345_9_T0_C7	17	Kern 1
EMB345_15_T0_C	19	1	grab	EMB345_15_T0_C	19	Kern 1
EMB345_16_T0_C	19	1	grab	EMB345_16_T0_C	19	Kern 1
EMB345_22_T1_H	20	1	grab	EMB345_22_T1_H	20	Kern 1
EMB345_23_T1_H	20	1	grab	EMB345_23_T1_H	20	Kern 1
EMB345_26_T1_H	21	1	grab	EMB345_26_T1_H	21	Kern 1
EMB345_27_T1_H	21	1	grab	EMB345_27_T1_H	21	Kern 1
EMB345_28_T1_C	21	1	grab	EMB345_28_T1_C	21	Kern 1
EMB345_29_T1_C	21	1	grab	EMB345_29_T1_C	21	Kern 1
EMB345_31_T1_H	22	1	grab	EMB345_31_T1_H	22	Kern 1
EMB345_32_T1_H	22	1	grab	EMB345_32_T1_H	22	Kern 1
EMB345_33_T1_C	22	1	grab	EMB345_33_T1_C	22	Kern 1
EMB345_34_T1_C	22	1	grab	EMB345_34_T1_C	22	Kern 1
EMB345_40_T2_H	27	1	grab	EMB345_40_T2_H	27	Kern 1
EMB345_41_T2_H	27	1	grab	EMB345_41_T2_H	27	Kern 1
EMB345_42_T2_C	27	1	grab	EMB345_42_T2_C	27	Kern 1
EMB345_43_T2_C	27	1	grab	EMB345_43_T2_C	27	Kern 1
EMB345_44_T2_H	28	1	grab	EMB345_44_T2_H	28	Kern 1
EMB345_45_T2_H	28	1	grab	EMB345_45_T2_H	28	Kern 1
EMB345_46_T2_C	28	1	grab	EMB345_46_T2_C	28	Kern 1
EMB345_47_T2_C	28	1	grab	EMB345_47_T2_C	28	Kern 1
EMB345_54_T3_H	31	1	grab	EMB345_54_T3_H	31	Kern 1
EMB345_55_T3_H	31	1	grab	EMB345_55_T3_H	31	Kern 1
EMB345_56_T3_C	31	1	grab	EMB345_56_T3_C	31	Kern 1
EMB345_57_T3_C	31	1	grab	EMB345_57_T3_C	31	Kern 1
EMB345_59_T3_H	01	1	grab	EMB345_59_T3_H	01	Kern 1
EMB345_60_T3_H	01	1	grab	EMB345_60_T3_H	01	Kern 1
EMB345_61_T3_C	01	1	grab	EMB345_61_T3_C	01	Kern 1
EMB345_62_T3_C	01	1	grab	EMB345_62_T3_C	01	Kern 1
AL616_17_BIGO2-	21	Chamber				

AL616_17_BIGO2-	21	Chamber				
EMB345_AL616_3	28	Chamber				
EMB345_AL616_3	28	Chamber				
Single trawl area						
EMB345_36_ST_T	26	1	grab	EMB345_36_ST_T	26	Kern 1
EMB345_37_ST_T	26	1	grab	EMB345_35_ST_T	25	Kern 1; Kern 2
EMB345_58_ST_T	31	1	grab	EMB345_58_ST_T	31	Kern 1
Fehmarnbelt MGF area						
EMB345_11_f8	18	3	grab	EMB345_11_f8	18	Kern 1; Kerne
EMB345_12_f2	18	3	grab	EMB345_12_f2	18	Kerne 1,2,3
EMB345_13_f13	18	3	grab	EMB345_13_f13	18	Kerne 1,2,3;
EMB345_48_f18	29	3	grab	EMB345_48_f18	29	Kern 1,2,3
EMB345_49_f17	29	3	grab	EMB345_49_f17	29	Kern 1,2,3
EMB345_50_f15	29	3	grab	EMB345_50_f15	29	Kern 1,2,3
EMB345_12_f2	18	Dredge				
EMB345_13_f13	18	Dredge				
EMB345_50_f15	29	Dredge				

*adjusted, was C2 first; **adjusted, was C3 first; *** BIGO Lander deployed by RV ALKOR, rest sediment from chambers after incubation and sampling for other components was preserved and transported to ELISABETH MANN BORGESE to see if there were apparent differences in macrofauna density between parallel chambers that may reflect any measured fluxes.

In the trawling experiment an undisturbed control area was sampled and compared at different time points with the disturbed area, also referred to as “high impact” or “HI”.

In agreement with the known distribution of habitats and biotopes in the German Baltic Sea (Marx et al., 2024) all samples and video imaging material collected from the Experimental area were typical for the HELCOM 'HUB' Underwater Biotope AB.H3L3 “Baltic aphotic muddy sediment dominated by ocean quahog (*Arctica islandica*)”. Noteworthy, this is also the case for stations in the MGF Fehmarnbelt focus area, confirming the relevance of the chosen experimental trawling area for the overall context of the MGF project. Only exception is sampling station f13 in the south-east of the REF area, falling into HUB AB.J3 “Baltic aphotic sand characterized by macroscopic infaunal biotic structures”, and the video transect EMB345_13_10 recorded on 18.7. that falls according to Marx et al., 2024 map into HUB AB.J3L3 “Baltic aphotic sand dominated by ocean quahog (*Arctica islandica*)”. Due to lack of time from EMB345 campaign in Fehmarnbelt we only managed to obtain underwater imaging on two short transects in the REF area.

Remarkable, however, is that preliminary visual examination of samples on board suggests generally much lower species richness, abundance and biomass of macrozoobenthos in the experimental area off Kühlungsborn comparing to samples from Fehmarnbelt, possibly due to nearly hypoxic condition occurring in the region somewhat more frequently than in the Fehmarnbelt MGF area (but see Zettler et al., 2017, where rather similar severity is suggested for both areas based on long-term estimates). There were many empty shells of *A. islandica* and only few alive individuals of *A. islandica* comparing to expectations in most samples off Kühlungsborn, both before and after trawling (**Fig. 5.15**). During EMB345 near-bottom O_2 concentration below 2 ml/l was measured only once (see **Appendix 1**), but it showed significant decrease immediately (both in control and HI areas) and a week after trawling (significant only in control). Over the cruise, it

was on average 2.4 ml/l in the experimental area, comparing to an average of 3.17 ml/l measured at Fehmarnbelt stations (**Fig. 5.16**). The O_2 concentration of 2.0 ml/l (2.8 mg/l) is accepted as a threshold below which conditions are considered hypoxic and widely harmful in ecosystem-level processes (Diaz and Rosenberg, 1995). Other dominant species in the samples included the bivalve *Macoma balthica*, several polychaetes such as *Scoloplos armiger* and *Nephtys hombergii*, and the common starfish *Asterias rubens* (**Fig. 5.15**).

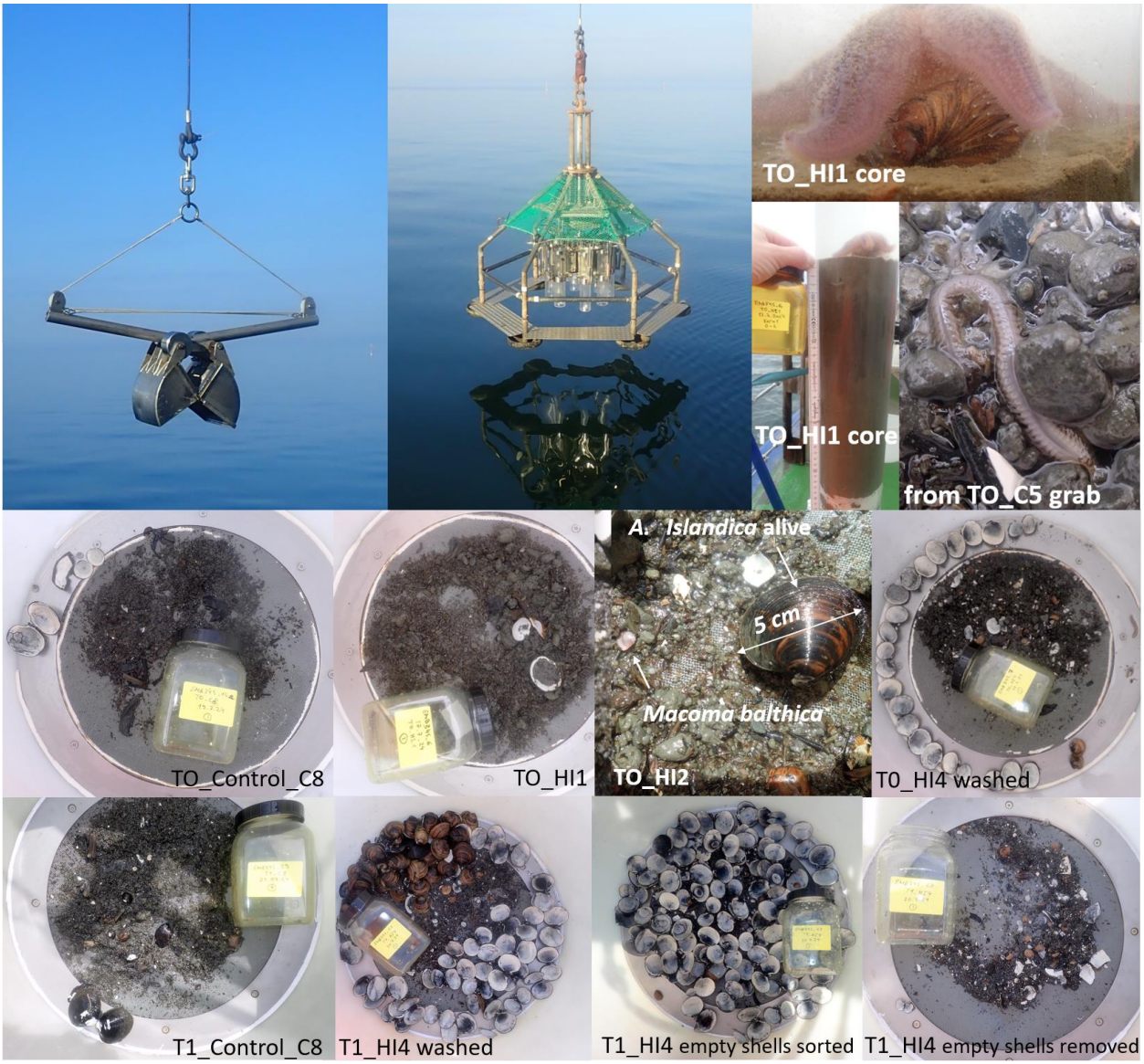

Fig. 5.15 Photos of Van Veen grab and multicorer and exemplary grab and core samples collected for macrozoobenthos analysis from both experimental control and high impact areas before and immediately after trawling (text on the pictures indicates the corresponding time and station).

Sediment median grain size showed no significant differences over time, but was significantly higher at HI stations comparing to control stations when sampled at T1 (**Fig. 5.18**; Kruskal-Wallis chi-squared = 4.66, df = 1, p-value = 0.031).

Total organic content (%) of surface sediment (determined by loss on ignition) showed no consistent patterns and no significant differences between any time points within control stations (Kruskal-Wallis chi-squared = 1.87, df = 3, p-value = 0.599), as well as within “High impact” (HI) area stations (Kruskal-Wallis chi-squared = 0.752, df = 3, p-value = 0.861).

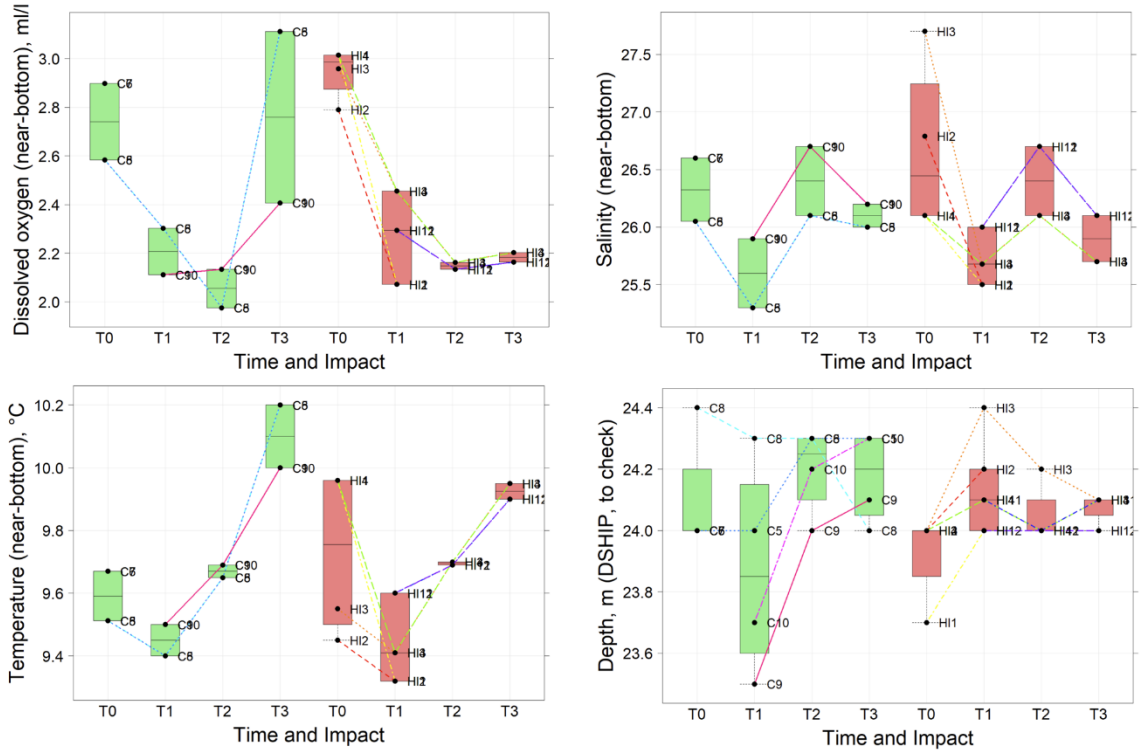

Fig. 5.16 Dissolved oxygen concentrations in near-bottom water, salinity and temperature measured by CTD. Water depth is plotted just for control, swell should be kept in mind.

Difference between "control" stations vs "high impact" stations were significant particularly and only for the T1 sampling time (**Fig. 5.18**), immediately after trawling (Kruskal-Wallis rank sum test p Kruskal-Wallis chi-squared = 5.533, df = 1, p-value = 0.019), also after filtering out the two station HI1 and HI2 that were not trawled directly (Kruskal-Wallis chi-squared = 4.1325, df = 1, p-value = 0.042).

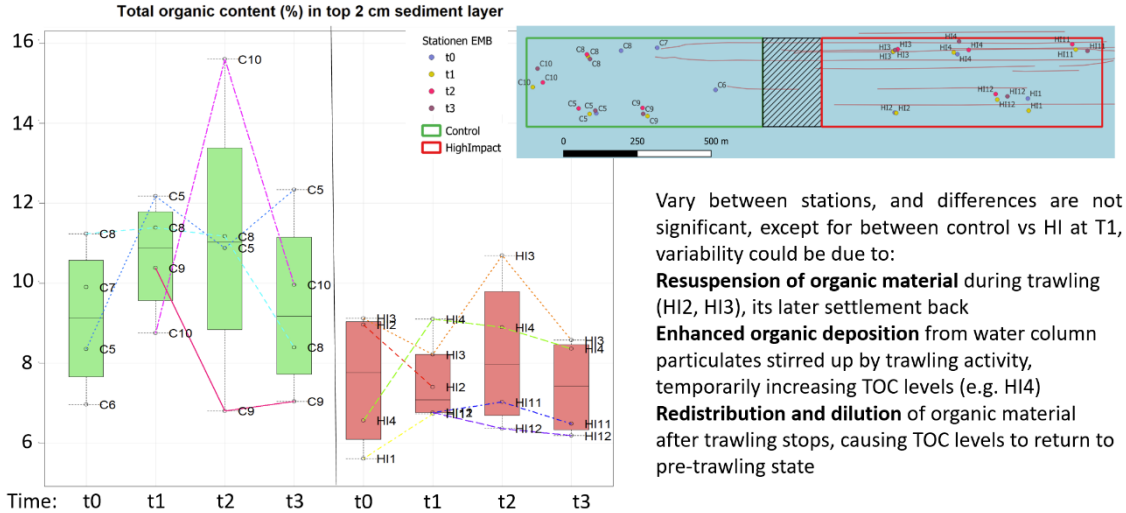

Fig. 5.17 Boxplots showing the variability and temporal dynamic of sediment parameters at stations within the control and HI areas, before and after trawling.

Habitat characteristics and visual effects of disturbance were investigated using a hand-held SeaViewer HD underwater video systems. In total during the EMB345 cruise 20 short transects, each with a duration of 10 to 40 minutes, were recorded (see **Fig. 5.14** and **Appendix 2** for full list and duration of transects). Their summed length of about 7 km (not yet corrected to visible

seafloor, with is presumably the case for 60-80 % of recorded video material). From board of RV ALKOR underwater video (UWV) survey was done with OFOS system (GEOMAR, S. Sommer), possibly our records can be complementary to those.

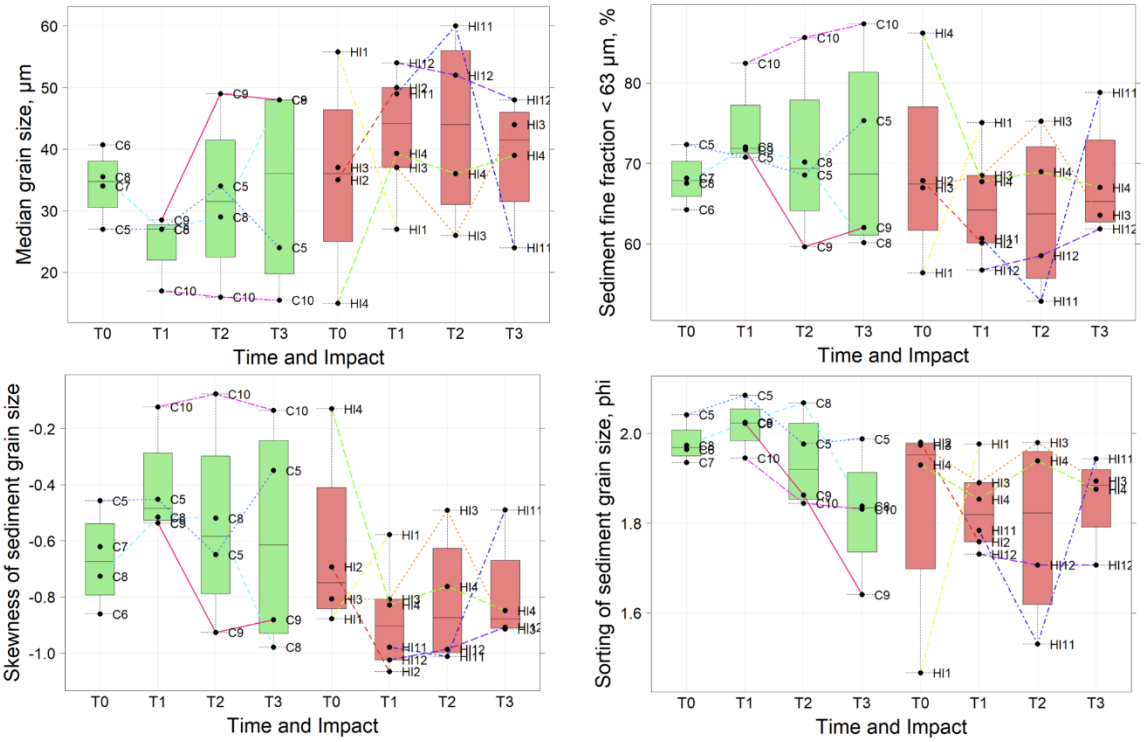

Fig. 5.18 Boxplots showing the variability and temporal dynamic of total organic content (%) for surface sediments (top 2 cm) at stations within the control and HI areas.

Underwater video images may allow coarse estimates of density of ocean quahog *Arctica islandica* (as under relatively good visibility conditions its syphons open at the sediment-water boundary for filtering are well seeing). The comparison of underwater video images before and after trawling with hydroacoustic backscatter characteristics derived from multibeam echosounder may help to relate the distinct backscatter patterns to the occurrence of mussel shells clusters, furrows and mounts from trawling activity and other features. For example, on the first look, despite clear trawling marks on the seafloor (from both trawl doors and foot rope), at T1 the day after disturbance similar macrofaunal density and composition as before trawling was observed on the sediment surface, in the direct proximity to trawl marks (see **Fig. 5.19**), including open syphons of ocean quahog *Arctica islandica*, common starfish *Asterias rubens*, flatfish, and Hydrozoa, as well as snakeblenny – (common german name Spitzschwanz-Schlangenstachelrücken, *Lumpenus lampretaeformis*, nationally recognised as endangered Red List species in the Baltic Sea).

To enhances the analysis of benthic communities and seafloor features from the UVW images, a Python script was developed (in cooperation by Ivan Kuznetsov https://github.com/kuivi/pychop) to synchronize and combine frames from two underwater videos recorded using different cameras (SeaViewer and GoPro). This script aligns frames based on drift (distance travelled by the vessel) and timestamps, accounting for any time discrepancies between the cameras by processing the ship log data to interpolate positions and headings and applying coordinates transformations. By extracting frames every N meters (e.g. 1 or 5) of drift and combining them into single images, visual observations from both cameras at specific locations can be compared.

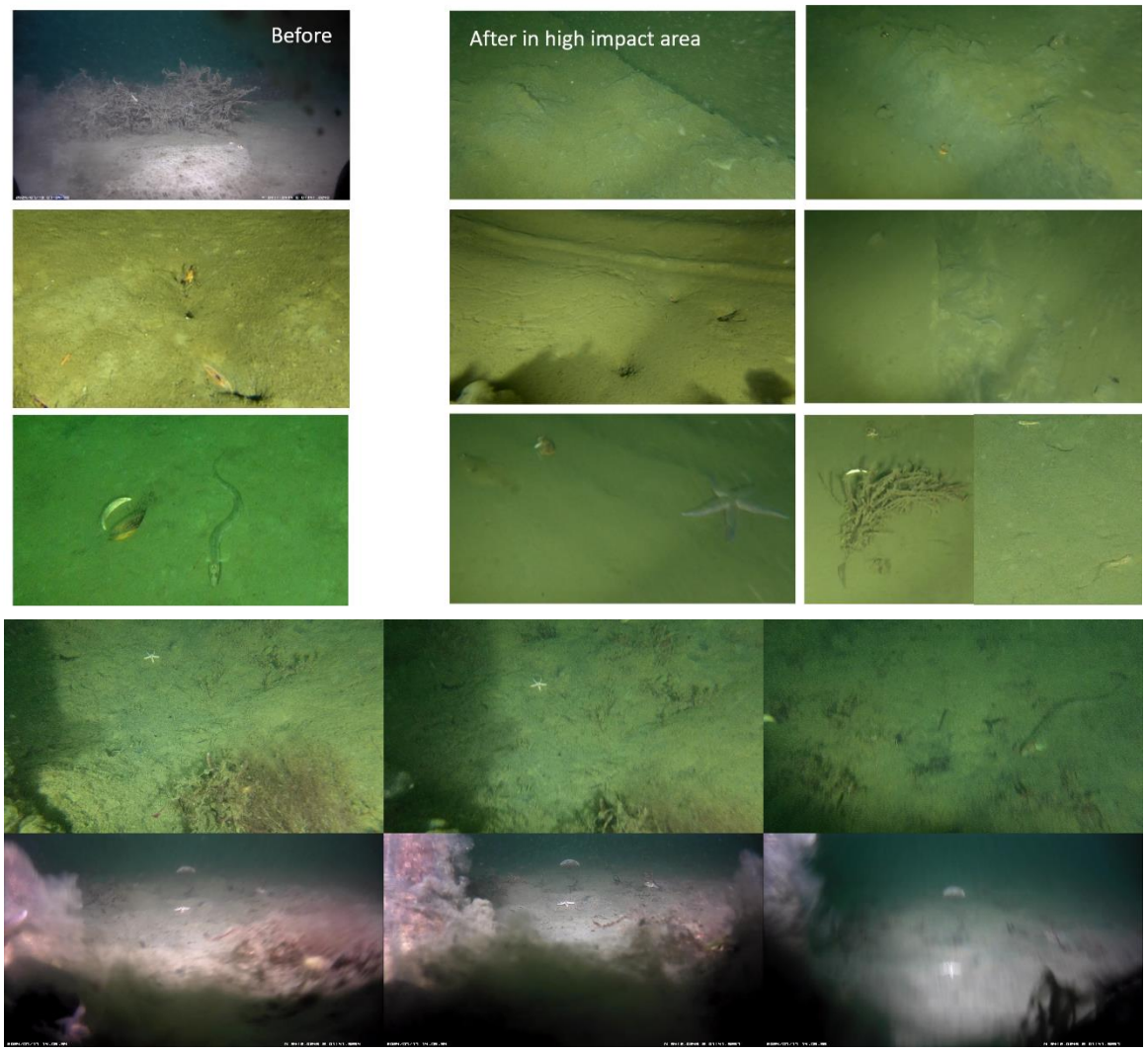

Fig. 5.19 Examples of still images from under-water video transects (see **Fig. 5.14**) illustrating the typical well visible fauna at the seafloor before trawling (upper row left), visible effects of trawling on bottom sediment habitat in the area impacted by trawl door and footrope (upper row right pane), and synchronized and combine the frames from SeaViewer and GoPro from T0_C7_9_5 transect (lower pane with 2 x 3 frames).

6. Ship's Meteorological Station

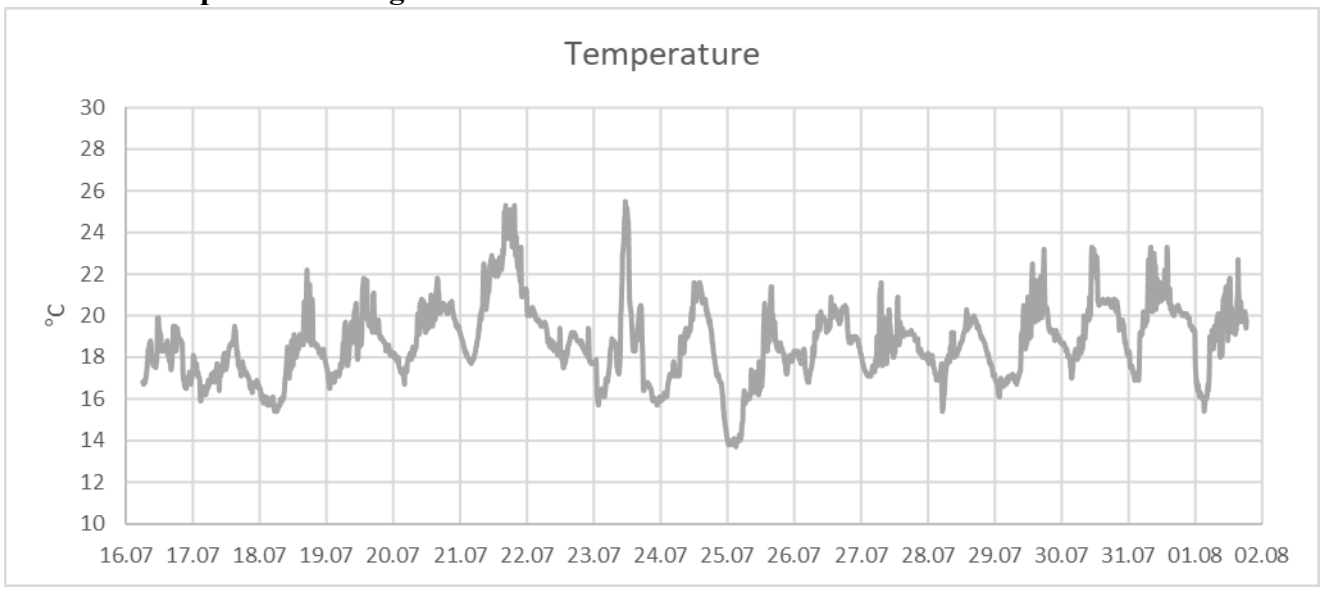

Fig. 6.1 Track of temperature changes during the cruise EMB345. Data derived from the ship's meteorological station.

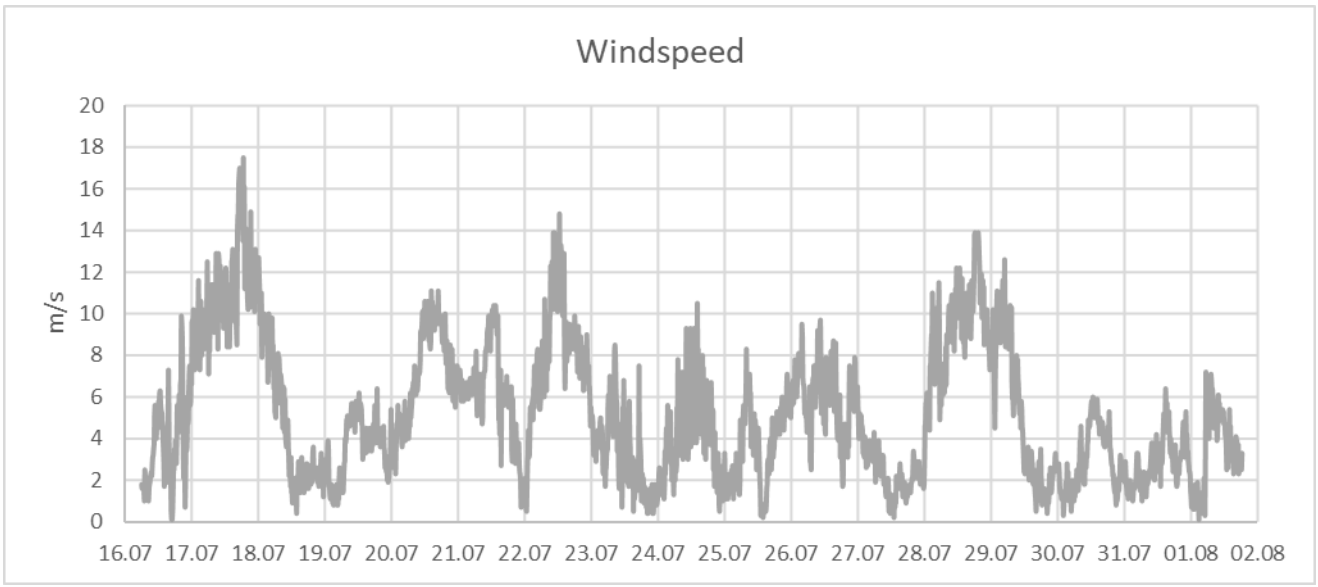

Fig. 6.2 Track of windpseed changes during the cruise EMB345. Data derived from the ship's meteorological station.

7. Station List EMB345

7.1. Station list experimental area Kühlungsborn

Table 7.1 Station list experimental area Kühlungsborn sorted by date. CTD= conductivity, temperature and depth rosette with water sampler; MUC= multicorer; VVG= Van Veen Grab, MB= multibeam; UWV= under water video glider.

Ship Event ID	Operational Label	Gear	Date	Latitude	Longitude
EMB345_1-1	HI2	CTD	2024-07-16	54.19868	11.70237
EMB345_1-2	HI2	CTD	2024-07-16	54.19866	11.70270
EMB345_1-3	HI2	MUC	2024-07-16	54.19866	11.70272
EMB345_1-4	HI2	VVG	2024-07-16	54.19858	11.70261
EMB345_2-1	HI3	CTD	2024-07-16	54.20043	11.70278
EMB345_2-2	HI3	MUC	2024-07-16	54.20050	11.70275
EMB345_2-3	HI3	VVG	2024-07-16	54.20047	11.70297
EMB345_3-1	HI Area	UWV	2024-07-16	54.19956	11.70232
EMB345_4-1	HI Area	CTD	2024-07-16	54.19867	11.70251
EMB345_5-1	Experimental Area	MB	2024-07-16	54.19468	11.71829
EMB345_6-1	HI1	CTD	2024-07-17	54.19885	11.70951
EMB345_6-2	HI1	VVG	2024-07-17	54.19867	11.70960
EMB345_6-3	HI1	MUC	2024-07-17	54.19893	11.70965
EMB345_7-1	HI4	MUC	2024-07-17	54.20035	11.70609
EMB345_7-2	HI4	VVG	2024-07-17	54.20012	11.70626
EMB345_7-3	HI4	VVG	2024-07-17	54.19996	11.70563
EMB345_7-4	HI4	VVG	2024-07-17	54.19992	11.70619
EMB345_7-5	HI4	UWV	2024-07-17	54.19984	11.70616
EMB345_8-1	C6	CTD	2024-07-17	54.19926	11.69395
EMB345_8-2	C6	VVG	2024-07-17	54.19968	11.69371
EMB345_8-3	C6	MUC	2024-07-17	54.19955	11.69348
EMB345_8-4	C6	MUC	2024-07-17	54.19938	11.69366
EMB345_9-1	C7	MUC	2024-07-17	54.20090	11.69043
EMB345_9-2	C7	VVG	2024-07-17	54.20031	11.69122
EMB345_9-3	C7	VVG	2024-07-17	54.20037	11.69085
EMB345_9-4	C7	VVG	2024-07-17	54.20031	11.69091
EMB345_9-5	C7	UWV	2024-07-17	54.20071	11.69059
EMB345_15-1	C8	CTD	2024-07-19	54.20079	11.68816
EMB345_15-2	C8	VVG	2024-07-19	54.20082	11.68868
EMB345_15-3	C8	MUC	2024-07-19	54.20085	11.68865
EMB345_16-1	C5	MUC	2024-07-19	54.19898	11.68724
EMB345_16-2	C5	VVG	2024-07-19	54.19901	11.68720
EMB345_16-3	C5	VVG	2024-07-19	54.19902	11.68725
EMB345_16-4	C5	UWV	2024-07-19	54.19900	11.68700
EMB345_17-1	HI Area	CTD	2024-07-19	54.20049	11.71012
EMB345_18-1	HI Area	UWV	2024-07-19	54.20074	11.70000
EMB345_18-2	North of HI Area	UWV	2024-07-19	54.20208	11.70669

EMB345_18-3	CTD Test	CTD	2024-07-19	54.16093	11.76984
EMB345_19-1	CTD Test	CTD	2024-07-19	54.16077	11.76680
EMB345_20-1	Single Trawl	MB	2024-07-19	54.19569	11.71683
EMB345_21-1	HI Area	MB	2024-07-20	54.19727	11.71144
EMB345_21-2	HI Area	CTD	2024-07-20	54.20206	11.71163
EMB345_21-3	HI Area	CTD	2024-07-20	54.19984	11.69619
EMB345_22-1	HI4	CTD	2024-07-20	54.20032	11.70632
EMB345_22-2	HI4	MUC	2024-07-20	54.20041	11.70590
EMB345_22-3	HI4	VVG	2024-07-20	54.20029	11.70601
EMB345_23-1	HI3	VVG	2024-07-20	54.20049	11.70255
EMB345_23-2	HI3	MUC	2024-07-20	54.20051	11.70277
EMB345_24-1	HI Area	UWV	2024-07-20	54.19963	11.70230
EMB345_25-1	HI Area	MB	2024-07-20	54.19786	11.71839
EMB345_26-1	HI1	CTD	2024-07-21	54.19865	11.70918
EMB345_26-2	HI1	VVG	2024-07-21	54.19857	11.70965
EMB345_26-3	HI1	MUC	2024-07-21	54.19856	11.70968
EMB345_27-1	HI2	MUC	2024-07-21	54.19864	11.70280
EMB345_27-2	HI2	VVG	2024-07-21	54.19864	11.70303
EMB345_27-3	HI2	UWV	2024-07-21	54.19792	11.70208
EMB345_28-1	C5	CTD	2024-07-21	54.19898	11.68692
EMB345_28-2	C5	VVG	2024-07-21	54.19894	11.68681
EMB345_28-3	C5	MUC	2024-07-21	54.19897	11.68690
EMB345_29-1	C8	MUC	2024-07-21	54.20074	11.68685
EMB345_29-2	C8	VVG	2024-07-21	54.20074	11.68678
EMB345_30-1	Control Area	UWV	2024-07-21	54.20012	11.68749
EMB345_31-1	HI11	CTD	2024-07-22	54.20029	11.71155
EMB345_31-2	HI11	VVG	2024-07-22	54.20034	11.71248
EMB345_31-3	HI11	VVG	2024-07-22	54.20039	11.71243
EMB345_31-4	HI11	MUC	2024-07-22	54.20036	11.71224
EMB345_32-1	HI12	MUC	2024-07-22	54.19802	11.70844
EMB345_32-2	HI12	VVG	2024-07-22	54.19815	11.70860
EMB345_32-3	HI12	MUC	2024-07-22	54.19893	11.70807
EMB345_32-4	HI12	VVG	2024-07-22	54.19901	11.70781
EMB345_33-1	C10	CTD	2024-07-22	54.19991	11.68446
EMB345_33-2	C10	VVG	2024-07-22	54.19979	11.68454
EMB345_33-3	C10	MUC	2024-07-22	54.19984	11.68464
EMB345_33-4	C10	MUC	2024-07-22	54.19985	11.68440
EMB345_34-1	C9	MUC	2024-07-22	54.19884	11.68990
EMB345_34-2	C9	VVG	2024-07-22	54.19890	11.68980
EMB345_35-1	HI Area	MB	2024-07-25	54.19839	11.71767
EMB345_35-2	HI Area	CTD	2024-07-25	54.19713	11.70178
EMB345_35-3	HI Area	CTD	2024-07-25	54.19745	11.71527
EMB345_35-4	HI Area	CTD	2024-07-25	54.19639	11.69511

EMB345_35-5	HI Area	UWV	2024-07-25	54.19659	11.69418
EMB345_35-6	HI Area	UWV	2024-07-25	54.19720	11.68810
EMB345_35-7	HI Area	MUC	2024-07-25	54.19624	11.69455
EMB345_36-1	Control Single Trawl	CTD	2024-07-26	54.19532	11.69438
EMB345_36-2	Control Single Trawl	VVG	2024-07-26	54.19519	11.69456
EMB345_36-3	Control Single Trawl	MUC	2024-07-26	54.19543	11.69444
EMB345_37-1	Single Trawl	VVG	2024-07-26	54.19628	11.69405
EMB345_38-1	HI3	VVG	2024-07-26	54.20066	11.70251
EMB345_39-1	HI Area	UWV	2024-07-26	54.20014	11.70266
EMB345_40-1	HI4	CTD	2024-07-27	54.20038	11.70580
EMB345_40-2	HI4	VVG	2024-07-27	54.20056	11.70611
EMB345_40-3	HI4	MUC	2024-07-27	54.20047	11.70669
EMB345_41-1	HI3	MUC	2024-07-27	54.20058	11.70300
EMB345_41-2	HI3	VVG	2024-07-27	54.20072	11.70278
EMB345_41-3	HI3	UWV	2024-07-27	54.20061	11.70250
EMB345_42-1	C5	CTD	2024-07-27	54.19925	11.68666
EMB345_42-2	C5	VVG	2024-07-27	54.19915	11.68708
EMB345_42-3	C5	MUC	2024-07-27	54.19915	11.68734
EMB345_43-1	C8	MUC	2024-07-27	54.20078	11.68687
EMB345_43-2	C8	VVG	2024-07-27	54.20073	11.68694
EMB345_43-3	C8	UWV	2024-07-27	54.20091	11.68675
EMB345_44-1	HI11	CTD	2024-07-28	54.20002	11.71269
EMB345_44-2	HI11	VVG	2024-07-28	54.20025	11.71249
EMB345_44-3	HI11	MUC	2024-07-28	54.20052	11.71207
EMB345_45-1	HI12	MUC	2024-07-28	54.19910	11.70800
EMB345_45-2	HI12	VVG	2024-07-28	54.19903	11.70812
EMB345_46-1	C9	VVG	2024-07-28	54.19893	11.68930
EMB345_46-2	C9	MUC	2024-07-28	54.19909	11.68966
EMB345_47-1	C10	MUC	2024-07-28	54.19998	11.68453
EMB345_47-2	C10	VVG	2024-07-28	54.19997	11.68447
EMB345_53-1	Hydroacoustics reference	VVG	2024-07-30	54.21204	11.69313
EMB345_53-2	Hydroacoustics reference	VVG	2024-07-30	54.21153	11.71128
EMB345_53-3	Hydroacoustics reference	VVG	2024-07-30	54.20437	11.71095
EMB345_53-4	Hydroacoustics reference	VVG	2024-07-30	54.20474	11.69257
EMB345_53-5	Hydroacoustics reference	VVG	2024-07-30	54.19615	11.71046

EMB345_54-1	HI4	CTD	2024-07-31	54.20068	11.70616
EMB345_54-2	HI4	VVG	2024-07-31	54.20076	11.70594
EMB345_54-3	HI4	MUC	2024-07-31	54.20074	11.70620
EMB345_55-1	HI3	MUC	2024-07-31	54.20055	11.70286
EMB345_55-2	HI3	VVG	2024-07-31	54.20061	11.70295
EMB345_55-3	HI3	UWV	2024-07-31	54.20045	11.70272
EMB345_56-1	C5	CTD	2024-07-31	54.19900	11.68675
EMB345_56-2	C5	VVG	2024-07-31	54.19903	11.68695
EMB345_56-3	C5	MUC	2024-07-31	54.19906	11.68720
EMB345_57-1	C8	MUC	2024-07-31	54.20063	11.68702
EMB345_57-2	C8	VVG	2024-07-31	54.20076	11.68727
EMB345_57-3	C8	UWV	2024-07-31	54.20090	11.68692
EMB345_58-1	Control Single Trawl	MUC	2024-07-31	54.19559	11.69443
EMB345_58-2	Control Single Trawl	VVG	2024-07-31	54.19554	11.69443
EMB345_59-1	HI11	CTD	2024-08-01	54.20040	11.71230
EMB345_59-2	HI11	VVG	2024-08-01	54.20039	11.71274
EMB345_59-3	HI11	MUC	2024-08-01	54.20030	11.71284
EMB345_60-1	HI12	MUC	2024-08-01	54.19902	11.70860
EMB345_60-2	HI12	VVG	2024-08-01	54.19905	11.70864
EMB345_60-3	HI12	UWV	2024-08-01	54.20058	11.70766
EMB345_61-1	C9	CTD	2024-08-01	54.19886	11.68961
EMB345_61-2	C9	VVG	2024-08-01	54.19889	11.68972
EMB345_61-3	C9	MUC	2024-08-01	54.19891	11.68967
EMB345_62-1	C10	MUC	2024-08-01	54.20041	11.68428
EMB345_62-2	C10	VVG	2024-08-01	54.20044	11.68442
EMB345_62-3	C10	UWV	2024-08-01	54.20054	11.68440

7.2. Station list Fehmarnbelt

Table 7.2 Station list the survey area at Fehmarnbelt sorted by date. CTD= conductivity, temperature and depth rosette with water sampler; MUC= multicorer; VVG= Van Veen Grab, MB= multibeam; UWV= under water video glider.

Ship Event ID	Operational Label	Gear	Date	Latitude	Longitude
EMB345_10-1	Fehmarnbelt MPA	MB	2024-07-17	54.54130	10.75138
EMB345_14-1	Fehmarnbelt MPA	MB	2024-07-18	54.54020	10.76832
EMB345_11-1	F8	CTD	2024-07-18	54.55127	10.76051
EMB345_11-2	F8	MUC	2024-07-18	54.55154	10.76044
EMB345_11-3	F8	MUC	2024-07-18	54.55172	10.76011
EMB345_11-4	F8	MUC	2024-07-18	54.55182	10.76050
EMB345_11-5	F8	VVG	2024-07-18	54.55165	10.76009
EMB345_11-6	F8	VVG	2024-07-18	54.55161	10.75972
EMB345_11-7	F8	VVG	2024-07-18	54.55177	10.75973
EMB345_12-1	F2	CTD	2024-07-18	54.55584	10.75912
EMB345_12-2	F2	MUC	2024-07-18	54.55587	10.75912
EMB345_12-3	F2	MUC	2024-07-18	54.55596	10.75879
EMB345_12-4	F2	MUC	2024-07-18	54.55589	10.75878
EMB345_12-5	F2	VVG	2024-07-18	54.55586	10.75884
EMB345_12-6	F2	VVG	2024-07-18	54.55579	10.75870
EMB345_12-7	F2	VVG	2024-07-18	54.55584	10.75875
EMB345_12-8	F2	VVG	2024-07-18	54.55597	10.75880
EMB345_12-9	F2	DRG	2024-07-18	54.55547	10.75780
EMB345_13-1	F13	CTD	2024-07-18	54.53889	10.72464
EMB345_13-2	F13	MUC	2024-07-18	54.53892	10.72462
EMB345_13-3	F13	MUC	2024-07-18	54.53894	10.72475
EMB345_13-4	F13	MUC	2024-07-18	54.53885	10.72469
EMB345_13-5	F13	MUC	2024-07-18	54.53885	10.72446
EMB345_13-6	F13	VVG	2024-07-18	54.53894	10.72449
EMB345_13-7	F13	VVG	2024-07-18	54.53895	10.72455
EMB345_13-8	F13	VVG	2024-07-18	54.53899	10.72441
EMB345_13-9	F13	Dredge	2024-07-18	54.54027	10.72385
EMB345_13-10	F13	UWV	2024-07-18	54.53765	10.72762
EMB345_48-1	F18	CTD	2024-07-29	54.54867	10.76783
EMB345_48-2	F18	VVG	2024-07-29	54.54885	10.76791
EMB345_48-3	F18	VVG	2024-07-29	54.54879	10.76801
EMB345_48-4	F18	VVG	2024-07-29	54.54886	10.76760
EMB345_48-5	F18	MUC	2024-07-29	54.54874	10.76713
EMB345_48-6	F18	MUC	2024-07-29	54.54870	10.76709
EMB345_48-7	F18	MUC	2024-07-29	54.54873	10.76777
EMB345_49-1	F17	MUC	2024-07-29	54.54144	10.68609

EMB345_49-2	F17	MUC	2024-07-29	54.54170	10.68593
EMB345_49-3	F17	MUC	2024-07-29	54.54147	10.68544
EMB345_49-4	F17	VVG	2024-07-29	54.54151	10.68569
EMB345_49-5	F17	VVG	2024-07-29	54.54143	10.68546
EMB345_49-6	F17	VVG	2024-07-29	54.54149	10.68557
EMB345_49-7	F17	CTD	2024-07-29	54.54079	10.68458
EMB345_50-1	F15	CTD	2024-07-29	54.54155	10.69503
EMB345_50-2	F15	CTD	2024-07-29	54.54128	10.69459
EMB345_50-3	F15	VVG	2024-07-29	54.54122	10.69456
EMB345_50-4	F15	VVG	2024-07-29	54.54132	10.69450
EMB345_50-5	F15	VVG	2024-07-29	54.54142	10.69508
EMB345_50-6	F15	MUC	2024-07-29	54.54151	10.69532
EMB345_50-7	F15	MUC	2024-07-29	54.54146	10.69471
EMB345_50-8	F15	MUC	2024-07-29	54.54149	10.69471
EMB345_50-9	F15	MUC	2024-07-29	54.54147	10.69493
EMB345_50-10	F15	MUC	2024-07-29	54.54141	10.69474
EMB345_50-11	F15	MUC	2024-07-29	54.54163	10.69436
EMB345_50-12	F15	DRG	2024-07-29	54.54211	10.69608
EMB345_50-13	F15	UWV	2024-07-29	54.54181	10.71283
EMB345_51-1	F15	CTD	2024-07-29	54.54083	10.70577
EMB345_52-1	F15	MB	2024-07-29	54.53806	10.72475

8. Data and Sample Storage and Availability

Data collected during the cruise EMB345 will be used in the 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. The data collected by all sub-projects will be critically checked and made available to the project partners via an internal database. 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	Contact
Event list	https://www.pangaea.de/expeditions/events/EMB345	Live	Klaus.Juergens@io-warnemuende.de
Microbial RNA/DNA sequencing	GFBio or NCBI or Genbank	In preparation	Judith.Piontek@io-warnemuende.de
eDNA	GFBio or NCBI or Genbank	In preparation	Judith.Piontek@io-warnemuende.de
Macrozoo-benthos	IOW “BenthosDB”	In preparation	Mayya.Gogina@io-warnemuende.de
Bathymetry	IOWDB	In preparation	Inken.Schulze@io-warnemeunde.de/ Peter.Feldens@io-warnemuende.de
Protists community	Pangaea	In preparation	Hartmut.Arndt@uni-koeln.de

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11. Abbreviations

Abbreviation	Meaning
ADCP	Acoustic Doppler Current Profiler
BMBF	Bundesministerium für Bildung und Forschung
BSH	Bundesamt für Schiffahrt und Hydrologie
C	Control area
DAM	Deutsche Allianz Meeresforschung
DZMB	Deutsches Zentrum für Marine Biodiversitätsforschung
eDNA	environmental DNA
EEZ	Exclusive Economic Zone
EMB	ELISABETH MANN BORGESE
FB	Fehmarnbelt
HF	High Frequency
HI	High Impact Area
HySo	Hydrosonde
IOW	Leibniz-Institute for Baltic Sea Research
LF	Low Frequency
MB	Multibeam
MBES	Multibeam Echosounder
MGF	Mobile bottom-contact fishing
MPA	Marine Protected Area
MUC	Multicorer
REF	Reference area
ST	Single trawl
SVP	Sound Velocity Profiles
URO	University of Rostock
UWV	Underwater Video System
VVG	Van Veen Grab

12. Appendices

Appendix 1 Full list of recorded abiotic parameters.

D	Station	o	m	z	S	S	o	s	c	T	Ti	S	Imp	latitude	longitude
1	EMB345_1_T	8	3	c	-	1	2	2	1	9	T0	H	HI	54°	11°
1	EMB345_2_T	9	3	c	-	1	2	2	1	9	T0	H	HI	54°	11°
1	EMB345_6_T	5	5	c	-	1	3	2	1	9	T0	H	HI	54°	11°
1	EMB345_7_T	6	1	c	-	1	3	2	1	9	T0	H	HI	54°	11°
1	EMB345_8_T	6	4	c	-	1	2	2	1	9	T0	H	cont	54°	11°
1	EMB345_9_T	9	3	c	-	1	2	2	1	9	T0	C	cont	54°	11°
1	EMB345_11_f	8	5	c	-	1	3	2	1	1	FB	f	MP	54°	10°
1	EMB345_12_f	9	3	c	-	1	3	2	1	1	FB	f	MP	54°	10°
1	EMB345_13_f	4	9	c	-	1	2	2	1	9	FB	f	RE	54°	10°
1	EMB345_15	1	3	c	-	1	2	2	1	9	T0	C	cont	54°	11°
1	EMB345_16	8	2	c	-	2	2	2	1	9	T0	C	cont	54°	11°
2	EMB345_22	9	3	c	-	1	2	2	1	9	T1	H	HI	54°	11°
2	EMB345_23	8	3	c	-	1	2	2	1	9	T1	H	HI	54°	11°
2	EMB345_26	6	2	c	-	1	2	2	1	9	T1	H	HI	54°	11°
2	EMB345_27	7	5	c	-	1	2	2	1	9	T1	H	HI	54°	11°
2	EMB345_28	1	2	c	-	2	2	2	1	9	T1	C	cont	54°	11°
2	EMB345_29	1	2	c	-	2	2	2	1	9	T1	C	cont	54°	11°
2	EMB345_31	6	4	c	-	1	2	2	1	9	T1	H	HI	54°	11°
2	EMB345_32	6	5	c	-	1	2	2	1	9	T1	H	HI	54°	11°
2	EMB345_33	8	1	c	-	1	2	2	1	9	T1	H	HI	54°	11°
2	EMB345_34	1	2	c	-	2	2	2	1	9	T1	C	cont	54°	11°
2	EMB345_37	7	5	c	-	1	2	2	1	9	ST	N	ST	54°	11°
2	EMB345_36	6	6	c	-	1	2	2	1	9	T2	H	HI	54°	11°
2	EMB345_40	8	3	c	-	1	2	2	1	9	T2	H	HI	54°	11°
2	EMB345_41	1	2	c	-	1	2	2	1	9	T2	H	HI	54°	11°
2	EMB345_42	1	3	c	-	1	1	2	1	9	T2	C	cont	54°	11°
2	EMB345_43	1	2	c	-	2	1	2	1	9	T2	C	cont	54°	11°
2	EMB345_44	7	6	c	-	1	2	2	1	9	T2	H	HI	54°	11°
2	EMB345_45	6	5	c	-	1	2	2	1	9	T2	H	HI	54°	11°
2	EMB345_46	6	4	c	-	1	2	2	1	9	T2	C	cont	54°	11°
2	EMB345_47	1	1	c	-	1	2	2	1	9	T2	C	cont	54°	11°
2	EMB345_48_f	1	3	c	-	1	3	2	1	1	FB	f	MP	54°	10°
2	EMB345_49_f	9	3	c	-	1	2	2	1	1	FB	f	REF	54°	10°
2	EMB345_50_f	5	5	c	-	1	2	2	1	1	FB	f	REF	54°	10°
3	EMB345_54	8	4	c	-	1	2	2	1	9	T3	H	HI	54°	11°
3	EMB345_55	8	4	c	-	1	2	2	1	9	T3	H	HI	54°	11°
3	EMB345_56	1	2	c	-	1	3	2	1	1	T3	C	cont	54°	11°
3	EMB345_57	8	4	c	-	1	3	2	1	1	T3	C	cont	54°	11°
3	EMB345_58	5	6	c	-	1	2	2	1	1	ST	C	ST	54°	11°
0	EMB345_59	6	2	c	-	1	2	2	1	9	T3	H	HI	54°	11°
0	EMB345_60	6	4	c	-	1	2	2	1	9	T3	C	cont	54°	11°
0	EMB345_61	7	4	c	-	1	2	2	1	1	T3	C	cont	54°	11°
0	EMB345_62	9	1	c	-	1	2	2	1	1	T3	C	cont	54°	11°

Appendix 2 List of recorded underwater video transects.

NN	Video Name_EMB345_cast	Transect	Date GoProUTC	Time	Start	Duration ca. min	Start SeaViewerUTC	Length, m	Length at seafloor, m (in progress)
1	T0_HI3_3_1		16/07/2024	15:58:45		24	15:59:05	389	
2	T0_HI4_7_5		17/07/2024	08:00:30		23	08:01:30	351	
3	T0_C7_9_5		17/07/2024	14:06:00		12	14:05:30	328	204
4	FB_REF_13_10		18/07/2024	14:44:00		14	14:45:00	283	
5	T0_C5_16_4		19/07/2024	07:30:00		16	07:30:40	111	89
6	T0_HI_18_1		19/07/2024	12:52:00		44	12:52:40	625	
7	T0_HI_18_2		19/07/2024	13:51:00		22	13:51:00	612	
8	T1_HI3_24_1		20/07/2024	17:35:30		15	17:36:00	257	

9	T1_HI2_27_3	21/07/2024 07:32:30	11	07:33:20	385
10	T1_C8_30_1	21/07/2024 12:38:00	20	12:38:30	289
11	ST_entlang_35_5	25/07/2024 11:40:00	12	11:40:20	216
12	ST_quere_NtoS_35_6	25/07/2024 12:05:30	10	12:06:00	202
13	HI_ST_quere_NtoS_39_1	26/07/2024 14:08:00	40	14:08:30	642
14	T2_HI3_NtoS_41_3	27/07/2024 07:22:00	22	07:22:30	372
15	T2_Control_43_3	27/07/2024 12:36:00	30	12:36:20	407
16	FB_REF_50_13	29/07/2024 14:09:30	18	14:14:30	375
17	T3_HI3_NtoS_55_3	31/07/2024 07:16:30	24	07:17:00	295
18	T3_C8_C5_57_3	31/07/2024 12:18:30	15	12:19:10	296
19	T3_HI_60_3	01/08/2024 07:33:30	24	07:34:00	228
20	T3_Control_62_1	01/08/2024 13:12:30	39	13:15:20	495