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Milucka, J.,

Graf, J., Marchant, H., Kitzinger, K., von Arx, J., Wendt, J., Decker, Ch., Elling, F., Gande, D., Stock, L., Ruhland, G.

# Report and preliminary results of R/V Poseidon cruise POS539

**Varna (Bulgaria) – Varna (Bulgaria)**
**06.11.2019 – 21.11.2019**

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# Report and preliminary results of R/V Poseidon cruise POS539

Varna (Bulgaria) - Varna (Bulgaria)

November 6 - November 21, 2019

**J. Milucka,**

J. Graf, H. Marchant, K. Kitzinger, J. von Arx, J. Wendt, Ch. Decker, F. Elling,

D. Gande, L. Stock, G. Ruhland

2020

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# 1 Participants

## 1.1 Scientific Party

| Name | Task | Institute |
|---|---|---|
| Milucka, Jana, Dr. | Chief Scientist | MPIMM |
| Decker, Charlotte | Microbiology/Student | MARUM |
| Elling, Felix, Dr. | Organic Chemistry/Scientist | MARUM/HU |
| Gande, Darjan | Microbiology/Student | MARUM/UB |
| Graf, Jon, Dr. | Biogeochemistry/Scientist | MPIMM |
| Kitzinger, Katharina, Dr. | Biogeochemistry/Scientist | MPIMM |
| Marchant, Hannah, Dr. | Biogeochemistry/Scientist | MPIMM |
| Ruhland, Götz | Oceanography/Technician | MARUM |
| Stock, Lennart | Polysaccharides/Student | MPIMM |
| von Arx, Jan | Greenhouse Gases/Student | MPIMM |
| Wendt, Jenny | Organic Chemistry/Technician | MARUM |

**Figure 1. Scientists and crew of the cruise POS539.**

## 1.2 Participating Institutions

| | |
|---|---|
| HU | Harvard University |
| MARUM | Center for Marine Environmental Sciences, University of Bremen |

| | |
|---|---|
| MPIMM | Max Planck Institute for Marine Microbiology |
| UB | University of Bremen |

## *2 Narrative of the Cruise*

The R/V Poseidon cruise POS539 took place between November 4th and November 22nd in the western Basin of the Black Sea (*ca*. 42°30'N to 44°N and 29° E to 31° E). The overarching aim of the campaign was to obtain sediment and water samples, including suspended particle material, from the various redox zones of the Black Sea. These samples will be used to investigate the activity and physiology of microorganisms involved in the conversion of nitrogen compounds and degradation of organic carbon under various oxygen conditions. The main topics of the cruise were: (a) to quantify the contribution of archaeal nitrifiers to the nitrogen and carbon cycles, b) to measure the production and consumption of the powerful greenhouse gases $CH_4$ and $N_2O$, c) to record palaeoenvironmental changes in high resolution, and d) to describe the complexity and identity of biopolymers.

R/V Poseidon left the port of Varna, Bulgaria, on November 6$^{th}$ at 9:00 local time, on schedule, and steamed east towards the first station. Due to the short distance to station research activities were commenced on the same day at ca. 15:00. Over the course of the next 14 days we retrieved water and sediment samples from 10 discrete shelf and slope stations. First, we performed a 'deep water' transect, which included three stations in the Western basin with water depths over 2000 m. Then, we continued with a second perpendicular transect from the NW slope to shelf, in which the stations gradually transitioned from ca. 2000 m towards ca. 80 m depth. Additionally, two stations were setup north and south of the shelf transect, respectively, for paleo-oceanographic studies.

Throughout the cruise the weather conditions were overwhelmingly good, only towards the end of the campaign gusty winds of 7 Bft were recorded. At all stations, water temperature dropped from *ca*. 17°C at the surface to *ca*. 8°C at 50 m depth and remained stable throughout the rest of the water column. Chlorophyll was only detected in the mixed surface layer (upper ca. 25 m) and no deep chlorophyll maximum was observed. This was expected due to the lower photosynthetic activity usually associated with late autumn. Water density and salinity increased with increasing depth of the water column, as a consequence of the vertical mixing of saline bottom Meditteranean waters with freshwater

originating from the riverine input. Oxygen concentrations dropped rapidly to zero at *ca*. 100-120 m, in accordance with the expected water properties. Below the oxycline, waters were anoxic and sulfidic (i.e. euxinic).

Experiments using stable isotopes were carried out on-board to determine the rates of nitrification, organic nitrogen utilization, methane turnover and $N_2O$ production in the water column. At all stations, *in situ* pumps were deployed to collect particulate suspended material. These samples will be used for analyzing lipids and DNA to constrain sources of lipid biomarkers, their isotopic composition, and their biosynthetic pathways to enhance paleoceanographic reconstructions. Additionally, sediments (up to *ca*. 5.5 m long) were recovered with the gravity corer. These were preserved for transport to Bremen where organic molecules such as DNA and lipids will be analyzed to reconstruct the past climate and environment.

Station activities were completed on November 20th at 14:00 local board time. For the rest of the day last analyses and incubations were finished and packing was completed. On November 21$^{st}$ at 10:30 local time, R/V Poseidon reached the port of Varna in Bulgaria, thus concluding the POS539 expedition. Analyses and results from the experiments will provide a basis for our understanding of the microbial control on the carbon and nitrogen cycle of the Black Sea.

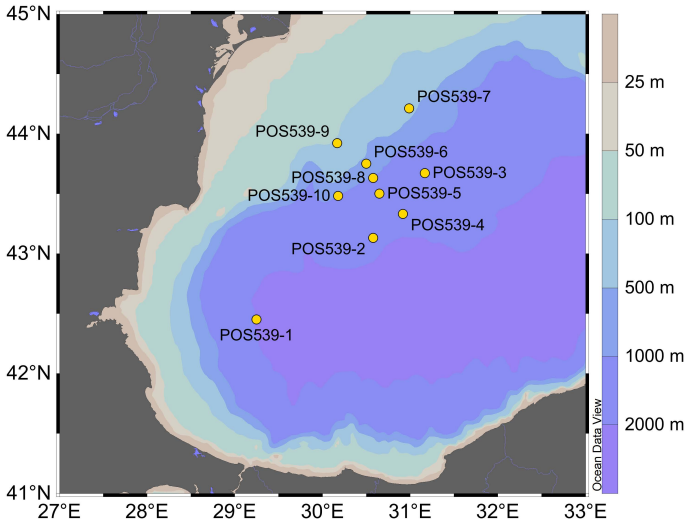
# *3 Research Programme*

## *3.1 Description of the Work Area*

The cruise area encompassed the western shelf and slope of the Black Sea basin. The cruise track stretched between *ca*. 42°30'N and 44°N and 29°E and 31°E (Figure 2). Ten stations were spread within this area, which were roughly arranged in two perpendicular transects. The first transect included stations GeoB23901-03 (W1, W2, W3) in the Western basin, with water depths over 2000 m. The second transect included stations GeoB23904, 05, 06, 08 and 09 (S1-S5) and stretched from the Western basin towards the shelf. The water depths of stations in this transect ranged between ca. 2000 m (GeoB23904) to ca. 80 m (GeoB23909). Two additional stations GeoB23907 (P2; 350 m) and GeoB23910 (P1; 1200 m) were sampled on the slope.

**Figure 2. An overview of station names and their corresponding GeoB number (left) and a map of the sampled stations in the northwestern part of the Black Sea (right).**

| Station nb. POSEIDON | Station nb. GeoB | Station name |
|---|---|---|
| POS539_01 | GeoB23901 | W1 |
| POS539_02 | GeoB23902 | W2 |
| POS539_03 | GeoB23903 | W3 |
| POS539_04 | GeoB23904 | S1 |
| POS539_05 | GeoB23905 | S2 |
| POS539_08 | GeoB23908 | S3 |
| POS539_06 | GeoB23906 | S4 |
| POS539_09 | GeoB23909 | S5 |
| POS539_10 | GeoB23910 | P1 |
| POS539_07 | GeoB23907 | P2 |




## *3.2 Aims of the cruise*

The aim of the cruise was to retrieve sediment and water samples, including suspended particle material, from the various redox zones of the Black Sea. These samples will be used to address the following aims:

(a) Determine the metabolic activity of archaeal ammonia oxidizers and their biogeochemical significance for the Black Sea carbon and nitrogen cycle.

(b) Determine rates of microbial turnover of climate relevant greenhouse gases (methane and nitrous oxide) and identify the responsible microorganisms.

(c) Apply innovative enzymatic assays to decode the complex composition of organic matter and determine the degradability and storage of polysaccharides at different redox conditions.

(d) Record the palaeoenvironmental transition from a freshwater habitat to the present mainly anoxic marine basin using biomolecular signatures, and obtain information on the structure and function of past microbial communities.

## *3.3 Agenda of the cruise*

To achieve the aims of the cruise at discrete stations water and sediment samples were retrieved using CTD - Rosette with 12 Niskin bottles (28 deployments at 9 stations),

pumpCTD (6 deployments at 6 stations), gravity corer (9 deployments at 5 stations) and multicorer (7 deployments at 6 stations). Details of the performed events and deployed instruments are included in the Station List (Chapter 5).

# *4 Preliminary Results*

## *4.1 Sampling with CTD-Rosette*

### 4.1.1 Biogeochemical profiles

At nine stations a CTD Rosette with twelve 10L Niskin bottles was deployed (Table 1). Water samples were taken for dissolved gases ($N_2O$ and $CH_4$) and nutrients. Gas samples were filled into acid washed 60 ml serum bottles using gas tight Viton tubing by overflowing the bottles, and were immediately killed by the addition of copper chloride. The samples were then sealed bubble free with rubber stoppers, crimped and will be analysed back in Bremen using a concentration cavity ring down mass spectrometer (Picarro). Additionally, 200 ml of water was fixed with formaldehyde (1% final concentration) and filtered onto two (50 ml and 150 ml) 0.22 µm GTTP filters. Fluorescence *in situ* hybridisation (FISH) will be carried out on these filters to visualize and quantify specific microorganisms. At stations GeoB23904, GeoB23906 and GeoB23909 additionally nutrient samples were taken from the CTD Rosette. For this, ca. 100 ml of water was collected from each depth. Ammonium samples were collected into OPA-washed and rinsed Falcon tubes, supplemented with the OPA reagent and measured onboard. Sulfide samples were collected into 50 ml Falcon tubes, immediately fixed with ZnAc and measured onboard. Leftover water was sterile-filtered into 15 ML Falcon tubes, frozen and transported to Bremen for further nutrient measurements.

**Table 1. List of CTD-Rosette deployments and depths of water taken with the Niskin bottles of the rosette sampler. Water samples were taken for determinations of nutrients, gases, pigments, polysaccharides and isotopic analyses.**

| Station nb. GeoB | Latitude | Longitude | Max. depth (m) | Depths sampled (m) |
|---|---|---|---|---|
| GeoB23901-1 | 42° 27,010' N | 029° 15,021' E | 2030m | 4x13, 4x99, 4x2030 m |
| GeoB23901-3 | 42° 27,011' N | 029° 15,081' E | 1900m | 3x15, 6x104, 3x1862 m |
| GeoB23901-6 | 42° 26,987' N | 029° 15,015' E | 2020 m | 5x85, 5x105, 1x494, 1x1980 m |

| | | | | |
|---|---|---|---|---|
| GeoB23901-7 | 42° 27,003' N | 029° 15,004' E | 200 m | 1x4, 1x19, 1x31, 1x44, 1x60, 1x74, 1x86, 1x104, 1x119, 1x135, 1x150, 1x164 m |
| GeoB23902-1 | 43° 08,010' N | 030° 35,006' E | 1900 m | 4x22, 4x118, 4x1863 m |
| GeoB23902-2 | 43° 08,009' N | 030° 35,011' E | 200m | 2x25, 5x117, 5x130 m |
| GeoB23902-3 | 43° 08,019' N | 030° 35,003' E | 200m | 1x24, 1x61, 1x91,5x102, 1x115,1x122, 1x134, 1x149 m |
| GeoB23902-5 | 43° 08,041' N | 030° 34,986' E | 1890m | 1x25, 1x62, 1x91, 1x105, 1x113, 1x122, 1x135, 1x150, 1x199, 1x296, 1x492, 1x1853 m |
| GeoB23903-2 | 43° 39,998' N | 031° 09,985' E | 1650 m | 6x37, 1x72, 1x101, 1x296, 2x835, 1x1618 m |
| GeoB23903-3 | 43° 40,014' N | 031° 10,015' E | 1650 m | 3x37, 4x87, 4x1618 m |
| GeoB23903-4 | 43° 40,002' N | 031° 10,016' E | 100m | 12x38 m |
| GeoB23903-6 | 43° 39,994' N | 031° 10,013' E | 200m | 1x27, 1x42, 1x51, 1x69, 1x76, 1x84, 1x96, 1x104, 1x112, 1x120, 1x149, 1x200 m |
| GeoB23904-2 | 43° 20,001' N | 030° 54,945' E | 1530m | 6x37, 1x85, 1x95, 1x120, 1x300, 1x800, 1x1501 m |
| GeoB23904-3 | 43° 19,992' N | 030° 54,949' E | 200m | 1x25, 1x36, 1x50, 1x68, 1x76, 1x84, 1x92, 1x102, 1x110, 1x120, 1x150, 1x200 m |
| GeoB23905-1 | 43° 30,011' N | 030° 38,986' E | 200m | 1x26, 1x41, 1x60, 1x74, 1x82, 1x90, 1x110, 1x116, 1x121, 1x129, 1x148, 1x198 m |
| GeoB23905-3 | 43° 29,932' N | 030° 39,058' E | 1280m | 7x27, 1x296, 1x491, 1x786, 2x1255 m |
| GeoB23905-4 | 43° 29,954' N | 030° 39,037' E | 1280 m | 3x121, 4x1256 m |
| GeoB23905-5 | 43° 29,952' N | 030° 39,017' E | 22 m | 12x23 m |
| GeoB23906-1 | 43° 45,009' N | 030° 30,185' E | 200 m | 1x28, 1x43, 1x63, 1x82, 1x92, 1x106, 1x116, 1x123, 1x131, 1x140, 1x151, 1x198 m |
| GeoB23906-2 | 43° 45,028' N | 030° 30,081' E | 730 m | 1x21, 6x28, 1x91, 1x119, 1x131, 1x199, 1x718 m |
| GeoB23906-3 | 43° 45,026' N | 030° 30,039' E | 150 m | 5x30, 1x117, 3x126, 3x150 m |
| GeoB23908-2 | 43° 38,095' N | 030° 34,992' E | 200m | 1x27, 1x42, 1x61, 1x80, 1x92, 1x99, 1x106, 1x114, 1x122, 1x129, 1x149, 1x198 m |
| GeoB23908-3 | 43° 38,011' N | 030° 35,027' E | 1080m | 4x28, 4x114, 4x1059 m |
| GeoB23908-4 | 43° 38,060' N | 030° 35,085' E | 500m | 7x23, 1x107, 1x119, 1x130, 1x296, 1x492 m |
| GeoB23908-6 | 43° 38,006' N | 030° 35,002' E | 120 m | 8x101, 3x114 m |
| GeoB23909-4 | 43° 55,002' N | 030° 09,989' E | 70 m | 1x18, 1x22, 1x27, 1x32, 1x37, 1x42, 1x47, 1x52, 1x57, 1x62, 1x66, 1x71 m |
| GeoB23909-5 | 43° 55,001' N | 030° 09,982' E | 70 m | 4x18, 2x38, 2x47, 2x57, 2x72 m |
| GeoB23910-4 | 43° 29,046' N | 030° 11,005' E | 1100 m | 1x27, 1x42, 1x62, 1x81, 1x109, 1x116, 1x124, 1x132, 1x140, 1x159, 1x492, 1x1080 m |

## 4.1.2 Microbial community and eDNA analyses

On three stations (GeoB23901, -03 and -08) we collected water samples to analyse for the microbial community composition and free-floating eDNA analysis. Water was collected

from depths that corresponded to oxygenated top water layer, chemocline layer and anoxic water below (Table 2). Approximately 4-5 litres of seawater in duplicates were first filtered through 0.45 µm and then through 0.2 µm nylon filters (90 mm diameter, Nalgene, sterile filter units). Filters were then placed in sterile Petri dishes, frozen at -20°C and transported on dry ice to Bremen.

**Table 2. Water depths sampled with a CTD-Rosette for microbial community analyses.**

| Station nb (GeoB) | Depth (m) | Depth characteristics |
|---|---|---|
| GeoB23901 | 15 | Oxic top layer |
| GeoB23901 | 104 | Chemocline |
| GeoB23901 | 1862.5 | Bottom anoxic layer |
| GeoB23903 | 37.5 | Oxic top layer |
| GeoB23903 | 72.3 | Above the chemocline |
| GeoB23903 | 101.5 | Below the chemocline |
| GeoB23908 | 29.8 | Oxic top layer |
| GeoB23908 | 117 | Chemocline |
| GeoB23908 | 126 | Below the chemocline |
| GeoB23908 | 150.02 | Anoxic layer |

On station GeoB23901, 2x 10L of sea-water from depth of 15 meters and 2x 10L from depth of 1862.5 meters were additionally collected into closed 10 L containers for eDNA degradation experimental setup. For nine consecutive days, 1L of water was sampled out of each container per day and headspace was filled with nitrogen gas in those from 1862.5 m depth so that the water remained anoxic. Sampled volume was then filtered onto sterile 0.45µm nitrocellulose filters and filters were frozen at -20°C in sterile 15 mL tubes. Samples were later transported to Bremen on dry ice.

### 4.1.3 Isotope incubations

To study the role of ammonia-oxidizing archaea in the biogeochemical processes of the Black Sea, isotope incubations were setup. For this, 50 L of seawater was sampled with the CTD-Rosette from three discrete depths from stations GeoB23901 (85, 104 and 105 m) and GeoB23902 (102, 116 and 130 m). Target depths were chosen based on the nitrite, nitrate and ammonia maxima. Incubations were set up in glass bottles that have been acid washed and rinsed beforehand. All bottles were kept at 4°C since it was not possible to store every bottle at its respective *in situ* temperature. Two duplicate 20 L incubations were each

amended with $D_2O$ (1 % v/v final concentration), $H^{13}CO_3^-$ (3-4 % v/v final concentration) and $NH_4^+$ (10 µM final concentration) and incubated in the dark. For lipid and molecular analysis, 10 L were sampled at each time point (T1 = 5 days and T2 = 10 days), filtered onto 0.1 µm pore size filters, and stored frozen. In addition, 50 ml of water was filtered and stored at 4°C for nutrient analyses. For cell counting, 45 ml of water was fixed for 12 to 24 hours with 32% formaldehyde solution (3% v/v final concentration) and afterwards filtered onto 0.2 µm membrane filters which were subsequently frozen.

As a reference for the incubations, 10 liters of water were directly taken from the Niskin bottles and likewise filtered for nutrients, TOC and lipid analysis. Additionally for the analysis of the initial $^{13}C$ label concentration, 7 ml of each incubation were taken and biological activity in these samples was stopped by the addition of mercury (II) chloride (10% w/v saturated solution). Additional 15 ml of each incubation were filtered and stored at 4°C for analyzing the $D_2O$ concentration.

Parallel to the dual SIP incubations 200 ml of each of the chosen depths were incubated with $^{15}NH_4^+$ and $H^{13}CO_3^-$ , harvested after 5 and 10 days, respectively, chemically fixed with formaldehyde and filtered on 0.2 µm membrane filters as described above for potential NanoSIMS or CARD-FISH analysis.

### 4.1.4 Microbial enrichments

*Enrichment of nitrifiers*

Several water samples were collected for the enrichment of nitrifying microorganisms under different incubation conditions. Water samples (100 ml) obtained from the chemocline with the CTD-Rosette were used to start on-board enrichments of ammonia-oxidizing archaea (supplemented with 50 µM $NH_4^+$ and 150 µg/ml streptomycin) and nitrite-oxidizing bacteria (supplemented with 50 µM $NO_2^-$; Table 3).

Additional water samples (500-1000 ml) were collected for the enrichment of ammonia oxidizing archaea at the MARUM in Bremen. For this, samples were transferred into sterile glass bottles with plastic caps and kept at room temperature.

Additionally, surface sediment obtained with the multicorer at station GeoB23909 was used for enrichments. The sediment was transferred into sterile glass bottles, closed with plastic caps and flushed with $N_2$ gas. These enrichments were stored at 4°C.

**Table 3. Water samples obtained for the enrichment of nitrifiers.**

| Station | Event | Bottle | Water depth (m) | Volume (ml) | Added substrates |
|---|---|---|---|---|---|
| GeoB23901 | 7 | 4 | 104.3 | 100 | 50 µM $NO_2^-$ |
| GeoB23901 | 7 | 3 | 134.7 | 100 | 50 µM $NH_4^+$, 150 µg/ml streptomycin |
| GeoB23901 | 7 | 3 | 134.7 | 100 | 50 µM $NO_2^-$ |
| GeoB23901 | 7 | 6 | 74.4 | 100 | 50 µM $NO_2^-$ |
| GeoB23901 | 7 | 5 | 86.4 | 100 | 50 µM $NO_2^-$ |
| GeoB23901 | 7 | 4 | 104.3 | 100 | 50 µM $NH_4^+$, 150 µg/ml streptomycin |
| GeoB23901 | 7 | 6 | 74.4 | 100 | 50 µM $NH_4^+$, 150 µg/ml streptomycin |
| GeoB23901 | 7 | 5 | 86.4 | 100 | 50 µM $NH_4^+$, 150 µg/ml streptomycin |
| GeoB23901 | 7 | 8 | 44 | 500 | None |
| GeoB23901 | 7 | 7 | 60 | 500 | None |
| GeoB23901 | 7 | 6 | 74 | 500 | None |
| GeoB23901 | 7 | 5 | 86 | 500 | None |
| GeoB23901 | 7 | 4 | 104.3 | 500 | None |
| GeoB23901 | 7 | 3 | 134.7 | 500 | None |
| GeoB23902 | 3 | 5 | 102 | 100 | 50 µM $NH_4^+$, 150 µg/ml streptomycin |
| GeoB23902 | 3 | 4 | 115 | 100 | 50 µM $NH_4^+$, 150 µg/ml streptomycin |
| GeoB23902 | 3 | 5 | 102 | 100 | 50 µM $NO_2^-$ |
| GeoB23902 | 3 | 4 | 115 | 100 | 50 µM $NO_2^-$ |

*Enrichment of ammonium and methane-oxidizing microorganisms*

Water column samples for enrichments were collected from stations GeoB23905, -06, and -08. From each station, water from 3 distinct depths was collected. The absolute depths varied among stations but they always represented the 'nitrate/nitrite zone', 'base of chemocline' and the sulfide-free 'methane/ammonium zone', respectively (see Table 4).

All bottles were filled off from the Niskin bottles using a long silicon tubing that reached all the way to the bottom of the bottles. Bottles were filled with as little water aeration as possible. They were left to overflow and were closed headspace-free with a butyl stopper and a screw cap. Immediately after filling, all bottles were transferred into a dark cool (*ca*. 8°C) incubator. All bottles were standing for up to 5 days without any supplements in the incubator. Then, bottles were supplemented with combinations of electron donors and acceptors (Table 4).

All water enrichments were transported to Bremen with a cool container and arrived on January 6$^{th}$. All bottles were visually inspected on January 7$^{th}$ and were then used for further enrichments.

**Table 4. Water samples and substrate additions used for the enrichments of ammonium and methane-oxidizing microorganisms.**

| Station nb | Water depth (m) | Added substrates |
|---|---|---|
| GeoB 23905 | 122 | 50 µM $NH_4^+$, 20 µM $NO_3^-$, 10 ml $CH_4$ |
| GeoB 23905 | 130 | 50 µM $NH_4^+$, 20 µM $NO_2^-$, 10 ml $CH_4$ |
| GeoB 23905 | 150 | 50 µM $NH_4^+$, 100 µl birnessite, 10 ml $CH_4$ |
| GeoB 23906 | 117 | 50 µM $NH_4^+$, 20 µM $NO_3^-$, 10 ml $CH_4$ |
| GeoB 23906 | 125 | 50 µM $NH_4^+$, 20 µM $NO_2^-$, 10 ml $CH_4$ |
| GeoB 23906 | 150 | 50 µM $NH_4^+$, 100 µl birnessite, 10 ml $CH_4$ |
| GeoB 23908 | 106 | 50 µM $NH_4^+$, 20 µM $NO_3^-$, 10 ml $CH_4$ |
| GeoB 23908 | 114 | 50 µM $NH_4^+$, 20 µM $NO_2^-$, 10 ml $CH_4$ |
| GeoB 23908 | 130 | 50 µM $NH_4^+$, 100 µl birnessite, 10 ml $CH_4$ |

### 4.1.5 Dissolved organic matter for characterization and radiocarbon analysis

To study the accumulation of dissolved organic matter in the deep Black Sea, water was collected using the CTD – Rosette (Table 5). Water samples were transferred from the Niskin bottles into plastic containers that had been acid-leached, rinsed with ultrapure water, and rinsed with sample water before use. Water samples were filtered through a 0.2 µm pore size filter. The filtrate was then acidified to pH 2 using ultrapure hydrochloric acid and 10 ml subsamples for dissolved organic carbon quantification were transferred into pre-combusted glass vials with Teflon-lined plastic caps. The remaining filtrate was passed through a solid phase extraction cartridge and eluted with methanol into pre-combusted glass vials with Teflon-lined plastic caps. Additional water samples were taken to perform organic matter degradation experiments at HU (Table 6).

**Table 5. Water samples obtained for dissolved organic matter analysis**

| Station | Event | Bottle | Water depth (m) | Volume filtered (ml) |
|---|---|---|---|---|
| GeoB23901 | 7 | 12 | 5 | 2000 |
| GeoB23901 | 7 | 10 | 19.2 | 2000 |
| GeoB23901 | 7 | 8 | 44.4 | 2000 |
| GeoB23901 | 7 | 5 | 86.4 | 4400 |
| GeoB23901 | 7 | 4 | 104.3 | 4400 |
| GeoB23901 | 7 | 3 | 134.7 | 3400 |
| GeoB23901 | 6 | 2 | 494 | 4400 |
| GeoB23901 | 6 | 1 | 1980 | 4400 |
| GeoB23902 | 3 | 12 | 24 | 5000 |

| | | | | |
|---|---|---|---|---|
| GeoB23902 | 3 | 11 | 61 | 5000 |
| GeoB23902 | 3 | 10 | 91 | 5000 |
| GeoB23902 | 5 | 9 | 105 | 5000 |
| GeoB23902 | 5 | 8 | 115 | 4300 |
| GeoB23902 | 5 | 6 | 135 | 4550 |
| GeoB23902 | 5 | 2 | 492.6 | 4550 |
| GeoB23902 | 5 | 1 | 1852.8 | 4500 |
| GeoB23903 | 3 | 1 | 1618 | 5000 |
| GeoB23903 | 3 | 3 | 835.2 | 5000 |
| GeoB23903 | 3 | 4 | 296.7 | 5000 |
| GeoB23903 | 3 | 8 | 37.5 | 5000 |
| GeoB23903 | 6 | 3 | 120 | 5000 |
| GeoB23903 | 6 | 7 | 84.4 | 5000 |
| GeoB 23905 | 1 | 12 | 26.3 | 5000 |
| GeoB 23905 | 1 | 5 | 116 | 5000 |
| GeoB 23905 | 1 | 1 | 198.4 | 5000 |
| GeoB 23905 | 3 | 3 | 785.9 | 4400 |
| GeoB 23905 | 3 | 2 | 1255.8 | 5000 |
| GeoB 23906 | 3 | 8 | 25 | 5000 |
| GeoB 23906 | 2 | 5 | 90 | 5000 |
| GeoB 23906 | 2 | 4 | 118 | 5000 |
| GeoB 23906 | 2 | 3 | 130 | 5000 |
| GeoB 23906 | 2 | 2 | 200 | 5000 |
| GeoB 23906 | 2 | 1 | 720 | 5000 |
| GeoB 23909 | 4 | 10 | 18 | 5000 |
| GeoB 23909 | 4 | 5 | 47 | 5000 |
| GeoB 23909 | 4 | 1 | 70 | 5000 |

**Table 6. Water samples for organic matter degradation experiments.**

| Station | Event | Bottle | Water depth (m) | Volume (ml) |
|---|---|---|---|---|
| GeoB 23910 | 4 | 1 | 1080 | 2000 |
| GeoB 23910 | 4 | 1 | 1080 | 2000 |
| GeoB 23910 | 4 | 1 | 1080 | 2000 |
| GeoB 23910 | 4 | 5 | 132 | 2000 |
| GeoB 23910 | 4 | 12 | 27 | 2000 |
| GeoB 23910 | 4 | 12 | 27 | 2000 |

### 4.1.6 Inorganic carbon and hydrogen isotopic analyses

Water samples for isotopic measurements were obtained using the CTD-Rosette (Table 7). Water for quantification and carbon isotopic analysis of dissolved inorganic carbon was collected in 50-ml falcon tubes by overflowing the tube from the bottom up directly from the Niskin bottle through a rinsed plastic hose. Duplicate sub-samples were then divided into i)

15-ml falcon tubes filled to the top without air bubbles with caps wrapped in Parafilm, ii) glass vial with Teflon-lines plastic caps (7 ml), and iii) evacuated exetainers (12 ml). Exetainers and falcon tubes were stored at 4 °C. Glass vials were stored at -20 °C.

Water for hydrogen isotopic analysis was collected in 15-ml falcon tubes by overflowing the tube as described above. The tubes were capped, wrapped in Parafilm and stored at 4 °C.

**Table 7. Water samples obtained for isotopic analysis**

| Station | Event | Bottle | Water depth (m) |
|---|---|---|---|
| GeoB23901 | 7 | 6 | 74 |
| GeoB23901 | 6 | 3 | 104 |
| GeoB23901 | 6 | 1 | 1980 |
| GeoB23902 | 3 | 4 | 115 |
| GeoB23902 | 5 | 3 | 296 |
| GeoB23902 | 5 | 1 | 1850 |
| GeoB23903 | 3 | 1 | 1618 |
| GeoB23903 | 3 | 3 | 835.2 |
| GeoB23903 | 3 | 4 | 296.7 |
| GeoB23903 | 3 | 8 | 37.5 |
| GeoB23903 | 6 | 6 | 96 |
| GeoB23903 | 6 | 7 | 84 |
| GeoB23903 | 6 | 8 | 76 |
| GeoB23904 | 1 | 4 | 120 |
| GeoB23904 | 1 | 5 | 95 |
| GeoB23904 | 1 | 6 | 85 |
| GeoB23904 | 1 | 7 | 37 |
| GeoB23904 | 1 | 3 | 300 |
| GeoB23904 | 1 | 2 | 800 |
| GeoB23904 | 1 | 1 | 1550 |
| GeoB23905 | 1 | 7 | 90 |
| GeoB23905 | 1 | 4 | 121 |
| GeoB23905 | 1 | 3 | 129.5 |
| GeoB23905 | 3 | 2 | 1255.8 |
| GeoB23905 | 3 | 3 | 785.9 |
| GeoB23905 | 3 | 5 | 296.5 |
| GeoB23905 | 3 | 6 | 27.7 |
| GeoB23906 | 2 | 6 | 25 |
| GeoB23906 | 2 | 5 | 90 |
| GeoB23906 | 2 | 4 | 118 |
| GeoB23906 | 2 | 3 | 130 |
| GeoB23908 | 4 | 6 | 23 |
| GeoB23908 | 4 | 5 | 106 |
| GeoB23908 | 4 | 4 | 115 |
| GeoB23908 | 4 | 3 | 130.7 |

| | | | |
|---|---|---|---|
| GeoB23908 | 4 | 2 | 296.8 |
| GeoB23908 | 4 | 1 | 492 |
| GeoB23909 | 5 | 1 | 70 |
| GeoB23909 | 5 | 5 | 47 |
| GeoB23909 | 5 | 10 | 18 |
| GeoB23910 | 4 | 4 | 140 |
| GeoB23910 | 4 | 5 | 132 |
| GeoB23910 | 4 | 6 | 124 |

### 4.1.7 Degradability and storage of polysaccharides

Samples for the analyses of polysaccharides were taken at all deep-water stations, four out of five slope-to-shelf stations and at the last sediment station (Table 8; Figure 3). At the stations framed with a red ring, a whole set of sampling was conducted. The last sediment station (yellow dotted ring) only included algae analysis with the AlgaeLabAnalyzer (Figure 5).

**Table 8: An overview of sampling for the analyses of polysaccharides.**

| Station MG | Station GEOMAR | Event number | Date | Start [UTC] | Latitude | Longitude | Bottom depth [m] |
|---|---|---|---|---|---|---|---|
| POS539_St-MG-01 | GeoB23901 | 01 | 06.Nov.19 | 14:50 | 42°27.05'N | 29°14.99'E | 2052 |
| POS539_St-MG-02 | GeoB23902 | 01/05 | 08./09.Nov.19 | 12:42 | 43°8'N | 30°35'E | 1930 |
| POS539_St-MG-03 | GeoB23903 | 03/04/06 | 10./11.Nov.19 | 12:36 | 43°40'N | 31°9.99'E | 1708 |
| POS539_St-MG-05 | GeoB23905 | 04/05 | 13.Nov.19 | 10:59 | 43°29.96'N | 30°39.05'E | 1332 |
| POS539_St-MG-06 | GeoB23906 | 01 | 14.Nov.19 | 11:01 | 43°45.01'N | 30°30.12'E | 824 |
| POS539_St-MG-07 | GeoB23908 | 02/03 | 17.Nov.19 | 06.01 | 43°38.08'N' | 30°35.04'E | 1119 |
| POS539_St-MG-08 | GeoB23909 | 04/05 | 18.Nov.19 | 11:00 | 43°55'N' | 30°9.99'E | 81 |
| POS539_St-MG-09 | GeoB23910 | 04 | 19.Nov.19 | 12:24 | 43°28.99'N' | 30°11'E | 1202 |

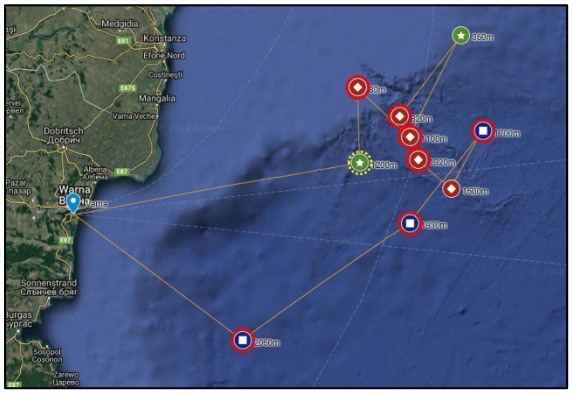



**Figure 3: Map of sampling stations for analyses of polysaccharides.**

The sampling from the CTD-Rosette encompassed three depths - the chlorophyll maximum, the lower zone of the chemocline and the anoxic zone close to the seafloor (Figure 4). At each depth, four bottles were deployed (Table 9). A sample of 50 mL per bottle was saved for algae analysis. From each depth, 40 L of seawater were filtered through pre-combusted 0.47 µm GF/F filters with 142 mm diameter (Figure 5). After filtration, the filters were frozen at -20°C for particulate organic matter (POC) analysis.

**Figure 4. Sampling from the CTD-Rosette into 10L plastic containers for polysaccharide analyses.**

The GF/F flow-through was used for further samples and analyses (Figure 5). For dissolved organic matter (DOC) analysis, 20 mL of the GF/F flow-through was collected in a pre-combusted glass vial and acidified to pH 2 using 40 µL of 25% HCl. The GF/F filters will serve to determine POC using an elemental analyzer. Laminarin in the POC fraction will be extracted

by heat extraction at 60°C for 60 mins and quantified using enzymatic digestion and subsequent reducing sugar assay (Becker et al. 2017). DOC will be quantified on a Shimadzu TOC analyzer by total combustion. Furthermore, three times 30 mL and 2 L (biological replicates) were collected from the GF/F flow-through and acidified to pH 2. These samples will serve for molecular level DOM analyses using PPL extraction methods (Dittmar et al. 2008). All DOC samples were stored at 4°C and shipped to Bremen in a cooling container. High molecular weight DOM (HMW-DOM) samples were taken from the GF/F flow through in three biological replicates.

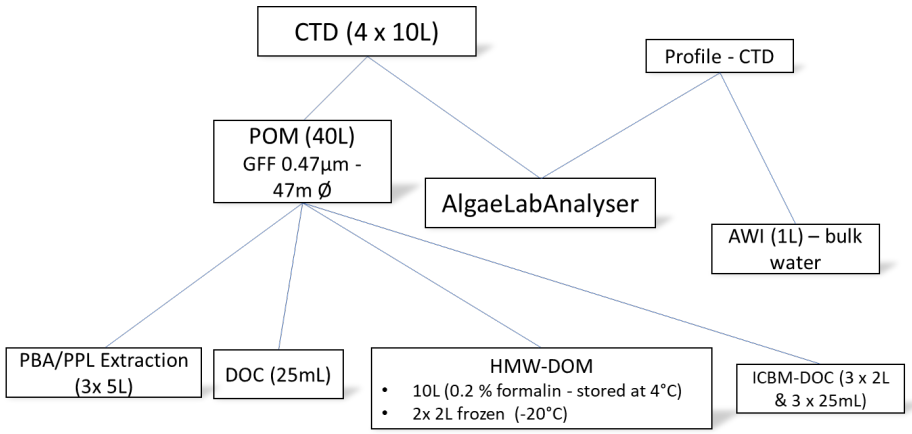
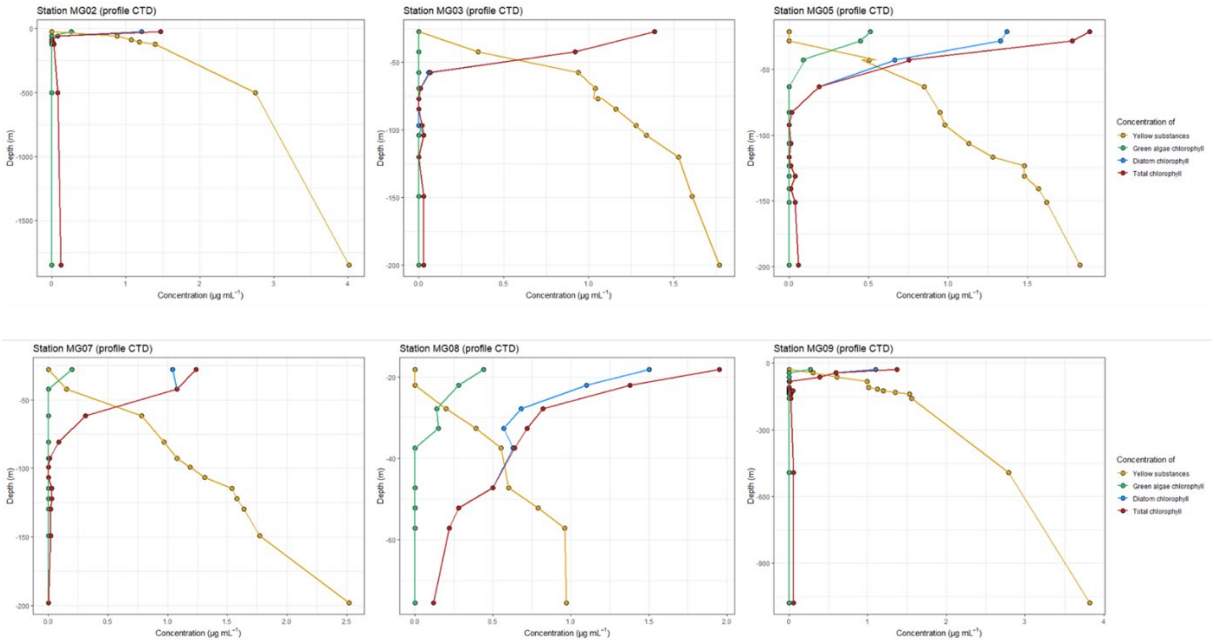



**Figure 5. Overview of the sampling protocol for polysaccharide analyses.**

Two times 2 L were frozen immediately at -20°C and then brought back to Germany on board of R/V Poseidon. 10 L of the GF/F flow-through from each depth were fixed with 50 mL formaldehyde (~0.2 % final concentration). The 10 L canisters were stored on deck in the shade and shipped to Bremen inside a 4°C cooling container.

HMW-DOM will be characterized using ELISA and epitope deletion in immunolabelling microarrays. 5 L of GF/F flow-through from each depth was used for glycan extraction. This analysis was performed in triplicates using phenyl boronate columns (Bio-Rad, Feldkirchen, Deutschland). In brief, water samples were alkalified to pH 9.5 and while glycans bound to phenyl boronate gel at high pH, non-bound residuals were washed off with MilliQ-water. Glycans were then eluted from phenyl boronate gel by decreasing the pH to approx. 4.5. Extracts of 80 mL were collected in two 50 mL Sarstedt tubes per sample and frozen at -20°C. The samples were brought back to Germany. Samples will be freeze-dried to remove the volatile extraction buffer and analyzed using liquid chromatography-mass spectrometry in combination with enzymatic digestion to unambiguously identify carbohydrate structures.

**Figure 6. Preliminary results obtained by the AlgaeLabAnalyzer showing at each station**.




DOM extraction was performed using SPE-PPL extraction cartridges (Priority Pollutant, Agilent, Santa Clara, USA) on 5 L GF/F flow-through following Dittmar et al. (2008), but abstaining from acidifying the samples. In brief, PPL cartriges were rinsed in methanol and samples applied using a peristaltic pump. Non-bound residuals were washed off with MilliQ-water. DOC was eluted with methanol and collected in a 12 mL precombusted glass vial. The samples were stored and shipped to Bremen in a 4°C cooling container.

Algae analysis was conducted using a spectrophotometric AlgaeLabAnalyser (bbe moldaenke, Schwentinental, Deutschland). The instrument measures the concentrations of chlorophyll, tannins and some algal groups (Figure 6). One water sample from every CTD-bottle (twelve per CTD cast) was analyzed. Algae samples were taken from a CTD-Rosette at nearly all stations. Also, from the CTD-Rosette, five 1 L samples from 5 different depths at two stations were frozen immediately at -20°C for carbon isotope analysis. Preliminary data showing the depth distribution of the individual fractions are shown in Figure 6.

**Table 9. Samples collected for polysaccharide analyses.**

| | **Date** | **06.Nov.19** | **08./09.Nov.19** | **10./11.Nov.19** | **13.Nov.19** |
|---|---|---|---|---|---|
| **Station** | MG | POS539_St-MG-01 | POS539_St-MG-02 | POS539_St-MG-03 | POS539_St-MG-05 |
| | GEOMAR | GeoB23901 | GeoB23902 | GeoB23903 | GeoB23905 |
| | Region | Water column 1 | Water column 2 | Water Column 3 | Slope 2 |
| **C o —** | Event no. | 01 | 01/05 | 03/04/06 | 04/05 |

| | | | | | |
|---|---|---|---|---|---|
| | POC (>0.47 µm) | 40 L – 3 depth | 40 L– 3 depth | 40 L– 3 depth | 40 L– 3 depth |
| | DOC (<0.47 µm, pH 2) | 20 mL – 3 depth | 20 mL/2 L (3 replicates)/ 30 mL (3 replicates) – 3 depth | 20 mL/2 L (3 replicates)/ 30 mL (3 replicates) – 3 depth | 20 mL/2 L (3 replicates)/ 30 mL (3 replicates) – 3 depth |
| | PBA extraction (glycans) | 100 mL – 3 replicates – 1 depth | 100 mL – 3 replicates – 3 depth | 100 mL – 3 replicates – 3 depth | 100 mL – 3 replicates – 3 depth |
| | HMW-DOM | 10 L(fixed) – 2 L (frozen) – 3 depth | 10 L(fixed) – 2x2 L (frozen) – 3 depth | 10 L(fixed) – 2x2 L (frozen) – 3 depth | 10 L(fixed) – 2x2 L (frozen) – 3 depth |
| | Bulk water | | 1 L – 5 depth - | | |
| | Algae analysis (green algae, diatoms, yellow substances) | 4 replicates – 3 depth | 4 replicates – 3 depth/ 7 depth | 4 replicates – 3 depth/ 11 depth | 4 replicates – 3 depth |

| | **Date** | **14./15.Nov.19** | **17.Nov.19** | **18.Nov.19** | **19.Nov.19** |
|---|---|---|---|---|---|
| **Station** | Station MG | POS539_St-MG-06 | POS539_St-MG-07 | POS539_St-MG-08 | POS539_St-MG-09 |
| | Station GEOMAR | GeoB23906 | GeoB23908 | GeoB23909 | GeoB23910 |
| | Region | Slope 4 | Slope 3 | Slope 5 | Sediment 2 |
| | Event no. | 01/03 | 02/03 | 04/05 | 04 |
| **Collected samples** | POC (>0.47 µm) | 20 L – 1 depth | 40L – 3 depth | 20L – 1 depth, 1 blank | |
| | DOC (<0.47 µm, pH 2) | 20 mL – 1 depth | 20 mL/2 L (3 replicates)/ 30 mL (3 replicates) – 3 depth | 20 mL – 1 depth – 1 blank | |
| | PPL extraction (DOC) | 6 mL – 1 depth – 2 replicates- pH2/8 | 6 mL – 3 depth – 3 replicates- pH2/5/8 | 6 mL – 1 depth/1 blank – 2 replicates- pH5 | |
| | HMW-DOM | | 10 L(fixed) – 2x2 L (frozen) – 3 depth | | |
| | Bulk water | | | 1 L – 5 depth | |
| | Algae analysis (green algae, diatoms, yellow substances) | 12 depth | 4 replicates – 3 depth/ 12 depth | 2 replicates – 1 depth/ 12 depth | 12 depth |

## *4.2 Sampling with pumpCTD*

### 4.2.1 Biogeochemical profiles

At stations GeoB23901, -02, -03, -05, -08, and -10, a pump cast-CTD system equipped with additional oxygen sensors and a PAR unit was deployed (Table 10).

**Table 10: List of pumpCTD deployments. Water samples were taken for analyses of nutrients, gases, DNA, RNA, and for stable isotopic incubations.**

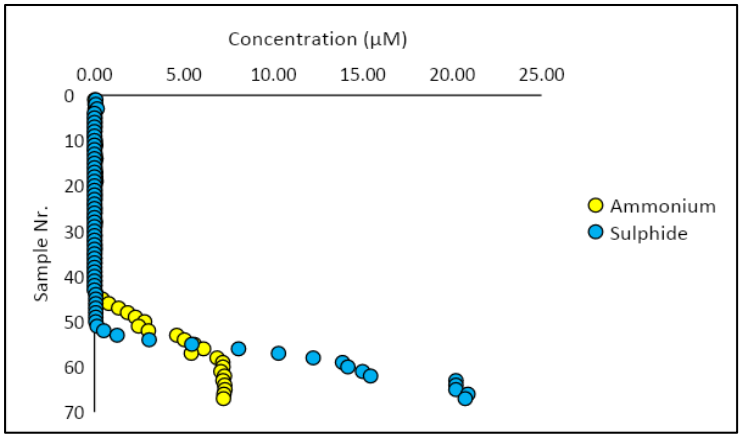
| GeoB station nb./Event nb. | Latitude | Longitude | Depth (m) |
|---|---|---|---|
| POS53910-5 | 43° 28,976' N | 030° 11,031' E | 1209.7 |

| | | | |
|---|---|---|---|
| POS53908-5 | 43° 38,009' N | 030° 35,004' E | 1120.8 |
| POS53905-2 | 43° 30,014' N | 030° 38,975' E | 1326.5 |
| POS53903-7 | 43° 39,950' N | 031° 10,006' E | 1714.7 |
| POS53902-6 | 43° 08,035' N | 030° 35,040' E | 1932.1 |
| POS53901-8 | 42° 26,987' N | 029° 14,970' E | 2057.4 |

This enabled high-resolution depth sampling to be carried out during the downcast in the upper 150 m of the water column. Samples were taken every *ca*. 3 m for subsequent determination of ammonium, sulphide, nitrogen oxides ($NO_3^-$, $NO_2^-$), phosphate, silicate, urea and cyanate concentrations. Ammonium concentrations were measured onboard using the fluorometric o-phthaldialdehyde (OPA) method. Sulphide concentrations were measured onboard spectrophotometrically using the diamine method after fixing the samples with zinc acetate. Samples for all other nutrients were filtered through 0.2 µm syringe filters and frozen for analysis in Bremen.

An abrupt oxycline at *ca*. 100-120 m was detected at all pCTD stations. Preliminary results (Figure 7) showed that that the upper oxic water column was depleted in both ammonium and sulphide. Below the onset of anoxia, both sulphide and ammonium concentrations increased rapidly with depth. This suggests that within the oxic-anoxic transition zone and slightly below, there could be rapid cycling of these compounds.

**Figure 7. A representative profile of sulfide (blue circles) and ammonium (yellow circles) from a deep station (> 2000 m depth) in the Black Sea basin.**



## 4.2.2 Incubations for determination of process rates

In contrast to Niskin bottles, sampling with a pumpCTD introduces much less oxygen to the collected water. This is an obvious advantage when working in oxygen-deplete waters of the Black Sea. Therefore, additional stable isotope incubations to quantify microbial process rates (Table 12) were set up with water collected from discrete depths within and below the oxycline using the pumpCTD (Table 11). PumpCTD was deployed and incubations were setup at 6 stations – GeoB23901, -02, -03, -05, -08, -10. All incubations were set up using seawater collected during the upcast of the pumpCTD system.

**Table 11. Incubation depths (m) from the pCTD.**

| Station | GeoB 23901 | GeoB 23902 | GeoB 23903 | GeoB 23905 | GeoB 23908 | GeoB 23910 | Position |
|---|---|---|---|---|---|---|---|
| D1 | 135 | 122,1 | 103,8 | 128,5 | 130,5 | - | Deep reference depth |
| D2 | 120 | 112,5 | 96,7 | 121,3 | 113,7 | 131,7 | Nitrate maximum |
| D3 | 105 | 104,4 | 84,2 | 115,2 | 109,1 | 124 | Nitrite maximum |
| D4 | - | 134,6 | 72,8 | 109,5 | 104,5 | 117,2 | Oxycline |

These incubations will help elucidate the connection between the carbon and the nitrogen cycle in the oxygen-depleted water column (Table 12). Additionally, aerobic and anaerobic ammonia oxidation rates, and the utilization of the simple organic nitrogen compounds cyanate and urea was investigated (Table 12).

**Table 12. Stable isotope incubation setup for POS539 for stations GeoB23901, -02, -03, -05, -08, -10.**

| Incubation ID | Process investigated | Added Tracers | Oxygen addition |
|---|---|---|---|
| **E1** | Nitrite oxidation | $^{15}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{14}N\text{-}NO_3^-$, $^{13}C$-DIC | 10 uM |
| **E2** | Anammox | $^{15}N\text{-}NH_4^+$, $^{14}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{13}C$-DIC | Anoxic |
| **E3** | Ammonia oxidation | $^{15}N\text{-}NH_4^+$, $^{14}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{13}C$-DIC | 10 uM |
| **E4** | Cyanate utilization | $^{15}N$-Cyanate, $^{14}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{13}C$-DIC | D3/D4 10 uM |
| **E5** | Cyanate utilization (control) | $^{15}N$-Cyanate, $^{14}N\text{-}NH_4^+$, $^{14}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{13}C$-DIC | D3/D4 10 uM |
| **E6** | Urea utilization | $^{15}N$-Urea, $^{14}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{13}C$-DIC | D3/D4 10 uM |
| **E7** | Urea utilization (control) | $^{15}N$-Urea, $^{14}N\text{-}NH_4^+$, $^{14}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{13}C$-DIC | D3/D4 10 uM |
| **E10** | Methane oxidation/denitrification | $^{15}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{13}C\text{-}CH_4$ | anoxic |

| | | | |
|---|---|---|---|
| **E11** | Methane and ammonium co-oxidation | $^{15}N\text{-}NH_4^+$, $^{14}N\text{-}N_2O$, $^{13}C\text{-}CH_4$ | anoxic |
| **E12** | Nitrous oxide reduction | $^{15}N\text{-}N_2O$, $^{13}C\text{-}DIC$ | anoxic |
| **E13** | Denitrification from nitrite | $^{15}N\text{-}NO_2^-$, $^{14}N\text{-}N_2O$, $^{13}C\text{-}DIC$ | anoxic |

Incubations (except E5 and E7) were set up in acid washed 256 ml glass serum bottles. These were filled directly from the pCTD using gas tight viton tubing and were overflown before being sealed with rubber stoppers and crimped. Subsequently, all bottles were degassed with helium for 20 minutes and the isotopically labelled substrates were added. Samples were then equilibrated for 5 minutes before being aliquoted anoxically into eight 12 ml gas-tight glass exetainers. These were then incubated close to *in situ* temperature (*ca*. 8°C) in the dark for up to 24 hours. E5 and E7 were instead set up in acid washed 500 ml Schott bottles but were then prepared the same way as all other incubations.
At five timepoints, biological activity in individual exetainers was stopped by the addition of 100 µl of mercury (II) chloride (10% w/v saturated solution). At the end of the incubation period (24 hours), the remaining three exetainers were pooled and seawater was fixed with formaldehyde (1% final concentration) before being filtered onto a gold-sputtered 0.22 µm GTTP filters for analysis of isotope uptake into single cells using nanoSIMS in Bremen. Killed exetainers for rate determination were taken back to Bremen for analyses using ratio mass spectrometry (IsoPrime).

### 4.2.3 DNA and RNA sampling

Water samples for DNA and RNA (for metagenome and metatranscriptome sequencing, respectively) were taken from all incubations depths (Table 11). From each depth, ~4 L water from the pCTD was directly filtered onto a 0.22 µm Sterivex filter cartridge using a Masterflex peristaltic pump. In total, 6 Sterivex filters were taken per depth, which served as triplicate samples for DNA and RNA extraction. Three filters for DNA extraction were directly frozen and stored at -20°C. The other three filters for RNA extraction were preserved in RNAlater™ preservation solution and also stored at -20°C. Filters usually appeared slightly greyish with a hint of green or brown except for station GeoB23908, where filters from D2-D4 had an intense dark red colour. The samples were shipped to the MPI on dry ice. Subsequent steps will include DNA and RNA extraction from the respective filters using DNeasy or RNeasy PowerWater kits followed by library preparation and sequencing using short read (Illumina) and long reads (PacBio) sequencing technologies at the Max Planck-

Genome-centre Cologne. The metagenomics datasets will be used for microbial community profiling as well as genome reconstructions. Functional gene transcription with focus on genes related to $CH_4$- and N-cycling will be investigated using the metatranscriptomic datasets.

## *4.3 Sampling of suspended particulate matter using in-situ pumps*

Suspended particulate matter was filtered using four In-situ Pumps (ISPs). One of the four ISPs was dead on arrival, likely due to an electrical defect. For most casts, the wire was equipped with three ISPs, operating at different depths. Two of the pumps broke down during the cruise, leading to only a single functional pump at the later stations. ISPs were deployed at 9 stations (Table 13). The pumps were fitted with three filters on two tiers of filter holders to capture different particle size classes. The first tier (pre-filter) was always fitted with an Isopore TCTP filter (10 µm pore size). The second tier was fitted with a GFF filter (0.7 µm pore size) on top of either a GF75 filter (0.3 µm pore size) or an Isopore GTTP filter (0.2 µm pore size). Target depths were chosen to sample intervals with high contrasting microbial diversity and sustaining distinct biogeochemical processes (chlorophyll maximum; upper, middle, and lower chemocline; shallow anoxic, and deep anoxic). All filters were removed from the ISP immediately after ISP recovery, were wrapped in pre-combusted aluminum foil (GFF, GF75, and GTTP filters) or in opaque plastic bottles (GTTP filters) and stored at -20 °C. To overcome the limitations from malfunctioning pumps, at some stations additional water was obtained from the CTD-Rosette and filtered by hand using vacuum pumps (see Table 13).

**Table 13. Summary of ISP casts.**

| Station | Event | water depth (m) | Volume filtered (l) | Filter 1 pore size (µm) | Filter 2 pore size (µm) | Filter 3 pore size (µm) | Type filter 1 | Type filter 2 | Type filter 3 | Notes |
|---|---|---|---|---|---|---|---|---|---|---|
| GeoB23901 | 2 | 2000 | 238 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23901 | 9 | 2000 | 272.97 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23901 | 9 | 100 | 87.96 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23901 | 9 | 75 | 200.8 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23902 | 4 | 1880 | 168.84 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23902 | 4 | 116 | 101.14 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23902 | 4 | 300 | 322.5 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| GeoB23903 | 1 | 1650 | 236.92 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23903 | 1 | 800 | 133.79 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23903 | 1 | 300 | 305.87 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23903 | 5 | 72 | 231.16 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23903 | 5 | 84 | 143.21 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23903 | 5 | 100 | 305.04 | 10 | 0.7 | 0.2 | Isopore TCTP | GFF | Isopore GTTP | |
| GeoB23903 | 3-4 | 37.5 | 78.1 | - | - | 0.2 | - | - | Isopore GTTP | Filtered from Niskin |
| GeoB23904 | 1 | 1550 | 491.92 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23904 | 1 | 800 | 514.58 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23904 | 1 | 300 | 422.24 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23904 | 4 | 85 | 741.83 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23904 | 4 | 98 | 721.73 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23904 | 4 | 120 | 751.91 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23904 | 2 | 37 | 52.8 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | Filtered from Niskin |
| GeoB23905 | 3 | 27.7 | 57.4 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | Filtered from Niskin |
| GeoB23905 | 6 | 300 | 602.51 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23905 | 6 | 800 | 497.48 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23905 | 6 | 1270 | 458.43 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23906 | 2 | 28.6 | 61.5 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | Filtered from Niskin |
| GeoB23906 | 4 | 118 | 471.98 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23906 | 4 | 130 | 752.41 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23908 | 4 | 23.1 | 65.17 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | Filtered from Niskin |
| GeoB23908 | 6 | 100.9 | 70 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | Filtered from Niskin |
| GeoB23908 | 1 | 500 | 450.55 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23908 | 1 | 300 | 615.25 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23908 | 7 | 115 | 480.44 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23908 | 7 | 105 | 690.95 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23909 | 6 | 70 | 772.18 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23910 | 6 | 145 | 373.16 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23910 | 7 | 125 | 395.35 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |
| GeoB23910 | 8 | 135 | 277.17 | 10 | 0.7 | 0.3 | Isopore TCTP | GFF | GF75 | |

## *4.4 Sampling of sediments using a multicorer*

Sampling of sediments using a multicorer was attempted at several sites. However, no sediments were recovered at all but one site, likely due to the softness of the sediments, which prevented triggering of the closure mechanism. At station GeoB23909-6, greyish sediments interspersed with and topped by mussel beds were recovered from 81 m water depth (Figure 8). Sediments were sliced onboard for lipid analysis, DNA analysis, and enrichment cultures at MARUM. One core was preserved whole under $N_2$ atmosphere for transport to MARUM.

**Figure 8: Sediments at GeoB 23909-6 recovered using a multicorer. The dark reddish band indicates the surface, which was covered by mussels.**

### 4.4.1 Microbial enrichments

Sediment samples for microbial enrichments were collected from station GeoB23909 upon a successful deployment of the multicorer (MUC). For this, one core (nb. 08) was sectioned onboard. Two 250 ml Schott bottles were filled with the sediment-overlying water collected with the sediment core. Then, 40 ml of sediment from the depth horizon between 17 and 19 cm and 19 and 21 cm was added to the first and the second bottle, respectively. The upper sediment layer still contained purplish shells with some mud, whereas the lower layer was largely shell free and predominantly mud. The bottles were closed off headspace-free with a butyl stopper and a screw cap. Immediately, a 50 ml helium headspace was created and the headspace was flushed for a few minutes with helium. Then, both bottles were transferred into a cool, dark incubator.

All sediment enrichment bottles were transported to Bremen with a cool container and arrived on January 6$^{th}$. All bottles were visually inspected on January 7$^{th}$. White layers and deposits were observed in the sediment samples. Headspace was measured for the presence of methane (GC) and methane was detected in all bottles.

## *4.5 Sampling of gravity cores*

### 4.5.1 Paleo-environmental analyses

On four stations (GeoB23903, -06, -07, -10; Table 14) the gravity corer was deployed. In total, 6 gravity cores were retrieved, with an average length of around 500 cm. This length

was sufficient to encompass the laminated top sediment layer, organic-rich sapropel layer and lacustrine-marine transition layer, which appeared clay-ish.

**Table 14. Overview of the recovered gravity cores.**

| Station nb. GeoB | Water depth (m) | Core recovered length (cm) | Core diameter (cm) |
|---|---|---|---|
| GeoB23903 | 1716 | 550 | 12.5 |
| GeoB23906 | 829 | 573.5 | 12.5 |
| GeoB23907 | 365.8 | 483 | 12.5 |
| GeoB23907 | 366.5 | 342.5 | 12.5 |
| GeoB23910 | 1216 | 547 | 12.5 |
| GeoB23910 | 1194 | 566.5 | 12.5 |

On deck, metal pipes (6 meters in length) were attached to the core weight unit. For each sampling event, fresh, clean plastic liner was supplied into the metal pipe and fixed on the bottom with the core catcher. Gear was then deployed from deck with help of the ship´s crane (Figure 9). Upon recovery of the gear, the unit was checked for possible over-penetration of the sediment bed by examining the length of the free space between beginning of the metal pipe and top of the weight. Core catcher was removed from the bottom of the metal pipe and around 15 cm of the sediment in it was packed in a bag and frozen at -20°C. After removal of the core catcher, bottom of the core liner was filled with supporting foam. Core liner with the sediment was then pulled out gradually from the metal pipe and cut into one-meter pieces. From each one-meter piece a subsample (2-3 ml sediment) was collected with a cutoff syringe, transferred into a glass vial, preserved with a NaOH solution, and closed gas-tight. These samples will be used for methane measurements at the MARUM. Then, a protective plastic cap was fitted on each side of the plastic liner piece containing the sediment.

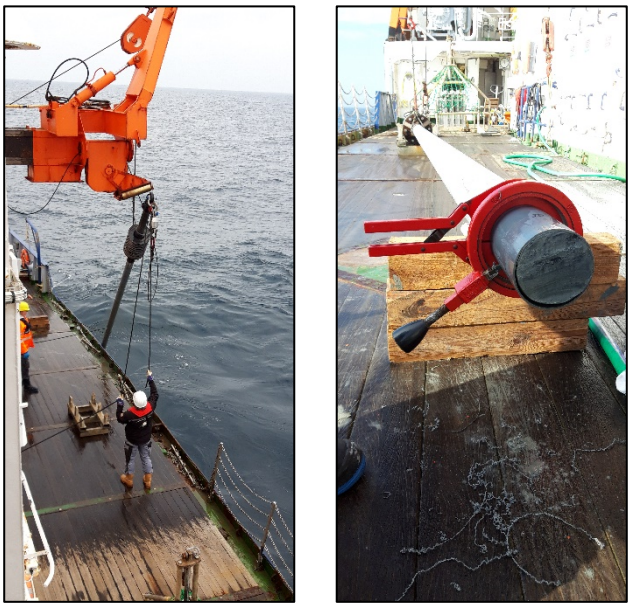


**Figure 9. Deployment of the gravity corer from deck (left) and a view of the recovered core (right).**

Each one-meter piece was labelled and individually packed in aluminium-enforced gas tight plastic bags and the atmosphere was exchanged with nitrogen gas. Packed core pieces were then stored at 4°C in a shipping container on board and shipped to MARUM for further storage and sub-sampling.

The samples arrived to Bremen on January the 6th, 2019 and were stored at MARUM GeoB core repository. Further processing by opening of the gravity cores and subsampling in clean conditions required for ancient DNA research will take place at the University of Bremen and MARUM to obtain samples for DNA extractions and biomarker analysis. Among others, DNA will be extracted from the obtained sediment samples using a protocol allowing for the retrieval of short ancient DNA fragments. The extracted DNA will be sequenced with shot-gun metagenomic sequencing approach. Metagenomic analyses will be performed to determine the authenticity of ancient DNA and taxonomy of the extracted fragments. Data will be later compiled together with biomarker analyses results and previous studies for paleoenvironmental reconstruction.

### 4.5.2 Microbial enrichments

On station GeoB23910, upon successful deployment and sectioning of a gravity corer (SL) the surface layer in both recovered gravity cores (SL1 and SL2) was scooped on deck with a clean lab spoon into a 250 ml Schott bottle (ca. 30-40 ml sediment). The sediment was topped up with water from the CTD bottle nb. 1 fired at deepest sampled depth (1080 m). The bottles were closed headspace-free with a butyl stopper and a screw cap. A 50 ml headspace was created with helium and the headspace was flushed with helium for a few

minutes. Then, both bottles were transferred into a cool, dark incubator. All sediment enrichment bottles were transported to Bremen with a cool container and arrived on January 6th. All bottles were visually inspected on January 7th. White layers and deposits were observed in the sediment samples. Headspace was measured for the presence of methane (GC) and methane was detected in all bottles.

## *5 Station List*

Station list for the CTD-Rosette (CTD) pumpCTD (pCTD), In Situ Pumps (ISP), the Multicorer (MUC) and the Gravity Corer (GC).

| Station nb. GeoB | Date/Time | Device | Latitude | Longitude | Depth (m) |
|---|---|---|---|---|---|
| GeoB23901-1 | 11/6/2019 14:43 | CTD | 42° 27,040' N | 029° 15,002' E | 2057.4 |
| GeoB23901-2 | 11/6/2019 16:40 | ISP | 42° 26,964' N | 029° 14,978' E | 2068.8 |
| GeoB23901-3 | 11/7/2019 4:55 | CTD | 42° 27,012' N | 029° 15,045' E | 2075.8 |
| GeoB23901-4 | 11/7/2019 6:25 | MUC | 42° 27,083' N | 029° 15,027' E | 2050.7 |
| GeoB23901-5 | 11/7/2019 8:20 | MUC | 42° 27,001' N | 029° 15,057' E | 2076.6 |
| GeoB23901-6 | 11/7/2019 10:37 | CTD | 42° 27,004' N | 029° 15,045' E | 2070 |
| GeoB23901-7 | 11/7/2019 12:32 | CTD | 42° 27,007' N | 029° 15,004' E | 2065.2 |
| GeoB23901-8 | 11/7/2019 13:50 | pCTD | 42° 26,987' N | 029° 14,970' E | 2057.4 |
| GeoB23901-9 | 11/7/2019 16:26 | ISP | 42° 26,995' N | 029° 15,039' E | 2063.8 |
| GeoB23902-1 | 11/8/2019 12:37 | CTD | 43° 07,998' N | 030° 34,996' E | 1931 |
| GeoB23902-2 | 11/8/2019 14:16 | CTD | 43° 08,005' N | 030° 35,009' E | 1930.8 |
| GeoB23902-3 | 11/8/2019 14:52 | CTD | 43° 08,007' N | 030° 35,004' E | 1930.2 |
| GeoB23902-4 | 11/8/2019 16:00 | ISP | 43° 08,018' N | 030° 34,983' E | 1930.1 |
| GeoB23902-5 | 11/9/2019 4:49 | CTD | 43° 08,047' N | 030° 34,960' E | 1929.9 |
| GeoB23902-6 | 11/9/2019 6:50 | pCTD | 43° 08,035' N | 030° 35,040' E | 1932.1 |
| GeoB23902-7 | 11/9/2019 11:00 | MUC | 43° 07,995' N | 030° 34,993' E | 1933 |
| GeoB23903-1 | 11/10/2019 4:30 | ISP | 43° 39,994' N | 031° 10,001' E | 1707.9 |
| GeoB23903-2 | 11/10/2019 11:05 | CTD | 43° 40,003' N | 031° 10,000' E | 1708.2 |
| GeoB23903-3 | 11/10/2019 12:35 | CTD | 43° 39,999' N | 031° 09,989' E | 1707.3 |
| GeoB23903-4 | 11/10/2019 13:53 | CTD | 43° 40,007' N | 031° 10,009' E | 1705.6 |
| GeoB23903-5 | 11/10/2019 16:00 | ISP | 43° 39,993' N | 031° 09,915' E | 1709.3 |
| GeoB23903-6 | 11/11/2019 4:15 | CTD | 43° 39,989' N | 031° 10,014' E | 1709.3 |
| GeoB23903-7 | 11/11/2019 6:09 | pCTD | 43° 39,950' N | 031° 10,006' E | 1714.7 |
| GeoB23903-8 | 11/11/2019 8:39 | GC | 43° 40,032' N | 031° 10,164' E | 1709.9 |
| GeoB23903-9 | 11/11/2019 10:50 | MUC | 43° 39,987' N | 031° 09,998' E | 1706.9 |
| GeoB23904-1 | 11/11/2019 14:39 | ISP | 43° 19,978' N | 030° 54,994' E | 1601.8 |
| GeoB23904-2 | 11/12/2019 6:02 | CTD | 43° 19,985' N | 030° 54,912' E | 1607.4 |
| GeoB23904-3 | 11/12/2019 7:30 | CTD | 43° 19,999' N | 030° 54,917' E | 1607.6 |
| GeoB23904-4 | 11/12/2019 8:22 | ISP | 43° 20,007' N | 030° 54,961' E | 1602.7 |

| | | | | | |
|---|---|---|---|---|---|
| GeoB23905-1 | 11/13/2019 4:02 | CTD | 43° 30,015' N | 030° 38,985' E | 1324.6 |
| GeoB23905-2 | 11/13/2019 5:59 | pCTD | 43° 30,014' N | 030° 38,975' E | 1326.5 |
| GeoB23905-3 | 11/13/2019 8:30 | CTD | 43° 29,937' N | 030° 39,024' E | 1330.9 |
| GeoB23905-4 | 11/13/2019 10:58 | CTD | 43° 29,961' N | 030° 39,050' E | 1328.6 |
| GeoB23905-5 | 11/13/2019 12:05 | CTD | 43° 29,950' N | 030° 39,021' E | 1328.4 |
| GeoB23905-6 | 11/13/2019 14:46 | ISP | 43° 30,031' N | 030° 38,922' E | 1346.2 |
| GeoB23906-1 | 11/14/2019 11:01 | CTD | 43° 45,010' N | 030° 30,120' E | 823.4 |
| GeoB23906-2 | 11/14/2019 11:57 | CTD | 43° 45,023' N | 030° 30,170' E | 826 |
| GeoB23906-3 | 11/14/2019 13:20 | CTD | 43° 45,018' N | 030° 30,033' E | 824.9 |
| GeoB23906-4 | 11/14/2019 13:47 | ISP | 43° 45,043' N | 030° 30,059' E | 819.9 |
| GeoB23906-5 | 11/15/2019 6:03 | MUC | 43° 44,992' N | 030° 29,980' E | 831.9 |
| GeoB23906-6 | 11/15/2019 6:59 | GC | 43° 45,044' N | 030° 30,092' E | 829.8 |
| GeoB23907-1 | 11/16/2019 6:05 | GC | 44° 12,813' N | 030° 59,600' E | 362.1 |
| GeoB23907-2 | 11/16/2019 7:15 | GC | 44° 12,813' N | 030° 59,593' E | 365.8 |
| GeoB23907-3 | 11/16/2019 8:06 | GC | 44° 12,754' N | 030° 59,594' E | 366.4 |
| GeoB23908-1 | 11/16/2019 14:00 | ISP | 43° 37,988' N | 030° 35,002' E | 1122.7 |
| GeoB23908-2 | 11/17/2019 6:01 | CTD | 43° 38,079' N | 030° 35,041' E | 1118.5 |
| GeoB23908-3 | 11/17/2019 6:56 | CTD | 43° 38,010' N | 030° 35,025' E | 1119.2 |
| GeoB23908-4 | 11/17/2019 8:01 | CTD | 43° 38,044' N | 030° 35,095' E | 1124.3 |
| GeoB23908-5 | 11/17/2019 11:06 | pCTD | 43° 38,009' N | 030° 35,004' E | 1120.8 |
| GeoB23908-6 | 11/17/2019 13:16 | CTD | 43° 38,018' N | 030° 34,999' E | 1121.1 |
| GeoB23908-7 | 11/17/2019 13:39 | ISP | 43° 38,000' N | 030° 34,995' E | 1121.5 |
| GeoB23909-1 | 11/18/2019 8:00 | GC | 43° 55,072' N | 030° 10,018' E | 81.9 |
| GeoB23909-2 | 11/18/2019 8:24 | GC | 43° 55,068' N | 030° 10,020' E | 34.7 |
| GeoB23909-3 | 11/18/2019 8:53 | MUC | 43° 55,041' N | 030° 10,018' E | 81.9 |
| GeoB23909-4 | 11/18/2019 10:59 | CTD | 43° 55,002' N | 030° 09,992' E | 82.3 |
| GeoB23909-5 | 11/18/2019 11:49 | CTD | 43° 55,003' N | 030° 09,991' E | 81.6 |
| GeoB23909-6 | 11/18/2019 12:18 | ISP | 43° 55,001' N | 030° 09,983' E | 82.4 |
| GeoB23910-1 | 11/19/2019 7:03 | MUC | 43° 29,020' N | 030° 10,995' E | 1194.4 |
| GeoB23910-2 | 11/19/2019 8:27 | GC | 43° 28,919' N | 030° 11,077' E | 1202.2 |
| GeoB23910-3 | 11/19/2019 11:03 | GC | 43° 29,086' N | 030° 10,950' E | 1196.4 |
| GeoB23910-4 | 11/19/2019 12:24 | CTD | 43° 28,993' N | 030° 10,995' E | 1204.8 |
| GeoB23910-5 | 11/19/2019 13:39 | pCTD | 43° 28,976' N | 030° 11,031' E | 1209.7 |
| GeoB23910-6 | 11/19/2019 16:13 | ISP | 43° 29,033' N | 030° 10,917' E | 1195.6 |
| GeoB23910-7 | 11/20/2019 4:45 | ISP | 43° 29,059' N | 030° 10,995' E | 1199.7 |

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