Leibniz Institute for Baltic Sea Research Warnemünde

Cruise Report

R/V Alkor

Cruise-No. AL483

This report is based on preliminary data

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Cruise No.: AL483

Dates of the cruise:
from 06.08.2016 to 25.08.2016

Areas of Research:
methane chemistry, molecular biology, planktology

Dates and names of Ports of call:
Ventspils (Latvia). 15-18 August 2016

Institute:
Institut für Ostseeforschung Warnemünde (IOW)

Co-operating institutions:
Georg-August University of Göttingen
Christian-Albrechts-Universität zu Kiel

Chief Scientist:
Dr. Oliver Schmale

Number of Scientists: 11

Projects:
ZOOM (ZOOplankton associated Methane production) funded by the DFG (GZ: SCHM2530/5-1, LA1466/10-1).

Graduate school "Phosphorus research" of the Leibniz Science Campus Phosphorus Research Rostock.

Cruise Report

This cruise report consists of 32 pages including cover.

1. Scientific crew

Chief scientist:	Schmale, Oliver
Scientists:	Loick-Wilde, Natalie Moser, Annabell Mothes, Stephanie Otto, Stefan Power, Nicole Christine Recknagel, A. Constantin Stawiarski, Beate Thiel, Volker Wäge, Janine Wittenborn, Anna Katharina

2. Research program

Methane is a known greenhouse gas that severely enhances climate change on earth, yet not all methane sources into atmosphere have been identified. A process that might be of importance is the production of methane by microorganisms within the anoxic guts of certain zooplankton species. This production takes place in the upper oxygenated water column and thus could have a direct impact on the methane flux between ocean and atmosphere. We hypothesize that highly productive regions like marginal seas, which have never been studied in detail in this context before, are areas of enhanced zooplankton-mediated methane production, which most probably causes the subthermocline methane anomaly that have been sporadically identified in the oxygenated water column of the Baltic Sea. On the proposed cruise we will combine methane chemistry, microbiology, and zooplanktology in a multidisciplinary approach to investigate zooplankton and particle-related methanogenesis in detail using the Baltic Sea as a model system. We plan to investigate the following key questions: (1) Is the subthermocline methane anomaly a widespread phenomenon in the Baltic Sea? (2) Does zooplankton have the potential to support the methane anomaly in the shallow water and how are copepod species and environmental factors like food composition influencing methane production? (3) Which microbes are involved in upper-water methane production and can we detect differences in methanogenic assemblages and their activities between copepod guts, fecal pellets, and other water column seston particles?

3. Narrative of cruise with technical details

The original work program of the cruise was subdivided into three main parts: The target of the first part of the cruise was a mass characterization along a transect from Rostock to the western Gotland Basin (Landsort Deep). The water mass characterizations included analysis regarding the spatial variability of the subsurface CH_4 maximum below the thermocline, and the qualitative study of the plankton community. Based on these results, the second target of the cruise was to identify positions of research areas along a methane or plankton gradient (A1 to A4), that should be studied in more detail during the course of the cruise. The third phase was dedicated to obtain insights into the mechanistic understanding of the processes involved in subthermocline CH_4 production. This work should be conducted at the CH_4 hot spot station as defined by the highest subthermocline methane anomaly. Because of the difficult weather situation during the cruise, we focused our work on spatial variability of the parameters (part 1) and the mechanistic understand of subsurface methane production at the hot spot station (part 3). The planed investigation along a methane or plankton gradient (A1 to A4, part 2) could for this reason not be performed.

Part 1. The spatial variability of the subsurface CH_4 maximum along a transect across the central Baltic Sea

Saturday, August 6th

R/V Alkor left the harbor of Rostock Marienehe on 6 August at 13:00 UTC, as scheduled, with 11 participants and fully functional instrumentation on board. The laboratories were arranged on the way to station Z01 located in the Bornholm Basin. The weather conditions were already difficult from the beginning of the cruise with Beaufort number (BF) of 6.

Sunday, August 7th

We arrived at station Z01 at 11:00 UTC with winds around 6 BF. We performed a CTD cast to test the CTD equipment, sampling procedure, and handling of the samples. An additional CTD cast was conducted for phytoplankton sampling in 10 m water depth for the enrichment of the plankton. Station Z02 in southern part of the eastern Gotland Basin (EGB) was reached in the evening at 20:00 UTC. Also here we performed a CTD cast and took water samples for gas chemical and molecular biological studies. Additional water samples were taken for phytoplankton sampling.

Monday, August 8th

We reached Z03 in the EGB at 02:00 UTC in the night. As it was done on the previous stations, we started with a CTD cast and sampled the water column in high resolution in the upper 70 m. Two WP2 net hauls for zooplankton sampling were carried out at the same station in the depth intervals 80-20 m and 20-0 m. The station was finished with a CTD cast for phytoplankton sampling at 12 m water depth. Z04 (equivalent to IOW monitoring station TF271) in the central part of the EGB was

reached at 10:30 UTC. A CTD cast with water sample collection was performed in the upper 70 m of the water column. Afterwards a WP2 net haul was conducted between 60-20 m water depth and 20-0 m water depth. Methane concentrations below the thermocline showed a clear anomaly and also the phytoplankton analysis indicated a distinct occurrence of Dinophysis and Temora in that specific depth interval. Weather conditions became even more complicate in this exposed part of the Baltic Sea with wind speeds up to 7 BF. After we finished the station work we proceeded to station Z05 about 40 nm north of station Z04 (arrival at 16:00 UTC). Here (station Z05), the wind speed and wave height only allowed for a CTD cast and the sampling of the water column in the upper 70 m. No additional casts or net hauls could be performed. The station work was interrupted and the ship took shelter from the strong sea on the eastern side of the Swedish island Gotska Sandön.

Tuesday and Wednesday, August 9th and August 10th

The meteorological situation on the Baltic Sea remained difficult during these two days (wind up to 7 BF). The scientific crew used these days for sample preparation, discussions and data evaluation. Based on these discussions we decided to shorten the transect (to neglect two stations in the southern part of the western Gotland Basin) and to proceed to the western Gotland Deep (Landsort Deep, equivalent to IOW monitoring station TF284) after the weather conditions improved. The meteorological forecast predicted winds to decrease down to 5 BF for the next days, and we decided to continue our field work the next day at the Landsort Deep.

Thursday, August 11th

On the next day the wind calmed down substantially and we left Gotska Sandön around midnight. Station Z06 (TF284) was reached at 06:00 UTC. We performed our sampling procedure as we did on the last stations along the transect with a CTD cast and water sampling in the upper 70 m of the water column. This was followed by two WP2 net hauls (depth intervals: 60-15 m and 15-0 m). Phytoplankton was sampled in the upper 10 m. First plankton analysis could show that Temora was only present in a a small number and Dinophysis could not be detected. A methane anomaly as it was identified below the thermocline in the EGB could not be detected at Z06. After station work we continued the transect anticlockwise and reached station Z07 at 11:00 UTC. Water samples were taken in the upper 70 m, and WP2 net hauls were carried out between 60-25 m and 25-0 m. Phytoplankton samples were taken with the CTD water sampler at 10 m. Afterwards we proceeded to station Z08, the northernmost station of our transect that was reached at 16:00 UTC. Water samples were taken in the upper 70 m with the rosette water sampler. Plankton samples were obtained with the WP2 net (60-20 m and 20-0 m) The same sampling strategy was conducted at station Z09 (reached at 21:00 UTC) in the eastern Gotland Basin.

Part 2. Studies to understand the mechanisms controlling the subsurface methane production by zooplankton at the hot spot station

Friday and Saturday, August 12th and August 13th

Station Z10 was reached at 02:00 UTC. This station position is similar to Z05 that was interrupted on August 8th. Under fair weather conditions (3 BF), we sampled the upper 70 m with the CTD water sampler. Phytoplankton samples were taken in the upper 20 m with an additional CTD cast. At 09:00 UTC we reached Z11 (equivalent to IOW monitoring station TF271 and Z04). Based on the first results of our studies we decided to define this station as our "hot spot" station and to perform more detailed investigations on that site to gain a mechanistic understanding of subsurface methane production (see above, part 3 of our work program). We started to work according the program with a CTD cast for water sampling in the upper 50 m. Afterwards, we took a number of WP2 net hauls in different water depths for qualitative and quantitative studies. We also obtained samples with living copepods from the depth interval between 45-25 m. Additional zooplankton samples were obtained with an Apstein net within the depth interval 0-25 m. Also phytoplankton (cyanobacteria) was sampled in that shallow depth interval with the Apstein net. Furthermore, phytoplankton samples were taken with the CTD water sampler in 10 and 20 m water depth. The caught copepods together with ^{13}C labeled phytoplankton was used for incubation experiments to gain zooplankton associated methane production rates. First on board results showed that Temora and Dinophysis were still present at the location as it was observed on Z04 before. In addition to the sampling program regarding these investigations, we obtained samples (based on CTD water stations) to investigate microbial methane oxidation at the thermocline and methane production by the decomposition of methylphosphonate (MPn). The wind strength increased continuously and we decided to end our station work at about 21:00 UTC and to steer closer to eastern coast of Gotland near the harbor of Slite.

Sunday, August 14th

The weather conditions became better in the night between Saturday and Sunday (5 BF) and we decided to continue our work on Sunday morning in the center of the EGB (IOW monitoring station TF271). We arrived at station Z12 at 06:00 UTC and started our station work with an Apstein net in the mixed layer depth (20-0 m) for phytoplankton sampling. The following CTD cast was targeted to sample the thermocline for lipid biomarker and molecular biological studies. Afterwards we sampled the water column at 22 m water depth with additional two CTD cast to obtain phytoplankton samples for incubation experiments. When that station work was finished, we took seven sediment cores (max. depth of 1 m) with the Frahmlot for molecular biological studies and lipid biomarker investigations. At 13:30 UTC we sampled again the water column in high resolution in the upper 50 m for gas and molecular biological studies. This work was followed by two WP2 net hauls (depth: 45-25 m, 20-0 m) to obtain living copepods. We finished station work at about 15:00 UTC and prepared our incubation experiments with the freshly caught copepods and radiolabeled phytoplankton.

Monday, August 15th

Weather forecast predicted increasing wind strength for the following days. Based on this information, we decided to take plankton samples that we planned to use in incubation experiments in the save harbor of Ventspils (Latvia). Station work on Monday started at 04:30 UTC with a CTD cast in the upper 35 m water depth for gas and molecular biological studies. An additional CTD was taken to obtain water from 15 and 25 m water depth for the upcoming plankton incubation experiments. After that station was finished, we took plankton samples with the Apstein net in the depth interval 20-0 m. This work was followed by four WP2 net hauls to obtain living copepods from 45-25 m and 20-0 m water depth. The station work was completed with two additional CTD casts for phytoplankton sampling at around 20 m water depth (depth of Dinophysis). In agreement with the captain, it was decided to steer to the nearby harbor of Ventspils (start at 10:00 UTC, arrival in the harbor at about 17:00 UTC) until weather conditions had improved.

Tuesday and Wednesday, August 16th and August 17th

Weather conditions in the field remained difficult during these days. Even in the harbor of Ventspils the wave height was substantially. However, we used these days for incubation experiments with different copepod and phytoplankton species. The relatively calm meteorological situation in the harbor also allowed microscopic work that we performed to select the different copepod species for later molecular biological analysis at the IOW main laboratory. Scientific discussions held during our stay helped to coordinate the field work for the following days.

Thursday, August 18th

We left the harbor of Ventspils at 05:00 UTC and reached the Gotland Deep (TF271) at 10:00 UTC. The first CTD cast on station Z13 was directed to take water samples in about 25 m water depth for incubation experiments. In these experiments the uptake of methylphosphonate and the supposed production of methane were monitored over the next four days. On the following CTD cast we obtained samples in the upper 40 m in high depth resolution for gas analysis and molecular biological studies. Additionally, three CTD casts were conducted for phytoplankton sampling in 21 m water depth (depth of Dinophysis). At the middle of the day we met R/V Elisabeth Mann Borgese at station Z13. On that vessel detailed studies regarding the turbulent mixing in the upper water column were conducted with a microstructure profiler (MicroRider). This dataset will be used in combination with our vertical methane distribution to calculate methane fluxes from the subthermocline methane maximum to the sea surface. In addition, tests with a prototype of an in-situ methane sensor were conducted. These results may also contribute to the methane measurements on discrete water samples carried out on R/V Alkor. Station work on that day ended at about 14:00 UTC and the scientific team prepared the plankton samples for incubation measurements and molecular biological studies.

Friday, August 19th

On this day, the first CTD cast at station Z13 was taken at 7:00 UTC for lipid biomarker studies (depth 0-18 m). On the second cast we gained phytoplankton samples from 21 m water depth for fecal pellet experiments. The following CTD at midday (13:00 UTC) was taken in high resolution around the thermocline for gas analysis, molecular biological studies, and methane oxidation rate measurements. After the samples were taken, the CTD was prepared for the next operation on which we gained water samples from 25 m water depth that was used in the incubation experiments. Two WP2 net hauls were taken afterwards in 45-25 m and 25-0 m water depth for qualitative and quantitative zooplankton studies. Living Copepods were obtained on the following three WP2 net hauls (45-25 m), and incubation experiments were set-up around 15:00 UTC with the freshly taken material.

Saturday, August 20th

The first CTD cast at Z13 was performed at 07:00 UTC for gas analysis on bottom water samples to study the impact of the inflow event (Major Baltic Infow 2014/15) on the evolution of the gas distribution. Other water samples were taken on that cast in 21 m water depth to obtain phytoplankton samples for incubation experiments with copepods. This station work was followed by an additional CTD cast, where we sampled the upper 50 m of the water column in high resolution for gas analysis. The last CTD was taken at 09:00 UTC for phytoplankton sampling in 25 m water depth. At about 09:00 UTC the tender of R/V Elisabeth Mann Borgese came alongside and journalists from “Die Welt” embarked R/V Alkor. The interview with the journalists ended at about 11:00 UTC and the visitors returned to R/V Elisabeth Mann Borgese. The scientific team continued their work on the samples taken in the morning and prepared a new set of incubation experiments with copepods and phytoplankton.

Sunday, August 21th

On Wednesday we continued our station work on Z13 at 11:00 UTC with a CTD cast for lipid biomarker studies at the redoxcline (sampled depth interval 100-60 m). Additional water samples were taken on that cast to obtain water for later incubation experiments with copepods. Four WP2 net hauls were taken at 14:00 UTC for qualitative and quantitative copepod studies, and to gain living copepods for further incubation experiments. At about 18:00 UTC we took the last CTD cast to sample the upper 50 m of the water column for gas analysis and molecular biological studies. Incubation experiments were set up in the evening with labeled phytoplankton and copepods.

Monday, August 22th

Still on Z13, the station work started at 06:00 UTC with a CTD cast. Water sampled on that cast in 10 m water depth was used for incubation experiments to continue our work on methane production by the decomposition of methylphosphonate. On the following CTD cast we sampled the upper water column in high resolution (0-50 m) for gas analysis. At 07:00 UTC we took again four WP2 net hauls for qualitative and

quantitative copepod studies, and to gain living copepods for further incubation experiments. Additional samples for lipid biomarker studies were taken in the mixed layer depth with the Apstein net (one net haul). After these net hauls we finalized our work at Z13 and steered to the next station about 44 nm southwards. Z14 was reached at 12:00 UTC. Two CTD casts were performed to sample the entire water column for gas analysis. The work on that station was finished around 13:00 UTC and we continued our travel southwards to station Z15.

Tuesday, August 23rd

We reached station Z15 at midnight (00:00 UTC). Investigations on the transect at the beginning of the cruise indicated that this region is not characterized by a subsurface methane accumulation. Following these observations, we defined this station as our background station (i.e. not influenced by subsurface methane production). We started the station work with a high resolution CTD cast in the upper 50 m for gas and molecular biological studies. Four WP2 net hauls were carried out for zooplankton studies. An additional CTD was taken at the end of the station work to obtain an entire methane profile from the water column. We left station Z15 at about 01:00 UTC. The last station we approached on our cruise was station Z16 (similar with station Z01 at the beginning of the cruise). Here we took three WP2 net holes to catch copepods in the depth interval 30-0 m. Three Apstein net hauls were taken in the same depth interval for the IOW monitoring program. Phytoplankton samples for the IOW monitoring program were obtained with a CTD cast in the upper 30 m water depth.

Wednesday and Thursday, August 24th and August 25th

Cruise AL483 was completed on Wednesday at approximately 14:00 UTC, when the R/V Alkor docked at Marienehe harbor. Demobilization was completed in the morning of 25 August 2016.

4. Scientific report and first results

4.1 Physical measurements in the water column (Oliver Schmale)

The key instrument of the physical observational program was our pumped SBE 911plus CTD system (Seabird Electronics, USA) consisting of a SBE 9plus CTD Unit with redundant sensor packs and a SBE 11plus deck unit. Conductivity, Temperature and Oxygen were each measures with twin SBE 4 conductivity sensors, SBE 3plus temperature sensors and SBE 43 oxygen sensors, each sensor assembly pumped via SBE 5T submersible pumps. Turbidity and fluorescence were observed with a type FLNTURTD sensor from Wetlabs (USA), whereas PAR and SPAR were recorded using the sensor types OSP200L4S and QSR-2200 from Biospherical Instruments (USA). For the detection of blue-green (phycocyanin) algae a Wetlabs (USA) ECO fluorometer was used. To measure the distribution of colored dissolved organic matter (CDOM) in the water profile, we used a Wetlabs (USA) ECO FL CDOM flourometer. All sensors were embedded in a SBE 32 Carousel Water

Sampler with a set of 16 x 10 L free-flow bottles from Hydrobios (Germany). Realtime data aquisition and post-measurement data processing were performed using SBE Seasoft V2 software package. All CTD casts obtained during AL451 are compiled in the station list.

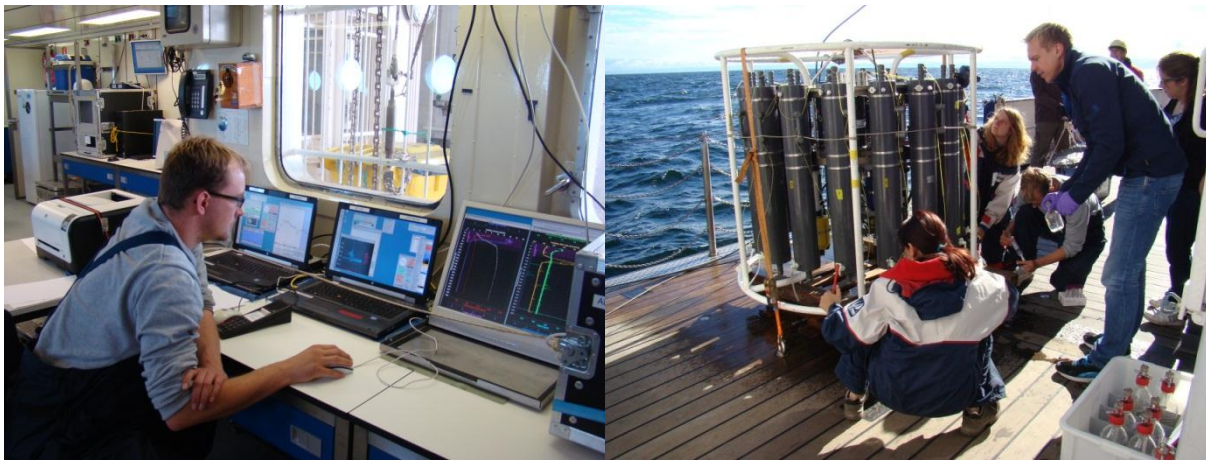


Figure 1. Left: CTD control desk in the R/V Alkor dry laboratory. Right: Water sampling at the CTD rosette system equipped with 16 free-flow bottles.

4.2 Methane chemistry in the water column (Oliver Schmale)

Water samples for water column methane analysis were taken with the CTD rosette system (see 4.1.). Methane concentrations were determined regularly to obtain information about the regional and temporal distribution pattern of methane. Methane oxidations rates and cell abundance of methane oxidizing bacteria was only conducted at three station. The target of this approach was to gain insights into the methane sink mechanisms in the surrounding of the thermocline. A compilation of the samples taken during the cruise is given in Tab. 1.

Methane Analyses

A 600 ml subsample from each water-column sample bottle was transferred into pre-evacuated 1100 ml glass bottles. The dissolved methane was extracted by the use of a vacuum degassing line. A subsample of the gas was transferred into 10 ml pre-evacuated crimp-top glass vials containing 4 ml of supersaturated salt solution (Schmale et al., 2010, 2012). The methane concentration was determined using a gas chromatograph (Trace GC, Thermo Fisher Scientific Inc.) equipped with a flame ionization detector. After the cruise the gas subsamples will be analyzed in the IOW home laboratory for ^{13}C CH_4 values using a MAT 253 isotope-ratio mass spectrometer (Thermo Fisher Scientific Inc.).

Methane oxidation rate measurements

Water samples were directly transferred from the sampler bottle into 600 ml incubation bottles, and sealed bubble-free with natural rubber septa. Methane was injected into the water samples, which were incubated near in situ temperatures for 3 days in the dark. The microbial activity was stopped by injection of 500 µl sodium hydroxide (50% (w/w)). After the cruise, methane oxidation rates will determined at the IOW home laboratory following the method of Jakobs et al. (2013, 2014).

Table 1. Samples taken in the context of water column methane chemistry. CH_4 = methane concentration analysis; MOB = analysis MOB cell numbers, MOx = quantification of methane turnover rates.

Date	Time (UTC)	ZOOM station	CTD file	CH_4	MOB	MOx
07.08.16	20:36	Z02_CTD02	V0003F02	x		
08.08.16	02:06	Z03_CTD02	V0005F02	x		
	10:50	Z04_CTD02	V0007F02	x		
	16:17	Z05_CTD01	V0008F01	x		
11.08.16	06:07	Z06_CTD01	V0009F01	x		
11.08.16	10:57	Z07_CTD02	V0010F02	x		
	16:05	Z08_CTD01	V0011F02	x		
	16:21	Z08_CTD02	V0011F02	x		
	21:08	Z09_CTD02	V0012F02	x		
12.08.16	02:42	Z10_CTD02	V0013F02	x		
	08:56	Z11_CTD02	V0014F02	x	x	x
	12:57	Z11_CTD04	V0015F01	x	x	x
15.08.16	04:48	Z12_CTD06	V0017F02	x		
18.08.16	11:01	Z13_CTD02	V0018F02	x	x	x
19.08.16	13:06	Z13_CTD08	V0019F03	x	x	x
20.08.16	08:00	Z13_CTD11	V0020F02	x		
21.08.16	10:57	Z13_CTD13	V0021F01	x		
	18:09	Z13_CTD14	V0021F03	x		
22.08.16	06:37	Z13_CTD16	V0022F02	x		
	12:18	Z14_CTD01	V0023F01	x		
	12:50	Z14_CTD02	V0023F02	x		
23.08.16	00:01	Z15_CTD01	V0024F01	x		
	00:52	Z15_CTD02	V0024F02	x		

Quantification of methane oxidizing bacteria

For bacteria quantification water samples were collected in 50-ml sterile plastic tubes. Samples were fixed with methanol-free formol (2%) for at least 2.5 to max. 4 hours. Subsequently, a 50-ml, 10-ml, and 1-ml subsample from each water sample were filtered on Nucleopore filters (Whatman, pore size 0.2 µm) with a maximum pressure of -100 mbar. Filters were stored in Eppendorf tubes at -20°C until further processing. After the cruise, the quantity of methane oxidizing bacteria (MOB) will be determined from filtered microbes using Catalysed Reporter Deposition Fluorescence In Situ

Hybdridization (CARD-FISH) followed by tyramide signal amplification (Schmale et al., 2016). In addition to CARD-FISH filters will be counter stained with 4´,6´-diamidino-2-phenylindole (DAPI). CARD-FISH and DAPI-stained cells will be enumerated using epifluorescence microscopy (Zeiss AxioImager.M2).

Preliminary results

Methane concentration profiles in the eastern Gotland Basin (EGB) show a distinct methane maximum below in the thermocline in about 25 m water depth (Fig. 2). In contrast to these observations, no methane enrichment could be observed in the upper water column of the western Gotland Basin (WGB). These results are consistent with our observations on the Meteor M87-4 cruise in summer 2012.

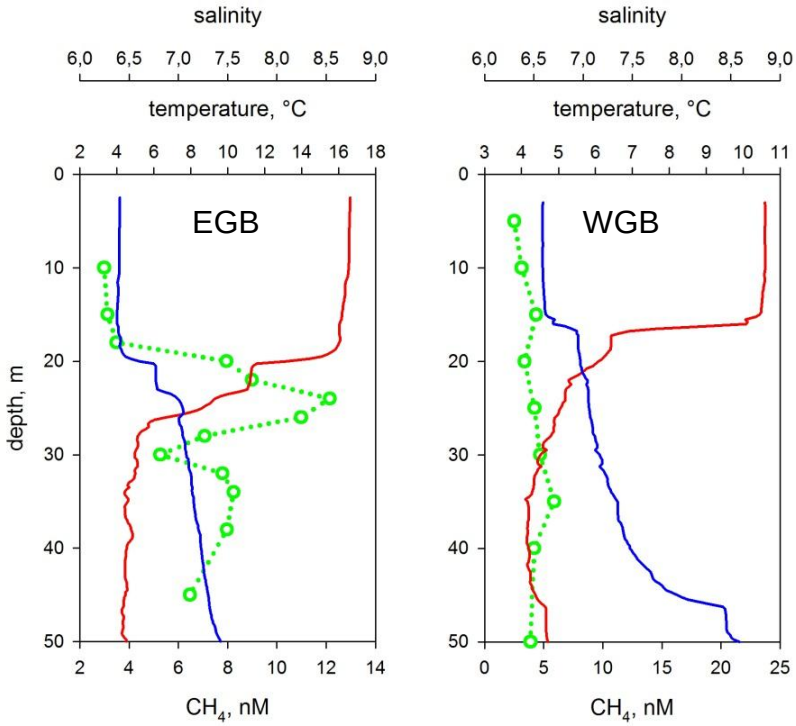


Figure 2. CTD and methane concentration profiles in the EGB and WGB.

4.3 Incubation experiments to determine zooplankton associated methane production rates (Beate Stawiarski)

The main goal of this contribution was to quantify methane production rates by two different copepod species, also in connection to their diet. The first objective was to identify the “Hotspot station” at which the subthermocline methane anomaly was most significant. At this station selected incubation experiments were conducted, where zooplankton was fed with ^{14}C labelled phytoplankton and the produced $^{14}CH_4$ was measured with a “purge and trap system” on board (Fig. 3, De Angelis and Lee, 1994). To complete the methane budget, parallel incubation experiments were conducted to quantify microbial methane oxidation within the feeding experiments (similar to the method described in chapter 4.2).

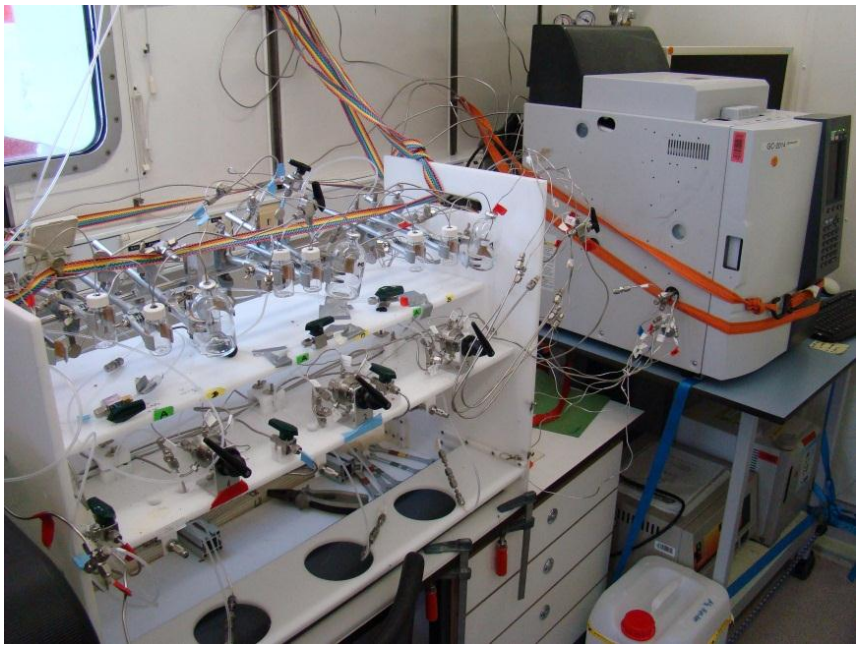


Figure 3. The purge and trap system in the IOW isotope container.

Phytoplankton samples

Phytoplankton stock cultures for potential incubation experiments were obtained at 9 stations along the transect (Tab. 2). The phytoplankton was sampled from two chlorophyll peaks in the water column, which were identified by two chlorophyll sensors attached to the CTD. The first phytoplankton culture was collected from the top chlorophyll maximum (0-10 m) with an Apstein net. The phytoplankton was resuspended in 500 ml of seawater and left for an hour to allow the *Nodularia sp.* (cyanobacteria) cells to float on top. The cells were then collected with a pipette and transferred into a 1 L bottle which was filled up with unfiltered surface seawater and 36.2µM NaH_2PO_4 was added. The *Nodularia sp.* culture was incubated in a mesocosm tub on deck which was continuously flushed with sea surface water (Fig. 4) at a temperature of ~18.5°C. The light intensity was measured to be 27- 87 µmol photons m^{-2} s^{-1}. A Datalogger (LI-COR LI-1000) equipped with two sensors calibrated for use in either water or air was used for these light measurements. The second phytoplankton culture was collected from a subsurface chlorophyll peak close to the thermocline (~20 m depth), where *Dinophysis sp.* was expected to be dominant. For this, up to 150 L of seawater were first obtained from a CTD rosette, then filtered through a 100 µm mesh to remove zooplankton and concentrated on a 20 µm mesh. Finally, the sample was resuspended in f/2 medium to make up 1L of concentrated phytoplankton culture. The *Dinophysis sp.* dominated culture was incubated in the cold room by the window at a constant temperature of 10°C. The light was measured to be 0.1 - 2.5 µmol photons m^{-2} s^{-1}. The light intensity in 20 m depth within the water column was 0.2- 0.4 µmol photons m^{-2} s^{-1}. Also, a laboratory strain of *Rhodomonas sp*. was incubated in f/2 medium in the mesocosm on deck.

Phytoplankton ^{14}C incubation

When a phytoplankton culture was selected for an incubation experiment (Tab. 2 and 3), it was split between three 1 L incubation bottles and filled up with the corresponding medium. One of these bottles was spiked with 0.5 mCi of ^{14}C-labelled $NaHCO_3$ (58.8 mmol mCi^{-1}, Fig. 4). The other two bottles received the equal amount of unlabelled $NaHCO_3$. All bottles were incubated for at least 3 days to allow cells to grow. The specific activity of the labeled culture was measured by filtering 1- 5 ml of the culture through a 0.45 µm cellulose nitrate filter (Millipore) and rinsed thoroughly with Milli-Q water. The filter was then dissolved in Filter Count Scintillation Cocktail (Perkin Elmer) by vortexing and measured on a liquid scintillation counter (Perkin Elmer, Tri-Carb 2800TR). Blanks were collected at t_0 after label was added to the incubation bottle. The *Rhodomonas sp.* strain was labelled on 11.08.2016, 13.08.2016 and 14.08.2016 for experiment 1, 5 and 7, respectively (Tab. 3).

Table 2. Phytoplankton stock cultures, sampling conditions and experimental conditions.

CTD file	ZOOM station	Sampl. gear	sampling date	time	depth (m)	amount (L)	experiment	Date of ^{14}C labelling	Start of the experim.
V002F03	Z01_CTD03	CTD	07.08.2016	12:15	10	100			
V0004F01	Z02_CTD03	CTD	07.08.2016	22:45	10	80			
V0006F02	Z03_CTD03	CTD	08.08.2016	05:45	10	70	2	12.08.16	15.08.16
V0006F02	Z03_CTD03	CTD	08.08.2016	05:45	25	70	2	12.08.16	15.08.16
V0007F03	Z04_CTD03	CTD	08.08.2016	14:00	10	60		09.08.16	-
V0007F03	Z04_CTD03	CTD	08.08.2016	14:00	20	80		09.08.16	-
V0009F02	Z06_CTD02	CTD	11.08.2016	09:00	10	80			
V0010F03	Z07_CTD03	CTD	11.08.2016	14:00	10	80			
	Z08_AP_01	Apstein	11.08.2016	19:30	0-20				
V0012F03	Z09_AP_01	CTD	11.08.2016	00:15	22,5	80			
	Z09_CTD03	Apstein	11.08.2016	23:45	0-20				
V0013F03	Z10_CTD03	CTD	12.08.2016	06:00	22,3	110			
	Z10_AP_01	Apstein	12.08.2016	06:00	0-20				
V0014F03	Z11_CTD03	CTD	12.08.2016	12:00	10	80			
V0014F03	Z11_CTD03	CTD	12.08.2016	12:00	20	55	3	13.08.16	16.08.16
	Z11_AP_02	Apstein	12.08.2016	15:00	0-20		3	13.08.16	16.08.16
V0015F03	Z11_CTD06	CTD	12.08.2016	18:00	10	80			
V0015F03	Z11_CTD06	CTD	12.08.2016	18:00	21	80	3	13.08.16	16.08.16
	Z12_AP_01	Apstein	14.08.2016	08:00	0-20		4	14.08.16	17.08.16
V0016F02	Z12_CTD02	CTD	14.08.2016	09:00	20	140		14.08.16	-
V0016F03	Z12_CTD03	CTD	14.08.2016	10:30	20	150		15.08.16	-
	Z12_AP_02	Apstein	15.08.2016	09:00	0-20		6	15.08.16	19.08.16
V0017F05	Z12_CTD09	CTD	15.08.2016	11:00	22-25	150	4	15.08.16	17.08.16
V0018F03	Z13_CTD03	CTD	18.08.2016	12:00	20	150	7	18.08.16	21.08.16
V0018F04	Z13_CTD04	CTD	18.08.2016	14:00	20	150	7	18.08.16	21.08.16
	Z13_AP_01	Apstein	18.08.2016	14:00	0-20				

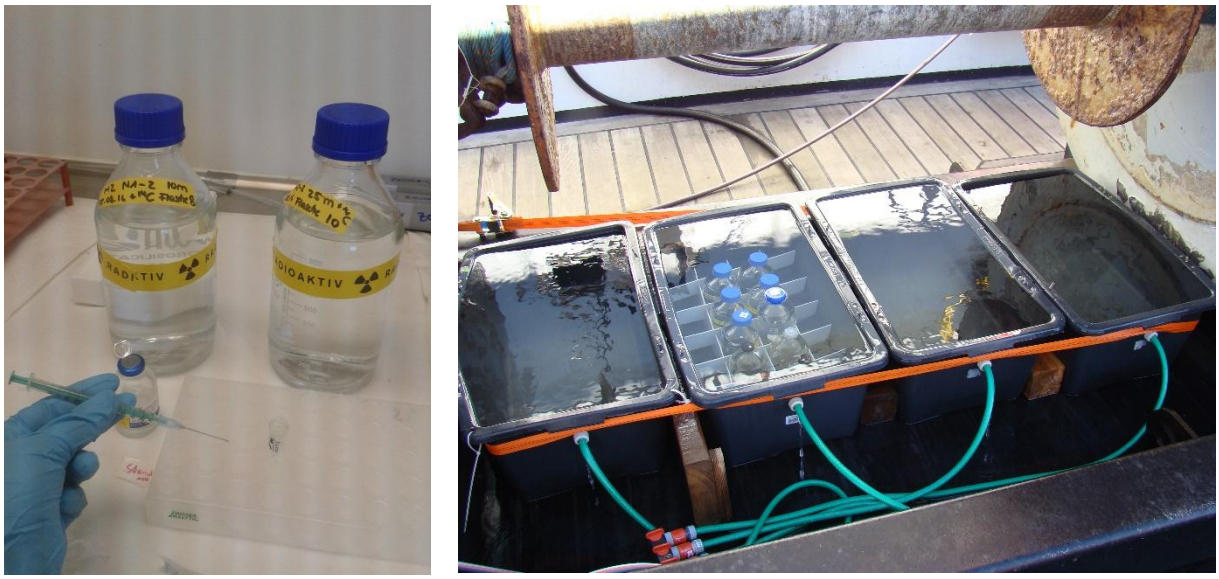


Figure 4. Left: 1L incubation bottles spiked with 0.5 mCi of ^{14}C-labelled $NaHCO_3$. Right: mesocosm tub on deck continuously flushed with sea surface water.

Zooplankton samples

Copepods for incubation experiments were collected with a WP2 net (towed 0.1 m s^{-1}) from two depth intervals after vertical migration started in the evening (Fig. 5). The upper water column (15-0 m) was sampled for *Acartia sp.* dominated copepods, but the deeper water column (35-15 m) was sampled for *Temora sp.* dominated copepods. The cod end was closed to reduce damage of living copepods and the content was transferred immediately into a bucket filled with seawater from 20 m depth. The copepods were left in the cold room at 10°C for an hour to allow damaged individuals to settle on the bottom of the bucket. Copepods for experiments were removed gently with a beaker from the bucket.

Figure 5. Left: Zooplankton sampling with the WP2 net. Middle: First screening of the plankton samples on board. Right: Temora sample under the microscope.

Methane production experiments

The first experiment was conducted on the 14.08.2016 with a well labelled culture of *Rhodomonas sp.* (Tab. 3). A previous study has shown that both copepod species produce methane if fed with *Rhodomonas sp.* (De Angelis & Lee, 1994). However, in

those experiments larger individuals from the Atlantic Ocean had been used. Hence, first conditions were selected to test which amount of Baltic Sea individuals was sufficient to detect significant amounts of methane using the purge and trap system. In this experiment, both *Temora sp.* and *Acartia sp.* dominated zooplankton was used.

In experiment 2 and 3 two types of food (*Nodularia sp.* and *Dinophysis sp.* dominated phytoplankton) were fed to either *Acartia sp.* or *Temora sp.* dominated zooplankton, respectively (Tab. 3). The phytoplankton culture from the *Dinophysis sp.* dominated depth did not take up the ^{14}C label sufficiently, possibly due to mixotrophic feeding. Hence, in experiment 4 a food mixture of *Dinophysis sp.* and *Nodularia sp.* was used. In experiment 5-7 incubation times were increased to produce higher methane concentrations and decrease the level of uncertainty of the methane production rates. These experiments were conducted for Rhodomonas (experiment 5), which was identified as a food source, which leads to significant methane production rates, *Nodularia sp.* (experiment 6), which led to less methane production and finally with a mixture of *Rhodomonas sp.* and *Dinophysis sp.* dominated phytoplankton (experiment 7) to compare the rates with the other diets.

Table 3. Setup of incubation experiments (exp.) and the samples taken for molecular biological analysis at the IOW home laboratory.

Exp	Date of exp.	Changed parameter	Dominant copepod	Dominant phytoplankton	Specific activity of phytoplankton	No. of copep.	Molecular samples
1	14.08.16	number of copepods	*Temora sp.*	*Rhodomonas sp.*	18,79	27, 57, 73	copepods; filters
			Acartia sp.			25, 50, 76	
2	15.08.16	food	*Acartia sp.*	*Nodularia sp.*	1,87	50	copepods; filters
				Dinophysis sp.	0,03	54	
3	16.08.16	food	*Temora sp.*	*Nodularia sp.*	5,5	75	copepods; filters
				Dinophysis sp.	0,08	55	
4	17.08.16	food (symbiosis)	*Temora sp.* *Acartia sp.*	*Nodularia sp.* – *Dinophysis sp.* - Mix	4,19	64	/
5	18.08.16	time	*Temora sp.*	*Rhodomonas sp.*	16,58	66	copepods; filters
6	19.08.16	time	*Temora sp.*	*Nodularia sp.*	8,09	50	copepods; filters
7	21.08.16	food (symbiosis)	*Temora sp.* *Acartia sp.*	*Rhodomonas sp.*- *Dinophysis sp.* - Mix	14,31	58	copepods; filters

Side parameters

To ensure that the copepods within the experiments were not food limited, unlabelled incubations were conducted under identical conditions (as in Tab. 3) and samples for particulate organic carbon (POM) were taken on precombusted GF/F filters at the beginning and at the end of each incubation. The first sample was removed from the incubation bottle before the copepods were added to it, and the second sample was taken after copepods were removed from the incubation bottle. Also a second series of unlabelled experiments was conducted to measure methane oxidation rates to

account for the potential loss of methane by microbial oxidation. For this, $^{14}CH_4$ was injected into the incubation bottles and the amount of produced CO_2 was measured (see method in chapter 4.2).

Further, samples of the unlabelled food and the copepod culture which was added to the incubation bottles were taken and fixed to later characterize the exact community composition.

To correlate methane concentration in the water column with phytoplankton and zooplankton community composition and biomass, fixed phytoplankton and zooplankton and chlorophyll *a* (chapter 4.4, Tab. 4) samples were taken along the transect for later analysis.

Preliminary results

The results show evidence for methane production by both copepod species. When Rhodomonas was fed, the methane concentration increased with increasing number of copepods and with increasing incubation time within the incubation bottles. The production rates (Copepod $^{-1}$ day^{-1}) were similar if the same food source was used in different experiments. Also, significantly more methane was produced using Rhodomonas sp., compared to *Nodularia sp.* (T-test, $p < 0.05$). However, *Temora sp.* seems to produce higher amounts of methane copepod^{-1} d^{-1} than *Acartia sp.*.

4.4 Microbiological studies on methanogenic archaea in the water column, copepods, and faecal pellets (Janine Wäge)

The goal of this contribution is to investigate our hypothesise that anoxic microbial fermentation within the guts of marine zooplankton, namely copepods, and their faecal microbial fermentation could be a potential CH_4-source. To test this, copepods and their faecal pellets, as well as water column samples were collected to be analysed for methanogenic archaea at the IOW. For this DNA and RNA will be extracted from the samples, followed by next generation sequencing and quantitative PCR analysis (Narihiro and Sekiguchi, 2011). Samples were collected along a transect in the Gotland Basin.

Archaea in the oxygenated water column

To test if methanogens are present in the water column, water samples were collected on polycarbonate filters (Fig. 7). Two size fractions, 3 µm and 0.22 µm, were sampled. The fractionation was carried out to investigate if the methanogens are attached to particles and/or if they can also occur in the free water column. The latter is less likely, as methanogens are strictly anaerobic, however previous research showed that some species can tolerate oxygen for several hours (Cynar and Yayanos, 1991). At each station two direct filters were sampled (1 L water on 0.22 µm filter). For the size fractionation one litre of water was filtered through a 3 µm filter and the filtrate was additionally filtered through a 0.22 µm filter. The fractionation was

carried out in duplicates. All filters were snap frozen in liquid nitrogen and stored at -80 °C. This was done for all stations (see Tab. 4) between 10-50 m, in 2.5 - 10 m intervals. Furthermore, filters were collected from the depth with the highest CH_4 measurement at the station with the highest CH_4 concentration at different days and day times. Additionally, samples for nutrients (PO_4, NO_3, NO_2, NH_4 und SiO_2), cell counts (Paraformaldehyde + Glutaraldehyde) and chlorophyll a analyses were collected (Tab. 4).

Figure 7. Filtration work in the wetlab of R/V Alkor.

Archaea in copepods

To test if methanogens are present in the guts of copepods, different samples were collected. Sampling was conducted with a WP2 net (100 µm mesh size), which was pulled with a speed of 0.1 m s^{-1}. Two different depth ranges were investigated: 45 m to 25 m (*Temora sp.* dominated) and 25 m to 0 m (*Acartia sp.* dominated). The collected copepods were transferred into a bucket with 15 L of water from the same station and same depth range. After approximately 60 min the dead and weak animals sank to the bottom of the bucket and were removed. 600 ml of the water was used to concentrate the copepods on a 20 µm gaze. The copepods were transferred into 200 ml 0.22 µm filtered seawater. Subsequently the water was filtered onto a 3 µm filter and then snap frozen in liquid nitrogen. Additionally, animals were picked by species (*Acartia sp.*, *Temora sp.* and *Centropages sp.*) under a stereo microscope and fixed in RNAlater. Furthermore, mixed species samples were fixed in RNAlater. For this the copepods were concentrated on a 20 µm gaze and were transferred into Eppendorf tubes. All samples are stored at -80 °C for further molecular analysis.

Archaea in faecal pellets

The aim of the experiment was to collect the faecal pellets from copepods, preferably without any other faeces, and to analyse them for methanogens later in the laboratory. As copepods only produce faecal pellets when they eat, we decided to use *Rhodomonas sp.* from a laboratory culture as food source. *Rhodomonas sp.* is known to be liked by copepods (De Angelis & Lee, 1994) and would not bias the

result with already existing faecal pellets in the food source. For the experiment zooplankton was collected with a WP2 net (100 µm mesh size) between 45-25 m at around 4 pm at Z12 and Z13 (Tab. 4). This is the time when the zooplankton moves to the upper layers to feed. The copepods were transferred into a 20 L bucket and left at 10 °C for 60 min. All dead and weak animals sank to the bottom and were removed with a tube. 100 ml of water with copepods was transferred from the bucket into a 500 ml glass dish and topped up with 270 ml 0.22 µm filtered seawater from the same depth. As food source 30 ml of laboratory cultured *Rhodomonas sp.* was added. In total seven Petri dishes with animals and three control dishes were prepared. The control dishes contained 100 ml of the copepod water, but no copepods. All Petri dishes were incubated in a cold room in the dark at 10 °C for 3 h. The faecal pellet production was checked under a stereo microscope. The animals were separated by a 200 µm gaze and transferred into an Eppendorf tube with ethanol. The water including the faecal pellets were filtered on a 3 µm polycarbonate filter and shock frozen. The same experiment was repeated with concentrated phytoplankton from the second chlorophyll maximum (~21 m) to see if the amount of faecal pellets is higher or lower with their natural food.

Table 4. List of samples collected for molecular analyses, including DNA-Filters and copepods, as well as background data (nutrients, chl a and cell counts (P+G)).

ZOOM Station	CTD file	Date	Sampling gear	Sample
Z01	V0002F02	07.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z02	V0003F02 V0003F03	07.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z03	V0005F02	08.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z04	V0007F02	08.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z05	V0008F01	08.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z06	V0009F01	11.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a; FISH
Z07	V0010F01	11.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z08	V0011F02	11.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z09	V0012F02	11.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z10	V0013F02	12.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a; FISH
Z11	V0014F02	12.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a
Z12	V0015F01	12.08.2016	CTD	DNA-Filter (A, B, C); nutrients; P+G; Chl a; FISH
Z12	V0016F05	14.08.2016	CTD	DNA-Filter (A, B, C)
Z12	V0017F02	15.08.2016	CTD	DNA-Filter (A, B, C)
Z13	V0018F01	18.08.2016	CTD	DNA-Filter (A, B, C); P+G; Chl a
Z13	V0018F02	18.08.2016	CTD	DNA-Filter (A, B, C); Chl a
Z13	V0019F03	19.08.2016	CTD	DNA-Filter (A, B, C); Chl a
Z13	V0020F02	20.08.2016	CTD	DNA-Filter (A, B, C); Chl a
Z13	V0021F03	21.08.2016	CTD	DNA-Filter (A, B, C); Chl a
Z13	V0022F02	22.08.2016	CTD	DNA-Filter (A, B, C); Chl a
Z14	V0024F01	23.08.2016	CTD	DNA-Filter (A, B, C); Chl a
Z10-12	/	12.08.2016	WP2 net	Copepods on 3 µm filters
Z12	/	14.08.2016	WP2 net	Faecel pellets on 3 µm filters; Copepods
Z12	/	14.08.2016	WP2 net	Faecel pellets on 3 µm filters; Copepods
Z12	/	15.08.2016	WP2 net	*Temora sp.*; *Acartia sp.*; *Centropages sp.*
Z13	/	18.08.2016	WP2 net	Faecel pellets on 3 µm filters; Copepods
Z13	/	19.08.2016	WP2 net	Faecel pellets on 3 µm filters; Copepods
Z13	/	19.08.2016	WP2 net	Mixed copepods
Z13	/	20.08.2016	WP2 net	Mixed copepods
Z13	/	21.08.2016	WP2 net	Mixed copepods
Z13	/	22.08.2016	WP2 net	Mixed copepods
Z14	/	23.08.2016	WP2 net	Mixed copepods

4.5 Organic geochemical investigations on zooplankton associated methane production (Volker Thiel)

These studies will investigate whether and how copepods from the Eastern Gotland Basin incorporate organic compounds from their food into their body lipids. (i) A particular focus will be on biomarkers for the major diet of *Temora longicornis,* as this copepod is suspected to induce the methane anomalies in the oxygenated water column of the Eastern Gotland Basin. (ii) A second major task, complementary to microbiological investigations conducted in ZOOM (see chapter 4.4), is to detect and identify lipids from methanogenic archaea that putatively thrive in the digestive tract of *T. longicornis*. (iii) Another important question is as to which extent organic compounds produced or transformed by copepods are exported from the water column and deposited, and preserved, in Gotland Deep sediments (e.g. Berndmeyer et al., 2013; Blumenberg et al., 2013).

Strategies

During the research cruise, samples dominated by three abundant copepod species of the Baltic Sea (*Temora longicornis*, *Paracalanus* sp.,.and *Acartia* sp.) were taken from different water depths using a WP2 net. Depending on their living depth their diet probably varies. *Temora longicornis* and *Acartia* sp. possibly consume dinoflagellates (*Dinophysis norvegica*) or diatoms (*Chaetoceros* sp.) while *Paracalanus* is supposed to thrive preferentially on ciliates. The importance of cyanobacteria for the copepod diet is not clear yet. Whereas cyanobacteria (*Nodularia* sp./ *Aphanizomenon* sp., Fig. 8) live mainly in the mixed layer, dinoflagellates and diatoms concentrate below the thermocline, and heterotrophic ciliates at the halocline. Concentrated samples of each of these potential food sources were retrieved separately using a WP2 net, or filtered from water samples taken from the respective depth intervals. In the lab, each of these samples will be analysed with GC-MS and pyrolysis-GC-MS (see below). It is known that lipids, especially fatty acids and alcohols, can be incorporated into the storage lipids of copepods. These components shall be identified by comparison of the lipid content of the zoo- and phytoplankton “end members” with the copepod neutral lipids.

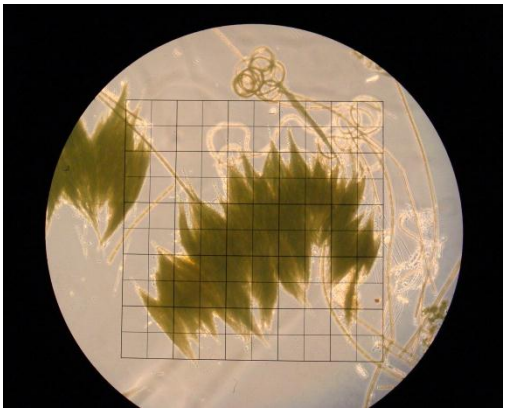


Fig. 8. Cells of the dominant cyanobacteria Nodularia spumigena (filaments) and Aphanizomenon sp. (bundles); 1 raster field = 125 x 125 µm^2.

Among the extractable organic compounds of *Temora longicornis* we will also search for hints for its methane production, namely in the form of lipid biomarkers specific for methanogenic archaea, such as the glycerol diether archaeol or the isoprenoid hydrocarbon 2,6,10,15,19-pentamethylicosane. It will also be attempted to track, and localize, these and other archaeal lipids on microscopic samples using ToF-SIMS.

To answer the third research question (sedimentary copepod organics) we took a sediment core using a Frahmlot (Fig. 9). The first 60 cm of the sediment were sampled in 1 to 5 cm steps. The oldest sediments sampled may be around 100 years old. Samples from selected depths will be extracted using the same procedure as for the biomass, and analysed for sterols, wax esters, or chitin derived from copepods that once lived in the water column of the Gotland Deep. Samples taken/prioritized for organic geochemical analyses are listed in Tab. 5 and 6.

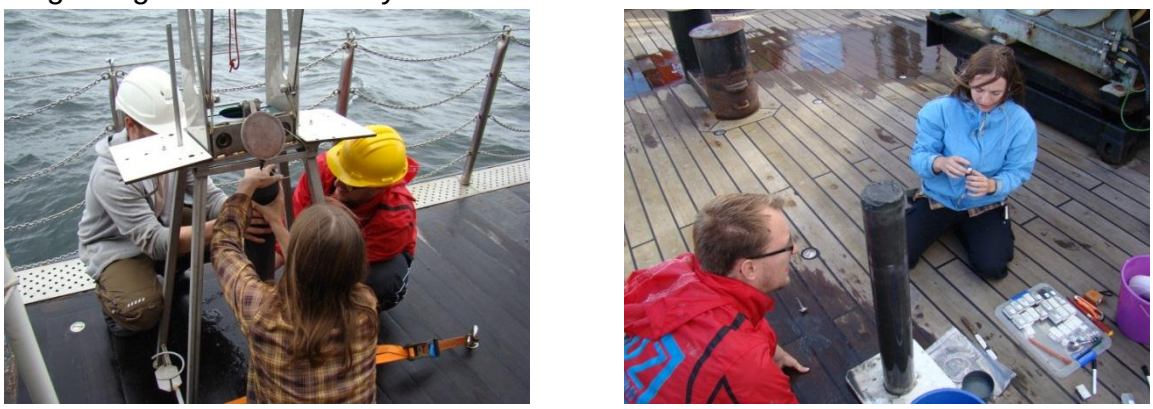


Figure 9. Left: Recovery of a sediment core taken in the eastern Gotland Basin. Right: Core cutting and sample preparation.

On the samples taken during our field work on R/V Alkor, we will perform the following work in the home laboratory:

Lipid extraction

Each sample will be extracted by dichloromethane and methanol using ultrasonic disruption. The neutral lipid fraction (NL) will be separated from the phospholipids fatty acids (PLFA) by phase extraction using SiOH glass columns. After drying and weighing the NL-fraction, fatty acyl moieties from wax esters and triacylglycerols will be converted into fatty acid methyl esters (FAME) using trimethylchlorsilane/methanol. After re-extraction of FAMEs, alcohols and hydrocarbons into n-hexane, an aliquot will be treated with Bis(trimethylsilyl)trifluoroacetamide (BSTFA) to derivatize the alcohols to their corresponding TMS-ethers. FAMEs, OH-TMS, and hydrocarbons will be analysed with gas chromatography–mass spectrometry (GC-MS). If the different compounds are not sufficiently separated from each other for a detection and quantification with GC-MS, it is envisaged to further separate the three lipid classes by column chromatography using SiOH glass columns. Hydrocarbons and FAMES will be analysed directly with GC-MS. Alcohols will be converted into TMS ethers before analysis.

Pyrolysis

To semiquantitatively analyse bulk carbohydrates (including chitin), proteins and lipids, samples or extraction residues will be sequentially pyrolysed online using a temperature programmable pyrolysis device, and the products analysed via GC-MS.

ToF-SIMS

To gain insight into the spatial patterns of lipids and other organic compounds in the animal's anatomy, selected copepod samples will be analysed using time-of-flight secondary ion mass spectrometry (ToF-SIMS). ToF-SIMS will provide at the µm-level mass spectra from the outermost molecular layers of cryosections, as well as native and formol-fixed samples of the candidate copepods.

Table 5. Samples taken from the water column for organic geochemical and complementary analyses (Univ. Göttingen). Sample prioritized for the planned M.Sc. thesis work are shaded gray.

	Probe	Biomarker	Lugol	Formol	Tof-Sims
Mixed Layer	Cyanobakterien: Nodularia Aphanizonemon	Z11-TF0271 AP 0-25m	Z11-TF0271 AP 0-25m Phyto Pl. + Formol		
	Zooplankton. Temora ~80% Arcatia ~20%	Z11-TF0271 AP 0-25m		Z11-TF0271 AP 0-25m Zoo Pl. + Formol	
	Plankton >100 µm		Z13- TF0271 0-18 m CTD-06		
	Phytoplankton >20 µm		Z13- TF0271 0-18 m CTD-06		
	0,7 µm Filter	Z13- TF0271 18, 12, 6, 0m CTD-06	Z13- TF0271 0-18 m CTD-06		
	Phytoplankton >20 µm		Z13-TF0271 AP 0-20m Cyanos+Lugol	Z13-TF0271 AP 0-20m Cyanos+Formol	Z13-TF0271 AP 0-20m Cyanos 20-50µm 3x mit N2 1x ohne N2
Unterhalb Thermokline	Dinophysis	Z12-TF0271 CTD-01 23m	Z12-TF0271 CTD-01 23m Dino + Lugol		
	Zooplankton >100 µm	Z12-TF0271 CTD-01 23m		Z12-TF0271 CTD-01 23m Zoo Pl. + Formol	
	0,7 µm Filter	Z12-TF0271 CTD-01 23m			
	0,2 µm Filter	Z12-TF0271 CTD-01 23m			
	Hintergrund: Copepoden >100µm	Z15-TF0250 WP2 20-45 Copepoden		Z-15-TF0250 WP2 20-45 Copepoden+Formol >100µm	
	Hintergrund: Diatomeen 20-100µm	Z15-TF0250 WP2 20-45 Diatomeen	Z-15-TF0250 WP2 20-45 Diatomeen+Lugol 20-100µm		
	Copepoden	Z13-TF0271 WP2 20-45m Temora		Z13-TF0271 WP2 20-45m Temora +Formol	Z13-TF0271 WP2 20-45m Temora 3x mit N2 1x ohne N2
	Temora	Z11-TF0271 WP2 25-45m		Z11-TF0271 WP2 25-45m Zoo Pl. + Formol	
	Temora	Z11-TF0271 WP2 25-60m		Z11-TF0271 WP2 25-60m Zoo Pl. + Formol	
Chemokline / Halokline	Paracalanus	Z11-TF0271 WP2 60-90m		Z11-TF0271 WP2 60-90m Zoo Pl. + Formol	
	Ciliaten Chemoautrophe 0,7 µm Filter	Z13-TF0271 CTD-13 60-100m			
	Zooplankton >100 µm			Z13-TF0271 CTD-13 60-100m Zoo Pl. + Formol	
unterhalb Chemokline / Halokline	Paracalanus ~50 % Oithonia ~50%	Z11-TF0271 WP2 90-200m		Z11-TF0271 WP2 90-200m Zoo Pl. + Formol	

Table 6. Sediment samples taken for organic geochemical analyses (Univ. Göttingen). Sample prioritized for the planned M.Sc. thesis work are shaded gray.

	Sample	Biomarker	Lugol	Formol	Tof-Sims
Sediment	Sediment: 0-1 cm	Z12-Tf0271 0 cm	Z12-TF0271 FL 0cm Floc + Lugol	Z12-TF0271 FL 0cm Floc + Formol	
	Sediment: 1-2 cm	Z12-TF0271 FL 1-2cml			
	Sediment: 2-3 cm	Z12-TF0271 FL 2-3cml	Z12-TF0271 FL 2-3cm Sed. + Lugol	Z12-TF0271 FL 2-3cm Sed. + Formol	
	Sediment: 3-4 cm	Z12-TF0271 FL 3-4 cm			
	Sediment: 4-5 cm	Z12-TF0271 FL 4-5 cm	Z12-TF0271 FL 5cm Sed. + Lugol	Z12-TF0271 FL 5cm Sed. + Formol	
	Sediment: 5-6 cm	Z12-TF0271 FL 5-6 cm			
	Sediment: 6-7 cm	Z12-TF0271 FL 6-7 cm			
	Sediment: 7-8 cm	Z12-TF0271 FL 7-8 cm			
	Sediment: 8-9 cm	Z12-TF0271 FL 8-9 cm			
	Sediment: 9-10 cm	Z12-TF0271 FL 9-10 cm			
	Sediment. 10-12 cm	Z12-TF0271 FL 10-12 cm			
	Sediment: 12-14 cm	Z12-TF0271 FL 12-14 cm	Z12-TF0271 FL ~13cm Sed. + Lugol	Z12-TF0271 FL ~13cm Sed. + Formol	
	Sediment: 14-16 cm	Z12-TF0271 FL 14-16 cm			
	Sediment: 16-18 cm	Z12-TF0271 FL 16-18cm			
	Sediment: 18-20 cm	Z12-TF0271 FL 18-20 cm			
	Sediment: 20-25 cm	Z12-TF0271 FL 20-25cm			
	Sediment: 25-30 cm	Z12-TF0271 FL 25-30 cm			
	Sediment: 30-35 cm	Z12-TF0271 FL 30-35 cm			
	Sediment: 35-40 cm	Z12-TF0271 FL 35-40 cm			
	Sediment: 40-45 cm	Z12-TF0271 FL 40-45cm			
	Sediment: 45-47,5 cm	Z12-TF0271 FL 45-47,5cm			
	Sediment: 47,5-52,5 cm	Z12-TF0271 FL 47,5-52,5 cm			
	Sediment. 52,5-57,5 cm	Z12-TF0271 FL 52,5-57,5cm			
	Sediment: 57,5-62,5 cm	Z12-TF0271 FL 57,5-62,5 cm	Z12-TF0271 FL ~60cm Sed. + Lugol	Z12-TF0271 FL ~60cm Sed. + Formol	
	Sediment: 62,5-65 cm	Z12-TF0271 FL 62,5-65 cm			

4.6 Studies to evaluate the potential of methylphosphonate as a methane precursor (Constantin Recknagel)

During the cruise two incubation experiments were carried out. The goal of these experiments was to evaluate whether there is a direct correlation of methylphosphonic acid and methane. In 2008 Karl et al. showed that when surface water from the North Pacific was incubated with methylphosphonic acid (MPn), the methane concentration in the head-space increased. In the experiments a stoichiometric conversion of MPn to methane was achieved when MPn was the only P-source present in the sample (Karl et al., 2008).

Incubation experiments

Two experiments were performed during the cruise of AL483. Both experiments were done using water samples taken in the eastern Gotland Basin (Z11 and Z13, Tab. 7). For the first experiment water samples from 10, 32.5 and 40 m were achieved with the CTD/rosette system. The different depths were chosen based on a screening of the vertical distribution pattern of dissolved methane. One sample was taken within in the methane maximum at 32.5 m water depth, and two other samples were taken above and below this maximum.

Four different conditions were evaluated for the three different water samples, each in triplicates. For each sample 200 mL water was placed in a 250 mL glass serum bottle and capped with a gas-tight septum and crimp-sealed with aluminium closures. After incubation overnight the samples were spiked with different concentrations of methylphosphonic acid. The four different conditions were the following:

(1) Control: Filtrated water (0.2 µm), spiked with 1 µMol MPn

(2) Blank: Unfiltrated water: spiked with 0 µMol MPn

(3) 5 µM: Unfiltrated water: spiked with 1 µMol MPn

(4) 20 µM: Unfiltrated water: spiked with 5 µMol MPn

Table 7. Incubation experiments for MPn related methane production.

ZOOM Station	Sampling gear	CTD-file	sampling date	depth (m)	amount (L)	experiment	start of the experiment
Z11	CTD	V0015F01	12.08.2016	10	5	1	13.08.2016
Z11	CTD	V0015F01	12.08.2016	32.5	5	1	13.08.2016
Z11	CTD	V0015F01	12.08.2016	40	5	1	13.08.2016
Z13	CTD	V0018F01	18.08.2016	10	25	2	19.08.2016
Z13	CTD	V0022F01	22.08.2016	10	25	collected for further experiments	-

The samples were incubated for four days either at seawater temperature and daylight (samples taken in 10 m) or at 4°C in the dark (samples taken in 32.5 and 40 m). During this time the methane production was regularly determined (about once a day) by measuring the methane concentration in the head-space of the samples. Therefore 1 mL of filtrated water was added to the sample to prevent mixing with ambient air and then 1 mL of the head-space was collected using a gas-tight syringe and injected into a GC-FID system (Fig. 10, see method in chapter 4.2). Furthermore regularly 2 mL of water were collected from the bottles for latter analysis of the MPn-concentration.

In addition, we performed for each depth an incubation experiment with two different concentrations of isotopically labeled MPn (^{13}C-MPn). The goal was to identify isotopically labeled methane in the head-space and to determine in this way a direct conversion of ^{13}C-MPn to ^{13}C-methane. Therefore the different water samples were spiked either with 0.2 or 0.8 µMol ^{13}C-MPn and incubated in the same way like the samples described before. At the different time points 5 mL of the head-space were collected and stored in a 20 mL glass-vial filled with saturated salt-water for latter analysis.

Figure 10. Methane analysis in the chemistry laboratory on R/V Alkor.

Preliminary results

As a result only in the samples which were collected at a water depth of 10 m an increase of the methane concentration was observed (see Fig. 11). Interestingly, we found the highest methane production rates in our filtered control sample. To explain this observation, further experiments in our home laboratory are needed.

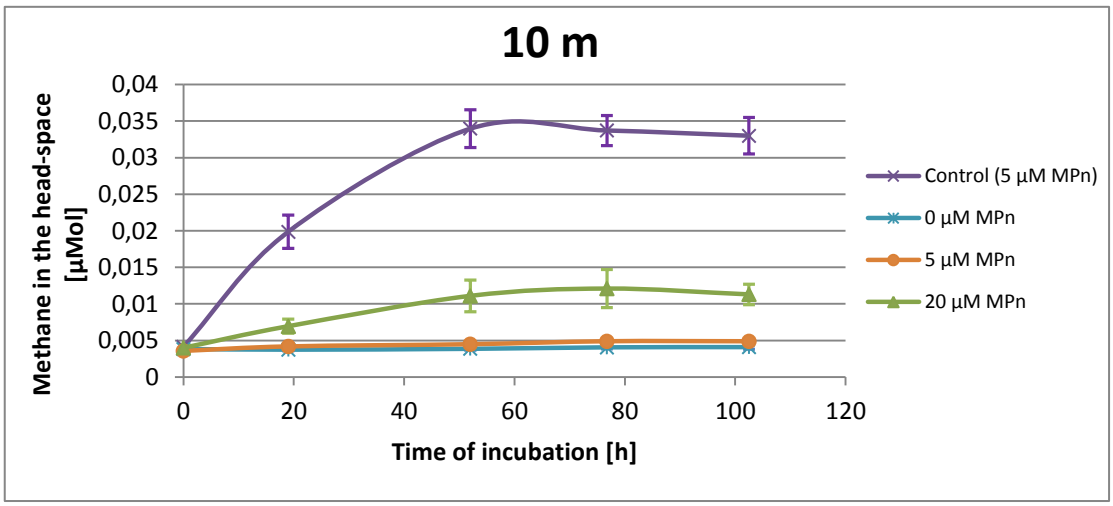


Figure 11. Methane evolution in the headspace over time (10 m sample).

Based on this result a second incubation experiment was performed to evaluate the influence of the light on the methane production rates. Therefore, water was collected from a depth of 10 m and treated in the same way as described before. The samples were stored at surface seawater temperature and at different light conditions namely daylight, 25% of daylight and darkness (see Fig. 12). Furthermore an experiment with isotopically labeled MPn was performed in the same way as described above.

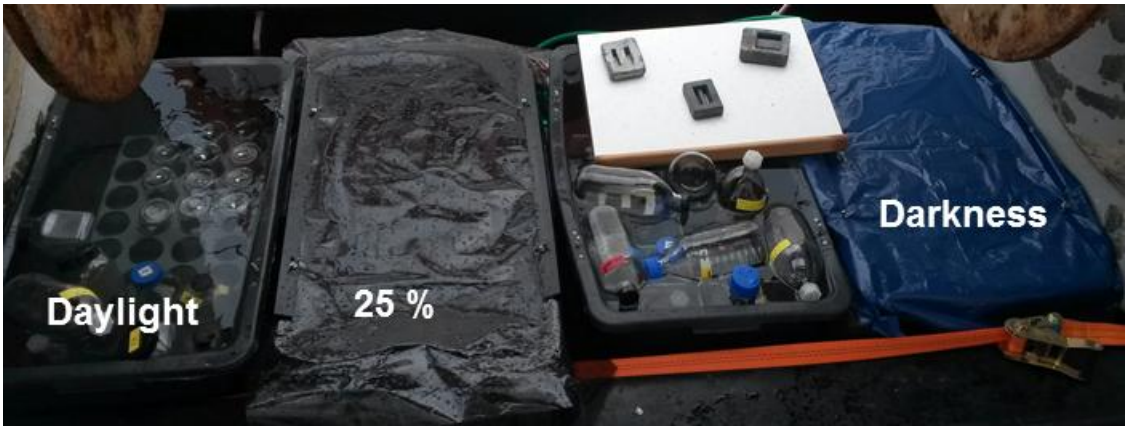


Figure. 12. Storage of the samples during the incubation with different light conditions

5. Moorings, scientific equipment and instruments

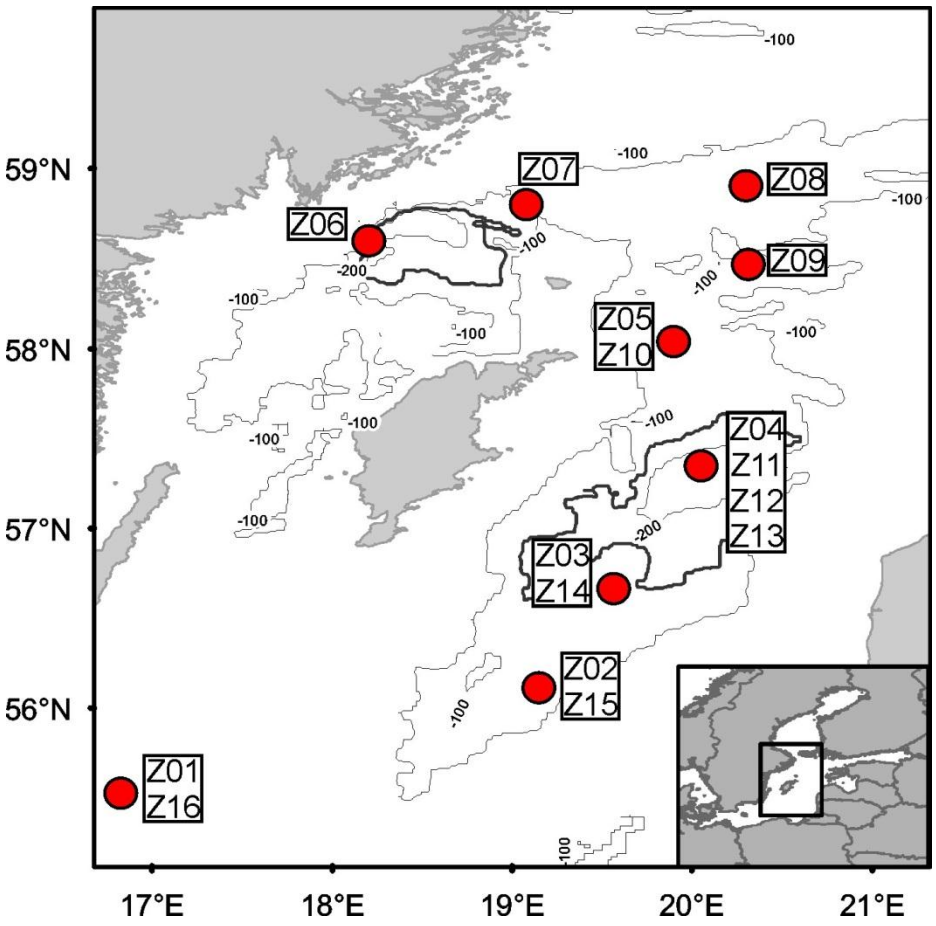
See chapter 4 in the specific method sections

6. Additional remarks

None

7. Appendix.

Station map



Station list

Date	Time (UTC)	Position		ZOOM station	CTD file
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	11:21	55 30.0070	16 50.0115	Z01_CTD02	V0002F02
	12:13	55 30.0070	16 50.0115	Z01_CTD03	V0002F03
	12:13	56 05.0067	19 10.0415	Z02_CTD01	V0003F01
	20:36	56 05.0067	19 10.0415	Z02_CTD02	V0003F02
	21:46	56 05.0067	19 10.0415	Z02_CTD03	V0004F01
08.08.2016	01:52	56 37.9983	19 34.9285	Z03_CTD01	V0005F01
	02:06	56 37.9983	19 34.9285	Z03_CTD02	V0005F02
	02:20	56 37.9983	19 34.9285	Z03_WP2_01	
	02:30	56 37.9983	19 34.9285	Z03_WP2_02	
	03:41	56 37.9983	19 34.9285	Z03_CTD03	V0006F02
	10:25	57 19.2209	20 03.0153	Z04_CTD01	V0007F01
	10:50	57 19.2209	20 03.0153	Z04_CTD02	V0007F02
	11:00	57 19.2209	20 03.0153	Z04_WP2_01	
	11:20	57 19.2209	20 03.0153	Z04_WP2_02	
	11:44	57 19.2209	20 03.0153	Z04_CTD03	V0007F03
	16:17	57 59.9811	19 54.0139	Z05_CTD01	V0008F01
11.08.2016	06:07	58 34.9638	18 13.9478	Z06_CTD01	V0009F01
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	07:29	58 34.9638	18 13.9478	Z06_CTD02	V0009F02
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	03:47	57 59.9823	19 53.9652	Z10_CTD03	V0013F03
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	08:56	57 19.1889	20 03.0068	Z11_CTD02	V0014F02
	09:00	57 19.1889	20 03.0068	Z11_WP2_01	

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	09:25	57 19.1889	20 03.0068	Z11_WP2_03	
	09:30	57 19.1889	20 03.0068	Z11_WP2_04	
	09:35	57 19.1889	20 03.0068	Z11_WP2_05	
	09:40	57 19.1889	20 03.0068	Z11_WP2_06	
	09:45	57 19.1889	20 03.0068	Z11_WP2_07	
	09:50	57 19.1889	20 03.0068	Z11_AP_01	
	10:00	57 19.1889	20 03.0068	Z11_AP_02	
	10:11	57 19.1889	20 03.0068	Z11_CTD03	V0014F03
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14.08.2016	06:00	57 19.1864	20 03.0349	Z12_AP_01	
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	11:20	55 29.9979	16 50.0039	Z16_WP2_03	
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	11:40	55 29.9979	16 50.0039	Z16_AP_02	
	11:50	55 29.9979	16 50.0039	Z16_AP_03	
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Abbreviations:

CTD	CTD cast (conductivity, temperature, depth) for physical water column analysis and to obtain water samples
WP2	Net hauls for plankton sampling
AP	Apstein net hauls forplankton sampling
FL	Frahmlot operations for sediment sampling

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