Cruise report 64PE408

NESSC Black Sea

Istanbul-Istanbul

January 28th-February 5th

Pelagia in Istanbul

Black Sea

Chief Scientist: Marcel van der Meer

Contents

1. Introduction 4
 1.1 Aim and background 4
 1.2 Scientific crew: 4
 2. NESSC Black Sea cruise 5
 2.1 Station 1 5
 2.2 Station 2 6
3. 64PE408: Black Sea, station 2, CTD 8
 3.1 Introduction 8
 3.2 Materials and Methods 8
  3.2.1 Sampling 8
  3.2.2 Sample treatment 8
 3.3 Evaluation 9
4. Filtering water for post-cruise nanoSIMS analyses of diatoms and coccoliths 11
5. Analysis of δD and δ18O of seawater with the Liquid Water Isotope Analyzer (LWIA) 12
 5.1 Running the LWIA onboard 12
 5.2 Irregularities 12
 5.3 Taking samples for post-cruise measurement of δD and $\delta^{18}O$ at NIOZ, Texel 13
  5.3.1 Discrete samples taken during transit 13
6. 64PE408 Multicores 15
 6.1 Station 1 – 15
 6.2 Station 2 – 15
7. Alkalinity 16
8. SIAM Black Sea cruise report 18
 8.1 Saturday 30-1-2016 18
 8.2 Sunday 31-1-2016 18
 8.3 Monday 01-02-2016 20
 8.4 Tuesday 2-2-2016 21
 8.5 Wednesday 3-2-2016 21
 8.6 Thursday 4-2-2016 22
9. In situ pumps summary 26
Appendix 1: 2,2 CTD sample list 28
Appendix 1, 2.2 NUTS sample list 29
Appendix 1: 2,2 DOC/TOC sample list 30
Appendix 1: 2,2 Chlorophyll sample list 30

Appendix 1: 2,2 POC/POP/PON sample list 31
Appendix 1: 2,19 CTD sample list 32
Appendix 1, 2.19 NUTS sample list 33
Appendix 1: 2,19 DOC/TOC sample list 34
Appendix 1: 2,19 Chlorophyll sample list 34
Appendix 1: 2,19 POC, POP, PON sample list 35
Appendix 2: 2,9 Clean CTD/GoFlow Anaerobic nuts 36
Appendix 2: 2,9 Clean CTD/GoFlow Anaerobic nuts sample list 37
Appendix 2: 2,15 Clean CTD/GoFlow Anaerobic nuts 38
Appendix 2: 2,15 Clean CTD/GoFlow Anaerobic nuts sample list 39
Appendix 3 PROTOCOLS 40
App 3.1 Water sampling from the regular CTD 41
App 3.1.1 Preserving water samples for FISH purposes and filtration 41
App 3.1.2 CARD-FISH fixation for sediment samples [Llobet-Brossa,Rosselló-Mora & Amann 1998] (Wageningen) 42
App 3.1.3 NH_4, NO_3, NO_2, Silicate and PO_4 42
App 3.1.4 DOC/TOC 43
App 3.1.5 DIC and δ13C of DIC 43
App 3.1.6 POC/PON/POP [Particulate organic carbon, nitrogen and phosphate] 44
App 3.1.7 Hydrogen isotopes 44
App 3.1.8 Alkalinity 44
App 3.1.9 Fixation of samples for Flow cytometry 44
App 3.1.10 Chlorophyll a 45
App 3.1.11 Protocol filtering seawater to obtain coccolithophore and diatom samples 45
App 3.1.12 Filtration Setup in wooden box – UU 47
General instructions for filtering: 48
App 3.1.13. Preparation samples for O_2 sensor calibration (first tap out of CTD bottle) 48
App 3.2.1 Methane in water samples 49
App 3.2.2. Sulfate/Sulfide/S/Fe/Metals etc in water samples 50
App 3.3.1 Core slicing with hydraulic slicer and bottom water sampling 52
App 3.3.2 Porewater extraction from cores (predrilled liner) 53
App 3.3.3 Porewater extraction and sediment sampling from gravity/piston core 54
App 3.3.4 Multicore methane sampling 55
App 3.4 Water sampling with In-situ pumps 56
App 3.5 “Anaerobic” In-situ pumps 57

Appendix 4: Chapter 8 directly obtained data 59
Appendix 4: Chapter 8 sample nomenclature and location 60
Appendix 5: Core description 61
Appendix 6 Casino summary 67

1. Introduction

1.1 Aim and background

This cruise is the second of a series of three cruises funded by the Netherlands Earth System Science Centre (NESSC) a Gravitation grant from the Dutch Ministry of Education, Culture and Science. This leg in the Black Sea is also partially funded by the gravitation grant SIAM (Soehngen Institute of Anaerobic Microbiology) as a collaborative effort between NIOZ (Prof. Jaap Sinninghe Damste) and the University of Wageningen (Prof. Fons Stams). The cruise is carried out by scientists from the NIOZ Royal Netherlands Institute for Sea Research, University of Utrecht and Wageningen University.

Specific goals of the project

To study the microbiology of the Black Sea in the water column and the sediment in high resolution by means of activity measurements, diversity estimations, and physicochemical characterization. In addition, we will collect samples for the determination of alkenone and alkenone producers both in the water column and sediments.

- We will estimate the microbial diversity and abundance of targeted groups by means of metagenomics and microscopy (FISH)
- We will collect samples for quantification of bacteria/viruses and phytoplankton in the water column by flow cytometry
- We will get material and start isolations of certain microbes of interest
- We will collect samples for DNA but also for lipid analysis to determine potential lipid biomarkers of microbial groups which could be then use to track the presence of that group in present and past systems
- We will estimate activity measurements of specific microbial processes
- We will complement this data with the analysis of specific compounds (physicochemical measurements) in the water column and sediments

Activities

- SPM collection from *in situ* pump for lipid and genetic analysis (16S rRNA gene amplicon pyrosequencing, DNA/RNA extraction selected quantitative PCR measurements, metagenomics in selected samples)
- CTD water for incubation experiments with labeled substrates on board
- CTD water for nutrient analyses
- Multicore: DNA/RNA, porewater, enrichments
- Piston core: porewater, lipid, DNA, microbial enrichments (Wageningen)

1.2 Scientific crew:

Marcel van der Meer, NIOZ	Chief scientist
Marianne Baas, NIOZ	In situ pumps
Anne Roepert, UU	CTD water filtration for nanosims, LGR etc. Gravity core description and sampling, and many other things

Ellen Hopmans	CTD filtrations
Gabriella Weiss, NIOZ	Multi-core, core slicing, alkalinity.
Saara Suominen, NIOZ	Incubation experiments
Daan	Microbiology/incubations
Irene	Microbiology/incubations
Rineke	Outreach, multi core slicing
Roald van der Heiden, Pelagia, NIOZ	Multi beam, 3.5 kHz, in situ pumps, sediment trap etc.

2. NESSC Black Sea cruise

Istanbul

Due to work on a bridge over the Bosporus our departure from Istanbul was delayed by one day. The pilot came on board on Friday 29-01-2016 at 23:15 local time and took us through the Bosporus without further delays.

Transit

30-01-2016: The transit went a bit faster than expected and just after lunch we arrived at station 1, formerly known as station 12. During transit the LGR was running, measuring the hydrogen and oxygen isotopic composition of seawater at the same time discrete samples were taken from the aquaflow system for measurements back in the lab.

2.1 Station 1

Multibeam

The first cast of after arrival was a multibeam cast combined with the 3.5 kHz system to get a good idea of the seafloor topography and sediment structure before starting to core. Station 1 was added to the Black Sea cruise especially for the coring since station 2, formerly known as station 1, has proven not to be so suitable for long cores. The original Station location was decided to fit the coring needs.

Multicore

Based on previous cruise reports (PHOXY) from the area 2X4 weights were removed from the top of the Multicorer. To cover all bases the multicore was loaded with 4 dividable cores, 4 regular slice cores and 4 archive cores. Fortunately we removed the weights since we retrieved relatively long cores with only 2 to 4 cm water on top, which made handling the core quite difficult. They were beautiful though. 3 cores were sliced in cm resolution for 60cm, one core was used as inoculum for microbial enrichments and the top 3 cm of 3 other cores were sampled specifically for alkenones.

Multicore in the hydraulic slicer.

Piston/gravity core

Because of the relatively soft sediment in combination with the predrilled liner a gravity core was taken instead of a piston core, same instrument, but now without the piston. The total length of the core was 502 cm. Through the predrilled holes an attempt was made to sample porewater using rhizons. This was more difficult than expected, the sediment here

looked like gelatine contained very little liquid water. However, after a few days it became clear that the valves connected to the rhizons used for this piston core were either the wrong type of valve (stoppers) or in the closed position making it impossible to extract pore water from the core. The core was sampled for inoculum for microbial enrichments, photographed and described. We predrilled the liner every 25 centimeter, for future reference, this is not very practical since the liner is cut every meter exactly on a hole covered with tape. Next time would be better to drill the first hole at 15 centimeters and then every 25 centimeters (see core description).

Transit

From Station 1 to Station, work at station 1 ended a bit earlier than expected and we arrived at Station 2 at 22:00 local time.

2.2 Station 2

In situ pump

At 23:00 we deployed 4 in situ pumps (cast 1), one anaerobic pump at 1000m and three regular pumps at 170, 250 and 500 meter all with a 0.3 μm filter. Unfortunately to save the debubbler from breaking it was left on the table and did not make it on to the anaerobic pump, which did not filter anything. The pumps were retrieved at 4 am on the 31st of January.

The next in situ pump deployment took place after a regular CTD cast (2) and a CTD cast (3) using the ultraclean CTD to collect 180 liter of water from 1000 meter and 6 liter from 500 meter for different incubation experiments.

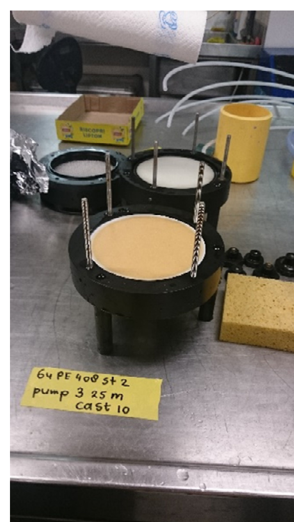
Cast 4, in situ pumps 4 pumps anaerobic pump at 500 meter, 3 regular in situ pumps at 105, 110, and 130 meters.

Cast 5, 3 regular in situ pumps at 5, 25, and 50 meters

Cast 6, 3 regular in situ pumps at 70, 80, 85 meters.

Cast 7, 3 regular in situ pumps at 1000, 1500,1980 meters, overnight to allow everyone to get some sleep.

Not all pumps pumped, some filters seemed clogged even with nothing visibly on the filter. Some filters were torn or cracked, some were bulging. Even though we continued to do the same thing more and more deployments became successful the next day, suggesting that it is either something with the filters or with the water. Initially it looked like deployments in anoxic water were more of a problem than those in oxic water, but this did not hold up after day 2.



In situ pump filter from 25m

01-02-2016: After two CTD casts starting at 8 in the morning, one (cast 8) to collect 220 liter of water for incubations and one (cast 9) to sample the water column anaerobically for different nutrients, sulfide etc, and water for microbial enrichments. More in situ pump deployments were carried out.

Cast 10; In situ pump (5, 25, 50m, 0.3 μm)

Cast 11; In situ pump (70, 80, 85m, 0.3 μm)

Cast 12; In situ pump (90, 95, 100m, 0.3 μm)

Cast 13; In situ pump (1000, 1500,1980 0.3 μm)

02-02-2016: Cast 14, Ultra clean CTD with go-flo bottles to collect 220 liter of water from approximately 50 meter for incubations. After this we had coffee and cake to celebrate Rineke's birthday. Cast 15 was the second deployment of the Ultra clean CTD to sample the water column anaerobically for different nutrients, sulfide etc.

Cast 16; In situ pump (105, 110, 130m, 0.3 μm)

Cast 17; Anaerobic in situ pump (1000m,0.3 μm) + 3X regular in situ pump (170, 250, 500m 0.3 μm)

Multicores, including predrilled archive cores.

Cast 18; In situ pump (1000, 1500, 1980m, 0.3 μm)

03-02-2016: Cast 19 was a regular CTD cast to collect water from 18 depths for nutrient analysis etc.

Cast 20; In situ pump (170, 250, 500m, 0.3 μm)

Cast 21; In situ pumps, including the anaerobic pump to collect samples from 5 meter depth, a depth we have very little material of. Deploying an extra pump saves an additional cast. (5, 90, 95, 100m, 0.3 μm)

The bridge under construction at the Black Sea end of the Bosporus

The last cast at station 2 was a multicore cast (22) to collect 3 cores for slicing (1 cm resolution), 3 predrilled cores for pore water sampling using rhizons (since Perspex is difficult to drill we used the plastic archive cores, 0.5 cm holes every 1 cm (2 cores) or every 2 cm (1 core) and 1 core predrilled with 2 cm holes every 5 cm (every 10 cm on both sides, overlapping) for methane sampling. On top of the 4 weights that were already removed for the first multi core cast an additional 2 weights from the center of the corer were removed. The multi core at this station was skipped since it was planned as trial multi core, multi coring at this site has been problematic in the past and transiting back to Istanbul to make the convoy through the Bosporus had priority. We left Station 2 at 18:30 Wednesday the 3de of February and were the last ship allowed to pass the Bosporus with the convoy the next morning. During transit and while anchored in front of the harbor CTD filtering continued as well as reorganizing samples and cleaning and organizing the lab space(s).

Back in Istanbul

3. 64PE408: Black Sea, station 2, CTD

Ellen Hopmans

3.1 Introduction

The water column at station 2 was sampled during 4 different casts using conductivity-temperature-depth (CTD) equipment. Two of these casts, nr. 2 and 19 were performed with the standard CTD-equipment, while casts 9 and 15 were performed using the CTD fitted with go-flow bottles enabling oxygen free sampling of the water column. In all cases 18 depths were sampled with special emphasis on the chemocline where sampling was performed at 5 m depth intervals.

3.2 Materials and Methods

3.2.1 Sampling

Casts 2 and 19: Bottles were closed according to the CTD sample lists (appendix. 1). Of cast 19, CTD bottle nr. 3 (depth 16) failed to close, no duplicate was available. In general, water was sampled in 4 ml glass vials and 12 ml exeteiners for (^{13}C-)DIC, and 125 ml and 2 L polyethylene bottles for processing for various analysis off board. For the 3 most shallow depths of both casts, an additional 2 L container was filled to allow for chlorophyll measurements. In case of cast 2, additional samples were taken in 100 ml glass bottles from CTD bottles 4, 15 (3x) and 21 for determination of oxygen levels to calibrate the O_2 sensors on the CTD, and water was sampled from each CTD bottle in 200 ml grease sealed glass bottles for alkalinity measurements on board (by titration or VINTA). In addition, A. Roepert collected material from the 3 most shallow depths to prepare filters for Nano-SIMS measurements (UU) (see chapter 4).
Sample waters from the polyethylene bottles were subdivided, preserved and stored for measurement at a later date off board of various nutrients, DOC and TOC, particulate organic carbon, nitrogen, and phosphorus, chlorophyll, and hydrogen isotopes. Samples were also prepared for quantitation of microbial abundance using Fluorescent In Situ Hybridization (FISH), and bacteria, viruses and phytoplankton by flow cytometry (off board). Overall the processing of the samples lasted 2 full days (processing times noted in appendix 1). Water samples were kept at 4 °C until processing.

Casts 9 and 12: Bottles were closed according to the anaerobic CTD sample list (appendix 2). All bottles closed for both casts. Bottles were not pressurized with N_2 to maintain anoxia during sampling, as only small volumes of water were sampled for each depth. Water was sampled from the go-flow bottles using connector tubing with 2 or 3-way valves, fitted with disposable needles. All tubing, connectors and needles were flushed and filled with water prior to sampling to ensure sampled water did not come into contact with oxygen. Water from each depth was collected in a vacuum blood tube for methane analysis and in 50 ml N_2 flushed glass bottles. To allow filling of the 50 ml bottle, the septum was pierced with an additional needle to allow venting of over-pressure. Samples were also collected by I. Sanchez (Chapter 8). Sample waters from the 50 ml bottles were subdivided, preserved and stored for analysis off board for sulfate, S, Fe and trace metals and sulfide. In general, samples were processed within 8 hr of sampling.

3.2.2 Sample treatment

Standard CTD: Sample waters from the polyethylene bottles were subdivided, preserved and stored for various analyses off board according to protocols provided by L. Villanueva (appendix 3). Only deviations from the provided protocols or noteworthy observations are noted below. Subsampling was performed in the order of the descriptions below; exact time of processing is noted in appendices relating to each cast.

DIC and ^{13}C-DIC: Water samples were preserved with saturated $HgCl_2$ solution. Before addition of $HgCl_2$, saturated $FeCl_2$ was added to samples to remove H_2S. Upon addition of $FeCl_2$ to H_2S containing water, a black FeS precipitate is formed immediately and the protocol prescribes drop-wise addition of the $FeCl_2$ until no visible reaction is observed.

It must be noted that the waters containing H_2S turned very black upon addition of $FeCl_2$, making it difficult to observe whether FeS was still being formed. $FeCl_2$ was therefore always added in excess. Samples were treated in order of decreasing depth. In the sample from depth 13 no visible reaction was observed and therefore no $FeCl_2$ was added to the remaining, more shallow, depths.

FISH: Samples were preserved and filtered according to the protocol. On the advice of Dr. I. Sanchez-Andrea, filters were not placed on a microscope slide and frozen immediately, but placed instead in a 25 mm id petri-dish and left to air-dry for 24 hr at room temperature before storage at -20°C.

Bacteria, viruses and phytoplankton: In case of cast 2, samples were treated according to protocol. At the time of cast 19, the supply of liquid N_2 was very low. It was thus decided to fix and collect all samples requiring flash freezing on 3/2/2016 to make best use of the remaining liquid N_2. However, no liquid nitrogen was left in the tank by the end of the day and therefore samples were frozen directly at -80°C after appr. 8 hr of storage at 4 C.

DOC/TOC: All samples were processed according to the protocol. However, due to lack of amber TOC vials, depths 4 and 6 were not sampled.

Hydrogen isotopes, N/P, Si: All samples were processed according to the protocol. One acrodisc filter was used per depth.

Chlorophyll: All samples were processed according to protocol.

POC/PON: In all cases, filtration proceeded easily and 2000 ml of water was filtered. In some cases there was not enough water available and a lesser amount was filtered (see appendix 1)

Go-flo CTD: Water was collected in vacuum blood tubes for methane analysis and 50 ml N2 flushed glass bottles. Water from the 50 ml bottles was subdivided, preserved and stored for analysis off board for sulfate, S, Fe and trace metals and sulfide according to protocols provided by L. Villanueva (appendix 3). Only deviations from the provided protocols or noteworthy observations are noted below. Subsampling was performed in the order of the descriptions below; exact time of processing is noted in appendices relating to each cast.

Methane: According to the protocol, the blood vacuum tubes had to be prepared with three drops of HCl before filling with water. It was impossible to measure out 3 drops of acid in a syringe and dosing it into the tubes without transfer of air or dosing too much acid as the syringe is emptied uncontrollably into the vacuum. Therefore, 3 drops of acid were dispensed into an eppendorf tube with a Pasteur pipette, sucked up into a syringe with as little air as possible and the syringe was allowed to empty into the vacuum tube. It was unfortunately unavoidable to transfer some air into the vacuum tubes during addition of the acid.

S/Fe/Mn/trace metals: In general, samples were prepared according to protocol. In some cases sample water was briefly exposed to air before fixing due to malfunctioning of the 50 ml bottle septum. These instances are noted in appendix 2. After removal of the first few ml of water from the 50 ml bottle, a vacuum would result in the bottle making it a very slow process to draw more water for further subsampling.

Sulfide: According to the protocol 1 ml of water had to be preserved with 50 µl Zn-acetate solution to fix the sulfide. For the samples of cast 9, the prescribed volumes were doubled to allow the use of 2 ml cuvettes (1 ml cuvettes were not present). However, at the initial try of determining the sulfide on board (By Dr. I. Sanchez-Andrea) it appeared the chemicals had degraded and had lost activity. It was therefore decided to store the preserved samples for later analysis off board. For the samples of cast 12, the 1 ml volume according to the original protocol was preserved.

3.3 Evaluation

In general, sampling and sample treatment for the standard CDT went according to plan. However, in order to allow a more efficient workflow it would be advisable to practice some of the protocols at the home laboratory before

start of the cruise to become familiar with materials and methods. Sample processing was also slowed down considerably due to a lack of fume hood space (as only one fume hood was available while multiple protocols, performed by several scientists, called for the use of a fume hood) and the lack of test tube racks and storage boxes for the samples being processed and for final storage. In some cases not enough materials were available or supplies were very tight (TOC vials, PBS buffer) or unusable materials (insulin syringes with needles too short to puncture the septum of blood vacuum tubes) were provided. Because of the large amount of depths to be sampled in duplicate, the inefficiency of the workflow, processing all samples took approximately two 16 hr work days in case of the regular CTD. As there was no transit time between stations, casts were scheduled in rapid succession with the result that in some cases processing of one set of samples was not finished before the next cast was deployed (in case of cast 2 and 9). On paper, all scientist were assigned a primary and secondary task. However in practice, due to the vast amount of work planned, all scientists were fully occupied by their primary tasks. In addition, the handling of the anaerobic CTD samples was not assigned to any of the scientists as primary task. It would therefore be advisable, for future cruises, to carefully examine the work load and either allow more time or arrange for more persons.

4. Filtering water for post-cruise nanoSIMS analyses of diatoms and coccoliths

Anne Roepert

For nanoSIMS analyses, diatoms and coccoliths were targeted to be sampled. For each depth, three different volumes ranging between approximately 500 ml to 2000 ml were filtered through 25 mm 5 µm polycarbonate filter to target diatoms. The filtrate was collected and three volumes ranging between 10 ml and 2000 ml were filtered through 25 mm 0.4 µm polycarbonate filters mounted onto a 25 mm GFF support filter to target coccoliths. For each depth, the remaining water in the jerrycan (between 4 – 7 litres) was filtered over one 47 mm 0.4 µm polycarbonate filter mounted onto a 47 mm GFF support filter as a backup.

During cruise 64PE408 in the Black Sea, nanoSIMS samples were only taken at station 2 from cast 2. The following table provides an overview over the volumes filtered.

Cruise number: 64PE408	
Date:	31-02-2016
Station:	2
Cast:	2

depth #	depth [m]	CTD bottle #
1	20	5
2	18	25
3	16	50

DEPTH 1	DIATOM FILTERS		COCCO FILTERS		BULK FILTER	
	volume [ml]	time [UTC]	volume [ml]	time [UTC]	volume [ml]	time [UTC]
V1	2000	15:33	50	15:45	1240	17:47 ***
V2	975	16:57	99	15:57 *		
V3	525	17:03	430	17:16 **		
			10	17:40		

DEPTH 2	DIATOM FILTERS		COCCO FILTERS		BULK FILTER	
	volume [ml]	time [UTC]	volume [ml]	time [UTC]	volume [ml]	time [UTC]
V1	2000	07:43	10	07:46	2820	8:35
V2	1000	08:15	50	07:52		
V3	475	08:20	100	08:05		

DEPTH 3	DIATOM FILTERS		COCCO FILTERS		BULK FILTER	
	volume [ml]	time [UTC]	volume [ml]	time [UTC]	volume [ml]	time [UTC]
V1	2000	09:25	9	09:30	5220	10:19
V2	1000	09:53	49	09:48		
V3	475	10:05	98	10:02		

* piece of black 'dust' on filter. Where introduced? Everything handled rinsed and caps on filtration unit and measurement cylinder during filtration…

** Filter 'clogged', only running drop by drop and coloured yellow!

*** Filter running drop by drop, completely yellow… Therefore, another filter was prepared with substantially less volume.

5. Analysis of δD and δ18O of seawater with the Liquid Water Isotope Analyzer (LWIA)

Anne Roepert, Gabriella Weiss, Marcel van der Meer and the rest of the scientific crew

5.1 Running the LWIA onboard

The LWIA was for this cruise specifically equipped with an in-house made flow-through cell allowing semi-continuous stable water isotope analyses during transits. Each sample was preceded by 3 preparatory injections which were followed by 6 sample injections that were analyzed.

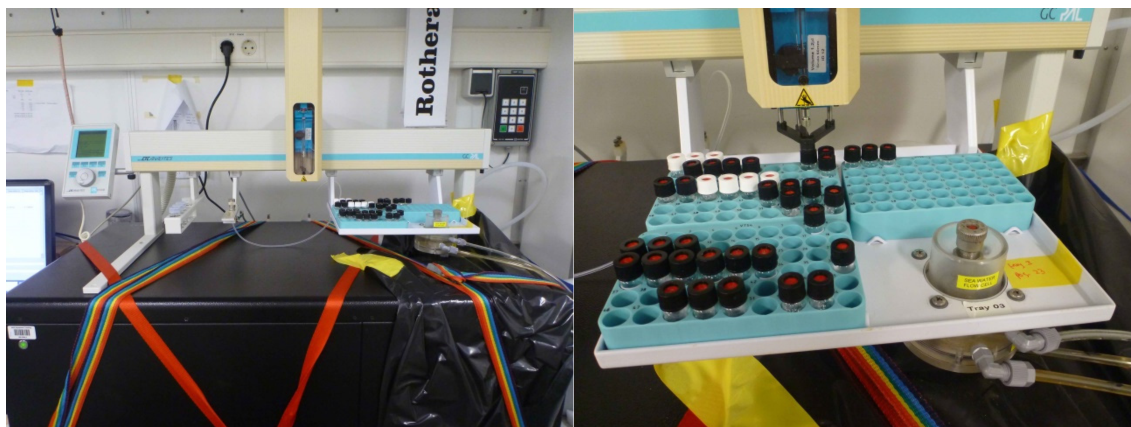


Figure 1. The LWIA mounted in the container. The flow-through cell is located on the tray in the right.

Before starting a run, the LWIA underwent a cleaning procedure to remove salt particles that accumulated during the previous run. In general, the cleaning procedure involved rinsing the syringe at the end and before the start of each run in the washing solution (bidest) at least 5 times. It was observed, that the motor of the syringe arm got stuck relatively often in the position of the washing solution. When this occurred, the syringe was ordered to move to 'check needle position' position (over the injector port) or to 'zero'. During vented cavity mode in the 'change septum' program, the injector port was taken off, rinsed with milliQ, dried, and the septum replaced. The tubing underneath the injector port was taken off at the port and on the back panel. The tubing was separated from the screen filter and rinsed with milliQ and dried. After all parts were completely dry, everything was put back together. To minimize the risk of a bent syringe, the needle position was checked with the 'check needle position' order and the injector port position adjusted so that the needle was centered. If needed, the septum of the flow-through cell was replaced or empty standards were replaced by new ones.

5.2 Irregularities

The analysis of water from the flow-through cell was started after leaving port in Istanbul on the 30th of January. Everything went well until on the morning of the 2nd of February, the needle was discovered to be bent and the plunger encrusted in salt. The LWIA underwent the cleaning procedure and the needle was replaced by a new one. From this moment on, any further attempts to run the LWIA resulted in red 'pres' flags displayed along with the measurements.

The problem was just realized towards the end of the cruise: a thick salt crust (mixed with pieces of septum) had built up inside the injector port which blocked it completely.

Table 1 Overview of runs of the LWIA during cruise 64PE408 in the Black Sea.

Date	Type	Data file name	Note
30-01-2016	samples, transit 1	lwia_2016-01-30_f0000.txt	Run started at 01:00 UTC.

31-01-2016	samples, station 2	lwia_2016-01-31_f0000.txt	Run started at 18:22 UTC.
01-02-2016	samples, station 2	lwia_2016-02-01_f0000.txt	Run started at 18:14 UTC. On 02-02-2016 at ~ 06:00 UTC the syringe was discovered to be bent. The plunger of the syringe was covered in salt.
02-02-2016	samples, station 2	lwia_2016-02-02_f0000.txt	Run started at 7:55 UTC. Pressure flags!
02-02-2016	standards	lwia_2016-02-02_f0001.txt	pressure flags
02-02-2016	standards	lwia_2016-02-02_f0002.txt	pressure flags.
02-02-2016	standards	lwia_2016-02-02_f0003.txt	Cleaned filter with N_2. Started at 17:26 UTC. Pressure flags.
02-02-2016	standards	lwia_2016-02-02_f0004.txt	Replaced septum. Started at 17:51 UTC. Pressure flags.
03-02-2016	standards	lwia_2016-02-03_f0000.txt	Cleaned needle seat. Pressure flags.
03-02-2016	standards	lwia_2016-02-03_f0001.txt	Started at 21:34 UTC. Pressure flags.
03-02-2016	standards	lwia_2016-02-03_f0002.txt	pressure flags.
03-02-2016	standards	lwia_2016-02-03_f0003.txt	pressure flags.

5.3 Taking samples for post-cruise measurement of δD and $\delta^{18}O$ at NIOZ, Texel

As a backup to the continuous onboard-measurements of hydrogen and oxygen isotopes from water with the LWIA, discrete samples were taken during transits (the aimed sampling interval was each hour) and from CTD bottles at each station.

5.3.1 Discrete samples taken during transit

Samples were taken from a split line of the aqua-flow pump connection that serves the flow-through cell of the LWIA. Sample glass vials (2 ml) were rinsed three times with sample and then filled overflowing. The time of sampling (UTC) was noted to be able to relate the samples to the data of the continuous CTD measurements. The sampling interval was aimed to be each hour. Transect samples were labelled T*i.j* with *i* being the transit number (towards the respectively numbered station) and *j* being a consecutive index of all samples taken during the respective transit. Samples were stored at 4°C. Table 2 lists all samples that were taken on this cruise.

Table 2 Samples taken for post-cruise δD and $\delta^{18}O$ measurements during cruise 64PE408.

TRANSIT 1		
ID	Date	Time*
T1.1	30-01-2016	00:58
T1.2	30-01-2016	02:00
T1.3	30-01-2016	02:57
T1.4	30-01-2016	03:56
T1.5	30-01-2016	04:56
T1.6	30-01-2016	05:57

TRANSIT 2		
ID	Date	Time*
T2.1	30-01-2016	18:53
T2.2	30-01-2016	19:52
T2.3	30-01-2016	20:58

Station 2 (time series)		
ID	Date	Time*
S2.1	31-01-2016	18:12
S2.2	01-02-2016	06:08
S2.3	01-02-2016	12:00
S2.4	01-02-2016	17:59
S2.5	02-02-2016	05:59
S2.6	02-02-2016	12:00

TRANSIT 3		
ID	Date	Time*
T3.1	03-02-2016	18:21
T3.2	03-02-2016	18:57
T3.3	03-02-2016	19:57
T3.4	03-02-2016	20:57
T3.5	03-02-2016	21:58
T3.6	03-02-2016	23:04

T1.7	30-01-2016	07:00
T1.8	30-01-2016	07:57
T1.9	30-01-2016	08:57
T1.10	30-01-2016	10:00
T1.11	30-01-2016	10:59
T1.12	30-01-2016	11:55

S2.7	03-02-2016	06:10
S2.8	03-02-2016	12:00

T3.7	04-02-2016	00:00
T3.8	04-02-2016	01:00
T3.9	04-02-2016	01:59
T3.10	04-02-2016	02:49
T3.11	04-02-2016	03:57
T3.12	04-02-2016	04:56
T3.13	04-02-2016	06:00
T3.14	04-02-2016	07:03

* Time is given in UTC.

6. 64PE408 Multicores

Gabriella Weiss

6.1 Station 1 –

Multicoring was successful at station 1. Weights were removed from the multicorer based on previous knowledge of sampling at this location, but still more weight could have been removed. Cores came up completely full with very little bottom water on top. Adding rubber stoppers to the cores was difficult in some cases and a few cores were lost. The cores with the least amount of bottom water were chosen for slicing in order for stoppers to be used and make transportation of cores possible. Three cores measuring ~55cm were sliced at 1cm resolution and stored in geochemical bags at -80°C for lipids and DNA analyses. 5mL of sediment was sampled from a separate core at 1cm resolution for 20cm and stored in pots at -20°C for future porosity/carbonate measurements. The top 6cm of three other cores were sampled for alkenone δD measurments and stored at -80°C. A final core was stored as an archive core at 4°C for Anne Roepert.

All cores were approximately 60cm in length, but only sliced until ~55cm because of the bottom stopper. The top 5-6cm of the cores was very soupy, fluffy sediment, but became more condensed around 6cm when coccolith ooze layers dominated the sediment. Scattered throughout the cores at varying depths were a number of fish bones. Around 25cm, coccolith ooze had seeped down and covered the space between the coring tube and the core. The inner part of the sediment became darker black and much drier than the previous layers. Around 40cm, the sediment changed to a more rich brown color and became more coherent and slightly more difficult to separate. From 48cm down, the outer edge contaminated with the coccolith ooze from the upper layers was removed prior to storing in a geochemical bag.

6.2 Station 2 –

Multicoring was successful at station 2. Cores were collected for porewater sampling and for slicing for future DNA and lipid analyses. Cores were approximately 45cm in length. The top 4 cm of the cores was very watery and fluffy sediment that was sampled in 1cm intervals using a cut-off syringe. At 9cm, the sediment became more condensed and sticky coccolith ooze layers. There was a lot of water between the edges of the coring tube and the cores, therefore the outside of each 1cm slice was removed before putting into geochemical bags. At 23cm, the sediment changed to dark brown with black layers throughout. A pinkish, purple layer was present on core 1 at 37-38cm, core 2 at 36-37cm and core 3 at 38-40cm. Following this pinkish, purple layer the sediment was very sandy and rocky for a couple mm and then switched to be thick grey clay. One archive core was stored for potential future analyses.

7. Alkalinity

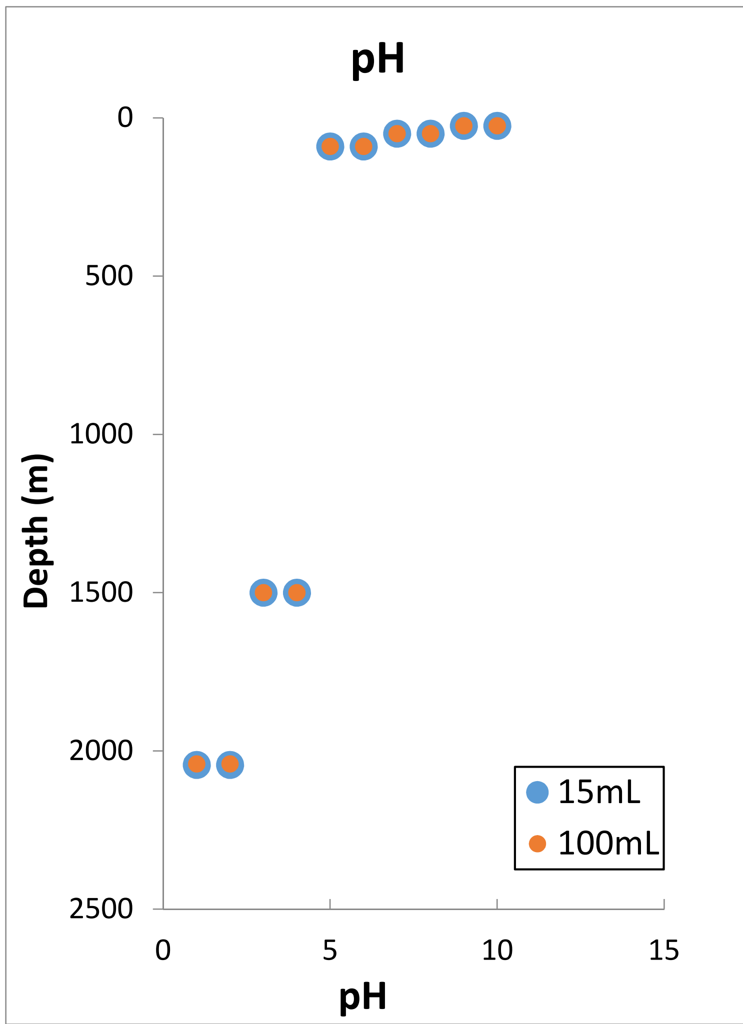
Gabriella Weiss

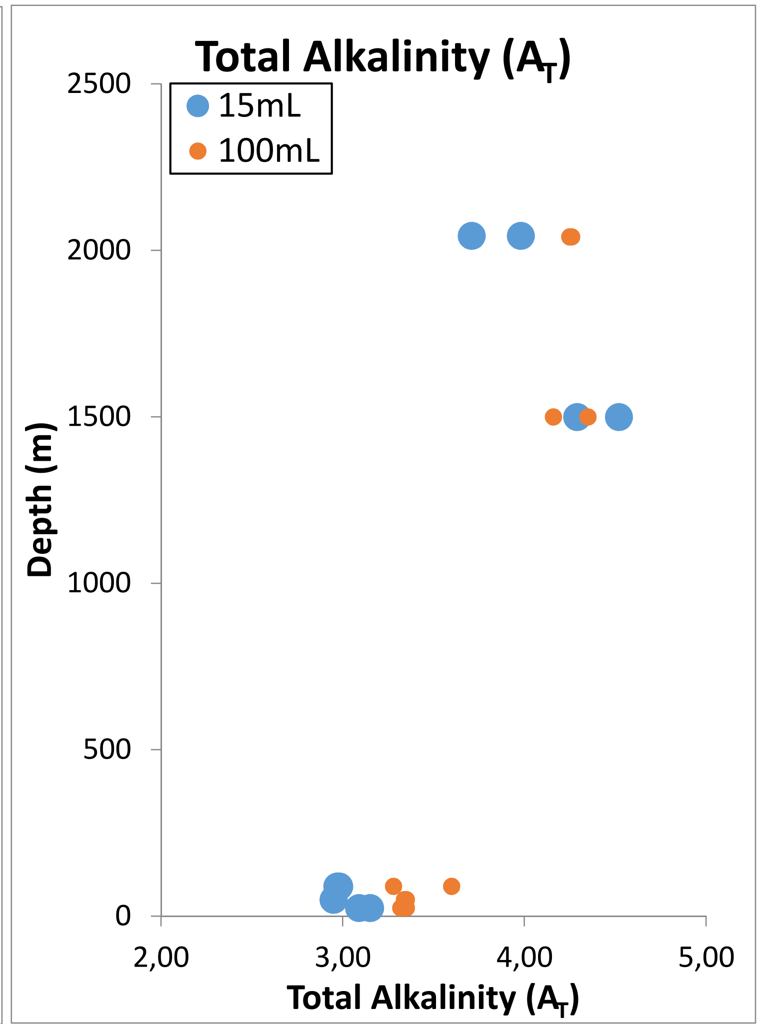
At station 2, samples were collected from the CTD (cast 2) for future analysis on the VINDTA in 250mL glass bottles, sealed with grease, stoppered and stored at 4°C. 125mL samples were taken at 5 depths (2044, 1500, 90, 50 and 25m) for total alkalinity measurements by titration. Titration was conducted using a DMP Titrino 785. Aliquots of 15mL were used for the titration and two samples were analyzed for each depth. Points were recorded manually as a result of technical difficulties. 0.01 M HCl was added until samples reached a pH of ~4.5. A_T values did not fall into an acceptable range for alkalinity, so titrations were repeated. 250mL samples were collected from the Ultra Clean CTD at the same depths two days later and aliquots of 100mL were titrated to a pH of 3.5 with 0.01 M HCl to determine total alkalinity and check the results of the previous titrations using larger volumes to increase accuracy. Total alkalinity (A_T) was determined using the equation:

$$F = (V + V_0)*10^{-pH}$$

Where V is the amount of HCl added and V_0 is the sample volume. A_T results are reported in milli molar (mM). See table 1 for A_T results. Following the second round of titrations, A_T values were still quite different than expected for the Black Sea. The molarity of the HCl was tested using reference material for oceanic CO_2 measurements, Batch 144. 20mL of Batch 144 reference material was titrated three times and all three times yielded very similar values. The molarity of the HCl was found to be 0.048 M. The A_T equation was corrected for this error and A_T values looked more reasonable. pH was the same for both days.

Sample	CTD Cast #	Depth (m)	Volume (mL)	Start pH	End pH	Amt HCl added (mL)	A_T (mM)	A_T corrected
1	2	2044	15	7.5	4.44	1.204	0.777	3.71
2	2	2044	15	7.61	4.46	1.308	0.837	3.98
3	2	1500	15	7.59	4.43	1.458	0.945	4.52
4	2	1500	15	7.54	4.42	1.418	0.906	4.29
5	2	90	15	7.56	4.47	0.976	0.624	2.98
6	2	90	15	7.55	4.45	0.964	0.623	2.97
7	2	50	15	8.00	4.46	0.958	0.618	2.95
8	2	50	15	7.95	4.43	0.962	0.617	2.95
9	2	25	15	8.21	4.44	1.028	0.664	3.15
10	2	25	15	8.17	4.42	1.01	0.648	3.09
1	15	2041	100	7.51	3.49	9.848	0.891	4.25
2	15	2041	100	7.46	3.49	9.88	0.891	4.26
3	15	1500	100	7.44	3.49	9.692	0.871	4.16
4	15	1500	100	7.48	3.49	10.142	0.910	4.35
5	15	90	100	7.56	3.49	7.866	0.688	3.28
6	15	90	100	7.56	3.49	7.826	0.753	3.60
7	15	50	100	8.06	3.49	7.996	0.701	3.35
8	15	50	100	8.08	3.49	7,954	0.699	3.34
9	15	25	100	8.07	3.49	7,938	0.696	3.32
10	15	25	100	8.10	3.49	7,964	0.701	3.35


pH
Depth (m)
0
500
1000
1500
2000
2500
0
5
10
15
pH
15mL
100mL


Total Alkalinity (A_T)
2500
2000
1500
1000
500
0
Depth (m)
15mL
100mL
2,00
3,00
4,00
5,00
Total Alkalinity (A_T)

8. SIAM Black Sea cruise report

Irene Sanchez Andrea, Daan M. van Vliet

For directly obtained data and sample nomenclature and location, see the appendix 4.

8.1 Saturday 30-1-2016

We are travelling to station 1. This station was previously referred to as station 12, for instance during the NIOZ cruise of 2013. Our schedule:

14:00 cast 1:multibeam + 3.5 kHz

15:30 cast 2: CTD (profile 1960 m)

17:00 cast 3: multicore (4 cores sliced (3 NIOZ, 1 Wageningen), from 12 sliced cores select 4 best.

19:00 cast 4: Gravity core (predrilled for pore water every 25 cm, needs to be opened to take pictures and sediment samples). Gravity core to avoid imploding the liner. Does need to be long enough.

20:30 Transit to Station 2, arrival 30/01/2016 around 24:00

The gravity core was successful, with 5 meters of depth (core description appendix 5). These were sectioned into semicores of 1 m each. We named the sections from bottom to top, 1 to 5 (1 is the deepest). We opened holes in the tapes with clean paper clips and put the redox probe inside, but this did not work so well. We put rhizons through the same holes. Afterwards, we opened bigger holes for the redox probe, and this time it worked better. The rhizons did not produce any noticeable amount of porewater after a couple of hours, so we left the 5 semicores overnight at 4 C inside anaerobic bags with anaerogen bags. Caroline Slomp already advised us that it could take up to seven hours to get porewater from deeper layers with rhizons, but a look in cruise reports of previous Black Sea cruises revealed that previously porewater was retrieved not with rhizons, but rather through centrifugation. In the current cruise, we had no centrifuge available.

8.2 Sunday 31-1-2016

The schedule:

00:30 cast 1: anaerobic in situ pump (1000 m, 0.3 um, 2 for NIOZ -80 C)

04:30 cast 2; CTD aprox 6:15 on deck (profile 2000m, sampling water 18 depths incl. oxic water for Anne Roepert. Depths (m): 5, 25, 50, 70, 80, 85, 90, 95, 100, 105, 110, 130, 170, 250, 500, 1000, 1500, 1980

07:15 cast 3: Ultra Clean GoFlo anaerobic CTD (500, 1000 m).

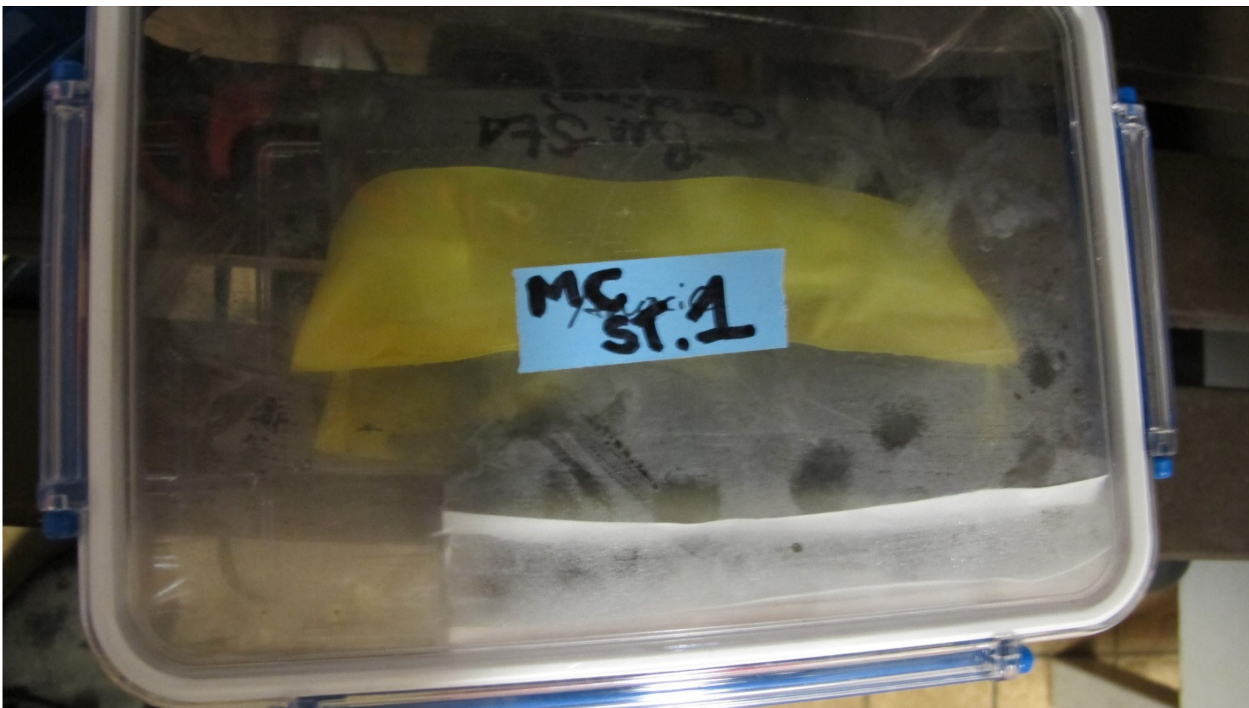
12:05 cast 4: anaerobic in situ pump (500 m, 0.3 um, 1 filter for nioz, 1 filter for WUR). On deck around 16:00.

22:30 cast 7: in situ pump (1000, 1500, 1980 m, 0.3 um, half of each filter for WUR). Retrieved next morning at 8:00.

At 5:00 am we started analyzing the cores. The rhizons did not produce any porewater, the sediment is too clay-ish. We cut open them in the middle with an angle grinder, slid one half over the other and positioned them both open. Normally, one half was cleaned and used for the geological description and the other half we used for sampling for enrichments, backups, FISH or DNA/RNA (Saara).

Semicore 4 (100-200cm) was directly transferred inside an anaerobic glovebag, the glovebag was flushed again, and an anaerogen bag was open inside to avoid trace oxygen. This was all done in our laboratory container, which was kept at 10 C. With cut-open syringes, sediment was transferred from core to 500 mL anoxic Black Sea (BS) medium in 1 L bottles. Three bottles were filled with approximately 200 mL sediment per bottle. The sediment used corresponded to 130-200 cm depth. Bottle stayed colorless confirming the anaerobiosis of the bag. Bottles were pressurized to 1.5 bar with nitrogen.

In the meantime, back up sediment was transferred from all the nine described depths (see excel file), to geological bags and closed. All the sediment bags were transferred to an anaerobic box and anaerogen bag was opened and put in the box to create an anoxic atmosphere. Box was kept at 4C.



Sediment samples of 0.5 mL were fixed in 1.5 mL of 6% PFA (for final concentration of 4%) for FISH analysis. Fixation lasted for about 7 hours at 4 C (ideally is 4h), then they were washed twice with PBS and resuspended in PBS:ethanol. Stored at -20 C.

Four sterile, anoxic bottles were filled with 800 mL of anoxic water from 500 m depth. The UltraClean GoFlo CTD system consisted of large sealed GoFlo bottles, which are pressurized with nitrogen on top and sampled at the bottom through valves. The tap was opened at the bottom, a needle was connected by a tube, and the bottles were filled. An extra needle was stuck through the bottle stopper to release pressure. These bottles were brought to our 10 C lab container for further operations. 0.8 mL of sterile resarzurine stock (1000x) was added to each bottle to check anoxic conditions. At the beginning the bottles were blue but after a few hours they became first pink, then colorless. To one of the bottles 1 mL of 1M Na_2S was added, corresponding to about 30 mL of buffer/reducing agent solution (1M carbonate, 30 mM sulfide), since this bottle did not turn fully colorless. It quickly did afterwards. 40 mL of buffer/reducing agent solution was added to one of the other three bottles. This bottle could then be used for insertion of an anoxic filter with concentrated biomass from 500 m depth, and subsequent pre-incubation of a month at the in situ temperature (around 9 C). All bottles were pressurized to 1.5 bar with nitrogen.

The anoxic filtration unit from 500 m depth was brought on deck, transferred to a bucket and taken inside the anaerobic glove bag in our 10 C laboratory. The glove bag was flushed twice and an anaerogen bag was open inside. The device was connected through a tube to a bottle and to a vacuum pump. After all the water on the top of the device was pumped through, the filter was opened, folded and introduced in the anoxic buffered sea water corresponding from 500 m water column. Our initial plan of scraping off the biomass was not successful.

8.3 Monday 01-02-2016

Schedule:

8:00 in situ pumps from yesterday arrive on deck.

10:30 cast 9: Ultra Clean GoFlo anaerobic CTD (all 18 depths). Arrives on deck around 11:30

When the GoFlo CTD arrived on deck, water from all 18 depths (see excel file) was transferred anoxically to anoxic, sterile 250 mL bottles, with the same procedure as yesterday. These bottles were transferred to our 10 C laboratory container. These bottles were then subsampled for polysulfide, sulfur compounds, volatile fatty acids (VFA) and redox potential/pH analyses. Subsampling for polysulfide analysis was done as soon as possible, directly after sampling. 5 mL was taken with a 5 mL syringe and filtered over a 0.2 um standard filter into a clean, anoxic 5 mL glass vial, giving a final volume of around 4 mL. Pressure was released by brief insertion of another needle. These samples were stored at -20 C. Secondly, exactly 10 mL of each water column sample was taken with a 10 mL syringe and transferred to a falcon tube (15mL) previously filled with 500 uL of ZnAc (5%), for sulfur compounds analysis. Simultaneously, 5 mL was subsampled in 5 mL plastic “pony” vials for VFA analysis, and 2.5 mL was subsampled into pony vials for redox potential and pH. The sulfur compound and VFA samples were stored at -20 C.

Redox potential was measured directly after subsampling (at 10 C), and pH some hours later. However, redox potential measurements proved to be inaccurate, needing more equilibration time (around 20 minutes) than was provided on this day. Also, pH measurements were erratic, shifting from 6.5 to 7.5. The pH measurements of Gabriella Weiss will be stored as data instead.

Sulfide analysis was unsuccessful. 2 mL of sample from all depths was mixed with 80 uL of reagent. The reagent provided, which should contain 4 g L^{-1} of diamine and 6 g L^{-1} of $FeCl_3$ hydrate in 18% w/v HCl, did not give satisfactory results when compared to previous analyses in the WUR lab. Instead of turning colorless, the blank stayed a brownish pink. Samples containing a sufficient amount of fixed sulfide – based on robust measurements at equal depths during previous cruises – did not develop a blue color. Sulfide analysis will be done in the WUR lab with frozen fixed samples instead.

At 13:30, filters with concentrated biomass from 5, 25 and 50 m depth arrived on deck. There was a miscommunication between WUR, Laura Villanueva, and Marcel, regarding the filters of which the WUR was to receive half of, as cultivation backups. In the general cruise description, it was mentioned that Wageningen could receive half a filter from each depth, while in the excel overview of the cruise apparently this was not included. Laura informed that she was under the impression we would only take half a filter as backup for the six lowest depths, depths 13 to 18, or 170 to 2000 m. We have kept to this last agreement, but before this was clear, half a filter from 5 m depth was already taken by us. This was done by placing the filter on aluminum foil with clean forceps, folding the

filter twice to form a quarter, folding it back once, cutting the filter in half over the folding line, and inserting half the filter quickly into a 250 mL bottle containing sea water from the corresponding depth. The bottle was then pressurized with 1.5 bar nitrogen. This procedure was also applied to the other depths that were sampled later.

8.4 Tuesday 2-2-2016

Schedule:

> 10:30 cast 15: Ultra Clean GoFlo anaerobic CTD (all 18 depths). Arrives on deck around 11:30
>
> 17:00 cast 17: in situ pump (170, 250, 500 m, 0.3 um, half of each filter for WUR). Arrives on deck around 22:30.

Around 8:00 the filters for depths 16, 17, 18 (1000, 1500 and 1980 m depth) arrived. They were folded, cut and stored in 250 mL bottles containing seawater from the corresponding depth.

GoFlo anoxic column water was sampled from all depths, in the same way as described yesterday, but now in anoxic, sterile 117 or 250 mL vials. These samples were regarded as “duplicate” samples of the samples of the day before. The primary samples were thus coded “I”, the duplicates “II”. The same subsampling as yesterday was performed, and the samples were stored accordingly. A better redox measurement was performed, with longer equilibration time (20 min). oxygen intrusion did not significantly affect the redox potential in this timespan, as experimentally verified.

All cultures (station 1 gravity core sediment cultures, station 2 seawater cultures) were covered with aluminum foil and stored in covered boxes to prevent light exposure and growth of phototrophs.

Filters with concentrated biomass from depths 14 and 15 was obtained and folded, cut and stored. The filtration unit for depth 13 (170 m depth) failed and will be repeated tomorrow. We decided not to use the 800 mL seawater culture amended with a filter from 500 m depth from the anoxic in situ pump, but to use the 250 mL backup culture amended with half a filter from the same depth instead for further cultivation efforts. This was decided since the conditions of the non-anoxic in situ pump are actually pretty anoxic, even though there is some exposure to air while folding and cutting. The problem with the filter from the anoxic in situ pump was that the conditions inside the glove bag were not sterile, and sediment residues may have come in contact with the filter. This is deemed more problematic than the slightly oxic conditions of the non-anoxic filter.

8.5 Wednesday 3-2-2016

Schedule:

> 11:00 cast 20: in situ pump (170, 250, 500 m, 0.3 um, WUR gets half of the 170 m filter) arrives on deck around 15:00.
>
> 17:00 cast 22: Multicore (3 cores for anoxic sediment sampling by WUR, 3 predrilled cores for porewater (2 for WUR, 1 for NIOZ), 3 sliced cores (NIOZ, also for WUR CARD-FISH samples), one archive core predrilled with large holes for methane). 2 additional weights were removed to lessen penetration into the sediment; at station 1 this was too much.

Half a filter with concentrated biomass was successfully obtained from depth 13 in the second try (170 m depth). The filter was folded, cut and stored as described previously.

An anaerobic glove bag was cleaned with MilliQ water and sterilized with halamid solution, so that it can be used for multicore sediment transfer to culture bottles with medium.

Multicore sampling was successful. We started sampling for methane. The core had 2.2 cm diameter holes, 5 in each side, 10 in total. Cut-syringes were introduced in the holes (from top to bottom) and the sediment extracted was introduced in a bottle prefilled with NaCl solution. The diameter of the bottle was not optimal and some sediment would smear out of the bottle. Bottles were closed with the rubber stoppers with the help of a needle to be able to close them with water to the bottle (minimizing gas space). The first two holes (1 and 2) were mainly water. The third one (located 16 cm from the top) was the first one with good sediment (pictures in Gabriella's camera). From there on, just worth to mention that hole 5 and 7 were a bit waterish too and the hole 11 corresponded exactly to a nice layer that we identified in the multicore slicing: dark sediment separated by a pink layer from the bottom grey layer. We presumed this could be purple bacteria buried.

For the rhizons, based on the methane extraction observation, we started to collect our sample 1 from the top 16 cm of the PVC core with 2 cm resolution. We sampled two cores in this way (NIOZ-WUR) and the third core (WUR) had samples with 4 cm resolution, keeping the nomenclature of the other cores, that is (MC-St2-Sx, 1-3-5-7-9-etc). Samples were taken from core A for sulfide, VFA, metals and silicates* and from core B sulfide again, nitrogen species, and DIC. We will give the samples for metals, silicates*, DIC and nitrogen species to Ellen.

-The rhizon from 19 sample (don't confuse it with cm as it is the nomenclature in the tubes from NIOZ), did not have enough sample for DIC analysis. To make it clear: our samples are from 1 to 20 with 2 cm separation so that is 40 cm. The labels from NIOZ were called 1 cm, 2 cm, etc.. so they should be renamed.

-The sample 7 for HS core A, might have a wrong concentration due to a bubble. Rhizons until depth 13 were sample in the evening and the rest left overnight.

-*Note: the samples taken for silica were frozen at -20C. They should have been kept at 4C, therefore they should not be used for this analysis. Since we were not really interested in those, we better keep this samples as back-up for any other analysis that might require more volume.

In the meantime, while Gabriella was slicing the core, subsamples were fixed in PFA for CARD-FISH. Samples were left overnight at 4C fixing.

The upper 8 points of the polysulfide core were done within one hour maintaining underpressure in the syringes (meaning that porewater would not be exposed to air). The lowest point was left overnight.

All the samples were frozen at -20C except for DIC that was given to Ellen at 4C.

8.6 Thursday 4-2-2016

Schedule:

0:00 transit back to Istanbul, arrival around 12:00.

Rhizons from depth 13 until 20 were collected and sampling finished. CARD-FISH samples were centrifuged, washed twice in PBS and resuspend in PBS:Ethanol. Stored at -20C. Nomenclature: MC-CF-St2-1/20- I(duplicate 1) or II (duplicate II).

The multicores were cleaned, emptied and photographed to try to link the exact position of the rhizon along the cores

-core A (HS, VFA, Me, P/Si): the grey mud starts at 2.7 cm from the grey clay

Figure 2: core A

-core B (Hs, N, DIC): we can see that our hole for sample 20 is exactly 3 cm above the pink layer, which extends until 5 cm and then they clay starts.

Figure 3: core B, ignore the numbers written, from number 15 on, put attention to the holes, the first hole on the right is sample 20

-core C: (polysulfide): the deepest hole is 4 cm above the grey clay Grey clay starts at 4.5 cm from the bottom

Figure 4: core C

9. In situ pumps summary

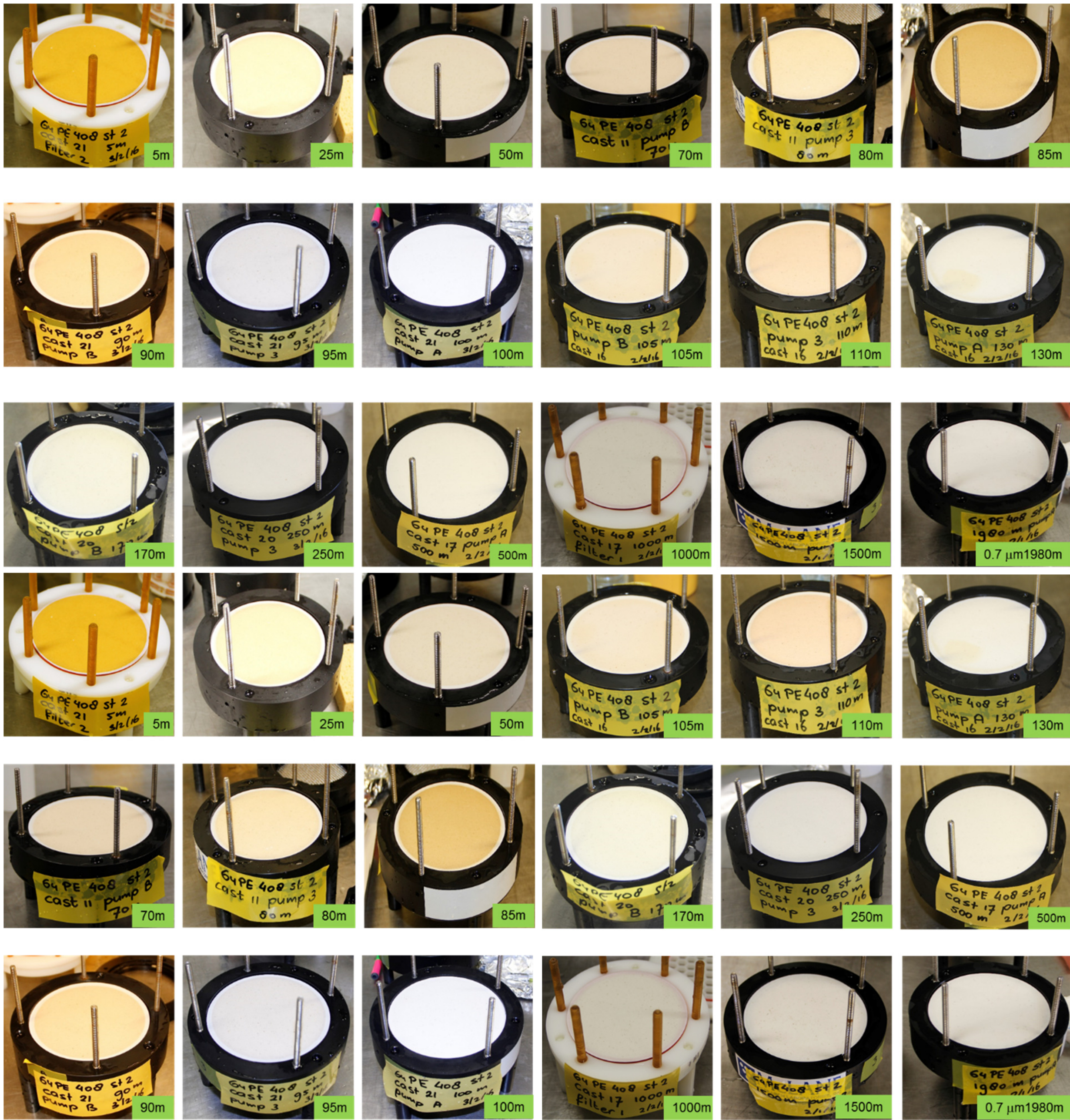
Marianne Baas

Depths	L	L (FM)	L	L (FM)	L	L (FM)
5		*	107.2	106		
25	61.84	74	nd**	245		
50	87.64	92	143.35	141		
70	246.83	317	166.58	165		
80	349.4	334	299.99	342		
85	351.81***	415	214.74	223		
90	133.63	144	179.11	184		
95	172.8	181	425.58	406		
100	169.83	169	80.66	81		
105	620.64**	528	126.5	125		
110	5.52	6	391.9**	382		
130	0	0	188.9	192		
170	-	-	105.61	108	533.04**	433
250	86.53	88	161.85	157	129.01	132
500	366.82	350	32.1	33	111.68	123
1000	257.61**	237	844.34**	658	693.06**	615
1500	449.26**	377	236.96**	210	429.94**	459
1980	35.09**	29	102.1	101	85.06**	80

5m AN	300.92	133	FH2
		146	FH1
500m AN	428.33	218	FH2
404		184	FH1
1000m AN	589.47	285	FH2
535		245	FH1

* filter imploded
** filter unit contains water after the normal manual pumping procedure
*** filters seems to have a "crack"

0.7 um and 1/2 filter Wageningen
1/2 filter Wageningen
0.7 um


5m
25m
50m
70m
80m
85m
90m
95m
100m
105m
110m
130m
170m
250m
500m
1000m
1500m
0.7 µm1980m
5m
25m
50m
105m
110m
130m
70m
80m
85m
170m
250m
500m
90m
95m
100m
1000m
1500m
0.7 µm1980m

Appendix 1: 2,2 CTD sample list

Ellen Hopmans

Cruise	64PE408	
Station	2	
Cast	2	
Date	31-01-2016	
Time	4:30 AM	UTC
Location	42°53.978'N	30°31.000"E
Max depth	2103	m
Surface T	8.3	(°C)

CTD bottle #	**Depth (m)**	**T (°C)**	**Depth #**	**Remark**
1	2044	9.114	18	
2	1500	9.038	17	
3	1000	8.958	16	
4	500	8.893	15	Oxygen
5	250	8.808	14	
6	170	8.724	13	
7	130	8.662	12	
8	110	8.597	11	
9	105	8.591	10	
10	100	8.578	9	
11	95	8.559	8	
12	90	8.535	7	
13	85	8.526	6	
14	80	8.509	5	
15	70	8.498	4	Oxygen (triplo)
16	50	7.825	3	Anne
17	50	7.825	3	Nuts
18	25	7.823	2	Anne
19	25	7.823	2	Nuts
20	5	7.820	1	Anne
21	5	7.820	1	Oxygen
22				
23				
24				

Appendix 1, 2.2 NUTS sample list

Depth #	CTD bottle #	CTD samples							
1	21								
2	19	**treatment**	**repetition**	**labelling**	**processing time***	**storage time***	**storage T (°C)**	**Who**	**Notes**
3	17	DIC	single	64PE408-DIC-1/18-A	6:20 UTC	6:58 UTC	4	ECH	FeCL2 added to depth 13-18, no visible rxn in depth 13 so stopped
4	15	13C-DIC	single	64PE408-13DIC-1/18-A	6:00 UTC	6:58 UTC	4	ECH	FeCL2 added to depth 13-18; also added to depth 7 by mistake
5	14	FISH	duplo	64PE408-FISH-1/18-A-I/II**	7:18 UTC	17:30 UTC; 1/2/16	-20	ECH	filtering took place from 15:30-18:30 UTC; on advise of I. Sanchez-Andrea, filters were placed in 25mm id petridishes, air dried for ca. 1 day and stored at -20 °C; final storage time/date indicated.
6	13	Bac/Vir	duplo	64PE408-bac/vir-1/18-A-I/II	8:32 UTC	11:30 UTC	-80	ECH	transferred from fridge to N2 for flash freezing at 9:22 UTC
7	12	Phytoplankton	duplo	64PE408-phyto-1/3-A-I/II	8:45 UTC	11:30 UTC	-80	ECH	transferred from fridge to N2 for flash freezing at 9:22 UTC
8	11	DOC/TOC	single	B1-18	8:55 UTC	9:18 UTC	4	ECH	see tab DOC_TOC for full list
9	10	dD	duplo	64PE408-dD-1/18-A-I/II	10:00-14:10 UTC	14:15 UTC	4	ECH	
10	9	N/P	duplo	64PE408-N/P-1/18-A-I/II	10:00-14:10 UTC	14:15 UTC	-20	ECH	
11	8	Si	single	64PE408-Si-1/18-A	10:00-14:10 UTC	14:15 UTC	4	ECH	
12	7	Chla	duplo	64PE408-Chla-1/3-A-I/II	19:00-20:00 UTC	20:00 UTC	-20	ECH	stored in ponyvial instead of eppendorf; for filtered volumes see tab Chla
13	6	POC	single	64PE408-POC-1/18-A	7:50-21:15 UTC; 1/2/16	7:50-21:15 UTC; 1/2/16	-20	ECH	for volumes and more exact processing times see tab POC/PON/POP
14	5	PON/POP	single	64PE408-PON/POP-1/18-A	7:50-21:15 UTC; 1/2/16	7:50-21:15 UTC; 1/2/16	-20	ECH	for volumes and more exact processing times see tab POC/PON/POP
15	4								
16	3								
17	2	*Unless indicated processing/storage took place on same day as cast							
18	1	** sample 8AII spilled and approximately 15 ml filtered instead of the prescribed 30 ml; also for sample 11AI the filter fell on the table with the sample side down.							

numbering of lid and bottle did not match on bottles with 2PO and 5PO. After discussion we went with label on lid & exchanged stickers on the bottle

Appendix 1: 2,2 DOC/TOC sample list

Processing time: 8:55 am UTC
Storage time: 9:18 am UTC

Depth nr.	CTD bottle #	DOC/TOC vial code
1	21	B1
2	19	B2
3	17	B3
4	15	B4
5	14	B5
6	13	B6
7	12	B7
8	11	B8
9	10	B9
10	9	B10
11	8	B11
12	7	B12
13	6	B13
14	5	B14
15	4	B15
16	3	B16
17	2	B17
18	1	B18

Appendix 1: 2,2 Chlorophyll sample list

Processing time: 19:00 pm UTC
Storage time: 19:45 pm UTC

Depth nr.	**CTD bottle #**	volume filtered (ml)	label
1	21	500	64PE408-Chla-1-A-I
1	21	500	64PE408-Chla-1-A-II
2	19	530	64PE408-Chla-2-A-I
2	19	470	64PE408-Chla-2-A-II
3	17	500	64PE408-Chla-3-A-I
3	17	500	64PE408-Chla-3-A-II

Appendix 1: 2,2 POC/POP/PON sample list

processing date: 2-1-2016

		POC				PON/POP			
Depth #.	**CTD bottle #**	**proc.time (UTC)**	**filter wgt (g)**	**volume filtered (ml)**	**label**	**proc.time (UTC)**	**filter wgt (g)**	**volume filtered (ml)**	**label**
1	21	07:50	0.1302	2000	64PE408 POC 1A	07:50	0.1344	2000	64PE408 PON/POP 1A
2	19	08:50	0.1305	2000	64PE408 POC 2A	08:30	0.1310	2000	64PE408 PON/POP 2A
3	17	13:50	0.1294	2000	64PE408 POC 3A	13:30	0.1302	2000	64PE408 PON/POP 3A
4	15	14:16	0.1290	2000	64PE408 POC 4A	14:05	0.1308	2000	64PE408 PON/POP 4A
5	14	14:50	0.1307	2000	64PE408 POC 5A	14:35	0.1312	2000	64PE408 PON/POP 5A
6	13	15:22	0.1339	2000	64PE408 POC 6A	15:05	0.1302	2000	64PE408 PON/POP 6A
7	12	15:50	0.1300	2000	64PE408 POC 7A	15:28	0.1340	2000	64PE408 PON/POP 7A
8	11	16:00	0.1297	2000	64PE408 POC 8A	15:55	0.1286	2000	64PE408 PON/POP 8A
9	10	16:45	0.1392	2000	64PE408 POC 9A	16:35	0.1325	2000	64PE408 PON/POP 9A
10	9	17:18	0.1313	2000	64PE408 POC 10A	17:00	0.1325	2000	64PE408 PON/POP 10A
11	8	17:50	0.1334	2000	64PE408 POC 11A	17:40	0.1320	2000	64PE408 PON/POP 11A
12	7	18:05	0.1313	2000	64PE408 POC 12A	17:58	0.1339	2000	64PE408 PON/POP 12A
13	6	18:25	0.1364	2000	64PE408 POC 13A	18:20	0.1307	2000	64PE408 PON/POP 13A
14	5	18:45	0.1318	2000	64PE408 POC 14A	18:43	0.1305	2000	64PE408 PON/POP 14A
15	4	20:10	0.1287	1975	64PE408 POC 15A	20:03	0.1301	1975	64PE408 PON/POP 15A
16	3	20:35	0.1172	2000	64PE408 POC 16A	20:30	0.1363	2000	64PE408 PON/POP 16A
17	2	21:00	0.1314	2000	64PE408 POC 17A	20:45	0.1309	2000	64PE408 PON/POP 17A
18	1	21:15	0.1305	2000	64PE408 POC 18A	21:15	0.1318	2000	64PE408 PON/POP 18A

Appendix 1: 2,19 CTD sample list

Cruise	64PE408	
Station	2	
Cast	19	
Date	2-3-2016	
Time		UTC
Location	42°53.993'N	30°30.950'E
Max depth	2095.3	m
Surface T	8.2	(°C)

CTD bottle	Depth (m)	T (°C)	Depth #	Remark
1	2045	9.114	18	
2	1500	9.038	17	
3	1000	8.958	16	did not close
4	500	8.893	15	
5	250	8.809	14	
6	170	8.724	13	
7	130	8.653	12	
8	110	8.605	11	
9	105	8.595	10	
10	100	8.573	9	
11	95	8.548	8	
12	90	8.540	7	
13	85	8.522	6	
14	80	8.502	5	
15	70	8.477	4	
16	50	8.500	3	
17	25	7.874	2	
18	5	7.880	1	
19				
20				
21				
22				
23				
24				

Appendix 1, 2.19 NUTS sample list

CTD samples

treatment	repetition	labelling	processing time*	storage time*	storage T (°C)	Who	Notes
DIC	single	64PE408-DIC-1/18-B	9:20 UTC	10:00 UTC	4	ECH	FeCL2 added to depth 13-18, no visible rxn in depth 13 so stopped
13C-DIC	single	64PE408-13DIC-1/18-B	9:20 UTC	10:00 UTC	4	ECH	FeCL2 added to depth 13-18, no visible rxn in depth 13 so stopped
FISH	duplo	64PE408-FISH-1/18-B-I/II**	12:30 UTC	17:30 UTC; 4/2/16	-20	ECH	filtering took place from 15:30-18:30 UTC; on advise of I. Sanchez-Andrea, filters were placed in 25mm id petridishes, air dried for ca. 1 day and stored at -20 °C; final storage time/date indicated.
Bac/Vir	duplo	64PE408-bac/vir-1/18-B-I/II	9:20 UTC	17:30 UTC	-80	ECH	Placed in fridge at 10:30 UTC; No N2 available for flash freezing; samples placed directly in -80 at indicated storage time.
Phytoplankton	duplo	64PE408-phyto-1/3-B-I/II	9:20 UTC	17:30 UTC	-80	ECH	Placed in fridge at 10:30 UTC; No N2 available for flash freezing; samples placed directly in -80 at indicated storage time.
DOC/TOC	single	B1-18	12:00 UTC	12:00 UTC	4	ECH	see tab DOC_TOC for full list
dD	duplo	64PE408-dD-1/18-B-I/II	10:00-13:50 UTC	14:00 UTC	4	ECH	
N/P	duplo	64PE408-N/P-1/18-B-I/II	10:00-13:50 UTC	14:00 UTC	-20	ECH	
Si	single	64PE408-Si-1/18-B	10:00-13:50 UTC	14:00 UTC	4	ECH	
Chla	duplo	64PE408-Chla-1/3-B-I/II	19:15-20:00 UTC	20:00 UTC	-20	ECH	stored in ponyvial instead of eppendorf; for filtered volumes see tab Chla
POC	single	64PE408-POC-1/18-B	6:35-12:13 UTC; 4/2/16	6:35-12:13 UTC; 4/2/16	-20	ECH	for volumes and more exact processing times see tab POC/PON/POP
PON/POP	single	64PE408-PON/POP-1/18-B	6:25-12:25 UTC; 4/2/16	6:25-12:25 UTC; 4/2/16	-20	ECH	for volumes and more exact processing times see tab POC/PON/POP

*Unless indicated processing/storage took place on same day as cast

** FISH 10-B-II missing due to spill

Appendix 1: 2,19 DOC/TOC sample list

Processing time: 12:00 UTC
Storage time: 12:00 UTC

Depth nr.	CTD bottle #	DOC/TOC vial code	
1	18	B19	
2	17	B20	
3	16	B21	
4	15	*	
5	14	B23	
6	13	*	
7	12	B25	
8	11	B26	
9	10	B27	
10	9	B28	
11	8	B29	
12	7	B30	
13	6	B31	
14	5	B32	
15	4	B33	
16	3		no sample
17	2	B35	
18	1	B36	

*Lack of amber vials, these depths were selected to be skipped

Appendix 1: 2,19 Chlorophyll sample list

Processing time: 19:15 pm UTC
Storage time: 20:00 pm UTC

Depth nr.	CTD bottle #	volume filtered (ml)	label
1	18	500	64PE408-Chla-1-B-I
1	18	500	64PE408-Chla-1-B-II
2	17	500	64PE408-Chla-2-A-I
2	17	500	64PE408-Chla-2-B-II
3	16	500	64PE408-Chla-3-B-I
3	16	500	64PE408-Chla-3-B-II

Appendix 1: 2,19 POC, POP, PON sample list

processing date: 2-4-2016

		POC				PON/POP			
Depth nr.	**CTD bottle #**	**proc.time (UTC)**	**filter wgt (g)**	**volume filtered (ml)**	**label**	**proc.time (UTC)**	**filter wgt (g)**	**volume filtered (ml)**	**label**
1	18	06:35	0.1324	2000	64PE408 POC 1B	06:25	0.1314	2000	64PE408 PON/POP 1B
2	17	06:52	0.1304	2000	64PE408 POC 2B	06:45	0.1312	2000	64PE408 PON/POP 2B
3	16	07:15	0.1464	2000	64PE408 POC 3B	07:07	0.1172	2000	64PE408 PON/POP 3B
4	15	07:35	0.1296	2000	64PE408 POC 4B	07:29	0.1301	2000	64PE408 PON/POP 4B
5	14	07:50	0.1278	2000	64PE408 POC 5B	07:45	0.1309	2000	64PE408 PON/POP 5B
6	13	08:10	0.1272	2000	64PE408 POC 6B	08:01	0.1288	2000	64PE408 PON/POP 6B
7	12	08:35	0.1350	2000	64PE408 POC 7B	08:28	0.1296	2000	64PE408 PON/POP 7B
8	11	08:50	0.1292	2000	64PE408 POC 8B	08:44	0.1283	2000	64PE408 PON/POP 8B
9	10	09:10	0.1321	2000	64PE408 POC 9B	09:01	0.1301	2000	64PE408 PON/POP 9B
10	9	09:32	0.1302	2000	64PE408 POC 10B	09:22	0.1318	2000	64PE408 PON/POP 10B
11	8	09:50	0.1293	2000	64PE408 POC 11B	09:40	0.1335	2000	64PE408 PON/POP 11B
12	7	10:05	0.1317	2000	64PE408 POC 12B	09:57	0.1325	2000	64PE408 PON/POP 12B
13	6	10:20	0.1322	1950	64PE408 POC 13B	10:14	0.1316	2000	64PE408 PON/POP 13B
14	5	11:27	0.1329	2000	64PE408 POC 14B	11:20	0.1311	2000	64PE408 PON/POP 14B
15	4	11:47	0.1308	1800	64PE408 POC 15B	11:37	0.1321	1975	64PE408 PON/POP 15B
16	3								
17	2	12:10	0.1303	1920	64PE408 POC 17B	12:01	0.1312	2000	64PE408 PON/POP 16B
18	1	12:25	0.1306	2050	64PE408 POC 18B	12:13	0.1317	2000	64PE408 PON/POP 17B

Appendix 2: 2,9 Clean CTD/GoFlow Anaerobic nuts

Cruise	64PE408	
Station	2	
Cast	9	
Date	2-1-2016	
Time	10:30 AM	UTC
Location	45°54.017'N	30°30.978'E
Max depth	2093.8	m
Surface T	8.3	(°C)

CTD bottle	Depth (m)	T (°C)	Depth nr	Remark
1	2063	9.112	18	
2	1500	9.039	17	
3	1000	8.961	16	
4	500	8.895	15	
5	250	8.808	14	
6	170	8.728	13	
7	130 (126.1)	8.657	12	
8	110 (108.6)	8.603	11	
9	105	8.579	10	
10	100	8.561	9	
11	95	8.549	8	
12	90	8.540	7	
13	85	8.528	6	
14	80	8.512	5	
15	70	8.503	4	
16	50	8.525	3	
17	25	7.914	2	
18	5	7.932	1	
19				
20				
21				
22				
23				
24				

Appendix 2: 2,9 Clean CTD/GoFlow Anaerobic nuts sample list

CTD bottle #	Depth #	CTD samples							
1	18								
2	17	**treatment**	**repetition**	**labelling**	**processing time UTC**	**storage time UTC**	**storage T (°C)**	**Who**	**Notes**
3	16	Methane	duplo	64PE408-Meth-1/18-A-I/II	10:30	11:30	4	ECH	2 tubes missing due to broken needles
4	15	Sulfate	duplo	64PE408-SO4-1/18-A-I/II	12:30	17:50	-20	GW/ECH	
5	14	S/Fe/Mn/Trace	duplo	64PE408-S/Fe/Mn-1/18-A-I/II	12:30	17:50	-20	GW/ECH	
6	13	Sulfide	duplo	64PE408-HS-1/18-A-I/II	12:30	17:50	to I. Sanchez for analysis	GW/ECH	Scaled up recipe x 2 to allow for measuring in 2 ml cuvettes. Reaction chemicals appeared to be degraded and assay did not work
7	12								so samples were stored for further analysis at Wageningen by I. Sanchez
8	11								
9	10								
10	9								
11	8								
12	7								
13	6								
14	5								
15	4								
16	3								
17	2								
18	1								

Appendix 2: 2,15 Clean CTD/GoFlow Anaerobic nuts

Cruise	64PE408	
Station	2	
Cast	15	
Date	2-2-2016	
Time	10:30 AM	UTC
Location	42°53.992'N	30°31.036'E
Max depth	2066.2 (2060)	m
Surface T	8.2	(°C)

CTD bottle	**Depth (m)**	**T (°C)**	**Depth nr**	**Remark**
1	2041	9.115	18	
2	1500	9.040	17	
3	1000	8.961	16	
4	500	8.895	15	
5	250	8.806	14	
6	170	8.724	13	
7	130	8.659	12	
8	110 (107.7)	8.608	11	
9	105	8.596	10	
10	100	8.568	9	
11	95	8.561	8	
12	90	8.541	7	
13	85	8.517	6	
14	80	8.498	5	
15	70	8.501	4	
16	50	8.529	3	
17	25	7.819	2	
18	5	7.844	1	
19				
20				
21				
22				
23				
24				

Appendix 2: 2,15 Clean CTD/GoFlow Anaerobic nuts sample list

Depth #.	CTD bottle #	CTD samples							
18	1	**treatment**	**repetition**	**labelling**	**processing time UTC**	**storage time UTC**	**storage T (°C)**	**Who**	**Notes**
17	2	Methane	duplo	64PE408-Meth-1/18-B-I/II	10:15	11:30	4	ECH	added acid 5 min after filling tubes with water for depth 16
16	3	Sulfate	duplo	64PE408-SO4-1/18-B-I/II	12:00	16:45	-20	ECH	
15	4	Sulfide	duplo	64PE408-HS-1/18-B-I/II	12:00	16:45	to I. Sanchez for analysis	ECH	
14	5	S/Fe/Mn/Trace	duplo	64PE408-S/Fe/Mn-1/18-B-I/II	12:00	16:45	-20	ECH	
13	6								
12	7								
11	8	bottle 8 started leaking in air through septum between subsampling for sulfide and S/Fe/Mn/Trace							
10	9	bottle 14: septum came off (faulty lid) before S/Fe/Mn/Trace							
9	10								
8	11								
7	12								
6	13								
5	14								
4	15								
3	16								
2	17								
1	18								

Appendix 3 PROTOCOLS

IMPORTANT notes regarding toxic waste handling!

- Pipette tips, tissues of other solids in touch with $HgCl_2$ should be stored in geochemical bags, stapled and dispose inside the black waste container identifying clearly that it's $HgCl_2$ waste
- Same thing applies for pipettes/waste in contact with Formaldehyde and glutaraldehyde (or other fixatives)
- All liquid waste filtrate through fixed samples with formaldehyde/glutaraldehyde etc should be stored in the Fixatives 20L carboy. This can be detoxified by adding some cleaning ammonia to it. Leave it in the bottle to be detoxified back at NIOZ
- To inactivate antibiotics or nitrapyrin from the incubation experiments, add a spoonful of activated carbon, shake the bottle and let it settled, repeat a couple of times. Let the activated carbon to settle and dispose the liquid through the sink. Leave the activated carbon at the bottom of the carboy to be detoxified back at NIOZ (alternatively it can be transferred to a smaller bottle for convenience)

NOTEs:

- Be sensitive using the material and cleaning products so that the rest of the legs do not run out of things!!
- Pay attention regarding the temperatures of storage of each sample
- Pipette fixatives and/or toxic compounds under the chemical hoods
- Work under the chemical hood when handling H_2S
- Minimize the time in which the samples are not stored at their expected temperatures (e.g. sediment slices and filters that should be stored at -80C can be temporarily stored in a box with ice until there's time to transport them downstairs to the -80C)
- Label everything properly according to the sampling code
- Write down volume filtered, possible mistakes, failures of the protocol etc to report back
- **REMEMBER:** Do not label bags or tubes with yellow tape if needs to be stored at -80C
- Cryovials should be label ONLY with the pens that say "cryovials", the other permanent pens won't work when the cryovials get preserved in liquid nitrogen

CARE & USE OF THE FLUORESCENCE MICROSCOPY AXIOPHOT

- The microscope should be assembled by **Marcel together with Saara and Gabriella** in the first leg of the cruise. **Saara and Gabriella** will be in charge of packing it at the end of the cruise (exactly as it was when opened)
- The microscope is disassembled at the moment so take extra care in not damaging the lens when you remove the tissue paper covering the apertures
- The boxes of the microscope are heavy and should be handled with the best of care
- **Use of the microscope:**
    - To use fluorescent light: Turn on fluorescence lamp box at least 15 minutes before using. **IMPORTANT**: If you turn off the lamp, wait at least 15 minutes before turn it on again. In any case it is better not to turn on/off continuously. Remember also to turn off the lamp at the end of microscopy session
    - If you use the 100x objective: Use immersion oil on the coverslip of the preparation. Wipe out the objective lens with a microscope tissue (NOT a regular one!).
    - Never go back from the 100x objective to the 40x when the coverslip already have oil on it! Otherwise the 40x objective will get oily and it's not ready for it

App 3.1 Water sampling from the regular CTD

App 3.1.1 Preserving water samples for FISH purposes and filtration

Material

- 50 ml Falcon tubes
- Formaldehyde 36%
- 1 ml Pipettor
- Pipette tips
- Filters (0.2 and 0.45 um)
- Small filtration unit
- Vacuum pump
- 1 L PBS 1X sterilized
- Container for solid waste
- Bottle for liquid waste
- Gloves
- Tweezers
- Microscope slides and storage box

Note:

IMPORTANT: Work under the chemical hood! Change gloves if they get wet!

1. Gloves and pipettes tips touching formaldehyde should be disposed in a closed container that has to be taken back to the lab
2. Formaldehyde has to be declared as a chemical and stored in the chemical closet
3. All the manipulations should be done under the hood in the dry lab
4. Do 3 replicates (filters) for each water sample if possible

Procedure

- 30 ml seawater in a 50 ml tube (aprox with lines of the falcon)
- Add 4 ml formaldehyde 36% (final concentration 4%)
- Mix by inversion
- Store the samples at 4C protected from light (1h min; 18 h maximum)
- filter the samples onto polycarbonate 25 mm 0.2 um filter mounted on a 0.45 um 25 mm nitrocellulose filter
- After sample filtration, wash with 10 ml PBS1x
- Air-dry filters
- Put filters on a microscope slide and store at -20C protected from light

DAPI staining and counterstaining with DAPI +Vectashield (OPTIONAL: Only if you want to check the density of the sample)

- Add 10 ul DAPI (10 ug/ml) to 10 ml sample
- Wait 10 minutes (cover ramp from light) and filter

Counterstaining (OPTIONAL: Only if you want to check the density of the sample)

- Add one drop of Vectashield +DAPI mixture (1.5 ug/ml)
- Wait for 5 min
- Cover with coverslip

App 3.1.2 CARD-FISH fixation for sediment samples [Llobet-Brossa,Rosselló-Mora & Amann 1998] (Wageningen)

Original

1. Fix sediment samples with fresh formaldehyde (end concentration 1 - 4%) for 1 -2 at RT or max 24 hours at 4°C
2. centrifuge at 16,000 g for 5 minutes; pour off supernatant and resuspend sample with 1 X PBS pH 7.6
3. repeat washing step 2 twice
4. store sediment sample in a 1:1 mix of PBS / ethanol at -20°C until further processing

Adapted for Black Sea cruise

1. Fix sediment samples with fresh formaldehyde (end concentration 4%, dilution with PBS) for 1 -2 at RT or max 24 hours at 4°C
2. centrifuge at 16,000 g for 5 minutes; pour off supernatant and resuspend sample with 1 X PBS pH 7.6
3. repeat washing step 2 twice
4. store sediment sample in a 1:1 mix of PBS / ethanol at -20°C until further processing

Requirements

(3x24 samples, aim at 100 samples)

- formaldehyde sufficient for 100 mL 4%
- PBS pH 7.6 sufficient for dilution of formaldehyde + 300 mL (take 1L in aliquots to be sure)
- PBS/Ethanol 100 mL at least (200 mL to be sure)

App 3.1.3 NH_4, NO_3, NO_2, Silicate and PO_4

Note: 2 pony vials per depth

- The acrodisc filters can be reused as long as they are flushed with water and not used from the surface to the bottom water (keep a filter for surface and another one for deep waters!)
- Same is applicable to the 10 ml syringe (try to recycle as much as possible!)

Material

- Polyethylene bottles 125 ml (or 1 L), filled with aprox. 0.1 M HCI or 1L-acid washed previously
- Pony vials with cap
- acrodisc filters 0.2 um
- Syringe 10ml
- Squeeze bottle with demi water
- Sample tray to store pony vials

Procedure

- Work as clean as possible. Avoid contact with skin and sample.
- Empty the polyethylene bottle and pre-rinse for at least 3 times with water from the CTD-bottle with the same number code as the polyethyleen bottle. Rinse the lid of the bottle as well.
- Fill the bottle until the edge.
- Code the pony files with at least Station number, cast number and ctd-bottle number.
- Put the pony vials in a sample tray.
- One pony vial for the analysis of silicate is needed and another one for the analysis of NO_x, NH_4 and PO_4
- Start, if possible, with the sea surface samples. Sample bottle of the CTD with the highest number and the lowest nutrient amount. Thus avoiding contamination.

- Rinse the syringe twice with 2 ml sample from the polyethylene bottle. Make sure that the whole syringe is rinsed.
- Take 12 ml from the sample bottle without air. Connect acrodisc filter to the syringe and rinse the filter with 6 ml sample.
- Rinse the pony vial with 2 ml filtrated sample.
- Fill pony vial till under the edge with filtrate. **Do not overfill the pony vial because off expansion of the water during storage at low temperature and loose caps.** Rinse the cap of the pony vial as well.
- Close the vial properly. Check if the vial is closed.
- Continue with next sample.
- After last sample rinse the syringe inside and outside with demi water.
- The filter can be used for more CTD stations, depends on the turbidity of the samples taken.
- Store the vials for silicate analysis at 4°C in the fridge. Not at a lower temperature; silicate can form polymers!
- Store the vials for the analysis of NO_x, NH_4 and PO_4 at - 20°C in the freezer.

App 3.1.4 DOC/TOC

- **Label the DOC/TOC vials with a piece of yellow tape and <u>ONLY a number</u> (record the sample, station corresponding to that number).**
- Fill up the DOC/TOC vials but **<u>ONLY</u> up to 3-4 cm below the rim**!!
- Add 10-12 drops of HCl 37% with Pasteur pipette
- Close well and mix
- Store at 4C

NOTE: Store dirty/broken Pasteur pipettes in a box

App 3.1.5 DIC and δ13C of DIC

How to make $HgCl_2$ saturated solution:

- 10 g $HgCl_2$ in 100 ml water or whatever if indicated in the containers with weighted solid $HgCl_2$
- Add 25 μl saturated solution into 4 ml vials or 60 ul in 12 ml exeteiners (under chemical hood)

Material

- 4 ml glass vials with screw caps
- Several glass bottles (100 ml) with 4.1 g NaCl tightly closed (only for porewater samples)
- MQ water sterile 100 ml content
- 100 ml cylinder
- Pipettor 10-100 ul and 1 ml pipettor
- Pipette boxes

Procedure

- **NOTE:** water/porewater samples with high sulfide contents (water column samples deeper than 100 m and all the porewater samples of the piston core and the multicore) have to be pretreated with saturated $FeCl_2x4H_2O$ solution before adding the $HgCl_2$. Add the water sample to the DIC vial or exeteiner, add drop by drop the saturated $FeCl_2x4H_2O$ solution and wait until you stop seeing formation of FeS precipitate. Then you can add the $HgCl_2$.
- Label the DIC vials
- The DIC vials have to be filled up completely until there is a heap of liquid on top
- Then, add the $HgCl_2$ (**gloves and operate under the hood)**
- Then screwed it right away as NO GAS should be contained.
- Wrap in tissue just in case it drips out
- Turn vials upside down and keep at 4C
- **For porewater:** If there is not enough liquid leftover for filling them up, the procedure is to add 1 mL of the porewater and fill up to the top with MilliQ with a concentration of NaCl of 41 g/L. The solution can be made freshly every day with MilliQ water that has been kept in tightly sealed bottles.

App 3.1.6 POC/PON/POP [Particulate organic carbon, nitrogen and phosphate]

IMPORTANT: Write down in your notebook the weight of the filter indicated in the plastic slide and the sampling code that will be also indicated on the plastic slide

Material

- Bottles to collect water from the CTD (acid cleaned) and **labeled as PO with a number indicating the bottle of the CTD (there are bottles labelled as e.g. 1 and 1', one for POC and the other for PON/POP)**
- Combusted and pre-weighted 47 mm, 0.7 µm pore size GFF
- Tweezers
- Filtering unit (3 cup ramp!)
- Vacuum pump
- tubing for the connection
- Squeeze bottle with MQ water

Note

- For each sample depth 2 L for POC and 2 L for PON/POP (**until the filter clogs!**)

Procedure

- The GFFs need to be combusted and weighted before use
- First wash collection bottles 3 times with sample water
- Collect about 2 L sea water from the CTD
- Rinse the filtering system with MQ
- Put the GFF into the filtering unit
- Shake the plastic bottle before filtering
- **Measure some volume with a cylinder. Add some to the filtering cup. Filter and report the volume filtered according to what is added to the filtering cup! Do not add a lot of volume in the filtering cup so that you can anticipate when the filter is going to clog. If it does clog, calculate how much it was already filtered by subtracting from what is left in the cylinder!**
- Rinse the filtering unit with MQ to wash all particles onto the filter and to wash out the salt
- Store the filter in the same plastic slide and label the slide with the proper code
- Store at -20C

App 3.1.7 Hydrogen isotopes

- Get water for the CTD.
- Filter with 0.2 um acrodisc filter in an exeteiner tube
- Fill tube completely and invert
- Store at 4C

App 3.1.8 Alkalinity

- Approximately 200 ml in a glass bottle (Gabriella), sealed with grease
- Measure on board (VINTA) or the porewater with the titrator (Gabriella)

App 3.1.9 Fixation of samples for Flow cytometry

Material:

- 1 ml pipettor (and 5 ml pipettor) and sterile pipette tips
- 100 ul pipettor (for fixatives) and pipette tips
- 2 ml cryovials

- 25% glutaraldehyde 25% EM stored at 4C
- 5 ml cryovials
- formaline:hexamine (18:10% v:w, stored at room temperature)
- Liquid Nitrogen
- Panties to flash freeze samples in a dewar vessel

Use only the permanent pens labeled as “cryovials” on the cryovials!!!

Bacteria and virus samples: 1 mL unfiltered sample in a pre-labeled 2 mL cryovial + 20 uL glutaraldehyde (25% EM-grade, stored at 4 degrees). Place for 30 minutes (max 1 hour) at 4C (fixation process) and then flash freeze in liquid nitrogen. After 2 h this can be transferred to -80C.

Phytoplankton samples: 3.5 mL untreated sample (using the 1-5 mL pipet) in a pre-labeled 5 mL cryovial + 100 ul formaline:hexamine (18:10% v:w, stored at room temperature). At 4C for 30 min (max 1 h) and then flash freeze in liquid nitrogen. After 2 h this can be transferred to -80C.

App 3.1.10 Chlorophyll a

Material

- 2L collection bottles
- Filters (25 mm GFFs precombusted) and filtration unit (small one)
- 15 ml falcon or Eppendorf tubes for storage

Procedure

- Collect from selected depths water from the CTD into 2L sampling bottles with aprox 1 L seawater
- Store bottles in the fridge (4 °C) until you are ready to filter them
- Set up your small filtration unit using 25 mm GF/Fs filters
- Shake the bottle that you are about to filter
- Filter seawater (the exact volume that you will be able to filter will depend on the phytoplankton biomass. Aim at filtering 500 ml, but your filter might clog and you might be forced to stop filtering.
- It is essential to write on your lab book how much seawater was collected
- Once filtration is over, using tweezers, insert filter in an Eppendorf
- Put sticker with all info onto the Eppendorf and secure the whole thing with cello tape
- Do it duplicate if possible
- Store in the freezer (-20 °C)

App 3.1.11 Protocol filtering seawater to obtain coccolithophore and diatom samples

Anne Roepert & Shaun Akse – UU Geochemistry

The aim of this protocol is to create a reproducible step-by-step guide to collect coccolithophore and diatom samples by filtration of seawater in the 2016 NESSC cruises. The filters will be used for nanoSIMS analyses, i.e. the density of material on the filter is supposed to be rather low, so that single cells/liths are exposed. The seawater is first filtered through the 5 µm PC filters to collect diatoms. The filtrate is filtered in a second step through 0,4 µm PC filters to collect the smaller coccoliths.
A bulk filter is taken as a backup containing more material.
Coccoliths are prone to dissolution in acidic waters. Therefore, the filters and filtration setup will be rinsed with a 0.05 M NH_4HCO_3 buffer (pH 7.8), instead of milliQ.

At each station three water depths are sampled:

1. Shallow, ~ 20 m water depth

2. Intermediate depth, between the surface and the deep chlorophyll maximum
3. Deep chlorophyll maximum

The approximate total volume of seawater required at each depth is 10 L.

Three different types of filters will be produced at each depth at each station.

- **Bulk filter**: Polycarbonate – 47mm (Diameter) - 0,4 µm (Pore size)
    - 1 volume per depth: 5 L or filtration until clogging.
- **Diatom filter**: Polycarbonate – 25mm – 5 µm
    - 3 volumes per depth
- **Coccolith filter**: Polycarbonate – 25mm – 0,4 µm
    - 3 volumes per depth

Table 3 Expected sample volumes for filtration

		filters for nanoSIMS aim at low cell density!	
	Bulk filter PC, 47 mm, 0,4µm pores	**Diatom filter** PC, 25 mm, 5 µm pores	**Coccolith filter** PC, 25 mm, 0,4µm pores
volume 1	5 L or until clogging	0.5 L	100 ml
volume 2	-	1 L	500 ml
volume 3	-	2 L	1 L

To check if the volumes need to be adapted to the local conditions, take a sample and investigate the cell density under the microscope. Therefore, filter the smallest volume first, put the filter on a glass slide, add a drop of buffer and a cover slip onto the filter and microscope. In case particle density is much too high or too low, adapt the volumes of the remaining filters.

Prepare 0.05 M NH_4HCO_3 buffer solution

(0.05 M NH_4HCO_3 = 4g/L NH_4HCO_3)

1. Work with gloves
2. Add two vials (8g each) of NH_4CO_3 to a 5 L jerrycan.
3. Rinse vials with milliQ, add to jerrycan.
4. Fill the jerrycan up to 4 L with milliQ.
5. Add stirring magnet and mix thoroughly (on magnetic stirrer).
6. Pour buffer solution into squeeze bottles.

Bulk filter

Per sampled water depth, one bulk filter is required.

1. For the collection of the bulk filter the Nalgene set-up on a Schott bottle is required and the 47mm GFF and PC filters. Make sure the filtration system is rinsed thoroughly with NH_4HCO_3 buffer.
2. Turn on the vacuum pump and open valves before applying first a GFF filter and then the PC filter with tweezers. Moisten filters with NH_4HCO_3 buffer.
3. Complete setup with upper cylinder, close valve and vent flask.
4. Fill cylinder with sample.
5. Open vacuum
6. As sample starts to filter, add additional seawater until desired volume is reached.

   a. The Schott bottle is full but the filtration of the sample is not yet finished (i.e. higher sample volume than volume of the Schott bottle)?
      i. Close off system from pump and vent.
      ii. Disconnect the Nalgene holder from the Schott bottle including the filter and eventually sample on top of the filter (keep upright).
      iii. Pour filtrate into waste, put back together and continue filtering.
7. When desired volume is filtered, rinse holder/filters at least three times (approx. 10 ml each time)with NH_4HCO_3 buffer.
8. While pump is still turned on, remove filter with tweezers and add to (large) petridish onto aluminum-foil square.
9. Finished? Close valve and vent security flask before turning off the pump.

App 3.1.12 Filtration Setup in wooden box – UU

Shaun Akse & Anne Roepert

To set up glassware safely onboard ship, we have constructed a wooden box in which 6 individual filtration flasks plus a safety flask (connected between the individual filtration flasks and the pump) can be positioned.

Set up the system as indicated in the figure. Use tubing that is suitable for vacuum pumping (should be firm enough to not collapse). Make sure to secure the wooden box with cargo straps

onboard ship. Fix tubing with tie wraps and glassware with rubber bands to the box.

The glass filtration flasks are suitable for filters of 25mm diameter filters. Note that there are different types of funnels and teflon support disks (all labelled in bags). Combine them only as labelled. Mixing up different types might lead to leakage.

For filters of 47 mm diameter, a Nalgene filtration setup can be connected onto a Schott bottle. The tubing towards the safety flask is connected as indicated in the figure.

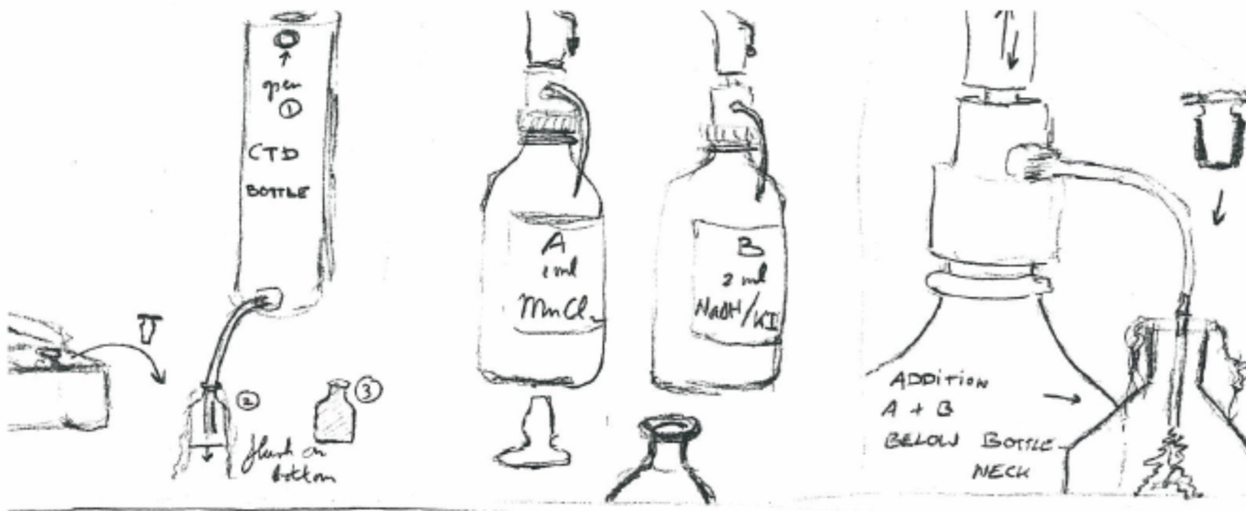
Valve 1: vent the safety flask. Valve 2: connect or close off filtration system. Valve 3: vent the filtration flask	*Nalgene setup* 1: open during filtration. 2: white ring to disassemble filtration unit. 3: connect to safety flask here!

General instructions for filtering:

1) Place filters onto holders **while there is an under-pressure** at that holder (connected to pump and pump on).
2) **Vent the flask** underneath the filter **before adding the sample**.
   This ensures that the sample covers the whole filter before getting pulled through.
3) Take off a filter from a holder **while the pump is still connected**.
   Otherwise your filter is lying very loose on the holder and in case of PC filters, might even fly away…
4) **Vent the system** (or at least the safety flask) **before turning off the pump**.
   Otherwise water from the pump will be sucked into the safety flask.

App 3.1.13. Preparation samples for O_2 sensor calibration (first tap out of CTD bottle)

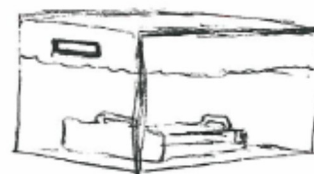
- Pre-label bottles with yellow tape. Use CTD bottle nr., not depth!
- Prime dispensers with demi-water, then place on reagent bottles and prime with reagent.
- Sample anoxic CTD bottles first
- Open CDT bottle tap and let water flush tap hose. Place tap hose on bottom of flask and fill while rotating. Overfill the bottle 3 x by letting it overflow. Rinse stopper with overflow water.
- Remove tap hose while water is running, leave bottle open.
- Add chemicals A & B: gently lift dispenser(no air in end of fill tube), positioning outflow of dispenser well below the bottle neck, deliver chemicals (bottle will overflow).
- Place stopper and mix, with thumb on stopper. Water will become cloudy.
- After 15 minutes mix again. Place rubber band.
- Place in crate under water.



mixing 20 seconds

AFTER 15 MINUTES
MIX AGAIN

STORAGE AT
LEAST 2 HOURS
UNDER WATER (20°C)
BEFORE MEASSURING



App 3.2 Water sampling from the GO-flo anaerobic bottles

App 3.2.1 Methane in water samples

Material

- Blood sample tubes preferentially glass, under vacuum
- **Tube connector that connects the GO-flo bottle and ends in a cut syringe barrel with a needle at the end (to be able to puncture the vacuum blood tubes)**
- Safety googles

Procedure

- Prepare vacuum blood test tubes with three drops of HCl 30% (fluka) through a 1 ml syringe with needle through the stopper
- Connect GO-flo bottle outlet with the CTD connector/barrel plus needle

- Puncture the vacuum blood test tube butyl stopper with the needle
- Mix contents
- Store at 4C

App 3.2.2. Sulfate/Sulfide/S/Fe/Metals etc in water samples

General Sampling procedure:

- Obtain water from the GO-flo in through the connection system + needle in a 50 ml N_2 flushed bottle
- **Note:** Bottles are N_2 over-pressurized so it might be necessary to insert another needle in the bottle to be able to fill it up!
- Go to a container/lab

a. <u>Sulfate</u>

- Prepare a pony vial with 40 µl 5% (w/v) Zn-acetate solution
- With a 1 ml syringe plus needles remove 1 ml water from the 50 ml N_2-flushed sampling bottle (put bottle upside down) and add to the pony vial
- Store at -20C

b. <u>S/Fe/Mn/Trace metals:</u>

Material:

- 10-100 ul pipettor
- 10-100 ul pipettes tips
- 1 ml pipettor
- 1 ml pipette tips
- 15 ml flacon tubes
- **HCl 30% suprapur** solution
- Tube rack

Procedure

- From the same 50 ml N_2-flushed collection bottle
- Prepare 15 ml labeled falcon tubes with **20 ul suprapur HCl (30%)**
- Get 2 ml seawater with a 1 ml syringe plus needle and add them to the 15 ml falcon tube
- Store at -20C

c. <u>Sulfide analysis</u>

- Take the rest of the 50 ml N_2-flushed collection bottle inside the anaerobic glove bag
- **Fix the water with Zn acetate: 1 ml water + 50 µl 5% Zn acetate solution (N_2-flushed one) in pony vials (add sticker label)**
- **NOTE: Keep the three amber bottles of the Diamine/$FeCl_3$ x $6H_2O$ solutions at 4C and equilibrate at room temperature before they need to be used**

<u>Procedure to measure sulfide on board</u>

Material:

- Collected sulfide samples and fixed with Zn acetate in pony vials
- Na_2S 1M (wageningen stock)
- N_2-flushed MQ water

- 15 and 50 ml falcon tubes
- Spectrophotometer
- Cuvettes for spectrophotometer
- Pipettor and tips to add solutions
- Anaerobic glove bag
- Pony vials for mixing with reagents

Procedure

- **1. Make standard curve**
  - Stock **Na_2S 1M**: Mix 250 µl 1M stock + 2.25 ml N_2-flushed MQw in a 15 ml falcon tube >> makes a **100 mM stock**
  - Pipette 250 µl 100 mM stock + 24.75 ml N_2-flushed MQw >> makes a **1 mM (1000 µM stock**)
  - **500 µM stock**: 1 ml 1000 µM stock + 1 ml water + 100 µl 5% Zn acetate
  - **250 µM stock**: 1 ml 500 µM stock + 1 ml water + 100 µl 5% Zn acetate
  - **100 µM stock**: 200 µl 1000 µM stock + 1800 µl water + 100 µl 5% Zn acetate
  - **100 µM stock**: 200 µl 1000 µM stock + 1800 µl water + 100 µl 5% Zn acetate
  - **50 µM stock**: 1 ml 100 µM stock + 1 ml water + 100 µl 5% Zn acetate
  - **25 µM stock**: 1 ml 50 µM stock + 1 ml water + 100 µl 5% Zn acetate
  - **12.5 µM stock**: 1 ml 25 µM stock + 1 ml water + 100 µl 5% Zn acetate
  - **6.25 µM stock**: 1 ml 12.5 µM stock + 1 ml water + 100 µl 5% Zn acetate
  - **1 µM stock:** 200 µl 100 µM stock + 1800 µl water + 100 µl 5% Zn acetate
  - **0.1 µM stock:** 200 µl 1 µM stock + 1800 µl water + 100 µl 5% Zn acetate

- **2. Note:** Sulfide concentrations expected in the black Sea water column range from 0.1-400 uM
  - **Water column**:
    - Surface to 110 m: less than 4 µM
    - 110-170 m: less than 4 µM
    - 170-250 m: less than 250 µM
    - Deeper: less than 1000 µM
  - **Porewater cores**:
    - Between 300-600 µM
  - **Porewater piston core**:
    - Sampling depths: 50 cm, 100 cm (less than 400 µM), 200 cm, 350 cm, 500 cm, 800 cm (less than 40 µM)

- **3. Measure standard and samples:**
  - Samples fixed with Zn acetate: 1 ml water + 50 µl 5% Zn acetate solution (N_2-flushed one) in pony vials (add sticker label)
  - Aliquot 1 ml of each standard indicated above in a pony vial
  - Make a blank with N_2-flushed water
  - Add 80 µl reagent solution (diamine/$FeCl_3$) according to the sulfide range indicated in the bottle to the standards, samples and blank
  - Mix gently. Let color develop for 20 min and measure at 670 nm (outside anaerobic glove bag).
  - The samples with high concentrations may develop color above 1.0 in the absorbance analysis after 20 minutes. If this is the case, dilute the samples with N_2-flushed water as indicated in the table and measure again

Sulfide range µM	Dilution required

1-40	No dilution
40-250	2:25 (2 parts of sulfide sample plus 23 parts of dilutant)
250-1000	1:50 (1 part added to 49 pars of dilutant)

App 3.3 Sediment sampling with Multicore

App 3.3.1 Core slicing with hydraulic slicer and bottom water sampling

Material

- Core slicer
- Multicore liners
- Rubber Stoppers
- Tubing (thick and thin)
- Metal spatulas
- Geochemical bags
- Stapples, stapples
- 125 ml bottles acid cleaned
- Filters (0.2um 25mm)
- 20 ml Plastic Syringe
- Labels
- Tissue
- Fridge-Freezer- -80C
- Pots porosity and carbonate

Procedure

- From the Multicorer, first place the stoppers on the top of the multicore liners that are full with sediment and unscrew them carefully from the multicorer.
- Then place a cut stopper underneath the liner and push sufficiently upwards taking care that there is no leakage and also not to disturb the core.
    - From the Multicorer, first place the stoppers on the top of the multicore liners that are full with sediment and unscrew them carefully from the multicorer.
    - Then place a cut stopper underneath the liner and push sufficiently upwards taking care that there is no leakage and also not to disturb the core.
- Take off the overlying water using the tubing or a **50-30 ml Syringe (precooled!!)**, aliquot some water in a 125 ml bottle for the "regular nutrients" and fill up a N2-flushed bottle for the "anaerobic ones", leaving approx. 3cm water above the sediment (**'bottom-water' sample).**
- Place the cores onto the core-slicer.
- Turn on the core slicer and individually move the sediment upwards using the hydraulic levers.
- Remove the remaining overlying water using the smaller tubing taking care not to disturb the sediment surface.
- Place the slicing table on top of the cores.
- Align all the cores so that the sediment surface is align with the top. Using a tissue, remove any surface water that is still present.

- Install the correct slicing program to be used on the slicer, not forgetting to press 'Nullen' on the side panel of the slicer before starting.
- Using the foot pedal, the cores are sliced every 1 cm (every 0.25 cm if possible in the upper part of the core):
- Using the plastic slicer, collect the slices into labeled geochemical bags using a metal spatula (clean between samples with water, ethanol and let them air dry) as aid (if needed). Repeat this step until the bottom of the core is reached.

- **Note:** for DNA and lipids store slices in geochemical bags, staple the bags and store at -80C
- **Porosity/Carbonate**: 5 ml wet sediment (with a syringe!) into the pots: storage at -20C

NOTE: Bottom water sample

- Get samples for the following analysis
  - N species, P and Si
  - DOC/TOC
  - DIC
  - Hydrogen isotopes
  - Anaerobic one: Sulfide, Sulfate, methane, Fe/S/Mn

App 3.3.2 Porewater extraction from cores (predrilled liner)

Materials to use:
-Core tube with pre-drilled (small) holes
-Yellow tape
-Syringes, stopcocks, luer locks, rhizons.
-Paperclip

Prior to sampling: cover holes with yellow tape. Preferable to collect several cores as back up.

1. Place the core in the support in the thermostated container

<u>2. Porewater sampling</u>. Can be done later, will not change very much over time.

a) Prepare all syringes with rhizons. Number the syringes according to the horizon to sample. Wear gloves all the time and work very clean. Put the core in a stable holder. Note the height of the sediment-water interface and number the holes according to that.

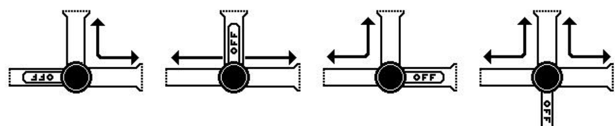
b) Work with two people. One punctures the yellow tape with a paperclip, the other directly puts the rhizon in. Don't be afraid to use some force to push rhizon through hole. Clean the hole and attach the syringe in such a way that the rhizon stays in the core perfectly horizontally (attach to the core with tape or with another kind of support), so it won't sample other depths. Keep stopcocks still in closed position (atmosphere to syringe open, to rhizon closed. OFF).

c) For next holes, alternate sides if you have small depth intervals.
d) If all syringes are in, open the connection between the syringe and the rhizon of each sample, starting from the top (this means change the OFF position into the direction of the atmosphere, see second picture at the bottom).

e) Pull the syringe barrel open, so vacuum is formed and put pieces of wood in to keep it open. Filling the syringes can take several hours. Up to 7 hours is no problem. If you are looking for redox sensitive elements, don't expand time too much to avoid risk of oxidation. Note: It's better to fill the syringe barrel completely if possible to avoid oxygen.

f) After syringes are full, close stopcocks OFF at syringe side. Remove from core.

g) Check and write down sample volume according to the ruler of the syringe barrel. Then use sampling plan to decide which vials can be filled with which sample volume.
Notes:
- Add the appropriate amount of HCl or NaOH according to the protocol and the volume that you have recovered in the tubes.
- If there is precipitation after sampling, shake the syringe very well and try to take a homogeneous sample. The precipitation will disappear in the vial after the addition of acid.
- If not all vials can be filled completely, also change the amount of acid added depending on amount of sample.



General scheme for the subsampling of the porewater samples.

Analysis	Vol. (ml)	Vial	Treatment	Code	Method	Storage
HS	0.5	Pony vial	0.5 ml water + 25 ul 5% Zn acetate solution	HS	Onboard	Analysis on board
Alkalinity	1.2	15 ml greiner, later transferred to alkalinity tube	None	ALK	Onboard titration	4°C
PO_4, Silica	1	Pony vial	4 µl 5 M HCl	PO4, Si	Back at NIOZ	4°C
S, Fe, Mn, trace metals, major elements	1 or 2	15ml greiner	10 µl suprapur 30% HCl per ml	ME	ICP-OES ICP-MS Utrecht	4°C
DIC	0.5	Glass vial 5 ml	- 4.4 ml saturated NaCl (freshly made) - 25 µl saturated solution HgCl2	DIC	Back at NIOZ	-20°C upside down
SO_4	0.5 ml	pony vial	20 µl 5% (w/v) Zn-acetate solution	IC	UU or NIOZ YE	-20°C
NH_4, NO_3, NO_2	Remaining sample, if >0.5	Pony vial	None	Bulk	Back at NIOZ	- 20°C

App 3.3.3 Porewater extraction and sediment sampling from gravity/piston core

Sampling depths: 50 cm, 100 cm, 200 cm, 350 cm, 500 cm, 800 cm

Note:

- The linen of the core should be drilled beforehand and taped with yellow tape
- Pre-cool a bottle at 4C to recover bottom water: Once the Gravity core is back on deck rapidly get a sample of bottom water as specified for CTD measurements
- Gravity cores are cut on 1 m sections on deck

Material

- Spatulas (metal) and aluminum foil
- Separators to divide different areas of the gravity core
- Ruler and camera to photograph the cores
- Rhizons (long and short)
- Containers for subsampling of the samples (or geochemical bags)
- Staples and stapler (if geo bags are used)

Procedure:

- Introduce the rhizon in the middle of the core to avoid contamination
- Apply vacuum with the syringe (10 ml syringe) and the wooden retainer
- Take samples and proceed to the listed analyses in order of priority: Sulfide, others
- To take samples for lipids or DNA from the gravity/piston core (also for isolations):
    - Open the core after removing the porewater
    - Photograph and determine the position of the sample/s to be taken (use ruler)
    - Choose sediment area inside the core to avoid contamination of the coring fluid (also it is recommended to sample from outside to inside the core to evaluate community composition and lack of contamination by the fluid)
    - Take big pieces of sediment and store in geochemical bags at -80C (lipids/DNA) or at 4C (enrichments, Wageningen)

App 3.3.4 Multicore methane sampling

Material needed:

- **Core tube with pre-drilled holes (2 cm diameter holes!)**
- Yellow tape, knife
- Cutoff 10ml syringes and 1 normal 10ml syringe
- 65ml glass bottles filled with saturated salt (NaCl) solution, incl. rubber stoppers and screw caps

Make sure to work as fast as possible in order to reduce the potential loss of methane!

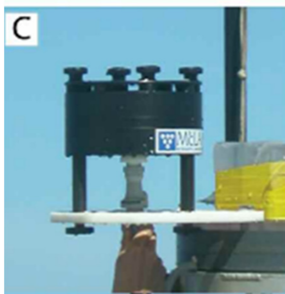
- For sampling porewater methane concentrations in the multicores, one subcore from each cast has to be pre-drilled with 2 cm diameter holes (two rows of 10 cm resolution holes on opposing sides of the tube, offset by 5 cm).
- Tap the holes with electrical tape or yellow tape prior to coring
- Prior to the coring, carefully tape the pre-drilled holes and make sure that there are no air-gaps left underneath the tape.
- Upon recovery, the subcore should be removed from the multicorer, stoppered, capped and transferred to a temperature-controlled container (the one with the slicer etc)
- Mark the sediment-water interface (SWI) on the yellow tape and start numbering the holes below the SWI.
- The subsequent sampling is best performed with two persons. One person cuts a cross into the tape while the second person slightly inserts a 10ml cutoff syringe. The syringe should only be inserted a few millimeters to stabilize it. Then, the cylindrical tube (outer part of the syringe) is slowly pushed into the core, while holding the plunger (inner part of the syringe). **Attention: Do not insert the whole syringe first and then pull out the plunger!**
- Precisely extract 10ml wet sediment and immediately transfer it into a 65ml glass bottle filled with saturated salt (NaCl) solution (**made fresh! 500 ml bottles with weighted NaCl add the appropriated amount of MQw**).
- The second person should seal the 65ml glass bottles with a black rubber stopper and a screw cap once the first person has injected all of the sediment into the saturated salt solution. **Attention: The bottles should be completely filled with the saturated salt solution and thus be overflowing when the sediment is injected (to avoid air, i.e. oxygen, to be trapped)!**
- After the closure, the bottles are shaken and stored upside down at 4C

Sampling procedure:

App 3.4 Water sampling with In-situ pumps

Material

- Pump
- Tool box
- 142 mm 0.7 µm GFFs
- Laptop
- Cable to connect laptop and pump
- Tweezers



Procedure

See pump manual

Each pump was programmed before deployment and material was collected on pre-ashed glass fiber filters (0.7 µm pore size, 142 mm diameter). The programming consists of a sample volume; an initial flow rate; a minimum flow rate; a time limit; the pump data period and a count-down timer. At station 2, three *in situ* pumps were deployed at 15 depths during 5 pumping sessions. At station 8, three *in situ* pumps were deployed at 3 depths in 1 pumping session. At station 9, two *in situ* pumps were deployed at 2 depths in 1 pumping session and at station 15, one *in situ* pump was deployed. At station 7, two *in situ* pumps were deployed at 2 depths in 1 pumping session. At station 14 two *in situ* pumps were deployed at 5 and 10 meters below sea surface.

The filters were collected immediately after the pumps were retrieved. The filters were photographed from above,

Notes:

- Fold the filter **once exactly!**
- Wrap in aluminum foil and label on the aluminum foil
- Label with black permanent pen and store in a geochemical bags (stapple the bag)
- Store at -80C

FULL BATTERIES: EVERY TIME THAT A "DEEP SAMPLING STATION" STARTS
ALSO FULL BATTERIES SHOULD BE ENOUGH TO PERFORM APROX 7 STATIONS 3 UPPER SAMPLING DEPTHS
- Each pump has 24 batteries!

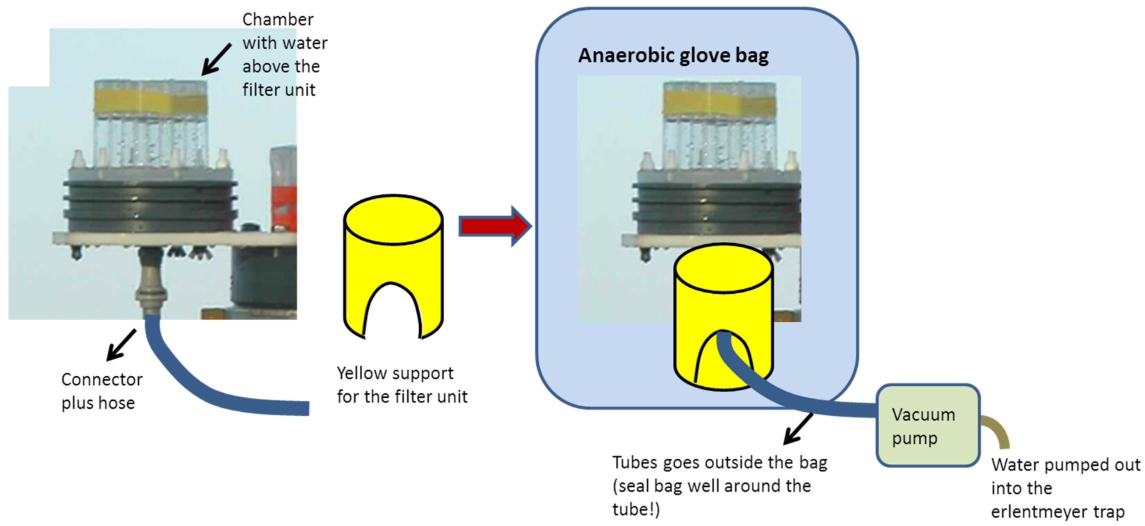
App 3.5 "Anaerobic" In-situ pumps

Information:

- These pumps are basically the same as the regular ones but the holder is different (mini- **MULVFS 142 mm filter holder**) because the material of the filter holder doesn't contain metals (ideal for sampling of metal species) and also allows the placement of several filters concatenated
- The pump we are borrowing has two of these MULVFS holders. These holders have a water camber made out of tubes o top of the filter unit so that once the filter holder comes back to deck, it is still covered by "anaerobic" water. The filter can be removed from the unit under anaerobic conditions (anaerobic glove bag) and even stored inside impermeable aluminum foil or seal inside a gas-impermeable bag inside the anaerobic glove bag

Procedure

- The computer program is the same as for the regular pumps
- Once the pump comes back to deck, disassemble the filter unit (with the water deposit on top), connect the filter support outlet (bottom) to the connector plus big hose made for the purpose and place the filter unit on the yellow tube support
- Bring the whole thing inside the anaerobic glove bag
- Make anaerobiosis inside the bag by flushing N_2 through a tube connected to the bag (and well-sealed around the tube so that there are no loses) followed by a cycle of vacuum (another tube coming out of the bag to a vacuum pump) followed by N_2 again (at least twice). Leave the bag full of N_2
- Then attach the hose connected to the in situ pump holder to the vacuum pump and drain the water contained in the chamber above the filter unit
- Open the filter unit (still inside the anaerobic glove bag), remove the filter, fold it once and store in aluminum foil (in this case normal one) and inside a geochemical bag or use it for enrichments right away.
- Store the filter at -80C right away if used for lipids/DNA/RNA



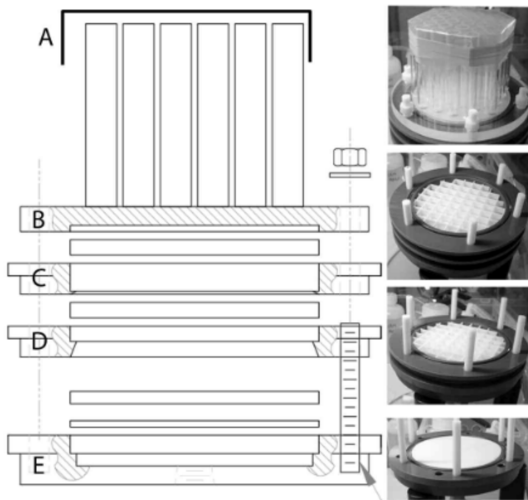
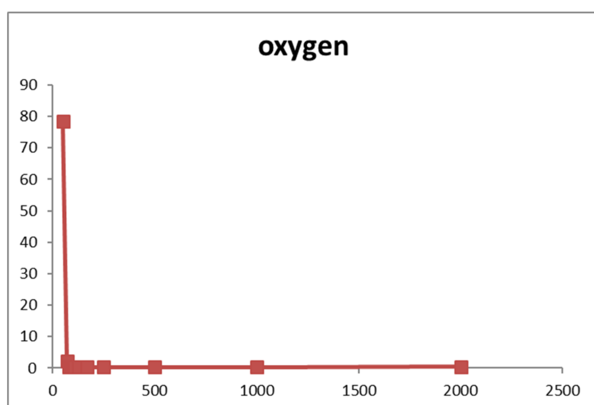
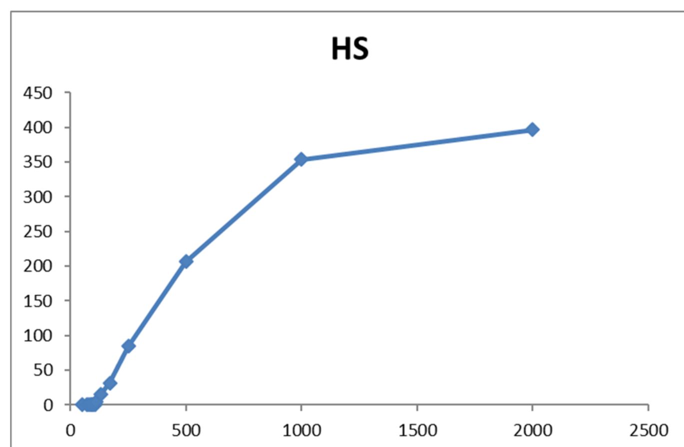


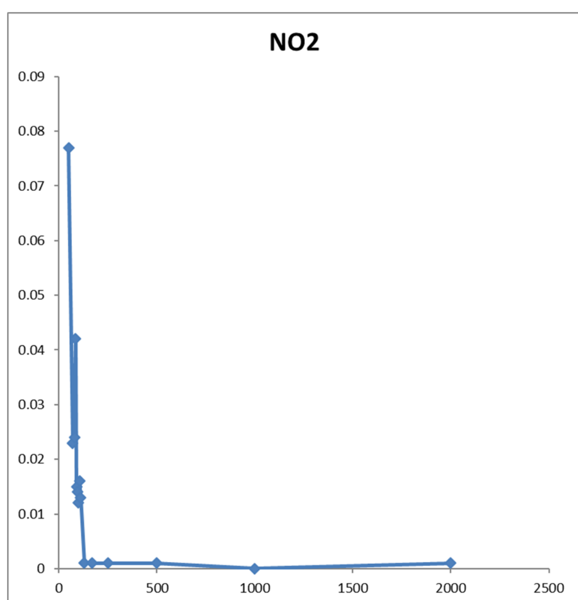
Fig. 2. Schematic of mini-MULVFS holder (Fig. 1B, type A2) with pictures of the baffle and filter support plates. The baffle logic follows that of the main MULVFS filter holder (Bishop and Wood 2008) with modifications to facilitate mounting on McLane pumps and handling in the laboratory.

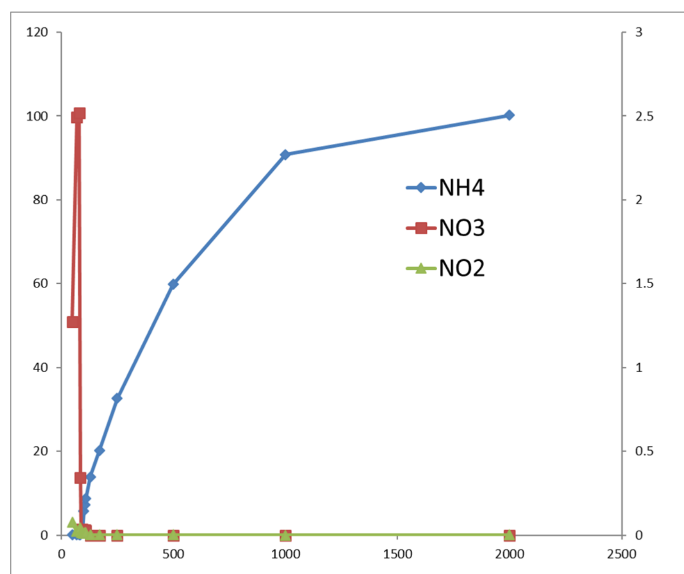
Appendix 4: Chapter 8 directly obtained data

depth	salinity	DIC	HS	NH4	NO3	NO2	PO4	Si	temperature	oxygen	pressure	fluorescence
50	19.4	3.292	0.1	0.083	1.273	0.077	0.738	26.56	7.908	78.37	51	0.102
70	20.1	3.326	0.15	0.053	2.493	0.023	1.121	45.8	8.418	2.194	71	0.053
80	20.4	3.269	0.1	0.058	2.518	0.024	1.137	45.725	8.494	0.509	81	0.064
85	20.5	3.273	0.15	0.063	0.344	0.042	0.787	55.36	8.504	0.333	86	0.056
90	20.6	3.295	0.1	0.423	0.035	0.015	2.121	57.84	8.513	0.321	91	0.058
95	20.8	3.3058	0.2	1.08	0.04	0.014	4.68	61.74	8.531	0.325	95	0.061
100	20.9	3.3188	0.2	5.73	0.03	0.012	7.155	64.74	8.543	0.323	101	0.0627
105	20.9	3.333	0.85	7.208	0.03	0.016	7.91	66.29	8.555	0.324	106	0.063
110	20.9	3.352	4.6	8.759	0.027	0.013	6.74	70.59	8.573	0.324	110	0.063
130	21.1	3.357	14.65	13.902	0	0.001	5.483	84.815	8.633	0.325	132	0.064
170	21.4	3.383	31.55	20.122	0	0.001	4.885	97.015	8.71	0.335	172	0.074
250	21.7	3.524	84.65	32.629	0	0.001	5.2	131.19	8.812	0.334	253	0.087
500	22.1	3.819	206.3	59.821	0	0.001	6.8	218.565	8.89	0.319	505	0.112
1000	22.3	4.117	353.8	90.764	0	0	7.915	292.04	8.961	0.3	1011	0.084
2000	22.3	4.229	397	100.164	0	0.001	8.425	324.44	9.109	0.464	2026	0.085









Appendix 4: Chapter 8 sample nomenclature and location

	station 1	Piston core	station 12	
	station 2	all	station 1	phox2

SULFIDE METHOD

1-40 uM	Original sa	water	solution 1
1:1	1000	0	80
1:10	100	900	80
1:20	50	950	80
1:50	20	980	80

extinction coefficient (L mol-1 cm-1)
2.95E+04

1-40 uM	Original sa	water	solution 1
1:1	2000	0	80
1:10	200	1800	80
1:20	100	1900	80
1:50	40	1960	80

WATER COLUMN

STATION 2							estimated			measured	1-2-2016	1-2-2016
Nomenclature	depth (m)	FISH	Polysulfid	BACK-UP	CK-UP wa	RICHMEN	HS (uM)	Dilution	Absorban	HS (uM)	Redox (mV	pH
1	5	yes	yes	yes, filter	nop	nop		1	0	0.00	142	7.97
2	25	yes	yes	nop	yep	nop		1		0.00	138	6.88
3	50	yes	yes	nop	yep	nop	0.1	1	0	0.00	132	6.6
4	70	yes	yes	nop	yep	nop	0.15	1	0.4	13.56	124	7.67
5	80	yes	yes	nop	yep	nop	0.1	1		0.00	123	6.83
6	85	yes	yes	nop	yep	nop	0.15	1		0.00	126	6.69
7	90	yes	yes	nop	yep	nop	0.1	1		0.00	120	7.61
8	95	yes	yes	nop	yep	nop	0.2	1		0.00	125	6.65
9	100	yes	yes	nop	yep	nop	0.2	1		0.00	119	6.62
10	105	yes	yes	nop	yep	nop	0.85	1		0.00	122	7.47
11	110	yes	yes	yes, filter	nop	nop	4.6	1		0.00	-35	7.48
12	130	yes	yes	yes, filter	nop	nop	14.65	1		0.00	-138	6.66
13	170	yes	yes	yes, filter	nop	nop	31.55	10	1.1	372.88	-228	6.9
14	250	yes	yes	yes, filter	nop	nop	84.65	10		0.00	-293	7.55
15	**500**	yes	yes	**yes, anoxi**	YES	**YES, ANOX**	206.3	10		0.00	-317	7.42
16	**1000**	yes	yes	**yes, anoxi**	nop	nop	353.8	20		0.00	-295	6.94
17	1500		yes					20		0.00	-305	6.87
18	1980	yes	yes	yes, filter	nop	nop	397	20		0.00	-314	6.92

POREWATER

STATION 2	Depth (cm	CARD-FISH, sul	Polysulfid	BACK-UP	ENRICHMENTS		HS (uM)	Dilution	Absorban	HS (uM)
bw	bw	yes	yes	region 1	yes		150	20	0.1	67.80
	0	yes	yes	1	yes		150	20	0.1	67.80
	1	yes	no	1	yes			20		0.00
	2	yes	yes	1	yes			20		0.00
	3	yes	no	1	yes			20		0.00
	4	yes	yes	1	yes			20		0.00
	5	yes	no	1	yes		400	20		0.00
	6	yes	yes	2	nop			20		0.00
	7	yes	no	2	nop			20		0.00
	8	yes	yes	2	nop			20		0.00
	9	yes	no	2	nop			20		0.00
	10	yes	yes	2	nop		400	20		0.00
	11	yes	no	3	nop					0.00
	12	yes	yes	3	nop					0.00
	13	yes	no	3	nop					0.00
	14	yes	yes	3	nop					0.00
	15	yes	no	3	nop		200	10		0.00
	16	yes	yes	4	nop			0		0.00
	17	yes	no	4	nop			0		0.00
	18	yes	yes	4	nop			0		0.00
	19	yes	no	4	nop			0		0.00
	20	yes	yes	4	nop		300	15		0.00

STATION 1	Depth (cm	CARD-FISH, sul	Polysulfid	BACK-UP	ENRICHMENTS		HS (uM)	Dilution	Absorban	HS (uM)	Core Secti	Distance
9	25						200	10		0.00	1	
8	30						400	20		0.00	1	
7	35						200	10		0.00	1	
6	40						300	15		0.00	1	
5	45						400	20		0.00	1	
4	**50**	**yes**	**yes**	**yes**	**nop**		250	12.5		0.00	1	
3	**100**	**yes**	**yes**	**yes**	**nop**		400	20		0.00	1	
2	150	yes	**yes**	**yes**	**nop**		800	40		0.00	2	
1	**200**	**yes**	**yes**	**yes**	**yes**		0			0.00	2	
	250	**yes**										
	300	**yes**										
	350	**yes**	**yes**	**yes**	**yes**		0			0.00	3	
	400	**yes**									4	
	500	**yes**	**yes**	**yes**	**nop**		0			0.00	5	

? sulfidic zone
methanogenic zone

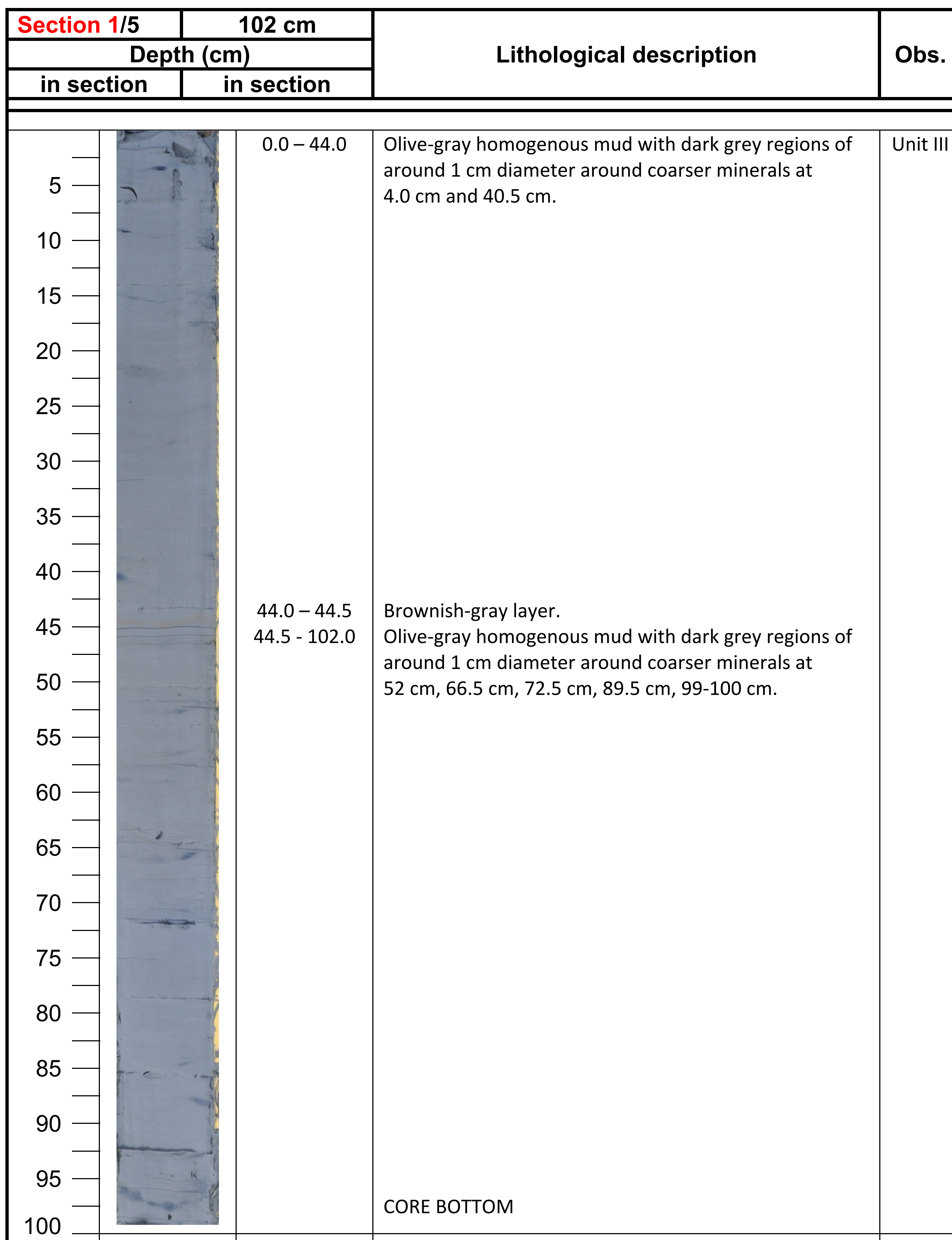
Nomencla	PW-HS-St1-50-I	PW (pore	HS (type a	St1 (statio	50 (cm de	I (first duplicate)
			HS	sulfide		
			S	sulfate, sulfite, thiosulfite, sulfur		
			Sx	polysulfide		
			VFA	fatty acids		
			CF	card-fish		
		WC (water column)		St1 (statio	1-18 (num	I (first duplicate)
		GC (gravity core)			150 (cm)	
			Bu	backup		
		MC1 (multicore, numberOfThe			1-4 (region)	

Core secti	Distance from t	Redox 1 (r	Distance f	Redox (mV)
1	**50**	**-60**	**75**	**-158**
2	**52**	**12**		**-406**
3	**51**	**-250**		**-345**
4	**52**	**-62**	**77**	**-373**
5	**51**	**-256**	**77**	**-221**

Depths (cr	REAL	Section	Sample	ENRICHME	BACKuo
50	40	**5**	**9**		yes
100	90	**5**	**8**		yes
150	140	**4**	**7**	yes	yes
200	190	**4**	**6**	yes	yes
250	240	**3**	5		yes
300	290	**3**	4		yes
350	340	**2**	3		yes
400	390	**2**	2		yes
500	490	**1**	1		yes

NESSC CRUISE 64PE408 – Black Sea - 2016			
R/V Pelagia	**Sampling equipment:**	**Gravity corer**	**Date: 30 January 2016**
Water depth:1969 m	**Latitude:** **Longitude:**	**42°56’12.0”** **30°01’54.8”**	**Station Nr. 1** **equivalent to PHOXY-12 (2013)**

Author: Anne Roepert (*Utrecht University*)

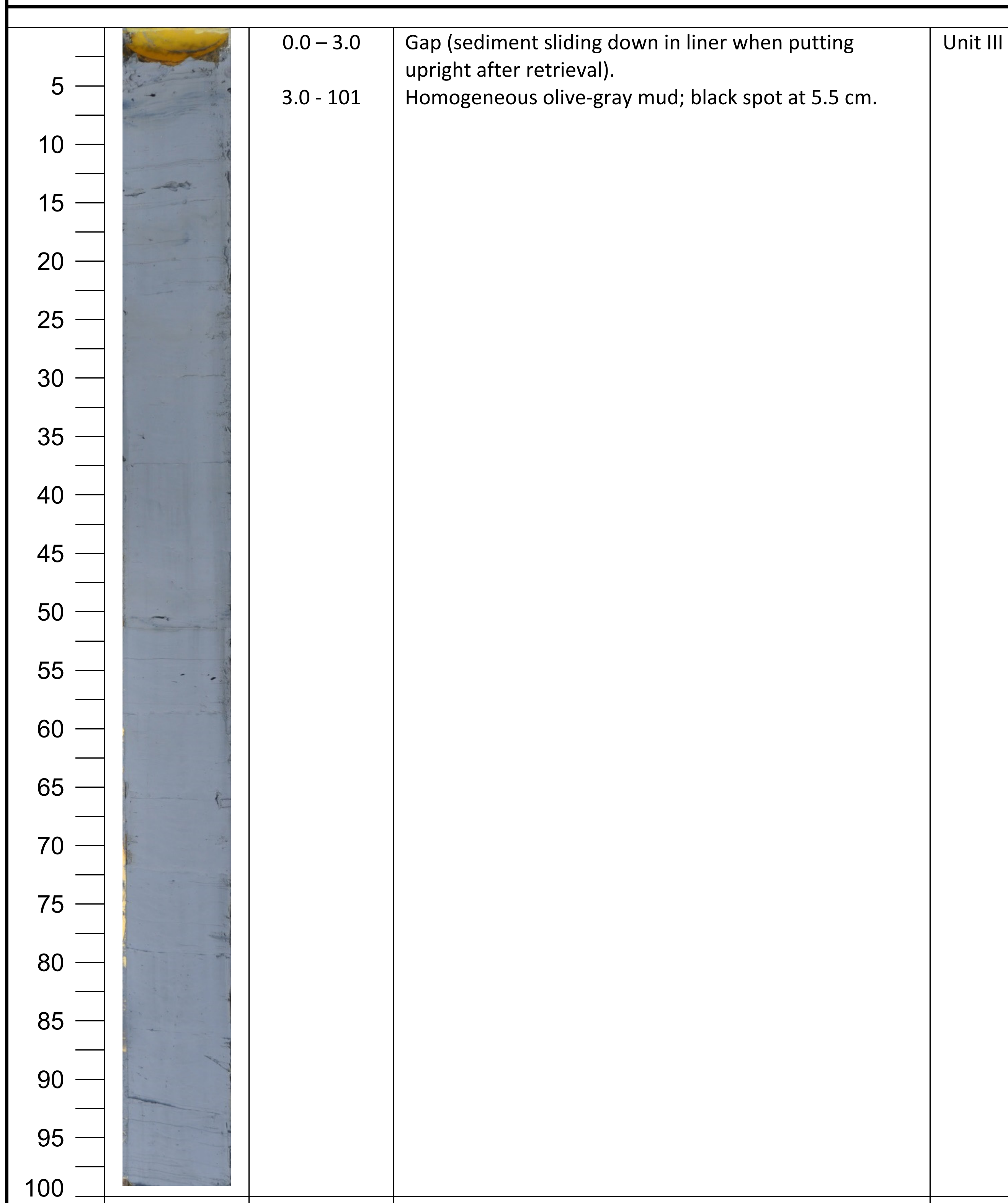


Section 1/5	**102 cm**	**Lithological description**	**Obs.**
Depth (cm) in section	**Depth (cm) in section**		
	0.0 – 44.0	Olive-gray homogenous mud with dark grey regions of around 1 cm diameter around coarser minerals at 4.0 cm and 40.5 cm.	Unit III
	44.0 – 44.5	Brownish-gray layer.	
	44.5 - 102.0	Olive-gray homogenous mud with dark grey regions of around 1 cm diameter around coarser minerals at 52 cm, 66.5 cm, 72.5 cm, 89.5 cm, 99-100 cm.	
		CORE BOTTOM	

NESSC CRUISE 64PE408 – Black Sea - 2016			
R/V Pelagia	Sampling equipment:	Gravity corer	Date: 30 January 2016
Water depth:1969 m	Latitude: Longitude:	42°56'12.0" 30°01'54.8"	Station Nr. 1 equivalent to PHOXY-12 (2013)

Author: Anne Roepert (*Utrecht University*)

Section 2/5	101 cm	Lithological description	Obs.
Depth (cm)			
in section	in section		
	0.0 – 3.0	Gap (sediment sliding down in liner when putting upright after retrieval).	Unit III
	3.0 - 101	Homogeneous olive-gray mud; black spot at 5.5 cm.	



NESSC CRUISE 64PE408 – Black Sea - 2016		
R/V Pelagia	Sampling equipment: Gravity corer	Date: 30 January 2016
Water depth:1969 m	Latitude: 42°56'12.0" Longitude: 30°01'54.8"	Station Nr. 1 equivalent to PHOXY-12 (2013)

Author: Anne Roepert (*Utrecht University*)

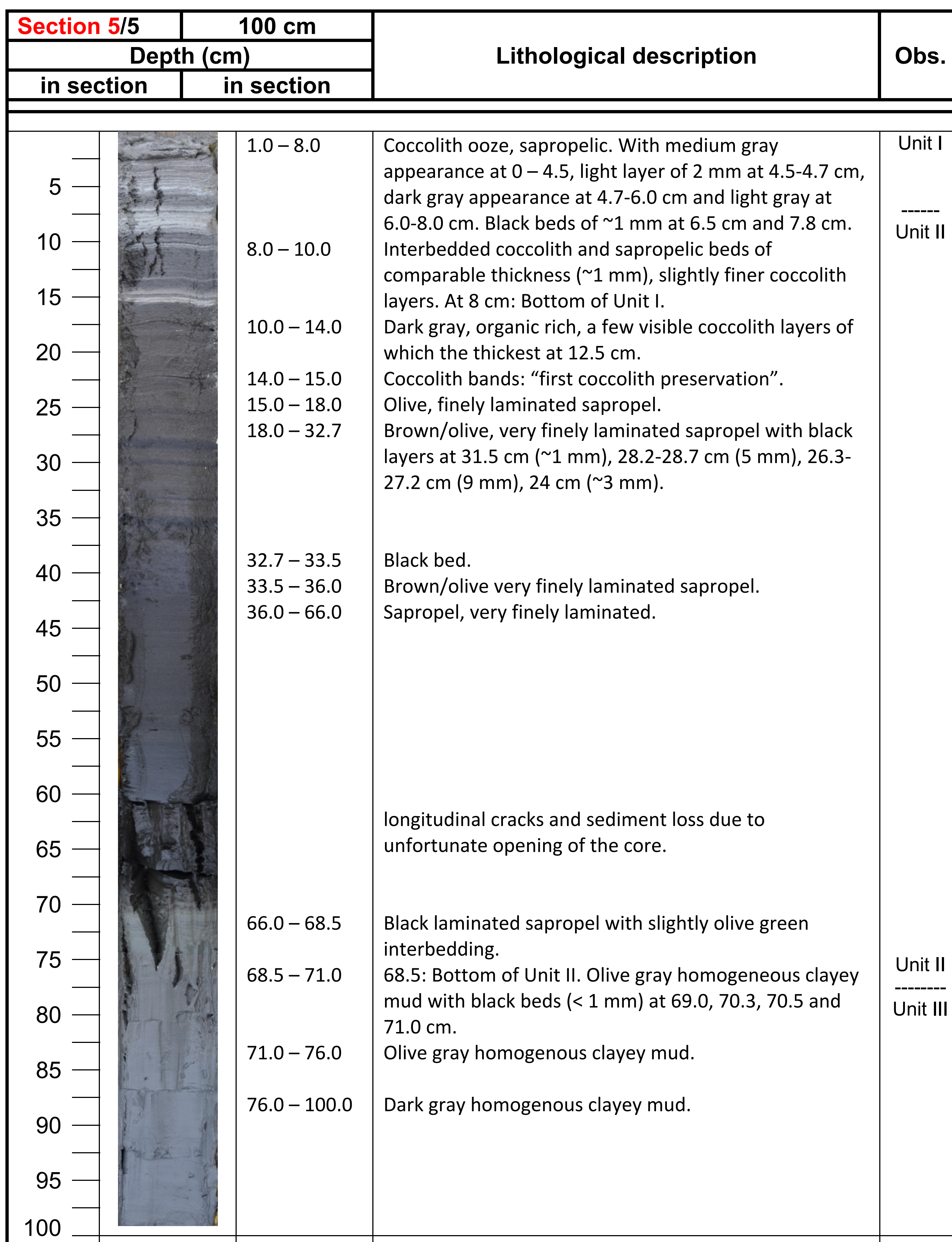
Section 3/5 — 100 cm Depth (cm) in section	Depth (cm) in section	Lithological description	Obs.
5 – 100 (scale)	0.0 – 12.5	Black cohesive mud, some coarser black minerals (sand fraction) Few local spots of light clayey mud.	Unit III
	12.5 – 14.0	Light gray mud with black local spots.	
	14.0 – 21.5	Black cohesive mud, very fine lighter discontinuous bands (sub-mm).	
	21.5-22.5	light gray clayey mud.	
	22.5-34.0	Black clayey mud with some light gray layers and some spotty light gray zones.	
	34.0 – 39.0	Transition from black / light gray clayey mud to olive clayey mud. Intermitted layers in black/gray.	
	39.0 – 55.0	Olive clayey mud, homogenous with local black zones around black coarse minerals.	
	55.0 – 56.0	Several black intermitted layers.	
	56.0 – 78.5	Olive clayey mud, homogenous.	
	78.5 – 80.0	Black intermitted layers.	
	80.0 – 100.0	Olive clay with areas of black layers at 84 cm (2 mm), 89 cm (2 mm), 91.5 cm (2 mm), 94 cm (3 mm), 95.5 cm (3 mm). They layers at 94 and 95.5 cm did not span the whole width of the liner.	

NESSC CRUISE 64PE408 – Black Sea - 2016			
R/V Pelagia	Sampling equipment:	Gravity corer	Date: 30 January 2016
Water depth:1969 m	Latitude: Longitude:	42°56'12.0" 30°01'54.8"	Station Nr. 1 equivalent to PHOXY-12 (2013)

Author: Anne Roepert (*Utrecht University*)

Section 4/5	101 cm	Lithological description	Obs.
Depth (cm) in section	in section		
	0.0 – 4.0	Dark gray laminated mud.	Unit III
	4.0 – 10.5	Light gray mud.	
	10.5 – 16.0	Dark gray laminated mud.	
	16.0 – 26.0	Light gray mud; at 25-26 cm some whitish intercalations.	
	26.0 – 33.0	Light gray mud with a few black spots.	
	33.0 – 36.0	Dark gray (anthrazite) beds and spots, not clearly laminated.	
	36.0 – 43.0	Light gray mud: 1 sand layer (< 1 mm) at 41.0 cm	
	43.0 – 45.0	Even lighter gray mud.	
	45.0 – 52.0	ca. 45° "plane" (spanning the whole core diameter) filled with sandy material, around it dark and light gray mud, no laminae visible.	
	52.0 – 53.0	Dark gray mud.	
	53.0 – 56.0	Light gray mud with 1 black ~1 mm layer.	
	56.0 – 64.0	Dark gray mud. At 59.4 cm light bed ~1 mm. At 62.0 cm dark bed ~0.5 mm.	
	64.0 – 65.0	Light gray mud.	
	65.0 – 65.7	Dark gray mud.	
	65.7 – 67.5	Light gray mud with black spots.	
	67.5 – 72.0	Dark gray mud.	
	72.0 – 101.0	Black clayey mud, strongly cohesive, clearly different than unit II sapropel.	

5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100



NESSC CRUISE 64PE408 – Black Sea - 2016			
R/V Pelagia	**Sampling equipment:**	**Gravity corer**	**Date: 30 January 2016**
Water depth:1969 m	**Latitude:** **Longitude:**	**42°56'12.0"** **30°01'54.8"**	**Station Nr. 1** **equivalent to PHOXY-12 (2013)**

Author: Anne Roepert (*Utrecht University*)

Section 5/5	**100 cm**		
Depth (cm) in section	**Depth (cm) in section**	**Lithological description**	**Obs.**
	1.0 – 8.0	Coccolith ooze, sapropelic. With medium gray appearance at 0 – 4.5, light layer of 2 mm at 4.5-4.7 cm, dark gray appearance at 4.7-6.0 cm and light gray at 6.0-8.0 cm. Black beds of ~1 mm at 6.5 cm and 7.8 cm.	Unit I ------ Unit II
	8.0 – 10.0	Interbedded coccolith and sapropelic beds of comparable thickness (~1 mm), slightly finer coccolith layers. At 8 cm: Bottom of Unit I.	
	10.0 – 14.0	Dark gray, organic rich, a few visible coccolith layers of which the thickest at 12.5 cm.	
	14.0 – 15.0	Coccolith bands: "first coccolith preservation".	
	15.0 – 18.0	Olive, finely laminated sapropel.	
	18.0 – 32.7	Brown/olive, very finely laminated sapropel with black layers at 31.5 cm (~1 mm), 28.2-28.7 cm (5 mm), 26.3-27.2 cm (9 mm), 24 cm (~3 mm).	
	32.7 – 33.5	Black bed.	
	33.5 – 36.0	Brown/olive very finely laminated sapropel.	
	36.0 – 66.0	Sapropel, very finely laminated. longitudinal cracks and sediment loss due to unfortunate opening of the core.	
	66.0 – 68.5	Black laminated sapropel with slightly olive green interbedding.	
	68.5 – 71.0	68.5: Bottom of Unit II. Olive gray homogeneous clayey mud with black beds (< 1 mm) at 69.0, 70.3, 70.5 and 71.0 cm.	Unit II -------- Unit III
	71.0 – 76.0	Olive gray homogenous clayey mud.	
	76.0 – 100.0	Dark gray homogenous clayey mud.	

A gravity core of a total length of 5.04 m was recovered at station 1. Obtaining pore water with rhizons from predrilled holes failed. The sections were opened, described for their lithology and sampled for microbiological purposes.

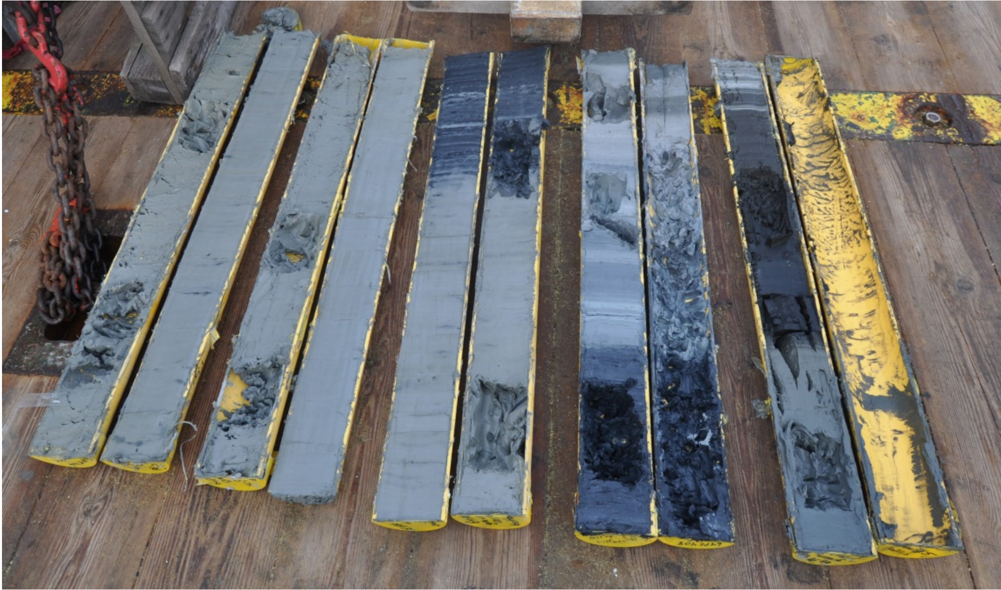


Figure 5 After sampling: sections 1 to 5 from left to right. Section tops in the background of the picture.

Appendix 6 Casino summary

Date	Time	Latitude(deg. min.milli)	Longitude(deg. min.milli)	Phase name	Phase type	Type	Device name	Device code	Action name	Action code	Operation Id	Station number	Strate
29/01/2016	20:48:33	N 40° 58.77084'	E 28° 53.61864'	TRANSIT1	TRANSIT	PHA							
30/01/2016	11:32:25	N 42° 54.0162'	E 30° 0.81534'	STATION1	STATION	PHA							
30/01/2016	11:35:36	N 42° 54.30948'	E 30° 0.95886'			OPE	Multibeam	EM302	Begin	BEGIN	408_EM3021	1	1_1
30/01/2016	12:13:28	N 42° 57.05982'	E 30° 2.44812'			OPE	Multibeam	EM302	End	END	408_EM3021	1	1_1
30/01/2016	12:33:05	N 42° 56.24334'	E 30° 1.99284'			OPE	CTD	CTD	Begin	BEGIN	408_CTD1	1	1_2
30/01/2016	13:11:24	N 42° 56.22132'	E 30° 1.9263'			OPE	CTD	CTD	Bottom	BOT	408_CTD1	1	1_2
30/01/2016	13:53:28	N 42° 56.21628'	E 30° 1.96554'			OPE	CTD	CTD	End	END	408_CTD1	1	1_2
30/01/2016	14:43:15	N 42° 56.19066'	E 30° 1.89564'			OPE	Multi Corer	MC12	Bottom	BOT	408_MC121	1	1_3
30/01/2016	17:16:42	N 42° 56.20266'	E 30° 1.89372'			OPE	Gravity Core	GC	Begin	BEGIN	408_GC1	1_4	
30/01/2016	17:17:00	N 42° 56.20266'	E 30° 1.89276'			OPE	Gravity Core	GC	Bottom	BOT	408_GC1	1_4	
30/01/2016	18:02:08	N 42° 56.11578'	E 30° 1.6995'	TRANSIT2	TRANSIT	PHA							
30/01/2016	20:37:18	N 42° 54.0288'	E 30° 30.77976'	STATION2	STATION	PHA							
30/01/2016	20:45:28	N 42° 54.01794'	E 30° 30.95832'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE1	2	2_1
31/01/2016	02:16:01	N 42° 54.00486'	E 30° 31.0107'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE1	2	2_1
31/01/2016	02:51:24	N 42° 53.99496'	E 30° 31.0047'			OPE	CTD	CTD	Begin	BEGIN	408_CTD2	2_2	
31/01/2016	03:29:01	N 42° 53.98584'	E 30° 31.02294'			OPE	CTD	CTD	Bottom	BOT	408_CTD2	2_2	
31/01/2016	04:40:54	N 42° 54.00354'	E 30° 30.97716'			OPE	CTD	CTD	End	END	408_CTD2	2_2	
31/01/2016	06:41:38	N 42° 54.0144'	E 30° 31.0209'			OPE	CTD Ultra clean	UCC	Begin	BEGIN	408_UCC1	2	2_3
31/01/2016	07:08:54	N 42° 54.05118'	E 30° 31.03188'			OPE	CTD Ultra clean	UCC	Bottom	BOT	408_UCC1	2	2_3
31/01/2016	07:36:12	N 42° 53.99532'	E 30° 31.0815'			OPE	CTD Ultra clean	UCC	End	END	408_UCC1	2	2_3
31/01/2016	09:21:40	N 42° 54.0165'	E 30° 31.03362'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE2	2	2_4
31/01/2016	14:07:26	N 42° 53.97894'	E 30° 31.02618'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE2	2	2_4
31/01/2016	15:30:01	N 42° 54.0003'	E 30° 31.06764'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE3	2_5	
31/01/2016	16:45:38	N 42° 54.00828'	E 30° 31.00692'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE3	2_5	start pump 17:45 stop 18:45
31/01/2016	18:08:12	N 42° 54.0801'	E 30° 31.0911'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE4	2	2-jun
31/01/2016	19:41:24	N 42° 54.05544'	E 30° 31.0656'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE4	2	2-jun
31/01/2016	20:49:15	N 42° 54.03738'	E 30° 31.03686'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE5	2	2_7
01/02/2016	06:03:03	N 42° 54.04932'	E 30° 31.04832'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE5	2	2_7
01/02/2016	07:03:38	N 42° 53.98182'	E 30° 30.93672'			OPE	CTD Ultra clean	UCC	Begin	BEGIN	408_UCC2	2	2_8
01/02/2016	07:18:25	N 42° 54.02808'	E 30° 30.99252'			OPE	CTD Ultra clean	UCC	Bottom	BOT	408_UCC2	2	2_8
01/02/2016	07:29:58	N 42° 54.021'	E 30° 30.9873'			OPE	CTD Ultra clean	UCC	End	END	408_UCC2	2	2_8
01/02/2016	08:12:26	N 42° 53.99382'	E 30° 30.96564'			OPE	CTD Ultra clean	UCC	Begin	BEGIN	408_UCC3	2	2_9
01/02/2016	08:58:03	N 42° 53.98794'	E 30° 30.96258'			OPE	CTD Ultra clean	UCC	Bottom	BOT	408_UCC3	2	2_9
01/02/2016	10:06:19	N 42° 53.9928'	E 30° 30.9945'			OPE	CTD Ultra clean	UCC	End	END	408_UCC3	2	2_9
01/02/2016	12:04:07	N 42° 54.03618'	E 30° 31.01052'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE6	2	2_10
01/02/2016	13:26:57	N 42° 54.00528'	E 30° 30.99186'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE6	2	2_10
01/02/2016	14:36:00	N 42° 54.01488'	E 30° 31.01208'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE7	2	2_11
01/02/2016	16:30:15	N 42° 53.99532'	E 30° 31.01016'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE7	2	2_11
01/02/2016	17:22:31	N 42° 54.01878'	E 30° 30.95934'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE8	2_12	
01/02/2016	19:58:31	N 42° 53.97432'	E 30° 30.9798'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE8	2	2_12
01/02/2016	20:58:26	N 42° 54.02652'	E 30° 31.00452'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE9	2	2_13
02/02/2016	06:00:50	N 42° 54.02928'	E 30° 31.01094'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE9	2	2_13
02/02/2016	06:58:09	N 42° 53.98224'	E 30° 31.02954'			OPE	CTD Ultra clean	UCC	Begin	BEGIN	408_UCC4	2	2_14
02/02/2016	07:10:59	N 42° 53.96772'	E 30° 30.98652'			OPE	CTD Ultra clean	UCC	Bottom	BOT	408_UCC4	2	2_14
02/02/2016	07:21:38	N 42° 53.9928'	E 30° 31.05048'			OPE	CTD Ultra clean	UCC	End	END	408_UCC4	2	2_14
02/02/2016	08:10:12	N 42° 54.05916'	E 30° 31.03026'			OPE	CTD Ultra clean	UCC	Begin	BEGIN	408_UCC5	2	2_15
02/02/2016	08:58:21	N 42° 54.03942'	E 30° 31.00626'			OPE	CTD Ultra clean	UCC	Bottom	BOT	408_UCC5	2	2_15
02/02/2016	10:09:24	N 42° 54.01368'	E 30° 30.99066'			OPE	CTD Ultra clean	UCC	End	END	408_UCC6	2	2_15
02/02/2016	11:26:48	N 42° 54.0021'	E 30° 30.9777'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE10	2_16	
02/02/2016	15:00:46	N 42° 54.04008'	E 30° 30.99798'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE10	2_16	
02/02/2016	16:47:38	N 42° 54.00642'	E 30° 31.01388'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE11		
02/02/2016	20:57:27	N 42° 54.05214'	E 30° 31.05'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE11	2_17	
02/02/2016	21:33:54	N 42° 54.10476'	E 30° 31.05486'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE12	2_18	
03/02/2016	05:54:54	N 42° 54.03162'	E 30° 30.97794'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE12	2_18	
03/02/2016	06:37:57	N 42° 53.99898'	E 30° 30.95532'			OPE	CTD with samples	CTDBOT	Begin	BEGIN	408_CTDBOT1	2	2_19
03/02/2016	07:14:40	N 42° 53.96706'	E 30° 30.9261'			OPE	CTD with samples	CTDBOT	Bottom	BOT	408_CTDBOT1	2	2_19
03/02/2016	08:23:00	N 42° 54.0645'	E 30° 30.92646'			OPE	CTD with samples	CTDBOT	End	END	408_CTDBOT1	2	2_19
03/02/2016	09:29:05	N 42° 54.01548'	E 30° 31.0548'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE13	2_20	
03/02/2016	12:04:29	N 42° 53.95854'	E 30° 31.00152'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE13	2_20	
03/02/2016	13:06:42	N 42° 54.00168'	E 30° 30.94368'			OPE	In Situ Pump	MCLANE	Begin	BEGIN	408_MCLANE14	2_21	
03/02/2016	14:53:47	N 42° 54.02742'	E 30° 30.96978'			OPE	In Situ Pump	MCLANE	End	END	408_MCLANE14	2_21	

03/02/2016	15:47:54	N 42° 53.99928'	E 30° 30.99852'			OPE	Multi Corer	MC12	Bottom	BOT	408_MC122	2_22
03/02/2016	16:36:48	N 42° 53.94888'	E 30° 30.80292'	TURNING1	TURNING	PHA						
04/02/2016	14:22:11	N 40° 58.78362'	E 28° 53.69412'	CALL1	CALL	PHA						