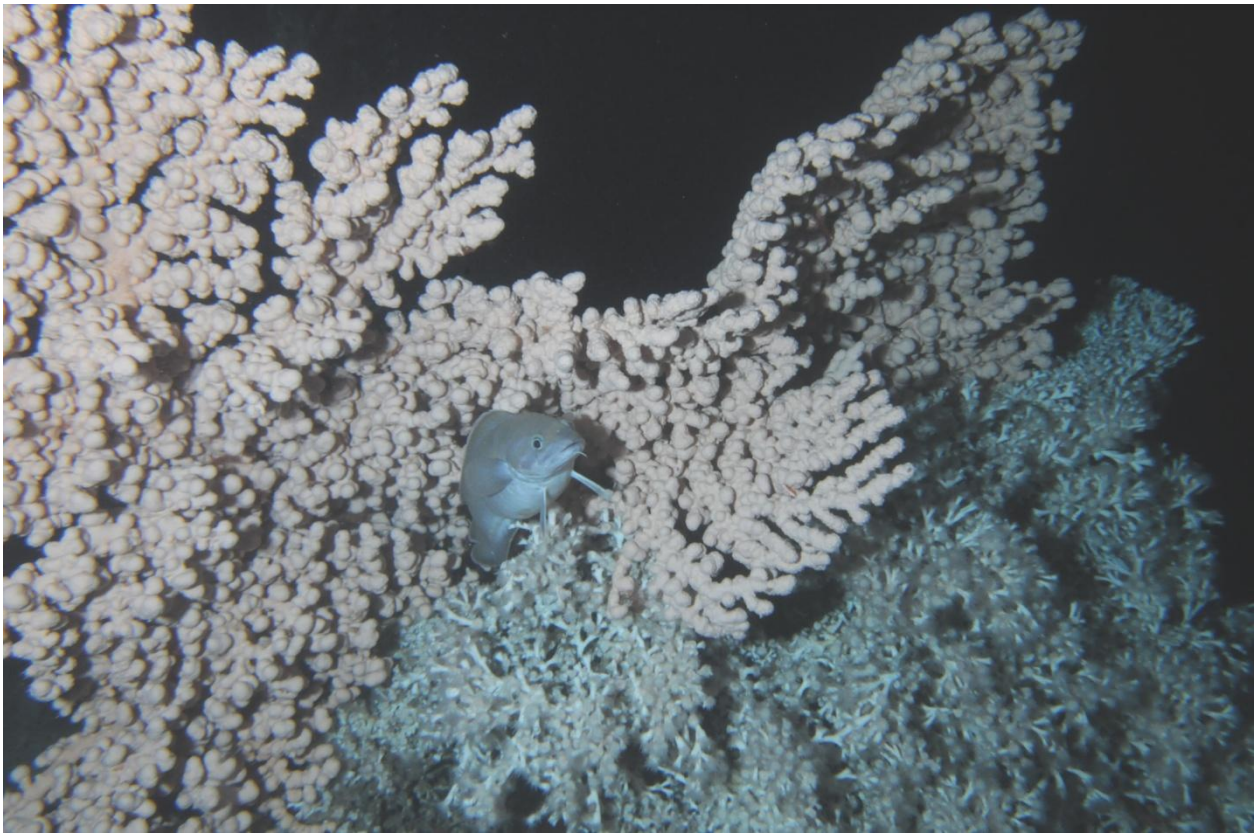
Environmental Monitoring Report

Morvin 2009-2010



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Summary

This report presents results from environmental monitoring during discharge of mud and drill cuttings during top hole drilling of four wells at the Morvin field in 2009 and 2010 (Block 6506/11 and production license 134b/c).

An observation platform equipped with acoustical and optical technology was developed that together with traditional environmental monitoring could be used to evaluate the effects of release of mud and drill cuttings on the environment in general and on corals in particular.

The sediment core samples reveal that one sample close to discharge point is contaminated with THC. There are also elevated levels of Barium in the downstream direction as expected. No other metal contamination was found.

The sediment trap upstream of the discharge point contained barium levels that were an order of magnitude lower than at the other sediment trap sites, although concentrations of Ba at this location were still considerably above the local background levels, so some minor contamination of that location is possible. The two downstream traps collected drill cuttings

Lipid class and fatty acid analyses of corals that had been exposed to drill cuttings and of corals that had not been exposed showed no significant differences between the two. Hence, the exposed corals did not have decreased amount of storage lipids compared corals from the unexposed control area and this suggest that there is no differences in the feeding rate between the two.

Image analyses revealed no significant behavioural differences between corals that were exposed to drill cuttings and unexposed corals. Detailed analyses of the time series from the exposed coral reef revealed that changes in current direction and speed were the main reasons for changes in coral polyp behaviour.

In conclusion, the mud and drill cuttings did reach the coral reefs in the downstream direction. However, our analyses do not reveal any immediate damage to the corals. It is recommended

that the coral reefs are revisited at a later stage to reveal long-term effects of the discharge of mud and drill cuttings.

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Contents

1. Introduction

The Morvin field is Located 20 kilometers west of the Åsgard B platform on the Halten Bank (see figure 1). The field is located in block 6506/11 and is regulated under production license 134b/c. The Morvin field is special in the sense that it is located in an area with high abundance of cold water coral reefs and therefore strongly regulated by the Climate and pollution agency (Klif). To minimize the effects on the corals, the mud and cuttings were released in a location expected to minimize the exposure to the corals by the use of a cutting transport system (CTS).

Figure 1 Location of the Morvin field (Map from Statoil).

The objective of this study was to develop an observation platform equipped with acoustical and optical technology that together with traditional environmental monitoring could be used to evaluate the effects of release of mud and drill cuttings on the environment in general and on corals in particular during top hole drilling of four wells at Morvin.

A multi-sensor platform was constructed based on the landers developed in the HERMES project (http://www.imr.no/korallobservatoriet, EU project Hotspot Ecosystems Research on the Margins of European Seas). The lander, having two scientific echosounders, is shown in figure 1 together with the camera satellite. The time-lapse camera was mounted on a satellite to be able to place it as close as possible to a coral reef. To allow for real time monitoring, the lander and satellite were connected to a surface buoy with a wireless Ethernet link to the rig. Hence, acoustic and optical data were accessible from anywhere on the Internet.

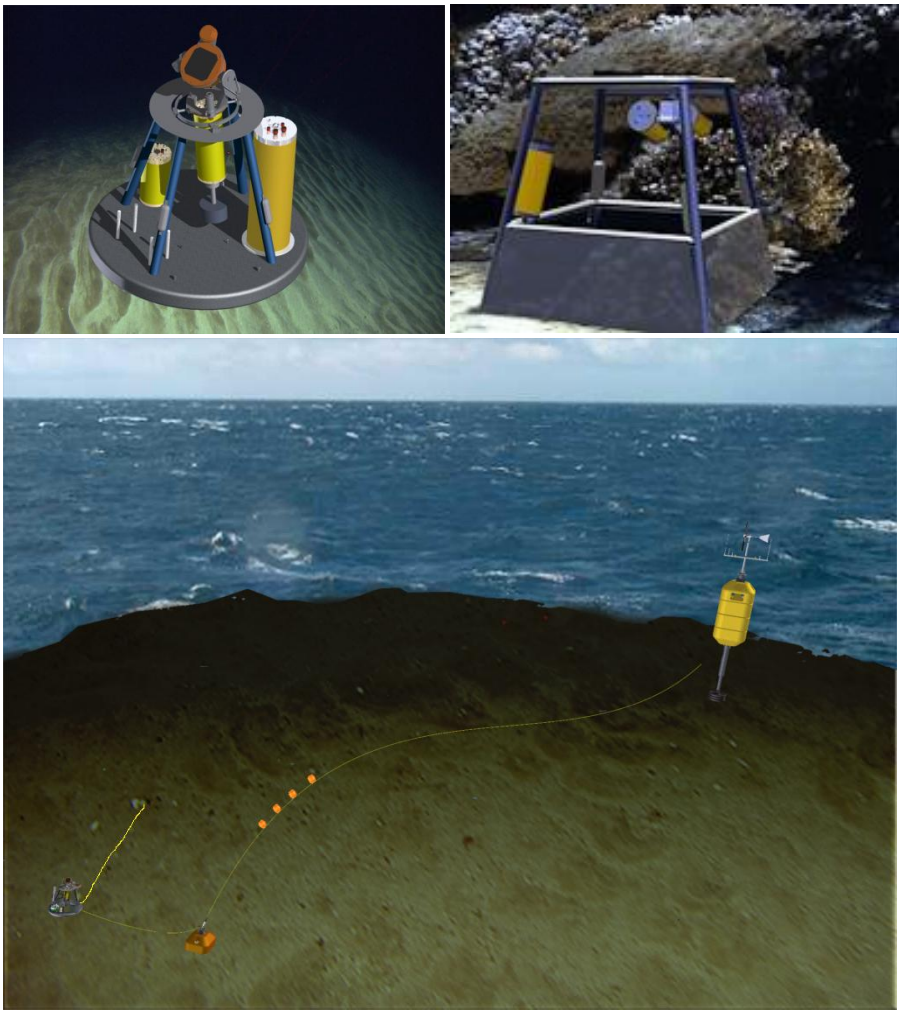


Figure 2 The acoustic lander, the camera satellite and the two connected to the surface buoy

The camera satellite was placed close to the MRRE reef (figure 3) to study behavioural changes of a selection of coral polyps when exposed to drill cuttings. Images were taken every 30 minutes.

The lander had two scientific echosounders, working at 38 and 120 kHz, which were motor controlled in order to scan the plume of drill cuttings. The echosounders are used to study the distribution and density of the plume.

A current profiler was mounted on the camera satellite to measure the current speed and direction close to bottom in order to better understand the density and direction of the plume.

Three sediment traps were placed along the expected current axis, one upstream and two downstream of the discharge point. The traps each had 21 bottles and were programmed to change bottle every 36 hours.

Sediment core samples were collected from two locations prior to and eight locations after the drilling period. The samples were collected using a remotely operated underwater vehicle (ROV).

Finally, samples of live corals were collected after being exposed to drill cuttings and compared to unexposed corals to reveal any changes in feeding rates between the two.

2. Field work

In this chapter an overview of the field work is given. Figure 3 shows the locations of the lander, camera satellite and the three sediment traps relative to Transocean Leader (rig) and the discharge point.

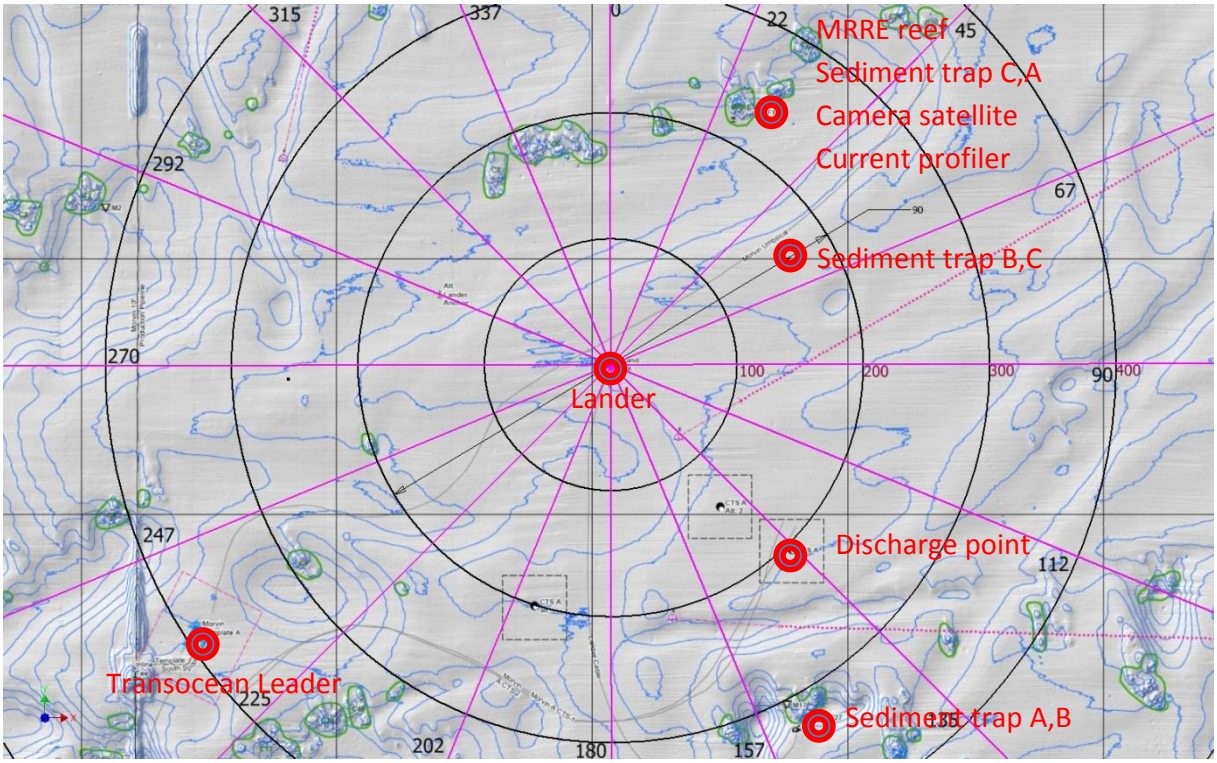


Figure 3 Overview of the Morvin field showing the rig Transocean Leader, the discharge point, the lander, the camera satellite and the three sediment traps. Green circles indicate the position of coral reefs.

Lander and camera satellite

The lander and camera satellite were deployed on 8.11.2009 using the vessel M/V Scandi Bergen. The location of the lander was chosen based on expected current direction in order to get an optimal acoustical view of the plume of drill cuttings. The camera satellite was placed close to the MRRE reef where the corals were most likely to be exposed to drill cuttings.

During a storm 18.11.2009, the cable between the lander and the surface buoy ruptured meaning that communication with the lander was lost and the battery capacity severly reduced. The lander and camera satellite were recovered and a new surface cable connected. The reduced battery capacity meant that the original acoustical monitoring plan had to be abandoned to secure enough power to the camera satellite. The lander and camera satellite were redeployed on November 26th. Problems with power and communication reduced the output of the satellite time-lapse camera to only 3 continues time series, 27.11.2009-28.11.2009,

30.11.2009-1.12.2009 and 9.2.2010. To compensate for the lack of satellite pictures from the MRRE reef, a ROV monitoring plan was established taking photos of selected coral colonies.

Current profiler

An Aanderaa RDCP600 current profiler was mounted on the frame of the camera satellite to measure the curent direction and speed close to the MRRE coral reef. The profiler was also equipped with sensors to measure temperature and turbidity. Due to a water leakage, the temperature and turbidity sensors did not provide reliable data.

Sediment traps

Three sediment traps were deployed between 8-9th of November 2009 using the vessel M/V Skandi Bergen. One trap was placed south and upstream of the discharge point, while the two others were placed north of the discharge point in the downstream direction (see figure 3). The traps were recovered on December 6th and redeployed on February 6th 2010 after a break in the drilling operation. There were problems with the trap programming described in the chapter on sediment traps.

Sediment core samples

Sediment core samples were taken prior to drilling in 3 positions in a radius of 100 m and 3 positions in a radius of 200 m from the discharge point using the ROV of the vessel Acergy Petrel. After completion of the drilling 25 new samples were taken using the ROV of R/V Edda Fauna.

Coral samples

Samples of live corals were collected using the ROV of M/V Edda Fauna from two reefs after completion of the drilling, the MRRE reef that had been exposed to drill cuttings and the M27 reef that had not. The MRRE reef can be found next to the camera satellite on the map in figure 3, while the M27 reef is outside the map about 600 m to the east of MRRE.

3. Current measurements

PI: Eirik Tenningen, Institute of Marine Research

3.1 Methods

The drill cuttings were discharged in a position believed to minimize the exposure on the surrounding coral reefs. Based on DNMI 2000 models, the prevailing current was predicted to flow in a northerly direction away from the nearest corals to the south of the discharge point. An Aanderaa RDCP600 current profiler was used for measuring the current flow. The instrument was mounted on the camera satellite close to the MRRE reef (see map in figure 3 in chapter 2).

3.2 Results and discussion

Figure 4 shows the current speed during the entire recording time of the RDCP. The line between November 23rd and November 26th indicates a lack of data during this period due to a service inspection.

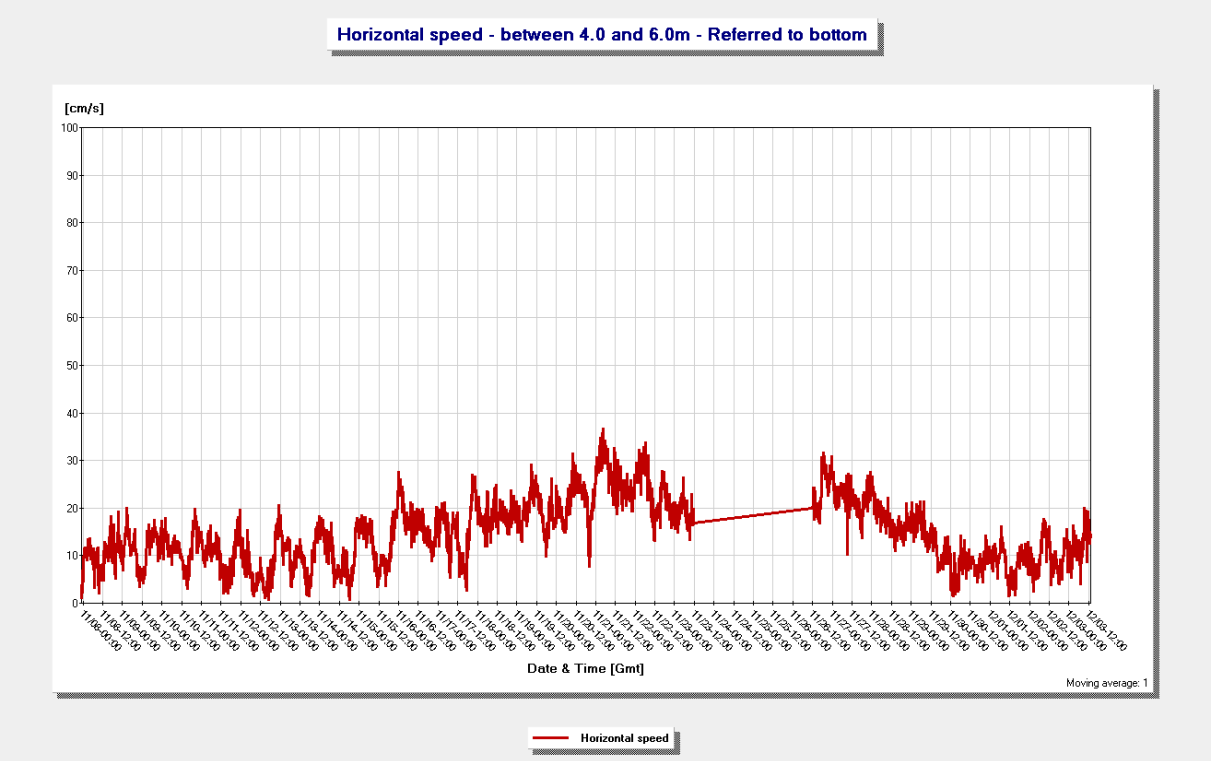


Figure 4 Current speed

The current speed reaches is maximum of approximately 35 cm/s on November 21st. Previous experiments indicate that the corals might reduce their degree of expansion when the current speed exceeds 20 cm/s. Figure 5 shows the current direction from the same period as figure 4.

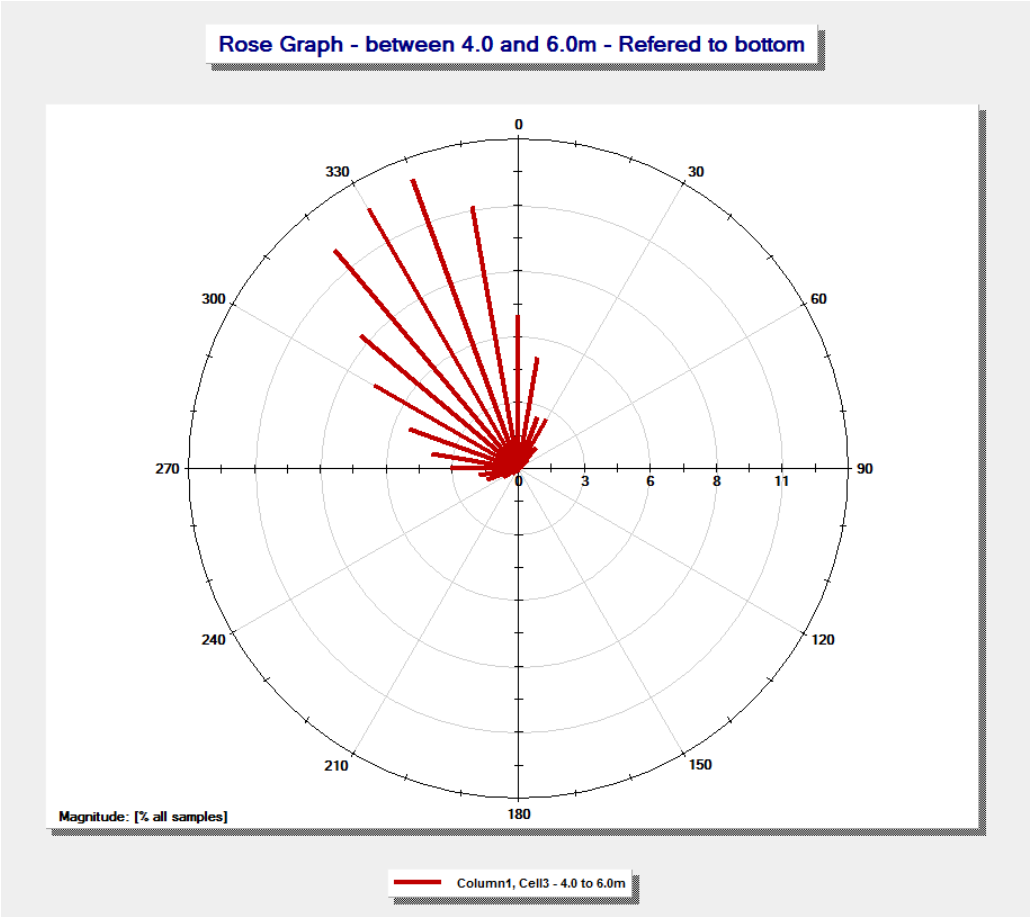


Figure 5 Current direction

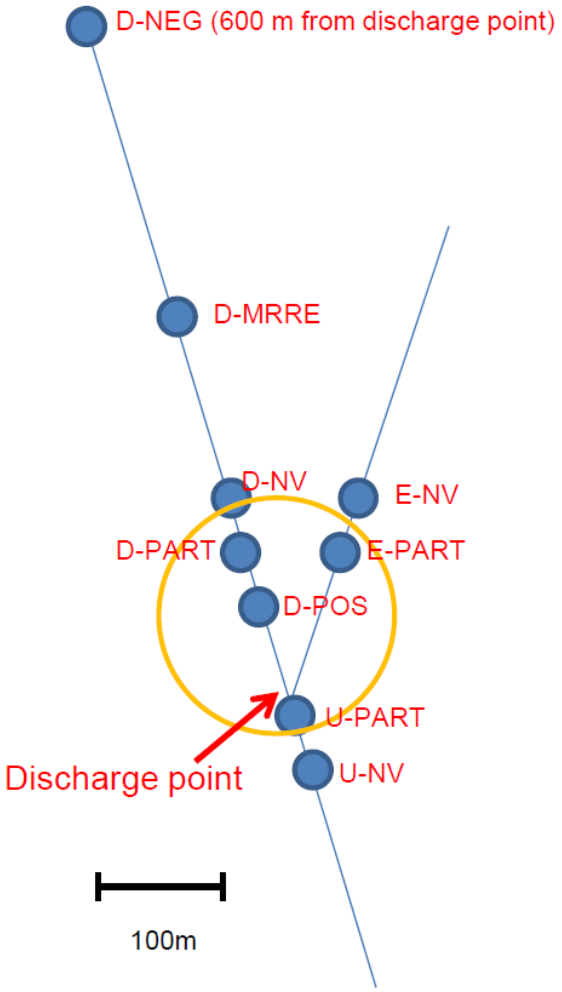
The prevailing current is, as predicted, in a north-northwesterly direction. Based on the current measurements there would be no influence from drill cuttings on the corals south of the release point.

4. Sediment core samples

PI: Jarle Klungsøyr, Institute of Marine Research

4.1 Methods

Push core samples were collected prior to and after drilling. All samples labeled RC8-xx and RC9-xx were collected close to the discharge point in the downstream (north) direction prior to the start of the drilling at 100 m and 200 m distance from the discharge point, respectively. The other samples were collected after completion of the drilling at the locations indicated by the map in figure 6 in a line along the measured prevailing current direction (N-NW direction) and with a shorter line in a more easterly direction.



Figur 6 Map showing the locations of the core samples taken after completion of the drilling. The yellow circle indicates expected area of sedimentation

The position codes are according to the codes below (described in Serpent's "Morvin March 2010 quick-look report"):

D = downstream
U = upstream
E = east
NEG = negative
NV = not visible
PART = partial
MRRE = the coral reef of that name
Therefore:
D – NEG: Downstream negative ~ 600 m from disturbance
D-MRRE: 6 m south of the MRRE coral reef
D-NV: Downstream no visible disturbance, 135 m from pipe approximately 10 m from the edge of the visible cuttings
D-PART: Downstream partial disturbance 100 m from pipe in partial disturbance zone
D-POS: Downstream positive sample – within full disturbance
U-NV: Upstream, no visible disturbance 40 m upstream of pipe
U-PART: Upstream, partial disturbance 25 m upstream of pipe
E-PART: East, partial disturbance 75 m from pipe
E-NV: East, no visible disturbance 110m from pipe

THC analysis

THC analyses were carried out using an accredited method established at IMR (Method O22). Dry sediment samples were extracted by Accelerated Solvent Extraction (ASE) using hexane:dichloromethane (1:1) as solvent, removal of sulphur by active copper, clean-up on silica Bond-Elute column and analysed by gas chromatography with flame ionization detector (GC-FID). Quantification limits were 1.0 mg/kg dry weight.

Metal analysis

For these analyses acidified aqueous sample solutions were obtained by dissolving 1 g of freeze-dried sediment in 7N HNO_3 in an autoclave at 120°C for 1 hour (Norwegian Standard NS 4770). The cadmium (Cd) analysis was done on a Perkin-Elmer SIMA 6000 atomic absorption spectrometer equipped with a graphite furnace (GFAAS). The mercury (Hg) analysis was done with a cold-vapour atomic absorption spectrometer (CVAAS) instrument CETAC M-6000A Hg Analyzer. Most of the reported elements were analysed using inductively coupled plasma atomic emission spectrometry (ICP-AES) type Perkin Elmer Optima 4300 Dual View. All results are reported as mg/kg dry sediment.

Total organic carbon analysis

For the TOC analyses a LECO CS 244 analyser was used. Aliquots (~200 mg) of the samples were treated with 10 % (volume) hydrochloric acid (HCl) at 60ºC to remove carbonate, and then washed with distilled water to remove HCl. We point out to the reader that the possible loss of organic material by acid leaching is not taken into account. The samples were dried overnight (50ºC) and then analysed.

4.2 Results and discussion

StatoilHydro commissioned Akvaplan-niva AS to carry out a regional environmental survey at Haltenbanken in 2009 (Akvaplan-Niva, 2010). In this study samples from 15 regional stations were collected to establish background concentrations for THC and metals (Ba, Cd, Cu, Cr, Hg, Pb, Zn) in the whole region. The results from this study are used to give comments to the results from this study at Morvin.

The results from the THC analysis are shown in table 1.

Table 1 THC concentration

Sample ID	Concentration [mg/kg]	
Morvin D-NEG 1 THC	**6,2**	
Morvin D-NEG 2 THC	**3,0**	
Morvin D-NEG 3 THC	**6,8**	
D- MRRE 1 THC	**3,6**	
D- MRRE 2 THC	**2,1**	
D-MRRE 3 THC	**3,4**	
D-NV 1 THC	**3,0**	
D-NV 2 THC	**4,0**	
D-NV 3 THC	**3,3**	
D-PART 1	**3,0**	
D-PART 2	**6,1**	
D-PART 3	**4,0**	
U-NV 1 THC	**4,3**	
U-NV 2 THC	**3,4**	
U-NV 3 THC	**3,4**	
U-PART 1 THC	**5,4**	
U-PART 2 THC	**7,1**	
U-PART 3 THC	**13,7**	
E-PART 1 THC	**6,1**	
E-PART 2 THC	**7,5**	
E-PART 3 THC	**7,0**	
E-NV 1 THC	**4,0**	
E-NV 2 THC	**3,7**	
E-NV 3 THC	**3,9**	
RC8- 1a	**5,0**	
RC8- 2a	**3,5**	
RC8- 3a	**3,5**	
RC9- 1a	**3,3**	
RC9- 2a	**3,4**	
RC9- 3a	**3,4**	
D-POS 1 THC	**178,1**	

The results show that the sediments are contaminated with THC in position D-POS which is the nearest to the release point in the downstream direction. This value (178.1 mg/kg) is significantly higher than for the rest of the samples (2.1-13.7 mg/kg). In comparison the 15 regional stations collected in 2009 showed THC concentrations in the range 1.8-4.1 mg/kg dw. THC concentrations higher than 50 mg/kg dw. may result in biological effects on benthic fauna (Bakke et al. 1990).

The results of the metal analysis are shown in table 2 and Appendix C. In the regional study the range of background concentration for the different elements were barium (Ba) 83-287 mg/kg dw., cadmium (Cd) 0.048-0.11 mg/kg dw., copper (Cu) 6.5-12.2 mg/kg dw., chromium (Cr) 16.4-34.6 mg/kg dw., lead 13.9-20.9 mg/kg dw., mercury (Hg) 0.023-0.237 mg/kg dw, zinc (Zn) 40.7-90.0 mg/kg dw (Akvaplan-Niva, 2010).

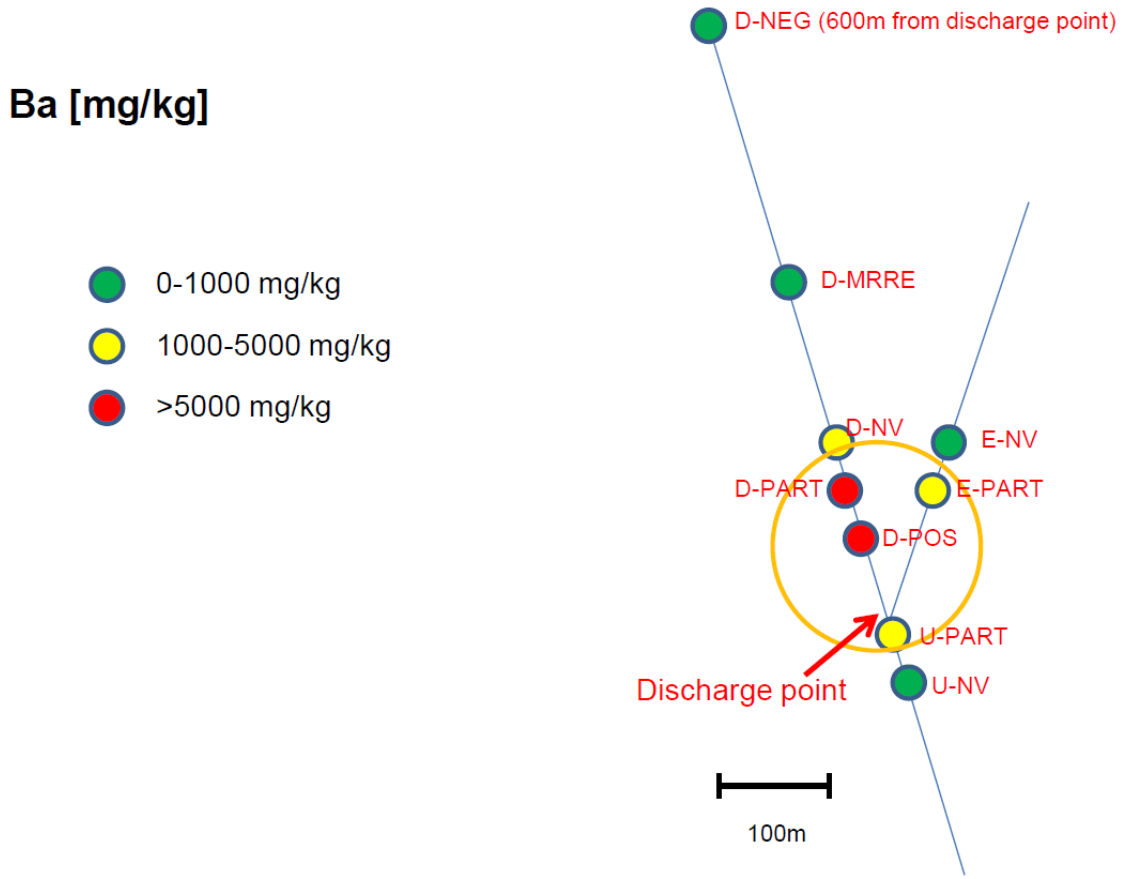
Assuming that natural background concentrations for Ba at Morvin is 300 mg/kg dw., 16 of 32 samples/positions showed elevated levels (Table 2). The highest concentrations were found in sample/position D-PART 2 HM. The analysis for Ba was very useful for tracing inputs of drilling mud at Morvin showing a north-northwesterly distribution as expected.

Table 2 shows that at Morvin all results for cadmium and most results for mercury were low and in the same range over the sampling area. Values for mercury were slightly above background concentration in sample D-MRRE 2 HM, D-PART 2HM, D-POS 1HM and D-POS 1THC. D-POS 1HM showed slightly elevated concentrations for Cu and Cr (Table 2). Cu and Zn were slightly elevated at D-POS 1THC.

Table 2 Metal analysis

Sample ID	Ba [mg/kg]	Cd [mg/kg]	Cu [mg/kg]	Cr [mg/kg]	Pb [mg/kg]	Zn [mg/kg]	Hg [mg/kg]
RC 8-1a	76,5	0,06	3,6	15,9	9,7	31,6	0,14
RC 8-2a	83,7	0,07	3,2	14,6	9,9	28,6	0,13
RC 8-3a	98,7	0,05	3,5	15,3	10,5	31	0,13
RC 9-1a	114	0,07	3,9	17,1	11,2	34,1	0,15
RC 9-1a	112	0,06	3,7	17	10,5	32,6	0,14
RC 9-1a	171	0,07	3,9	16,8	11,4	32,7	0,15
D-NEG 1 HM	285	0,08	4,3	17,3	13,8	35	0,19
D-NEG 2 HM	302	0,05	3,6	15,5	11,1	31,8	0,15
D-NEG 3 HM	237	0,04	3,9	16	11,6	33	0,15
D-MRRE 1 HM	219	0,07	3,7	16,2	11,3	33,1	0,17
D-MRRE 3 HM	193	0,05	3,7	16,2	11,4	32,3	0,14
D-MRRE 2 HM	177	0,08	3,5	15,6	9,5	30,7	0,38
D-NV 1 HM	1110	0,05	3,5	15,5	10,5	30,7	0,14
D-NV 2 HM	1380	0,06	3,8	15,6	10,6	31,2	0,16
D-NV 3 HM	1140	0,05	3,2	14,3	9,2	28,8	0,13
D-PART 1 HM	2630	0,08	5,1	18,9	12,7	36,1	0,26
D-PART 2 HM	8840	0,07	9,2	20,4	14	39,7	0,35
D-PART 3 HM	7280	0,05	5,9	18,5	12,1	36,2	0,2
D-POS 1 HM	8160	0,08	28,4	41	18,7	64	0,31
D-POS 1THC	7960	0,06	50,9	24,4	54,8	30,7	0,85
U-NV 1 HM	191	0,07	4,9	17,8	12,8	36,8	0,16
U-NV 2 HM	178	0,06	4	18,1	11,2	35,2	0,15
U-NV 3 HM	211	0,09	4,1	17,7	11	34,4	0,14
U-PART 1 HM	1350	0,08	6,1	23,1	11,4	40,8	0,17
U-PART 2 HM	1080	0,07	7,8	24,3	12,2	45,1	0,17
U-PART 3 HM	3370	0,09	10,3	29,1	11,9	51,7	0,18
E-PART 1 HM	1530	0,05	5,3	20,2	10,9	37	0,15
E-PART 2 HM	1690	0,08	6,3	23,5	10,7	40,7	0,16
E-PART 3 HM	1790	0,09	5,8	20,7	11,1	37,6	0,17
E-NV 1 HM	225	0,05	6,6	24,2	10,7	42,2	0,13
E-NV 2 HM	261	0,06	7	24,4	11,6	44,1	0,15
E-NV 3 HM	574	0,07	4,5	17,9	12	34,7	0,15

The sediments are contaminated with barium in the northwesterly downstream direction with the higher values close to the discharge point. There is also a slightly elevated level of barium in position U-Part which is close to, but upstream of, the discharge point. The positions D-NEG, D-MRRE and U-NV are at typical background levels. Figure 7 visualizes the barium values listed in table 2.



Figur 7 Barium values relative to the Morvin discharge point

The sediment core samples do not show any significant contamination with other metals than barium.

Finally, table 3 shows the results from the total organic carbon analysis.

Table 3 Total organic carbon analysis

Sample ID	TOC
	[%]
RC 8 - 1a	0,35
RC 8 - 2a	0,33
RC 8 - 3a	0,32
RC 9 - 1a	0,34
RC 9 - 2a	0,34
RC 9 - 3a	0,37
D-NEG 1 HM	0,40
D-NEG 2 HM	0,35
D-NEG 3 HM	0,37
D-MRRE 1 HM	0,38
D-MRRE 3 HM	0,35
D-MRRE 2 HM	0,31
D-NV 1 HM	0,36
D-NV 2 HM	0,47
D-NV 3 HM	0,30
D-PART 1 HM	0,41
D-PART 2 HM	0,36
D-PART 3 HM	0,31
D-POS 1 HM	0,39
D-POS 1 THC	0,22
U-NV 1 HM	0,43
U-NV 2 HM	0,37
U-NV 3 HM	0,41
U-PART 1 HM	0,35
U-PART 2 HM	0,47
U-PART 3 HM	0,47
E-PART 1 HM	0,34
E-PART 2 HM	0,36
E-PART 3 HM	0,38
E-NV 1 HM	0,32
E-NV 2 HM	0,35
E-NV 3 HM	0,36

TOC-values are at the same level prior to and after the drilling operation. TOC is typically in the order of g/kg and THC in mg/kg so elevated THC values do not necessarily result in elevated TOC values.

Conclusion

These analyses reveal that one sample close to the discharge point is contaminated with THC. There are also elevated levels of Barium in the downstream direction. No other metal contamination was found.

5. Sediment trap analysis

PIs: Autun Purser and Laurenz Thomsen, Jacobs University Bremen

In this chapter the main results from the sediment trap analysis are presented. The full report can be found in Appendix B.

An array of sediment traps was deployed around the drill cutting discharge point for two periods of drilling during late 2009 and early 2010. During each drilling event three traps were deployed. Figure 3 in chapter 2 shows the location of the traps from 9.11.2009-6.12.2009 and 6.2.2010-23.2.2010. Traps were deployed just prior to commencement of drilling operations, and retrieved after drilling was complete.

5.1 Methods

The sediment traps used for both deployments were three identical K.U.M. K/MT 234 Sediment traps, each fitted with 21 bottles of 400ml. All three traps were fitted with custom made electronics and programming devices constructed by IMR.

During each deployment, each sediment trap was programmed to rotate the sample bottle every 36 hours, to provide a maximum 31.5 day coverage period.

Material collected from the sediment traps was analysed at Jacobs University, Bremen. The following parameters were assessed for each sample (where sufficient quantity of material collected), and the methodologies detailed:

Current profiler:

The flow direction and flow velocity of the seawater in the bottom layers of the ocean was measured by a current profiler during the first drilling period (see figure 3 in chapter 2 for location of current profilers).

Total sample mass:

Total sample mass for each sample was investigated as described in Bodungen et al. (1991).

Particulate organic material:

Carbon was measured in all samples, Nitrogen levels were measured whenever sample bottles contained >25mg dry weight of material.

Subsamples of the material in each bottle was filtered onto filter paper. Filters were acidified after the method of Pike and Moran (1997) to remove particulate inorganic carbon. After acidification, samples were dried in a 60°C oven and subsequently analysed in a EURO EA Elemental Analyser.

Amino acid analysis and degradation indices:

Amino acid analysis was carried out by reverse-phase HPLC using a slightly modified method of Cowie and Hedges (1992) and Van Mooy et al. (2002), as described in Garcia and Thomsen (2008).
From the amino acid composition, the degradation index (DI) was calculated after Dauwe et al. (1999). The ratios of aspartic acid (asp) and glutamic acid (glu) to their decompositional products β-alanine (bala) and γ-aminobutyric acid (gaba), as well as the joint percentage of bala and gaba (%[bala+gaba]) on all amino acids were calculated. These indicators have been widely used to verify variations in organic matter decomposition stage, both within the water column and in marine sediments (Lee and Cronin, 1982; Cowie and Hedges, 1992; Dauwe and Middelburg, 1998).

Amino acid analysis was carried out for all samples.

Physical parameters

Particle size:

Particle size distributions were measured for all samples. Median particle sizes for all sample bottles were determined (by total particle volume in each sample). Size was determined using the LISST-ST instrument following the procedures described in Pedocchi and Garcia (2006).

Settling velocity:

It was not possible to measure settling velocities from material collected in bottles containing <25mg material, as the quantity of particles was insufficient. For samples where sufficient material was available, >100 particles were analysed to determine settling rates. This was carried out by filming material sinking through a settling cylinder and tracking particle movement over time with the ImageJ software application (Abramoff, et al. 2004).

Critical shear velocity:

This has been determined for all samples containing >25mg of material. The methodology used is described in Thomsen and gust (2000). Resuspension thresholds for both the fine material and coarser material was determined for each sediment trap bottle.

Metals and trace elements:

Bottles containing >25mg material were suitable for these analyses. Where sufficient material was present, Ba, Cd, Cr, Cu, Pb, Zn, Fe, K, Mg, Mn, Ni and Sr were measured.

An ICP-OES machine was used for these analyses, with reference HBVO2. For samples containing low concentrations of material, results were checked with voltammetry.

5.2 Results and discussion

Due to problems in trap functioning, during deployment 2 in particular, only results from the first deployment are presented in detail in this chapter. Results from the second deployment can be found in Appendix B.

For monitoring the initial Nov-Dec 2009 drilling event, sediment trap A was deployed at 01:55 on 8.11.2009, trap B at 01:42 and trap C at 02:31 on 9.11.2009 (see figure 3 in chapter 2 for trap locations in relation to drill cutting discharge point).

Flow conditions:

During the initial drilling event in Nov-Dec 2009, the current meter measured a seawater flow in a near uniform direction away from Trap A, in a north-northwesterly direction (see chapter 3). Given that this sediment trap was on the far side of the drill cutting discharge point it could be used to represent a 'drilling control'.

Trap problems:

Although material was collected by each trap during the initial drilling event, there seems to have been some significant problems with the rotation of the sample bottles in the traps. These problems are outlined below and should be taken into consideration when reviewing the results of the sample analyses. Only the analyses of sample bottles which contained >10 mg dry weight of material will be discussed in detail in this report, under the assumption that the other bottles failed to open for any appreciable period of time.

Trap A

During the first drilling event, only one trap bottle, A21, collected >10 mg of material. Bottle A21 contained 27.2 g of material. It is highly unlikely that trap A accurately recorded the deposition of material over time during deployment 1. From the total organic carbon mass collected in the other trap bottles over 1.5 day periods, it would appear that trap A turned rapidly to bottle A21 following deployment, and collected all material from 8th Nov to 6th Dec into that one bottle.

Trap B and C

Trap B and C did not rotate sediment trap bottles correctly. Every second and third bottles were missed (these containing <10mg material on recovery, as with trap A bottles A1-A20), the other bottles each contained in excess of 1000 mg of material (figure 8). We assume in the presentation of results that each of these bottles represents a 1.5 day collection period, and therefore collection ended 10.5 days after trap deployment for trap B and C.

Total sample mass:

During the initial drilling period, the total sample mass collected in the sample bottles varied greatly, both between traps and over time (figure 8).

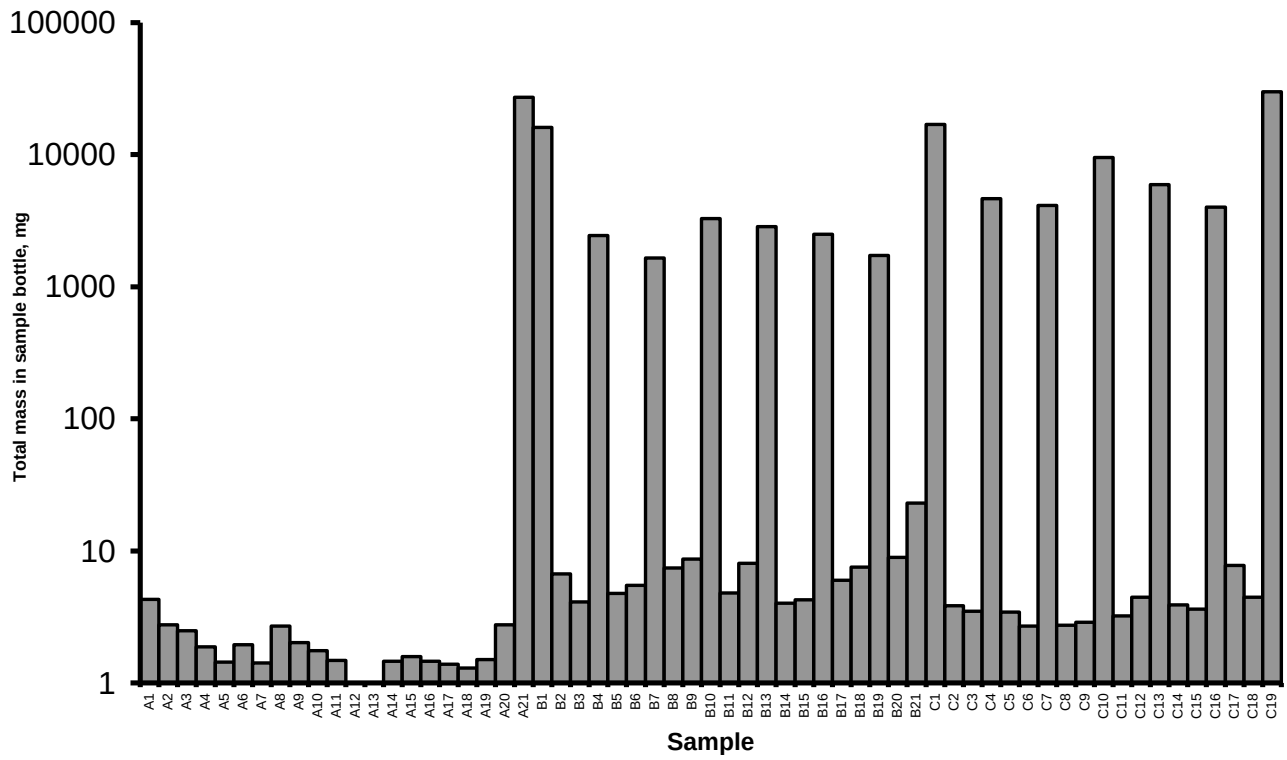


Figure 8 Total sample masses per bottle collected during initial drilling event (Nov-Dec 2009), plotted with a logarithmic scale

During the first drilling event, the first and last bottles of trap C (C1 and C19) the first bottle from trap B (B1) and bottle A21 from trap A have by far the most material within them (figure 8). This could reflect extra material deposited in the traps as a function of the deployment or recovery process. The absence of an elevated concentration of material in the final trap bottle from trap B may be because the final bottle was B21, which is not one of the bottles which collected > 10 mg material (i.e. not one of every third bottle, 1, 4, 7, 10, 13, 16 and 19).

Amino acid analysis and degradation indices:

Concentrations of total hydrolizable amino acids (THAA) ranged between 10 and 25 mmol/kg. Highest concentrations were found in samples A21, the trap which was located at the reference station. Highest THAA values at locations exposed to drill cuttings were found in bottle B19. The THAA concentrations and degradation index values are within the range of values reported for more labile coastal and ocean margin settings (DI between -1 [refractory] and +1 [labile, fresh]) (Dauwe et al., 1999). The degradation indices for samples B4, B7, B16, C4, C16 and C19 indicate periods when less labile organic matter entered the traps. As sampling took place during winter, this observation indicates that the organic material in the water column during times of drilling operations varied in composition and represent winter conditions**.** The data indicate that drill cuttings have no negative impact on the degradation of organic material.

Physical parameters:

Particle size:

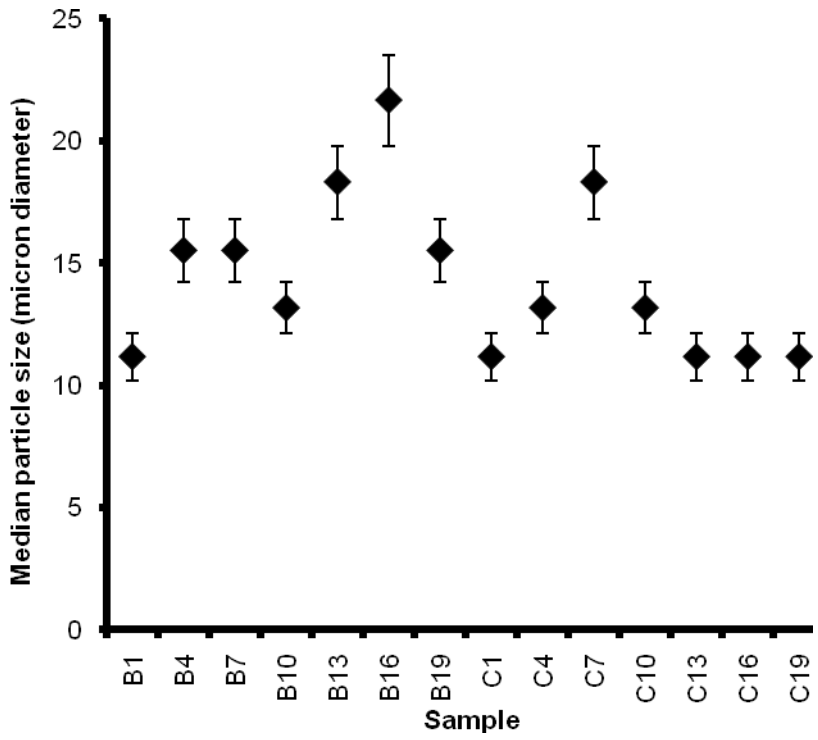


Figure 9 Median particle sizes for the samples containing >25mg of material collected during initial drilling event (Nov-Dec 2009). ***These samples are considered as valid for further analyses since all other samples indicate trap malfunctioning (too little mass, see figure 8)***

Particle size distributions show two trends: Traps bottles which were not rotated into position correctly (those containing <10 mg total material, i.e. A1-A20, B2, B3, B5, B6, B8, B9, B11, B12, B14, B15, B17, B18, B20, B21, C2, C3, C5, C6, C8, C9, C11, C12, C14, C15 and C18) showed median particle diameters of 50 – 300 µm. This could indicate short exposure times for these particular trap bottles. During these short periods during which the trap rotated past these sparcely populated bottles, only the larger aggregated organic material with high settling velocities could enter the trap**,** while small particles with low settling velocties could not do so before the trap rotated the bottle away from the intake.

Those trap samples which result from correctly working 36 h opening times (trap samples containing >25 mg of material, i.e. B1, B4, B7, B10, B13, B16, B19, C1, C4, C7, C10, C13, C16, C19) show much smaller median diameters (figure 9). These trap samples are therefore dominated by high numbers of finer drill cuttings, resulting in a general shift of the particle size spectrum towards smaller particles during periods of drilling.

Critical shear velocities:

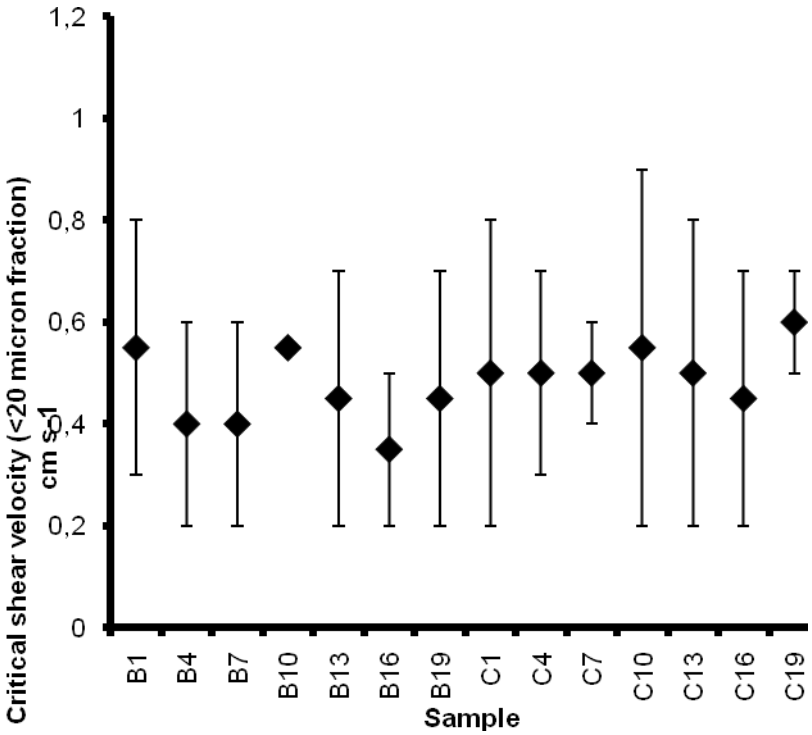


Figure 10 Critical shear velocity of <20 micron material for bedload transport collected during initial drilling event (Nov-Dec 2009).

Data on mean critical shear velocity show that bedload transport of the particles which entered the trap varied between 0.4 and 0.6 cm/s. This corresponds to free stream velocities of ≈ 8-10 cm s^{-1}. Under these flow conditions, the particles would roll along the seafloor.

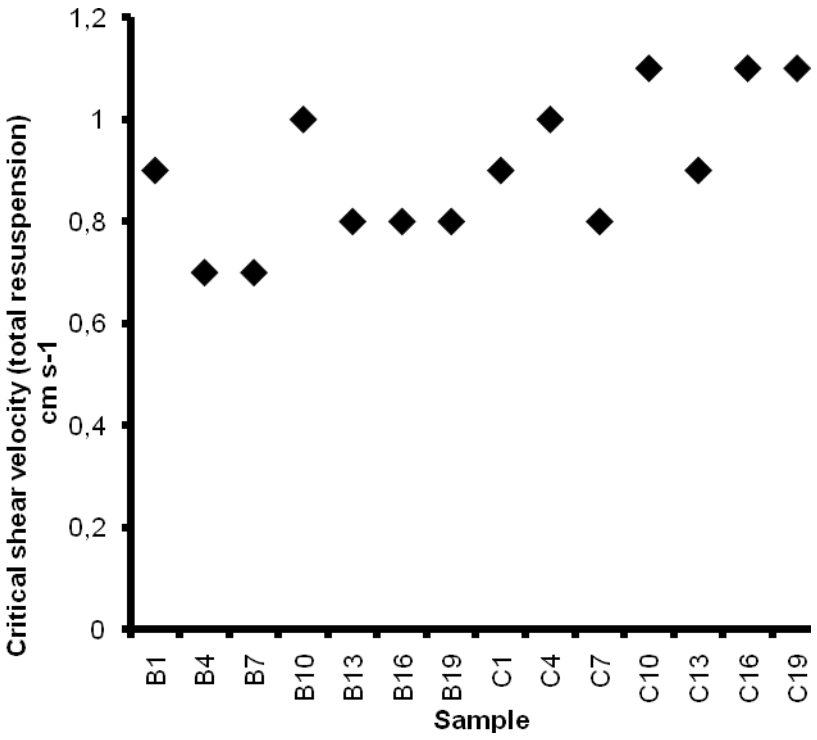


Figure 11 Critical shear velocity for full resuspension of material collected during initial drilling event (Nov-Dec 2009).

Data on mean critical shear velocity show that full resuspension of particles which entered traps B and C occurred between 0.7 and 1.2 cm s^{-1}. That corresponds to free stream velocities of ≈ 10 -20 cm s^{-1}

There is a trend of increasing shear velocities from station B to C. This indicates that with increasing distance from the drilling site, the particle composition changed. The further the station was away from the drilling site, the less easy resuspension of settling material would be. One explanation for this observation could be that biofilms were built up on the drill cuttings during their transport within the bottom boundary layer, resulting in less resuspendable particles.

Conclusion

During drilling operations the mix of drill cuttings and organic material settling to the seafloor would be resuspended under flow velocities of 10 – 20 cm s^{-1}. This would indicate that under flow conditions often present at the study site the material would be readily resuspeded and dispersed in a downstream direction.

Settling velocities:

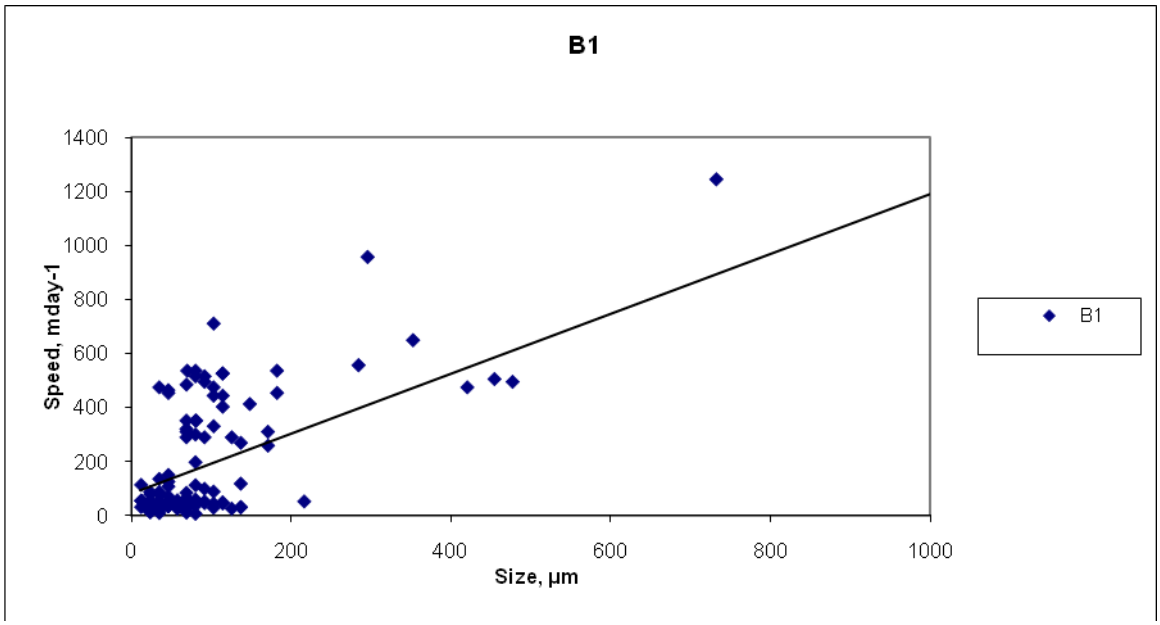


Figure 12 Settling velocity

Trap sample B1 a good example for the general trend observed: The majority of particles in that sample are small (figure 12) and represent the drill cuttings. However a second class of particles with settling velocities of 300 to 1300 m day^{-1} are present. These represent most probably organic-mineral aggregates which settled out of the water column into the traps. Their number is not high enough to change the median particle size but they are a significant component of the vertical flux of particles at the study site.

Interestingly settling velocities and particle sizes of the aggregated fraction, which did not dominate the trap samples in number, increased from location B to C and over time from start to end of trap deployment. This can again be explained with a seabed process, in which the aggregated particle fraction undergoes several resuspension loops between locations of trap B and C. Each resuspension loop results in a compaction of the aggregates (and therefore excess density) which increases the particles settling velocity.

Regarding exposure and dispersion, this would mean that with increasing distance from the drilling site, the drill cuttings increasingly aggregate with the organic material, which also form biofilms. This results in an increase of critical shear velocity and settling velocity of the particles. For more details on this process see Thomsen 2002 and 2004.

Metals and trace elements:

The following graphs show the ICP-OES measurements for the metals and trace metals analysed. All background levels are according to Akvaplan-Niva, 2010.

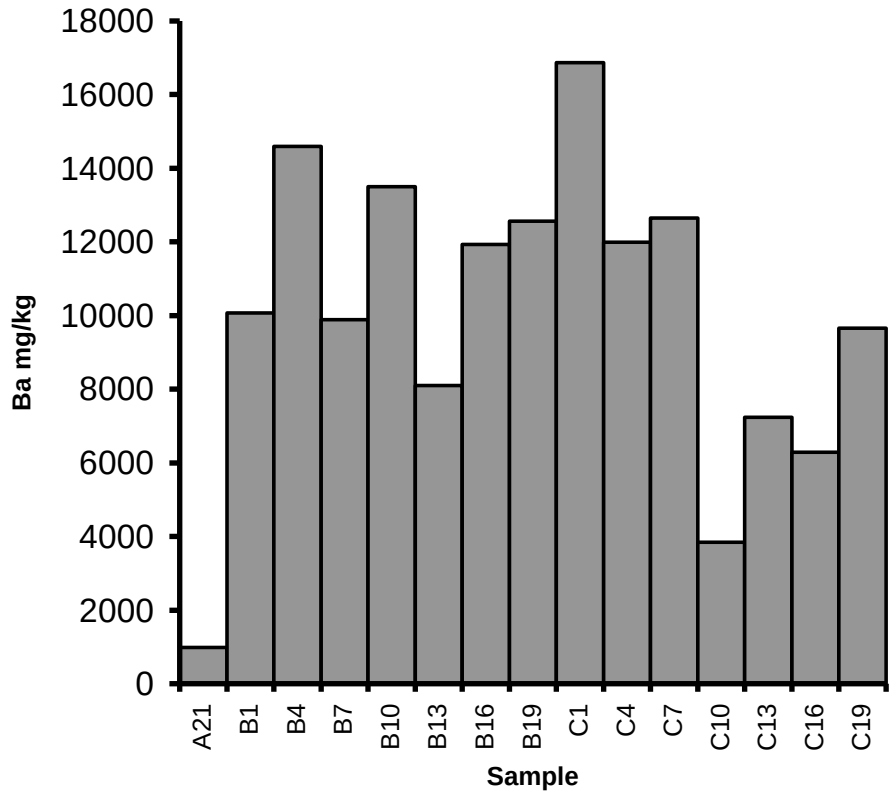


Figure 13 Barium concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Barium concentrations are far higher (by at least an order of magnitude) in trap bottles from traps B and C than in bottle A21. The quantity in A21 however, (981.4 mg/kg) is higher than the range of 83-287 mg/kg background concentration measured in the region. This could indicate that some drill cutting material reached the trap, perhaps by transport within the water column following discharge and prior to settling. Although the drill cuttings were released at the seabed, the turbidity at release and small size of the particles may have resulted in a small percentage of the material being resuspended into water masses overlying those measured by the current profiler – and potentially be transported in another direction (i.e. toward trap A) before again settling.

Chromium concentration did not vary with sediment trap. The levels are a little higher than the typical background levels for the area (16.4 – 34.6 mg/kg), but below those of general marine sediments (~99.8- 112 mg/kg, Mess-3 reference material, NRCC). Copper values are also slightly higher than the background values.

Lead values were generally similar across sediment traps and samples, although slightly higher in bottle A21. Background levels of 13.9 – 20.9 mg/kg compare well with these observations.

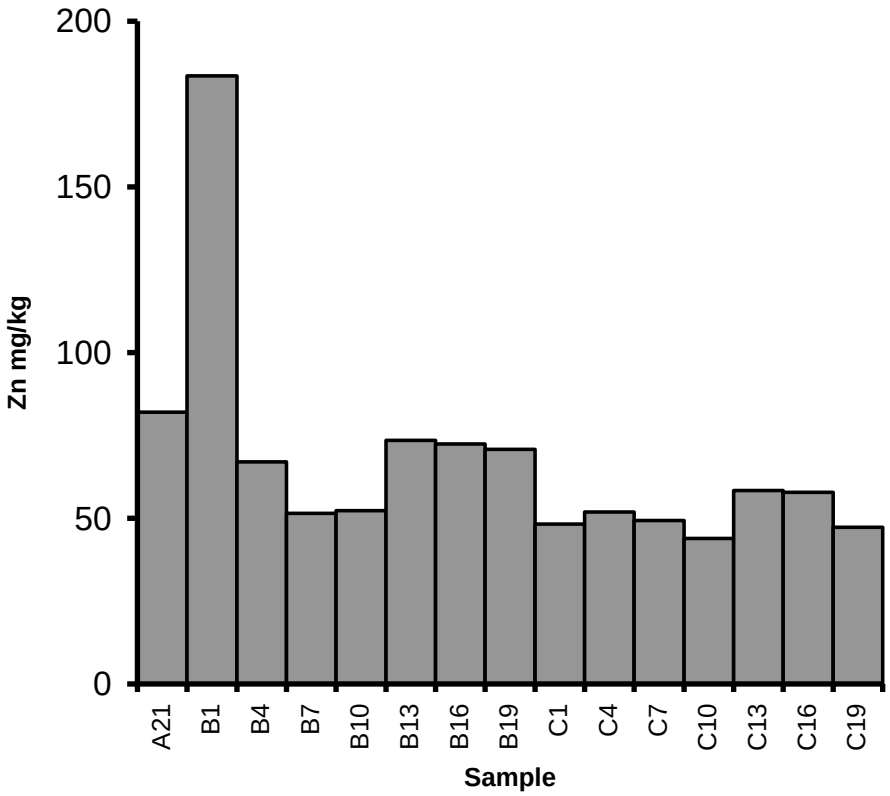


Figure 14 Zinc concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

A background zinc concentration of 40.7 – 90.0 mg/kg for the region corresponds with the results from the majority of sediment traps. The elevated concentration observed in sediment trap B1 is unlikely to be related with the drilling operation, as Zn concentrations in sediments highly contaminated with drill cuttings (chapter 3) was not observed to differ from this background range.

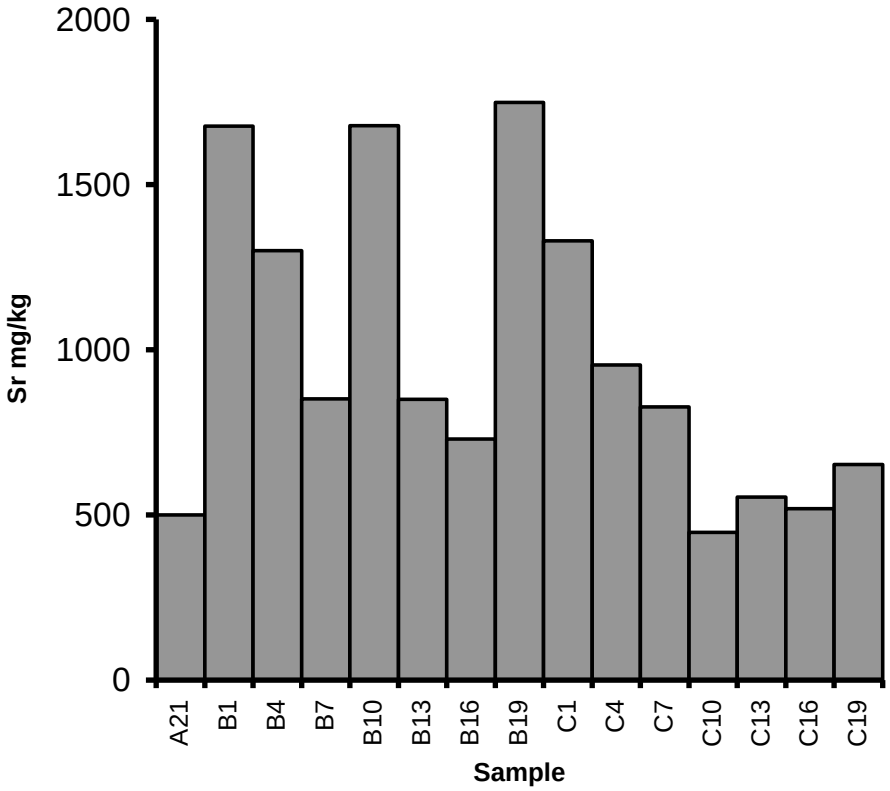


Figure 15 Strontium concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Strontium concentrations appear to be slightly higher in samples from traps B and C than trap A. Measured concentrations are generally above typical marine sediments (MESS-3 reference, NRCC). This is not unexpected given the high calcium carbonate scleractinian coral abundance in the region of drilling (Thomson & Livingston, 1970). The periodic peaks observed at B and C could reflect local elevated concentrations relating to periodic flow and resuspension conditions.

General conclusion :

During drilling operations in November/December 2009, traps B and C were exposed to drill cuttings and trap A may have been exposed to some low concentrations of drill cuttings. The drill cuttings of small size entered the trap bottles in sufficient concentration to change the overall particle size spectrum from large particles (> 100 µm to small particles < 25 µm). It is impossible to give precise numbers for the particle concentration of the fine material in the water column during drilling operations since only sediment trap samples, and no in-situ water samples were available for analysis. As drilling occurred during winter, only a little organic material was present in the water column. Although of low concentration, this material was of a more labile quality than the drill cuttings and settled into the sediment traps in the form of aggregates of 100 to 400 µm median size. These aggregated particles had aggregated with drill cuttings since the settling velocities of material from the traps was an order of magnitude higher than that reported for aggregates originating from within natural benthic boundary layer conditions (Thomsen et al., 2002).

The data on critical shear velocity of the material indicate that once these drill cutting/ organic particle material arrived on the seafloor, bedload transport would occurr under low flow conditions

of ≈ 10cm/s. Full resuspension of this material would occur under flow velocities of 10 – 16 cm/s. These currents are found regularly at the study site (see chapter 3). With increasing distance from the drill cutting discharge point, the drill cuttings increasingly aggregate with the organic material and form biofilms. This results in an increase of critical shear velocity required to resuspend settled material and an increased settling velocity of the particle with distance from the drill cutting discharge point.

Sedimentation:

It is difficult to derive conclusions on particle accumulation during drilling operations. In order to do so, for the initial drilling event more data on mass accumulations at reference station A would be required. Only trap bottle A21 collected material and it is uncertain the exact period of flux this bottle represents. The uncertainty regarding the rotation timing of traps B and C during this first deployment would make any conclusions on the influence of drill cuttings on mass accumulation rates on the seabed very tenuous. Natural and drill cutting enhanced mass accumulation rates for the second drilling period cannot be made, given the absence of flow data and poor bottle rotation performance of the sediment traps.

Barium levels were an order of magnitude lower at the control site (Sediment trap A) than at the other sediment trap sites during the initial drilling event, although concentrations of Ba at this location were still above the local background levels, so some minor contamination of that location is possible.

6. Lipid class and fatty acid profiles in cold-water coral *Lophelia pertusa*

PI: Sonnich Meier, Institute of Marine Research

As part of the investigation on the effects of mud and cuttings discharges on deep water corals, lipid analyses were conducted. This was done to study if cold water corals in the vicinity of the platform reduced their feeding during and after the drilling operation. The following null hypothesis was tested:

H0: Coral that are exposed to particles from the drilling activity will eat less and therefore have lower amount of storage lipids and different fatty acids profile compared to other coral colonies.

6.1 Methods

A detailed description of the analysis methods is given in appendix A. Briefly, Corals were collected using ROV and frozen on dry ice and held at – 80°C until analysed. The lipids were extracted by Folch method and the lipid classes were separated by high-performance thin-layer chromatography (HPTLC) into six fractions:

- Polar lipids (PL, a mixture of all the membrane phospholipids, PC, PE, PS and PI).
- Cholesterol
- Free fatty acids (FFA)
- Triacylglycerol (TAG, storage lipid)
- Unknown fraction (probably monoalkyldiacyl glycerol, MADAG)
- Wax esters (WE, storage lipid)

Methyl esters of the fatty acids (FAME) from total lipids and the lipid classes were prepared and analysed on gas chromatography (GC-FID).

There were analysed 6 samples from each coral colony and 2-3 colonies from each area. The difference between lipid % and lipid classes composition were tested by Analysis of Variance (ANOVA) with Tukey (HSD) post hoc tests. All statistical analyses were carried out using XLSTAT software (Addinsoft, U.S.). Principal Component Analysis (PCA) was carried out on the FA profiles using Sirius (Version 7.1, Bergen, Norway). The fatty acid datasets were normalised.

6.2 Results and discussion

There were no difference in the amount of lipid extracted from the exposed corals (MRRE C1 and C2) and the reference samples (M27 C1 and C2) (Table 4). One sample from the unexposed reef (M27 C3) had significant lower lipid levels, but this coral sample was heavily covered with natural sediment and there were low amount of soft tissue in the coral. This sample was therefore excluded from the comparison between the sampling sites.

Lipid classes

The lipid extracted from the corals was clearly dominated by storage lipids, approximately 50 % of the FAs were found in the wax ester fraction and the triacylglycerol around 30 %. Hence the energy storage lipid contributed to more than 80 % of the total amount of FA. There were significant higher levels of WE and lower levels of TAG in MRRE C2 compared with the other samples sites. MRRE C1 had relatively lower levels of polar lipid (PL). Due to the lack of previous data on lipid class analysis of coral we cannot tell if this is significantly different compared to natural variation. However, since all corals from both areas had high levels of storage lipids (WE and TAG) there is from this data no support of the theory that exposed corals had been eating less food than corals from the control area.

Table 4 Amount of lipid (% of ash free dry weight) and lipid classes composition (% of FA in each lipid class relative to total FA) for reference samples (M27) and exposed samples (MRRE). Different letters = significant difference between sampling sites (ANOVA, $p < 0.05$).

		Lipid classes composition (FA% of totally FA)				
	Lipid %	WE	TAG	PL	Unknown	FFA
M27 C1	17.6 ± 3.2^{a}	52.8 ± 0.7^{b}	30.7 ± 1.1^{a}	9.4 ± 1.6^{a}	5.8 ± 0.2^{a}	1.3 ± 1.5^{b}
M27 C2	14.8 ± 3.8^{a}	52.5 ± 2.2^{b}	31.1 ± 2.6^{a}	8.9 ± 2.6^{a}	5.6 ± 0.7^{a}	1.9 ± 0.3^{ab}
M27 C3	5.9 ± 2.5^{b}	51.5 ± 1.7^{b}	32.4 ± 1.3^{a}	5.1 ± 1.8^{ab}	7.1 ± 0.6^{a}	3.9 ± 0.6^{a}
MRRE C1	16.5 ± 2.5^{a}	57.1 ± 4.9^{b}	31.5 ± 4.6^{a}	3.5 ± 1.1^{b}	6.3 ± 0.9^{a}	1.6 ± 0.4^{ab}
MRRE C2	13.8 ± 4.1^{ab}	63.5 ± 3.8^{a}	20.9 ± 1.5^{b}	9.7 ± 5.8^{a}	4.3 ± 0.6^{b}	1.7 ± 0.2^{ab}

Fatty acids composition

There were not found any clear differences in the fatty acids composition between the two samplings sites (figure 16). The corals from MRRE C2 had higher levels of saturated FA (SFA) and lower levels of mono unsaturated FA (MUFA) in both WE and TAG compared with the others corals. This can suggest a lower input of food particles related to copepods (*Calanus finmarchicus*), but there were not found differences between MRRE C1 and control (M27).

There were very large differences in the fatty acid composition between the different lipid classes. Figure 17 shows a principal component analysis (PCA) of the FA profiles of WE, TAG, Unknown and PL fraction. The score plots show that the different lipid classes are clearly clustered by its FA profile, but there is no separation between corals from different samplings areas. Details about the FA profiles are given in appendix A.

The FA compositions that were found in these analyses are similar to what have been reported earlier in cold water corals from the North Atlantic (Dodds et al., 2009).

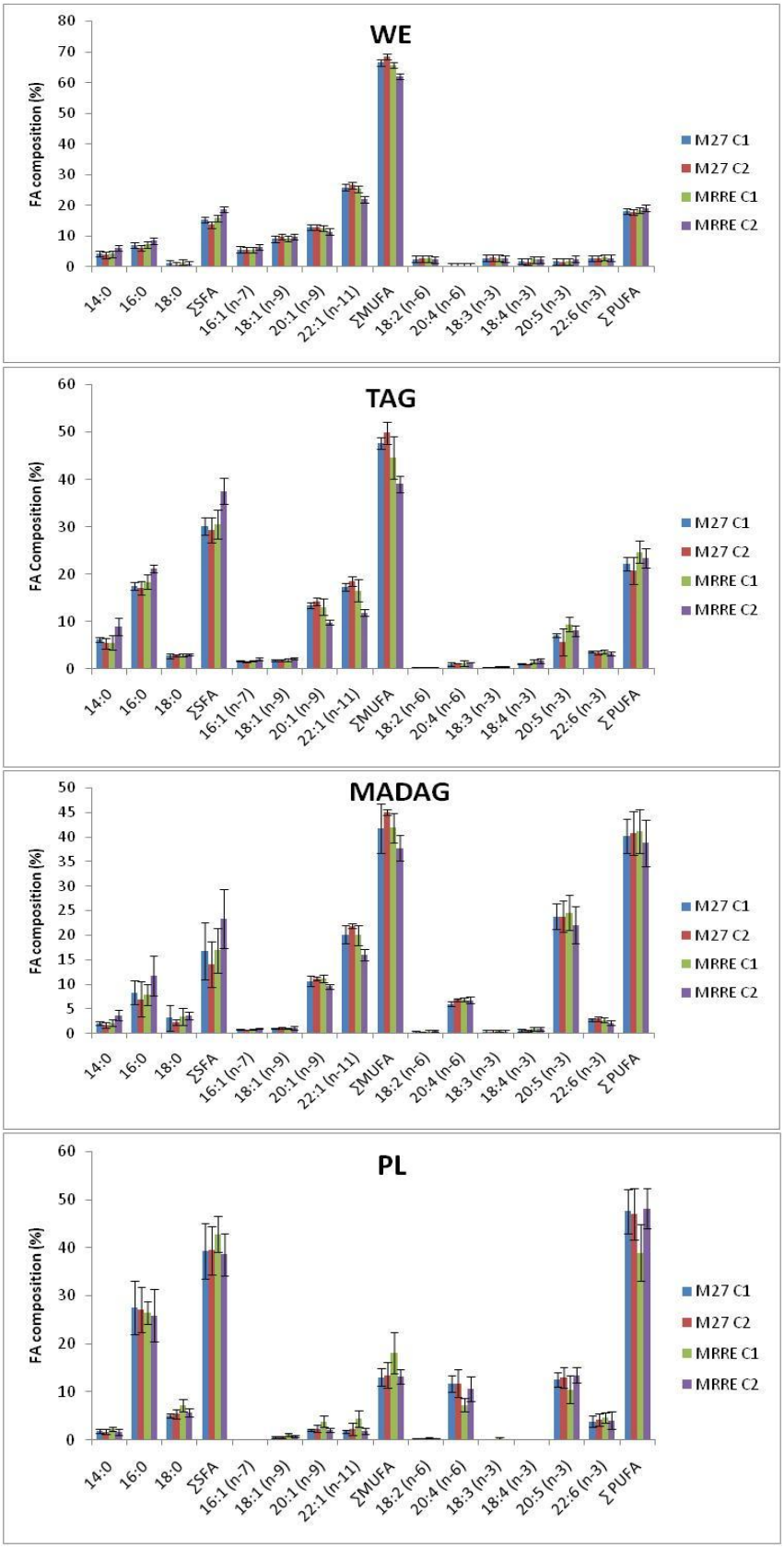


Figure 16 Fatty acids composition of the 13 dominating FAs (% of total FAs) in Wax ester (WE), triacylglycerol (TAG), monoalkyldiacylglycerol (MADAG) and polar lipids. Exposed corals (MRRE) and control corals (M27).

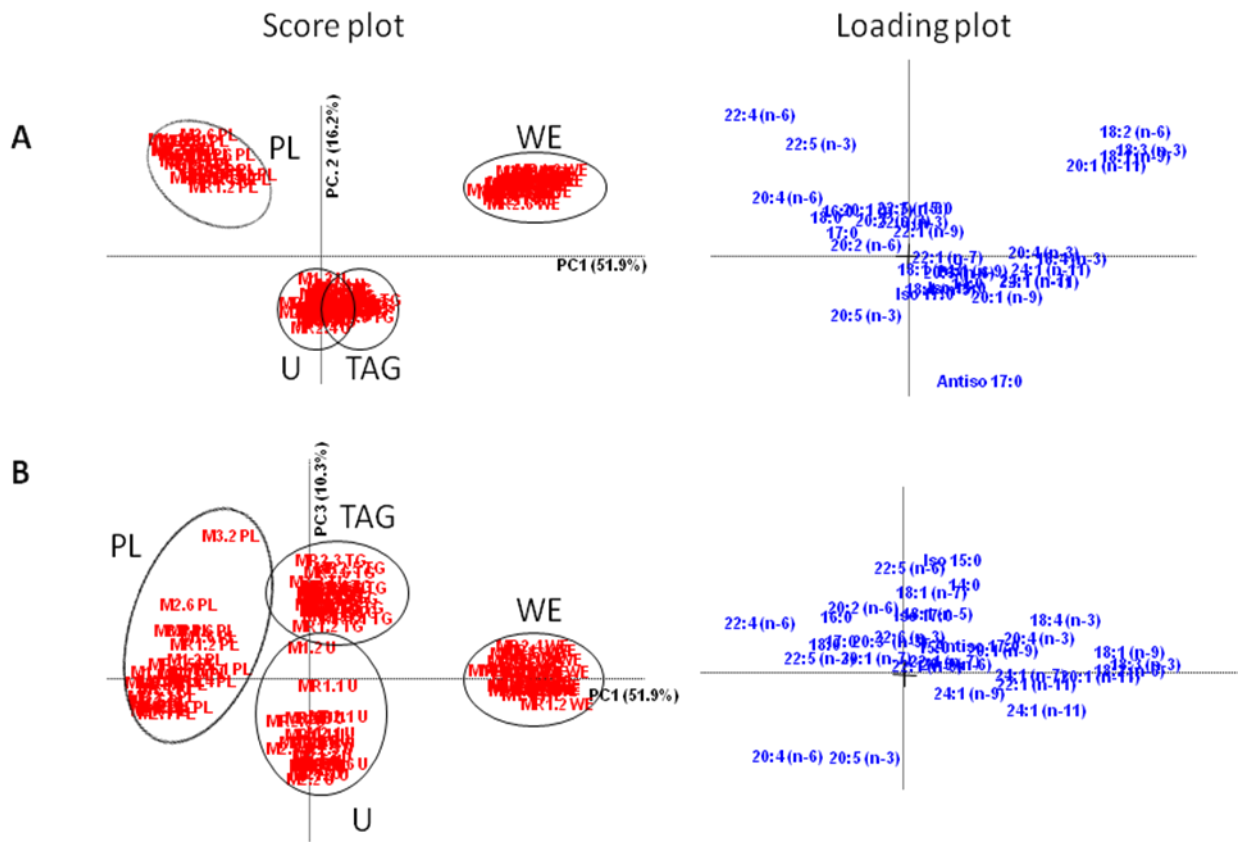


Figure 17 Principal component analysis (PCA) of the FA profiles of WE, TAG, Unknown fraction and PL fraction of coral form both M27 (marked M) and MRRE (Marked MR).

A. Score and loading plot for PC1 and PC2 (explain 68 % of the total variance). B. Score and loading plot for PC1 and PC3 (explain 62 % of the total variance)

A variation in lipid composition was found between the sampling points. However, the internal variation in MRRE and M27 was higher than the variation between the two. No significant difference in lipid amount or lipid class composition was found between the reef that had been exposed to drill cuttings (MRRE) and the unexposed reference reef (M27). Therefore we can reject the null hypothesis; the exposed corals did not have decreased amount of storage lipids than corals from the control area and this suggest that there is no differences in the feeding rate between the exposed reef (MRRE) and the reference reef (M27).

7. Image analysis

PIs: Pål Buhl-Mortensen and Eirik Tenningen, Institute of Marine Research

Deep-water coral (*Lophelia pertusa*) polyp behaviour can be used as an indicator of environmental stress. The polyp behaviour reflects feeding patterns, and probably other physiological processes. Retracted polyps are not feeding. Strong current may be one factor that could lead to polyp retraction. In such conditions the polyps are not able to catch food particles and the corals may save energy by being retracted. To study possible effects from exposure to drill cuttings we monitored polyp behaviour near the Morvin drilling site.

7.1 Methods

Images of coral colonies were taken at close distance to provide information about the expansion state of polyps. The images were first used for real-time monitoring during the drilling phase. We were then looking for any abnormal changes in behaviour or generation of mucus (slime that the coral produces under stress) on corals exposed to plumes of drill cuttings. Three sets of time series were post processed for more detailed results on behavioural patterns. Two time series were taken from the onboard camera of the ROV and one from the camera satellite, connected with cable to a lander. The ROV time series were used for a general comparison of behavioural patterns between exposed and unexposed coral colonies, whereas the satellite time series was used to study behavioural patterns within one colony over shorter time intervals.

For the ROV time series we used a standardized sub-sampling of 51 images (34 from MRRE and 17 from M17). In each image approximately 40 polyps were identified and classified with regard to state of polyp expansion. We used three classes of polyp expansion state:

- Expanded
- Partly expanded
- Retracted

Partly expanded is the state where the tentacles are outside the calyx (polyp skeleton), but not stretched out into a full crown. Retracted refers to the state where there are no visible tentacles.

For the satellite time series 125 images were analysed using the same classes as for the ROV time series. This time series covered a period of 61 hours from 26.11.2009 to 28.11.2009. An example of an analysed image is given in figure 18. For each image three sub sample areas at fixed locations were selected, labeled A, B and C. The polyps were classified as expanded, partly expanded or retracted in each of the 125 images.

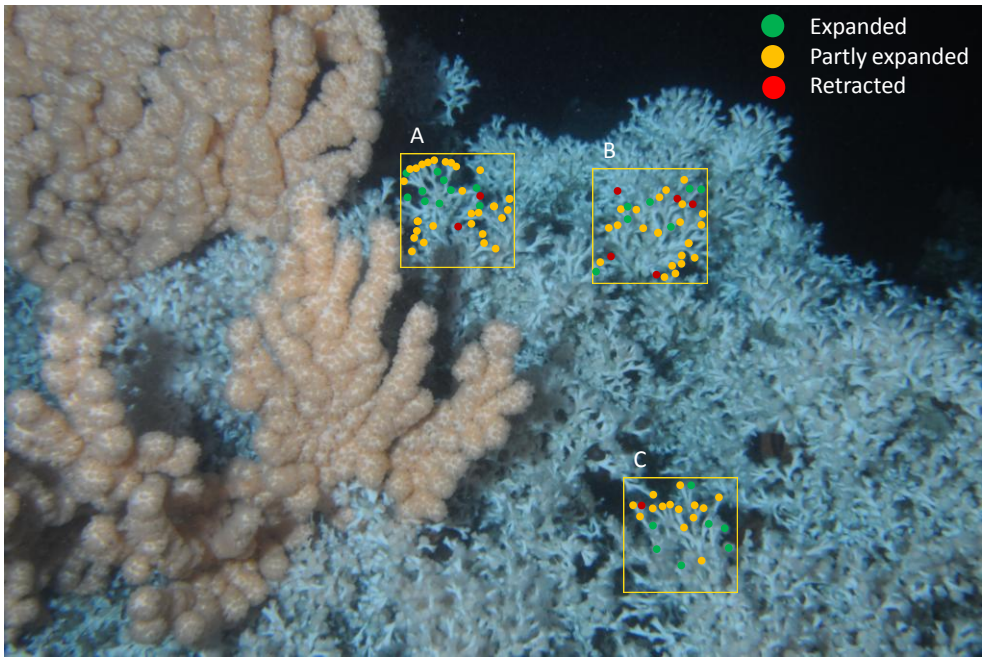


Figure 18 Image taken with the camera satellite, showing the location of three subsample areas used for classification of polyp's expansion state. Green dots indicate polyps fully expanded, whereas orange and red indicate partly expanded and retracted, respectively.

Polyp behaviour has previously been used as an indicator of environmental stress. Serigstad et al (2001) exposed corals to a thin oil solution and showed that the percentage of expanded polyps was significantly lower for the exposed corals during the exposure period. Roberts and Anderson (2002) presented results from image analyses of polyp expansion to reveal responses of coral polyps to environmental perturbations such as sedimentation.

7.2 Results and discussion

Figure 19 presents the time series named "coral 1" from the MRRE reef which was exposed to drill cuttings. Figure 20 presents the time series named "coral 1" from the M17 reef which is not expected to have been exposed to drill cuttings. For each image the portion of polyps that were totally expanded, partly expanded and totally retracted were identified. For both time series more polyps were expanded than retracted, except for the period after 3.12 at MRRE. Unfortunately, the M17 time series ended prior to 3.12.

The time between images in these two series is too long to describe patterns at an interval of less than one day.

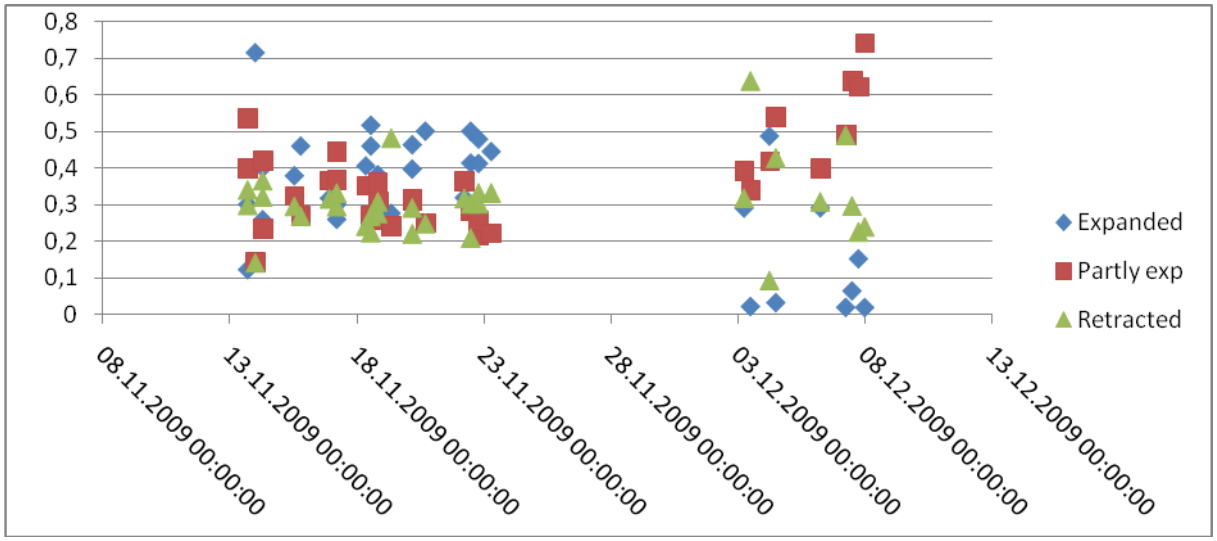


Figure 19 Proportion of polyps in different states of expansion at the MRRE location.

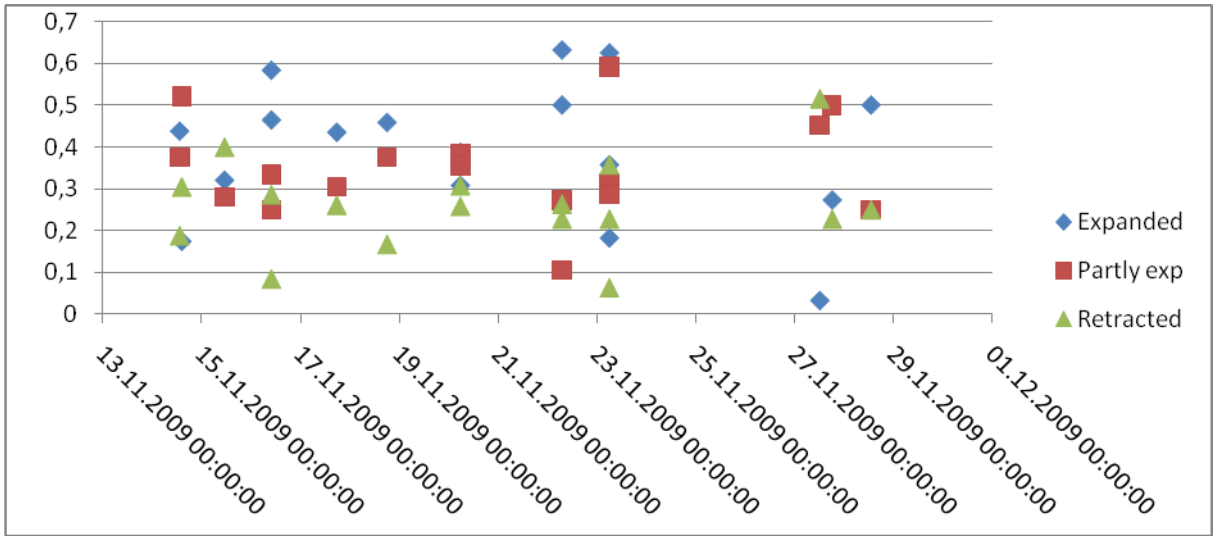


Figure 20 Proportion of polyps in different states of expansion at the M17 location.

Limitations in the image quality and lack of continuity in the time series makes it difficult to conclude on any effects imposed by the drilling activity. However, there is a tendency of a shift towards fewer expanded and more retracted polyps at MRRE towards the end of the time series following a long period of exposure to drill cuttings. Again, the lack of M17 data from this period makes it difficult to verify whether this is natural behaviour or an effect of the exposure.

Figure 21 shows a comparison between the expanded, partly expanded and retracted polyps of MRRE and M17 for the period 13-28 November. The fraction of expanded polyps is identical for the two, while the fraction of retracted polyps is slightly higher for the exposed MRRE reef.

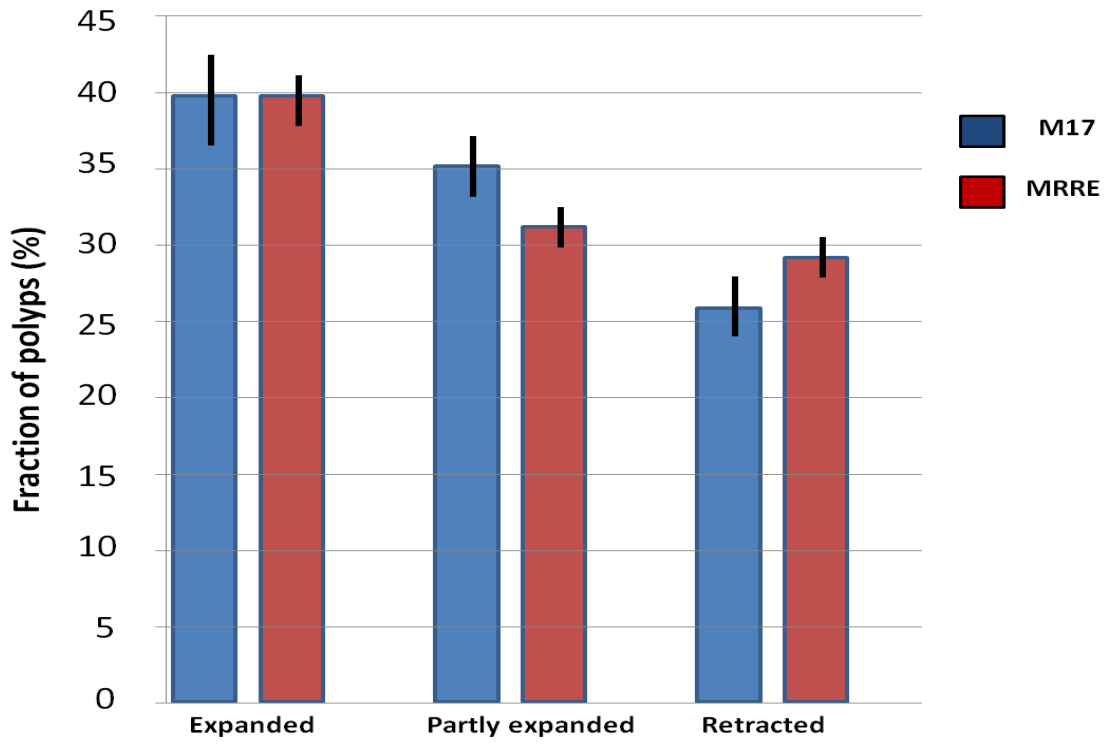


Figure 21 Comparison between corals that have been exposed (MRRE) to drill cuttings and corals that have not been exposed (M17). The vertical lines indicate 95 % confidence limits.

Figure 22 presents the camera satellite time series together with current data. While the number of retracted polyps is fairly constant across the time series, there is a clear shift towards more expanded and fewer partly expanded (named partly retracted in the figure).

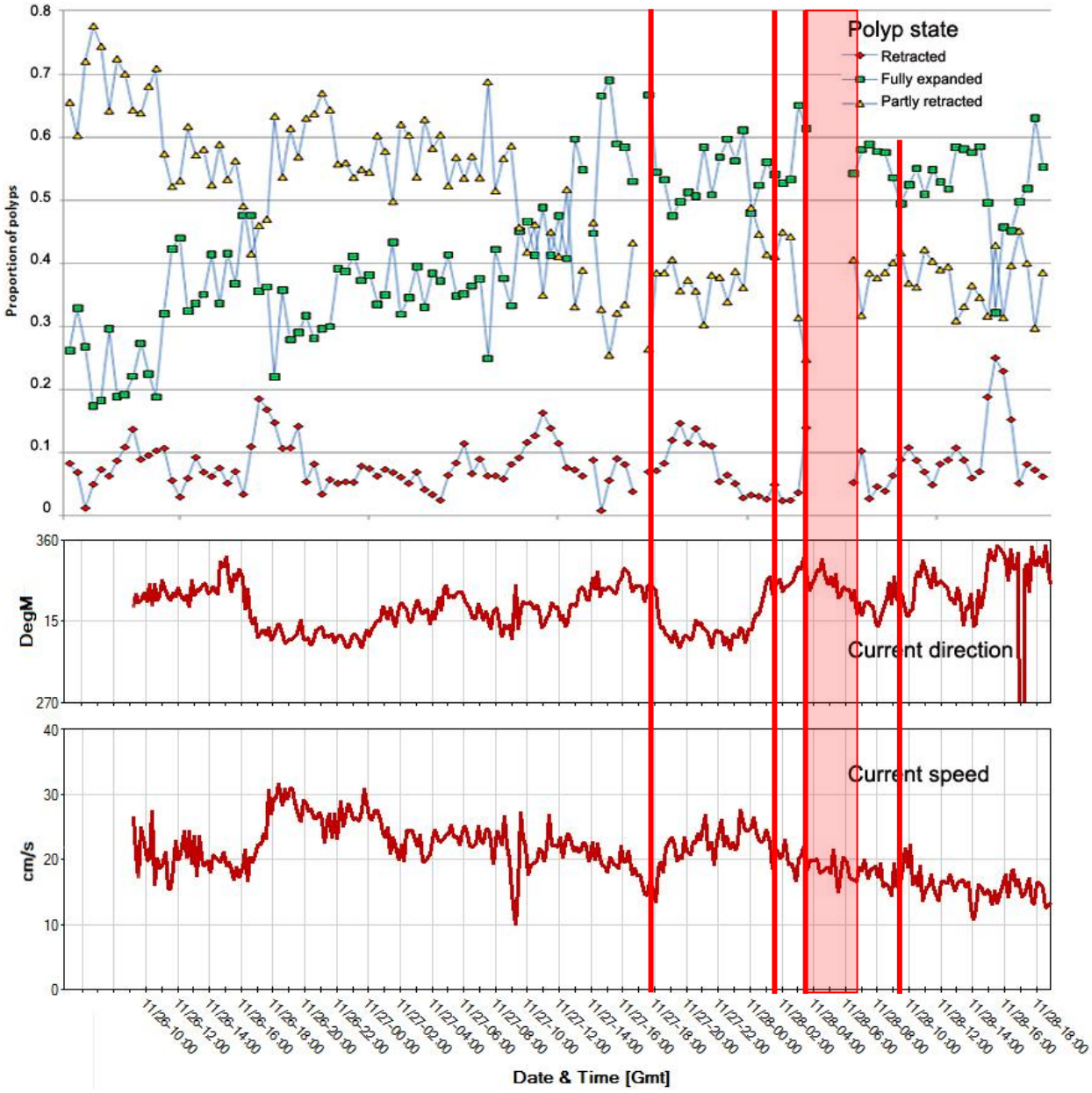


Figure 22 Polyp state and water currents (horizontal speed [cm/s] and direction [degrees]) 4-6 m above bottom. Red vertical lines indicate time of exposure.

During this time series, we have four periods of drill cutting exposure. The first exposure occurs on the 27.11 between 17.15-17.45. This occurs after the change between expanded and partly expanded polyps and can therefore not cause the shift. Immediately after the exposure, the polyps seem to be more expanded than just prior to the exposure.

On the 28th of November there are three periods of exposure at 01.15, 03.15-06.45 and at 9.15 with the longer period indicated by lack of polyp expansion data in the figure. Prior to this period there is a tendency towards fewer partly expanded polyps and more expanded, but immediately after the exposure, these levels are normal again.

The change in proportion of polyps being expanded, partly expanded and retracted can be related to changes in current speed and direction. On November 26th at approximately 16.00 there is shift in current direction from about 350 degrees to about 310 degrees. There is a rise in current speed from about 20 cm/s to 30 cm/s. Prior to this change the proportion of polyps expanded is rising and the proportion of polyps partly expanded is decreasing. Following the change in current speed and direction these proportions are stable until there is a sudden drop in current speed on November 27th at 09.00. From here on, the proportion of expanded polyps is higher than the proportion of partly expanded polyps.

Based on our image analyses we cannot see any significant immediate effects of the corals being exposed to drill cuttings. Revisiting the corals on a regular basis over the next years is strongly recommended to reveal any long term effects.

8. General conclusions

This study was set up using both traditional and experimental environmental monitoring. The acoustic lander should enable real-time monitoring of the density and distribution of the plume of drill mud and cuttings while the time-lapse camera should reveal any behavioural changes of corals being exposed to this plume. Further, samples of cold-water corals were collected to investigate differences in feeding behaviour in corals that had been exposed to drill cuttings and corals that had not. Together with more traditional monitoring as sediment push core and trap samples this would give a good overview of the environmental impact of the release of drill cuttings.

Unfortunately, the outcome of the study was reduced due to a series of technical problems. The acoustic lander monitoring had to be abandoned due to the early loss of the communication buoy. The camera satellite did not function according to specifications resulting in limited time series from the coral reef, and finally, serious problems with the electronics of the sediment traps resulted in samples that were difficult to interpret.

The choice of discharge point at Morvin was done on the basis of a predicted current in a northerly direction. Our current measurements, although limited in time, show that the prevailing current during release of drill mud and cuttings was in a north-northwesterly direction away from the closer coral reefs. This is also supported by the elevated levels of Barium in this direction, found both in the sediment core and trap samples.

The sediment core samples reveal that one sample close to discharge point is contaminated with THC. No significant metal contamination was found in the sediment core or trap samples.

Lipid class and fatty acid analyses of corals that had been exposed to drill cuttings and of corals that had not been exposed showed no significant differences between the two. Hence, the exposed corals did not have decreased amount of storage lipids compared corals from the unexposed control area and this suggest that there is no differences in the feeding rate between the two.

Image analyses revealed no significant behavioural differences between corals that were exposed to drill cuttings and unexposed corals. Detailed analyses of the time series from the exposed coral reef revealed that changes in current direction and speed were the main reasons for changes in coral polyp behaviour.

In conclusion, the plume of mud and drill cuttings did reach the coral reefs in the downstream direction. However, our analyses do not reveal any immediate damage to the corals.

It is recommended that the coral reefs are revisited at a later stage to reveal any long-term effects of having been exposed to mud and drill cuttings.

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Appendix A Lipid class and fatty acid profiles in cold-water coral *Lophelia pertusa.*

Methods

Sampling:

Corals were collected using ROV and quickly placed in aluminum foil and frozen on dry ice. The samples were hold on dry ice (-70 C) until shipping to the laboratory in Bergen, where the samples were stored at -80 C until analysed.

There was analyzed 6 parallels coral polyp from each sample area.

Lipid extraction:

Total lipid was extracted by a modified Folch method with chloroform/methanol (2:1, v/v.) (Meier *et al.,* 2006).

Coral polyp materials were grounded and sub-samples of 2 g wet weight of samples were extracted with 20 ml chloroform/methanol (2:1, v/v.). Non-lipid material was removed by washing the extract with 0.88% KCl (aq). The extract was dried with MgSO4 (s) and filtered through a glass filter funnel. The lipid content was determined from this extract by evaporating the solvent until constant weight.

Lipid Classes separation and fatty acids analysis:

The lipid classes are separated by high-performance thin-layer chromatography (HPTLC) according to the methods in Olsen *et al.,* 1989. The HPTLC plates were developed with hexane:diethyl ether: acetic acid (80:30:2 v/v). There have been analyzed six different fractions (see figure A1), the lipids were visualized by UV and the different spots are scraped from the silica plate with a razor blade and placed into a 15 ml glass tube for metanolysis:

- Polar lipids (a mixture of all the membrane phospholipids, PC, PE, PS and PI).
- Cholesterol
- Free fatty acids (normally low in biological tissue, high levels indicate hydrolysis and bad sample handling)
- Triacylglycerol (storage lipid)

- Unknown fraction (probably monoalkyldiacyl glycerol)
- Wax esters (storage lipid)

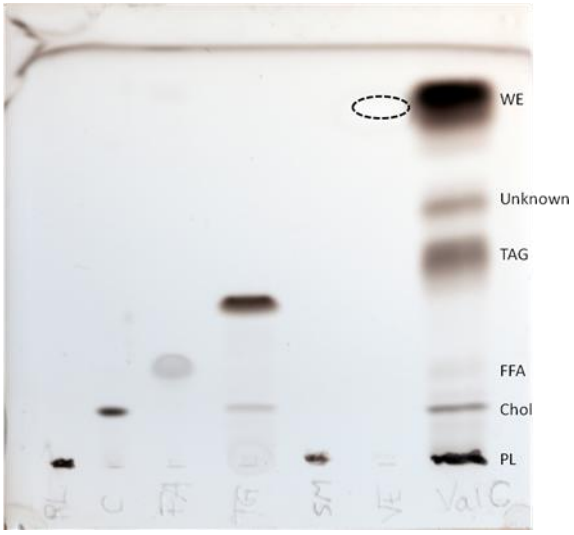

Figure A1 Separation of coral lipid and lipid standards by HPTLC. Lane 1: egg yolk phosphotidycholin (polar lipid, PL); lane 2: cholesterol (Chol), lane 3: Free Nonadecanoic acid (FFA, 19:0); lane 4: Trilinolenoylglycerol (TAG), lane 4: sphingomyaline; lane 5: L: Leuryl palmitate (wax ester (WE)); lane 6: lipid extract from coral (M27 C1 s6). The separated lipid classes were detected by spraying the plate with 3 % cupric acetate in 8 % phosphoric acids followed by charring at 160 C for 20 min. The leuryl palmitate (WE) had low color respond, but the spotted line shows the eluation position.

Methyl esters of the fatty acids (FAME) from total lipids and the lipid classes were prepared and analysed on gas chromatography (GC-FID) as described by (Meier et al., 2006).

The FAME is quantified using nonadecanoic acid (19:0) as internal standard. Fatty alcohols from the wax esters are quantified in the same GC analysis using nonadecanol (19:0 alk) as internal standard.

FAME and fatty alcohols were separated after metanolysis using solid phase column (500 mg aminopropyl-SPE). The FAME fraction was eluated with 3 ml hexane + 2 ml hexane:ethyl acetate (9:1 v/v) and the fatty alcohols were eluated with 4 ml chloroform.

To establish correct identification of the FAME, one sample of each lipid class was analyzed on GC-MS (se appendix B).

From this analysis we have determined:

- Total lipid amount (% of ash-free dry mass).
- Lipid classes distribution (% of fatty acids in each lipid classes relative to total amount of fatty acids).
- Fatty acids profile from total lipid and all lipid classes.
- Fatty alcohols profile of the wax ester.

Dry weight and ash measurement

Grounded coral polyp material was dried over night at 110 °C for determination of water content, thereafter the samples were burned at 450° C for 6 h to determine the ash weight. The lipids are quantified relative to ash free dry weight (total sample weight – water – ash)

Statistical analyses

The difference between lipid % and lipid classes composition were tested by Analysis of Variance (ANOVA) with Tukey (HSD) post hoc tests. All statistical analyses were carried out using XLSTAT software (Addinsoft, U.S.).

Principal Component Analysis (PCA) was carried out on the FA profiles using Sirius (Version 7.1, Bergen, Norway). The fatty acid datasets were normalised.

Results and discussion

Lipid amount

There were no difference in the amount of lipid extracted from the exposed corals (MRRE C1 and C2) and the reference samples (M27 C1 and C2). One sample (M27 C3) had significant lower lipid levels, but this coral sample was heavily covered with black sediment and there were low amount of soft tissue in the coral. This sample was therefore excluded from the comparison between the sampling sites.

Lipid classes:

The lipid extracted from the corals was clearly dominated by storage lipids, approximately 50 % of the FAs were found in the wax ester fraction and the triacylglycerol around 30 %. Hence the energy storage lipid contributed to more than 80 % of the total amount of FA.

Table A1 Amount of lipid (% of ash free dry weight) and lipid classes composition (% of FA in each lipid class relative to total FA) for reference samples (M27) and exposed samples (MRRE). Different letters = significant difference between sampling sites (ANOVA, p < 0.05).

		Lipid classes composition (FA% of totally FA)				
	Lipid %	WE	TAG	PL	Unknown	FFA
M27 C1	17.6 ± 3.2^{a}	52.8 ± 0.7^{b}	30.7 ± 1.1^{a}	9.4 ± 1.6^{a}	5.8 ± 0.2^{a}	1.3 ± 1.5^{b}
M27 C2	14.8 ± 3.8^{a}	52.5 ± 2.2^{b}	31.1 ± 2.6^{a}	8.9 ± 2.6^{a}	5.6 ± 0.7^{a}	1.9 ± 0.3^{ab}
M27 C3	5.9 ± 2.5^{b}	51.5 ± 1.7^{b}	32.4 ± 1.3^{a}	5.1 ± 1.8^{ab}	7.1 ± 0.6^{a}	3.9 ± 0.6^{a}
MRRE C1	16.5 ± 2.5^{a}	57.1 ± 4.9^{b}	31.5 ± 4.6^{a}	3.5 ± 1.1^{b}	6.3 ± 0.9^{a}	1.6 ± 0.4^{ab}
MRRE C2	13.8 ± 4.1^{ab}	63.5 ± 3.8^{a}	20.9 ± 1.5^{b}	9.7 ± 5.8^{a}	4.3 ± 0.6^{b}	1.7 ± 0.2^{ab}

Fatty acids profiles:

There were very large differences in the fatty acid composition between the different lipid classes. Table A2-A8 givs the fatty acids composition in the different lipid classes.

Storages lipids.

The composition in the WE fraction was dominated by mono unsaturated FA (MUFA). 22:1(n-11), 20:1 (n-9) and 18:1 (n-9) contributed with 43-49 % of the total FA and mono unsaturated fatty alcohols 20:1-A and 22:1-A contributed with 75-77% of the total fatty alcohols. There were low levels of poly unsaturated FA (PUFA) in the wax esters. 22:6 (n-3) was the highest PUFA and found to be up to 3 % of the total FA. For the saturated FA (SFA), 16:0 and 14:0 were dominating (6-8% and 4-6 % of total FA, respectively) (table A3 and A4).

The FA composition in the TAG are also dominated by the MUFA, 22:1 (n-11) and 20:1 (n-9) contribute with 22-32% of the total FA, but there are higher levels of SFA in the TAG compared with the WE (16:0 contribute with 17-21 % of totally FA). In the PUFA 20:5(n-3) is dominating (6-9 % of total FA) (table A5). The fatty acids and fatty alcohols profiles from the WE is very dominated by copepods lipid biomarkers (MUFAs) and this indicate a high input of calanoid copepods in the diet of thise corals (Dodds et al, 2009)

Polar lipids.

The fatty acid composition of the polar lipids is dominated by PUFA and SFA (16:0 contribute with 17-27 % of the total FA) (table A7). The coral polar lipids have a very special PUFA composition with unusual high levels of PUFA (n-6) (13-20 %): compared to PUFA (n-3) (25-37%). The n6/n3 ratio was as high as 0.7. Cold water marine animals usually have high levels of PUFA (n-3) and the ratio of n6/n3 is typically found to be under 0.1 (Berge and Barnathan, 2005). This indicates that the corals have a special lipid biosynthesis and that they have a high degree of modification of the composition of the polar membrane lipids. There were identified large High levels of dimethyl acetals (DMAs)

were identified showing that the coral have high levels of plasmalogens (ether-linked glycerophospholipidslipids) (Brosche et al., 1985). The DMAs is not quantified but the levels of 16:-DMA, 18:0-DMA and 18:1-DMA were approximately around 10 % of total amount of FA. The FA composition of the polar lipids from the MRRE C1 samples had lower levels of PUFA and higher levels of MUFA compared with the other samples. Again, it is difficult to evaluate if this is an abnormal situation or can be described by normal variation. To our knowledge, the FA composition of polar lipids in *Lophelia pertusa* has not been described earlier in the scientific literature.

Unknown fraction:

The identity of this unknown fraction is not confirmed by standards or mass spectra analysis, but Hamoutene et al. (2008) and Imbs et al, (2010) reported that deep sea coral contains monoalkyldiacyl glycerol (MADAG) and this agree very well with the eluation on the HPTLC. A special FA composition was found in this fraction with high levels of MUFA 20:1 (n-9) and 22:1 (n-11) and PUFA 20:4 (n-6) and 20:5 (n-3) (table A6).

Table A2. Fatty acid composition (relative to total fatty acids) in total lipid (TL) from coral. Mean±SD

	M27 C1	M27 C2	M27 C3	MRRE C1	MRRE C2
14:0	4.03 ± 0.13	3.80 ± 0.22	3.64 ± 0.25	4.05 ± 0.93	5.56 ± 0.47
Iso 15:0	0.16 ± 0.01	0.16 ± 0.02	0.16 ± 0.00	0.16 ± 0.03	0.20 ± 0.02
15:0	0.43 ± 0.02	0.41 ± 0.02	0.41 ± 0.03	0.41 ± 0.06	0.51 ± 0.02
16:0	10.69 ± 0.73	10.32 ± 0.77	9.01 ± 0.24	10.03 ± 0.82	11.38 ± 1.03
Iso 17:0	0.52 ± 0.01	0.51 ± 0.02	0.53 ± 0.00	0.55 ± 0.02	0.41 ± 0.21
17:0	0.46 ± 0.01	0.45 ± 0.01	0.48 ± 0.00	0.43 ± 0.02	0.46 ± 0.03
18:0	1.64 ± 0.15	1.55 ± 0.09	1.59 ± 0.04	1.47 ± 0.10	1.57 ± 0.23
20:0	0.36 ± 0.01	0.36 ± 0.02	0.36 ± 0.00	0.34 ± 0.01	0.39 ± 0.05
21:0	0.22 ± 0.11	0.15 ± 0.09	0.08 ± 0.01	0.07 ± 0.01	0.16 ± 0.12
22:0	0.13 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	0.12 ± 0.01	0.14 ± 0.01
∑SFA	**18.92 ± 1.09**	**18.10 ± 0.97**	**16.62 ± 0.48**	**17.83 ± 1.71**	**21.06 ± 1.32**
14:1 (n-5)	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.00	0.10 ± 0.02	0.14 ± 0.02
16:1 (n-9)	0.21 ± 0.03	0.20 ± 0.01	0.21 ± 0.02	0.22 ± 0.03	0.25 ± 0.03
16:1 (n-7)	3.58 ± 0.07	3.52 ± 0.17	3.40 ± 0.22	3.86 ± 0.51	4.49 ± 0.42
16:1 (n-5)	1.01 ± 0.01	1.00 ± 0.03	1.02 ± 0.05	0.88 ± 0.10	1.19 ± 0.08
17:1 (n-9)	0.42 ± 0.01	0.41 ± 0.01	0.42 ± 0.02	0.41 ± 0.03	0.46 ± 0.00
18:1 (n-9)	5.32 ± 0.10	5.32 ± 0.22	6.00 ± 0.07	5.60 ± 0.33	6.35 ± 0.27
18:1 (n-7)	1.34 ± 0.12	1.35 ± 0.07	1.65 ± 0.00	1.32 ± 0.07	1.22 ± 0.05
18:1 (n-5)	2.44 ± 0.14	2.44 ± 0.16	2.83 ± 0.03	2.11 ± 0.12	2.36 ± 0.28
20:1 (n-11)	0.94 ± 0.04	0.98 ± 0.04	1.01 ± 0.04	1.07 ± 0.06	0.94 ± 0.05
20:1 (n-9)	11.59 ± 0.29	11.98 ± 0.55	12.22 ± 0.32	12.66 ± 1.08	9.77 ± 0.72
20:1 (n-7)	0.44 ± 0.02	0.42 ± 0.01	0.43 ± 0.00	0.38 ± 0.03	0.42 ± 0.06
20:1 (n-5)	0.41 ± 0.03	0.39 ± 0.02	0.40 ± 0.00	0.29 ± 0.02	0.37 ± 0.06
22:1 (n-11)	20.14 ± 0.41	20.96 ± 0.89	21.88 ± 0.85	21.72 ± 2.17	17.46 ± 1.23
22:1 (n-9)	2.05 ± 0.05	2.10 ± 0.12	2.11 ± 0.01	2.30 ± 0.27	2.04 ± 0.08
22:1 (n-7)	0.14 ± 0.04	0.15 ± 0.01	0.15 ± 0.03	0.16 ± 0.01	0.15 ± 0.01
24:1 (n-11)	1.22 ± 0.09	1.27 ± 0.07	1.46 ± 0.03	1.31 ± 0.17	0.97 ± 0.15
24:1 (n-9)	0.80 ± 0.03	0.82 ± 0.06	0.90 ± 0.02	0.78 ± 0.05	0.76 ± 0.02
24:1 (n-7)	0.23 ± 0.04	0.24 ± 0.02	0.23 ± 0.01	0.23 ± 0.03	0.21 ± 0.03
∑MUFA	**52.35 ± 1.09**	**53.66 ± 1.92**	**56.43 ± 1.58**	**55.40 ± 3.26**	**49.55 ± 2.50**
18:4(n-1)	0.39 ± 0.02	0.38 ± 0.02	0.36 ± 0.01	0.47 ± 0.07	0.49 ± 0.05
16:2 (n-4)	0.37 ± 0.05	0.36 ± 0.02	0.29 ± 0.01	0.34 ± 0.06	0.47 ± 0.03
18:2 (n-6)	1.59 ± 0.06	1.59 ± 0.08	1.53 ± 0.06	1.71 ± 0.07	1.63 ± 0.13
20:2 (n-6)	0.80 ± 0.06	0.83 ± 0.03	1.04 ± 0.03	0.75 ± 0.14	0.62 ± 0.04
20:3 (n-6)	0.43 ± 0.05	0.39 ± 0.04	0.46 ± 0.00	0.38 ± 0.03	0.39 ± 0.06
20:4 (n-6)	2.63 ± 0.44	2.97 ± 0.53	2.84 ± 0.43	1.88 ± 0.08	2.85 ± 0.81
22:4 (n-6)	1.82 ± 0.17	1.66 ± 0.28	1.75 ± 0.20	1.00 ± 0.10	1.52 ± 0.53
22:5 (n-6)	0.13 ± 0.04	0.15 ± 0.01	0.03 ± 0.00	0.14 ± 0.06	0.14 ± 0.02
18:3 (n-3)	1.71 ± 0.08	1.66 ± 0.13	1.59 ± 0.05	1.82 ± 0.13	1.83 ± 0.17
18:4 (n-3)	1.57 ± 0.09	1.39 ± 0.17	1.13 ± 0.06	2.11 ± 0.52	2.17 ± 0.18
20:3 (n-3)	0.42 ± 0.01	0.40 ± 0.01	0.47 ± 0.01	0.33 ± 0.04	0.36 ± 0.03
20:4 (n-3)	1.59 ± 0.08	1.51 ± 0.13	1.41 ± 0.04	1.67 ± 0.16	1.62 ± 0.14
20:5 (n-3)	7.32 ± 0.16	7.27 ± 0.40	6.68 ± 0.79	7.31 ± 0.51	7.63 ± 0.44
21:5 (n-3)	0.34 ± 0.02	0.31 ± 0.01	0.29 ± 0.02	0.32 ± 0.03	0.33 ± 0.01
22:5 (n-3)	3.72 ± 0.14	3.54 ± 0.21	3.37 ± 0.49	2.56 ± 0.24	3.59 ± 0.41
22:6 (n-3)	3.90 ± 0.10	3.82 ± 0.23	3.73 ± 0.21	3.98 ± 0.34	3.77 ± 0.25
∑ PUFA	**28.72 ± 0.56**	**28.24 ± 1.37**	**26.95 ± 2.06**	**26.77 ± 1.61**	**29.39 ± 1.44**
∑ PUFA (n-6)	**7.39 ± 0.45**	**7.59 ± 0.71**	**7.64 ± 0.60**	**5.86 ± 0.21**	**7.15 ± 1.19**
∑ PUFA (n-3)	**20.57 ± 0.55**	**19.91 ± 1.05**	**18.66 ± 1.47**	**20.10 ± 1.67**	**21.29 ± 0.56**
(n-6)/(n-3)	**0.36 ± 0.03**	**0.38 ± 0.04**	**0.41 ± 0.00**	**0.29 ± 0.04**	**0.34 ± 0.05**

Table A3. Fatty acids composition in wax ester of coral.

	M27 C1	M27 C2	M27 C3	MRRE C1	MRRE C2
14:0	4.22 ± 0.22	3.89 ± 0.46	3.83 ± 0.01	4.18 ± 0.93	6.18 ± 0.47
Iso 15:0	0.14 ± 0.07	0.18 ± 0.03	0.17 ± 0.00	0.21 ± 0.03	0.17 ± 0.09
15:0	0.57 ± 0.13	0.73 ± 0.23	0.59 ± 0.03	0.71 ± 0.13	0.77 ± 0.13
16:0	7.04 ± 1.07	6.17 ± 0.27	7.65 ± 0.48	7.26 ± 1.42	8.46 ± 0.50
Iso 17:0	0.39 ± 0.05	0.45 ± 0.07	0.38 ± 0.04	0.45 ± 0.05	0.49 ± 0.05
17:0	0.36 ± 0.07	0.36 ± 0.06	0.35 ± 0.03	0.34 ± 0.05	0.41 ± 0.03
18:0	1.25 ± 1.53	0.65 ± 0.04	2.57 ± 0.47	1.54 ± 1.68	0.76 ± 0.07
20:0	0.28 ± 0.07	0.22 ± 0.01	0.25 ± 0.00	0.25 ± 0.03	0.32 ± 0.09
21:0	0.76 ± 0.12	0.74 ± 0.13	0.54 ± 0.02	0.56 ± 0.26	0.78 ± 0.10
22:0	0.15 ± 0.07	0.14 ± 0.03	0.09 ± 0.00	0.15 ± 0.04	0.22 ± 0.09
∑SFA	**15.31 ± 2.72**	**13.72 ± 1.06**	**16.57 ± 1.09**	**15.81 ± 3.10**	**18.75 ± 1.05**
14:1 (n-5)	0.23 ± 0.17	0.40 ± 0.05	0.41 ± 0.06	0.40 ± 0.08	0.35 ± 0.18
16:1 (n-9)	0.37 ± 0.08	0.39 ± 0.06	0.32 ± 0.05	0.37 ± 0.05	0.40 ± 0.07
16:1 (n-7)	5.63 ± 0.24	5.48 ± 0.22	5.44 ± 0.11	5.52 ± 0.55	6.44 ± 0.32
16:1 (n-5)	1.11 ± 0.09	1.07 ± 0.07	1.07 ± 0.04	0.89 ± 0.14	1.38 ± 0.12
17:1 (n-9)	0.78 ± 0.10	0.65 ± 0.32	0.74 ± 0.03	0.71 ± 0.12	0.79 ± 0.10
18:1 (n-9)	9.11 ± 0.51	9.87 ± 0.21	9.77 ± 0.59	9.26 ± 0.69	9.79 ± 0.94
18:1 (n-7)	1.08 ± 0.04	1.17 ± 0.03	1.14 ± 0.05	1.08 ± 0.08	1.09 ± 0.06
18:1 (n-5)	1.87 ± 0.13	2.09 ± 0.06	2.01 ± 0.01	1.68 ± 0.13	1.75 ± 0.93
20:1 (n-11)	1.35 ± 0.27	1.63 ± 0.04	1.55 ± 0.01	1.60 ± 0.13	1.22 ± 0.12
20:1 (n-9)	12.83 ± 0.64	12.84 ± 0.41	12.36 ± 0.10	12.63 ± 0.68	11.47 ± 0.45
20:1 (n-7)	0.33 ± 0.03	0.35 ± 0.02	0.33 ± 0.01	0.32 ± 0.03	0.31 ± 0.04
20:1 (n-5)?	0.41 ± 0.04	0.35 ± 0.14	0.38 ± 0.02	0.31 ± 0.02	0.37 ± 0.07
22:1 (n-11)	25.92 ± 0.93	26.58 ± 0.81	26.05 ± 0.52	25.35 ± 2.38	21.99 ± 1.57
22:1 (n-9)	1.91 ± 0.09	2.06 ± 0.07	1.89 ± 0.02	2.40 ± 0.27	2.02 ± 0.18
22:1 (n-7)	0.30 ± 0.13	0.35 ± 0.08	0.14 ± 0.01	0.26 ± 0.08	0.29 ± 0.05
24:1 (n-11)	1.80 ± 0.10	1.60 ± 0.41	1.98 ± 0.03	1.53 ± 0.58	1.15 ± 0.19
24:1 (n-9)	1.05 ± 0.05	1.07 ± 0.04	1.06 ± 0.03	1.00 ± 0.07	0.85 ± 0.11
24:1 (n-7)	0.45 ± 0.07	0.51 ± 0.09	0.37 ± 0.02	0.42 ± 0.05	0.40 ± 0.05
∑MUFA	**66.51 ± 1.95**	**68.46 ± 0.78**	**67.02 ± 0.06**	**65.73 ± 3.79**	**62.08 ± 1.04**
18:4(n-1)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16:2 (n-4)	0.25 ± 0.19	0.23 ± 0.18	0.43 ± 0.01	0.17 ± 0.15	0.34 ± 0.27
18:2 (n-6)	2.59 ± 0.14	2.63 ± 0.13	2.60 ± 0.16	2.64 ± 0.17	2.30 ± 0.13
20:2 (n-6)	0.36 ± 0.02	0.38 ± 0.02	0.39 ± 0.02	0.33 ± 0.03	0.33 ± 0.02
20:3 (n-6)	0.65 ± 0.37	0.56 ± 0.15	0.35 ± 0.00	0.43 ± 0.08	0.66 ± 0.22
20:4 (n-6)	0.16 ± 0.05	0.25 ± 0.07	0.18 ± 0.00	0.22 ± 0.07	0.26 ± 0.09
22:4 (n-6)	0.25 ± 0.11	0.14 ± 0.06	0.32 ± 0.02	0.13 ± 0.05	0.22 ± 0.09
22:5 (n-6)	0.14 ± 0.03	0.15 ± 0.06	0.08 ± 0.00	0.13 ± 0.03	0.13 ± 0.04
18:3 (n-3)	2.83 ± 0.15	2.95 ± 0.10	2.74 ± 0.17	2.87 ± 0.15	2.63 ± 0.22
18:4 (n-3)	1.80 ± 0.20	1.64 ± 0.22	1.41 ± 0.05	2.30 ± 0.56	2.33 ± 0.13
20:3 (n-3)	0.34 ± 0.05	0.28 ± 0.04	0.34 ± 0.00	0.27 ± 0.02	0.33 ± 0.09
20:4 (n-3)	1.82 ± 0.16	1.74 ± 0.17	1.60 ± 0.10	1.83 ± 0.18	1.81 ± 0.15
20:5 (n-3)	1.68 ± 0.17	1.66 ± 0.15	1.42 ± 0.27	1.75 ± 0.41	2.56 ± 0.25
21:5 (n-3)	0.41 ± 0.11	0.43 ± 0.22	0.36 ± 0.02	0.45 ± 0.08	0.42 ± 0.06
22:5 (n-3)	2.02 ± 0.19	2.07 ± 0.05	1.67 ± 0.17	1.83 ± 0.17	1.95 ± 0.16
22:6 (n-3)	2.76 ± 0.27	2.71 ± 0.21	2.53 ± 0.17	3.11 ± 0.37	2.89 ± 0.25
∑ PUFA	**18.07 ± 1.44**	**17.82 ± 0.66**	**16.41 ± 1.15**	**18.46 ± 1.51**	**19.17 ± 0.52**
∑ PUFA (n-6)	**4.15 ± 0.48**	**4.11 ± 0.19**	**3.90 ± 0.22**	**3.88 ± 0.19**	**3.91 ± 0.25**
∑ PUFA (n-3)	**13.67 ± 1.05**	**13.48 ± 0.75**	**12.08 ± 0.92**	**14.41 ± 1.64**	**14.93 ± 0.39**
(n-6)/(n-3)	**0.30 ± 0.02**	**0.31 ± 0.03**	**0.32 ± 0.01**	**0.27 ± 0.04**	**0.26 ± 0.02**

Table A4 Fatty alcohol composition in wax ester from coral.

	M27 C1	M27 C2	M27 C3	MRRE C1	MRRE C2
14:0-A	0.98 ± 0.41	0.93 ± 0.37	1.11 ± 0.13	0.94 ± 0.35	0.85 ± 0.30
16:0-A	9.08 ± 0.80	8.51 ± 0.29	7.97 ± 0.34	8.04 ± 0.44	9.68 ± 1.47
18:0-A	1.13 ± 0.15	1.12 ± 0.16	1.09 ± 0.03	1.08 ± 0.14	1.24 ± 0.24
16:1(n-7)-A	1.92 ± 0.14	1.82 ± 0.04	1.66 ± 0.11	1.82 ± 0.11	1.70 ± 0.05
16:1(n-5)-A	0.27 ± 0.07	0.25 ± 0.03	0.23 ± 0.01	0.25 ± 0.04	0.29 ± 0.07
18:1(n-9)-A	3.39 ± 0.16	3.16 ± 0.18	3.21 ± 0.09	2.99 ± 0.13	2.93 ± 0.10
18:1(n-7)-A	1.17 ± 0.14	1.04 ± 0.06	1.03 ± 0.01	0.98 ± 0.07	1.07 ± 0.03
18:1(n-5)-A	0.65 ± 0.04	0.63 ± 0.03	0.60 ± 0.02	0.52 ± 0.03	0.57 ± 0.08
∑20:1-A*	27.11 ± 0.42	26.95 ± 0.43	26.48 ± 0.04	27.13 ± 0.23	26.92 ± 0.66
∑22:1-A*	47.90 ± 1.91	49.78 ± 0.52	50.39 ± 1.02	50.07 ± 1.41	48.62 ± 1.62
24:1-A	3.89 ± 0.15	3.61 ± 0.32	4.28 ± 0.27	3.94 ± 0.63	3.76 ± 0.37
18:2(n-6)-A	1.07 ± 0.08	0.98 ± 0.13	0.88 ± 0.06	1.02 ± 0.10	1.08 ± 0.03
18:3 (n-3)-A	1.45 ± 0.32	1.22 ± 0.09	1.06 ± 0.02	1.23 ± 0.12	1.29 ± 0.09

*∑20:1-A is dominated by 20:1 (n-9)-A, ∑22:1-A is dominated by 22:1 (n-11)-A

Table A5 Fatty acids composition in triacylglycerol (TAG) from coral.

	M27 C1	M27 C2	M27 C3	MRRE C1	MRRE C2
14:0	6.21 ± 0.55	5.44 ± 1.10	5.18 ± 0.34	5.54 ± 1.50	8.90 ± 1.86
Iso 15:0	0.33 ± 0.04	0.29 ± 0.04	0.31 ± 0.02	0.28 ± 0.06	0.41 ± 0.06
15:0	0.29 ± 0.30	0.57 ± 0.06	0.56 ± 0.05	0.58 ± 0.08	0.50 ± 0.38
16:0	17.47 ± 0.85	17.21 ± 1.43	16.77 ± 0.42	18.41 ± 1.54	21.27 ± 0.82
Iso 17:0	0.91 ± 0.03	0.91 ± 0.02	0.92 ± 0.04	0.92 ± 0.04	1.04 ± 0.04
17:0	0.82 ± 0.03	0.86 ± 0.06	0.87 ± 0.06	0.86 ± 0.05	1.02 ± 0.07
18:0	2.79 ± 0.47	2.79 ± 0.19	3.54 ± 0.03	2.89 ± 0.31	3.03 ± 0.20
20:0	0.51 ± 0.04	0.56 ± 0.18	0.49 ± 0.03	0.50 ± 0.16	0.58 ± 0.05
21:0	0.10 ± 0.02	0.06 ± 0.05	0.05 ± 0.06	0.07 ± 0.04	0.11 ± 0.07
22:0	0.29 ± 0.10	0.28 ± 0.13	0.16 ± 0.02	0.23 ± 0.15	0.27 ± 0.07
∑SFA	**30.12 ± 1.77**	**29.36 ± 2.70**	**29.25 ± 0.70**	**30.65 ± 3.06**	**37.58 ± 2.69**
14:1 (n-5)	0.01 ± 0.01	0.04 ± 0.04	0.05 ± 0.01	0.03 ± 0.01	0.05 ± 0.05
16:1 (n-9)	0.22 ± 0.09	0.17 ± 0.07	0.26 ± 0.04	0.21 ± 0.08	0.50 ± 0.57
16:1 (n-7)	1.65 ± 0.09	1.58 ± 0.10	1.62 ± 0.05	1.73 ± 0.16	2.10 ± 0.30
16:1 (n-5)	1.29 ± 0.06	1.25 ± 0.09	1.25 ± 0.05	1.09 ± 0.08	1.50 ± 0.09
17:1 (n-9)	0.33 ± 0.05	0.29 ± 0.03	0.36 ± 0.03	0.29 ± 0.07	0.37 ± 0.06
18:1 (n-9)	1.80 ± 0.18	1.84 ± 0.19	2.54 ± 0.05	1.86 ± 0.34	2.27 ± 0.26
18:1 (n-7)	2.25 ± 0.13	2.34 ± 0.19	2.61 ± 0.22	1.97 ± 0.16	2.23 ± 0.30
18:1 (n-5)	3.77 ± 0.12	3.82 ± 0.29	4.48 ± 0.46	3.06 ± 0.28	3.72 ± 0.38
20:1 (n-11)	0.32 ± 0.08	0.27 ± 0.12	0.34 ± 0.04	0.18 ± 0.12	0.23 ± 0.08
20:1 (n-9)	13.44 ± 0.56	14.29 ± 0.80	14.73 ± 2.01	13.12 ± 1.66	9.85 ± 0.47
20:1 (n-7)	0.49 ± 0.02	0.49 ± 0.03	0.56 ± 0.07	0.41 ± 0.03	0.46 ± 0.03
20:1 (n-5)	0.46 ± 0.05	0.46 ± 0.03	0.57 ± 0.05	0.37 ± 0.03	0.44 ± 0.02
22:1 (n-11)	17.37 ± 0.78	18.53 ± 0.98	14.39 ± 5.41	16.60 ± 2.37	11.89 ± 0.68
22:1 (n-9)	2.12 ± 0.08	2.23 ± 0.11	2.39 ± 0.36	1.93 ± 0.23	1.68 ± 0.09
22:1 (n-7)	0.29 ± 0.13	0.33 ± 0.16	0.20 ± 0.04	0.30 ± 0.12	0.29 ± 0.12
24:1 (n-11)	0.76 ± 0.06	0.78 ± 0.05	0.95 ± 0.12	0.61 ± 0.10	0.53 ± 0.09
24:1 (n-9)	0.74 ± 0.04	0.81 ± 0.08	0.82 ± 0.12	0.60 ± 0.05	0.71 ± 0.09
24:1 (n-7)	0.32 ± 0.05	0.35 ± 0.04	0.30 ± 0.03	0.30 ± 0.05	0.23 ± 0.03
∑MUFA	**47.64 ± 1.28**	**49.84 ± 2.34**	**48.42 ± 2.09**	**44.65 ± 4.52**	**39.06 ± 1.75**
18:4(n-1)	0.21 ± 0.03	0.16 ± 0.05	0.18 ± 0.03	0.22 ± 0.05	0.34 ± 0.06
16:2 (n-4)	0.06 ± 0.07	0.17 ± 0.13	0.06 ± 0.00	0.23 ± 0.16	0.19 ± 0.16
18:2 (n-6)	0.18 ± 0.20	0.30 ± 0.01	0.38 ± 0.21	0.37 ± 0.09	0.30 ± 0.16
20:2 (n-6)	1.32 ± 0.10	1.36 ± 0.10	1.83 ± 0.24	1.26 ± 0.21	1.03 ± 0.08
20:3 (n-6)	0.50 ± 0.22	0.59 ± 0.07	0.66 ± 0.06	0.53 ± 0.04	0.63 ± 0.10
20:4 (n-6)	0.96 ± 0.40	1.16 ± 0.04	0.05 ± 0.01	1.21 ± 0.64	1.45 ± 0.08
22:4 (n-6)	1.71 ± 0.08	1.66 ± 0.14	2.14 ± 0.38	1.19 ± 0.19	1.36 ± 0.13
22:5 (n-6)	0.19 ± 0.08	0.18 ± 0.09	0.21 ± 0.01	0.15 ± 0.06	0.19 ± 0.10
18:3 (n-3)	0.36 ± 0.06	0.36 ± 0.04	0.45 ± 0.01	0.46 ± 0.06	0.47 ± 0.06
18:4 (n-3)	1.15 ± 0.12	1.03 ± 0.05	0.86 ± 0.06	1.69 ± 0.43	1.73 ± 0.42
20:3 (n-3)	0.43 ± 0.18	0.49 ± 0.05	0.63 ± 0.08	0.41 ± 0.05	0.39 ± 0.05
20:4 (n-3)	1.25 ± 0.08	1.20 ± 0.10	1.16 ± 0.17	1.24 ± 0.13	1.21 ± 0.12
20:5 (n-3)	7.10 ± 0.37	5.67 ± 2.82	6.78 ± 1.00	9.43 ± 1.49	8.12 ± 1.12
21:5 (n-3)	0.31 ± 0.02	0.30 ± 0.03	0.30 ± 0.03	0.31 ± 0.05	0.32 ± 0.06
22:5 (n-3)	2.83 ± 0.15	2.67 ± 0.27	2.87 ± 0.51	2.30 ± 0.26	2.39 ± 0.17
22:6 (n-3)	3.67 ± 0.20	3.49 ± 0.42	3.74 ± 0.44	3.69 ± 0.47	3.24 ± 0.40
∑ PUFA	**22.24 ± 1.39**	**20.80 ± 2.89**	**22.32 ± 2.79**	**24.70 ± 2.31**	**23.37 ± 2.02**
∑ PUFA (n-6)	**4.86 ± 0.48**	**5.26 ± 0.31**	**5.29 ± 0.47**	**4.71 ± 0.70**	**4.96 ± 0.26**
∑ PUFA (n-3)	**17.10 ± 0.94**	**15.21 ± 2.89**	**16.80 ± 2.29**	**19.53 ± 2.56**	**17.88 ± 2.15**
(n-6)/(n-3)	**0.28 ± 0.02**	**0.36 ± 0.09**	**0.32 ± 0.02**	**0.25 ± 0.06**	**0.28 ± 0.04**

Table A6 Fatty acids composition in unknown fraction from coral.

	M27 C1	M27 C2	M27 C3	MRRE C1	MRRE C2
14:0	2.13 ± 0.37	1.72 ± 0.60	1.93 ± 0.33	2.15 ± 0.62	3.64 ± 1.09
Iso 15:0	0.13 ± 0.16	0.06 ± 0.06	0.22 ± 0.07	0.17 ± 0.13	0.08 ± 0.07
15:0	0.36 ± 0.13	0.27 ± 0.05	0.37 ± 0.08	0.43 ± 0.12	0.63 ± 0.21
16:0	8.28 ± 2.41	6.94 ± 3.57	7.01 ± 1.43	7.88 ± 2.10	11.71 ± 4.01
Iso 17:0	0.60 ± 0.06	0.57 ± 0.10	0.53 ± 0.04	0.52 ± 0.11	0.71 ± 0.12
17:0	0.65 ± 0.10	0.70 ± 0.29	0.59 ± 0.05	0.75 ± 0.23	1.04 ± 0.34
18:0	3.14 ± 2.69	2.27 ± 0.55	3.95 ± 0.96	3.43 ± 1.70	3.59 ± 0.82
20:0	0.58 ± 0.20	0.53 ± 0.19	0.55 ± 0.04	0.58 ± 0.10	0.99 ± 0.23
21:0	0.27 ± 0.10	0.29 ± 0.09	0.26 ± 0.06	0.12 ± 0.18	0.17 ± 0.24
22:0	0.36 ± 0.11	0.37 ± 0.23	0.33 ± 0.15	0.52 ± 0.25	0.34 ± 0.36
∑SFA	**16.81 ± 5.84**	**14.07 ± 4.63**	**16.15 ± 3.27**	**16.89 ± 4.52**	**23.37 ± 6.02**
14:1 (n-5)	0.03 ± 0.03	0.03 ± 0.04	0.02 ± 0.01	0.03 ± 0.04	0.03 ± 0.03
16:1 (n-9)	0.14 ± 0.19	0.07 ± 0.05	0.16 ± 0.04	0.13 ± 0.14	0.09 ± 0.04
16:1 (n-7)	0.80 ± 0.09	0.69 ± 0.06	0.72 ± 0.07	0.78 ± 0.06	0.97 ± 0.10
16:1 (n-5)	0.49 ± 0.10	0.42 ± 0.11	0.34 ± 0.03	0.32 ± 0.09	0.58 ± 0.16
17:1 (n-9)	0.11 ± 0.05	0.16 ± 0.10	0.00 ± 0.00	0.19 ± 0.13	0.28 ± 0.13
18:1 (n-9)	1.01 ± 0.08	1.06 ± 0.12	1.23 ± 0.05	1.00 ± 0.13	1.15 ± 0.37
18:1 (n-7)	0.91 ± 0.27	0.83 ± 0.14	0.93 ± 0.01	0.82 ± 0.07	0.95 ± 0.12
18:1 (n-5)	2.09 ± 0.09	1.99 ± 0.11	2.23 ± 0.03	1.68 ± 0.18	2.55 ± 0.26
20:1 (n-11)	0.39 ± 0.07	0.37 ± 0.15	0.49 ± 0.02	0.29 ± 0.13	0.30 ± 0.13
20:1 (n-9)	10.63 ± 1.04	11.13 ± 0.40	9.78 ± 0.43	11.21 ± 0.81	9.54 ± 0.55
20:1 (n-7)	0.45 ± 0.06	0.41 ± 0.02	0.42 ± 0.01	0.39 ± 0.05	0.45 ± 0.04
20:1 (n-5)	0.37 ± 0.12	0.35 ± 0.06	0.41 ± 0.06	0.42 ± 0.08	0.49 ± 0.14
22:1 (n-11)	20.15 ± 1.92	21.87 ± 0.43	19.97 ± 0.75	19.98 ± 2.00	15.99 ± 1.22
22:1 (n-9)	2.22 ± 0.22	2.28 ± 0.21	2.29 ± 0.06	2.03 ± 0.26	1.66 ± 0.24
22:1 (n-7)	0.32 ± 0.15	0.31 ± 0.14	0.20 ± 0.01	0.31 ± 0.08	0.31 ± 0.11
24:1 (n-11)	1.35 ± 0.14	1.41 ± 0.12	1.51 ± 0.05	1.03 ± 0.14	1.01 ± 0.16
24:1 (n-9)	1.17 ± 0.22	1.35 ± 0.30	1.09 ± 0.02	0.95 ± 0.18	1.29 ± 0.27
24:1 (n-7)	0.37 ± 0.10	0.35 ± 0.17	0.32 ± 0.03	0.36 ± 0.08	0.17 ± 0.11
∑MUFA	**41.84 ± 5.05**	**45.09 ± 0.54**	**42.10 ± 1.17**	**41.92 ± 2.98**	**37.78 ± 2.65**
18:4(n-1)	0.12 ± 0.10	0.07 ± 0.06	0.19 ± 0.02	0.13 ± 0.11	0.08 ± 0.08
18:2 (n-6)	0.29 ± 0.22	0.22 ± 0.13	0.31 ± 0.08	0.37 ± 0.25	0.43 ± 0.18
20:2 (n-6)	0.67 ± 0.09	0.64 ± 0.13	0.75 ± 0.01	0.62 ± 0.09	0.57 ± 0.09
20:3 (n-6)	0.57 ± 0.27	0.49 ± 0.20	0.42 ± 0.06	0.35 ± 0.22	0.88 ± 0.95
20:4 (n-6)	6.03 ± 0.41	6.81 ± 0.33	7.40 ± 0.09	6.85 ± 0.42	6.79 ± 0.68
22:4 (n-6)	0.34 ± 0.05	0.35 ± 0.03	0.38 ± 0.06	0.19 ± 0.07	0.24 ± 0.05
22:5 (n-6)	0.08 ± 0.18	0.13 ± 0.18	0.26 ± 0.03	0.07 ± 0.16	0.06 ± 0.13
18:3 (n-3)	0.38 ± 0.26	0.37 ± 0.37	0.42 ± 0.04	0.47 ± 0.18	0.37 ± 0.25
18:4 (n-3)	0.61 ± 0.21	0.50 ± 0.12	0.29 ± 0.04	0.81 ± 0.38	0.86 ± 0.42
20:3 (n-3)	0.27 ± 0.11	0.32 ± 0.27	0.08 ± 0.10	0.33 ± 0.23	0.58 ± 0.36
20:4 (n-3)	0.78 ± 0.10	0.76 ± 0.15	0.58 ± 0.01	0.87 ± 0.12	0.81 ± 0.18
20:5 (n-3)	23.85 ± 2.66	23.84 ± 3.17	24.71 ± 1.53	24.59 ± 3.52	22.07 ± 3.84
21:5 (n-3)	0.37 ± 0.23	0.38 ± 0.29	0.42 ± 0.02	0.40 ± 0.25	0.15 ± 0.27
22:5 (n-3)	2.81 ± 0.21	2.71 ± 0.35	2.57 ± 0.25	2.01 ± 0.33	2.09 ± 0.29
22:6 (n-3)	2.76 ± 0.34	2.94 ± 0.45	2.83 ± 0.20	2.71 ± 0.46	2.13 ± 0.49
∑ PUFA	**40.18 ± 3.48**	**40.84 ± 4.43**	**41.75 ± 2.10**	**41.19 ± 4.45**	**38.85 ± 4.75**
∑ PUFA (n-6)	**7.99 ± 0.46**	**8.64 ± 0.39**	**9.51 ± 0.13**	**8.45 ± 0.47**	**8.97 ± 1.18**
∑ PUFA (n-3)	**31.83 ± 3.11**	**31.82 ± 4.30**	**31.91 ± 1.95**	**32.20 ± 4.78**	**29.07 ± 5.18**
(n-6)/(n-3)	**0.25 ± 0.01**	**0.28 ± 0.03**	**0.30 ± 0.01**	**0.27 ± 0.05**	**0.32 ± 0.07**

Table A7 Fatty acid composition in polar lipids (PL) from coral.

	M27 C1	M27 C2	M27 C3	MRRE C1	MRRE C2
14:0	1.87 ± 0.43	1.71 ± 0.46	1.57 ± 0.37	2.25 ± 0.43	1.62 ± 0.56
Iso 15:0	0.11 ± 0.06	0.10 ± 0.04	0.12 ± 0.17	0.18 ± 0.14	0.08 ± 0.04
15:0	0.54 ± 0.27	0.60 ± 0.15	0.49 ± 0.10	0.72 ± 0.17	0.59 ± 0.15
16:0	27.47 ± 5.58	27.11 ± 4.63	17.08 ± 0.85	26.53 ± 2.38	25.89 ± 5.42
Iso 17:0	0.44 ± 0.05	0.47 ± 0.07	0.53 ± 0.07	0.62 ± 0.23	0.41 ± 0.10
17:0	1.19 ± 0.06	1.24 ± 0.12	1.23 ± 0.04	1.71 ± 0.37	1.43 ± 0.24
18:0	5.14 ± 0.41	5.41 ± 0.92	5.66 ± 0.77	7.13 ± 1.25	5.72 ± 0.87
20:0	1.32 ± 0.12	1.34 ± 0.17	1.92 ± 0.48	1.78 ± 0.43	1.48 ± 0.31
21:0	0.47 ± 0.11	0.51 ± 0.10	0.16 ± 0.01	0.55 ± 0.60	0.50 ± 0.19
22:0	0.81 ± 0.34	0.97 ± 0.37	2.33 ± 0.29	1.34 ± 0.33	0.93 ± 0.49
∑SFA	**39.37 ± 5.73**	**39.47 ± 5.07**	**31.08 ± 2.67**	**42.82 ± 3.80**	**38.64 ± 4.38**
14:1 (n-5)	0.03 ± 0.04	0.03 ± 0.01	0.03 ± 0.03	0.03 ± 0.03	0.02 ± 0.01
16:1 (n-9)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16:1 (n-7)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16:1 (n-5)	0.01 ± 0.04	0.01 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
17:1 (n-9)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.16	0.07 ± 0.16
18:1 (n-9)	0.49 ± 0.18	0.57 ± 0.26	1.19 ± 0.29	1.10 ± 0.31	0.72 ± 0.24
18:1 (n-7)	1.30 ± 0.17	1.22 ± 0.50	2.15 ± 0.29	1.10 ± 0.45	1.04 ± 0.22
18:1 (n-5)	1.51 ± 0.30	1.62 ± 0.35	2.17 ± 0.06	2.04 ± 0.34	1.93 ± 0.26
20:1 (n-11)	0.20 ± 0.03	0.20 ± 0.07	0.30 ± 0.01	0.25 ± 0.08	0.23 ± 0.08
20:1 (n-9)	2.07 ± 0.29	2.35 ± 0.75	3.09 ± 0.73	3.87 ± 1.20	2.07 ± 0.35
20:1 (n-7)	0.88 ± 0.16	0.81 ± 0.12	0.63 ± 0.09	0.69 ± 0.12	0.90 ± 0.15
20:1 (n-5)	1.04 ± 0.13	0.90 ± 0.09	0.93 ± 0.19	0.95 ± 0.12	1.01 ± 0.07
22:1 (n-11)	1.79 ± 0.32	2.35 ± 1.26	3.12 ± 0.97	4.36 ± 1.74	1.84 ± 0.59
22:1 (n-9)	2.62 ± 0.34	2.51 ± 0.16	2.37 ± 0.55	2.50 ± 0.23	2.39 ± 0.44
22:1 (n-7)	0.22 ± 0.03	0.23 ± 0.03	0.28 ± 0.10	0.37 ± 0.14	0.23 ± 0.09
24:1 (n-11)	0.13 ± 0.07	0.16 ± 0.11	0.20 ± 0.03	0.17 ± 0.17	0.08 ± 0.08
24:1 (n-9)	0.71 ± 1.12	0.46 ± 0.30	0.49 ± 0.05	0.63 ± 0.28	0.62 ± 0.45
24:1 (n-7)	0.03 ± 0.04	0.06 ± 0.05	0.10 ± 0.05	0.05 ± 0.04	0.05 ± 0.04
∑MUFA	**13.03 ± 1.86**	**13.50 ± 2.59**	**17.06 ± 3.17**	**18.18 ± 4.33**	**13.20 ± 1.53**
18:4(n-1)	0.07 ± 0.03	0.03 ± 0.02	0.09 ± 0.04	0.05 ± 0.02	0.07 ± 0.03
16:2 (n-4)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:2 (n-6)	0.22 ± 0.10	0.29 ± 0.09	0.46 ± 0.10	0.48 ± 0.14	0.32 ± 0.10
20:2 (n-6)	1.17 ± 0.24	1.27 ± 0.27	2.32 ± 0.09	1.53 ± 0.18	1.32 ± 0.32
20:3 (n-6)	0.36 ± 0.42	0.19 ± 0.14	0.27 ± 0.05	0.43 ± 0.21	0.18 ± 0.18
20:4 (n-6)	11.63 ± 1.70	11.73 ± 2.91	6.04 ± 0.91	7.24 ± 1.40	10.68 ± 2.58
22:4 (n-6)	6.33 ± 1.45	5.26 ± 1.06	5.01 ± 0.09	3.61 ± 0.30	5.14 ± 1.22
22:5 (n-6)	0.13 ± 0.14	0.19 ± 0.16	0.37 ± 0.22	0.31 ± 0.34	0.28 ± 0.27
18:3 (n-3)	0.09 ± 0.06	0.11 ± 0.07	0.33 ± 0.06	0.25 ± 0.24	0.07 ± 0.05
18:4 (n-3)	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.15 ± 0.07	0.09 ± 0.04
20:3 (n-3)	0.56 ± 0.07	0.55 ± 0.10	1.06 ± 0.07	0.66 ± 0.12	0.67 ± 0.10
20:4 (n-3)	0.22 ± 0.04	0.24 ± 0.08	0.37 ± 0.01	0.39 ± 0.05	0.32 ± 0.13
20:5 (n-3)	12.62 ± 1.52	13.04 ± 2.16	11.46 ± 3.72	10.51 ± 2.87	13.47 ± 1.60
21:5 (n-3)	0.41 ± 0.08	0.48 ± 0.12	1.26 ± 1.10	0.49 ± 0.36	0.52 ± 0.25
22:5 (n-3)	9.95 ± 2.22	9.40 ± 1.77	14.01 ± 1.36	8.29 ± 2.07	11.00 ± 2.80
22:6 (n-3)	3.83 ± 1.16	4.22 ± 1.31	8.74 ± 1.40	4.60 ± 1.03	4.03 ± 1.82
∑ PUFA	**47.59 ± 4.63**	**47.03 ± 5.32**	**51.85 ± 5.84**	**39.00 ± 5.95**	**48.16 ± 4.15**
∑ PUFA (n-6)	**19.84 ± 2.07**	**18.92 ± 3.29**	**14.47 ± 0.63**	**13.60 ± 1.24**	**17.91 ± 2.74**
∑ PUFA (n-3)	**27.68 ± 4.39**	**28.09 ± 4.66**	**37.29 ± 5.24**	**25.35 ± 4.83**	**30.18 ± 6.14**
(n-6)/(n-3)	**0.73 ± 0.14**	**0.70 ± 0.20**	**0.39 ± 0.04**	**0.55 ± 0.07**	**0.63 ± 0.22**

Table A5 Fatty acid composition in free fatty acids (FFA) from coral.

	M27 C1	M27 C2	M27 C3	MRRE C1	MRRE C2
14:0	3.04 ± 2.15	1.68 ± 0.48	1.78 ± 0.38	3.23 ± 1.36	2.99 ± 1.30
Iso 15:0	0.05 ± 0.06	0.06 ± 0.07	0.37 ± 0.07	0.05 ± 0.06	0.05 ± 0.07
15:0	0.78 ± 0.60	0.35 ± 0.23	0.56 ± 0.15	0.93 ± 0.62	0.67 ± 0.57
16:0	15.32 ± 6.04	9.35 ± 2.04	12.17 ± 2.93	23.05 ± 9.29	15.71 ± 6.85
Iso 17:0	0.22 ± 0.19	0.20 ± 0.20	0.43 ± 0.08	0.48 ± 0.31	0.24 ± 0.20
17:0	1.52 ± 0.83	1.06 ± 0.31	0.75 ± 0.14	2.00 ± 0.91	1.59 ± 0.57
18:0	7.72 ± 3.29	5.02 ± 1.92	8.49 ± 2.37	10.27 ± 5.44	9.37 ± 5.61
20:0	0.89 ± 0.54	0.47 ± 0.15	0.52 ± 0.10	0.72 ± 0.36	0.67 ± 0.33
21:0	1.28 ± 1.15	0.52 ± 0.65	0.24 ± 0.33	0.25 ± 0.38	0.73 ± 0.70
22:0	0.15 ± 0.16	0.11 ± 0.16	0.13 ± 0.02	0.16 ± 0.24	0.05 ± 0.11
∑SFA	**31.02 ± 13.85**	**18.95 ± 4.61**	**25.64 ± 6.61**	**41.39 ± 15.93**	**32.22 ± 14.49**
14:1 (n-5)	0.12 ± 0.07	0.10 ± 0.15	0.04 ± 0.01	0.13 ± 0.20	0.05 ± 0.04
16:1 (n-9)	0.37 ± 0.23	0.49 ± 0.78	0.45 ± 0.11	0.38 ± 0.50	0.55 ± 0.41
16:1 (n-7)	1.07 ± 0.48	0.63 ± 0.16	0.90 ± 0.05	0.86 ± 0.26	1.25 ± 0.41
16:1 (n-5)	0.12 ± 0.16	0.38 ± 0.12	0.36 ± 0.07	0.35 ± 0.12	0.25 ± 0.27
17:1 (n-9)	0.20 ± 0.31	0.09 ± 0.14	0.43 ± 0.10	0.27 ± 0.47	0.27 ± 0.46
18:1 (n-9)	3.04 ± 1.77	3.67 ± 3.66	1.57 ± 0.01	1.35 ± 0.23	2.94 ± 1.15
18:1 (n-7)	1.82 ± 3.75	1.16 ± 1.32	0.93 ± 0.08	1.15 ± 0.72	0.83 ± 1.26
18:1 (n-5)	1.28 ± 0.43	1.19 ± 0.43	1.57 ± 0.30	1.92 ± 0.52	1.43 ± 0.14
20:1 (n-11)	0.41 ± 0.17	0.26 ± 0.13	0.31 ± 0.07	0.20 ± 0.12	0.29 ± 0.24
20:1 (n-9)	2.65 ± 1.04	2.56 ± 1.01	2.34 ± 0.12	3.79 ± 1.47	2.29 ± 0.36
20:1 (n-7)	0.50 ± 0.20	0.33 ± 0.05	0.50 ± 0.19	0.73 ± 0.19	0.52 ± 0.11
20:1 (n-5)	0.81 ± 0.29	0.54 ± 0.09	0.66 ± 0.13	1.24 ± 0.49	0.94 ± 0.75
22:1 (n-11)	3.53 ± 1.51	3.22 ± 1.81	3.36 ± 0.06	5.80 ± 2.89	2.49 ± 0.82
22:1 (n-9)	1.40 ± 0.59	0.96 ± 0.21	1.12 ± 0.04	1.71 ± 0.81	1.14 ± 0.12
22:1 (n-7)	0.17 ± 0.23	0.08 ± 0.13	0.20 ± 0.08	0.16 ± 0.24	0.13 ± 0.16
24:1 (n-11)	0.06 ± 0.14	0.23 ± 0.20	0.30 ± 0.00	0.10 ± 0.14	0.06 ± 0.13
24:1 (n-9)	0.41 ± 0.46	0.69 ± 0.79	0.39 ± 0.03	1.45 ± 1.27	0.90 ± 0.98
24:1 (n-7)	0.01 ± 0.01	0.13 ± 0.18	0.06 ± 0.01	0.49 ± 1.04	0.06 ± 0.13
∑MUFA	**17.95 ± 6.00**	**16.71 ± 4.96**	**15.50 ± 1.42**	**22.09 ± 6.54**	**16.40 ± 3.27**
18:4(n-1)	0.23 ± 0.14	0.15 ± 0.14	0.05 ± 0.04	0.07 ± 0.13	0.21 ± 0.21
16:2 (n-4)	0.07 ± 0.03	0.13 ± 0.11	0.05 ± 0.00	0.17 ± 0.06	0.11 ± 0.04
18:2 (n-6)	1.02 ± 0.34	0.89 ± 0.18	0.97 ± 0.18	0.98 ± 0.22	0.91 ± 0.55
20:2 (n-6)	1.42 ± 0.51	1.07 ± 0.29	1.21 ± 0.15	1.02 ± 0.27	1.02 ± 0.11
20:3 (n-6)	0.60 ± 0.95	0.26 ± 0.15	0.16 ± 0.17	0.12 ± 0.21	0.30 ± 0.19
20:4 (n-6)	16.58 ± 7.81	24.36 ± 3.74	23.31 ± 5.17	12.36 ± 7.00	18.69 ± 7.21
22:4 (n-6)	8.66 ± 2.94	8.39 ± 1.10	8.50 ± 0.84	5.80 ± 2.70	7.92 ± 2.11
22:5 (n-6)	0.23 ± 0.22	0.41 ± 0.41	0.43 ± 0.26	0.15 ± 0.34	0.20 ± 0.38
18:3 (n-3)	0.31 ± 0.20	0.31 ± 0.19	0.56 ± 0.07	0.26 ± 0.43	0.32 ± 0.14
18:4 (n-3)	0.06 ± 0.06	0.04 ± 0.03	0.08 ± 0.07	0.00 ± 0.01	0.02 ± 0.02
20:3 (n-3)	0.35 ± 0.22	0.34 ± 0.11	0.24 ± 0.32	0.21 ± 0.20	0.37 ± 0.10
20:4 (n-3)	0.41 ± 0.26	0.52 ± 0.14	0.35 ± 0.04	0.35 ± 0.23	0.61 ± 0.64
20:5 (n-3)	9.43 ± 4.82	14.26 ± 3.22	12.06 ± 2.36	7.39 ± 5.28	8.72 ± 3.74
21:5 (n-3)	0.11 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.31	0.00 ± 0.00
22:5 (n-3)	8.24 ± 3.27	8.66 ± 1.89	7.12 ± 0.63	5.01 ± 2.83	9.54 ± 4.70
22:6 (n-3)	3.30 ± 1.53	4.52 ± 1.07	3.77 ± 0.23	2.48 ± 1.56	2.45 ± 0.84
∑ PUFA	**51.02 ± 18.42**	**64.34 ± 8.83**	**58.87 ± 8.03**	**36.51 ± 19.12**	**51.39 ± 15.63**
∑ PUFA (n-6)	**28.50 ± 9.45**	**35.40 ± 3.65**	**34.58 ± 5.26**	**20.42 ± 9.52**	**29.05 ± 9.08**
∑ PUFA (n-3)	**22.21 ± 9.57**	**28.66 ± 6.12**	**24.18 ± 2.80**	**15.85 ± 10.13**	**22.02 ± 7.97**
(n-6)/(n-3)	**1.83 ± 1.35**	**1.27 ± 0.23**	**1.43 ± 0.05**	**1.43 ± 0.37**	**1.38 ± 0.34**

Method validation

For testing the reproducibility and variation in the methods we analyzed 5 parallels of one coral sample (M27 C1-S6). There was good reproducibility for both the lipid extraction (RSD =7 %) and the TLC separation/ GC-analysis (RSD 1.5-17). Accordingly, the variation in the FA profile was found to be low (See table A9).

The low amount of free fatty acids (FFA) in all samples shows that there have been little hydrolysis of the other lipid classes and the samples therefore are considered high quality.

Table A9 Reproducibility of lipid (n=5) and dry weight determination (n=2)

Water (%)	Ash (%)	Dry weight tissue (%)	Lipid (%)
29.9 ± 0.8 (RSD=2.7)	65.5 ± 0.6 (RSD=0.9)	4.6 ± 0.2 (RSD=4.7)	21.1 ± 1.5 (RSD=7.0)

Table A10 Reproducibility of lipid class distribution analyses (n=5). The table shows the distribution of fatty acids in the different lipid classes (% fatty acids relative to totally fatty acids). Mean value± standard deviation (SD), (RSD=relative standard deviation)

WE (FA)	TAG	PL	Unknown	FFA
53.17 ± 1.00 (RSD=1.8)	30.29 ± 0.47 (RSD=1.5)	11.03 ± 0.92 (RSD=8.3)	5.91 ± 0.26 (RSD=4.3)	0.49 ± 0.08 (RSD=17.0)

Table A11 Fatty acid distribution in different lipid classes of coral (M27 C1-S6) (FA % relative to total FA). Method validation with 5 parallels. TL=total lipid, WE=wax ester, TAG=triacylglycerol, PL=polar lipids, Unknown lipid (see TLC), FFA=free fatty acids. (mean±SD)

	TL	WE	TAG	PL	Unknown	FFA
14:0	4.26 ± 0.07	4.34 ± 0.08	6.74 ± 0.16	1.77 ± 0.21	2.37 ± 0.04	7.29 ± 1.32
Iso 15:0	0.17 ± 0.02	0.20 ± 0.04	0.34 ± 0.01	0.08 ± 0.04	0.17 ± 0.01	0.16 ± 0.15
15:0	0.47 ± 0.00	0.54 ± 0.07	0.61 ± 0.01	0.63 ± 0.05	0.37 ± 0.01	1.86 ± 0.23
16:0	11.14 ± 0.31	6.43 ± 0.14	17.86 ± 0.36	29.59 ± 1.45	6.60 ± 0.29	26.32 ± 4.02
Iso 17:0	0.54 ± 0.02	0.32 ± 0.01	0.91 ± 0.01	0.42 ± 0.02	0.55 ± 0.02	0.37 ± 0.17
Antiso 17:0	0.26 ± 0.02	0.12 ± 0.01	0.39 ± 0.01	0.00 ± 0.00	0.27 ± 0.03	0.05 ± 0.10
17:0	0.46 ± 0.02	0.29 ± 0.02	0.81 ± 0.03	1.11 ± 0.03	0.61 ± 0.02	3.13 ± 0.43
18:0	1.91 ± 0.04	0.71 ± 0.02	2.60 ± 0.03	5.35 ± 0.48	2.23 ± 0.05	11.99 ± 1.09
20:0	0.37 ± 0.02	0.37 ± 0.04	0.48 ± 0.02	1.23 ± 0.08	0.56 ± 0.03	1.92 ± 0.28
21:0	0.34 ± 0.02	0.67 ± 0.03	0.11 ± 0.01	0.43 ± 0.03	0.35 ± 0.03	3.45 ± 0.45
22:0	0.12 ± 0.05	0.05 ± 0.01	0.20 ± 0.04	0.55 ± 0.02	0.32 ± 0.03	0.26 ± 0.25
∑SFA	**20.03 ± 0.43**	**14.03 ± 0.33**	**31.05 ± 0.61**	**41.18 ± 2.04**	**14.41 ± 0.31**	**56.79 ± 6.70**
14:1 (n-5)	0.10 ± 0.01	0.20 ± 0.16	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.11 ± 0.10
16:1 (n-9)	0.15 ± 0.04	0.47 ± 0.06	0.20 ± 0.01	0.00 ± 0.00	0.11 ± 0.04	0.55 ± 0.34
16:1 (n-7)	3.56 ± 0.06	5.68 ± 0.09	1.71 ± 0.03	0.00 ± 0.00	0.92 ± 0.18	1.55 ± 0.26
16:1 (n-5)	1.02 ± 0.02	1.07 ± 0.02	1.29 ± 0.03	0.00 ± 0.00	0.38 ± 0.03	0.09 ± 0.21
17:1 (n-9)	0.44 ± 0.01	0.78 ± 0.05	0.33 ± 0.01	0.00 ± 0.00	0.15 ± 0.12	0.58 ± 0.37
18:1 (n-9)	5.20 ± 0.06	8.79 ± 0.17	1.69 ± 0.04	0.39 ± 0.08	1.05 ± 0.06	1.19 ± 0.68
18:1 (n-7)	1.28 ± 0.02	1.04 ± 0.02	2.14 ± 0.08	1.16 ± 0.11	1.48 ± 0.16	9.44 ± 1.75
18:1 (n-5)	2.41 ± 0.04	1.79 ± 0.04	3.64 ± 0.03	1.21 ± 0.04	2.00 ± 0.06	0.82 ± 0.33
20:1 (n-11)	0.88 ± 0.06	1.59 ± 0.10	0.40 ± 0.05	0.19 ± 0.02	0.49 ± 0.09	0.66 ± 0.16
20:1 (n-9)	11.78 ± 0.28	12.27 ± 0.23	12.66 ± 0.18	1.86 ± 0.05	9.81 ± 0.48	2.61 ± 0.75
20:1 (n-7)	0.44 ± 0.03	0.32 ± 0.00	0.45 ± 0.01	0.96 ± 0.07	0.41 ± 0.06	0.70 ± 0.11
20:1 (n-5)	0.45 ± 0.03	0.42 ± 0.02	0.45 ± 0.02	1.20 ± 0.06	0.34 ± 0.03	1.06 ± 0.55
22:1 (n-11)	19.83 ± 0.22	25.24 ± 0.54	16.36 ± 0.29	1.59 ± 0.10	18.98 ± 1.08	4.63 ± 1.03
22:1 (n-9)	2.06 ± 0.02	1.77 ± 0.04	1.99 ± 0.04	2.70 ± 0.19	2.10 ± 0.13	1.52 ± 0.44
22:1 (n-7)	0.05 ± 0.08	0.10 ± 0.01	0.16 ± 0.03	0.16 ± 0.04	0.09 ± 0.02	0.00 ± 0.00
24:1 (n-11)	1.09 ± 0.03	1.70 ± 0.06	0.67 ± 0.02	0.08 ± 0.08	1.20 ± 0.07	0.00 ± 0.00
24:1 (n-9)	0.75 ± 0.03	1.05 ± 0.04	0.69 ± 0.03	0.31 ± 0.08	0.99 ± 0.08	0.42 ± 0.19
24:1 (n-7)	0.15 ± 0.04	0.38 ± 0.02	0.28 ± 0.03	0.02 ± 0.02	0.31 ± 0.05	0.03 ± 0.07
∑MUFA	**51.63 ± 0.36**	**64.66 ± 0.66**	**45.14 ± 0.58**	**11.84 ± 0.42**	**32.66 ± 18.30**	**25.95 ± 1.13**
18:4(n-1)	0.36 ± 0.05	0.78 ± 0.11	0.22 ± 0.01	0.07 ± 0.04	0.20 ± 0.04	0.49 ± 0.19
16:2 (n-4)	0.47 ± 0.02	0.47 ± 0.02	0.04 ± 0.00	0.00 ± 0.00	0.14 ± 0.05	0.10 ± 0.02
18:2 (n-6)	1.58 ± 0.02	2.65 ± 0.07	0.32 ± 0.01	0.07 ± 0.09	0.26 ± 0.20	0.40 ± 0.20
20:2 (n-6)	0.79 ± 0.01	0.36 ± 0.01	1.31 ± 0.03	0.98 ± 0.06	0.67 ± 0.05	1.25 ± 0.25
20:3 (n-6)	0.52 ± 0.07	1.42 ± 0.28	0.63 ± 0.01	0.33 ± 0.05	0.91 ± 0.32	2.44 ± 2.29
20:4 (n-6)	2.48 ± 0.06	0.13 ± 0.01	1.15 ± 0.02	11.55 ± 0.73	6.40 ± 0.26	3.01 ± 2.21
22:4 (n-6)	1.88 ± 0.12	0.31 ± 0.02	1.74 ± 0.03	7.94 ± 0.61	0.41 ± 0.03	4.04 ± 2.01
22:5 (n-6)	0.15 ± 0.04	0.20 ± 0.02	0.22 ± 0.04	0.11 ± 0.03	0.05 ± 0.00	0.04 ± 0.09
18:3 (n-3)	1.69 ± 0.05	2.93 ± 0.15	0.34 ± 0.00	0.10 ± 0.04	0.62 ± 0.36	0.21 ± 0.19
18:4 (n-3)	1.63 ± 0.04	2.04 ± 0.11	1.30 ± 0.05	0.03 ± 0.04	0.99 ± 0.33	0.04 ± 0.05
20:3 (n-3)	0.43 ± 0.01	0.35 ± 0.02	0.51 ± 0.01	0.57 ± 0.03	0.17 ± 0.01	0.00 ± 0.00
20:4 (n-3)	1.56 ± 0.02	1.96 ± 0.02	1.34 ± 0.04	0.20 ± 0.02	0.93 ± 0.17	0.00 ± 0.00
20:5 (n-3)	7.15 ± 0.14	1.80 ± 0.10	7.55 ± 0.21	12.92 ± 1.33	26.15 ± 1.18	1.46 ± 1.67
21:5 (n-3)	0.38 ± 0.17	0.54 ± 0.22	0.32 ± 0.01	0.00 ± 0.00	0.41 ± 0.12	0.67 ± 0.60
22:5 (n-3)	3.49 ± 0.11	2.20 ± 0.04	2.89 ± 0.04	8.71 ± 0.50	3.13 ± 0.13	2.56 ± 2.02
22:6 (n-3)	3.77 ± 0.08	3.16 ± 0.02	3.94 ± 0.08	3.32 ± 0.31	3.34 ± 0.18	0.55 ± 0.73
∑ PUFA	**28.34 ± 0.40**	**21.30 ± 0.38**	**23.81 ± 0.47**	**46.91 ± 2.42**	**44.77 ± 1.56**	**17.26 ± 7.22**
∑ PUFA (n-6)	**7.40 ± 0.17**	**5.07 ± 0.31**	**5.36 ± 0.05**	**20.98 ± 0.49**	**8.69 ± 0.22**	**11.18 ± 3.24**
∑ PUFA (n-3)	**20.11 ± 0.37**	**14.98 ± 0.37**	**18.19 ± 0.41**	**25.86 ± 2.10**	**35.74 ± 1.49**	**5.49 ± 4.30**
(n-6)/(n-3)	**0.37 ± 0.01**	**0.34 ± 0.03**	**0.29 ± 0.00**	**0.81 ± 0.05**	**0.24 ± 0.01**	**4.53 ± 5.64**
Lipid classe distribution (%)	53.17 ± 1.00	30.29 ± 0.47	11.03 ± 0.92	5.91 ± 0.26	0.49 ± 0.08	

Table A12 Fatty alchol distribution in total lipid (TL) and wax ester fraction (WE) of coral (M27 C1-S6) (Fatty alcohols % relative to total fatty alcohols). Method validation with 5 parallells. (mean±SD).

	TL	WE
14:0-A	1.41 ± 0.09	1.33 ± 0.07
16:0-A	9.88 ± 0.52	9.70 ± 0.24
18:0-A	1.12 ± 0.15	1.24 ± 0.13
20:0-A	0.25 ± 0.02	0.59 ± 0.25
16:1(n-7)-A	2.11 ± 0.12	2.06 ± 0.07
16:1(n-5)-A	0.23 ± 0.09	0.27 ± 0.02
18:1(n-9)-A	3.63 ± 0.16	3.71 ± 0.10
18:1(n-7)-A	1.32 ± 0.19	1.18 ± 0.05
18:1(n-5)-A	0.62 ± 0.08	0.67 ± 0.02
∑20:1-A*	26.87 ± 1.02	26.69 ± 0.59
∑22:1-A*	45.42 ± 1.49	46.19 ± 0.62
24:1-A	3.89 ± 0.15	3.95 ± 0.24
18:2(n-6)-A	1.17 ± 0.07	1.06 ± 0.04
18:3 (n-3)-A	2.09 ± 1.54	1.35 ± 0.14

*∑20:1-A is dominated by 20:1 (n-9)-A, ∑22:1-A is dominated by 22:1 (n-11)-A

Table A13 Identification of fatty acids, fatty alcohols (marked with green), dimethylacetal (DMA), methyl alkenyl ether (MAE) (marked with blue) and sterols from coral samples. The peaks are identified by GC-MS. Corresponding chromatograms are given in figure A1-A5. Retention time index (ECL-values) are calculated relative to the saturated FA (marked with bold letters)

Peak	Identitet	RT (min)	ECL-value	M+	Base peak	plasmalogen M-31 eller 32	PUFA Beregnet omega	Beregnet alpha
1	**14:0**	**10.89**	**14.00**	**242**	**74**			
2	isopren 16:0	11.03	14.06	270	87			
3	iso 15:0	11.92	14.49	256	74			
4	**15:0**	**12.94**	**15.00**	**256**	**74**			
5	14:0-ALK	14.29	15.57		55			
6	16:0 DMA	14.53	15.67	284	75	255		
7	Pristan methyl ester no1	14.76	15.77	312	88			
8	Pristan methyl ester no2	14.83	15.79	312	88			
9	**16:0**	**15.35**	**16.00**	**270**	**74**			
10	DMA	15.79	16.19		75	269		
11	16:1 (n-9)	15.79	16.19	268	55			
12	16:1 (n-7)	15.92	16.24	268	55			
13	MAE?	16.09	16.30		71			
14	DMA	16.18	16.33		75	236		
15	16:1 (n-5)	16.22	16.35	268	55			
16	18:0 MAE	16.38	16.42	282	71	250		
17	iso 17:0	16.61	16.51	284	74			
18	15:0-Alk	16.78	16.57	228	55			
19	DMA?Phtalate	16.99	16.66	194	163			
20	antiso 17:0	16.99	16.66	284	74			
21	**17:0**	**17.86**	**17.00**	**284**	**74**			
22	DMA	17.94	17.02		75			
23	17:1 (n-9)	18.42	17.20	282	55			
24	16:0-Alk	19.46	17.58		55			
25	18:0 DMA	19.66	17.65	283	75	252		
26	16:1-Alk	20.21	17.85		55			
27	**18:0**	**20.60**	**18.00**	**298**	**74**			
28	DMA 18:1 (n-9)	20.68	18.02		71/75			
29	18:1 (n-11)	21.02	18.14	296	55			
30	18:1 (n-9)	21.10	18.17	296	55			
31	18:1 (n-7)	21.28	18.23	296	55			
32	18:1 (n-5)	21.66	18.37	296	55			
33	18:2 (n-6)	22.33	18.60	294	67			

Table A13 continued

Peak	Identitet	RT (min)	ECL-value	M+	Base peak	plasmalogen M-31 eller 32	PUFA Beregnet omega	Beregnet alpha
34	**19:0**	**23.44**	**19.00**	**312**	**74**			
35	18:3 (n-3)	24.12	19.23	292	79		108	236
36	18:4 (n-3)	24.95	19.53	290	79		108	194
37	18:0-Alk	25.08	19.58		82			
38	18:4 (n-1)	25.29	19.65		79			
39	18:1 (n-9)-Alk	25.68	19.79		82			
40	18:1(n-7)-Alk	25.86	19.86		82			
41	18:1(n-5)-Alk	26.19	19.97		82			
42	**20:0**	**26.24**	**20.00**	**326**	**74**			
43	20:1 (n-11)	26.61	20.12	324	55			
44	20:1 (n-9)	26.78	20.18	324	55			
45	20:1 (n-7)	26.98	20.26	324	55			
46	18:2 (n-6)-Alk	27.12	20.30		55			
47	20:1 (n-5)/+steroid	27.36	20.39	324	55	steroid topper	368	353
48	19:0 -Alk	27.93	20.59		83			
49	20:2 (n-6)	28.02	20.63	322	67			
50	20:3 (n-6)	28.75	20.88	320	79		150	222
51	18:3 (n-3) Alk	28.78	20.89		79			
52	**21:0**	**29.07**	**21.00**	**340**	**74**			
53	20:4 (n-6)	29.35	21.10	318	79		150	180
54	20:3 (n-3)	29.82	21.26	320	79		108	264
55	20:4 (n-3)	30.56	21.53	318	79		108	222
56	20:5 (n-3)	31.19	21.75	316	79		108	180
57	20:1 (n-9)-Alk	31.36	21.81		55			
58	**22:0**	**31.90**	**22.00**	**354**	**74**			
59	22:1 (n-11)	32.29	22.15	352	320/55			
60	22:1 (n-9)	32.40	22.19	352	320/55			
61	22:1 (n-7)	32.64	22.28	352	320/55			
62	21:5 (n-3)	34.14	22.82		79		108	194
63	22:4 (n-6)	35.01	23.15		79		150	208
64	22:1 (n-11)-Alk	36.73	23.79		55/82			
65	22:5 (n-3)	36.78	23.81	342	79		108	208
66	22:6 (n-3)	37.55	24.09	342	79		108	166
67	24:1 (n-11)	37.63	24.12	348	55			
68	24:1 (n-9)	37.82	24.20	348	55			
69	24:1-Alk	42.03	25.82	320	79			
70	Sterol 6, cholestdienes	43.44	25.98	368				
71	Sterol 7, cholestdienes	43.78	25.97	368	368			
72	Methoxycholesterol	47.69	27.00	400	368			
73	Cholesterol	60.15	25.58	386	386			

Figure A3 Chromatogram from GC-MS analysis of FAME from wax esters

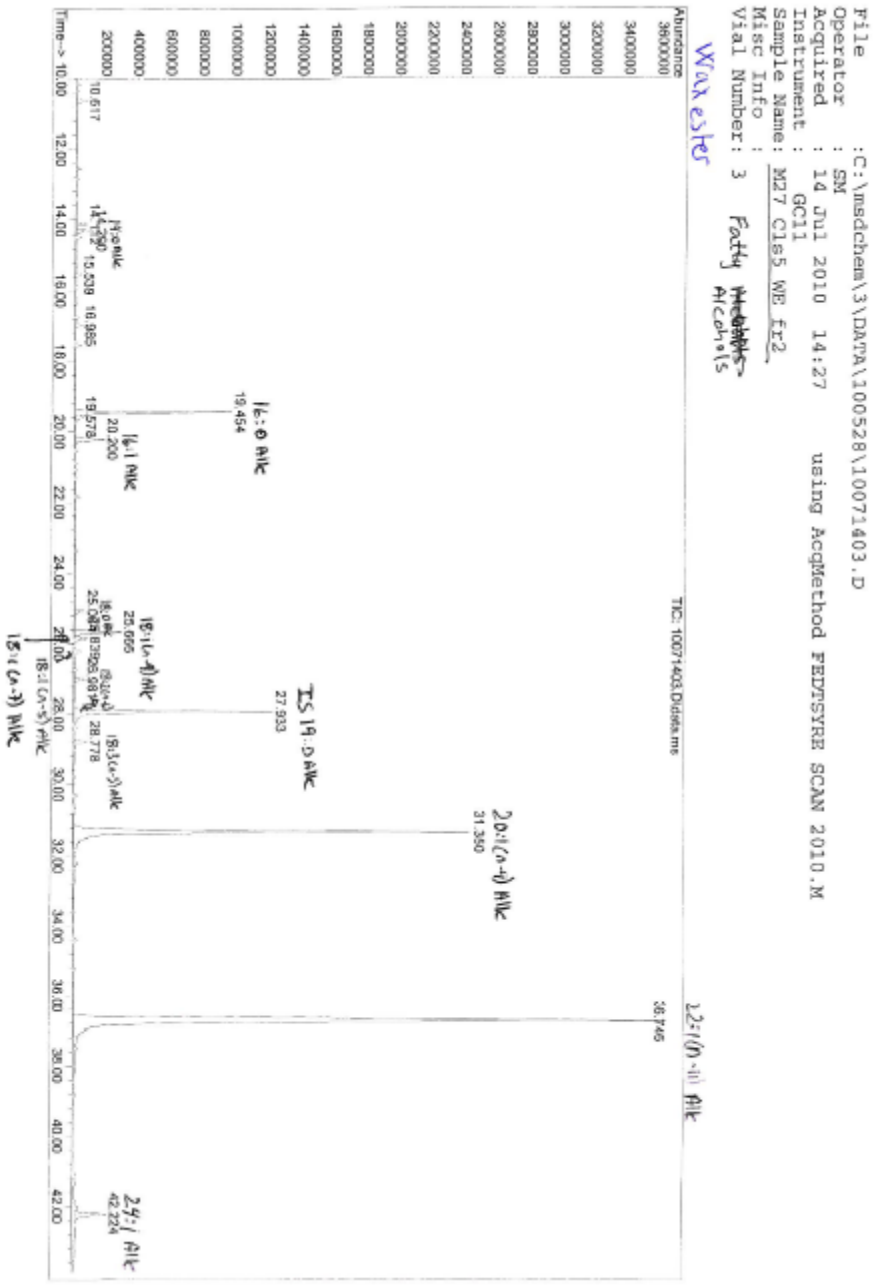

Figure A4 GC-MS analysis of Fatty alcohols from wax esters

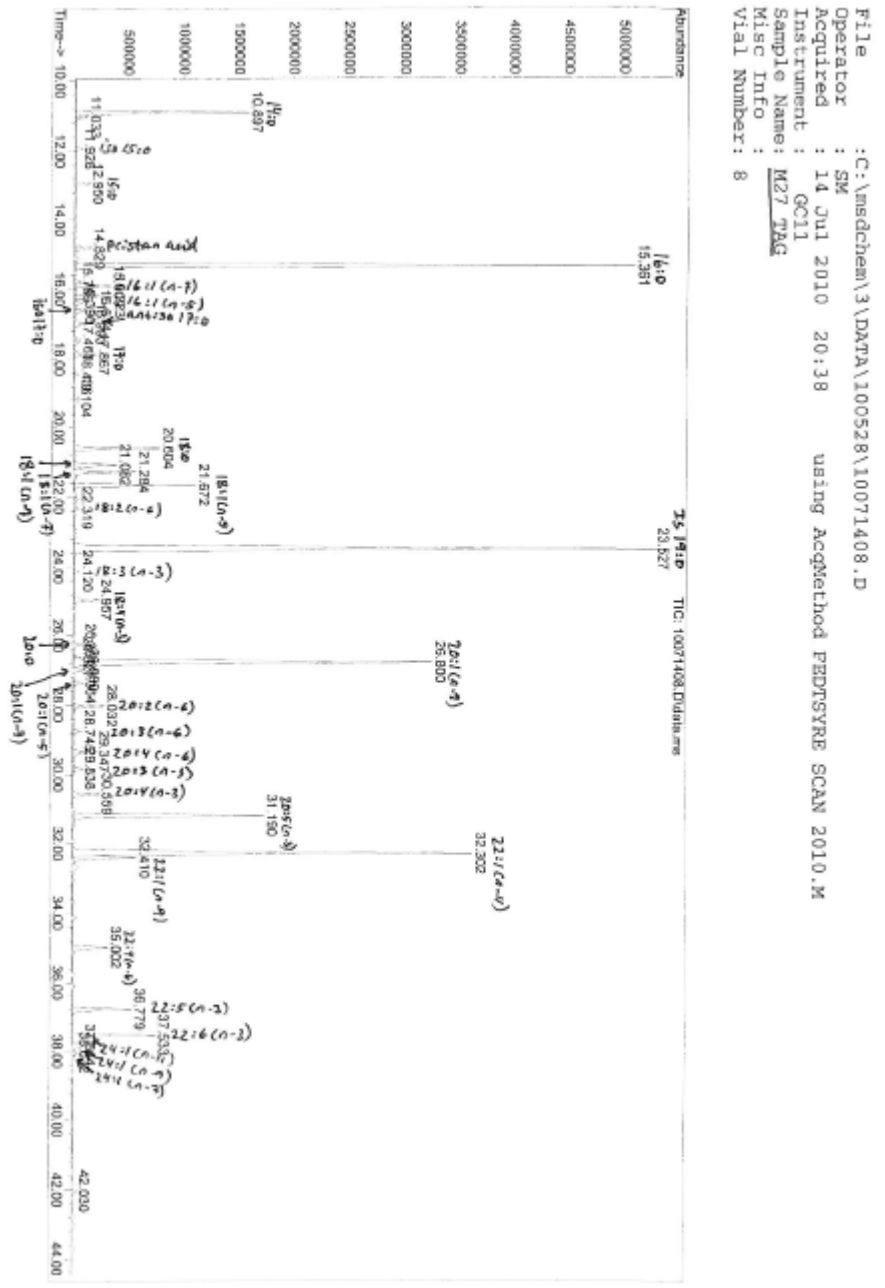

Figure A5 Chromatogram from GC-MS analysis of FAME from triacylglycerol

Figure A6 Chromatogram from GC-MS analysis of FAME from polar lipids

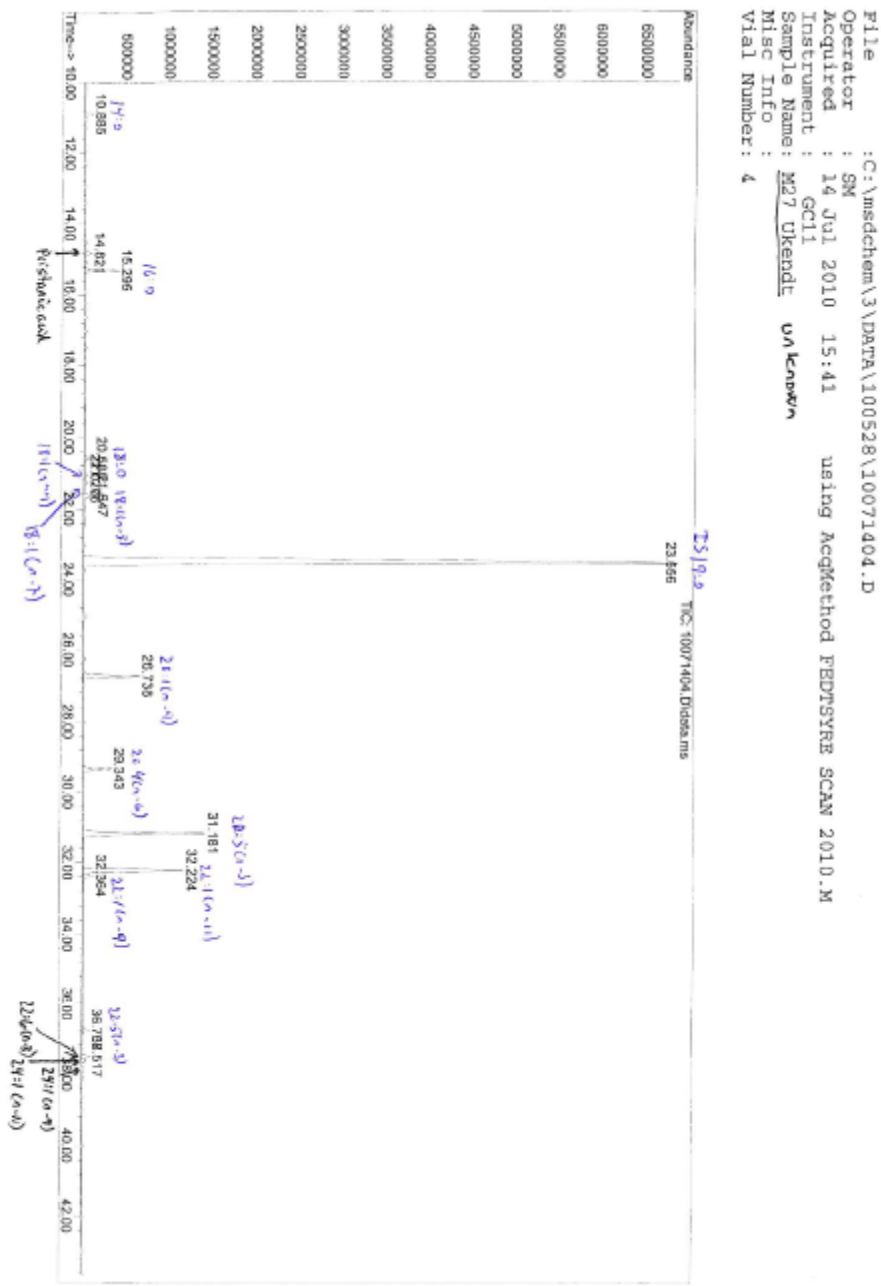

Figure A7 Chromatogram from GC-MS analysis of FAME from unknown fraction

Figure A8 Chromatogram from GC-MS analysis of FAME from free fatty acids

Figure A9 Chromatogram from GC-MS analysis of sterols from cholesterol

Appendix B Sediment trap analysis

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Overview

An array of sediment traps was deployed around the drill cutting discharge point for two periods of drilling during late 2009 and early 2010. During each drilling period, three traps were deployed. From November 9th to December 6th, to cover the initial period of drilling, traps were deployed to the locations indicated on **Figure 1**. **Figure 2** shows the locations of traps to cover the second period of drilling, in February 2010. Traps were deployed just prior to commencement of drilling operations, and retrieved after drilling was complete.

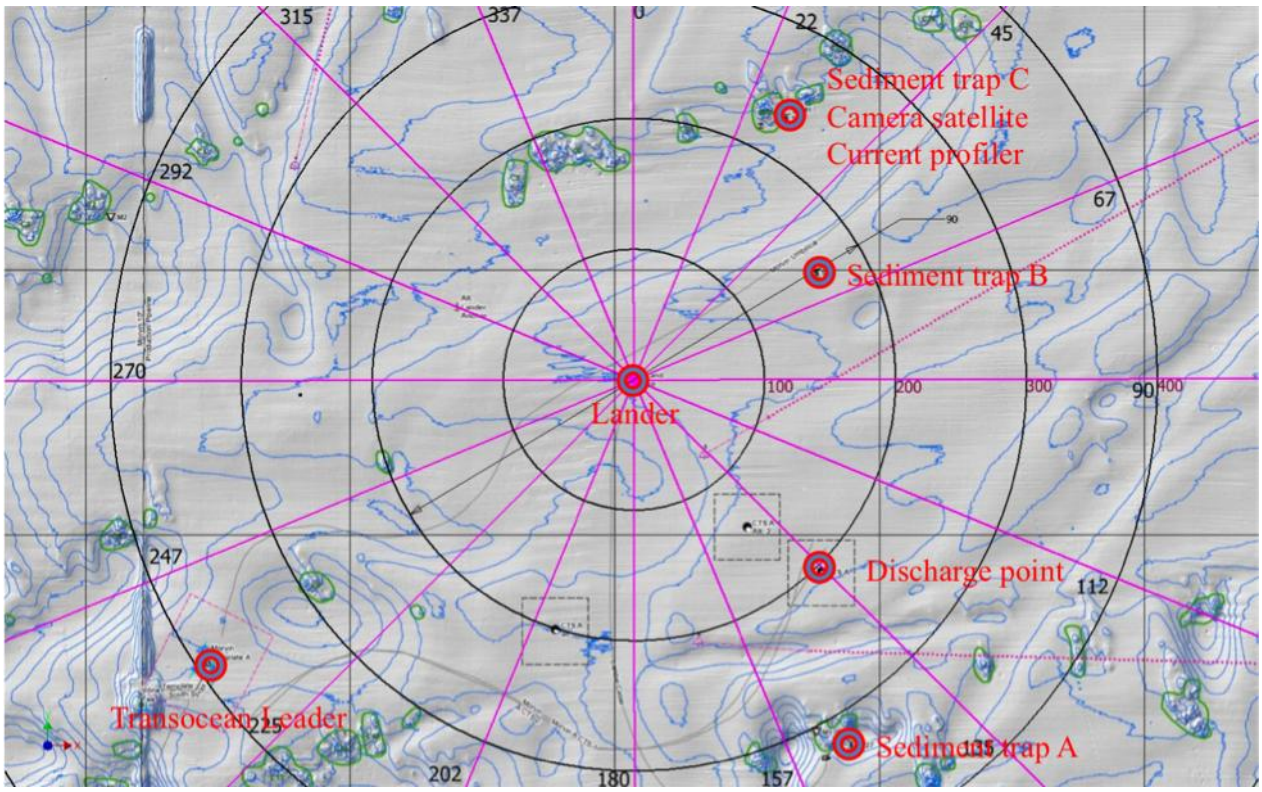

Figure 23. Sediment trap positions during initial drilling period (Nov-Dec 2009). Sediment trap A south and upstream of the discharge point and traps B and C north and downstream of the discharge point. Flow direction determined by current profiler, positioned close to sediment trap C.

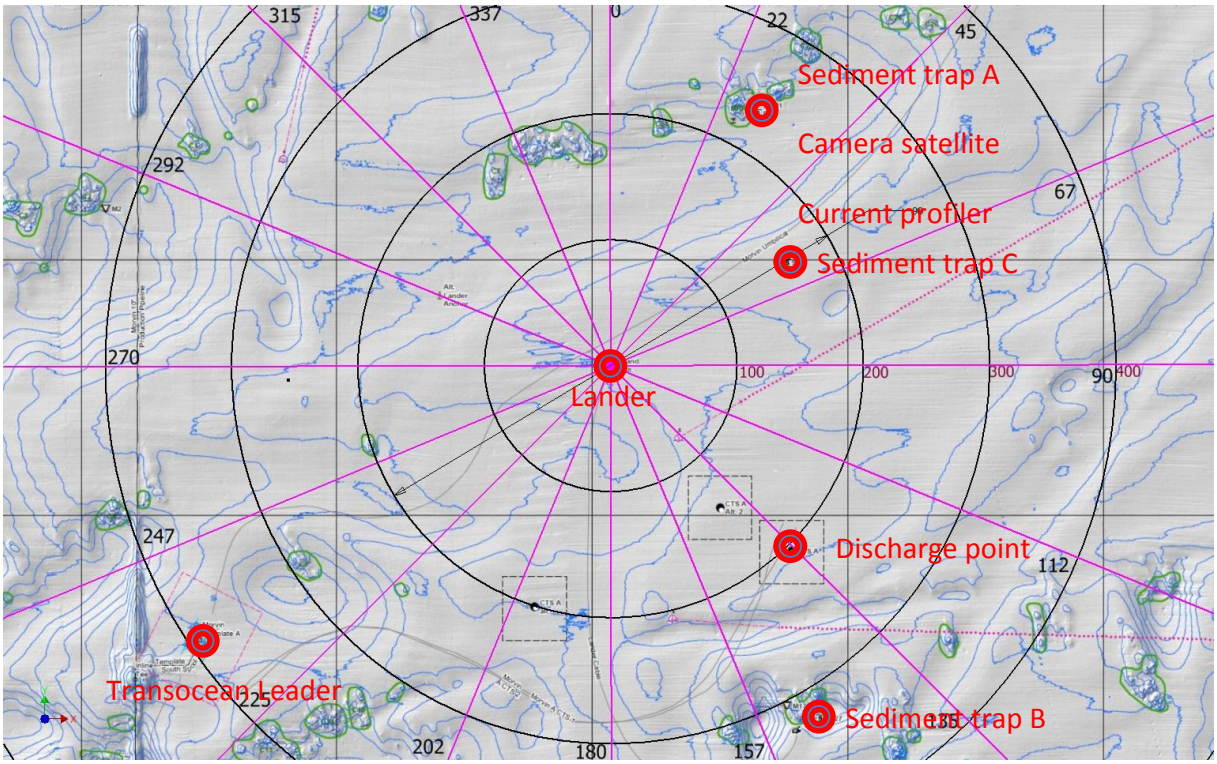

Figure 2. Sediment trap positions during second drilling period (Feb 2010). Current direction was not measured successfully during the second drilling period so it is uncertain as to which traps were exposed to drill cutting material.

Sediment trap design and deployment durations

The sediment traps used for both deployments were three identical K.U.M. K/MT 234 Sediment traps, each fitted with 21 400ml sediment trap bottles. All three traps were fitted with custom made electronics and programming devices constructed by IMR.

During each deployment, each sediment trap was programmed to rotate the sample bottle every 36 hours, to provide a maximum 31.5 day coverage period.

Methods

Material collected from the sediment traps analysed at Jacobs University Bremen. The following parameters were assessed for each sample (where sufficient quantity of material collected), and the methodologies detailed:

Current profiler:

The flow direction and flow velocity of the seawater in the bottom layers of the ocean was measured by current profilers during each drilling period (see **figures 1** and **2** for locations of current profilers).

Total sample mass:

Total sample mass for each sample was investigated as described in Bodungen et al. (1991).

Particulate organic material:

Carbon was measured in all samples, Nitrogen levels were measured whenever sample bottles contained >25mg dry weight of material.

Subsamples of the material in each bottle was filtered onto filter paper. Filters were acidified after the method of Pike and Moran (1997) to remove particulate inorganic carbon. After acidification, samples were dried in a 60°C oven and subsequently analysed in a EURO EA Elemental Analyser.

Amino acid analysis and degradation indices:

Amino acid analysis was carried out by reverse-phase HPLC using a slightly modified method of Cowie and Hedges (1992) and Van Mooy et al. (2002), as described in Garcia and Thomsen (2008).
From the amino acid composition, the degradation index (DI) was calculated after Dauwe et al. (1999). The ratios of aspartic acid (asp) and glutamic acid (glu) to their decompositional products β-alanine (bala) and γ-aminobutyric acid (gaba), as well as the joint percentage of bala and gaba (%[bala+gaba]) on all amino acids were calculated. These indicators have been widely used to verify variations in organic matter decomposition stage, both within the water column and in marine sediments (Lee and Cronin, 1982; Cowie and Hedges, 1994; Dauwe and Middelburg, 1998).

Amino acid analysis was carried out for all samples.

Physical parameters:

Particle size:

Particle size distributions were measured for all samples. Median particle sizes for all sample bottles were determined (by total particle volume in each sample). Size was determined using the LISST-ST instrument following the procedures described in Pedocchi and Garcia (2006).

Settling velocity:

It was not possible to measure settling velocities from material collected in bottles containing <25mg material, as the quantity of particles was insufficient. For samples where sufficient material was available, >100 particles were analysed to determine settling rates. This was carried out by filming material sinking through a settling cylinder and tracking particle movement over time with the ImageJ software application (Abramoff, et al. 2004).

Critical shear velocity:

This has been determined for all samples containing >25mg of material. The methodology used is described in Thomsen and gust (2000). Resuspension thresholds for both the fine material and coarser material was determined for each sediment trap bottle.

Metals and trace elements:

Bottles containing >25mg material were suitable for these analyses. Where sufficient material was present, Ba, Cd, Cr, Cu, Pb, Zn, Fe, K, Mg, Mn, Ni and Sr were measured.

An ICP-OES machine was used for these analyses, with reference HBVO2. For samples containing low concentrations of material, results were checked with voltammetry.

Results and discussion

The results of sample analyses differed between the Nov-Dec 2009 and Feb 2010 drilling periods, and because of this the results and discussion will be split into two sections, presenting findings from each drilling period in turn.

Initial drilling event : Nov-Dec 2009. Sediment trap deployment 1.

For monitoring the initial Nov-Dec 2009 drilling event, sediment trap A was deployed in position SF27 at 01:55 on November 11th, trap B in position SF2 at 01:42 on November 9th and trap C in position SF1 at 02:31 on November 9th (see **Figure 1** for trap locations in relation to drill cutting discharge point).

Flow conditions:

During the initial drilling event in Nov-Dec 2009, the current meter measured a seawater flow in a near uniform direction away from Trap A, in a northwesterly direction. Given that this sediment trap was on the far side of the drill cutting discharge point it could be used to represent a 'drilling control'.

Trap problems:

Although material was collected by each trap during the initial drilling event, there seems to have been some significant problems with the rotation of the sample bottles in the traps. These problems are outlined below and should be taken into consideration when reviewing the results of the sample analyses. Only the analyses of sample bottles which contained >10 mg dry weight of material will be discussed in detail in this report, under the assumption that the other bottles failed to open for any appreciable period of time.

Trap A

During the first drilling event , only one trap bottle, A21, collected >10 mg of material. Bottle A21 contained 27200 mg of material. It is highly unlikely that trap A accurately recorded the deposition of material over time during deployment 1. From the total organic carbon mass collected in the other trap bottles over 1.5 day periods, it would appear that trap A turned rapidly to bottle A21 following deployment, and collected all material from 11th Nov to 6th Dec into that one bottle. The amount of material in bottles A1-A20 is too low to reflect a reasonable flux of material on the Norwegian Margin at the time of deployment, and probably represents very minor diffusion of material into the bottles whilst the trap was in-situ on the seafloor, or possibly dust from the ship prior to deployment.

Trap B

Trap B did not rotate sediment trap bottles correctly. Every second and third bottles were missed (these containing <10mg material on recovery, as with trap A bottles A1-A20), the other bottles each contained in excess of 1000 mg of material (**Fig. 3**). 21 bottles were delivered for analysis from this trap, of which material >1000 mg was present in B1, B4, B7, B10, B13, B16 and B19. Whether the trap stopped collecting after bottle B21, or continued to rotate (with B1 again therefore being the next exposed bottle – three places from B19) or stopped after one rotation is uncertain. We assume in the presentation of results that each of these bottles represents a 1.5 day collection period, and therefore collection ended 10.5 days after trap deployment.

Trap C

Trap C did not rotate sediment trap bottles correctly either. Every second and third bottles were missed (these containing <10mg material, as with trap A bottles A1-A20). The other bottles from this trap each contained in excess of 1000 mg of material (**Fig. 3**). 19 bottles were delivered for analysis from this trap, of which material >1000 mg was present in C1, C4, C7, C10, C13, C16 and C19. Whether the trap stopped collecting after bottle C21, or continued to rotate (with C1 again therefore being the next exposed bottle – three places from C19) or stopped after one rotation is uncertain. We assume in the presentation of results that each of these bottles represents a 1.5 day collection period, and therefore collection ended 10.5 days after trap deployment.

Analysis of material collected in sediment traps deployed Nov-Dec 2009, (the initial drilling period).

Total sample mass – Nov-Dec 2009:

During the initial drilling period, the total sample mass collected in the sample bottles varied greatly, both between traps and over time (**Fig. 3**).

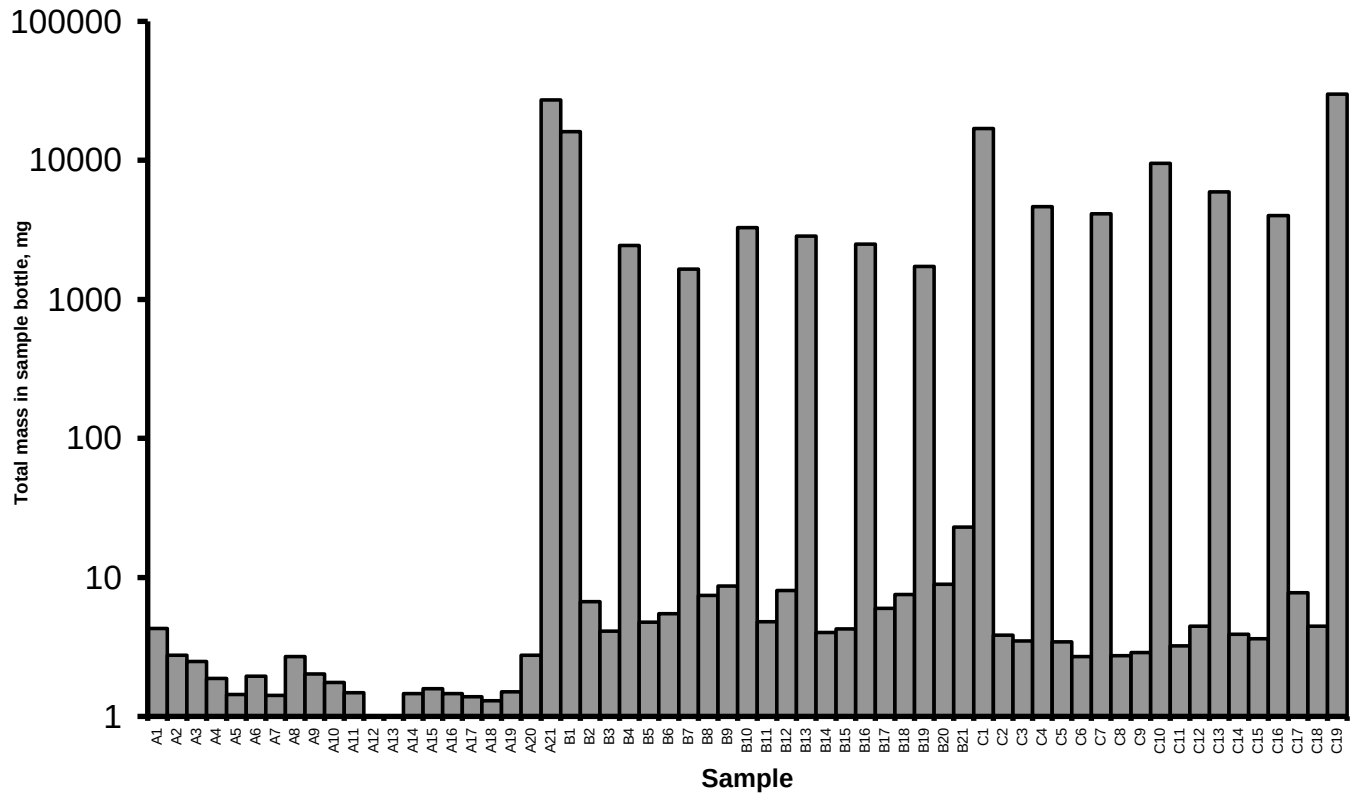

Figure 3 Total sample masses per bottle collected during initial drilling event (Nov-Dec 2009), plotted with a logarithmic scale

During the first drilling event, the first and last bottles of trap C (C1 and C19) the first bottle from trap B (B1) and bottle A21 from trap A have by far the most material within them (**Fig. 3**). This could reflect extra material deposited in the traps as a function of the deployment or recovery process. The absence of an elevated concentration of material in the final trap bottle from trap B may be because the final bottle was B21, which is not one of the bottles which collected > 10 mg material (i.e. not one of every third bottle, 1, 4, 7, 10, 13, 16 and 19).

Particulate organic material – Nov-Dec 2009:

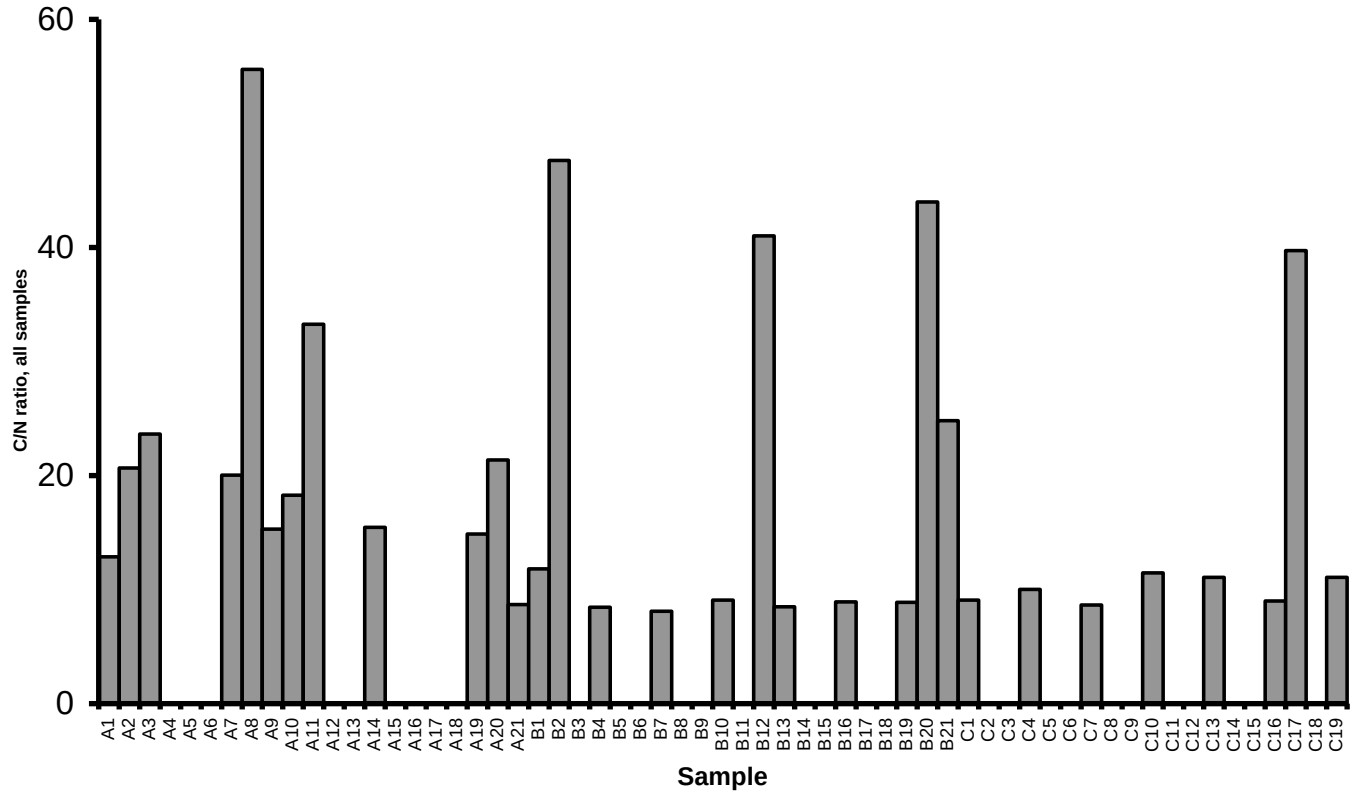

Figure 4 C/N ratios for all samples collected during initial drilling event (Nov-Dec 2009), where sufficient material was available for analysis.

Figure 4 shows that during the initial drilling event there was a difference in C/N ratios between all bottles in sediment trap A (except for bottle A21) and those from every 3rd bottle collected by traps B and C. This is a further indication that these trap bottles did not collect material from the water column, and the small volume of material within more likely represents some minor contaminant.

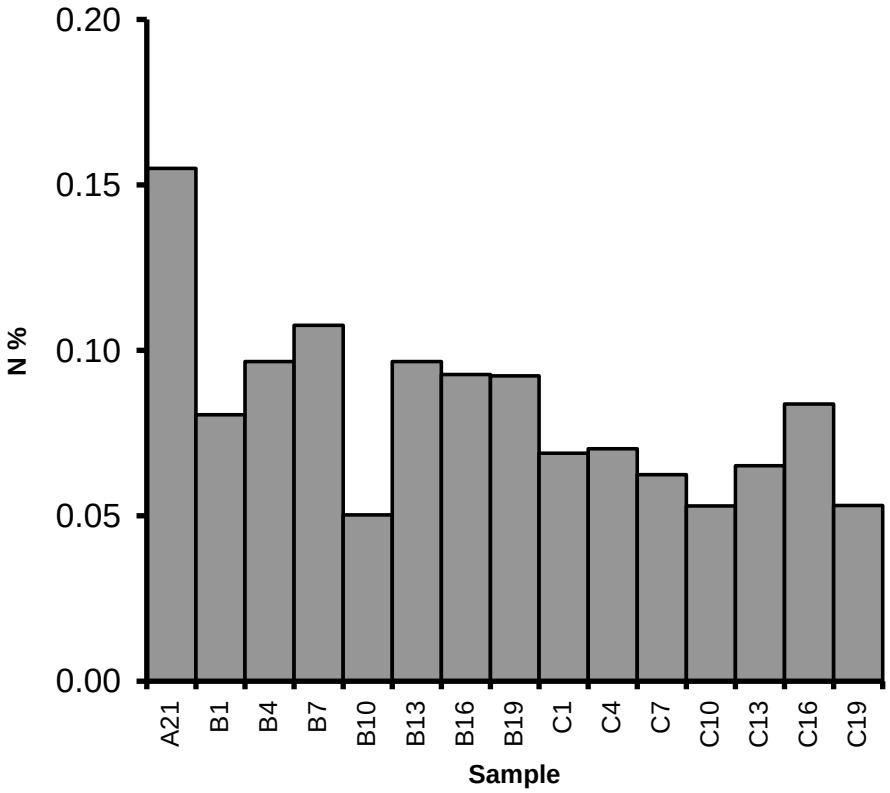

Figure 5 Nitrogen % for the samples containing >25mg of material collected during initial drilling event (Nov-Dec 2009).

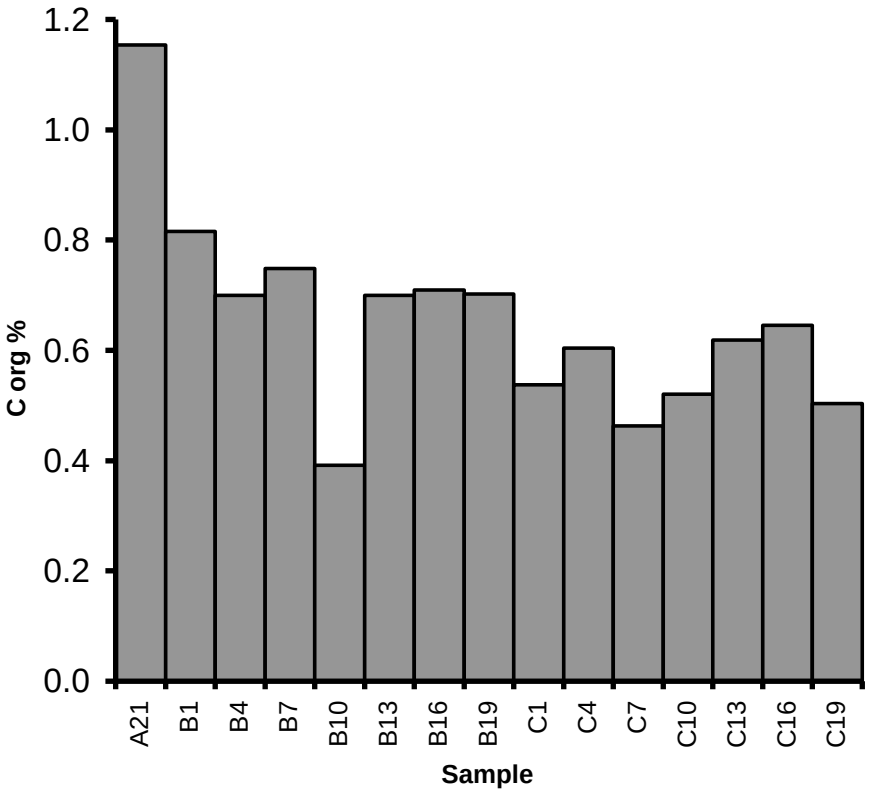

Figure 6 Carbon org % for the samples containing >25mg of material collected during initial drilling event (Nov-Dec 2009).

Site A which acts as reference site, contains highest percentage of organic carbon. This indicates that is has not been exposed to drill cuttings, or if there has been some exposure it is to a lesser degree than traps B and C, where lithogenic material has increased total mass and therefore decreased % Corg.

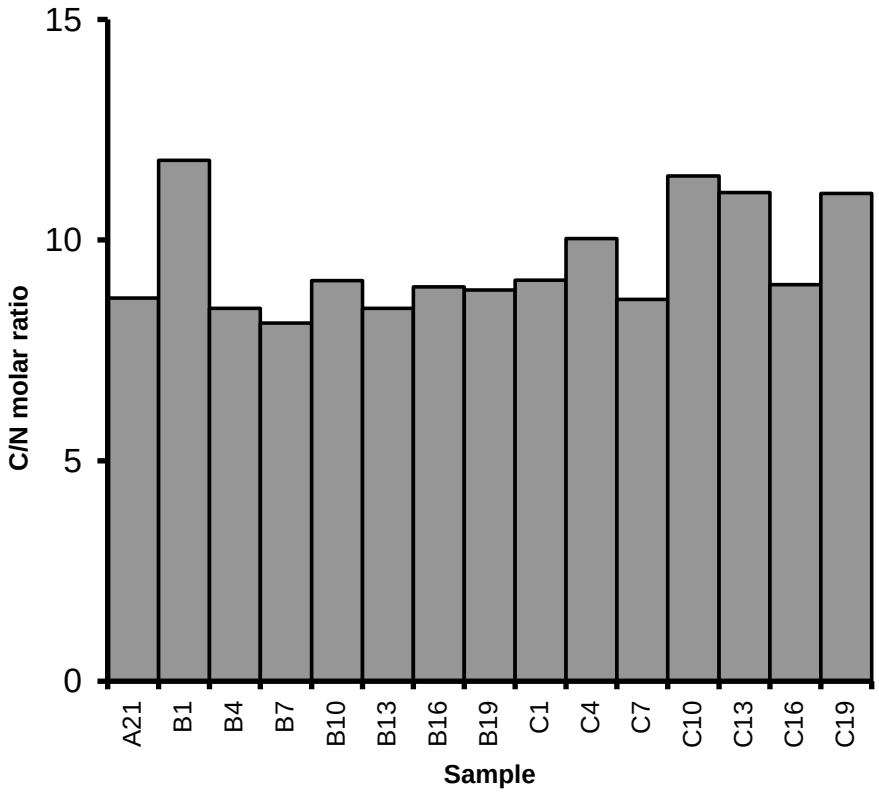

Figure 7 Carbon/Nitrogen molar ratio for the samples containing >25mg of material collected during initial drilling event (Nov-Dec 2009).

Relatively fresh organic material (C/N ~8) entered the trap bottles during the deployment period.

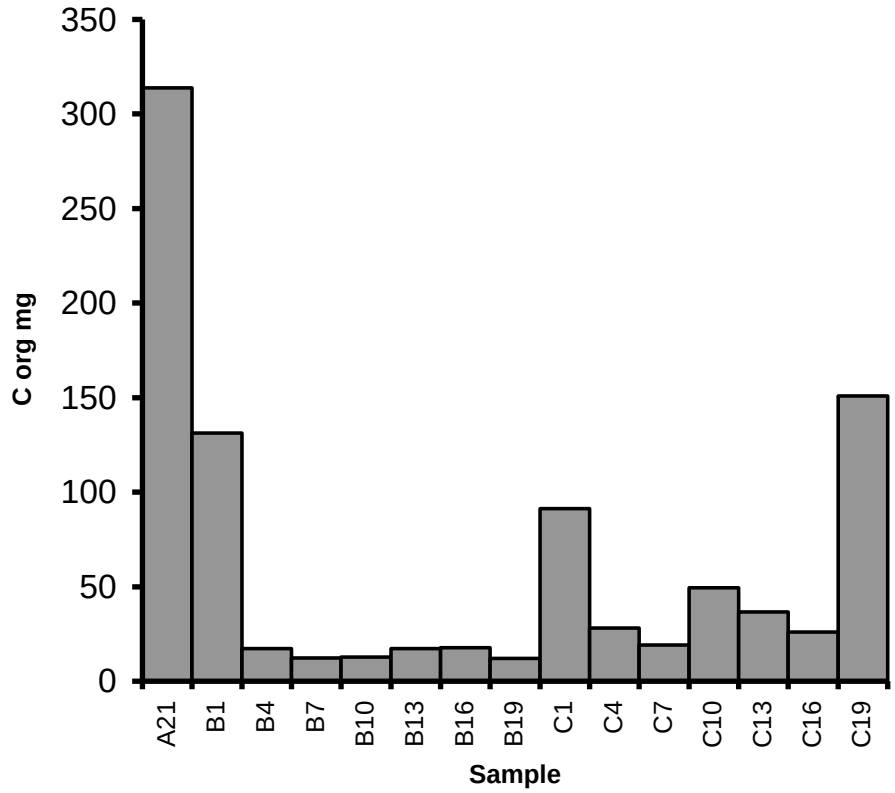

Figure 8 Carbon org mg for the samples containing >25mg of material collected during initial drilling event (Nov-Dec 2009).

The mass of carbon collected was far higher in bottle A21 than in any of the other bottles. Given the problems with trap rotation discussed above this is not a surprising observation, as this bottle appears to have been exposed and collecting material for the longest period. As this drilling event took place during winter, the water column contained only a low quantity of organic matter, hence the low C org mg concentrations observed in the other bottles.

Figs. 5 – 8 indicate that trap bottle A21 contained the most organic carbon, both by volume and carbon %.

Amino acid analysis and degradation indices – Nov-Dec 2009:

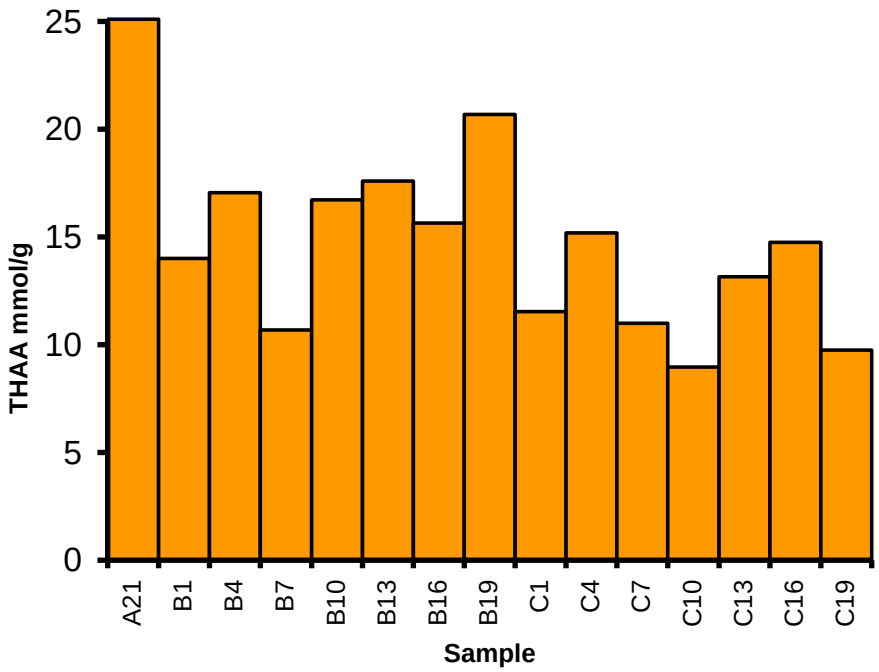

Figure 9 THAA mmol/g amino acid degradation index of material collected during initial drilling event (Nov-Dec 2009).

Concentrations of total hydrolizable amino acids (THAA) (**fig. 9**) ranged between 10 and 25 mmol/kg. Highest concentrations were found in samples A21, the trap which was located at the reference station. Highest THAA values at locations exposed to drill cuttings were found in bottle B19. The THAA concentrations and degradation index values (**Fig.10**) are within the range of values reported for more labile coastal and ocean margin settings (**DI between -1 [refractory] and +1 [labile, fresh])** (Dauwe et al., 1999). The degradation indices for samples B4, B7, B16, C4, C16 and C19 indicate periods when less labile organic matter entered the traps. As sampling took place during winter, this observation indicates that the organic material in the water column during times of drilling operations varied in composition and represent winter conditions**. The data do not indicate that drill cuttings have a negative impact on the degradation of organic material.**

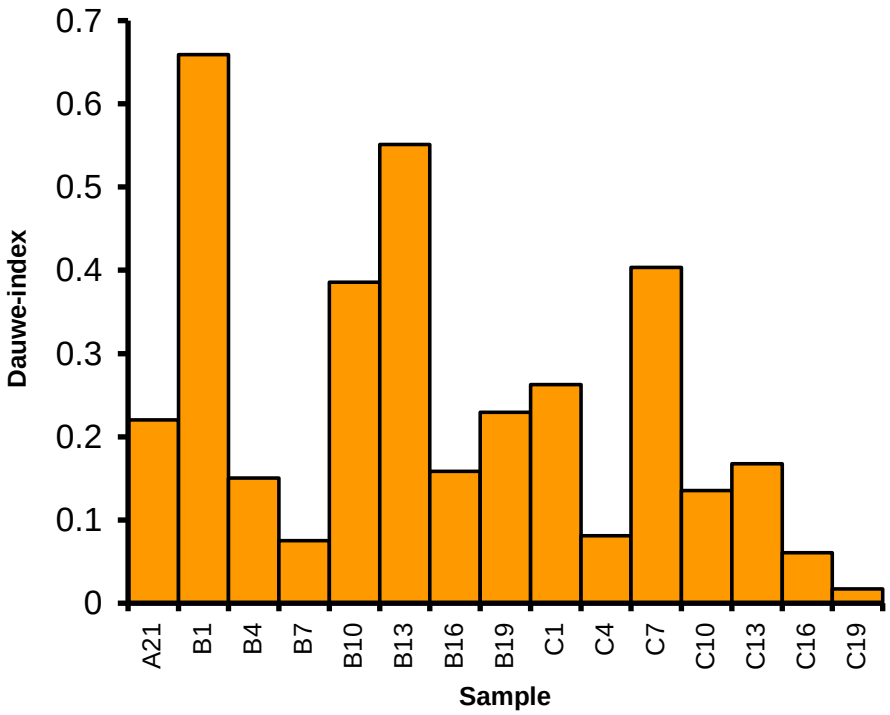

Figure 10 Dauwe amino acid degradation index of material collected during initial drilling event (Nov-Dec 2009).

Physical parameters – Nov-Dec 2009:

Particle size:

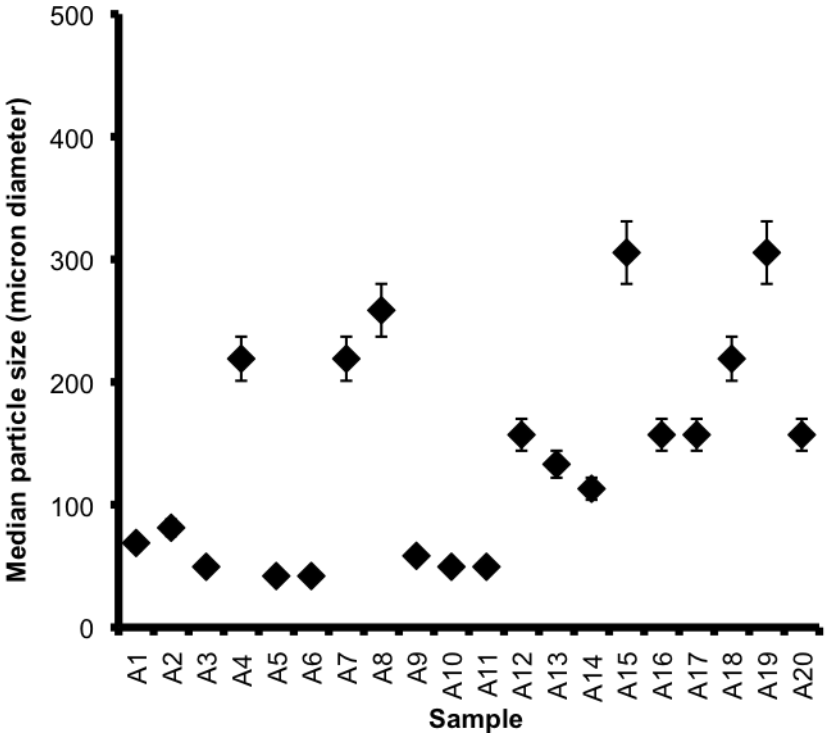

Figure 11 Median particle sizes in trap A of material collected during initial drilling event (Nov-Dec 2009).

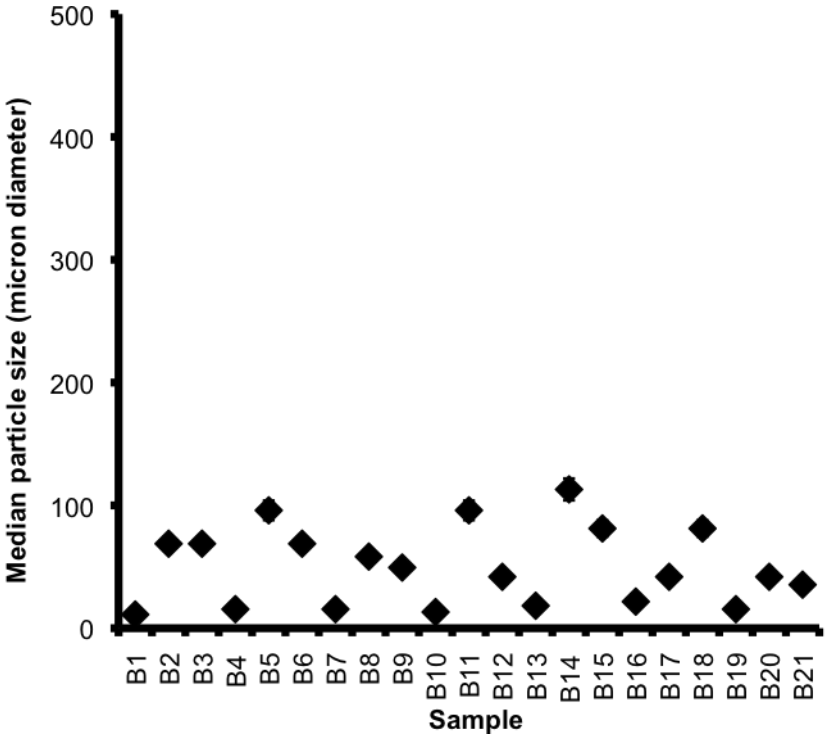

Figure 12 Median particle sizes in trap B collected during initial drilling event (Nov-Dec 2009).

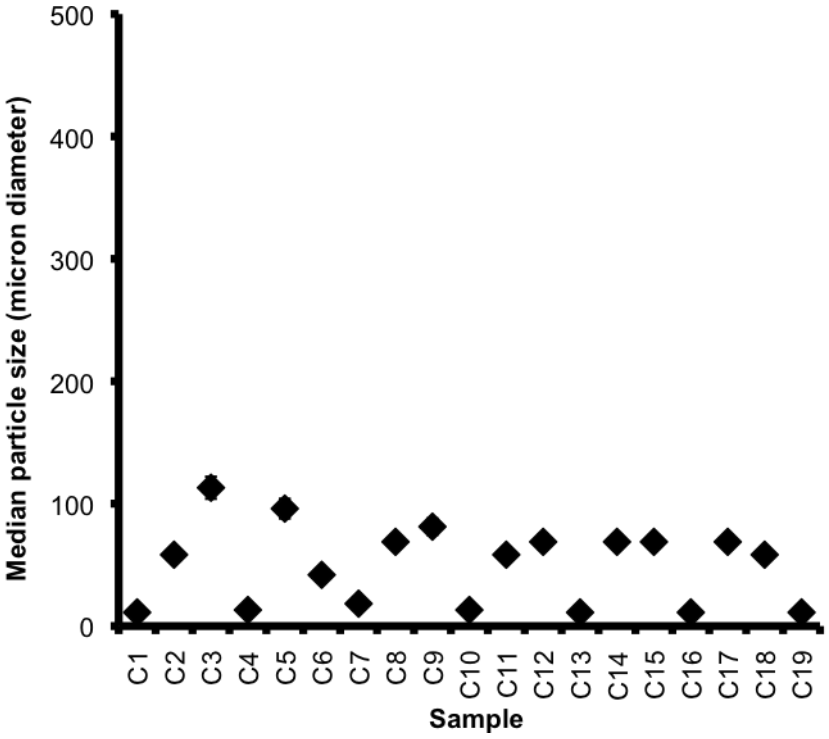

Figure 13 Median particle sizes in trap C collected during initial drilling event (Nov-Dec 2009).

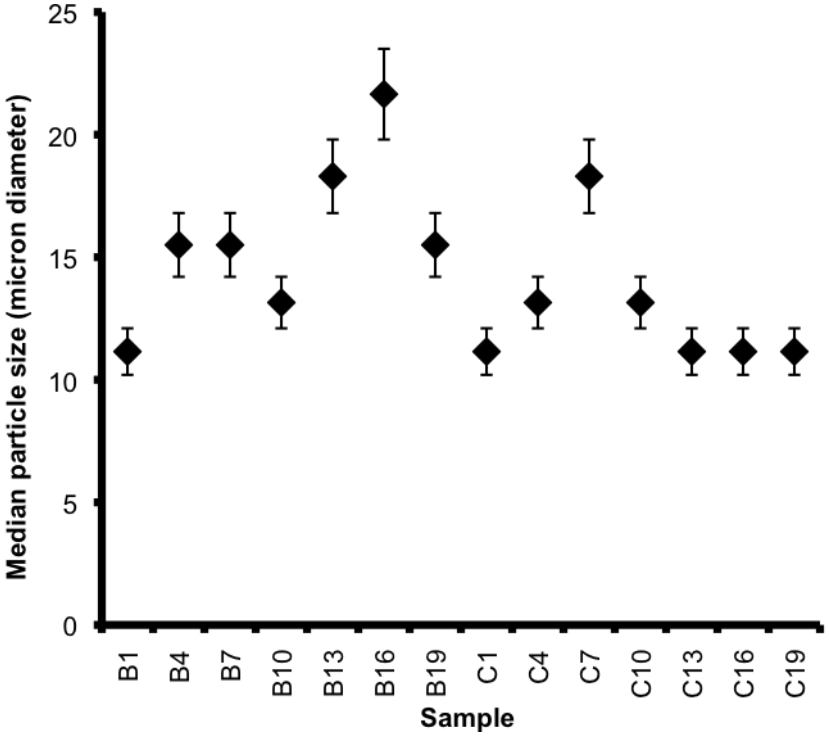

Figure 14 Median particle sizes for the samples containing >25mg of material collected during initial drilling event (Nov-Dec 2009). *These samples are considered as valid for further analyses since all other samples indicate trap malfunctioning (too little mass, see figure 3)*

Particle size distributions in figures 11 – 14 show two trends: Traps bottles which were not rotated into position correctly (those containing <10 mg total material, i.e. A1-A20, B2, B3, B5, B6, B8, B9, B11, B12, B14, B15, B17, B18, B20, B21, C2, C3, C5, C6, C8, C9, C11, C12, C14, C15 and C18 in **Figs. 11-13**) showed median particle diameters of 50 – 300 µm. This could indicate short exposure times for these particular trap bottles. During these short periods during which the trap rotated past these sparcely populated bottles, only the larger aggregated organic material with high settling velocities could enter the trap**,** while small particles with low settling velocties could not do so before the trap rotated the bottle away from the intake.

Those trap samples which result from correctly working 36 h opening times (trap samples containing >25 mg of material, i.e. B1, B4, B7, B10, B13, B16, B19, C1, C4, C7, C10, C13, C16, C19) show much smaller median diameters (**Fig. 14). These trap samples are therefore dominated by high numbers of finer drill cuttings, resulting in a general shift of the particle size spectrum towards smaller particles during periods of drilling.**

Critical shear velocities

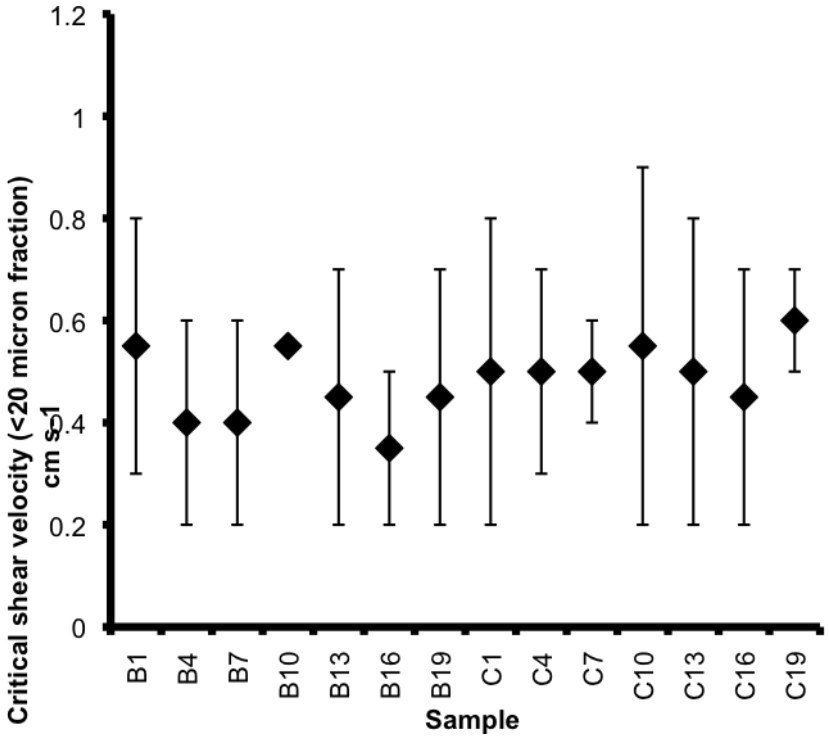

Figure 15 Critical shear velocity of <20 micron material for bedload transport collected during initial drilling event (Nov-Dec 2009).

Data on mean critical shear velocity show that bedload transport of the particles which entered the trap varied between 0.4 and 0.6 cm/s. This corresponds to free stream velocities of ≈ 8-10 cm s^{-1}. Under these flow conditions, the particles would roll along the seafloor.

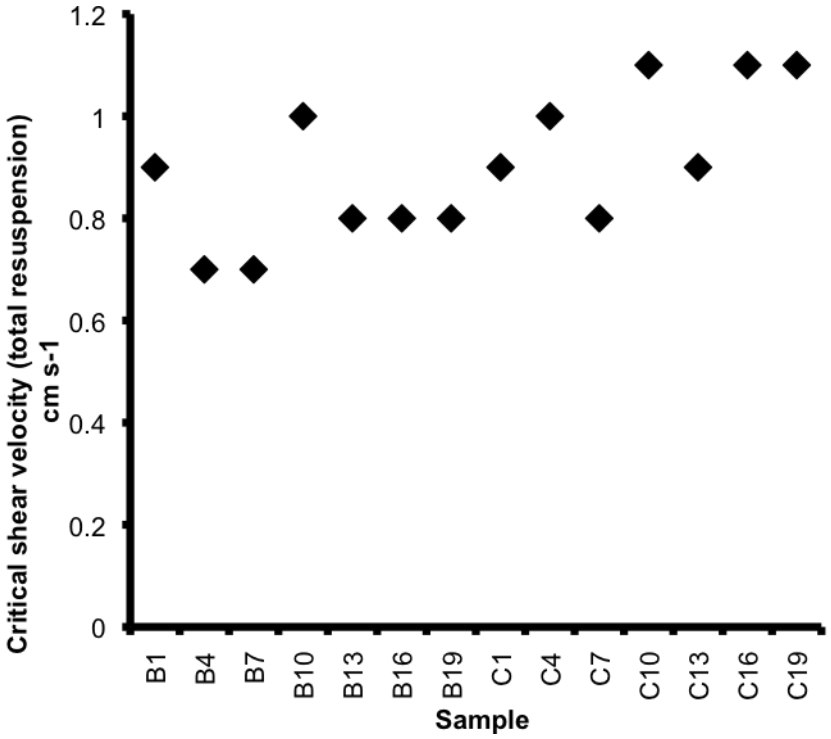

Figure 16 Critical shear velocity for full resuspension of material collected during initial drilling event (Nov-Dec 2009).

Data on mean critical shear velocity show that full resuspension of particles which entered traps B and C occurred between 0.7 and 1.2 cm s^{-1}.That corresponds to free stream velocities of ≈ 10 -20 cm s^{-1}

There is a trend of increasing shear velocities from station B to C. This indicates that with increasing distance from the drilling site, the particle composition changed. The further the station was away from the drilling site, the less easy resuspension of settling material would be. One explanation for this observation could be that biofilms were built up on the drill cuttings during their transport within the bottom boundary layer, resulting in less resuspendable particles.

CONCLUSION: During drilling operations the mix of drill cuttings and organic material settling to the seafloor would be resuspended under flow velocities of 10 – 20 cm s^{-1}. This would indicate that under flow conditions often present at the study site the material would be readily resuspeded and dispersed in a downstream direction.

Settling velocities:

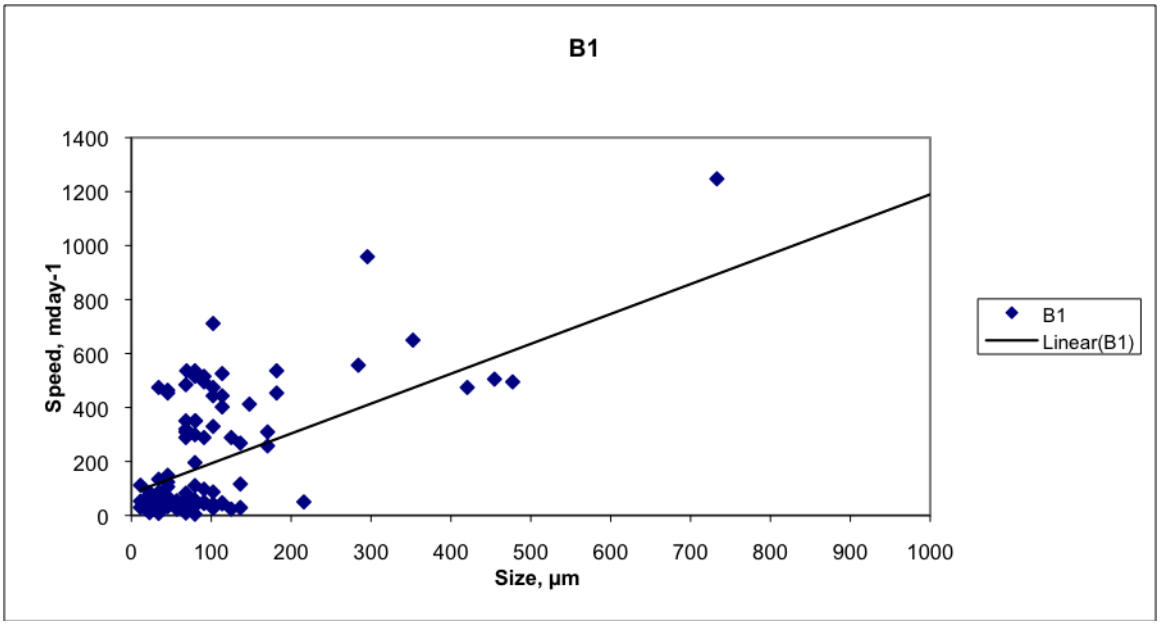

Figure 17 Settling velocity of material collected within bottle B1 during initial drilling event (Nov-Dec 2009).

Trap sample B1 a good example for the general trend observed: The majority of particles in that sample are small (**fig. 17**) and represent the drill cuttings. However a second class of particles with settling velocities of 300 to 1300 m day^{-1} are present. These represent most probably organic-mineral aggregates which settled out of the water column into the traps. Their number is not high enough to change the median particle size but they are a significant component of the vertical flux of particles at the study site.

Interestingly settling velocities and particle sizes of the aggregated fraction, which did not dominate the trap samples in number, increased from location B to C and over time from start to end of trap deployment. This can again be explained with a seabed process, in which the aggregated particle fraction undergoes several resuspension loops between locations of trap B and C. Each resuspension loop results in a compaction of the aggregates (and therefore excess density) which increases the particles settling velocity.

Regarding exposure and dispersion, this would mean that with increasing distance from the drilling site, the drill cuttings increasingly aggregate with the organic material, which also form biofilms. This results in an increase of critical shear velocity and settling velocity of the particles. For more details on this process see Thomsen 2002 and 2004.

Metals and trace elements – Nov-Dec 2009:

The following graphs show the ICP-OES measurements for the metals and trace metals analysed.

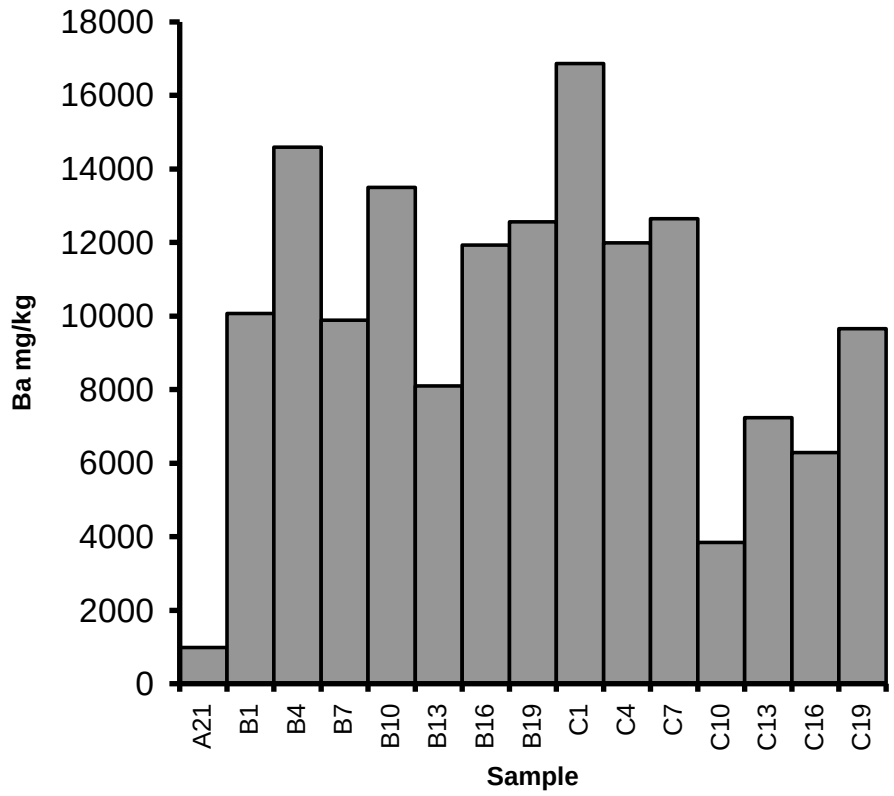

Figure 18 Barium concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Barium concentrations are far higher (by at least an order of magnitude) in trap bottles from traps B and C than in bottle A21. The quantity in A21 however, (981.4 mg/kg) is higher than the range of 83-287 mg/kg background concentration measured in the region (Akvaplan-niva Report no. 4664-03, 2010). This could indicate that some drill cutting material reached the trap, perhaps by transport within the water column following discharge and prior to settling. Although the drill cuttings were released at the seabed, the turbidity at release and small size of the particles may have resulted in a small percentage of the material being resuspended into water masses overlying those measured by the current profiler – and potentially be transported in another direction (i.e. toward trap A) before again settling.

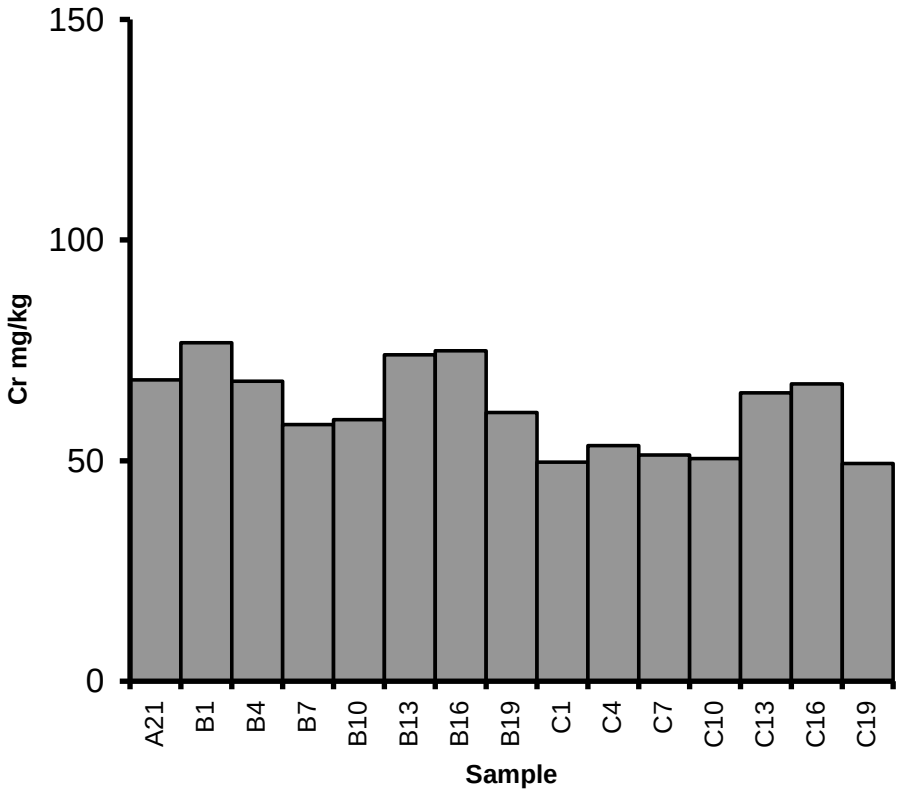

Figure 19 Chromium concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Chromium concentration did not vary with sediment trap. The levels are a little higher than those given in the recent Akvaplan-niva report (Report no. 4664-03, 2010) for the area (16.4 – 34.6 mg/kg), but below those of general marine sediments (~99.8- 112 mg/kg, Mess-3 reference material, NRCC).

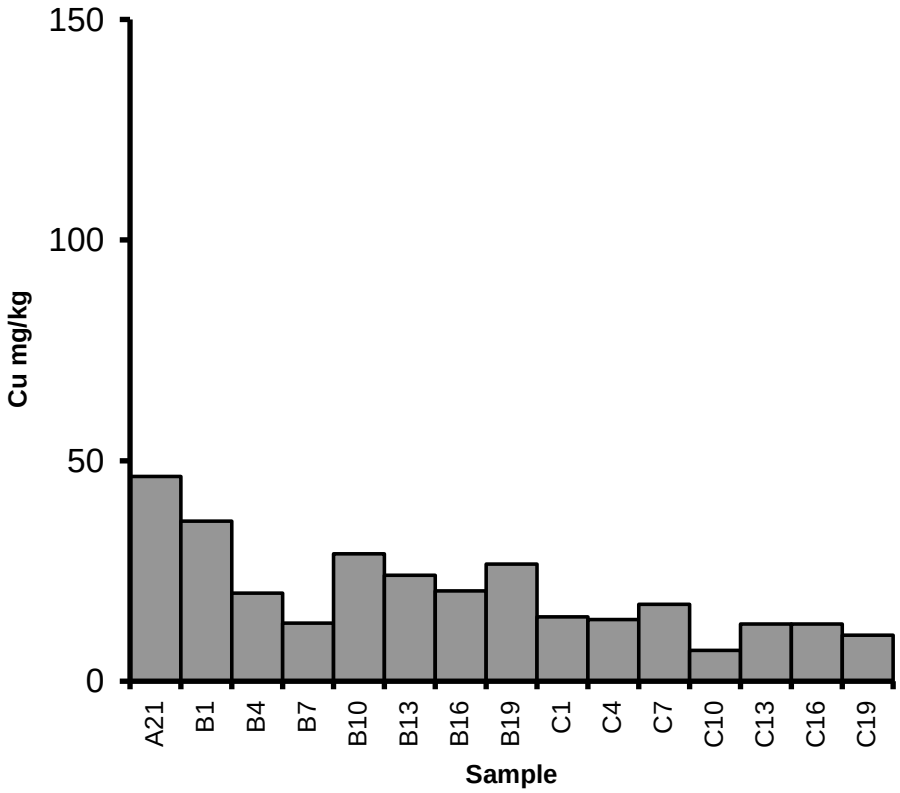

Figure 20 Copper concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Copper concentrations did not vary greatly between sediment trap bottles, with observed concentrations generally above background concentrations for the region detailed in the recent Akvaplan-niva report of 6.5 – 12.2 mg/kg (Report no. 4664-03, 2010).

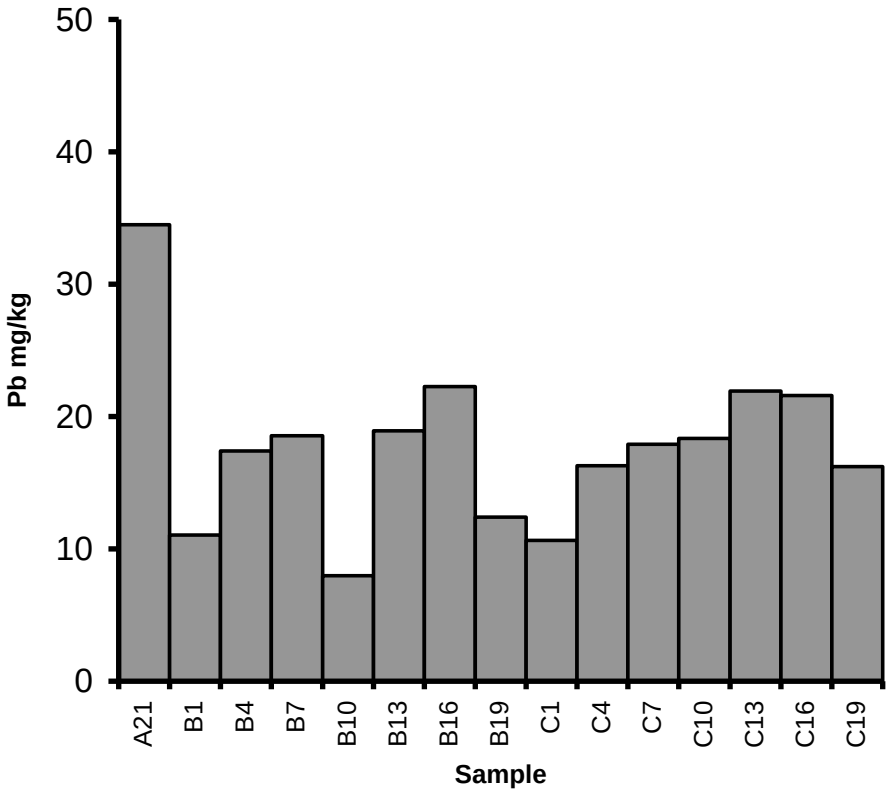

Figure 21 Lead concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Lead values were generally similar across sediment traps and samples, although slightly higher in bottle A21. Background levels given in the Akvaplan-niva report (Report no. 4664-03, 2010) of 13.9 – 20.9 mg/kg compare well with these observations.

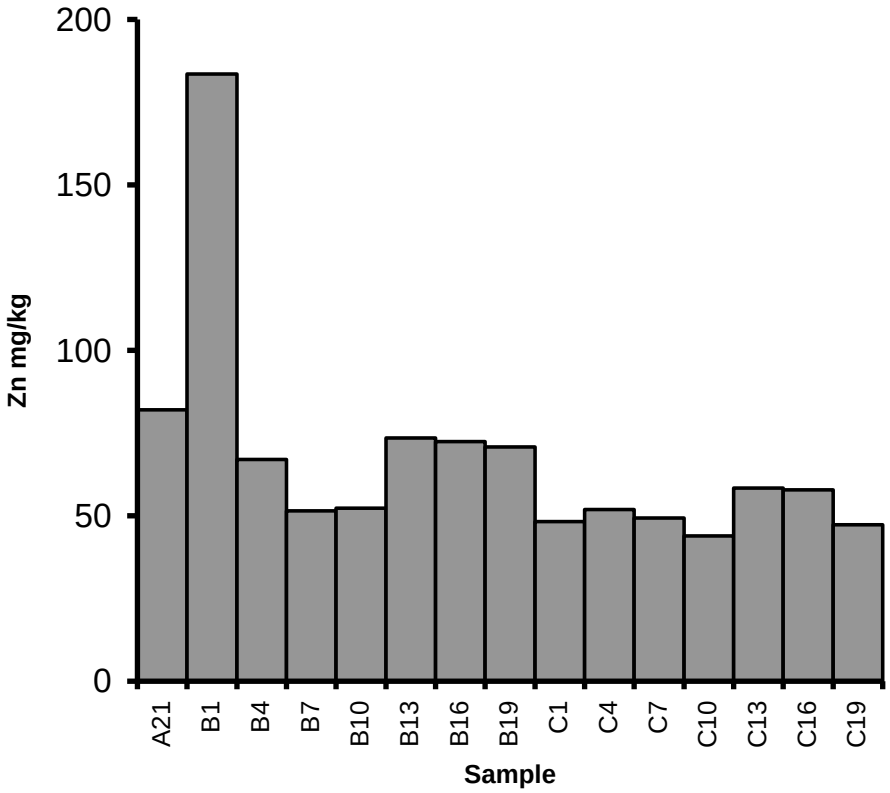

Figure 22 Zinc concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

A background zinc concentration of 40.7 – 90.0 mg/kg (Akvaplan-niva report no. 4664-03, 2010) for the region corresponds with the results from the majority of sediment traps. The elevated concentration observed in sediment trap B1 is unlikely to be related with the drilling operation, as Zn concentrations in sediments highly contaminated with drill cuttings (described in the chapter on core samples) was not observed to differ from this background range.

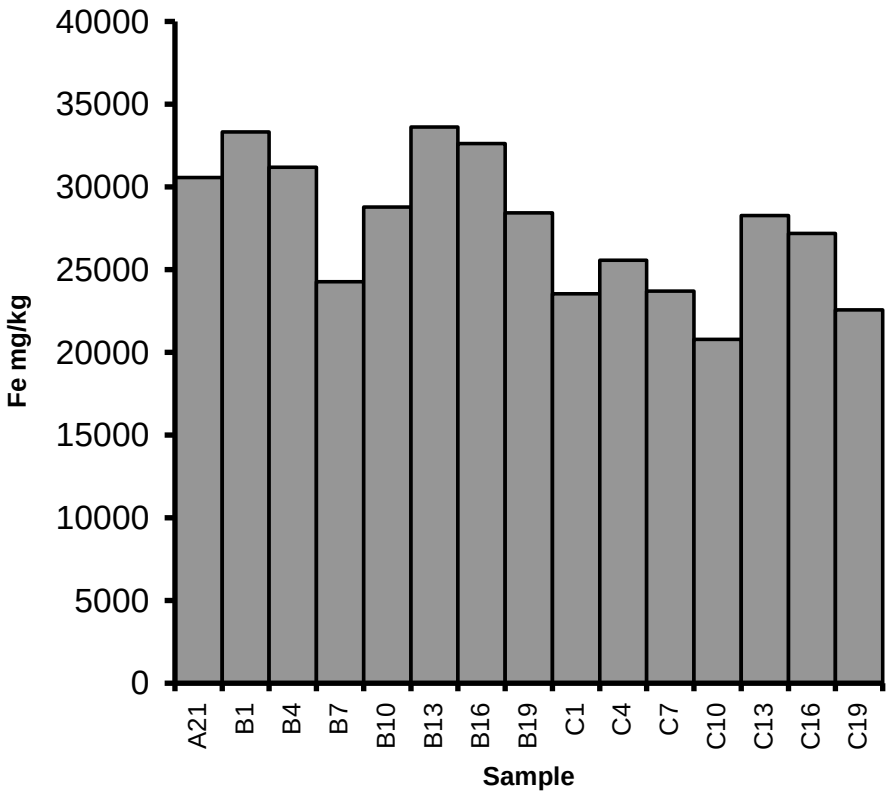

Figure 23 Ferrous metal concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

There was little variation in Fe concentration between samples, and all values are reasonable for marine sediments (MESS-3, NRCC).

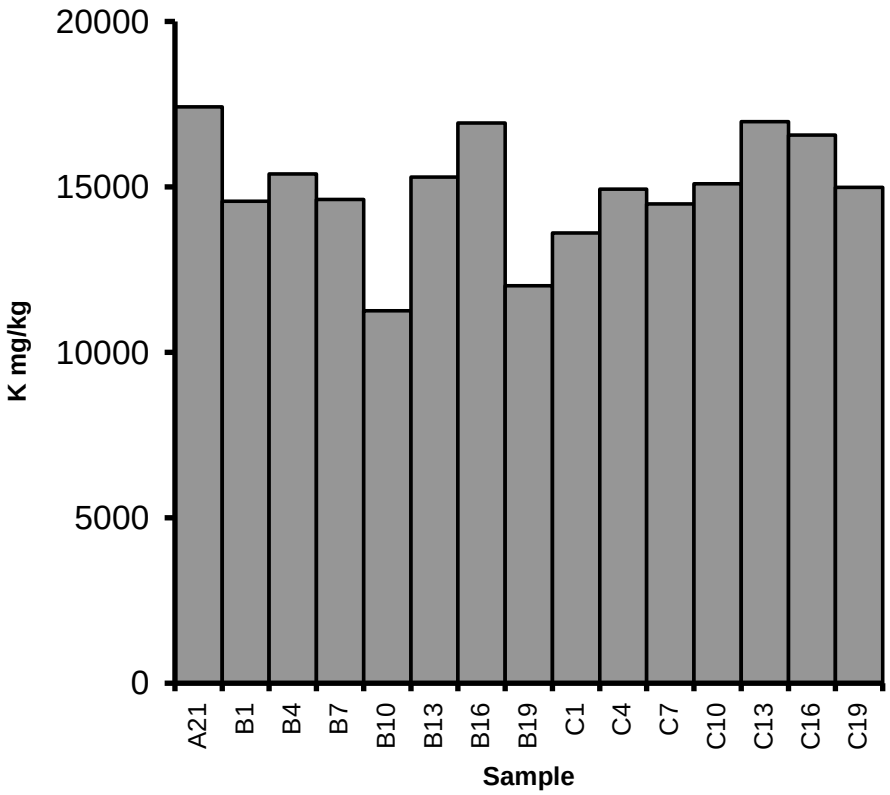

Figure 24 Potassium concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

There was little variation in K concentration between samples, and all values are reasonable for marine sediments (MESS-3, NRCC).

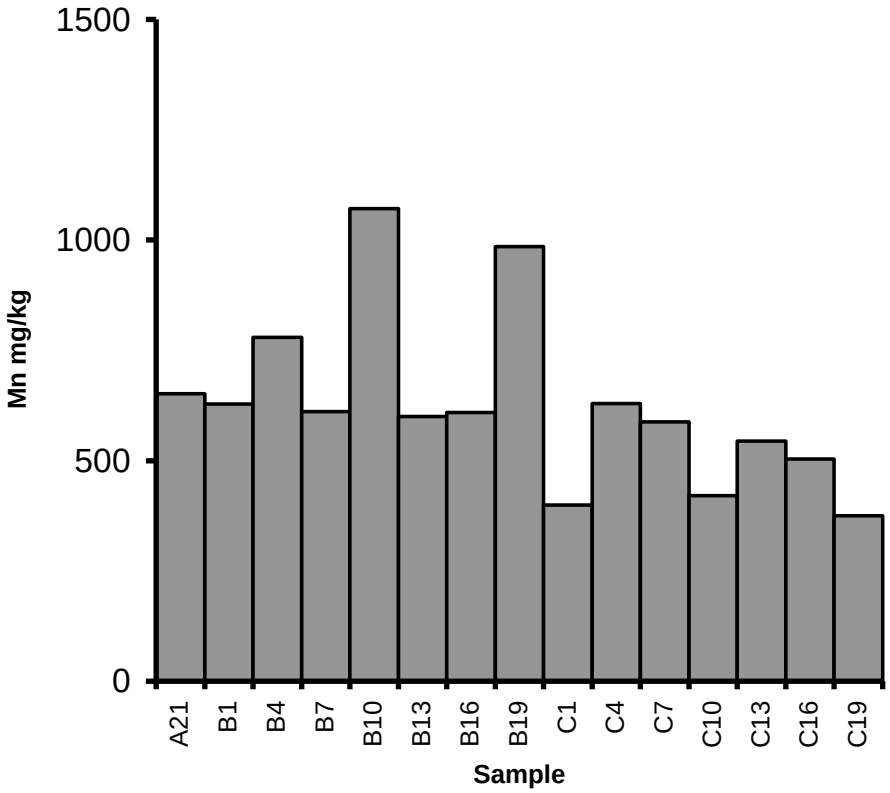

Figure 25 Manganese concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Concentrations of MN did not vary significantly from expected background levels (MESS-3, NRCC).

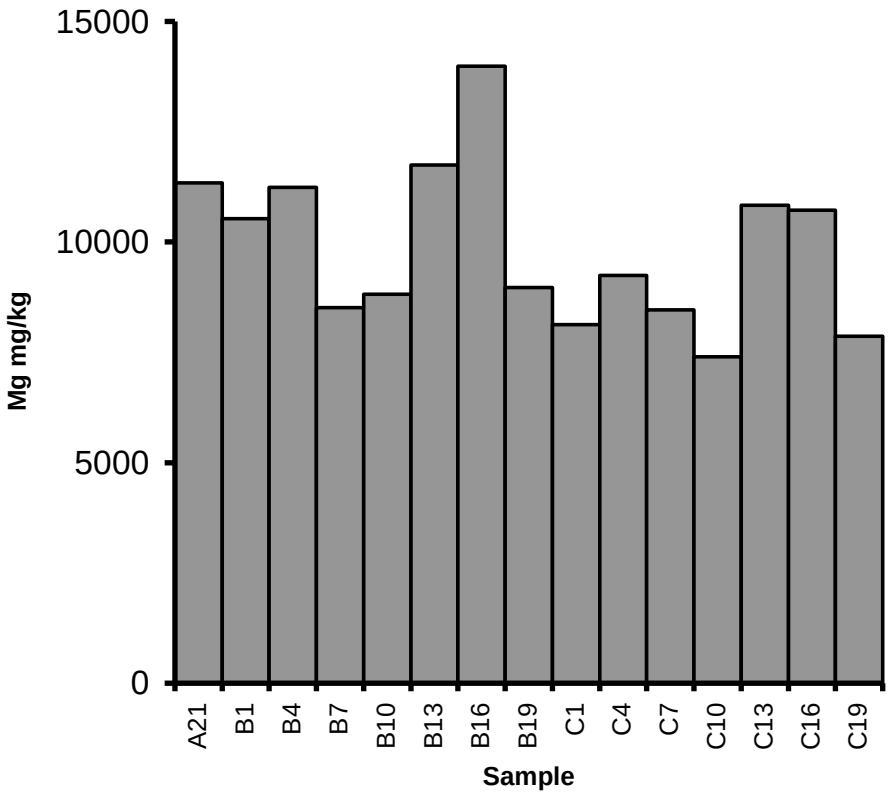

Figure 26 Magnesium concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Concentrations of Mg did not vary significantly from expected background levels (MESS-3, NRCC).

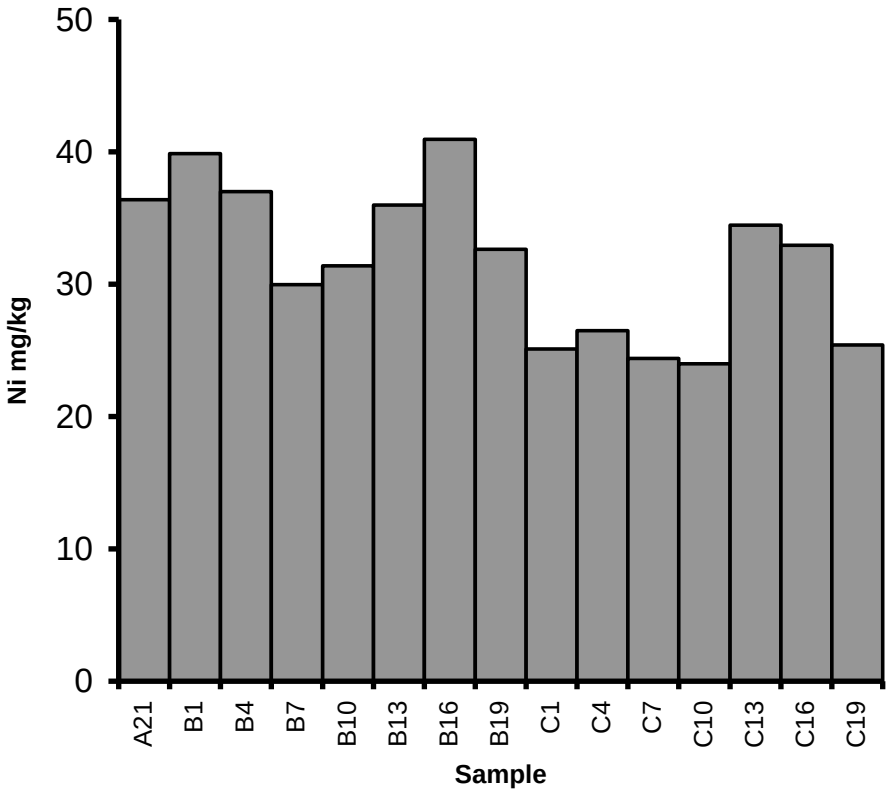

Figure 27 Nickel concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

No great variation was observed in Ni concentrations between trap samples, with observed concentrations just under those commonly measured in marine sediments (MESS-3 reference, NRCC – 46 – 51.1 mg/kg).

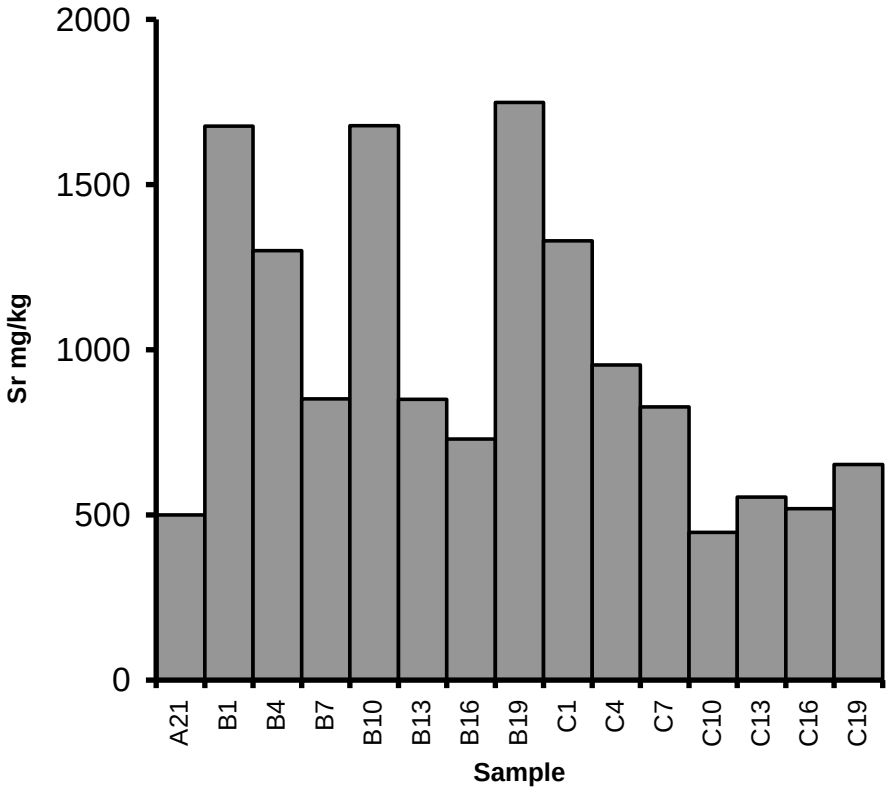

Figure 28 Strontium concentration in sediment trap bottles collected during initial drilling event (Nov-Dec 2009).

Strontium concentrations appear to be slightly higher in samples from traps B and C than trap A. Measured concentrations are generally above typical marine sediments (MESS-3 reference, NRCC). This is not unexpected given the high calcium carbonate scleractinian coral abundance in the region of drilling (Thomson & Livingston, 1970). The periodic peaks observed at B and C could reflect local elevated concentrations relating to periodic flow and resuspension conditions.

Second drilling event : Feb 2010. Sediment trap deployment 2.

Positions of the sediment traps deployed to monitor discharge during the Feb 2010 drilling event are given on **Fig. 2.** Traps were deployed on Feb 6th and retrieved at the end of the month.

Flow conditions:

During the second period of drilling, no current flow data was collected successfully, making the identification of a 'drilling control' sediment trap difficult.

Trap problems:

As during the initial drilling period, sediment traps deployed to monitor discharge during the second drilling period failed to rotate correctly. Determining when particular bottles were actually open during the second drilling period is very difficult, these problems are outlined below and should be

taken into consideration when reviewing the results of the sample analyses. Only the analyses of sample bottles which contained >10 mg dry weight of material will be discussed in detail in this report, under the assumption that the other bottles failed to open for any appreciable period of time.

Trap A

During the second drilling period, this trap again failed to rotate correctly. All material was collected in bottle A1, with a total mass of 42320 mg collected in that one bottle (**Fig. 29**). Whether this bottle represents the whole flux of material during the deployment period is uncertain.

Trap B

Only two bottles collected material during this deployment, B1 and B2, again indicating a rotation problem. Whether these two bottles each represent 1.5 days of material or more is unclear.

Trap C

Thirteen bottles collected material during deployment 2 – Bottles C1 –C9 and C11-C14. There was a considerable variation across the bottles in collected mass and other parameters, as discussed further in the relevant report sections.

Analysis of material collected in sediment traps deployed Feb 2010, (the second drilling period).

Total sample mass – Feb 2010:

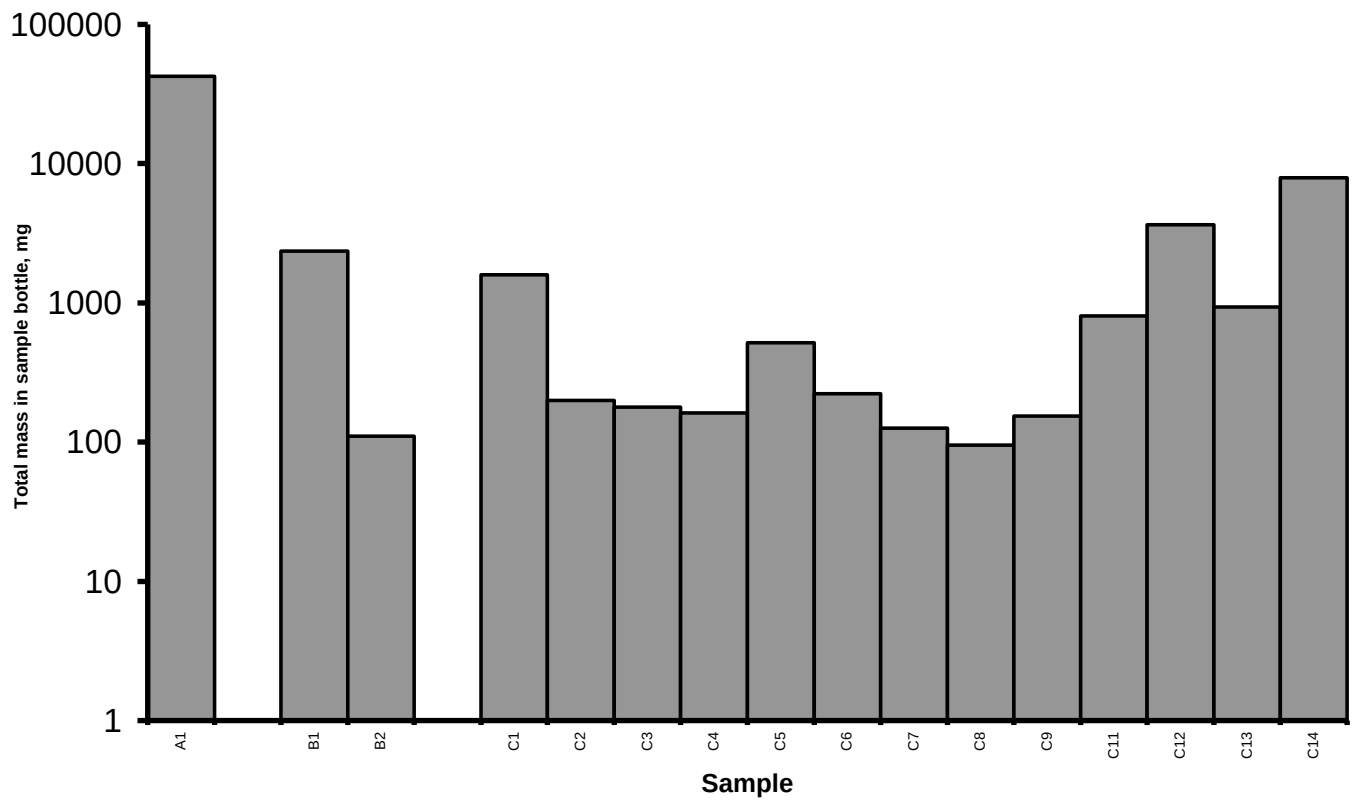

Figure 29 Total sample masses per bottle collected during second drilling event (Feb 2010), plotted with a logarithmic scale

What happened with traps A and B is unclear. Trap C seems to have perhaps rotated correctly, with the number of bottles containing samples reflecting the deployment period (**Figure 29**). There is less mass however in the majority of these bottles than was collected during the initial drilling campaign (**Fig. 3**). This could indicate one of the following: 1) undersampling by trap C during the second drilling event, 2) that during the first drilling event, traps B and C returned to deliver additional material to every third bottle repeatedly throughout the deployment period, or 3) Less material settling in the water column during the second drilling event.

Particulate organic material – Feb 2010:

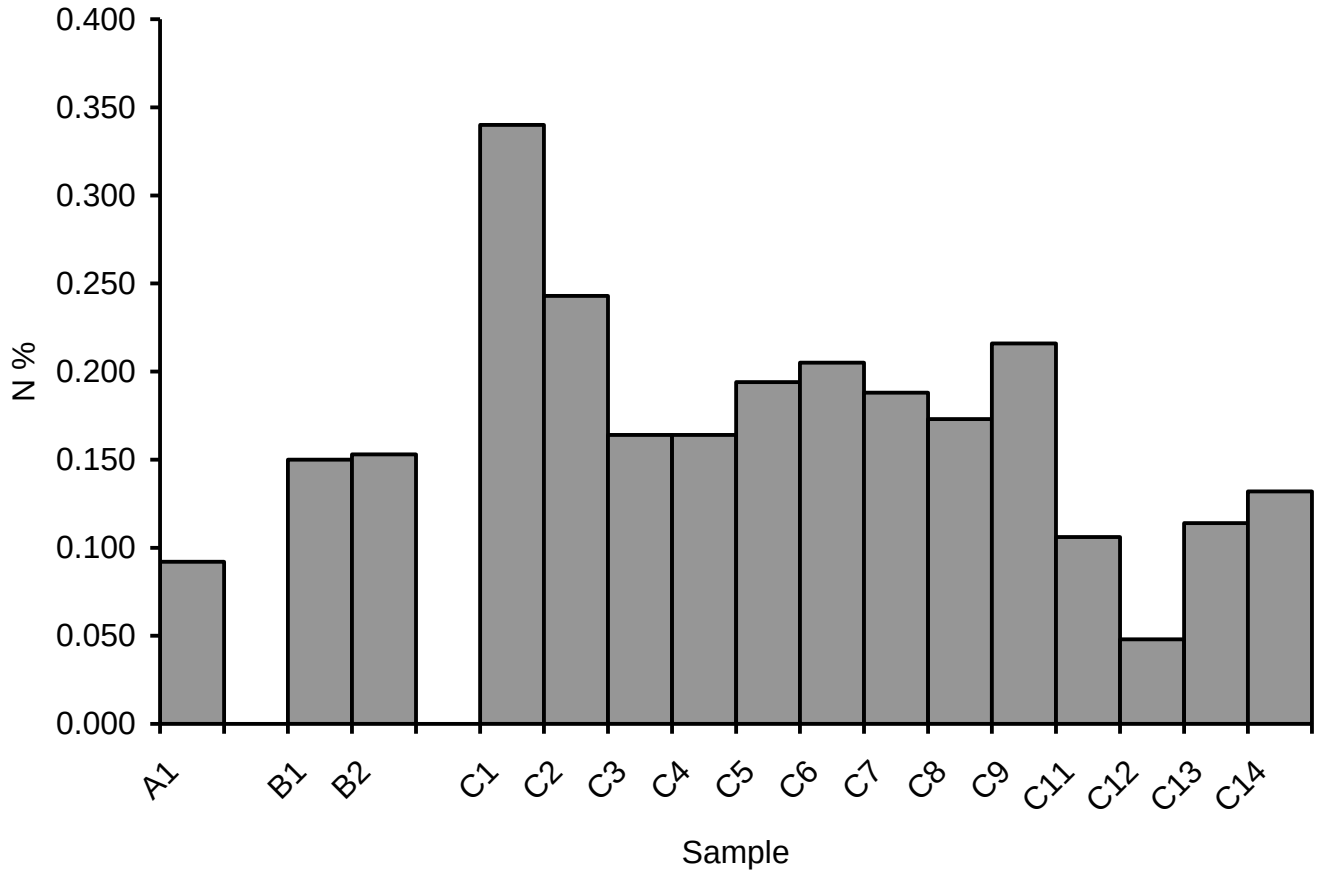

Figure 30 Nitrogen % for the sample material collected during second drilling event (Feb 2010).

Nitrogen % values measured were generally slightly higher during the second drilling event than during the first.

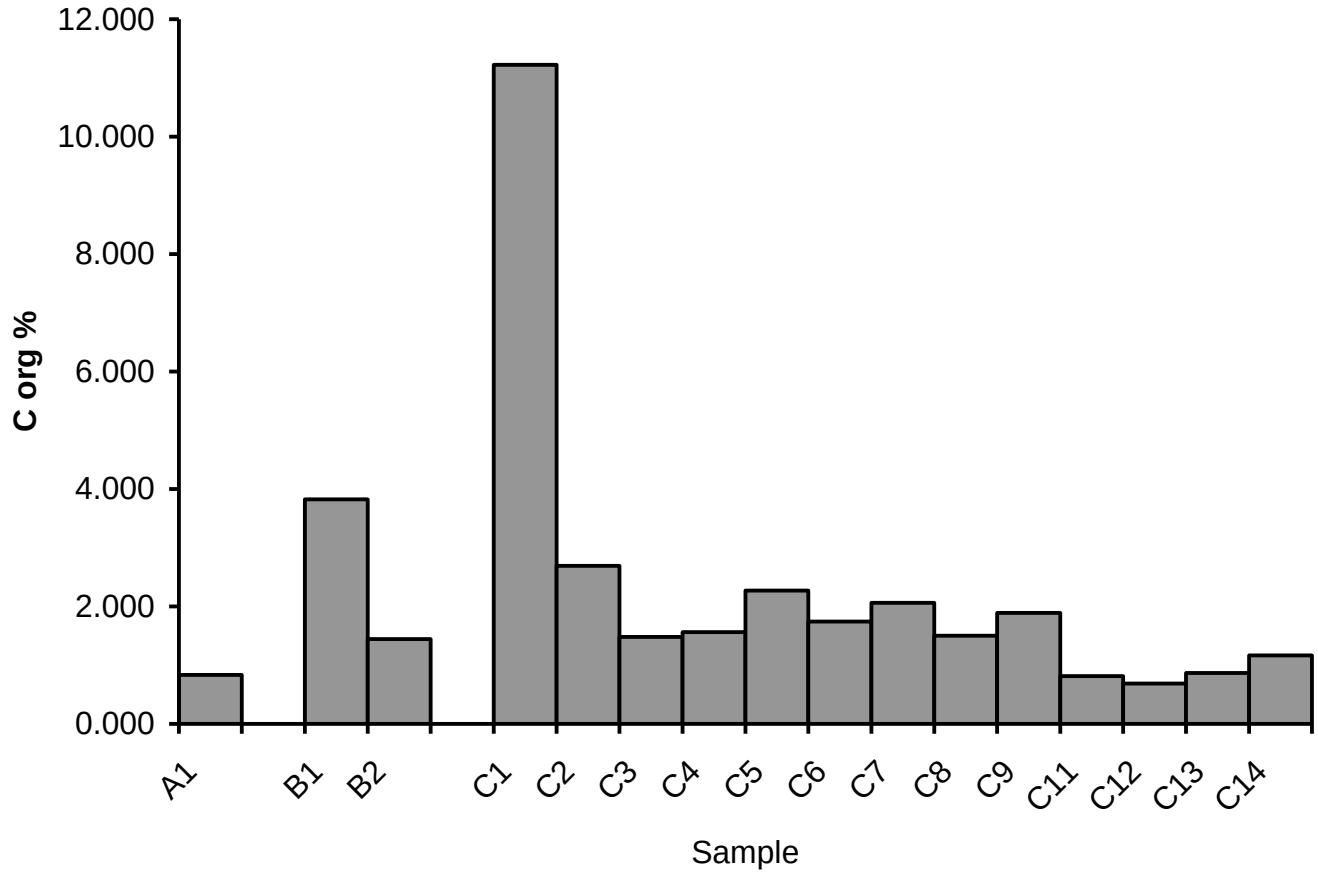

Figure 31 C org % for the sample material collected during second drilling event (Feb 2010).

The lowest % organic carbon concentrations was observed in bottles A1 and C11 – C14. These bottles also contain some of the highest total masses (**Fig. 29**). This is an indication of drill cuttings making up more of the collected mass in these bottles. As also indicated on **Fig. 29**, total mass is also high in bottles B1 and C1, but in these bottles C org % is also observed in **Fig. 31** to be at its highest. Given that these bottles represent the first bottles to be exposed by traps B and C this elevated C org % and mass concentration may well be the result of the deployment process.

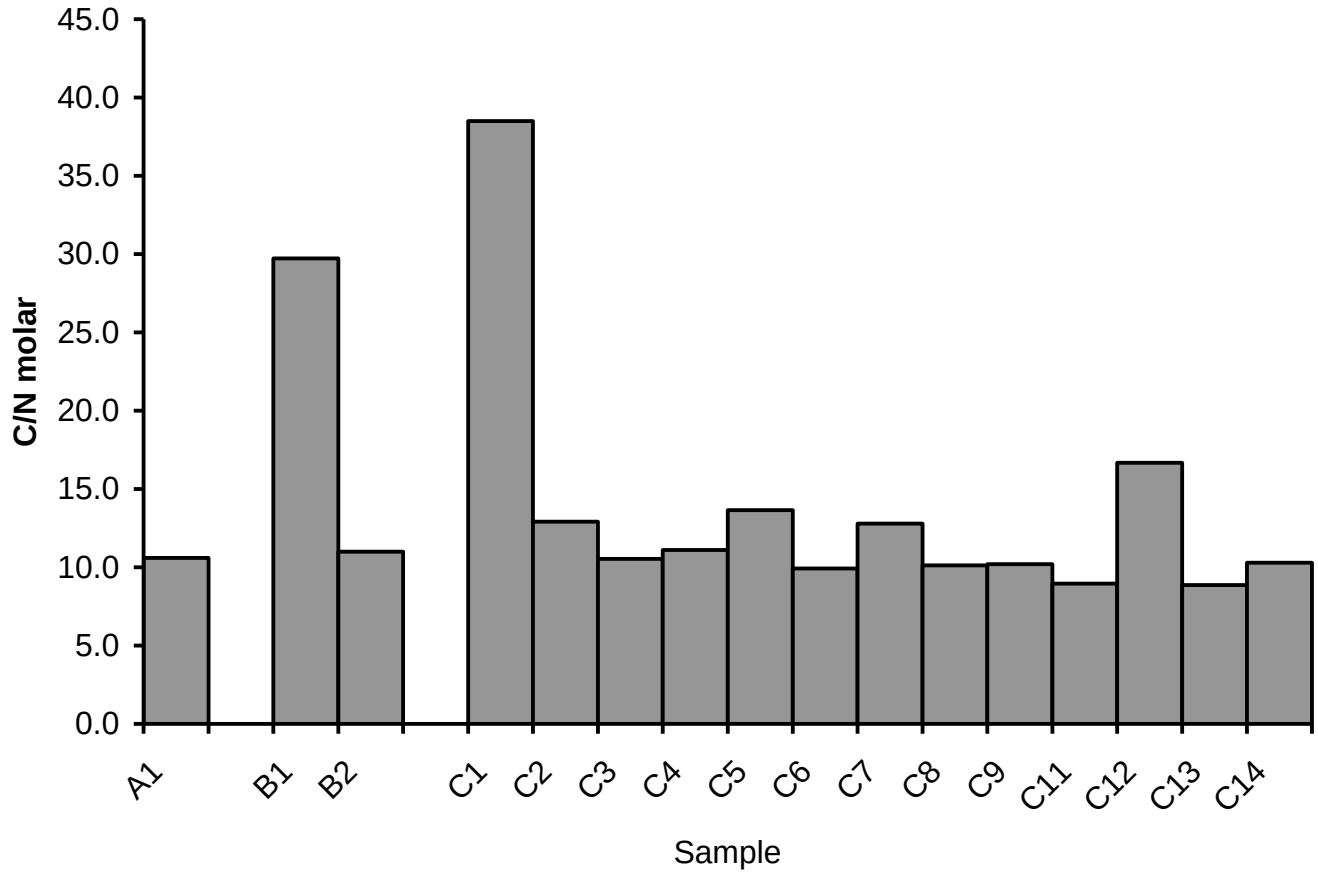

Figure 32 C/N Molar ratio for the sample material collected during second drilling event (Feb 2010).

As during the first drilling event (**Fig. 7**) relatively organic material of C/N ~10 entered the sediment traps during most of the deployment period, as indicated in **Fig. 32**. High C/N levels observed in samples B1 and C1 again probably reflect some artifact of the trap deployment process.

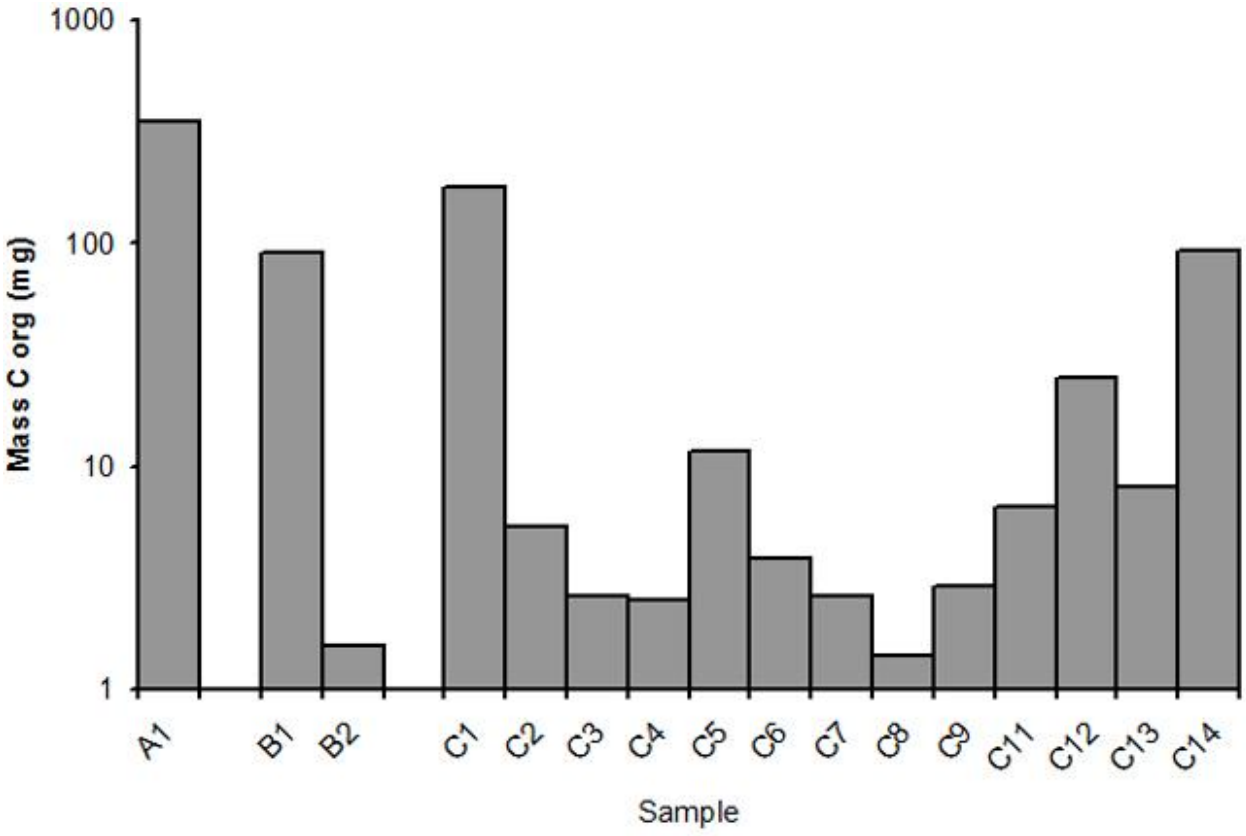

Figure 33 Mass of organic carbon of the sample material collected during second drilling event (Feb 2010).

Again, as observed during the first drilling event (Fig. 8), the total mass of organic carbon collected during the second drilling event (Fig. 33) tends to be much larger in the sample bottles marking the start and end of deployments (in this case, bottles A1, B1, C1 and C14), probably indicating a deployment / retrieval process or trap rotation problem rather than some actual temporal change settling organic carbon concentration in the water column.

Amino acid analysis and degradation indices – Feb 2010:

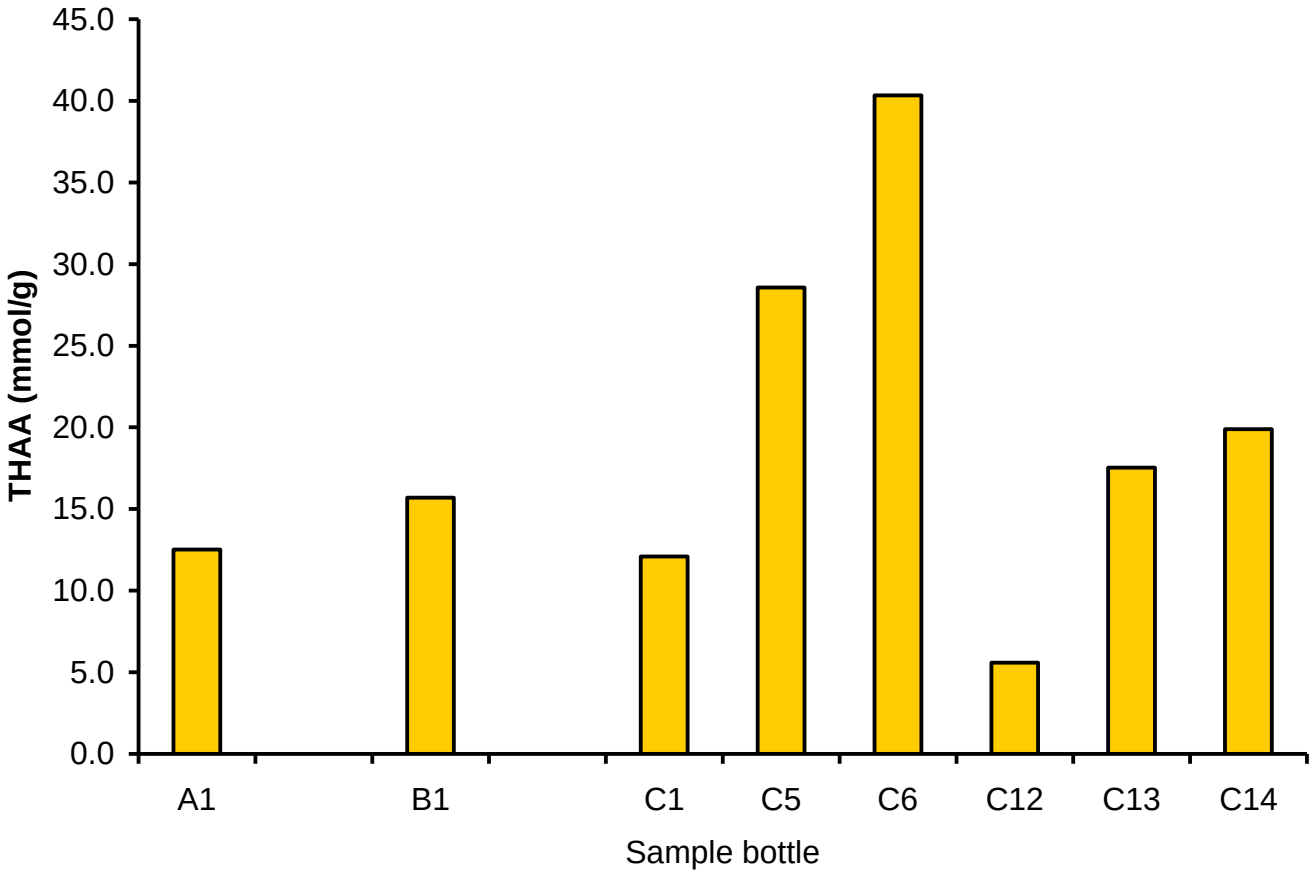

Figure 34 THAA mmol/g amino acid degradation index of material collected during second drilling event (Feb 2010).

Concentrations of total hydrolizable amino acids (THAA) (**fig. 34**) ranged between 5 and 45 mmol/kg. Besides samples C5 and C8 the THAA concentrations were in the same range as reported for the first deployment.

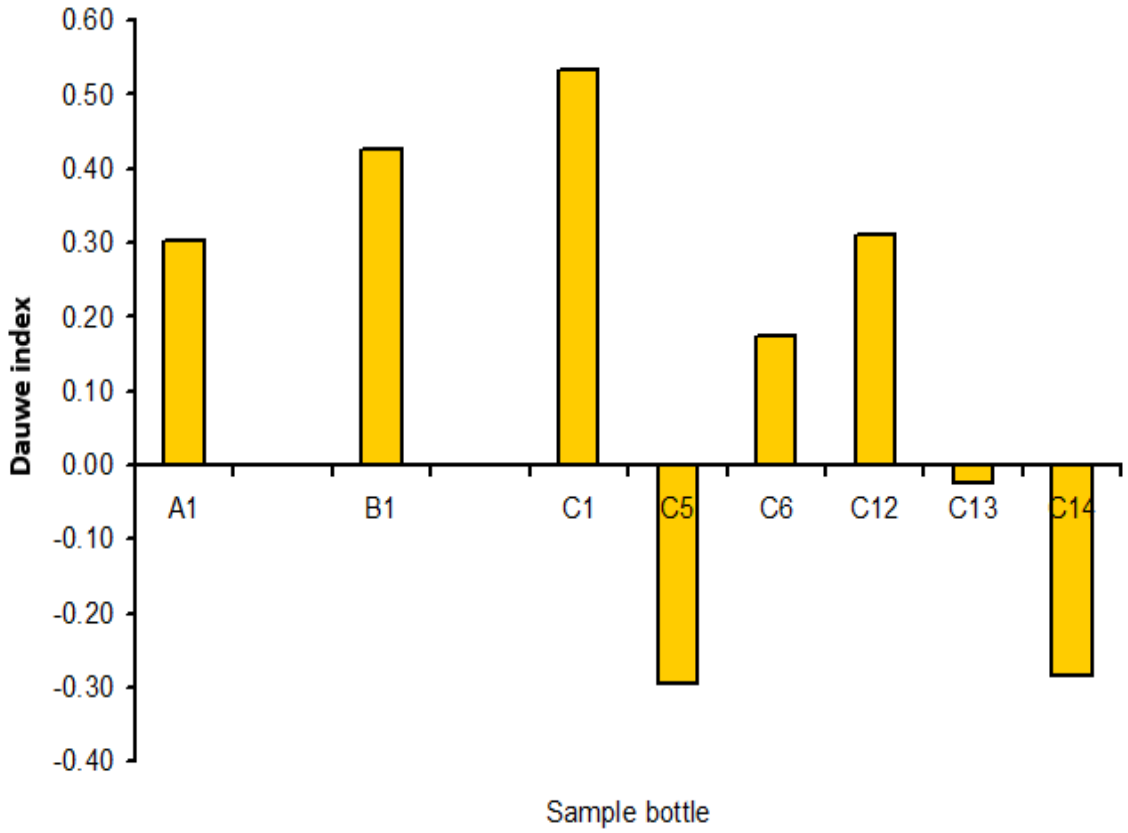

Figure 35 Dauwe amino acid degradation index of material collected during second drilling event (Feb 2010).

The Degradation index from samples collected during the second drilling period (**fig.** 35) are different to those observed during the first. There are three periods with more degraded material reaching the sediment traps, (indicated by negative degradation indices in samples C5, C13, C14) which most likely represents a difference in quantity of fresh material within the water column during the two drilling operations.

Metals and trace elements – Feb 2010:

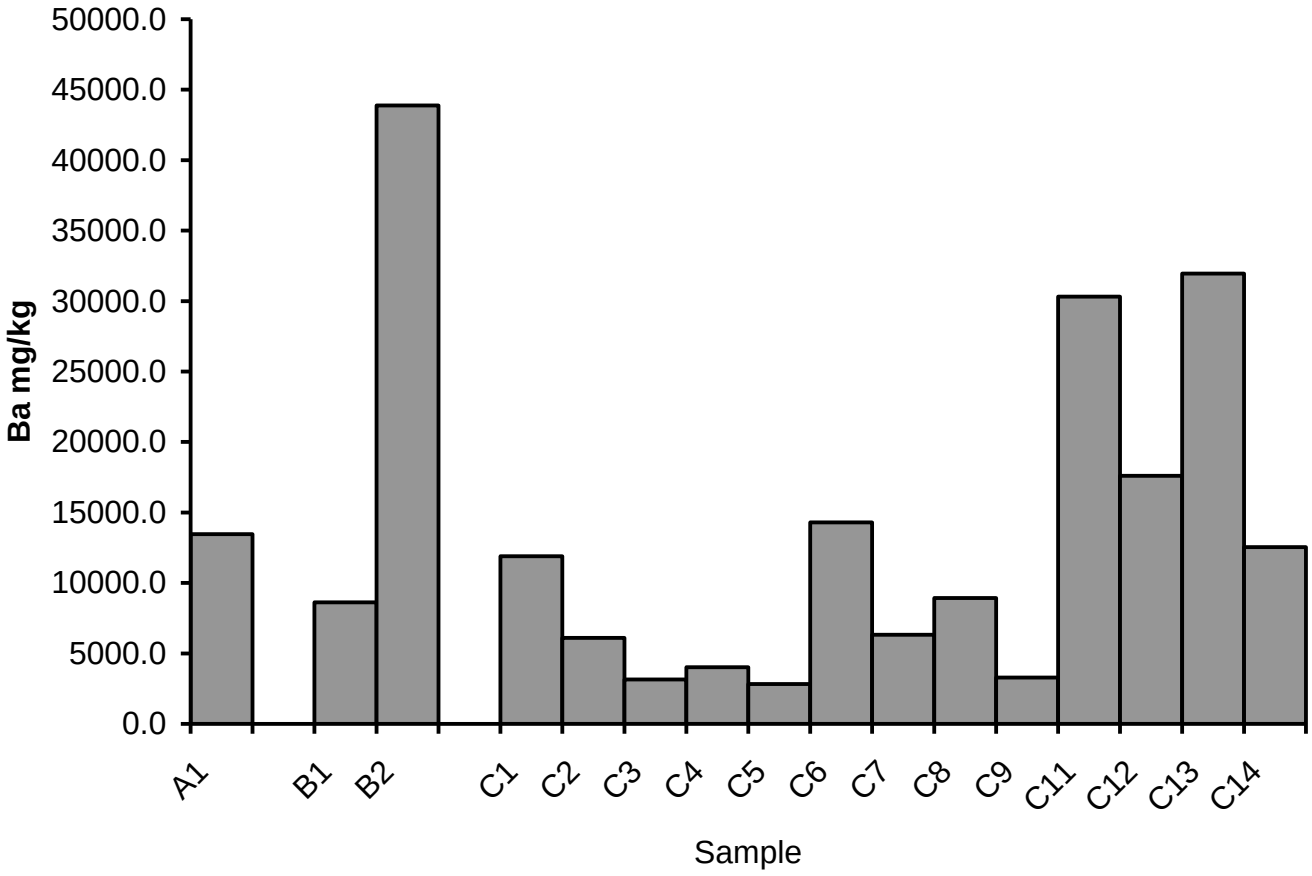

Figure 36 Barite concentration within the sample material collected during second drilling event (Feb 2010).

The figure shows elevated Barite concentration across the region, indicating that currents were not uniformly in one direction, and some drill cutting contamination occurred at all sites. Assuming trap C rotated correctly, it would appear that the peak of drill cutting deposition at trap C occurred in late February, with the highest concentrations of barite collected in bottles C11 – C13. Unfortunately, with the failure of traps A and B to rotate correctly, it is not possible to identify the peak deposition periods at these trap sites.

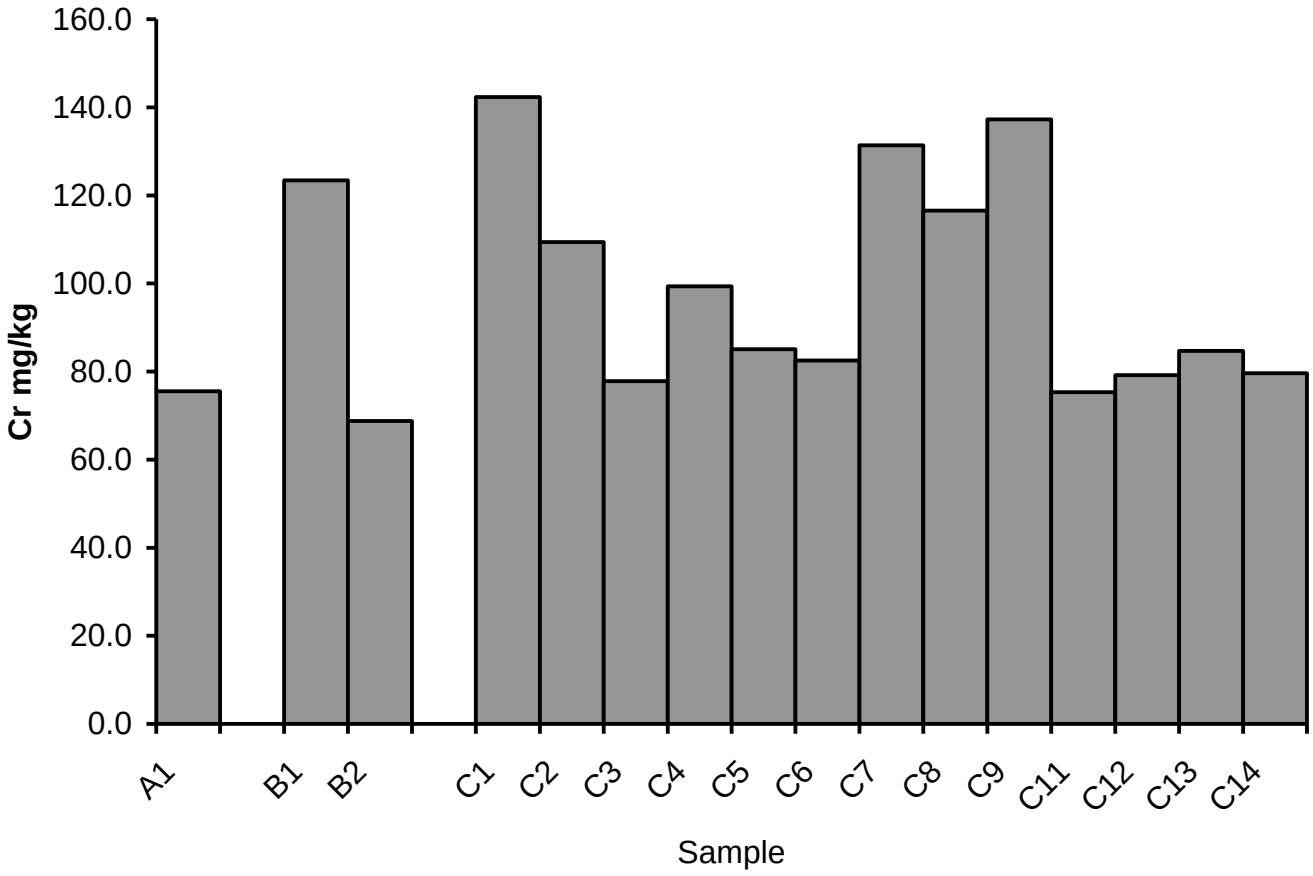

Figure 37 Chromium concentration within the sample material collected during second drilling event (Feb 2010).

As during the first drilling event, chromium concentration did not vary greatly with sediment trap. The levels observed were again a little higher than those given in the recent Akvaplan-niva report (Report no. 4664-03, 2010) for the area (16.4 – 34.6 mg/kg), but generally comparable with those of general marine sediments (~99.8- 112 mg/kg, Mess-3 reference material, NRCC).

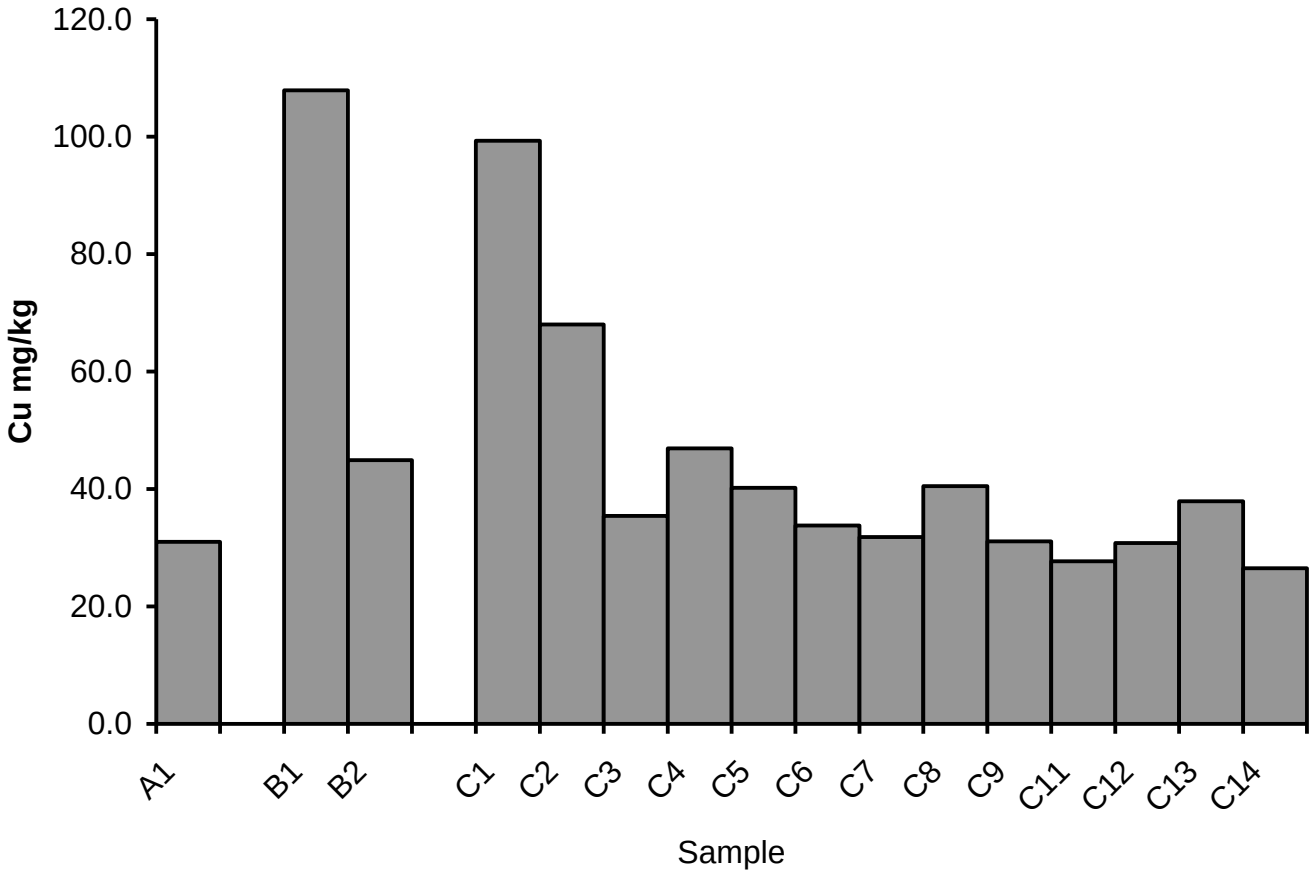

Figure 38 Copper concentration within the sample material collected during second drilling event (Feb 2010).

Observed copper concentrations within sediment trap bottles were generally slightly higher during the second drilling event (**Fig. 38**) than the first (**Fig. 20**). Greatest concentrations were observed in bottles B1 and C1, perhaps representing some contamination during trap deployment.

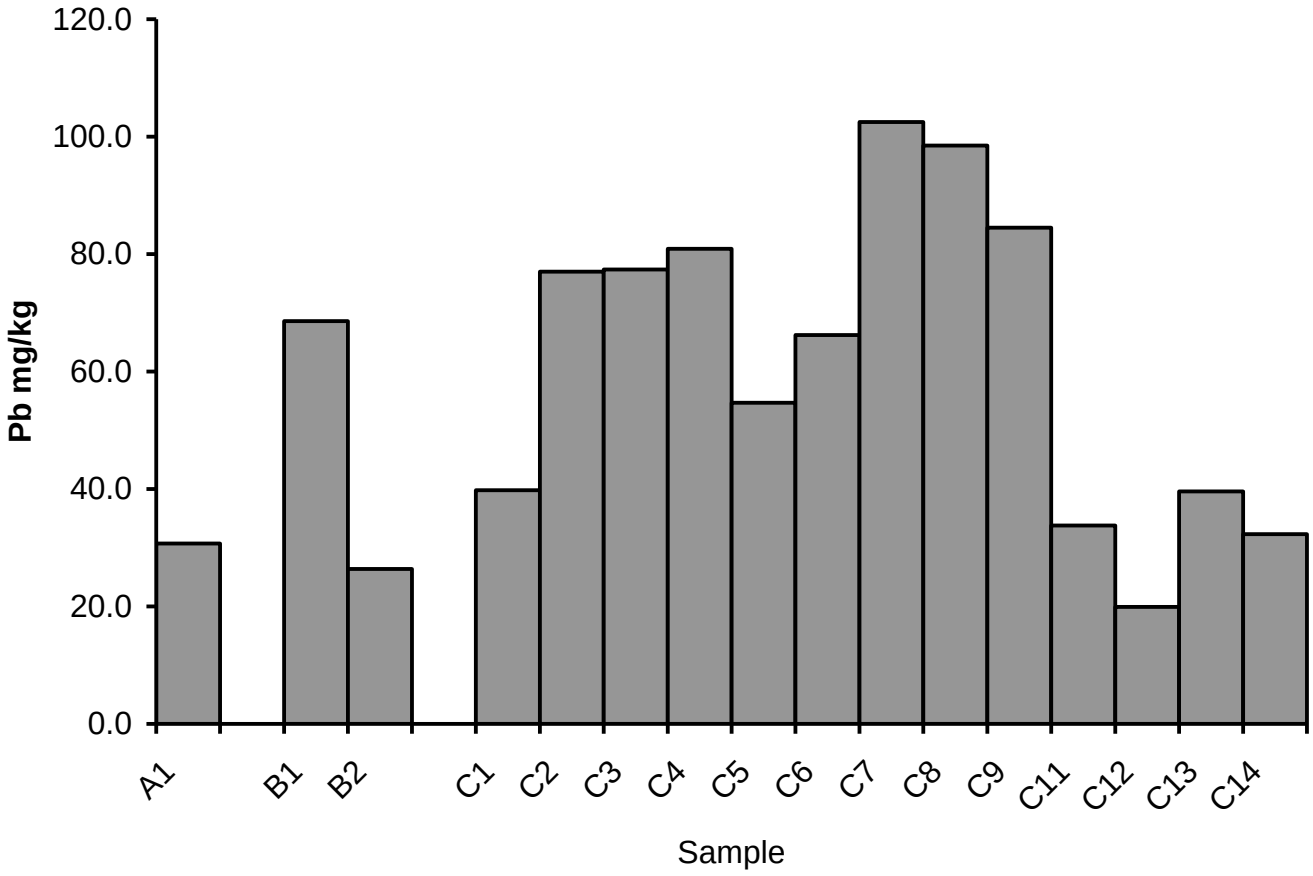

Figure 39 Lead concentration within the sample material collected during second drilling event (Feb 2010).

Lead concentrations collected during the second deployment varied across sample bottles (**Fig. 39**). Highest concentrations were observed in bottles C2-C9, which were the bottles showing the lowest Barite concentrations (**Fig. 36**). This trend was also observed during the first deployment, with bottle A21 containing the lowest Barite concentration (**Fig. 18**) and highest Pb concentration (**Fig. 21**).

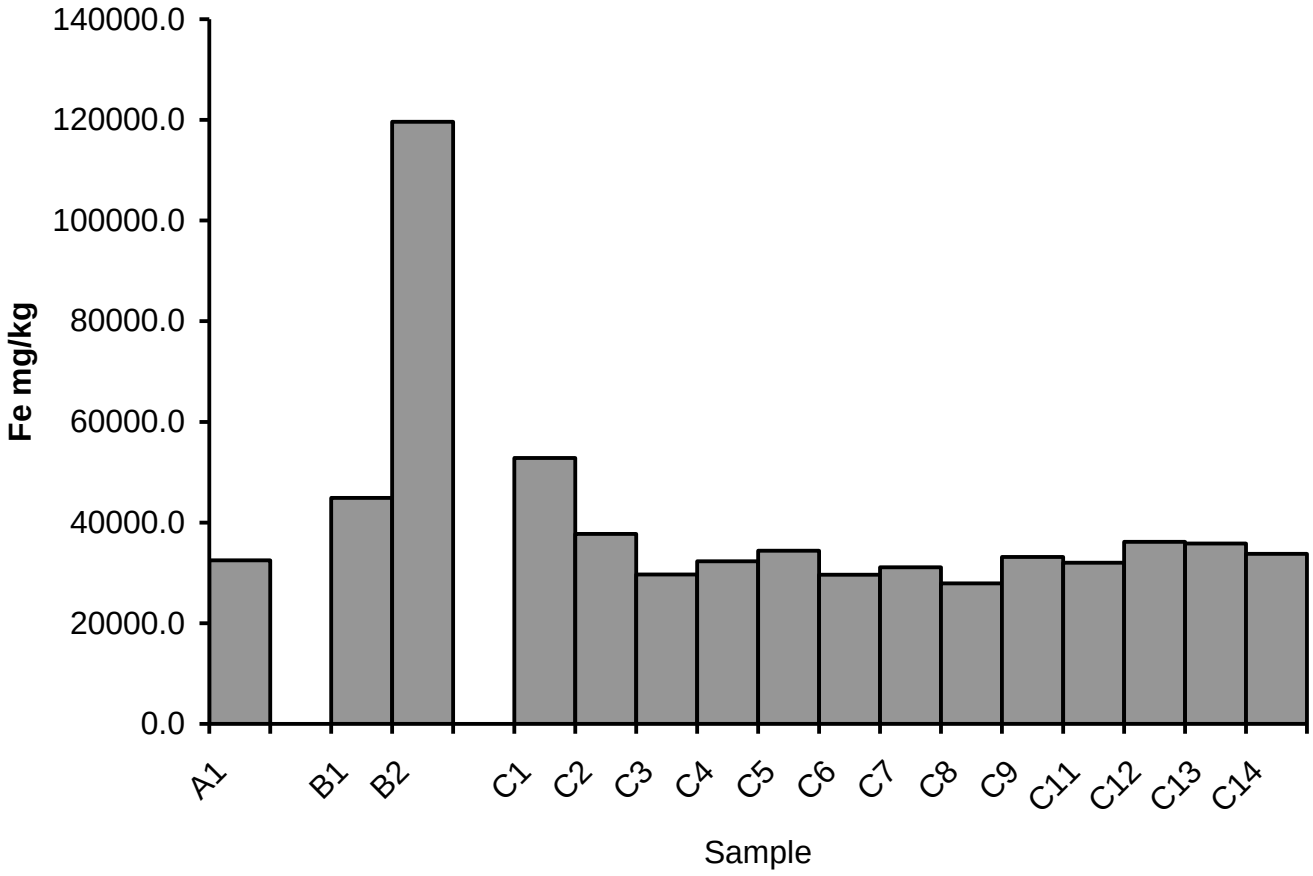

Figure 40 Ferrous metal concentration within the sample material collected during second drilling event (Feb 2010).

Ferrous metal concentrations in sediment trap bottles collected during the second drilling event (**Fig. 40**) were generally similar to those collected during the first drilling event (**Fig. 23**). Sediment trap bottle B2 collected during the second drilling event contained a ~4x greater Fe concentration than any of the other bottles, from either deployment. This high value could be a consequence of the rotation failure of trap B.

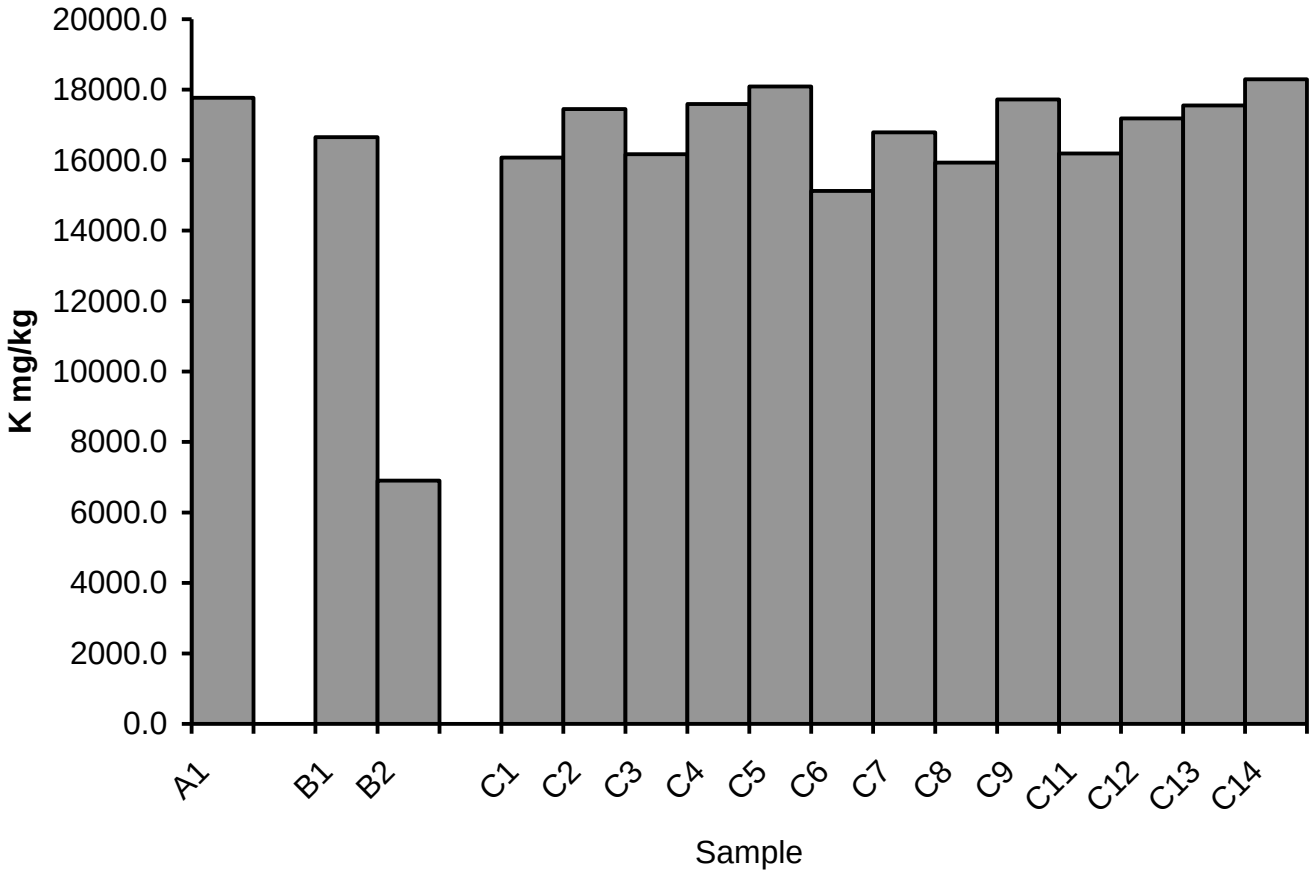

Figure 41 Potassium concentration within the sample material collected during second drilling event (Feb 2010).

There was little variation in K concentration between samples collected during drilling event 2, and all values are reasonable for marine sediments (MESS-3, NRCC). The concentration within trap B2 was the lowest, perhaps again as a consequence of the trap rotation failure.

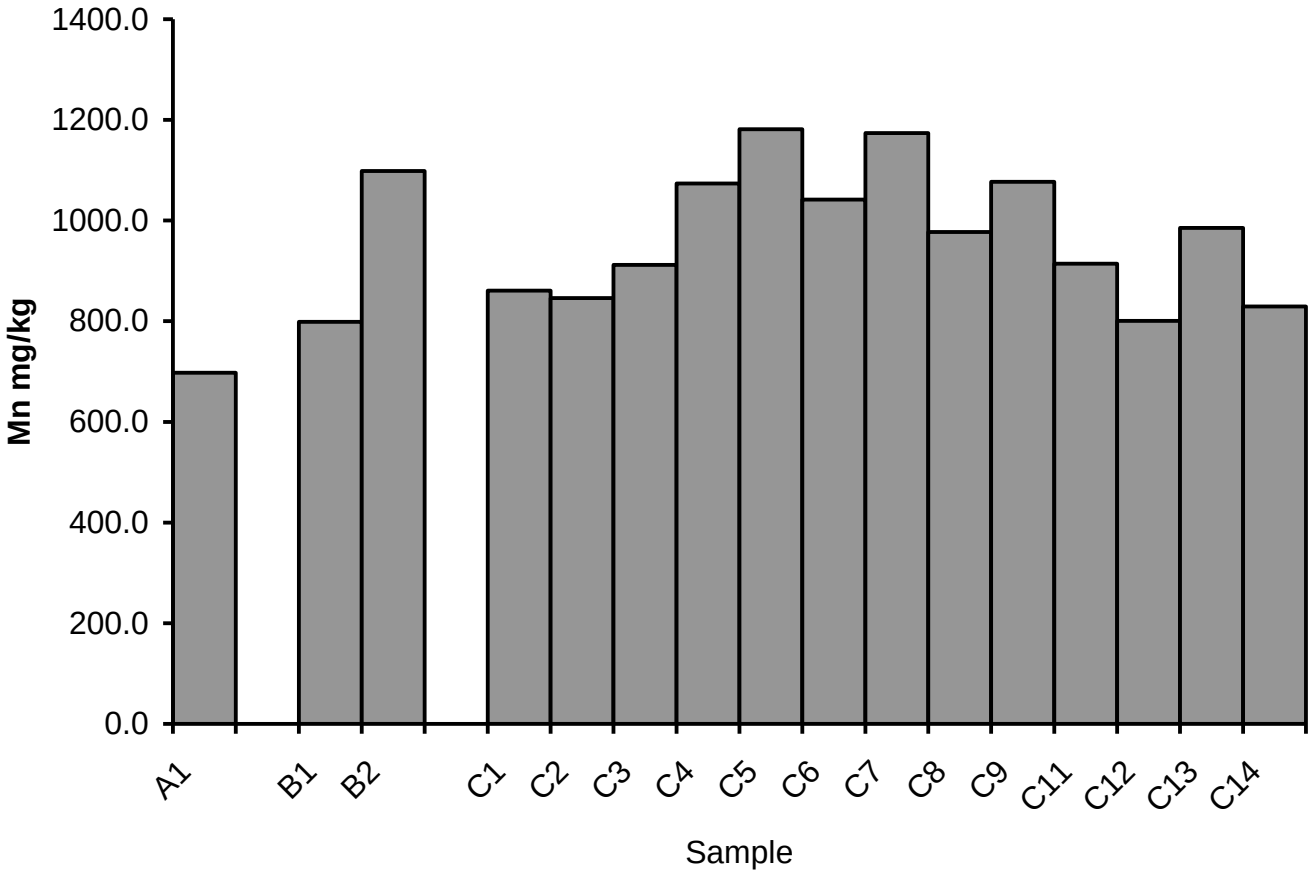

Figure 42 Manganese concentration within the sample material collected during second drilling event (Feb 2010).

Concentrations of MN did not vary significantly from expected background levels (MESS-3, NRCC) during the second drilling event.

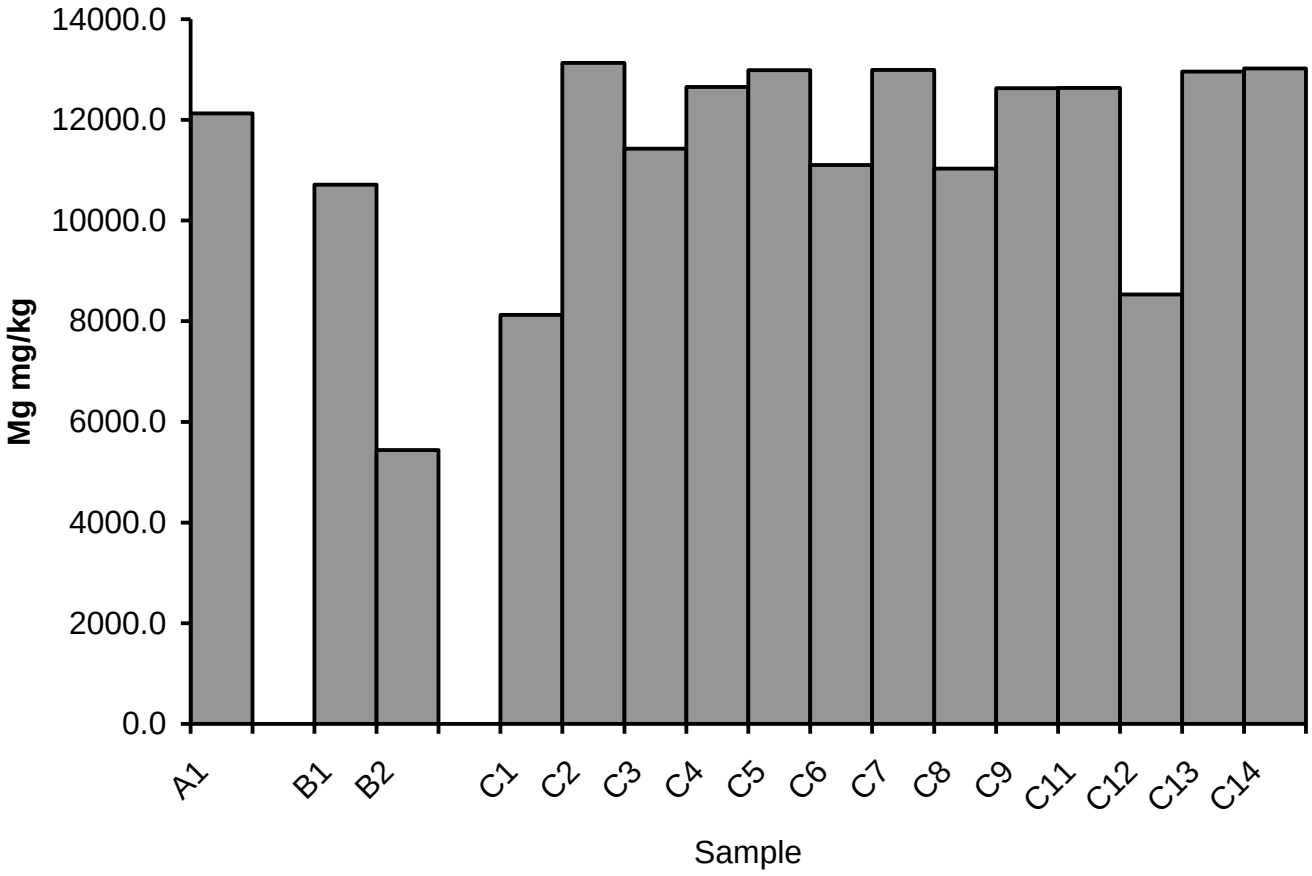

Figure 43 Magnesium concentration within the sample material collected during second drilling event (Feb 2010).

Concentrations of Mg did not vary significantly from expected background levels (MESS-3, NRCC) during the second drilling event.

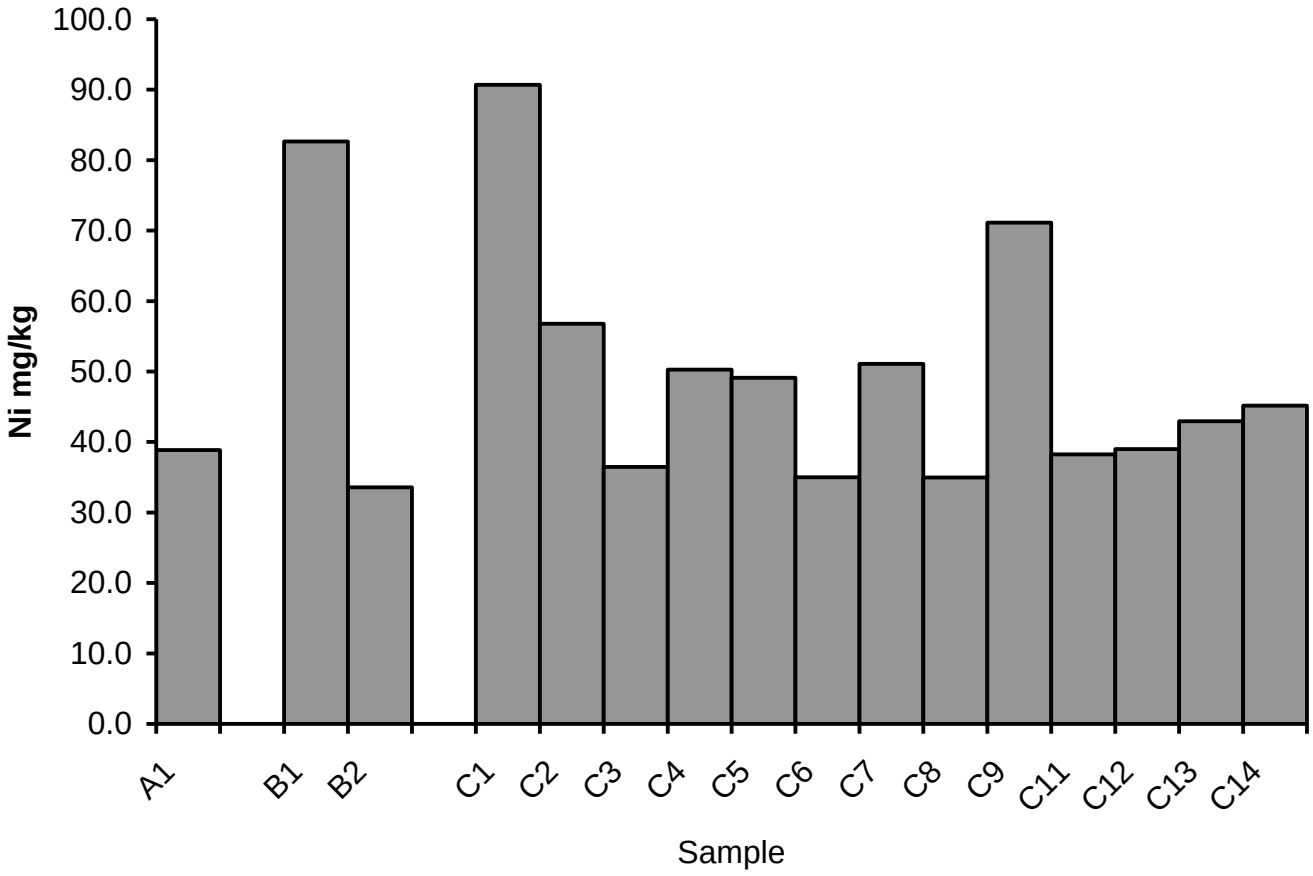

Figure 44 Nickel concentration within the sample material collected during during second drilling event (Feb 2010).

Some variation was observed in Ni concentrations between trap samples, with observed concentrations generally just under those commonly measured in marine sediments (MESS-3 reference, NRCC – 46 – 51.1 mg/kg). Trap bottles B1, C1 and C9 contained the highest concentrations of Nickel, with concentrations of ~70-95 1 mg/kg.

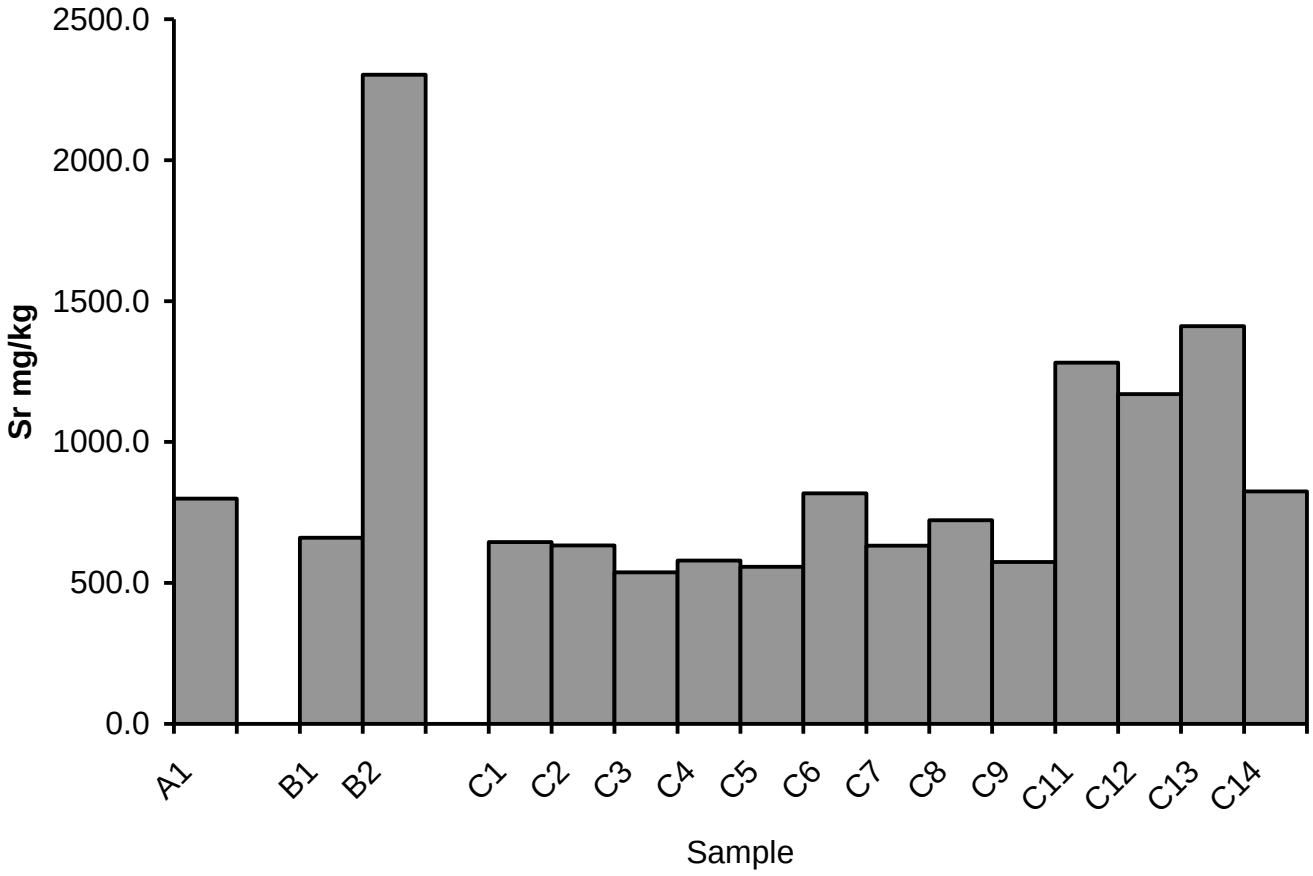

Figure 45 Strontium concentration within the sample material collected during second drilling event (Feb 2010).

Strontium concentrations, as during the first drilling event, were observed to be higher than concentrations are generally observed in marine sediments (MESS-3 reference, NRCC). This is not unexpected given the high calcium carbonate scleractinian coral abundance in the region of drilling (Thomson & Livingston, 1970).

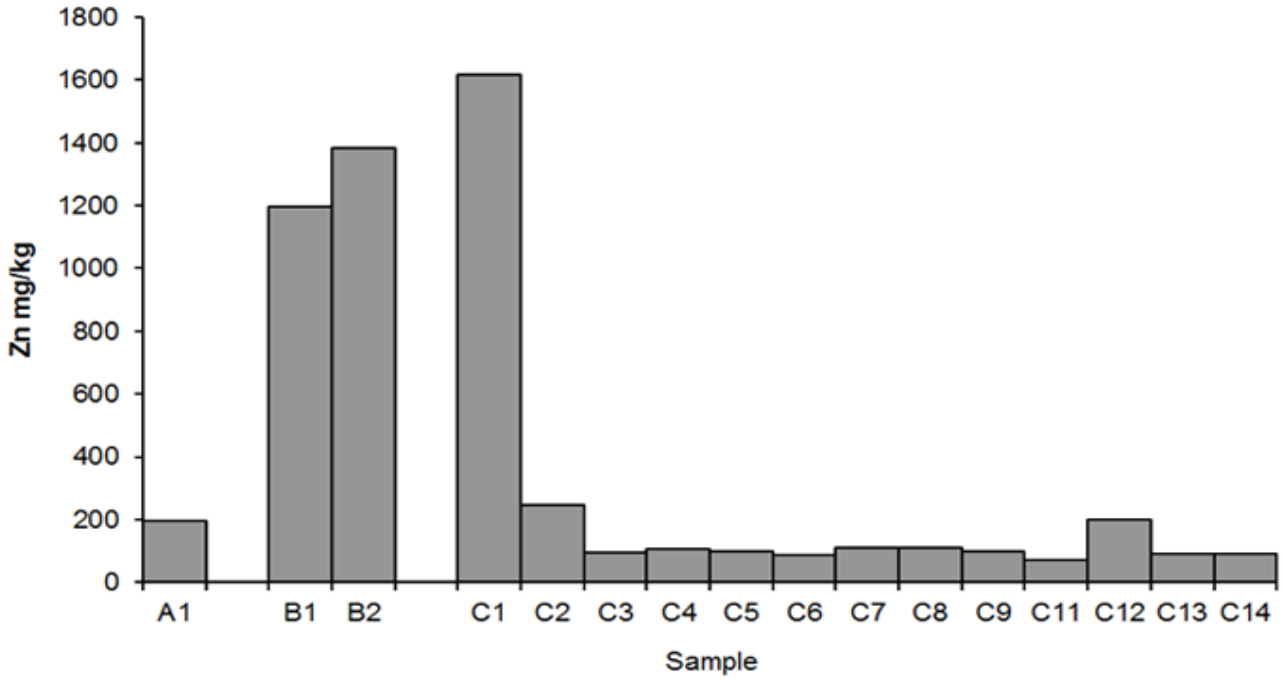

Figure 46 Zinc concentration within the sample material collected during second drilling event (Feb 2010).

A background zinc concentration of 40.7 – 90.0 mg/kg (Akvaplan-niva report no. 4664-03, 2010) for the region corresponds with the results from the majority of sediment trap samples collected during the second drilling event (**Fig. 46)**. The elevated concentration observed in sediment trap samples B1, B2 and C1 is unlikely to be related with the drilling operation, as Zn concentrations in sediments highly contaminated with drill cuttings (as measured at several sites in May 2009 and March 2010 by Acergy Petrel and Edda Fauna, presented in a recent IMR report) was not observed to differ from this background range (discussed In association with **Fig. 22**). The elevated concentrations measured in these bottles is more likely the result of contamination on deployment / retrieval of traps.

General conclusion :

During drilling operations in November/December 2009, traps B and C were exposed to drill cuttings. Trap A may have been exposed to some low concentrations of drill cuttings. The drill cuttings of small size entered the trap bottles in sufficient concentration to change the overall particle size spectrum from large particles (> 100 µm to small particles < 25 µm). It is impossible to give precise numbers for the particle concentration of the fine material in the water column during drilling operations since only sediment trap samples, and no in-situ water samples were available for analysis. As drilling occurred during winter, only a little organic material was present in the water column. Although of low concentration, this material was of a more labile quality than the drill

cuttings and settled into the sediment traps in the form of aggregates of 100 to 400 µm median size. These aggregated particles had aggregated with drill cuttings since the settling velocities of material from the traps was an order of magnitude higher than that reported for aggregates originating from within natural benthic boundary layer conditions (Thomsen et al., 2002).

The data on critical shear velocity of the material indicate that once these drill cutting/ organic particle material arrived on the seafloor, bedload transport would occurr under low flow conditions of ≈ 10cm/s. Full resuspension of this material would occur under flow velocities of 10 – 16 cm/s. These currents are most probably found regularly at the study site. With increasing distance from the drill cutting discharge point, the drill cuttings increasingly aggregate with the organic material and form biofilms. This results in an increase of critical shear velocity required to resuspend settled material and an increased settling velocity of the particle with distance from the drill cutting discharge point.

Sedimentation:

It is difficult to derive conclusions on particle accumulation during drilling operations. In order to do so, for the initial drilling event more data on mass accumulations at reference station A would be required. Only trap bottle A21 collected material and it is uncertain the exact period of flux this bottle represents. The uncertainty regarding the rotation timing of traps B and C during this first deployment would make any conclusions on the influence of drill cuttings on mass accumulation rates on the seabed very tenuous. Natural and drill cutting enhanced mass accumulation rates for the second drilling period cannot be made, given the absence of flow data and poor bottle rotation performance of the sediment traps.

Barium levels were an order of magnitude lower at the control site (Sediment trap A) than at the other sediment trap sites during the initial drilling event, although concentrations of Ba at this location were still above the local background levels, so some minor contamination of that location is possible.

Table 1. Preliminary table for sediment transport modeling for particles containing drill cuttings during time of drilling operations (at 5°C, salinity of 36, u_{100} = flow velocity at 100 cm above seafloor).

d (µm)	**Ws (cm s^{-1})**	**U_{*c} (cm s^{-1})**	**u_{100} (cm s^{-1})**
10 (drill cuttings)	**0.013**	**0.5 – 0.6**	**8 - 10**
20 (drill cuttings)	**0.03**	**0.5 – 0.6**	**8 - 10**
100 (organo-mineral aggregates)	**0.5**	**0.7**	**11**

200 (organo-mineral aggregates)	**0.7**	**0.9**	**14**
400 (organo-mineral aggregates)	**1.2**	**1**	**16**

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Appendix C Sediment core samples

This appendix contains the results from the sediment core metal analysis

Table C1 Metal analysis

Sample ID	Si	Al	Fe	Ti	Mg	Ca	Na	K	Mn
	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]
RC 8-1a	<250	7770	11200	432	5140	54800	6120	2990	225
RC 8-2a	272	7390	10500	399	4970	55200	5890	2870	174
RC 8-3a	<250	7870	11100	428	5240	58700	6520	3100	222
RC 9-1a	<250	8800	12800	490	5960	56500	7250	3350	369
RC 9-1a	<250	8390	12000	455	5690	57200	6840	3170	289
RC 9-1a	258	8580	12200	460	5860	56900	8130	3290	305
D-NEG 1 HM	<250	9060	13100	474	6330	66700	10800	3620	417
D-NEG 2 HM	<250	8140	11700	447	5520	54900	7090	3080	372
D-NEG 3 HM	<250	8160	12100	433	5610	59000	7230	3170	341
D-MRRE 1 HM	<250	8520	12300	444	5660	59800	7230	3190	283
D-MRRE 3 HM	<250	8220	12000	431	5630	55900	8090	3130	273
D-MRRE 2 HM	<250	8030	11600	439	5790	54500	6720	3020	300
D-NV 1 HM	<250	8030	11700	431	5460	55500	6770	2990	339
D-NV 2 HM	276	7870	11500	426	5470	53900	7160	2980	320
D-NV 3 HM	293	7350	10800	405	5190	55700	6700	2850	221
D-PART 1 HM	<250	9650	13500	523	6500	52300	8290	3540	412
D-PART 2 HM	<250	10300	14600	561	8200	50200	17300	3930	438
D-PART 3 HM	<250	9450	13500	546	6560	47300	7930	3380	383
D-POS 1 HM	541	19800	27300	921	17300	24900	28600	6630	775
D-POS 1THC	353	5640	20400	103	6700	12600	24700	2080	2290
U-NV 1 HM	<250	9480	13400	470	6780	64700	7720	3450	240
U-NV 2 HM	<250	9290	13300	477	6350	59400	7480	3490	334
U-NV 3 HM	259	9270	13200	483	6570	59500	7720	3370	362
U-PART 1 HM	<250	11100	15600	596	7690	49000	10500	3950	411
U-PART 2 HM	<250	12600	17300	658	8350	51600	11500	4450	419
U-PART 3 HM	<250	14900	20200	773	10500	40700	19100	5300	360

E-PART 1 HM	<250	10200	14000	541	6660	49700	8070	3630	369
E-PART 2 HM	<250	11500	15900	667	8380	55600	12500	4330	490
E-PART 3 HM	<250	10500	14500	591	7390	53500	10900	3930	337
E-NV 1 HM	<250	12000	16500	623	7650	39700	6500	4280	371
E-NV 2 HM	<250	12900	16800	623	7990	48100	7260	4600	311
E-NV 3 HM	<250	9100	13400	496	6830	48800	11400	3460	393

Table C2 Metal analysis continued

Sample ID	P	Cu	Zn	Pb	Ni	Co	V	Mo	Cd	Cr
	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]
RC 8-1a	476	3,6	31,6	9,7	12,2	3,88	25,5	<1	<0.1	15,9
RC 8-2a	456	3,2	28,6	9,9	10,4	3,38	22,9	<1	<0.1	14,6
RC 8-3a	476	3,5	31,0	10,5	11,9	3,93	24,4	<1	<0.1	15,3
RC 9-1a	521	3,9	34,1	11,2	13,4	4,72	27,2	<1	<0.1	17,1
RC 9-1a	479	3,7	32,6	10,5	12,9	4,42	25,9	<1	<0.1	17,0
RC 9-1a	487	3,9	32,7	11,4	12,4	4,23	26,3	<1	<0.1	16,8
D-NEG 1 HM	506	4,3	35,0	13,8	14,0	4,81	28,8	<1	<0.1	17,3
D-NEG 2 HM	507	3,6	31,8	11,1	11,6	4,35	25,1	<1	<0.1	15,5
D-NEG 3 HM	503	3,9	33,0	11,6	12,2	4,41	26,1	<1	<0.1	16,0
D-MRRE 1 HM	481	3,7	33,1	11,3	12,0	4,26	26,0	<1	<0.1	16,2
D-MRRE 3 HM	544	3,7	32,3	11,4	12,7	4,14	26,3	<1	<0.1	16,2
D-MRRE 2 HM	503	3,5	30,7	9,5	12,8	4,29	25,4	<1	<0.1	15,6
D-NV 1 HM	482	3,5	30,7	10,5	11,4	3,75	24,7	<1	<0.1	15,5
D-NV 2 HM	486	3,8	31,2	10,6	12,0	3,66	25,3	<1	<0.1	15,6
D-NV 3 HM	456	3,2	28,8	9,2	11,7	3,10	22,8	<1	<0.1	14,3
D-PART 1 HM	505	5,1	36,1	12,7	14,9	3,67	29,4	<1	<0.1	18,9
D-PART 2 HM	530	9,2	39,7	14,0	13,4	0,61	31,1	<1	<0.1	20,4
D-PART 3 HM	539	5,9	36,2	12,1	13,3	1,11	29,2	<1	<0.1	18,5
D-POS 1 HM	503	28,4	64,0	18,7	26,4	5,05	55,1	<1	0,11	41,0
D-POS 1THC	151	50,9	30,7	54,8	4,8	<0.1	16,5	<1	<0.1	24,4
U-NV 1 HM	580	4,9	36,8	12,8	15,6	4,68	29,7	<1	<0.1	17,8
U-NV 2 HM	530	4,0	35,2	11,2	13,4	4,90	29,2	<1	<0.1	18,1
U-NV 3 HM	502	4,1	34,4	11,0	14,2	4,74	27,8	<1	<0.1	17,7
U-PART 1	510	6,1	40,8	11,4	18,7	5,22	33,8	<1	<0.1	23,1

HM										
U-PART 2 HM	564	7,8	45,1	12,2	18,7	5,76	37,2	<1	<0.1	24,3
U-PART 3 HM	570	10,3	51,7	11,9	20,7	5,52	44,9	<1	<0.1	29,1
E-PART 1 HM	527	5,3	37,0	10,9	15,2	4,61	30,7	<1	<0.1	20,2
E-PART 2 HM	500	6,3	40,7	10,7	16,7	5,16	33,8	<1	<0.1	23,5
E-PART 3 HM	503	5,8	37,6	11,1	14,3	4,35	31,3	<1	<0.1	20,7
E-NV 1 HM	536	6,6	42,2	10,7	15,7	5,99	36,7	<1	<0.1	24,2
E-NV 2 HM	541	7,0	44,1	11,6	18,2	5,92	36,7	<1	<0.1	24,4
E-NV 3 HM	529	4,5	34,7	12,0	14,6	4,85	28,7	<1	<0.1	17,9

Table C3 Metal analysis continued

Sample ID	Ba	Sr	Zr	B	Be	Li	Sc	Ce	La	Y	As
	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]
RC 8-1a	76,5	195	5,1	23	0,22	11,1	2,27	22,5	10,9	6,37	3,0
RC 8-2a	83,7	196	4,8	22	0,22	10,6	2,11	21,6	10,6	6,14	<2
RC 8-3a	98,7	209	5,1	24	0,22	11,4	2,24	23,5	11,6	6,48	2,4
RC 9-1a	114	207	5,6	25	0,24	12,6	2,48	26,7	13,3	7,17	3,0
RC 9-1a	112	213	5,4	24	0,21	12,1	2,38	24,4	11,7	6,56	2,6
RC 9-1a	171	214	5,4	25	0,24	12,3	2,41	24,2	11,7	6,56	2,7
D-NEG 1 HM	285	247	5,5	29	0,27	12,8	2,54	24,3	11,8	7,01	3,3
D-NEG 2 HM	302	208	5,3	25	0,24	11,6	2,32	23,7	11,4	6,72	3,8
D-NEG 3 HM	237	218	5,3	25	0,26	11,7	2,35	24,4	11,8	6,62	2,7
D-MRRE 1 HM	219	221	5,4	25	0,25	12,3	2,39	25,2	12,0	6,63	3,0
D-MRRE 3 HM	193	207	5,3	25	0,23	11,8	2,33	24,4	11,7	6,72	2,7
D-MRRE 2 HM	177	199	5,3	24	0,22	11,3	2,28	23,6	11,3	6,67	2,8
D-NV 1 HM	1110	231	5,3	24	0,22	11,4	2,28	22,3	11,5	6,66	2,5
D-NV 2 HM	1380	236	5,3	24	0,22	11,2	2,29	23,6	11,6	6,64	2,9
D-NV 3 HM	1140	228	4,9	22	0,22	10,5	2,12	21,6	10,6	6,22	2,4
D-PART 1 HM	2630	278	6,2	26	0,25	13,5	2,66	25,7	12,9	7,25	3,2
D-PART 2 HM	8840	498	7,1	34	0,25	13,9	2,83	26,0	14,1	7,52	4,0
D-PART 3 HM	7280	409	6,8	23	0,21	13,2	2,60	27,4	14,5	7,09	3,0
D-POS 1 HM	8160	543	19,3	79	0,39	24,8	4,96	39,5	21,2	10,5	6,1
D-POS 1THC	7960	1370	6,4	18	0,27	4,69	1,20	13,3	7,71	4,72	2,6
U-NV 1 HM	191	256	5,7	28	0,26	14,7	2,63	24,7	12,0	7,22	2,9
U-NV 2 HM	178	228	5,7	25	0,23	13,1	2,60	25,5	12,3	6,95	2,2

U-NV 3 HM	211	226	5,7	25	0,23	13,1	2,50	24,6	11,9	7,01	<2
U-PART 1 HM	1350	227	7,8	26	0,25	15,8	3,02	28,8	14,2	7,58	3,2
U-PART 2 HM	1080	231	9,0	29	0,29	18,1	3,39	33,1	16,2	8,74	3,6
U-PART 3 HM	3370	271	12,1	32	0,32	21,5	3,96	35,7	18,0	9,43	4,6
E-PART 1 HM	1530	228	7,4	26	0,25	14,0	2,81	27,0	13,4	7,42	3,1
E-PART 2 HM	1690	253	8,5	29	0,25	15,7	3,09	28,3	14,4	7,85	3,3
E-PART 3 HM	1790	249	7,6	28	0,24	14,6	2,82	26,7	13,5	7,55	2,6
E-NV 1 HM	225	144	8,9	27	0,30	16,6	3,23	31,8	15,6	8,18	3,5
E-NV 2 HM	261	179	9,3	29	0,33	18,0	3,43	31,9	15,8	8,61	2,5
E-NV 3 HM	574	203	5,7	26	0,24	13,0	2,54	26,1	13,3	7,30	3,2