Queen Mary University of London

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SCHOOL OF BIOLOGICAL AND CHEMICAL SCIENCES

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ETNP (Eastern Tropical North Pacific); 28th December 2013- 10th January 2014

Title: Nitrous oxide and nitrogen gas production in the ETNP – a process and community based study.

Cruise Report

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Nitrous oxide and nitrogen gas production in the ETNP – a process and community based study.

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Reference: Queen Mary University of London; School of Biological and Chemical Sciences; JC097 Cruise Report

Abstract: The Eastern Tropical North Pacific (ETNP) oxygen minimum zone (OMZ) is the largest zone of oxygen depleted waters in the global ocean. Nevertheless, little is known about what governs nitrogen release from these waters. This is the second cruise we undertake in the ETNP, following our first cruise (D373) and, as opposed to the offshore sites of the first cruise, this time we sampled seawater from sites closer to the coast line (off the west coast of Guatemala). The overall aim of the project is to identify the key processes of N_2 and N_2O release in the atmosphere from the waters of the OMZ of ETNP, both offshore and inshore. Firstly, we perform high resolution water profiles (nutrient and gases), to fully characterise the water column of this area of the ocean. Subsequently, we carry out experimental manipulations to measure the production of N_2 and N_2O via processes such as denitrification and anammox, from both the water column and seabed sediments. In addition, samples were obtained for molecular analysis of the communities involved in the metabolism of these gases. Understanding these processes will help us to predict the conditions under which N_2O accumulates and will further clarify the mechanisms of fine-tuning of the nitrogen cycle in the global ocean. Moreover, we set up experiments for the measurement of methane production and consumption from the water column and sediments, which will offer insights into the main sinks and sources of methane in the ocean.

Keywords: OMZ, ETNP, JC097 research cruise, nitrous oxide, nitrogen, stable isotopes, methane oxidation, anaerobic methane oxidation, methane production, N cycle, C cycle

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1. Cruise personnel

The list of the ship's crew and scientific personnel is given in the table below.

Table 1. List of cruise personnel.

Name	Role	Organisation
Mark Trimmer	PSO	Queen Mary University of London
Ian Andrew Sanders	technician	Queen Mary University of London
Panagiota-Myrsini Chronopoulou	scientist	Queen Mary University of London
Felicity Claire Shelley	scientist	Queen Mary University of London
Victoria Warren	scientist	Queen Mary University of London
James William Pritchard	scientist	Queen Mary University of London
Michail Vitalevich Zubkov	scientist	NOC
Manuela Hartmann	scientist	NOC
Emma Louise Cavan	scientist	NOC
Dougal Mountfield	NOC technician	NOC
Alan Michael Sherring	NOC technician	NOC
Nicholas Jan Rundle	NOC technician	NOC
William Robert Platt	NOC technician	NOC
Mark Maltby	IT support	NOC
Peter Charles Sarjeant	Master	NERC
Philip Douglas Gauld	Chief Officer	NERC
Malcolm Harold Graves	2nd Officer	NERC
Paul Graham Munro	3rd Officer	NERC
George Grant Parkinson	Chief Engineer	NERC
Michael Murray	2nd Engineer	NERC
Michael Gerard Murren	3rd Engineer	NERC
Lawrence Porrelli	3rd Engineer	NERC
Sebastian Martin Ulbricht	ETO	NERC
Mark Alan Rogers	ETO	NERC
Paula Anne McDougall	PCO	NERC
Martin Andrew Harrison	CPOS	NERC
Philip Allison	CPOD	NERC
David Anthony Price	POD	NERC
Mark Stephen Moore	SG1A	NERC
John Hopley	SG1A	NERC
Jarrod David Welton	SG1A	NERC
David Mackenzie	SG1A	NERC
Brian Conteh	ERPO	NERC
Peter Anthony Lynch	H/CHEF	NERC
Christopher Brian Keighley	CHEF	NERC
Peter Wayne Robinson	STWD	NERC
Carl Piper	A/ STWD	NERC

2. Scientific objectives

2.1. Research background

Oxygen minimum zones (OMZs), which are characterized as O_2 deficient layers in the ocean water column, are known for their key role in global nitrogen (N) cycling and for being the main areas of nitrogen loss (as N_2 and N_2O) to the atmosphere (Paulmier and Ruiz-Pino, 2009). Hence, they have dual links to the atmosphere through the warming potential of N_2O and the effects of N regulation on carbon fixation. Until recently, however, the microbial metabolisms responsible for this flux of N_2O and N_2 remained unclear. Previously N_2O production had been ascribed to either nitrification in the oxycline, denitrification deeper in the OMZ or a coupling of both (Codispoti and Christensen, 1985; Dore et al., 1998; Naqvi et al., 1998). Using high-resolution profiles and ^{15}N isotope tracers we were the first to actually measure N_2O production in the central Arabian Sea and demonstrated that most (>95 %) of the N_2O produced could be explained by nitrite (NO_2^-) reduction to N_2O i.e., one pathway (Nicholls *et al.* 2007). However, it is not as simple as this. One pathway of N_2O formation requires some complexity to generate the high and low concentrations of N_2O characteristic of the OMZ in the central Arabian Sea. Again, our ^{15}N tracers uncovered some of this by showing that the ratio of N_2 to N_2O production during the reduction of NO_2^- ($NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) is not fixed and appears to be 'flexible'. For example, where water column N_2O concentration is high, we measured a low ratio of N_2 to N_2O production and vice versa where water column N_2O concentration was low. Although this 'flexible' ratio explains the majority of N_2O and helps redefine our understanding of N_2O production in oxygen minimum zones, why this ratio should change is unknown. In addition, despite anaerobic ammonium oxidation (anammox) accounting for a large proportion of N removal (as N_2) in other anoxic water basins (Kuypers et al., 2005; Dalsgaard et al., 2003), neither anammox nor classic denitrification could fully account for the N_2 production we measured in the Arabian Sea: our data suggest an alternative coupling potentially via organic N (Nicholls et al., 2007; Trimmer and Purdy, 2012). Having already undertaken one research cruise in the Eastern Tropical North Pacific (ETNP), with this second research cruise in the area we aim to further study N cycle across more sites of the OMZ of ETNP in both the water column and sediments.

2.2. Cruise specific objectives

The overall aim of this project is to take our previous knowledge further and study nitrogen cycle in a non- well characterized OMZ, the Eastern Tropical North Pacific (ETNP). During this cruise, which is a continuation of a cruise we undertook in December 2011- January 2012, we obtained samples from inshore sites (close to the west coast of Guatemala) and we aim to investigate what are the sinks of nitrogen close to the coast versus offshore (comparison with data derived from last ETNP cruise). This time we are looking at both the water column and seabed sediments. Our project has a dual character, i.e. looking at the processes involved in the release of nitrogen from the OMZ back to the atmosphere, and linking these processes to bacterial gene diversity and expression across a gradient of OMZ intensity in the Eastern Tropical North Pacific (ETNP). Moreover, this time we are not only looking at the release of nitrogen to the atmosphere, but also at nitrogen assimilation by microorganisms through the process of nitrogen fixation and nitrification. The particular objectives of this second ETNP cruise are summarized below.

- Characterization of the water column (gas, nutrient, sulphide and pH profiles) close to the coast of Guatemala.
- Experimental manipulations of O_2 and N_2O tensions in the water column at 6 targeted sites to test the effect of oxygen on N_2O production.

- Screening for N_2 production coupled to ^{15}N organic-N. This is to test a newly proposed pathway of N cycle, whereby the oxidation of organic-N is coupled to the reduction of NO_2^- directly to the formation of N_2 gas.
- Molecular analysis of the active communities involved in the metabolism of the above mentioned gases.
- Measurement of water column denitrification at different temperatures.
- Determination of nitrogen fixation potential in the water column at different temperatures.
- Measurement of water column nitrification.
- Measurement of N_2 production as a function of O_2 consumption in seawater samples from targeted sites.
- Measurement of denitrification in seabed slurries, after subjecting them to a range of temperatures.
- Determination of N_2 production in seabed slurries, via anammox.
- Measurement of N_2 production in intact sediment samples (cores).

In addition to N cycle, we also set up experiments to investigate aspects of the C cycle, such as methane oxidation and production processes. The overall aim is to characterise sources and sinks of methane in this part of the global ocean. Our particular objectives are given below:

- Measurement of aerobic methane oxidation in the water column of ETNP, after addition of $^{13}CH4$ in seawater from various depths across the OMZ.
- Monitoring methane production at selected depths.
- Measurement of methane production from intact sediment cores.
- Assessment of nitrite-dependent anaerobic methane oxidation in the water column.

Parallel to investigating processes of the N and C cycles, we also looked at general aspects of microbial and planktonic metabolism. In particular:

- On-board measurement of biological respiration in surface plankton samples.
- Flow cytometry of water samples.
- Determination of ^{3}H-leucine take-up in water samples under anoxic conditions.
- Measurement of carbon fixation rates in planktonic concentrated samples.
- Determination of the abundance of microplankton in seawater.
- Analysis of Chlorophyll, particulate organic carbon (POC) and lipids in water samples.
- Analysis of different water fractions for POC/N/P and lipids.

3. Cruise overview

JC097 took place between 28/12/2013 and 10/02/2014 in the waters of ETNP. The port of departure was Panama Balboa and the return port was Manzanillo, Mexico. Scientific work was conducted at 6 sites (Figure 1), the exact coordinates of which are shown in Table 2.

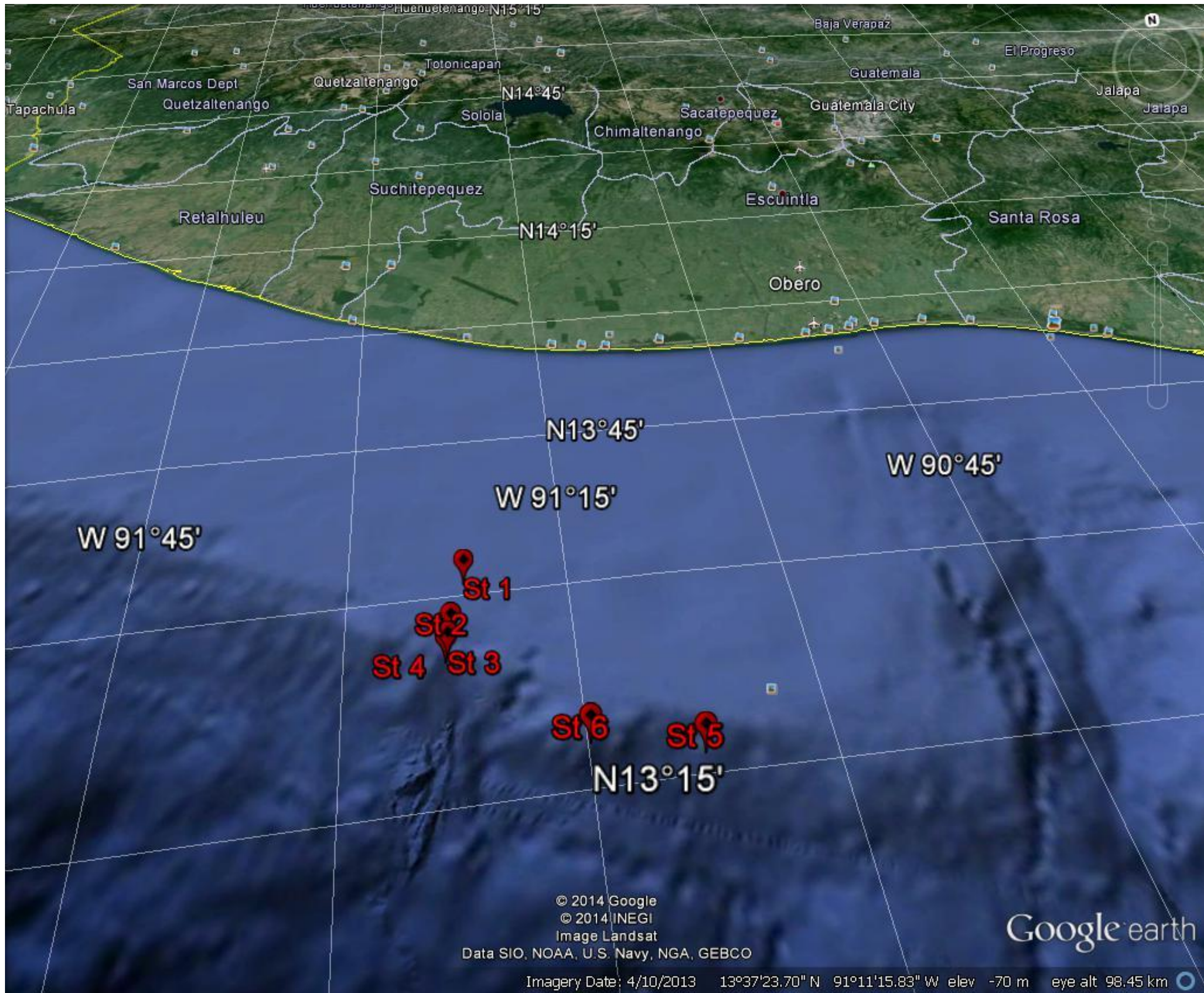


Figure 1. JC097 sampling sites. The 6 sites depicted here were the main sampling sites for most of the experiments. High resolution vertical water profiles were also obtained from in between these sites.

Table 2. Coordinates of the sampling sites.

Site	Latitude	Longitude	CTDs	Total casts
St 1	13°31'2.00"N	91°21'63.00"W	2 to 10	9
St 2	13°26'32.00"N	91°22'61.00"W	23 to 33	11
St 3	13°24'64.00"N	91°22'75.00"W	35 to 46	12
St 4	13°24'43.00"N	91°22'83.00"W	53 to 65	13
St 5	13°16'25.00"N	91°08'06.00"W	66 to 84	19
St 6	13°17'51.00"N	91°14'51.00"W	85 to 97	13

The casts not shown in the above table were either transects for surface oxygen measurements or casts only for vertical water profiles in between the sampling sites.

4. Diary cruise narrative

The following diary is based on scientific rough log sheets provided by the bridge.

<u>28-31.12.13</u>

Passage towards the first sampling site

<u>01.01.14</u>

Continuation of passage towards the first site

CTD winch problems- fix and test deployment

<u>02.01.14</u>

SWATH survey

CTD001- to 89 m and back on deck; scientists sampling for full water profile

CTD002- to 125 m and back on deck; scientists sampling for full water profile

Box corer deployment; hydraulic hose failure and repair; resumed veering (130 m) and brought back on deck.

SWATH survey

<u>03.01.14</u>

SWATH survey (continued)

Arrived at Site 1

CTD003- to 110 m and back on deck; scientists sampling 4 targeted depths

Snow Catcher to 40 m

Net in water- 60 m

Snow Catcher to 90 m

CTD004- to 115 m and back on deck; scientists sampling anoxic water from 1 depth

SWATH survey

Snow Catcher to 40 m (twice)

Small craft approaching to starboard-science suspended

Snow Catcher to 90 m

SWATH survey

<u>04.01.14</u>

Snow Catcher to 40 m (twice)

Small craft approaching to starboard-science suspended

Snow Catcher to 90 m

SWATH survey

CTD005- to 90 m and back on deck; scientists sampling 2 targeted depths

Box corer to 118 m

Box corer to 123 m

Micro-net to 60 m

Mega corer; wire 122 m

Mega corer; wire 123 m

<u>05.01.14</u>

SWATH survey

On site for CTD

CTD006- to 117 m and back on deck; scientists sampling 3 targeted depths

CTD007- SAPs (80 & 95 m)

SAPs at 85 m

Snow Catcher to 60 m

Recovery of SAPs

Snow Catcher to 80 m

Micro-net to 60 m (twice)

SAPs on board

SWATH survey

<u>06.01.14</u>

SWATH survey (continued)

Back on site

CTD008- to 115 m and back on deck; scientists sampling 5 targeted depths

Micro-net to 60 m (three times)

Mega corer; 132 m

CTD009- to 117 m and back on deck; scientists sampling to check for changes in oxygen concentration within the experimental bottles

SWATH survey

<u>07.01.14</u>

SWATH survey (continued)

CTD010- to 115 m and back on deck; scientists sampling 5 targeted depths

CTD011 to CTD019- transect for oxygen profile-no bottles fired

CTD020- to 50 m and back on deck; scientists sampling for water profiling

CTD021/CTD022- transect for oxygen profile-no bottles fired

SWATH survey

<u>08.01.14</u>

SWATH survey (continued); on the way to site 2

CTD023- to 180m and back on deck; scientists sampling for water profiling

Micro-net to 60 m

CTD024- to 9m and back on deck; scientists sampling 1 targeted depth

Micro-net to 60 m

<u>09.01.14</u>

CTD025- to 135m and back on deck; scientists sampling 2 targeted depths

Micro-net to 60 m

Snow Catcher to 60 m

Snow Catcher to 150 m

Micro-net to 60 m (twice)

CTD026- to 175m and back on deck; scientists sampling 1 targeted depth

<u>10.01.14</u>

Snow Catcher to 40 m

Snow Catcher to 150 m

Shifting back on site for CTD

CTD027- to 175m and back on deck; scientists sampling 7 targeted depths

Micro-nets and mega corers alternating for the rest of the day

CTD028- to 150 m and back on deck; scientists sampling 2 targeted depths, matching mega corers depths

<u>11.01.14</u>

CTD029- to 135 m and back on deck; scientists sampling 2 targeted depths

CTD030- SAPs (125 & 140 m)

CTD031- SAPs (135 & 152 m)

Snow Catcher to 120 m

Recovery of first set of SAPs and CTD back on deck

Micro-net to 60 m

Recovery of second set of SAPs and CTD

<u>12.01.14</u>

CTD032- to 135 m and back on deck; scientists sampling 4 targeted depths

Micro-net to 60 m

CTD033- to 135 m and back on deck; scientists sampling 3 targeted depths

Micro-net test

Micro-net to 60 m

Micro-net to 155 m

Move to max seabed 900 m

CTD034- to 900 m and back on deck; scientists sampling for water profiling

<u>13.01.14</u>

Mega corer to 912 m

Mega corer to 975 m

Micro-net to 60 m

Micro-net to 110 m

Mega corer to 450 m

Micro-net

Mega corer to 355 m

Micro-net to 200 m

Mega corer to 140 m

Mega corer to 110 m

14.01.14

Repositioning the vessel; arrived at site 3

CTD035- to 365 m and back on deck; scientists sampling for water profiling

CTD036- to 205 m and back on deck; scientists sampling for water profiling

Micro-net to 350 m

Repositioning the vessel for micro-net

Micro-net to 60 m

Micro-net to 200 m

15.01.14

Repositioning the vessel towards sampling site

CTD037- to 250 m and back on deck; scientists sampling 5 targeted depths

Micro-net to 60 m

Snow Catcher to 200 m

Micro-net to 60 m

CTD038- to 380 m and back on deck; scientists sampling 1 targeted depth

16.01.14

Snow Catcher to 220 m

Repositioning the vessel; back on site

Snow Catcher to 50 m

Repositioning the vessel; back on site

Assessing fishing activity; decision made to remain on site

CTD039- to 373 m and back on deck; scientists sampling 4 targeted depths

Micro-net to 60 m

Mega corer to 370 m

Mega corer to 375 m

Mega corer to 375 m (twice)

Mega corer to 400 m

Micro-net to 60 m

Move towards 5,339 m contour

CTD040- to 5315 m and back on deck; scientists sampling for water profiling

<u>17.01.14</u>

Repositioning the vessel; back on site

CTD041- to 360 m and back on deck; scientists sampling 4 targeted depths

Snow Catcher

CTD042- SAPs (125 & 195 m)

Snow Catcher to 170 m

Recovery of SAPs; CTD on deck

CTD043- SAPs (135 & 235 m)

Recovery of SAPs; CTD on deck

<u>18.01.14</u>

CTD044- to 235 m and back on deck; scientists sampling 8 targeted depths

Micro-net

Repositioning vessel to 3,000 m seabed

CTD045- to 3010 m and back on deck; scientists sampling for water profiling

Micro-net to 60 m

Micro-net to 300 m

Repositioning vessel for sea-soar

Sea-soar deployed to 700 m; sailing at 4 Kt; sea-soar recovered

CTD046- to 235 m and back on deck; scientists sampling 11 targeted depths

Micro-net

<u>19.01.14</u>

Manoeuvring to avoid fishing marks

On CTD station (off site 3)

CTD047- to 485 m and back on deck; scientists sampling for water profiling

CTD048- to 585 m and back on deck; scientists sampling for water profiling

CTD049- to 692 m and back on deck; scientists sampling for water profiling

CTD050- to 882 m and back on deck; scientists sampling for water profiling

CTD051- to 1497 m and back on deck; scientists sampling for water profiling

<u>20.01.14</u>

Repositioning the vessel towards Mega Corer sampling station

Mega corer to 480 m

Micro-net

Micro-net to 200 m

Micro-net to 80 m

Micro-net to 100 m

Micro-net to 300 m

Manoeuvring to avoid fishing marks

Repositioning to sampling site

CTD052- to 450 m and back on deck; scientists sampling 3 targeted depths

Micro-net

<u>21.01.14</u>

Approaching site 4

CTD053- to 300 m and back on deck; scientists sampling 3 targeted depths

Micro-net to 60 m

Repositioning to 1000 m seabed

CTD054- to 350 m and back on deck; scientists sampling 5 targeted depths

Micro-net to 60 m

Snow Catcher to 40 m

Snow Catcher to 350 m

Micro-net to 60 m

Fishing vessel observing; fishing vessel clear

Micro-net to 60 m

CTD055- to 600 m and back on deck; scientists sampling 2 targeted depths

CTD056- to 430 m and back on deck; scientists sampling 1 targeted depth

<u>22.01.14</u>

Snow Catcher to 40 m

Snow Catcher to 350 m

Vessel moving to 500 m site

CTD057- to 430 m and back on deck; scientists sampling 6 targeted depths

Micro-net

Mega corer to 690 m

Mega corer to 690 m

Mega corer to 495 m

Mega corer to 200 m

Mega corer to 465 m

Mega corer to 430 m

Long line from fishing vessel fouled in ship's bow area

Mega corer to 505 m

Mega corer to 397 m

Micro-net

CTD058- to 400 m and back on deck; scientists sampling 2 targeted depths

Manoeuvring awaiting indication from fishing V/L

<u>23.01.14</u>

Diversion to avoid fishing marks

Heading towards Puerto Quetzal to peak up equipment parts, navy observer and divers to detach fishing nets from the ship's propeller

Diversion to avoid fishing lines

Return to track

CTD059- SAPs (200 & 215 m) and fire Niskins at 5 targeted depths

<u>24.01.14</u>

Stern thruster and starboard propeller isolated

EK60 Science in progress

Proceed to CTD site

CTD060- to 466 m and back on deck; scientists sampling 10 targeted depths

Micro-net

CTD061- to 225 m and back on deck; scientists sampling 2 targeted depths

Snow Catcher to 120 m

Micro-net to 350 m

Micro-net to 200 m

Micro-net to 100 m

V/L repositioning

Micro-net to 350 m

CTD062- SAPs (120 & 225 m) and fire Niskins at 5 targeted depths

Recovery of SAPs and CTD

SWATH survey

<u>25.01.14</u>

Proceed to Puerto Quetzal to drop off the C/Chef that got silk

Reposition to site

CTD063- to 500 m and back on deck; scientists sampling for water profiling

CTD064- to 330 m and back on deck; scientists sampling for water profiling

CTD065- to 480 m and back on deck; scientists sampling for water profiling

<u>26.01.14</u>

SWATH survey

Reach site 5

Mega corer to 475 m

Mega corer to 433 m

Mega corer to 620 m

Mega corer to 520 m

Mega corer to 397 m

Micro-net

Mega corer to 210 m

Mega corer to 584 m

Snow Catcher to 40 m

Closing net to 150 m

Mega corer to 581 m

Mega corer to 667 m

Mega corer to 320 m

SWATH survey

27.01.14

SWATH survey

CTD066- to 350 m and back on deck; scientists sampling 6 targeted depths

Micro-net

Repositioning to 2000 m seabed

CTD067- to 1970 m and back on deck; scientists sampling 5 targeted depths

Snow Catcher to 70 m

Snow Catcher to 350 m

CTD068- to 500 m and back on deck; scientists sampling 1 targeted depth

CTD069- to 490 m and back on deck; scientists sampling 1 targeted depth

CTD070- to 1998 m and back on deck; scientists sampling 6 targeted depths

Micro-net

Repositioning to 2000 m sampling station

28.01.14

Repositioning to 2000 m seabed

Snow Catcher to 70 m

Snow Catcher to 350

Proceed to next station

CTD071- to 712 m and back on deck; scientists sampling 7 targeted depths

Micro-net

Mega corer to 600 m (twice)

Mega corer to 620 m

Snow Catcher to 360

Mega corer to 660 m

Mega corer to 648 m

Mega corer to 630 m

Snow Catcher to 120

CTD072- to 415 m and back on deck; scientists sampling 1 targeted depth

CTD073- to 390 m and back on deck; scientists sampling 1 targeted depth
CTD074- to 395 m and back on deck; scientists sampling 1 targeted depth
EK60 survey overnight

29.01.14
Complete EK60 survey
Reposition
CTD075- to 350 m and back on deck; scientists sampling 6 targeted depths
CTD076- SAPs (90 & 220 m)
Recovery of SAPs/CTD
Snow Catcher to 220
Snow Catcher to 350
CTD077- SAPs (332 & 388 m) and Niskin fired at 2 targeted depths
Recovery of SAPs/CTD
CTD078- to 485 m and back on deck; scientists sampling 1 targeted depth
SWATH survey

30.01.14
SWATH survey complete
CTD079- to 500 m and back on deck; scientists sampling 6 targeted depths
Micro-net
Snow Catcher to 60 m
CTD080- to 388 m and back on deck; scientists sampling 5 targeted depths
Closing net to 150 m
Closing net to 350 m
CTD081- to 60 m and back on deck; scientists sampling 1 targeted depth
CTD082- to 60 m and back on deck; scientists sampling 6 targeted depths
SWATH survey

31.01.14
SWATH survey (continue)
CTD hydraulic hose problem
Snow Catcher
CTD problem resolved
Snow Catcher to 270 m
CTD083- to 150 m and back on deck; scientists sampling 4 targeted depths
CTD084- to 490 m and back on deck; scientists sampling for water profiling
Mega corer to 500 m
Mega corer to 565 m
Mega corer to 520 m

Repositioning V/L

Mega corer to 500 m

SWATH survey

01.02.14

SWATH survey (continue)

Reaching site 6

CTD085- to 150 m and back on deck; scientists sampling 5 targeted depths

Snow Catcher to 205 m

CTD086- to 350 m and back on deck; scientists sampling for water profiling

Snow Catcher to 100 m

CTD087- to 144 m and back on deck; scientists sampling for water profiling

V/L on station for mega core

Mega corer to 351 m

Mega corer to 350 m

Mega corer to 364 m

02.02.14

Repositioning V/L back to sampling site 6

CTD088- to 150 m and back on deck; scientists sampling 4 targeted depths

Snow Catcher

Repositioning V/L to 3000 m seabed

CTD089- to 1500 m and back on deck; scientists sampling 4 targeted depths

Snow Catcher (twice)

Repositioning V/L to 500 m seabed

CTD090- to 120 m and back on deck; scientists sampling for water profiling

CTD091- SAPs (65 & 228 m) and Niskin fired at 4 targeted depths

Snow Catcher at 3000 m seabed site

03.02.14

Repositioning V/L to 500 m seabed

CTD092- to 350 m and back on deck; scientists sampling 11 targeted depths

04.02.14

CTD093- to 220 m and back on deck; scientists sampling 7 targeted depths

CTD094- SAPs (80 & 220 m) and Niskin fired for water profiling

05.02.14

CTD095- Niskin valves did not seal

CTD096- SAPs (132 & 85 m) and Niskin fired at targeted depths

Snow Catcher (twice)

Repositioning V/L to 150 m seabed

Mega corer to 150 m

Repositioning V/L to 550 m seabed

Mega corer to 550 m

Repositioning V/L for CTD

CTD097- to 1000 m and back on deck; scientists sampling 7 targeted depths

Steer to sea-soar site

06.02.14

Se-soar for the whole day as we make our way to Guatemala to drop off the navy observer

07-10.02.14

Heading towards port of Manzanillo/Mexico

5. Sampling sites activities log

Below is a log of all the sampling activities per site.

Site 1

The activities that took place at site 1, and the exact co-ordinates and times are shown in Table 3.

Table 3. Activities log- Site 1.

Activity ID	Lat(N)	Long(W)	Date	Start time	Description
CTD002	13 31.022'	91 21.62'	02/01/2014	16:57	Vertical seawater profiles, 2-117 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, pH, H_2S
CTD003	13 31.02'	91 21.626'	03/01/2014	14:07	Targeted depths 20, 60, 107 m, Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by precon in the lab for ^{15}N2O gas production and water samples taken for filtration and molecular analysis back in the lab; flow cytometry
CTD004	13 30.82'	91 21.92'	04/01/2014	00:03	Targeted depths 115 m; water collection for IPT experiment
CTD005	13 31.01'	91 21.67'	04/01/2014	11:50	Targeted depths 20, 90 m; flow cytometry
CTD006	13 31.00'	91 21.64'	05/01/2014	08:06	Targeted depths 20, 85, 95 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab; flow cytometry
CTD007	13 31.05'	91 21.60'	05/01/2014	17:09	SAPs (80, 95 m & 85, 105 m)
CTD008	13 31.01'	91 21.68'	06/01/2014	11:54	Targeted depths 75, 90, 95, 115 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab; flow cytometry
CTD009	13 31.00'	91 21.73'	06/01/2014	00:10	Targeted depth 117 m; check oxygen levels in experimental botttles
CTD010	13 31.03'	91 21.61'	07/01/2014	11:58	Targeted depths 75, 85, 90, 95, 115 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production

Times are in GMT.

Site 2

The activities that took place at site 2, and the exact co-ordinates and times are shown in Table 4.

Table 4. Activities log- Site 2.

Activity ID	Lat(N)	Long(W)	Date	Start time	Description
CTD023	13 26.31'	91 22.50'	08/01/2014	15:50	Vertical seawater profiles, 9-175 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, pH, H_2S
CTD024	13 26.34'	91 22.53'	08/01/2014	00:19	Targeted depth 9 m, water collection for N_2 fixation experiments
CTD025	13 26.32'	91 32.56'	09/01/2014	07:54	Targeted depths 50, 135 m, Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by precon in the lab for ^{15}N2O gas production and water samples taken for filtration and molecular analysis back in the lab
CTD026	13 26.33'	91 22.51'	10/01/2014	00:20	Targeted depths 174 m; water collection for IPT experiment
CTD027	13 26.32'	91 22.58'	10/01/2014	11:49	Targeted depths 30, 40, 50, 60 m; flow cytometry
CTD028	13 26.31'	91 22.54'	10/01/2014	00:02	Targeted depths 40, 157 m; water collection for analysis of POC/N/P and lipids
CTD029	13 26.31'	91 22.51'	11/01/2014	13:45	Targeted depths 60, 125, 135 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab; flow cytometry
CTD030	13 26.32'	91 22.51'	11/01/2014	17:02	SAPs (125, 140 m)
CTD031	13 26.32'	91 22.49'	11/01/2014	22:36	SAPs (135, 152 m)
CTD032	13 26.32'	91 22.50'	12/01/2014	11:48	Targeted depths 20, 50, 60, 120 m; flow cytometry
CTD033	13 26.32'	91 22.50'	12/01/2014	12:51	Targeted depths 140, 145, 155 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab

Times are in GMT.

Site 3

The activities that took place at site 3, and the exact co-ordinates and times are shown in Table 5.

Table 5. Activities log- Site 3.

Activity ID	Lat(N)	Long(W)	Date	Start time	Description
CTD035	13 24.68'	91 22.69'	14/01/2014	12:38	Vertical seawater profiles, 135-365 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, pH, H_2S; flow cytometry
CTD036	13 24.64'	91 22.75'	14/01/2014	15:20	Vertical seawater profiles, 5-205 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, pH, H_2S; flow cytometry
CTD037	13 24.64'	91 22.75'	15/01/2014	14:00	Targeted depths 90, 125, 225, 235, 245 m, Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by precon in the lab for ^{15}N2O gas production and water samples taken for filtration and molecular analysis back in the lab; flow cytometry; leucine uptake
CTD038	13 24.64'	91 22.73'	16/01/2014	00:13	Targeted depths 380 m; water collection for IPT experiment
CTD039	13 24.64'	91 22.74'	16/01/2014	11:54	Targeted depths 20, 45, 50, 220 m; flow cytometry
CTD040	12 58.20'	91 26.75'	16/01/2014	23:06	Vertical seawater profiles, 200-5310 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis
CTD041	13 24.67'	91 22.71'	17/01/2014	14:18	Targeted depths 115, 125, 235, 245 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab; leucine uptake; N-DAMO
CTD042	13 24.64'	91 22.76'	17/01/2014	17:03	SAPs (125, 195 m)

CTD043	13 24.67'	91 22.73'	17/01/2014	22:17	SAPs (135, 235 m)
CTD044	13 24.64'	91 22 73'	18/01/2014	11:57	Targeted depths 20, 60, 125, 135,, 170, 195, 235 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab; flow cytometry; water collection for analysis of POC/N/P and lipids
CTD045	13 09.92'	91 24 97'	18/01/2014	17:17	Vertical seawater profiles, 45-3000 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis
CTD046	13 06.07'	91 13.29'	19/01/2014	00:20	Targeted depths 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 m, water collection for N_2 fixation experiments; flow cytometry

Times are in GMT.

Site 4

The activities that took place at site 4, and the exact co-ordinates and times are shown in Table 6.

Table 6. Activities log- Site 4.

Activity ID	Lat(N)	Long(W)	Date	Start time	Description
CTD053	13 24.43'	91 22.83'	21/01/2014	12:30	Targeted depths 60, 185, 250; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by precon in the lab for ^{15}N2O gas production and water samples taken for filtration and molecular analysis back in the lab; leucine uptake
CTD054	13 21.28'	91 22.78'	21/01/2014	15:10	Targeted depths 15, 20, 65, 105, 350 m; water collection for analysis of POC/N/P and lipids
CTD055	13 21.23'	91 22.81	21/01/2014	20:14	Targeted depths 25, 500 m, collection of water to test methane production; biological respiration measurement
CTD056	13 24.44'	91 22.77'	22/01/2014	00:08	Targeted depths 380 m; water collection for IPT experiment
CTD057	13 24.44'	91 22.80'	22/01/2014	11:46	Targeted depths 5, 10, 20, 30, 40, 50 m; flow cytometry
CTD058	13 24.04'	91 24.04'	23/01/2014	01:17	Targeted depths 25, 400 m, collection of water to test methane production; biological respiration measurement
CTD059	13 21.20'	91 22.68'	23/01/2014	23:12	Targeted depths 195, 200, 205, 215 m; SAPs (200, 215 m), aerobic methane oxidation
CTD060	13 24.44'	91 22.82'	24/01/2014	11:45	Targeted depths 2, 10, 20, 30, 40, 50, 110, 120, 130, 250 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab; flow cytometry; leucine uptake
CTD061	13 24.44'	91 22.82'	24/01/2014	14:04	Targeted depths 185, 225 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab
CTD062	13 24.52'	91 22.69'	24/01/2014	22:50	SAPs (120, 225 m)
CTD063	13 16.28'	91 08.06'	25/01/2014	20:37	Vertical seawater profiles, 316-500 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis
CTD064	13 16.31'	91 08.05'	25/01/2014	22:44	Vertical seawater profiles, 140-324 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis
CTD065	13 16.31'	91 08.04'	26/01/2014	00:52	Vertical seawater profiles, 4-148 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis; collection of water samples for methane cryofocusing

Times are in GMT.

Site 5

The activities that took place at site 5, and the exact co-ordinates and times are shown in Table 7.

Table 7. Activities log- Site 5.

Activity ID	Lat(N)	Long(W)	Date	Start time	Description
CTD066	13 16.25'	91 08.06'	27/01/2014	12:11	Targeted depths 20, 40, 52, 90, 132, 228, 350; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by precon in the lab for ^{15}N2O gas production and water samples taken for filtration and molecular analysis back in the lab; flow cytometry; leucine uptake
CTD067	13 13.72'	91 17.90'	27/01/2014	15:40	Targeted depths 50, 60, 200, 350, 1965 m; water collection for analysis of POC/N/P and lipids
CTD068	13 17.527'	91 10.643'	27/01/2014	21:38	Targeted depths 490 m; water samples for methane cryofocusing
CTD069	13 17.642'	91 20.186'	27/01/2014	22:37	Targeted depths 487 m; water samples for methane cryofocusing
CTD070	13 10.449'	91 12.485'	28/01/2014	00:10	Targeted depths 10, 20, 30, 40, 50, 60 m; DIN skalar analysis; water for N_2 fixation
CTD071	13 16.11'	91 08.52'	28/01/2014	11:48	Targeted depths 5, 10, 20, 30, 40, 50, 712 m; flow cytometry, water collection for IPT experiment
CTD072	13 16.875'	91 07 97'	28/01/2014	22:45	Targeted depths 415 m; collection of water to target methane
CTD073	13 16 933'	91 08.102'	29/01/2014	00:10	Targeted depths 390 m; collection of water to target methane
CTD074	13 16.945'	91 07.973'	29/01/2014	01:32	Targeted depths 395 m; collection of water to target methane
CTD075	13 16 29'	91 07.94'	29/01/2014	12:19	Targeted depths 67, 90, 132, 205, 220, 350 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab; leucine uptake
CTD076	13 16.32'	91 07.82'	29/01/2014	14:16	SAPs (90, 220 m)
CTD077	13 16.38'	91 08.07'	29/01/2014	21:37	SAPs (90, 220 m); Targeted depths 332, 412 m for N-DAMO
CTD078	13 17.13'	91 09.07'	30/01/2014	01:00	Targeted depths 484 m; collection of water to target methane
CTD079	13 16.29'	91 08.06'	30/01/2014	11:50	Targeted depths 5, 10, 15, 20, 25, 30 m; flow cytometry
CTD080	13 16.217'	91 08.113'	30/01/2014	15:59	Targeted depths 47, 67, 90, 226, 388 m; aerobic methane oxidation; methanogenesis
CTD081	13 16.240'	91 08.031'	30/01/2014	22:47	Targeted depth 40 m; collection of water to monitor oxygen consumption and spike with 15N isotopes to measure $^{15}N_2$ production back in the lab
CTD082	13 13.281'	91 07.977'	31/01/2014	00:15	Targeted depths 7, 10, 20, 30, 40, 50, 60 m; DIN skalar analysis; water for N_2 fixation
CTD083	13 16.26'	91 07.96'	31/01/2014	19:23	Targeted depth 45, 50, 60, 67 m; collection of water to monitor oxygen consumption and spike with 15N isotopes to measure $^{15}N_2$ production back in the lab
CTD084	13 16.27'	91 08.03'	31/01/2014	21:13	Vertical seawater profiles, 304-488 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis

Times are in GMT.

Site 6

The activities that took place at site 6, and the exact co-ordinates and times are shown in Table 8.

Table 8. Activities log- Site 6.

Activity ID	Lat(N)	Long(W)	Date	Start time	Description
CTD085	13 16.25'	91 08.04'	01/02/2014	11:54	Targeted depth 28, 60, 67, 70, 74 m; collection of water to monitor oxygen consumption and spike with 15N isotopes to measure $^{15}N_2$ production back in the lab
CTD086	13 16.24'	91 08.03'	01/02/2014	15:11	Vertical seawater profiles, 136-350 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis
CTD087	13 16.23'	91 08.05'	01/02/2014	17:16	Vertical seawater profiles, 8-144 m: Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis
CTD088	13 17.51'	91 14.51'	02/02/2014	12:01	Targeted depths 90, 132, 220, 350; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by precon in the lab for ^{15}N2O gas produ

					ction and water samples taken for filtration and molecular analysis back in the lab; leucine uptake
CTD089	13 04.18'	91 11.52'	02/02/2014	15:26	Targeted depths 10, 20, 40, 100, 350 m; water collection for analysis of POC/N/P and lipids
CTD090	13 16.18'	91 07.97'	02/02/2014	21:24	Vertical seawater profiles, 25-120 m: DIN skalar analysis
CTD091	13 16.21'	91 07.95'	02/02/2014	23:08	Targeted depths 65, 228, 256, 264 m; aerobic methane oxidation; methanogenesis; SAPs(65, 228 m)
CTD092	13 16.20'	91 07.16'	03/02/2014	11:45	Targeted depth 5, 10, 120, 27, 40, 67, 70, 74, 120, 220, 350 m; collection of water to monitor oxygen consumption and spike with 15N isotopes to measure $^{15}N_2$ production back in the lab; flow cytometry; leucine uptake
CTD093	13 16.25'	91 07.97'	04/02/2014	12:01	Targeted depths 67, 90, 132, 205, 220, 350 m; Isotopes added to seawater samples/ samples incubated on-board/ to be analysed by mass spectrometry in the lab for $^{15}N_2$ gas production and water samples taken for filtration and molecular analysis back in the lab; collection of water to monitor oxygen consumption and spike with 15N isotopes to measure $^{15}N_2$ production back in the lab; water for nitrification experiments
CTD094	13 16.27'	91 07.99'	04/02/2014	14:12	Vertical seawater profiles, 136-350 m: DIN skalar analysis
CTD095	13 13.29'	91 08.04'	05/02/2014	CTD0 95	failed
CTD096	13 16.243'	91 07.982'	05/02/2014	14:04	Targeted depth 74, 80 m; collection of water to monitor oxygen consumption and spike with 15N isotopes to measure $^{15}N_2$ production back in the lab; flow cytometry; SAPs (85, 132 m)
CTD097	13 13.44'	91 10.08'	05/02/2014	23:13	Targeted depths 92, 120, 350, 500, 700, 900, 1000 m; flow cytometry; leucine uptake

Times are in GMT.

6. Operations

Water samples mentioned in this section derived from deployments of the following equipment: 20 L Niskin bottles on a CTD rosette Sea-Bird 24, snow catcher, micro-net and closing net. *In situ* filtration of seawater was done with Stand Alone Pumps (SAPs). Sediment samples derived from deployments of a seabed coring system (mega corer) fitted with 6 cores. CTD sensors were calibrated and maintained by the responsible NOC technicians. Log sheets with all the relevant details are available.

6.1. Vertical water profiles

Introduction

For the purposes of the current project it was essential to have a good knowledge of how the concentrations of oxygen, DIN and DIC change at the various depths and across the width of the area under investigation. During our first ETNP cruise we did high resolution vertical water profiles well offshore the Guatemalan coast. With this second cruise we wish to target sampling sites much closer to the coast line. For this reason, we used several CTD casts at each sampling site and in between the sites with a view to gaining insights into the water characteristics of the area. This allowed us to have an overview of the concentrations of O_2, PO_4^{3-}, NO_2^-, NO_3^-, NH_4^+, CO_2, CH_4, N_2O from the surface to the bottom of each station, and based on these profiles and the rest of CTD parameters (e.g. density, chlorophyll content) we decided the exact depths from which we sampled for the planned experimental manipulations.

Materials &Methods

Concentrations of NO_3^-, NO_2^-, $PO_4^{3-}$and NH_4^+ were measured using a segmented flow auto analyser (Skalar, Netherlands) and standard colorimetric techniques in duplicate samples of unfiltered water (15 mL), collected in PTFE bottles directly from the Niskin bottles (after overfilling the former 3 times).

The distribution of N_2O, CH_4 and CO_2 in the water column was determined by sub-sampling water from a Niskin bottle into the bottom of exetainers (12 ml glass vials) and allowing it to overflow at least

three times before sealing. Headspace concentrations were measured after equilibration with analytical grade helium and injection into an Agilent 6890 gas chromatograph equipped with parallel µECD and FID (Nicholls et al., 2007).

Preliminary Results

The concentrations of DIN (NO_3^-, NO_2^- and NH_4^+) and the N deficit derived from CTD casts on and in between the sampling sites (17 high resolution vertical water profiles in total). The exact coordinates of these profiles are shown in Table 9 and the data obtained in Figure 2. N deficit (representing the amount of fixed nitrogen that has been removed, i.e. due to denitrification) was calculated according to the equation $16 \times PO_4^{3-} - [(NO_3^- + NO_2^- + NH_4^+)]$ - (Gruber and Sarmiento, 1997; Chang et al., 2010).

All measurements were made on duplicate samples using a Skalar auto-analyser. Calibration of the instrument was conducted based on known amount of standard solutions.

The concentrations of gases (N_2O, CH_4 and CO_2) were measured using a GC/ECD (for N_2O) or GC/FID (for CH_4 and CO_2). Calculations were based on the concentrations of a standard gas mixture (supplied by BOC), containing a known amount of all the analysed gases. A standard gas sample was run with each batch of samples to be measured. Oxygen data was obtained from the CTD profile casts. Results are shown in Figure 3.

Table 9. Coordinates of the CTD casts for the high-resolution vertical water profiles.

Profile ID	CTD	Lat(N)	Long(W)	Date	Start time	Depth (m)
1	CTD001	13 35.40'	91 20.96'	02/01/2014	14:08	1-89
2	CTD002	13 31.022'	91 21.62'	02/01/2014	16:57	2-117
3	CTD020	13 41.64'	91 20.19'	07/01/2014	23:06	2-46
4						9-175
	CTD023	13 26.31'	91 22.50'	08/01/2014	15:50	
5	CTD034	13 22.66'	91 22.91'	13/01/2014	00:19	4-833
6	CTD035	13 24.68'	91 22.69'	14/01/2014	12:38	135-365
6	CTD036	13 24.64'	91 22.75'	14/01/2014	15:20	5-205
7	CTD040	12 58.20'	91 26.75'	16/01/2014	23:06	200-5310
8	CTD045	13 09.92'	91 24 97'	18/01/2014	17:17	45-3000
9	CTD047	13 24.44'	91 22.77'	19/01/2014	13:55	25-485
10	CTD048	13 24.10'	91 22.80'	19/01/2014	16:46	25-580
11	CTD049	13 23.57'	91 22.85'	19/01/2014	19:38	10-692
12	CTD050	13 21.65'	91 23.19'	19/01/2014	22:24	22-882
13	CTD051	13 18.68'	91 23.73'	20/01/2014	00:49	50-1492
14	CTD063	13 16.28'	91 08.06'	25/01/2014	20:37	316-500
14	CTD064	13 16.31'	91 08.05'	25/01/2014	22:44	140-324
14	CTD065	13 16.31'	91 08.04'	26/01/2014	00:52	4-480
15	CTD084	13 16.27'	91 08.03'	31/01/2014	21:13	304-488
15	CTD085	13 16.25'	91 08.04'	01/02/2014	11:54	28-74
15	CTD086	13 16.24'	91 08.03'	01/02/2014	15:11	136-350
16	CTD094	13 16.27'	91 07.99'	04/02/2014	14:12	7-122
17	CTD090	13 16.18'	91 07.97'	02/02/2014	21:24	5-120

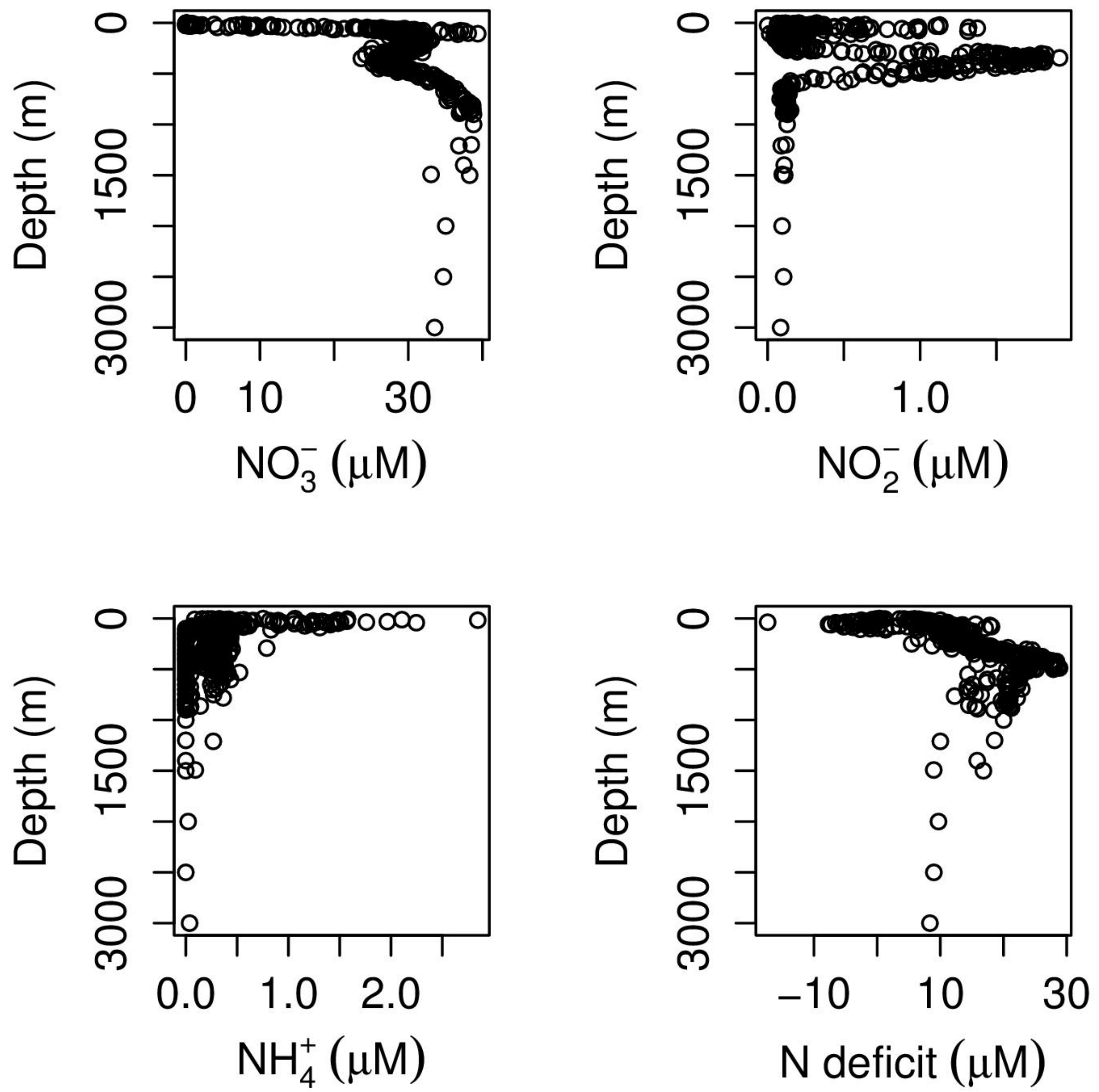


Figure 2. Concentration of dissolved inorganic nitrogen (DIN) in the water column. The data shown here has derived from 17 different high resolution profiles at various sampling sites off the west coast of Guatemala.

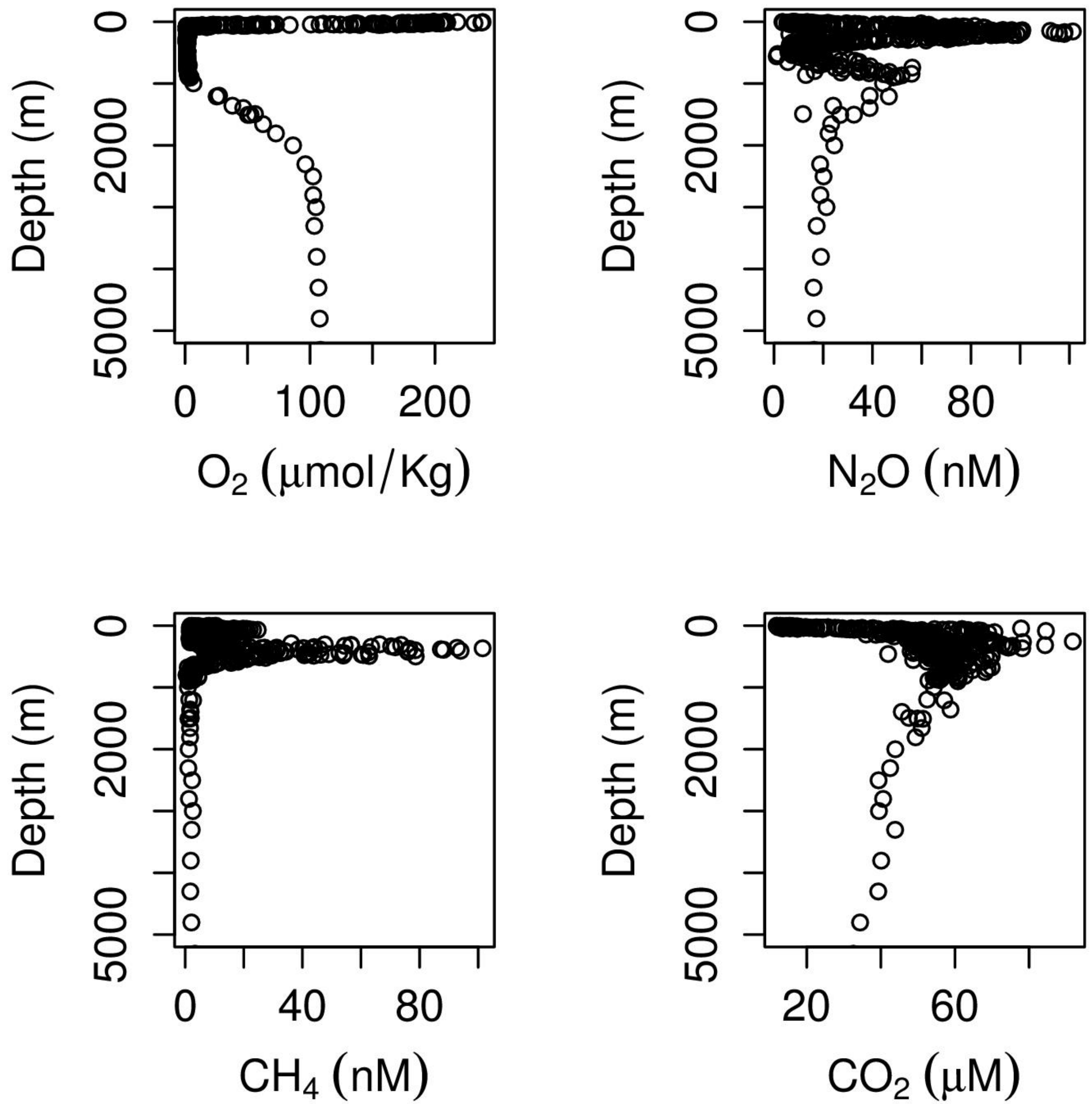


Figure 3. Concentration of dissolved oxygen (O_2), nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2) in the water column. The data shown here has derived from 17 different high resolution profiles at various sampling sites off the west coast of Guatemala.

6.2. Measuring rates of nitrous oxide production in ocean water using stable isotopes

Introduction

Our previous work in the offshore waters of the ETNP OMZ demonstrated that the various oxygen treatments had a clear effect in the production of N_2O: N_2O production practically stopped in fully oxic water, increased in hypoxic water, and was similar to ambient conditions in 16μM O_2. In addition, most of the N_2O produced offshore was ^{45}N- rather than ^{46}N-N_2O indicating a non-direct link to the $^{15}NO_2^-$ pool and a non-typical denitrification. With this second cruise, our aim is to investigate how similar treatments affect the N_2O production closer to the coast line and whether there is an effect when using different stable isotopes, i.e. $^{15}NO_2^-$, $^{15}NH_4^+$ and $^{15}NH_2OH$.

Materials & Methods

The procedure follows Nicholls et al. (2007) with one major modification. Water samples from 2 depths per station were collected from the CTD into 4 Litre polypropylene Nalgene bottles and additional samples were collected to act as references for the natural 15N abundance of nitrous oxide in the water. The Nalgenes were then gently degased (1 Bar) for 20 minutes with a variety of non-toxic gases, namely oxygen, nitrogen and nitrous oxide, to obtain the following conditions: 100% air saturation, medium oxygen levels (oxygen to 7,500 psi), low oxygen (oxygen to 3,750 psi), and no oxygen (degased with oxygen-free nitrogen). Once degased, the water was dispensed under pressure (1 Bar) into 150 ml glass serum bottles, and ^{15}N-NO_2^-, ^{15}N-NH_4^+ or ^{15}N-NH_2OH stable isotope tracers were added to a final concentration of 10 μM. The oxygen concentration was measured in each serum bottle using a calibrated electrode, the bottle sealed and then incubated for 48 h at 12°C. Subsequently, microbial activity was stopped by injecting 500 μl of 37% formaldehyde through the septa (with venting) in the fume. The bottles were then safely stowed for transport back to the lab.

All samples were analysed on a continuous flow isotope ratio mass spectrometer (IRMS) (Finnigan MAT DeltaPlus,Thermo-Finnigan) and the mass charge ratios for m/z 44,m/z 45, and m/z 46 (44N2O, 45N2O, and 46N2O) measured, using a trace gas preconcentrator unit (PreCon, Thermo-Finnigan).

Preliminary Results

In contrast to the N_2O production offshore being dominated by 45N-N_2O (about 80% of the total N_2O production), in the sites closer to the coast we observe a dominance of 46N-N_2O. Another key finding here is that enrichment with ^{15}N-NH_2OH increases both the 45N- and 46N-N_2O production by at least two orders of magnitude, especially in the 100% Air saturation treatment (see Figure 4). If we exclude the ^{15}N-NH_2OH treatment and concentrate on the ^{15}N-NO_2^- and ^{15}N-NH_4^+, we observe that the production of N_2O is favoured with the ^{15}N-NH_4^+ enrichment in comparison to the ^{15}N-NO_2^-, and that similarly to what happens offshore, the production is maximum for lower oxygen levels (with anoxia-OFN treatment giving the maximum production, Figure 5).

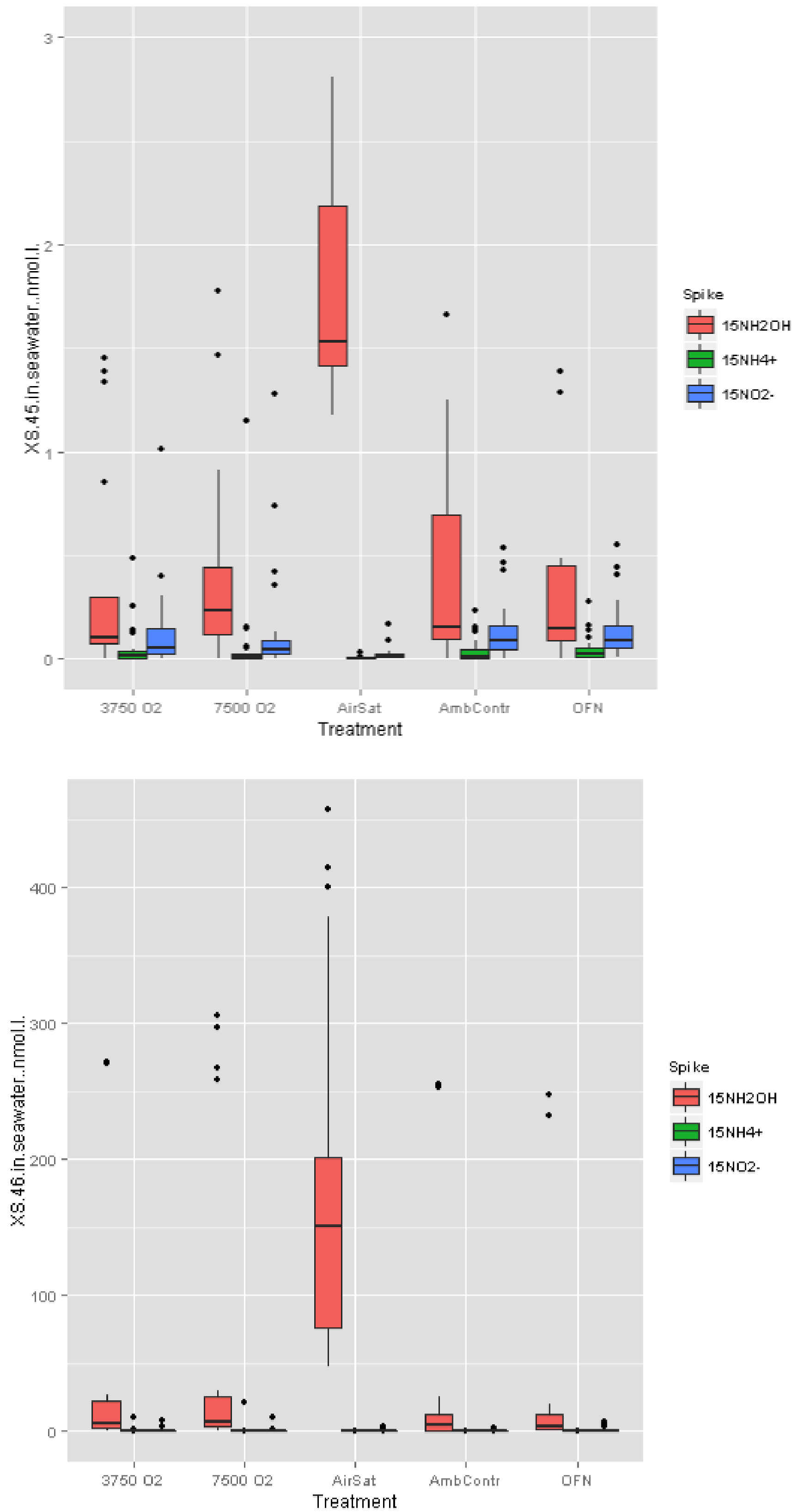


Figure 4. Excess production (in nmol/l of water) of N_2O. Production of 45N-N_2O (up) and 46N-N_2O (down) at various oxygen levels and with three different isotopic enrichments ($^{15}NO_2^-$, $^{15}NH_4^+$ and $^{15}NH_2OH$). Each box is defined by the 25th and 75th percentiles (spread) with the vertical line measuring 1.5 times the spread. The thick horizontal line in each box gives the median value for each treatment.

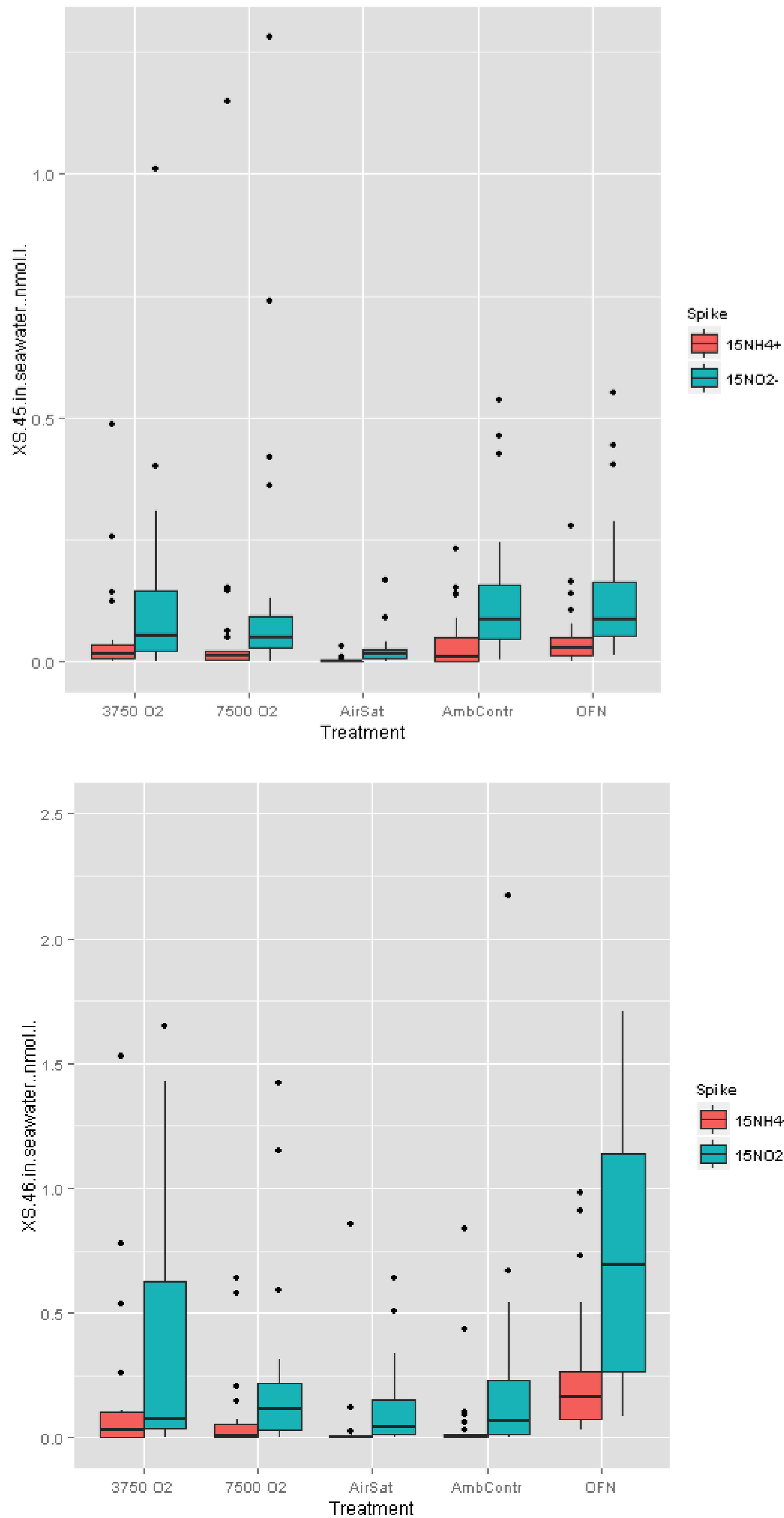


Figure 5. Excess production (in nmol/l of water) of N_2O. Production of 45N-N_2O (up) and 46N-N_2O (down) at various oxygen levels and with two different isotopic enrichments ($^{15}NO_2^-$, $^{15}NH_4^+$, excluding $^{15}NH_2OH$). Each box is defined by the 25th and 75th percentiles (spread) with the vertical line measuring 1.5 times the spread. The thick horizontal line in each box gives the median value for each treatment.

6.3. Measuring the production of N_2 using organic N stable isotopes

Introduction

In our last cruise we were able to measure 15N-N_2 production only at one site/one depth offshore. We therefore concluded that nitrogen is released back to the atmosphere as N_2O, possibly via some form of nitrifier denitrification. However, evidence from the Arabian Sea suggests a direct coupling of ^{14}N from organic nitrogen and $^{15}NO_2^-$, that is, potentially, a form of heterotrophic anammox - completely separate to the production of N_2 from the reduction of N_2O (Trimmer and Purdy, 2012). With this cruise we aim to further investigate this potential, using dual labelled organic substances (i.e.$^{15}N(^{13}C)$ –glycine, ^{15}N (^{13}C)-glutamic acid) as well as the classic anammox reactions ($^{15}NH_4^+$ and $^{14}NO_2^-$ + $^{15}NH_4^+$ or $^{15}NO_2^-$ + $^{14}NH_4^+$) and $^{15}NH_2OH$.

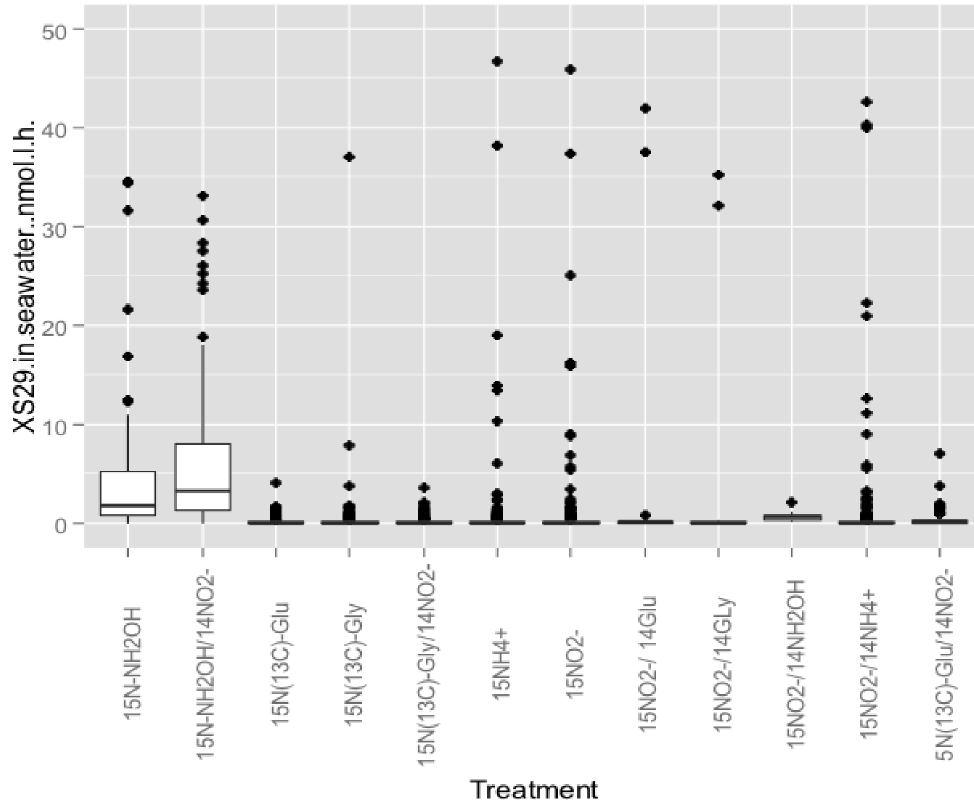
Materials & Methods

This experiment was conducted at five selected depths at each site. Seawater was collected from the CTD into 1 L glass (serum) bottles. Subsequently, bottles were pressurised under helium (1 bar) and 12 ml of seawater was transferred into exetainers (gas-tight vials). Exetainers were injected with 50 µl of stable isotopes to a final concentration of 10 µM (for $^{15}NO_2^-$, $^{14}NO_2^-$, $^{15}NH_4^+$) or 5 µM (for $^{15}N(^{13}C)$ – glycine, ^{15}N (^{13}C)-glutamic acid) and incubated for a pre-defined time period (3, 6, 12, 24 and 48 h) at 12 °C. Finally, microbial activity was stopped by injecting 50 µl of $ZnCl_2$ (50%). Exetainers were stored safely in designated carton boxes.

Samples were analysed for $^{29}N_2$ and $^{30}N_2$ gas production using an IRMS.

Preliminary Results

As with the N_2O production the N_2 production is also maximum (more than two orders of magnitude) when samples were enriched with $^{15}NH_2OH$ (Figure 6). If we exclude the $^{15}NH_2OH$ treated samples and look closer at the rest of the treatments, there seems to be a small production on average in all of them and a big variation in between the sites and depths (Figure 7). A more detailed analysis is required in order to assess the significance of such production. However, it is clear that N_2 production does happen to some extent inshore, whereas we could not measure it offshore (first cruise data).



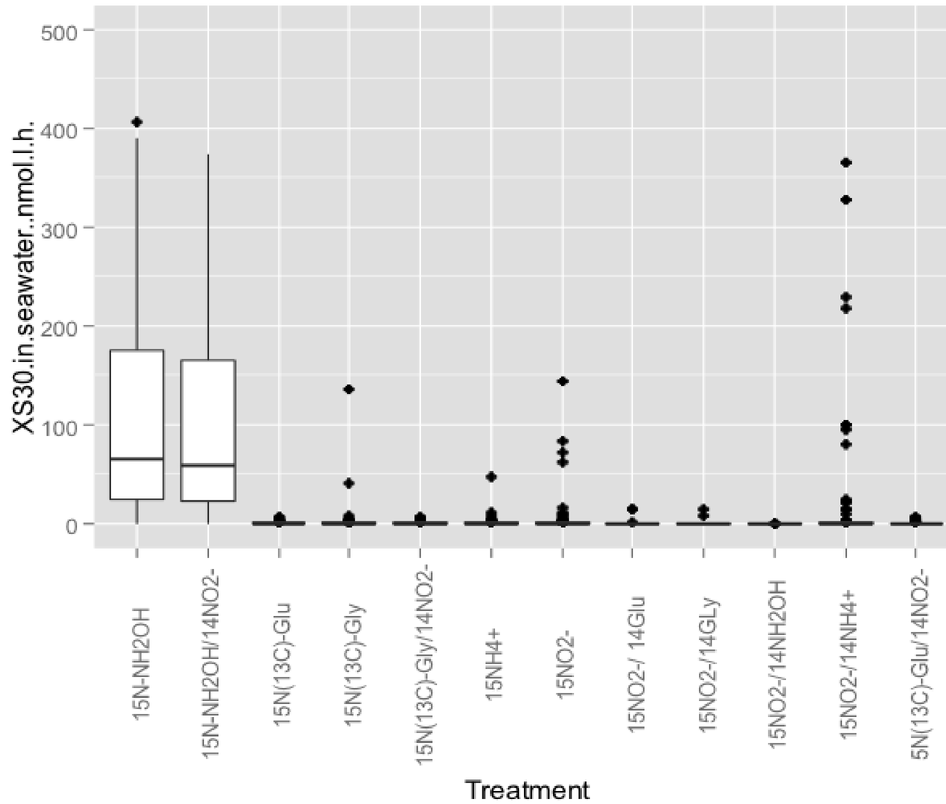


Figure 6. Excess N_2 production in the water column. Production of 29N-N_2 (up) and 30N-N_2 (down) with addition of various stable isotopes. Each box is defined by the 25th and 75th percentiles (spread) with the vertical line measuring 1.5 times the spread. The thick horizontal line in each box gives the median value for each treatment.

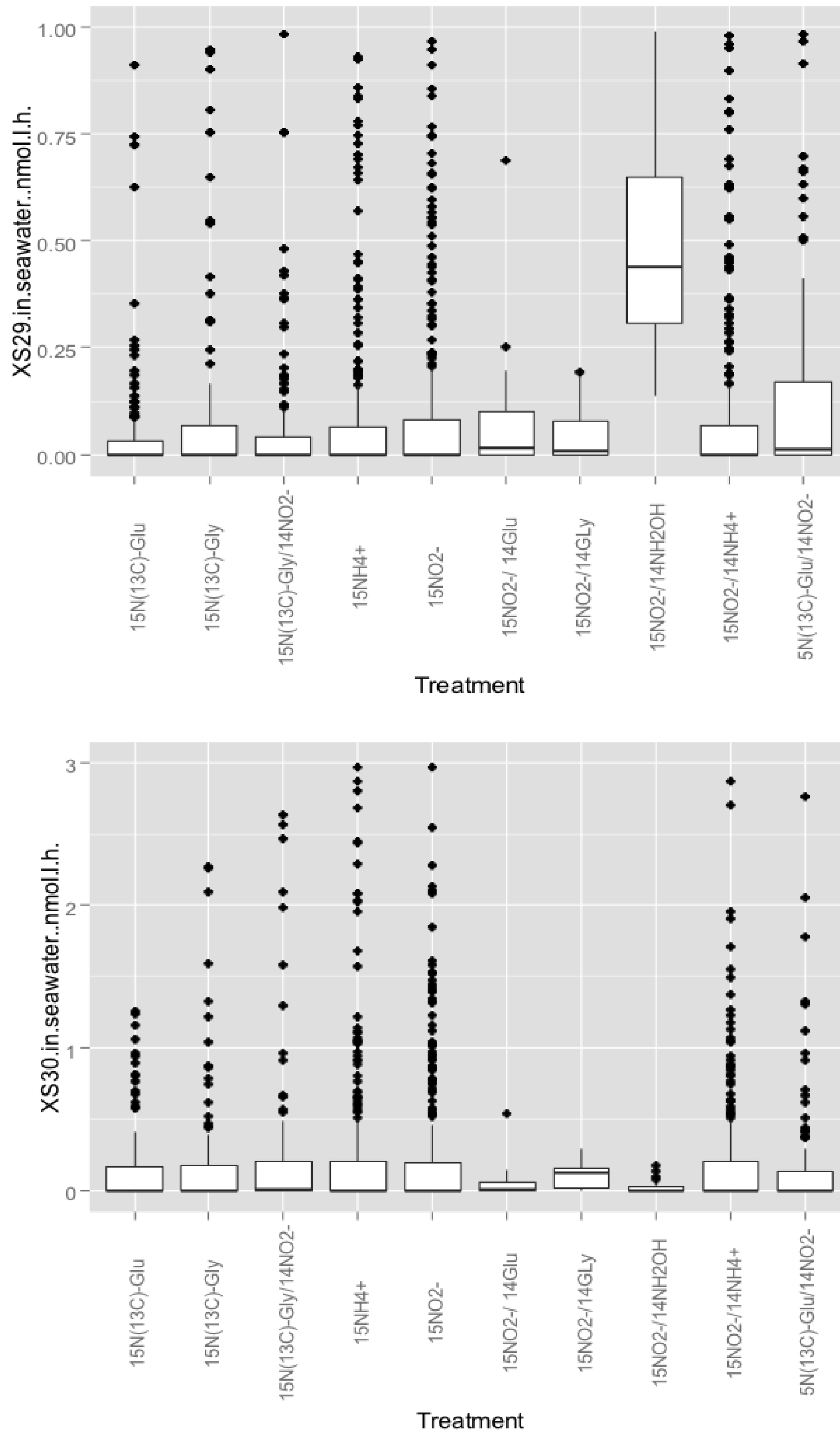


Figure 7. Excess N_2 production in the water column, excluding the $^{15}NH_2OH$ treatment. Production of 29N-N_2 (up) and 30N-N_2 (down) with addition of various stable isotopes. Each box is defined by the 25th and 75th percentiles (spread) with the vertical line measuring 1.5 times the spread. The thick horizontal line in each box gives the median value for each treatment.

6.4. Molecular characterisation of the microbial communities involved in N cycling processes

Introduction

Apart from looking at the processes of N cycle, it is equally important to gain good insights into the microorganisms involved in these processes. For this purpose, prokaryotic cells (Bacteria and Archaea) were collected from the same samples used for the experiments described in paragraphs 6.2 and 6.3. Following the chemical analysis of these samples, there will be molecular analysis of selected samples from the most active depths/ sites, with a view to target 16S and functional genes (i.e. *nirS*, *nirK* and *nosZ, amoA* genes). We want to know if there will be changes in the gene copy numbers reflecting the changes observed in the production of N_2O and N_2 and identify which microbes are involved in the production of these gases.

Materials & Methods

Water samples were collected from the CTD/Niskin rig into 1 L glass serum bottles. Samples were transported into the ship's lab and 15N stable isotopes were added to a final concentration as in their respective production experiments. For the N_2O production experiment, samples for molecular analysis were obtained from sites 1, 3 and 5 at only one depth, and they were enriched with nitrite, ammonium or hydroxylamine. For the N2 production experiment, samples for molecular analysis were obtained at all sites/ depths and enriched as shown in Table 10. After a 48 h incubation, water was filtered through 0.2 µM Supor filters using a gentle vacuum. After filtration samples were stored in 2 ml plastic vials and flash frozen (using liquid nitrogen) before being stored at -80 °C for transport back to England. Nucleic acids (DNA and RNA) were extracted and will be used for next generation sequencing, in order to gain insights into the identity of the microbes involved in the processes of interest. Additionally, there will be quantitative PCR analysis, in order to target and quantify functional genes of N cycle.

Table 10. Enrichments of water samples for molecular analysis of the N_2 production experiment.

		Treatment					
Site	**Depth (m)**	**Ambient**	**Nitrite**	**Nitrite & Ammonium**	**Hydroxylamine**	**Glycine**	**Glutamic acid**
1	85	×	×	×	×	×	×
1	95	×	×	×			
1	115	×	×	×			
1	105	×	×	×	×	×	×
1	75	×	×	×			
2	135	×	×	×	×	×	×
2	125	×	×	×			
2	145	×	×	×			
2	155	×	×	×	×	×	×
2	140	×	×	×			
3	125	×	×	×	×	×	×
3	115	×	×	×			
3	135	×	×	×			
3	235	×	×	×	×	×	×
3	195	×	×	×			
4	120	×	×	×	×	×	×
4	130	×	×	×			
4	110	×	×	×			
4	225	×	×	×	×	×	×
4	185	×	×	×			
5	220	×	×	×	×	×	×
5	205	×	×	×			

5	132	×	×	×			
5	90	×	×	×	×	×	×
5	67	×	×	×			
6	220	×	×	×	×	×	×
6	132	×	×	×			
6	85	×	×	×			
6	80	×	×	×	×	×	×
6	74	×	×	×			

6.5. Measurement of denitrification in the water column at different temperatures

In this cruise we looked at N_2 gas production not only via anammox and coupling with organics oxidation but also via the process of denitrification. For this purpose, seawater was collected from the CTD into 1 L glass serum bottles from the same depths as for the anammox experiment. Subsequently, bottles were pressurised under helium (1 bar) and 12 ml of seawater transferred into exetainers. Exetainers were injected with 50 µl of $^{15}NO_2^-$ to a final concentration of 10 µM and incubated for 0, 3, 6, 12, 24 or 48 h at 6, 12, 15, 20 or 24 ^oC. Finally, microbial activity was stopped by injecting 50 µl of $ZnCl_2$ (50%). Exetainers were stored safely in designated carton boxes and transferred to the lab for measurement of ^{15}N-N_2 production on IRMS. The results are currently being analysed.

6.6. Assessment of nitrogen fixation potential in the water column at different temperatures

In addition to investigating the release of nitrogen to the atmosphere, we also looked at the other side of the N cycle, more particular at the potential of converting the atmospheric N_2 to ammonium, a form that can be readily assimilated by organisms. To do so, we collected seawater (from about 5-10 m depth) from the CTD into 1 L glass serum bottles, during evening hours (i.e. when N fixation is known to peak). Alternatively, concentrated water (i.e. water with increased amount of biomass) was collected from the micro-net deployments and 1 ml of this water was diluted with water obtained from the CTD at the same depth into 35 ml glass serum bottles. These bottles were sealed with no headspace and amended with 3 ml of $^{15}N_2$ (for the 1 l bottles) or 0.5 ml $^{15}N_2$ (for the 35 ml bottles) via a gas tight syringe, displacing water from inside. They were then incubated for a 0, 3, 6, 12 or 18 h at 15, 20, 24, 30 or 35 ^oC. Finally the serum bottles were opened and filtered onto pre-weighed 0.2 µM Supor filters, which were then frozen until processing back on land. A known part of the filter will be used to determine the amount of ammonium produced, using IRMS.

6.7. Measurement of nitrification in the water column at different temperatures

We also looked at the next step of the N assimilation pathway, which is the conversion of ammonium (or ammonia) to nitrite (NO_2^-), followed by further oxidation to nitrate (NO_3^-). For this purpose, water was collected from the CTD into exetainers (5 replicates for each temperature) from around 27 m depth at two sampling points. Then a 2 ml helium/air headspace was introduced to the exetainers, in order to obtain ambient water oxygen concentrations and $^{15}NH_4^+$ was injected through the septa to a final concentration of 1, 2 or 4 µM. Exetainers were then incubated at 12, 15, 20, 24 or 35 ^oC for 0, 3, 6, 12, 24, or 48 h and microbial activity was stopped. Exetainers were transported to the lab, where the produced NOx (NO_2^- or NO_3^-) will be converted to N_2, which can be measured on IRMS.

6.8. Measurement of denitrification in sediment slurries at different temperatures

In parallel to monitoring the water column n cycle processes, we also looked at the same process in the seabed sediments. For the measurement of denitrification in sediments, sediment was collected from the top 0-2 cm and 2-4 cm of the cores (from mega corer deployments) into sealable bags without enclosing air. The bags were immediately transferred to the anaerobic glove box and sediment was transferred into exetainers. Degased water (with OFN) from the same sampling site was added to each exetainer, so that 2 ml of headspace was left, and $^{15}NO_2^-$ was injected to a final

concentration of 10 µM. After incubation at 6, 12, 15, 20 or 24 ^oC for 0, 0.25, 0.5, 1, 2, 4, or 8 h, the microbial activity was stopped with injection of 200 µl 37% formaldehyde. The exetainers were transferred to the lab and measured for production of ^{15}N-N_2 production on IRMS. The results are currently being analysed.

6.9. Measurement of N_2 and N_2O production from intact sediment cores (Isotope Pairing Technique)

The procedure follows a procedure described by Trimmer et al. (2006). Perspex tubes (3.4cm x 25cm) were deepened into the sediment cores until they were half filled with sediment, sealed from the bottom with a rubber bung and placed into a tank filled with site water. Stable isotope tracer ($^{15}NO_3^-$) was then added to give a final concentration of 100 µM. The oxygen concentration was measured in each core using a calibrated electrode, and the core sealed and then incubated for 4 h with constant stirring provided by an electronic magnetic stirrer. After 4 h the core was unsealed and the oxygen concentration measured again. The sediment and overlying water was homogenised and a 12.5 ml was sampled into a gas-tight vial (exetainers) containing 200 µl formaldehyde (37% solution CH_2O), prepared in fume hood to stop microbial activity. This resulted in a final solution for formaldehyde of 1.8%. The gas-tight vials were then safely stowed for transport back to the home port, where they were measured for N_2 production on IRMS and N_2O production on precon. The results are currently being analysed.

6.10. Measurement of aerobic methane oxidation in the water column

Introduction

As with the first cruise, in this second ETNP cruise we aim to take further our knowledge on methane oxidation in the waters of this area of the ocean.

Materials & Methods

Water samples were collected from the CTD into exetainers. Triplicate samples were spiked with $^{13}CH_4$ gas to a final concentration of approximately 300 nM. Two additional samples were obtained at each depth to serve as controls; one was spiked with the same amount of $^{13}CH_4$ as the samples and fixed at the start of the experiment, the other one was not spiked and was fixed back in the lab (at the same time with the samples). Moreover, triplicate samples from 3 depths were sacrificed at various time points to assess methane oxidation over time (time series experiment) and samples from 2 different depths were spiked with increasing concentrations of $^{13}CH_4$ (dose response experiment).The vials were left to incubate at 12^oC for various time points, the last time point being after about six months (on return to the lab). Microbial activity was stopped with injection of 100 µl concentrated HCl, samples were headspaced with 2 ml helium and measured for production of ^{13}C-CO_2 with the IRMS.

In parallel, 5 x 1 L bottles were obtained from 47 m and 226 m and filtered through 0.2 µM Supor filters using a gentle vacuum. . Alternatively, water from 200 and 215 m was filtered through the SAPs filters. The filters were immediately frozen with liquid nitrogen and transferred to the UK for DNA extraction and further molecular analysis. The functional gene encoding for the methane monooxygenase enzyme (*pmoA*), present in all aerobic methanotrophs, was amplified and samples were sent for next generation sequencing (MiSeq) to gain insights into the identities of this microorganisms in our samples.

Preliminary results

Table 11 shows the water depths at which water column methane oxidation was observed. There was only one point (200 m water depth) where we measured substantial methane oxidation, and two more (65 and 228 m) where there was some methane oxidation observed.

Time series experiments were conducted at 215 m, from 997 m seabed, and 67 m and 226 m from 560 m seabed. For two of these depths (215 and 226 m) we obtained evidence of increased methane oxidation (as reflected in the production of ^{13}C-CO_2) with time (Figure 8).

Table 11. Methane oxidation measured in the water column at various sampling sites.

CTD	Seabed depth (m)	Sampling depth (m)	Methane Oxidation?
CTD59	997	195	no
CTD59	997	200	yes
CTD59	997	205	no
CTD59	997	210	no
CTD59	997	215	no
CTD80	560	67	no
CTD80	560	226	no
CTD91	500	65	subtle
CTD91	500	228	subtle

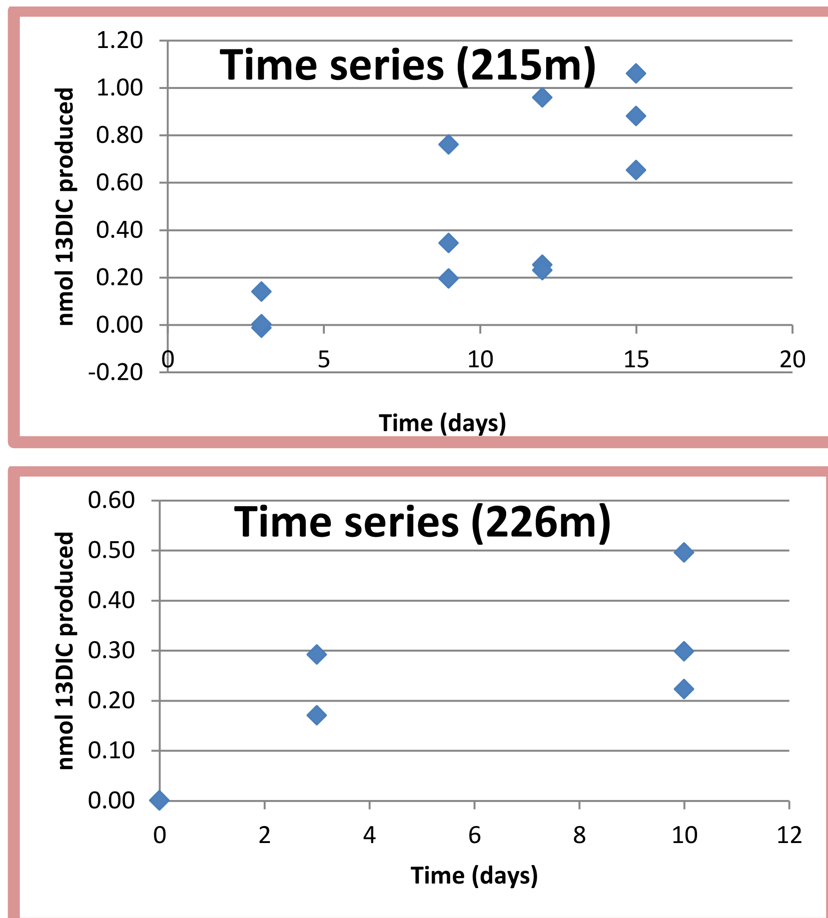


Figure 8. Water column methane oxidation; time series experiments. Oxidised methane was measured as the end ^{13}C-CO_2 product from 215 m (up) and 226 m (down) water depth.

6.11. Nitrite Dependent Anaerobic Methane Oxidation (N-DAMO)

Introduction

The aim of this experiment was to target any occurring nitrite driven anaerobic methane oxidation processes in the OMZ of ETNP. A new pathway of oxygen production that couples anaerobic oxidation of methane with the reduction of nitrite to nitrogen has recently been suggested (Ettwig et al., 2010). We therefore, set up an experiment to check for the occurrence of such process in our samples.

Materials & Methods

Seawater samples were collected from five selected depths (235 m/ site 3; 332 m and 412 m/ site 5; 264 m and 256 m/ site 6). Seawater was sampled from the CTD into 1 L serum bottles and subsampled (under helium pressure) in exetainers. Subsequently, a 2 ml OFN headspaced was introduced to ensure anoxic conditions and exetainers were spiked with $^{15}NO_2^-$ and $^{13}CH_4$. They were then incubated at 12^oC for predefined time points (the final time point being after return to the lab, about six months). Microbial activity was stopped with the addition of 100 µl concentrated HCl. Samples were transferred back in the UK and were analysed using IRMS for production of ^{13}C-CO_2 and ^{15}N-N_2.

In addition, three 1 L bottles were obtained from 264 m and 256 m and filtered through 0.2 µM Supor filters using a gentle vacuum. The filters were immediately frozen with liquid nitrogen and transferred to the UK for DNA extraction and further molecular analysis. For the rest of the depths, molecular samples were obtained with the use of SAPs. SAP filters were treated in the same way. The functional gene encoding for the methane monooxygenase in anaerobic microbes was amplified and samples were sent for next generation sequencing (MiSeq) to gain insights into the identities of this microorganisms.

Preliminary results

Table 12 summarises the results obtained from this experiment. Although $^{13}CO_2$ was produced at all depths, the total ^{30}N-N_2 produced does not match the expected, which should have been 4 moles of ^{30}N-N_2 produced for every 3 moles of $^{13}CH_4$ oxidised. We therefore cannot conclude that the methane oxidation measured at the studied depths in nitrite driven.

Table 12. Anaerobic methane oxidation measurements.

Depth	**Treatment**	**nmol 13DIC average**	**SE**	**nmol $^{29}N_2$ average**	**SE**	**nmol $^{30}N_2$ average**	**SE**
235	13CH4	**3**	1.3	**0.5**	0.38	**0.4**	0.19
332	13CH4	**10**	0.2	**-0.2**	0.07	**-0.1**	0.09
412	13CH4	**12**	4.0	**-6.1**	0.02	**-1.8**	0.10
264	13CH4	**3**	1.2	**0.1**	0.18	**0.3**	0.08
256	13CH4	**11**	0.0	**0.2**	0.10	**0.2**	0.16
235	13CH4+15NO2-	**2**	1.1	**16.0**	9.81	**32.2**	10.15
332	13CH4+15NO2-	**12**	3.5	**7.2**	0.72	**4.4**	0.96
412	13CH4+15NO2-	**15**	0.8	**4.1**	1.04	**5.2**	1.53
264	13CH4+15NO2-	**18**	0.1	**0.4**	2.63	**14.5**	7.80
256	13CH4+15NO2-	**2**	0.0	**-3.9**	0.61	**6.4**	0.00

6.12. Methane production in the water column

Anoxic water (about 20 L in total) from two selected sites (at 400 and 388 m) was filtered directly from the Niskin bottles through 8 µm pore size filters with gravity. The tubing was clamped at the end of the filtration and the filter units were transferred in the anoxic glove box. Subsequently, the filters were placed in exetainers and the exetainers were filled with degased water (using OFN) from the same depths. Exetainers with just water and no filter were also prepared, whereas exetainers with a sterile filter (no biomass) were used as reference. All exetainers were headspaced with 2 ml OFN, to ensure oxygen free conditions and incubated until arrival in England (about 5 months). Samples were measured for methane production using GC-FID. Some methane production was observed but we need to analyse the data in more detail.

6.13. Methanogenesis in sediments

To quantify the changing potential for methanogenesis with depth into the sediment, anoxic slurries were prepared and the methane concentration was tracked over time. Sediment cores were recovered from the seabed using the Mega Corer. The water overlying the sediment cores was sampled for methane analysis using a 60 ml syringe and tubing and allowed to flow into an exetainer. To sample the sediment from discrete depth intervals, the core was carefully extruded starting with the surface layer. A truncated syringe was used to transfer ~4 ml of wet sediment into 12 ml exetainers and then an additional 3 ml of bottom water (overlying the cores) was added. The exetainers were then capped and the headspace and water was purged with helium for 2 minutes to make them anoxic. The headspace methane concentration was then measured using GC-FID as above and the slurries were then left to incubate at 12°C in the dark. The methane concentration was measured 8 times over the following 12 days. Methanogenesis was only observed at the top layer of the sediment. DNA from this layer was extracted to look into the microbial community involved in methane production. The gene encoding for the methyl coenzyme M reductase α-subunit (*mcrA*) was targeted and amplified fragments of it sent for next generation sequencing (MiSeq), in order to retrieve identities of the Archaea involved in this process.

In addition to measuring methanogenesis potential in slurries, we also measure it in intact sediments. Intact sediment cores were sealed from above ensuring no contamination with surrounding water during transport from the ocean floor to the surface. Any cores with a compromised sediment water interface or air contamination were discarded. 12 cores were subsampled carefully from the cores into Perspex tubes (3.4cm x 25cm) ensuring as little disturbance as possible to the overlying water to prevent air contamination to maintain anoxic conditions, sealed from above with a rubber bung and transferred to an incubation tank at 12°C. Within 2 h of sediment sampling the rubber bung is carefully removed to avoid air contamination, the overlying water bubbled with oxygen free nitrogen (OFN) for 2 min whilst checking the oxygen concentration with an O2 sensor (OX-50, Unisense, Aarhus, Denmark) to ensure anoxic conditions were maintained. Previous experiment using fully oxygenated cores had demonstrated that 2 min was sufficient to degas the sediment core of oxygen and remove > 90% CH_4 which was determined using the GC-FID. A subsample of overlying water is taken (15 ml) using a syringe and overflowed into a 12 ml exetainer. Degased bottom water (OFN bubbling 20 min) from each site collected from the mega-core deployment is used to top up core whilst continually checking O2 concentration to ensure no oxygen contamination. After 24 h the bung was removed and a sample of water was taken from sediment core and overflowed into an exetainer. Methane concentrations at T0 and Tf were measured using GC-FID and CH_4 flux was calculated as the increase between T0 and Tf. The results are currently being analysed.

6.14. Characterisation of microbial plankton communities

Objective

To determine abundance and phylogenetic composition of dominant microbial groups within planktonic communities of the photic and oxygen minimum layers.

Materials & Methods

Seawater from various depths was sampled by 20 L Niskin bottles mounted on the rosette probe (Seabird CTD system, Table 13). Sampled water was decanted in acid-washed 50 mL centrifuge tubes, fixed with 1% paraformaldehyde, stained with the DNA-specific dye – SYBR Green I (Sigma) and analysed by flow cytometry (FACSort, Becton Dickinson) within 4 hours. Alternatively fixed samples were flash frozen in liquid nitrogen and stored at -80°C for analyses ashore. Cell concentrations of different bacterioplankton groups, pico- and nano- sized protists were determined.

Table 13. Sampled stations for flow cytometry.

CTD no.	Station no.	Lat	Long	Date	Time [GMT]
1	1	13°35.39	91°21.62	2.1.14	14:40
2	1	13°35.39	91°21.62	2.1.14	16:57
23	2	13°26.32	91°22.51	8.1.14	15:50
35	3	13°24.73	91°22.66	14.1.14	12:40
36	3	13°24.73	91°22.66	14.1.14	15:20
47	3 WP1	13°24.44	91°22.77	19.1.14	13:55
48	3 WP2	13°24.10	91°22.80	19.1.14	16:46
49	3 WP3	13°23.57	91°22.85	19.1.14	19:38
50	3 WP4	13°21.65	91°23.19	19.1.14	22:24
51	3 WP5	13°18.68	91°23.73	19.1.14	0:49
63	4	13°24.28	91°08.06	25.1.14	20:37
64	4	13°24.28	91°08.06	25.1.14	22:44
65	4	13°24.28	91°08.06	25.1.14	0:51
84	5	13°16.27	91°08.03	31.1.14	21:13
85	6	13°16.25	91°08.06	1.2.14	11:54
87	6	13°16.23	91°08.05	1.2.14	17:16
88	6	13°17.51	91°14.51	2.2.14	12:02
92	6	13°16.28	91°08.03	3.2.14	11:55
94	6	13°16.27	91°07.99	4.2.14	14:12
97	6	13°13.44	91°10.06	5.2.14	23:05

Preliminary results

Bacterioplankton concentrations apart from being the highest in the photic layer showed a reproducible secondary peak in the middle part of oxygen minimum layer (e.g. Figure 9). Three distinct groups, differentiated by their 90° light scatter and green fluorescence – characteristic DNA content, dominated bacterioplankton communities of both the photic and oxygen minimum layers.

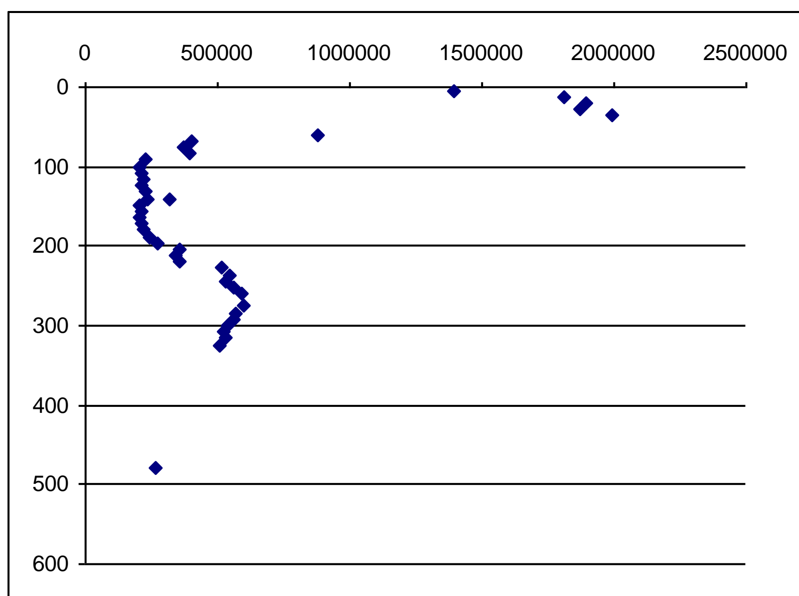


Figure 9. Vertical distribution of bacterioplankton.

6.15. Leucine uptake rates of bacterial groups in the oxygen minimum zone

Objective

- To determine group-specific uptake rates of ^{3}H-leucine using flow cytometric sorting
- To collect concentrated seawater samples for phylogenetic affiliation of the dominant bacterioplankton groups (flow sorted for rate measurements) using fluorescence *in situ* hybridisation (TSA-FISH).

Materials & Methods

To minimise oxygen-contamination samples from different depths were collected in glass bottles, which were capped and quickly transferred into a glove box with oxygen free nitrogen atmosphere (Table 14). After addition of 3 nM of ^{3}H-leucine samples were incubated at room temperature for 8 hours. Incubations were terminated by cell fixation with 1% paraformaldehyde. Fixed cells were flow sorted within 10 h (when possible) or flash-frozen immediately for later analyses.

Table 14. Samples collected for ^{3}H-leucine experiments.

CTD no.	Station no.	Lat	Long	Date	Time [GMT]	bottle no.	bottle depth [m]
37	3	13°24.64	91°22.75	15.1.14	14:10	5	195
41	3	13°24.67	91°22.71	17.1.14	14:18	5	235
53	3	13°24.43	91°22.83	20.1.14	12:30	1	250
66	4	13°16.26	91°08.06	27.1.14	12:11	3	228
86	5	13°26.24	91°08.05	01.2.14	15:11	24	136
86	5	13°26.24	91°08.05	01.2.14	15:11	13	224
86	5	13°26.24	91°08.05	01.2.14	15:11	6	280
86	5	13°26.24	91°08.05	01.2.14	15:11	1	350
88	6	13°17.51	91°14.51	02.2.14	12:02	24	90

88	6	13°17.51	91°14.51	02.2.14	12:02	5	132
88	6	13°17.51	91°14.51	02.2.14	12:02	3	220
88	6	13°17.51	91°14.51	02.2.14	12:02	1	350
92	6	13°16.28	91°08.03	03.2.14	11:55	7	74
92	6	13°16.28	91°08.03	03.2.14	11:55	5	120
92	6	13°16.28	91°08.03	03.2.14	11:55	3	220
92	6	13°16.28	91°08.03	03.2.14	11:55	1	350
97	6	13°13.44	91°10.06	05.2.14	23:05	22	92
97	6	13°13.44	91°10.06	05.2.14	23:05	17	120
97	6	13°13.44	91°10.06	05.2.14	23:05	13	350
97	6	13°13.44	91°10.06	05.2.14	23:05	9	500
97	6	13°13.44	91°10.06	05.2.14	23:05	8	700

6.16. Phylogenetic composition of the dominant bacterial groups

Seawater samples (50mL) were fixed with 1% PFA and concentrated on 0.2µm pore-size polycarbonate filters. Concentrated samples were flash frozen with liquid nitrogen and stored at -80°C. To characterise phylogenetic composition of bacterioplantkon that dominated the identified flow cytometric groups we will flow sort the bacterial cells for TSA-FISH ashore.

Initial scintillation counts carried out on board the ship (Packard Tri-Carb 3100) revealed different leucine uptake rates of the three flow sorted groups. To enhance precision of metabolic rates of identified bacterioplankton groups the tracer samples of flow sorted cells will be reanalysed ashore using low background counters. Combining these data with molecular analyses will enable us to link prokaryotic community composition and function.

6.17. Single-cell carbon fixation rates of microplanktonic (20-200µm) eukaryotes

Objectives

- To estimate group-specific carbon fixation rates of large eukaryotes using $^{14}CO_2$ labelling in combination with laser cutting microscopy
- To determine the abundance of these eukaryotes using FlowCam microscopy

Materials & Methods

To collect sufficient number of microplankton cells plankton net casts were carried out at the stations indicated in Table 15. After addition of sodium ^{14}C-bicarbonate tracer plankton net concentrates (20-100 µm) were incubated at ambient temperature and mean light conditions for up to 10h using an LED light array. Samples were fixed with 1% glutaraldehyde and filtered on 8µm pore size polycarbonate filter using gravity filtration. Carbon fixation rates of total filters were monitored by scintillation counting of processed subsamples (Packard TriCarb 3100) to control that single cell uptake rates can be measured.

Table 15. Light ^{14}C-bicarbonate uptake experiments.

Plankton net no.	Station no.	Lat	Long	Date	Time [GMT]	Depth [m]
4	1	13°31.02	91°21.65	06.1.14	14:00	60
12	2	13°26.33	91°22.51	10.1.14	12:30	60
15	2	13°26.33	91°22.50	12.1.14	15:00	60
24	3	13°24.67	91°22.66	16.1.14	12:30	60
26	3	13°24.64	91°22.68	18.1.14	12:30	60
38	4	13°24.45	91°22.8	22.1.14	12:30	60
40	4	13°24.44	91°22.89	24.1.14	12:30	60
47	5	13°18.62	91°17.42	27.1.14	13:15	60
49	5	13°16.14	91°08.42	28.1.14	12:30	60
50	5	13°16.35	91°08.03	30.1.14	12:30	60
56	6	13°16.03	91°08.19	03.2.14	12:30	50

6.18. Determination of community composition of microplankton

To determine abundance of microplankton organisms in the water column plankton net samples were collected and analysed using a FlowCam (Table 16). The FlowCam was modified by replacing the peristaltic pump with a syringe pump to allow high and precise flow rates. Where possible 49 ml of every size fraction (100-20, 100-180 and >180µm) were analysed in duplicate.

After the cruise, single cell carbon fixation rates will be determined using a laser cutting microscope to excise single cells. The detailed data set will allow estimation of carbon fixation rates by microplankton organisms. In combination with molecular and FlowCam analyses this approach will link eukaryotic community composition and function.

Table 16. Deployments of the size-fractionating microplankton nets with and without the closure ball valve.

Plankton net no.	Station No.	Date	Time [GMT]	Lat°N	Long°W	Deployed depth [m]
1	1	03.01.14	19:50	13°30.98	91°21.67	60
2	1	04.01.14	18:30	13°31.02	91°21.81	60
3	1	05.01.14	20:00	13°30.97	91°21.68	60
4	1	06.01.14	14:00	13°31.02	91°21.65	60
5	1	06.01.14	18:30	13°31.02	91°21.65	60
6	2	08.01.14	15:00	13°26.32	91°22.51	60
7	2	08.01.14	20:30	13°26.36	91°22.61	60
8	2	08.01.14	01:30	13°26.59	91°22.52	60
9	2	09.01.14	15:00	13°26.32	91°22.50	60
10	2	09.01.14	19:00	13°26.18	91°22.54	60
11	2	09.01.14	24:00	13°26.44	91°22.28	60
12	2	10.01.14	12:30	13°26.33	91°22.51	60
13	2	10.01.14	18:30	13°26.45	91°22.42	60
14	2	11.01.14	20:30	13°26.32	91°22.51	60
15	2	12.01.14	15:00	13°26.33	91°22.50	60

16	2	12.01.14	21:10	13°26.	91°22.50	60
17	2	13.01.14	06:00			60
18	5	13.01.14				60
19	3	14.01.14	15:30	13°24.64	91°22.68	180-130
20	3	14.01.14	20:00	13°24.64	91°22.68	60
21	3	15.01.14	00:30	13°24.64	91°22.68	130-50
22	3	15.01.14	15:30			60
23	3	15.01.14	20:00			60
24	3	16.01.14	12:30			60
25	3	16.01.14	18:15			60
26	3	18.01.14	12:30			60
27	3	18.01.14	18:45	13°24.64	91°22.68	60
28		18.01.14	19:30	13°10.11	91.25.07	250-150
29		18.01.14	16:50	13°16.41	91°14.70	60
30		20.01.14	14:30	13°24.33	91°23.21	250-140
31		20.01.14		13°24.33	91°23.21	140-50
32		20.01.14		13°24.33	91°23.21	50-1
33		20.01.14	18:30			50-1
34		20.01.14	20:15	13°24.45	91°24.71	250-140
35		21.01.14	00:30	13°06.21	91°14.43	60
36		21.01.14	14:30	13°24.43	91°23.83	60
37		21.01.14	19:30	13°21.25	91°22.81	60
38		22.01.14	12:30	13°24.45	91°22.8	60
39		23.01.14	01:00	13°25.29	91°25.30	60
40	4	24.01.14	12:30	13°24.44	91°22.89	60
41	4	24.01.14	18:30	13°24.45	91°22.89	250-100
42	4	24.01.14	19:30	13°24.45	91°22.89	100-50
43	4	24.01.14	20:30	13°24.25	91°22.95	50-0
44	4	25.01.14	06:00	13°24.18	91°22.97	250-100
45	5 WP 3	26.01.13	16:30	13°16.91	91°08.31	60
46	5	26.01.14	20:15	13°16.41	91°08.22	100-50
47	5	27.01.14	13:15	13°18.62	91°17.42	60
48	5	28.01.14	00:30	13°10.45	91°13.2	60
49	5	28.01.14	12:45	13°16.14	91°08.42	40
50	5	30.01.14	12:30	13°16.35	91°08.03	40
51	5	30.01.14	18:30	13°16.29	91°08.05	250-100
52	5	30.01.14	19:30	13°16.29	91°08.05	100-50
53	5	30.01.14	20:00	13°16.29	91°08.05	50-0
54	5	30.01.14	20:30	13°16.29	91°08.05	250-100
55	5	31.01.14	00:30	13°13.83	91°08.06	40
57		03.02.14	02:30	13°16.37	91°08.14	60
56	5	03.02.14	12:45	13°16.03	91°08.19	50
58		04.02.14	13:15	13°04.19	91°11.48	60
59	6 (3000m)	04.02.14	21:30	13°04.19	91°11.48	60
60	6 (3000m)	04.02.14	22:00	13°04.17	91°11.46	2700-1000
61	6 (1500m)	06.02.14	00:00	13°13.21	91°11.99	1350-900

6.19. Measurement of biological respiration in the water column

Using a fractionating micronet we sampled the top 60m of the water column and took plankton samples from the 20-100 µm and 100-180 µm fractions. Usually, about 400-500 ml was obtained from the small fraction and 150-250 ml from the larger fraction. They were then incubated in micro-respiration vials and at temperatures ranging from 4-37°C and the oxygen was logged for 5 hours at a time. Unisense micro-electrodes were used along with the micro-respiration kit to hold and stir the vials. All was performed in a 25 l water bath which controlled the incubation temperature.

6.20. Estimation of POC, Chlorophyll and lipids content in the water column

Introduction

The marine snow catchers (MSCs) (Figure 10) are used to collect sinking particles in 3 sinking rate fractions, suspended, slow and fast sinking. The MSCs allow us to quantify how much organic material produced in the surface either reaches the sediment or is remineralised. It's thought in the eastern tropical north Pacific oxygen minimum zone (OMZ) organic carbon is transferred very efficiently to depth, but quite why remains a mystery. The aim of this work was to estimate particulate organic carbon (POC) flux and use other variable such as lipid concentration, opal, particulate inorganic carbon and particulate organic nitrogen and phosphorous to understand why the biological carbon pump is so efficient where oxygen is low.

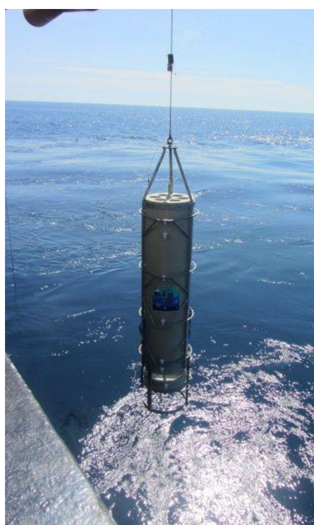


Figure 10. Marine snow catcher.

Materials & Methods

The MSC was deployed at 6 stations (Figure 11), firstly at the coast (water depth = 100 m) to further offshore (water depth = 3000 m) with a maximum deployment depth of 350 m. It was deployed at 4 depths during the day and at 2 depths during the night. The MSC is deployed with both ends open and a messenger fired to close the plungers and brought immediately to deck to be left to settle. Ideally it should stand for 2 hours in custom-made deck frames. After 2 hours the suspended fraction can be sampled, then this is drained and the slow sinking fraction is sampled. After this the tray with the fast sinking particles can be removed. Estimates of POC were made using on the fast sinking materials, sinking rates calculated using sinking chamber and flow cam and the proportion of

zooplankton fecal pellets to aggregates counted. On the slow sinking 2 L of water was filtered to measure POC, PIC, POP, PON, Lipids and BSi and for the suspended fraction POC and lipids. Water column POC, lipids and chlorophyll were also measured at MSC sampling stations using the CTD. Additionally echosounder (EK60) data were collected to look for evidence of zooplankton/fish diel vertical migrations.

Additional MSC deployments were done to estimate respiration rates on particles at Queen Mary University of London.

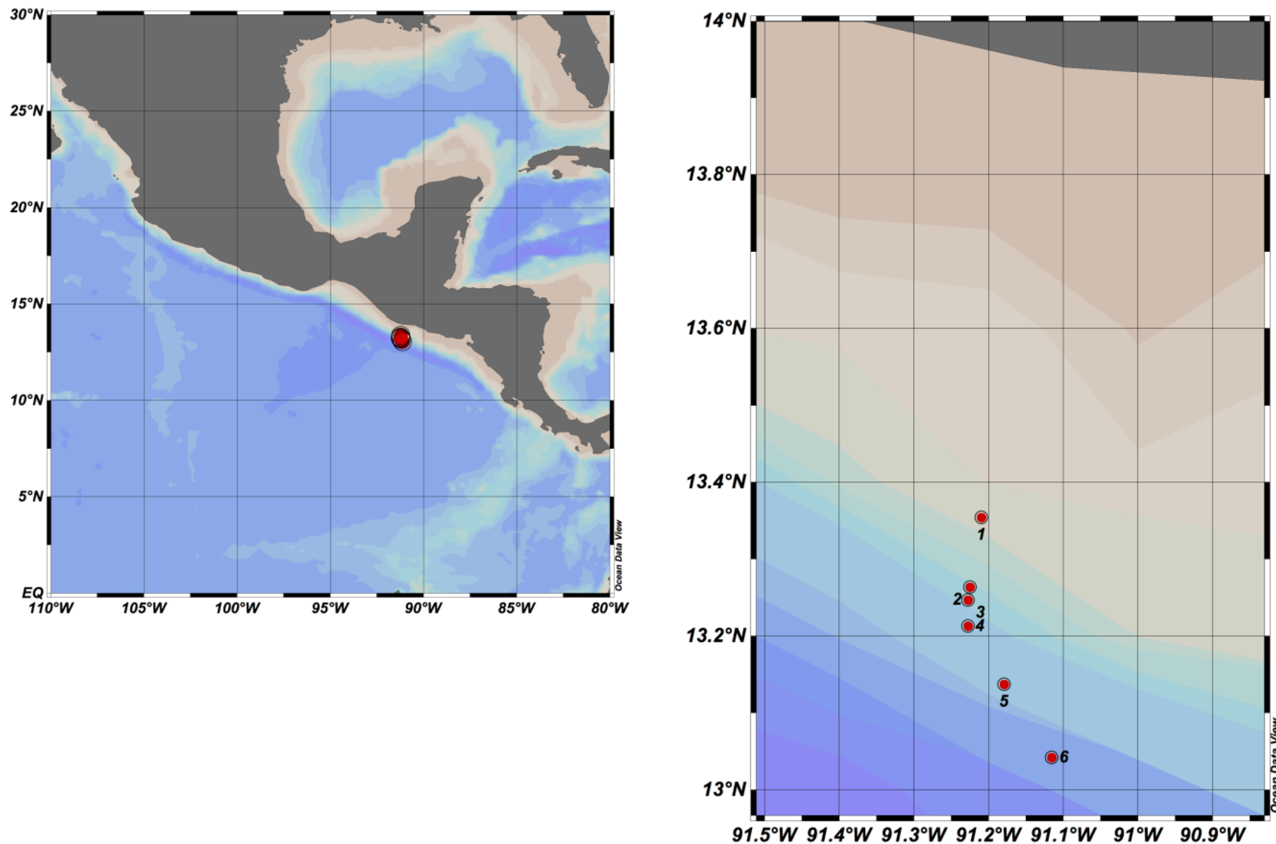


Figure 11. Sampling stations for marine snow catchers.

The parameters below were analysed:

Chl – Analysis on board using Turner Fluorometer

POC – Done at Jacobs Bremen University, Germany by Annika Moje, using CN analyser

PIC – Analysis at NOCS

BSi – Analysis at NOCS following Brown et al. (2003)

POP – Analysis at NOCS following Raimbault et al. (1999)

Lipids – Analysis at University of Liverpool with Prof. George Wolff

Sinking rates (flow cam) – Analysis on board and finished at NOCS, new method developed with Mike Zubkov

Preliminary results

Chlorophyll (μg/L) ->

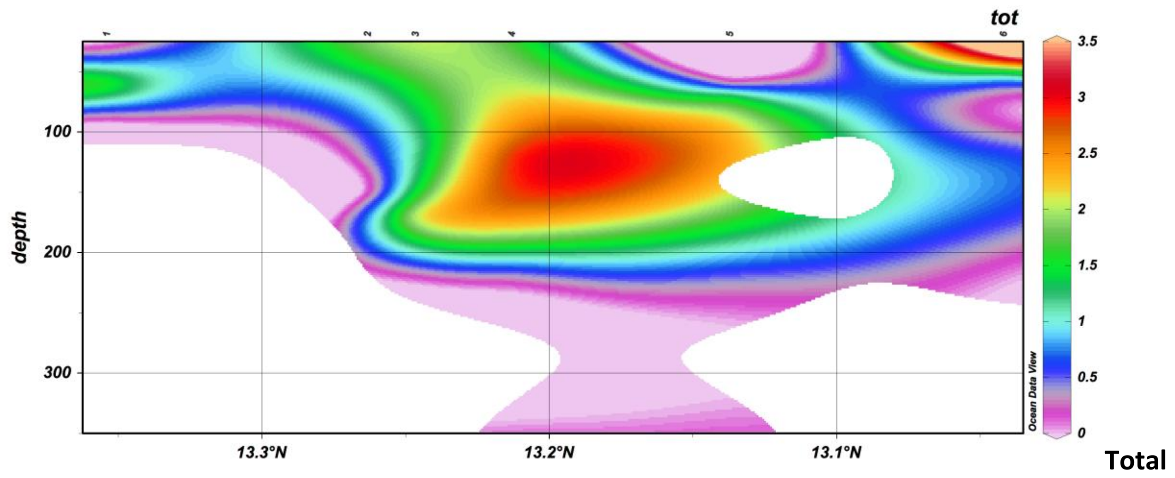


Figure 12. Chlorophyll content across the sampling sites.

POC flux (mg C m^{-2} d^{-1}) ->

Agg = Aggregate

FP = Fecal pellet

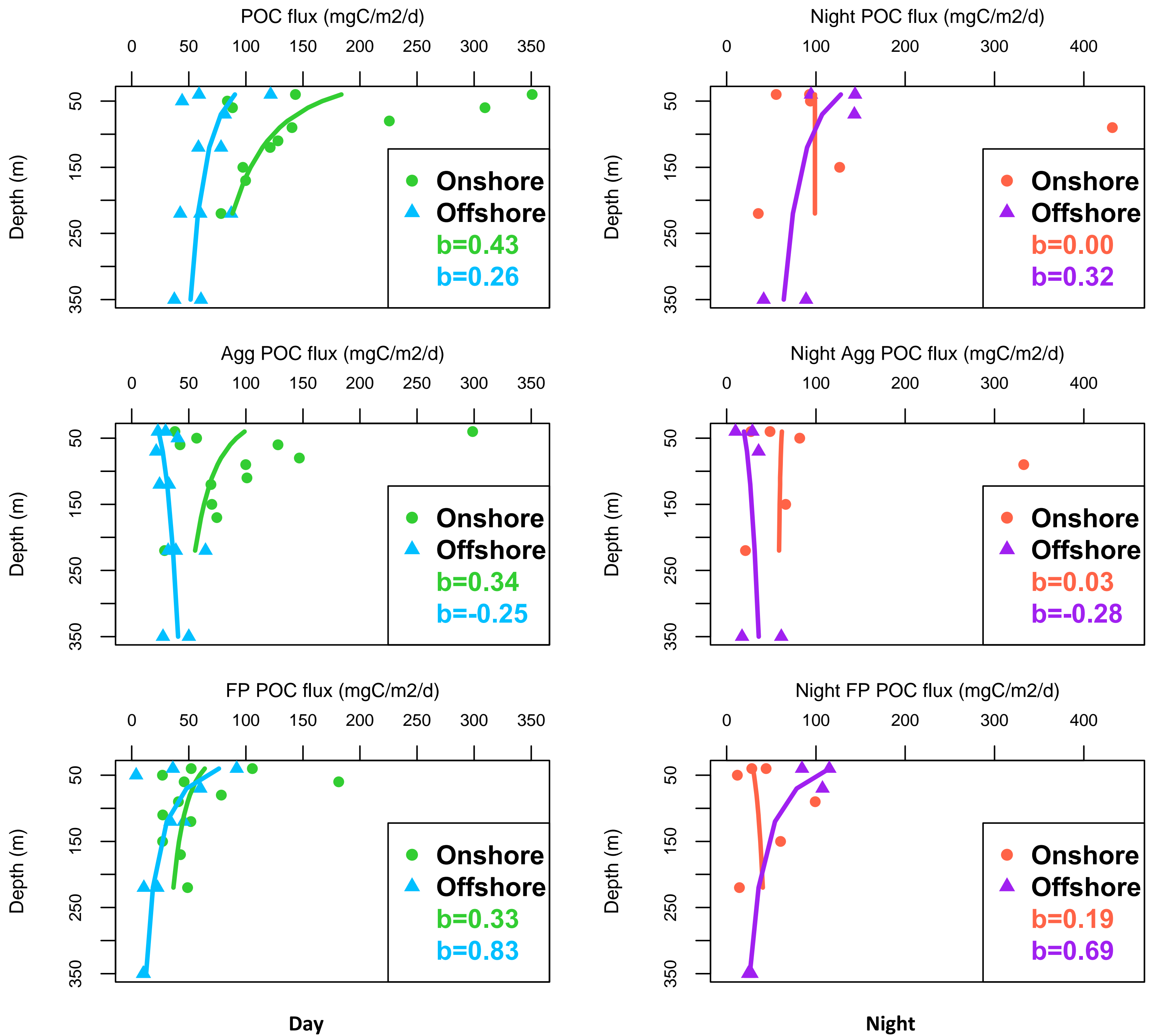


Figure 13. Particulate organic carbon (POC) fluxes.

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