QUEEN MARY UNIVERSITY OF LONDON

SCHOOL OF BIOLOGICAL AND CHEMICAL SCIENCES

RRS DISCOVERY- CRUISE D373

ETNP (Eastern Tropical North Pacific); December 2011- January 2012

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

Cruise Report (6 months)

Principal Scientist: Mark Trimmer

September 2012

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; D373 Cruise Report

Abstract: With this research cruise we aim to study N cycle in a non- yet characterized OMZ, the OMZ of Eastern Tropical North Pacific (ETNP). The overall aim of this project is to link N cycling processes to bacterial gene diversity and expression across a gradient of OMZ intensity in the (ETNP), firstly by characterization of the water column (e.g. N_2O, CO_2, CH_4 profiles) and then experimental manipulations to test if the ratio of N_2 to N_2O production is 'fixed' or 'flexible', screening for N_2 production coupled to ^{15}N organic-N and a molecular analysis of the active communities involved in the metabolism of these gases. In order to determine the extent of variation and potential within the water column, we performed experiments at 6 different sites, outside, on the edge and within the core OMZ. Understanding this response 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.
In addition, we set up experiments for the measurement of anaerobic methane oxidation, aerobic methane oxidation, methane production, and tracing of the oxidised carbon into end products of methane oxidation by ^{13}C-labelling in seawater samples. This will offer insights into carbon cycling in this area of the ocean.

Keywords: OMZ, ETNP, D373 research cruise, nitrous oxide, 15N-nitrogen, organic nitrogen, methane oxidation, anaerobic methane oxidation, methane production, N cycle, C cycle

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1. Cruise personnel

The list of the ships 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
Susanna Tuulia Maanoja	scientist	Queen Mary University of London
Simon Williams	scientist	University of Warwick
Laura Anne Bristow	scientist	University of Southern Denmark (SDU)
Dougal Mountfield	NOC technician	NOC
Darren Young	NOC technician	NOC
Zoltan Nemeth	IT support	NOC
Peter Charles Sarjeant	Master	NERC
Philip Douglas Gauld	Chief Officer	NERC
Malcolm Harold Graves	2nd Officer	NERC
Nigel Garnett	3rd Officer	NERC
Edward McLaughlin	Chief Engineer	NERC
Cambell Morrison	2nd Engineer	NERC
John Robert Harnett	3rd Engineer	NERC
Edin Silajdzic	3rd Engineer	NERC
Philip Mathews	ETO	NERC
David Ralph Hartshorne	PCO	NERC
Stuart Clive Cook	CPOD	NERC
Martin Andrew Harrison	CPOS	NERC
Steven Duncan	POD	NERC
Mark Stephen Moore	SG1A	NERC
John Brodowski	SG1A	NERC
Steven Gallagher	SG1A	NERC
Richard Deal	SG1A	NERC
Emlyn Gordon Williams	MM1A	NERC
Peter Anthony Lynch	H/Chef	NERC
Dean Anthony Hope	Chef	NERC
Jacqueline Waterhouse	STWD	NERC

2. Scientific objectives

2.1. Background rationale

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 N2O produced could be explained by nitrite (NO_2^-) reduction to N_2O (Nicholls et al., 2007a, b). 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 N2O 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 (Dalsgaard et al., 2003; Kuypers et al., 2005; Thamdrup et al., 2006) 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).

2.2. Cruise specific objectives

With this research cruise we aim to take our previous knowledge further and study N cycle in a non- yet characterized OMZ, the Eastern Tropical North Pacific (ETNP). The overall aim of this project is to link N cycling to bacterial gene diversity and expression across a gradient of OMZ intensity in the Eastern Tropical North Pacific (ETNP). The cruise objectives are summarized below.

- Characterization of the water column (e.g. N_2O, CH_4, CO_2 profiles)
- Experimental manipulations of O_2 and N_2O concentrations in the water column across the OMZ of ETNP to test if the ratio of N_2 to N_2O production is 'fixed' or 'flexible'.
- 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. The use of dual labelled isotopes will enable to trace the metabolism of ^{13}C and ^{15}N through both dissimilative and assimilative pathways (including SIP of nucleic acids).
- Molecular analysis of the active communities involved in the metabolism of the above mentioned gases.
- Analysis of zooplankton, phytoplankton and bacterioplankton collected with SAPs filters at each site.

- Analysis of NO_3^-, NO_2^- & NH_4^+ after addition of $^{15}NO_3^-$ or $^{15}NO_2^-$ to discrete seawater samples with a view to determining N cycling pathways.
- Analysis of natural abundance isotopes of NO_3 and NO_2 from selected samples

In order to determine the extent of variation and potential within the water column, we have performed experiments at sites outside, on the edge and within the core OMZ. 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.

In addition to N cycle, we also set up experiments to investigate aspects of the C cycle, such as methane oxidation and production processes. These are summarized below.

- Measurement of the rates of aerobic methane oxidation in ETNP, after addition of 12CH4 in seawater from various depths across the OMZ.
- Monitoring methane production in selected depths from 3 different sites.
- Measuring production of $^{13}CO_2$ from added $^{13}CH_4$ gas to water samples.
- Tracing of the oxidised $^{13}CH_4$ in bacterial biomass.
- Measurement of nitrite-driven Anaerobic Methane Oxidation (AMO), following addition of $^{15}NO_2^-$ and $^{13}CH_4$ in seawater of selected depths from 5 different sites.

3. Cruise overview

D373 took place between 07/12/2011 and 28/01/2012 in the waters of ETNP. The port of departure was Panama Balboa and the return port was Salina Cruz, Mexico (NB disembarkation was originally planned to take place in Panama Balboa, on 20th December but due to unforeseen circumstances- see section 4- this had to change). The ship track from the port of departure and CTD stations are shown in Figure 1. Scientific work was conducted at 6 sites across longitude 92° 30'W with latitudes as shown in Table 2. In addition, there were transects in between sites in order to collect oxygen data across this uncharacterized OMZ (Table 3).

Figure 1. D373 ship track, including port of departure and sampling sites. Numbers in red correspond to CTD stations.

Table 2. Sampling stations across 92W.

Station	Lat	CTD casts	# CTDs
1	12N	1 to 7	7
2	13N	8 to 16	9
3	11N	33 to 43	11
4	10N	55 to 66	12
4.5*	9.5N	86 to 87	2
5	9N	75 to 85	11
6	8N	95 to 102	8

* At station 4.5 SAPs were attached on the CTD wire and the only sampling activity was the collection of plankton on SAPs filters.

Table 3. Transects across 92W.

Transect	Lat	CTD casts	# CTDs
1	13 to 11N	17 to 32	16
2	11 to 10N	44 to 54	11
3	10 to 9N	67 to 74	8
4	9 to 8N	88 to 94	7

4. Diary cruise narrative

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

<u>07.12.11</u>

At Panama Balboa port, preparing for outbound passage

<u>08-11.12.11</u>

Passage towards the first station. On 10.12.12 (24:00) clocks were retarded 1h to UTC.

<u>11.11.11</u>

V/L arrived at Station 1.

CTD001- no bottles fired; to be repeated

CTD002- to 800 m, back on deck, scientists sampling- vertical profiles

CTD003- to 400 m, back on deck, scientists sampling- vertical profiles

<u>12.12.12</u>

CTD004- to 3893 m, back on deck, scientists sampling- vertical profiles

CTD005- to 700 m, back on deck, scientists sampling- targeted depths

<u>13.12.12</u>

CTD006- to 264 m, back on deck, scientists sampling- targeted depths

CTD007- to 264 m, back on deck, scientists sampling- targeted depths

SAPs in water; SAPs back on deck

<u>14.12.12</u>

SAPs in water, starboard A-Frame; SAPs back on deck

Reload SAPs on board

V/L approaching on port quarter (claimed to be fishing boat)- Keep clear in Spanish on VHF

Science differed to ensure fishing v/l intention is to keep clear

SAPs in water, starboard A-Frame; SAPs back on deck

<u>15.12.11</u>

Stopped for scanfish deployment. V/L at 4 Kts, scanfish in water

V/L increased speed to 6 Kts; cable out to 1000 m; Delays due to technical issues with winch.

Scanfish not fully deployed due to delays.

V/L at 8 Kts, 1000 m tow astern; steering following survey track

Complete turn to starboard to avoid net; completed turn

End of scanfish survey, scanfish on deck.

<u>16.12.11</u>

V/L slow steaming, towards survey start position

Scanfish deployment, V/L at 4 Kts

Increase speed to 6 Kts, cable out to 1100 m.

Increase speed to 8 Kts

Commenced port turn; turn completed.

Scanfish reported lost; V/L turning on reciprocal track; efforts to locate scanfish in the water.

V/L heading towards station 2

<u>17.12.11</u>

Passage; arrived at Station 2.

CTD008- to 1200 m, sampling complete at 540 m, back on deck, scientists sampling- vertical profiles

CTD009- to 520 m, back on deck, scientists sampling- vertical profiles

CTD010- to 60 m, back on deck, scientists sampling- vertical profiles

<u>18.12.11</u>

CTD011- to 4040 m, back on deck, scientists sampling- vertical profiles

CTD012- to 1000 m, back on deck, scientists sampling- targeted depths

<u>19.12.11</u>

CTD013- to 200 m, back on deck, scientists sampling- targeted depths

CTD014- to 200 m, back on deck, scientists sampling- targeted depths

CTD015- to 4030 m, back on deck, scientists sampling- targeted depths

<u>20.12.11</u>

Heading at SAPs station

SAPs 1 in water to 1250 m, SAPs back on board and secure

Manoeuver to reposition for next SAPs

SAPs in water to 15 m, SAPs recovered for adjustments, SAPs deployed to 35 m, SAPs back on deck

<u>21.12.11</u>

CTD016- to 1020 m, back on deck, scientists sampling- targeted depths

Heading south full speed for CTD station

CTD017- to 150 m (transect), back on deck

CTD018- to 150 m (transect), back on deck

CTD019- to 150 m (transect), back on deck

CTD020- to 150 m (transect), back on deck

CTD021- to 150 m (transect), back on deck, set onto next transect station

V/L hove to on station and CTD022 in water - to 150 m (transect), back on deck, set onto next transect station

CTD023- to 150 m (transect), back on deck

Slow steaming through night

<u>22.11.11</u>

CTD024- to 150 m (transect), back on deck, secure and commence steaming

CTD025- to 150 m (transect), back on deck, secure and commence steaming

CTD026- to 150 m (transect), back on deck, secure and commence steaming

CTD027- to 150 m (transect), back on deck, secure and commence steaming

CTD028- to 150 m (transect), back on deck, secure and commence steaming

CTD029- to 150 m (transect), back on deck, secure and commence steaming

CTD030- to 150 m (transect), back on deck, secure and commence steaming

CTD031- to 150 m (transect), back on deck, secure and commence steaming

CTD032- to 150 m (transect), back on deck, secure and commence steaming

Set heading for Station 3

<u>23.12.11</u>

Arrived at Station 3

CTD033- to 1000 m (sampling started at 900 m up to 440 m), back on deck, scientists sampling- vertical profiles

CTD034- to 420 m, back on deck, scientists sampling- vertical profiles

Emergency drill

CTD035- to 180 m, back on deck, scientists sampling- vertical profiles

<u>24.12.11</u>

CTD036- to 2635 m, back on deck, scientists sampling- vertical profiles

CTD037- to 650 m, back on deck, scientists sampling- targeted depths

CTD038- to 650 m, back on deck, scientists sampling- targeted depths

<u>25.12.11</u>

No deployments conducted on this day; Christmas celebrations

V/L lying ahull

Slow steaming towards Station 3

<u>26.12.11</u>

CTD039- to 200 m, back on deck, scientists sampling- targeted depths

CTD040- to 200 m, back on deck, scientists sampling- targeted depths

CTD041- to 2910 m, back on deck, scientists sampling- targeted depths

<u>27.12.11</u>

SAPs in water to 1130 m, SAPs back on board and secure

Manoeuver to reposition for next SAPs

SAPs in water to 45 m, filtering at 32 m and 45 m, SAPs back on deck

<u>28.12.11</u>

CTD042- to 650 m, back on deck, scientists sampling- targeted depths

CTD043- to 800 m, back on deck, scientists sampling- targeted depths

V/L preparing for CTD-transects

CTD044- to 150 m (transect), back on deck, secure and commence steaming

CTD045- to 150 m (transect), back on deck, secure and commence steaming

CTD046- to 150 m (transect), back on deck, secure and commence steaming

CTD047- to 150 m (transect), back on deck, secure and commence steaming

<u>29.12.11</u>

V/L hoving to into current, stemming current

CTD048- to 300 m (transect), back on deck, secure and commence steaming

CTD049- to 300 m (transect), back on deck, secure and commence steaming

CTD050- to 300 m (transect), back on deck, secure and commence steaming

CTD051- to 300 m (transect), back on deck, secure and commence steaming

CTD052- to 300 m (transect), back on deck, secure and commence steaming

CTD053- to 300 m (transect), back on deck, secure and commence steaming

CTD054- to 300 m (transect), back on deck, secure and commence steaming

End of CTD-transects; heading to Station 4

V/L move to preventing unwanted drift south

<u>30.12.11</u>

At Station 4

CTD055- to 900 m (sampling started at 860 m up to 584 m), back on deck, scientists sampling- vertical profiles

CTD056- to 568 m (sampling up to 292 m), back on deck, scientists sampling- vertical profiles

CTD057- to 280 m (sampling up to 148m), back on deck, scientists sampling- vertical profiles

31.12.11

Radar performances of both radars tested and were satisfactory

CTD058- to 3710 m, back on deck, scientists sampling- vertical profiles

CTD059- to 860 m, back on deck, scientists sampling- targeted depths

CTD060- to 700 m, back on deck, scientists sampling- targeted depths

01.01.12

CTD061- to 200 m, back on deck, scientists sampling- targeted depths

CTD062- to 250 m, back on deck, scientists sampling- targeted depths

CTD063- to 3702 m, back on deck, scientists sampling- targeted depths

Fire system reset, all investigated, clear

V/L hove to overnight

02.01.12

SAPs to 1080 m, back on board

Both SAPs in water to 50 m (filtering from 38 m and 50 m), SAPs recovered

03.01.12

V/L hove to in vicinity of site

CTD064- to 700 m, back on deck, scientists sampling- targeted depths

CTD065- to 250 m, back on deck, scientists sampling- targeted depths

CTD066- to 860 m, back on deck, scientists sampling- targeted depths

CTD066- to 860 m, back on deck, scientists sampling- targeted depths

V/L preparing for CTD-transect

CTD067- to 500 m (transect), back on deck, secure and commence steaming

CTD068- to 500 m (transect), back on deck, secure and commence steaming

Slow steaming to next transect station

04.01.12

CTD069- to 500 m (transect), back on deck, secure and commence steaming

CTD070- to 500 m (transect), back on deck, secure and commence steaming

V/L hove to on station for CTD

CTD071- to 500 m (transect), back on deck, secure and commence steaming

V/L hove to on station for CTD

CTD072- to 500 m (transect), back on deck, secure and commence steaming

CTD073- to 500 m (transect), back on deck, secure and commence steaming

CTD074- to 500 m (transect), back on deck, secure and commence steaming

05.01.12

Passage towards Station 5

At Station 5

CTD075- to 800 m (sampling up to 558 m), back on deck, scientists sampling- vertical profiles

CTD076- to 568 m (sampling started at 558 m up to 294 m), back on deck, scientists sampling- vertical profiles

CTD077- to 283 m (sampling up to 7 m), back on deck, scientists sampling- vertical profiles

06.01.12

CTD078- to 3603 m (sampling up to 43 m), back on deck, scientists sampling- vertical profiles

CTD079- to 800m, back on deck, scientists sampling- targeted depths

CTD080- to 600m, back on deck, scientists sampling- targeted depths

07.01.12

CTD081- to 67 m, back on deck, scientists sampling- targeted depths

CTD082- to 200 m, back on deck, scientists sampling- targeted depths

CTD083- to 3600 m, back on deck, scientists sampling- targeted depths

08.01.12

At site for SAPs

SAPs in water, pump at 650 m, Back on board

1st SAPs deployed to 20 m, 2nd SAPs in water, SAPs fully deployed to 55 m (filtering at 28 m and 55 m), back on deck

Move on site for CTD

09.01.12

CTD084- to 550 m, back on deck, scientists sampling- targeted depths

CTD085- to 718 m, back on deck, scientists sampling- targeted depths

V/L in station for SAPs (Station 4.5)

CTD086 at 20 m and connecting SAPs

1st SAPs in water, veered to 700 m, hauled to 645 m

1st SAPs at surface, exchange of pumps, CTD087 deployed to 340 m, stopped at 300 m

2nd SAPs in water

Station 4.5 completed

10.01.12

V/L at CTD position

CTD088- to 650 m (transect), back on deck, secure and commence steaming

CTD089- to 650 m (transect), back on deck, secure and commence steaming

CTD090- to 650 m (transect), back on deck, secure and commence steaming

V/L hove to on station for CTD

CTD091- to 698 m (transect), back on deck, secure and commence steaming

V/L hove to on station for CTD

CTD092- to 650 m (transect), back on deck, secure and commence steaming

V/L hove to on station for CTD

CTD093- to 650 m (transect), back on deck, secure and commence steaming

CTD094- to 650 m (transect), back on deck, secure and commence steaming

11.01.12

At Station 6, V/L lying ahull

CTD095- to 683 m (sampling started at 640 m up to 443 m), back on deck, scientists sampling- vertical profiles

CTD096- to 424 m (sampling up to 217 m), back on deck, scientists sampling- vertical profiles

CTD097- to 208 m (sampling up to 5 m), back on deck, scientists sampling- vertical profiles

12.01.12

CTD098- to 3000 m (sampling up to 82 m), back on deck, scientists sampling- vertical profiles and targeted depths

CTD099- to 640 m, back on deck, scientists sampling- targeted depths

CTD0100- to 540 m, back on deck, scientists sampling- targeted depths

13.01.12

CTD101- to 82 m, back on deck, scientists sampling- targeted depths

Sternline/propeller to recover fouled lines

Science operations suspended until further notice

V/L on station for CTD

CTD102- to 200 m, back on deck, scientists sampling- targeted depths

Sampling completed- end of science

Problem: Loss of engine lubricant started on the night of 12.01.12. This resulted to an early end of sampling activities and heading back to the planned disembarkation port (Panama Balboa). However, due to legal issues related to sailing through the Costa-Rican EEZ while pouring oil in the seawater, RRS Discovery had to stop in international waters outside Costa-Rica's EEZ. RRS Discovery had to wait for a tag vessel for about 5 days. This was followed by a tag operation (lasting approximately 1 week) with the involvement of two tag vessels, which brought RRS Discovery in the port of Salina Cruz/ Mexico (final disembarkation port) on 28.01.12.

5. Station/activities log

A detailed list of all the CTD casts throughout the cruise in given in Appendix 1, and a list of all the surface meteorology activities in

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

Station 1

The activities that took place at station 1, and the exact co-ordinates and times are shown in Table 4.

Table 4. Activities log- Station 1.

Date	Lat(N)	Long(W)	Activity ID	Start time	Bottom time	End Time	Description
11.12.11	12 0.346	92 30.220	CTD002	16:04	16:28	17:51	Vertical seawater profiles, 432-800 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C sample of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
11.12.11	12 0.388	92 30.304	CTD003	21:07	21:20	22:40	Vertical seawater profiles, 9-400 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
12.12.11	12 0.024	92 29.972	CTD004	14:05	15:26	17:44	Vertical seawater profiles, 5-3893 m, Gases (CO2, CH4, N2O) GC analysis onboard, DIN skalar analysis onboard, samples collected from certain depths (30, 2618, 3893 m) and spiked with 12CH4 and 13CH4 for CH4 oxidation experiment on board measured with GC, isotopic values of CO2 and biomass to be measured onshore, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
12.12.11	11 59.883	92 29.913	CTD005	20:41	21:01	21:35	Targeted depths (180, 390, 700) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases, samples collected from 180 m and spiked with 12CH4 and 13CH4 for CH4 oxidation experiment on board measured with GC, isotopic values of CO2 and biomass to be measured onshore, δ13C samples of CH4/IRMS analysis onshore, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS, seawater filtered at 390 and 700 m for onshore DNA and RNA extraction and analysis
13.12.11	11 59.980	92 30.106	CTD006	14:15	14:32	14:58	Targeted depths (12, 46, 264) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases, seawater filtered at 12, 46 and 264 m for onshore DNA and RNA extraction and analysis
13.12.11	12 0.224	92 30.048	CTD007	18:05	18:16	18:50	Targeted depths (12, 46, 264) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2O gas, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS
14.12.11	12 0.00	92 30.00	SAP-1-30m	16:47		19:00	SAPs at 30 m, filters to be analysed in the lab
14.12.11	12 0.00	92 30.00	SAP-1-180m	16:40		19:05	SAPs at 180 m, filters to be analysed in the lab

14.12.11	12 0.00	92 30.00	SAP-2-30m	23:00	00:48	SAPs at 30 m, filters to be analysed in the lab
14.12.11	12 0.00	92 30.00	SAP-2-180m	22:50	00:55	SAPs at 180 m, filters to be analysed in the lab
14-16.12.11	12 0.00	92 30.00	Scanfish			Scanfish deployment to obtain oxygen data in between sites, NB Scanfish was lost in the ocean

Times are in GMT.

Station 2

The activities that took place at station 2, and the exact co-ordinates and times are shown in Table 5.

Table 5. Activities log- Station 2.

Date	Lat(N)	Long(W)	Activity ID	Start time	Bottom time	End Time	Description
17.12.11	13 0.019	92 30.138	CTD008	14:06	14:32	16:04	Vertical seawater profiles, 540-1000 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2, sewater filtered at 1000, 840, 700 and 540 m for onshore DNA extraction and analysis
17.12.11	12 59.881	92 30.193	CTD009	18:24	18:41	19:53	Vertical seawater profiles, 60-520 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2 sewater filtered at 400, 240, 100 and 60 m for onshore DNA extraction and analysis
17.12.11	13 0.031	92 29.954	CTD010	21:59	22:06	22:36	Vertical seawater profiles, 5-60 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
18.12.11	13 0.126	92 30.021	CTD011	14:06	15:29	17:20	Vertical seawater profiles, 5-4055 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
18.12.11	13 0.029	92 29.913	CTD012	19:18	19:54	20:37	Targeted depths (540, 750, 1000) m, NH4 fluorometric analysis (540 m), Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases, 13CH4+ and 15NO2- istotope additions to seawater samples/ onboard incunbation and IRMS analysis onshore, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS, seawater filtered at 750 and 540 m for onshore DNA and RNA extraction and analysis
19.12.11	12 59.938	92 29.932	CTD013	14:07	14:20	14:49	Targeted depths (40, 100, 200) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases
19.12.11	12 59.885	92 29.932	CTD014	16:20	16:31	16:57	Targeted depths (40, 100, 200) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2O gas, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS, seawater filtered at 200 and 100 m for onshore DNA and RNA extraction and analysis
19.12.11	12 54.852	92 30.243	CTD015	18:25	19:47	21:36	Targeted depths (25, 35, 100, 540, 1250, 4030 m), Isotopes added to seawater samples/

							samples incubated onboard/ to be analysed by mass spectometry in the lab for N2O gas, samples collected from targeted depths and spiked with 12CH4 and 13CH4 for CH4 oxidation experiment on board measured with GC, isotopic values of CO2 and biomass to be measured onshore
20.12.11	13 0.00	92 30.00	SAP-3-1250m	14:41		17:43	SAPs at 1250 m, filters to be analysed in the lab
20.12.11	13 0.00	92 30.00	SAP-4-23m	20:15		21:59	SAPs at 23 m, filters to be analysed in the lab
20.12.11	13 0.00	92 30.00	SAP-4-35m	20:07		22:01	SAPs at 35 m, filters to be analysed in the lab
21.12.11	12 59.999	92 29.999	CTD016	14:06	14:31	15:35	Targeted depths (40, 100, 540) m, NH4 fluorometric analysis (560-1020 m), Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases, 13CH4+ and 15NO2- istotope additions to seawater samples/ onboard incunbation and IRMS analysis onshore, seawater samples collected at 100 m and 540 m for incubation using 13C-labelled TMA and Urea and fixation of cells for fluorescent in-situ hybridisation (FISH)

Times are in GMT.

Station 3

The activities that took place at station 3, and the exact co-ordinates and times are shown in Table 6.

Table 6. Activities log- Station 3.

Date	Lat(N)	Long(W)	Activity ID	Start time	Bottom time	End Time	Description
23.12.11	10 59.954	92 29.985	CTD033	14:03	14:30	15:50	Vertical seawater profiles, 440-900 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
23.12.11	11 0.000	92 29.863	CTD034	18:28	18:41	19:39	Vertical seawater profiles, 190-420 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
23.12.11	11 0.000	92 30.067	CTD035	22:51	22:59	00:00	Vertical seawater profiles, 7-180 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
24.12.11	10 59.989	92 29.909	CTD036	14:11	15:06	16:29	Vertical seawater profiles, 31-2635 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
24.12.11	10 59.898	92 30.023	CTD037	18:54	19:11	19:39	Targeted depths (420, 650) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases
24.12.11	10 59.982	92 30.054	CTD038	20:50	21:06	21:32	Targeted depths (420, 650) m, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-,

							NO2- & NH4+ by IRMS, seawater filtered at both depths for onshore DNA and RNA extraction and analysis
26.12.11	10 59.755	92 29.683	CTD039	15:31	15:41	16:12	Targeted depths (39, 100, 200) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases
26.12.11	10 59.781	92 29.771	CTD040	18:19	18:28	18:50	Targeted depths (39,100,200) m, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS, seawater filtered at 100 and 200 m for onshore DNA and RNA extraction and analysis
26.12.11	10 59.960	92 29.840	CTD041	20:50	22:14	23:35	Targeted depths (39, 300, 1130, 1360, 2760, 2910 m), Gases (CO2, CH4, N2O) GC analysis, δ13C samples of CH4/IRMS analysis onshore, samples collected from depths 39, 300, 1130 and 2910 m and spiked with 12CH4 and 13CH4 for CH4 oxidation experiment on board measured with GC, isotopic values of CO2 and biomass to be measured onshore
27.12.11	11 0.00	92 30.00	SAP-5-1130m	14:49		17:49	SAPs at 1130 m, filters to be analysed in the lab
27.12.11	11 0.00	92 30.00	SAP-6-32m	20:00		21:38	SAPs at 32 m, filters to be analysed in the lab
27.12.11	11 0.00	92 30.00	SAP-6-45m	17:58		21:40	SAPs at 45 m, filters to be analysed in the lab
28.12.11	10 59.997	92 29.844	CTD042	14:03	14:21	14:56	Targeted depths (100, 200, 420, 250) m; Organic N isotopes added to seawater samples/filtered onboard/ filters transferred to the lab for molecular analysis, 13CH4+ and 15NO2- istotope additions to seawater samples/ onboard incunbation and IRMS analysis onshore
28.12.11	11 0.024	92 29.860	CTD043	16:09	16:29	17:02	Targeted depths (39,800) m, seawater filtered at both depths for onshore DNA and RNA extraction and analysis

Times are in GMT.

Station 4

The activities that took place at station 4, and the exact co-ordinates and times are shown in Table 7.

Table 7. Activities log- Station 4.

Date	Lat(N)	Long(W)	Activity ID	Start time	Bottom time	End Time	Description
30.12.11	9 59.880	92 30.068	CTD055	14:04	14:38	15:55	Vertical seawater profiles, 584-860 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
30.12.11	10 0.170	92 29.913	CTD056	18:08	18:23	19:32	Vertical seawater profiles, 292-568 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2

30.12.11	10 0.012	92 30.007	CTD057	22:51	23:02	23:45	Vertical seawater profiles, 148-280 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
31.12.11	10 0.004	92 30.037	CTD058	14:01	15:13	17:23	Vertical seawater profiles, 4-3710 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
31.12.11	10 0.094	92 30.074	CTD059	20:05	20:25	21:05	Targeted depths (436, 700, 860) m, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS, water samples collected from depth 436 m and sealed for measuring methane production onshore seawater filtered at 436 and 700 m for onshore DNA and RNA extraction and analysis
31.12.11	10 0.063	92 29.976	CTD060	22:42	22:56	23:25	Targeted depths (436, 700) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases
01.01.12	9 59.983	92 30.027	CTD061	14:05	14:16	14:43	Targeted depths (40, 60, 200) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2O gas
01.01.12	10 0.001	92 30.033	CTD062	16:12	16:23	16:50	Targeted depths (40, 60, 250) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS, seawater filtered at 60 and 250 m for onshore DNA and RNA extraction and analysis
01.01.12	10 0.012	92 30.001	CTD063	18:01	19:18	20:46	Targeted depths (37, 268, 1080, 1155, 2435, 3710 m), Gases (CO2, CH4, N2O) GC analysis, δ13C samples of CH4/IRMS analysis onshore, samples collected from depths 37, 268, 1080 and 3710 m and spiked with 12CH4 and 13CH4 for CH4 oxidation experiment on board measured with GC, isotopic values of CO2 and biomass to be measured onshore
02.01.12	10 0.00	92 30.00	SAP-7-1080m	14:54		17:24	SAPs at 1080 m, filters to be analysed in the lab
02.01.12	10 0.00	92 30.00	SAP-8-50m	20:09		21:46	SAPs at 50 m, filters to be analysed in the lab
02.01.12	10 0.00	92 30.00	SAP-8-38m	20:12		21:44	SAPs at 38 m, filters to be analysed in the lab
03.01.12	10 0.026	92 30.177	CTD064	14:03	14:24	15:12	Targeted depths (45, 60, 436, 700) m, Organic N isotopes added to seawater samples/filtered onboard/ filters transferred to the lab for molecular analysis, 13CH4+ and 15NO2- istotope additions to seawater samples/ onboard incunbation and IRMS analysis onshore
03.01.12	9 59.942	92 29.904	CTD065	16:03	16:15	16:34	Targeted depth 250 m, Organic N isotopes added to seawater samples/filtered onboard/ filters transferred to the lab for molecular analysis
03.01.12	9 59.944	92 29.887	CTD066	17:09	17:32	18:16	Targeted depths (50, 436, 700, 860) m, 13CH4+ and 15NO2- istotope additions to seawater samples/ onboard incunbation and IRMS analysis onshore , seawater filtered 50 and 860 m depths for onshore DNA and RNA extraction and analysis, seawater samples collected at 436 m and 700 m for incubation using 13C-labelled TMA and Urea and fixation of cells for fluorescent in-

							situ hybridisation (FISH)

Times are in GMT.

Station 5

The activities that took place at station 5, and the exact co-ordinates and times are shown in Table 8.

Table 8. Activities log- Station 5.

Date	Lat(N)	Long(W)	Activity ID	Start time	Bottom time	End Time	Description
05.01.12	8 59.983	92 29.980	CTD075	14:01	14:24	15:46	Vertical seawater profiles, 558-800 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
05.01.12	8 59.970	92 30.014	CTD076	17:24	17:43	18:02	Vertical seawater profiles, 294-558 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
05.01.12	9 00.020	92 29.959	CTD077	21:05	21:18	22:29	Vertical seawater profiles, 7-283 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
06.01.12	8 59.967	92 30.020	CTD078	14:04	15:22	17:43	Vertical seawater profiles, 43-3603 m, Gases (CO2, CH4, N2O) GC analysis, DIN skalar analysis, NH4 fluorometric analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO3 and NO2
06.01.12	8 59.995	92 32.003	CTD079	19:17	19:38	20:15	Targeted depths (450, 600, 800) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS, seawater filtered at 450 and 600 m for onshore DNA and RNA extraction and analysis
06.01.12	8 59.987	92 29.961	CTD080	21:30	21:47	22:20	Targeted depths (450, 600) m, Organic N isotopes added to seawater samples/filtered onboard/ filters transferred to the lab for molecular analysis
07.01.12	8 59.937	92 30.018	CTD081	14:02	14:10	14:30	Targeted depths (30, 67) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2O gas

07.01.12	8 59.966	92 30.100	CTD082	15:55	16:06	16:28	Targeted depths (67, 200) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N2 and N2O gases, 15NO3- or 15NO2- stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO3-, NO2- & NH4+ by IRMS, seawater filtered at 67 and 200 m for onshore DNA and RNA extraction and analysis
07.01.12	8 59.948	92 30.064	CTD083	17:40	18:52	20:26	Targeted depths (31, 290, 640, 1048, 3600 m), ð13C samples of CH4/IRMS analysis onshore (1048 m), samples collected from depths 37, 268, 1080 and 3710 m and spiked with 12CH4 and 13CH4 for CH4 oxidation experiment on board measured with GC, isotopic values of CO2 and biomass to be measured onshore
08.01.12	9 0.00	92 30.00	SAP-9-640m	14:31		16:36	SAPs at 640 m, filters to be analysed in the lab
08.01.12	9 0.00	92 30.00	SAP-10-28m	18:57		20:28	SAPs at 28 m, filters to be analysed in the lab
08.01.12	9 0.00	92 30.00	SAP-10-55m	18:54		20:30	SAPs at 55 m, filters to be analysed in the lab
09.01.12	9 00.081	92 30.032	CTD084	14:03	14:24	15:05	Targeted depths (67, 200, 450, 550) m, Organic N isotopes added to seawater samples/filtered onboard/ filters transferred to the lab for molecular analysis, 13CH4+ and 15NO2- istotope additions to seawater samples/ onboard incunbation and IRMS analysis onshore
09.01.12	9 00.007	92 30.071	CTD085	16:03	16:25	17:02	Targeted depths (40, 450, 600, 718) m, water samples collected from depth 450 m and sealed for measuring methane production onshore, seawater filtered 40 and 718 m depths for onshore DNA and RNA extraction and analysis, seawater samples collected at 450 m and 600 m for incubation using 13C-labelled TMA and Urea and fixation of cells for fluorescent in-situ hybridisation (FISH)

Times are in GMT.

Station 4.5

The activities that took place at station 4.5, and the exact co-ordinates and times are shown in Table 9. At this station there were only SAPs deployments.

Table 9. Activities log- Station 4.5.

Date	**Lat(N)**	**Long(W)**	**Activity ID**	**Start time**	**Bottom time**	**End Time**	**Description**
09.01.12	9 30.030	92 30.118	SAP-11-290m	23:55		01:15	SAPs at 290 m, filters to be analysed in the lab
09.01.12	9 30.028	92 30.631	SAP-11-645m	21:32		23:28	SAPs at 645 m, filters to be analysed in the lab

Station 6

The activities that took place at station 6, and the exact co-ordinates and times are shown in Table 10.

Table 10. Activities log- Station 6.

Date	Lat(N)	Long(W)	Activity ID	Start time	Bottom time	End Time	Description
11.01.12	7 59.975	92 30.019	CTD095	13:59	14:18	15:45	Vertical seawater profiles, 433-640 m, Gases (CO_2, CH_4, N_2O) GC analysis, DIN skalar analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH_4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO_3 and NO_2
11.01.12	8 00.009	92 30.020	CTD096	18:03	18:15	19:27	Vertical seawater profiles, 217-424 m, Gases (CO_2, CH_4, N_2O) GC analysis, DIN skalar analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH_4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO_3 and NO_2
11.01.12	7 39.988	92 30.004	CTD097	21:24	21:34	22:35	Vertical seawater profiles, 5-208 m, Gases (CO_2, CH_4, N_2O) GC analysis, DIN skalar analysis, samples taken for onshore DON skalar analysis, δ13C samples of CH_4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO_3 and NO_2
12.01.12	8 00.005	92 30.002	CTD098	13:58	15:00	16:47	Vertical seawater profiles, 82-3000 m, Gases (CO_2, CH_4, N_2O) GC analysis, DIN skalar analysis, samples taken for onshore DON skalar analysis, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N_2 and N_2O gases (388, 540) m, δ13C samples of CH_4/IRMS analysis onshore, samples for onshore analysis of natural abundance isotopes of NO_3 and NO_2
12.01.12	8 00.024	92 29.882	CTD099	18:32	18:49	19:25	Targeted depths (388, 540, 640 m), $^{13}CH_4^+$ and $^{15}NO_2^-$ istotope additions to seawater samples/ onboard incunbation and IRMS analysis onshore, water samples collected from depth 388 m and sealed for measuring methane production onshore, $^{15}NO_3^-$ or $^{15}NO_2^-$ stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO_3^-, NO_2^- & NH_4^+ by IRMS, seawater filtered 388 and 540 m depths for onshore DNA and RNA extraction and analysis
12.01.12	7 59.941	92 29.964	CTD100	20:47	21:03	21:31	Targeted depths (388, 540) m, Organic N isotopes added to seawater samples/filtered onboard/ filters transferred to the lab for molecular analysis
13.01.12	07 59.993	92 29.972	CTD101	14:02	14:08	14:33	Targeted depths (33, 82) m, Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N_2 and N_2O gases
13.01.12	7 59.961	92 29.987	CTD102	17:25	17:38	18:09	Targeted depths (55, 82, 185 m), Isotopes added to seawater samples/ samples incubated onboard/ to be analysed by mass spectometry in the lab for N_2 gas, samples collected from depths 55 and 185 m and spiked with $^{12}CH_4$ and $^{13}CH_4$ for CH_4 oxidation experiment on board measured with GC, isotopic values of CO_2 and biomass to be measured onshore, $^{15}NO_3^-$ or $^{15}NO_2^-$ stable isotope additions to seawater samples and incubation onboard/discrete samples returned for onshore analysis of NO_3^-, NO_2^- & NH_4^+ by IRMS, seawater filtered 82 and 185 m depths for onshore DNA and RNA extraction and analysis

Times are in GMT

6. Operations

All samples mentioned in this section derived from deployments of 20 L Niskin bottles on a CTD rosette Sea-Bird 24. All sensors were calibrated and maintained by the responsible NOC technicians (Dougal Mountfield and Darren Young), and log sheets with all the relevant details are available.

6.1. Vertical water profiles

<u>Introduction</u>

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. However, the OMZ of ETNP is a largely uncharacterised area of the global ocean and very little has been published. To the best of our knowledge, the only available data (as to oxygen and nitrite concentrations) comes from transects shown in figure 2 (85°W transect) and 3 (88°W transect). For this reason, the first CTD casts of each site were carried out 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 we decided the exact depths from which we sampled for the planned experimental manipulations.

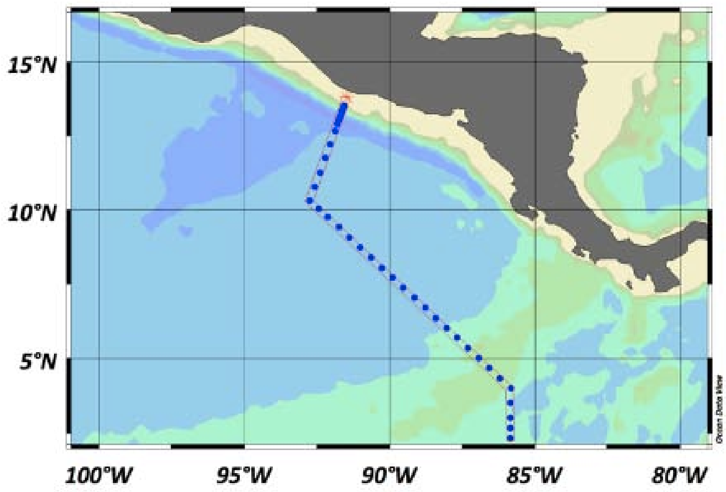

Figure 2. 85°W transect (chart provided by B. Thamdrup). Across this transect there is available data on oxygen and nitrite concentrations.

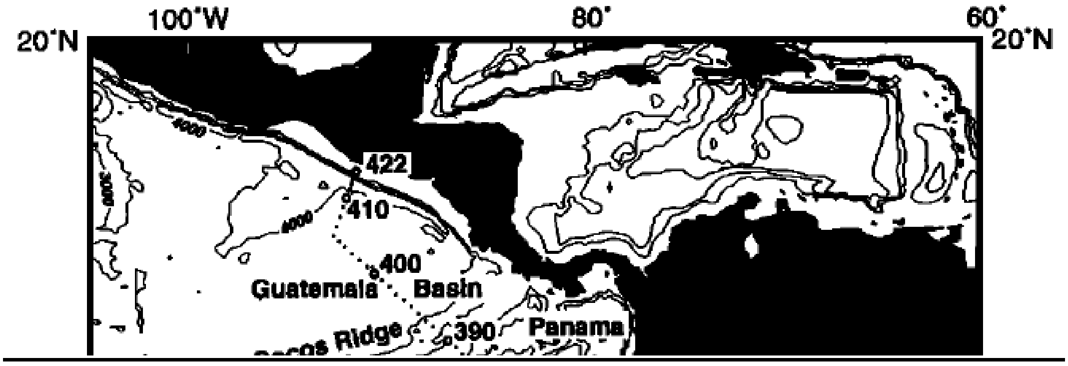

Figure 3. 88^{0}W transect. Adapted from a paper by (Tsuchiya and Talley, 1998), which gives oxygen data for stations indicated by the dotted line.

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 samples of unfiltered water (60 mL), collected in PTFE bottles directly from the Niskin bottles (after overfilling the former 3 times). In addition, water samples (15 ml) were collected for determination of bulk DON (potassium persulphate coupled to UV oxidation) offshore. Sub-micro molar NH_4^+ measurements were made in 80 ml water sample, using a Turner Designs TD-700 fluorimeter and the method of (Holmes et al., 1999).

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 a serum bottle (125 mL) 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).

For the O_2 profiles the data derived from all the CTD deployments at each depth/ station have been compiled using Matlab.

Preliminary Results

The concentrations of DIN (NO_3^- and NO_2^-), PO_4^{3-} and the N deficit are shown in figure 4. N deficit (representing the amount of fixed nitrogen that has been removed, i.e. due to denitrification) was calculated according to the equation $[(NO_3^- + NO_2^-)] - 16\times PO_4^{3-}$ (Gruber and Sarmiento, 1997; Chang et al., 2010). However, $[NH_4^+]$ should be added to calculations.

All measurements were made on single samples using a Skalar auto-analyser. Calibration of the instrument was conducted based on known amount of standard solutions. Measurements of NH_4^+ concentration were conducted using both Skalar and a Turner fluorimeter. Figure 5 shows measurements of the Turner fluorimeter for stations 1-4 (NB the fluorimeter was not functioning properly after station 5).

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. Results are shown in figure 6.

Oxygen saturation (%) for each station is shown in figure 7. Additional oxygen data was obtained in between the sampling stations, using the CTD sensors. Compilation of this data should provide us a detailed map of oxygen concentration of this previously uncharacterised area of the ocean.

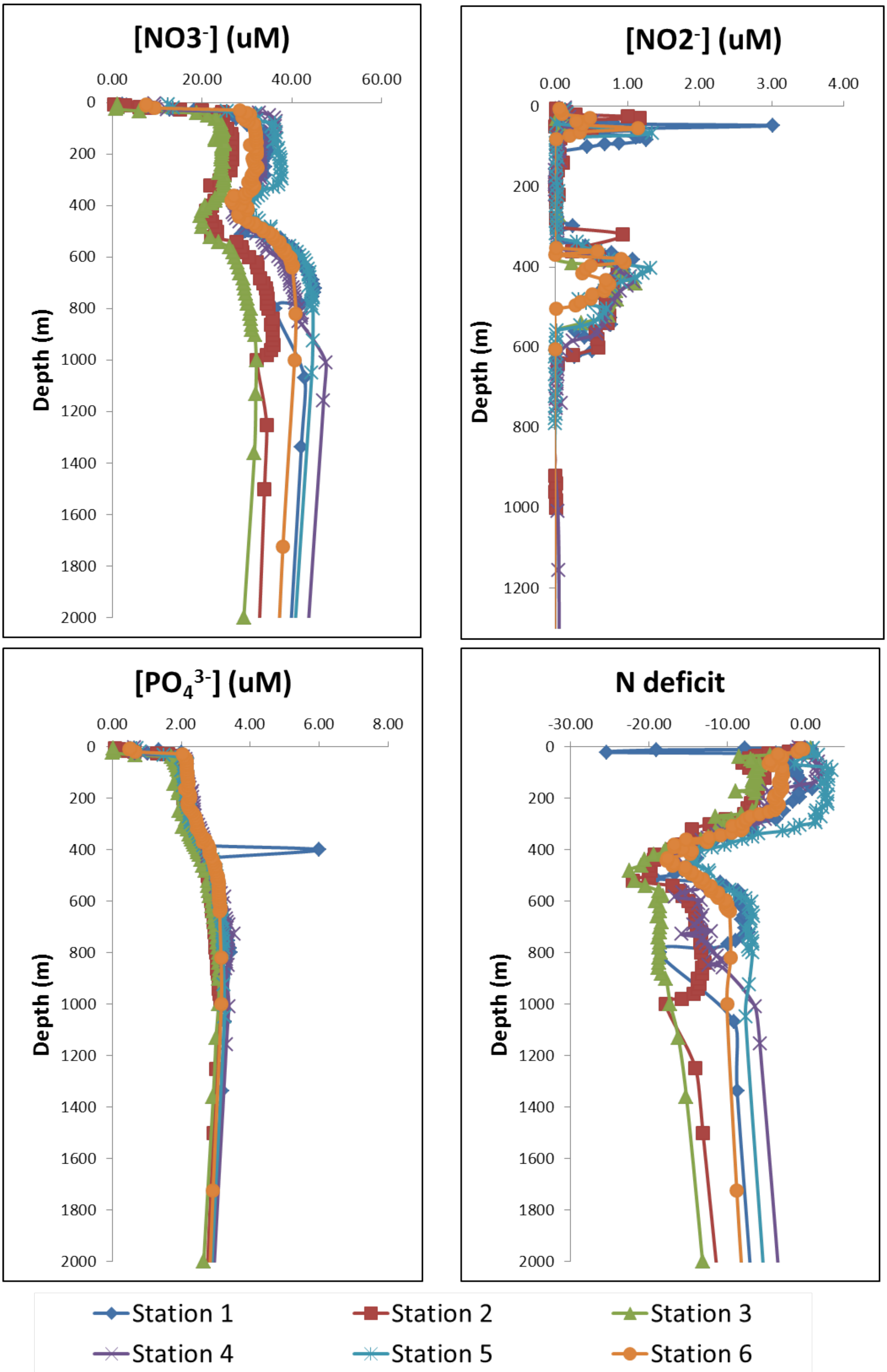

Figure 4. Concentrations of NO_3^-, NO_2^-, PO_4^{3-} and N deficit in the water column at all stations. All measurements were made using a Skalar flow auto-analyser.

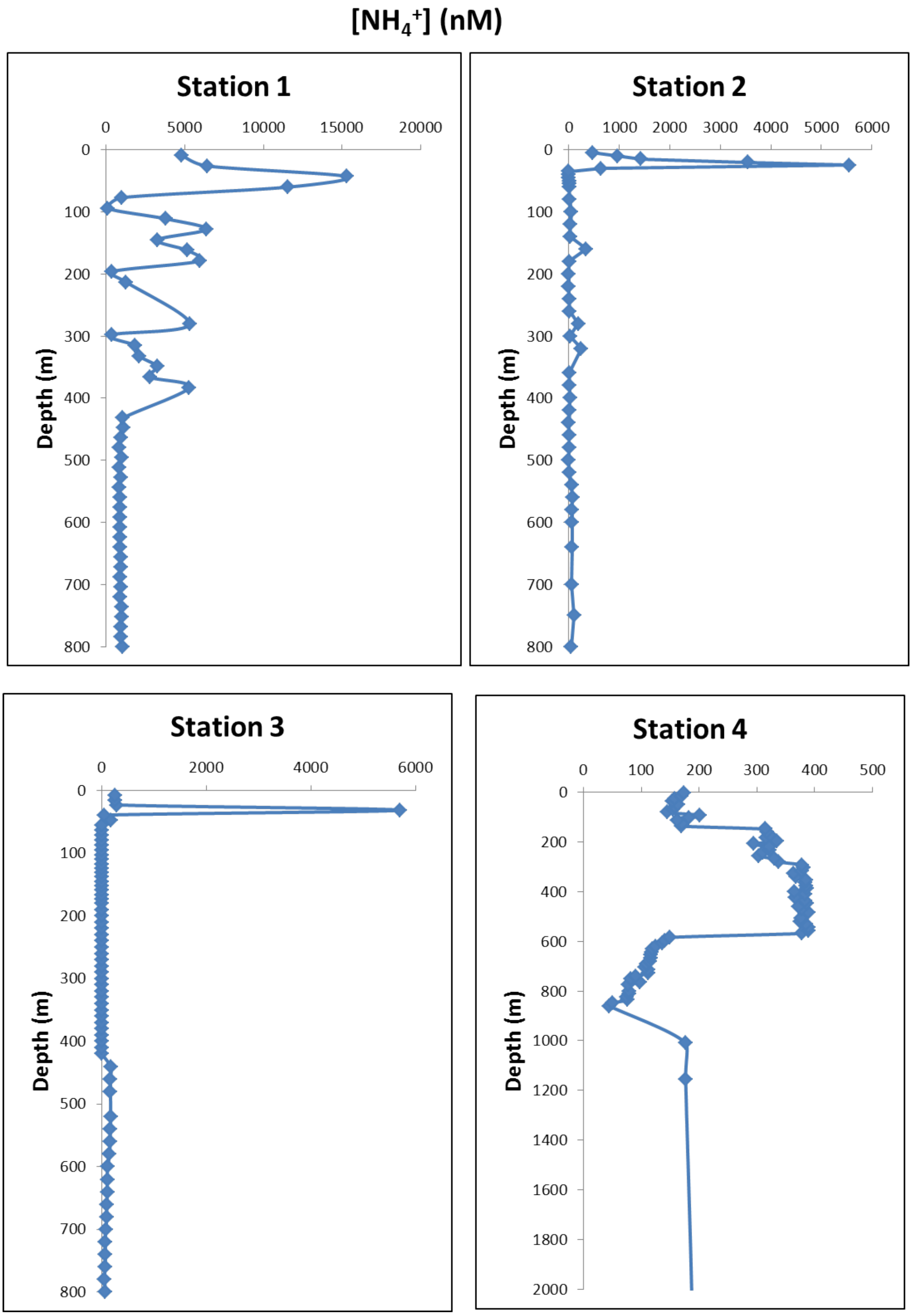

Figure 5. Profiles of NH4+ across various depths of four stations. Measurements were made using a Turner fluorimeter. Four or two replicate samples were measured at station 1, and only one at the other stations.

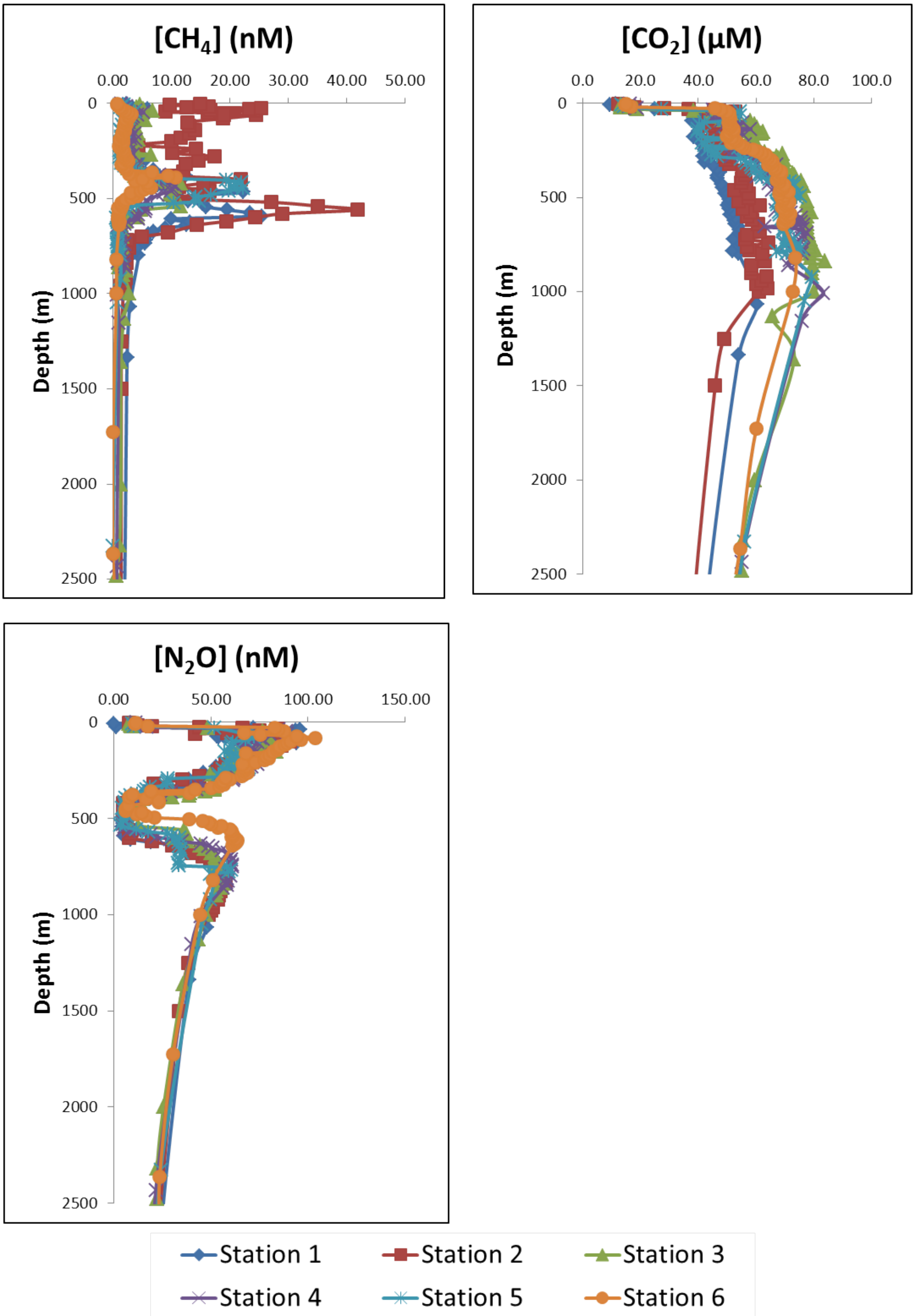

Figure 6. Concentrations of gases at various depths of all stations. Numbers are the mean of duplicate samples, measured on GC.

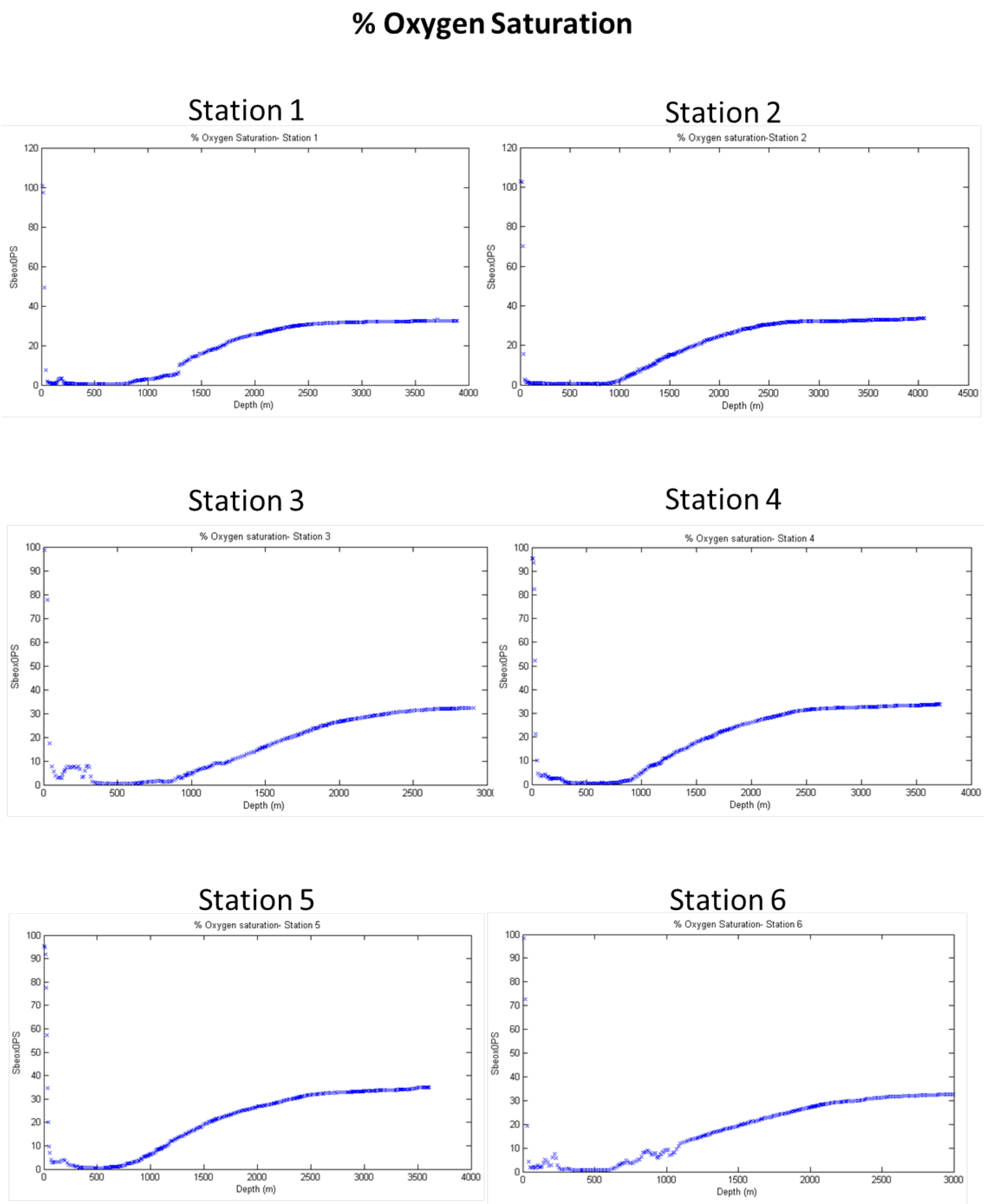

Figure 7. Oxygen saturation (%) profiles at 6 different stations. Measurements were made by CTD deployments.

6.2. Measuring rates of nitrous oxide production in ocean water using stable isotope $^{15}N\text{-}NO_2^-$

Introduction

The peaks and troughs in N_2O that we detected in the OMZ of the Arabian Sea (Nicholls et al., 2007) indicate that the production and reduction of N_2O are not occurring at the same rates at all depths. Examination of the ratio of $^{30}N_2$ to $^{46}N_2O$ produced from experiments with $^{15}N\text{-}NO_2^-$ showed that the ratio of N_2 to N_2O production was not constant and there was a significant negative correlation with the ambient concentration of N_2O ($r = 0.97$, $p = 0.03$), i.e., where *in situ* N_2O concentrations were high, there was a relatively low ratio of N_2 to N_2O produced. Hence, whilst the reduction of NO_2^- has long been hypothesised as a source of N_2O deep in the OMZ – previous results from the Arabian Sea suggest that NO_2^- reduction can explain N_2O production throughout the OMZ, and at any one depth it is potentially modulation of N_2O reduction that generates peaks and troughs in N_2O. Our aim is to measure N_2O production in another OMZ, namely ETNP, and investigate how this is modulated throughout the OMZ.

Materials & Methods

The procedure follows Nicholls et al. (2007) with one major modification. Water samples from 5 depths per station were collected from the CTD into 4 Litre polypropylene Nalgene bottles and additional samples were collected using 1 Litre glass serum bottles to act as references for the natural 15N abundance of nitrous oxide in the water. The Nalgenes were then gently degassed (1 Bar) for 20 minutes with a variety of non-toxic gases, namely: oxygen and nitrogen (0.75% and 99.25%, respectively); nitrous oxide and nitrogen (0.0002% and 99.999%, respectively); oxygen, nitrous oxide and nitrogen (as before) or helium (99.999%). Once degassed, the water was then dispensed under pressure (1 Bar) into 1 litre glass serum bottles, 1 mL of $^{15}N\text{-}NO_2^-$ (1mM) stable isotope tracer was then added to give 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 72 h. Subsequently, microbial activity was stopped by injecting formaldehyde (6 mL of 37% solution CH2O) through the septa (with venting) in the fume hood to give a final solution for formaldehyde of 0.2%. The bottles were then safely stowed for transport back to the home port.

All samples are to be 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).

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

Introduction

The last couple of years our knowledge of the N cycle is continuously enriched. 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 experiment we aim to further investigate our hypothesis, using a range of labelled and dual labelled organic substances (i.e. ^{15}N- urea, $^{15}N(^{13}C)$ –urea, ^{15}N (^{13}C)-TMA) as well as the classic anammox reactions ($^{14}NO_2^-$ + $^{15}NH_4^+$ or $^{15}NO_2^-$ + $^{14}NH_4^+$). This should allow us to trace the metabolism of ^{13}C and ^{15}N through both dissimilative and assimilative pathways.

Materials & Methods

This experiment was conducted at four selected depths at each station. 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 ul of stable isotopes to a final concentration of 10 µM (for $^{15}NO_2^-$, $^{14}NO_2^-$,$^{15}NH_4^+$) or 50 µM (for ^{15}N- urea, ^{14}N- urea, ^{15}N (^{13}C)-TMA, ^{14}N-TMA) and incubate for a pre-defined time period. Finally, microbial activity was stopped by injecting 50 µl of $ZnCl_2$ (50%). Exetainers were stored safely in designated carton boxes.

Samples are currently being analysed for N_2 gas production using an IRMS, and measured for $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$.

6.4. Molecular characterization of the microbial community involved in N cycle processes.

Introduction

Apart from looking at the processes of N cycle, it is equally important to gain good insights into the microorganisms involved. For this purpose, bacterial cells 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* genes). Our hypothesis is that there will be changes in expression where the reduction of N_2O is either stimulated or inhibited.

As we have used ^{13}C-labelled organic-N compounds there will also be Stable Isotope Probing (SIP) experiments to separate the extracted nucleic acids for analysis with bacterial and archaeal specific PCR to identify the organisms involved in the degradation of added organic-N compounds. In addition to this, samples of water from selected depths will be analysed with FISH probes for a range of known anammox organisms (Schmid et al., 2005) and for members of the communities identified as potential partners in the SIP analysis.

Materials & Methods

Water samples were collected from the CTD/Niskin rig and transported into the ship's lab for filtration through 0.2 µM polycarbonate filters using a gentle vacuum or via CellTraps™ via a peristaltic pump. Volume of water may vary but did not exceed 10 L. Water was transported in 10-20 L Carboys/Nalgene bottles before being filtered small volumes (250 ml) at a time. CellTraps™ will filter about 2 L at a time or be filtered directly from the sampling vessel and required no pouring into a smaller container. In between sites sampling vessels were washed with 10% hydrochloric acid (HCl). 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) will be extracted and subjected to pyrosequencing and/or quantitative PCR to target functional genes of N cycle.

For the FISH (Fluorescence In-Situ Hybridisation) experiment seawater samples from two selected depths of stations 2, 4 and 5 were transferred to the ship's main lab from the CTD in small volumes (~200 ml) and 4% Paraformaldehyde added (final concentration 1%) and stored overnight at 4°C before freezing at -20°C. Bacterial cells are to be observed using a fluorescence microscope in the lab.

6.5. Determination of nitrite oxidation rates using stable isotopes and measurement of natural abundance isotopic composition of nitrite and nitrate

Introduction

The overal purpose of this experiment is the determination of nitrite oxidation rates, which will elucidate further our knowledge of the N cycling pathways in the OMZ of ETNP. This will be measured and compared in ambient conditions and after addition of $^{15}NO_3^-$ or $^{15}NO_2^-$ to discrete seawater samples.

Materials & Methods

For the determination of nitrite oxidation rates, 150ml water samples were collected directly from the CTD and a 15N nitrite (stable isotope) spike was added; to a single sample from each depth 1.6ml of 37% formaldehyde was added to stop microbial activity. Samples were subsequently drawn from these spiked samples and filtered through a 0.22µm filter and frozen, samples were taken immediately after the spike was added and then after 6, 12 and 24 hours. After each sample was taken the headspace in the sample bottle was degassed with helium and an injection of air made where necessary to amend the oxygen concentration in the sample. In order to determine the rates of nitrite oxidation it is important to know the isotopic composition of the ambient pool of nitrite and nitrate. For this purpose, samples were collected directly from the CTD and filtered through a GF/F, an aliquot of this sample is preserved with 6M sodium hydroxide as in (Casciotti and McIlvin, 2007) and frozen.

6.6. Measurement of methane oxidation rates and tracing of the oxidised carbon into end products of methane oxidation by ^{13}C-labelling in seawater samples

Introduction

Along with N cycle procedures, we aim to investigate aspects of the C cycle. For this purpose we set up experiments to measure methane oxidation rates, as well as trace the final products of methane oxidation.

Materials & Methods

The procedure follows (Kelley, 2003) with minor modifications. Water samples were collected from the CTD. A headspace of helium was inserted to sample vials and the ambient concentration of CH4 and CO_2 was measured with a GC-FID using a standard gas mixture for calibration. A headspace of helium and oxygen mixture is inserted in incubation samples and the samples for measuring methane oxidation rates were spiked with 180 – 220 µl of 0.2 – 1 % $^{12}CH_4$ gas. Samples for stable isotope labelling were spiked with 900 µl of 5 % $^{13}CH_4$ gas or with 300 µl of supersaturated $^{13}CH_4$ solution. Control samples were treated with 25 – 550 µl of concentrated (37 %) HCl and all samples were incubated at 10oC. Samples spiked with $^{12}CH_4$ were measured with the GC-FID every 48 h or less frequently until the methane was been used up. These samples were used as a reference for 13C-labelled samples, which were killed with concentrated HCl when the methane was assumed to be used up. Labelled samples were stored for stable isotope analysis in England. In addition, there were samples collected from each station and spiked with $^{13}CH_4$ with a view to trace the oxidised $^{13}CH_4$ to $^{13}CO_2$ in the bacterial cell biomass.

When back in UK, all samples were killed with concentrated HCL (marking the last time point of the experiments). Samples spiked with $^{12}CH_4$ gas have already been processed, using GC-FID. Samples spiked with $^{13}CH_4$ will be analysed using IRMS. Samples for the measurement of assimilated $^{13}CO_2$ in the bacterial biomass, were filtered through GF/C filters (0.2 µm pore size) and filters stored in -20oC.

6.7. Methane production

Anoxic water of selected depths at 3 stations was sampled from the Niskin bottles into 125 of 150 ml serum bottles (after 3 times overflow). Control samples were treated with 1 ml of 37% formaldehyde, in order to stop microbial activity. The rest of the samples were incubated until arrival in England, where they were all treated with 37% formaldehyde. After helium headspacing (16% of final volume), samples were measured for methane production using GC-FID. Initial analysis of the results does not reveal obvious methane production, but we need to analyse the data in more detail.

6.8. Anaerobic Methane Oxidation (AOM)

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 dinitrogen 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 two selected depths at four stations. Seawater was sampled from the CTD into 1 L serum bottles and subsampled (under He pressure) in exetainers. Subsequently, exetainers were spiked with $^{15}NO_2^-$ and $^{13}CH_4$ and after a predefined incubation period, microbial activity was stopped with the addition of 37% formaldehyde. Samples were transferred back in the UK and will be analysed using IRMS.

6.9. Zooplankton, phytoplankton and bacterioplankton analysis

Introduction

Along with the investigation of processes involved in the N and C cycles we took the opportunity to collect planktonic cells of all sizes, with a view to investigate how these organisms are involved in N and C cycling.

Materials & Methods

Large volumes of seawater from selected depths of all stations were filtered using Stand Alone Pumps (SAPs). For the collection of zooplankton cells mesh ø293 mm disk filters of 200 µm pore size were used, for phytoplankton cells ø293 mm disk filters of 20 µm pore size (both supplied by SEFAR NITEX), and bacterioplankton cells were collected on ø 293 mm polycarbonate filters, 0.2 µm pore size. All filters were immediately frozen (in liquid nitrogen) after collection and stored at -80 ^{o}C. Zooplankton and phytoplankton will be analysed for ^{13}C assimilation using IRMS. Bacterial cells will be used for DNA extraction and subsequent analysis.

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Appendix 1.

Below is a detailed list of all the CTD casts, with exact co-ordinates and times.

stn	*Nm*	*lat*	*lon*	*day*	*start*	*cast*	*end*	*depth*	*start lat*	*start lon*	*cast lat*	*cast lon*
1	**CTD001**	**12 00.21N**	**92 30.19W**	**345**	**14:40**	**15:10**	**15:35**	**1284**	**12.00358**	**-92.50322**	**12.00416**	**-92.50383**
1	**CTD002**	**12 00.34N**	**92 30.22W**	**345**	**16:02**	**16:27**	**17:48**	**900**	**12.00568**	**-92.50372**	**12.00674**	**-92.50426**
1	**CTD003**	**12 00.38N**	**92 30.30W**	**345**	**21:06**	**21:19**	**22:36**	**400**	**12.00631**	**-92.50499**	**12.00736**	**-92.50512**
1	**CTD004**	**12 00.02N**	**92 29.97W**	**346**	**14:06**	**15:26**	**17:36**	**3893**	**12.00062**	**-92.49954**	**12.00062**	**-92.50049**
1	**CTD005**	**11 59.88N**	**92 29.92W**	**346**	**20:40**	**21:00**	**21:33**	**700**	**11.99800**	**-92.49859**	**11.99852**	**-92.49902**
1	**CTD006**	**12 00.02N**	**92 30.13W**	**347**	**14:21**	**14:31**	**14:55**	**264**	**12.00031**	**-92.50216**	**12.00162**	**-92.50329**
1	**CTD007**	**12 00.22N**	**92 30.05W**	**347**	**18:04**	**18:16**	**18:47**	**264**	**12.00368**	**-92.50079**	**12.00435**	**-92.50056**
2	**CTD008**	**13 00.02N**	**92 30.12W**	**351**	**14:06**	**14:32**	**16:00**	**1200**	**13.00036**	**-92.50208**	**12.99890**	**-92.50391**
2	**CTD009**	**12 59.84N**	**92 30.21W**	**351**	**18:28**	**18:40**	**19:50**	**520**	**12.99800**	**-92.50319**	**12.99731**	**-92.50539**
2	**CTD010**	**13 00.03N**	**92 29.95W**	**351**	**21:59**	**22:05**	**22:34**	**60**	**13.00555**	**-92.53600**	**13.00071**	**-92.49942**
2	**CTD011**	**13 00.11N**	**92 30.01W**	**352**	**14:05**	**15:29**	**17:15**	**4055**	**13.00188**	**-92.50018**	**13.00416**	**-92.50139**
2	**CTD012**	**13 00.03N**	**92 29.92W**	**352**	**19:18**	**19:54**	**20:35**	**1000**	**13.00050**	**-92.49866**	**13.00353**	**-92.49553**
2	**CTD013**	**12 59.93N**	**92 29.93W**	**353**	**14:13**	**14:20**	**14:45**	**200**	**12.99888**	**-92.49884**	**12.99916**	**-92.49880**
2	**CTD014**	**12 59.88N**	**92 29.93W**	**353**	**16:23**	**16:30**	**16:55**	**200**	**12.99801**	**-92.49882**	**12.99820**	**-92.49914**
2	**CTD015**	**12 59.85N**	**92 30.25W**	**353**	**18:28**	**19:47**	**21:40**	**4030**	**12.99757**	**-92.50414**	**12.99725**	**-92.50731**
2	**CTD016**	**13 00.00N**	**92 30.00W**	**355**	**14:10**	**14:31**	**15:32**	**1020**	**13.00006**	**-92.50004**	**13.00021**	**-92.49956**
T1	**CTD017**	**12 53.17N**	**92 29.96W**	**355**	**17:31**	**17:38**	**17:43**	**150**	**12.88613**	**-92.49938**	**12.88598**	**-92.49972**
T2	**CTD018**	**12 46.60N**	**92 30.08W**	**355**	**18:39**	**18:46**	**18:53**	**150**	**12.77663**	**-92.50138**	**12.77679**	**-92.50161**
T3	**CTD019**	**12 40.01N**	**92 30.04W**	**355**	**20:01**	**20:08**	**20:14**	**150**	**12.66677**	**-92.50068**	**12.66640**	**-92.50105**
T4	**CTD020**	**12 33.29N**	**92 30.04W**	**355**	**21:23**	**21:27**	**21:33**	**150**	**12.55478**	**-92.50070**	**12.55500**	**-92.50091**
T5	**CTD021**	**12 26.68N**	**92 30.03W**	**355**	**22:39**	**22:46**	**22:52**	**150**	**12.44474**	**-92.50052**	**12.44481**	**-92.50062**
T6	**CTD022**	**12 20.01N**	**92 29.99W**	**355**	**23:59**	**00:05**	**00:13**	**150**	**12.33343**	**-92.49982**	**12.33334**	**-92.49957**
T7	**CTD023**	**12 13.34N**	**92 30.02W**	**356**	**01:14**	**01:22**	**01:30**	**150**	**12.22239**	**-92.50026**	**12.22211**	**-92.49985**
T8	**CTD024**	**12 06.65N**	**92 30.04W**	**356**	**13:59**	**14:06**	**14:12**	**150**	**12.11090**	**-92.50069**	**12.11047**	**-92.50102**
T9	**CTD025**	**11 53.29N**	**92 30.06W**	**356**	**15:54**	**16:02**	**16:07**	**150**	**11.88824**	**-92.50093**	**11.88800**	**-92.50247**
T10	**CTD026**	**11 46.74N**	**92 30.33W**	**356**	**17:11**	**17:19**	**17:25**	**150**	**11.77902**	**-92.50552**	**11.77881**	**-92.50573**
T11	**CTD027**	**11 39.92N**	**92 30.16W**	**356**	**18:27**	**18:34**	**18:41**	**150**	**11.66538**	**-92.50268**	**11.66545**	**-92.50297**
T12	**CTD028**	**11 33.30N**	**92 30.06W**	**356**	**19:42**	**19:49**	**19:54**	**150**	**11.55504**	**-92.50098**	**11.55546**	**-92.50133**
T13	**CTD029**	**11 26.65N**	**92 30.06W**	**356**	**20:54**	**21:01**	**21:07**	**150**	**11.44411**	**-92.50104**	**11.44460**	**-92.50101**
T14	**CTD030**	**11 19.96N**	**92 30.13W**	**356**	**22:10**	**22:17**	**22:21**	**150**	**11.33274**	**-92.50211**	**11.33252**	**-92.50380**
T15	**CTD031**	**11 13.42N**	**92 30.09W**	**356**	**23:22**	**23:29**	**23:33**	**150**	**11.22362**	**-92.50148**	**11.22313**	**-92.50371**
T16	**CTD032**	**11 06.64N**	**92 30.04W**	**357**	**00:38**	**00:42**	**00:48**	**150**	**11.11069**	**-92.80077**	**11.11054**	**-92.50201**
3	**CTD033**	**10 59.96N**	**92 29.98W**	**357**	**14:04**	**14:28**	**15:49**	**1000**	**10.99925**	**-92.49947**	**10.99928**	**-92.49947**
3	**CTD034**	**11 00.01N**	**92 29.87W**	**357**	**18:28**	**18:41**	**19:39**	**420**	**11.00009**	**-92.49778**	**10.99827**	**-92.49793**
3	**CTD035**	**11 00.01N**	**92 30.06W**	**357**	**22:51**	**22:59**	**00:00**	**180**	**11.00010**	**-92.50096**	**10.99973**	**-92.50132**
3	**CTD036**	**10 59.99N**	**92 29.91W**	**358**	**14:11**	**15:06**	**16:29**	**2635**	**10.99983**	**-92.49850**	**10.99904**	**-92.49968**
3	**CTD037**	**10 59.90N**	**92 30.02W**	**358**	**18:55**	**19:11**	**19:39**	**650**	**10.99828**	**-92.50038**	**10.99760**	**-92.50132**
3	**CTD038**	**10 59.98N**	**92 30.05W**	**358**	**20:50**	**21:06**	**21:32**	**650**	**10.99972**	**-92.50084**	**10.99954**	**-92.50164**

3	CTD039	10 59.74N	92 29.66W	360	15:31	15:41	16:12	200	10.99571	-92.49429	10.99692	-92.49653
3	CTD040	10 59.78N	92 29.77W	360	18:19	18:28	18:50	200	10.99635	-92.49614	10.99663	-92.49706
3	CTD041	10 59.96N	92 29.84W	360	20:50	22:14	23:35	2910	10.99936	-92.49732	10.99412	-92.50226
3	CTD042	10 59.99N	92 29.82W	362	14:02	14:21	14:56	650	10.99991	-92.49696	11.00045	-92.49884
3	CTD043	11 00.02N	92 29.84W	362	16:09	16:29	17:02	800	11.00038	-92.49727	11.00049	-92.49875
W1	CTD044	10 55.11N	92 30.02W	362	18:28	18:32	18:38	150	10.91853	-92.50028	10.91876	-92.50098
W2	CTD045	10 50.06N	92 29.99W	362	19:40	19:46	19:51	150	10.83434	-92.49990	10.83518	-92.50022
W3	CTD046	10 45.05N	92 29.97W	362	20:59	21:04	21:10	150	10.75083	-92.49957	10.75116	-92.50004
W4	CTD047	10 40.01N	92 30.05W	362	22:14	22:21	22:27	150	10.66681	-92.50055	10.66679	-92.50123
W5	CTD048	10 35.00N	92 29.98W	363	13:58	14:11	14:24	300	10.58332	-92.49961	10.58229	-92.50015
W6	CTD049	10 29.93N	92 30.09W	363	15:13	15:23	15:35	300	10.49887	-92.50145	10.49851	-92.50204
W7	CTD050	10 24.91N	92 30.03W	363	16:25	16:37	16:48	300	10.41515	-92.50056	10.41469	-92.50071
W8	CTD051	10 19.95N	92 30.01W	363	17:32	17:43	17:54	300	10.33254	-92.50009	10.33216	-92.50087
W9	CTD052	10 15.01N	92 29.92W	363	18:48	18:57	19:07	300	10.25009	-92.49865	10.24975	-92.49798
W10	CTD053	10 10.07N	92 29.98W	363	20:04	20:15	20:25	300	10.16788	-92.49970	10.16822	-92.49980
W11	CTD054	10 04.98N	92 29.97W	363	21:30	21:40	21:51	300	10.08304	-92.49944	10.08260	-92.49899
4	CTD055	09 59.90N	92 30.06W	364	14:04	14:38	15:55	900	9.99829	-92.50093	9.99686	-92.50231
4	CTD056	10 00.18N	92 29.91W	364	18:08	18:23	19:32	568	10.00294	-92.49845	10.00066	-92.50044
4	CTD057	10.00.02N	92 29.99W	364	22:51	23:02	23:45	280	10.00034	-92.49984	9.99902	-92.50149
4	CTD058	10 00.01N	92 30.03W	365	14:01	15:13	17:23	3710	10.00014	-92.50042	10.00044	-92.50305
4	CTD059	10 00.09N	92 30.06W	365	20:05	20:25	21:05	860	10.00143	-92.50098	10.00258	-92.50273
4	CTD060	10 00.07N	92 30.03W	365	22:42	22:56	23:25	700	10.00120	-92.50044	10.00267	-92.50277
4	CTD061	09 59.98N	92 30.03W	001	14:05	14:16	14:43	200	9.99975	-92.50042	9.99949	-92.50055
4	CTD062	10 00.00N	92 30.03W	001	16:12	16:23	16:50	250	9.99997	-92.50050	10.00013	-92.50049
4	CTD063	10 00.01N	92 30.00W	001	18:01	19:18	20:46	3702	10.00019	-92.50003	9.99962	-92.50051
4	CTD064	10 00.02N	92 30.16W	003	14:03	14:24	15:12	700	10.00040	-92.50273	10.00220	-92.50561
4	CTD065	09 59.94N	92 29.89W	003	16:03	16:15	16:34	250	9.99899	-92.49815	10.00019	-92.50023
4	CTD066	09 59.93N	92 29.85W	003	17:09	17:32	18:16	1732	9.99887	-92.49757	10.00038	-92.50021
P1	CTD067	09 55.07N	92 30.08W	003	22:30	22:46	23:01	500	9.91789	-92.50126	9.92150	-92.50562
P2	CTD068	09 50.06N	92 30.11W	004	00:10	00:26	00:40	500	9.83428	-92.50175	9.83653	-92.50571
P3	CTD069	09 44.87N	92 30.15W	004	14:06	14:26	14:41	500	9.74786	-92.50258	9.74823	-92.50358
P4	CTD070	09 40.10N	92 30.09W	004	15:50	16:15	16:28	500	9.66831	-92.50157	9.66904	-92.50296
P5	CTD071	09 32.07N	92 29.97W	004	18:18	18:41	18:56	500	9.53455	-92.49951	9.53706	-92.49830
P6	CTD072	09 24.09N	92 30.10W	004	20:27	20:48	21:03	500	9.40154	-92.50170	9.40311	-92.50472
P7	CTD073	09 16.03N	92 29.98W	004	22:37	22:54	23:12	500	9.26713	-92.49962	9.26755	-92.50107
P8	CTD074	09 08.04N	92 29.99W	005	00:37	00:51	01:06	500	9.13393	-92.49907	9.13446	-92.50146
5	CTD075	08 59.98N	92 29.98W	005	14:01	14:24	15:46	800	8.99974	-92.49959	8.99999	-92.49959
5	CTD076	08 59.98N	92 30.01W	005	17:24	17:43	18:02	558	8.99963	-92.50013	8.99902	-92.50045
5	CTD077	09 00.03N	92 29.95W	005	21:05	21:18	22:29	283	9.00044	-92.49917	8.99975	-92.49963
5	CTD078	08 59.98N	92 30.01W	006	14:04	15:22	17:43	3603	8.99968	-92.50011	9.00030	-92.50100
5	CTD079	09 00.00N	92 30.00W	006	19:17	19:38	20:15	800	9.00001	-92.50001	8.99903	-92.50102
5	CTD080	08 59.99N	92 29.96W	006	21:30	21:47	22:20	600	8.99988	-92.49929	8.99824	-92.50020
5	CTD081	08 59.94N	92 30.01W	007	14:02	14:10	14:30	67	8.99906	-92.50020	8.99797	-92.50124
5	CTD082	08 59.98N	92 30.09W	007	15:55	16:06	16:28	200	8.99961	-92.50151	8.99891	-92.50229
5	CTD083	08 59.95N	92 30.06W	007	17:40	18:52	20:26	3600	8.99918	-92.50099	8.99961	-92.50055
5	CTD084	09 00.07N	92 30.03W	009	14:03	14:24	15:05	550	9.00125	-92.50044	9.00269	-92.50278

5	CTD085	09 00.00N	92 30.04W	009	16:03	16:25	17:02	718	8.99998	-92.50060	9.00048	-92.50230
4.5	CTD086	09 30.03N	92 30.11W	009	21:24	21:53	23:29	700	9.50045	-92.50189	9.50124	-92.50473
4.5	CTD087	09 30.03N	92 30.65W	009	23:31	23:45	01:19	340	9.50055	-92.51075	9.50095	-92.51190
W1	CTD088	08 52.52N	92 30.01W	010	14:01	14:20	14:31	650	8.87540	-92.50022	8.87505	-92.50128
W2	CTD089	08 44.89N	92 30.10W	010	15:39	15:58	16:15	650	8.74822	-92.50163	8.74712	-92.50246
W3	CTD090	08 37.45N	92 30.00W	010	17:13	17:32	17:47	650	8.62410	-92.49998	8.62316	-92.50039
W4	CTD091	08 29.91N	92 30.00W	010	18:48	19:05	19:24	650	8.49858	-92.49993	8.49813	-92.49869
W5	CTD092	08 22.41N	92 30.00W	010	20:21	20:38	20:56	650	8.37354	-92.49998	8.37264	-92.49980
W6	CTD093	08 14.92N	92 30.04W	010	21:57	22:13	22:29	650	8.24865	-92.50072	8.24751	-92.50098
W7	CTD094	08 07.53N	92 30.01W	010	23:31	23:49	00:06	650	8.12549	-92.50017	8.12472	-92.50103
6	CTD095	07 59.97N	92 30.02W	011	13:59	14:18	15:45	683	7.99957	-92.50031	7.99946	-92.50103
6	CTD096	08 00.01N	92 30.01W	011	18:03	18:15	19:27	424	8.00018	-92.50023	7.99951	-92.50087
6	CTD097	07 59.99N	92 30.00W	011	21:24	21:34	22:35	208	7.99980	-92.50001	7.99971	-92.50036
6	CTD098	08 00.01N	92 30.00W	012	13:58	15:00	16:47	3000	8.00011	-92.50008	8.00052	-92.49967
6	CTD099	08 00.02N	92 29.98W	012	18:32	18:49	19:25	640	8.00036	-92.49817	8.00010	-92.49748
6	CTD100	07 59.94N	92 29.97W	012	20:47	21:03	21:31	540	7.99903	-92.49943	7.99914	-92.49875
6	CTD101	07 59.99N	92 29.97W	013	14:02	14:08	14:33	82	7.99987	-92.49955	7.99982	-92.49964
6	CTD102	07 59.96N	92 29.99W	013	17:25	17:38	18:09	185	7.99937	-92.49976	7.99911	-92.50000

Letters T, W and P indicate transects, i.e. CTD used only to get oxygen data in between sites.

Appendix 2.

Below is a detailed list of all the surface meteorology activities.

Surfmet Log Sheet D373

Julian Day	Time	Activity	Crate	Bottle	Lat	Lon	Salin
343	01:00	Wetsensors cleaned	-	-	7.438098	-82.540314	29.1976
345	09:00	salinity sample	121-144	121	11.606422	-91.634423	33.3205
345	21:00	salinity sample	121-144	122	12.005766	-92.504961	33.1547
346	09:00	salinity sample	121-144	123	12.202474	-92.631505	33.1034
346	21:00	salinity sample	121-144	124	11.998521	-92.499022	33.1399
347	09:00	salinity sample	121-144	125	12.175046	-92.653779	33.1399
347	21:00	salinity sample	121-144	126	12.021022	-92.502236	33.0896
348	09:00	salinity sample	121-144	127	12.065431	-92.478299	33.6595
348	21:00	salinity sample	121-144	128	12.004811	-92.480020	33.0842
348	21:00-21:03	Transm. & Fluorimeter cleaned					
349	09:00	salinity sample	121-144	129	11.840847	-92.440283	33.1039
349	21:00	salinity sample	121-144	130	11.789299	-92.024179	33.4212
350	09:00	salinity sample	121-144	131	11.930006	-92.480764	33.1499
350	21:00	salinity sample	121-144	132	11.854723	-92.898125	33.4192
351	09:00	salinity sample	121-144	133	12.614873	-92.581991	33.3804
351	21:00	salinity sample	121-144	134	13.005746	-92.536335	33.3727
352	09:00	salinity sample	121-144	135	12.927101	-92.504818	33.4486
352	13:50-14:10	Transm. & Fluorimeter cleaned					
352	21:00	salinity sample	121-144	136	13.011346	-92.493059	33.4169
353	09:00	salinity sample	121-144	137	13.070555	-92.546184	33.6276
353	21:00	salinity sample	121-144	138	12.994725	-92.513497	33.3649
354	09:00	salinity sample	121-144	139	12.981590	-92.628032	33.3991
354	09:01-09:04	Transm. & Fluorimeter cleaned					
354	21:00	salinity sample	121-144	140	13.005057	-92.497279	33.4518
355	09:00	salinity sample	121-144	141	12.968843	-92.382508	33.5385
355	21:00	salinity sample	121-144	142	12.576844	-92.499058	33.3714
356	09:00	salinity sample	121-144	143	12.153960	-92.442537	33.3017
356	21:00	salinity sample	121-144	144	11.444539	-92.501036	33.3435
357	14:00	salinity sample	73-96	73	10.999479	-92.499279	33.5538
358	02:00	salinity sample	73-96	74	11.025632	-92.478840	33.6023
358	14:00	salinity sample	73-96	75	10.999635	-92.499075	33.6002
359	01:50	salinity sample	73-96	76	11.042645	-92.538597	33.5838
359	13:50	salinity sample	73-96	77	10.997518	-92.758919	33.6423
360	02:27	salinity sample	73-96	78	11.000596	-92.775576	33.6351
360	14:00	salinity sample	73-96	79	11.002752	-92.502755	33.9539
361	01:50	salinity sample	73-96	80	10.963166	-92.476817	34.1356

361	13:50	salinity sample	73-96	81	11.001033	-92.499130	34.0915
362	01:50	salinity sample	73-96	82	10.951041	-92.472692	34.1003
362	13:50	salinity sample	73-96	83	10.999897	-92.596059	34.0333
362	13:50-13:57	Transm. & Fluorimeter cleaned					
363	01:50	salinity sample	73-96	84	10.644516	-92.494857	34.1003
363	14:00	salinity sample	73-96	85	10.583175	-92.499544	33.9974
364	01:50	salinity sample	73-96	86	10.040088	-92.457990	34.1598
364	13:50	salinity sample	73-96	87	9.998839	-92.501098	34.1718
365	02:00	salinity sample	73-96	88	10.005058	-92.502407	34.1422
365	13:45	salinity sample	73-96	89	9.998675	-92.507167	34.0455
365	22:48-23:10	Transm. & Fluorimeter cleaned					
001	02:00	salinity sample	73-96	90	10.008042	-92.480218	33.9832
001	13:50	salinity sample	73-96	91	10.000158	-92.499873	33.9808
002	01:50	salinity sample	73-96	92	10.000573	-92.461120	34.0504
002	14:00	salinity sample	73-96	93	10.000326	-92.500137	33.9837
003	01:50	salinity sample	73-96	94	10.003954	-92.528247	34.1159
003	12:50-13:01	Transmissometer Optics Cleaned					
003	13:50	salinity sample	73-96	95	9.998520	-92.499781	33.62229
004	01:50	salinity sample	73-96	96	9.851130	-92.511505	33.6913
004	14:00	salinity sample	25-48	25	9.747849	-92.500912	33.7733
005	01:50	salinity sample	25-48	26	9.141392	-92.495228	34.2779
005	13:50	salinity sample	25-48	27	8.999848	-92.500440	34.0871
005	13:51-13:58	Transm. & Fluorimeter cleaned					
006	01:50	salinity sample	25-48	28	9.023254	-92.528326	34.099
006	13:50	salinity sample	25-48	29	8.999570	-92.501278	34.0685
007	01:50	salinity sample	25-48	30	9.013964	-92.552002	34.112
007	14:05	salinity sample	25-48	31	8.998648	-92.500569	34.002
008	01:50	salinity sample	25-48	32	8.948691	-92.602872	33.9824
008	13:50	salinity sample	25-48	33	9.000112	-92.500629	33.8176
008	13:50-14:10	TSG, Transm. & Fluorimeter cleaned					
008	16:42-16:58	Non-Toxic tap closed (plumbing works)					
009	01:50	salinity sample	25-48	34	9.008046	-92.499718	33.9285
009	13:50	salinity sample	25-48	35	8.999913	-92.500190	33.9771
010	01:58	salinity sample	25-48	36	9.477794	-92.530974	34.161
010	14:00	salinity sample	25-48	37	8.875445	-92.500009	34.0024
011	01:50	salinity sample	25-48	38	8.035913	-92.508463	33.9502
011	13:50	salinity sample	25-48	39	7.999658	-92.499759	33.9197
011	13:52	Transm. & Fluorimeter cleaned					
012	01:50	salinity sample	25-48	40	8.000198	-92.516938	33.8112
012	13:50	salinity sample	25-48	41	7.999515	-92.502701	33.7939
013	01:50	salinity sample	25-48	42	7.995008	-92.506347	33.8231
013	13:50	salinity sample	25-48	43	7.999986	-92.499948	33.8317
014	01:50	salinity sample	25-48	44	8.202622	-92.848423	33.7991
014	14:00	salinity sample	25-48	45	8.556995	-92.125959	34.0098
015	01:50	salinity sample	25-48	46	8.549022	-89.000115	34.276
015	14:00	salinity sample	25-48	47	8.497340	-89.155943	34.3651