UK Ocean Acidification Research Program

RRS *Discovery 366/367*

6th June to 12th July 2011

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Preface

The data presented in this Cruise Report are provisional and should not be used or reproduced without permission. In some cases they are fully calibrated and in other cases not. Further details can be obtained from the originators (see Scientific Reports). In due course the full data set will be lodged with the British Oceanographic Data Centre.

Cruise participants in cruise D366/D367.

Acknowledgements

We thank NERC for funding our research programme and providing us with ship time. We thank the crew and officers of the RRS *Discovery* for their excellent assistance at sea.

1- Introduction

Since the onset of the industrial revolution, the partial pressure of CO_2 (pCO_2) in the atmosphere has increased by more than 30% from 280 µatm to the present value of 390 µatm[1]. The atmospheric CO_2 increase has been partially buffered by a net increase in CO_2 flux into the ocean. The ocean has a considerable capacity to store CO_2; approximately 40% of the anthropogenic CO_2 released between 1800 and 1994 has been absorbed by the ocean[2].

As CO_2 reacts with seawater, it initially forms carbonic acid and generates strong changes in carbonate chemistry through well-known chemical reactions. The increase of pCO_2 in the surface ocean decreases the concentration of carbonate ions ($[CO_3^{2-}]$), increases the concentration of bicarbonate ions ($[HCO_3^-]$), lowers the pH and the saturation state ($\Omega_{CaCO3} = [CO_3^{2-}] \times [Ca^{2+}]/K_{sp}$) of the major shell-forming calcium carbonate ($CaCO_3$) minerals[3]. The increase in atmospheric pCO_2 from pre-industrial to current concentrations has resulted in a decrease in average surface ocean pH of ca. 0.1 unit, from pH 8.2 to 8.1. The decrease in surface ocean pH under the IS92A CO_2 emission scenario ("business as usual") will be another 0.3-0.4 units by the end of the 21st century, followed by a maximum reduction of ~0.8 units by the year 2300 (ref 4). The carbonate ion concentration is predicted to fall to about half of pre-industrial levels by the year 2100 (ref 5), leading to a halving of Ω_{CaCO3}. Average surface ocean dissolved inorganic carbon (DIC) concentrations are expected to increase from ca. 2026 (present) to 2144 µmol kg^{-1}. Carbonate ion and Ω_{CaCO3} values are naturally lower towards the poles and surface waters in polar regions will become under-saturated (i.e. corrosive) with respect to the $CaCO_3$

minerals aragonite and calcite before other regions (e.g. aragonite within decades[6] or possibly even sooner as a result of their low temperatures with consequent higher CO_2 gas solubility, and hence low pH and carbonate ion concentrations.

This report is for the first cruise of the NERC funded Ocean Acidification Programme. The goal of our Ocean Acidification Consortium project is to quantitatively investigate the links between changes in the ocean carbonate system (Ocean Acidification; OA) and organism physiology and morphometry (including $CaCO_3$ shells), biogeochemical rates, plankton biodiversity and community structure, food webs and climate-relevant processes. Our goal will be realized primarily through three cruises to the UK shelf region, Arctic and Southern Oceans, focussing on in-situ observations across natural carbonate chemistry gradients, and by carrying out on-deck CO_2 perturbation incubations. In addition, land-based laboratory experiments will be undertaken. The work will make a distinctive UK contribution by adopting an approach that complements existing/planned international OA research. To our knowledge this study will represent the first attempt to link pelagic ocean carbonate system variations with sea-surface biology, biogeochemical rates and climate processes in such a comprehensive manner.

The overall aim of this project is to obtain a quantitative understanding of the impact of OA on the surface ocean biology and ecosystem, and on the role of the surface ocean within the overall Earth System.

Our high-level objectives are:

1. To ascertain the impact of OA on planktonic organisms (in terms of physiological impacts, morphology, population abundances and community composition).
2. To quantify the impacts of OA on biogeochemical processes affecting the ocean carbon cycle (both directly and indirectly, such as via availability of bio-limiting nutrients).
3. To quantify the impacts of OA on the air-sea flux of climate active gases (DMS and N_2O in particular).

The primary hypotheses (more specific hypotheses are listed in section 3) which we will test are:

(H1) *A decline in pH and Ω_{CaCO3} as a result of rising atmospheric CO_2 concentrations will affect the rate and quality of formation of $CaCO_3$ shells by planktonic calcifiers.*

(H2) *Carbonate chemistry changes will influence biogeochemical rates per unit biomass, such as photosynthesis, respiration, calcification and nitrification.*

(H3) *Community structure will change and calcifying organisms will make up less of the total community (and consist of less strongly calcified genotypes) under lower pH/Ω_{CaCO3} conditions.*

(H4) *OA will impact on climate through reductions in ballasting by $CaCO_3$, production of albedo-altering DMS and production of the greenhouse gas N_2O.*

(H5) *High CO_2 will alter zooplankton:phytoplankton and phytoplankton:bacteria ratios through production of increasingly carbon-rich particulate and dissolved organics (food quality and DOC).*

(H6) *Some place-to-place differences in in-situ parameters are due to carbonate chemistry gradients rather than to alternative environmental gradients*.

In addition to these objectives three days of Discovery shiptime devoted to sampling the cold-water coral Lophelia pertusa for the benthic UKOA consortium (cruise D367) were integrated with cruise D366. Scleractinian cold-water coral species like L. pertusa form amongst the most three-dimensionally complex and biodiverse habitats on continental shelves, slopes and seamounts7 and their aragonitic skeletons are particularly vulnerable to shallowing of the aragonite saturation horizon projected over the next century[5,6,7,8]. Live corals were sampled at the start of the cruise from the Mingulay Reef Complex in the Sea of the Hebrides to allow ship-board measurements of respiration and growth on freshly-collected specimens. At the end of the cruise further samples were taken to establish an 18-month laboratory-based experiment following coral metabolism and growth under elevated pCO_2 and temperature.

References: 1 Hofmann DJ et al (2009) *Atmos. Environ.* 43:2084-2086. **2** Zeebe RE et al (2008) *Science* 321:51-52. **3** Zeebe RE and Wolf-Gladrow D (2001) *CO2 in Seawater*, Elsevier Amsterdam **4** Caldeira K and ME Wickett (2003) *Nature* 425:365–365. **5** Orr JC et al (2005) *Nature* 437:681-686. **6** Steinacher M et al (2009) *Biogeosciences* 6:515-533. **7** Roberts et al (2006) Science 312:543-547. **8** Guinotte et al (2006) Front. Ecol. Envr. 4: 141-146

2- Narrative cruise overview D366/D367

The RRS *Discovery* departed for cruise D366/D367 from Liverpool, UK, at 0830 h on Monday 6th June 2011 and docked in Liverpool, UK, after a period of 34 days at sea at 1700 h on Saturday 9th July. Cruise D367 was directly linked to D366, and formed part of the benthic UK Ocean Acidification consortium. Cruise 366 was the first cruise of the UK Ocean Acidification project: 'Ocean Acidification Impacts on Sea-Surface Biology, Biogeochemistry and Climate'. The subsequent two cruises for this consortium will be in the Arctic and Antarctic Oceans. The main aims of the D366 cruise were to assess the influence of oceanic carbonate chemistry conditions on microbial communities and biogeochemical processes through observational work, and of enhanced pCO_2 levels on the microbial community in ship-board bioassay experiments. The main aim of the D367 cruise was to collect deep water corals for both ship-board and land-based experimental pCO_2 perturbation work. The D366/D367 cruise is part of the NERC/DEFRA/DECC funded UK Ocean Acidification programme. There were no sharp time boundaries between activities underpinning the D366 and D367 cruises, but cruise D366 roughly took up 29-31 days of the 34 days sea-time, and D367 roughly 3-5 days.

Cruise participants are listed in Table 1.1.

The research vessel RRS *Discovery* was our platform; the vessel performed well, with only minor down-time due to malfunctioning equipment. We lost ca. 1-2 days as a consequence of winch problems (cable and hydraulic issues). In addition, we had to delay stations because of high seas; this caused a delay of ca. 1-2 days.

During the cruise all scientific work was recorded on Greenwich Mean Time (GMT).

We occupied 69 stations in European shelf waters thereby conducting 75 CTD casts, sampled the Mingulay reef for corals and undertook 9 biological pCO_2 incubation experiments. Of the nine experiments, five were large collaborative experiments, 3 were experiments of limited scope involving nutrient enrichments in addition to pCO_2 amendments, and one involving a more gradual increase in pCO_2 partial pressure. The incubations were conducted in a specially built laboratory container, which will be available for the two subsequent OA cruises.

Table 1.1: Scientific personnel

Name	**Institution**
Eric Achterberg	University of Southampton
Steve Archer	Plymouth Marine Laboratory
Lizeth Avendano	University of Southampton
Dorothee Bakker	University of East Anglia
Cecilia Belastreri	Marine Biological Association
Laura Bretherton	University of Essex
Ian Brown	Plymouth Marine Laboratory
Darren Clark	Plymouth Marine Laboratory
Cynthia Dumousseaud	University of Southampton
Sebastian Hennige	Herriot-Watt University
Ross Holland	University of Southampton
Frances Hopkins	Plymouth Marine Laboratory
Evelyn Lawrenz	University of Essex
Gareth Lee	University of East Anglia
Alex Poulton	University of Southampton
Victoire Rerolle	University of Southampton
Sophie Richier	University of Southampton
Tingting Shi	University of Southampton
Mark Stinchcombe	University of Southampton
David Suggett	University of Essex
Toby Tyrell	University of Southampton
Laura Wicks	Herriot-Watt University

The cruise track during D366 is shown in Figure 1.1, covering a distance of 4730 nautical miles. A total of 69 stations were occupied from 6th June – 7th July 2011 (Table 1.2). We typically occupied two stations per day, one pre-dawn and one close to lunch time. All CTD deployments were made with a stainless steel frame. On days where bioassay experiments were initiated, the sample handling for these experiments precluded pre-dawn stations on many occasions. The underway sample collection commenced in the Irish Sea on June 7th and continued until 1600 h July 6th.

Dates, times and locations of stations together with detailed information of scientific activities on station are provided in the CTD report and the narrative cruise diary (Appendix B). Dates, times and positions of underway samples are also provided in Appendix C.

Table 1.2 stations list

Station	Month	Date	Time	Latitude	Longitude
1	6	6	11:24	53°6 N	3°33 W
2	6	6	17;30	54°41 N	3°88 W
3	6	7	04:30	54°66 N	5°35 W
4	6	7	12:00	55°53 N	6°68 W
5	6	7	16:00	55°76 N	7°26 W
6	6	8	05:11	56°79 N	7°40 W
6	6	8	13:25	56°81 N	7°40 W
6	6	8	19:40	56°81 N	7°38 W
6	6	9	02:18	56°82 N	7°39 W
7	6	9	13:00	56°35 N	7°85 W
8	6	9	16:00	56°13 N	8°13 W
9	6	10	09:20	54°36 N	10°74 W
10	6	10	16:00	53°70 N	11°30 W
11	6	11	04:30	52°13 N	11°71 W
12	6	11	12:00	51°25 N	11°33 W
13	6	12	10:00	50°61 N	8°69 W
14	6	12	15:00	50°81 N	7°91 W
15	6	13	04:30	51°61 N	5°71 W
16	6	13	18:10	53°6 N	5°7 W
17	6	14	05:50	52°47 N	5°89 W
18	6	14	13:05	51°23 N	6°05 W
19	6	15	04:30	50°02 N	4°38 W
20	6	15	13:55	49°85 N	5°33 W
21	6	16	09:00	49°89 N	7°88 W
22	6	16	14:00	49°56 N	8°26 W
23	6	17	15:00	48°58 N	9°48 W
24	6	19	04:30	46°49 N	7°20 W
25	6	19	12:00	45°46 N	7°16 W
26	6	21	04:15	46°17 N	7°22 W
27	6	21	13:00	47°02 N	7°61 W
28	6	22	04:15	48°00 N	7°19 W

Table 1.2 Stations list (continued)

Station	Month	Date	Time	Latitude	Longitude
29	6	22	14:44	48°81 N	5°13 W
30	6	23	09:00	49°86 N	5°27 W
31	6	23	13:55	50°08 N	4°61 W
32	6	24	04:25	50°02 N	4°36 W
33	6	24	15:00	50°33 N	2°33 W
34	6	25	06:00	50°41 N	0°15 E
35	6	25	17:45	51°64 N	2°04 E
37	6	26	04:15	52°99 N	2°50 E
38	6	26	12:00	53°65 N	4°18 E
39	6	27	04:30	54°30 N	7°3 E
40	6	27	12:00	54°36 N	5°15 E
41	6	28	08:00	57°20 N	3°48 E
43	6	28	12:00	57°44 N	3°95 E
45	6	28	15:20	57°68 N	4°41 E
47	6	28	18:45	57°91 N	4°86 E
48	6	29	03:30	57°66 N	4°58 E
49	6	29	08:30	56°99 N	4°99 E
52	6	29	14:00	57°45 N	5°53 E
55	6	30	08:00	58°49 N	10°00 E
56	6	30	12:04	58°2 N	9°2 E
57	6	30	16:00	57°86 N	8°33 E
59	7	2	03:00	56°49 N	3°60 E
60	7	2	11:00	57°24 N	4°03 E
61	7	3	03:30	59°67 N	4°12 E
62	7	3	11:00	59°89 N	2°51 E
63	7	4	08:00	59°94 N	1°78 W
64	7	4	13:00	59°98 N	2°65 W
65	7	5	03:30	59°98 N	5°98 W
66	7	5	14:35	59°41 N	7°78 W
67	7	6	04:00	58°60 N	10°49 W

68	7	6	14;00	57°43 N	11°16 W
69	7	7	08:38	56°82 N	7°38 W

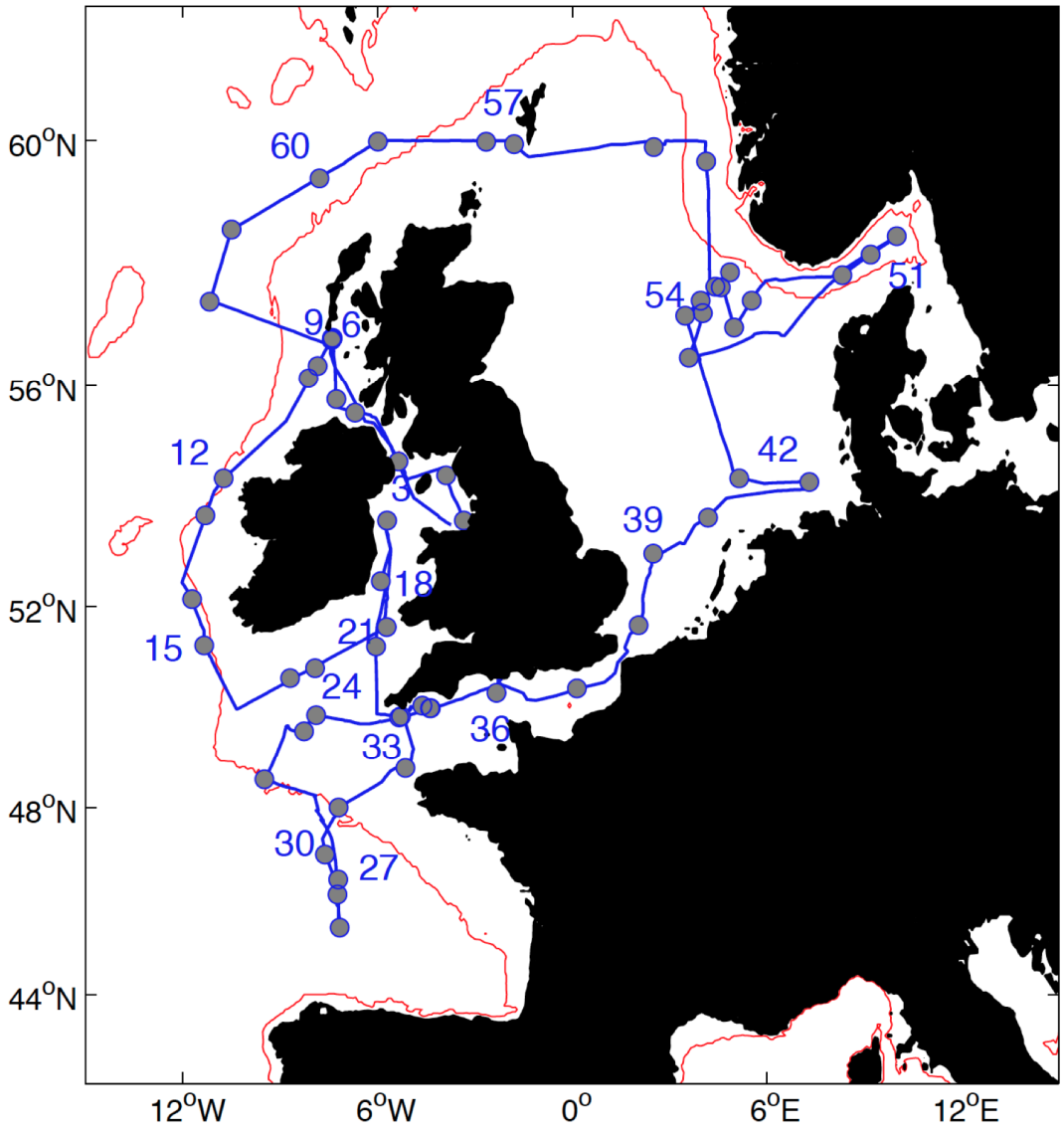


Figure 1.1: Station map for cruise D366, with station numbers indicated adjacent to station position.

We sailed on Monday June 6th following a slight delay due to checks on the ship's steering mechanisms. We sailed out into Liverpool Bay to immediately undertake our first station at 1130 h GMT. This went smooth and we proceeded to our next station, planned for 1700 h (GMT) June 6. Mingulay Reef was reached on Wednesday June 8th and this allowed us to sample for the first bioassay experiment with water collected from the Niskin bottles on the CTD frame and conduct a standard CTD operation with subsequent chemical and biological sampling. Following these activities we started to collect coral material using a videocamera and a van Veen grab. A number of CTD casts were also undertaken on Wednesday June 8th, primarily to provide carbonate chemistry data for the coral researchers. The collected corals were used for ship-board pCO_2 incubation experiments.

The coral collection went very well, and after 24 h all required material was collected. We therefore proceeded with the boat transfer of scientists on Thursday morning June 9th. Two scientists left the vessel following completion of their work, whilst two new scientists joined the vessel after their overnight stay at Barra. Following the successful boat transfer we departed at 0730 h for sampling of the European shelf waters. Two stations were occupied north of Ireland on Thursday June 9th.

On Friday morning June 10th we sampled at 0500 h from the first pCO_2 perturbation experiment (t= 48 h), the subsequent sampling was undertaken on Sunday morning June 12th (t= 96 h).

CTD sampling and initiation of pCO_2 experiments were the routine tasks during the cruise. In the Bay of Biscay some station delay occurred due to poor weather and winch problems. More weather related delay occurred in the vicinity of the Skagerrak. During the cruise we also had 2 further boat transfers of spares for the de-ionised water system.

In the vicinity of Portland, we were involved in the search for a missing scallop diver. After a three hour search, the man was spotted by the ship's crew whilst climbing on the cliffs. The man was lucky to be found safe and well.

A trace metal clean tow fish was deployed throughout the cruise commencing at the start of the cruise with samples being collected daily for experiments investigating the effects of pH changes on trace metal speciation. Water from the epoxy coated fish was pumped directly into a clean chemistry, using a Teflon pump system through acid washed PVC tubing. The system performed well, with one recovery to undertake repairs to the hose.

At sea, dissolved copper speciation was determined using stripping voltammetry from samples collected using the tow fish. Furthermore, samples collected from the CTD casts or underway surface seawater supply (non-toxic) were also analysed on-board for nutrients, chlorophyll a, oxygen, salts, and total alkalinity and dissolved inorganic carbon (using a Vindta 3C), DMS, N_2O and FRRF. A full range of samples were collected from the CTD casts and underway seawater supply (non-toxic) for land-based biological and chemical analyses. Experimental work was performed on the cruise to measure nitrification and dinitrification rates, calcification and primary production. Full details of all the work at sea are provided in cruise reports.

The sampling from the underway surface seawater supply (non-toxic) was stopped at 1600 h on July 6th. The last station was sampled on 7th July on the Mingulay reef. On July 7th and 8th we undertook further collection of coral material and the Mingulay reef site. Following collection of all the required material, we departed for Liverpool at 1000 h, July 8th. We arrived in Liverpool docks on Saturday 9th, and all scientists had disembarked by 1500 h July 10th.

No incidents occurred on the cruise.

We ran a scientific blog for the cruise on the NOC website (www.noc.ac.uk).

A more detailed description of events and activities is provided within the narrative diary provided in Appendix B.

We thank NERC for funding our research programme and providing us with ship time. We thank the crew and officers of the RRS *Discovery* for their excellent assistance at sea.

3- Scientific reports

3.1- NMF-SS Sensors & Moorings Cruise Report

3.1.1- CTD system configuration

1) One CTD system was prepared; the main water sampling arrangement was a NOC 24-way stainless steel frame system, (s/n SBE CTD4 (1415)), and the initial sensor configuration was as follows:

Sea-Bird 9plus underwater unit, s/n 09P-46253-0869

Sea-Bird 3P temperature sensor, s/n 03P-2919, Frequency 0 (primary)

Sea-Bird 4C conductivity sensor, s/n 04C-2571, Frequency 1 (primary)

Digiquartz temperature compensated pressure sensor, s/n 100898, Frequency 2

Sea-Bird 3P temperature sensor, s/n 03P-4151, Frequency 3 (secondary, vane mounted)

Sea-Bird 4C conductivity sensor, s/n 04C-3054, Frequency 4 (secondary, vane mounted)

Sea-Bird 5T submersible pump, s/n 05T-2279, (primary)

Sea-Bird 5T submersible pump, s/n 05T-30002, (secondary, vane mounted)

Sea-Bird 32 Carousel 24 position pylon, s/n 32-34173-0493

Sea-Bird 11plus deck unit, s/n 11P-34173-0676

2) The auxiliary input initial sensor configuration was as follows:

Sea-Bird 43 dissolved oxygen sensor, s/n 43-1882 (V0)

Tritech PA200 altimeter, s/n 6196.118171 (V2)

Chelsea MKIII Aquatracka fluorometer, s/n 88-2050-095 (V3)

Chelsea 2-pi PAR irradiance sensor, DWIRR, s/n PAR 06 (V4)

Chelsea 2-pi PAR irradiance sensor, UWIRR, s/n PAR 07 (V5)

WETLabs light scattering sensor, red LED, 650nm, s/n BBRTD-169 (V6)

Chelsea MKII 25cm path Alphatracka transmissometer, s/n 07-6075-001 (V7)

3) Additional instruments:

Ocean Test Equipment 20L ES-120B water samplers, s/n's 27-33, 36-41, 43, 44, 46, 48-59

Sonardyne HF Deep Marker beacon, s/n 245116-001

NOC 10 kHz acoustic bottom finding pinger, s/n B6

TRDI WorkHorse 300kHz LADCP, s/n 13329 (downward-looking)

NOC WorkHorse LADCP battery pack, s/n WH001

4) Sea-Bird 9*plus* configuration file D366_st_NMEA.xmlcon was used for all CTD casts, with D366_st_noNMEA.xmlcon used for the back-up, simultaneous logging desktop computer. Both PAR sensors were removed for any cast deeper than 500 metres. The LADCP command file used for all casts was WHMD366.txt.

3.1.2- Other instruments

1) Autosal salinometer---One salinometer was configured for salinity analysis, and the instrument details are as below:

Guildline Autosal 8400B, s/n 68958, installed in Stable Laboratory as the primary instrument, Autosal set point 24C.

2) Fast Repetition Rate Fluorometer---One FRRF system was installed as follows:

Chelsea MKI, s/n 182042---Configured for underway sampling, located in Water Bottle Annexe.

3.2- Bioassay experiment

Sophie Richier

(University of Southampton, National Oceanography Centre Southampton)

During this cruise we performed 5 bioassay experiments designed to evaluate the response to artificial carbonate system manipulation of multiple organisms and processes. The *in situ* carbonate chemistry conditions at each bioassay location are listed in Table 1.

Methods

Experimental set up

The incubation was performed within a purpose-built experimental laboratory contained allowing acute temperature and light control. The temperature in the container was set to match the *in situ* at the time of the water collection. The light (100 µE. m^{-2}. s^{-1}) was provided by LED panels and remains constant through the cruise. A 14/8 h light/dark cycle was applied.

Each of the 5 bioassay was run for 4 days with 2 time points: T1 (48h) and T2 (96h). In order to provide enough water to process the various analyses and to have triplicate of each measurement, 9 bottles were dedicated to each time point. A total of 72 bottles were set up for each bioassay and arranged on shelves in the container.

Sampling

At pre-dawn, surface water (~ 5m deep) was collected from Niskin bottles (24 X 20L) out of one single cast and immediately dispatched in transparent acid-cleaned 4L polycarbonate bottles on deck. The bottles were then wrapped individually in zip lock bags to avoid contamination pending carbonate chemistry manipulations.

Carbonate chemistry manipulation

As soon as the CTD was back on deck, aliquots of water (n=3) were sampled and processed for DIC and TA (see C. Dumousseaud report).
Unfiltered water containing the unperturbed full site of microbial groups was then manipulated to achieve 4 different target pCO_2 levels (Ambient, 550, 750 and 1000 µatm). The manipulation of the carbonate system was achieved through additions of $NaHCO_3^-$ (1M) + HCl (1M) (Borowitzka, 1981; Gattuso and Lavigne, 2009; Schulz et al., 2009) calculated from the *in situ* carbonate chemistry parameters using a Matlab program (Mark Moore). The manipulation was immediately validated on board. Following manipulation of pCO_2, the bottles were sealed using septa lids to remove potential air bubbles trapped in the bottleneck during closure.

Date	*Bioassay experiment*	*Cast*	*DIC (µmol.kg^{-1})*	*TA (µmol.kg^{-1})*	*Calculated pCO_2*
08.06.11	*E01*	*6*	*2091.0*	*2312.5*	*338.5*
14.06.11	*E02*	*21*	*2095.1*	*2321.1*	*336.5*
21.06.11	*E03*	*31*	*2083.8*	*2346.1*	*341.9*
26.06.11	*E04*	*42*	*2034.7*	*2294.7*	*406.9*
02.07.11	*E05*	*63*	*2084.6*	*2310.8*	*370.0*

Table 1: Bioassay set up and conditions.

Bioassay breakdown

The bottles dedicated to a specific time point were removed from the container at the same time and dispatched in three groups defined by the nature of analyses performed afterwards (e.g. gas analyses). From each batch of bottles (a, b and c), water for the carbonate chemistry was sampled first to avoid rapid equilibrium with ambient condition once the bottle is opened. The remaining volume in each bottle was then shared between people according to their requirement. The samples were

either processed on board or stored at the appropriate temperature (RT, -20°C, -80°C) pending analyses back in the laboratory.

References

Borowitzka., 1981 Mar. Biol., 62 (1), 17-23

Gattuso, J.-P and Lavigne, H., 2009 Biogeosciences Discuss., 6, 4413-4439.

Schulk et al., 2009 Biogeosciences 6, 2145-2153.

3.3- Carbonate Chemistry from on-board experiments

Cynthia Dumousseaud

(School of Ocean and Earth Science, National Oceanography Centre Southampton)

Objectives:

The objectives on this cruise were to provide carbonate chemistry measurements from the bioassays in order to determine the initial conditions and to monitor the carbonate chemistry throughout the experiments. Dissolved Inorganic Carbon (DIC) and Total Alkalinity (TA) samples were collected from the bioassay CTDs before any experiment bottles were filled. The samples were analysed immediately in order to determine the initial conditions and to calculate the amount of bicarbonate and hydrochloric acid solutions to add for each treatment. DIC and TA were also measured in each treatment just after spiking in order to check the initial targets.

Sampling protocol:

The sampling procedure used for the initial Dissolved Inorganic Carbon and Total Alkalinity measurements followed Dickson et al. (2007). For the initial conditions, 3 samples were collected from 3 different Niskin bottles in 250 ml Schott Duran borosilicate glass bottles with glass stopper. Samples were taken straight after the Niskin bottle was opened. A piece of silicone tubing was used for the sampling and care was taken to prevent any air bubbles being trapped in the sample. The bottle was air-tight sealed with a glass stopper and the samples were analysed immediately (within 1 hour of sampling).

The samples from each time-point were collected in 40 ml EPA vials and immediately poisoned with a saturated solution of mercuric chloride (8 µl). The samples were then analysed within two days, except for the last two experiments (6 and 7) for which samples will be analysed upon return to NOC, Southampton.

Samples collected:

Samples for initial DIC and TA were collected from each bioassay cast (Table 1). Samples for DIC and TA were collected from each experiment time-point bottles.

Sample analysis:

The instrument used for the determination of DIC was the Apollo AS-C3 (Apollo SciTech, USA; Figure 1). The system uses a LI-COR (7000) CO_2 infrared analyser as a detector, a mass-flow-controller to precisely control the carrier gas (N_2) flow, and a digital pump for transferring accurate amounts of reagent and sample. Phosphoric acid (10%) was used to convert all the CO_2 species. The sample volume was set to 0.75 ml for the whole cruise. The system generally achieved a precision of 0.1% or better. Certified Reference Materials (batch 109) from A.G. Dickson (Scripps Institution of Oceanography) were used as standards to calibrate the system at the beginning of each day of analysis.

The instrument used for the determination of TA was the Apollo AS-ALK2 (Apollo SciTech, USA; Figure 1). The system is equipped with a combination pH electrode (8102BNUWP, Thermo Scientific, USA) and temperature probe for temperature control (Star ATC probe, Thermo Scientific, USA) connected to a pH meter (Orion 3 Star benchtop pH meter, Thermo Scientific, USA). Each seawater sample was titrated with hydrochloric acid 0.1 M using an open-cell titration (Dickson et al. 2007). All TA samples were analyzed at 25 ºC (±0.1 ºC) with temperature regulation using a water-bath (GD120, Grant, UK). The acid is added in small increments and the electromotive force monitored for every step until the carbonic acid equivalence point is reached (protonation of carbonate and bicarbonate ions). The system conducts an automated Gran titration. Certified Reference Materials (batch 109) from A.G. Dickson (Scripps Institution of Oceanography) were used as standards to standardize the acid at the beginning of each day of analysis.

Preliminary results:

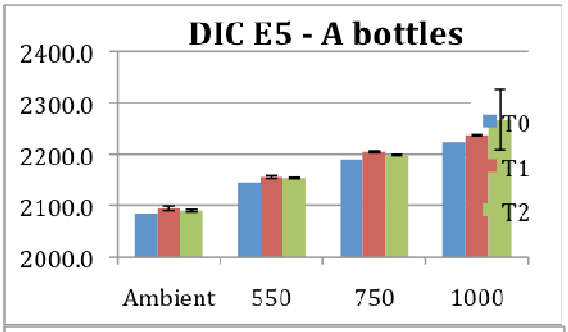
All DIC and TA samples were analysed on board except for Bioassays 6 and 7 for which the analysis will be done back at NOC, Southampton. No major

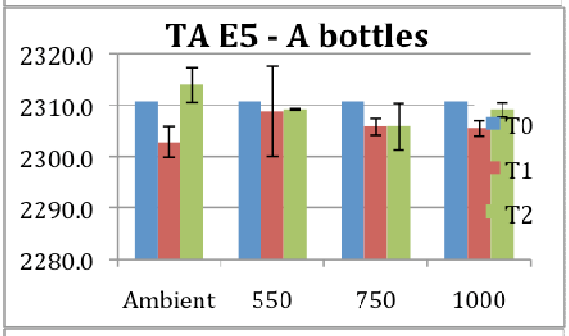
problem was encountered with the analysis. Figure 2 shows the preliminary results for the incubation experiment #5.

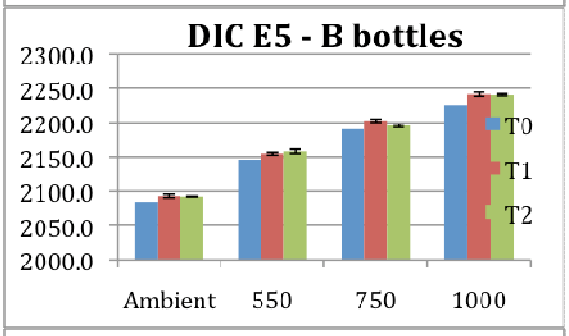
References:

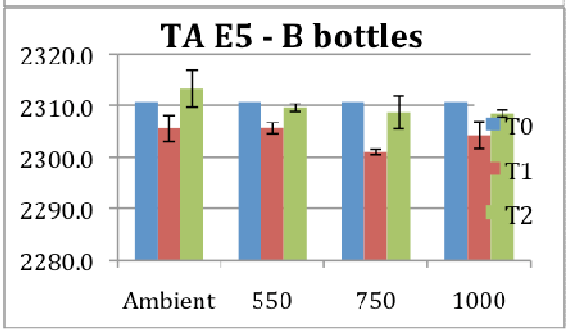
Dickson, A.G., Sabine, C.L., Christian, J.R. (Eds.) 2007. Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, IOCCP report No. 8, 191 pp.

Figure 1: Apollo AS-C3 (left) and AS-ALK2 (right) used for the determination of Dissolved Inorganic Carbon and Total Alkalinity.


DIC E5 - A bottles
2400.0
2300.0
2200.0
2100.0
2000.0
T0
T1
T2
Ambient
550
750
1000


TA E5 - A bottles
2320.0
2310.0
2300.0
2290.0
2280.0
T0
T1
T2
Ambient
550
750
1000


DIC E5 - B bottles
2300.0
2250.0
2200.0
2150.0
2100.0
2050.0
2000.0
T0
T1
T2
Ambient
550
750
1000


TA E5 - B bottles
2320.0
2310.0
2300.0
2290.0
2280.0
T0
T1
T2
Ambient
550
750
1000

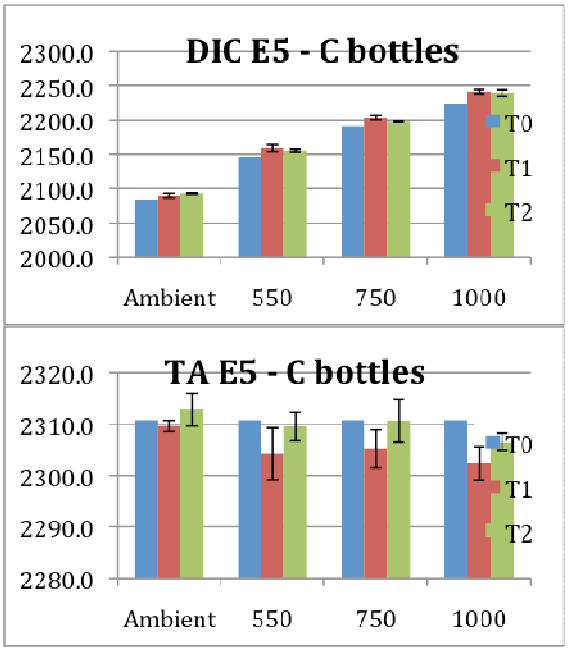


Figure 2: DIC in µmol kg^{-1} (left) and TA in µmol kg^{-1} (right) for each of the treatments and time-points for Bioassay #5 (the bars represent the average of the values of the triplicates and the error bars the standard deviation for the triplicates).

Table 1: List of the casts and Niskin bottles sampled for DIC/TA on each bioassay

Experiment number	Station	Cast	Date	Niskin bottles sampled	Comments
1	006	006	8/6/11	7, 11, 18	
2	017	021	14/6/11	8, 14, 20	
2b	024	029	19/6/11	23, 24	
3	026	031	21/6/11	2, 6, 19	
4	036	042	26/6/11	1, 6, 19	
4b	048	054	29/6/11	23, 24	
5	058	064	2/7/11	5,10, 22	
5b	061	067	3/7/11		T_0 not used for spiking
6	062	068	3/7/11		T_0 not used for spiking
7	066	072	5/7/11		T_0 not used for spiking

3.4 In situ Observations of Dissolved Inorganic Carbon, Alkalinity and the Partial Pressure of Carbon Dioxide

Dorothee C.E. Bakker and Gareth Lee

(School of Environmental Sciences, University of East Anglia, Norwich, UK)

Rationale and Objectives

A quantitative understanding the impact of ocean acidification on the surface ocean biology and ecosystem, and on the role of the surface ocean within the overall Earth System are key to the UKOARP (UK Ocean Acidification Research Programme) project 'Ocean Acidification Impacts on Sea-Surface Biology, Biogeochemistry and Climate'. An accurate assessment of the carbonate chemistry and the factors controlling it is essential for addressing this aim for UK shelf waters.

The *in situ* carbonate chemistry measurements on D366 have the aim to assess the inorganic carbonate chemistry in the waters around the UK, both on and off the continental shelf. Two accurate carbonate chemistry measurements are needed for calculation of the other carbonate parameters, such as the carbonate ion concentration and the omega for aragonite and calcite. On D366 we have made accurate measurements of dissolved inorganic carbon (DIC) and total alkalinity (TA) for depth profiles and of pH (by Victoire Rerolle) and the partial pressure of CO_2 (pCO_2) in surface waters. Overdetermination of the carbonate system in surface water with parallel measurements of DIC, alkalinity, pH (by Victoire Rerolle) and pCO_2 for shallow CTD samples and for underway samples will enable a thorough assessment of the accuracy of the four carbonate chemistry parameters on D366.

Methods

Dissolved Inorganic Carbon and Total Alkalinity

Sampling from the CTD Rosette for DIC and TA - Water samples for the determination of DIC and alkalinity were drawn from the 20 l Niskin bottles on the CTD rosette and collected in 250 ml glass bottles with ample rinsing and overflowing to avoid gas exchange with the air. Two replicate 250 ml samples were collected per Niskin. Most CTD stations and most depths were sampled. Leaking Niskins were not sampled. About 60 CTD casts with typically 6 to 8 Niskins per cast were sampled and analysed on board, equivalent to about 420 samples. The samples were poisoned with a saturated mercuric chloride solution (50 µl per 250 ml sample).

Surface water sampling for DIC and TA - Two hourly water samples for the determination of DIC and alkalinity were drawn from the non-toxic surface water supply in the deck laboratory. Parallel sampling was undertaken for nutrients and salinity (4 times per day). The series was extended to also include samples for the microscopic determination of plankton species during the second half of the cruise. The DIC and alkalinity samples were collected in 250 ml glass bottles with ample rinsing and overflowing to avoid gas exchange with the air. Two replicate 250 ml samples were collected every two hours, equivalent to 323 samples throughout the cruise. The samples were poisoned with a saturated mercuric chloride solution (50 µl per 250 ml sample).

Dissolved Inorganic Carbon and Total Alkalinity measurements -Water samples were analysed for DIC and TA on two VINDTA instruments. The VINDTA combined DIC/alkalinity instruments (#4 and #7, version 3C) operate at 25°C (Mintrop, 2004). Most CTD samples were analysed on one instrument (#7) and most underway samples on the second instrument (#4).

The DIC concentration was determined by coulometry after the method of Johnson et al. (1987). Generally all samples from two stations were run on one coulometer cell (#7). Two to three CRMs (Certified Reference Material, batch 107) were used per coulometric cell and station. The average of the two DIC replicates was used.

The alkalinity measurements were made by potentiometric titration. The acid consumption up to the second endpoint is equivalent to the titration alkalinity.

The systems use a Metrohm Titrino 719S for adding acid, an ORION-Ross pH electrode and a Metrohm reference electrode. The burette, the pipette (volume approximately 100 ml), and the analysis cell have a water jacket around them. The titrant (0.1 M hydrochloric acid, HCl) was made at UEA. The average of the two alkalinity replicates was used.

The VINDTA instruments performed well during the cruise. Problems included a broken Peltier element (#4), failing level sensors for pipette full and cell full (#4, #7), a malfunctioning temperature sensor (#4) and rough sea state reducing the accuracy of TA and DIC analyses. The DIC and TA data are undergoing quality control.

Partial pressure of CO_2 in surface water and marine air

Continuous measurements of the partial pressure of CO_2 (pCO_2) in surface water and marine air were made throughout the cruise by infrared detection on a LI-COR 7000. The ship's seawater supply provided water for underway sampling from 5 m depth at the bow to the laboratory container on the aft deck. Temperature and salinity of the intake water were determined by the ship's sensors.

Seawater flowed through an equilibrator. Part of the water went to waste via a bypass. The water flow was quite variable and rather difficult to control. The water supply contained a large number of bubbles. The equilibrator sucked in air if the flow rate was above ~2.5 l/min with bubbles escaping via the equilibrator. The equilibrator was operated at a flow rate of 0.8 to 1.8 l min^{-1}. The equilibrator flooded once and ran dry twice, probably due to water usage elsewhere on board. We strongly recommend use of a flow controller for future gas sampling from the surface water supply for ensuring a steady water flow. Recording the water flow rate would be highly useful.

Marine air was pumped through tubing from the monkey island. Two Pt-100 probes accurately determined the water temperature in the equilibrator. A long vent kept the headspace of the equilibrator close to atmospheric pressure. The CO_2 content and the moisture content of the headspace were determined by an infrared LI-COR 7000 analyser. The analysis of the CO_2 content in the headspace was interrupted at regular intervals for that of the CO_2 content in marine air and in four CO_2 standards. Samples from the equilibrator headspace and marine air were partly dried to 10°C below the ambient temperature in an electric cool box. The

standards bought from BOC of 0, 250, 350 and 450 µmol CO_2 mol^{-1} in a nitrogen and oxygen mixture had been calibrated against certified NOAA standards prior to the cruise and will be recalibrated after the cruise at UEA. The analyses were carried out for a flow speed of 100 ml min^{-1} through the LI-COR at a slight overpressure. A final analysis for each parameter was made at atmospheric pressure with no flow. The flow and overpressure did not have a discernable effect on the CO_2 and moisture measurements, once the pressure had been corrected for. The correction by Takahashi et al. (1993) will be used to correct for warming of the seawater between the ship's water intake and the equilibrator. The pCO_2 measurements will be time stamped by our own GPS positions. The pCO_2 data await data quality control.

Data availability

The final DIC, alkalinity, and pCO_2 data will be stored with other cruise data at the British Oceanographic Data Centre (http://www.bodc.ac.uk/). The data will become publicly accessible once the results have been published. The carbonate parameters will also be submitted to the international, public CO_2 database at the Carbon Dioxide Information Analysis Center (http://cdiac.esd.ornl.gov/oceans/).

Recommendations

- A steady water supply to the laboratories and laboratory containers with flow control would prevent some of the problems affecting our underway pCO_2 measurements. Cruise participants might consider bringing a flow controller for any applications requiring a steady continuous water supply.
- Reliable closure of Niskins on the CTD rosette with a reduced failure rate.

References

Johnson, K.M., P.J. LeB. Williams, L. Brändström and J.McN. Sieburth (1987) Coulometric total carbon dioxide analysis for marine studies: automatization and calibration. Marine Chemistry 21, 117-133.

Mintrop, L. (2004) VINDTA, Versatile Instrument for the Determination of Titration Alkalinity. Manual for versions 3S and 3C. Version 2.0. MARine ANalytics and DAta (MARIANDA), Kiel, Germany, 45 pp.

Takahashi, T., J. Olafsson, J.G. Goddard, D.W. Chipman, S.C. Sutherland (1993) Seasonal variation of CO_2 and nutrients in the high-latitude surface oceans: a comparative study. Global Biogeochemical Cycles 7, 843-878.

Acknowledgements – We thank these cruise participants for their help with the underway sampling: Jeremy Young, Mark Stinchcombe, Toby Tyrrell and Victoire Rerolle.

3.4- pH measurements

Victoire Rerolle

(School of Ocean and Earth Science, NOCS, University of Southampton)

Introduction

The carbonate system is a key component of the chemical perspective of oceanography as it plays an important role in the oceans' capacity to take up atmospheric CO_2. Dissolved inorganic carbon (DIC) is present in seawater in three forms (CO_{2aq}, HCO_3^- and CO_3^{2-}) which are in equilibrium on timescale longer than a few minutes. In oceanography, the carbonate system can be determined by four parameters: DIC, pCO_2, alkalinity and pH.

This project aims to measure seawater pH. This cruise was an opportunity to test the spectrophotometric pH sensor that I am developing for my PhD. Two pH sensors were used: one automated sensor running continuously on the non-toxic water supply and a second to analyse discrete samples from CTD casts.

Method

Sampling – Profiles of pH were sampled from the Stainless Steel CTD (see Table 1 for list of the stations and depths sampled). Water for pH was sampled after oxygen and before DIC and alkalinity. A piece of silicone tubing was used for the sampling and care was taken to prevent any air bubbles being trapped in the sample. The sample was stored in a 20 mL borosilicate vial bottle, which was first rinsed with the sample in order to remove traces of a previous sample. The tubing was inserted at the bottom of the bottle which was then filled and water was left to overflow by two or three bottle volume. Samples were left in water bath (20 degC) for 20 minutes to equilibrate before analysis.

pH sensor- pH is measured by adding a colored indicator to the seawater sample and measuring the color of the mix. The indicator used is Thymol Blue. The pH sensor has been developed at the NOCS (Sensor group).

Underway measurements- The automated pH system was running continuously on the non-toxic water supply from the 06/06/2011 to the 07/07/2011. Measurements were only interrupted for system performance checking and maintenance.

Discrete sample measurements- Measurements were performed at 20°C. Temperature was controlled using a thermostated water bath. Analysis took half an hour per sample to rinse and then analyze the sample three times.

The performance of the system is evaluated by running certified Tris buffer provided by the Scripps Institution of Oceanography. The consistency of the data will be checked thanks to continuous pCO_2 measurements (see Ian Brown and Dorothee Baker), DIC/Alkalinity sampled on the underway supply every two hours (see Dorothee Baker) and trends in other parameters such as chlorophyll, temperature, salinity and nutrients.

Table 1: List of the stations and depths sampled for pH analysis.

CTD	NISKIN	DEPTH
35	1	90
	3	75
	5	50
	8	35
	14	16
	18	12
	22	8
	23	5
36	3	60
	6	38
	9	30
	12	25
	15	20
	18	15
51	1	
	5	
	9	
	13	
	17	
	21	
58	1	
	5	
	10	
	13	
	19	
	21	
61	1	550
	7	200

	21	5
	23	2
41	1	45
	5	35
	10	24
	13	15
	17	8
	22	4
46	1	36
	5	25
	9	20
	13	13
	17	8
	21	2
47	2	55
	5	35
	9	30
	14	20
	17	15
	21	5
49	1	50
	5	38
	9	28
	13	20
	17	13
	21	6

	11	50
	13	23
	21	10
62	1	
	7	
	13	
	16	
63	1	
	7	
	10	
	13	
	16	
	19	
66	1	
	7	
	14	
	16	
	19	
68	1	
	7	
	12	
	19	
	21	
75	1	170
	6	160
	10	150
	14	140
	23	120

3.5- Microplanktonic Microbial Diversity

Ross Holland

(Ocean Biogeochemistry and Ecosystems, National Oceanography Centre)

Objectives

Cruise objectives were to characterise, identify and enumerate microplanktonic groups along the cruise track through an intensive underway and CTD sampling regime and to provide flow cytometric support and analysis for bioassay experiments.

Underway Sampling

Samples were taken from the ships non toxic seawater supply, and fixed immediately with paraformaldehyde (0.1% final concentration) by an automated liquid handling robot (Tecan UK, Reading). Samples were drawn at half hourly intervals from 09:00 09/06/11 until 0700 07/07/11.

CTD Sampling

Samples were taken from all available depths and casts between CTD 11 and CTD 74, and were fixed immediately in paraformaldehyde (0.1% final concentration.)

Bioassay sampling

Samples were taken from all available replicates of bioassay treatments at all time points of bioassays 1 – 5. Samples were fixed immediately in paraformaldehyde (0.1% final concentration.)

Flow Cytometric Analysis of Microbial Diversity

Within 12 hours of sampling, samples were stained with the DNA stain SYBr Green I and incubated for an hour in the dark at ambient temperature. Samples were then analysed in a Partec Cyflow Space Flow Cytometer (Partec UK, Canterbury) equipped with a 20mW 488nm solid state laser.

The following groups were identified and enumerated:

- Heterotrophic Bacterioplankton (Low nucleic acid),
- Heterotrophic Bacterioplankton (High nucleic acid)
- Viruses,
- Synechococcus Cyanobacteria
- Picophytoplankton,
- Nanophytoplankton,
- Heterotrophic Flagellates (Protists)

Two analysis protocols were used. Cyanobacteria and Heterotrophic Bacterioplankton were resolved on bivariate dotplots of SSC against DNA (green) fluorescence and SSC against phycoerithryn (orange) fluorescence (to facilitate the enumeration of synechococcus Cyanobacteria.)

Nano and Pico phytoplankton and heterotrophic flagellates were resolved on bivariate dotplots of DNA (green) fluorescence against chlorophyhl (red) fluorescence

Absolute counts were obtained by adding 0.5 micron multifluorescent microspheres at a known concentration, calibrated using the TruCount feature of the Partec instrument, to each sample.

3.6- Inorganic nutrient analysis

Mark Stinchcombe

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(mark.stinchcombe@noc.ac.uk)

Cruise Objectives

Our objective on cruise D366 around the United Kingdom was to measure the concentrations of the inorganic nutrients: total oxidised nitrogen (TON), silicate and phosphate using segmented flow analysis. This analysis was performed on board and no samples were kept for analysis at the NOC.

Method

Analysis for micro-molar concentrations of nitrate and nitrite (hereinafter TON), phosphate and silicate was undertaken on a Skalar San+ segmented flow autoanalyser following methods described by Kirkwood (1996). Samples were drawn from Niskin bottles on the CTD into 25ml sterilin coulter counter vials and kept refrigerated at approximately 4^{o}C until analysis, which commenced within twelve hours. Overall 38 runs with a total 2362 samples were analysed. This is a total of 1294 CTD samples, 323 underway samples, 496 bioassay samples and 57 from other sources.

An artificial seawater matrix (ASW) of 40g/litre sodium chloride was used as the intersample wash and standard matrix. The nutrient free status of this solution was checked by running Ocean Scientific International (OSI) low nutrient seawater (LNS) on every run. A single set of mixed standards were made up by diluting 5mM solutions made from weighed dried salts in 1litre of ASW into plastic 1litre volumetric flasks that had been cleaned by soaking in MilliQ water (MQ). The concentration of the standards were tested on every run by analysing diluted OSI certified standards,

one high concentration sample (10µM for TON and silicate, 1.95µM for phosphate) and one low concentration sample (1µM for TON and silicate and 0.1µM for phosphate). Data processing was undertaken using Skalar proprietary software and was performed within twelve hours of the run being finished. The wash time and sample time were 90 seconds; the lines were washed daily with 10% Decon and MQ.

Performance of the Analyser

On a couple of occasions during the cruise we had some fairly rough seas. The ship was moving quite a bit and this affected the analyser. The light source is a filament bulb and during times of ship motion the filament can move creating very noisy baselines. This calms down when there is a reduction in the ships motion but unfortunately there is not much we can do to stop this other than replacing the light sources with LEDs but this is expensive and so is unlikely to happen in the near future. At one point towards the start of the cruise there was a sudden drop in signal ouput in the phosphate and silicate channels. This was linked to the bulb, the light output had dramatically decreased. Both bulbs were then changed and the problem was resolved.

The cruise track took us close to Norway and part way up the Skaggerak. The surface water here had very low salinity values, as low as 22. These waters also produced strange readings in the phosphate channel. The samples were showing troughs along the baseline instead of peaks. This type of signal usually indicates contamination in the ASW that is used as the inter-sample wash. However we also analyse LNS on all runs and this wasn't showing up a contamination. To be sure I changed the ASW anyway and opened up two fresh batches of LNS. Again the troughs were still visible in the phosphate baseline but with no indication of contamination in the ASW. I can only put this anomaly down to the low salinity value changing the optical density of the water. I shall have to some tests back at the NOC when the analysers get back to see if I can replicate this behaviour. The troughs were only seen in the very surface waters, the rest of the profile was fine.

In general the analyser worked very well on this cruise. Over the winter both out Skalar systems had a hardware upgrade and both were serviced by Skalar. The most notable upgrade was from a 16-bit interface unit to a 32-bit. This seems to have greatly increased our precision and our detection limits also seem to be much lower. The software package had also been upgraded. One problem we have seen in recent years is that the software would fail several times throughout a cruise. It would freeze and then have to be reinstalled. Sometimes this could happen twice a day. During this cruise the software did not crash once. This is possibly down to the software upgrade but also could be linked to the fact we are now running the analysers of new laptops so all in all the equipment upgrade has been a great success and was long over-due.

Data

All the samples were analysed on board. No samples were stored for analysis back at the NOC. There will need to be some quality checking though of data. One aspect which we will process at the NOC is a bulk nutrient correction. This has been introduced since the publication of the Go-Ship manual (Hydes et al, 2010). The bulk nutrient correction involves running a high concentration sample in every run from the same, stable source. All the values are then correlated and the average value obtained. Each individual run can then have its bulk nutrient value compared to the average value and the data in the run can be corrected accordingly. This helps to iron out any variations that might be seen in the data set between runs.

During the cruise uncorrected data was plotted using Ocean Data View (Sclitzer, R., Ocean Data View, http://odv.awi.de, 2010). The underway data was plotted in this way because it allows easy interpretation of spatial patterns. Figures 1 to 3 show the TON, phosphate and silicate data respectively.

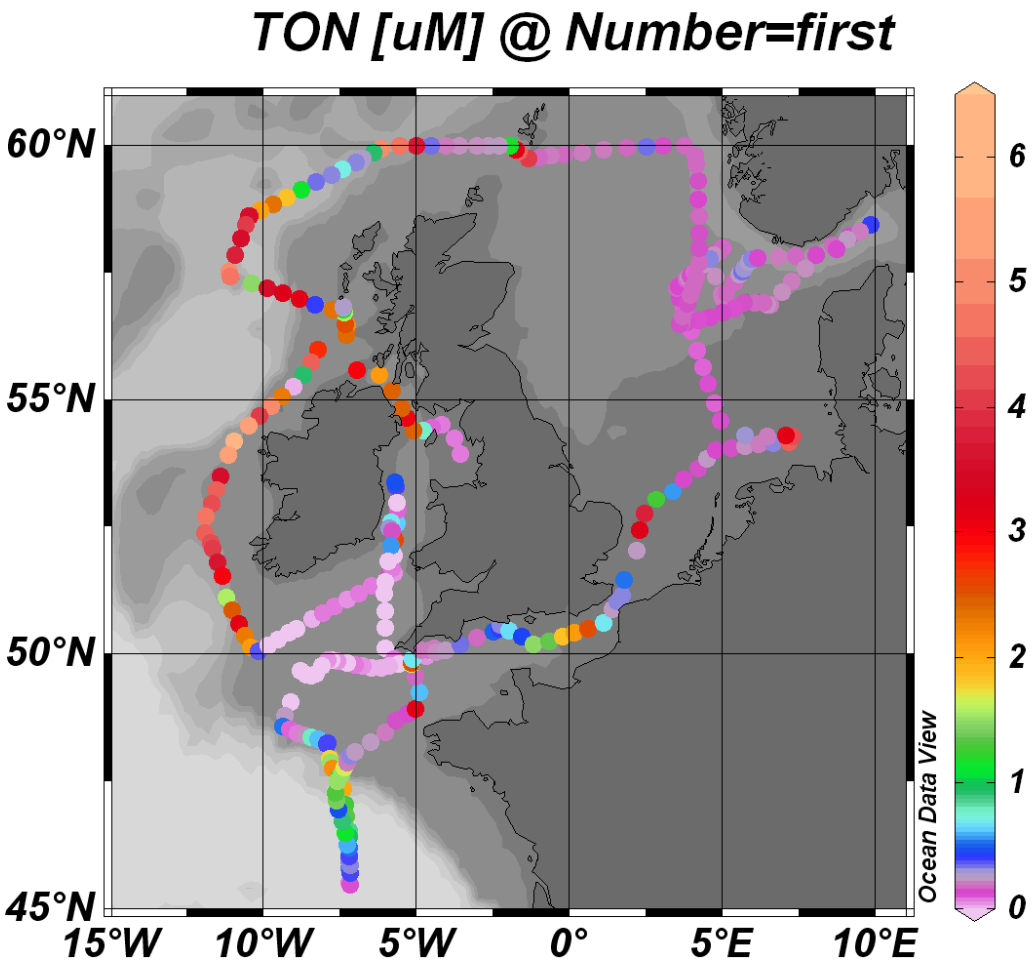

Figure 1: Underway TON data. Underway samples were taken every two hours with the exception of a period of 3 days whilst we were doing a transect of a coccolithophore bloom in and just outside the Skaggerak. High TON values were seen in the North Atlantic waters but very low values were seen in the Irish Sea, Celtic Sea and the North Sea. Higher values through the Straits of Dover coincide with low phosphate values indicating this region could well be phosphate limited.

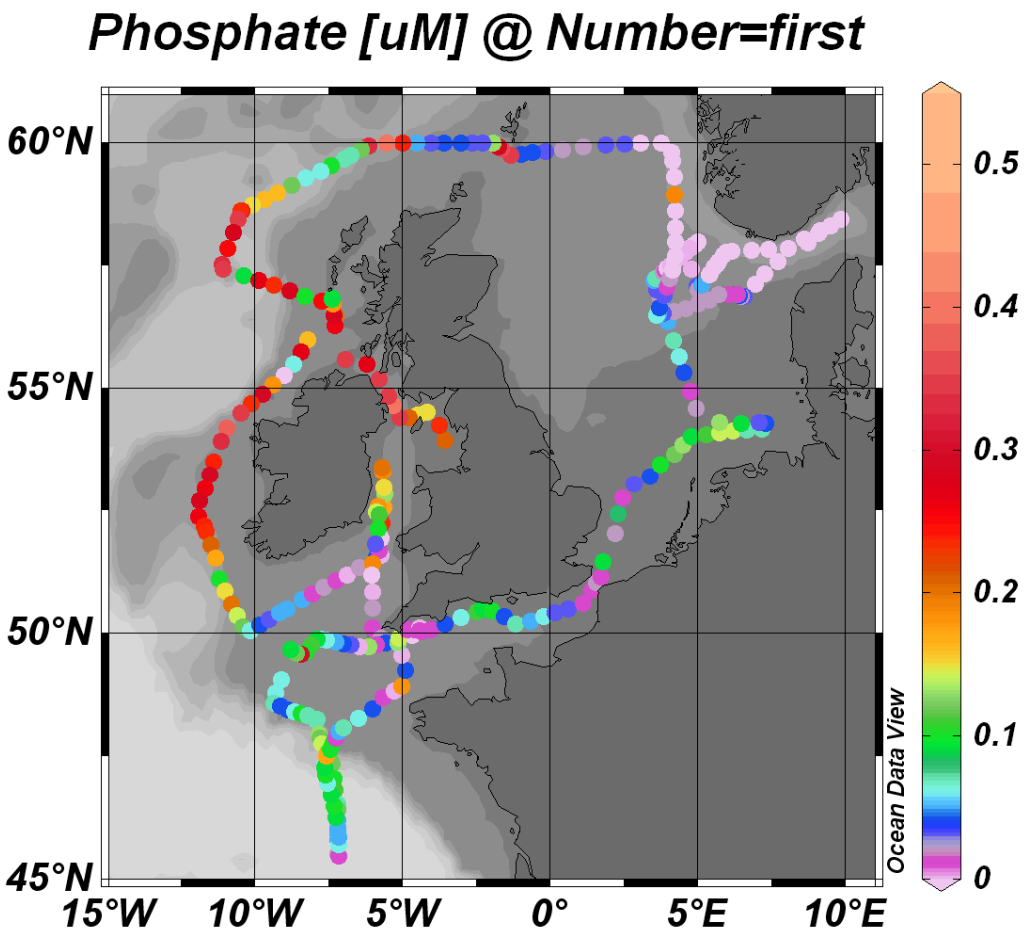

Figure 2: Underway phosphate data. Note the very low values (0 uM) in the Skaggerak and around the coast of Norway. This is due to the low salinity of these waters as discussed in the main text. Low values around the Straits of Dover indicate the waters here are likely phosphate limited. There are higher values in the North Atlantic, especially to the west of Ireland.

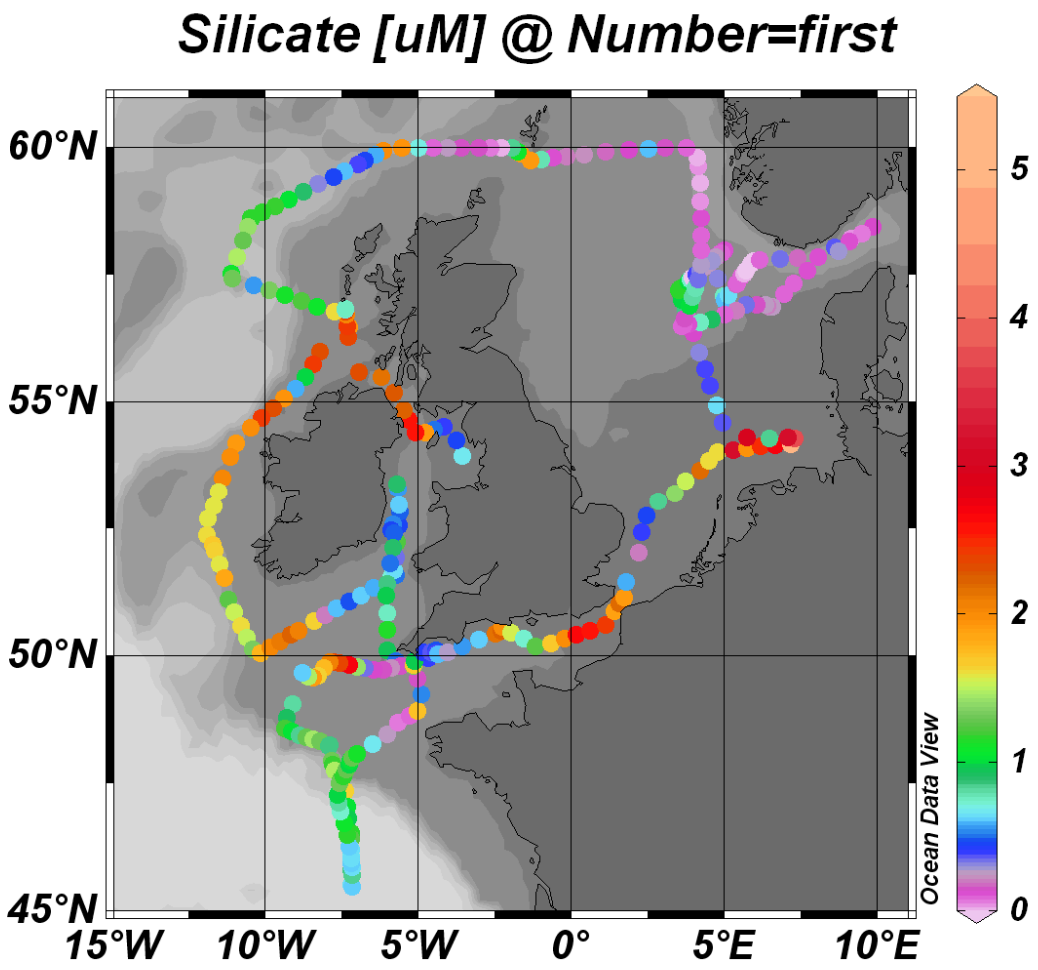

Figure 3: Underway silicate data. Silicate values were not really limiting at the majority of places around the UK. The only area of low silicate values was around Norway and the Skaggerak. In general though there were higher values in the North Atlantic compared the Irish Sea, Celtic Sea though the English Channel also had some high values.

3.7- In situ Dissolved Oxygen Analysis

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Cruise objectives

The objectives of the dissolved oxygen analysis were to provide a calibration data set for the oxygen sensor mounted on the frame of the CTD for cruise D366 around the United Kingdom. For this, a Winkler titration with amperometric end point detection was performed on a number of water samples drawn from the Niskin bottles mounted on the CTD frame.

Methods

Water for the determination of the dissolved oxygen concentration was only taken from the stainless steel CTD casts and they were the first samples to be drawn from the Niskin bottles. Where possible, each depth was sampled in duplicate. This was made possible because in the vast majority of casts there were less than twelve depths. On the few occasions there were more than twelve depths then the sampling depths were chosen based on the oxygen profile provided by the CTD package. Any steep gradients in oxygen concentration were avoided. Any Niskins within the top 10m were generally not sampled as wave action can produce tiny bubbles in the samples and the oxygen trace can be highly irregular in this region unless there were only a few depths sampled in that particular profile.

The water was drawn through short pieces of silicon tubing into clear, pre-calibrated, narrow-necked glass bottles. The temperature of the water at the time of sampling was measured using an electronic thermometer probe. The temperature would be used to calculate any temperature dependant changes in the bottle volumes. Each of the samples was fixed immediately using 1ml of manganese

chloride and 1ml of alkaline iodide, shaken thoroughly and left to settle for approximately thirty minutes. After this time they shaken again and then left for at least an hour before analysis but all were analysed within a day.

It should be noted that there were no dissolved oxygen samples analysed before station 7, cast 11 as there was no-one on board to undertake the analysis. There was also no dissolved oxygen analysis between station 41, cast 47 and station 57, cast 63 as we were undertaking a very intensive sampling programme along two transects through a coccolithophore bloom. There were serious time restraints, both in terms of sampling and of analysis, and so it was felt dissolved oxygen sampling could be dropped from these stations. Finally, there was also no dissolved oxygen from station 69, cast 75 as this was a dedicated cast for coral sampling and so dissolved oxygen was not required. It is felt that there is enough data to successful calibrate the sensor for the whole cruise and so these missing stations will not have an adverse affect on the quality of the calibration.

The samples were analysed in the main laboratory following the procedure outlined in Holley and Hydes (1995). The samples were acidified using 1 ml of sulphuric acid immediately before titration and stirred using a magnetic stirrer. The Winkler whole bottle titration method with amperometric endpoint detection with equipment supplied by Metrohm UK Ltd was used to determine the oxygen concentration.

During the first days on the ship the sodium thiosulphate was made up with 50g/l. The sodium thiosulphate needs at least one day to stabilise. The normality of the sodium thiosulphate titrant was checked using a potassium iodate standard. This was repeated several times throughout the cruise and especially once the reagents had been changed. The reagents were changed twice during this cruise. Sodium thiosulphate standardisation was carried out by adding the reagents in reverse order with, stirring in between, and then 10ml of a 0.01N potassium iodate solution using an automated burette. The sample was titrated and the volume of sodium thiosulphate required was recorded. This was repeated until at least four measurements agreed to within 0.003ml of each other. The average of these titrations was used to calculate the volume of sodium thiosulphate which was then

used in the calculation of the final dissolved oxygen calculation. The volumes of sodium thiosulphate required in this standardisation process can be seen in Table 1.

Table 1: Standardisation of the sodium thiosulphate was performed seven times on the cruise. This table shows the final volumes with the averages that were used during the calculation of dissolved oxygen. All values are millilitres.

Date	1	2	3	4	5	6	Average	Standard Deviation
13th June	0.5110	0.5115	0.5110	0.5105	0.5110		**0.5110**	**0.0004**
15th June	0.5120	0.5125	0.5120	0.5110	0.5130	0.5120	**0.5121**	**0.0007**
17th June	0.5155	0.5160	0.5160	0.5160			**0.5159**	**0.0003**
20th June	0.5120	0.5120	0.5115	0.5115			**0.5118**	**0.0003**
24th	0.5110	0.5110	0.5105	0.5115			**0.5110**	**0.0004**

June								
29th June	0.5105	0.5090	0.5095	0.5105			**0.5099**	**0.0007**
3rd July	0.5095	0.5100	0.5095	0.5105	0.5100		**0.5099**	**0.0004**

A blank measurement was also carried out to account for the oxygen in the reagents. The reagents were added in reverse order, as for the sodium thiosulphate standardisation, and then 1ml of the potassium iodate standard was added using an automated burette. This was titrated and the volume of sodium thiosulphate required was recorded. 1ml of potassium iodate was again added to the same sample and it was titrated again. This was repeated a third time. The average of the second and third volumes of sodium thiosulphate was subtracted from the first. This process was repeated at least four times. The average blank value is then used in the calculation of the final dissolved oxygen calculation. The volumes of sodium thiosulphate required in this blanking process can be seen in Table 2.

Table 2: A blank measurement was performed seven times on the cruise. This table shows the final volumes with the averages that were used during the calculation of dissolved oxygen. All values are millilitre

Date	**Volume of sodium thiosulphate**			**A – ((B + C) / 2)**	**Average Blank**	**Standard Deviation**
	A	**B**	**C**			
13th June	0.0500	0.0515	0.0520	**-0.0017**	**-0.0019**	**0.0001**
	0.0495	0.0515	0.0515	**-0.0020**		

	0.0500	0.0520	0.0515	**-0.0017**		
	0.0490	0.0510	0.0510	**-0.0020**		
15th June	0.0555	0.0525	0.0525	**0.0030**	**0.0031**	**0.0001**
	0.0550	0.0525	0.0510	**0.0033**		
	0.0545	0.0515	0.0510	**0.0033**		
	0.0540	0.0515	0.0505	**0.0030**		
17th June	0.0530	0.0495	0.0525	**0.0020**	**0.0019**	**0.0001**
	0.0530	0.0515	0.0510	**0.0018**		
	0.035	0.0520	0.0510	**0.0020**		
	0.0530	0.0515	0.0510	**0.0018**		
20th June	0.0535	0.0520	0.0515	**0.0018**	**0.0014**	**0.0003**
	0.0530	0.0515	0.0515	**0.0015**		
	0.0525	0.0515	0.0515	**0.0010**		
	0.0525	0.0510	0.0515	**0.0013**		
	0.0525	0.0510	0.0515	**0.0013**		
24th June	0.0515	0.0510	0.0515	**0.0003**	**0.0006**	**0.0003**
	0.0520	0.0510	0.0510	**0.0010**		
	0.0520	0.0510	0.0515	**0.0008**		
	0.0520	0.0515	0.0515	**0.0005**		
	0.0515	0.0510	0.0510	**0.0005**		
29th June	0.0505	0.0500	0.0500	**0.0005**	**0.0007**	**0.0004**
	0.0515	0.0510	0.0505	**0.0007**		

	0.0515	0.0500	0.0505	**0.0012**		
	0.0515	0.0510	0.0510	**0.0005**		
3rd July	0.0535	0.0515	0.0505	**0.0025**	**0.0024**	**0.0004**
	0.0530	0.0525	0.0495	**0.0020**		
	0.0525	0.0505	0.0510	**0.0017**		
	0.0530	0.0515	0.0495	**0.0025**		
	0.0535	0.0505	0.0505	**0.0030**		
	0.0530	0.0510	0.0500	**0.0025**		

Oxygen sensor calibration:

Calibration of the oxygen sensor will be done by BODC after the cruise so it is not possible to provide any calibration comparisons between the bottle data and the sensor data at this stage. However it is possible to show how the bottle oxygen data correlates to other factors to that there are not other aspects which are affecting the oxygen profile. Figures 1 to 5 show oxygen concentration plotted against temperature, depth, Niskin, oxygen bottle and bottle volume. None of these variables show a correlation with oxygen concentration so we can be confident that they are not affecting our results.

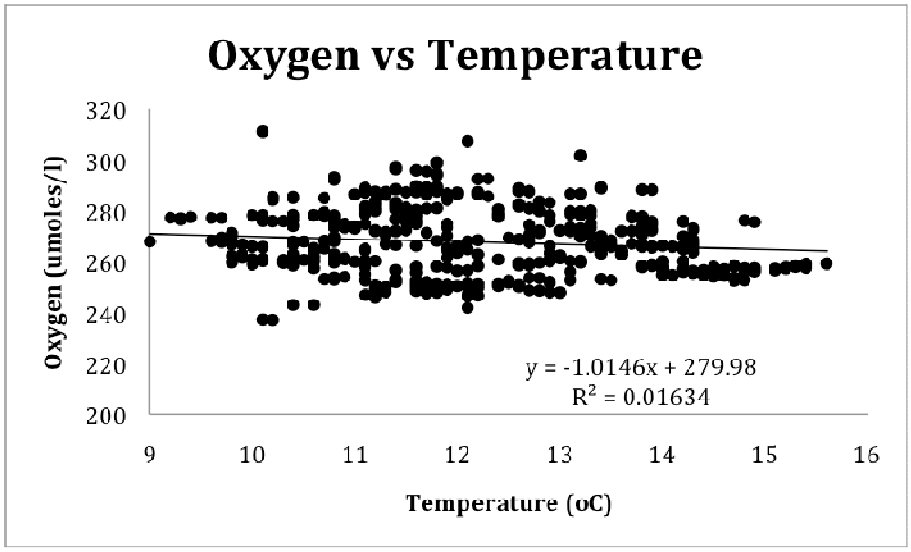

Figure 1: Oxygen concentration plotted against fixing temperature. There is no correlation between oxygen concentration and temperature throughout the cruise.

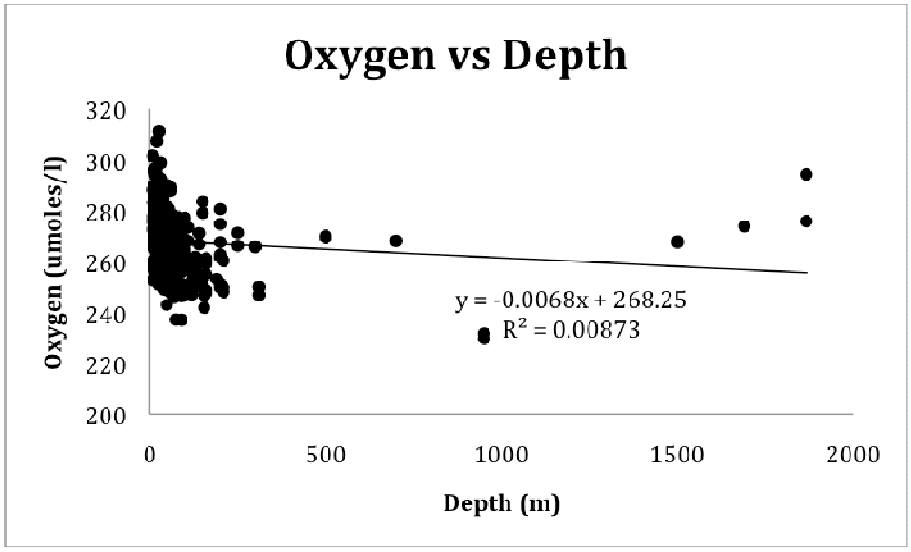

Figure 2: Oxygen concentration plotted against depth. Again there is no correlation seen between these variables.

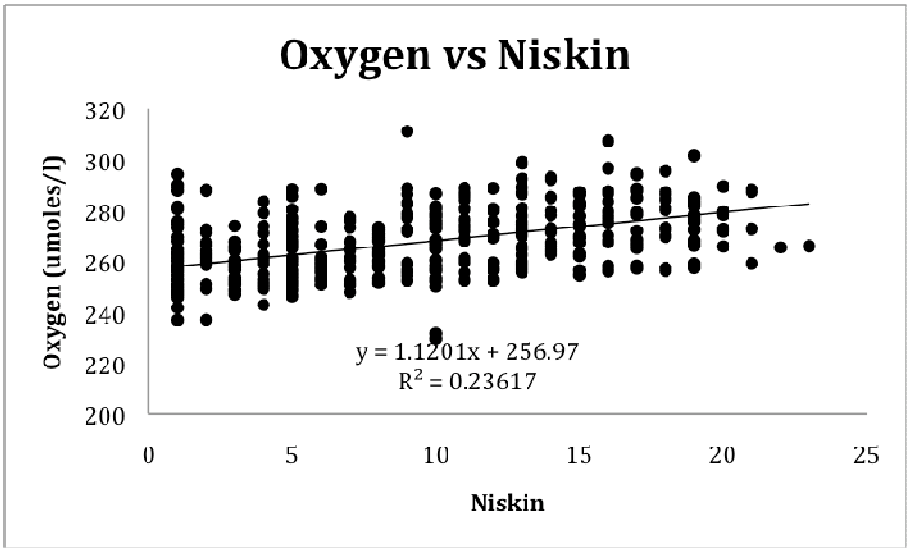

Figure 3: Oxygen concentration plotted against Niskin bottle. Again there is very little correlation between the Niskin bottle number and the oxygen concentration.

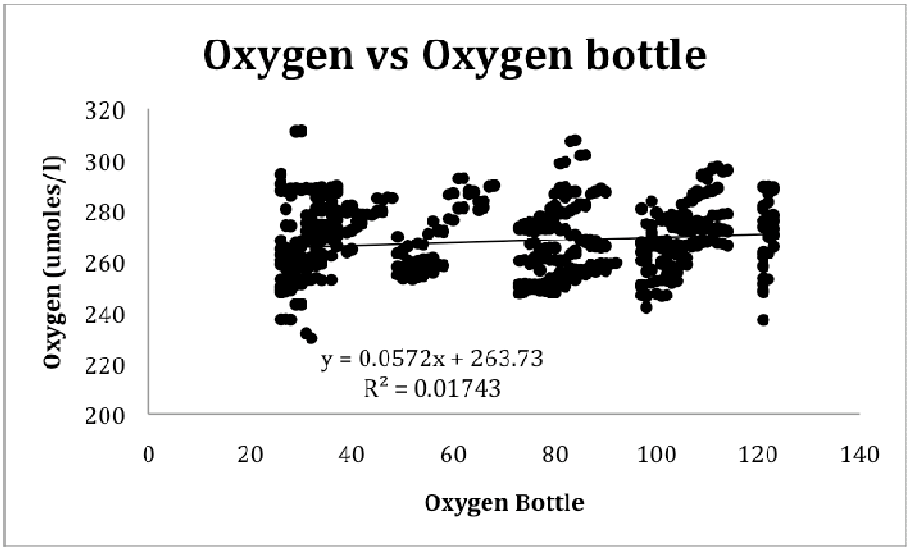

Figure 4: Oxygen concentration plotted against the oxygen bottle number that the sample was taken in. No correlation is seen.

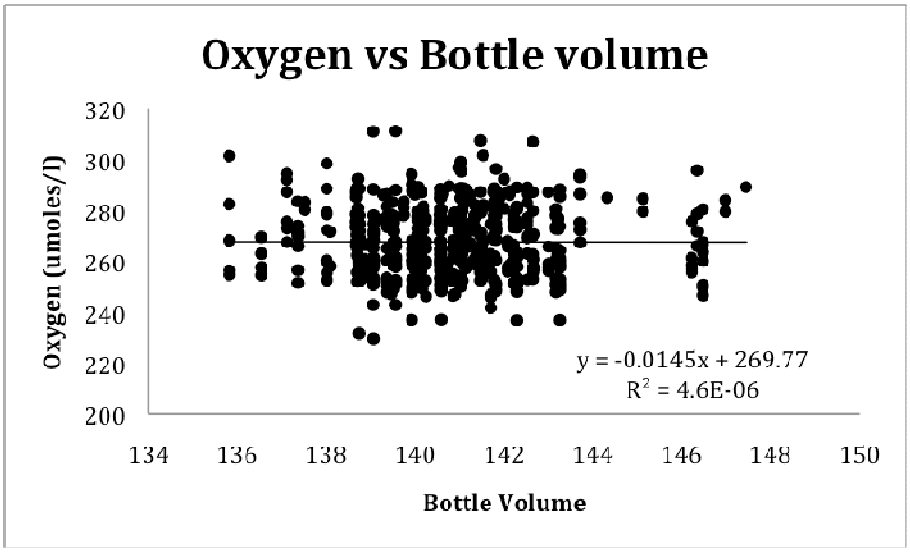

Figure 5: Oxygen concentration plotted against the volume of the bottle used to collect the sample. Again no correlation is seen.

3.8- Plankton filtration

David J. Suggett *(University of Essex, Colchester, UK)*

Sophie Richier *(University of Southampton, National Oceanography Centre Southampton, UK)*

Aliquots of water were taken from the CTD (and in the case of chlorophyll *a* and POC/N/P, also from the bioassays as described in the relevant section of this cruise report) for filtration and analyses of the following properties, which characterise biomass and/or physiology of the planktonic communities:

Total & size fractionated Chlorophyll *a*— Aliquots of 100-200 mL from 5-8 depths were filtered onto 25 mm Glass Fibre (GF) filters or 10µm pore size polycarbonate filters (to yield a total and >10µm size fraction, respectively and therefore by difference a <10 µm size fraction). All filters were extracted in 90% acetone for 24 h, and chlorophyll*a* quantified with a Turner Designs *Trilogy* fluorometer. Final chlorophyll *a* concentrations were obtained via dilutions of a solution of pure chlorophyll a (Sigma, UK) in 90% acetone and a solid standard.

Phytoplankton pigments and Particulate ABsorption (PAB) samples— Aliquots of 1 L from 1-2 depths were filtered on 25 mm GFF filters and stored at -80^{o}C for later analysis by HPLC (pigments) and spectrophotometry (PABs) at University of Essex (Suggett); samples for HPLC were flash frozen in liquid nitrogen prior to -80^{o}C storage.

Particulate organic carbon/nitrogen/phosphorous (POC/N/P)— Aliquots of 1 L from 1-2 depths were filtered on 25 mm GFF filters and oven dried (30^{o}C) for 8-12 hours; filters for POC/PON were pre-combusted at 400°C whilst those for POP were acid soaked (and repeat milliQ rinsed). Samples were dry stored for later POC/N/P quantification at University of Southampton (Richier).

3.9- Underway (continuous) Fast Repetition Rate (FRR) fluorometry

David J. Suggett *(University of Essex, Colchester, UK)*

Fast Repetition Rate (FRR) fluorometry provides a rapid and non-invasive means to examine the physiological properties of phytoplankton. This technique takes advantage of a bio-optical 'waste' product produced by all algae (fluorescence from chlorophyll*a*) and special FRR protocols have been designed to quantify how phytoplankton harvest (the effective absorption cross section, σ_{PSII}) and subsequently utilise (the photochemical efficiency, F_v/F_m, and electron turnover time, τ_{QA}) absorbed light (Kolber et al. 1998). Recent developments have shown that broad scale FRR patterns can relate to physiology (acclimation/stress) and community (adaptation) (Suggett et al. 2009). Therefore, specific goals of this cruise were to determine:

1. Do broad scale patterns exist between σ_{PSII} and F_v/F_m around UK/European waters consistent with past smaller scale analyses from temperate systems?
2. To what extent can these patterns be attributed to community versus physiology?
3. Whether potential patterns conform with broad scale changes in hydrography, e.g. temperature and nutrients availability (including DIC)?

A *Chelsea Technologies* Inc. *Fasttracka* I FRR fluorometer was connected to the non-toxic underway seawater supply and programmed to deliver single turnover (ST) sequences of 100 1.1 µs saturation flashes at 2.8 µs intervals followed by 20 1.1 µs relaxation flashes at 98.8 µs (see Moore et al. 2006). A series of 16 sequences were collected per acquisition per 30s interval. Acquisitions were then binned at 5-minute intervals into a single induction curve to maximise signal: noise (Suggett et al. 2004). The instrument was set to automatically change gain at 20% lower and 85% upper

signal thresholds to similarly ensure signal:noise was maximised, in particular across highly variable hydrographical regions. Non-linearity in instrument response was characterised using extracts of chlorophyll a at both the beginning and end of the cruise. Each induction curve was fit with the biophysical model of Kolber et al., (1998) using modified v6 software (c/o Sam Laney) to yield photophysiological parameters specific to photosystem II of the photosynthetic light reaction: minimum and maximum fluorescence yields (F_o, F_m, instrument units), photochemical efficiency (F_v/F_m, dimensionless), effective absorption cross section (σ_{PSII}, A^2 $quanta^{-1}$) and the turnover time of the primary electron acceptor Q_A (τ_{QA}, ms).

References

Kolber, Z.S., Prásil, O., Falkowski, P.G. 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochimica et Biophysica Acta 1367, 88-106.

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Suggett, D.J., MacIntyre, H.L., Geider, R.J. 2004. Evaluation of biophysical and optical determinations of light absorption by photosystem II in phytoplankton. Limnology and Oceanography Methods 2, 316-332.

Suggett, D.J., Moore, C.M., Hickman, A.E., Geider, R.J. 2009. Interpretation of Fast Repetition Rate (FRR) fluorescence: signatures of community structure v physiological state. Marine Ecology Progress Series 376: 1-19.

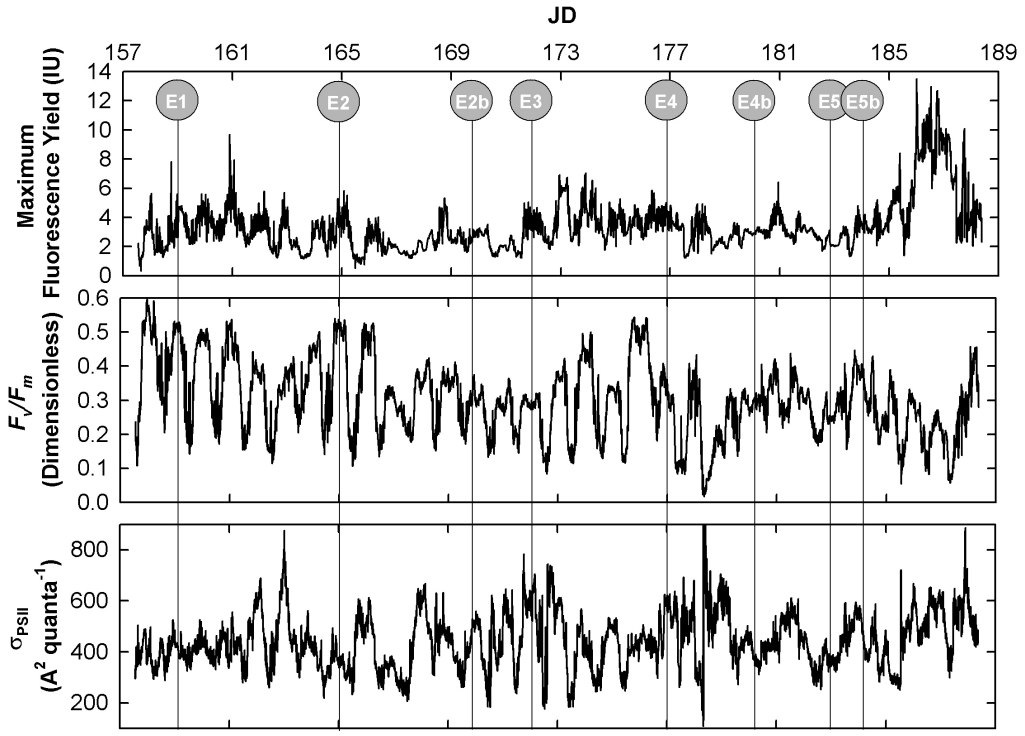

Figure 1: Continuous record of underway FRR fluorescence data (5 minute bins) for maximum fluorescence yield (F_m, instrument units, IU), Photochemical efficiency (F_v/F_m, dimensionless) and the effective absorption cross section (σ_{PSII}, A^2 $quanta^{-1}$). Positions at which the 5 main bioassay experiments were performed are also shown.

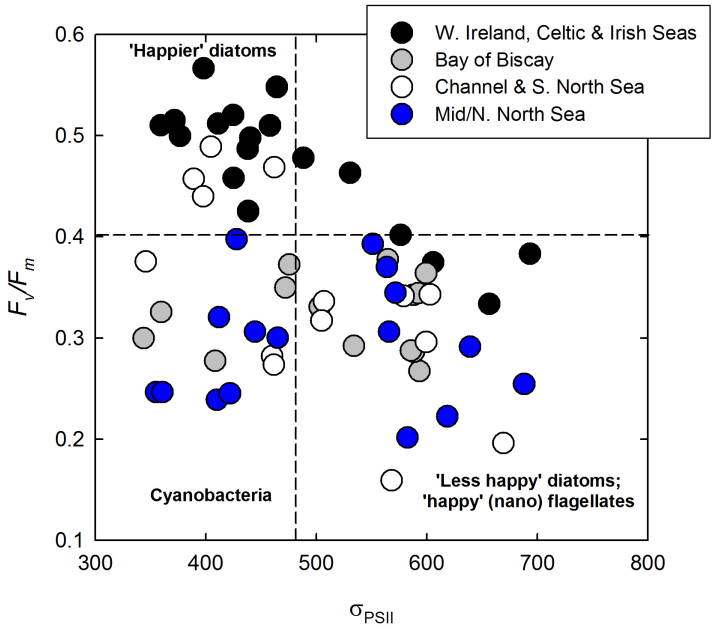

Figure 2: Summary of night-time values for F_v/F_m (dimensionless) and σ_{PSII} (A^2 $quanta^{-1}$) (binned into two points per dark period), plotted relative to one another to demonstrate the co-variability as driven by physiological state (steady state versus stressed) and community structure (*sensu* Suggett et al. 2009).

3.10- Photo-adaptation

Dr. Evelyn Lawrenz *(University of Essex, Colchester, UK)*

Background

Measurements of phytoplankton physiology and primary productivity are not routinely made as part of oceanographic studies because they often require incubation of samples in a laboratory setup or employ radioactive tracers, which limits use of these techniques in the field. Thus, relatively little information on primary productivity exists on large spatial and temporal scales. To assess the impacts of ocean acidification and global climate change on phytoplankton (and marine ecosystems as a whole) and to develop successful management strategies of marine resources, primary productivity measurements on a much wider spatial and temporal scales are required. Such measurements have become available with the development of Fast Repetition Rate Fluorometry (FRRF) (Kolber eta l. 1998), a rapid *in situ* and non-invasive active fluorescence approach to measuring primary productivity and key variables related to algal physiology. Such variables include simultaneous measurements of the effective photosystem 2 (PSII) absorption cross section (σ_{PSII}) and the maximum PSII photochemical efficiency (F_v/F_m), which in turn can then be used to calculated electron transport rates (ETR, in units of mol electrons (g Chl^{-1}) h^{-1}) (Kolber and Falkowski 1993).

Objectives

Objective 1: Assess the variability in σ_{PSII}, F_v/F_m and primary productivity of natural phytoplankton populations with varying community composition and from a wide range of ambient CO_2 concentrations occurring along the cruise track.

Objective 2: Assess the effect of ocean acidification on photophysiology (i.e. σ_{PSII} and F_v/F_m) and primary productivity in natural phytoplankton populations exposed to increasing CO_2 concentrations using.

Methods

To address objective 1, water samples from 59 stations were collected with the CTD from the surface (5-10 m) and the deep chlorophyll maximum (DCM). Minimizing exposure to ambient light, samples were transferred into black PE bottles for one hour of dark acclimation at *in situ* temperatures. A FastTreckaII FRR fluorometer attached to a FastAct unit (Chelsea Technologies Group, Ltd.) was used in bench-top configuration to measure fluorescence yields and photosynthesis versus irradiance relationships (PE curves). The FRRF was programmed to generate 100 single turnover (ST) saturation flashlets in 1 µs intervals for each of the 32 acquisition sequences using a sequence interval of 200 ms. Saturation flashes were followed by 20 50 µs relaxation flashes at 200 ms intervals. PE curves consisted of 10 180 s light steps ranging from 0-505 µmol photons m^{-2} s^{-1} for samples from the DCM and from 0-1401 µmol photons m^{-2} s^{-1} for surface samples, resulting in a total incubation time of 30 min per PE curve. To prevent sedimentation of large cells inside the fluorometer cuvette, samples were gently aerated using a setting of 3 on the mixing pump and a delay of 2s. Temperatures of the FastAct water jacket were kept at the corresponding *in situ* values of the water sample.

To address objective 2, samples were collected at the beginning of each CO_2-enrichment experiment (To) and after 48 and 96 h of incubation (see corresponding section). Samples were dark-acclimated for one hour and measured in the FRRF for 2 min as described above using the same protocol, but doing all measurements in the dark, i.e. no PE curves were acquired.

Results

Quality control and data analysis are still ongoing.

References

Kolber, Z.S., Prásil, O., Falkowski, P.G. 1998. Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochimica et Biophysica Acta 1367, 88-106.

3.11- Primary production (total and >10 μm) and Calcite Production

Dr. Alex J. Poulton *(OBE/NOC, Southampton, UK)*

Sampling

(1) Predawn CTD casts – Measurements were made on water samples collected from five light depths (55%, 20%, 14%, 5% and 1% of surface irradiance) during 14 predawn (0300-0600) CTD casts. Water samples were incubated in on-deck incubators on the aft deck, with light levels replicated using combinations of neutral density and misty blue light filters and in situ temperatures were replicated by continuously flushing the incubators with sea-surface water.

(2) OA Bioassays – Measurements were made for the Tzero, T48 and T96 time points for all five (D366 E01-E05) of the bioassays. All samples were incubated in the OA container on the aft deck.

Methodology

(1) Primary Production (total) and Calcite Production – Daily (dawn-to-dawn, 24-hrs) rates of primary production (PP) and calcite production (CP) were determined at 14 CTD stations following the methodology of Balch et al. (2000). Water samples (70-ml, 3 light, 1 formalin-killed) were collected from the light depths, spiked with 20-40 mCi of ^{14}C-labelled sodium bicarbonate and incubated on deck. Incubations were terminated by filtration through 25-mm 0.4-μm Nuclepore polycarbonate filters, with extensive rinsing with fresh filtered seawater to remove any labeled ^{14}C-DIC. Filters were then placed in glass vials with gas-tight septum and a bucket containing a Whatman GFA filter soaked with 200-ml phenylethylamine (PEA) attached to the lid. Phosphoric acid (1-ml, 1%) was injected through the septum into the bottom of the vial to convert any labelled ^{14}C-PIC to ^{14}C-CO_2 which was then caught in the PEA soaked filter. After 20-24 hrs, GFA filters were removed and placed in fresh vials and 10-ml of Ultima-Gold liquid scintillation cocktail was added to both vials: one containing the polycarbonate filter (non-acid labile production, organic or primary production) and one containing the GFA filter (acid-labile production, inorganic

production or calcite production). Activity in both filters was then determined on a Tri-Carb low level liquid scintillation counter and counts converted to uptake rates using standard methodology.

(2) >10 μm Primary production - Daily rates of size-fractionated primary production (>10 μm) was also measured from the 14 production CTD casts. Triplicate water samples were collected from each light depth (70-ml), spiked with 4-5 mCi ^{14}C-labelled sodium bicarbonate and incubated on deck. Incubations were terminated after 24 hours with filtering through 25-mm 10-μm Nuclepore polycarbonate filters, with extensive rinsing with fresh filtered seawater to remove any labeled ^{14}C-DIC. Filters then had 1 ml of 1% phosphoric acid added (20-24 hrs) to remove any trace amounts of ^{14}C-DIC or ^{14}C-PIC. Ultima-Gold liquid scintillation cocktail (10-ml) was then added and activity on the filters was determined on a Tri-Carb low level liquid scintillation counter and counts converted to uptake rates using standard methodology.

References

Balch, W.M., Drapeau, D.T., Fritz, J.J., 2000, Monsoonal forcing of calcification in the Arabian Sea, Deep-Sea Research II, 47, 1301-1337.

Table 1: List of CTDs sampled for primary production (total and >10 μm) and calcite production.

CTD cast number	*Date*	*Depths (m)*
C07	8 June 2011	6, 10, 14, 20, 30
C15	11 June 2011	5, 12, 16, 25, 35
C19	13 June 2011	10, 13, 18,22, 25

C24	15 June 2011	3, 8, 12, 18, 22
C29	19 June 2011	5, 18, 22, 33, 46
C32	21 June 2011	10, 18, 22, 33, 50
C34	22 June 2011	5, 12, 15, 22, 35
C38	24 June 2011	3, 8, 11, 15, 25
C43	26 June 2011	2, 5, 10, 15, 20
C45	27 June 2011	2, 6, 10, 15, 22
C54	29 June 2011	5, 12, 26, 32, 40
C65	2 July 2011	12, 12, 17, 26, 36
C67	3 July 2011	4, 8, 12, 18, 35
C71	5 July 2011	5, 12, 18, 25, 40

3.13- Phytoplankton community composition

Dr. Alex J. Poulton *(OBE/NOC, Southampton, UK)*

Light microscopy – Water samples were preserved with 2-3% acidic Lugol's solution from 1-2 depths (mixed layer, chlorophyll maximum where present) from 65 CTD casts and from each treatment bottle from the 5 bioassay experiments. In the case of CTD sampling, 100-ml samples were collected and preserved, while 250-ml samples were collected from the bioassays. Phytoplankton community composition will be assessed using light microscopy (following Poulton et al. 2007) for diatoms, dinoflagellates, and planktonic ciliates.

References

Poulton, A.J., Moore, C.M., Seeyave, S., Lucas, M.I., Fielding, S., Ward, P., 2007, Phytoplankton community composition around the Crozet Plateau, with emphasis on diatoms and *Phaeocystis*, Deep-Sea Research II 54, 2085-2105.

3.14- Photosynthesis versus irradiance

Laura Bretherton *(University of Essex, Colchester, UK)*

Background

Ocean acidification (Raven *et al.* 2005) has been a growing concern over the last few decades, and is now recognised by the International Panel on Climate Change (IPCC) as a significant consequence of elevated atmospheric CO_2 (IPCC 2007). As ocean acidification increases the amount of inorganic carbon available, it will no doubt affect primary productivity in some way. Marine productivity accounts for around 50% of global primary production (Falkowski and Raven 2007) and is driven mainly by marine algae. Laboratory work on cultures has indicated a variation in response between different species with regards to stimulation of photosynthesis under elevated pCO_2 (Rost *et al.* 2003; Langer *et al.* 2006), which translates to mesocosm studies that found a community shift (Tortell *et al.* 2002; Tortell *et al.* 2010). Different communities may exhibit different responses to ocean acidification, especially considering that differences in community could arise from variation in environmental conditions (e.g. areas of naturally high or low pH, DIC etc.).

The cruise track for D366 passed through areas of different environmental conditions, which favoured different community structures. The objectives for cruise D366 were:

i. Investigate the differences in photophysiology and carbon fixation of phytoplankton communities around the UK and European shelf seas; and,
ii. Find out if different community compositions react differently to ocean acidification in terms of carbon fixation and photophysiology.

Methods

Samples of 1L volumes were taken either from a CTD cast, or from bioassay bottles (see relevant section on bioassay set up) and divided into 12 60mL bottles. Each

bottle was spiked with ^{14}C and stacked into a blacked out column that was placed inside an incubator. An LED panel shone light through the column, and light levels were adjusted by putting 15% neutral density filters in between the bottles. After 2-3 hours, the bottles were removed and filtered onto 0.4µm Cyclopore polycarbonate filters. The filters were acid fumed (32% HCl) in a desiccator for several hours before being placed into vials filled with scintillation cocktail and loaded into a scintillation counter. The data curves were fitted using the Jassby and Platt (1976) model.

Results

Data analysis is still ongoing.

References

Falkowski, P. G. and Raven, J. A. (2007). *Aquatic Photosynthesis*. Princeton, Princeton University Press.

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Jassby, A. D. and Platt, T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.* **21**(4): 540-547.

Langer, G., Geisen, M., Baumann, K.-H., Klas, J., Riebesell, U., Thoms, S. and Young, J. (2006). Species-specific responses of calcifying algae to changing seawater carbonate chemistry. *Geochem. Geophys. Geosys.* **7**: Q09006.

Raven, J. A., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C. and Watson, A. (2005). Ocean acidification due to increasing atmospheric carbon dioxide.

Rost, B., Riebesell, U., Burkhardt, S. and Sultemeyer, D. (2003). Carbon acquisition of bloom-forming marine phytoplankton. *Limnol. Oceanogr.* **48**(1): 55-67.

Tortell, P. D., DiTullio, G. R., Sigman, D. M. and Morel, F. M. M. (2002). CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage. *Marine Ecology Progress Series* **236**: 37-43.

Tortell, P. D., Trimborn, S., Li, Y., Rost, B. and Payne, P. D. (2010). Inorganic carbon utilization by Ross Sea phytoplankton across natural and experimental CO_2 gradients. *J. Phycol.* **46**: 433-443.

3.15- Dissolved Organic Carbon (DOC)

Tingting Shi *(School of Ocean and Earth Science, National Oceanography Centre, Southampton, University of Southampton)*

Objectives

The objective of this work on the cruise was to collect seawater samples for DOC measurements from both CTD casts and bioassay experiments. CTD sampling covered several depths from surface to deep water to help understand the vertical distribution of DOC in the study areas. Sampling from bioassay experiments were made to investigate the effects of pCO_2 perturbations on DOC production.

Sampling

CTD casts:

Seawater samples were taken from every depth of 57 CTD casts. Water taken from the surface to 300 m (or shallower depending on seafloor depth) was filtered using pre-combusted (450 ℃, 4 h) GF/F filters (Fisher) to remove the particulate carbon and most organisms in the seawater. Samples were filled into pre-combusted glass ampoules and acidified to pH<2 with 40 µL 50% HCl immediately after collection. The ampoules were sealed and stored in fridge (4 ℃).

Bioassay experiments:

Samples were taken from 3 replicates of 4 treatments at both T=48 h and T=96 h time points of bioassay experiment 1 to 5. All the samples were filtered and acidified. The ampoules were sealed and stored at 4 ℃.

Methodology:

DOC samples will be analysed using the high temperature combustion technique. The principle of this technique is to combust the dissolved organic carbon compounds in the samples into CO_2 and measure the amount of generated CO_2. Filtered and acidified seawater samples are to be sparged with oxygen to remove

dissolved inorganic carbon from the water and then injected into a combustion column. The non-purgeable organic carbon in the sample is combusted at 680 °C and converted to CO_2, which can be detected by a non-dispersive infrared detector (NDIR). A Shimadzu TOC-TDN instrument will be used (TOC V CPN).

3.16- Transparent Exopolymer Particles (TEP)

Tingting Shi *(School of Ocean and Earth Science, National Oceanography Centre, Southampton, University of Southampton)*

Objectives

The objective of this study was to collect seawater samples for TEP measurements from both CTD casts and bioassay experiments. CTD sampling covered several depths from surface to deep water to investigate the vertical distribution of TEP in the studied areas. Sampling from bioassay experiments was undertaken to investigate the effects of pCO_2 perturbations on TEP production.

Sampling

CTD casts:

Seawater was taken from 56 CTD casts. Four to six depths were chosen from each CTD cast. TEP were collected by filtering the seawater through 0.45 µm pore-size polycarbonate filters (25 mm in diameter) at constant 200 mBar vacuum. Three replicates were filtered for one seawater sample. The particles retained on the filters were stained with 500 µL of 0.02% aqueous Alcian Blue in 0.06% acetic acid (pH = 2.5). The dye was pre-filtered with 0.2 µm pore-size polycarbonate filters before use. After being stained, filters were rinsed once with Milli-Q water, and then put into 15 mL neutral centrifuge tubes and stored in freezer at - 20 ℃.

Bioassay experiments:

Seawater was taken from 3 replicates of 4 treatments at both T=48 h and T=96 h time points of bioassay experiment 1 to 5. All the water samples were filtered to collect 3 replicates of TEP samples. The filters were stained and stored at - 20 ℃.

Methodology:

TEP will be analysed using a colorimetric technique. The particles can be detected by staining with Alcian Blue, a cationic copper phthalocyanine dye that combines

with carboxyl ($-COO^-$) and half-ester sulphate ($-OSO_3^-$) reactive groups of acidic polysaccharides. The amount of Alcian Blue adsorbed onto the filter is directly related to the weight of the polysaccharide retained on the filter. Analysis of TEP samples will be carried out on land. The filters will be soaked in 6 mL of 80% sulphuric acid for 2 h to dissolve the adsorbed Alcian Blue. The absorbance of the solution at 787 nm (absorption maximum) will be measured using U-1800 spectrophotometer (Hitachi).

3.17- Trace metal speciation analysis

Lizeth Avendaño *(School of Ocean and Earth Science, National Oceanography Centre, Southampton, University of Southampton)*

Cruise objectives

To investigate the influence of ocean acidification on trace metal speciation (Fe,Cu) in surface seawater samples, using the Competitive Ligand Equilibration Adsorptive Cathodic Stripping Voltammetry (CLE-ACSV) method. Related chemical measurements are also being investigated in order to provide complementary information.

Sampling protocol

Surface seawater (ca. 3-4 m depth) was collected daily from a towed fish deployed from the side of the ship and filtered inside a clean container using a filter capsule (Sartobran 300, Sartorius) for all the experiments, except for Dissolved Inorganic Carbon (DIC) and Total Alkalinity (TA) measurements. These last were collected at the same time as the rest of the samples from the underway seawater supply using 250 ml Schott Duran borosilicate glass bottles with glass stopper and preventing air bubbles trapped in the sample. The bottles for DIC and TA were immediately poisoned with a saturated solution of mercuric chloride (50 μl) to prevent any biological activity and positively sealed and stored at room temperature for their posterior analysis onshore. The first two weeks of seawater samples were used for the determination of the parameters and conditions necessary to undertake the metal titrations onboard and to test all of the chemical reagents to be used. All of the onshore analysis of this work will be performed in the NOC, Southampton.

Seawater from the filter capsule was collected onto 250 ml LDPE bottles (Nalgene) for the determination of trace metal (Fe, Cu) speciation. Samples for the determination of Fe speciation were immediately frozen after collection for their analysis onshore. Samples were also collected for the determination of total

dissolved trace metals using 60 ml LDPE bottles. These bottles were immediately acidified to pH 2 (80 μl) using UpA HCl and stored at room temperature for their analysis onshore. Collection of seawater onto 25 ml sterilin coulter counter vials for inorganic nutrient analysis was also performed and samples were kept refrigerated at approximately 4°C until analysis on-board. Seawater from the towed fish was also collected without a filtration capsule onto 3 x 60 ml LDPE bottles for a pH buffer experiment with analysis onshore. These samples were pipetted with 600 ul of a 1M HEPES buffer solution of pH 8.1, 7.6 and 7.2, respectively and stored at 12 °C until their filtration with a 0.45 μm size filter after 24 h, and acidified afterwards with 80 μl of UpA HCl.

Sample analysis

Labile Cu concentrations were determined by CLE-CSV with salicylaldoxime (SA, Campos and Vandenberg, 1994) as the competing ligand. Three samples for Cu speciation were treated immediately after collection with 1M HEPES buffer solution (0.01 M final concentration) in order to provide a pH of 8.1, 7.6 and 7.2, respectively, and also with a 0.01 M solution of SA (10 μM final concentration). Each sample was left to equilibrate for a minimum period of 1 h and then an aliquot of 15 ml was pipetted onto a set of 14 polyestyrene cups. These cups were spiked with a 1 μM Cu(II) standard solution to give a concentration range between 0 and 30 nM and left for equilibration overnight at room temperature (~21 °C). The samples were analyzed next day after a minimum period of 12 h equilibration using two voltammetry systems each consisting of a potentiostat (PGStat 10 Autolab Echochemie, Netherlands) with a static mercury drop electrode (Metrohm), a KCl reference electrode and a counter electrode of glassy carbon. The Cu ligand titration was performed using the following procedure: 1) removal of oxygen for 5 min with nitrogen (oxygen free grade) gas, after which 5 fresh mercury drops were formed, 2) a deposition potential of -1 V applied for 60 s whilst the solution was stirred, and 3) at the end of the adsorption period the stirrer was stopped and the potential was scanned from -0.1 to -0.4 V using square wave method at a frequency of 100 Hz. The stripping current (peak height) from the reduction of the adsorbed CuSA/$Cu(SA)_2$ complex was recorded. pH of each of the titration points was recorded with a Metrohm pH meter after measurement.

Inorganic nutrient analysis were performed using a segmented flow autoanalyzer on-board by Mark Stinchcombe.

Samples collected

A total of 21 days of sampling from the towed fish (12 x 250 ml LDPE bottles for trace metal speciation, 1 or 2 x 60 ml LDPE bottles for total dissolved trace metals, 1 bottle for DIC/TA measurements, 1 vial for nutrient analysis and 3 x 60 ml LDPE bottles with unfiltered seawater) were undertaken.

Posterior work

The data obtained from the Cu titrations performed on-board this cruise will be analyzed using mathematical calculation spreadsheets at the NOC, Southampton, for the determination of Cu speciation at the different pH levels tested. Samples taken for Fe speciation will be conducted also in the NOC following Gledhill and Vandenberg (1994) protocol. DIC/TA samples will be analyzed using the Apollo AS-C3 and AS-ALK2 systems, respectively. Total trace metal samples and unfiltered buffer treated samples will be analyzed with ICPMS technique.

References

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Campos, M.L. A.M., van den Berg, C.M.G. Determination of copper complexation in seawater by cathodic stripping voltammetry and ligand competition with salycilaldoxime. *Analytica Chimica Acta*. **1994**, 284 : 481-496.

3.18- Ammonium measurements in water column and incubation experiments on cruise D366

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Introduction

My contribution towards the research activities on the cruise consisted of undertaking ship-board measurements of ammonium in water column samples at all stations and in pCO_2 perturbation experiments.

Materials and methods

Samples for water column measurements of ammonium were taken from the 20 L Niskin bottles deployed on the stainless steel CTD rosette frame. Samples were taken on a daily basis, and all CTD stations were covered. Samples for ammonium were collected in polypropylene vials and reagent added, with subsequent fluorimetric analysis 24 h later. The method by Kerouel, Aminot (1997) was followed, allowing nanomolar ammonium concentrations to be determined. Typically 6-12 depths were covered for a CTD cast.

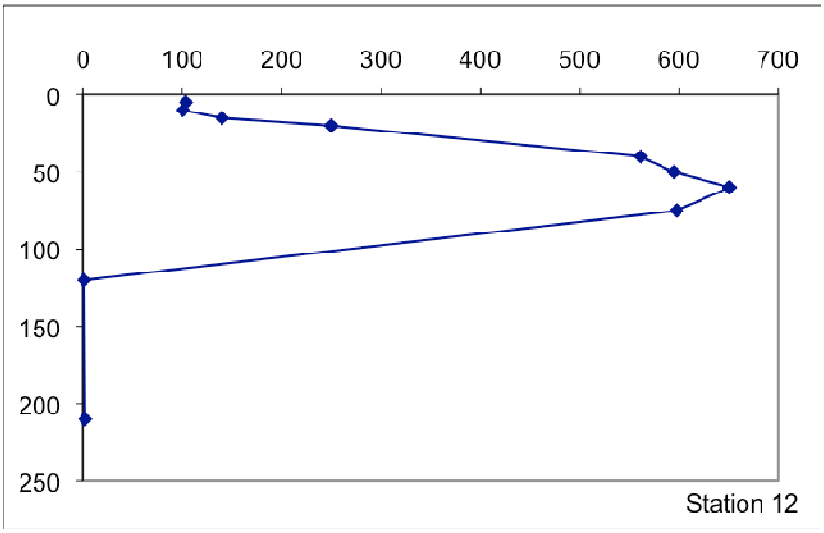
Ammonium measurements were also undertaken in the pCO_2 perturbation experiments. For this purpose, ca. 20 ml of sample was poured from the incubation bottles into a polypropylene vial and reagent was added. The same protocol as for the water column samples was followed.

Results

Ammonium measurements at sea were successful. The concentrations were typically lower in the surface mixed layer (typically 20-400 nM) with enhanced

concentrations (typically between 400-900 nM, but as high as 2 to 3 µM) at depth below the mixed layer as a result of bacterial breakdown of phytoplankton material. At deeper stations, the ammonium concentrations decreased to < 10 nM at depths below 200 m.

Figure 1 shows an example of a depth profile for station 12, with depth in metres on y axis and ammonium concentrations in nM on x axis.



Acknowledgements

We want to thank the captain, officers and crew of the RRS *Discovery* for support during the cruise. The researchers all did a great job and made this cruise a great success.

References

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3.19- Coccolithophore assemblage composition and morphology

Dr. Jeremy R. Young *(University College London)*

Dr. Toby Tyrrell *(NOCS, Southampton, UK)*

Sampling

(1) CTD casts – Samples were collected from all CTD casts other than those exclusively for bioassays, at from 4 to 8 water depths. The number of water depths and the depths selected varied depending on the nature of the water profile but typically two samples were taken from each of the surface mixed layer, thermocline and sub-thermocline water. In total 397 samples were taken.

(2) Bioassays – Samples were collected from the Tzero, T48 and T96 time points for all five (D366 E01-E05) of the regular bioassays and the three main nutrient addition bioassays (2B 4B and 5B). From each regular bioassay three replicate samples were taken from each of the four CO_2 conditions, i.e. 12 samples per time point and 25 samples total per bioassay. For the nutrient addition bioassays samples were taken at Tzero and T48 with triplicate samples from each of four nutrient conditions and two CO2 conditions, again giving 25 samples total per bioassay. In total 200 bioassay samples were taken.

(3) *Underway samples* – During the latter part of the cruise (from 21st June onwards) samples were collected as part of the underway sampling collection set organized by Dorothee Bakker. Typically samples were collected at two hourly intervals from the non-toxic seawater supply system. During the bloom sampling (28th-30th June) samples were taken at hourly intervals. In total 198 samples were taken. In addition after the main underway sampling ended, on arrival at Mingulay on 7th July, sampling of the non-toxic water supply was continued at 3 hourly intervals.

(4) *Culture isolation samples* – water samples for culture isolation were collected during the last week of the cruise. Thee were variously collected from the CTD cast bottles and non-toxic water supply with suitable samples being selected following a

light microscope reconnaissance of the assemblage composition. In total 19 samples were taken.

Methodology

(1) Coccolithophore assemblage samples – for each of the CTD, underway and bioassay samples 100-250ml of water was filtered, onto 25mm diameter 0.8µm mesh filter membranes, by vacuum filtration, without prefiltration. Samples were rinsed with ammonia-buffered milli-Q water immediately after filtration. Two filters were taken per sample, one on polycarbonate filters (Whatman nuclepore or cyclopore) for scanning electron microscopy and a second on cellulose acetate filters (Sartorius) for R microscopy. Samples were then transferred to plastic petrislides and oven dried at 40°C for 2 to 4 hours. Light microscopy preparations were then made the same day. A portion of filter was mounted on a glass microscope slide using a low viscosity UV-setting adhesive (Norland Optical Adhesive 74). For the CTD cast and bioassay samples, the water was filtered immediately after collection. For the underway samples water was stored in a refrigerator after collection and processed in batches twice a day.

(2) *Culture isolation samples* – these plankton samples were concentrated using vacuum filtration onto a 25mm filter disk. Filtration was stopped when about 25ml of water remained unfiltered and this water was then pipetted into a 50ml plastic tube. The filter disk was removed while still wet and rapidly placed into the plastic tube. In some case two or three filtrations per sample were carried out in order to increase the volume filtered.

Most filtration was onto 0.8µm filters but for samples targeting *Coccolithus pelagicus* 10µm filters were used. In most cases the water was prefiltered through a 60µm nylon mesh, in order to remove zooplankton and large dinoflagellates.

(3) Assemblage counts. The coccolithophore assemblage was analysed by light microscopy using a Leitz Ortholux polarizing microscope at x1000 magnification. Counts were made of coccolithophores present per filter are and converted to specimens per litre. In most cases this was essentially a count of *Emiliania huxleyi*. More diverse assemblages were encountered in the Atlantic waters as encountered

west of Ireland, in the Bay of Biscay and West of Scotland. Additionally the abundance and main groups of dinoflagellates and diatoms were noted.

(4) Planned post-cruise work. Directly post-cruise LM counts will be completed for all samples collected, and SEM study of a few diverse samples undertaken to check taxon identifications. These counts will then be used to select subsets of samples for further analysis using LM-based image analysis and scanning electron microscopy. These analyses will be focused on *Emiliania huxleyi* and will be used to determine the distribution of morphotypes, and degree of calcification of coccoliths and to provide counts of coccoliths numbers.

3.20- Particulate Inorganic Carbon (PIC) and Biogenic Silica (BSi) Analysis

Dr. Alex J. Poulton *(OBE/NOC, Southampton, UK)*

Dr. Jeremy R. Young *(University College London)*

Dr. Toby Tyrrell *(NOCS, Southampton, UK)*

Samples were taken for Particulate Inorganic Carbon (PIC) and Biogenic Silica (BSi) Analysis in parallel with the coccolithophore morphology samples and from the same water depths from both the CTD casts and the bioassay samples. BSi samples were taken from all samples, PIC samples were taken from most samples, excluding those from areas such as the English Channel in which sediment derived particles were very likely to dominate the particulate matter (Daniels et al., submitted) (as also confirmed by light microscopy observations). 250ml samples were collected, by vacuum filtration onto 25mm polycarbonate filter membranes. The membranes were then transferred the plastic tubes and oven dried.

Reference

C. J. Daniels, L. Pettit, A. J. Poulton, T. Tyrrell (submitted). The influence of lithogenic material on particulate inorganic carbon measurements of coccolithophores in the Bay of Biscay. *Limnology & Oceanography.*

3.21- Collection of DNA elutions, filters in RNA later and coccolithophores cultures

Cecilia Balestreri *(MBA, Plymouth, UK)*

Cruise objective

Collect samples from bioassay experiments and CTD stations. These will be analysed and used to assess genetic variability within extant phytoplankton populations, with particular interest in *Emiliania huxleyi* adaptive potential. We want to verify the impact of ocean acidification on planktonic organisms.

Sampling

Water has been collected into Nalgene bottles (previously washed with acid solution, HCl 1.5%, and rinsed three times with MilliQ water).

(1) CTD casts – Water samples (4L) were collected from one light depth during 18 predawn CTD casts (*Table 1*).

(2) Bioassays experiments – 72 bottles were collected for each bioassay experiment and they were divided into 4 treatments:

- Ambient (control samples)
- 550 µatm of CO_2
- 750 µatm of CO_2
- 1000 µatm of CO_2

750 ml of water was collected (three bottles for each treatment) from the T0, T48 and T96 time points for all five (D366 E01-E05) of the bioassays (*Table 2*). All samples were incubated in the temperature-and light controlled bioassay container on the aft deck.

Methodology

(1) Filtration – Water from each bottle, split into five samples.

Each aliquot was filtered using a vacuum pump and a filter rig (previously washed with acid solution, HCl 1.5%, and rinsed three times with MilliQ water).

- Two aliquots were filtered using 0.45 µm filters. The filters were collected in cryovial tubes and 1.5 ml of 'RNA later' was added.

- Two aliquots were filtered using 0.45 µm filters. Each filter was rinsed into a petri dish with 2 ml of PBS buffer solution and the final solution was collected into an eppendorf tube.

- One aliquot was filtered using a 3 µm nitrocellulose filter. The filter was rinsed into a petri dish with 2 ml of sea-water collected from the bottle and the final solution was collected in a Facs flow cytometry tube.

(2) DNA extraction and RNA collection – The filters in RNA later were cooled overnight at 4°C and subsequently put in the freezer at -20°C. They will be analysed post-cruise.

The solution from the previously collected eppendorf tubes was used for DNA extraction. A 'QIAGEN DNeasy kit' for DNA extraction was used (kit-protocol). The final DNA elutions were collected into eppendorf tubes and were freeze at -20°C. They will be analysed post-cruise.

(3) Flow cytometry counting and sorting – The solutions from the Facs tube were analysed using a FACSORT flow cytometer and the numbers of cells inside each sample were recorded. The flow cytometer was then used to sort coccolithophores. The cells isolated in this way were transferred into culture vessels containing nutrient media. These vessels were incubate at 12°C. They will be analysed post-cruise.

Table 1: List of CTDs sampled

CTD cast number	*Date*	*Depths (m)*
C16	11 June 2011	5
C24	15 June 2011	8
C35	22 June 2011	5
C37	23 June 2011	15
C44	26 June 2011	3
C46	27 June 2011	13
C47	28 June 2011	5
C49	28 June 2011	6
C50	28 June 2011	
C51	28 June 2011	6
C53	28 June 2011	2
C55	29 June 2011	2
C59	29 June 2011	30
C66	2 July 2011	5
C69	4 July 2011	5
C70	4 July 2011	2
C72	5 July 2011	6
C74	6 July 2011	5

Table 2:. Bioassay experiments list

Bioassay experiment number	*Date*
E1 – T0	8 June 2011
E1 – T 48	10 June 2011
E1 – T 96	12 June 2011
E2 – T0	14 June 2011
E2 – T 48	16 June 2011
E2 – T 96	18 June 2011
E3 – T0	21 June 2011
E3 – T 48	23 June 2011
E3 – T 96	25 June 2011
E4 – T0	26 June 2011
E4 – T 48	28 June 2011
E4 – T 96	30 June 2011
E5 – T0	2 June 2011
E5 – T 48	4 July 2011
E5 – T 96	6 July 2011

3.22- Dissolved oxygen and community respiration onboard measurements

Sophie Richier

(University of Southampton, National Oceanography Centre Southampton)

Background

Dissolved oxygen (O_2) in seawater is produced by photosynthesis and consumed by respiration and photochemical reactions in the surface waters. Equilibrium between dissolved O_2 in seawater and O_2 in the atmosphere is maintained through air-sea gas exchange. The aim of this work is to quantify respiration of O_2 in surface waters.

Methods

Dissolved O_2 was determined by automated Winkler titration with photometric end-point detection (Carritt & Carpenter, 1966). The concentration of thiosulphate was calibrated every 3 days. Respiration experiments were carried out according to Robinson et al. (2002).

In brief, seawater samples were collected from 12 bottles (triplicates of 4 conditions) after 96 h incubation out of each bioassay.

Two 125 ml glass O_2 bottles, rinsed once with collected water, were filled from each of the 12 incubation bottles. One half was placed in the dark in the container for 24 hours under controlled temperature. The other half was taken at the same time and fixed immediately for at least 6h in the dark (T0 subsamples).

Dark incubated O_2 bottles were removed after the 24-hour incubation, fixed and left for at least 6h in the dark before analysis for O_2.

Community respiration (CR) was calculated as O_2 consumption in the Dark samples (Dark – T0). In total, 5 experiments were carried out for the determination of

community respiration along the transect and the station summary is listed in Table 2.

Table 2: Sample collecting for respiration measurement from bioassay bottles at T96h.

Date	Time (GMT)	Bioassay experiment	Bioassay incubation bottle
12.06.11	05:00	E01	7,8,9,25,26,27,43,44,45,61,62,63
18.06.11	05:00	E02	7,8,9,25,26,27,43,44,45,61,62,63
25.06.11	04:00	E03	7,8,9,25,26,27,43,44,45,61,62,63
30.06.11	04:00	E04	7,8,9,25,26,27,43,44,45,61,62,63
06.07.11	04:00	E05	7,8,9,25,26,27,43,44,45,61,62,63

References

Carritt, D.E. and Carpenter, J.H., 1966. Comparison and evaluation of currently employed modifications of the Winkler method for determining dissolved oxygen in seawater; a NASCO Report. Journal of Marine Research, 24: 286-319.

Robinson, C. et al., 2002. Plankton respiration in the Eastern Atlantic Ocean. Deep-Sea Research Part I - Oceanographic Research Papers, 49(5): 787-813.

Serret, P., Robinson, C., Fernandez, E., Teira, E. and Tilstone, G., 2001. Latitudinal variation of the balance between plankton photosynthesis and respiration in the eastern Atlantic Ocean. Limnology and Oceanography, 46(7): 1642-1652.

3.23- RNA and Protein sample collection

Sophie RICHIER *(University of Southampton, National Oceanography Centre Southampton)*

Primary production and key metabolic proteins in marine microbial communities

Background

The diversity of marine microbial communities is poorly understood, however, microbial processes catalyze biochemical cycles on global scales. Despite this diversity the proteins that perform the chemistry of these reactions are highly conserved.

Primary production in the ocean is usually quantified through basic methods in oceanography (e.g. chlorophyll content, photosynthetic efficiency (Fv/Fm), satellite pictures). We propose here to investigate the photosynthetic process at a physiological and molecular level in order to better understand the role of nutrients availability on photosynthetic activity. Quantification of both key metabolic genes and related proteins in samples taken around the UK, will provide such information.

Methods

Sampling for proteins and RNA

Water samples were collected from CTD (Table 3) from the surface and chlorophyll maximum. In the absence of a deep chlorophyll maximum, water samples were taken from the bottom of the mixed layer depth. For protein samples a volume of water ranging from 1L to 2.0 L (depending on biomass in seawater) was filtered in triplicate for 45 min through GF/F filters (0.7 µm, 25mm, Whatman). The filters were then snap-frozen in liquid nitrogen and stored at - 80^{o}C. Filters will be used for protein extractions on return to the NOCS.

For RNA samples, water was collected from the CTD out of the same niskin bottle

then the one for protein.

The water was filtered on sterivex columns for 15 to 25 min and snap frozen in liquid nitrogen for storage at -80 °C.

Table 3: List of the casts, niskin bottles and volume collected for RNA and protein samples.

Date	Cast	Depth (m)	Volume filtered (l)	Samples
15.06.11	25	12,4	1	Protein
16.06.11	27	30,10	1	Protein
17.06.11	28	40,10	1	Protein
21.06.11	33	15,10	1.5	Protein
22.06.11	35	12,5	1	Protein
23.06.11	37	17,3	1	Protein
24.06.11	38	15,3	1	Protein
	39	3	1	Protein
26.06.11	44	3	1	Protein
27.06.11	45	6	1.2	Protein
	46	6	1.2	Protein
28.06.11	47	31,6	1.5, 1.0	RNA/Protein
	49	21,6	1.5, 1.0	RNA/Protein
	51	23, 5.5	1.5, 1.0	RNA/Protein

29.06.11	55	8	2.0, 1.2	RNA/Protein
	56		1	Protein
	58	30.5	2.0, 1.0	RNA/Protein
30.06.11	61	23.6	1	Protein
	62	15, 1-2	1	Protein
02.07.11	65	17, 12.8	1.2	Protein
03.07.11	66	28,5	1.2	Protein
04.07.11	70	8	1.2	Protein
05.07.11	71	5	1.2	Protein

Changes in gene and protein expression in the natural community in response to ocean acidification

Background

As the research community explores the impact of ocean acidification (OA) on marine ecosystems (Royal Society, 2005), a key link to forecasting the effects of this altered seawater chemistry is understanding the response at the organismal level. A potential productive path for the OA research community is to leverage genomics tools (transcriptomic and proteomic) to understand the mechanisms that might be driving altered skeleton formation in marine calcifying organisms, and in addition, to reveal whether potential compensation in the key pathways for biomineralization and other processes is possible. The measurements of all mRNAs in the natural microbial community as well as proteins have emerged in ocean sciences to assess the physiological response of organisms to abiotic environmental conditions. These

techniques have the potential to highlight pathways that are changing in response to elevated pCO_2 (Zehr et al., 2008).

Methods

Water samples were collected from six biossay bottles at each T2 time point (Table 4).

For proteomic analyses, 1.5 L was filtered in duplicate for 45 min through GF/F filters (0.7 µm, 25mm, Whatman).

For RNA, 2.0L of water was filtered in duplicate for 15-20 min through sterivex column (0.2 µm). The filters and sterivex columns were then snap-frozen in liquid nitrogen and stored in a -80 ^{o}C freezer pending analyses.

Table 4: List of incubation bottles and volume collected from the bioassay bottles after 96h incubation.

Bioassay experiment	Sample	Filter type	Volume (l)	Bioassay Incubation bottle	Filtration time
E01	RNA	Sterivex	1.5	73,74,76,77	01:05
E01	Protein	GF/F	2	73-78	03:20
E02	RNA	Sterivex	1	73,74,76,77	00:30
E02	Protein	GF/F	1	73-78	00:45
E03	RNA	Sterivex	1.5	73,74,76,77	00:20
E03	Protein	GF/F	1.5	73-78	00:45
E04	RNA	Sterivex	1.5	73,74,76,77	00:20
E04	Protein	GF/F	1.5	73-78	01:00
E05	RNA	Sterivex	2	73,74,76,77	00:25

E05	Protein	GF/F	1.5	73-78	00:45

References

Royal Society (2005) Ocean Acidification due to increasing atmospheric carbone dioxide., Vol. Royal Society, London.

Zehr J.P., Hewson, I., Moisander, P.H., 2008 Molecular biology techniques and applications for ocean sensing. Ocean Sci. Discuss., 5, 625-657.

3.24- Assessing the impacts of OA on dimethyl sulphide (DMS), dimethyl sulphoniopropionate (DMSP) and associated processes

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Background

Oceanic emission of the trace gas dimethyl sulfide (DMS) is the major source of reduced sulfur into the marine boundary layer, influencing atmospheric chemistry (von Glasow et al. 2004) and contributing to the radiative properties of oceanic clouds (Ayers et al 1991, Charlson et al. 1987, Korhonen et al 2008). DMS is an enzymatic breakdown product of dimethylsulfoniopropionate (DMSP) synthesised by phytoplankton. Both DMS and DMSP also contribute significant proportions of the carbon and sulphur flux through microbial foodwebs (Simo et al. 2004) and may play important roles as infochemicals, influencing predator prey interactions (Wolfe et al. 1997). In consequence alterations in atmospheric pCO_2 concentrations that lead to increased sea surface temperature, changes in upper-ocean stratification and decreasing ocean pH are likely to influence the extent of DMS and DMSP production, with potential impacts on climate, ocean biogeochemistry and microbial foodweb structure and function.

A number of previous studies have recorded responses in net DMS and DMSP production in relation to varied pCO_2, including high-latitude mesocosm experiments (Hopkins et al, 2010, Wingenter et al. 2007) and ship-board incubation experiments (Lee et al. 2009). However, there remains limited understanding of the mechanisms behind the observed pH-related changes in DMS and DMSP concentrations. Our overarching objective was to improve our understanding of the processes that may alter net DMS production and hence, its emission to the atmosphere, in the face of changing ocean pH.

Specific objectives

1. To determine the spatial variability in water column DMS and DMSP concentrations in relation to varied pCO2, pH and microbial community composition.

2. To quantify DMSP production rates in relation to varied pCO_2 exposure in bioassay experiments and relate this to phytoplankton community composition.

3. To quantify the biological loss rates of DMS in relation to varied pCO_2 exposure in bioassay experiments and thereby determine the rates of gross production of DMS and the efficiency of transformation of DMSP to DMS.

Methods

1. *DMS and DMSP concentrations: CTD profiles*

Seawater samples for DMS and DMSP were directly taken from Niskin bottles, and collected in 250 ml amber glass-stoppered bottles. The bottle was rinsed three times before being filled gently from the bottom through the Tygon tubing, and then allowed to over-flow 2 – 3 times. Once full, the glass stopper was securely placed on the bottle, ensuring the presence of no headspace. Samples were kept in a coolbox and analysed within 2 hours.

For analysis, 20ml of seawater was gently drawn from the amber bottle into a glass syringe through ¼" nylon tubing. The samples were gently filtered through a stainless steel Millipore filtration unit containing 25mm GF/F filter, directly into a 10ml glass syringe. The addition of air/bubbles was kept to a minimum at all times. 5ml of filtered seawater was injected into a glass purge tower. The sample was purged with He gas for 7.5 minutes at 90 ml/min, and the sample stream was dried by passing through a stainless steel counterflow nafion drier, at a flow rate of ~180 ml/min. The sample was trapped in a 1/16" PTFE loop held in liquid nitrogen. Once purging was complete, the sample loop was rapidly submerged in boiling water, injecting the sample into a Varian 3800 GC with pulsed flame photometric detector (PFPD). The

oven was held at 60°C until DMS eluted at ~3.3 minutes, and for the remainder of the 5 minute runtime the oven ramped to 250°C.

DMS calibrations were performed using liquid DMS standard diluted 3 times in MilliQ, to give working standards in the range 0.03 – 3.3 ng S ml^{-1}. Four to five point calibrations were performed every 2 – 4 days throughout the cruise.

Samples for total DMSP were taken from the same amber bottles used for DMS analysis. Once the DMS sample had been removed, the bottle was gently rotated 3 times, and 7ml of seawater was removed using a pipette, and transferred into an 8ml glass vial. 35µl of 50% H_2SO_4 was added to fix the sample for later analysis. All DMSPt samples will be analysed upon return to PML.

Table 1 lists the CTD casts and depths from which samples for DMS and DMSPt were taken.

Table 1. List of CTDs sampled for DMS and DMSPt.

CTD cast #	*Date*	*Depth (m)*	*Parameters*
03	7 June 2011	40,30,20,15,15,5,2	DMS DMSPt
04	7 June 2011	35,25,15,15,12,10,5,25,15,10,2	DMS DMSPt
06	8 June 2011	45,38,30,20,14,10,6	DMS DMSPt
11	9 June 2011	100,60,40,25,20,15,10	DMS DMSPt
15	11 June 2011	160,80,60,35,25,16,5	DMS DMSPt
16	11 June 2011	50,40,20,10,5	DMS DMSPt
19	13 June 2011	50,50,25,22,18,13,10,5	DMS DMSPt

20	13 June 2011	30,26,22,15,10,5	DMS DMSPt
22	13 June 2011	60,45,30,25,20,15,10,5	DMS DMSPt
23	14 June 2011	90,75,48,36,23,18,7,3	DMS DMSPt
24	14 June 2011	60,22,18,12,8,3	DMS DMSPt
30	19 June 2011	80,65,50,33,22,18,5	DMS DMSPt
34	21 June 2011	90,55,45,35,22,15,12,5	DMS DMSPt
35	22 June 2011	90,75,50,30,16,8,2	DMS DMSPt
36	23 June 2011	60,38,30,25,20,15,5,2	DMS DMSPt
37	23 June 2011	56,40,16,15,13,8,3,2	DMS DMSPt
38	24 June 2011	60,45,35,25,15,11,7,3	DMS DMSPt
43	26 June 2011	20,15,10,5,5,5,2	DMS DMSPt
44	26 June 2011	30,25,20,15,10,3	DMS DMSPt
45	26 June 2011	30,22,15,10,6,2	DMS DMSPt
46	26 June 2011	36,25,20,13,8,2	DMS DMSPt
47	28 June 2011	55,35,30,20,15,5	DMS DMSPt
49	28 June 2011	50,38,28,20,13,6	DMS DMSPt
51	28 June 2011	55,42,30,22,13,6	DMS DMSPt
54	29 June 2011	72,52,40,32,26,12,5	DMS DMSPt
55	29 June 2011	49,40,28,16,8,2	DMS DMSPt
58	29 June 2011	55,40,30,20,11,5	DMS DMSPt
61	30 June 2011	100,50,23,19,15,10,3	DMS DMSPt
62	30 June 2011	100,62,40,15,8,2	DMS DMSPt

63	30 June 2011	50,30,20,8,1.8	DMS DMSPt
65	2 July 2011	50,36,26,17,12,12,12	DMS DMSPt
66	2 July 2011	42,36,28,26,20,15,5	DMS DMSPt
67	3 July 2011	100,50,35,18,12,8,4	DMS DMSPt
68	3 July 2011	75,50,35,18,12,8,4	DMS DMSPt
69	4 July 2011	55,35,30,20,15	DMS DMSPt
71	5 July 2011	80,60,40,25,18,12,5	DMS DMSPt
74	6 July 2011	80,50,35,20,5	DMS DMSPt

2. Experimental incubations

a. DMSP production rates

Specific synthesis rates of DMSP were determined using a stable isotope-based approach, involving tracing the incorporation of ^{13}C into DMSP by proton transfer reaction-mass spectrometry (PTR-MS) (Stefels et al 2010).

DMSP production was determined in sub-incubations of the main bioassay experiments at T0, T48 and T96 hours, as detailed in the Table below.

Phytoplankton group-specific DMSP intracellular content was determined using a flow cytometric – gas chromatography approach (Archer et al. 2010).

b. DMS loss and production rates

DMS loss rates were determined by the addition of ^{13}C-labelled DMS to dark incubations of seawater.

^{13}C-DMS was prepared by reducing ^{13}C-dimethyl sulphoxide (DMSO) using sodium borohydride ($NaBH_4$). 100µl of ^{13}C-DMSO was diluted in 900µl of MilliQ water in a

glass purge tower. 1g of $NaBH_4$ was added and allowed to react for 5 minutes. Next, the mixture was purge at 20 ml/min for 20 minutes. The ^{13}C-DMS was collected in a 1/16" PTFE loop submerged in liquid nitrogen. In order to determine the quantity of 13C-DMS produced, the sample loop was weighed before and after purging. The 13C-DMS was rinsed out of the loop directly into a 20ml glass serum vial by syringing 15ml of MilliQ through the sample loop. The serum vial was crimped sealed and the ^{13}C-DMS solution was stored in the dark at 4°C. The primary solution underwent 2 serial dilutions in MilliQ to produce a working solution.

Experimental incubations were performed at T0, T48 and T96 of the five bioassay experiments (E01 – E05), during which concentrations of both ^{13}C-DMS and DMS were monitored to determine both dark loss, and dark net production of DMS. 800ml of seawater was directly siphoned from the bioassay bottle into a 1L Tedlar bag. Once filling was complete, all bubbles/headspace were removed from the bag. Each Tedlar bag was spiked with the working solution of ^{13}C-DMS to give concentrations of 0.1 – 0.2 ng S ml^{-1}. After spiking, the Tedlar bags were left for one hour to allow complete homogenisation of the tracer. The Tedlar bags were incubated in the dark, in the bioassay incubation container. 20ml samples were withdrawn using a glass syringe at T0, and at 3 further time-points over a 12 hour period. The samples were gently filtered through a stainless steel Millipore filtration unit containing 25mm GF/F filter, directly into a 10ml glass syringe. The addition of air/bubbles was kept to a minimum at all times. 5ml of filtered seawater was injected into a glass purge tower. The sample was purged with He gas for 5 minutes at 60 ml/min, and the sample stream was dried by passing through a stainless steel counterflow nafion drier, at a flow rate of ~120 ml/min. The sample was trapped in a 1/16" PTFE loop held in liquid nitrogen. Once purging was complete, the sample loop was rapidly submerged in boiling water, injecting the sample into an Agilent 5973N gas chromatograph with mass spectral detector, using a 60m DB-VRX capillary column. The oven was held at 60°C for 8 minutes, and for the remainder of the 12 minute runtime run the oven ramped to 220°C. DMS and ^{13}C-DMS eluted at ~5.08 minutes. In order to monitor system sensitivity and drift, 250 µl of a 5 ppmv deuterated DMS (d6) gas standard was injected upstream of each sample. DMS-d6 eluted at ~5.04 minutes. Table 2 lists the bioassay experiments, CO_2 treatments and bioassay bottle numbers from which dark ^{13}DMS-loss and DMS net production rates were determined.

Table 2: Bioassay experiments, CO_2 treatments and bioassay bottle numbers from which dark ^{13}DMS-loss and DMS net production rates and DMSP production rates were determined.

Bioassay Experiment	*CO_2 treatments*	*Bioassay Bottle # T48*	*Bioassay Bottle # T96*
E01	Ambient, 750µatm	1, 2, 3, 37, 38, 39	10, 11, 12, 46, 47, 48
E02	Ambient, 750µatm	1, 2, 3, 37, 38, 39	10, 11, 12, 46, 47, 48
E03	Ambient, 750µatm	1, 2, 3, 37, 38, 39	10, 11, 12, 46, 47, 48
E04	Ambient, 750µatm	1, 2, 3, 37, 38, 39	10, 11, 12, 46, 47, 48
E05	Ambient, 750µatm	1, 2, 3, 37, 38, 39	10, 11, 12, 46, 47, 48

Preliminary result

DMS in natural waters along the cruise track

Over the course of the cruise DMS concentrations ranging from 0 – 21.3 nM were encountered. As way of summary, the cruise track has been divided into 8 regions and the general DMS characteristics of each of these regions will be briefly described.

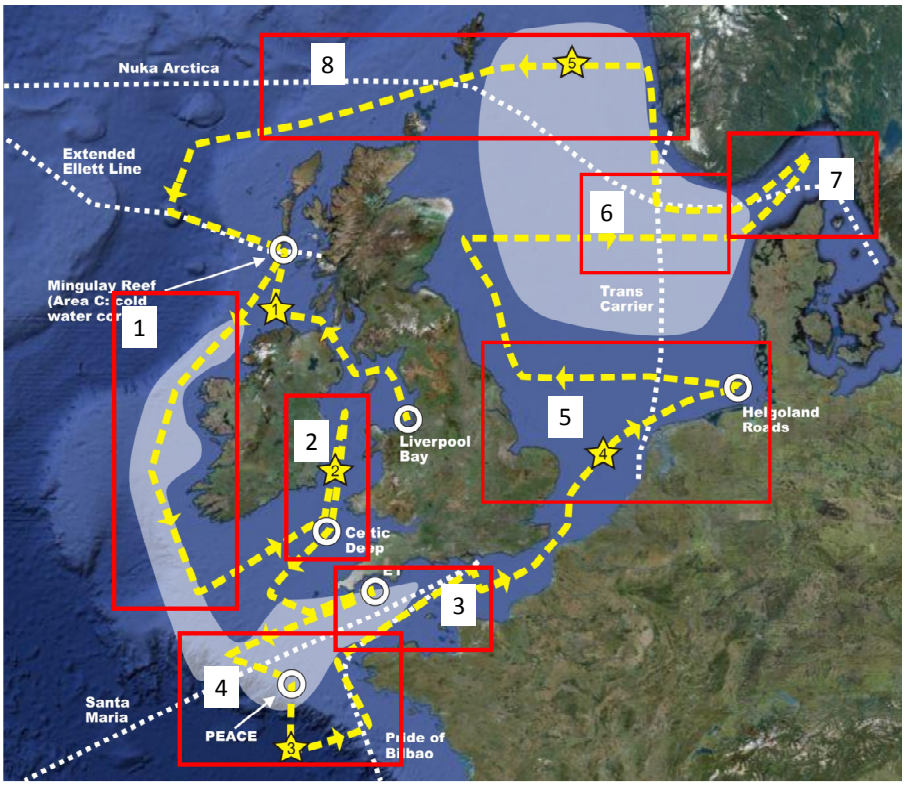

Figure 1: The cruise track showing areas with specific DMS characteristics described here. 1. Eastern Atlantic, 2. Irish Sea, 3. English Channel, 4. Bay of Biscay, 5. Southern North Sea, 6. North Sea coccolithophore bloom, 7. Skaggerak, 8. Northern North Sea and Atlantic.

1. Eastern Atlantic

DMS concentrations ranging from 0 – 8 nM were encountered over the part of the cruise track. Profiles generally showed a sub-surface maximum, indicating the presence of a defined mixed layer. Figure 2 shows the DMS profile from CTD06, a typical profile from this region.

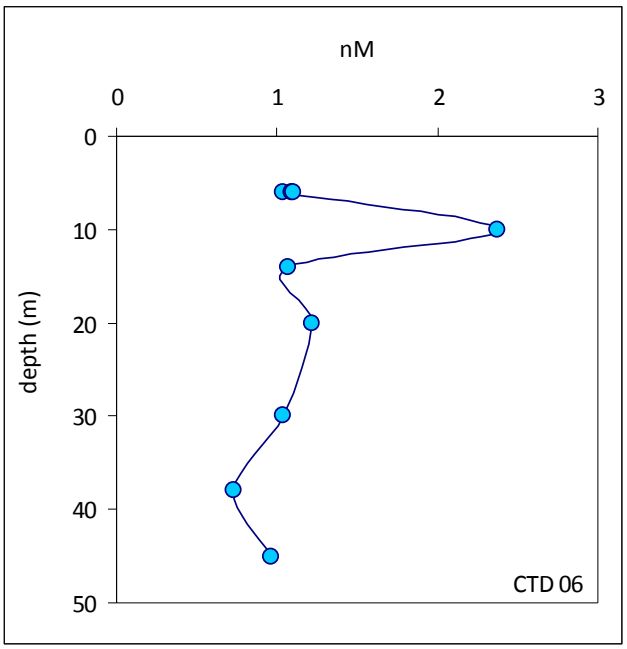

Figure 2: DMS concentrations (nM) with depth measured from CTD06: Eastern Atlantic.

2. Irish Sea

Conditions in the Irish Sea were generally well-mixed, with DMS concentrations ranging from 1.5 – 6 nM. Figure 3 shows a typical DMS depth profile from this region, with a slight subsurface maximum.

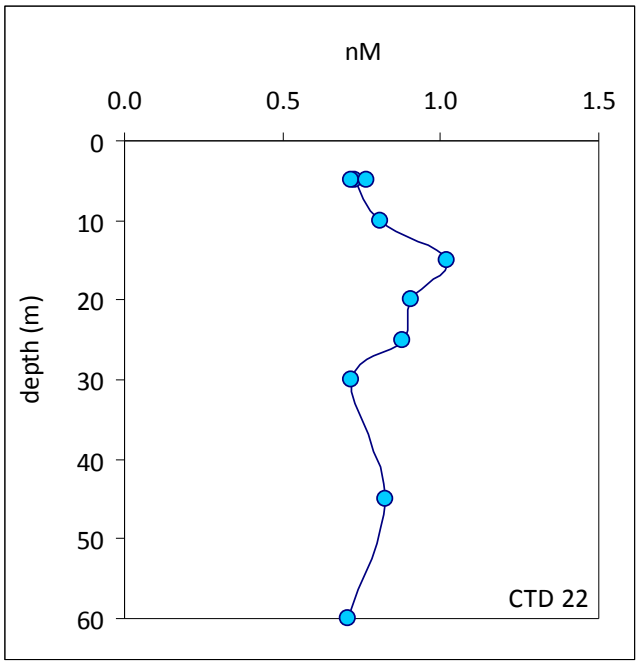

Figure 3: DMS concentrations (nM) with depth measured from CTD22: Irish Sea.

3. English Channel

The English Channel saw the occurrence of some higher concentrations of coccolithophores, resulting in some elevated DMS concentrations. Concentrations ranged from 0.2 – 8 nM. A high degree of patchiness was apparent however, as the high surface concentrations were not consistently observed. This is shown in Figure 4 – two consecutive CTD (35 and 36) casts with quite different profiles. The strong surface maximum in CTD35 may indicate the presence of coccolithophores in the surface layer.

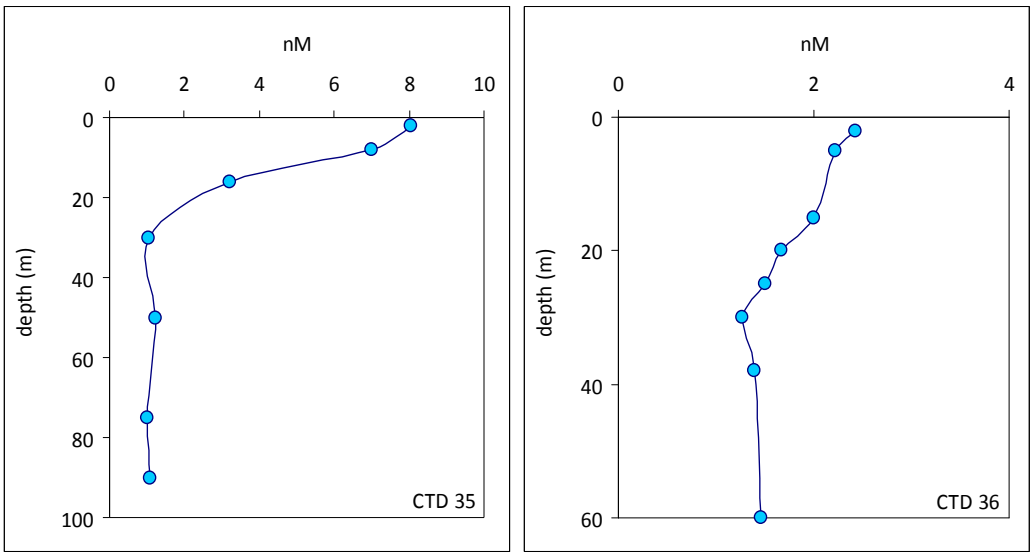

Figure 4: DMS concentrations (nM) with depth measured from CTD35 and CTD36: English Channel.

4. Bay of Biscay

In the Bay of Biscay, both off-shelf and on-shelf sites were sampled, with some apparent distinct differences in DMS concentration profiles. Off the shelf, low concentrations were observed – in fact, the lowest seen on the cruise. However, on the shelf, at the PEACE site, surface concentrations were elevated in comparison. Figure 5 shows DMS profiles from CTD30 and CTD34, demonstrating the difference between these two water types.

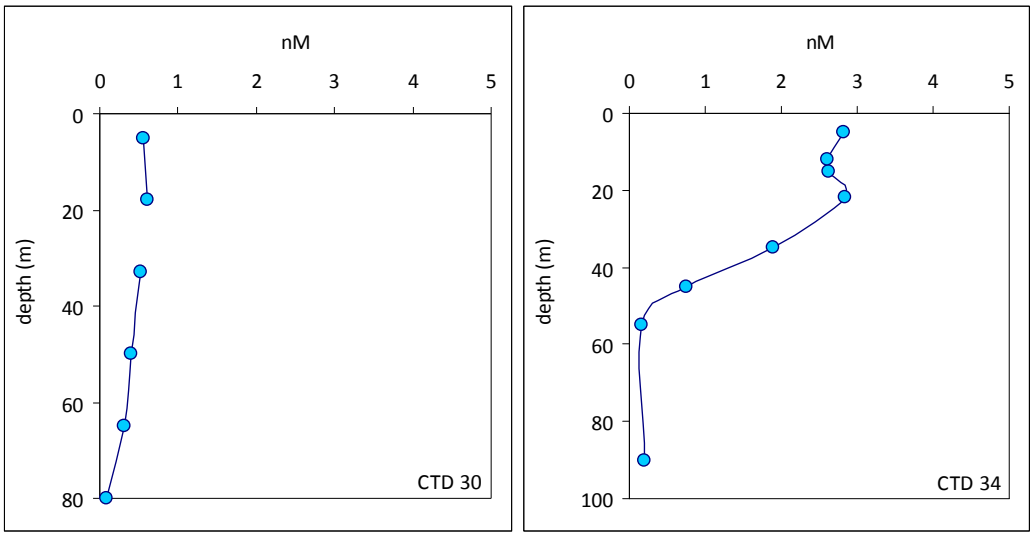

Figure 5: DMS concentrations (nM) with depth measured from CTD30 and CTD34: Bay of Biscay.

5. Southern North Sea

In the Southern North Sea, shallow, warm, well-mixed water was encountered, with DMS concentrations ranging from 1 – 5.5 nM. Concentrations increased when a *Noctiluca* bloom was encountered. Figure 6 shows two consecutive CTD casts (43 and 44), the first in non-bloom waters, and the second within a *Noctiluca* bloom.

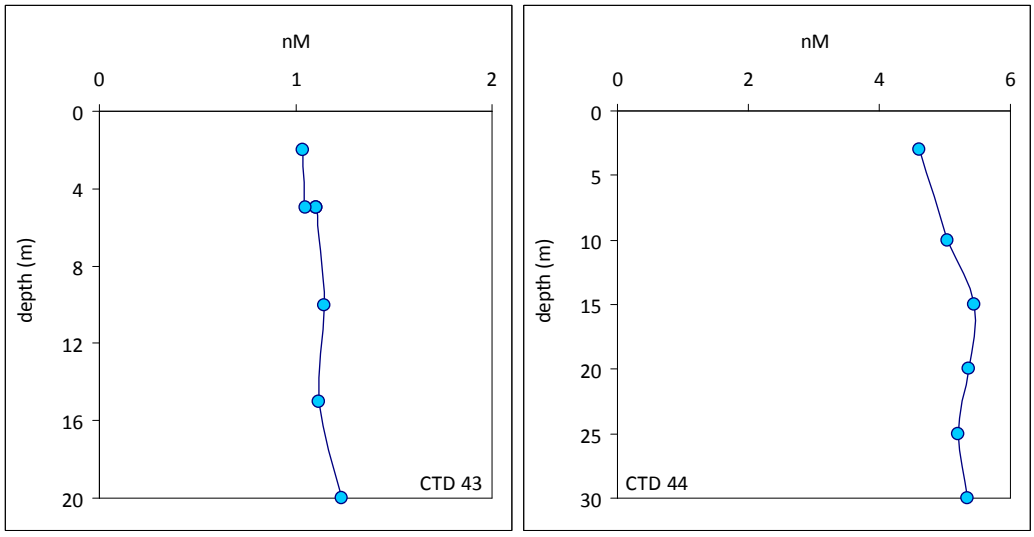

Figure 6. DMS concentrations (nM) with depth measured from CTD43 and CTD44: Southern North Sea.

6. North Sea coccolithophore bloom

A relic coccolithophore bloom was surveyed for 2-3 days. On 28 June 2011, DMS samples were analysed from the underway seawater system at 1 hourly intervals for 10 hours, shown in Figure 7.

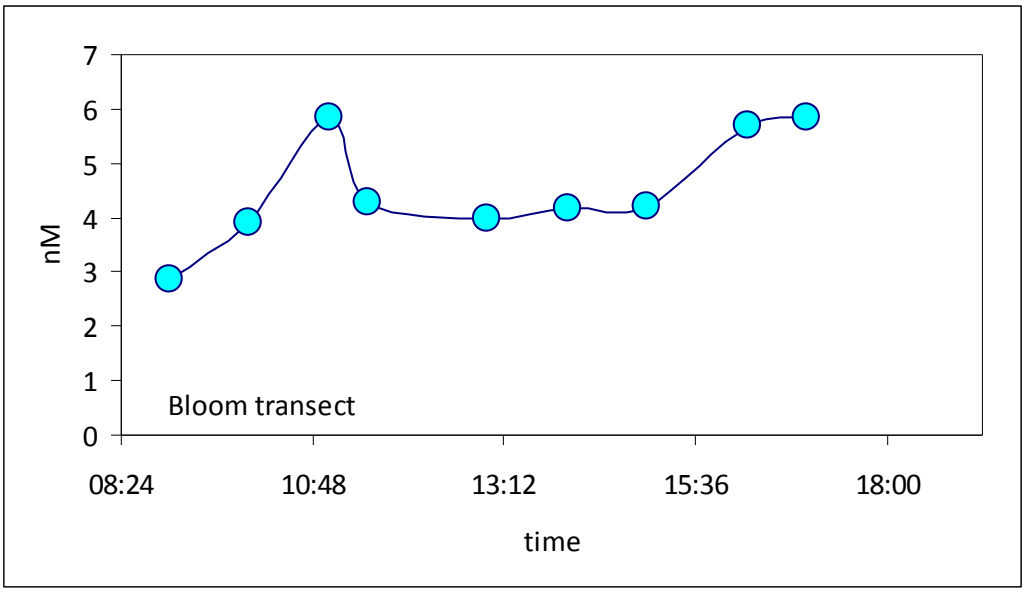

Figure 7: DMS concentrations (nM) during the bloom survey on 28th June 2011.

A series of CTD profiles were carried out during the course of the bloom survey, two examples of which are given in Figure 8 (CTD51 and 55). CTD51 shows elevated DMS concentrations in the surface, typical of the presence of coccolithophores in the surface layers. By contrast, CTD55 shows a deep (40m) DMS maximum, suggesting the deterioration and sinking of the bloom.

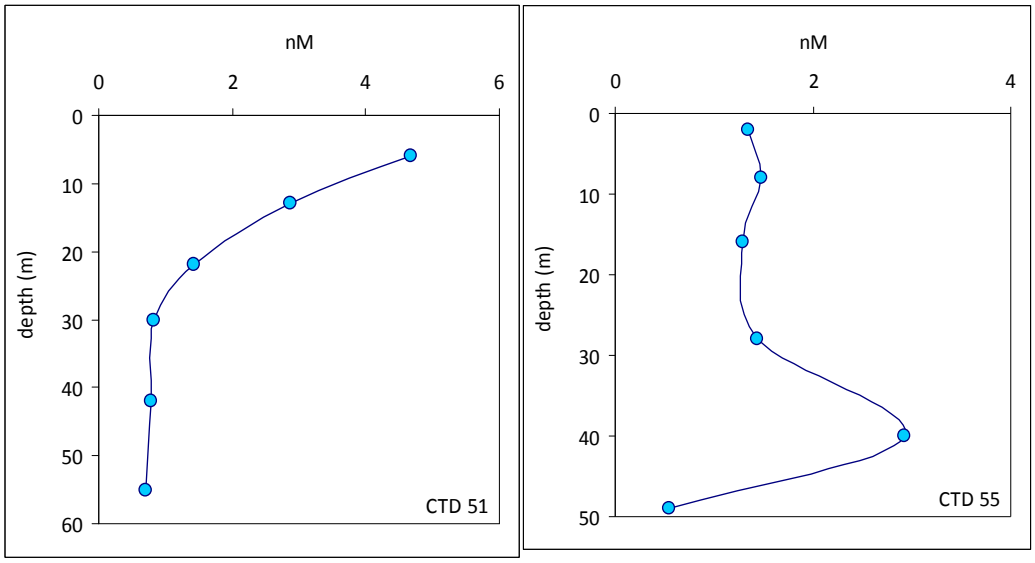

Figure 8: DMS concentrations (nM) with depth measured from CTD51 and CTD55: North Sea coccolithophore bloom.

7. Skaggerak

In the Skaggerak, strongly stratified waters were encountered, with a shallow, warm saline surface layer. DMS profiles from the CTD casts reflected this strong stratification, with sharply elevated concentrations in a narrow upper layer, as shown in Figure 9. Below 40m depth, DMS concentrations were below the method detection limit.

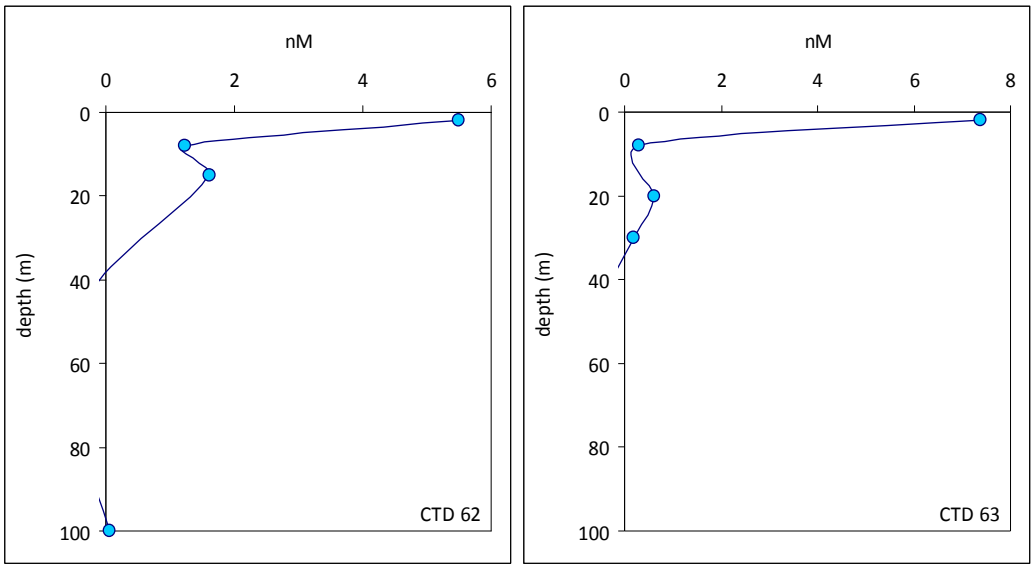

Figure 9: DMS concentrations (nM) with depth measured from CTD51 and CTD55: Skaggerak.

8. Northern North Sea and Atlantic

During the most northerly part of the cruise track, the highest DMS concentrations of the cruise were observed, with a maximum of 22 nM. As shown in Figure 10, DMS concentrations showed a gradual and steady increase from 40 – 50m to the surface.

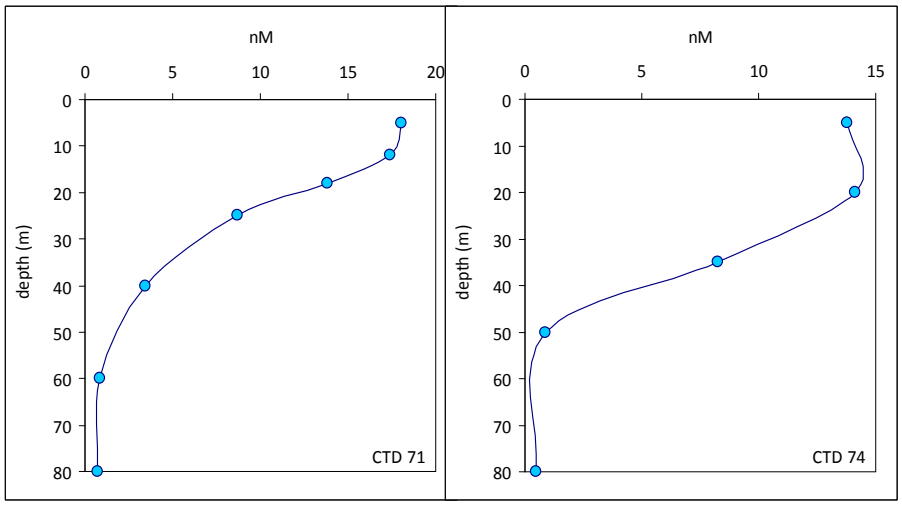

Figure 10: DMS concentrations (nM) with depth measured from CTD51 and CTD55: Northern North Sea.

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3.25- N-cycling rate measurements during CTD casts and ocean acidification bioassay experiments

Darren Clark *(Plymouth Marine Laboratory, Prospect Place, Plymouth, PL1 3DH)*

Objectives

To measure rates of N-cycling during CTD casts and ocean acidification bioassay experiments.

Sampling

i) Bioassay experiments.

Samples were taken for nitrate, nitrite and ammonium assimilation, ammonium regeneration, ammonium oxidation, and nitrite oxidation at each of the three time points, and at each of the four PCO_2 levels. In addition, estimations of ammonium, nitrite and nitrate will be made – detection limits in the region of 1 nmol-N.L^{-1} are expected. Estimations of PON and POC will be available, and an estimation of labile DON is anticipated for bioassay experiments 3-5. The latter is dependant upon the utility of a newly developed method

ii) CTD samples.

Water was sampled from the 55% sPAR depth for CTDs 3, 15, 19, 24, 28, 34, 38, 47, 55, 67, 71. This water was used for deck incubations, with estimations of nitrate, nitrite, and ammonium assimilation during 24 hour incubations (i.e. the full light dark cycle). In addition, short-term (~4 hour) incubations were undertaken during the encompassing the solar noon to provide an estimation of maximum rates of nitrate, nitrite and ammonium assimilation. Rates of ammonium regeneration, ammonium oxidation and nitrate oxidation were also made during 24 hour deck incubations. By considering all data, and using a

mass balance approach were appropriate, it is anticipated that estimations of bacterial N-regeneration, N-assimilation by phytoplankton, and the release of NO_2^- and DON from phytoplankton will be achieved.

3.26- Nitrous Oxide and Methane

Ian Brown *(Plymouth Marine Laboratory, Prospect Place, Plymouth, PL1 3DH)*

Nitrous oxide and methane are biogenically produced trace gases whose atmospheric concentrations are increasing at a rate in the order of 0.7 ppbv y^{-1}. Both gases are radiatively active, contributing approximately 6% and 15% of "greenhouse effect" respectively, whilst N_2O contributes to stratospheric ozone depletion and CH_4 limits tropospheric oxidation capacity.

The oceans are generally considered to be close to equilibrium relative to the atmosphere for both gases, however oceanic source/sink distributions are largely influenced by oxygen and nutrient status and regulatory processes are complicated and are currently not well understood. Little is know of the impacts of ocean.

Aim: *to examine spatial variability in methane production and Nitrous oxide along the cruise tract and in the bioassay CO_2 manipulations*

Methods

Samples were collected from CTD at stations identified below. 1 litre samples were equilibrated with compressed air and headspace analysis performed onboard using FID-gas chromatography and ECD-gas chromatography for CH_4 and N_2O respectively. Atmospheric concentrations were determined by the same methods using a pumped supply from the ships monkey island. Sample from the bioassay were sampled in the same way.

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CTD cast #	*Date*	*Depth (m)*	*Parameters*
03	7 June 2011	40,30,20,15,15,5,2	N2O, CH4
04	7 June 2011	35,25,15,15,12,10,5,25,15,10,2	N2O, CH4
06	8 June 2011	45,38,30,20,14,10,6	N2O, CH4
11	9 June 2011	100,60,40,25,20,15,10	N2O, CH4
15	11 June 2011	160,80,60,35,25,16,5	N2O, CH4
16	11 June 2011	50,40,20,10,5	N2O, CH4
19	13 June 2011	50,50,25,22,18,13,10,5	N2O, CH4
20	13 June 2011	30,26,22,15,10,5	N2O, CH4
22	13 June 2011	60,45,30,25,20,15,10,5	N2O, CH4
23	14 June 2011	90,75,48,36,23,18,7,3	N2O, CH4
24	14 June 2011	60,22,18,12,8,3	N2O, CH4
30	19 June 2011	80,65,50,33,22,18,5	N2O, CH4
34	21 June 2011	90,55,45,35,22,15,12,5	N2O, CH4
35	22 June 2011	90,75,50,30,16,8,2	N2O, CH4
36	23 June 2011	60,38,30,25,20,15,5,2	N2O, CH4

37	23 June 2011	56,40,16,15,13,8,3,2	N2O, CH4
38	24 June 2011	60,45,35,25,15,11,7,3	N2O, CH4
43	26 June 2011	20,15,10,5,5,5,2	N2O, CH4
44	26 June 2011	30,25,20,15,10,3	N2O, CH4
45	26 June 2011	30,22,15,10,6,2	N2O, CH4
46	26 June 2011	36,25,20,13,8,2	N2O, CH4
47	28 June 2011	55,35,30,20,15,5	N2O, CH4
49	28 June 2011	50,38,28,20,13,6	N2O, CH4
51	28 June 2011	55,42,30,22,13,6	N2O, CH4
54	29 June 2011	72,52,40,32,26,12,5	N2O, CH4
55	29 June 2011	49,40,28,16,8,2	N2O, CH4
58	29 June 2011	55,40,30,20,11,5	N2O, CH4
61	30 June 2011	100,50,23,19,15,10,3	N2O, CH4
62	30 June 2011	100,62,40,15,8,2	N2O, CH4
63	30 June 2011	50,30,20,8,1.8	N2O, CH4
65	2 July 2011	50,36,26,17,12,12,12	N2O, CH4
66	2 July 2011	42,36,28,26,20,15,5	N2O, CH4
67	3 July 2011	100,50,35,18,12,8,4	N2O, CH4
68	3 July 2011	75,50,35,18,12,8,4	N2O, CH4
69	4 July 2011	55,35,30,20,15	N2O, CH4
71	5 July 2011	80,60,40,25,18,12,5	N2O, CH4
74	6 July 2011	80,50,35,20,5	N2O, CH4

3.27- *Lophelia pertusa* sampling and experiments

Laura Wicks Sebastian Hennige and J Murray Roberts *(Heriot Watt University)*

Background

Cold-water corals are among the most three-dimensionally complex deep-sea habitats known and are associated with high local biodiversity (Roberts et al. 2006). However, their remoteness and the relatively short history of ecological research in these habitats mean that to date we have little information on carbon and energy flow through these systems, and how future changes in ocean chemistry and temperature will affect these ecosystems.

Ocean acidification, whereby increases in CO_2 in the atmosphere have led to a decline in the pH of our oceans, will affect the availability of carbonate ions that form the skeletal structures of many marine organisms via calcification. Cold-water corals have large and robust calcium carbonate ($CaCO_3$) skeletons that form complex three-dimensional structures which act as habitats for more than 1300 species (Roberts et al. 2006, 2009). Many cold-water coral habitats are found at high latitudes and deeper depths, which exhibit lower saturation state of calcium carbonate (Guinotte et al. 2006). However, it is anticipated that more than 70% of the cold-water coral bioherms known today will be exposed to waters undersaturated with respect to aragonite by the end of the century (Guinotte et al. 2006). Therefore, not only might calcification of cold-water corals be hampered but the crucial balance between processes that promote reef framework growth and processes that degrade the structure (bioerosion and dissolution) may be altered.

To assess the effect of future warming and ocean acidification on cold-water corals, live *Lophelia pertusa* colonies were collected from the only known inshore coral reef in UK waters, which was discovered east of the Hebridean island of Mingulay in 2003 (Roberts et al. 2005, 2009). *Lophelia pertusa* colonies from the Mingulay Reef Complex were used in short term ocean acidification and warming experiments during the cruise, and further colonies will be transported to Heriot Watt University (HWU) for long-term experiments. Additionally, information on the environmental

conditions (in terms of CTD data) was collected at Mingulay Reef. Water samples were taken from the reef site for determination of the carbonate chemistry of the seawater surrounding the reef complex.

Aims

1. To collect benthic samples of live coral and fauna associated with dead coral rubble and bottom water samples
2. To collect CTD data and water samples for carbonate chemistry analysis on the Mingulay Reef complex
3. To conduct onboard experiments, assessing physiological responses of *L. pertusa* to ocean acidification and warming.

Sample numbering

The contents of each grab was given a unique sampling number. For coral rubble and nubbins (preserved in ethanol, formalin or at -20℃):

HWU20110707/001A

where 20110707 is the date in reverse, and 001A is the grab number for the day of sampling.

Faunal sampling

8-9th June 2011

A total of 14 video-assisted van Veen grab samples were taken at different stations on Reef area 1 of the Mingulay Reef complex. A video camera two lights were attached to the winch cable 50 cm from the grab and were used to allow sampling of specific habitats. A total of 9 grab samples contained live *Lophelia pertusa* large enough for experimental purposes, which were stored in the mobile aquarium. From these 9 grab samples small fragments of *L. pertusa* were stored in ethanol for genetic analysis, in formalin for histological analysis and at -20℃ for lipid analysis. Five grabs contained only benthic samples which were stored in 4% buffered

formalin. The fauna present in each grab will be sorted and identified in the laboratory. One fragment of recently dead *L. pertusa* was preserved at -20°C for ^{14}C dating.

7th-8th July 2011

Fourteen video-assisted van Veen grab samples were taken at different stations on Reef area 1 of the Mingulay Reef complex. Thirteen of the grab samples contained live *L. pertusa* large enough for experimental purposes, which were stored in the two mobile aquariums to be transported back to Heriot-Watt University. From these 13 grab samples small fragments of *L. pertusa* were stored in ethanol for genetic analysis, in formalin for histological analysis and at -20°C for lipid analysis. One grab contained only benthic samples that were stored in 4% buffered formalin. The fauna present in each grab will be sorted and identified in the laboratory. Ten fragments of recently dead *L. pertusa* was preserved at -20°C for ^{14}C dating.

Shipboard experiments

Main experiment:

The aim of this experiment was to assess the impact of short-term ocean acidification (1 month) upon the metabolism and calcification (growth) rate of *Lophelia pertusa.* Collected *L. petusa* was split between two coral containers ('hotels'). Container 1 replicated 'ambient' conditions and was considered a control container for the purpose of this experiment. Container 2 was bubbled with 750 ppm CO_2 gas, to replicate future ocean acidification conditions.

Measurements of physiological parameters were conducted on *L. pertusa* fragments from the control and acidified hotel at time zero, after 7, 14 and 20 days.

Growth: This was assessed in two ways

1) radionuclide labelling
2) the alkalinity anomaly technique.

1: Replicate corals were removed form both hotels and incubated in 50 ml falcon tubes for 3 hours prior to the experiment. At Time zero, tubes were spiked with ^{14}C. Aliquots were taken for total activity assessment. After 6 hours, another aliquot was

taken, and the skeletons were rinsed and frozen for analysis back at HWU. Preliminary analysis on the water which contained coral fragments has confirmed that ^{14}C was taken up by the coral, and analysis of frozen skeletons back at HWU will determine the fraction taken up by tissue and by skeleton (growth rate).

2: The decrease in total alkalinity (TA) during coral incubation can be used to determine the change in calcium carbonate and hence used to determine the growth rate of corals. Samples were taken for TA from the hotel water at time zero, and from coral chambers isolated from the main container at the end of a 3-hour incubation in purpose-built stirred containers (n = 8). This will be processed back at HWU to determine calcification rate and hence growth.

Metabolism: It is unknown whether acidified conditions will increase the respiration rate of corals. If this is the case, then corals will have less energy available for growth and reproduction in future acidified conditions. To assess metabolism, respiration rate was measured on replicate corals form both containers at both time points. For this, corals were placed in custom-built tubes with stir bars and optode sensors. Respiration was assessed during 3 hours incubation. Results will be normalized back at HWU to coral weight and ash free dry weight. Coral nubbins used in this experiment were frozen for this reason.

Side experiments

Feeding:

Currently the feeding ecology of *L. pertusa* is poorly understood. The corals at Mingulay get a regular downwelling of surface water (Davies et al. 2009) so it is likely they feed on plankton from the surface. To enable assessments of the affect of ocean acidification on feeding ecology, knowledge of food preferences of fresh *L. pertusa is* crucial to establish a 'baseline'.

To this end we cultured *Skeletonema marinoi* and *Artemia* (brine shrimp) for feeding trials. For this experiment, food stocks were added to corals in stirred chambers, and the decrease in food availability was measured every hour for 3 hours. Filters of the food source were also taken for CHN analysis at HWU, so food

uptake can be combined with nutrition. This experiment was done with two food sources both singularly and combined, and performed on 'starved' corals, and 'well fed corals' to assess whether they employ an opportunistic feeding strategy.

Very short-term acidification:

A series of 1-day incubations at 280 (pre-industrial), 390 and 500 ppm CO_2 were performed on corals in aquaria, to assess the impacts of very short-term bubbling (and hence changes in pH) upon coral metabolism and growth (through alkalinity anomaly technique). Current literature shows discrepancies between short and long-term experiments, and these very short-term incubation experiments were designed in part to address this problem. Samples were taken for TA and skeletons were preserved at -20℃, to analyse at HWU for growth rates, and for metabolic normalization respectively.

Temperature experiments:

Future conditions will likely include a rise in sea temperatures on the Mingulay reef. As such, it is important to know how these corals respond to increases in temperature with regard to growth and metabolism. Two treatments were conducted; a rapid temperature increase and a slow temperature increase. Again, current literature does not account for a 'stress' versus 'acclimation' response, so comparing growth and respiration data form both treatments will assess whether there is a shock response, and also how corals can cope with increased projected temperatures, and what this will mean to their energetic budget for reproduction and growth. Samples were taken for TA and skeletons were preserved at -20℃, to analyse at HWU for growth rates, and for metabolic normalization respectively.
The activity log is described in the Appendix D

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Roberts JM, Brown CJ, Long D, Bates CR (2005) Acoustic mapping using a multibeam echosounder reveals cold-water coral reefs and surrounding habitats. Coral Reefs 24: 654-669

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Sample list

Sample no.	Description
201100607/001A	Video-assisted grab, live coral into aquarium (1 small basket), fragments in EtOH, 4% formalin and -20°C
201100607/002A	Video-assisted grab, live coral into aquarium (2 large basket), fragments in EtOH, 4% formalin and -20°C
201100607/003A	Video-assisted grab, live coral into aquarium (3 large basket), fragments in EtOH, 4% formalin and -20°C. 1 benthic sample into 4% formalin
201100607/004A	Video-assisted grab, live coral into aquarium (2 large basket), fragments in EtOH, 4% formalin and -20°C
201100607/005A	Video-assisted grab, coral rubble into 4% formalin
201100607/006A	Video-assisted grab, live coral into aquarium (2 large basket), fragments in EtOH, 4% formalin and -20°C. 4 tubes benthic samples in 4% formalin
201100607/007A	Video-assisted grab, live coral into aquarium (3 large basket), fragments in EtOH, 4% formalin and -20°C. 1 benthic sample in 4% formalin
201100607/008A	Video-assisted grab, live coral into aquarium (2 large basket), fragments in EtOH, 4% formalin and -20°C. 2 benthic sample in 4% formalin
201100607/009A	Video-assisted grab, benthic sample in 4% formalin
201100607/010A	Video-assisted grab, live coral into aquarium (2 large basket), fragments in EtOH, 4% formalin and -20°C. 1 benthic sample in 4% formalin
201100607/011A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in EtOH, 4% formalin and -20°C.
201100608/001A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in EtOH, 4% formalin and -20°C
201100608/002A	Video-assisted grab, benthic sample in 4% formalin. 1 dead fragment at -20°C
201100707/001A	Video-assisted grab, live coral into aquarium (1 small basket), fragments in EtOH, 4% formalin and -20°C
201100707/002A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in EtOH, 4% formalin and -20°C
201100707/003A	Video-assisted grab, live coral into aquarium (1 small basket), fragments in EtOH, 4% formalin and -20°C. 1 benthic sample in 4% formalin
201100707/004A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in

	EtOH, 4% formalin and -20°C. 1 benthic sample in 4% formalin. 1 dead fragment at -20°C
201100707/005A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in EtOH, 4% formalin and -20°C.
201100707/006A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in EtOH, 4% formalin and -20°C. 1 benthic sample in 4% formalin.
201100707/007A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in EtOH, 4% formalin and -20°C. 1 benthic sample in 4% formalin.
201100707/008A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in EtOH, 4% formalin and -20°C. 1 benthic sample in 4% formalin.
201100707/009A	Video-assisted grab, live coral into aquarium (1 small basket), fragments in EtOH, 4% formalin and -20°C. 1 benthic sample in 4% formalin.
201100707/010A	1 benthic sample in 4% formalin.
201100707/011A	Video-assisted grab, live coral into aquarium (1 small basket), fragments in EtOH, 4% formalin and -20°C.
201100708/001A	Video-assisted grab, live coral into aquarium (1 small basket), fragments in EtOH, 4% formalin and -20°C.
201100708/002A	Video-assisted grab, live coral into aquarium (1 large basket), fragments in EtOH, 4% formalin and -20°C.
201100708/003A	Video-assisted grab, live coral into aquarium (2 large baskets), fragments in EtOH, 4% formalin and -20°C.

3.28- Cruise Blog

Dr. Toby Tyrrell *(NOCS, Southampton, UK)*

Dr. Sebastian Hennige *(Heriott-Watt University, Edinburgh, UK)*

Dr. Jeremy Young *(UCL, UK)*

All members of the scientific party

Pre-Cruise Preparation

A volunteer was sought amongst the scientific crew, to take photographs for the cruise blog, and Seb Hennige offered his services. Additional photographic equipment (fish-eye lens, memory) was purchased. A decision had to be made as to who would put up the blog (a KE officer had not at that time been appointed to the consortium, and this could not easily be done straight from the ship) and it was decided that this would be done through NOCS, where the comms group have a track record of publishing such blogs in a timely manner.

Writing of Blogs

Most articles were written by individual scientists, with additional text and editing for style and readability. The blog tried to explain the individual pieces of science and the reasons for carrying them out, as well as to convey some of the excitement and interest (and hard work) of what it was like for the scientists carrying out the work. Scientific nomenclature was reworded in plain language wherever possible. Each article was kept to a limit of about one A4 page of text. By the end of the cruise the science of every scientist on board had been described to at least some degree, with only one exception. Some text was added to each blog to give an idea of where we were along the cruise track and any highlights. Each article was accompanied by 5 or so photos or other images.

Publication of Blogs

31 daily blog entries were submitted to NOCS Comms, with the first article on 06 June and the last on 07 July. Entries were usually submitted on the same day or, more usually, early on the following day. Blog entries were published with only a very short delay by Eileen Crockford at NOCS Comms, usually appearing online (highlighted on the NOC front page, linked from UKOARP website and elsewhere) late on the day of submission. Delays occurred over some weekends when NOCS Comms staff were understandably not at work although even then Eileen Crockford sometimes came in especially to put up blog entries. An estimate of readership (from 'page hits'), suggests an average of about 300 viewings per day. An example published blog entry is shown below.

National Oceanography Centre
NATURAL ENVIRONMENT RESEARCH COUNCIL

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Marine Life Talk – 4 August 2011
August 4, 2011

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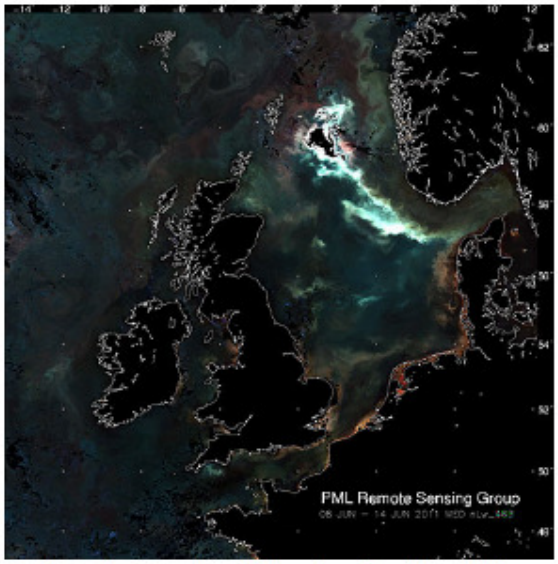
RRS Discovery cruise 366: 28 June 2011

June 28, 2011

Ever since the first week of the cruise, we have been intrigued by satellite images showing a large and intense coccolithophore bloom in the northern North Sea (see picture). Coccolithophores (see electron microscope image below and to the right) are microscopic phytoplankton, so small as to be completely invisible to the naked eye, no matter how sharp your vision is. They build tiny calcium carbonate shields ('coccoliths') and it is the light-scattering properties of billions upon billions of these coccoliths in the water that give the water its bright appearance from above. Imagine grinding up diamonds into tiny fragments and stirring those around in some water, with the light ricocheting and reflecting off the myriad fragments. This gives some idea of what's happening in coccolithophore-rich waters and why their appearance is rather unusual.

D366



Pseudo-true colour composite satellite image from 8th-14th June, with bloom (bright waters) SW of Norway.

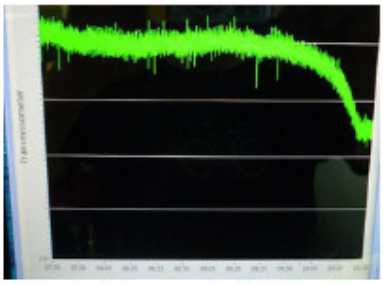
We kept looking enviously at the satellite images that we receive on the ship, sent to us daily from NEODAAS, a specialist group in Plymouth. However, we started the cruise on the western (wrong) side of the UK, and knew it would be weeks before we would arrive on the eastern side, and that the blooms typically only last a few weeks. Therefore we didn't expect it to still be there by now.

Thanks to heavy cloud cover during most of the last few weeks, we weren't always able to see whether or not the bloom was still there, but then just a couple of days ago we received an image. The bloom was still there!

As discussed in an earlier blog, the possible impact of ocean acidification on coccolithophores is of considerable concern and debate, and is being actively researched. This bloom offers us an opportunity to find out more. We will make the most of the great array of expertise present on the ship to probe several aspects of the bloom here, including what is causing it, whether such blooms are still likely to occur in the future when seawater is more acidic (we plan to use bloom water for the next bioassay), and how the coccolithophores and the carbon chemistry of seawater affect each other.

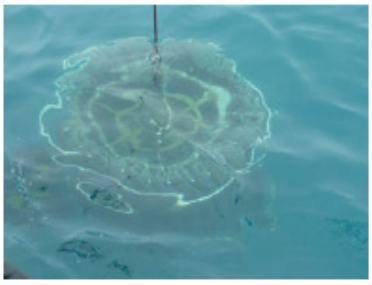
Once we realised that the bloom was still going to be

there, we plotted a track directly across it. At first, as we steamed into where we thought the bloom should be, we saw nothing, and wondered if the satellite image was somehow misleading. Then one of us, Alex from yesterday's blog, noticed that one instrument on the ship was starting to show an anomalous reading. The signal from the beam transmissometer (which measures how much of an emitted beam of light successfully crosses a fixed distance of seawater) was gradually declining, due to the coccoliths scattering some of the light. We were entering the bloom! Then, over the next hour or two, the colour of the water became increasingly bizarre when viewed from the deck of the ship, just like on other occasions when these blooms have been encountered at sea. It began to look, from the water colour at least, as if we were in tropical waters near a coral beach, rather than being in the normally greyish North Sea. The bloom waters are more aquamarine in colour, almost as if some milk has been stirred into the sea.



The first sign that we were entering the bloom, from the transmissometer. Time goes from left (past) to right (present). The declining light transmission is caused by the increasing presence of coccoliths in the water as we enter the bloom

We have been busy through the rest of the day completing our transect and collecting lots of data across the bloom. We can already tell from the areas that we have surveyed so far that there are large numbers of single coccoliths but only a few living coccolithophore cells, almost as if we have come across a battlefield strewn with armour and corpses, but with the fighting all over. In this sense what has been called a bloom in this blog is actually misnamed because the coccolithophores have declined and are no longer rapidly proliferating. This may limit our ability to understand how the bloom formed, but we may nevertheless be able to make significant advances. Already the data that we have been collecting is leading us to reject some hypotheses we had entertained beforehand and to formulate some new ones.



Above: Aquamarine tint to the water colour

Comparison of water colour on two days, left-hand side on a normal day, right-hand side in the bloom

Occurring intermittently and infrequently out at sea, not many get to encounter these blooms. It feels like a privilege to be able to see this curious natural phenomenon first-hand. We will see more of it over the next few days.

To find out more about these blooms, see: http://www.noc.soton.ac.uk/soes/staff/tt/eh/index.html

Previous Blog Post - *Next Blog Post*

Related News
RRS Discovery cruise 366: 24 June 2011
RRS Discovery cruise 366: 16 June 2011

Appendix A: Technical detail report

S/S CTD

Cast D366029 had only one PAR sensor installed (DWIRR).

Cast D366030 was split into two files (030 and 030a), as “birdcaging” and damage to CTD winch wire resulted in extensive downtime of approximately 5.5 hours. Four water samplers were triggered on cast 030, and the remainder closed on 030a. The profile was split at approximately 3564m on the up cast.

Total number of casts - 75 S/S frame

Casts deeper than 500m - 3 S/S frame

Deepest cast - 4835m S/S frame

Autosal

No issues reported

FRRF

No issues reported

LADCP

Because of the above mentioned problems with the damaged CTD wire on cast D366030_LADCP, the resulting profile contained approximately 5.5 hours of data at one depth, and 11.5 hours in total.

Cast D366032_LADCP was split into two profiles, as the first of the two memory cards was full after ensemble 910. No corrections/errors in the files. Second profile was from ensemble 912 to 2905.

No log file for cast D366072_LADCP

J. Benson/J. Burris

11 July 2011

Appendix B: RRS Discovery detailed cruise narrative D366/D367

June 3-July 10 2011

Friday 3-6-2011

Arrival in Liverpool in evening by majority of science party. Scientists checked into hotels. Off-loading of containers had commenced during Friday. Installation of instruments in ship's laboratories and container laboratories had commenced.

Saturday 4-6-2011

Researchers and PSO arrived at vessel in morning. Installation of labs and equipment commenced. All going smooth.

Sunday 5-6-2011

Mobilisation continued. No major issues, all going smooth.

Science party stayed on ship at night.

Monday 06-06-2011

Ship departed at 0830 h. Calm seas in Liverpool Bay.

Station 1 Liverpool Bay 53º36.73N 3º20.75W; water depth ca. 16 m

Times (GMT)

1130 h Stainless CTD (16 m) Cast 01

Station 2 Irish Sea; 54º24.65N 3º53.04W water depth ca. 34 m

Times (GMT)

1700 h Stainless CTD (34 m) Cast 02

Steam at 7 knots until arrival at station (time dependent station).

Weather in Irish Sea excellent and majority of science party is feeling well. Ship is making good progress.

Tuesday 07-06-2011

Station 3 Irish Sea; 54º40.83N 5º21.46W; water depth ca. 130 m

0430 h Stainless CTD (130 m) Cast 03

0900 h Bioassay meeting in plot

1030 h Safety Drill

Station 4 Irish Sea; 55º32.38 N 6º41.85 W; water depth ca. 80 m

1200 h Stainless CTD (80 m) Cast 04

Station 5 Irish Sea; 55º46.20 N 7º16.409 W; water depth ca. 60 m

1600 h Stainless CTD (60 m) Cast 05

Steam to Mingulay Reef.

Estimated arrival time Wednesday 0200 h.

Wednesday 08-06-2011 Mingulay Reef 56º47.688N 7º24.300W

Station 6

0200 h Bioassay CTD Mingulay Reef ; water depth ca. 180 m Cast 06

0500 h Stainless CTD Mingulay Reef (180 m) Cast 07

0630 h Video Grab sampling of deep coral material all day Wednesday

1330 h Stainless CTD Mingulay Reef (ca. 180 m) Cast 08. Strong currents, ship drifting off reef.

1930 h Stainless CTD Mingulay Reef (140 m) Cast 09 (**56º49.452 N 7º23.874 W**).

Thursday 09-06-2011 Barra

0218 h Stainless CTD Mingulay Reef (140 m) Cast 10 (**56º49.434 N 7º23.921 W**).

0230 Coral collection finished

0730 h Boat transfer of scientists

Depart for European Shelf transect

Station 7

1250 h Stainless CTD (180 m) Cast 11 (**56º21.869 N 7º51.743 W**).

Station 8

1600 h Stainless CTD (120 m) Cast 12 (**56º08.314 N 8º04.777 W**).

Friday 10-06-2011 Atlantic Ocean off Ireland

0500 h Breakdown t= 48 of first bioassay experiment

Station 9

0930 h Stainless CTD (250 m) Cast 13 (**54º21.951 N 10º44.566 W**).

Station 10

1600 h Stainless CTD (205 m) Cast 14 (**53º42.028 N11º19.991 W**).

Saturday 11-06-2011 Atlantic off Ireland

Station 11

0430 h Stainless CTD (330 m) Cast 15 (**52º08.259 N 11º42.872 W**).

Station 12

1200 h Stainless CTD (225 m) Cast 16 (**51º15.461 N 11º20.097 W**).

Sunday 12-06-2011 Atlantic off Ireland

0400 h onwards (most researchers up for 0500 h) Breakdown t= 96 of first bioassay experiment

Station 13

1000 h Stainless CTD (120 m) Cast 17 (**50º37.036 N 8º41.492 W**).

Station 14

1500 h Stainless CTD (120 m) Cast 18 (**50º40.835 N 7º55.119 W**).

Monday 13-06-2011 Atlantic off Ireland

Station 15

0430 h Stainless CTD (90 m) Cast 19 (**51º36.484 N 5º42.924 W**).

Station 16 Irish Sea Stratified waters off Dublin (53º36.585N 5º42.952W)

1816 h Stainless CTD (80 m) Cast 20

Tuesday 14-06-2011 Irish Sea mixed water (52º28.237N 5º54.052W)

Station 17

0200 h Bioassay CTD ; water depth ca. 70 m Cast 21

0430 h Stainless CTD (75 m) Cast 22 **(52º28.369N 5º53.789W)**

Station 18 (51º14.737N 6º03.409W) Celtic Deep Smart Buoy site

1000 h Stainless CTD (100 m) Cast 23

Wednesday 15-06-2011 Western Approaches

Station 19

0430 h Stainless CTD (78 m) Cast 24 **E1 Western Channel Observatory (50º01.737 N 4º22.844 W)**

0900 h Falmouth boat transfer

Station 20

1400 h Stainless CTD (86 m) Cast 25 **(49º50.963N 5º20.361W)**

Thursday 16-06-2011 Celtic Sea

0400 h onwards (most researchers up for 0500 h) Breakdown t= 48 h of second bioassay experiment

Station 21

0900 h Stainless CTD (100 m) Cast 26; **CD173 Station B2 (49º53.616 N 7º53.098W)**

Station 22

1400 h Stainless CTD (130 m) Cast 27 **(49º34.541N 8º16.782W)**

Friday 17-06-2011 Bay of Biscay

Poor weather conditions did not allow for a station to be sampled until the afternoon

Station 23

1500 h Stainless CTD (173 m) Cast 28; **CD173 Station (48º35.353N 9º29.314W)**

Saturday 18-06-2011 Bay of Biscay

0400 h onwards (most researchers up for 0500 h) Breakdown t= 96 of second bioassay experiment

Poor weather conditions did not allow for a station to be sampled until the afternoon. However, problems with the bow thrusters prevented us from undertaking a CTD station.

Sunday 19-06-2011 Bay of Biscay

Station 24

0430 h Stainless CTD (200 m in 4600 m) Cast 29 **(46º29.794 N 7º12.355 W)**

Station 25 Deep Station

1200 h Stainless CTD (4811 m) Cast 30 **(45º30.067 N 7º11.701 W)**

CTD very slowly recovered after winch cable problems

Monday 20-06-2011 Bay of Biscay

Re-termination of new CTD winch cable, and load tests with new cable. Also scrolling tests with the second winch system.

Hydraulic leak occurred but was fixed swiftly.

Tuesday 21-06-2011 Bay of Biscay

Station 26 46º12.137 N 7º13.253W

0200 h Bioassay CTD; water depth ca. 4721 m, cast 200 m deep; Cast 31;

0400 h Stainless CTD; water depth ca. 4721 m, cast 200 m deep ; Cast 32

Station 27 47º01.771N 7º37.340W

1300 h Stainless CTD (4370 m; cast depth 200 m) Cast 33;

Wednesday 22-06-2011 Celtic Sea

Station 28

0430 h Stainless CTD (170 m) Cast 34 **PEACE Station (48º00.124 N 7º11.529 W)** Cast 34

Station 29

1440 h Stainless CTD (100 m) Cast 35 **(48º49.410 N 5º08.956 W) Usshant Station** Cast 35

Thursday 23-06-2011 Atlantic Approaches

0400 h onwards (most researchers up for 0500 h) Breakdown t= 48 h of third bioassay experiment

Station 30 Falmouth Station

0900 h Stainless CTD (90 m) Cast 36 **(49º51.828 N 5º16.455 W)**

Boat transfer for spares

Station 31 Falmouth Station

1400 h Stainless CTD (73 m) Cast 37 **(50º05.220 N 4º37.018 W)**

Friday 24-06-2011 Atlantic Approaches

Station 32 Plymouth Station E1 Western Channel Observatory

0430 h Stainless CTD (75 m) Cast 38 **(50º01.559 N 4º21.642 W)**

Position sampling

Station 33 Portland Station (50º20.985 N 2º20.889W)

Position Sampling

1508 h Stainless CTD (55 m) Cast 39

Saturday 25-06-2011 Channel

0400 h onwards (most researchers up for 0500 h) Breakdown t= 96 h of third bioassay experiment

Station 34 Channel (50º25.267 N 00º08.732E) ***Position sampling***

0600 h Stainless CTD (54 m) Cast 40

Station 35 Channel (51º58.796 N 2º04.491 E)

Position sampling

1800 h Stainless CTD (54 m) Cast 41

Sunday 26-06-2011

Station 36 Southern North Sea (52º59.661 N 2º29.841 E) ***Time/Position Sampling***

0200 h Bioassay CTD; water depth ca. 30 m, Cast 42;

Station 37

0415 h Stainless CTD; water depth ca. 30 m ; Cast 43

Station 38 North Sea (53º39.359 N 4º11.849 E) ***Time sampling***

1200 h Stainless CTD (40 m) Cast 44

Monday 27-06-2011

Station 39 North Sea; Helgoland (54º18.354 N 7º18.536 E) ***Position sampling***

0430 h Stainless CTD; water depth ca. 43 m ; Cast 45

Station 40 North Sea (54º22.328 N 5º09.565 E) ***Time Sampling***

1200 h Stainless CTD (45 m) Cast 46

Tuesday 28-06-2011 Northern North Sea

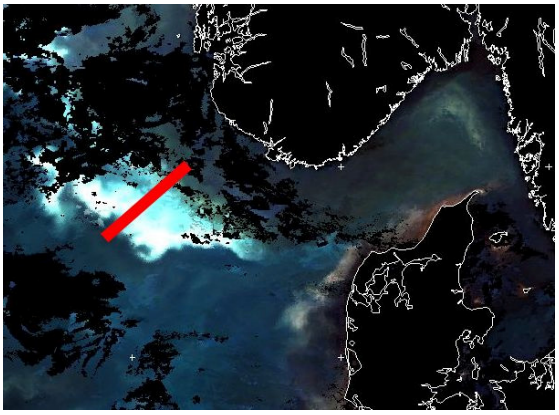
0400 h onwards (most researchers up for 0500 h) Breakdown t= 48 h of fourth bioassay experiment.

We will conduct a transect across a coccolithophore bloom today (57°12°N,3°30°E to 58°N,5°E) from 08:00 to ca. 18:00

We will occupy three detailed biogeochemical stations (0800 h, 1130 h and 1500 h), and four physics/nutrient stations 1000 h, 1330 h, 1630 h, 1800 h.

CTD depths to ca. 50 m

Underway sampling every hour 08:00 to 18:00 except when on station



Station 41 Out of bloom Station

0800 h Stainless CTD (64 m) Cast 47 **(57°12.053 N 3°29.366 E)** Biogeochemistry

Station 42 Edge of bloom Station

1030 h Stainless CTD (70 m) Cast 48 Physics/nutrients **(57°24.919 N 3°53.756 E)**

Station 43 Bloom Station

1200 h Stainless CTD (72 m) Cast 49 Biogeochemistry **(57°26.971 N 3°57.510 E)**

Station 44 Bloom Station

1340 h Stainless CTD (74 m) Cast 50 Physics/nutrients **(57°34.663 N 4°12.084 E)**

Station 45 Bloom Station

1520 h Stainless CTD (75 m) Cast 51 Biogeochemistry **(57°41.625 N 4°25.108 E)**

Station 46 Bloom Station

1630 h Stainless CTD (85 m) Cast 52 Physics/nutrients **(57°46.132 N 4°38.484 E)**

Station 47 Out of Bloom Station

1800 h Stainless CTD (103 m) Cast 53 Physics/nutrients (**57°55.466 N 4°51.882 E**)

Wednesday 29-06-2011 Northern North Sea

Second day of high resolution bloom sampling.

Station 48 Bloom Station

0330 h Stainless CTD (87 m) Cast 54 **(57°45.726 N 4°35.434 E)** Biogeochemistry

Station 49 Out of bloom Station

0830 h Stainless CTD (58 m) Cast 55 **(57°00.023 N 4°59.834 E)** Biogeochemistry

Station 50 Edge of bloom Station

1000 h Stainless CTD (60 m) Cast 56 Physics/nutrients **(57°06.038 N 5°07.535 E)**

Station 51 Bloom Station

1200 h Stainless CTD (60 m) Cast 57 Physics/nutrients **(57°18.591 N 5°21.929 E)**

Station 52 Bloom Station

1400 h Stainless CTD (91 m) Cast 58 Biogeochemistry **(57°27.059 N 5°32.836 E)**

Station 53 Bloom Station

1530 h Stainless CTD (100 m) Cast 59 Physics/nutrients **(57°34.983 N 5°41.412 E)**

Station 54 Out of Bloom Station

1744 h Stainless CTD (214 m) Cast 60 Physics/nutrients (**57°46.130 N, 5°57.020 E**)

Thursday 30-06-2011 Northern North Sea

0300 h onwards (most researchers up for 0400 h) Breakdown t= 96 h of fourth bioassay experiment.

Station 55 Skaggerak Station (**58°30 N, 10°00.222 E**)

0800 h Stainless CTD (550 m) Cast 61

Station 56 Skaggerak Station (**58°12.181 N, 09°12.423 E**)

1200 h Stainless CTD (670 m) sampled to 200 m Cast 62

Station 57 Bloom Station (**57°52.689 N, 08°20.123 E**)

1600 h Stainless CTD (520 m) sampled to 200 m. Cast 63

Friday July 1

Poor weather conditions (Force 8-9) with a high wave state did not allow us to undertake a CTD deployment at 0300 h.

The wave state did not improve during the day, with Force 7.

We therefore could not do any work on the Friday and delayed all our activities to Saturday.

Saturday July 2

Station 58 North Sea (56º30.293 N 3º39.506 E)

Bioassay Number 5. Stratified low nutrient waters

0100 h Bioassay CTD; water depth ca. 70 m, Cast 64;

Station 59 North Sea (56º29.611 N 3º36.541 E)

0300 h Stainless CTD; water depth ca. 70 m ; Cast 65

Station 60 North Sea (57º14.532 N 4º01.884 E) ***Time sampling***

1100 h Stainless CTD (63 m) Cast 66

Sunday July 3

Station 61 Northern North Sea

0330 h Stainless CTD (270 m) Cast 67 (**59°40.721 N, 4°07.633 E**) ***Time sampling***

Station 62

1100 h Stainless CTD (117 m) Cast 68 (**59°59.011 N, 2°30.896 E**) ***Time sampling***

1200 h Test deployment of video instrument for deep water coral collection

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Monday July 4

Breakdown bioassay E5 t= 48

0300 h onwards (most researchers up for 0400 h)

Station 63 Northern North Sea

0800 h Stainless CTD (100 m) Cast 69 **(59°56.236 N, 1°46.986 W**) ***Time sampling***

Station 64

1300 h Stainless CTD (100 m) Cast 70 **(60°00.012 N, 2°39.929 W**) ***Time sampling***

Tuesday July 5 Atlantic Ocean

Station 65

0330 h Stainless CTD (1050 m) Cast 71 **(59°59.298 N, 5°58.997 W**) ***Position sampling***

Station 66

1438 h Stainless CTD (1145 m) Cast 72 sampled to 200 m **(59°25.485 N, 7°47.831 W**) ***Position sampling***

Wednesday July 6 Atlantic Ocean

Breakdown bioassay E5 t= 96 h

0300 h onwards (most researchers up for 0400 h)

Station 67

0455 h Stainless CTD (1891 m) Cast 73 **(58°36.137 N, 10°29.986 W**) ***Position sampling***

Station 68

1400 h Stainless CTD (622 m) Cast 74 **(57°27.124 N, 11°09.59 W**) ***Position sampling***

Thursday July 7

Mingulay Coral Sampling (56º49.638N 7º23.292W)

Station 69 Mingulay

0838 h Stainless CTD (190 m) Cast 75

Coral Collection

Friday July 8 Coral Sampling

Mingulay Coral Sampling

Coral collection

Departure for Liverpool

Saturday July 9 Arrival in Liverpool

Arrival back in Liverpool for disembarkation

1600 h arrival in Liverpool

Sunday July 10 Disembarkation

Disembarkation by researchers in Liverpool

1430 h Last scientist (PSO) to disembark in Liverpool

Appendix C: Underway samples - day, time, location and parameter measured

UW	Date	Hour	Minute	Latitude	Longitude	Parameter sampled
41	6/12	0	6	50.0500	-10.1833	Tp, Salinity, DIC, TA, Nut, S
42	6/12	2	3	50.1690	-9.8682	Tp, Salinity, DIC, TA, Nut.
43	6/12	4	1	50.2765	-9.5460	Tp, Salinity, DIC, TA, Nut.
44	6/12	6	24	50.4058	-9.1825	Tp, Salinity, DIC, TA, Nut, S
45	6/12	8	5	50.4985	-8.9153	Tp, Salinity, DIC, TA, Nut.
46	6/12	12	12	50.6802	-8.4177	Tp, Salinity, DIC, TA, Nut, S
47	6/12	14	1	50.8000	-8.0667	Tp, Salinity, DIC, TA, Nut.
48	6/12	18	3	50.9247	-7.6933	Tp, Salinity, DIC, TA, Nut, S
49	6/12	20	12	51.0667	-7.2667	Tp, Salinity, DIC, TA, Nut.
50	6/12	22	3	51.1833	-6.9000	Tp, Salinity, DIC, TA, Nut.
51	6/13	0	6	51.3333	-6.4833	Tp, Salinity, DIC, TA, Nut, S
52	6/13	3	8	51.5387	-5.9135	Tp, Salinity, DIC, TA, Nut.
53	6/13	4	0	51.5900	-5.7568	Tp, Salinity, DIC, TA, Nut.
54	6/13	6	12	51.6833	-5.8167	Tp, Salinity, DIC, TA, Nut, S
55	6/13	8	3	51.9317	-5.7400	Tp, Salinity, DIC, TA, Nut.
56	6/13	10	4	52.2333	-5.7083	Tp, Salinity, DIC, TA, Nut.
57	6/13	12	18	52.5667	-5.6500	Tp, Salinity, DIC, TA, Nut, S
58	6/13	14	2	52.8333	-5.6167	Tp, Salinity, DIC, TA, Nut.
59	6/13	16	18	53.2800	-5.6650	Tp, Salinity, DIC, TA, Nut.
60	6/13	20	6	53.3833	-5.7167	Tp, Salinity, DIC, TA, Nut, S
61	6/13	22	24	52.9667	-5.6500	Tp, Salinity, DIC, TA, Nut.
62	6/14	0	7	52.5833	-5.8333	Tp, Salinity, DIC, TA, Nut, S
63	6/14	2	7	52.4650	-5.9033	Tp, Salinity, DIC, TA, Nut.
64	6/14	4	4	52.4683	-5.9017	Tp, Salinity, DIC, TA, Nut.
65	6/14	6	3	52.4050	-5.8217	Tp, Salinity, DIC, TA, Nut, S
66	6/14	8	10	52.1097	-5.8250	Tp, Salinity, DIC, TA, Nut.
67	6/14	10	1	51.8100	-5.9283	Tp, Salinity, DIC, TA, Nut.
68	6/14	12	5	51.4000	-6.0333	Tp, Salinity, DIC, TA, Nut, S
69	6/14	14	5	51.1767	-6.0550	Tp, Salinity, DIC, TA, Nut.
70	6/14	16	1	50.8433	-6.0367	Tp, Salinity, DIC, TA, Nut.
71	6/14	18	0	50.5110	-6.0248	Tp, Salinity, DIC, TA, Nut.
72	6/14	20	3	50.1160	-6.0168	Tp, Salinity, DIC, TA, Nut, S
73	6/14	22	4	49.9000	-5.7333	Tp, Salinity, DIC, TA, Nut.
74	6/15	0	14	49.8500	-5.1833	Tp, Salinity, DIC, TA, Nut, S
75	6/15	2	24	49.9550	-4.7083	Tp, Salinity, DIC, TA, Nut.
76	6/15	4	7	50.0267	-4.3617	Tp, Salinity, DIC, TA, Nut.
77	6/15	6	4	50.0400	-4.5517	Tp, Salinity, DIC, TA, Nut, S
78	6/15	8	7	50.0627	-4.9322	Tp, Salinity, DIC, TA, Nut.

UW	Date	Hour	Minute	Latitude	Longitude	Parameter sampled
79	6/15	10	1	50.0588	-4.9256	Tp, Salinity, DIC, TA, Nut.
80	6/15	12	1	50.0055	-5.0210	Tp, Salinity, DIC, TA, Nut, S
81	6/15	16	4	49.7998	-5.5963	Tp, Salinity, DIC, TA, Nut.
82	6/15	18	20	49.7678	-5.9190	Tp, Salinity, DIC, TA, Nut, S
83	6/15	20	6	49.7272	-6.1688	Tp, Salinity, DIC, TA, Nut.
84	6/16	22	5	49.7200	-6.4808	Tp, Salinity, DIC, TA, Nut.
85	6/16	0	7	49.7550	-6.7562	Tp, Salinity, DIC, TA, Nut, S
86	6/16	2	13	49.7733	-7.0142	Tp, Salinity, DIC, TA, Nut.
87	6/16	4	3	49.8172	-7.2685	Tp, Salinity, DIC, TA, Nut.
88	6/16	6	23	49.8553	-7.5817	Tp, Salinity, DIC, TA, Nut, S
89	6/16	8	1	49.8853	-7.7977	Tp, Salinity, DIC, TA, Nut.
90	6/16	10	12	49.8758	-7.9238	Tp, Salinity, DIC, TA, Nut.
91	6/16	12	1	49.7533	-8.0978	Tp, Salinity, DIC, TA, Nut, S
92	6/16	18	31	49.6053	-8.3033	Tp, Salinity, DIC, TA, Nut, S
93	6/16	21	10	49.5786	-8.4677	Tp, Salinity, DIC, TA, Nut.
94	6/17	0	11	49.5862	-8.6168	Tp, Salinity, DIC, TA, Nut, S
95	6/17	6	2	49.6655	-8.8147	Tp, Salinity, DIC, TA, Nut, S
96	6/17	10	9	49.0582	-9.1372	Tp, Salinity, DIC, TA, Nut.
97	6/17	11	56	48.7765	-9.3235	Tp, Salinity, DIC, TA, Nut.
98	6/17	14	11	48.5802	-9.4883	Tp, Salinity, DIC, TA, Nut, S
99	6/17	18	15	48.5792	-9.3737	Tp, Salinity, DIC, TA, Nut, S
100	6/17	20	2	48.5093	-9.1580	Tp, Salinity, DIC, TA, Nut.
101	6/17	22	12	48.4412	-8.8898	Tp, Salinity, DIC, TA, Nut.
102	6/18	0	8	48.4042	-8.6775	Tp, Salinity, DIC, TA, Nut, S
103	6/18	2	7	48.3637	-8.4497	Tp, Salinity, DIC, TA, Nut.
104	6/18	4	2	48.3158	-8.2178	Tp, Salinity, DIC, TA, Nut.
105	6/18	6	4	48.2522	-7.9160	Tp, Salinity, DIC, TA, Nut, S
106	6/18	8	0	48.2468	-7.9023	Tp, Salinity, DIC, TA, Nut.
107	6/18	9	58	48.2345	-7.9380	Tp, Salinity, DIC, TA, Nut.
108	6/18	12	0	47.9400	-7.8342	Tp, Salinity, DIC, TA, Nut, S
109	6/18	13	58	47.8722	-7.8063	Tp, Salinity, DIC, TA, Nut.
110	6/18	16	7	47.7308	-7.6735	Tp, Salinity, DIC, TA, Nut.
111	6/18	18	33	47.7502	-7.7500	Tp, Salinity, DIC, TA, Nut, S
112	6/18	20	15	47.5647	-7.5567	Tp, Salinity, DIC, TA, Nut.
113	6/18	22	3	47.3388	-7.4068	Tp, Salinity, DIC, TA, Nut.
114	6/19	0	7	47.0478	-7.3303	Tp, Salinity, DIC, TA, Nut, S
115	6/19	1	57	46.8018	-7.3128	Tp, Salinity, DIC, TA, Nut.
116	6/19	4	1	46.5168	-7.2063	Tp, Salinity, DIC, TA, Nut.
117	6/19	6	3	46.4053	-7.1988	Tp, Salinity, DIC, TA, Nut, S
118	6/19	8	7	46.0701	-7.1990	Tp, Salinity, DIC, TA, Nut.
119	6/19	10	0	45.7904	-7.2042	Tp, Salinity, DIC, TA, Nut.

UW	Date	Hour	Minute	Latitude	Longitude	Parameter sampled
120	6/19	12	0	45.5015	-7.1975	Tp, Salinity, DIC, TA, Nut, S
121	6/19	15	30	45.4770	-7.1710	Tp, Salinity, DIC, TA, Nut.
122	6/19	15	30	45.4770	-7.1710	Tp, Salinity, DIC, TA, Nut.
123	6/20	12	54	45.7048	-7.1807	Tp, Salinity, DIC, TA, Nut, S
124	6/20	14	25	45.8478	-7.1928	Tp, Salinity, DIC, TA, Nut.
125	6/20	16	7	45.9360	-7.2230	Tp, Salinity, DIC, TA, Nut.
126	6/21	0	14	46.0297	-7.2140	Tp, Salinity, DIC, TA, Nut, S
127	6/21	2	10	46.1978	-7.2235	Tp, Salinity, DIC, TA, Nut.
128	6/21	6	5	46.2644	-7.2576	Tp, Salinity, DIC, TA, Nut, S
129	6/21	7	57	46.4820	-7.3261	Tp, Salinity, DIC, TA, Nut, cocco counts
130	6/21	10	1	46.7055	-7.4277	Tp, Salinity, DIC, TA, Nut, cocco counts
131	6/21	11	57	46.9355	-7.5604	Tp, Salinity, DIC, TA, Nut, cocco counts, S
132	6/21	14	36	47.1238	-7.6351	Tp, Salinity, DIC, TA, Nut, cocco counts
133	6/21	16	0	47.2727	-7.6705	Tp, Salinity, DIC, TA, Nut, cocco counts
134	6/21	18	28	47.4965	-7.5793	Tp, Salinity, DIC, TA, Nut, cocco counts, S
135	6/21	20	4	47.6324	-7.4762	Tp, Salinity, DIC, TA, Nut, cocco counts
136	6/21	22	1	47.7650	-7.3670	Tp, Salinity, DIC, TA, Nut, cocco counts
137	6/21	23	13	47.8563	-7.2795	Tp, Salinity, DIC, TA, Nut, cocco counts
138	6/22	0	5	47.9330	-7.2330	Tp, Salinity, DIC, TA, Nut, cocco counts, S
139	6/22	2	3	48.0050	-7.1918	Tp, Salinity, DIC, TA, Nut, cocco counts
140	6/22	4	18	48.0020	-7.1887	Tp, Salinity, DIC, TA, Nut, cocco counts
141	6/22	6	2	48.0733	-7.0195	Tp, Salinity, DIC, TA, Nut, cocco counts, S
142	6/22	8	4	48.2683	-6.5201	Tp, Salinity, DIC, TA, Nut, cocco counts
143	6/22	10	8	48.4624	-6.0242	Tp, Salinity, DIC, TA, Nut, cocco counts
144	6/22	12	0	48.6812	-5.6864	Tp, Salinity, DIC, TA, Nut, cocco counts, S
145	6/22	13	56	48.8206	-5.2954	Tp, Salinity, DIC, TA, Nut, cocco counts
146	6/22	16	2	48.9118	-5.0443	Tp, Salinity, DIC, TA, Nut, cocco counts
147	6/22	18	7	49.2441	-4.9066	Tp, Salinity, DIC, TA, Nut, cocco counts, S
148	6/22	20	7	49.5540	-5.0532	Tp, Salinity, DIC, TA, Nut, cocco counts
149	6/22	22	4	49.7972	-5.1638	Tp, Salinity, DIC, TA, Nut, cocco counts
150	6/23	0	7	49.8273	-5.1798	Tp, Salinity, DIC, TA, Nut, cocco counts, S
151	6/23	10	8	49.8998	-5.1362	Tp, Salinity, DIC, TA, Nut, cocco counts, S
152	6/23	12	5	50.0595	-4.8433	Tp, Salinity, DIC, TA, Nut, cocco counts, S
153	6/23	16	6	50.0611	-4.5914	Tp, Salinity, DIC, TA, Nut, cocco counts
154	6/23	18	16	50.1027	-4.4327	Tp, Salinity, DIC, TA, Nut, cocco counts, S
155	6/24	0	1	50.0475	-4.4532	Tp, Salinity, DIC, TA, Nut, cocco counts, S
156	6/24	4	4	50.0268	-4.3585	Tp, Salinity, DIC, TA, Nut, cocco counts
157	6/24	6	10	50.0762	-4.0578	Tp, Salinity, DIC, TA, Nut, cocco counts, S
158	6/24	8	0	50.1817	-3.5899	Tp, Salinity, DIC, TA, Nut, cocco counts
159	6/24	10	4	50.3163	-3.0308	Tp, Salinity, DIC, TA, Nut, cocco counts

UW	Date	Hour	Minute	Latitude	Longitude	Parameter sampled
160	6/24	12	0	50.4408	-2.5023	Tp, Salinity, DIC, TA, Nut, cocco counts, S
161	6/24	13	53	50.4972	-2.2955	Tp, Salinity, DIC, TA, Nut, cocco counts
162	6/24	16	22	50.4884	-2.2913	Tp, Salinity, DIC, TA, Nut, cocco counts
163	6/24	19	0	50.5705	-2.2680	Tp, Salinity, DIC, TA, Nut, cocco counts, S
164	6/24	20	27	50.4502	-1.9802	Tp, Salinity, DIC, TA, Nut, cocco counts
165	6/24	22	13	50.3420	-1.5639	Tp, Salinity, DIC, TA, Nut, cocco counts
166	6/25	0	7	50.1870	-1.1717	Tp, Salinity, DIC, TA, Nut, cocco counts, S
167	6/25	2	4	50.2490	-0.6638	Tp, Salinity, DIC, TA, Nut, cocco counts
168	6/25	4	5	50.3283	-0.2235	Tp, Salinity, DIC, TA, Nut, cocco counts
169	6/25	5	59	50.4210	0.1500	Tp, Salinity, DIC, TA, Nut, cocco counts, S
170	6/25	8	15	50.4811	0.6116	Tp, Salinity, DIC, TA, Nut, cocco counts
171	6/25	10	6	50.5990	1.1078	Tp, Salinity, DIC, TA, Nut, cocco counts
172	6/25	12	1	50.8688	1.3865	Tp, Salinity, DIC, TA, Nut, cocco counts, S
173	6/25	13	10	51.0323	1.5618	Tp, Salinity, DIC, TA, Nut, cocco counts
174	6/25	14	4	51.1394	1.7072	Tp, Salinity, DIC, TA, Nut, cocco counts
175	6/25	14	4	51.1394	1.7072	Tp, Salinity, DIC, TA, Nut, cocco counts
176	6/25	16	4	51.4495	1.7712	Tp, Salinity, DIC, TA, Nut, cocco counts
177	6/25	20	22	52.0162	2.1865	Tp, Salinity, DIC, TA, Nut, cocco counts, S
178	6/26	22	22	52.4318	2.2709	Tp, Salinity, DIC, TA, Nut, cocco counts
179	6/26	0	3	52.7575	2.4433	Tp, Salinity, DIC, TA, Nut, cocco counts, S
180	6/26	6	3	53.0308	2.8410	Tp, Salinity, DIC, TA, Nut, cocco counts, S
181	6/26	8	5	53.1862	3.3715	Tp, Salinity, DIC, TA, Nut, cocco counts
182	6/26	9	59	53.4363	3.7044	Tp, Salinity, DIC, TA, Nut, cocco counts
183	6/26	12	1	53.6560	4.1976	Tp, Salinity, DIC, TA, Nut, cocco counts, S
184	6/26	14	13	53.8423	4.4850	Tp, Salinity, DIC, TA, Nut, cocco counts
185	6/26	15	58	54.0099	4.7842	Tp, Salinity, DIC, TA, Nut, cocco counts
186	6/26	18	5	54.0577	5.2792	Tp, Salinity, DIC, TA, Nut, cocco counts, S
187	6/26	20	6	54.0938	5.7210	Tp, Salinity, DIC, TA, Nut, cocco counts
188	6/26	22	4	54.1296	6.1704	Tp, Salinity, DIC, TA, Nut, cocco counts
189	6/27	0	4	54.1500	6.6362	Tp, Salinity, DIC, TA, Nut, cocco counts, S
190	6/27	2	13	54.1662	7.1575	Tp, Salinity, DIC, TA, Nut, cocco counts
191	6/27	4	3	54.2908	7.2850	Tp, Salinity, DIC, TA, Nut, cocco counts
192	6/27	5	58	54.3027	7.0721	Tp, Salinity, DIC, TA, Nut, cocco counts, S
193	6/27	7	58	54.2902	6.4487	Tp, Salinity, DIC, TA, Nut, cocco counts
194	6/27	10	9	54.2996	5.7231	Tp, Salinity, DIC, TA, Nut, cocco counts
195	6/27	14	0	54.5923	4.9202	Tp, Salinity, DIC, TA, Nut, cocco counts
196	6/27	16	0	54.9364	4.7376	Tp, Salinity, DIC, TA, Nut, cocco counts
197	6/27	18	11	55.3115	4.5286	Tp, Salinity, DIC, TA, Nut, cocco counts, S
198	6/27	20	8	55.6383	4.3491	Tp, Salinity, DIC, TA, Nut, cocco counts, S
199	6/27	22	6	55.9715	4.1710	Tp, Salinity, DIC, TA, Nut, cocco counts

UW	Date	Hour	Minute	Latitude	Longitude	Parameter sampled
200	6/28	0	28	56.3601	3.9621	Tp, Salinity, DIC, TA, Nut, cocco counts, S
201	6/28	2	12	56.6180	3.8208	Tp, Salinity, DIC, TA, Nut, cocco counts
202	6/28	4	0	56.8128	3.7140	Tp, Salinity, DIC, TA, Nut, cocco counts
203	6/28	6	4	57.0107	3.6018	Tp, Salinity, DIC, TA, Nut, cocco counts, S
204	6/28	7	58	57.2010	3.4900	Tp, Salinity, DIC, TA, Nut, cocco counts
205	6/28	8	57	57.2313	3.5537	Tp, Salinity, DIC, TA, Nut, cocco counts
206	6/28	10	6	57.3722	3.8208	Tp, Salinity, DIC, TA, Nut, cocco counts
207	6/28	11	2	57.4147	3.8900	Tp, Salinity, DIC, TA, Nut, cocco counts
208	6/28	12	30	57.4527	3.9472	Tp, Salinity, DIC, TA, Nut, cocco counts
209	6/28	13	0	57.5112	4.0752	Tp, Salinity, DIC, TA, Nut, cocco counts
210	6/28	14	5	57.5827	4.2000	Tp, Salinity, DIC, TA, Nut, cocco counts, S
211	6/28	15	0	57.6610	4.3497	Tp, Salinity, DIC, TA, Nut, cocco counts
212	6/28	16	8	57.7200	4.4633	Tp, Salinity, DIC, TA, Nut, cocco counts
213	6/28	17	1	57.7711	4.6431	Tp, Salinity, DIC, TA, Nut, cocco counts
214	6/28	18	0	57.8642	4.7467	Tp, Salinity, DIC, TA, Nut, cocco counts
215	6/28	18	59	57.9300	4.8720	Tp, Salinity, DIC, TA, Nut, cocco counts
216	6/28	20	0	57.9840	4.9780	Tp, Salinity, DIC, TA, Nut, cocco counts, S
217	6/28	21	6	57.9293	4.8845	Tp, Salinity, DIC, TA, Nut, cocco counts
218	6/28	22	25	57.8398	4.7120	Tp, Salinity, DIC, TA, Nut, cocco counts
219	6/29	0	8	57.7143	4.4718	Tp, Salinity, DIC, TA, Nut, cocco counts, S
220	6/29	2	10	57.6740	4.3878	Tp, Salinity, DIC, TA, Nut, cocco counts
221	6/29	3	40	57.7643	4.5877	Tp, Salinity, DIC, TA, Nut, cocco counts
222	6/29	6	4	57.4308	4.7703	Tp, Salinity, DIC, TA, Nut, cocco counts, S
223	6/29	8	0	57.0865	4.9528	Tp, Salinity, DIC, TA, Nut, cocco counts
224	6/29	9	6	57.0015	5.0030	Tp, Salinity, DIC, TA, Nut, cocco counts
225	6/29	10	0	57.1005	5.1263	Tp, Salinity, DIC, TA, Nut, cocco counts
226	6/29	11	0	57.1793	5.2202	Tp, Salinity, DIC, TA, Nut, cocco counts
227	6/29	12	0	57.3098	5.3653	Tp, Salinity, DIC, TA, Nut, cocco counts, S
228	6/29	12	58	57.3360	5.3977	Tp, Salinity, DIC, TA, Nut, cocco counts
229	6/29	13	59	57.4598	5.5475	Tp, Salinity, DIC, TA, Nut, cocco counts
230	6/29	15	0	57.5165	5.6170	Tp, Salinity, DIC, TA, Nut, cocco counts
231	6/29	16	2	57.5800	5.6797	Tp, Salinity, DIC, TA, Nut, cocco counts
232	6/29	17	5	57.7057	5.8570	Tp, Salinity, DIC, TA, Nut, cocco counts
233	6/29	18	4	57.7683	5.9493	Tp, Salinity, DIC, TA, Nut, cocco counts
234	6/29	19	2	57.7883	6.1300	Tp, Salinity, DIC, TA, Nut, cocco counts, S
235	6/29	21	5	57.8088	6.8203	Tp, Salinity, DIC, TA, Nut, cocco counts
236	6/29	22	59	57.8323	7.3927	Tp, Salinity, DIC, TA, Nut, cocco counts, S
237	6/30	1	4	57.8525	8.0500	Tp, Salinity, DIC, TA, Nut, cocco counts
238	6/30	3	1	58.0338	8.5898	Tp, Salinity, DIC, TA, Nut, cocco counts
239	6/30	4	58	58.2175	9.1345	Tp, Salinity, DIC, TA, Nut, cocco counts, S

UW	Date	Hour	Minute	Latitude	Longitude	Parameter sampled
240	6/30	7	14	58.4443	9.8338	Tp, Salinity, DIC, TA, Nut, cocco counts
241	6/30	10	57	58.3037	9.4797	Tp, Salinity, DIC, TA, Nut, cocco counts, S
242	6/30	13	18	58.1530	9.0580	Tp, Salinity, DIC, TA, Nut, cocco counts
243	6/30	14	59	57.9673	8.7222	Tp, Salinity, DIC, TA, Nut, cocco counts
244	6/30	17	15	57.7965	8.1232	Tp, Salinity, DIC, TA, Nut, cocco counts, S
245	6/30	19	8	57.5882	7.6918	Tp, Salinity, DIC, TA, Nut, cocco counts
246	6/30	21	10	57.3368	7.2574	Tp, Salinity, DIC, TA, Nut, cocco counts
247	6/30	23	9	57.1258	6.9360	Tp, Salinity, DIC, TA, Nut, cocco counts, S
248	7/1	1	21	56.8762	6.5628	Tp, Salinity, DIC, TA, Nut, cocco counts
249	7/1	3	4	56.8668	6.4698	Tp, Salinity, DIC, TA, Nut, cocco counts
250	7/1	5	8	56.8873	6.4098	Tp, Salinity, DIC, TA, Nut, cocco counts, S
251	7/1	6	57	56.9093	6.3417	Tp, Salinity, DIC, TA, Nut, cocco counts
252	7/1	9	2	56.9152	6.0458	Tp, Salinity, DIC, TA, Nut, cocco counts
253	7/1	11	16	56.9002	5.6834	Tp, Salinity, DIC, TA, Nut, cocco counts, S
254	7/1	13	5	56.7958	5.3617	Tp, Salinity, DIC, TA, Nut, cocco counts
255	7/1	14	58	56.7088	5.0048	Tp, Salinity, DIC, TA, Nut, cocco counts
256	7/1	17	6	56.6275	4.5882	Tp, Salinity, DIC, TA, Nut, cocco counts, S
257	7/1	19	4	56.5702	4.2078	Tp, Salinity, DIC, TA, Nut, cocco counts
258	7/1	21	2	56.5168	3.8077	Tp, Salinity, DIC, TA, Nut, cocco counts
259	7/1	22	58	56.5075	3.6505	Tp, Salinity, DIC, TA, Nut, cocco counts, S
260	7/2	1	5	56.5035	3.6533	Tp, Salinity, DIC, TA, Nut, cocco counts
261	7/2	3	3	56.4930	3.6065	Tp, Salinity, DIC, TA, Nut, cocco counts
262	7/2	5	0	56.6374	3.6892	Tp, Salinity, DIC, TA, Nut, cocco counts, S
263	7/2	7	14	56.8812	3.8502	Tp, Salinity, DIC, TA, Nut, cocco counts
264	7/2	9	0	57.0591	3.9464	Tp, Salinity, DIC, TA, Nut, cocco counts
265	7/2	11	0	57.2420	4.0312	Tp, Salinity, DIC, TA, Nut, cocco counts, S
266	7/2	13	4	57.4094	4.1202	Tp, Salinity, DIC, TA, Nut, cocco counts
267	7/2	15	9	57.6944	4.2384	Tp, Salinity, DIC, TA, Nut, cocco counts
268	7/2	17	11	57.9826	4.2299	Tp, Salinity, DIC, TA, Nut, cocco counts, S
269	7/2	19	5	58.2664	4.2276	Tp, Salinity, DIC, TA, Nut, cocco counts
270	7/2	21	9	58.6108	4.2174	Tp, Salinity, DIC, TA, Nut, cocco counts
271	7/2	23	1	58.9327	4.2037	Tp, Salinity, DIC, TA, Nut, cocco counts, S
272	7/3	1	8	59.2957	4.1918	Tp, Salinity, DIC, TA, Nut, cocco counts
273	7/3	2	59	59.6210	4.1362	Tp, Salinity, DIC, TA, Nut, cocco counts
274	7/3	5	6	59.8090	4.1095	Tp, Salinity, DIC, TA, Nut, cocco counts, S
275	7/3	7	1	59.9975	3.7652	Tp, Salinity, DIC, TA, Nut, cocco counts
276	7/3	9	3	59.9963	3.0502	Tp, Salinity, DIC, TA, Nut, cocco counts
277	7/3	9	57	59.9458	2.8108	Tp, Salinity, DIC, TA, Nut, cocco counts
278	7/3	11	29	59.9795	2.5170	Tp, Salinity, DIC, TA, Nut, cocco counts, S
279	7/3	13	0	59.9703	2.4937	Tp, Salinity, DIC, TA, Nut, cocco counts

UW	Date	Hour	Minute	Latitude	Longitude	Parameter sampled
280	7/3	14	58	59.9583	1.8561	Tp, Salinity, DIC, TA, Nut, cocco counts
281	7/3	17	11	59.9205	1.1170	Tp, Salinity, DIC, TA, Nut, cocco counts, S
282	7/3	19	25	59.8638	0.4065	Tp, Salinity, DIC, TA, Nut, cocco counts
283	7/3	21	12	59.8265	-0.1705	Tp, Salinity, DIC, TA, Nut, cocco counts
284	7/3	23	0	59.7962	-0.6200	Tp, Salinity, DIC, TA, Nut, cocco counts, S
285	7/4	1	1	59.7708	-0.9828	Tp, Salinity, DIC, TA, Nut, cocco counts
286	7/4	3	2	59.7515	-1.3398	Tp, Salinity, DIC, TA, Nut, cocco counts
287	7/4	5	0	59.8750	-1.6672	Tp, Salinity, DIC, TA, Nut, cocco counts, S
288	7/4	6	58	59.9215	-1.7650	Tp, Salinity, DIC, TA, Nut, cocco counts
289	7/4	9	18	59.9858	-1.9427	Tp, Salinity, DIC, TA, Nut, cocco counts
290	7/4	11	3	59.9987	-2.2893	Tp, Salinity, DIC, TA, Nut, cocco counts, S
291	7/4	13	1	59.9993	-2.6617	Tp, Salinity, DIC, TA, Nut, cocco counts
292	7/4	15	0	60.0005	-3.0410	Tp, Salinity, DIC, TA, Nut, cocco counts
293	7/4	17	13	60.0012	-3.6068	DIC, TA, Nut, cocco counts, S
294	7/4	19	5	59.9992	-4.0522	Tp, Salinity, DIC, TA, Nut, cocco counts
295	7/4	21	9	60.0015	-4.5300	Tp, Salinity, DIC, TA, Nut, cocco counts
296	7/4	23	2	60.0002	-4.9952	Tp, Salinity, DIC, TA, Nut, cocco counts, S
297	7/5	1	13	60.0028	-5.5627	Tp, Salinity, DIC, TA, Nut, cocco counts
298	7/5	5	24	59.9305	-6.1659	Tp, Salinity, DIC, TA, Nut, cocco counts, S
299	7/5	7	1	59.8561	-6.4078	Tp, Salinity, DIC, TA, Nut, cocco counts
300	7/5	9	16	59.7473	-6.7598	Tp, Salinity, DIC, TA, Nut, cocco counts
301	7/5	11	7	59.6650	-6.9877	Tp, Salinity, DIC, TA, Nut, cocco counts, S
302	7/5	13	0	59.5352	-7.4325	Tp, Salinity, DIC, TA, Nut, cocco counts
303	7/5	15	3	59.4262	-7.7945	Tp, Salinity, DIC, TA, Nut, cocco counts
304	7/5	17	13	59.2795	-8.2787	Tp, Salinity, DIC, TA, Nut, cocco counts, S
305	7/5	19	8	59.1250	-8.7859	Tp, Salinity, DIC, TA, Nut, cocco counts
306	7/5	21	3	58.9832	-9.2555	Tp, Salinity, DIC, TA, Nut, cocco counts
307	7/5	23	7	58.8510	-9.6831	Tp, Salinity, DIC, TA, Nut, cocco counts, S
308	7/6	1	1	58.7255	-10.0957	Tp, Salinity, DIC, TA, Nut, cocco counts
309	7/6	3	8	58.6155	-10.4467	Tp, Salinity, DIC, TA, Nut, cocco counts
310	7/6	5	16	58.6068	-10.5037	Tp, Salinity, DIC, TA, Nut, cocco counts, S
311	7/6	7	13	58.4443	-10.5922	Tp, Salinity, DIC, TA, Nut, cocco counts
312	7/6	9	0	58.1714	-10.7500	Tp, Salinity, DIC, TA, Nut, cocco counts
313	7/6	11	4	57.8418	-10.9386	Tp, Salinity, DIC, TA, Nut, cocco counts, S
314	7/6	13	5	57.5213	-11.1147	Tp, Salinity, DIC, TA, Nut, cocco counts
315	7/6	15	4	57.4338	-11.0948	Tp, Salinity, DIC, TA, Nut, cocco counts
316	7/6	17	30	57.2995	-10.3902	Tp, Salinity, DIC, TA, Nut, cocco counts, S
317	7/6	19	21	57.1953	-9.8803	Tp, Salinity, DIC, TA, Nut, cocco counts
318	7/6	21	13	57.0937	-9.3762	Tp, Salinity, DIC, TA, Nut, cocco counts
319	7/6	23	9	56.9858	-8.8438	Tp, Salinity, DIC, TA, Nut, cocco counts, S

320	7/7	1	5	56.8780	-8.3115	Tp, Salinity, DIC, TA, Nut, cocco counts
321	7/8	3	10	56.7657	-7.7522	Tp, Salinity, DIC, TA, Nut, cocco counts
322	7/9	5	2	56.8188	-7.3937	Tp, Salinity, DIC, TA, Nut, cocco counts, S
323	7/10	7	10	56.8257	-7.3917	Tp, Salinity, DIC, TA, Nut, cocco counts

The abbreviations of the parameters sampled are the following:

Tp:temperature; Nut: nutrients; Cocco counts: coccoliths count and S: salinity bottle.

Appendix D: Activity log *Lophelia pertusa*

DATE	TIME	POS N	POS W	DEPTH	ACTIVITY	SAMPLE NO	
08/06/2011	0703	56'49.2837	7'23.0592	146	GRAB IN WATER		
08/06/2011	0722				ON BOTTOM, CANT RECORD ON MINI DVI		
08/06/2011	0725	56'49.2837	7'23.0592	146	GRAB FIRED	HWU20110608/	001A
08/06/2011	0743	56'49.2974	7'23.5633	153	GRAB IN WATER		
08/06/2011	0759				TAPE START		
08/06/2011	0851	56'49.3156	7'23.4738	163	GRAB FAILED TO FIRE. RETURN TO DECK. UMBILICAL & GRAB SNAGGED ON LOST LOBSTER POT AND CLUMP WEIGHT CHAIN. CAMERA FEED LOST. UMBILICAL DAMAGED JUST BEFORE TERMINATION. FRAME ALSO BENT BUT STILL FUNCTIONAL. SWAPPED TO SPARE UMBILICAL		
08/06/2011							
08/06/2011	1043	56'49.2029	7'23.3559	137	GRAB IN		
08/06/2011	1101	56'49.1739	7'23.0269	117	VIDEO ON		
08/06/2011	1133	56'49.0894	7'23.1874	147	GRAB TRIGGERED ON LIVE CORAL	HWU20110608/	002A
08/06/2011	1134				VIDEO OFF		
08/06/2011	1216	56'49.0516	7'23.0130	161	GRAB IN		
08/06/2011	1238	56'49.0023	7'23.3440	121	RECORD		
08/06/2011	1246	56'49.0777	7'24.2191	160	BRINGING GRAB UP OFF THE REEF AND TOO FAST.		

					VIDEO OFF		
08/06/2011	1325	56'49.0035	7'23.	137	CTD IN WATER, STATION 7		
08/06/2011	1339	56'49.0006	7'23.0149	211	CTD ON BOTTOM		
08/06/2011					WATER SAMPLES FOR CARBONATE CHEM & NUTRIENTS 130-140M		
08/06/2011	1429	56'49.4026	7'23.4521	184	GRAB IN		
08/06/2011	1447	56'49.1717	7'23.5569	131	GRAB FIRED- LARGE FRAMEWORK AND BENTHIC FAUNA	HWU20110608/	003A
08/06/2011	1520	56'49.3561	7'24.4885	167	GRAB IN		
08/06/2011	1539	56'49.2475	7'23.5661	130	GRAB AT BOTTOM		
08/06/2011	1554	56'49.1452	7'23.0315	167	GRAB FIRED	HWU20110608/	004A
08/06/2011	1628	56'49.3521	7'23.3951	174	GRAB IN		
08/06/2011	1648	56'49.2558	7'23.4156	143	GRAB AT BOTTOM, VIDEO ON		
08/06/2011	1726	56'49.1004	7'24.0535	185	GRABBED RUBBLE	HWU20110608/	005A
08/06/2011	1756	56'49.0444	7'23.5701	186	GRAB IN		
08/06/2011	1813	56'49.3376	7'23.0055	167	GRAB AT BOTTOM		
08/06/2011	1814	56'49.2206	7'23.0064	164	GRABBED	HWU20110608/	006A
08/06/2011	1838	56'49.2195	7'24.0566	123	GRAB IN		
08/06/2011	1847	56'49.0185	7'24.0079	124	BOTTOM REACHED, VIDEO ON		
08/06/2011	1858	56'49.1575	7'23.0846	124	GRABBED	HWU20110608/	007A
08/06/2011	2018	56'49.4098	7'23.5701	186	GRAB IN		
08/06/2011	2038	56'49.3631	7'23.5830	174	GRAB AT BOTTOM		
08/06/2011	2045	56'49.3383	7'23.5871	164	GRABBED	HWU20110608/	008A
08/06/2011	2125	56'49.3201	7'23.4659	165	BOTTOM		

08/06/2011	2146	56'49.0305	7'23.4774	162	GRAB FIRED	HWU20110608/	009A
08/06/2011	2200	56'49.2927	7'23.4741	154	GRAB IN		
08/06/2011	2213	56'49.2853	7'23.4840	158	BOTTOM		
08/06/2011	2230	56'49.2688	7'23.4915	153	GRABBED	HWU20110608/	010A
08/06/2011	2242	56'49.2688	7'23.4960	154	GRAB IN		
08/06/2011	2244	56'49.2418	7'23.5423	142	BOTTOM		
08/06/2011	2254	56'49.0206	7'23.5750	150	GRAB FIRED, VIDEO OFF	HWU20110608/	011A
08/06/2011	2320	56'49.3651	7'23.4769	173	GRAB IN		
08/06/2011	2335	56'49.3262	7'23.5170	162	GRABBED	HWU20110608/	012A
08/06/2011	2348	56'49.2883	7'23.5673	151	GRAB IN		
09/06/2011	0002	56'49.2577	7'24.0118	143	BOTTOM		
09/06/2011	0019	56'49.0149	7'24.0611	142	GRAB FIRED, VIDEO STOPPED	HWU20110609/	001A
09/06/2011	0058	56'49.3372	7'23.5677	164	GRAB IN		
09/06/2011	0113			129	GRAB AT BOTTOM		
09/06/2011	0141	56'48.5892	7'24.3566	162	GRAB TAKEN, TARGET DEAD CORAL. VIDEO STOP. SAMPLE DEAD CORAL ROBBLE AND SEDIMENT	HWU20110609/	002A
					CTD THEN END OPS		
07/07/11	0606	56'49.13659	7'23.60029	141	GRAB IN		
	0617	56'49.226747	7'23.57403	121	GRAB AT BOTTOM – VIDEO RECORDING		
	0645				GRAB UP AS DRIFTED OFF REEF		
	0703	56'49.14662	7'24.11528	198	GRAB IN		
	0723	56'49.26689	7'23.89681	166	GRAB AT BOTTOM – VIDEO RECORDING	HWU20110707/	001A

	0735	56'49.32597	7'23.855	143	GRAB FIRED – COMMENCE RETRIEVAL		
	0740	56'49.36155	7'23.68250	135	GRAB IN		
	0758	56'49.43689	7'23.60843	141	GRAB AT BOTTOM – VIDEO RECORDING		
	0803	56'49.48	7'23.55	151	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	002A
	0832	56'49.23552	7'23.88646	173	GRAB IN		
	0841	56'49.28153	7'23.81950	139	GRAB AT BOTTOM – VIDEO RECORDING		
	0918	56'49.5690	7'23.47534	169	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	003A
					CTD		
	1046	56'49.21157	7'24.16606	138	GRAB IN		
	1100	56'49.25565	7'24.07184	145	GRAB AT BOTTOM – VIDEO RECORDING		
	1107	56'49.32090	7'24.03946	135	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	004A
	1117	56'49.35124	7'24.07667	134	GRAB IN		
	1128	56'49.38199	7'24.08317	138	GRAB AT BOTTOM – VIDEO RECORDING		
	1206				MISFIRED		
	1227	56'49.36300	7'24.05006	132	GRAB IN		
	1236	56'49.31640	7'24.08300	141	GRAB AT BOTTOM – VIDEO RECORDING		
	1246	56'49.26221	7'24.17279	121	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	005A
	1310	56'49.53928	7'23.63011	169	GRAB IN		
	1324	56'49.47374	7'23.76076	161	GRAB AT BOTTOM – VIDEO RECORDING		
	1330	56'49.44600	7'23.04354	154	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	006A
	1350	56'49.49226	7'23.75842	157	GRAB IN		
	1402	56'49.47222	7'23.62453	158	GRAB AT BOTTOM – VIDEO RECORDING		
	1422	56'49.30894	7'23.90319	133	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	007A

	1440	56'49.53300	7'23.62556	167	GRAB IN		
	1452	56'49.49061	7'23.76903	157	GRAB AT BOTTOM – VIDEO RECORDING		
	1500	56'49.41960	7'23.92758	134	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	008A
	1520	56'49.53501	7'23.62703	167	GRAB IN		
	1532	56'49.48477	7'23.74572	160	GRAB AT BOTTOM – VIDEO RECORDING		
	1550				MISFIRED – COMMENCE RETRIEVAL		
	1600	56'49. 43641	7'23.64624	147	GRAB IN		
	1619	56'49. 42260	7'23.65886	138	GRAB AT BOTTOM – VIDEO RECORDING		
	1621				MISFIRED – COMMENCE RETRIEVAL		
	1644	56'49. 45705	7'23.73231	145	GRAB IN		
	1655	56'49. 40703	7'23.02991	150	GRAB AT BOTTOM – VIDEO RECORDING		
	1730	56'49. 30231	7'23.30500	124	RELOCATING – NO CORAL SEEN		
	1801	56'49. 53582	7'23.55864	164	GRAB IN		
	1809	56'49. 51748	7'23.58455	160	GRAB AT BOTTOM – VIDEO RECORDING		
	1814				MISFIRED – COMMENCE RETRIEVAL		
	1825	56'49. 47536	7'23.69065	153	GRAB IN		
	1834	56'49. 48452	7'23.70291	156	GRAB AT BOTTOM – VIDEO RECORDING		
	1841	56'49. 50333	7'23.60017	159	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	009A
	1915	56'49. 47420	7'23.74703	154	GRAB AT BOTTOM – VIDEO RECORDING		
	1933	56'49. 40442	7'23.66576	154	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110707/	010A
	1944	56'49. 53561	7'23.62125	164	GRAB IN		
					RELOCATING – GRAB RETRIEVED		
	2036	56'49. 48163	7'23.82146	154	GRAB IN		

	2047	56'49. 45888	7'23.71172	144	GRAB AT BOTTOM – VIDEO RECORDING		
	2127				RELOCATING – GRAB RETRIEVED		
	2207	56'49. 34300	7'23.04722	141	GRAB AT BOTTOM – VIDEO RECORDING		
	2213	56'49. 36534	7'23.80876	137	GRAB FIRED – COMMENCE RETRIEVAL		
	0303	56’49.43982	7’24.00639	135	GRAB IN		
	0312	56’49.36810	7’24.10404	130	GRAB AT BOTTOM – VIDEO RECORDING		
	0352				RELOCATING AS OFF REEF		
	0424	56’49.53296	7’24.06907	155	GRAB AT BOTTOM – VIDEO RECORDING		
	0426	56’49.51362	7’24.12498	150	GRAB MISFIRED – COMMENCE RETRIEVAL – MUD		
	0502	56’49.41039	7’23.73959	134	GRAB AT BOTTOM – VIDEO RECORDING		
	0504	56’49.38752	7’23.77524	141	RELOCATING AS OFF REEF		
	0539	56’49.44277	7’23.83044	148	GRAB AT BOTTOM – VIDEO RECORDING		
	0540	56’49.43733	7’23.88205	147	RELOCATING AS OFF REEF		
	0612	56’49.52433	7’23.62226	161	GRAB AT BOTTOM – VIDEO RECORDING		
	0619	56’49.52698	7’23.67379	164	GRAB FIRED – COMMENCE RETRIEVAL – EMPTY AS WINCH LINE CAUGHT IN GRAB		
	0645	56’49.50248	7’23.56275	156	GRAB AT BOTTOM – VIDEO RECORDING		
	0710				RELOCATING AS OFF REEF		
	0737	56’49.25510	7’23.00510	177	GRAB AT BOTTOM – VIDEO RECORDING		
	0758	56’49.35327	7’23.92816	142	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110708/	001A
	0834	56’49.31573	7’23.90793	157	GRAB AT BOTTOM – VIDEO RECORDING		
	0836	56’49.33806	7’23.91043	152	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110708/	002A
	0900	56’49.53844	7’23.75411	175	GRAB AT BOTTOM – VIDEO RECORDING		

	0907				RELOCATING AS OFF REEF		
	0944	56’49.28474	7’24.03761	158	GRAB IN		
	0947	56’49.30744	7’24.01591	147	GRAB FIRED – COMMENCE RETRIEVAL	HWU20110708/	003A
	1005				END OPS		