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RRS *Discovery* Cruise 369

09 AUG -15 SEP 2011

Influence of advection and sedimentation on linking microbial phosphorus, carbon and nitrogen cycling in the North Atlantic subtropical gyre (LINK)

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ABSTRACT

This physics-led biogeochemical cruise to the North Atlantic subtropical gyre was designed to field test a prevailing hypothesis that open ocean ecosystems are in steady state. We aimed to study microbial community composition, spatial distribution and functioning in the photic and twilight zones, and to put this information into context with physical and chemical characterisation of the sampling region, with additional information on carbon export from the euphotic zone.

Underway sampling followed by flow cytometry was used to assess mesoscale spatial variability of microorganisms. Coarse-scale (CTD) and fine-scale (PumpCast profiler) vertical distribution of microorganisms was determined, with dominant microbial prokaryotic and eukaryotic groups quantified by flow cytometry. Abundance of larger microplankton organisms was assessed using the size-fractionating net (Micronet) and FlowCam microscope. Group-specific uptake of bicarbonate, phosphate and different nitrogen compounds was determined and group-specific production and grazing assessed using flow sorting. The ambient turnover rates of phosphate, organic phosphorus and labile dissolved organic matter, e.g. amino acids, was bioassayed. Microbial respiration in the twilight zone was studied using radioactive tracing in a deep-water *in situ* incubator.

Physical and chemical parameters were measured during the cruise in order to put microbial community data into context. A ship mounted ADCP was used to collect information about physical, mesoscale spatial context; by combining with turbulence profiler data, SeaSoar profiler data, and data collected during intensive CTD sampling for dissolved inorganic nutrients, we aim to estimate mesoscale nutrient fluxes into the photic zone. PELAGRA neutral buoyancy sediment traps were used to estimate biogenic sedimentation. Samples were collected for particulate calcite, opal, and POM measurements, together with the isotopic composition of PON.

The cruise achieved its main goal of collecting data that will allow us to link the functioning of oligotrophic oceanic microbial communities with their fluidic environment, in particular with the propagation of eddies through strongly stratified photic and twilight waters. Close collaboration between physicists, biologists and chemists ensured cohesion in sampling efforts resulting in a rare collection of multidisciplinary scientific evidence. Further analyses of collected samples and data in conjunction with computer modelling have a strong synthesis potential to qualitatively advance our knowledge of the pelagic ecosystem biogeochemistry in subtropical oceanic gyres – the most extensive regions on Earth (>40% of Earth's surface) of global climatic importance.

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NOCS	National Oceanography Centre, Southampton
MPI	Max Planck Institute for Marine Microbiology, Bremen
PML	Plymouth Marine Laboratory
NMFSS	National Marine Facilities - Sea Systems

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3	Nigel Garnett	2nd Officer
4	Sam Cook	3rd Officer
5	Edward McLaughlin	Chief Engineer
6	Gordon Gray	2nd Engineer
7	John Harnett	3rd Engineer
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12	Martin Harrison	CPO(S)
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14	Steve Duncan	CPO(D)
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16	Emlyn Williams	Motorman.1A
17	Peter Lynch	Head Chef
18	Dean Hope	Chef
19	Jacqueline Waterhouse	Steward

1. Overview

Itinerary

Sailed Santa Cruz de Tenerife	14:40 GMT on 9th August 2011
Began first survey	08:06 GMT on 15th August 2011
End of science	07:25 GMT on 12th September 2011
Departed survey region	07:25 GMT on 12th September 2011
Arrived Santa Cruz de Tenerife	18:00 GMT on 15th September 2011

Objectives

The primary objective of this physics-led biogeochemical cruise to the North Atlantic subtropical gyre (Figure 1) was to study microbial community composition, spatial distribution and functioning in the photic and twilight zones, and to put this information into context with physical and chemical characterisation of the sampling region, with additional information on carbon export from the euphotic zone.

Underway automatic sampling followed by flow cytometry was used for assessing mesoscale spatial variability of microorganisms. Coarse-scale (CTD) and fine-scale (PumpCast profiler) vertical distribution of microorganisms was determined, with dominant microbial prokaryotic and eukaryotic groups quantified by flow cytometry. Samples were collected and processed for subsequent molecular identification of flow cytometrically sorted dominant microbes. Abundance of larger microplankton organisms was assessed using the size-fractionating net (Micronet) and FlowCam microscope. Microbial respiration in the twilight zone was studied using radioactive tracing in a deep-water *in situ* incubator. Group-specific uptake of bicarbonate, phosphate and different nitrogen compounds was determined and group-specific production and grazing assessed using flow sorting. The ambient turnover rates of phosphate, organic phosphorus and labile dissolved organic matter, e.g. amino acids, was bioassayed.

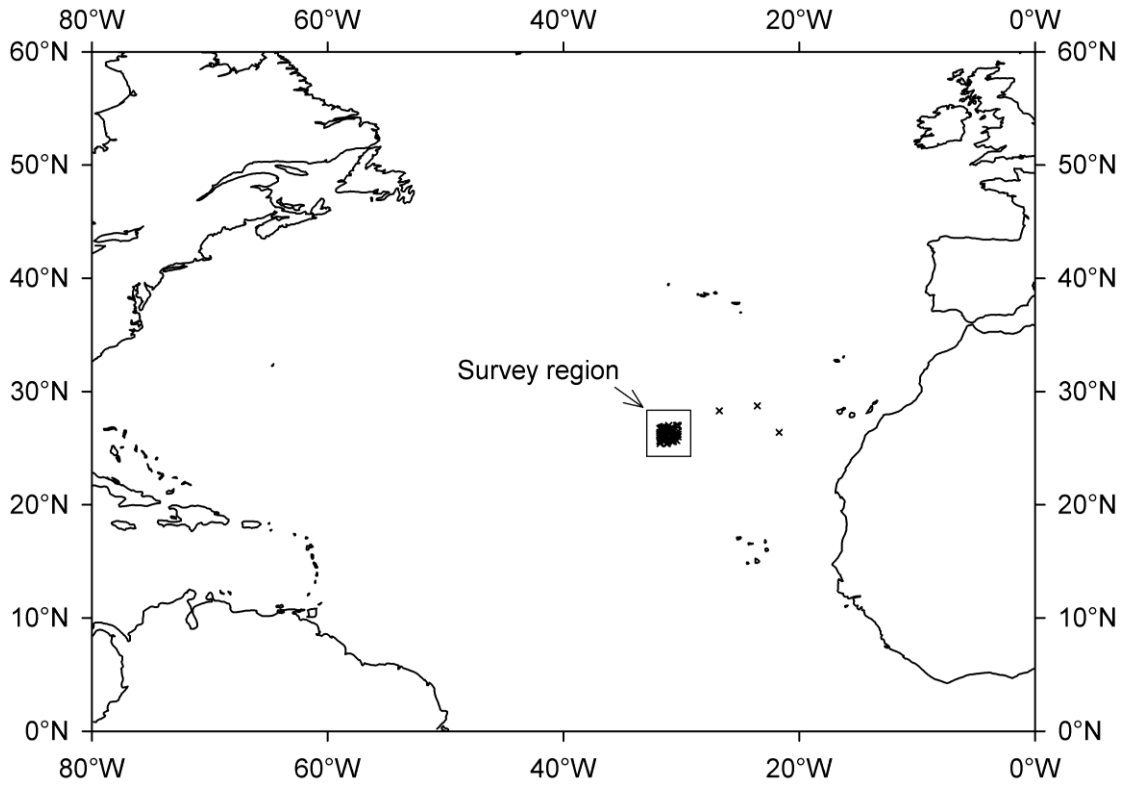

Figure 1: Study region with CTD stations marked (crosses). See the following six figures for higher resolution maps of the study site with CTD stations.

In order to put the microbial community data into context, physical and chemical parameters were measured during the cruise. A ship mounted ADCP was used to collect information about physical, mesoscale spatial context and, when combined with turbulence profiler data, SeaSoar profiler data and data collected using intensive CTD sampling for dissolved inorganic nutrients, may estimate mesoscale nutrient fluxes into the photic zone. PELAGRA neutral buoyancy sediment traps were used to estimate biogenic sedimentation. Samples were collected for particulate calcite, opal, and POM measurements, together with the isotopic composition of PON.

Narrative

RRS *Discovery* left Santa Cruz de Tenerife at 15:40 on the 9th of August 2011 and headed westwards towards the study site for six days, testing the equipment en route: stainless steel frame CTD (11th August) and titanium frame CTD (13th August), Seasoar (11th, 12th & 13th August) , PumpCast (13th August), Micronet (13th August) and turbulence profiler (14th August).

The cruise was divided into six mesoscale (~100 x 100 miles) surveys, which were carried out using alternately SeaSoar and CTD instruments. The aim of the surveys was to map physical features (e.g. eddoes) in the region, to provide context for the biological and export studies. During SeaSoar surveys, the SeaSoar was retrieved twice per day (early morning and pre-dusk) whilst CTD casts were done for sample collection. The survey lay within the region 25.4 - 27°N; 30.2 – 31.8°W (Figure 1). Deployment and recovery of the PELAGRA neutral buoyancy sediment traps and other non-routine equipment was scheduled for 'odds & ends' days in between the six surveys. In the first instance, three PELAGRA traps were deployed at 10:43 on 14th August before the initial survey.

The first, latitudinal, mesoscale survey was accompanied by the SeaSoar (Figure 2), and commenced at 08:06 on 15th August at the south-east corner of the survey area. The survey was interrupted at 14:03 the same day to locate and retrieve an early-surfacing PELAGRA trap. The survey continued until 20:23 on 18th August, with daily early morning (~05:30-07:30) and pre-dusk (~19:30-21:30) breaks for stainless steel CTD deployments. Three PELAGRA traps were deployed on the night of the 18th August, before recovering the remaining two PELAGRA traps from the first deployment in the early morning of the 19th August.

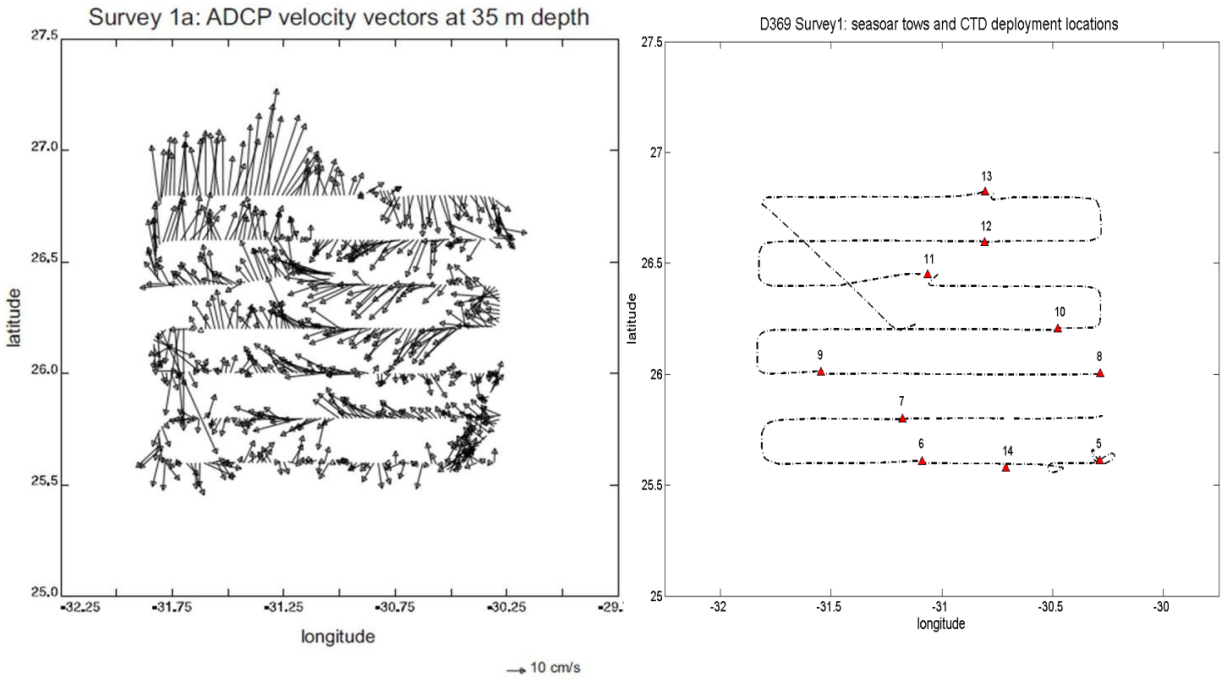

Figure 2: First mesoscale survey using SeaSoar. ADCP velocity vectors at 35 m depth (left) and SeaSoar path (dashed line) with CTD stations (triangles, right).

The 19th of August was the first of our odds & ends days, beginning with a standard CTD deployment (06:00), unsuccessful PumpCast deployment (07:00), Micronet (10:00), Deep Sea Incubator deployment to 1000 m (13:00), successful PumpCast deployment (15:25) and further CTD

deployment (17:00). We then returned to the PELAGRA trap deployment site to recover two early risers, before steaming to the beginning of the second survey area.

The second mesoscale survey covered longitudinal transects with an intensive CTD grid (Figure 3); this began at 05:53 on the 20th August in the south-east corner of the survey region. CTD casts were done every 2-4 hours along the survey grid until the survey ended short in the early morning of the 24th of August, at which point we deviated from the grid to recover a PELAGRA trap that had resurfaced.

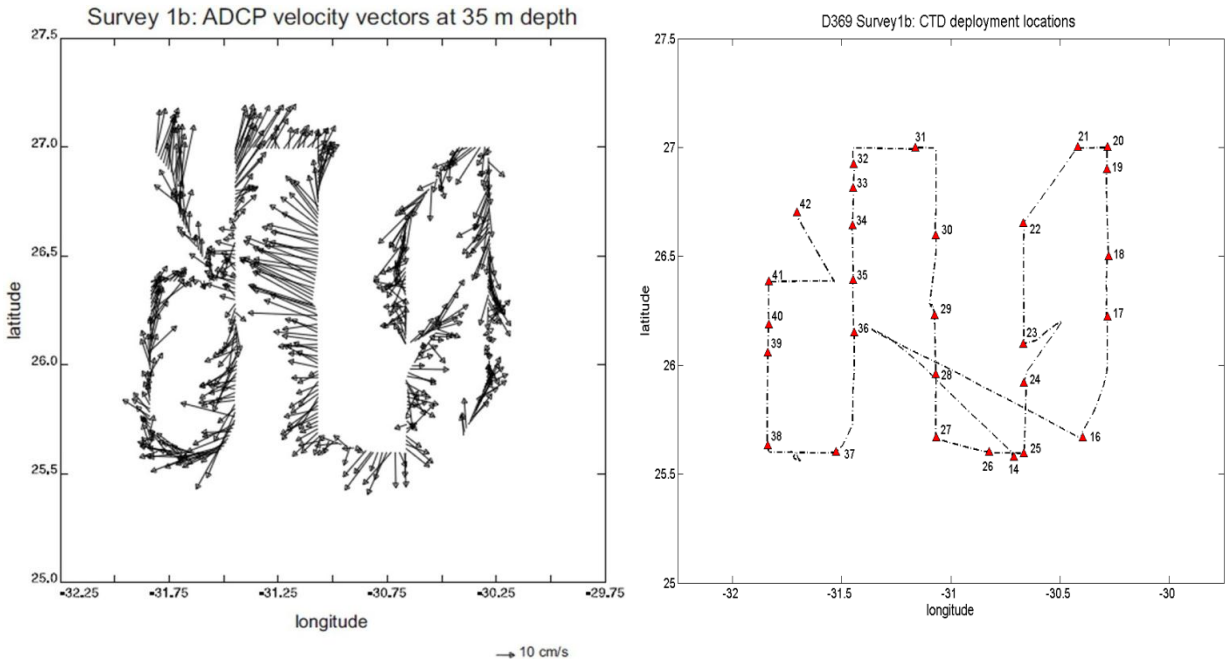

Figure 3: Second mesoscale survey of intensive CTD sampling. ADCP velocity vectors at 35 m depth (left) and cruise path (dashed line) with CTD stations (triangles, right).

The 24th of August was the second day of odd & ends, starting with a standard CTD (05:50), followed by PumpCast (09:00), Deep Sea Incubator deployment to 300 m (11:00), SAPS (12:25), and titanium CTD deployment to 2000 m (16:45). At this point we continued to the north-west corner of the study region to complete the survey with the SeaSoar in tow, arriving in the early morning of the 25th of August. At this point, the CTD was deployed (05:35) followed by the Micronet (07:20) before commencing the third survey.

SeaSoar was in tow for the third survey, beginning in the north-east corner at 08:20 on the 25th August and heading eastwards along a latitudinal transect (Figure 4). As before, the SeaSoar survey was interrupted twice daily for CTD casts. Through the night of the 26th August we recovered three PELAGRA traps, before returning to the third transect to resume the survey. The survey was

completed at 18:16 on the 28th August, at which point we towed the SeaSoar to the north-west of the region in preparation of the fourth survey, which would cover an eddy identified in the area.

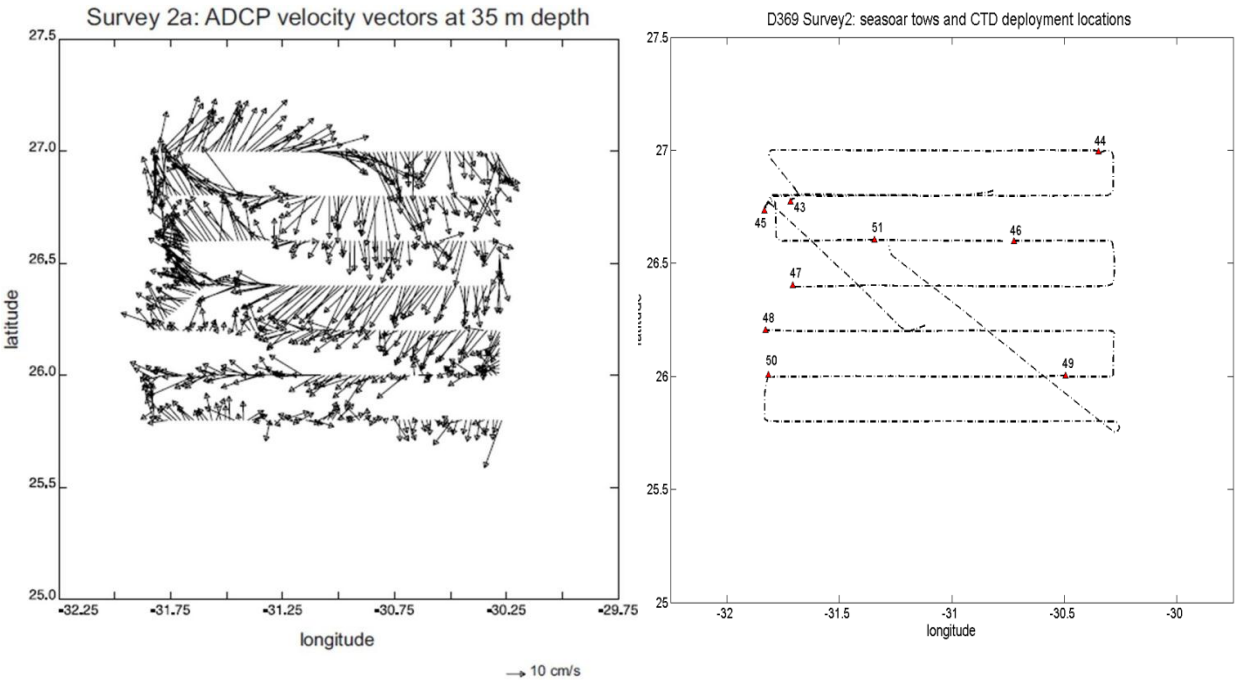

Figure 4: Third mesoscale survey using SeaSoar. ADCP velocity vectors at 35 m depth (left) and SeaSoar path (dashed line) with CTD stations (triangles, right).

Three PELAGRA traps were deployed in the centre of the eddy at 03:50-05:05 on the 29th August. We remained in this position for the third odds & ends day, which consisted of a morning CTD (06:00), PumpCast (08:10), Micronet (09:15), Deep Sea Incubator (11:10), noon CTD (12:45), and turbulence profiler (14:00-17:40).

The fourth survey was an ADCP/CTD triangulation survey of the eddy, beginning at 19:40 on the 29th of August and ending in the morning of the 3rd of September (Figure 5). The eddy survey using ADCP was punctuated by four CTD casts, two turbulence profiling series and one Micronet per day, and deviations to recover a surfaced PELAGRA trap in the morning of the 30th August and the remaining two in the late morning of the 2nd of September.

This lead onto the fourth odds & ends day (2nd September) beginning with a CTD at 06:00, followed by PumpCast (08:45), two Micronet deployments (10:30), another CTD (13:30), Deep Sea Incubator deployment to 1000 m (15:00) and the release of four PELAGRA traps (16:30-20:50).

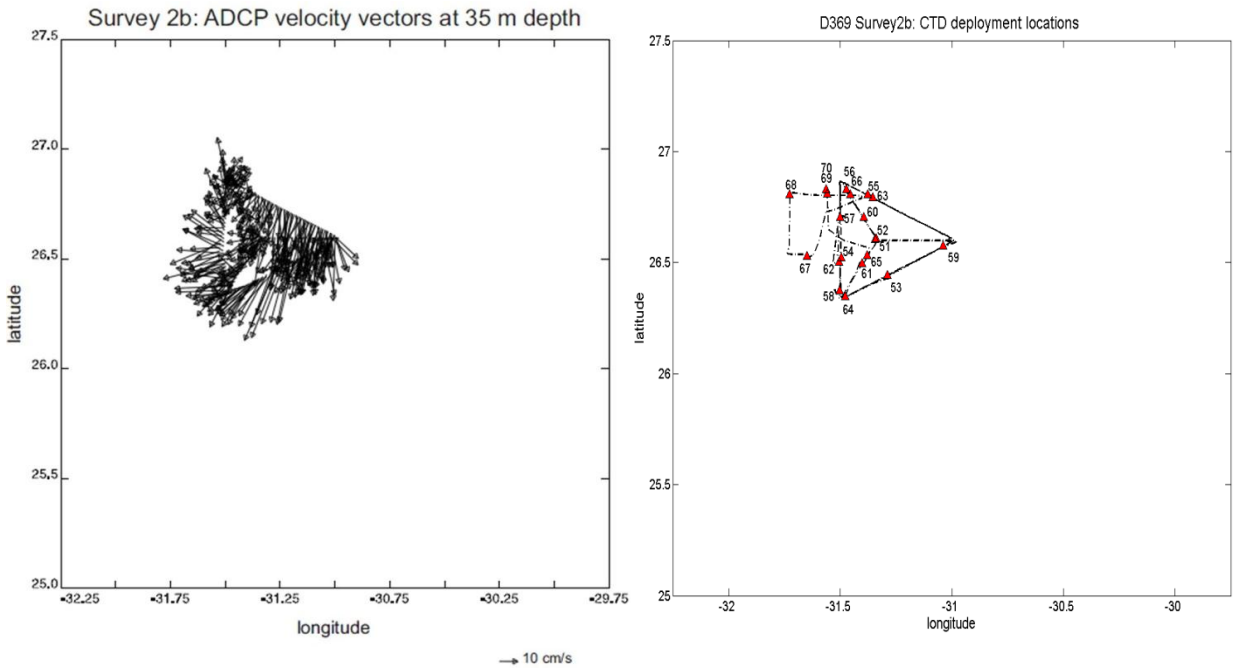

Figure 5: Fourth mesoscale survey of intensive CTD sampling. ADCP velocity vectors at 35 m depth (left) and cruise path (dashed line) with CTD stations (triangles, right).

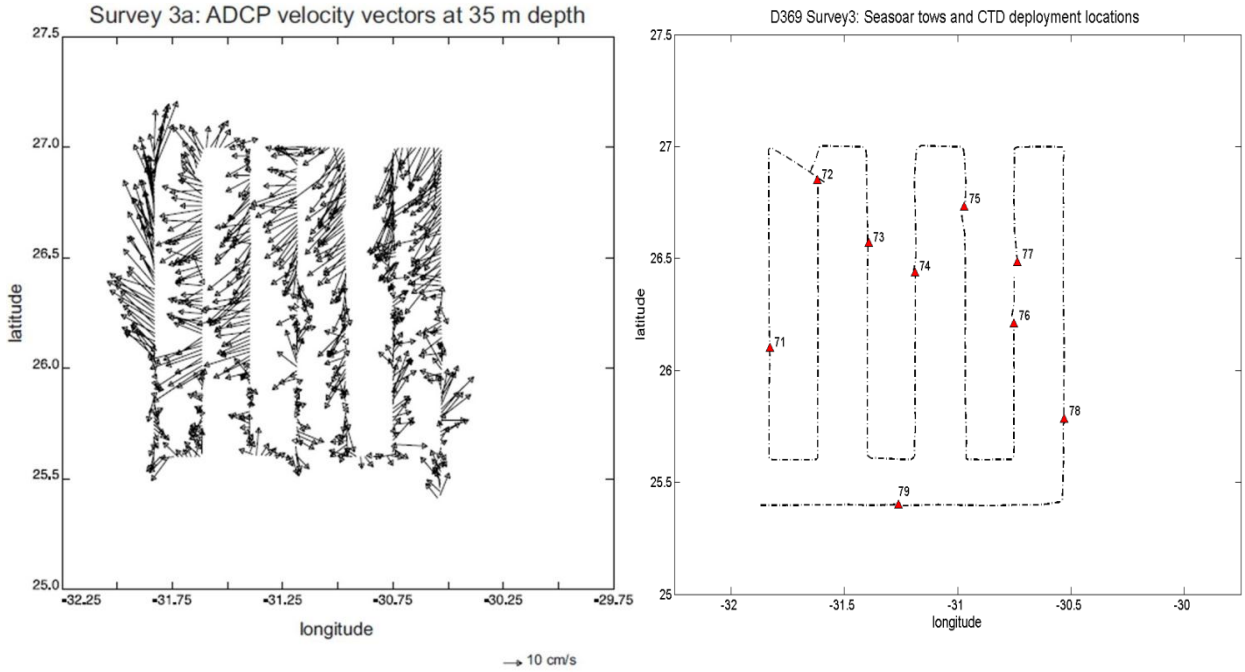

Figure 6: Fifth mesoscale survey using SeaSoar. ADCP velocity vectors at 35 m depth (left) and SeaSoar path (dashed line) with CTD stations (triangles, right).

Once the PELAGRA traps were safely deployed, we commenced with the fifth mesoscale survey, which was the last to incorporate the SeaSoar. The survey began back in the north-west corner of the study region, at 20:50 on the 3rd of September (Figure 6). This survey covered longitudinal transects thus we set off southwards, stopping twice per day for CTD casts. In the evening of the 4th of September we repositioned the ship to recover two resurfaced PELAGRA traps at around midnight, before continuing with the survey. The survey ended in the morning of the 8th of September, at which time the ship was positioned at the south-western point of the second triangulation CTD/ADCP survey that was done in the south-west of the study region.

In this position we deployed the CTD (11:45) and Deep Sea Incubator (13:05), before commencing the sixth and final mesoscale survey at 15:20 on the 8th of September (Figure 7). By evening, we were en route to two resurfaced PELAGRA traps, which were recovered between 04:00 and 06:15, before returning to the northern apex to resume the triangulation survey. The 9th of September was the fifth odds & ends day, constituting a CTD cast (11:45), PumpCast (13:55) and turbulence profiles (15:00), before continuing the CTD/ADCP survey. Turbulence profiles were done twice per day during the survey; Micronet casts were done on the 10th and 11th September, and the Deep Sea Incubator was also deployed to 1000 m on the 11th of September. This final survey was completed in the south-east apex of the triangle, at 07:25 on the 12th of September, and so concluded science for D369.

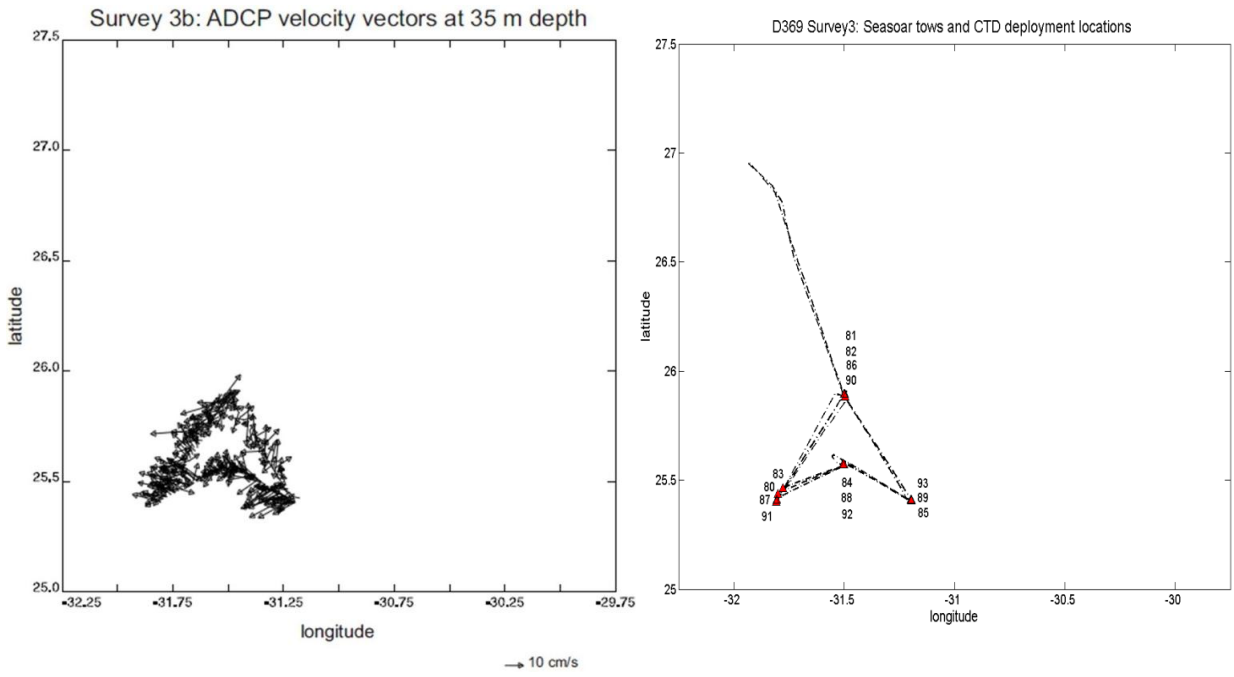

Figure 7: Sixth mesoscale survey of intensive CTD sampling. ADCP velocity vectors at 35 m depth (left) and cruise path (dashed line) with CTD stations (triangles, right).

2. Technical Support

SeaSoar Operations

Dougal Mountifield

Summary

The SeaSoar system that was deployed during the cruise was configured as on D350/351 (2010) but two SBE 43 Dissolved Oxygen sensors are now available for SeaSoar use. The Seasoar system had extensive use during the cruise with 6 trial and 24 survey deployments including one aborted tow due to failure of the Chelsea TG Fasttracka II causing a short circuit on the Seasoar power system. A total of three ~4 day surveys were completed.

211 h (8.8 days) was spent undulating on survey covering 1920 nm. In addition a total of 18 h was accumulated on trial towing covering an additional 164nm. The deployment time from system power-up to first dive was approximately 20-30 minutes. The recovery time was of similar duration. Hence each tow had a nominal overhead of 1 hour not profiling. Hence for the 30 deployments, 30 h (or ~8% of total Seasoar operation time) was lost in deployment and recovery.

Initial problems were encountered with the fish not diving greater than 200 m. Over four trial tows, with different wing trim angles, and the replacement of the hydraulic unit, no improvement was achieved. It was eventually established that the fish was inverting due to the proximity of the Turner Cyclops optics sensors to the rudder impacting roll control. These and the ODIM LOPC were removed and the fish was then towed at 8.5 - 9 knts on 750 m of faired cable and undulated between the surface and ~390 m.

The optics package (chlorophyll, phycoerythrin, CDOM and turbidity) was re-fitted to the vehicle for the second survey in a new location on the top of the vehicle behind the SUV6. The Turner Cyclops instruments were initially used at a gain of 1. This was later increased to a gain of 10, and a very small noisy signal was produced in Chlorophyll only. The CDOM signal had a long period oscillation of unknown origin. The optical sources on these instruments are insufficiently intense for use in oligotrophic water. The new CTG Unilux turbidity meters had their analogue output disabled as supplied. The instrument was reprogrammed to enable the analogue output, but it also had insufficient sensitivity for blue water work.

A short trial tow was undertaken with the LOPC refitted to establish its effect on roll attitude. The fish did not roll, but the LOPC may compromise maximum depth achievable.

Following the considerable problems were experienced with the CTG Minipack CTD-f's during D350/1, Chelsea TG were commissioned to completely overhaul all five units up to new standard and undertake a program of stress-testing, prior to final calibration. All five Minipack units were deployed and although the instruments continued to operate, the calibrations and stability of the instrumentation was very poor. Salinity offsets of ~0.2 - 0.5PSU were seen on all instruments, one unit had a temperature error of > 10 deg C, and all the fluorimeter calibrations were dubious, often with large offsets. All instruments also suffered from hysteresis, non-linearity and drift in salinity. Considerable efforts by the science party to produce correcting calibrations resulted in at best 2 units being useable, although drift problems remained for the first few profiles after power-up.

System developments prior to D369

Developments to the Seasoar system prior to D369 include a completely new topside system using new industrial PCs and an in-house topside interface unit providing g.SHDSL communications, towcable tension display and logging, and hydraulic current control in one 4U rack module. A new control software system was developed in Labview using PID control (Figure 8). The new Seasoar controller software also provides navigation data from GPS, log and gyro via UDP which is logged to disc merged with all control parameters, system status and fish depth. The topside interface now only requires the deck and tension cables to be plugged in during mobilisation. A complete spare topside system is also now available.

The new controller software generates a triangle waveform for flight control, creates an error signal from the difference between the setpoint and SeaSoar pressure, and uses a PID algorithm to generate a control signal. It displays these parameters and the towcable tension on graphs. It has fail-safe emergency up (+10 mA MOOG valve current) if there is a pressure comms error for > 4secs, a tension comms error, failure of the tension 4-20 mA current loop, or if tension exceeds the user set limit.

The valve current can be clamped to an upper and lower limit to prevent excessive control forces.

The user controls are minimum pressure, maximum pressure, climb rate and dive rate. The user can also tune the PID control loop with the three coefficients Kc, Ti and Td. The system was tuned on the trial tows and default values of Kc=0.020, Ti=6.000 and Td=0.180 used for the remainder of the

cruise. Peak control currents were <2 mA and most of the time the current was of the order of 0.1 mA. Tow cable tensions where smooth and peak tension was approx 1300 kg.

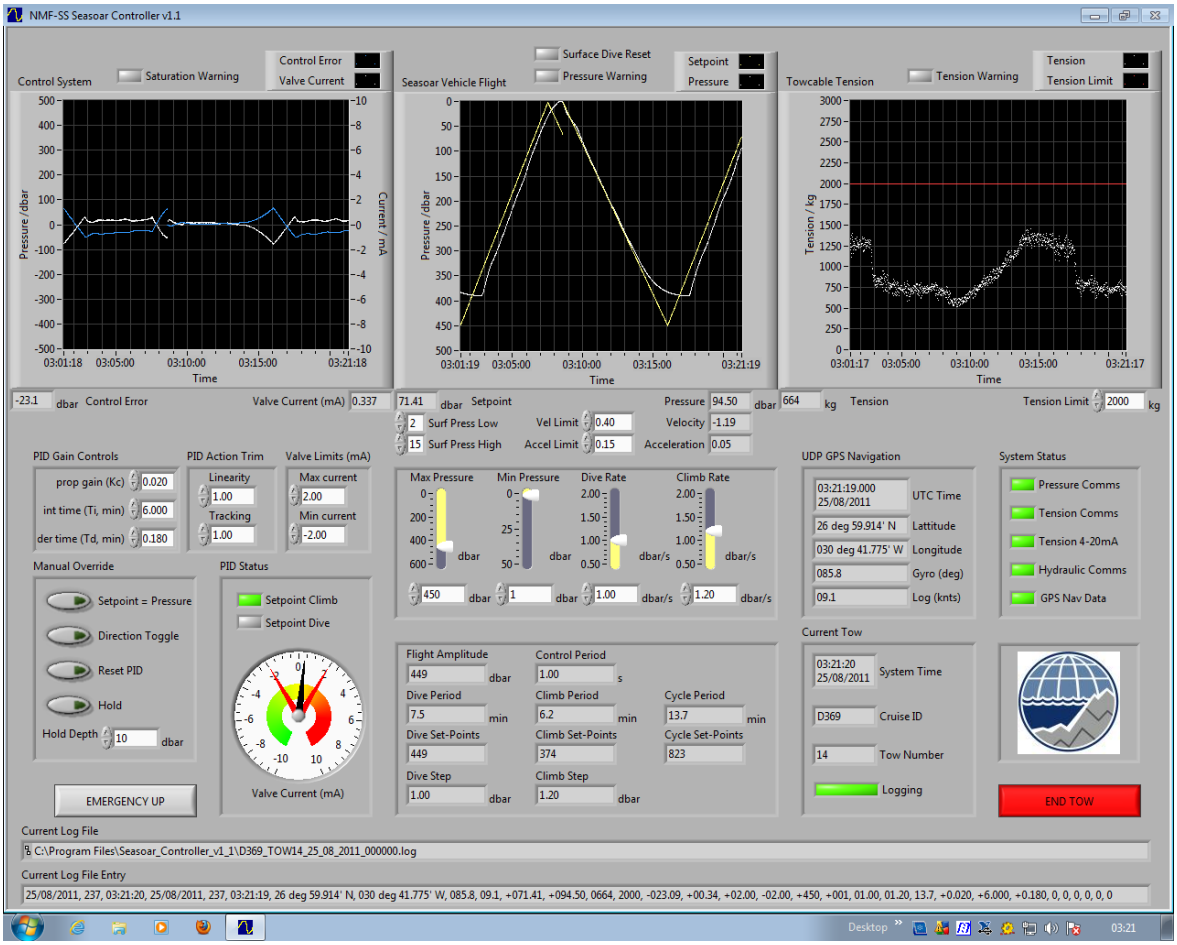

Figure 8: Screen grab of SeaSoar controller software.

Manual override controls are provided for setpoint = pressure (to reduce large control changes), direction toggle (to toggle the setpoint ramp from between climb and dive), reset PID (to prevent integral windup), and hold (for holding the SeaSoar fish in level flight). A software emergency up button is also provided.

During the first survey the fish dive rate immediately after the turn at the surface was often exceeding 8 m/s which was compromising T-S data. The immediate work around was to click setpoint = pressure at approx 3 m after the turn at the surface. This removed the integrated error accumulated up to the surface turn and therefore reduced the down-wing signal to a more neutral control regime. The software was modified during the cruise to remove a bug with the pressure buffer and to introduce an automated set-point = pressure surface dive reset based on fish dive velocity and acceleration within a pressure window. Defaults for the surface dive reset were velocity exceeding 0.35 dbar/s and

acceleration exceeding 0.15 dbar/s^2 in a 2-15 m pressure window. The surface dive reset function worked very well.

The new topside interface system is complemented with new deck cables and a new junction box on the winch. The winch has also recently had its control box replaced, integrating the new load-cell amplifier purchased after D350/1 and a local tow-cable tension display. This worked very well. The winch has also now had its brake replaced.

SeaSoar tow-fish configuration including spares

Sensors deployed bracketed in bold. Please refer to the deployment history table at the end of this report for details of sensor changes.

PENGUIN Submersible Linux Computer – s/n's **[PENGUIN1]** & PENGUIN2

Chelsea TG Minipack CTD-f - s/n [210011], [210012], [210035], [210039] & [04-4330-003]

Chelsea TG Fastracka-II FRRF-II s/n **[07-6139-001]** & **[07-6480-002]**

Chelsea TG Glowtracka Bioluminescence sensor – s/n **[07-6244-002]**

Maurer Instruments Ltd Flow Meter Model SR150 – s/n **[2885]**

Chelsea TG Hemispherical PAR sensor 0046-3097 – s/n 46/2835/08 & **[46/2835/09]**

Turner Cyclops mini fluorimeter – Chlorophyll "C" – s/n **[2100432]**

Turner Cyclops mini fluorimeter – Phycocyanin "P" – s/n **[2100433]**

Turner Cyclops mini fluorimeter – Phycoerythrin "E" – s/n **[2100594]**

Turner Cyclops mini fluorimeter – CDOM "U" – s/n **[2100595]**

Chelsea TG Unilux Nephelometer 2125-021-PL-D s/n 005 & **[006]**

Aanderaa Optode 3975 Dissolved Oxygen – s/n **[891]**

Seabird SBE43 Dissolved Oxygen Sensor – s/n **[2061]** & **[2068]**

NOC/Valeport SUV-6 UV Nutrient Sensor – no serial number marked

New Valeport SUV-6 UV Nutrient Sensor – serial number to be confirmed.

ODIM LOPC Optical Plankton Counter (660 m pressure case) – s/n 10690 & **[10693]**

SeaSoar PENGUIN instrument configuration

The following instruments were logged using the four serial ports in PENGUIN:

/dev/ttyS0 - Chelsea TG Minipack CTD-f (9600 baud)

/dev/ttyS1 – ODIM LOPC Optical Plankton Counter (115,200 baud)

/dev/ttyS2 – NOC/Valeport SUV-6 UV Nutrient Sensor (19,200 baud)

/dev/ttyS3 - Chelsea TG Fastracka-II FRRF-II (115,200 baud)

All the remaining instruments were logged using the auxiliary 0-5VDC inputs of the Chelsea Minipack CTD-f as follows:

Table 1: Y-Cable A.

Cable #	**Minipack Channel**	**Instrument**
1	10	UNUSED
1	11	UNUSED
2	12	Cyclops CDOM
3	13	Cyclops Phycoerythrin
4	14	Chelsea TG Unilux Turbidity
5	15	Cyclops Chlorophyll

Table 2: Y-Cable B

Cable #	**Minipack Channel**	**Instrument**
1	17	Optode Oxygen Conc
1	18	Optode Oxygen Temperature
2	19	SBE43 Oxygen
3	20	Chelsea Glowtracka
4	21	Chelsea PAR
5	22	UNUSED

SeaSoar deployment notes

The topside PSU voltage was set at ~75 V to yield approximately the PENGUIN PSU clamping voltage of 56 V at the fish end. The resistance of the power conductor loop in the tow cable was approximately 25 Ω. Total power supply current was found to be ~0.63A with the wire on the winch and 0.8 A with 750 m of wire streamed.

New vertical deployment frames were used that have fork slots to enable them to be easily moved around deck with a pallet truck. The new frames also have a 0.5 m deck bolt matrix to allow better positioning relative to the deployment block.

For the initial trial deployment the block was hung from the crane to allow more headroom, control and finer positioning of the block, however this significantly increased the deployment time and was too complicated. The Lebus deployment snatchblock was eventually hung from the pendulum arm on the after gantry and the pendulum ram was extended to give more headroom during deployment and more clearance from the transom during recovery.

The further failure of all five Chelsea Minipack CTD-f instruments is particularly disappointing as the manufacturer was recently commissioned with refurbishing and stress testing them. In my view, these are no longer supportable.

In general it is optimal to keep any instrumentation away from the rudder of the SeaSoar vehicle to reduce the probability of stalling it and losing roll-stability.

The new direct archive feature of the Chelsea Fastpro software for the Fasttracka II is a welcome addition and worked very well.

Fasttracka-II s/n 07-6139-001 has a RTC bug. The time sync feature in Fastpro does not work with it. It has no battery backup of its RTC, so the clock required setting after each power-up with a terminal. There is a second bug with this unit, with the date reported with 'sy ti' after setting the clock. The instrument errantly reports the year as 2017 after it is set to 2011. The time stamps in the data files were written correctly.

Fasttracka-II s/n 07-6480-002 was deployed for the first time since it was supplied new. It failed within 1 hour of immersion with a short-circuit on its power supply. This instrument may have flooded, and requires assessment by the manufacturer (Chelsea TG).

The signal from the SBE43 oxygen sensor was of a highly variable quality. Sometimes it produced a believable though somewhat noisy profile, but most of the time it was just noise. A thicker walled hose was fitted to prevent the hose from being crushed, but no improvement was seen. The sensor was removed and the plenum removed for inspection, but no damage was visible. The spare sensor was used with no change in performance. It is suggested that this sensor be used in conjunction with a SBE5T pump in the future.

The optics instrument suite has insufficient sensitivity for use in oligotrophic water. An alternative optics suite should be investigated, preferably with a serial output.

The previously encountered problems remain with PENGUIN +15VDC power, RTC/BIOS battery backup, Glowtracka sample rate and limited IO connectivity available for new instruments. A complete redesign of the PENGUIN system is already underway. A desk study has been completed, components selected and parts for a prototype bench-top system purchased. The new system will have 14 serial ports (increased from the current 4), fast onboard analogue inputs (none currently available), a higher capacity power system running at 150VDC (increased from the current 56V), onboard 3-axis accelerometer, magnetometer and gyro, and software controlled switchable power for up to 20 instruments (up from the current 4, of which only one is useable). A new data logging and instrument display system is proposed using Labview.

KST real-time plotting of SeaSoar data

During the cruise a Linux package called KST was used to display real-time graphs of Minipack data and its auxiliary instruments (oxygen and optics) on emperor (Figure 9). The package can be configured to tail a file and then display any parameter as a scalar or a graph. KST also allows user defined formulae which can also be graphed. This formula function was used to calculate salinity in real-time, which allowed the creation of an on-the fly T-S plot. This was very useful for online quality control of the stability of the Minipack CTD data.

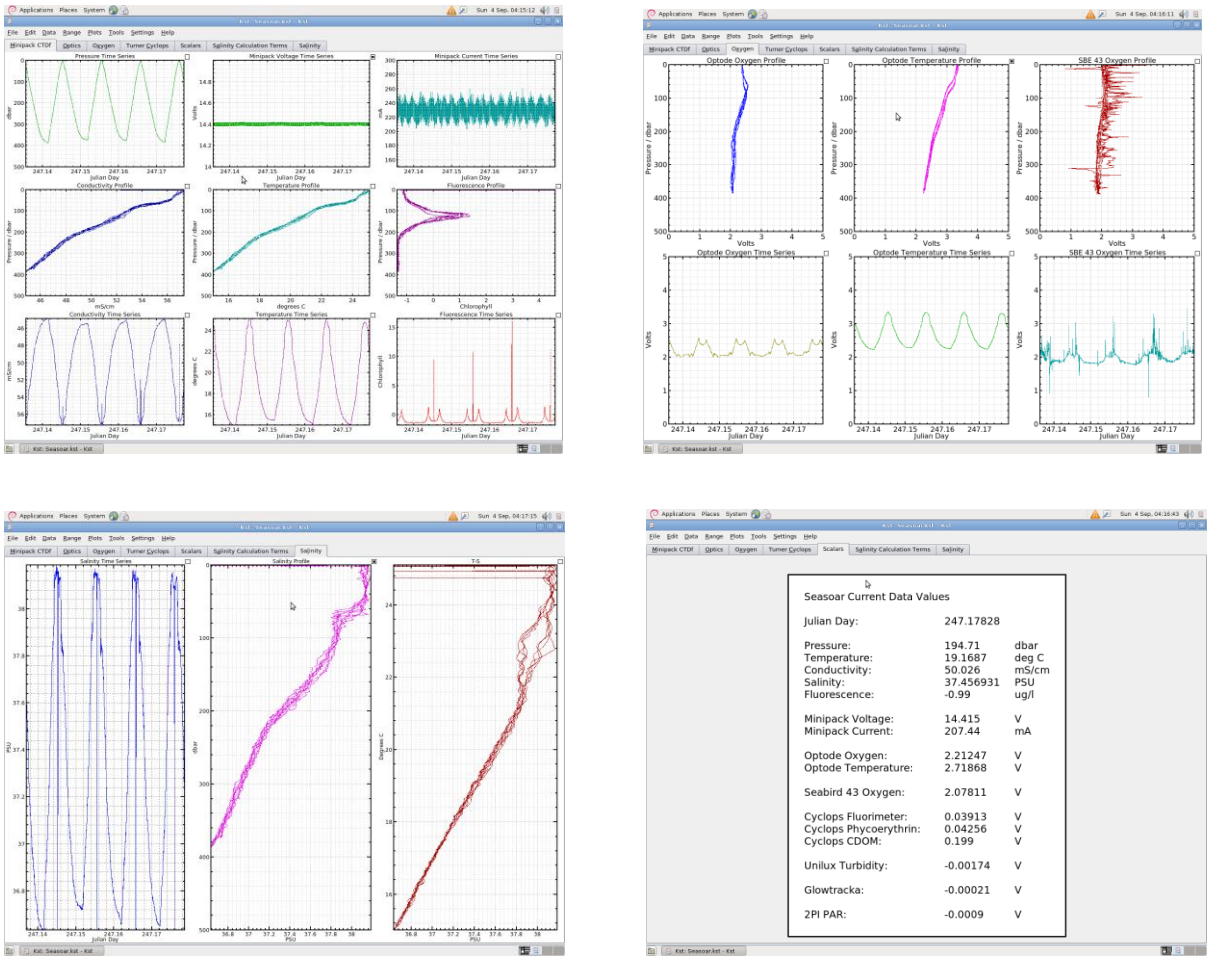

Figure 9: Screen grabs of real-time plotting of SeaSoar data.

Table 3: SeaSoar tow log.

Tow	Julian Day	Date	Start Log	Start Undulation	End Undulation	End Log	Daps Files	Comment
1	223	11/08/2011	10:03	10:10	18:04	18:35	minipack257.000 suv6258.000	Minipack s/n: 210035.Trial tow. Wings 15 deg up/15 down. Initialially good, but won't go deep.
2	224	12/08/2011	12:08	12:50	15:02	16:07	minipack256.000 suv6257.000	Trial tow. Wings 12 deg up/18 down. Still bad diving.
3	225	13/08/2011	14:41	15:21	19:24	20:12	minipack260.000 suv6261.000	Trial tow. Wings 14 deg up/16 down. New Hydraulic Unit. Still bad.
4	226	14/08/2011	13:31	13:31	15:57	16:27	minipack291.000 suv6292.000	Trial tow. Wings 16 deg up/14 down. Suspect vehicle inverting @200m.
5	227	15/08/2011	08:12	09:15	13:35	14:05	minipack262.000 suv6257.000 minipack252.000 suv6253.000 minipack263.000 suv6264.000	Start of 1st Survey proper. LOPC & Cyclops/Unilux removed from tail. Old Controller. Good dives.
6	227	15/08/2011	21:41	22:12	05:03	05:54	minipack264.000 suv6262.000	New Controller. Good flight 0-400m.
7	228	16/08/2011	07:57	08:28	19:03	19:37	minipack280.000 suv6283.000	1 Impellor blade broken during tow.
8	228	16/08/2011	21:31	22:09	04:55	05:36	minipack575.000 suv6577.000	Pressure bug in new controller observed.
9	229	17/08/2011	07:53	08:27	19:13	19:35	minipack875.000 suv6876.000	Initial Dive after turn at surface to rapid.
10	229	17/08/2011	21:27	21:56	04:55	05:25	minipack1188.000 suv61187.000	Clicking setpoint = pressure after turn at 3m to slow dive.
11	230	18/08/2011	07:50	08:18	20:00	20:27	minipack1461.000 suv61462.000	End of 1st Survey
12	233	21/08/2011	12:00	12:20	13:20	13:47	minipack307.000 suv6308.000	Test Tow. Minipack s/n: 210011. LOPC refitted for this tow. 20-330m Deepest 356m
13	235	23/08/2011	12:20	12:35	13:30	14:02	minipack367.000 suv6368.000	Test Tow. Minipack s/n: 210012 tested on deck +10 deg temp offset (not deployed). Minipack s/n: 210039. No LOPC. Optical Sensors mounted on body. Controller v1.1
14	236	24/08/2011	18:47	19:18	05:02	05:40	minipack258.000 minipack371.000 suv6259.000	Start of 2nd Survey. Minipack s/n: 04-4350-003 for rest of cruise
15	237	25/08/2011	08:00	08:52	19:16	19:38	minipack644.000 suv6645.000	
16	237	25/08/2011	21:34	22:05	04:58	05:23	minipack936.000 suv6937.000	
17	238	26/08/2011	07:38	08:03	19:20	19:44	minipack1207.000 suv61208.000	
18	239	27/08/2011	07:33	07:56	19:16	19:37	minipack512.000 suv6513.000	SBE43 2061 removed. 2068 fitted. Optics Cleaned.
19	239	27/08/2011	21:26	21:52	04:54	05:16	minipack823.000 suv6822.000	FRRF2 s/w crash.
20	240	28/08/2011	07:40	07:50	02:27	02:55	minipack1099.000 suv61100.000 minipack1343.000 suv61344.000	End of 2nd Survey. FRRF2 time drift. FRRF2 crash.
21	246	03/09/2011	21:00	21:35	04:58	05:29	minipack281.000 suv6282.000	Start of 3rd Survey. Old SUV6 removed. New SUV6 fitted. 2Hz data.
22	247	04/09/2011	07:29	07:50	20:12	20:33	minipack557.000 suv6558.000	Fish Held at 100m @ 15:30 to avoid large piece of wood on surface.
23	248	05/09/2011	00:12	00:40	05:03	05:29	minipack934.000 suv6933.000	Ti impellor replaced with Al unit after 2nd blade loss and further cracks.
24	248	05/09/2011	07:27	07:55	20:15	20:34	minipack1137.000 suv61139.000	
25	248	05/09/2011	22:20	22:47	05:06	05:28	minipack1470.000 suv61471.000	
26	249	06/09/2011	08:24	08:28	20:14	20:33	minipack271.000 suv6272.000	FRRF2 comms problems. New FRRF2 and cable fitted after this tow.
27	249	06/09/2011	23:20	23:31	00:40	00:40	minipack266.000 suv6267.000	Logging failed during deployment. Total failure @ 00:40. Inboard 01:10
28	250	07/09/2011	07:23	07:45	20:20	20:35	minipack399.000 suv6400.000	New FRRF2 had failed short circuit on previous tow. Old FRRF2 fitted.
29	250	07/09/2011	22:19	22:49	05:05	05:23	minipack731.000 suv6732.000	
30	251	08/09/2011	07:21	07:40	10:51	11:15	minipack983.000 suv6984.000	End of 3rd Survey. Final Tow

Computing and Instrumentation

Zoltan Nemeth

RVS LEVEL C System

Level C - The level C system is a Sun Solaris 10 UNIX Workstation discovery1 also known as ABCGATE. The RVS software suite is available on this machine. This suite of software allows the processing, editing and viewing of all data within the RVS data files. This system also has monitors that allow us to ensure that the level C is receiving data from the level B.

Ifremer Techsas System

The Ifremer data logging system is the system that will inevitably replace the existing Level A + B system while for the most part the Level C will remain as the main system for outputting, viewing and editing the acquired data.

The Techsas software is installed on an industrial based system with a high level of redundancy. The operating system is Red Hat Enterprise Linux Edition Release 3. The system itself logs data on to a RAID 0 disk mirror and is also backed up from the Level C using a 200GB / 400GB LTO 2 Tape Drive. The Techsas interface displays the status of all incoming data streams and provides alerts if the incoming data is lost. The ability exists to broadcast live data across the network via NMEA.

The storage method used for data storage is NetCDF (binary) and also pseudo-NMEA (ASCII). At present there are some issues on some data streams with file consistency between the local and network data sets for the ASCII files. NetCDF is used as the preferred data type as it does not suffer from this issue.

The Techsas data logging system was used to log the following instruments:

1) Trimble GPS 4000 DS Surveyor (converted to RVS format as gps_4000)
2) Chernikeef EM speed log (converted to RVS format as log_chf)
3) Ships Gyrocompass (converted to RVS format as gyro)
4) Simrad EA500 Precision Echo Sounder (ea500)

5) NMFD Surface-water and Meteorology (surfmet) instrument suite
6) ASHTECH ADU-5 Altitude Detection Unit (gps_ash)
7) NMFD Winch Cable Logging And Monitoring CLAM (winch)
8) Fugro Seastar 9200 G2 XP Differential (gps_g2)
9) Seabird SBE45 MicroTSG (seabird)

Fugro Seastar DGPS Receiver

The Fugro Seastar G2 is a Glonass and GPS receiver that is used to provide 10CM accuracy and also receives differential from the Fugro differential system. This signal is then buffered out to multiple systems including the Trimble 4000 DS. The Seastar was purchased as an upgrade to the old Seastar and G12 combination. The system is designed to cope wit the future expected solar activity that is expected to disable part of the existing GPS network. The system is also capable of receiving corrections via Internet if necessary.

NetCDF files for this system s9200G2s-FUGRO.gps

RVS Stream gps_g2

Forms part of the bestnav stream

Trimble 4000 DS Surveyor

The Trimble 4000DS is a single antenna survey-quality advanced GPS receiver with a main-masthead antenna. It uses differential corrections from the Fugro Seastar unit to produce high quality differential GPS (DGPS) fixes. It is the prime source of scientific navigation data aboard RRS *Discovery* and is used as the data source for Navigation on the ships display system (SSDS). This antenna is directly on top of the mast and suffers from negligible interference from other items on the mast. It is also almost directly at the centre point of the ship making it an ideal navigation system.

The Techsas NetCDF File ends with the following extensions :

Position-4000.gps

Satelliteinfo-4000.hps

RVS Stream gps_4000

Forms part of the bestnav stream

Ashtec ADU-2

This is a four antenna GPS system that can produce attitude data from the relative positions of each antenna and is used to correct the VMADCP for ship motion. Two antennae are on the Bridge Top and two on the boat deck.

The Ashtec system worked reliably throughout the cruise with some gaps that are quite usual with this system due to the amount of calculations necessary. No Large data gaps are present. The ADU-2 forms part of the bestnav system which is an assembly of multiple GPS signals including the gyronmea and emlog stream in order to calculate the best possible position, speed heading pitch and roll of the ship. The Ashtech is not as reliable as the Fugro Seastar G2 and the 4000DS mainly due to its low position on the ship it is hard for this system to maintain locks on satellites when the ship is maneuvering and the bridge and main mast come into its direct line of sight with the satellites.

The Techsas NetCDF File ends with the following extensions :

ADUPOS-PAPOS.gps

gppat-GPPAT.att

RVS Stream gps_ash

Forms part of the bestnav stream

Gyronmea

The Gyronmea is a file that receives its data from the Ships gyro compass located on the bridge. There are two such Gyros on the bridge and we are able to use either one of them as a source of heading. The selected Gyro is logged by the TECHSAS system and is used as part of the bestnav calculation.

The NetCDF File for Techsas ends with gyro-GYRO.gyr

RVS data stream gyro

RDI 150KHz Vessel Mounted ADCP (VMADCP)

The RDI Ocean Surveyor was setup by the science party at the start of the cruise with a bottom track and water track file that is included with the dataset. The configuration was changed when we left the shelf and went to deeper water. The Ocean surveyors are fed with data from the ships GPS, Gyro and ADU systems in order so that the system can calculate true speeds and direction of the currents below the ship.

100 Bins

8 Meter Bin Size

4 meter Blank

5.3 Meter Transducer Depth

Low Resolution (Long Range)

Ping as fast as possible.

Bottom Tracking only for the 1st 3 days whilst in shallower water.

RDI 75KHz Vessel Mounted ADCP (VMADCP)

The RDI Ocean Surveyor was setup by the science party at the start of the cruise with a bottom track and water track file that is included with the dataset. The configuration was changed when we left the shelf and went to deeper water. The Ocean surveyors are fed with data from the ships GPS, Gyro and ADU systems in order so that the system can calculate true speeds and direction of the currents below the ship.

100 Bins

8 Meter Bin Size

4 meter Blank

5.3 Meter Transducer Depth

Low Resolution (Long Range)

Ping as fast as possible.

Bottom Tracking only for the 1st 3 days whilst in shallower water.

Chernikeef EM log

The Chernikeef EM log is a 2-axis electromagnetic water speed log. It measures both longitudinal (forward-aft) and transverse (port – starboard) ships water sped.

The EM log was not calibrated prior to the cruise and was reading at 0.0 knots when alongside.

The system was logged by the TECHSAS logging system.

DYLog-LOGCHF-DYLog

RVS Stream chernikeef

Simrad EA500 Precision Echo Sounder (PES)

The PES system was used throughout the cruise, with a variation between use of the Fish and use of the hull transducer. The fish is more accurate than the hull transducer as it is capable of being deployed deeper and is also decoupled from the noise of the ship.

The PES outputs its data to a stream called ea500 on the Level C System.

Surfmet System

This is the NMFD surface water and meteorology instrument suite. The surface water component consists of a flow through system with a pumped pickup at approx 5 m depth. TSG flow is approx 25 litres per minute whilst fluorometer and transmissometer flow is approx 3 L/min. Flow to instruments is degassed using a debubbler with 40 L/min inflow and 10/L min waste flow.

The meteorology component consists of a suite of sensors mounted on the foremast at a height of approx 10 m above the waterline. Parameters measured are wind speed and direction, air temperature, humidity and atmospheric pressure. There is also a pair of optical sensors mounted on gimbals on each side of the ship. These measure total irradiance (TIR) and photo-synthetically active radiation (PAR).

The Non Toxic system was enabled as soon as we were far enough away from land.

The port PAR sensor was changed prior to sailing as it was out of cal.

I cleaned the Transmissometer and the Fluorometer in the wet lab before the start of the cruise.

Techsas NetCDF Files for Surfmet

Surf-SURFMET.SURFMETv2

MET-SURFMET.SURFMETv2

Light-SURFMET.SURFMETv2

SBE45-SBE45.TSG

Surfmet rvs stream is the raw data captured from the TECHSAS System

Surftemp rvs stream cleaned by the technician, remove spikes e.t.c.

The temp_h temp_m and cond data in the surfmet file is a direct copy of the seabird data however it can be delayed in time. For that reason, always use the data from the seabird instead of the surfmet for protsg and salinity calibrations.

These files contain:

Temp_h (Housing Temperature from the SBE45 in the wetlab)

Temp_m (Marine Temperature from the Hull intake)

Cond (Conductivity from the SBE45 in the wet lab)

Trans (Raw Voltage from Transmissometer)

Fluo (Raw Voltage from Fluorometer)

Speed (Wind Speed from Gill Windsonic Anemometer)

Direct (Wind Direction from Gill Windsonic Anemometer)

Airtemp (Air Temperature from Vaisala HMP45A)

Humid (Air Temperature from Vaisala HMP45A)

Pressure (Air Pressure from Vaisala PTB100)

PPAR (Photosynthetic Active Radiation from SKE510 PAR Sensor on PORT Gimbal)

SPAR (Photosynthetic Active Radiation from SKE510 PAR Sensor on STBD Gimbal)

PTIR (Total Incidental Radiation from CM6B TIR Sensor on PORT Gimbal)

STIR (Total Incidental Radiation from CM6B TIR Sensor on STBD Gimbal)

Seabird is the raw log of the SBE45 and SBE38 through the SBE45 Junction Box.

Temp_h (Housing Temperature of SBE45 TSG)

Temp_m (Remote or Marine Temperature from Inlet pipe)

Cond (Conductivity in SBE45 TSG)

Salin (Calculated Salinity from Instrument)

Sndspeed (Calculated Sound Velocity from Instrument)

Processed Data files

Relmov – Relmov is the relative motion file for this cruise. This is generated using the ships gyro and ships Chernikeef Log data to extract a movement in a given direction. This is then used by bestnav when and where necessary to calculate fixes if GPS fixes were not available.

Bestnav – Bestnav uses all 3 GPS Systems logged, gps_4000, gps_g2, gps_ash and creates a best suite stream by providing an as complete account of the ships track as possible. This is done by reading all 3 GPS streams with gps_4000 being primary, gps_g2 as secondary and gps_ash as tertiary. The system looks for gaps of a certain length in the primary and when it finds those gaps it requests that the next gps down fill in the gaps. If no GPS data is available it asks RELMOV to fill in until data is available again. Then the system calculates back over itself to ensure that the extrapolated positions are correct using the GPS data available around the gap.

Bestdrf – Bestdrf is a product of bestnav. When run bestnav uses the relmov data which contains a predicted vn and ve based upon direction and speed through the water. The Bestdrf file is the accurate drift velocity of what actually occurred based on the GPS changes between each record.

Protsg *- Protsg is the Processed Thermosalinograph data. The raw data is taken from the seabird stream or seatemp stream if cleaned and then ran through a salinity calculation. The data varies slightly from the raw seabird salin variable as they use a slightly different algorithm for the calculation of salinity.*

Pro_wind – This program is designed to remove the relative variables from the wind data logged by surfmet. By removing any fixed offsets in the system and removing the affect of ship motion pro_wind is a true representation of ships wind data.

Intdep – Intdep is a Interpolated data set that extrapolates data where none was logged based on a 2 min band pass filter. Intdep is then passed to which takes Carters tables into account.

Prodep – Prodep is an automated process that access the bestnav position fix data and then uses a pre programmed Carters table of corrections and corrects the echo sounder data for that given time.

Network Services

Networking hopefully worked well throughout the cruise despite a losing the internet link on the SDU. We spoke to Cruise –ip they suggested to use 37W satellite and not the 22W.

As we will no longer be within the Cruise-IP satellite coverage footprint there will be no internet access apart from a limited email service offered by AMS3.

AMS3 is the same e-mail system as used by the British Antarctic Survey to relay messages. In order to be able to access your email using AMS3 during the time of Cruise-IP outage, it will be necessary to sign up with your choice of account well before we lose internet coverage. If you wish to continue to send and receive e-mails, you may do this by providing me with your email address, username (not your password), and mail server address (only IMAP is supported, not POP3). Once I have entered your e-mail settings into the AMS3 system, it is then necessary for you to login using your normal password. AMS3 will then attempt to download your inbox over a day or so. If your inbox is very full, i.e. thousands of messages, or over 50 MB, you will requested to either clean out your inbox, or move the messages to a folder on your mail server, or sign up to a cabin account instead. You must reduce your inbox size before you can sign up to an AMS3 account.

The cabin accounts are shared e-mail accounts that are reused at the end of the cruise by the next occupant of that cabin. The passwords are fixed, and you cannot change them, therefore these accounts are not to be considered secure.

Data Storage

DISCOFS is an advanced Network Attached Storage device. All scientific cruise data was stored on this device under the Cruises/D369 folder, and organised with a standard template of folders

All cruise data was stored on this storage area.

All CTD, ADCP and LADCP data was backed up to DISCOFS on acquisition.

Data Backups

For the backup we are using Syncback software. SyncBack can save files on external hard drives, in ZIP archives, on network drives, on CDs or transfer them via FTP.
Syncback is installed on the Dell fileserver (DISCOFS) and it is used to backup Level C, Techsas and other cruise data computers.

To backup level C and Techsas we mount drives (P: R: and T:) to rvs/pro_data, rvs/raw_data and Techsas which are configured as NFS shares on level C and the Techsas machine.
mount 192.168.62.201:/rvs/pro_data P:\

Underway/pro_data folder
mount 192.168.62.201:/rvs/raw_data R:\

Underway/raw_data folder
mount 192.168.62.x:/Techsas T:\

Techsas folder

To backup the ADCP, CTD and SBWR we share a drive on these machines and backed it to the server using Syncback.

OS75KHZ/raw_data, OS150khz/raw_data, SBWR, CTD

Data Archiving

The Data archive will be provided on 500GB USB Hard Drives

1 x HDD to BODC, disk to be returned once data extracted.

1 x HDD to PSO

1 x HDD to NOCS held by NMFSS for 6 Months

Surfmet

Thermosalinograph cleaned 05/08/2011 08:30 (GMT)

Transmissometer & Fluorometer cleaned 05/08/2011 08:30

Transmissometer & Fluorometer cleaned 15/08/2011 05:00

Transmissometer & Fluorometer cleaned 24/08/2011 13:30

Transmissometer & Fluorometer cleaned 24/08/2011 04:15

Transmissometer & Fluorometer cleaned 30/08/2011 06:00

15/08/2011@05:00 Transmissometer- Indicated transmissibility lower than 2 it should normally indicate of 4.4 to 4.6 it was corrected after the system cleaned and flashed.

24/08/2011@04:15 Transmissometer- Indicated transmissibility lower than 4.2 it should normally indicate of 4.4 to 4.6 it was corrected after the system cleaned and flashed.

30/08/2011@04:15 Transmissometer- Indicated transmissibility lower than 4.2 it should normally indicate of 4.4 to 4.6 it was corrected after the system cleaned and flashed.

Echosounder & PES fish

PES fish deployed for deep water 11/08/2011@06:50 28 43.777N, 23 33.975W

Based on the collected ea500 data, I made a bathymetric chart with GMT (Figure 10).

PES fish recovered with damage cable and fairing on completion of deep water operations. 112581028 – 15/09/2011@10:28.

Generic Mapping Tools

GMT 4.5.7 64bit windows version with GSView 4.91 has been installed on the Blackbox2ng (under c:/programs/gmt; c:/program files/ghostgum folders)

I produced two plots every day (the CTD casts position map, and the RRS *Discovery* tracklog); the CTD cast position map contains the SeaSoar tows too (Figure 11).

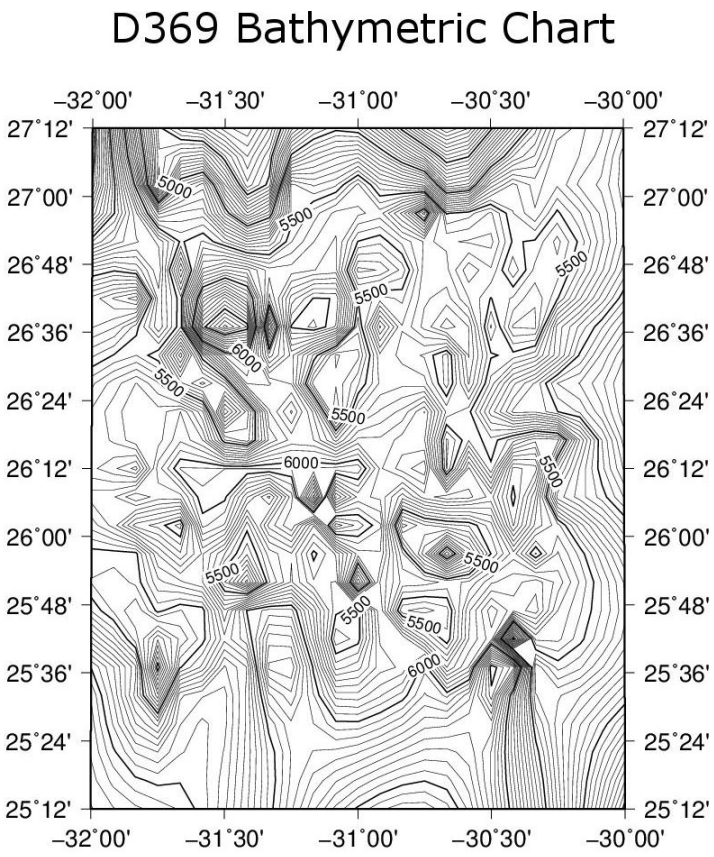

Figure 10: Bathymetric chart of study region.

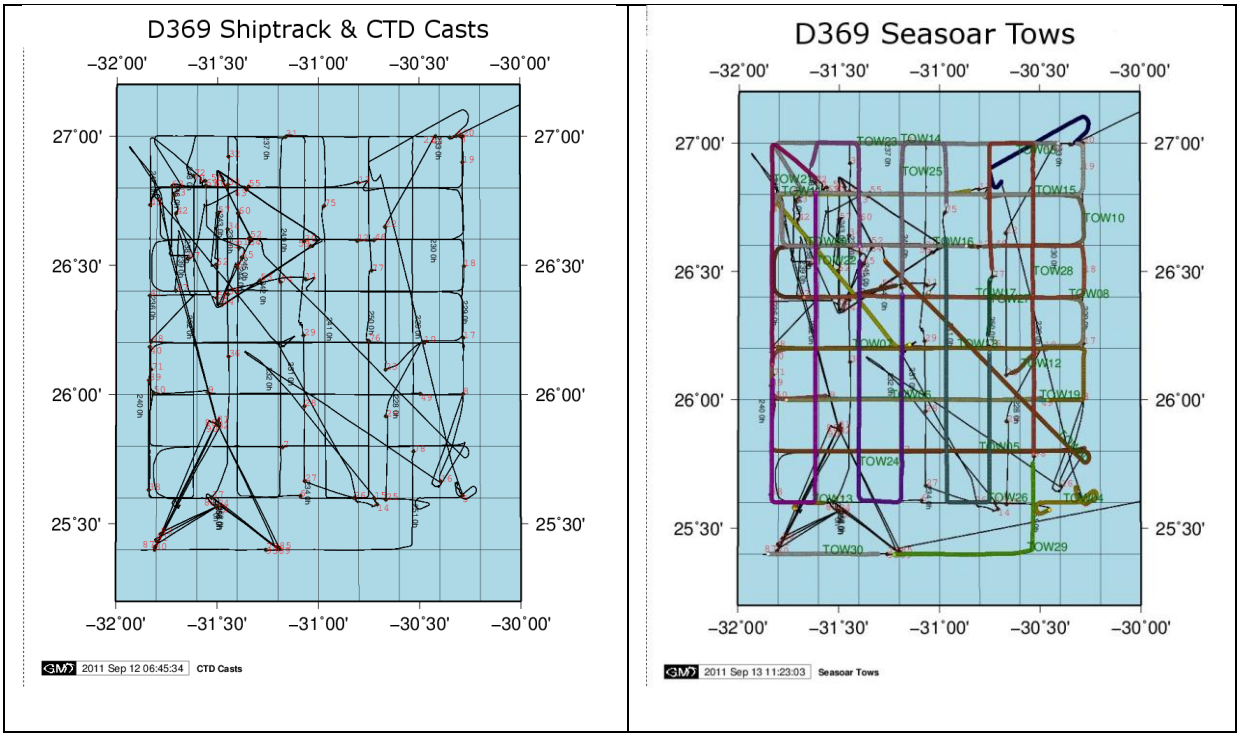

Figure 11: D369 ship track and CTD stations (left) and SeaSoar tows (right).

Table 4: Surfmet Sensor Information D369

Manufacturer	Sensor	Serial no	Comments	Calibration Expires
Seabird	SBE45	0229*	TSG	13/04/12
Seabird	SBE38	0490*	Remote Temp.	04/10/11
Wetlabs	Fluorometer	Ws3s-248*		13/12/12
Wetlabs	Transmissometer	CST-1132PR*		23/03/12
Vaisala	Barometer PTB100A	S3440012*		21/03/12
Vaisala	Temp/humidity HMP45A	E1055002*		29/09/11
SKYE	PAR SKE510	28563*	PORT	11/06/11
SKYE	PAR SKE510	28559	STBD	22/07/12
Kipp and Zonen	TIR CMB6	962301*	PORT	26/04/13
Kipp and Zonen	TIR CMB6	962276*	STBD	04/10/12
Sensors without cal				
Seabird	P/N 90402 SBE45 JB	66	Junction Box	
Gill	Windsonic Option 3	071123		

SPARES

Manufacturer	Sensor	Serial no	Comments	Calibration Expires
Seabird	SBE45	0232	TSG	08/12/11
Seabird	SBE38	0476	Remote Temp.	01/04/12
Wetlabs	Fluorometer	WS3S-247		20/04/12
Wetlabs	Transmissometer	CST-113R		23/06/11
Vaisala	Barometer PTB100A	Z4740021		23/03/11
Vaisala	HMP45A Temp/humidity	B4950011		10/04/12
SKYE	PAR SKE510	28557		07/06/13
Sensors without cal				
Seabird	P/N 90402 SBE45 JB	65	Junction Box	
Gill	Windsonic Option 3	071121		

Sensors marked * were fitted prior to D369

3. Scientific Investigations

VM-ADCP

Vessel Mounted ADCP (VM-ADCP) and navigation data

Violetta Paba, Rebecca Green, Simon van Gennip and John Allen

Introduction

The RRS *Discovery* is usually equipped with two hull mounted Ocean Survey broadband ADCPs. An RDI broad band 150 kHz (Ocean Surveyor) phased array style VM-ADCP is mounted in the hull 1.75 m to port of the keel, 33 m aft of the bow at the waterline, at an approximate depth of 5.3 m. A 75 kHz ADCP is also mounted in the hull, in a second well 4.15 m forward and 2.5 m to starboard of the 150 kHz well.

During refit in Amsterdam just prior to cruise D365 it was discovered that the 75 kHz VM-ADCP had significant damage to the transducer phase, possibly caused by barnacle fouling. As watertight integrity of the transducer face was almost certainly threatened, the unit was sent back to RDI in California for repair. Some weeks prior to D369, RDI pronounced the 75 kHz ADCP as irreparable. As the old unit had been sent to RDI in working but damaged condition, it was returned to the ship and refitted whilst alongside in Tenerife. Sadly the transportation and examination of the instrument had conspired to render it unusable with very low beam intensity on 2 or 3 beams. As pointed out strongly in the D365 cruise report, not having a spare transducer on shelf for eventualities like this is a major oversight on the part of the NERC, we will not however labour this point again here.

This section describes the operation and data processing paths for the VM-ADCP. The navigation data processing is described first since it is key to the accuracy of the ADCP current data. All integrated underway data were logged using the Ifremer TechSAS data logging system.. As on D365, Ellett line 2011, the 'live' RVS data format streams have overcome the problem discussed in previous reports of insufficient significant figure resolution in position data using *nclistit*. These live streams do not convert the netcdf format to RVS data format, instead, they log TechSAS broadcast messages independently.

Methods

Navigation

The ship's primary position instrument is a Fugro SeaStar 9200G2 system. The positional accuracy for the SeaStar 9200 system, tested whilst tied up alongside in Tenerife, was ± 0.14 m S.D. For this cruise, our back up system would be the new Ashtech ADU5 3-D gps system, replacing the previous ADU2 system, and whose positional accuracy was in parallel determined to be ± 0.9-1.5 m S.D.

Both the SeaStar 9200 and the Ashtech ADU5 systems have sufficient precision to enable the calculation of ship's velocities to much better than 1 cm s^{-1} over 2 minute ensemble periods and therefore below the instrumental limits (~ 1 cm s^{-1}) of the RDI VM-ADCP systems. Using the Fugro SeaStar 9200G2 system as its primary navigation source, the NMFSS Bestnav combined (10 second) cleaned navigation process was operational and working well on D369.

Navigation and gyro data were transferred daily or twice daily from the RVS format file streams to pstar navigation files, e.g. abnv36901, gpC36901 and gyr36901.

Scripts:

navexec0: transferred data from the RVS *bestnav* stream to PSTAR, calculated the ships velocity, appended onto the absolute (master) navigation file and calculated the distance run from the start of the master file. Output: abnv3691

gyroexec0: transferred data from the RVS *gyro* stream to Pstar, a nominal edit was made for directions between 0-360° before the file was appended to a master file.

gpCexec0: transferred data from the RVS *gps_g2* stream to Pstar, edited out pdop (position dilution of precision) greater than 7 and appended the new 24 hour file to a master file. The master file was averaged to create an additional 30 second file and distance run was calculated and added to both.

Heading

The ship's attitude was determined every second with the ultra short baseline 3D GPS Ashtech ADU5 navigation system. The Ashtech data were used to calibrate the gyro heading information as follows:

ashexec0: transferred data from the RVS format stream *gps_ash* to pstar.

ashexec1: merged the ashtech data from ashexec0 with the gyro data from gyroexec0 and calculated the difference in headings (hdg and gyroHdg); ashtech-gyro (a-ghdg).

ashexec2: edited the data from ashexec1 using the following criteria:

heading	0 < hdg < 360 (degrees)
pitch	-5 < pitch < 5 (degrees)
roll	-7 < roll < 7 (degrees)
attitude flag	-0.5 < attf < 0.5
measurement RMS error	0.00001 < mrms < 0.01
baseline RMS error	0.00001 < brms < 0.1
ashtech-gyro heading	-7 < a-ghdg < 7 (degrees)

The heading difference (a-ghdg) was then filtered with a running mean based on 5 data cycles and a maximum difference between median and data of 1 degree. The data were then averaged to 2 minutes and further edited for

-2 < pitch <2

0 < mrms < 0.004

The 2 minute averages were merged with the gyro data files to obtain spot gyro values. The ships velocity was calculated from position and time, and converted to speed and direction. The resulting a-ghdg should be a smoothly varying trace that can be merged with ADCP data to correct the gyro heading. Diagnostic plots were produced to check this. During ship manoeuvres, bad weather or around data gaps, there were spikes which were edited out manually (plxyed, Figure 12).

Ashtech ADU5 3D GPS coverage was excellent; it was nonetheless surprising that there were no gaps at all in the data stream throughout the cruise.

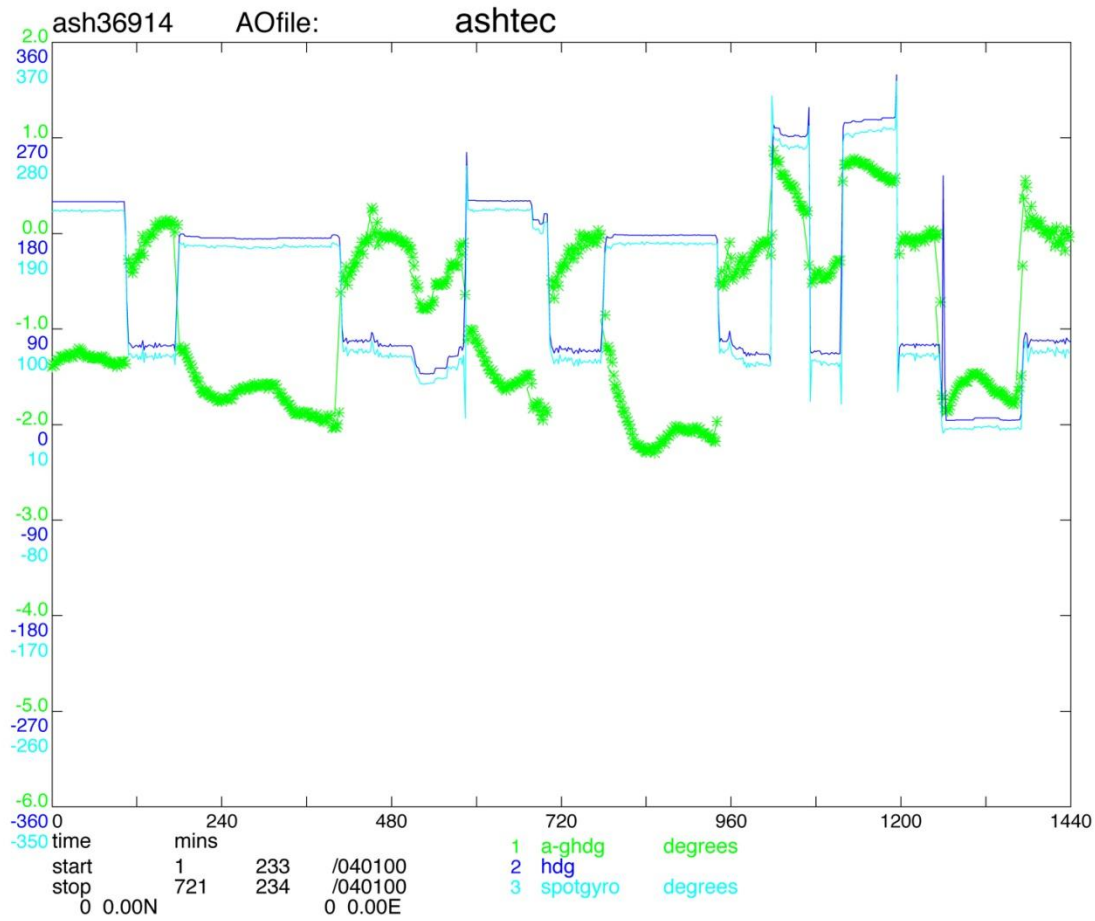

Figure 12: Example of the onscreen output of daily navigation hdg data generated by gyro (light blue line), ashtech ADU5 (dark blue line) and the difference between them (green line).

VMADCP data

This section describes the operation and data processing paths for the VM-ADCP, and closely follows that used on RRS *Discovery* 365 but with a different selection of vertical bin length and number of bins.

150 kHz VMADCP data processing

The RDI Ocean Surveyor 150 kHz Phased Array VM-ADCP was configured to sample over 120 second intervals with 96 bins of 4 m length and a blank beyond transmit of distance of 4 m. The instrument is a broad-band phased array ADCP with 153.6 kHz frequency and a 30° beam angle.

The deck unit had firmware upgrades to VMDAS 23.17 following the March 2008 refit. The controlling PC ran RDI software VmDAS v1.46.

Recent changes to the network COM ports on RRS *Discovery* occurred during the 2010 refit and the following is now applicable for both ADCPs when in operation (Table 5).

Table 5: Changes of COM ports during RRS *Discovery* 2010 refit

COM PORT	**Baud Rate**	**Data Stream**
COM1	9600	ADCP
COM2	4800	NMEA1 ($GPGGA – Position) ($HEHDT – Gyro)
COM3	9600	NMEA2 ($GPPAT – Ashtech)

Gyro heading, and GPS Ashtech heading, location and time were fed as NMEA messages into the serial ports of the controlling PC and VmDAS was configured to use the Gyro heading for co-ordinate transformation. VmDAS logs the PC clock time, stamps the data (start of each ensemble) with that time, and records the offset of the PC clock from GPS time. This offset was applied to the data in the PSTAR processing path, see below, before merging with navigation.

The 2 minute averaged data were written to the PC hard disk in files with a .STA extension, e.g. D369os150001_000000.STA, D369os150002_000000.STA etc. Sequentially numbered files were created whenever data logging was stopped and re-started. The software was set to close the file once it reached 100 MB in size, though on D369 files were closed and data collection restarted typically daily, but twice daily to observe currents carefully during the CTD survey of the anticyclonic eddy, such that the files never became that large. All files were transferred to the unix directory /D369_pstar/os150/raw setup on the ship's new Dell file server. This transfer included the plethora of much larger ping by ping data files, these can be useful in the event of major failure of the ship's data handling systems as they record all the basic navigation and ships heading/attitude data supplied by NMEA message.

The VM-ADCP was configured to run in 'Narrowband' range over resolution mode. Bottom tracking was used when leaving Santa Cruz, Tenerife, and the ship deviated around the island of La Gomera to

obtain a few hours of water depth within bottom tracking range (file 01). At the time of writing it is expected that bottom tracking will be used as we return to port.

The VM-ADCP processing path followed an identical route to that developed in 2001 for the 75 kHz ADCP (RRS *Discovery* cruise 253). In the following script descriptions, "##" indicates the daily file number.

s150exec0: data read into Pstar format from RDI binary file (psurvey2). Water track velocities written into "adp" files, bottom track into "bot" files if in bottom track mode. Velocities were scaled to cm/s and amplitude by 0.45 to db. The time variable was corrected to GPS time by combining the PC clock time and the PC-GPS offset. An offset depth for the depth bins was provided in the user supplied information, 9 m for the 150 kHz instruments, this equated to the sum of the water depth of the transducer in the ship's hull (~5 m in RRS *Discovery*) and the blank beyond transmit distance used in the instrument setup (see earlier). Output Files: (adp369##.raw, bot369##.raw).

s150exec1: data edited according to status flags (flag of 1 indicated bad data). Velocity data replaced with absent data if variable "2+bmbad" was greater than 25% (% of pings where >1 beam bad therefore no velocity computed). Time of ensemble moved to the end of the ensemble period (120 secs added with pcalib). Output files: (adp369##, bot369##).

s150exec2: this merged the adcp data (both water track and bottom track files, where they existed) with the ashtech a-ghdg created by ashexec2. The adcp velocities were converted to speed and direction so that the heading correction could be applied and then returned to east and north. Note the renaming and ordering of variables. Output files: (adp369##.true, bot369##.true).

s150exec3: applied the misalignment angle, ø, and scaling factor, A, discussed below, to both files. Variables were renamed and re-ordered to preserve the original raw data. Output Files: (adp369##.cal, bot369##.cal).

s150exec4: merged the adcp data (both files) with the bestnav (10 sec) NMFSS combined navigation imported to pstar through navexec0 (abnv3691). Ship's velocity was calculated from spot positions taken from the abnv3691 file and applied to the adcp velocities. The end product is the absolute velocity of the water. The time base of the ADCP profiles was then shifted to the centre of the 2 minute ensemble by subtracting 60 seconds and new positions were taken from abnv3691. Output Files: (adp369##.abs, bot365##.abs).

150 kHz VM-ADCP calibration

A calibration of the 150 kHz VM-ADCP was achieved using bottom tracking data available from our departure from Santa Cruz, Tenerife, when the ship deviated around the island of La Gomera to obtain a few hours of water depth within bottom tracking range (file 01). No further calibration was deemed necessary from inspection of the processed data during the cruise. Using straight, steady speed sections of standard two minute ensemble profiles over reasonably constant bottom depth the following calibrations for mis-alignment angle, ϕ, and necessary amplification (tilt), A, were derived by comparing GPS derived component vectors of the vessel speed and direction with processed VM-ADCP bottom track determined component vectors of the vessel speed and direction:

150 kHz:	ϕ	A
mean	-0.6122	1.00811
s.d	± 0.2628	± 0.0027

This was similar to the calibration used on D365.

Results and Discussion

Initial data inspection included absolute velocity vectors at 35 m. Water track files were appended daily, averaged in a 4 km regular grid, and plotted along the ship track. Visual comparison of these plots allowed rough assessment of the data consistency. Altimetry measurements were downloaded daily and compared with ADCP velocities at 35 meters. The detailed location of the various surveys during the cruise was based on this data, which let us focus on an interesting anticyclonic eddy formation centred around 26°45' °N and 31°20' W, and background water masses. The vectors at 35 m were more useful for locating the centre of the eddy, as the resolution of the altimetry measurements turned out to be too coarse to agree well with our *in situ* suite of observations. ADCP current vectors at 35 m were thus used to calculate speed and direction of the eddy, enabling us to predict its displacement for each forthcoming survey (Figure 13).

Once the centre of the eddy was determined, a Lagrangian survey was carried out twice within a more restricted area. Figure 14 shows the relatively lower and random velocities around the centre of the eddy, roughly located at 31.5°W, 26.75°N.

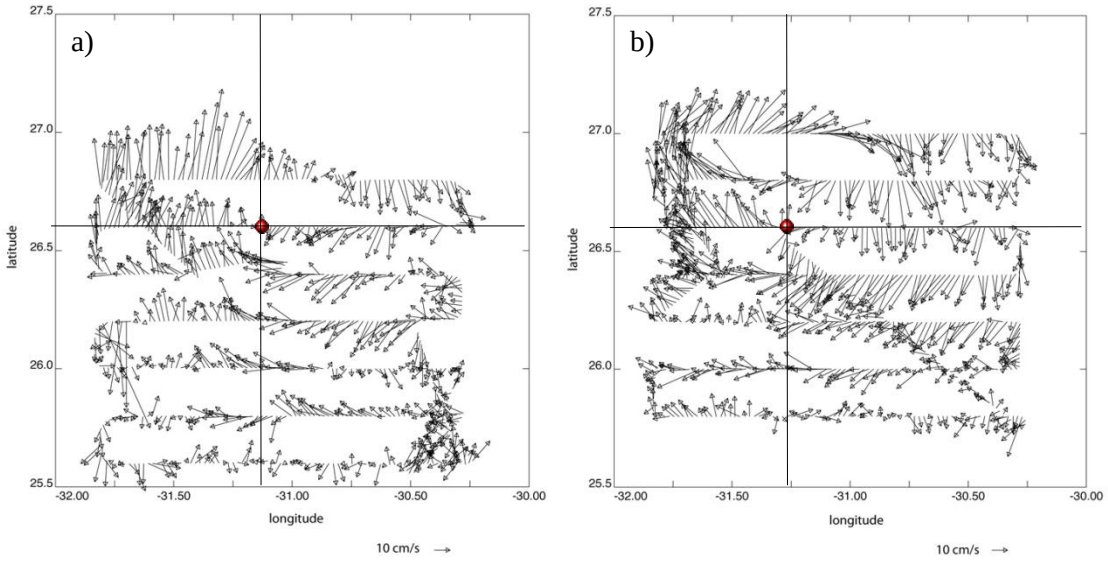

Figure 13: ADCP velocity vectors at 35 meters depth for survey 1 (a) and survey 2 (b). The red dot marks the location of the centre of the eddy, which changes from 31.15°W in survey 1 to 31.25°W in survey 2; thus a westward velocity of 1.3 cm/s was calculated.

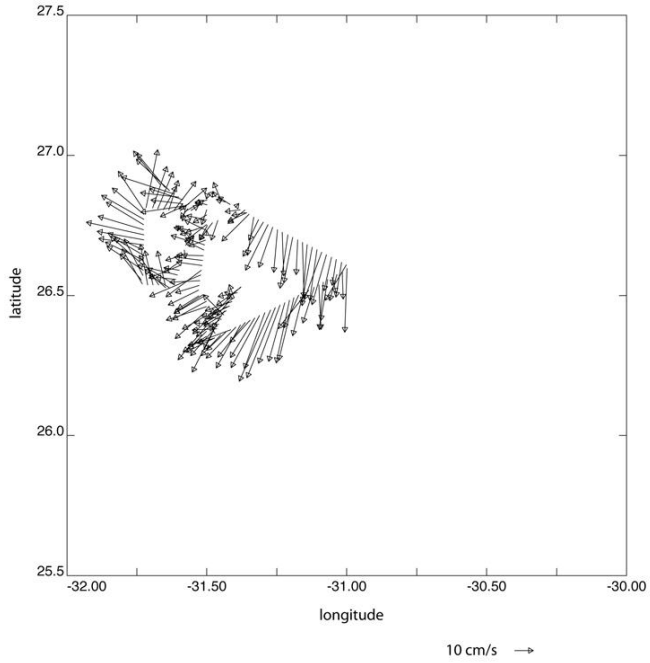

Figure 14: ADCP current vectors at 35 meters depth within the centre of the eddy, roughly located at 31.55°W, 25.58°N. A clockwise rotation can be observed on top of the typical randomness in current velocities found within the centre of anticyclonic eddies.

SeaSoar CTD Data

Rebecca Green, Simon van Gennip, Violetta Paba and John Allen

An overall objective of D369 under Oceans2025, was to investigate the mechanism for nutrient transport to the upper ocean in the North Atlantic subtropical gyre. To achieve spatial 'mapping' of an inevitably small but tangible area of this vast biome, an approximately 150x150km box was selected, centred around 26°30 N 31°00 W, so as to be away from the influence of topographic features and the subtropical convergence zone.

Three SeaSoar surveys of the area were planned; each four consecutive days long, separated by four day CTD surveys. Each SeaSoar survey was made up of approximately eight SeaSoar tows, separated by the requirement to carry out two water sampling CTDs a day. Each leg was ~150 km long conducted in a 'radiator pattern,' with the first two surveys in an east to west direction, the first starting in the southeast corner and the second in the northwest corner. The third and final survey was carried out in a north to south direction, such that any later interpretation would not have any systematic bias due to the anisotropic sampling resolution.

The deployment of SeaSoar for SeaSoar survey 1 started on 14/08/2011 but the first few tows were rather unsuccessful with shallow or no profiles as it proved difficult to obtain a stable flight pattern with the SeaSoar vehicle with its initial instrument layout. Having removed much of the external instrumentation, discussed later, the first survey continued at leg 2 (see Table 6 detailing individual final gridded SeaSoar surveys).

The SeaSoar instrument package is still centred around a Chelsea Technologies Group (CTG) minipack CTDf, as it has been since 2001 (Allen, *et al.*, 2002). The first survey deployment used Minipack S/N 210035 CTD unit, which produced excellent temperature and conductivity results for most of the survey. However, this started to fail towards the end of the final leg; something appeared to happen to the electronics and the conductivity sensor started drifting rapidly. It deteriorated until there was such a considerable pressure hysteresis such that the last few profiles were not recoverable. Luckily this was when the SeaSoar was being towed back towards the centre of the survey area, and did not affect the survey to a great extent. However, later analysis showed that the fluorimeter in the minipack had developed a serious pressure hysteresis, strangely only apparent generally on downcasts, about half way through the survey. This required significant editing out of downcasts.

Prior to the second SeaSoar survey, different Minipack CTD-f systems were tested. Minipack S/N 210011 was used for one test tow and produced badly offset T/S profiles. It was established to be at least 0.4 PSU high in conductivity; an offset which furthermore had some non-linearity. Minipack

S/N 210039 was trialled on another test tow. Unfortunately there was clearly a significant offset between the undulations, which required a time delay correction in excess of 1.5 seconds and resulted in an unacceptably noisy salinity profile. Minipack S/N 210019 produced an on deck temperature 10°C higher than ambient temperature and was thus not deployed. Finally, Minipack S/N 04-4350-003 was tested on JDay 236; this produced barely acceptable results but had to be used for Surveys 2 and 3. There was still a large offset apparent of 0.4-0.6PSU, and some non linearity – similar to S/N 210011.

Data

The 'C21' SeaSoar system (Allen, *et al.*, 2002), used for the first time on D253 (May/June 2001), carries a Chelsea Technologies Group (CTG) Minipack CTDF (Conductivity, Temperature, Depth and Fluorescence) instrument which is considerably more compact than CTD instruments traditionally carried by the SeaSoar vehicle. A substantial payload space is available in the SeaSoar for a multidisciplinary suite of additional instruments. Prior to RRS *Discovery* cruise D369, the SeaSoar vehicle had been prepared to carry the (NOC/Valeport) SUV-6 UV Nutrient Sensor, a PAR sensor, a Brooke Ocean laser optical plankton counter (LOPC), a second generation CTG Fast Repetition Rate Fluorometer (FRRFII), two oxygen sensors, three further fluorometric pigment sensors, a backscatter sensor and a bioluminescence sensor.

Prior to the first survey, the SeaSoar vehicle could not be flown correctly which resulted in the first survey being carried out with neither the fluorometric pigment sensors, the backscatter sensor, nor the LOPC. This was a surprise as this payload had been successfully flown the previous year, during *Discovery* D351. Between surveys 1 and 2, a new position was found for the optical sensors on the top of the body of the vehicle, and these were replaced. However, sadly the LOPC was never put back on the vehicle as tests had shown that it was not possible to get the required depth range out of the vehicle with this setup, despite having been achieved a year earlier. This was a great shame, and the dynamics of the vehicle will need to be considered again in the future.

During SeaSoar deployments data were recovered, in real time, from the PENGUIN data handling system on SeaSoar. In the case of the MiniPack and SUV-6 instruments the files were buffered for transfer in PENGUIN and the master data files were recorded on the EMPEROR Linux PC in the main lab. For the FRRFII, the freely available software ‘socat’ was used to provide a virtual RS232 link bridging the instrument to its parent software on a dedicated PC laptop in the main lab: all the EMPEROR and PENGUIN data handling is discussed in detail in the technical support section. Thus data were logged in three types of file, two DAPS files, one containing the CTDF measurements and

its associated additional analogue channels, the second containing the SUV-6 UV Nutrient Sensor data, and the proprietary PC files for the FRRFII. The FRRFII and SUV-6 UV Nutrient Sensor data were not dealt with during the cruise and will not be mentioned further in this report.

All of the variables output by the MiniPack CTDF were calibrated using pre-set calibrations stored in the instrument firmware. The sensors are sampled in the MiniPack at 16 Hz, but the data are 1 Hz averaged prior to the output data stream from the MiniPack. The variables output were:

- Conductivity ($mScm^{-1}$)
- Temperature (°C)
- Pressure (dbar)
- ΔT ($°Cs^{-1}$), temperature change over the one second averaging period.
- Chlorophyll (mgm^{-3})

Each of these were output at one second intervals and a time/date stamp was added by the DAPS handling software on PENGUIN. The time rate of change of temperature, ΔT ($°Cs^{-1}$), is the difference between the first and the last sample in the one second average of temperature. In addition to the MiniPack fluorimeter, the SeaSoar package was fitted with three Turner Designs CYCLOPS-7 Submersible Fluorometric instruments, PN2100-000 with sensors as detailed below:

Turner Designs chlorophyll sensor, serial no. 2100432
Turner Designs Phycoerythrin sensor, serial no. 2100594
Turner Designs CDOM "U" sensor, serial no. 2100595

Sadly these provided little useful data during the cruise; it is presumed that they are not sensitive enough for such extreme oligotrophic waters. These were connected to the MiniPack analogue instrument channels, as were a Seabird SBE43 oxygen sensor, an Anderraa Optode oxygen sensor, a PAR sensor, a CTG backscatter sensor, and a CTG GlowTracker bioluminescence sensor. Sadly the SBE-43 oxygen sensor failed to produce any useful data on the SeaSoar during this cruise. It is believed that something about the SeaSoar attitude in the water resulted in little flow of water through the tubing heading to the sensor head. Its successful use 3 years earlier, on D321, in the same configuration may have been just fortuitous: in the future this unit probably needs to be pumped.

Table 6: Final gridded survey SeaSoar legs during D369.

Survey 1	**Start date**	**Start datacycle**	**Stop Date**	**Stop datacycle**	**Total leg distance run/km**		
	227	**1**	**230**	**8619**	**882**		
Leg	**Start Day**	**Start datacycle**	**Stop Day**	**Stop datacycle**	**Distance run/km**		
					Start	**End**	**Total**
2	227	1	227	663	2072	2144	72
3	227	1225	228	2652	2216	2378	162
4	228	2704	228	4131	2384	2552	168
5	229	4183	229	5610	2564	2732	168
6	229	5713	230	7089	2750	2906	156
7	230	7192	230	8568	2924	3080	156
Survey 2	**Start date**	**Start datacycle**	**Stop Date**	**Stop datacycle**	**Total leg distance run/km**		
	236	**1**	**241**	**12036**	**1080**		
Leg	**Start Day**	**Start datacycle**	**Stop Day**	**Stop datacycle**	**Distance run/km**		
					Start	**End**	**Total**
8	236	1	237	1428	4530	4668	138
7	237	1480	237	2905	4680	4842	162
6	237	3061	238	4437	4866	5022	156
5	238	4449	238	5814	5028	5184	156
4	239	6529	239	7905	5274	5430	156
3	239	8008	240	9333	5448	5598	150
2	240	9538	240	10965	5628	5790	162
Survey 3	**Start date**	**Start datacycle**	**Stop Date**	**Stop datacycle**	**Total leg distance run/km**		
	246	**1**	**251**	**11730**	**1092**		
Leg	**Start Day**	**Start datacycle**	**Stop Day**	**Stop datacycle**	**Distance run/km**		
					Start	**End**	**Total**
A	246	52	247	1428	6906	7062	156
B	247	1531	248	2958	7080	7242	162
C	248	3100	248	4420	7260	7416	156
D	248	4550	249	5910	7434	7590	156
E	249	6070	249	7395	7614	7764	150
F	249	7549	250	8900	7788	7944	156
G	250	9100	250	10440	7968	8124	156

Processing steps

During the CTD surveys there were 2 CTD stops per day; one in the early morning and one in the evening. These provided a natural break for processing individual DAPS files. The following processing route was carried out twice a day:

pgexec0: Reads raw DAPS data into a PSTAR recognisable format, sets up the data name and header information. Copies time from Julian Day to seconds – N.B DAPS time is not necessarily an integer value of seconds thus sporadically two records may appear to have the same time. Through using the –square command in ptime, this situation is avoided.

pgexec1: Using output from pgexec0, simple editing and calibration of the data is performed. The time constant is the only calibration constant required, defined by the user according to the quality of the SeaSoar data in T/S diagrams. A number of steps were involved, primarily:

- Temperature correction applied using *pcalc*
- Interpolation of pressure to remove absent data, using *pintrp*
- Salinity and density calculated with *peos83*

plpred: This interactive pstar editor was used in order to remove the worst downcast spikes for the fluorimeter and salinity measurements. The first survey had significant spiking to remove. This was due to the high SeaSoar dive rates, which had been tuned out of the flight profile by the time of surveys two and three.

pgexec3: A new exec used to calculate the oxygen saturation, apply the SeaBird SBE-43 oxygen calibrations in stages, and perform a quick Optode A calibration. Oxygen calibration will be discussed later.

pgexec4: A new exec to correct the non-linear salinity offsets experienced during surveys 2 and 3: to be discussed later.

pgexec5: A final new exec to calibrate Optode oxygen data according to a known correction for temperature and salinity, and a significant pressure correction to bring it into line with the CTD oxygen sensor.

pcopya/plpred: Bad data was cropped out by selectively picking out datacycle numbers and deleting the records at these points. This was particularly significant at the surface, when the SeaSoar was being deployed and recovered.

pgrids: A single, gridded file was produced for each survey of geolocated (merged with navigation data), interpolated data. A 6km x 8dbar regular gridding format was used for all three surveys.

peos83: Used following pgrids in order to recalculate potemp and sigma0, using pressure, temperature, and salinity.

Temperature correction

There is a small delay in the response of the temperature sensor, which must be corrected for two reasons. Primarily, to obtain the correct temperature corresponding to conductivity measurements, to make accurate calculations of salinity. Moreover, to obtain an accurate determination of temperature for points in space and time.

Surprisingly, according to the Minipack users manual, the time response of the temperature and conductivity cells should be taken into account by the electronics in the CTDf unit. However, experience has shown this is rarely found to be the case. A lag in temperature is apparent in the data in two ways. There is a difference between up and down profiles of temperature (most apparent in the derived salinity) because the time rate of change of temperature has opposite signs on the up and down casts. The second manifestation is the "spiking" of salinity as the sensors traverse maxima in the gradients of temperature and salinity. The rate of ascent and descent of SeaSoar is greater (up to 2-4 ms^{-1} at the beginning of descent and ascent) than that of a lowered CTD package, thus the effects of the temperature lag are more pronounced. Thus, the following correction was applied to the temperature during ***pgexec1*** before evaluating the salinity

$$T_{corr} = T_{raw} + \tau.\Delta T$$

where ΔT is the temperature difference over the CTDs one second averaging interval, and output as a variable, τ is the time constant, normally set to some significant fraction of the one second averaging interval.

The best value of τ was chosen so as to minimise the difference between up and down casts and noise in the salinity profile.

Due to the minipack variation and drift, the best values for τ changed both between and within surveys. This value was altered according to the drift on T-S plots.

Initially the best value in survey 1 was found to be τ = 0.18 seconds, but this had to be changed to 0.37 seconds on the second tow, which lasted until the minipack had sensor failure halfway through the last tow.

Survey 2, using Minipack S/N 04-4350-003 had a fairly constant τ of 0.7 seconds until midway through the survey, when a fit of 0.8 seconds appeared more suitable. The final survey started with a best fit τ of 0.5 seconds, finishing with a value of 0.6 seconds to provide the cleanest profiles and the best fit between up and down undulations.

Table 7: Salinity calibration.

Survey	**Pstar file number**	**Offset applied**	**Non-linearity correction made? (see text)**	**Value**
1 *Master File: ss3691.distoxcor Gridded File: gr3691*	sa369001	-0.020	×	-
	sa369002	-0.020	×	-
	sa369003	-0.020	×	-
	sa369004	-0.020	×	-
	sa369005	-0.020	×	-
	sa369006	-0.020	×	-
	sa369007	-0.020	×	-
2 *Master File: ss3692.distoxcor Gridded File: gr3692*	sa369008	-0.415	✓	0.00836
	sa369009	-0.440	✓	0.00836
	sa369010	-0.450	✓	0.00836
	sa369011	-0.450	✓	0.00836
	sa369012	-0.450	✓	0.00836
	sa369013	-0.450	✓	0.00836
	sa369014	-0.465	✓	0.00836
	sa369015	-0.465	✓	0.00836
3 *Master File: ss3693.distoxcor Gridded File: gr3693*	sa369016	-0.555	✓	0.006
	sa369017	-0.560	✓	0.006
	sa369018	-0.555	✓	0.006
	sa369019	-0.565	✓	0.006
	sa369020	-0.555	✓	0.006
	sa369021	-0.575	✓	0.006
	sa369022	-0.555	✓	0.006
	sa369023	-0.555	✓	0.006
	sa369024	-0.575	✓	0.006
	sa369025	-0.575	✓	0.006

Salinity calibration

Calibration of Minipack CTD data was clearly necessary and so comparison was made with the well constrained T/S profiles from the traditional vertical CTD stations.

Survey 1 had a relatively stable and modest offset from the CTD stations, however the Minipack S/N 04-4350-003 used in surveys 2 and 3 had a large and non-linear offset in derived salinity. This non-linearity required correction through a cubic relationship to measured salinity and thus calibration of the salinity data for surveys 2 and 3 took the form:

Corrected salinity = ((salinity – 36)^3 * non linearity constant) – offset constant) + salinity (Table 7)

Having applied these calibrations, we assess there to be a 0.0 offset in salinity between the SeaSoar and CTD platforms, to an accuracy of ~ 0.02 psu. Whilst lower than the 0.01 accuracy we would normally strive for, this is mainly a result of the very strong vertical salinity and temperature gradients, typical of the North Atlantic subtropical gyre.

Fluorescence calibration

The fluorescence values for the SeaSoar towed vehicle were compared to the CTD measurements in order to obtain a suitable cross-calibration between platforms.

A quadratic formula was obtained by comparing the magnitude and size of corresponding fluorescence peaks of each survey, between SeaSoar and CTD profiles. These gave us the following calibrations that were subsequently applied to the SeaSoar raw fluorescence data.

Survey 1:

Fluorescence = -0.0019 fluorescence ^{2} + 0.0521 fluorescence + 0.0131

Survey 2:

Fluorescence = -0.0005 fluorescence ^{2} + 0.051 fluorescence + 0.0698

Survey 3:

Fluorescence = -0.0005 fluorescence ^{2} + 0.051 fluorescence + 0.0698

As discussed earlier, from about halfway through survey 1 the fluorimeter component of the Minipack CTDf S/N 210035 showed a strange but significant pressure hysteresis, principally on downcasts. This resulted in the requirement to delete most of the downcast fluorimeter data from this point. No similar problems were encountered with Minipack S/N 004-4350-003.

Oxygen optode calibration

A time lag in the Optode A (oxygen raw voltage) was clearly apparent between up and down profiles. Oxygen was accelerated in time by 10 seconds in a separate file, and then combined back into the master file.

The Anderra oxygen sensor provides a dissolved oxygen measurement based on an optical method. An initial calibration was carried out using the new script *pgexec3,* this applies the equation:

OPoxygen(μmol/L) = (Optode A (volts) / Optode A (100%)) * oxygen solubility

The oxygen solubility for the given temperature and salinity is also calculated in *pgexec3* using the standard equation of state. The Optode A voltage at 100% saturation was previously determined, by examining values between 3 and 5 m, as approximately 2.37 volts for all 3 surveys.

Following this initial rough calibration, suitable for early plotting, *pgexec5* was run to further calibrate firstly for the known effects of temperature and salinity on the Optode sensor oxygen measurements, and secondly for the previously unknown effects of pressure on a rapidly towed undulating platform such as SeaSoar.

The first calibration stage in *pgexec5*, for temperature and salinity effects, was taken from (Thierry, *et al.*, 2011):

$$OPoxyTS = OPoxygen * \exp(\text{measured salinity} - S0) * (B0 + B1T_s + B2\,T_s^2 + B3\,T_s^3) + C0(S^2 - S0^2)$$

Where S0= 35.0, B0 = -6.24097E-3, B1 = -6.93498E-3, B2 = -6.90358E-3, B3 = -4.29155E-3, CO = -3.11680E-7

T_s was calculated = $\ell n \frac{298.15-T}{273.15+T}$ where T is measured minipack CTDf temperature, °C.

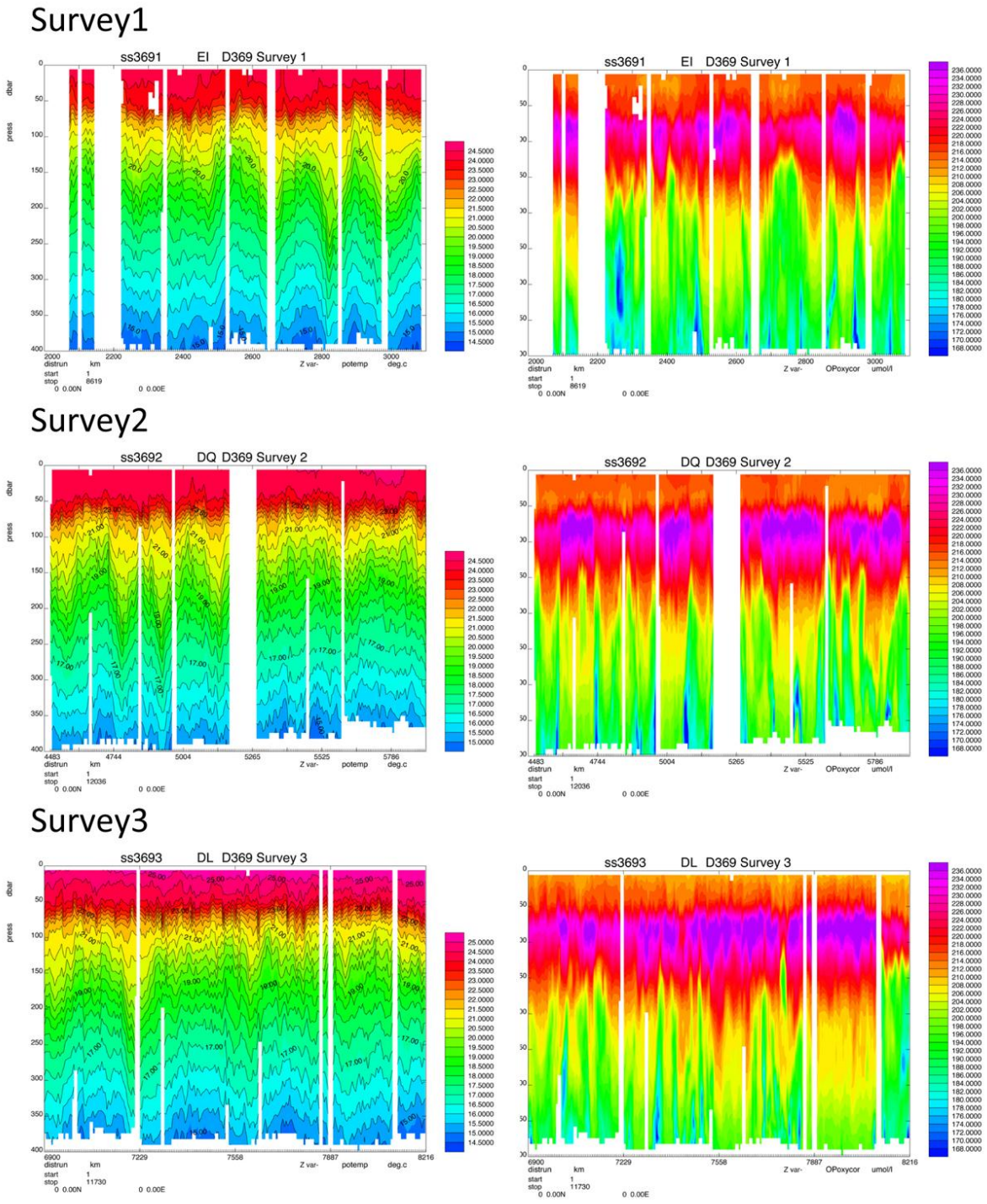

Figure 15: Potential temperature (°C) and oxygen (µmol/L) contoured against distance run for all SeaSoar surveys 1, 2 & 3 showing fine scale mesoscale variability.

The second stage for pressure compensation in *pgexec5* took the form:

$$\text{OPoxycor} = \text{OPoxyTS}\ \left(1 - A * \frac{pressure}{1000}\right)$$

The oxygen profiles from the Optode sensor on the SeaSoar vehicle, and the SeaBird SBE-43 on the CTD frame were carefully compared, both as potential temperature/oxygen profiles, and oxygen/pressure profiles. It was clear from this comparison that there was a significant pressure effect on the optode oxygen value. This may well be unique to its application on a rapidly undulating towed vehicle like SeaSoar. By comparison of the CTD and SeaSoar oxygen data, a simple linear pressure correction term, as given above, seemed most appropriate at this stage. For all 3 surveys, a value of A of 0.362 bought the SeaSoar oxygen in line with the CTD to an estimated accuracy of better than 5μmol/L, we consider this a good achievement for a vehicle such as SeaSoar (Figure 15).

Finally, following oxygen calibration against bottle values for the SeaBird SBE-43 sensor on the stainless steel CTD, described in the appropriate section of this report, we then offset the SeaSoar oxygen data for all 3 surveys by +7.0 μmol/L.

Echosounder

Violetta Paba, Rebecca Green, Simon van Gennip and John Allen

RRS *Discovery* is equipped with a SIMRAD EA500 Echosounder system. Acoustic bathymetry measurements were made for the majority of the cruise using a 10 kHz transducer in the Precision Echosounder (PES) 'fish' suspended over the port side of the ship. The PES provides a quieter environment for determining water depth in deep water and poor sea states than the alternative hull mounted transducer. The output from the echosounder was displayed on two SIMRAD VDU's located in the main lab. The speed of sound in the water was set to 1500 m/s.

The raw data was logged by techsas and provided as an RVS format *ea500* datastream containing values of uncorrected depth and time. In addition, the *ea500* data-stream was processed on the NMFSS workstations. A correction was made, taking into account the variability of sound speed in water, 'Carter' tables, and errors in the data-stream were visually identified and removed. A new off-line data-stream named *prodep* was created, and appended to every 24 hours, containing uncorrected depth, corrected depth and measurement time.

During the cruise, these data-streams were then processed as follows every 24 hours using a series of UNIX shell scripts utilising PEXEC routines.

simexec0: This script took the raw data from the two data streams creating files of raw data (*sim369nn*) and corrected data (*sim369nn.cal*).

simexec1: This script edited out depths less than 10 m in both the raw and corrected depth files in order to remove anomalous zero values.

Every 24 hours, corrected files (*sim369nn.cal*) were *papend* 'ed to a master bathymetry file *sim369tot*, and a 5 minute averaged file was also created, using *pavrge* (*sim3691.5 min*), from which header depths were obtained in the routine CTD processing.

Turbulence Profiles

Simon van Gennip, Violetta Paba, Rebecca Green and John Allen

Dedicated turbulence profiling was conducted during the last two CTD surveys. To gain a more complete and contrasting picture of the oligotrophic gyre, a dynamically active region was targeted for survey 1 and a much less active one was chosen for survey 2.

Our primary instruments were a pair of MSS90L free-fall microstructure profilers (Sea and Sun Technology GmbH and ISS Wassermesstechnik). The profiler is cylindrical in shape with two PNS shear probes and several other sensors (including conductivity, fast response temperature and pressure, and the more recent probe was also fitted with a fluorimeter). The shear probes make direct measurements of cross-axial velocity fluctuations using a piezoceramic beam. Data from the sensors are recorded continuously on a PC laptop, connected to the descending profiler via a slack tether and winch system. Turbulence in the ocean has pronounced spatial and temporal variation. To obtain robust estimates of dissipation rates, each station required a series of 5 or more profiles taken over the course of 1.5 h. The manufacturers' recommended drop speed of approximately 0.5 ms^{-1} is a compromise which allows profiles to be taken as rapidly as possible whilst minimising noise effects. Carrying fast response conductivity and temperature sensors allows the mapping of turbulence in parallel with microstructure in the upper water column hydrography.

The turbulence profiler was deployed on station generally following the main CTD cast; around 6 profiles were carried out each time. Midday and midnight CTD stations were chosen as we expected these to have the greatest contrast in terms of air/sea exchanges of heat.

Two different profilers were used, the MSS035 and the new MSS050. The MSS profilers were equipped with the following sensors:

- 2 velocity microstructure shear sensors (PNS-3 SHE 6086 and SHE 6092 for MSS035, PNS-6 SHE 098 and SHE 099 for MSS050)
- a microstructure temperature sensor (NTC),
- standard CTD sensors for precision measurements (PRESS, TEMP, COND),
- a turbidity (light scattering) sensor (MSS035 only), a fluorimeter (MSS050 only), a vibration control sensor (ACC),
- a two component tilt sensor (TILTX, TILTY) and surface detection sensor (SD)

Two shear sensors are fitted on the MSS profilers to provide both duplicate measurements, and provide a comparison in case of failure of a sensor (mode of failure is generally a lack of sensitivity).

During the first three stations (053, 055, 057) the new MSS050 was used. Communications problems that didn't seem to occur with the old profiler MSS035, forced us to swap to this instrument. These problems eventually re-occurred and were traced to the tether cable termination, which was duly rebuilt by Jon Short.

However, during CTD station 063, the MSS035 shear sensor 6086 (SHE 1) showed increasingly suspect variability compared with shear sensor 6092 (SHE2). As a result, the profiler was replaced once more with the new one (MSS050) which worked well for the remainder of the cruise. It should be noted that further communications problems occurred from time to time, these were all traced to poor USB connections with either the laptop or the blue profiler interface unit.

Data Acquisition

During the deployments, conducted with the winch located at the port stern side of the ship, the ship speed was held at 0.3-0.5 knots where possible. We aimed at obtaining profiles to around 350 m, close to the pressure limit for the syntactic buoyancy rings that act to control the profiler's free fall speed. In order to do so, the profiler was deployed to a depth of approximately 270 m with a few turns of slack cable always visible in the water. When the profiler reached 270 m, the winch operator would be instructed to begin hauling in the tether cable, and the profiler normally reached a depth of around 310 to 390 m depending on how much slack cable had been paid out, and vessel speed. The sea state was calm all cruise and provided optimal conditions for deployment.

A list of profiles is given in Table 8. No significant data processing was completed on board, although one or two early casts were processed in order to get diagnostic information from Alex Forryan, back at the NOCS.

Lowered ADCP

The stainless steel CTD frame was fitted with a 1200 KHz downward looking Acoustic Doppler current profiler (LADCP) and a 600 KHz upward looking LADCP. In the future, the data from these ADCPs are expected to enable us to estimate high frequency turbulence dissipation rates by the inertial dissipation method, and will allow us to further investigate the use of a second order structure function technique to look at the variation in measured velocity fluctuations between points along the acoustic beams; two independent estimates of turbulent dissipation to support the direct shear probe measurements.

Table 8: Summary of turbulence profiles.

CTD cast number	Profiler	Folder	Jday	Lat (deg:min)	Lon (deg:min)	Start cast (GMT)	Filename (rawdata .mrd)	Max depth (m)	Comments
369005	MSS035	MSS035/D369005	226	25 38.83 N	030 18.87 W	10:13:10	D3690001	280	MSS035 she1 6086, she2 6092 (not 6091 as recorded in file)
		MSS035/D369005		25 39.02 N	030 18.82 W	10:28:11	D3690002	298	
369052	MSS035	MSS035/D369052	241	26 36.40 N	031 20.59 W	14:39:40	D3690003	301	MSS035 she2 6086, she2 6092
		MSS035/D369052		26 36.35 N	031 20.62 W	14:49:50	D3600004	370	
		MSS035/D369052		26 36.25 N	031 20.64 W	15:05:16	D3690005	317	
		MSS035/D369052		26 36.15 N	031 20.61 W	15:18:24	D3690006	320	
		MSS035/D369052		26 36.06 N	031 20.60 W	15:31:05	D3690007	327	
		MSS035/D369052		26 35.95 N	031 20.60 W	15:44:10	D3690008	347	
	MSS050	MSS050/D369052		26 35.11 N	031 20.62 W	17:09:36	D3690001	345	MSS050 she1 098, she2 099
		MSS050/D369052		26 34.98 N	031 20.60 W	17:24:38	D3690002	307	
369053	MSS050	MSS050/D369053	242	26 26.49 N	031 17.17 W	01:29:13	D3690003	325	
		MSS050/D369053		26 26.39 N	031 17.08 W	01:44:22	D3690004	330	
		MSS050/D369053		26 26.26 N	031 17.07 W	01:58:55	D3690005	329	
		MSS050/D369053		26 26.18 N	031 16.99 W	02:14:55	D3690006	319	
		MSS050/D369053		26 25.99 N	031 16.95 W	02:30:11	D3690007	311	
		MSS050/D369053		26 25.83 N	031 16.90 W	02:42:45	D3690008	348	

CTD cast number	Profiler	Folder	Jday	Lat (deg:min)	Lon (deg:min)	Start cast (GMT)	Filename (rawdata .mrd)	Max depth (m)	Comments
369055	MSS050	MSS050/D369055	242	26 48.32 N	031 22.42 W	13:41:12	D3690009	327	Comms, break down, tried MSS035, same problem
369057	MSS050	MSS050/D369057	243	26 42.39 N	031 29.82 W	01:42:25	D3690010	335	Re-termination has cured comms problems
		MSS050/D369057		26 42.42 N	031 29.75 W	01:56:06	D3690011	322	
		MSS050/D369057		26 42.46 N	031 29.61 W	02:09:47	D3690012	323	
		MSS050/D369057		26 42.51 N	031 29.46 W	02:23:10	D3690013	327	
		MSS050/D369057		26 42.55 N	031 29.34 W	02:37:07	D3690014	324	
		MSS050/D369057		26 42.56 N	031 29.23 W	02:51:35	D3690015	260	cast aborted
		MSS050/D369057		26 42.56 N	031 29.04 W	03:09:59	D3690016	170	cast aborted
369059	MSS035	MSS035/D369059	243	26 34.57 N	031 02.32 W	13:28:21	D3690009	355	MSS035 she2 6086, she2 6092
		MSS035/D369059		26 34.52 N	031 02.22 W	13:43:45	D3690010	346	
		MSS035/D369059		26 34.50 N	031 02.14 W	14:00:24	D3690011	355	
		MSS035/D369059		26 34.45 N	031 02.07 W	14:15:11	D3690012	357	
		MSS035/D369059		26 34.37 N	031 02.00 W	14:30:34	D3690013	130	cast aborted
		MSS035/D369059		26 34.32 N	031 01.96 W	14:40:00	D3690014	362	
		MSS035/D369059		26 34.22 N	031 01.19 W	14:55:13	D3690015	361	
369061	MSS035	MSS035/D369061	244	26 29.73 N	031 24.21 W	01:42:00	D3690016	312	

CTD cast number	Profiler	Folder	Jday	Lat (deg:min)	Lon (deg:min)	Start cast (GMT)	Filename (rawdata .mrd)	Max depth (m)	Comments
		MSS035/D369061		26 29.91 N	031 24.25 W	01:56:34	D3690017	250	comms failure at 250 m
		MSS035/D369061		26 30.02 N	031 24.30 W	02:11:24	D3690018	318	
		MSS035/D369061		26 30.00 N	031 24.35 W	02:24:51	D3690019	330	Shear 1 looked bad (great variability) till past 100 m
		MSS035/D369061		26 30.15 N	031 24.41 W	02:38:09	D3690020	312	Shear 1 again as above
		MSS035/D369061		26 30.22 N	031 24.42 W	02:49:36	D3690021	352	Shear 1 went crazy 70-90 m, and shear two noisy
		MSS035/D369061		26 30.31 N	031 24.47 W	03:04:43	D3690022	357	Shear 1 only this time between 50 and 100 m
369063	MSS035	MSS035/D369063	244	26 47.76 N	031 21.41 W	13:36:28	D3690023	351	
		MSS035/D369063		26 47.86 N	031 21.44 W	13:50:35	D3690024	350	
		MSS035/D369063		26 47.98 N	031 21.50 W	14:05:00	D3690025	353	high variability in she1, only top 100 m , << previous night
		MSS035/D369063		26 48.10 N	031 21.57 W	14:20:00	D3690026	351	
		MSS035/D369063		26 48.18 N	031 21.66 W	14:33:55	D3690027	355	she1 increasingly suspect
		MSS035/D369063		26 48.23 N	031 21.74 W	14:49:00	D3690028	367	
369065		MSS035/D369065	245	26 31.74 N	031 22.72 W	01:28:38	D3690029	341	

CTD cast number	Profiler	Folder	Jday	Lat (deg:min)	Lon (deg:min)	Start cast (GMT)	Filename (rawdata .mrd)	Max depth (m)	Comments
		MSS035/D369065		26 31.69 N	031 22.68 W	01:43:35	D3690030	334	
		MSS035/D369065		26 31.64 N	031 22.64 W	01:58:02	D3690031	330	
		MSS035/D369065		26 31.62 N	031 22.59 W	02:12:50	D3690032	331	
		MSS035/D369065		26 31.62 N	031 22.54 W	02:26:30	D3690033	319	
		MSS035/D369065		26 31.64 N	031 22.43 W	02:40:52	D3600034	338	
369067	MSS050	MSS050/D369067	245	26 31.65 N	031 38.88 W	13:31:15	D3690017	383	MSS050 she1 098, she2 099
		MSS050/D369067		26 31.65 N	031 38.84 W	13:46:57	D3690018	357	
		MSS050/D369067		26 31.67 N	031 38.88 W	14:03:56	D3690019	295	
		MSS050/D369067		26 31.86 N	031 38.79 W	14:16:19	D3690020	372	
		MSS050/D369067		26 31.99 N	031 38.80 W	14:31:00	D3690021	334	
		MSS050/D369067		26 32.10 N	031 38.83 W	14:43:27	D3690022	343	
369068	MSS050	MSS050/D369068	245	26 48.64 N	031 43.60 W	22:16:32	D3690023	330	
		MSS050/D369068		26 48.69 N	031 43.54 W	22:30:06	D3690024	355	
		MSS050/D369068		26 48.71 N	031 43.47 W	22:45:17	D3690025	350	
		MSS050/D369068		26 48.74 N	031 43.37 W	23:00:00	D3690026	355	
		MSS050/D369068		26 48.78 N	031 43.26 W	23:14:30	D3690027	356	
		MSS050/D369068		26 48.82 N	031 43.13 W	23:30:06	D3690028	384	
369082	MSS050	MSS050/D369082	252	25 53.68 N	031 29.72 W	15:13:55	D3600029	363	
		MSS050/D369082		25 53.65 N	031 29.62 W	15:29:14	D3690030	347	

CTD cast number	Profiler	Folder	Jday	Lat (deg:min)	Lon (deg:min)	Start cast (GMT)	Filename (rawdata .mrd)	Max depth (m)	Comments
		MSS050/D369082		25 53.64 N	031 29.55 W	15:43:13	D3690031	349	trying to follow calm Langmuir lead
		MSS050/D369082		25 53.82 N	031 29.52 W	15:58:30	D3690032	329	staying in a lead
		MSS050/D369082		25 53.98 N	031 29.48 W	16:13:30	D3690033	329	
		MSS050/D369082		25 54.16 N	031 29.40 W	16:29:12	D3690034	342	
369084	MSS050	MSS050/D369084	253	25 34.06 N	031 29.79 W	01:21:21	D3690035	312	
		MSS050/D369084		25 34.06 N	031 29.69 W	01:35:05	D3690036	341	
		MSS050/D369084		25 34.06 N	031 29.62 W	01:49:36	D3690037	357	
		MSS050/D369084		25 34.05 N	031 29.59 W	02:04:50	D3690038	353	
		MSS050/D369084		25 34.04 N	031 29.52 W	02:20:05	D3690039	320	
		MSS050/D369084		25 34.04 N	031 29.31 W	02:34:51	D3690040	323	
369086	MSS050	MSS050/D369086	253	25 53.48 N	031 29.77 W	13:59:42	D3690041	373	
		MSS050/D369086		25 53.50 N	031 29.61 W	14:15:34	D3690042	366	
		MSS050/D369086		25 53.48 N	031 29.48 W	14:30:31	D3690043	371	
		MSS050/D369086		25 53.45 N	031 29.35 W	14:46:40	D3690044	395	
		MSS050/D369086		25 53.45 N	031 29.24 W	15:03:30	D3690045	368	
		MSS050/D369086		25 53.41 N	031 29.07 W	15:19:08	D3690046	352	
369088	MSS050	MSS050/D369088	254	25 34.60 N	031 31.48 W	01:33:25	D3690047	315	wind to starboard so more vessel speed
		MSS050/D369088		25 34.75 N	031 31.73 W	01:48:59	D3690048	317	

CTD cast number	Profiler	Folder	Jday	Lat (deg:min)	Lon (deg:min)	Start cast (GMT)	Filename (rawdata .mrd)	Max depth (m)	Comments
		MSS050/D369088		25 34.94 N	031 31.97 W	02:04:04	D3690049	310	
		MSS050/D369088		25 35.21 N	031 32.21 W	02:18:52	D3690050	295	
		MSS050/D369088		25 35.57 N	031 32.43 W	02:34:31	D3690051	358	increased hauling point to 300 m
		MSS050/D369088		25 35.84 N	031 32.69 W	02:51:45	D3690052	164	Comms problem, recover for a 7th cast
		MSS050/D369088		25 36.06 N	031 32.90 W	03:04:51	D3690053	369	
369090	MSS050	MSS050/D369090	254	25 53.12 N	031 30.47 W	15:12:32	D3690054	320	
		MSS050/D369090		25 53.32 N	031 30.68 W	15:34:54	D3690055	365	
		MSS050/D369090		25 53.44 N	031 30.99 W	15:43:59	D3690056	336	
		MSS050/D369090		25 53.56 N	031 31.26 W	16:06:59	D3690057	350	
		MSS050/D369090		25 53.66 N	031 31.59 W	16:15:48	D3690058	239	Comms problem, recover for 6th cast
		MSS050/D369090		25 53.68 N	031 39.81 W	16:30:28	D3690059	311	
369092	MSS050	MSS050/D369092	255	25 34.37 N	031 30.43 W	01:25:06	D3690060	322	
		MSS050/D369092		25 34.52 N	031 30.38 W	01:39:21	D3690061	346	
		MSS050/D369092		25 34.69 N	031 30.35 W	01:55:44	D3690062	325	
		MSS050/D369092		25 34.86 N	031 30.41 W	02:11:38	D3690063	311	
		MSS050/D369092		25 35.00 N	031 30.41 W	02:26:22	D3690064	308	
		MSS050/D369092		25 35.16 N	031 30.43 W	02:40:52	D3690065	337	

CTD Data

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Data Acquisition

Three 4-day CTD surveys were conducted during the D369 cruise, each after a SeaSoar survey. In total 93 CTDs were deployed; 90 were carried out using the stainless steel cast, generally to 500 m, and 3 using the titanium cast (ctd369004, ctd269043, ctd369086), two of which were for deeper deployments. Before reaching the area of interest, 12 CTDs, from 001 to 012, were deployed on the way. A list of all CTDs is available in the table below.

Survey 1

During the first SeaSoar survey, covering a 150 x 150km box, CTD casts were deployed between each tow at around dawn and dusk. In total 8 CTDs, cast numbers 007 to 014, were deployed.

The following 4-day CTD survey was conducted over the same area but following a North-South direction instead. The 5 by 5 grid of CTDs started from the south-east corner (25°36N 30°17) towards north-west corner (27°N 31°50) making 5 north-south transects separated by approximately 20 miles.

The survey started the 20th August at 6am and finished on the 23rd August at midnight; six to seven CTDs were deployed per day, reaching a total of 28 casts, CTD 015 to 042.

Survey 2

During the second SeaSoar survey, CTDs were deployed between each tow. In total 10 CTDs were carried out, these included CTD 043 to 050.

The two SeaSoar surveys allowed for a detailed dynamic picture of the region oceanographically. The locations of dynamic features of interest were noted and the study of their trajectories allowed for predictions of their future location for the second CTD survey. Thus the second CTD survey focussed on the centre of an anticyclonic eddy. It was opted to first reach the centre of the eddy (26°36N 31°20W) and then follow a triangular track whose vertices all lied on a circle of approximately 35km radius from the eddy centre. Their coordinate were (26°36N 30°59W), (26°20N 31°30W) and

(26°52N 31°30W). Such an approach was to allow us to study the eddy for four days whilst using the ADCP data to ensure that we remained in the slowly westward propagating eddy core.

The survey started the 29th August at Midnight and finished the 2nd of September at 1am. CTD casts were deployed 4 times a day at dusk and dawn, midnight and midday. Twenty CTDs were deployed during the survey, including CTD 051 to 070.

Survey 3

Following the third and final SeaSoar survey, containing CTD numbered 071 to 079. As before, a final CTD survey was designed to obtain a contrasting picture of oligotrophic waters in a less dynamically active region than the previously sampled anticyclonic eddy. Again the survey was sampled centred around the coordinate (25°34N 31°30W) and followed a triangular trajectory. The coordinate (25°53.5N 31°30W) formed the top of the triangle, (25°24N 31°48.5W) and (25°24N 31°11.5) formed the two base vertices.

The survey started on the 8th of September and finished on the 12th of September. In total 16 CTDs were deployed, CTD 080 up to 093, amongst which one was a titanium cast (ctd369086).

Table 9: List of CTD sampling stations.

Date	Time	CTD number	Lat.	Lon.
11/08/2011	07:13:52	ctd369001	28°43.90	-23°33.52
12/08/2011	06:24:46	ctd369002	28°17.67	-26°47.29
13/08/2011	05:55:56	ctd369003	27°00.13	-30°17.90
13/08/2011	08:36:01	ctd369004	27°00.08	-30°18.95
14/08/2011	06:00:43	ctd369005	25°36.58	-30°17.33
14/08/2011	19:57:17	ctd369006	25°36.44	-31°05.34
15/08/2011	05:59:00	ctd369007	25°47.78	-31°10.63
15/08/2011	19:56:13	ctd369008	26°00.08	-30°17.07

Date	Time	CTD number	Lat.	Lon.
16/08/2011	05:58:41	ctd369009	26°00.49	-31°32.78
16/08/2011	19:53:12	ctd369010	26°12.19	-30°28.66
17/08/2011	05:57:12	ctd369011	26°26.81	-31°03.91
17/08/2011	19:54:25	ctd369012	26°35.70	-30°48.46
18/08/2011	05:54:22	ctd369013	26°49.19	-30°48.22
19/08/2011	05:53:33	ctd369014	25°34.49	-30°42.62
20/08/2011	05:58:51	ctd369016	25°39.90	-30°23.84
20/08/2011	10:56:03	ctd369017	26°13.21	-30°16.97
20/08/2011	15:59:12	ctd369018	26°29.82	-30°16.75
20/08/2011	19:54:17	ctd369019	26°53.93	-30°17.13
20/08/2011	21:57:12	ctd369020	26°59.96	-30°17.02
20/08/2011	23:52:04	ctd369021	26°59.97	-30°25.15
21/08/2011	05:52:35	ctd369022	26°38.92	-30°40.17
21/08/2011	10:55:28	ctd369023	26°05.78	-30°40.06
21/08/2011	15:57:52	ctd369024	25°54.94	-30°39.95
21/08/2011	19:54:15	ctd369025	25°35.60	-30°39.97
21/08/2011	21:55:13	ctd369026	25°35.98	-30°49.45
21/08/2011	23:57:40	ctd369027	25°39.93	-31°04.03
22/08/2011	02:55:16	ctd369028	25°57.32	-31°04.12
22/08/2011	05:55:08	ctd369029	26°13.69	-31°04.41
22/08/2011	10:55:02	ctd369030	26°35.59	-31°04.23
22/08/2011	15:57:16	ctd369031	26°59.82	-31°09.70

Date	Time	CTD number	Lat.	Lon.
22/08/2011	19:54:12	ctd369032	26°55.30	-31°26.67
22/08/2011	21:55:19	ctd369033	26°48.73	-31°26.84
22/08/2011	23:56:13	ctd369034	26°38.42	-31°27.02
23/08/2011	02:54:09	ctd369035	26°23.24	-31°26.90
23/08/2011	05:55:20	ctd369036	26°08.82	-31°26.61
23/08/2011	10:56:21	ctd369037	25°36.06	-31°31.58
23/08/2011	15:59:21	ctd369038	25°37.84	-31°50.27
23/08/2011	19:55:13	ctd369039	26°03.30	-31°50.23
23/08/2011	21:56:15	ctd369040	26°11.04	-31°50.05
23/08/2011	23:56:14	ctd369041	26°22.96	-31°50.00
24/08/2011	05:53:48	ctd369042	26°42.04	-31°42.29
24/08/2011	16:43:42	ctd369043	26°46.28	-31°42.97
25/08/2011	05:53:40	ctd369044	26°59.68	-30°20.90
25/08/2011	19:55:03	ctd369045	26°44.01	-31°50.02
26/08/2011	05:52:41	ctd369046	26°35.81	-30°43.46
26/08/2011	19:56:15	ctd369047	26°24.15	-31°42.37
27/08/2011	05:55:05	ctd369048	26°12.19	-31°49.63
27/08/2011	19:54:17	ctd369049	26°00.19	-30°29.75
28/08/2011	05:50:58	ctd369050	26°00.37	-31°48.87
29/08/2011	05:55:49	ctd369051	26°36.17	-31°20.61
29/08/2011	12:59:39	ctd369052	26°36.47	-31°20.41
29/08/2011	23:56:21	ctd369053	26°26.56	-31°17.34

Date	Time	CTD number	Lat.	Lon.
30/08/2011	05:54:01	ctd369054	26°31.22	-31°29.71
30/08/2011	11:58:29	ctd369055	26°48.32	-31°22.55
30/08/2011	20:56:14	ctd369056	26°49.67	-31°28.31
30/08/2011	23:55:16	ctd369057	26°42.17	-31°29.95
31/08/2011	05:52:42	ctd369058	26°22.43	-31°30.23
31/08/2011	11:59:00	ctd369059	26°34.46	-31°02.27
31/08/2011	20:55:54	ctd369060	26°42.05	-31°23.72
31/08/2011	23:55:56	ctd369061	26°29.60	-31°24.17
01/09/2011	05:54:27	ctd369062	26°30.14	-31°30.37
01/09/2011	11:58:04	ctd369063	26°47.56	-31°21.32
01/09/2011	20:56:13	ctd369064	26°20.84	-31°28.60
01/09/2011	23:56:35	ctd369065	26°31.79	-31°22.76
02/09/2011	05:54:17	ctd369066	26°48.28	-31°27.23
02/09/2011	11:59:49	ctd369067	26°31.61	-31°38.87
02/09/2011	20:56:00	ctd369068	26°48.23	-31°43.58
03/09/2011	05:52:14	ctd369069	26°48.49	-31°33.52
03/09/2011	13:28:52	ctd369070	26°49.60	-31°33.72
04/09/2011	05:54:42	ctd369071	26°05.86	-31°49.61
04/09/2011	20:56:08	ctd369072	26°50.77	-31°37.16
05/09/2011	05:48:39	ctd369073	26°34.07	-31°23.56
05/09/2011	20:56:03	ctd369074	26°26.13	-31°11.23
06/09/2011	05:44:40	ctd369075	26°43.78	-30°58.27

Date	Time	CTD number	Lat.	Lon.
06/09/2011	20:55:12	ctd369076	26°12.42	-30°45.06
07/09/2011	05:46:37	ctd369077	26°28.79	-30°44.15
07/09/2011	20:56:12	ctd369078	25°46.76	-30°31.78
08/09/2011	05:44:53	ctd369079	25°23.95	-31°15.62
08/09/2011	11:58:12	ctd369080	25°23.96	-31°48.52
08/09/2011	19:55:12	ctd369081	25°53.55	-31°30.01
09/09/2011	11:58:29	ctd369082	25°53.58	-31°29.93
09/09/2011	19:55:15	ctd369083	25°27.58	-31°46.70
09/09/2011	23:56:10	ctd369084	25°34.12	-31°29.95
10/09/2011	05:49:54	ctd369085	25°24.20	-31°11.65
10/09/2011	11:52:12	ctd369086	25°53.57	-31°30.01
10/09/2011	19:55:15	ctd369087	25°24.52	-31°48.35
10/09/2011	23:55:17	ctd369088	25°34.26	-31°30.39
11/09/2011	05:56:01	ctd369089	25°24.50	-31°11.77
11/09/2011	12:02:21	ctd369090	25°52.87	-31°29.76
11/09/2011	19:56:22	ctd369091	25°26.02	-31°47.97
11/09/2011	23:55:12	ctd369092	25°34.16	-31°30.33
12/09/2011	05:50:53	Ctd369093	25°24.56	-31°11.27

Data Processing

Data Processing using the SeaBird Software on the data-logging PC

Following each cast the logging was stopped and the data saved to the deck unit PC. The logging software output four files per CTD cast in the form D369nnn with the following extensions: .dat (raw data file), .con (data configuration file), .bl (contained record of bottle firing locations), and .hdr (a header file).

The raw data files were then processed using SeaBird's own CTD data processing software, SBE.DataProcessing-Win32: v.7.20g, by the NMFSS technicians. SeaBird CTD processing routines were used following the new BODC required standard as follows.

DatCnv: The Data Conversion routine, DatCnv, read in the raw CTD data file (D369nnn.dat). This contained the raw CTD data in engineering units output by the SeaBird hardware on the CTD rosette. DatCnv requires a configuration file that defines the calibrated CTD data output so that it is in the correct form to be read into the pstar format on the UNIX system. The output file (D369nnn.cnv) format was set to binary and to include both up and down casts. A second output file (D369nnn.ros) contained bottle firing information, taking the output data at the instant of bottle firing. All processing was carried out in ascii format.

BottleSum: To create a bottle rosette position file (D369nnn.btl) from the combination of the .cnv and .ros files from DatCnv. BODC require 5 seconds of data aligned to each bottle firing and a reporting of mean, min, max and standard deviation. This requirement does not fit particularly with the UNIX processing that follows later and required an extra step to be created that will be discussed later.

WildEdit: A de-spiking routine, run on pressure and the 2 temperature sensors; the input and output files again were D369nnn.cnv. The data was scanned twice calculating the standard deviation of a set number of scans, setting values that are outside a set number of standard deviations (sd) of the mean to bad data values. To BODC standards, the scan range was set to 100, with 2 sd's on the first pass and 20 sd's on the second.

Filter: To process to BODC standards, filter is run on pressure to smooth out any instrument response time issues. The time constant was set to 0.15 seconds.

AlignCTD: This program read in D369nnn.cnv and was set to shift the Oxygen sensor relative to the pressure data by 2 seconds compensating for lags in the sensor response time. The output was written over the input file.

CellTM: The effect of thermal 'inertia' on the conductivity cells was removed using the routine Celltm. It should be noted that this routine must only be run after Wildedit or any other editing of bad data values. This routine uses the temperature variable to adjust the conductivity values, and if spikes exist in the former they are amplified in the latter. The algorithm used was:

$$dt = t_i - t_{i-7}$$
$$ctm_i = -b * ctm_{i-7} + a * \partial c \partial t * dt$$
$$c_{cor,i} = c_{meas,i} + ctm_i$$
$$a = \frac{2\alpha}{7\Delta * \beta + 2}$$
$$b = 1 - 2a/\alpha$$
$$\partial c \partial t = 0.8 * (1 + 0.006 * (t_i - 20))$$

where α, the thermal anomaly amplitude was set at 0.03 and β, the thermal anomaly time constant was set at 1/7 (the SeaBird recommended values for SBE911+ pumped system). Δ is the sample interval (1/24 second), dt is the temperature (t) difference taken at a lag of 7 sample intervals. $c_{cor,i}$ is the corrected conductivity at the current data cycle (i), $c_{meas,i}$ the raw value as logged and ctm_i is the correction required at the current data cycle, $\partial c \partial t$ is a correction factor that is a slowly varying function of temperature deviation from 20 °C.

SeaBird processed CTD files were manually backed up onto the UNIX network, via ftp to the file location /D369_pstar/ctd/SBEprocessed, so that data processing could be continued using PEXEC routines.

Data Processing on the UNIX system

The following c-shell UNIX scripts were used to process the data.

ctd0: This script read in the SeaBird processed ascii file (.cnv) and converted it into pstar format, also setting the required header information. The latitude and longitude of the ship when the CTD was at the bottom were typed in manually and added to the header. The output file contained the data averaged to 24hz. The output file was ctd369nnn.24hz.

ctd1: This script operated on the .24hz file and used the PEXEC program *pmdian* to remove residual spikes from all of the variables. The data were then averaged into a 1hz file using *pavrge*. Absent data values in the pressure data were interpolated across using *pintrp*. Salinity, potential temperature, sigma0 and sigma2 (referenced to 2000 db) were calculated using *peos83* and finally a 10 second averaged file was also created. The output files were ctd369nnn.1hz and ctd369nnn.10s respectively.

ctd2: This script carried out a head and tail crop of the .1hz file to select the relevant data cycles for just the up and down casts of the CTD. Before running ctd2, the .1hz files were examined in *mlist* to determine the data cycles for i.) the shallowest depth of the CTD rosette after the initial soaking at 10 m, ii.) the greatest depth, and iii.) the last good point before the CTD is removed from the water. These values were then manually entered at the correct screen prompts in ctd2. The data were then cut out with *pcopya* and the files ctd369nnn.ctu created. Finally, the data were averaged into two decibar pressure bins creating the files ctd369nnn.2db.

ctd3: The script ctd3 was used to produce the users preferred raw plots of the data in the .ctu files.

btledit: This script modified the file D369nnn.btl, now created to an overly complex BODC required standard, containing information of the cast number, bottle firing location, and depth information (mean, minimum, maximum and standard deviations). The script conserves, only the mean value information associated with bottle number, such that it was compatible with well established WOCE developed standard bottle sampling files in the following steps. The newly created file, D369nnn.btledit, was then used in fir0.

fir0: This script converted the .btledit file into pstar format. It then took the relevant data cycles from the .10s averaged file (secondary output from ctd1) and pasted it into a new file, fir369nnn, to contain the mean values of all variables at the bottle firing locations.

samfir: This script created the WOCE standard sampling file, sam369nnn containing selected variables from fir369nnn so that the results from the bottle sampling analysis could be added.

Salinity residuals

Once salinity bottle data had been processed and excel files created for each CTD cast then the following scripts were executed.

sal0: Read in the sample bottle excel files, that had been saved as tab delimited text only files, and converted some PC unique characters into UNIX friendly characters. Then sal0 created pstar format files with *pascin*: output file sal309nn.bot

passal: Pasted bottle file (sal369nnn.bot) values into sam369nnn files under the variable name botsal.

papend: Appended sam369nnn to the sam master file samStStot, grouping all sam369nnn files of previous casts.

parith: Computes the residual values bot-sal and bot-sal2, the difference between salinity measured from samples bottles and in situ measurements (salin and salin2).

Oxygen residuals

A similar procedure was operated for the oxygen calibration. CTD casts were regularly sampled for oxygen calibration. Niskin bottles of specific depth were selected and sampled for each cast. Oxygen

contents were then measured and values stored in tab delimited text only excel files under the format oxy369nnn.txt.

oxy0: Read in the oxygen sample bottle excel files and converted some PC unique characters into UNIX friendly characters. Then oxy0 created pstar format files with *pascin*: output file oxy309nnn.bot.

pasoxy: Paste bottle file (oxy369nnn.bot) values into sam369nnn files under the variable name bot-oxy..

papend: Appends sam369nnn to master file samStStot, grouping sam369nnn files of previous casts together.

parith: Computes the residual value bot-oxy, difference between salinity measured from sample bottles and oxygen sensor measurements.

Salinity Calibration

SeaBird claim that the correct in-situ calibration for their conductivity sensors is a linear function of conductivity with no offset. Plots of conductivity difference against conductivity added support to this and therefore *parith* and *allav* were used to calculate the mean square of the conductivity values and the mean product of the bottle and CTD conductivity values; to solve thus,

$$conductivity = A*(primaryconductivity)$$

$$conductivity = B*(secondary\ conductivity)$$

where

$$A = \frac{\sum Cond_{bot} Cond_{ctd}}{\sum (Cond_{ctd})^2} = \frac{\overline{Cond_{bot} Cond_{ctd}}}{\overline{(Cond_{ctd})^2}}$$

and

$$B = \frac{\sum Cond2_{bot} Cond2_{ctd}}{\sum (Cond2_{ctd})^2} = \frac{\overline{Cond2_{bot} Cond2_{ctd}}}{\overline{(Cond2_{ctd})^2}}$$

and $cond2_{bot}$ is the sample bottle conductivity determined with the secondary temperature variable.

For the instruments on the stainless steel frame the following calibration was carried out on board:

ctdcondcal: This script was used to calibrate the .ctu and .2db files and re-calculate salinity, potential temperature and sigma0/sigma2. A and B were set to 1.00001216 and 1.00006058 respectively.

Residual conductivity differences were 0.0000 with a standard deviation of 0.0011 for both primary and secondary conductivity sensors (Figure 16). However it is worth noting that there appears to be a small residual drift in the conductivity2 sensor. This drift is only at the 0.002 level over the duration of the cruise and therefore no correction has been applied.

For the instruments on the Titanium frame there were only three casts and therefore three opportunities to compare with bottle samples. Residuals indicated that both primary and secondary conductivity were good to within ± 0.003.

Oxygen calibration

Oxygen residuals were obtained by plotting the difference between bottle oxygen measurements and sensor oxygen values versus station number, pressure and oxygen. The script *convoxy* was used to convert Seabird SBE43 instrument oxygen values from mL/L to µmol/L and µmol/kg.

For the Seabird SBE43 oxygen instrument on the stainless steel CTD frame, a line of best fit indicated oxygen values to be low by 7 µmol/L. This small offset was corrected using *ctdoxycal*.

$$oxygen = oxygen + 7$$

A larger offset was noted for the Seabird SBE43 oxygen instrument on the titanium CTD frame, with oxygen values low by around 25 µmol/L. However, due to the limited number of deployments it was not considered valuable to carry out a calibration to bottle samples at this stage. If required in the future, calibration should be made by comparison with oxygen profiles from the stainless steel CTD frame.

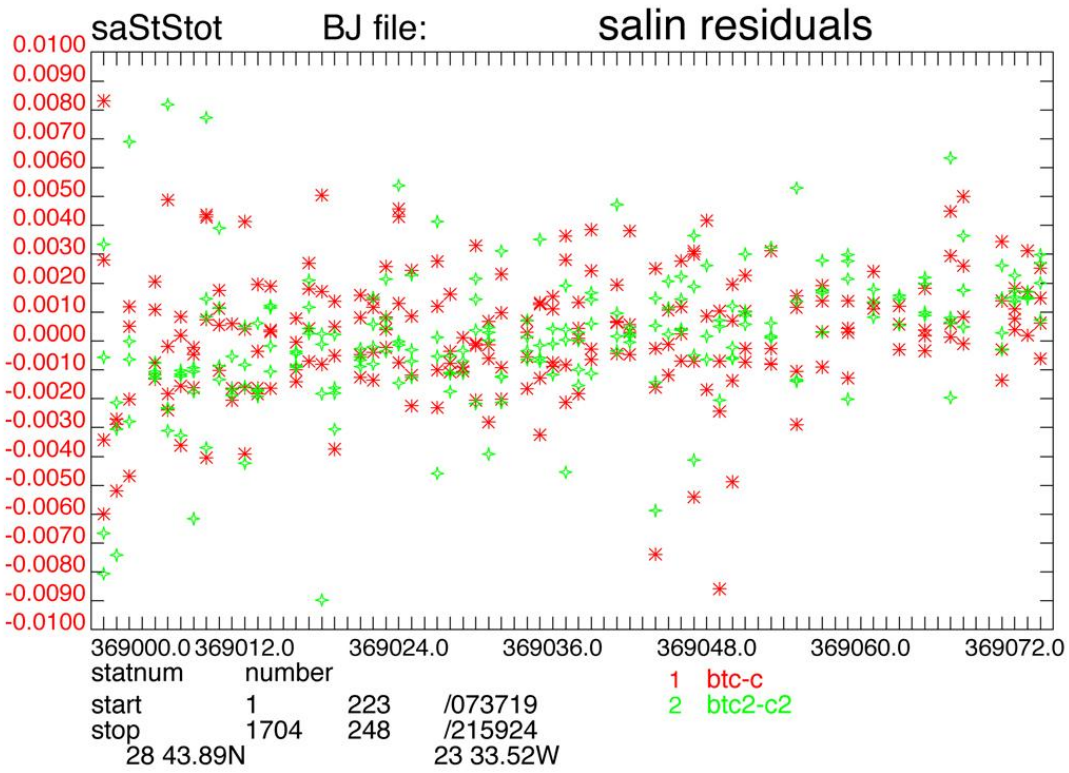

Figure 16: Residual conductivity.

Bottle salinity samples

Salinity samples were drawn from the Niskin bottles mounted on the CTD rosette from a selection of depths spanning the salinity range and wherever a weak salinity stratification was observed. Samples were taken using 200 mL glass sample bottles that were rinsed three times in the sample water, filled to the shoulder and sealed with a disposable plastic insert and the bottle's own screw cap. Samples

were also taken from the ThermoSalinoGraph (TSG) between CTDs and every 4 hours during SeaSoar surveys to calibrate the continual TSG measurements.

The salinometer for on-board salinity determination was put in the constant temperature lab; a model 8400B Autosal salinometer serial no. 68958 fitted with a peristaltic pump. Once a crate of sample bottles had been filled they were moved into the constant temperature lab to stand for 24 hours prior to analysis. Standardisation was performed using IAPSO Standard Seawater batch P153 before the analysis of each crate. The salinometer operations and the recording of the salinity data were controlled by the NMFSS Autosal 2009 software, version 8.5. This created excel friendly spreadsheets.

The salinometer behaved well throughout the cruise, with a single exception concerning the very first crate processed. This was a crate of salinity bottle from the first five CTDs. The salinometer was standardized, as is advised, at the beginning of every processing session. At the end of each processing session a standard salt was run through with the fictitious bottle number 9999 just to check for any drift of the salinometer during the processing. After this first crate of salinities, the standard seawater recorded a value of salinity 0.0065 low. During the procedure to calibrate the CTD to bottle samples it became clearly apparent that this was not a drift but an offset and thus it was concluded that something had gone wrong during the standardization of the salinometer on this first salinity bottle processing session. No subsequent problems were encountered.

Following salinometer processing, the data was copied from the un-networked salinometer PC to a USB stick. From here the data was transferred onto the network and imported into excel via a Mac laptop. For underway samples, a spreadsheet of bottle numbers and sample times obtained from the raw log sheets were matched with corresponding bottle salinities. Each time a new file was created it was appended onto the master excel file. For CTD samples, a spreadsheet of bottle salinities and the corresponding Niskin bottle from which they were taken (derived from the raw CTD log sheets) was created for each CTD cast.

Thermosalinograph and Surfmet Data

John Allen

Instruments

Underway surface meteorology and thermosalinograph measurements were recorded by the RVS Surfmet system throughout *Discovery* cruise 369. The details of the instruments used are given in the earlier computing and instrumentation section, however, the parameters measured were:

Non-toxic supply

Intake water temperature (temp_m)

TSG housing water temperature (temp_h)

Conductivity

Fluorescence (Chla)

Turbidity (transmissometer)

Meteorology

Seal level pressure

Air temperature/humidity

Photosynthetically available radiation (PAR) - port/starboard sensors

Total Incident Radiation (TIR) - port/starboard sensors

Wind speed and direction

Processing

Processing of the underway data was undertaken daily which entailed running several PSTAR routines as detailed below.

surfmet0: This script was used to convert the data from RVS format to PSTAR format using datapup. Resultant file was smt369**.raw

surfmet1: This ensured absent Surfmet data values were set to -999. The script also calculated TSG salinity using housing temperature, conductivity and a pressure value set to zero. Laboratory calibration of meteorological variables was applied also at this point. The Surfmet system applies the laboratory temperature sensor calibrations, as given in the earlier technical section, before the data reaches the RVS surfmet stream that we read in with *smtexec0*.

surfmet2: The master Ashtech file and navigation file were merged with smt369** at this point. This allowed accurate heading data to be incorporated into the underway dataset. The data were also averaged to 2 minute values here. This step creates the file smt369**.hdg

surfmet3: This routine computed vessel speed and subtracted it from relative winds to obtain true wind speed and direction. Resultant file was smt369**.met

Temperature calibration

A full inspection of TSG temperature against surface CTD values will be carried out later.

Salinity calibration

Salinity samples were taken from the underway source every ~4 h during SeaSoar surveys. A master Excel file of sample times and corresponding bottle salinities, as described in the previous Salinity Bottle Samples section, was read into PSTAR. The new file was then merged, using *pmerge*, with the existing smt369nn files to directly compare underway salinity (salin) and bottle salinity (botsal) in order to determine and apply a calibration to the underway salinity data. The initial comparisons were good and an offset of -0.154 applied to salinity. This calibration is good to ± 0.017 S.D. (Figure 17).

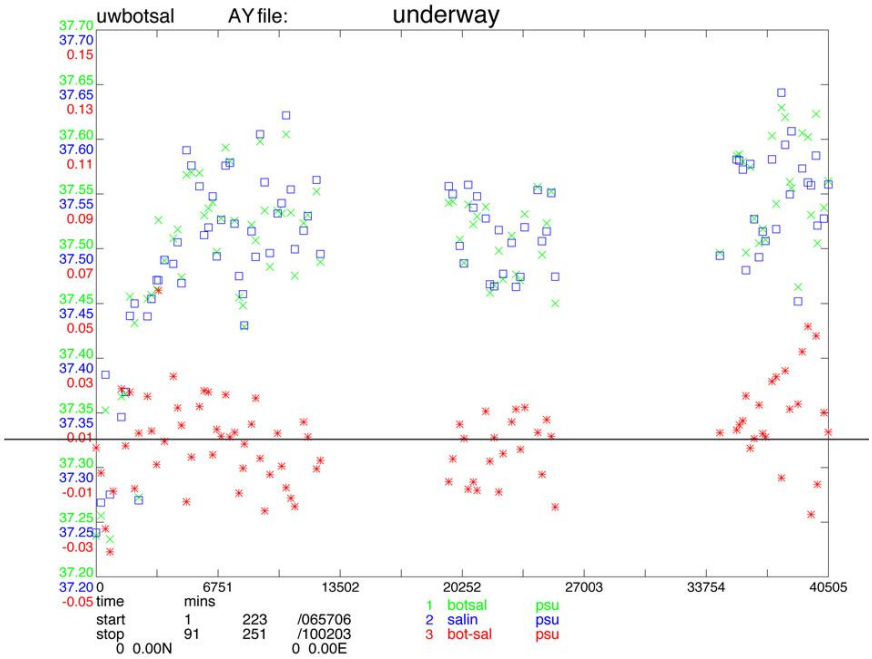

Figure 17: Salinity from TSG and underway bottle samples following calibration. Residuals, in red, showing zero offset in salinity to a ±0.017 S.D.

Micromolar Dissolved Inorganic Nutrients

Sinhue Torres-Valdes

Analysis

Seawater was collected for the analysis of micro-molar concentrations of dissolved nutrients; nitrate and nitrite (hereafter nitrate), phosphate and silicate. Samples for inorganic nutrient analysis were collected directly into 30 mL plastic pots. Pots were rinsed with sample water at least three times before withdrawing the sample. Samples were stored in a fridge at approximately 4°C until sampling for 2 or more stations was completed; analyses were thus carried out for typically 2-5 stations at a time. Samples were also analysed from the 6 pump casts. Analysis was performed for all even numbers from depth up to 150-100 m. After this, samples were analysed every 4 (e.g., 150, 146, 142 and so on) and every 10 from 100 m to the surface. This is because from about 150 m depth, concentrations of nitrate and phosphate are undetectable with the micro-molar analyser.

In general samples were analysed within 2-12 hours of sample collection using a segmented continuous-flow Skalar San^{plus} autoanalyser set up for analysis and data logging with the Flow Access Software version 2.0.25. This system follows the method described by Kirkwood (1996), with the exception that the pump rates through the phosphate line were increased by a factor of 1.5, which improves reproducibility and peak shape

For the D369 (LINK) cruise the analysis was calibrated using the set of standards shown in Table 10. Table 10 shows target and actual standards concentration. Target concentrations are values aimed at when preparing working standards (i.e., every day used standards). Actual concentrations are values corrected by taking into account *i)* the weight of the dry chemical used to prepare a given standard (Table 11) and, *ii)* the calibrated volume of the pipettes used for diluting stock standards (i.e., high concentration standards).

~5 mmol L^{-1} stock standard solutions prepared in Milli-Q water were used to produce working standards. Working standards were prepared in a saline solution (40 g NaCl in 1 L of Milli-Q water, here after artificial seawater), which was also used as a diluent for the analysis.

Table 10: Set of calibration standards (*Std*) used for dissolved inorganic nutrient analysis. Bold numbers are target concentrations, otherwise actual concentrations. Concentration units are µmol L^{-1}.

	Nitrate		**Phosphate**		**Silicate**	
Std 1	**25.0**	24.53	**1.6**	3.92	**15.0**	14.72
Std 2	**12.5**	12.27	**1.2**	2.94	**10.0**	9.82
Std 3	**5.0**	4.92	**0.8**	1.96	**5.0**	4.92
Std 4	**1.0**	1.00	**0.4**	0.98	**1.0**	1.02
Std 5	**0.5**	0.51	**0.1**	0.49	**0.5**	0.51
Std 6	**0.1**	0.10	**0.05**	0.05	**0.1**	0.10

Table 11: Compounds used to prepare stock standard solutions, weight dissolved in 1 L of Milli-Q water and Molarity of the solution.

Compound	**Weight (g)**	**Molarity 1 L stock solution**
KH_2PO_4	0.6849	**5.0272**
Na_2SiF_6	0.9454	**5.0061**
$NaNO_3$	0.4273	**5.0329**
$NaNO_2$	0.3454	**5.0274**

Observations

General observations

Previous to the cruise all labware was washed with 10% HCl and rinsed with MQ water and rinsed several times before use once onboard.

Typically, the autoanalyser was washed through with 10% Deacon 90 then Milli-Q water for at least 15-30 minutes each after each run. New pump tubing were fitted at the start of the cruise, along with a new cadmium column. All tubing was then changed twice during the cruise.

New batches of artificial seawater were prepared almost every 2 weeks. These were analysed prior to being used in order to check for contamination and consistency. Nano-molar analyses carried out by Dr. Matt Patey showed PO4 and NO3 concentrations of 1.6-3.6 and 31-33 nmol $L^{-1}$for phosphate and nitrate, respectively (i.e., lower than the detection limits of the micro-molar nutrient analysis).

Time series of baseline, instrument sensitivity, calibration curve correlation coefficient and nitrate reduction efficiency were compiled to check the performance of the autoanalyser over the course of the cruise and are shown in Figure 18 to Figure 22.

Problems

On the 25th August the Flow Access software crashed for no apparent reason. Analyses were not being recorded. However, the problem was solved by reinstalling it.

Originally, linear calibration settings for the three autoanalyser channels (i.e., NO3+NO2, Si(OH)4 and PO4) were specified by the Skalar Company engineer providing maintenance to the autoanalyser. This provided the best fitting for calibration standards used for silicate and phosphate, but did not produced the same results for nitrate measurements. As a result, all runs previous to CTD cast 50 were re-evaluated and concentrations re-calculated using a 2nd order calibration equation (which is set in the Flow Access software). The nitrate channel behaved erratically in analyses for casts 3-9, 12-13 and 14. Upon manual revision of runs, it would seem the problem was solved, though results will require further inspection. The baseline of the nitrate channel changed all of a sudden for no apparent reason during analysis of casts 3, 4 and the first pump cast. Some of the samples for this run may be have to be recalculated manually by obtaining raw peak height data directly from the raw analysis file.

NB: It must be noted that most of the analyses yield slightly negative concentrations of phosphate and nitrate in samples taken at depths typically shallower than 120 m. This is normal, given that the oligotrophic waters surveyed contain nutrients at concentrations lower than the artificial water used for the analyses, but which are resolved by the nano-molar system.

Performance of the Analyser

The performance of the autoanalyser was monitored by producing time series plots of the following parameters: standards concentration, baseline, calibration slope (instrument sensitivity), calibration correlation coefficient, nitrate reduction efficiency, low nutrient seawater and certified standards. These are plotted against run/analysis number (Figure 18 to Figure 22).

The precision of the method was determined by monitoring the variations of the complete set of standards measured throughout the cruise. Results of the standards measurements are summarised in

Table 12 and shown in Figure 18. Note precision improves as concentrations increase (

Table 12). Triplicate analyses were performed on the first samples, and duplicate analysis was preformed for the deepest samples (>150 m). Also, randomly selected samples were analysed in triplicate. The limits of detection of this method were estimated as twice the standard deviation of the concentration of lowest standard of each nutrient. Detection limits were 0.01 µmol L^{-1} for PO_4^{3-}, 0.17 µmol L^{-1} for NO_3^- and 0.03 µmol L^{-1} for $Si(OH)_4$.

Low Nutrient Seawater, Certified Ocean Scientific International (OSIL) standards and surface water from the South Atlantic Subtropical gyres were measured in duplicate in every run in order to test *i)* artificial seawater for contamination, *ii)* the noise in the measurements of low nutrient concentrations and *iii)* consistency from run to run (additional to working standards).

Table 12: Mean and variation of all standards measured, and precision of the analysis at each concentration (µmol L^{-1}).

	NO_3^-	Prec.	PO_4^{3-}	Prec.	$Si(OH)_4$	Prec.
Std 1	24.57 ± 0.17	0.7%	1.74 ± 0.01	0.6%	14.70 ± 0.03	0.2%
Std 2	12.34 ± 0.09	0.7%	1.26 ± 0.01	0.7%	9.85 ± 0.02	0.2%
Std 3	4.87 ± 0.09	1.8%	0.80 ± 0.01	0.9%	4.95 ± 0.03	0.6%
Std 4	0.98 ± 0.07	3.3%	0.40 ± 0.01	1.6%	1.00 ± 0.01	0.6%
Std 5	0.49 ± 0.03	7.1%	0.11 ± 0.01	5.3%	0.50 ± 0.01	1.5%
Std 6	0.15 ± 0.03	17.1%	0.05 ± 0.01	12.4%	0.11 ± 0.01	12.4%

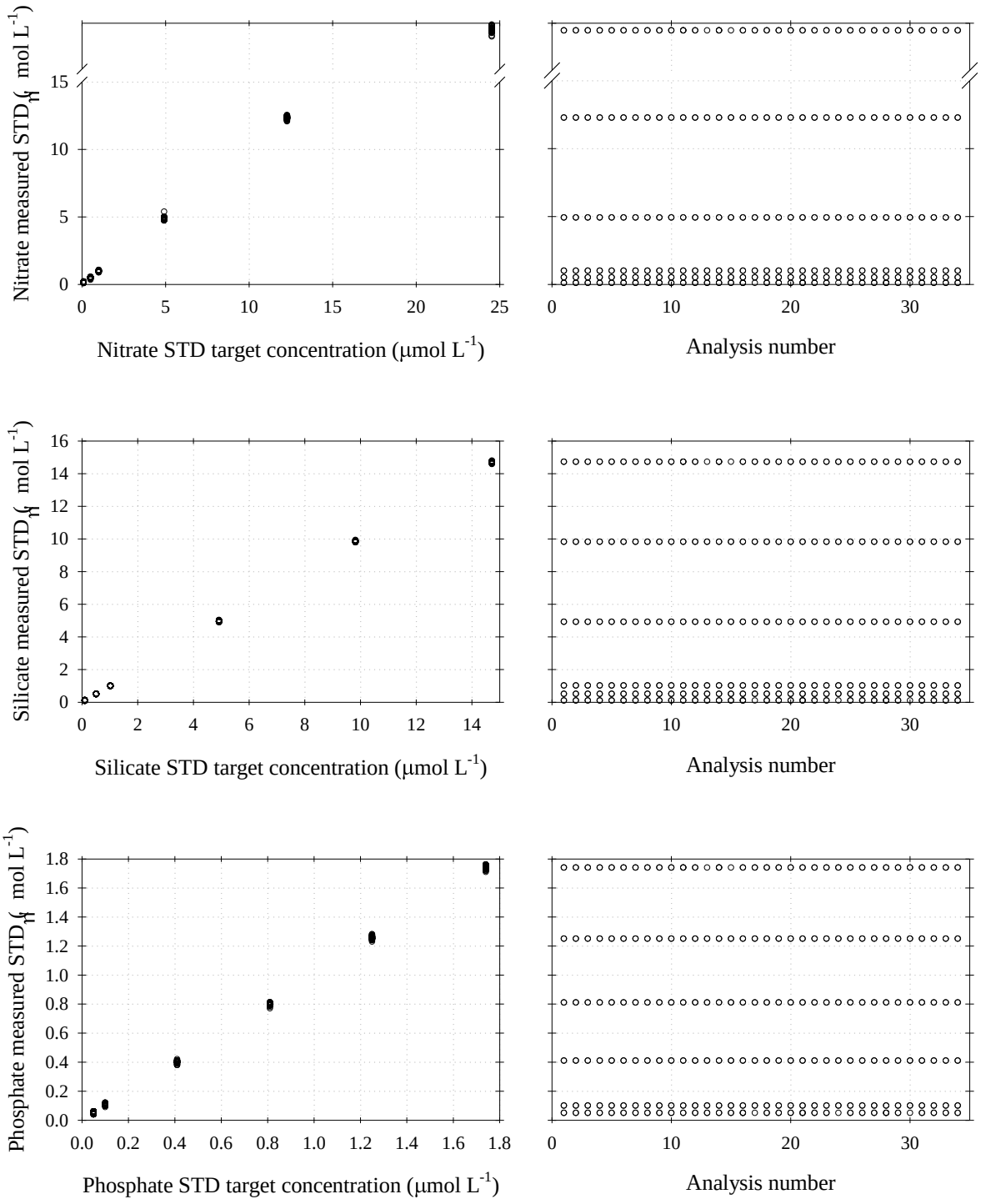

Figure 18: Complete set of 'measured' standards plotted against the 'target' concentration (left side panels). 'Measured' standards plotted against respective analysis number (right side panels).

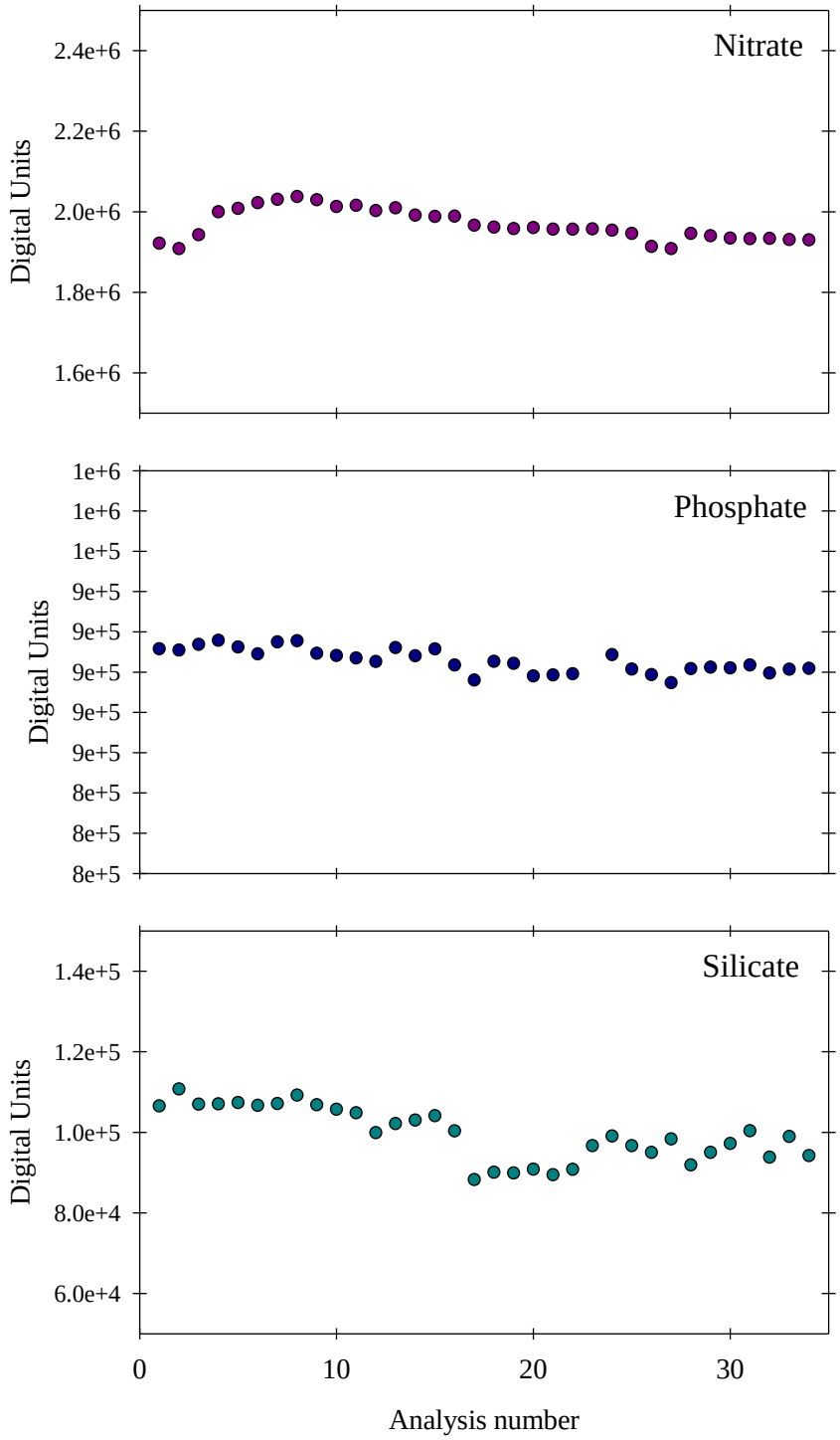

Figure 19: Baselines time series. The baselines for nitrate and phosphate were fairly consistent throughout the cruise, with the silicate baseline being more variable. Although the variability observed does not affect the results, it can be a good indicator of the quality of artificial seawater and reagents used.

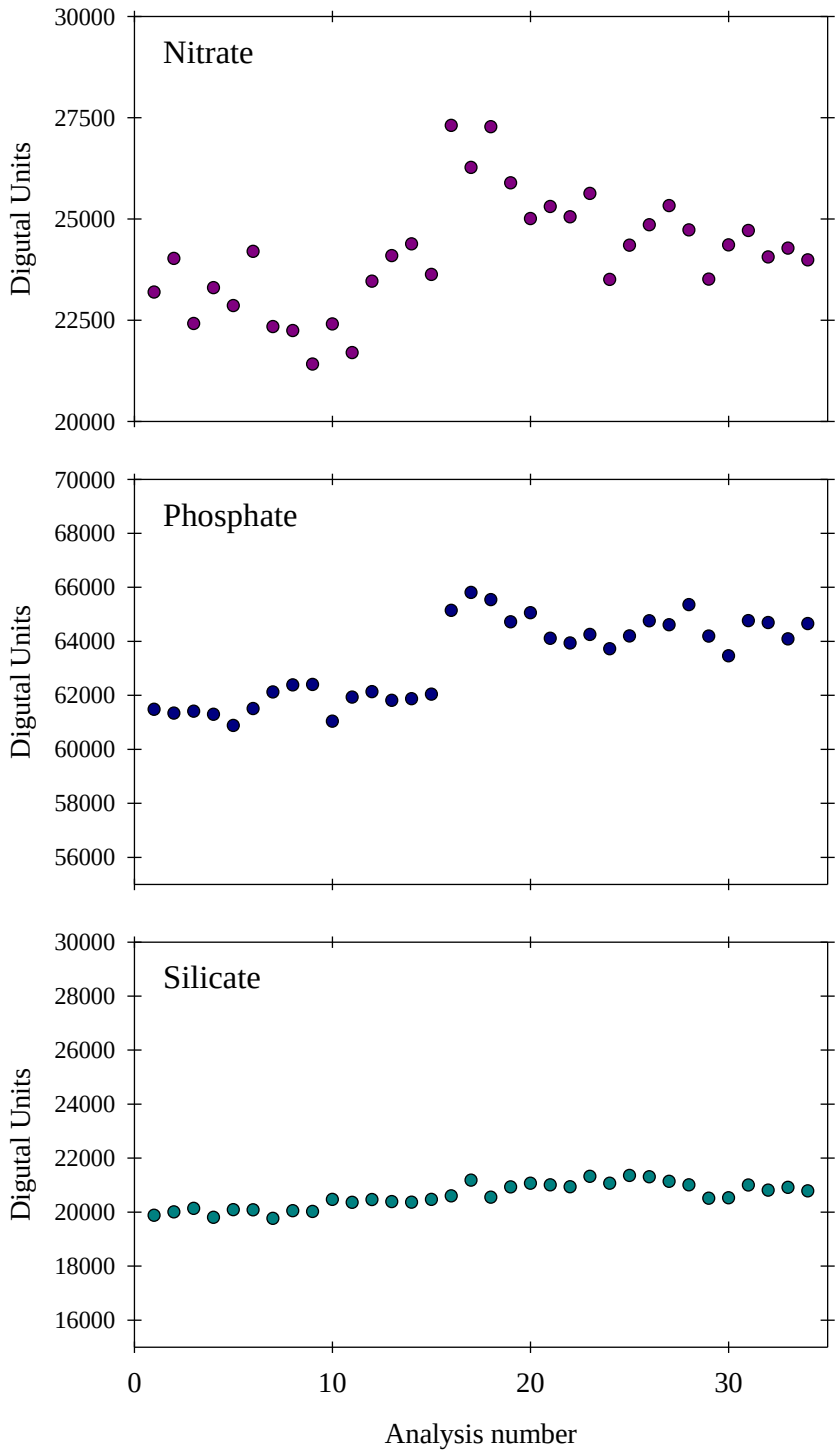

Figure 20: Calibration slopes time series. These show the sensitivity of the three different autoanalyser channels (i.e., nitrate, and phosphate and silicate), with increasing values (in digital units) indicating better sensitivity. Slopes for nitrate and phosphate showed a sudden increase in run number 16, which coincided with new tubing being installed in the instrument. This did not affect the silicate slope. The nitrate slope decreases gradually after.

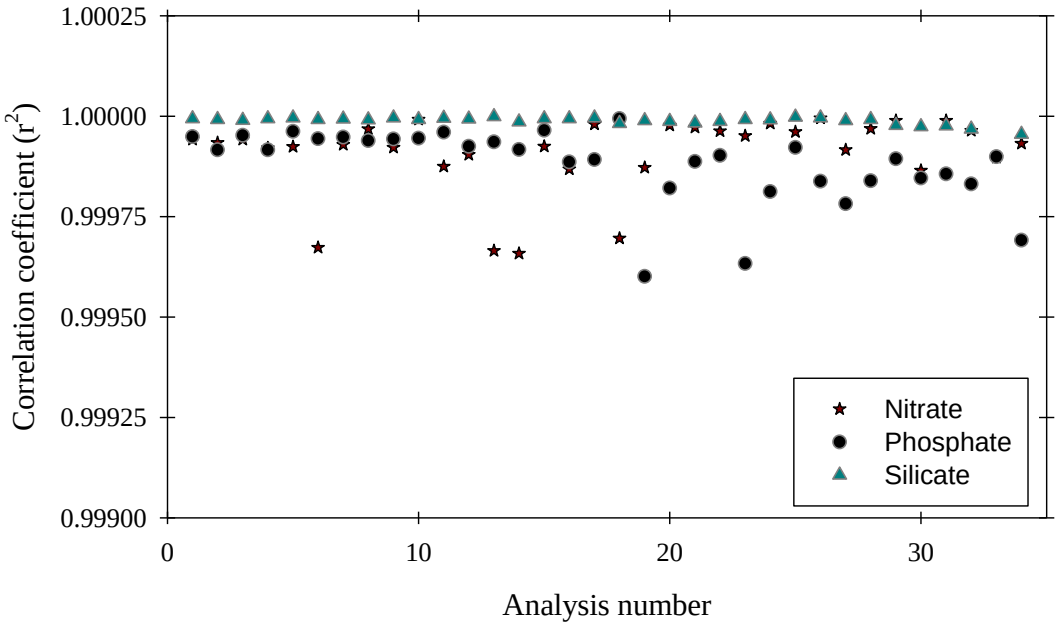

Figure 21: Calibration correlation coefficients. All r^2 were better than 0.999.

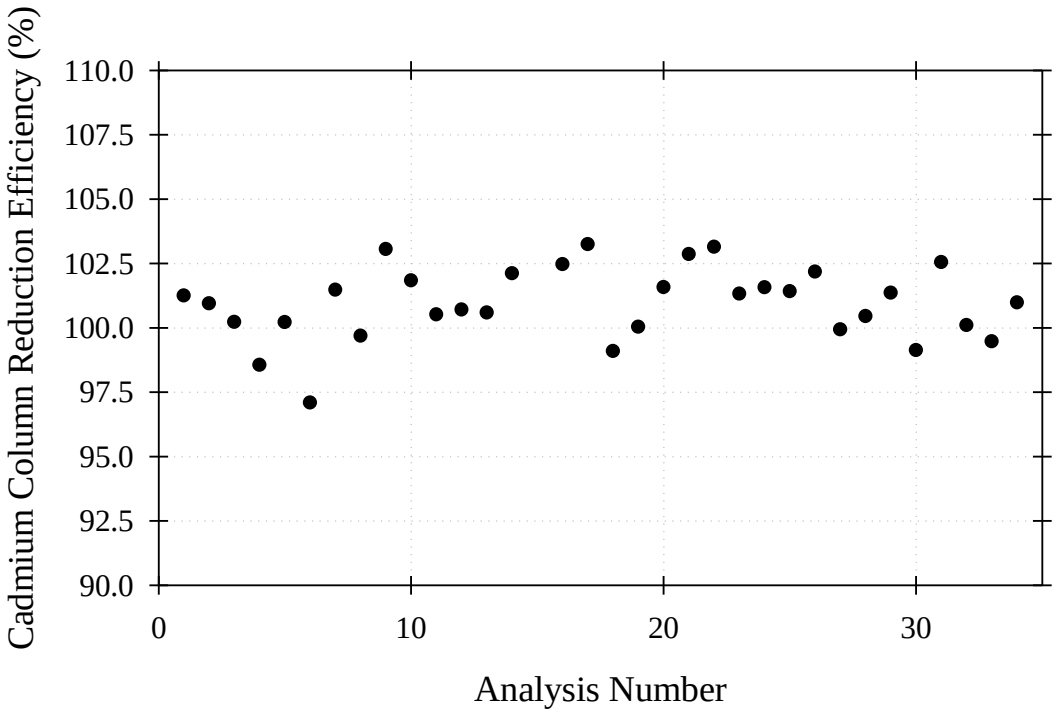

Figure 22: The efficiency of the cadmium column in reducing nitrate to nitrite is tested by measuring a nitrite standard of similar concentration to the top nitrate standard (approximately 25 µmol L^{-1}). This figure shows the ratio of nitrate to nitrite for all analysis carried out (expressed in %). The nitrite standard prepared however was slightly different than the nitrate standard, thus resulting in a ratio higher than 1 (or higher than 100%). Knowing this, the nitrate to nitrite ratio is expected to remain relatively constant while the reduction efficiency of the cadmium column is 100%.

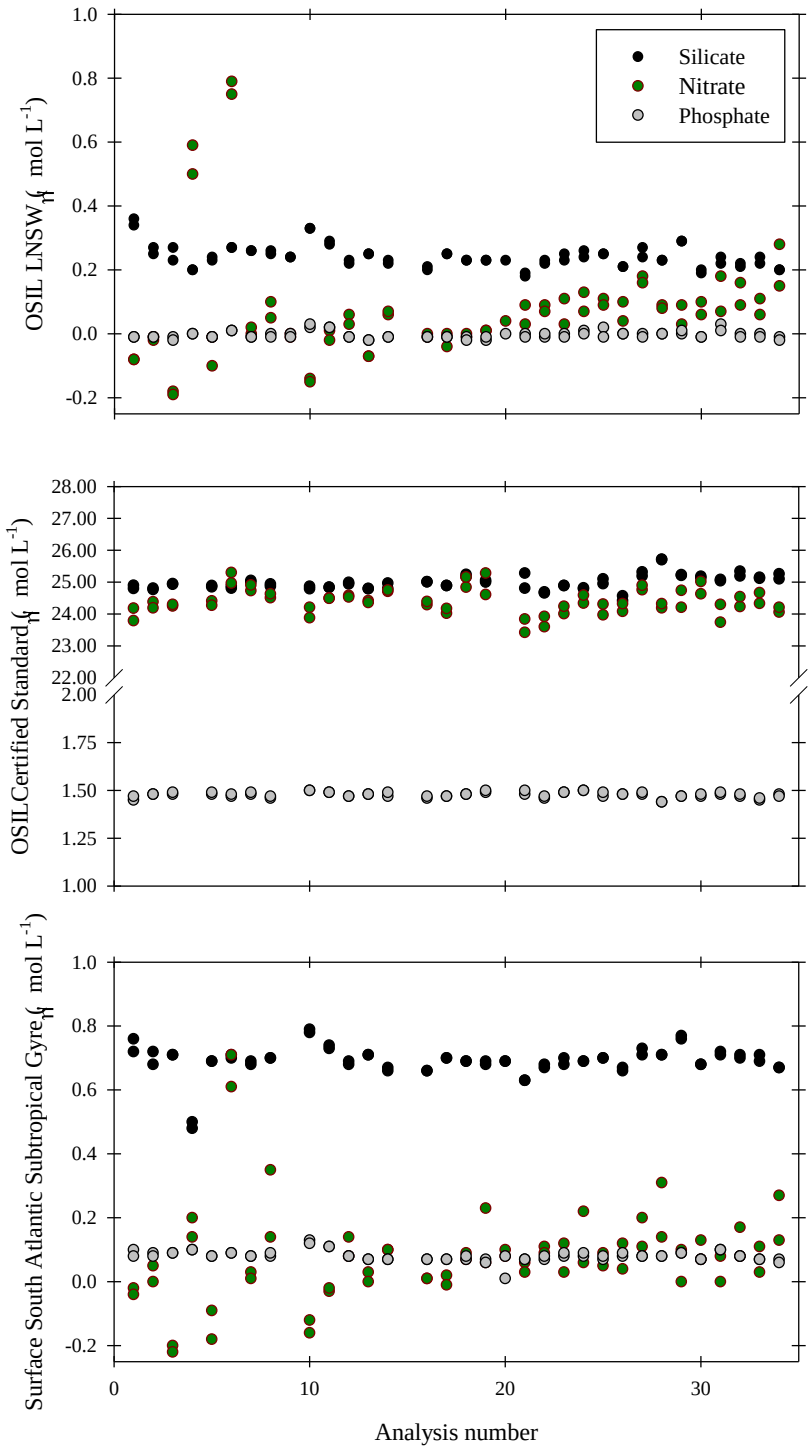

Figure 23: Low Nutrient Seawater (LNSW_OSIL), OSIL certified standards (CS) and South Atlantic subtropical gyre (SASG) surface water time series. Average concentrations for *i)* LNSW were 0.1±0.2 1 µmol-N L^{-1}, 0.24±0.041 µmol-Si L^{-1} and 0.00±0.1 µmol-P L^{-1}, *ii)* for CS were 24.4±0.4 1 µmol-N L^{-1}, 24.99±0.23 1 µmol-Si L^{-1} and 1.48±0.01 µmol-P L^{-1}, and *iii)* for SASG water were 0.1±0.2 1 µmol-N L^{-1}, 0.69±0.05 1 µmol-Si L^{-1} and 0.08±0.2 µmol-P L^{-1}. Black dots represent silicate, green dots represent nitrate and grey dots represent phosphate concentrations.

Nanomolar Dissolved Inorganic Nutrients

Matthew Patey

Overview

Analysis for dissolved nitrate + nitrite (NO_{2+3}^-) (hereinafter nitrate) and dissolved phosphate (PO_4^{3-}), or soluble reactive phosphate (SRP - the phosphorus fraction measured by the Molybdenum Blue technique), at nanomolar concentration were undertaken on a purpose-built, segmented-flow autoanalyser following a method described in Patey *et al.* (2008), with some changes to reagent flow rates. Two liquid waveguide capillary flow cells (LWCCs) were used to provide a two-metre path-length, enabling the detection of nanomolar concentrations. A single tungsten-halogen lamp (HL2000-HP, Ocean Optics) provided illumination for both LWCCs, with a bifurcated fibre-optic cable being used to divide the light between the two channels. Two Ocean Optics USB spectrometers monitored the absorbance in each cell (USB2000 for phosphate and a USB4000 for nitrate / nitrite).

For 7 CTD profiles (see Table 13), nitrite was also measured, enabling the nitrite contribution to the nitrite + nitrate signal to be determined and thus the true nitrate concentration to be derived. Since only two channels were available, this was achieved by re-running the samples (together with an additional set of nitrite standards) at the end of the day with the cadmium column bypassed so that nitrate reduction does not occur.

Samples were introduced manually by switching a sample line between a reference solution (low nutrient surface seawater) and sample or standard solution and the resulting change in absorbance was monitored. Absorbance versus time was continuously recorded for each channel and stored electronically in a plain text format. Calibration curves and results were generated manually from the peak heights using spreadsheet software.

Note: throughout this report (and for all the results generated) molar concentrations are reported in concentrations per unit volume (i.e. nM, μM and mM refer to $nmol.l^{-1}$, $\mu mol.l^{-1}$, $mmol.l^{-1}$, respectively).

Sampling approach

All apparatus coming into contact with the samples or reagents was cleaned by soaking in 1 M HCl and rinsing thoroughly with ultrapure water from a MilliQ system (hereafter referred to as MQ). Samples were taken from the CTD rosette using 60 mL acid-washed LDPE bottles, rinsing three times before filling, and were stored in the fridge until analysis. Analysis was typically undertaken within 12 hours, although for a few profiles sampled during the evening were not measured until the following afternoon (typically 18 – 20 hours later).

For each CTD profile, typically 10 - 12 samples were taken, covering every, or almost every, unique depth. However, during periods of intense sampling (with 3 or more CTD profiles per day), this was reduced to between 5 and 7 samples per day. A list of all stations sampled is presented in Table 13.

Table 13: List of casts sampled for nanomolar nutrient measurements.

Date	**CTD cast no.**	**Date**	**CTD cast no.**	**Date**	**CTD cast no.**
11/8/2011	1	22/8/2011	27,28,29,30,31,32,33	2/9/2011	65,66,67,68
12/8/2011	2	23/8/2011	34,35,36,37,38,39,40	3/9/2011	69,70
13/8/2011	3	24/8/2011	41,42	4/9/2011	71
14/8/2011	5,6	25/8/2011	44	5/9/2011	73
15/8/2011	7,8	26/8/2011	46	6/9/2011	75
16/8/2011	9,10	27/8/2011	48	7/9/2011	77
17/8/2011	11,12	28/8/2011	50	8/9/2011	79*,80*,81*
18/8/2011	13	29/8/2011	51	9/9/2011	82,83*
19/8/2011	14	30/8/2011	54,55,56,57	10/9/2011	84*,85*,86*,87*
20/8/2011	16,17,18,19,20,21	31/8/2011	58,59,60	11/9/2011	88,89,90,91
21/8/2011	22,23,24,25,26	1/9/2011	61,62,63,64		

Profiles for which nitrite was also measured (in addition to nitrate + nitrite and phosphate) are indicated with an asterisk.

Wash solution

In order to avoid refractive index changes and other effects resulting from mixing solutions of different salinity, low nutrient seawater was used as a wash solution (to form the baseline from which all sample and standard peaks are measured) and standard matrix. For the wash solution, surface seawater was collected from the CTD and used unfiltered. Knowledge of the nutrient concentration in this seawater is critical for accurate sample determination (particularly for nitrate and nitrite measurements) and its concentration was measured (relative to MQ water) at the beginning and end of each day's analysis.

Since this seawater was not aged, its nutrient concentrations were sometimes variable, typically decreasing with time as a result of bacterial nutrient uptake. By gently heating the seawater in a microwave for 10 minutes on a defrost setting, the stability of this baseline solution was improved. As an additional benefit, a small increase in nutrient concentration usually resulted from the microwave heating (typically 1 – 3 nM nitrate or phosphate), which makes the low surface nutrient concentrations easier to pick out (as small negative peaks in the baseline).

Standard matrix and salt-effect correction

Calibration of the instrument was performed by the preparation of standard additions. As with the wash solution, surface seawater was used in order to match the sample matrix as closely as possible. As part of the calibration, it is necessary to calculate the nutrient concentration in the seawater used to prepare the standards. This is done by making a comparison with purified water (MQ) with the assumption that the purified water is completely free from nutrients. As a result of the different refractive index of seawater and pure water, and also due to solution turbidity it is necessary to apply a salt-effect offset to the calibration of a seawater solution relative to pure water. This offset is measured in accordance with a method described by Zhang & Chi (2002), whereby one of the colour-producing reagents is replaced with a blank, so that the refractive index effects can be measured in the absence of any generation of the coloured reaction products. In place of the molybdate reagent for phosphate analysis, a 0.48 M H_2SO_4 solution was used, while purified (MQ) water was used in place of the NED reagent used for nitrate analysis.

Measurement of the salt effect was carried out on 9 occasions during the cruise. For nitrate/nitrite

measurements the increase in signal upon changing from pure water to seawater is small due to dilution of the seawater by a buffer solution. The mean increase was 1.1 ± 0.2 nM (± 1 standard deviation). For phosphate analysis, the lack of any buffer dilution results in a much larger salt-effect, with a mean value of – 9.6 ± 2.4 nM (± 1 standard deviation) from the 9 determinations.

To avoid the need to apply large salt-effect corrections to the phosphate data, surface seawater was treated to remove dissolved phosphate. Low phosphate seawater (LPSW) was prepared by a process described by Li & Hansell (2008), in which $FeCl_3$ solution was added to surface seawater and the resulting $Fe(OH)_3$ precipitate allowed to settle. After about 4 days the supernatant was siphoned off slowly (ca. 15 $mL.min^{-1}$) and then filtered by gravity through a 0.2 μm Sartobran 300 cartridge filter. On the nine days during which a measurement of the salt effect was made, the phosphate concentration in this solution (relative to purified (MQ) water) was measured to be -1.0 ± 1.1 $nmol.L^{-1}$ SRP (± 1 standard deviation). All calculations have assumed a phosphate concentration of zero in the standard matrix.

Calibration

Separate stock standards for each of the three nutrients measured were prepared by dissolving pre-weighed quantities of inorganic nutrients in MQ water. Chemicals were of analytical reagent grade (see Table 14). These three solutions were kept refrigerated and used for the duration of the cruise. Intermediate standards of approximately 10 μM were prepared from the mM stock solutions, also in MQ water. These solutions were kept refrigerated and re-prepared every 2 weeks.

Table 14: Compounds and weights used to prepare stock standard solutions

Compound	Weight (g)	Molarity of 1L stock (mM)
KH_2PO_4	0.1366	1.0039
KNO_3	0.1998	1.9761
$NaNO_2$	0.1414	2.0493

Working standard solutions were prepared freshly every day. Mixed nitrate and phosphate standards

were prepared using phosphate stripped seawater as the standard matrix. For phosphate, 5 working standards were prepared in the range 2 – 96 nM, giving a 6-point calibration with the inclusion of the zero standard. For nitrate, 4 working standards were prepared in the range 4 – 96 nM, with the 5th standard being used for a 96 nM nitrite standard to check the performance of the Cd reduction column. On days when nitrite was measured, four separate standards were prepared with approximately 8, 16, 32 and 64 nM NO_2^- added to low nutrient surface seawater.

Calibration was performed each day prior to measuring any samples. During the day's analysis the standards were stored in the fridge so that they could be re-used throughout the day. All standards were re-measured as samples at the end of the day in order to verify the precision of the technique and to check for baseline drift. Table 15 lists the mean concentrations measured for the sets of standards measured at the end of each day.

Reference materials

On one occasion during the cruise, the nitrate and phosphate standard additions were checked against certified OSIL nutrient standards. A mixed standard in was diluted from the OSIL nutrient standards to give a mixed standard containing 20 nM added nitrate and phosphate. The measured concentrations were NO_3^- = 20.2 nM and PO_4^{3-} = 19.0 nM.

Table 15: List of average measured standard concentration throughout the cruise.

Added NO_3^- (nM)	Measured NO_3^- (nM)	Added PO_4^{3-} (nM)	Measured PO_4^{3-} (nM)
0	0.3 ± 0.6 (n=52)	0	0.7 ± 0.4 (n=49)
		2.0	2.2 ± 0.3 (n=33)
4.0	4.0 ± 0.6 (n=29)	4.0	3.8 ± 0.5 (n=24)
		8.0	7.8 ± 0.4 (n=25)
15.8	15. ± 0.6 (n=29)	16.1	15.4 ± 0.4 (n=9)
31.6	31.0 ± 0.7 (n=29)	32.1	31.0 ± 0.7 (n=29)
63.2	63.0 ± 2.0 (n=9)	64.3	64.5 ± 0.8 (n=11)
94.9	95.2 ± 1.7 (n=29)	96.4	96.3 ± 1.3 (n=28)

Data are from measurements not used for the generation of any calibration curve.

Comparison with an OSI standard does not adequately test the accuracy of the method, since the background concentration in the solution used to dilute the standard also needs to be measured. Reference materials for nanomolar nutrient analysis are not yet widely established, largely due to difficulties with the stability of solutions containing very low nutrient concentrations. However, to gain some idea of the day-to-day consistency of the measurements, during the second half of the cruise, the same bottle of aged low nutrient seawater (again from OSI – batch LNS19) was measured at least once per day. Over time, a slight increase in nitrate concentration, from 7 to 9 nM, was observed, whilst phosphate concentration decreased (Figure 24). Some of these changes may be due to actual changes in the seawater, perhaps due to gradual chemical or biological contamination of the seawater. However the changes are likely to be partly due to analytical errors, particularly the more abrupt day-to-day changes. For nitrate measurements a critical step is measuring the concentration of the seawater wash solution, while for phosphate, any contamination of the LPSW std matrix will result in an a lowering of apparent sample concentrations.

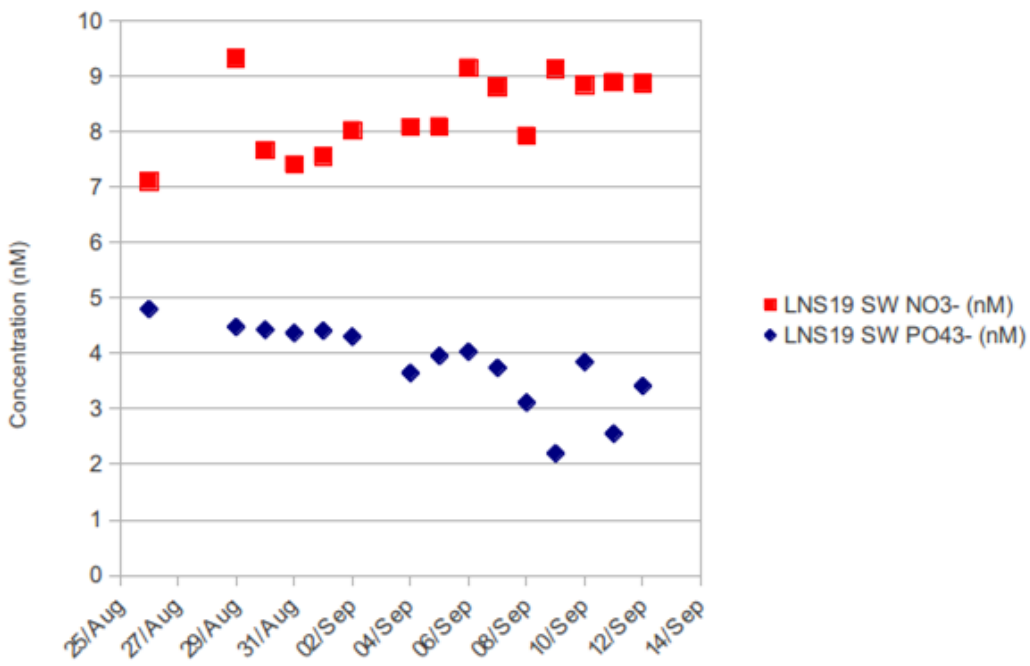

Figure 24: Concentration of OSI seawater (batch LNS19) measured over time.

Data have not been adjusted to compensate for these variations except in the case of data measured on 31 Aug, 1 Sep, and 12 Sep, when some analytical problems with the LNS (wash baseline) were encountered. On these days, a decreasing wash nutrient concentration (apparent when repeated measurements of 2 or more standards relative to the baseline are both shifted by the same offset) was

observed, most probably due to bacterial uptake in the wash solution. The effect was small, but significant (typically < 2 nM change over a several hour period). On these days, repeated measurements of the OSI seawater were used to monitor the change in the baseline concentration, and all samples were adjusted so that the OSI seawater concentrations matched those measured on neighbouring days.

Linearity and detection limit

Although the instrument was typically only calibrated up to 96 nM, very straight calibration curves were obtained (typically $R^2 > 0.999$ for up to 96 nM), and during the cruise the system was verified to be perfectly linear up to at least 700 nM. Comparison with micromolar data generated by S. Torres suggests that linearity for both nitrate and phosphate begins to be lost somewhere around 800 nM. The detection limit of the system varies slightly from day to day, but was determined on 25/8/2011 to be 0.5 nM NO_3^- and 0.4 nM PO_4^{3-}. This was based on 3x the standard deviation of 9 repeated measurements of the lowest standard (LPSW).

Cd column efficiency

The Cd column reduction efficiency was checked daily by comparing the known concentration of a 96 nM nitrite standard with the value measured, based on the nitrate calibration curve. The theoretical value as a proportion of the measured value (i.e. the nitrate reduction efficiency) varied between 95 and 106%, with most values lying between 99 and 102% and a mean value of 100.8% (Figure 25).

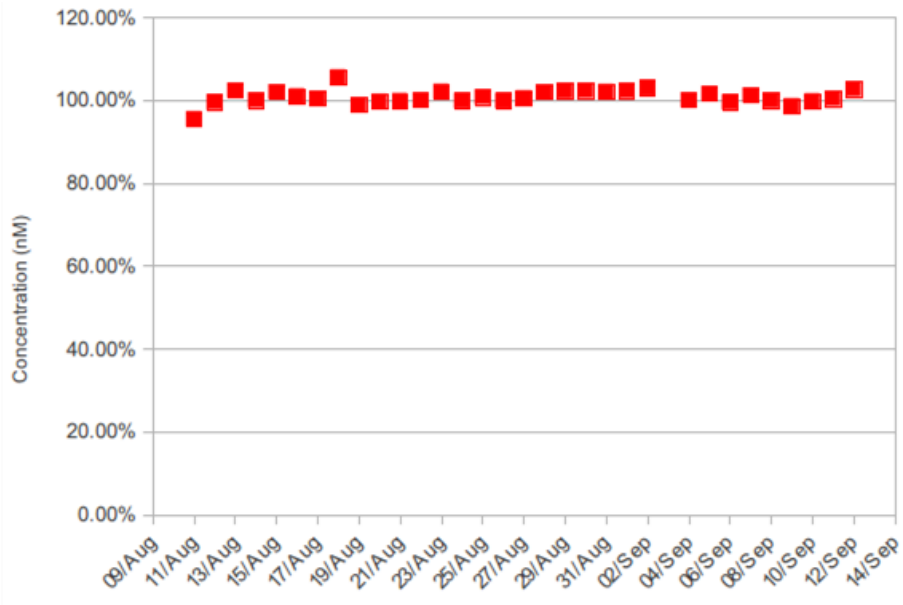

Figure 25: Cd reduction column efficiency determined daily during the cruise.

Abundance and Composition of Microbial Plankton Communities by Flow Cytometry

Glen Tarran and Ross Holland

Objective

To determine the distribution, abundance and community structure of nano- and picophytoplankton, heterotrophic bacteria and heterotrophic nano- and picoplankton from CTD casts and the ship's pumped seawater supply by flow cytometry.

Phytoplankton community structure and abundance by flow cytometry.

Fresh seawater samples were collected in clean 125 mL polycarbonate bottles from a Seabird CTD system containing a 24 bottle rosette of 10 or 20 L Niskin bottles from practically all CTD casts. Samples were stored in a refrigerator and analysed within 3 hours of collection. Fresh samples were measured using a Becton Dickinson FACSort flow cytometer which characterised and enumerated *Prochlorococcus* sp. and *Synechococcus* sp. (cyanobacteria) and pico- and eukaryote phytoplankton, based on their light scattering and autofluorescence properties. The data were immediately stored on disk and the cell numbers of the different groups recorded and entered into an Excel spreadsheet to calculate abundance per millilitre. Table 16 summarises the CTD casts sampled and analysed during the cruise.

Samples for bacteria and heterotrophic flagellate enumeration from CTD casts were kept refrigerated and fixed with paraformaldehyde within half an hour of surfacing. Both CTD and Underway samples (see below) were stained with the DNA stain SYBR Green I (Sigma) in order to separate particles in suspension based on DNA content and light scattering properties. Samples were analysed flow cytometrically within 24 hours of surfacing. Each stained sample was run twice through a Becton Dickinson FACSort flow cytometer; once to analyse sub-micron particles and once to analyse particles greater than 1 μm in diameter. Data were saved and will be analysed ashore.

Table 16: CTD casts sampled for phytoplankton, heterotrophic bacteria and heterotrophic flagellate community structure & abundance

Date	CTD no.	Time (GMT)	Lat, °N	Lon, °W	Depths (m)
11-Aug	1	08:13	28.73	23.56	5 10 15 28 30 49 60 65 80 100 125 250
12-Aug	2	07:37	28.30	26.79	5 20 30 40 50 60 70 80 90 100 110 120 130 140 160 180 200 250
13-Aug	3	07:06	27.00	30.30	5 20 40 55 70 90 110 120 140 180
14-Aug	5	07:15	25.61	30.28	5 20 30 40 50 60 70 80 90 100 110 120 130 140 160 180 200 250
14-Aug	6	21:27	25.64	31.09	5 20 30 40 50 60 70 80 90 100 110 115 120 125 130 135 140 145 150 160 180 200 250
15-Aug	7	07:18	25.79	31.18	5 20 30 40 50 60 70 80 90 100 110 115 120 130 140 160 180 200 250
15-Aug	8	21:20	26.00	30.28	5 20 30 40 50 60 70 80 90 100 110 115 120 125 130 140 160 180 200 220
16-Aug	9	07:30	26.01	31.55	5 20 30 40 50 70 80 90 100 110 115 120 130 140 160 180 200 250
16-Aug	10	21:25	26.20	30.47	5 20 30 40 50 60 70 80 90 100 110 115 120 125 130 140 160 180 200 220
17-Aug	11	07:25	26.44	31.06	5 20 30 40 50 60 80 90 100 110 120 130 140 160 180 200 250
17-Aug	12	21:21	26.60	30.81	5 20 30 40 50 60 70 80 90 100 110 120 125 130 135 140 160 180 200 250
18-Aug	13	07:28	25.82	30.80	5 20 30 40 50 60 70 80 90 100 110 120 130 140 160 180 200 250
19-Aug	14	06:59	25.57	30.71	5 20 35 50 60 70 80 90 100 120 140 150 160 180 200 250
20-Aug	16	06:52	25.66	30.40	5 20 50 80 90 110 120
20-Aug	17	11:58	26.22	30.28	5 20 50 80 90 110 125
20-Aug	18	16:52	26.50	30.28	5 20 50 80 90 110 130
20-Aug	19	20:56	26.90	30.29	5 20 50 75 90 110 120
20-Aug	20	22:35	27.00	30.28	5 20 50 80 90 110 150
21-Aug	21	02:26	27.00	30.42	5 20 50 90 100 110 135
21-Aug	22	06:50	26.65	30.67	5 20 50 90 100 110 135
21-Aug	23	11:55	26.10	30.68	5 20 50 90 100 110 135
21-Aug	24	16:52	25.92	30.67	5 20 50 80 90 110 125
21-Aug	25	20:56	25.59	30.67	5 20 50 72 90 110 125
21-Aug	26	22:35	25.60	30.82	5 20 50 65 90 110 112
22-Aug	27	00:55	25.67	31.07	5 20 50 80 90 110 130

Date	CTD no.	Time (GMT)	Lat, °N	Lon, °W	Depths (m)
22-Aug	28	04:08	25.96	31.07	5 20 50 78 90 110 115
22-Aug	29	06:53	26.23	31.07	10 20 50 73 90 110 120
22-Aug	30	12:10	26.59	31.07	5 20 50 85 90 110 136
22-Aug	31	16:53	27.00	31.16	5 20 50 72 90 110 138
22-Aug	32	20:57	26.92	31.45	5 20 50 90 110 135
22-Aug	33	22:35	26.81	31.45	5 20 50 83 90 110 133
23-Aug	34	00:57	26.64	31.45	5 20 50 83 90 110 135
23-Aug	36	07:00	26.15	31.44	5 20 50 86 90 110 114
23-Aug	37	11:59	26.60	31.53	5 20 50 90 40 110 130
23-Aug	38	16:57	25.63	31.84	5 20 50 75 90 106 110
23-Aug	39	21:01	26.05	31.84	5 20 50 70 90 110 120
23-Aug	40	22:34	26.18	31.83	5 20 50 70 90 110 125
24-Aug	41	01:06	26.38	31.83	5 20 50 80 90 110 135
24-Aug	42	07:02	26.70	31.71	5 20 40 65 90 100 123
25-Aug	44	07:21	27.00	30.35	5 10 20 40 50 60 71 90 100 110 120 130 141 160 180 250 500
25-Aug	45	21:29	26.73	31.83	5 20 30 40 50 60 85 100 120 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
26-Aug	46	07:17	26.60	30.72	5 10 20 30 40 50 60 80 89 100 112 130 140 160 180 200 250 500
26-Aug	47	21:26	26.40	31.70	5 20 30 50 60 70 80 90 100 105 110 115 120 125 130 140 160 180 200 500
27-Aug	48	07:18	26.20	31.83	5 10 20 30 40 50 60 70 81 90 100 110 120 130 140 160 180 200 220 500
27-Aug	49	21:18	26.00	30.50	5 20 30 50 60 80 100 120 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
28-Aug	50	07:14	26.00	31.81	5 10 20 30 40 50 60 65 80 90 100 106 120 130 140 160 180 200 220 500
29-Aug	51	07:20	26.60	31.34	5 10 20 30 40 50 60 70 81 90 100 110 122 130 140 160 180 200 250 500
30-Aug	54	07:18	26.52	31.49	5 20 30 50 70 76 90 110 130 140 144 180 200
30-Aug	55	13:21	26.80	31.37	5 20 40 60 70 80 90 110 120 150 160 200
30-Aug	56	22:21	26.83	31.47	5 20 40 60 75 90 100 110 120 125 140 160 200
31-Aug	57	01:24	26.78	31.50	5 20 50 60 75 90 100 110 115 120 140 160 200

Date	CTD no.	Time (GMT)	Lat, °N	Lon, °W	Depths (m)
31-Aug	58	07:15	26.37	31.50	5 20 40 60 70 88 100 110 120 126 140 160 200
31-Aug	59	13:18	26.57	31.04	5 20 40 50 60 75 100 110 137 140 160 200
31-Aug	60	22:10	26.70	31.39	5 20 40 60 80 100 110 120 130 140 160 200
01-Sep	61	01:10	26.49	31.40	5 20 40 60 75 90 100 110 120 150 200
01-Sep	62	07:16	26.50	31.50	5 20 40 60 70 90 110 120 136 140 150 160 200
01-Sep	63	13:20	26.79	31.36	5 20 40 60 70 80 90 110 120 140 150 160 200
01-Sep	64	22:21	26.35	31.48	5 20 40 60 80 100 110 120 130 150 180 200
02-Sep	65	01:14	26.53	31.38	5 20 40 60 75 90 100 110 130 150 200
02-Sep	66	07:22	25.81	31.46	5 20 40 60 80 100 110 120 140 150 154 160 200
02-Sep	67	13:25	26.53	31.65	5 20 30 50 75 80 100 110 120 135 150 160 200
02-Sep	68	22:04	26.80	31.73	5 20 40 60 80 90 100 110 120 134 140 160 200
03-Sep	69	07:11	26.80	31.56	5 20 40 50 60 70 80 90 100 130 148 180 200
04-Sep	71	07:14	26.10	31.83	5 20 30 40 50 60 72 80 90 100 110 120 130 140 160 180 200 250 500
04-Sep	72	22:23	26.85	31.62	5 20 30 40 50 60 70 75 90 100 105 110 115 130 138 150 160 200 500
05-Sep	73	07:12	26.57	31.43	5 10 20 30 40 50 60 70 80 88 100 110 120 130 148 160 200 500
06-Sep	75	07:09	26.73	30.97	5 20 30 40 50 60 70 85 90 100 110 120 130 142 150 160 200 300 500
06-Sep	76	22:12	26.21	30.75	5 20 30 50 60 75 90 100 110 120 135 140 160 200 500
07-Sep	77	07:08	26.48	30.73	5 20 30 40 50 60 70 80 90 100 110 120 136 140 150 160 200 500
07-Sep	78	22:07	25.78	30.53	5 20 30 40 50 60 70 77 90 100 110 120 130 140 150 160 200 500
08-Sep	79	07:07	25.40	31.26	5 20 30 40 50 60 70 80 91 100 110 120 128 140 150 160 200 500
08-Sep	80	13:08	25.40	31.81	5 20 30 50 67 90 100 112 140 160 200 500
08-Sep	81	21:21	25.89	31.50	5 20 40 60 80 90 110 120 130 140 160 200
09-Sep	82	13:24	25.89	31.50	5 20 40 40 65 86 90 110 126 140 160 180 200 500
09-Sep	83	20:51	25.46	31.78	5 20 50 75 90 110 130 150 200 300 500
10-Sep	84	00:56	25.57	31.50	5 20 50 80 90 100 110 150 200 300 500
10-Sep	85	07:04	25.40	31.19	5 20 40 50 65 85 100 121 140 160 180 200

Date	CTD no.	Time (GMT)	Lat, °N	Lon, °W	Depths (m)
10-Sep	86	13:31	25.89	31.50	5 20 40 65 75 100 115 140 159 200 300 500
10-Sep	87	21:08	25.41	31.81	5 20 50 65 75 90 105 115 125 150 175 200 500
11-Sep	88	01:08	25.57	31.50	5 20 50 65 80 90 100 110 115 150 175 200 500
11-Sep	89	07:22	25.40	31.19	10 20 30 40 50 60 70 80 90 100 110 120 128 140 150 160 200 500
11-Sep	90	13:14	25.88	31.49	10 20 30 40 50 60 70 83 90 100 125 150 200 500
11-Sep	91	20:59	25.43	31.79	5 20 50 65 80 90 100 110 115 150 175 200 500
12-Sep	92	01:04	25.57	31.50	5 20 50 65 75 90 100 110 125 150 200 500
12-Sep	93	07:16	25.40	31.19	2 20 30 40 50 70 81 90 100 110 120 140 150 160 200 500

Underway samples were collected on numerous occasions (see Table 17) from the ship's non-toxic seawater supply by an automated liquid handling robot (Tecan Miniprep 60, Tecan, Reading, UK). Samples were fixed instantly with paraformaldehyde and analysed flow cytometrically within 24 hours.

Table 17: Summary of underway sampling strategy

Start Date & Time	**End Date & Time**	**Sampling Frequency**
20/08/2011 20:00	21/08/2011 00:00	20 minutes
21/08/2011 20:00	22/08/2011 00:00	20 minutes
22/08/2011 18:00	22/08/2011 23:00	20 minutes
28/08/2011 17:00	02/09/2011 20:30	30 minutes
07/09/2011 20:00	12/09/2011 06:30	30 minutes

Alkaline Phosphatase Detection

Samuel Lew

ELF-97 alkaline phosphatase detection reagent was applied to filtered underway samples and 30 µm net hauls and visualised via fluorescent microscopy. The ELF stain was originally intended for flow cytometric analysis to assay the level of phosphatase on the single microbial cell but as flow cytometer availability was low, microscopy was the next best approach. Interestingly, the ELF dye labelled a pennate diatom but no other labelling was observed in either the large filtered fraction at 30 µm or the microbial fraction filtered at 0.2 µm. Various approaches were considered including direct application of the ELF dye to live filter media and pre-filtered incubations. Time series and concentration series were conducted for staining optimisation. Still signal was only detected for one diatom. No further analysis was made. It was possible that the ELF stain was rendered inactive due to accidental freezing upon delivery.

Membrane Potential

Samuel Lew

Concentration and time series were conducted using CTD samples from the deep chlorophyll maximum in order to optimise the detection and measurement of membrane potential using Di-4 ANEPPS. No signal was detected at any concentration or time point.

Further protocol optimisation was performed with the dual response probe DiOC2 (Baclight membrane potential detection kit). Detection of both emission wavebands in the red and green spectra was achieved. Optimisation included concentration and times series analyses at multiple depths (20 m, 50 m, oxygen maximum, 90 m, deep chlorophyll maximum). At depths above 90 m, the signal to noise ratio was too high to measure the signal in sufficient detail. Therefore, a diurnal cycle from the 9th to the 12th of September, sampling on the dawn, noon, dusk and midnight CTD at 90 m, deep chlorophyll maximum and 200 m was conducted.

Preliminary analysis indicates that detection of the smaller microbial component was successful although confirmation of the population with counter DNA staining is necessary but requires a UV laser to utilise DAPI which emits in a window outside of the DiOC2 dye emission. A smaller signal was also detected from the *Prochlorococcus* population; however, the red signal indicative of hyperpolarisation was small. Complete analysis of the data will be conducted at NOCS.

Bacterial Phosphorus Uptake

Diel rhythmicity and light effect on phosphorus uptake by dominant bacterioplankton groups in the North Atlantic subtropical gyre

Paola Gomez-Pereira, Isabelle Mary and Carolina Grob

Objectives

1. Measure inorganic phosphorus concentrations and uptake rates prokaryotes in the upper 100 m water layer.
2. Study diurnal variations in inorganic phosphorous uptake by the dominant prokaryotic groups.
3. Evaluate the effect of light on inorganic phosphorus uptake by the dominant prokaryotic groups.
4. Evaluate the light enhanced uptake of amino acid by prokaryotes inhabiting the deep chlorophyll maximum.
5. Compare uptake rates of [alpha ^{33}P]- Adenosine 5' triphosphate and [2,5, 8-^{3}H] Adenosine 5' triphosphate
6. Taxonomically identify and quantify the dominant prokaryotic in order to link community composition and function (in collaboration with B. Fuchs and J. Wulf)
7. Evaluate the expression of key genes related with circadian clock and light harvest.

Sample collection

Samples were collected into acid-washed 1 L thermo flask. Thermo flasks were washed three times with seawater sample before collection. Sample was processed within 1 hour after collection. Samples for RNA extraction were collected in acid-washed 10-20 L carboy using acid soaked silicone tubing and filtered immediately. Samples analysed are listed in Table 18 and Table 19 below.

Inorganic phosphorous concentration and uptake rate, diurnal variations and light enhanced uptake by major prokaryotic populations (Objectives 1, 2 and 3)

Ambient concentrations as well as uptake rates of inorganic and organic phosphate by total microbial plankton were measured using isotopic dilution time-series incubations at tracer concentrations (Zubkov, *et al.*, 2004, Zubkov, *et al.*, 2007). [^{33}P]-Phosphoric acid (specific activity 3000 Ci mmol^{-1}) was added at a concentration of 0.05, 0.08 or 0.15 nM and diluted with non-labelled Na_2HPO_4 or phosphoric acid using dilution series in a range between 0.4 and 4.8 nM. Samples were fixed after 10, 20, 30 and 40 minutes or 15, 30, 45 and 60 minutes with 1% (w/v) paraformaldehyde final concentration. Samples were filtered onto 0.2 µm pore size polycarbonate filters and washed twice with 3 ml of MQ water. Radioactivity retained on the filters was measured as counts per minute using a liquid scintillation counter. Results were obtained within approximately 6 h of sampling and used to evaluate the dilution series to be used in the following experiment.

For flow cytometric sorting of isotopically labelled cells, 4.8 to 8 mL of samples was incubated in Pyrex glass bottles. Bottles were washed and filled in the dark, only illuminated by a dim green light, in order to avoid effects of ambient lights during experiment set up. Bottles were placed in a 6 L water tank illuminated by a white warm LED light at different light intensities. A second water tank was kept in the dark by covering it with two layers of black bags. Temperature was kept constant at the ambient water temperature by circulating water through the water tanks connected with a refrigerated thermostatic bath with a pump. Temperature was set up at 0.5°C less of sea-water temperature in order to compensate heating produced by LED lights. Light intensities were 250 µmol m^{-2} s^{-1} for surface samples and 60-70 µmol m^{-2} s^{-1} for samples below 50 m. Samples were fixed after 3-4 h with 1% PFA. Samples were further used for flow cytometry cell sorting of labelled prokaryotes.

Cells were stained with SYBR Green I DNA stain prior of analysis by flow cytometry. Different number of *Prochlorococcus*, LNA and, occasionally total bacterioplankton cells, were sorted, filtered onto 0.2 µm polycarbonate filters and the radioactive retained on the filters counted in scintillation counter. Four proportional numbers of cells were sorted, and the mean cellular tracer uptake was determined as the slope of the linear regression of radioactivity against the number of sorted cells. Experiments described above were performed in all stations and depth listed in Table 18.

Table 18: Stations sampled for inorganic P uptake and microbial diversity.

Sample	Date	Time	Depth (m)	Sample	Date	Time	Depth (m)
CTD1	11/08/2011	6AM	20	CTD36	23/08/2011	6AM	85
CTD2	12/08/2011	6AM	20	CTD37	23/08/2011	11AM	94
CTD3	13/08/2011	6AM	20	CTD38	23/08/2011	4PM	75
CTD4	14/08/2011	6AM	80	CTD39	23/08/2011	8PM	70
CTD4	14/08/2011	6AM	20	CTD40	23/08/2011	10PM	70
CTD4	14/08/2011	6AM	20	CTD41	24/08/2011	0AM	80
CTD5	14/08/2011	8PM	20	CTD54	30/08/2011	6AM	20
	14/08/2011	8PM	80	CTD55	30/08/2011	12PM	20
	14/08/2011		80	CTD56	30/08/2011	9PM	20
CTD7	15/08/2011	6AM	20	CTD57	31/08/2011	12AM	20
	15/08/2011		50	CTD58	31/08/2011	6AM	20
CTD8	15/08/2011	8PM	20	CTD59	31/08/2011	12PM	20
	15/08/2011		110	CTD60	31/08/2011	9PM	20
CTD9	16/08/2011	6AM	20	CTD61	01/09/2011	0AM	20
	16/08/2011		115	CTD62	01/09/2011	6AM	20
CTD10	16/08/2011	8PM	20	CTD63	01/09/2011	12PM	20
	16/08/2011		80	CTD64	02/09/2011	9PM	20
CTD11	17/08/2011	6AM	110	CTD65	02/09/2011	12AM	20
	17/08/2011		20	CTD66	02/09/2011	6AM	20
CTD12	17/08/2011	6AM	20	CTD67	02/09/2011	12PM	20
			110	CTD68	02/09/2011	9PM	20

Sample	Date	Time	Depth (m)	Sample	Date	Time	Depth (m)
CTD13	18/08/2011	dawn	20	CTD72	04/09/2011	9PM	20
			110		04/09/2011		110
CTD16	20/08/2011	6AM	80	CTD74	05/09/2011	9PM	20
CTD17	20/08/2011	11AM	80		05/09/2011		100
CTD18	20/08/2011	4PM	80	CTD76	06/09/2011	9PM	20
CTD19	20/08/2011	8PM	75		06/09/2011		100
CTD20	20/08/2011	10PM	80	CTD79	08/09/2011	6AM	50
CTD21	21/08/2011	12AM	100	CTD80	08/09/2011	12PM	50
CTD22	21/08/2011	6AM	80	CTD81	08/09/2011	9PM	20
CTD23	21/08/2011	11AM	80	CTD82	09/09/2011	12PM	20
CTD24	21/08/2011	4PM	80	CTD83	09/09/2011	8PM	20
CTD25	21/08/2011	8PM	72	CTD84	10/09/2011	12AM	20
CTD26	21/08/2011	10PM	65	CTD85	10/09/2011	6AM	20
CTD27	22/08/2011	12AM	80	CTD86	10/09/2011	12PM	20
CT28	22/08/2011	3AM	78	CTD87	10/09/2011	9PM	20
CTD29	22/08/2011	6AM	73	CTD88	11/09/2011	12AM	20
CTD30	22/08/2011	11AM	86	CTD89	11/09/2011	6AM	20
CTD31	22/08/2011	4PM	72	CTD90	11/09/2011	12PM	20
CTD32	22/08/2011	8PM	90	CTD91	11/09/2011	9PM	20
CTD33	22/08/2011	10PM	78	CTD92	12/09/2011	12AM	20
CTD34	23/08/2011	12AM	O2 max	CTD93	12/09/2011	6AM	20
CTD35	23/08/2011	3AM	74				

Evaluate the light enhanced uptake of amino acid by prokaryotes inhabiting the deep chlorophyll maximum (Objective 4)

L-[^{35}S] methionine (specific activity 1000 Ci mmol^{-1}) was added at a concentration of 0.05 nM and diluted with non-labelled methionine using dilution series in a range between 0.05 nM and 1 nM. The L-[4,5-^{3}H] leucine (specific activity 140 Ci mmol^{-1}) was added at concentration between 0.1 nM and 1 nM. In order to evaluate cell specific uptake and their light enhanced uptake at different depths, samples were incubated with labelled methionine and leucine. The uptake of methionine and leucine was evaluated in few occasions due to low uptake rates. The uptake rates of amino acids was low, and therefore the uptake per cell and their light enhanced uptake could not be determined and it was not possible to fulfil objective 4.

Compare uptake rates of [alpha 33P]- Adenosine 5' triphosphate and [2,5' 8-3H] Adenosine 5' triphosphate (objective 5)

The uptake rates of adenosine 5' triphosphate were evaluated using the tracers [alpha 33P]-Adenosine 5' triphosphate in which the phosphorous part of the molecule is labelled and [2,5' 8-^{3}H] Adenosine 5' triphosphate in which the adenosine part of the molecule is labelled. [alpha ^{32}P]- ATP (specific activity 3000 Ci mmol^{-1}) was added at a concentration of 0.1 nM or 0.3 nM and diluted with non-labelled ATP using dilution series in a range between 0.1 nM – 1 nM. [2,5' 8-^{3}H] ATP (specific activity 51.5 Ci mmol^{-1}) was added at a concentration of 0.1- 1 nM. Occasionally, [2,5' 8-^{3}H] ATP was diluted with non-labelled ATP using a dilution series in a range of 0.2 to 0.5 nM. Samples were fixed and filtered as described above.

For the determination of the light enhanced uptake of *Prochlorococcus* and LNA by flow cytometric sorting of isotopically labelled cells, 8 mL of samples was incubated in Pyrex glass bottles in the light and in the dark as described above using a concentration of 0.8 nM of [2,5' 8-^{3}H] ATP and 0.3 nM [alpha ^{33}P]-ATP and processed as described for objectives 1, 2 and 3. Due to the low specific activity of the former, samples were incubated for 10 h. In order to check that the uptake was linear, subsamples were taken every 1 or 2 h. Experiments performed are listed in Table 19.

Preliminary experiments of light intensities and qualities were performed using [alpha 33P]-ATP as a tracer, as indicated in Table 19.

Taxonomically identify and quantify the dominant prokaryotic populations (in collaboration with B. Fuchs and J. Wulf)

Samples were collected in all stations were tracer experiments were performed. Populations of which phosphate uptake was measured will be identified in selected stations. Four aliquots of 1.6 mL sample were fixed with 1% paraformaldehyde or 1% formaldehyde for 1 h at room temperature, or at 4°C for 1-4 h. In CTDs 54-68, in those samples from 6 AM and 12 AM, LNA and *Prochlorococcus* populations were sorted on board by B. Fuchs. Identification of sorted cells will be performed at the National Oceanography Centre when equipment returns. B. Fuchs and J. Wulf will collaborate with reagents.

Table 19: Stations sampled for ATP uptake and microbial diversity.

Sample	**Date**	**Depth (m)**	**Time**
CTD09	26/07/2007	20	6AM
CTD44	25/08/2011	110	6AM
CTD46	26/08/2011	20	6AM
CTD48	27/08/2011	20	6AM
CTD48	27/08/2011	109	6AM
CTD50	28/08/2011	106	6AM
CTD50	28/08/2011	20	6AM
CTD52	29/08/2011	20	6AM
CTD72	04/09/2011	20	9PM
CTD74	05/09/2011	20	9PM
CTD76	06/09/2011	20	9PM
CTD78	07/09/2011	20	9PM
CTD86	10/09/2011	20	12PM
CTD93	12/09/2011	20	6AM

Evaluate the expression of key genes related with circadian clock and light harvest (objective 7)

During the diurnal cycle, RNA samples were collected to evaluate the expression of key genes related with circadian clock and light harvest of major prokaryotic populations (*Prochlorococcus*, SAR11). Between 2 and 3 L of seawater were pre-filtered on 5 μm filters and collected on 0.2μm filters in less than 30 min in order to preserve mRNA. Filters were then flash-frozen and store at -80°C for further analysis.

Similarly, RNA samples were collected during light/dark incubation experiments to relate the expression of genes related to light harvest with light stimulation of phosphorus uptake by microbial populations. 500 mL of sample were incubated in light or dark for 3 h and collected as described above.

Preliminary observations

Phosphorous concentration varied in a range between 0.5 nM and 3.4 nM while its uptake rate was between 0.1 - 4.8 nM day^{-1}. Changes in the diurnal cell specific uptake were observed in *Prochlorococcus* and LNA. Particularly the uptake rate of *Prochlorococcus* cells was higher in the early morning during the third diurnal survey (CTD78-CTD93). Inorganic phosphorous uptake was, in general, higher in the light than in the dark. The difference between light and dark uptake was higher at surface for LNA, while for *Prochlorococcus* it was higher below 50 m.

Amino acid uptake rates were very low; therefore cell specific uptake could not be determined.

In the occasion were both measurements were done in parallel, no difference was detected between the concentration of ATP evaluated with the tracer ^{33}P-ATP or [2,5' 8-^{3}H]-ATP. However, the uptake rate of the later was higher than of the former. The uptake of ^{33}P-ATP was between 0.09 and 0.14 nM day^{-1} while for [2,5' 8-^{3}H]-ATP it was between 0.16 and 0.38 nM day^{-1}. In four experiments the light enhanced uptake of both substrates were evaluated in parallel. In three of them, the uptake of both substrates was enhanced by light.

Molecular analyses (gene expression, FISH identification) will be performed back to the laboratory at NOCS or University Blaise Pascal (Clermont-Ferrand, France). No preliminary results are available at this time.

Eukaryotic Phosphorus Uptake

Uptake of inorganic and organic phosphorus by pigmented and non-pigment eukaryotes

Manuela Hartmann, Ludwig Jardillier and Mikhail Zubkov

Aims & objectives

Aim: To assess metabolic activities of dominant eukaryotic groups within the planktonic communities in the phosphate-depleted North Atlantic gyre. To use molecular approaches to identify these groups taxonomically in order to link community composition and function.

Objectives

- To estimate turnover rates of dissolved organic and inorganic phosphorus using ATP and phosphate tracers (in collaboration with Paola Gomez-Pereira and Isabelle Mary) and uptake rates of specific eukaryotic groups.
- To collect concentrated seawater samples for molecular analysis of the phylogenetic composition of the groups (flow sorted for rate measurements) using 18S-rDNA clone libraries and fluorescence *in situ* hybridisation (TSA-FISH).

Estimations of turnover rates and concentrations of dissolved organic and inorganic bioavailable phosphate

Ambient concentrations as well as uptake rates of the inorganic and organic phosphate by total bacterioplankton were measured using isotopic dilution time-series incubations (Zubkov, *et al.*, 2004, Zubkov, *et al.*, 2007). Microbial inorganic and organic phosphorus dynamics were determined in the phosphate-depleted North Atlantic gyre (Table 20), stations marked with a star indicate measurements of organic phosphorus) to estimate ambient concentrations and turnover rates of the bioavailable fraction of these nutrients. All seawater samples were processed within an hour of collection. In

addition, the relative contributions by dominant groups of eukaryotes to the phosphate cycle were determined using flow cytometric cell sorting.

Table 20: Sampling stations including CTD no., dates, bottle no. and depth. At stations marked with a * ambient concentrations of bioavailable, organic phosphate were determined.

CTD no.	**Date**	**Time (GMT)**	**Bottle no.**	**Bottle Depth (m)**
58	31/08/11	06:00	21	20
62*	01/09/11	06:00	22	20
66*	02/09/11	06:00	22	20
71	04/09/11	06:00	22	20
73	05/09/11	06:00	22	20
75	06/09/11	06:00	22	20
79*	08/09/11	06:00	22	20
89*	11/09/11	06:00	22	20
90*	11/09/11	12:00	22	20
91	11/09/11	20:00	22	20
92	12/09/11	24:00	22	20
93*	13/09/11	06:00	22	20

Collection of eukaryotic cells for molecular analyses of phylogenetic composition of dominant groups

In order to understand the contribution of photosynthetic picoeukaryotes (PPEs) to carbon fluxes and the microbial food web, it is necessary to quantify the dominating phylogenetic groups within that cluster. Clone libraries will be constructed using eukaryotic 18S rDNA primer pairs as well as prokaryotic 16S rDNA primer pairs targeting specifically chloroplastidic DNA of photosynthetic eukaryotes. This approach will be combined with TSA-FISH to assess the distribution, the abundance and the contribution of specific PPE classes to the total phytoplankton biomass. Moreover, the results of the molecular approach will be compared to those of the tracer experiments.

2 L of seawater sample were concentrated using a 30 µm pore-size mesh, to screen out larger organisms, combined with a Celltrap^{TM} ceramic filtration unit, 1.6 mL of this concentrate were fixed with RNA later, Betaine or 1% PFA. Samples for analyses of RNA and DNA were immediately flash frozen and stored at -80°C. Samples fixed with 1% PFA were incubated for 1 hour at 4°C, subsequently flash frozen with liquid and stored at -80°C.

Preliminary observations

Initial scintillation counts were carried out on board the ship (Packard Tri-Carb 3100). The bioavailable, inorganic phosphate concentrations ranged between 0.2-1.7 nM and the estimated turnover time for phosphate varied between 5-63 hours. Concentrations of 0.5-3.2 nM and an estimated turnover time of 332-1353 hours were measured for organic phosphate. After the cruise, the collected tracer samples of flow sorted cells we be analysed in detail on low background counters due to the sensitivity limitations of the scintillation counters on board. The detailed data set will allow estimation of rates of metabolic activity of bacterioplankton and phytoplankton, as well as production and mortality. Moreover, completion of molecular analysis will enable us to link prokaryotic and eukaryotic community composition and function.

Carbon Fixation

Single-cell carbon fixation of large (>20µm) microbial eukaryotes

Manuela Hartmann, Ludwig Jardillier and Mikhail Zubkov

Aims & objectives

Aim: To assess per cell carbon fixation rates of large microbial eukaryotes. To determine community composition using FlowCam analyses. To use molecular approaches to identify the organisms taxonomically to species level in order to link function and community composition.

Objectives

- To estimate carbon fixation rates of less abundant, large eukaryotes in the oligotrophic North Atlantic gyre using radiolabelling in combination with laser dissecting microscopy (in collaboration with the Max-Planck-Institut Bremen, Dr. Bernhard Fuchs).
- To determine the abundance of these eukaryotes using a semi-automatic, microscopic approach (FlowCam)
- To collect samples of different size fractions for molecular analysis of single cells using 18S-rDNA clone libraries in combination with laser dissecting microscopy.

Carbon fixation rates

Sodium ^{14}C-bicarbonate was used in a series of experiments to trace photosynthetic fixation by microbes. Plankton net cast were carried out at the stations indicated in Table 21. Plankton net concentrated and size-fractionated samples (20, 40, 100 and 180 µm) were incubated for up to 4h in a light array adjusted to ambient light conditions, subsequently fixed with glutaraldehyde (1% final concentration) and filtered on 10 µm pore size polycarbonate filter by gravity filtration. Carbon fixation rates of total filters were monitored using a scintillation counter (Packard TriCarb 3100) to ensure that single cell uptake can be measured. Per cell carbon fixation rates will be estimated in

collaboration with the Max-Planck-Institut Bremen (Dr. Bernhard Fuchs) using a Nikon laser dissecting microscope in order to target and excise specifically selected cells on the filters.

Determination of community composition of large eukaryotes

In order to measure abundance of large eukaryotic cells in the top layer of the photic zone (100 m) plankton net concentrated samples were collected and analysed with a FlowCam (stations see Table 21). The FlowCam technique was optimised by replacing the machines internal peristaltic pump with a syringe pump to allow stable and precise flow rates. If possible 49 mL of every size fraction (20, 40, 100 and 180 µm) were analysed in duplicates.

Table 21: Sampling stations including CTD no., dates, time and depth.

CTD no.	**Date**	**Time (GMT)**	**Depth (m)**
14	19/08/11	13:00	100
44	25/08/11	07:00	100
51	29/08/11	11:30	100
62	01/09/11	07:00	100
66	02/09/11	07:00	100
69	03/09/11	07:00	100
85	10/09/11	07:00	100

Collection of single eukaryotic cells for molecular analyses of exact phylogenetic identification

Due to the fixation of the cell morphological features do not allow an accurate identification of fixed single cells. Hence, molecular analyses of cells excised from filters using a laser dissecting microscope will be carried out. Clone libraries will be constructed using eukaryotic 18S rDNA primer pairs. Between 30 and 60 mL of every size fraction (20, 40, 100 and 180 µm, stations see Table 21) were filtered on 10 µm polycarbonate filters by gravity filtration, dried at room temperature for 20 min and then flash frozen and stored at -80°C.

Post-cruise analyses

After the cruise, the collected tracer samples will be transported to the Max-Planck-Institut in Bremen and single cell carbon fixation rates determined using a laser dissecting microscope to excise single cells. The detailed data set will allow estimation of rates of carbon fixation of large eukaryotic microbes. In combination with molecular and FlowCam analysis this approach will enable us to link eukaryotic community composition and function.

Tracking Changes in Community Composition

Carolina Grob

Large volume, long term incubations: simulating a mixing event

In order to evaluate potential changes in open-ocean community composition due to mixing events a series of mesocosm experiments were conducted on the after deck of the RRS *Discovery* using large volume incubators, i.e., two ~1 ton NutriLine Food-IBC polyethylene containers (Werit UK Ltd, Manchester).

In each case, 20 L of water from 500 m, i.e., with much higher nutrient concentrations than surface waters, were collected from the CTD right before starting the experiments. This deep water was used to simulate a mixing event by adding it to ~1 ton of surface water (~2 m) pumped directly into the incubators from the environment using a non-toxic system. One incubator was filled up with ~1 ton of surface water to be used as control, whereas the 20 L of water from 500 m was added to a different incubator, the mixing event simulation experiment one, right before filling it up in the same way as the control. Both incubators were sampled either once or twice a day to take samples for flow cytometry and variable fluorescence (FRRF) analyses. Samples for DNA were also collected using CellTrap® at the beginning and at the end of the 88 to 95 h long experiments for later molecular analyses to determine changes in community structure. In each case about 5 L of water sampled from the incubators was filtered using the CellTraps.

Table 22: Summary of mesocosm experiments. Samples for mixing event simulation experiments were taken from 500 m right before filling up the incubators.

Date	**CTD**	**Lat**	**Lon**	**Time when incubators were full (T0)**
20/08/11	16	25°39.7'N	30°23.787'W	14 h
27/08/11	48	26°12.094'N	31°49.631'W	12 h
31/08/11	59	26°34.44'N	31°02.20'W	22 h
05/09/11	73	26°33.966'N	31°23.588'W	22 h

Metagenomics of Synechococcus, Prochlorococcus and photosynthetic picoeukaryotes populations at selected stations

Seawater (20 L each time) was collected from the surface (5 m) and the Deep Chlorophyll Maximum (DCM, ~1% of surface light) at 3 different stations within the North Atlantic subtropical gyre (Table 23) and collected in Cell Traps (0.22 µm) after pre-filtering through 63 µm mesh and 10 µm PC filter membranes from 10 L carboys. Water was also collected from the surface and the deep Oxygen Maximum at two selected stations and processed as indicated in Table 23. In all cases CellTraps were used to collect DNA samples from ~7-9 L of seawater each time to have duplicates for each sampled depth and station. DNA will be extracted from populations of *Synechococcus*, *Prochlorococcus* and photosynthetic picoeukaryotes populations sorted using flow cytometry and amplified using a commercial kit for further analyses.

Table 23: Summary of samples taken for metagenomics work.

Date	**CTD**	**LAT**	**LON**	**DNA sample taken**
06/09/11	76	26°12.35'N	30°45.05'W	Surf (btl 24), DCM (135 m, btl 7)
07/09/11	78	25°46.67'N	30°31.83'W	Surf (btl 24), O_2 max (77 m, btl 15)
08/09/11	81	25°53.55'N	31°30.00'W	Surf (btl 23, 24), DCM (130 m, btl 8, 9)
10/09/11	87	25°24.37'N	31°48.36'W	Surf (btl 23, 24), O_2 max (75 m, btl 15,16)
11/09/11	91	25°25.81'N	31°47.64'W	Surf (btl 23, 24), DCM (110 m, btl 9, 10)

Samples were collected in duplicate after pre-filtering through 63 µm mesh and 10.0 µm PC filters. Between 7 and 9 L were filtered in each case. Surf stands for surface. DCM and O_2 max stand for Deep Chlorophyll and Oxygen Maximum, respectively. Btl stands for bottle number.

Picoplankton Activity

Molecular identification of total vs. active picoplankton populations in the Northern Atlantic Gyre

Ludwig Jardillier and Isabelle Mary

Aim

To determine the coarse-scale (CTD) and fine-scale (pump profiler) vertical distribution and diversity of functional picoplanktonic groups (prokaryotes and eukaryotes) through the water column.

Microbial diversity

Bacterioplankton and picophytoplankton are essential components of the ocean. Bacterioplankton play key roles in many biogeochemical processes. Understanding which bacterioplankton communities dominate and what they respond to remains a fundamental ecological question. An important first step towards understanding the roles of various prokaryotes in the ocean is determining the numbers and relative abundances of different bacterioplankton groups. In this study, we intend to better characterize the phylogenetic and functional relevance of cytometrically defined groups along the water column (0-2000 m) in the Northern Atlantic Gyre. For instance, low nucleic acid (LNA) population is largely dominated by the Alphaproteobacteria SAR11 clade in surface Atlantic waters (Mary, *et al.*, 2006) but may be comprised of SAR11 cells belonging to distinct subclades or ecotypes (Field, *et al.*, 1997) in deeper waters, as well as *Crenarchaeota* cells (Schattenhofer, *et al.*, 2009). Moreover, picophytoplankton has been shown to contribute significantly to the primary production in the ocean. This group comprises prokaryotes (*Synechococcus* and *Prochlorococcus*) as well as eukaryotes, the later contributing significantly to the CO_2 fixation despite its abundance lower than the prokaryotic counterpart (Jardillier, *et al.*, 2010). The diversity of these groups has extensively been studied over the last decade. However, new taxa are frequently discovered, even in well-studied regions of the Ocean (Kim, *et al.*, 2010). Also, the ecology of these groups has essentially been assessed through the study of their rDNA. We here set up a new protocol to allow coupling sorting of pigmented/non pigmented cells by flow cytometry technology and fine phylogenetic analysis of their composition through their rRNA. This new approach allows us to target specifically active microbes.

Sample collection

Vertical profiles of seawater were collected from the CTD, deep incubator or pump cast (Table 24). Briefly, 10 L of seawater were pre-filtered through 30 or 5 µm pore-size membranes, to remove large plankton and particles, then small cells were collected using cell concentration devices or onto 0.2 µm pore-size membranes (sterivex). Each sample was preserved with or without buffer to preserve cells, RNA or DNA. All samples were flash-frozen immediately after processing and stored at -80°C. Additional samples were preserved with 1% PFA and stored at -80°C for flow cytometry analysis.

Results

Molecular analyses will be performed back to the laboratory at University Blaise Pascal (Clermont-Ferrand, France) and University Paris-Sud (Paris, France). No preliminary results are available at this time.

Table 24: Stations sampled for microbial diversity, including CTD no., dates, and depth.

Station	**Depth (m)**	**Date**
CTD 042	250, 500, 750, 1000, 1250, 1500, 1750, 2000	24/08/2011
Deep sea incubator	1000	29/08/2011
CTD 052	5, 20, 50, 80 (O2 max), 100, 133 (DCM), 170, 200	29/08/2011
CTD070	5, 20, 50, 70 (O2 max), 100, 130 (DCM), 170, 200	03/09/2011
CTD080	20, O2 max, DCM, 200	08/08/2011
CTD082	5, 20, 50, O2 max, 100, DCM, 170, 200	09/09/2011
CTD086	300, 400, 500, 583, 667, 750, 810, 917	10/09/2011
Pump cast	0-200	11/09/2011
Deep sea incubator	1000	11/09/2011

Molecular Biology

Bernhard Fuchs, Jörg Wulf and Andreas Ellrott

Several methodological achievements in molecular biology have been reached during the cruise D369 into the Northern Atlantic Gyre. First, the fluorescence *in situ* hybridisidation (FISH) method has been successfully established during the cruise enabling the quantification of specific bacterioplankton populations already on board. Several parameters have been optimised to confidently visualise the main global players *Prochlorococcus* and SAR11.

In collaboration with Prof. Mike Zubkov's group, a routine was developed to sort specific flow cytometrically defined bacterioplankton populations for further phylogenetic analyses like cloning and FISH. Sorted populations were identified and quantified – and this is novel - already on board during the cruise. Two populations were in particular focus of our combined studies, the LNA (low nucleic acid containing-) and the HNA (high nucleic acid containing-) groups. Whereas the former consisted almost exclusively by the SAR11 clade, the latter was dominated by the *Prochlorococcus* family. HNA could be subdivided into several sub-populations corresponding to different cell sizes. They apparently all harbour different other phylogenetic groups besides members of the genus *Prochlorococcus*. For example in the HNA1 group tiny *Gammaproteobacteria* and *Bacteroidetes* have been detected, whereas in the HNA3 group large *Gammaproteobacteria* were dominating.

To resolve any potential fine structured layering of the water column the newly developed PumpCast-CTD was brought on board. All six deployments were successful and high resolution profiles could be recorded for salinity, temperature, oxygen and chlorophyll a fluorescence down to a depth of 230 m. Form each deployment 260 – 360 samples could be retrieved corresponding to depth layers of app. 0.5 - 1 m. Although the water column showed steep gradients in many of the parameters, a fine structured layering of the microbial community could not be detected so far. Further detailed analyses of specific taxonomic groups need to be done to unravel potential partitioning into different ecological niches across the gradients.

As a small exploratory project the microbial community residing on Micronet catches have been examined. First on board FISH experiments showed that in all of the size fractions gained from the Micronet (20, 40, 100, 180 µm) very large copiotrophic Bacteria of the Class *Gammaproteobacteria* and *Bacteroidetes* colonised plankton debris like faecal pellets, broken algae or copepods and crustaceans. This encourages further detailed analyses of the identity of the copiotrophs and on the spatial distribution of the bacterial groups on different plankton material.

Table 25: Summary of samples

Cast no.	Date	Filter material	Filter pore size (µm)	Filter diameter (mm)	Volume filtered (mL)	Number of filters	Purpose
1	11.08.11	Polycarbonate	0.2	47	20,14	11	FISH
2	12.08.11	Polycarbonate	0.2	47	20	1	FISH
4	13.08.11	Polycarbonate	0.2	47	20	13	FISH
7	16.08.11	Polycarbonate	0.2	47	20	12	FISH
11	17.08.11	Polycarbonate	0.2	47	20 / 20,08	9	FISH
13	18.08.11	Polycarbonate	0.2	47	20 / 20,08	9	FISH
deep incubator	19.08.11	Celluloseacetate	0.2	142	120000	1	DNA
deep incubator	19.08.11	Celluloseacetate	5	142	120000	1	DNA
16	20.08.11	Polycarbonate	0.2	47	20 / 20,08	5	FISH
17	20.08.11	Polycarbonate	0.2	47	20 / 20,08	6	FISH
18	20.08.11	Polycarbonate	0.2	47	20 / 20,08	6	FISH
19	20.08.11	Polycarbonate	0.2	47	20	8	FISH

Cast no.	Date	Filter material	Filter pore size (µm)	Filter diameter (mm)	Volume filtered (mL)	Number of filters	Purpose
20	20.08.11	Polycarbonate	0.2	47	20	8	FISH
22	21.08.11	Polycarbonate	0.2	47	20	8	FISH
23	21.08.11	Polycarbonate	0.2	47	20	7	FISH
24	21.08.11	Polycarbonate	0.2	47	20	8	FISH
25	22.08.11	Polycarbonate	0.2	47	20	8	FISH
37	23.08.11	Polycarbonate	0.2	47	20	7	FISH
38	23.08.11	Polycarbonate	0.2	47	20	6	FISH
39	23.08.11	Polycarbonate	0.2	47	20	8	FISH
40	23.08.11	Polycarbonate	0.2	47	20	8	FISH
41	24.08.11	Polycarbonate	0.2	47	20	8	FISH
PCTD 3	24.08.11	Polycarbonate	0.2	47	20	24	FISH
43	24.08.11	Polycarbonate	0.2	47	20	12	FISH
48	27.08.11	Polycarbonate	3	47	800	4	FISH

Cast no.	Date	Filter material	Filter pore size (µm)	Filter diameter (mm)	Volume filtered (mL)	Number of filters	Purpose
50	28.08.11	Polycarbonate	0.2	47	20,16	4	FISH
PCTD 4	29.08.11	Polycarbonate	0.2	47	20	67	FISH
Micronet	30.08.11	Polycarbonate	2	47	15	6	FISH
55	30.08.11	Polycarbonate	0.2	47	20	6	FISH
56	30.08.11	Polycarbonate	0.2	47	20	5	FISH
57	31.08.11	Polycarbonate	0.2	47	20	6	FISH
58	31.08.11	Polycarbonate	0.2	47	20	6	FISH
Micronet	31.08.11	Polycarbonate	2	47	200	5	FISH
59	31.08.11	Polycarbonate	0.2	47	20	6	FISH
59	31.08.11	Polycarbonate	2	47	1000	1	FISH
60	31.08.11	Polycarbonate	0.2	47	20	6	FISH
61	01.09.11	Polycarbonate	0.2	47	20	6	FISH
61	01.09.11	Polycarbonate	0.2	47	20	6	FISH

Cast no.	Date	Filter material	Filter pore size (µm)	Filter diameter (mm)	Volume filtered (mL)	Number of filters	Purpose
62	01.09.11	Polycarbonate	0.2	47	20	6	FISH
Micronet	01.09.11	Polycarbonate	2	47	200	5	FISH
63	01.09.11	Polycarbonate	0.2	47	20	6	FISH
64	01.09.11	Polycarbonate	0.2	47	20	6	FISH
65	02.09.11	Polycarbonate	0.2	47	20	6	FISH
66	02.09.11	Polycarbonate	0.2	47	20	6	FISH
Micronet	02.09.11	Polycarbonate	2	47	200	5	FISH
67	02.09.11	Polycarbonate	0.2	47	20	6	FISH
68	02.09.11	Polycarbonate	0.2	47	20	6	FISH
Micronet	03.09.11	Polycarbonate	2	47	250	5	FISH
PCTD 5	03.09.11	Polycarbonate	2	47	20	86	FISH
70	03.09.11	Polycarbonate	3	142	80000	1	DNA
70	03.09.11	Celluloseacetate	0.2	142	80000	1	DNA

Cast no.	Date	Filter material	Filter pore size (µm)	Filter diameter (mm)	Volume filtered (mL)	Number of filters	Purpose
74	05.09.11	Polycarbonate	3	142	93000	1	DNA
74	05.09.11	Celluloseacetate	0.2	142	93000	1	DNA
76	06.09.11	Polycarbonate	3	142	82000	1	DNA
76	06.09.11	Celluloseacetate	0.2	142	82000	1	DNA

N_2 Fixation & Nitrate Assimilation

A single cell approach on estimation of microbial N2 fixation and Nitrate assimilation

Abdul Sheik

It is well recognized that oceanic nitrogen fixation and nitrate assimilation has a pivotal role in providing fixed nitrogen compounds to surface water communities. However, the absolute rates of microbial N_2 fixation and nitrate assimilation are still poorly understood. Recently, the presence of marine viruses in the sea has been greatly acknowledged. The extent to which marine viruses mediate global C and N cycling remains one of the challenges in biogeochemistry. In a step towards understanding the rates of N_2 and nitrate assimilation of microbes and the pattern of virus abundances, stable isotopic incubations of $^{15}N_2$ and $^{15}NO_{3-}$ couple with $^{13}C\text{-}HCO_{3-}$ substrates were performed at 4 different stations. Time series isotopic incubations were performed at 12 h and 24 h time intervals, to study the effect of diurnal light cycles on microbial assimilation together with the dynamics of virus abundances. Further, we collected nucleic acid samples of DNA and RNA and viruses to quantify the nitrogen fixation genes and virus abundances over the stations.

There was no data generated during this cruise. In the coming months, bulk carbon and nitrogen assimilation of different N sources will be analysed using Isotope ratio mass spectrometer (IRMS). We will quantify the trends in nitrogen fixation genes using qPCR approach. The abundances of viruses will be performed using Flow Cytometry. During incubations, we will identify and quantify the abundances of microbial communities using Fluorescent *in-situ* hybridisation (FISH). Using high-resolution single cell technique of nano-scale secondary ion mass-spectrometer (nanoSIMS), we will quantify the single cell assimilation of microbial communities identified as per FISH approach. The assimilation trends of microbes will be compared to the virus abundances to verify the virus-mediated microbial regeneration of C and N.

Nitrogen Fixation, Nitrate & Carbon uptake

Stuart Painter and Sinhue Torres

During this cruise we measured rates of nitrogen fixation and nitrate and carbon uptake by the resident phytoplankton community via a series of simulated in-situ on-deck incubations using standard stable isotope techniques ($^{15}N_2$, $^{15}NO_3^-$, ^{13}C). A typical sampling strategy saw us collect water every other day from 4 light depths from the early morning CTD cast. The depths sampled corresponded to irradiance depths of 55%, 33%, 14% and 1% of surface irradiance (E_o). Incubations for N_2 fixation lasted 24 hours whilst those for nitrate and carbon uptake were limited to 6 hours or less. We also collected discrete chlorophyll samples from these same depths, which will be returned to NOC for analysis. After the first week we also included incubation bottles for surface water samples (97%) when water was available from the CTD, thanks to the availability of space in an on-deck incubator being used by Abdul Sheik (MPI).

For logistical reasons we were not able to measure N_2 fixation everyday (due to the 24 hour incubation length) so during the intervening days we undertook high resolution observations of nitrate and carbon uptake in the vicinity of the deep chlorophyll maximum. To accomplish this we sampled 6 depths spread across the lower 15% of the euphotic zone (corresponding to 15%, 10%, 5%, 1%, 0.5%, 0.1% of surface irradiance) and incubated samples using a laboratory incubator (Fytoscope FS-130) within which the irradiance and temperature regime was closely controlled. Incubations typically lasted 6 hours or less. Discrete chlorophyll samples were also collected from these depths.

No results were obtained during the cruise and the post cruise analysis may take 12-18 months to complete. Listed in Table 26 is a summary of the CTD casts sampled and the measurements made.

Table 26: Summary of CTD sampling and measurements

Jday / Date	**CTD Cast**	**Bottle No.**	**Measurements**	**Notes**
223 11/8/11	1 (early morning) – TEST CTD CAST	2,11,13,20	i) Bulk and size fractionated N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all except btl 2) iv) Discrete chlorophyll (all bottles)	No water left from bottle 2 to allow $NO_3/^{13}C$ uptake measurement at this depth (1% E_o)
224	2	4,6,8,13,18,19	i) High resolution $^{13}C/NO_3$ uptake	

Jday / Date	**CTD Cast**	**Bottle No.**	**Measurements**	**Notes**
12/8/11	(early morning)		(all bottles) ii) Discrete chlorophyll (all bottles)	
225 13/8/11	3 (early morning)	8,18,20,21	i) Bulk and size fractionated N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	
226 14/8/11	5 (early morning)	3,5,7,14,17,18	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	
227 15/8/11	7 (early morning)	10,17,21,22,24	i) Bulk and size fractionated N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	
228 16/8/11	9 (early morning)	5,7,9,15,17,19	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	
229 17/8/11	11 (early morning)	9,19,21,22,24	i) Bulk and size fractionated N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	Only 1 $^{15}N/^{13}C$ bottle at 14% depth
230 18/8/11	13 (early morning)	5,7,9,14,17,18,24	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	Additional surface bottle sampled
231 19/8/11	14 (early morning)	2,3,10,12,15,16	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	
232 20/8/11	16 (early morning)	6,15,20,24	i) Bulk and size fractionated N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	Start CTD survey 1

Jday / Date	**CTD Cast**	**Bottle No.**	**Measurements**	**Notes**
	17 (midmorning)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
	18 (afternoon)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
	19 (evening)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
233 21/8/11	22 (early morning)	8,16,18,22	i) $NO_3/^{13}C$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	
	23 (mid morning)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll iii) T0-$\delta^{15}N$	
	24 (afternoon)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll iii) T0- $\delta\ ^{15}N$	
	25 (evening)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
234 22/8/11	29 (early morning)	8,16,18,22	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
	30 (mid morning)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
	31 (afternoon)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll iii) Bulk N_2 fixation	Surface N_2 fixation measurement only
	32 (evening)	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
235 23/8/11	36 (early morning)	8,16,18,22	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll iii) Bulk N_2 fixation	Surface N_2 fixation measurement only
	37	8,22	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll iii) Bulk N_2 fixation	Surface N_2 fixation measurement only

Jday / Date	**CTD Cast**	**Bottle No.**	**Measurements**	**Notes**
	38	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
	39	8	i) $NO_3/^{13}C$ uptake ii) Discrete chlorophyll	
236 24/8/11	42 (early morning)	12,21,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	
237 25/8/11	44 (early morning)	7,18,20,222,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	Start SeaSoar survey 2
238 26/8/11	46 (early morning)	5,7,9,15,17,18	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	
239 27/8/11	48 (early morning)	8/9,18,20,22,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	
240 28/8/11	50 (early morning)	5,7,10,14,17,18	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	
241 29/8/11	51 (early morning)	10,18,20,22,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	
242 30/8/11	54 (early morning)	9,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) Discrete chlorophyll (all bottles)	Start CTD survey 2
	55 (midday)	8,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) Discrete chlorophyll (all bottles) iv) Nitrogenase protein	
	56	8,24	i) Bulk N_2 fixation (all bottles)	

Jday / Date	CTD Cast	Bottle No.	Measurements	Notes
	(evening)		ii) T0- $\delta^{15}N$ (all bottles) iii) Discrete chlorophyll (all bottles) iv) Nitrogenase protein	
243 31/8/11	57 (midnight)	8,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) Discrete chlorophyll (all bottles) iv) Nitrogenase protein	
	58 (early morning)	8,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) Discrete chlorophyll (all bottles) iv) Nitrogenase protein	
	59 (midday)	8,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) Discrete chlorophyll (all bottles) iv) Nitrogenase protein	
	60 (evening)	8,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) Discrete chlorophyll (all bottles) iv) Nitrogenase protein	
244 1/9/11	61 (midnight)	8,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) Discrete chlorophyll (all bottles) iv) Nitrogenase protein	
245 2/9/11	66 (early morning)	6,17,19,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	
246 3/9/11	69 (early morning)	2,3,9,11,14,15	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles) iii) T0- $\delta^{15}N$ (all bottles)	
	70 (afternoon)	2,4,6,10,14,16	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	

Jday / Date	CTD Cast	Bottle No.	Measurements	Notes
			iii) T0- $\delta^{15}N$ (all bottles)	
247 4/9/11	71 (early morning)	10,18,20,22,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	Start SeaSoar survey 3
248 5/9/11	73 (early morning)	5,8,9,15,17,18	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles) iii) T0- $\delta^{15}N$ (all bottles)	
249 6/9/11	75 (early morning)	8,18,20,21,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	Surface SF N_2 fixation bottle also added
250 7/9/11	77 (early morning)	4,6,9,15,17,18	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles) iii) T0- $\delta^{15}N$ (all bottles)	
251 8/9/11	79	8,18,20,21,24	i) Bulk N_2 fixation (all bottles) ii) T0- $\delta^{15}N$ (all bottles) iii) $NO_3/^{13}C$ uptake (all bottles) iv) Discrete chlorophyll (all bottles)	
252 9/9/11	82	5,7,9,15,17,(24)	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles) iii) surface N_2 fixation	No 15% bottle today (no water)
253 10/9/11	85	2,4,9,13,14,15	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	
254 11/9/11	89	4,6,9,16,18,19	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	
255 12/9/11	93	5,9,13,16,18,19	i) High resolution $^{13}C/NO_3$ uptake (all bottles) ii) Discrete chlorophyll (all bottles)	

Deploying PELAGRA Drifting Sediment Traps

Sam Ward

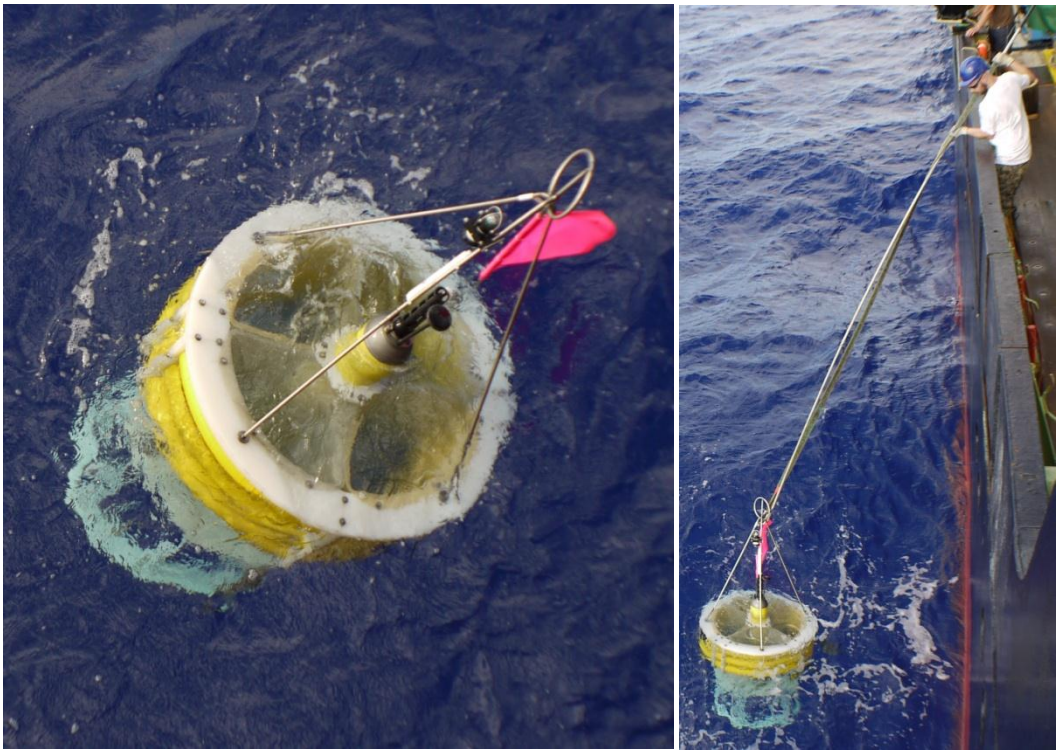

Figure 26: Deployment of PELAGRA trap.

The PELAGRA traps have recently undergone some minor software modifications to the upgrades which were made to the Apex floats last year. These upgrades were tested on D360 (Autosub trails cruise) and the faults have been resolved between then and D369.

The issues which still need addressing are:

- The short span of data logging from the Apex Seabird CTD as it is not collecting for the whole mission. (.msg files)
- Traps P2, P7 and P8 are not receiving the 002.log file which shows the traps latest position and Apex Data

The issues with the sampling cups have been addressed and they are now working efficiently.

There have been a few issues with the hydrostatic releases (Depressor and Abort Releases) which will be explained during this report.

	Pelagra Trap Number	Station Number	Deployment Date and Time	Deployment Position (Long)	Deployment Position (Lat)	Surface Date and Time	Surface Position (Long)	Surface Position (Lat)	Recovery Position Date and Time	Recovery Position (long)	Recovery Position (Lat)
Deployment 1	P4	3	14/08/2011 11:00	25˚39.4' N	030˚18.9' W	18/08/2011 11:53	25˚34.44' N	030˚33.96' W	19/08/2011 05:20	25˚34.00' N	030˚44.01' W
	P6	3	14/08/2011 11:30	25˚39.6' N	030˚18.9' W	18/08/2011 12:10	25˚34.98' N	030˚38.82' W	19/08/2011 04:27	25˚35.01' N	030˚48.04' W
	P2	3	14/08/2011 12:00	25˚39.6' N	030˚19.1' W	15/08/2011 00:03	25˚39.72' N	030˚20.82' W	15/08/2011 17:18	25˚39.03' N	030˚26.03' W
Deployment 2	P8	11	18/08/2011 22:00	26˚11.1' N	031˚10.8' W	19/08/2011 04:45	26˚11.28' N	031˚11.76' W	19/08/2011 23:17	26˚09.03' N	031˚21.01' W
	P7	11	18/08/2011 22:30	26˚11.6' N	031˚11.6' W	19/08/2011 05:11	26˚11.76' N	031˚12.66' W	19/08/2011 22:35	26˚09.09' N	031˚21.07' W
	P5	11	18/08/2011 23:00	26˚11.8' N	031˚12.2' W	23/08/2011 12:30	26˚19.38' N	031˚23.52' W	24/08/2011 03:39	26˚23.02' N	031˚32.00' W
Deployment 3	P4	26	22/08/2011 07:30	26˚16.2' N	031˚04.8' W	26/08/2011 08:15	26˚20.82' N	031˚24.12' W	27/08/2011 02:45	26˚33.04' N	031˚36.04' W
	P6	26	22/08/2011 08:00	26˚16.0' N	031˚05.5' W	26/08/2011 08:24	26˚27.09' N	031˚30.48' W	27/08/2011 01:00	26˚30.05' N	031˚39.07' W
	P2	26	22/08/2011 08:30	26˚16.8' N	031˚05.7' W	26/08/2011 09:04	26˚35.07' N	031˚34.32' W	26/08/2011 23:18	26˚38.00' N	031˚41.06' W
Deployment 4	P4	P1	29/08/2011 04:00	26˚36.0' N	031˚19.9' W	02/09/2011 04:56	26˚32.22' N	031˚35.34' W	02/09/2011 11:55	26˚31.06' N	031˚38.08' W
	P6	P1	29/08/2011 04:30	26˚35.8' N	031˚19.7' W	02/09/2011 05:04	26˚44.58' N	031˚32.28' W	02/09/2011 10:10	26˚44.01' N	031˚30.01' W
	P2	P1	29/08/2011 05:00	26˚35.8' N	031˚19.6' W	29/08/2011 09:59	26˚35.64' N	031˚20.76' W	30/08/2011 09:32	26˚38.09' N	031˚30.01' W
Deployment 5	P4	P1	03/09/2011 19:00	26˚49.0' N	031˚35.3' W	08/09/2011 19:52	26˚49.26' N	031˚50.22' W	09/09/2011 06:14	26˚48.06' N	031˚48.06' W
	P6	P1	03/09/2011 19:30	26˚49.2' N	031˚35.2' W	08/09/2011 20:04	26˚56.64' N	031˚56.94' W	09/09/2011 04:31	26˚57.05' N	031˚56.01' W
	P7	P1	03/09/2011 20:00	26˚49.6' N	031˚35.1' W	04/09/2011 02:28	26˚49.92' N	031˚35.22' W	04/09/2011 23:25	26˚51.07' N	031˚38.07' W
	P2	P1	03/09/2011 20:30	26˚50.1' N	031˚35.0' W	04/09/2011 01:26	26˚50.46' N	031˚35.04' W	05/09/2011 00:09	26˚52.09' N	031˚39.01' W

Table 27: Deployment, surface and recovery positions.

Deployment One

Please refer to Table 27 and Figure 27 for positions.

P2

Depth: 50 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 9 minutes

Target Temp: 23.625°C

Target Sal: 37.48

Ballast added: 4.087 kg

P2 Deployment information

The sink time was too short which caused P2 to surface (Figure 28). This was because it did not have sufficient time to pump its self back down to depth.

P4

Depth: 350 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 184 minutes

Target Temp: 15.47°C

Target Sal: 36.14

Ballast added: 4.1 kg

P4 Deployment information

From the piston data P4 was about 100 grams too heavy. P4 hit its required depth and delivered samples (Figure 29). P4 collected 3 Samples at 350 m and 1 intentional blank sample.

P6

Depth: 150 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 27 minutes

Target Temp: 19.015°C

Target Sal: 36.81

Ballast added: 4.092 kg

P6 Deployment information

From the piston data P6 was about 150 grams too heavy. P6 stayed between 200-250 m and could not pump its self up to depth (Figure 30). P6 collected 3 samples between 200-250 m and 1 intentional blank sample.

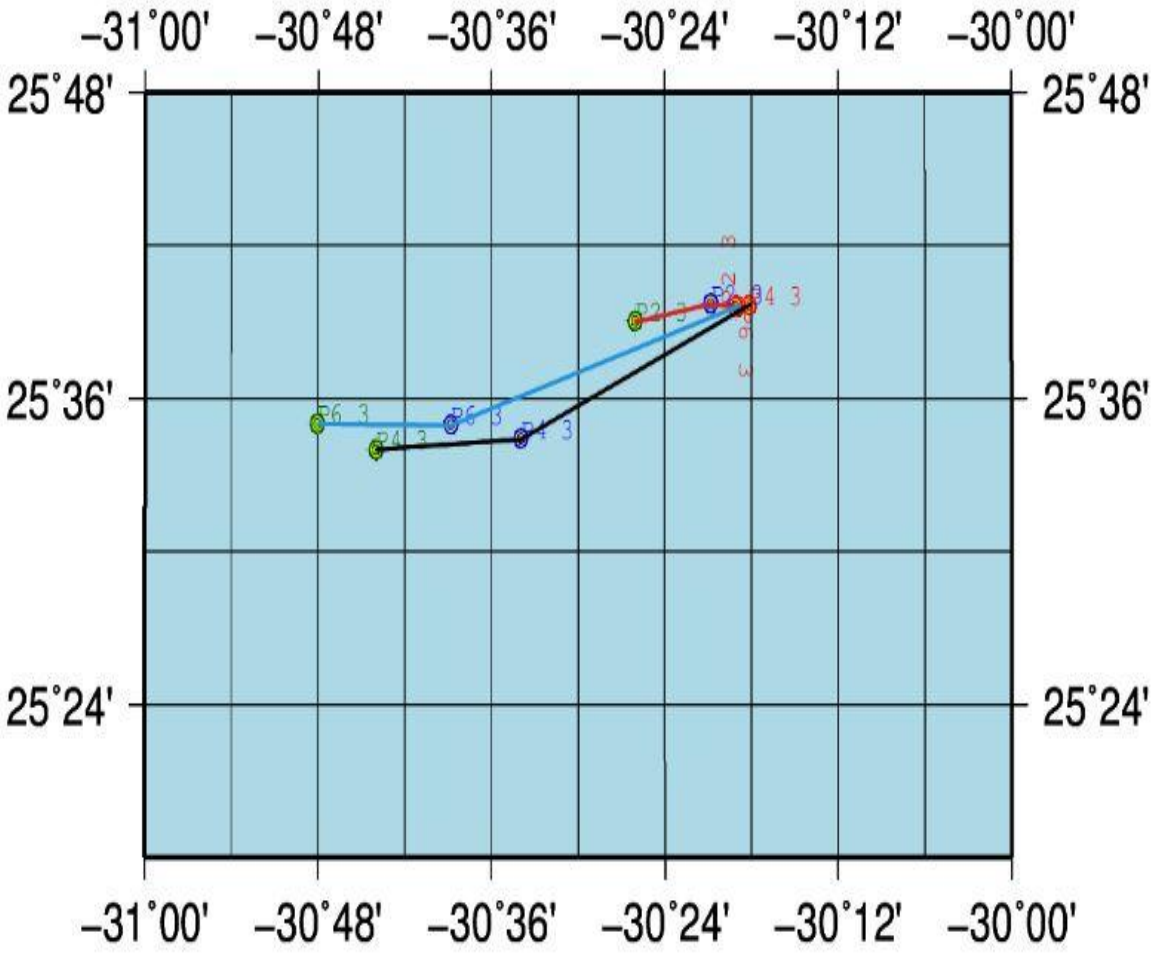

Figure 27: Deployment 1 surface and recovery. P2, P4, P6

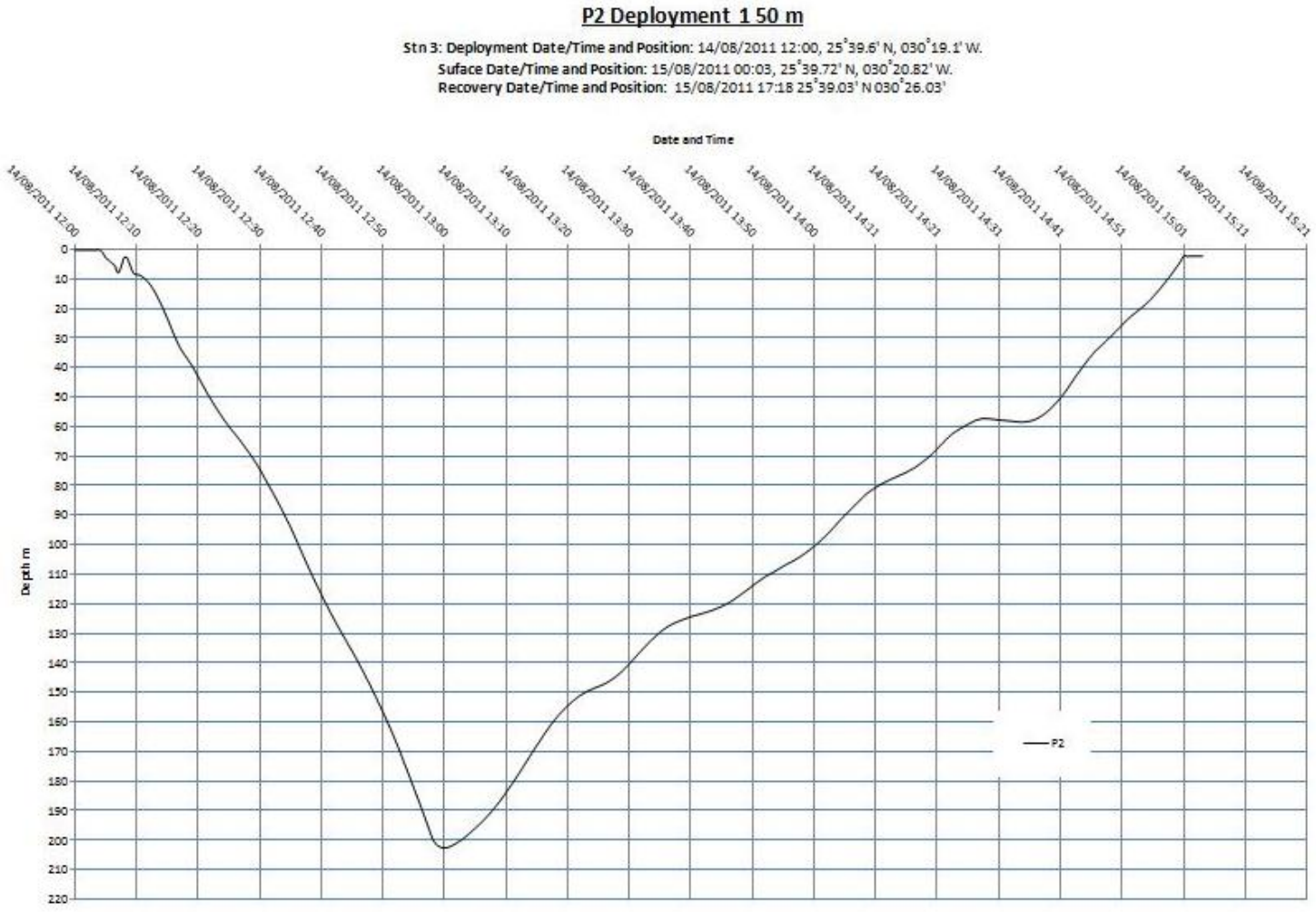

Figure 28: P2 Deployment 1, 50 m.

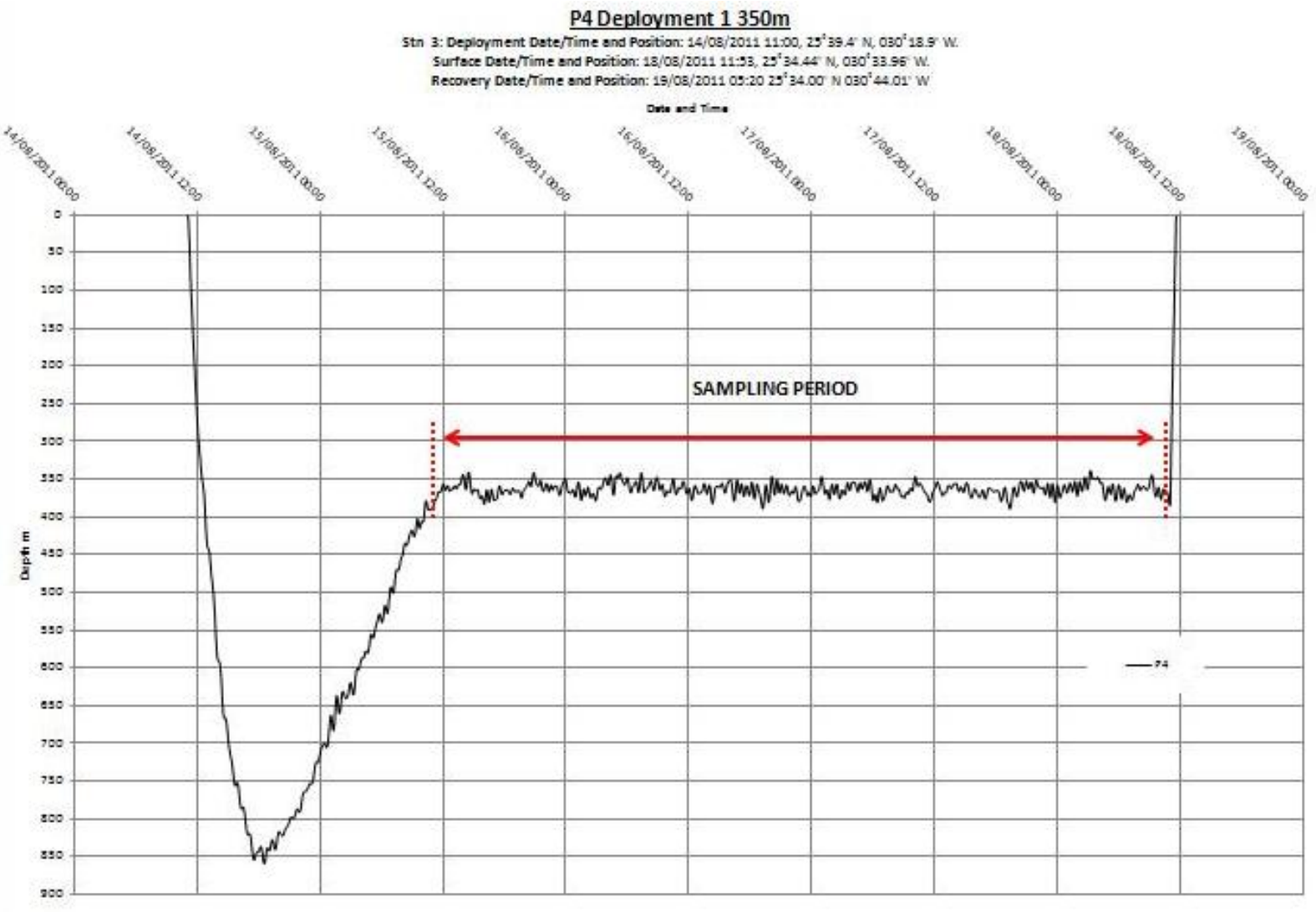

Figure 29: P4 Deployment 1, 350 m.

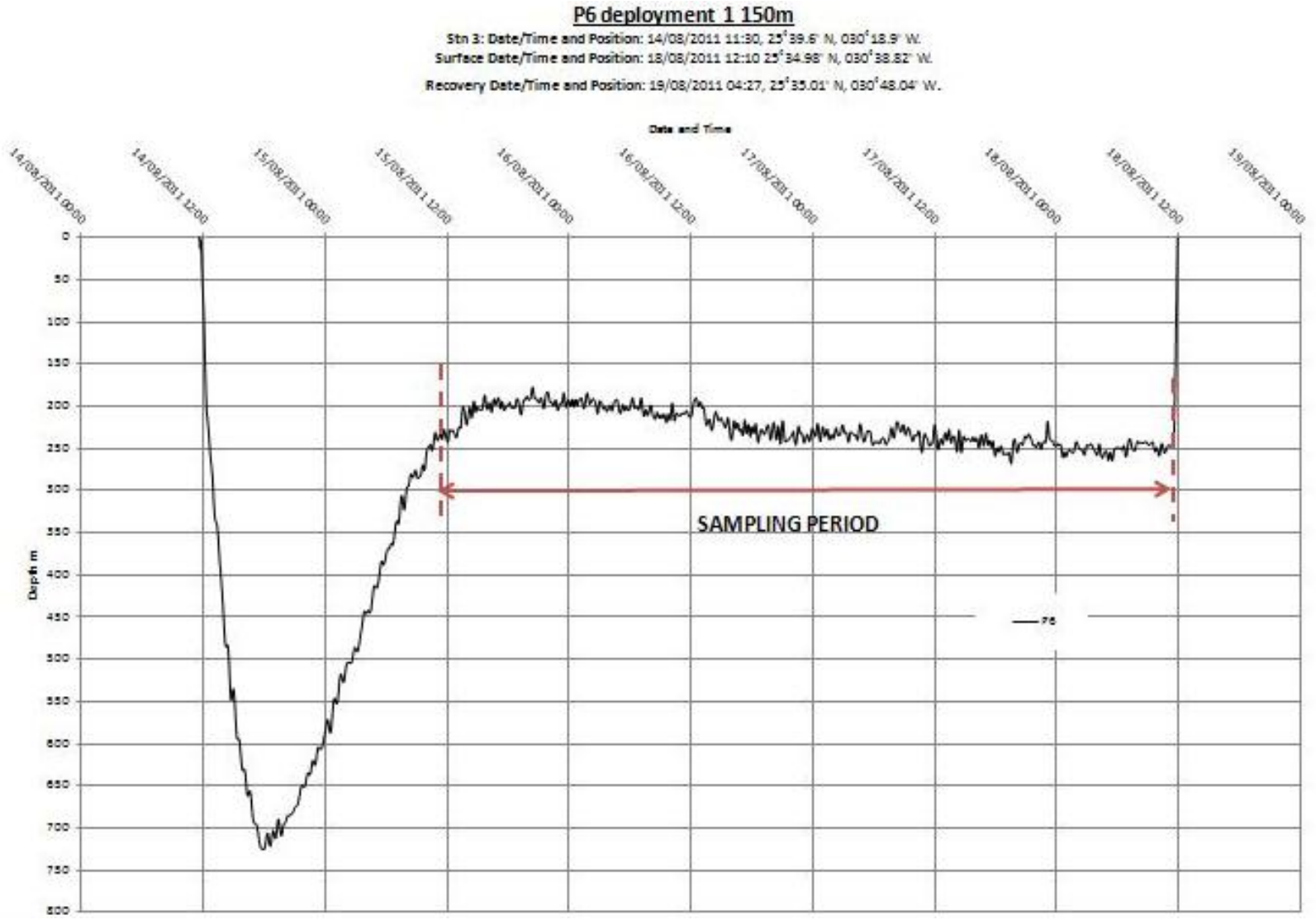

Figure 30: P6 Deployment 1, 150 m.

Deployment Two

Please refer to Table 27 and Figure 31 for positions.

P5

Depth: 50 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 115 minutes

Target Temp: 22.9°C

Target Sal: 37.4

Ballast added: 4.078 kg

P5 Deployment information

From the piston data P5 was about 345 grams too heavy. P5 stayed at 750 m and could not pump its self up to depth (Figure 32). P5 also sunk below its emergency abort depth and managed to pump its self up. The worrying issue was that the emergency release did not trigger!

P5 collected 3 Samples at 750 m and 1 intentional blank sample.

P7

Depth: 150 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 27 minutes

Target Temp: 20.25°C

Target Sal: 37.08

Ballast added: 4.08 kg

P7 Deployment information

From the data P7 was about 150 grams too heavy. P7 was over ballasted and dropped its emergency abort weight and surfaced (Figure 33).

P8

Depth: 350 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 168 minutes

Target Temp: 15.6°C

Target Sal: 36.18

Ballast added: 4.03 kg

P8 Deployment information

From the deployment data P8 didn't drop its depressor weight, so it was therefore 0.386 kg too heavy and sunk until it dropped its emergency abort weight and surfaced (Figure 34).

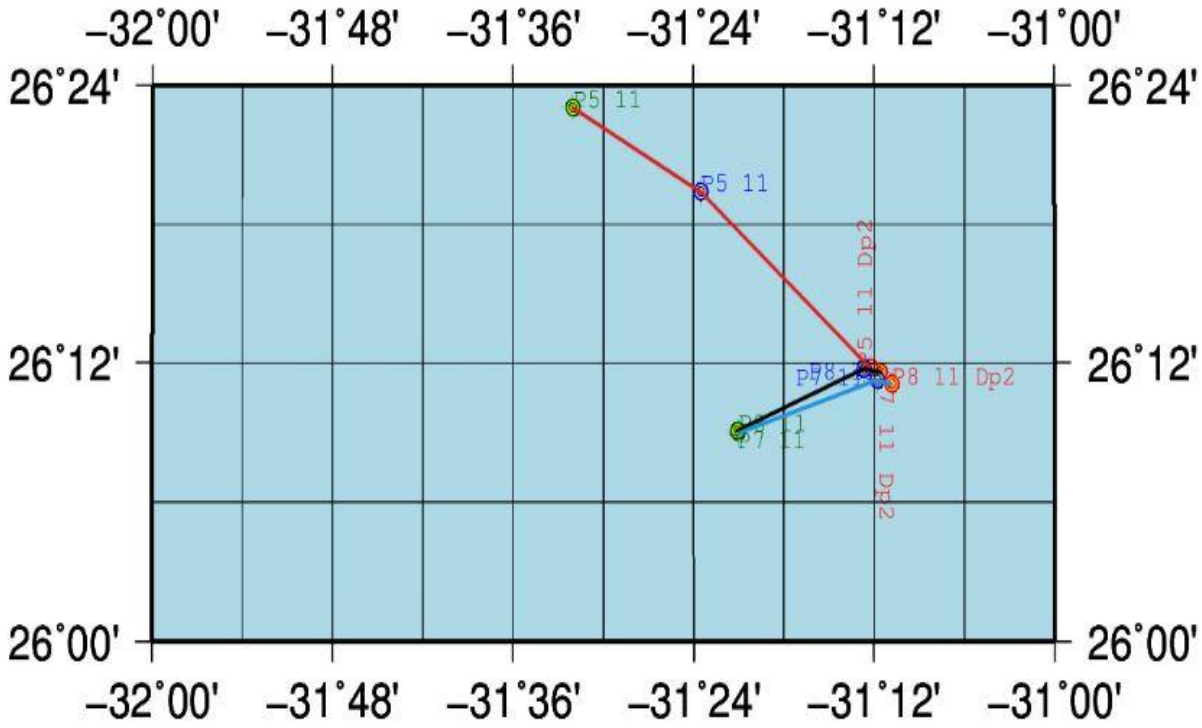

Figure 31: Deployment 2 surface and recovery. P5, P7, P8.

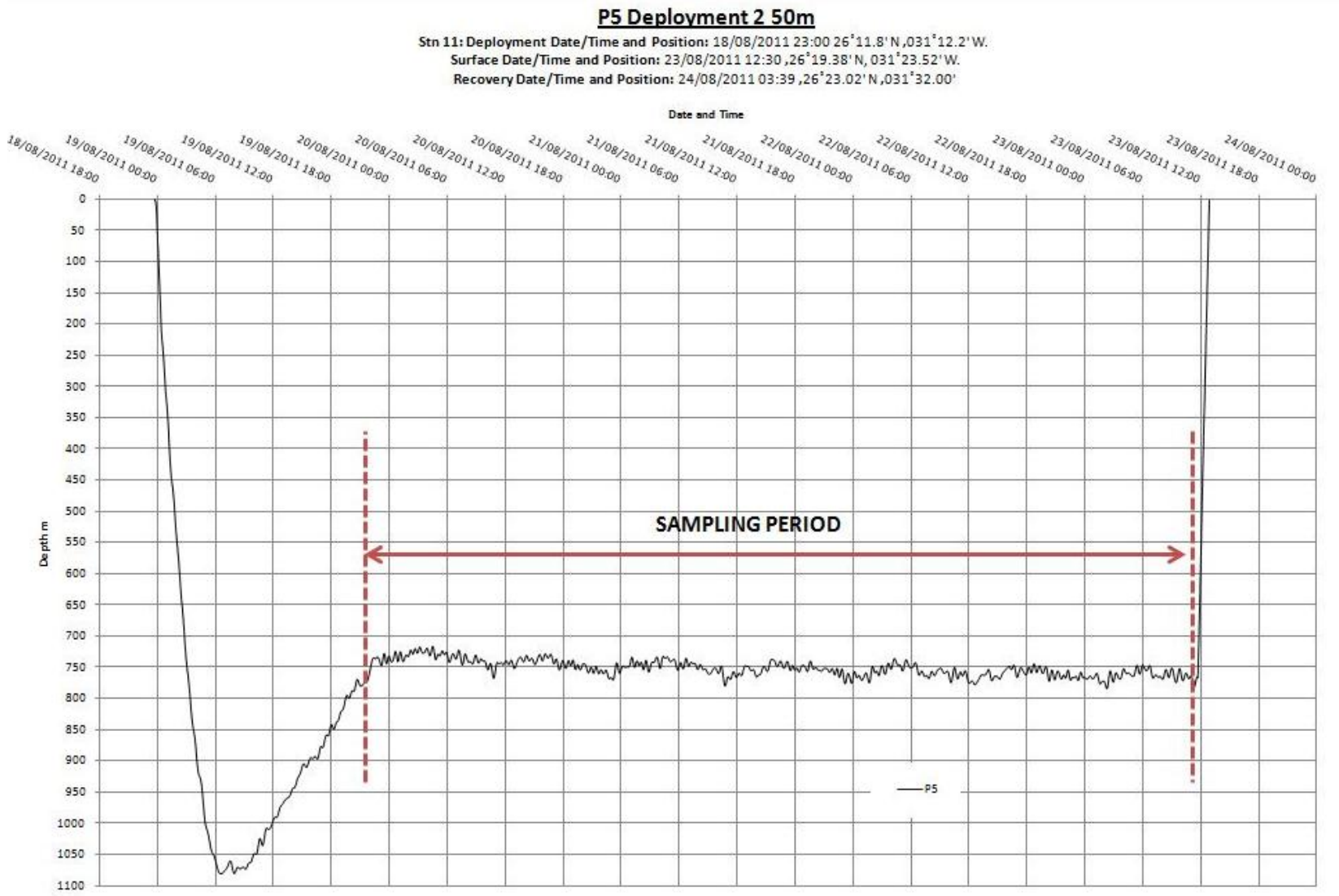

Figure 32: P5 Deployment 2, 50 m.

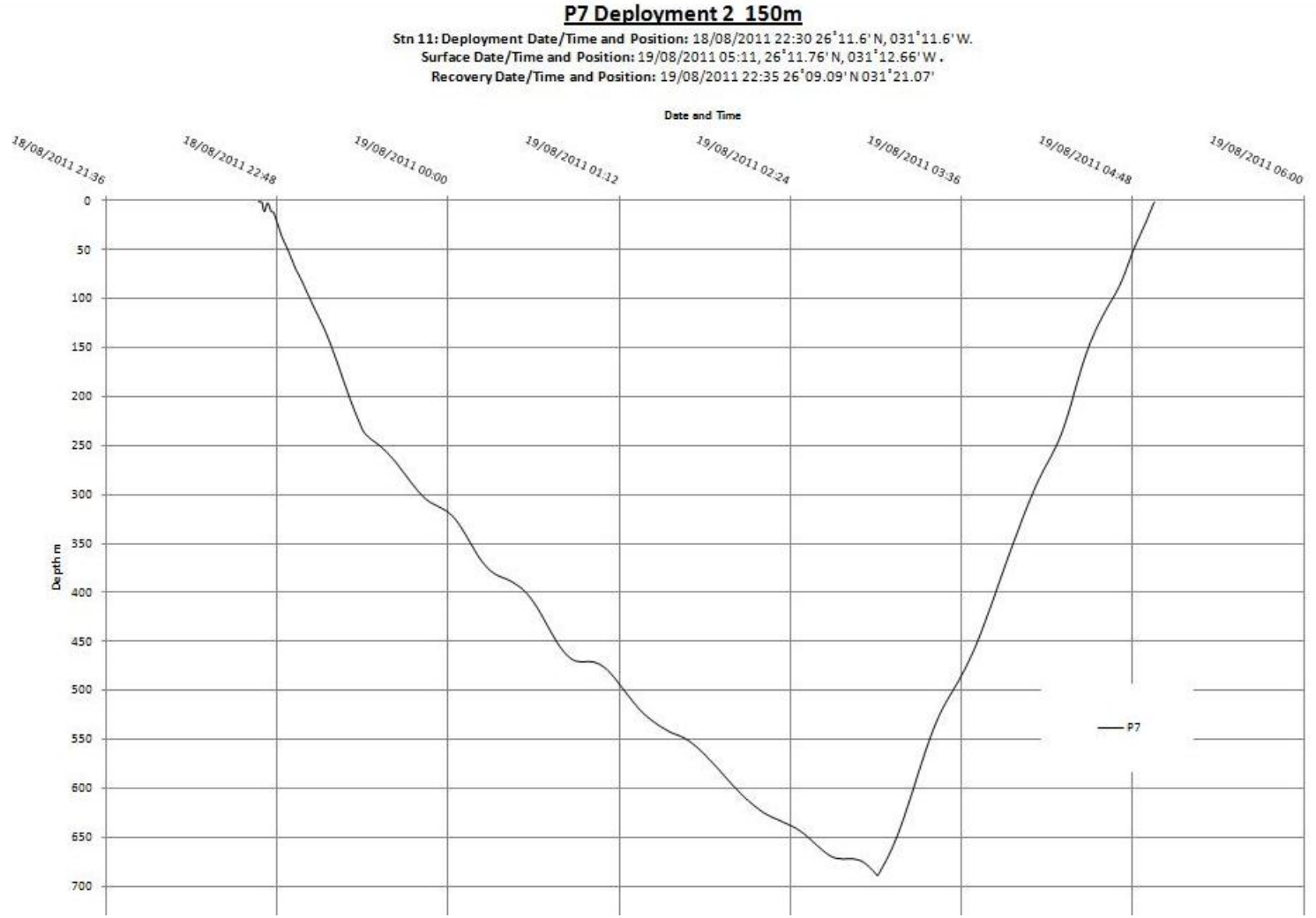

Figure 33: P7 Deployment 2, 150 m.

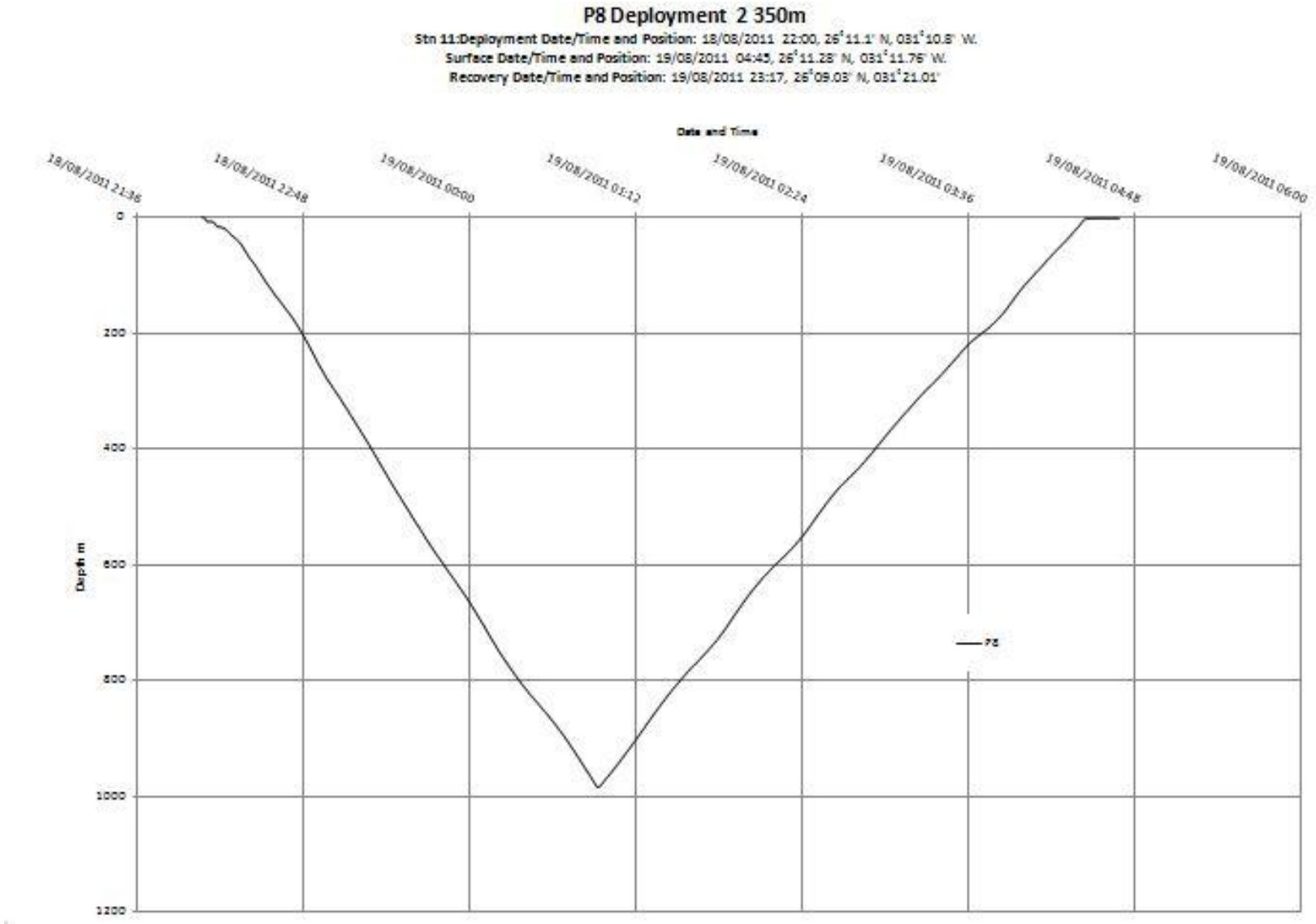

Figure 34: P8 Deployment 2, 350 m.

Deployment Three

Please refer to Table 27 and Figure 35 for positions.

P2

Depth: 50 m (Ballasted for 70 m)

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 115 minutes

Target Temp: 22.9°C

Target Sal: 37.4

Ballast added: 4.087 – 0.1 = 3.999 kg

P2 Deployment information

This was a successful deployment but P2 was still around 15 grams too heavy according to the piston data (Figure 36).

P2 collected 3 Samples at 65 m and 1 intentional blank sample.

P4

Depth: 350 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 184 minutes

Target Temp: 15.6°C

Target Sal: 36.18

Ballast added: 4.075 – 0.1 = 3.975 kg

P4 Deployment information

This was a successful deployment but P4 was still around 15 grams t0o heavy according to the piston data (Figure 37).

P4 collected 3 Samples at 350 m and 1 intentional blank sample.

P6

Depth: 150 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 27 minutes

Target Temp: 20.25°C

Target Sal: 37.08

Ballast added: 4.108 – 0.1 = 4.008 kg

P6 Deployment information

This was a successful deployment but P6 was still around 50 grams too heavy according to the piston data (Figure 38).

P6 collected 3 Samples at 150 m and 1 intentional blank sample.

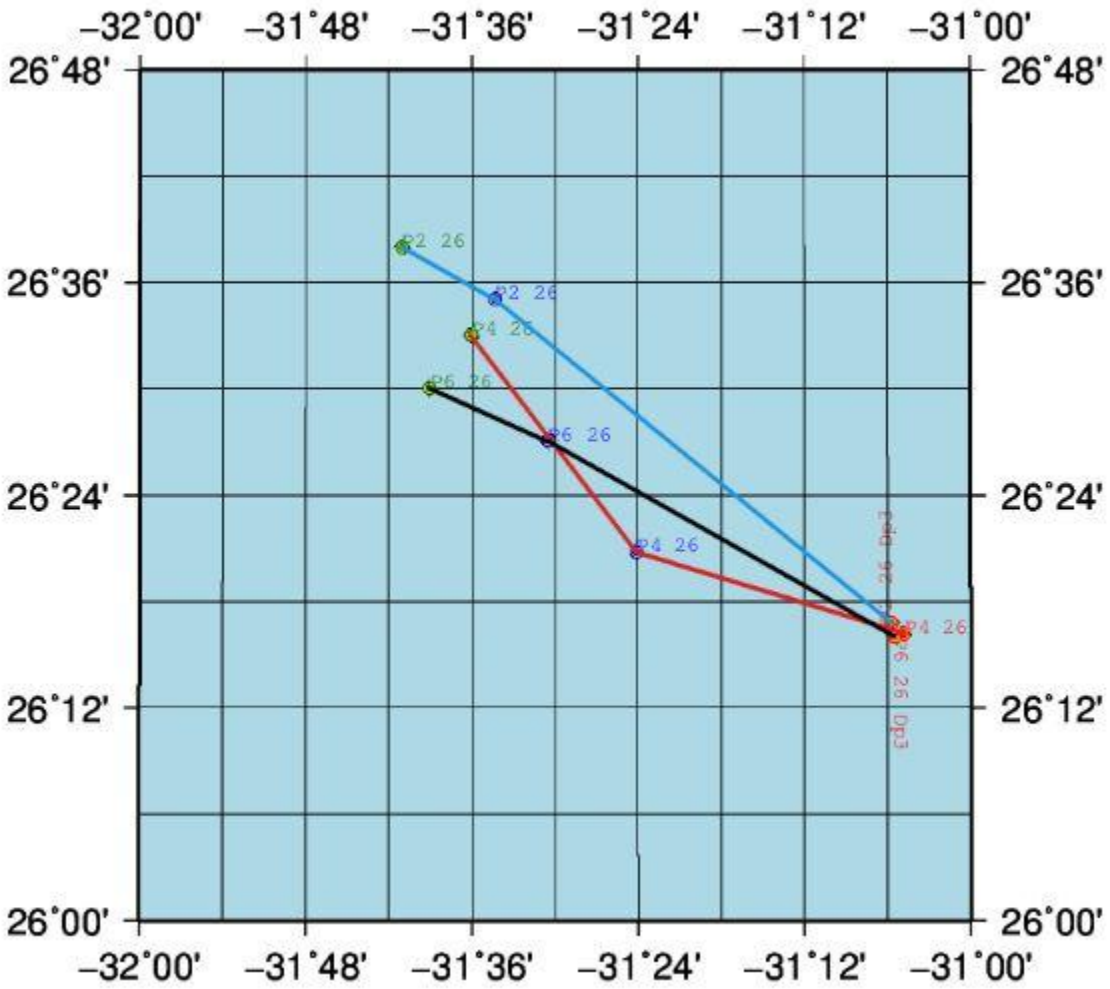

Figure 35: Deployment 3 surface and recovery. P2, P4, P6.

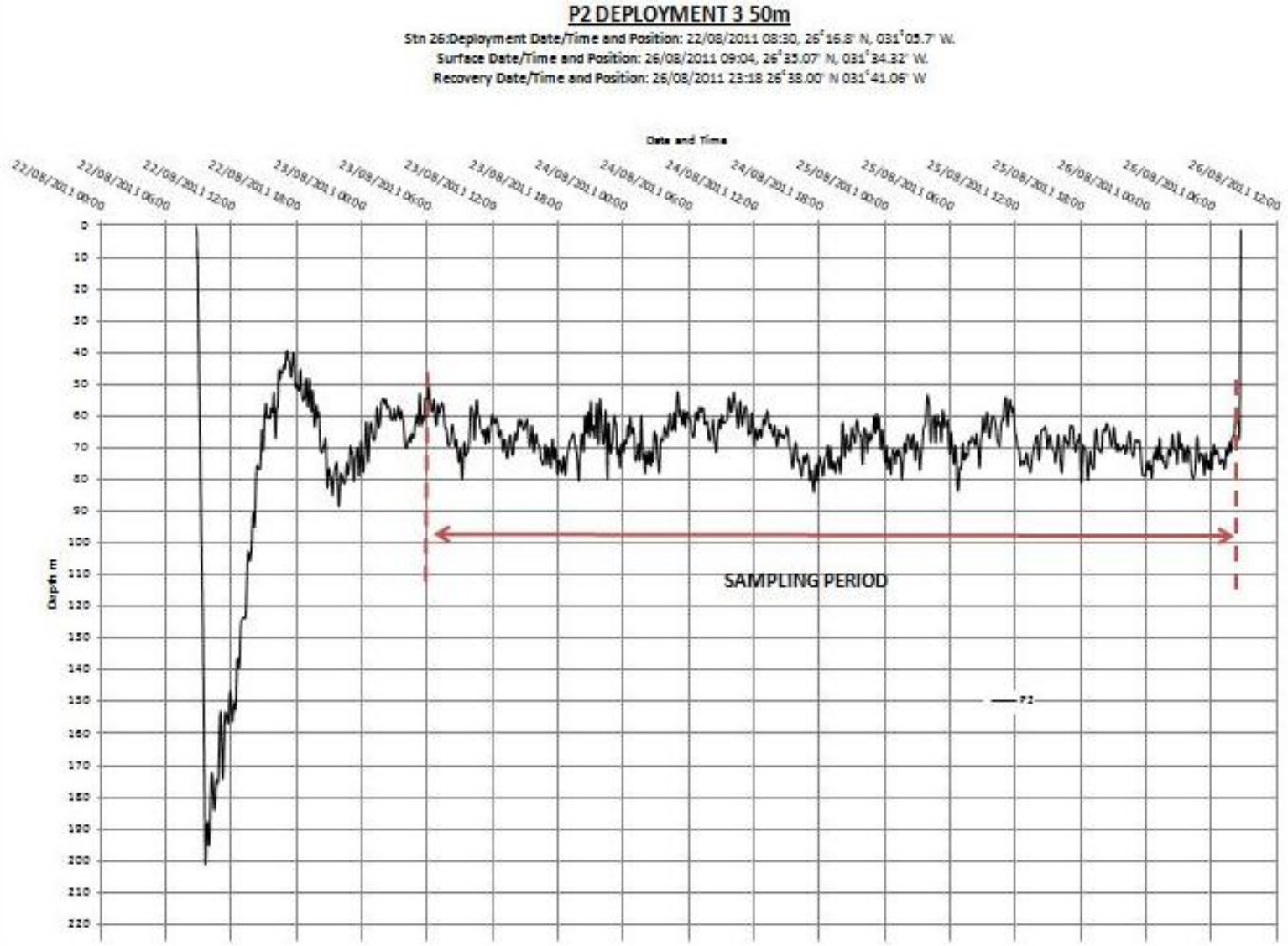

Figure 36: P2 Deployment 3, 50 m.

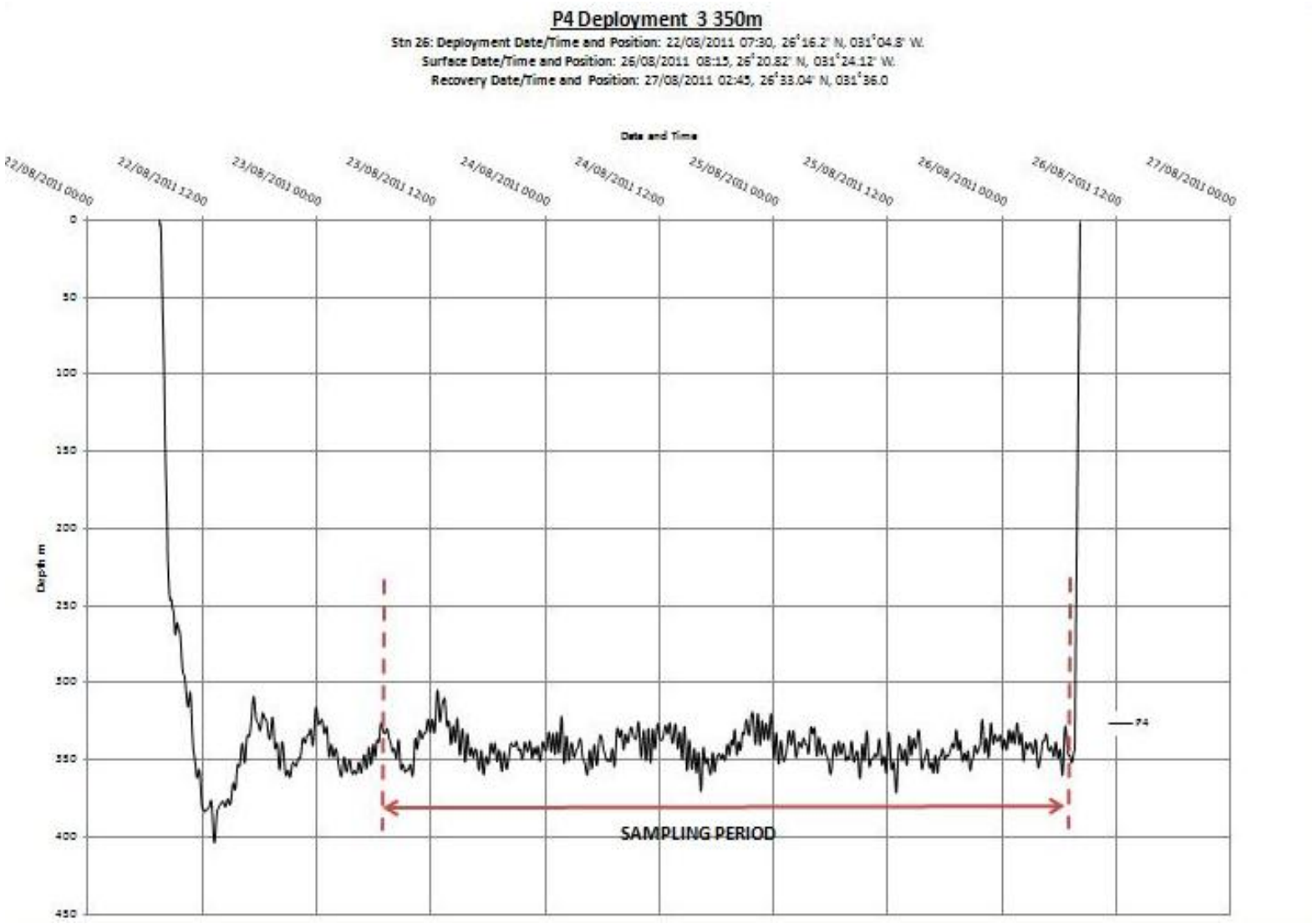

Figure 37: P4 Deployment 3, 350 m.

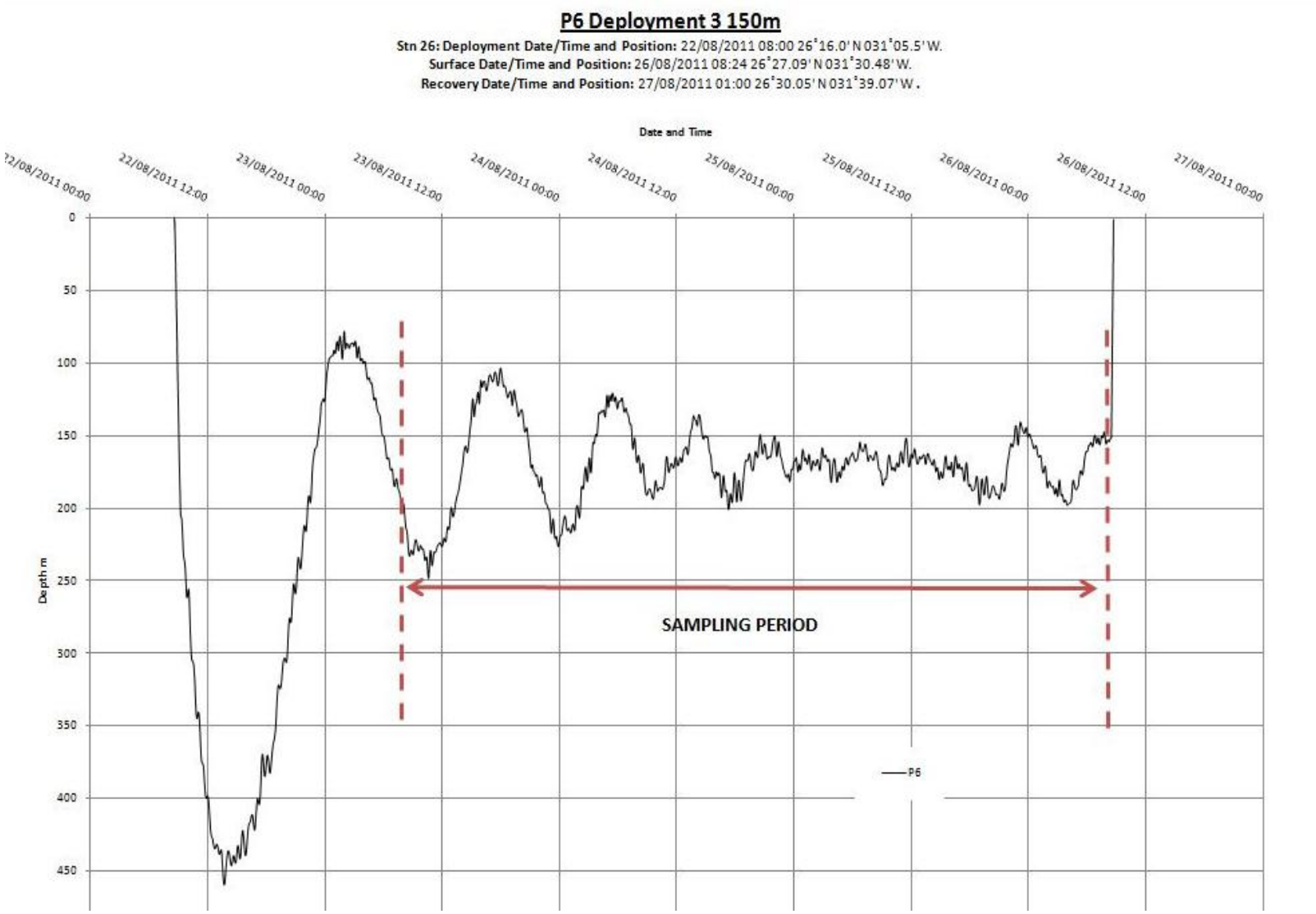

Figure 38: P6 Deployment 3, 150 m.

Deployment Four

Please refer to Table 27 and Figure 39 for positions.

P2

Depth: 50 m (ballasted to 75 m)

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 115 minutes

Target Temp: 21.9°C

Target Sal: 37.28

Ballast added: 4.087 – 0.085 = 4.002 kg

P2 Deployment information

P2 did not release its depressor weight and sunk until it released its emergency abort weight and surfaced (Figure 40). This was strange as the two deployments before P2's depressor release worked fine.

P4

Depth: 350 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 184 minutes

Target Temp: 16.4°C

Target Sal: 36.32

Ballast added: 4.083 – 0.085 = 3.998 kg

P4 Deployment information

This was a successful deployment (Figure 41).

P4 collected 3 Samples at 350 m and 1 intentional blank sample.

P6

Depth: 150 m

Sampling period: 72 hours

Stabilization period: 24 hours

Down time: 96 hours

Sink time: 27 minutes

Target Temp: 20.7°C

Target Sal: 37.18

Ballast added: 4.113 – 0.15 = 3.963 kg

P6 Deployment information

This was a successful deployment (Figure 42).

P6 collected 3 Samples at 150 m and 1 intentional blank sample.

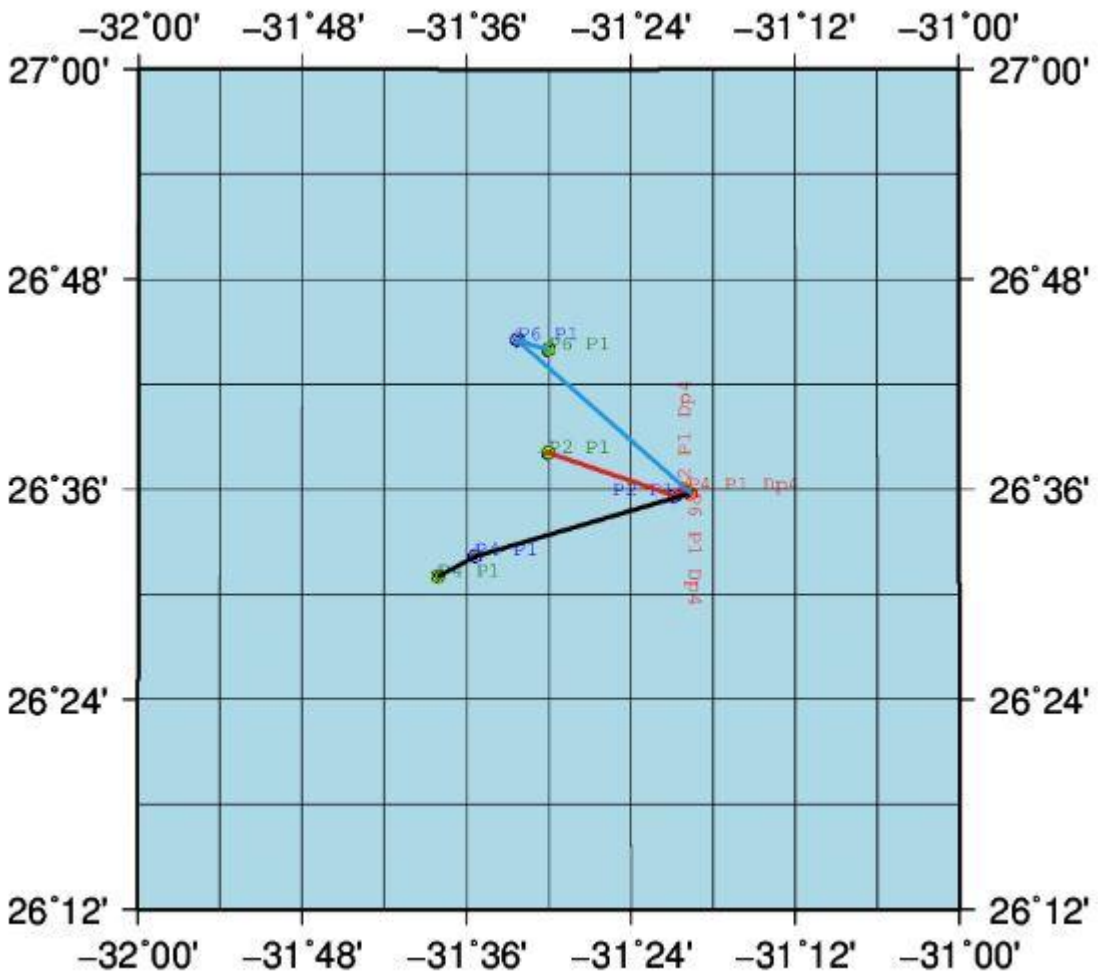

Figure 39: Deployment 4 surface and recovery. P2, P4, P6.

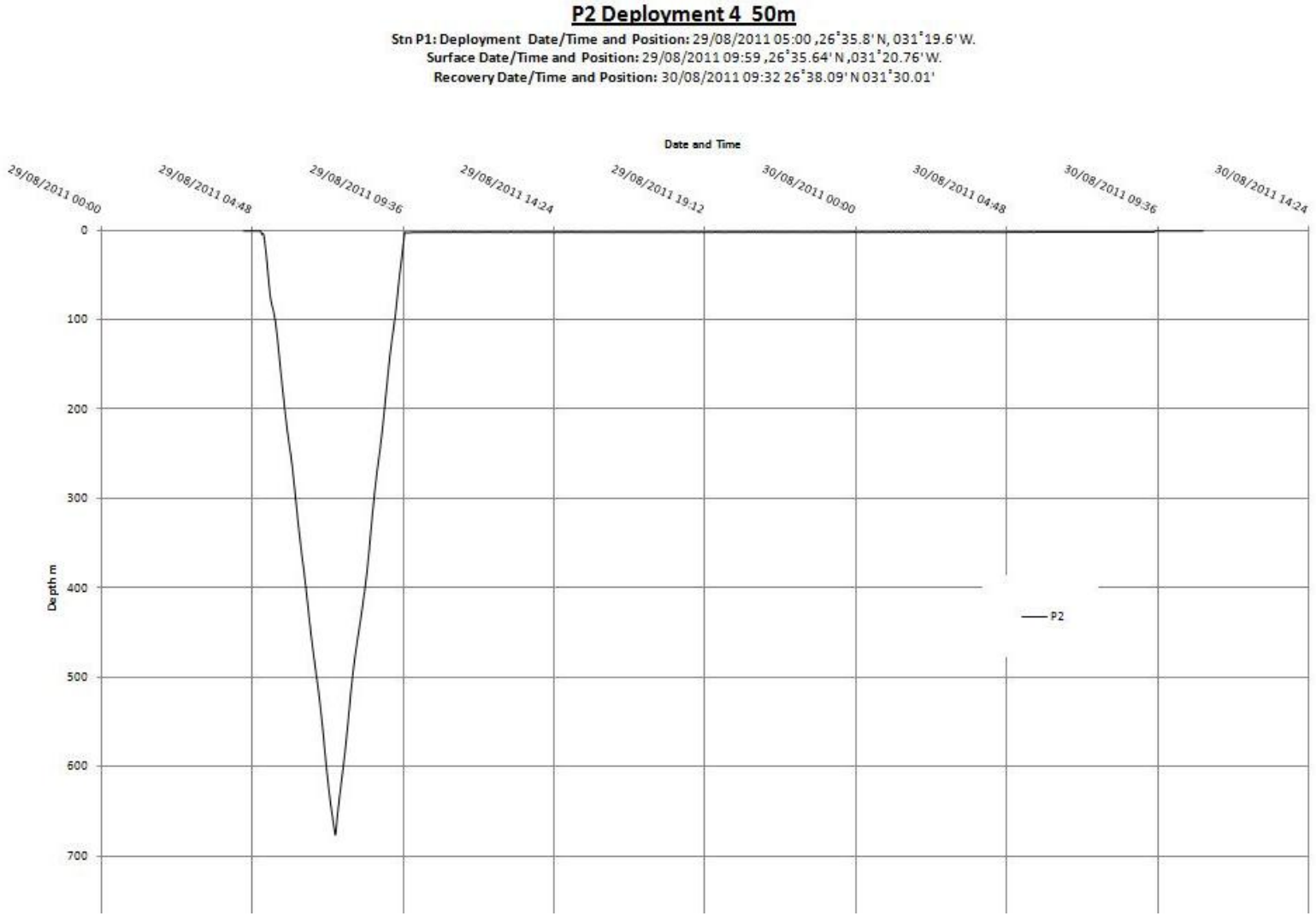

Figure 40: P2 Deployment 4, 50 m.

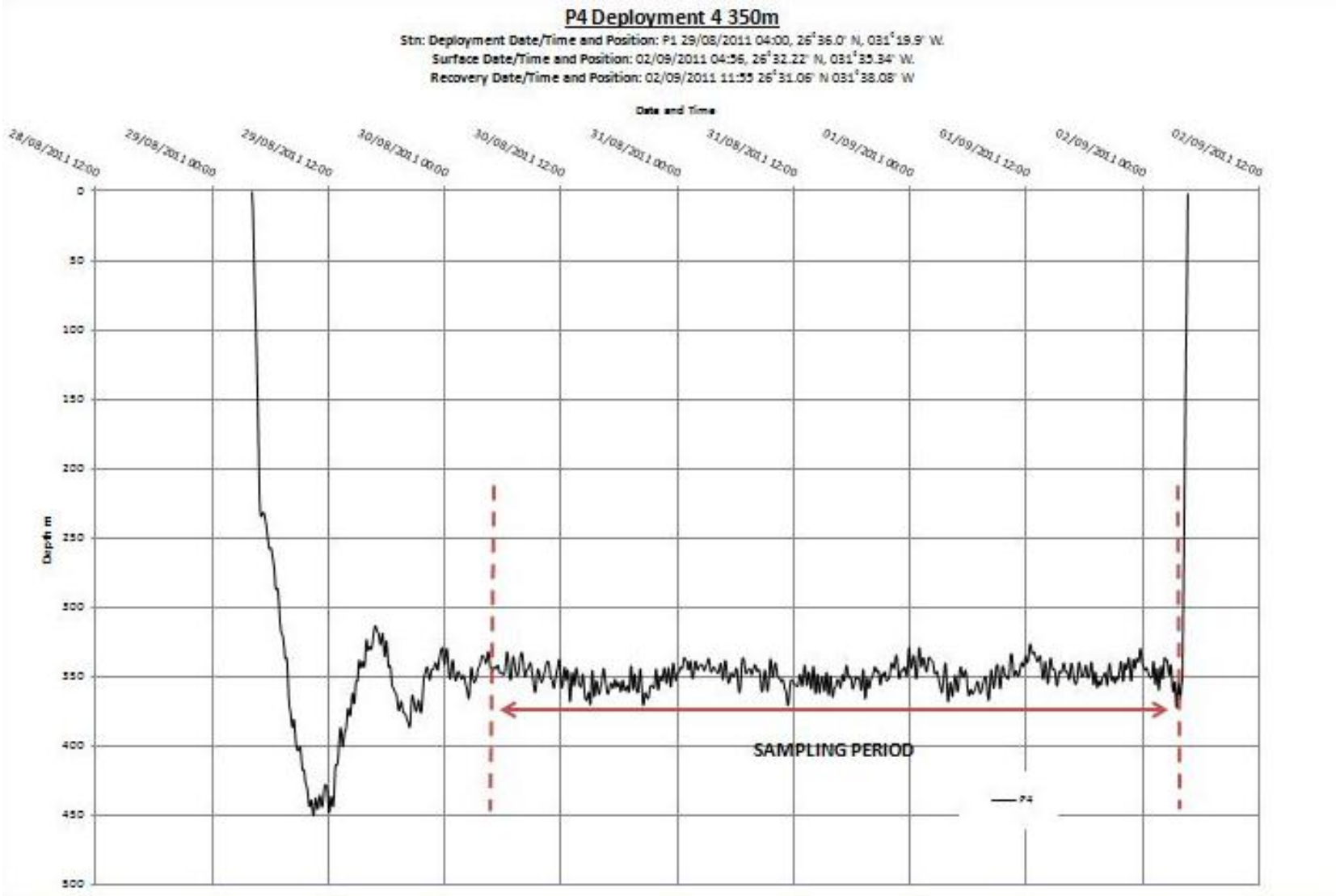

Figure 41: P4 Deployment 4, 350 m.

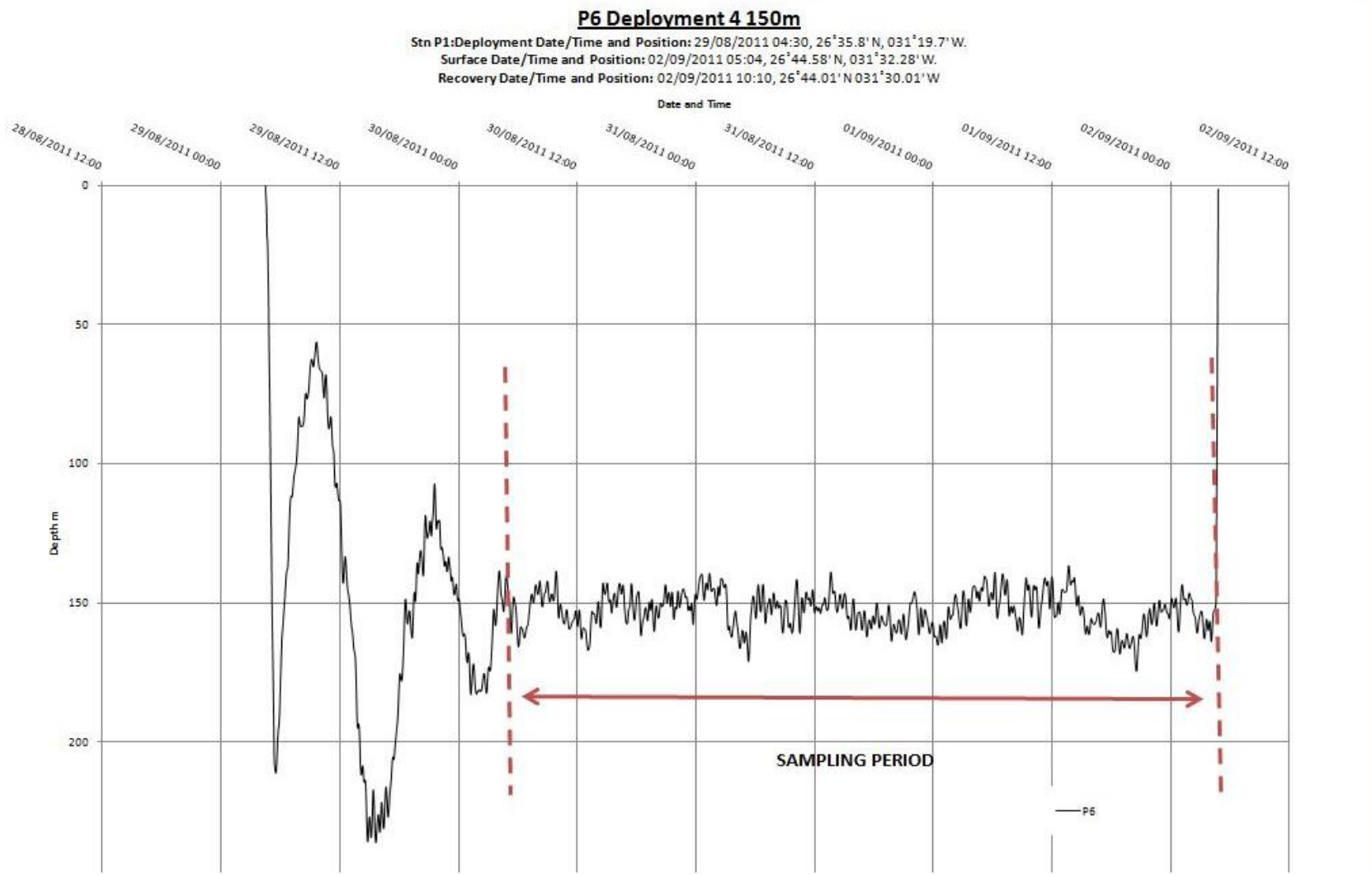

Figure 42: P6 Deployment 4, 150 m.

Deployment Five

Please refer to Table 27 and Figure 43 for positions.

P2

Depth: 80 m

Sampling period: 96 hours

Stabilization period: 24 hours

Down time: 120 hours

Sink time: 115 minutes

Target Temp: 22.8°C

Target Sal: 37.35

Ballast added: 4.077 – 0.1 = 3.977 kg

P2 Deployment information

P2 did release its depressor weight in this deployment, which suggests that the cable tie which connects the weight to the release may have been court in deployment 3. However, unfortunately P2 was under ballasted and could not pump its self down fast enough and surfaced (Figure 44). This deployment was in an Eddy and it was suggested that the dramatic Temperature and Salinity change in the upper layers could affect the ballasting calculations.

P7

Depth: 100 m

Sampling period: 96 hours

Stabilization period: 24 hours

Down time: 120 hours

Sink time: 90 minutes

Target Temp: 21.6°C

Target Sal: 37.26

Ballast added: 4.056 – 0.15 = 3.906 kg

P7 Deployment information

P7 released its depressor weight and could not pump its self down to depth in time (Figure 45). This was because the sink time was not long enough and the trap was to buoyant and surfaced.

P4

Depth: 350 m

Sampling period: 96 hours

Stabilization period: 24 hours

Down time: 120 hours

Sink time: 184 minutes

Target Temp: 16.6°C

Target Sal: 36.31

Ballast added: 4.085 – 0.085 = 4 kg

P4 Deployment information

This was a successful deployment (Figure 46).

P4 collected 3 Samples at 350 m and 1 intentional blank sample.

P6

Depth: 150 m

Sampling period: 96 hours

Stabilization period: 24 hours

Down time: 120 hours

Sink time: 27 minutes

Target Temp: 20.9°C

Target Sal: 37.21

Ballast added: 4.111 – 0.15 = 3.961 kg

P6 Deployment information

This was a successful deployment (Figure 47).

P6 collected 3 Samples at 150 m and 1 intentional blank sample.

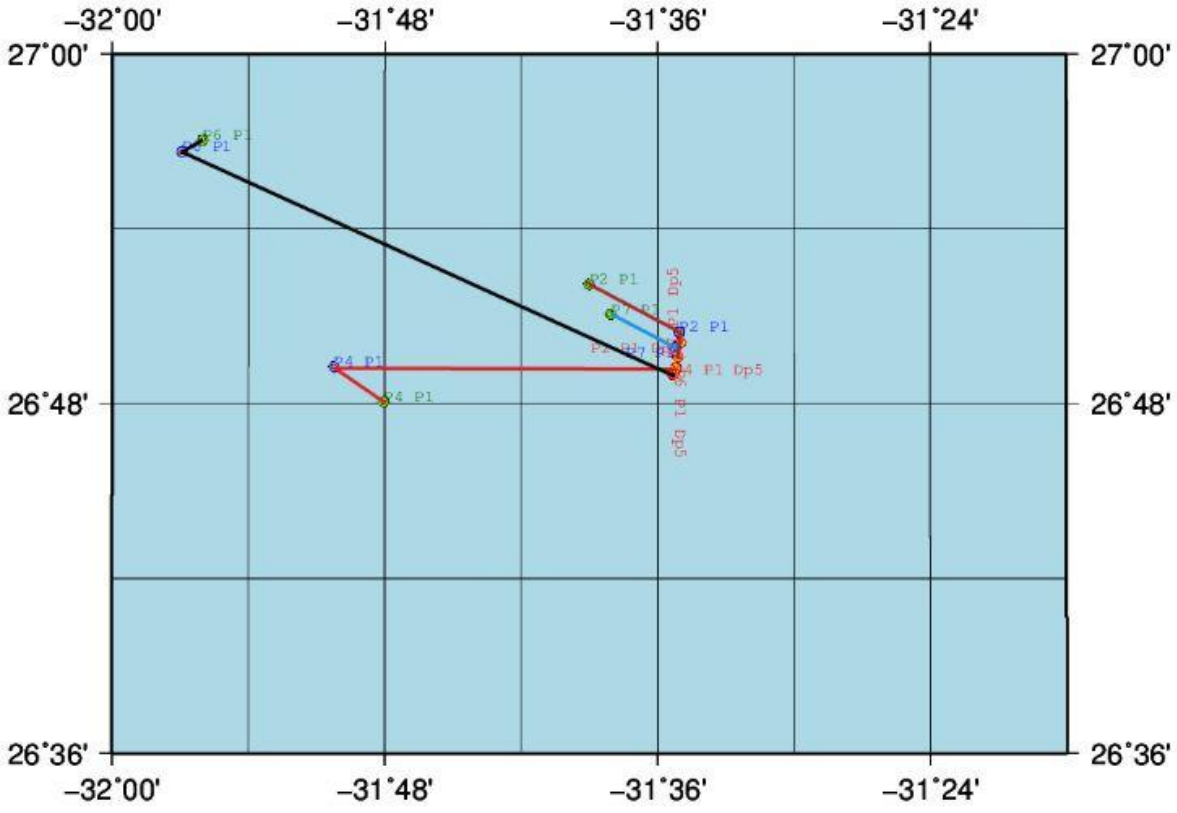

Figure 43: Deployment 5 surface and recovery. P2, P4, P6, P7.

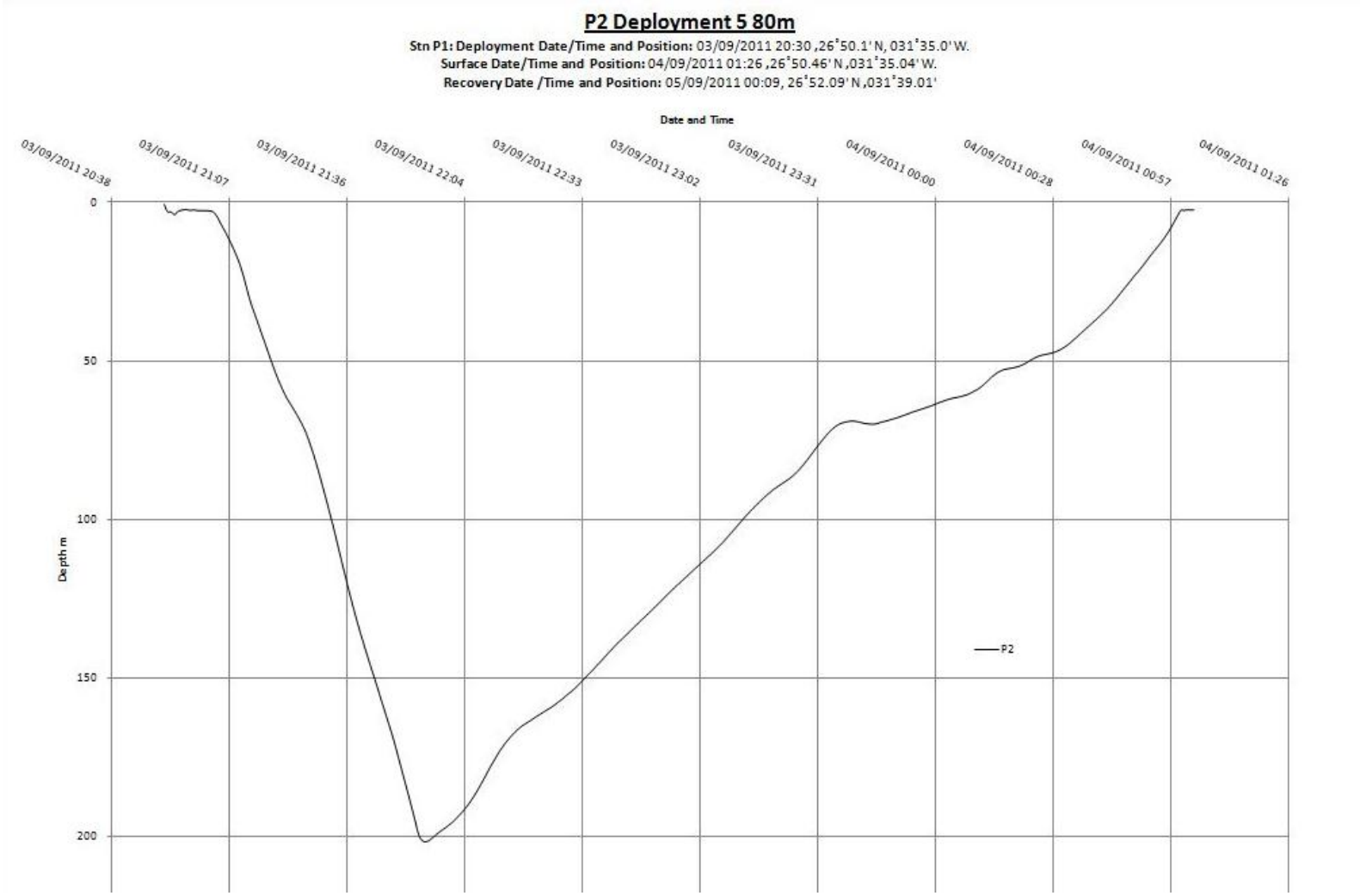

Figure 44: P2 Deployment 5, 80 m.

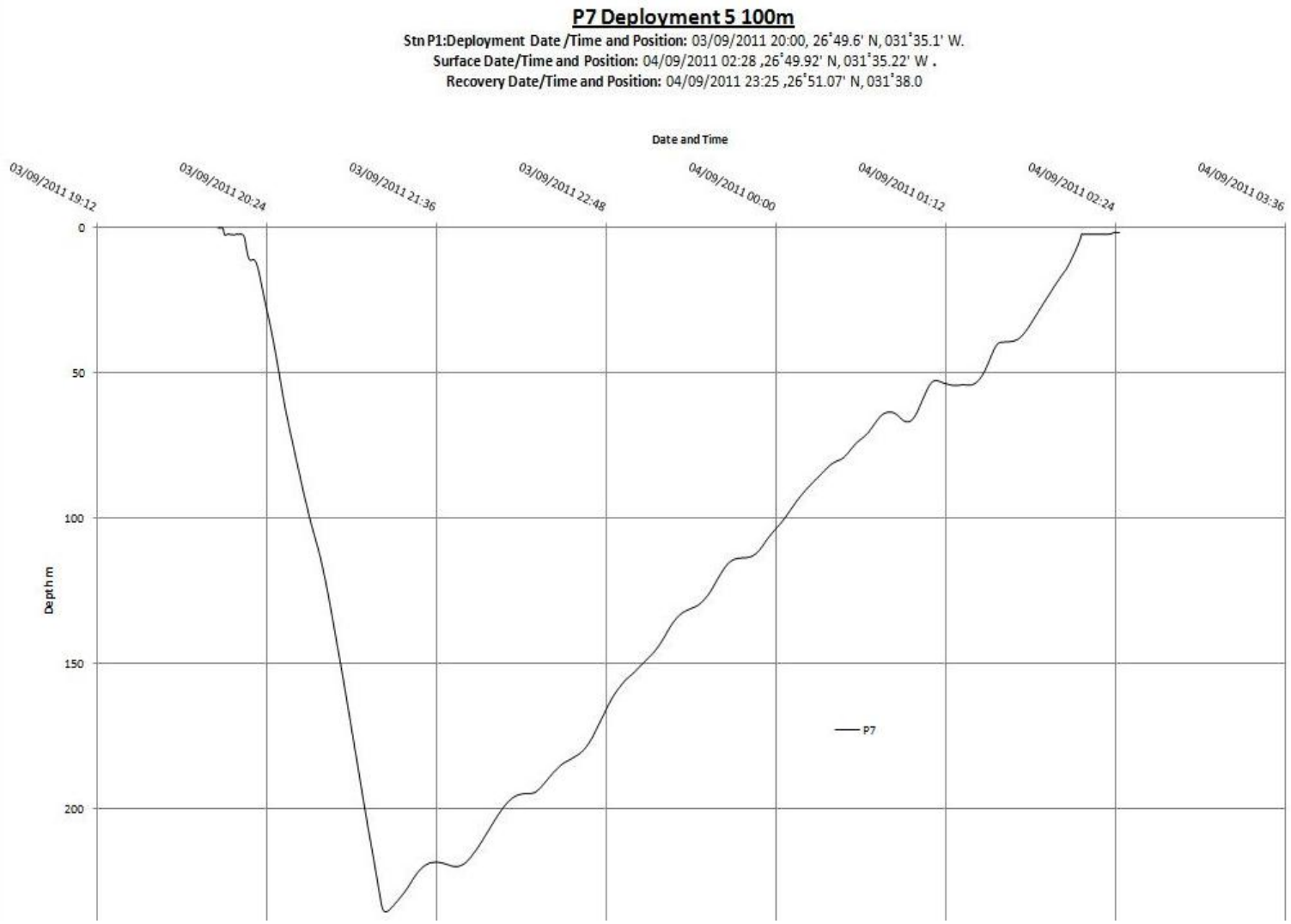

Figure 45: P7 Deployment 5, 100 m.

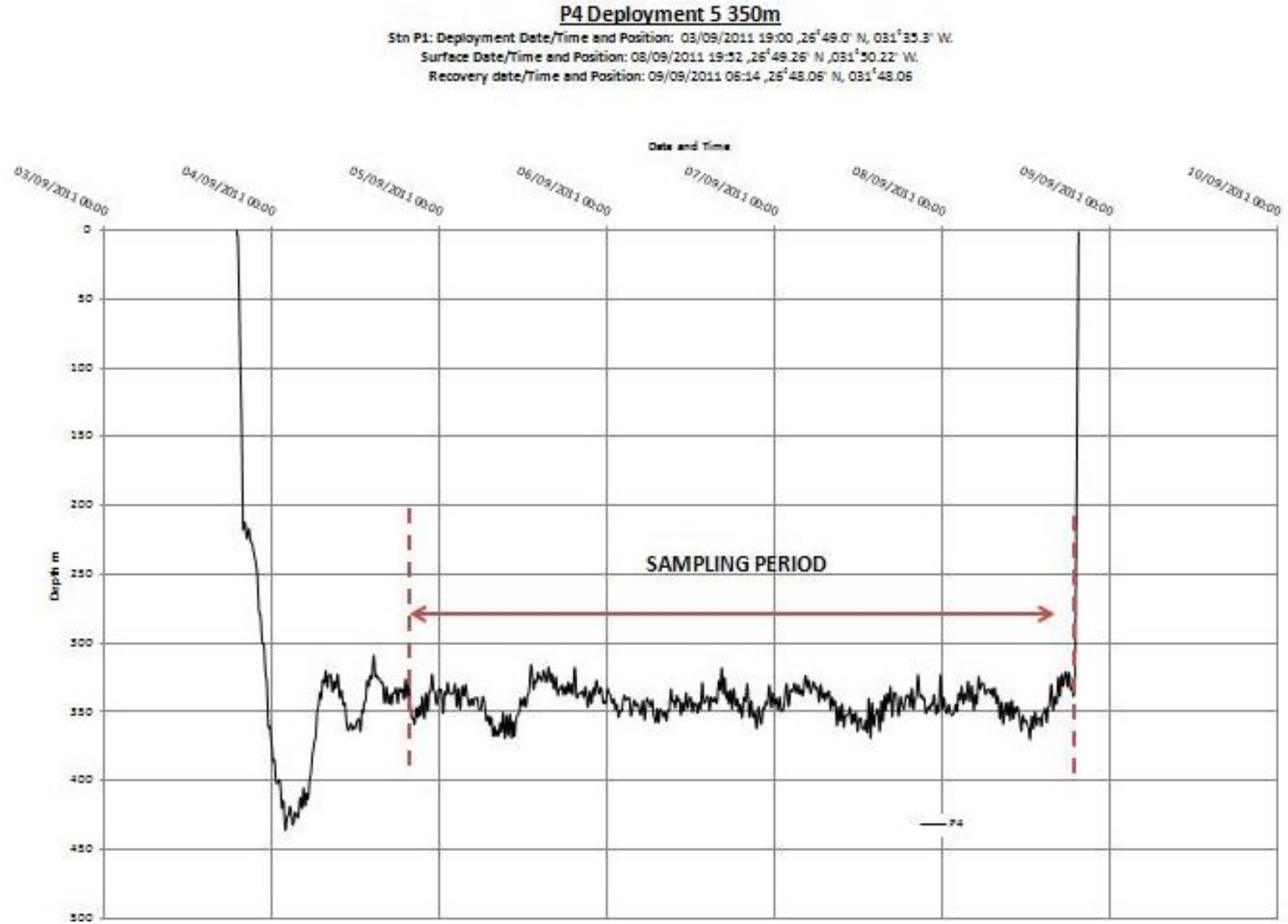

Figure 46: P4 Deployment 5, 350 m.

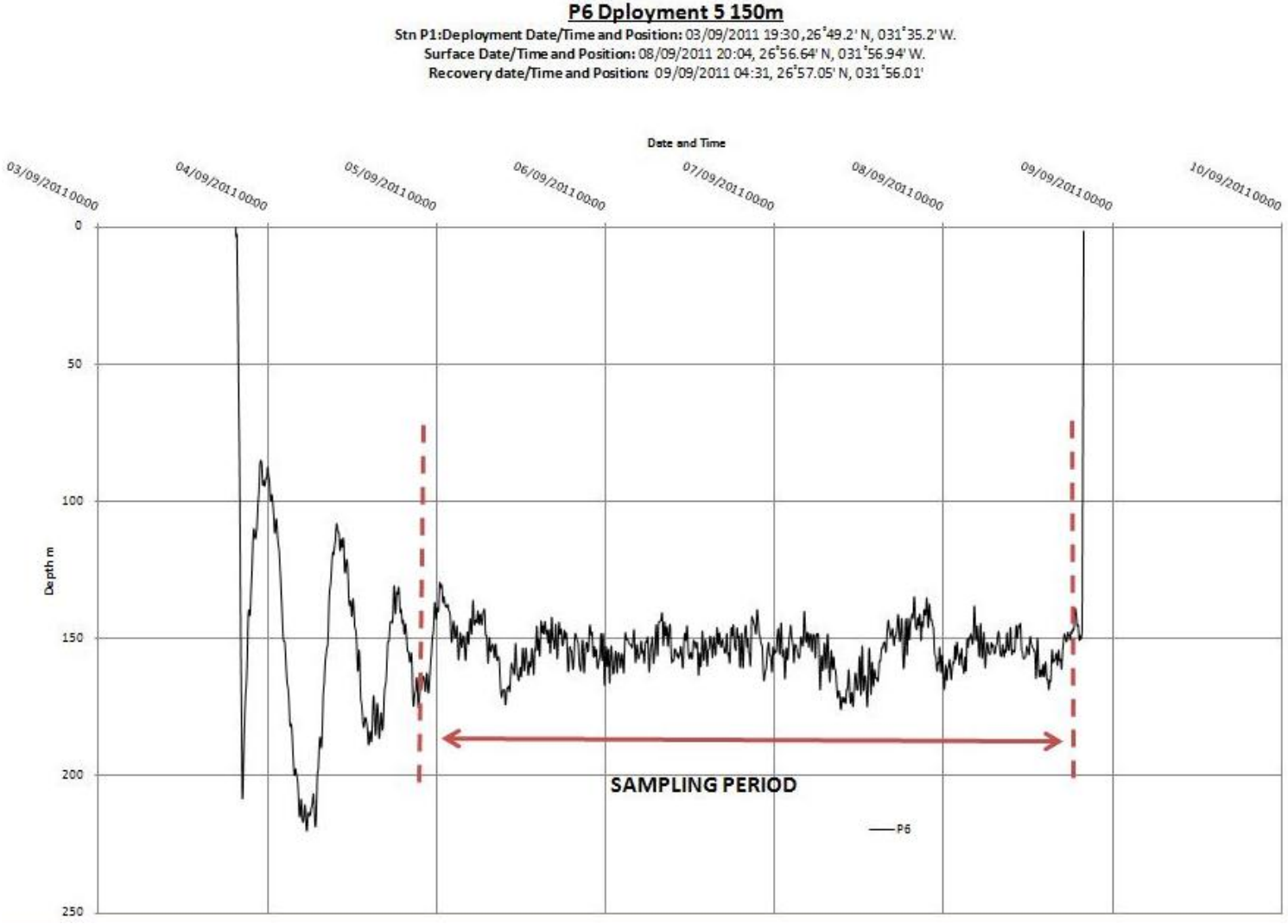

Figure 47: P6 Deployment 5, 150 m.

Downward Particle Fluxes

Application of 234Th:238U disequilibria technique and deployment of PELAGRA sediment traps.

Katsiaryna Pabortsava

Objectives

Using ^{234}Th:^{238}U disequilibria technique and deployments of neutrally buoyant PELAGRA sediment traps, measure vertical fluxes of particulate organic carbon and nitrogen (POC and PON), particulate inorganic carbon (PIC), biogenic silica (BSi, opal) and particulate concentrations of metals (Fe, Al, Ca, Sr, Mn) in the North Atlantic Oligotrophic Gyre to estimate how much primary production is exported from the surface of the ocean to its interior.

^{234}Th:^{238}U disequilibria approach

One way to understand marine particle dynamics in the upper ocean is to use ^{234}Th as a natural tracer of particle formation, transport, and dissolution. ^{234}Th is a naturally occurring isotope, produced by radioactive α-decay of ^{238}U. Unlike conservative ^{238}U ($t_{1/2}$=4.5x10^9 yr), ^{234}Th has a short half-life ($t_{1/2}$=24.1 days) and a strong scavenging affinity, i.e. it is readily scavenged onto particles surfaces and exported with them out of the euphotic zone. In the absence of ^{234}Th uptake onto particles, a secular equilibrium between ^{234}Th and ^{238}U is expected (^{234}Th=^{238}U). Since ^{234}Th is scavenged and then removed from the surface of the ocean as particles descend, a deficiency in ^{234}Th relative to ^{238}U occurs in the upper water column (^{234}Th > ^{238}U, also known as ^{234}Th : ^{238}U disequilibrium) (Santschi, *et al.*, 2006, Verdeny, *et al.*, 2009, Maiti, *et al.*, 2010). An opposite process takes place at depth where particles are solubilised and demineralised supplying ^{234}Th back into the water column and causing ^{234}Th excess relative to ^{238}U (^{234}Th < ^{238}U) (Maiti, *et al.*, 2010).

Assuming steady state of the system (no advective or diffusive turbulent transport), ^{234}Th flux from surface to depth can be calculated from its activity profile integrated from surface to depth *z* where ^{234}Th and ^{238}U secular equilibrium is reached:

$$Th\ flux = \int_0^z \lambda_{Th}(A_U - A_{Th})\, dz$$

where λ_{Th} is ^{234}Th decay constant ($\lambda_{Th} = 0.20876d^{-1}$); A_U is ^{238}U activity (dpm m^{-3}) calculated from the salinity; A_{Th} is ^{234}Th activity (dpm m^{-3}) (measured by beta-counting). Detailed description of ^{234}Th analytical procedures is given in van der Loeff *et al.* (2006) .

To derive particulate fluxes, ^{234}Th fluxes are multiplied by the known concentration ratio of a particle-associated element (e.g. C, N, P, etc.) to ^{234}Th on large particles collected with either sediment traps or *in situ* filtration systems (e.g. Stand alone pumping system, SAPS). For example, ^{234}Th-derived POC flux (export) is calculated as follows in van der Loeff *et al.* (2006):

$$POC\ flux = 234Th\ flux * {}^{POC}/_{234Th}$$

Determination of ^{234}Th in seawater was performed by scavenging of this nuclide by its co-precipitation with MnO_2 from 4 L of seawater collected at 10 different depths on a stainless CTD rosette. This is so called 'small volume technique' modified from 20 L-method developed by Rutgers van der Loeff and Moore (1999). This method not only allowed immediate on-board beta-counting of ^{234}Th activity, but also enhanced both spatial and temporal resolution of particle export.

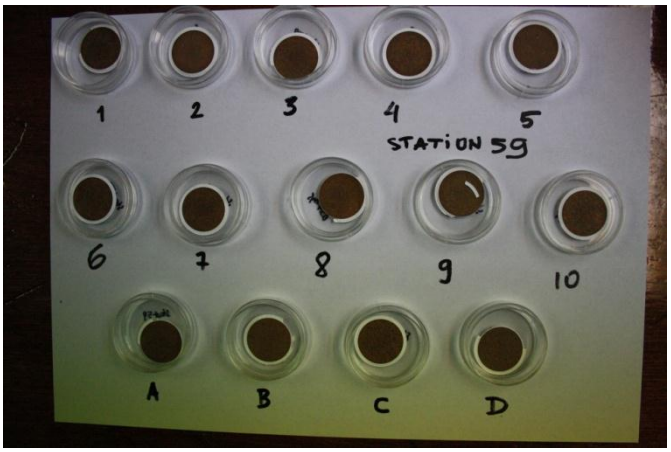

Figure 1: Example of QMA filters with precipitated MnO2 and scavenged Th-234.

Within ~1 hour of collection, seawater samples were acidified to pH 1-2 with concentrated HNO_3 at 1.5 mL/L to separate ^{234}Th from parental ^{238}U and shaken vigorously. 50 µL (0.35Bq/g) of ^{230}Th yield tracer was then added to each sample bottle. The samples were vigorously shaken again and left to equilibrate for 6-8 h. After equilibration, 7-8 mL of HN_4OH was added per sample to bring the pH to 8.0-8.1. To form suspension of MnO_2, 50 µL (7.5 mg/L) of $KMnO_4$ and 50 µL (7.5 mg/L) of $MnCl_2$ were subsequently added to seawater samples. The MnO_2 precipitate was then allowed to scavenge

^{234}Th for 6-8 h. The bottles where precipitation of MnO_2 took place were then attached to a specially designed filter-holders and the content was precipitated onto ashed 25 mm QMA (Whatman) filter (Figure 48). Filter precipitates were dried for 12-24 h at 60°C and then mounted onto Risø beta-counter filter holder under layer of Mylar film and Al foil in order to shield alpha-particles and low energy beta-emitters. ^{234}Th was quantified by counting daughter ^{234m}Pa ($t_{1/2}$=1.2 min) on a low-level Argon gas-flow 5-sample GM-25 beta-counter manufactured by Risø National Laboratories (Roskilde, Denmark). The counter utilizes an anti-coincidence shield above 25 mm-diameter sample windows. The unit is completely surrounded by lead bricks to reduce background count rates. To assess efficiency of beta-counter, 5 NISKIN CTD bottles were sampled simultaneously at a single depth of 1000 m in the open ocean. For experimental blank 4 L of Milli-Q water with all the reagents and spike was filtered. Counting of all the samples was performed until the counting error reached <3% (~1000 counts). The activity of parental ^{238}U was calculated from water salinity according to Chen *et al*. (1986):

$$^{238}U = 0.07081 \times S(‰)$$

Initial counting will be followed by a final background radiation count after >7 half-lives of ^{238}Th decay (~6 months).

After final background count, the MnO_2 filters will be dismounted and prepared for quantification of ^{234}Th recovery by ICP-MS analysis. Mn precipitate will be dissolved in 8 M HNO_3/10% H_2O_2 solution followed by addition of ^{229}Th spike. Anion exchange chromatography on AG1-X8 resin will be used to purify Th isotopes. Prior to analyses by ICP-MS, sample elute will be diluted in HNO_3 matrix, evaporated in several stages and brought up to volume of 2 mL with 10% HNO_3/1%HF solution. Finally, ^{229}Th:^{230}Th ratio will be measured by multicollector ICP-MS (Pike, *et al.*, 2005).

Neutrally buoyant PELAGRA sediment trap deployments

Interception of sinking particles by sediment traps is one of the few direct methods of spatial, temporal and vertical quantification of export flux in the ocean as well as sampling the particles for physical and chemical analyses (Buesseler, *et al.*, 2000).

To obtain [element]/^{234}Th ratio on sinking particles for ^{234}Th:^{238}U disequilibrium approach, as well as to compare Th-234 derived particle fluxes with direct fluxes measurements, neutrally buoyant PELAGRA (Particle Export measurements using Lagrangian trap) sediment traps (Figure 49) were deployed at the study site at nominal depths of 50, 150 and 350 m. PELAGRA traps are equipped with 4 separate collection funnels (0.115 m, 2 each) leading to a separate 500 mL Nalgene collection

cup filled with the preservative of choice (here 5% formaldehyde buffered with sodium Tetraborate). PELAGRA uses an APEX float (Autonomous Profiling EXplorer) which maintains trap's location at intended depth, density and pressure horizons and allows floating in a near-Lagrangian manner (Lampitt, *et al.*, 2008). It is designed to measure the attenuating flux in the mesopelagic (100-600 m depths). The great advantage of PELAGRAs over typical NBSTs is that it only collects the particles when it reaches its equilibrium pressure and density depth. Furthermore, the sample cups in PELAGRA move under and away from the collecting funnel sealing the content of the cups before and after the collection periods. This prevents contamination of the sample from any debris from the ship entering the trap (Lampitt, *et al.*, 2008). PELAGRAs are virtually free of motion relatively to surrounding water, thus there is no shear and no 'odour' plume from the collected material to attract grazers to the trap (Lampitt, *et al.*, 2008). PELAGRA is also equipped with a GPS beacon which facilitates location and recovery of the trap (Lampitt, *et al.*, 2008).

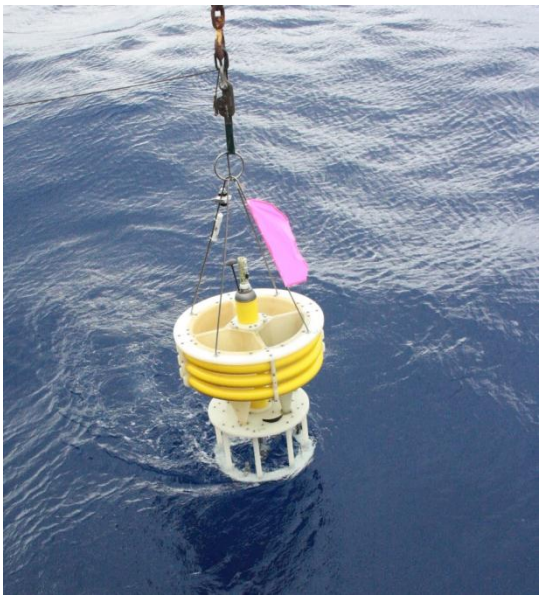

Figure 2: PELAGRA sediment trap being deployed

Three or four PELAGRA traps were deployed on each deployment day out of six traps on board. The cups of the traps were opened simultaneously after 24 h of trap stabilisation period, and the sinking particles were collected over the period of about 4 days. One cup on each trap was dedicated to a procedural blank (cup has never been opened during deployment) to determine 'leakage' of the preservative. The remaining two or three traps were deployed within 24 h after recovery of the previous set of PELAGRA traps.

Preparation of preservative

Formaldehyde preservative was used for all PELAGRA deployments. Preservative was needed to stop microbial and zooplankton activity in the samples. Formaldehyde was chosen over other 'poisons' (e.g. sodium azide, mercury chloride) as it is not only a poison but also a fixative – it makes zooplankton bodies more rigid and hence easier to remove when processing the samples. Such method made samples less prone to contamination from zooplankton remnants whilst picking.

To prepare preservative, particle-free deep water (1000 m) was collected in the beginning of the cruise from the Titanium CTD. Prior the cruise, formaldehyde was buffered with 5 g of Sodium Tetraborate per 1 L of Formaldehyde. An obvious excess of chelating resin Chelex 100 (chloride form, mesh size 100-200 µm) was added to buffered formaldehyde in order to scavenge metals present in Formaldehyde and sodium tetraborate. The brine was left for a minimum of 2 weeks and then filtered onto 0.4 µm polycarbonate filter to remove the resin. Finally, the preservative was prepared as follows:

- 1 L of buffered 'metal-free' formaldehyde in 19 L of deep sea water (1000 m)
- NaCl was added to raise the salinity of the preservative (usually about 180 g in 20 L of preservative) so that it was denser than seawater in the deployment area.

Here the protocol had to be readjusted (originally, 100 g of NaCl was added to the same amount of ingredients), as the PELAGRA traps were used in such warm waters for the first time (hence, greater salinity/density of the surface waters). To minimize the amount of salt added, the seawater used for preparation of preservative was frozen, and after being defrosted, its denser layer was siphoned out.

Sample processing

After recovery of the PELAGRA traps, each cup was checked for pH and 1 mL of concentrated formaldehyde brine (37%) was added to all but the blank cups (in the land laboratory the procedural blanks will also be treated with an appropriate amount of formaldehyde brine as they need careful splitting first). Example of just recovered cups with the collected trap material is shown in Figure 50. Depending on the amount of material present in the cups, 2 or 3 cups from the same trap were pooled into a 4 L LDPE bottle via 350 µm mesh to remove big zooplankton swimmers. Swimmers were kept in separate jars filled with the same preservative for further analysis in the land laboratory. The pooled

sample was then split into subsamples using a rotary splitter. Split sizes ranged from 1/8 to 1/64. Splits were used for Th-234, Mass/POC/PON, PIC, BSi and Metal analyses.

Samples for Th-234 analysis were filtered immediately onto pre-combusted (~12 h at 450°C) 25 mm QMA filters, dried at 60°C in the oven and then activity of Th-234 was counted on Riso Beta Counter as described above. 'Metal' samples were filtered onto 0.4 µm polycarbonate filters (previously soaked in 1% HCl for 1-2 days followed by thorough Milli-Q rinse) and stored at -20°C for further analysis on land. Filtrate was kept separately to determine dissolved concentration of metals in the samples.

For Mass/POC/PON, PIC, BSi samples, zooplankton swimmers were removed manually under dissecting microscope using forceps and a plastic pipette. Picked swimmers were kept in separate jars to estimate their contribution to particle fluxes. 'Swimmers-free' samples were then filtered onto pre-weighed, ashed (~12 h at 450°C) 25 mm GF/F filters for Mass/POC/PON (Whatman), and onto 25 mm polycarbonate filters (0.4 µm mesh Whatman) for PIC and opal. Filters were stored at -20°C for further analysis on land. Unused splits, archive samples, and procedural blanks were kept at +4°C for further analysis on land.

Blanks for each of the parameters were prepared by filtering 200 mL (for Mass/POC/PON, Metals, and Th-234) and 100 mL (BSi and PIC) of the preservative (not used in the actual deployment) onto appropriate filter type. Procedural blanks coming from the cup on the trap will be processed in the laboratory on land.

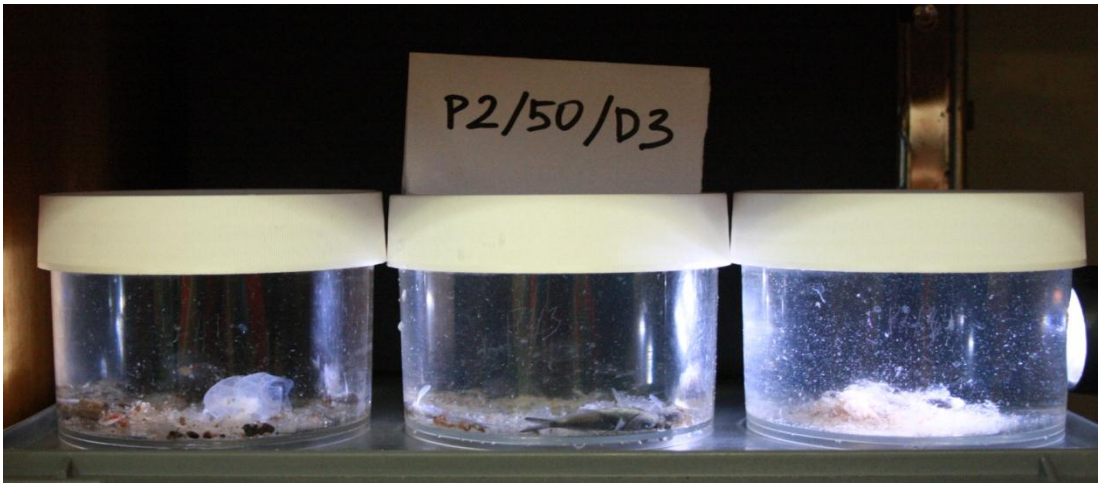

Figure 50: Example of 'trapped' material found in the PELAGRA cups after 4 days of sampling

SAPS Deployment

In situ stand alone pumping systems (SAPS) were deployed at three different depths: 10, 100 and 500 m below the base of the mixed layer depth. Sinking particles were collected onto a pre-filter (53µm Nitex mesh) and a main filter (1 µm Nitex mesh) with SAPS deployed at 10 and 100 m below the base of the mixed layer depth, and onto 53 µm Nitex mesh at 500 m below the depth of the mixed layer depth. Filter housings and Nitex meshes were acid cleaned prior to deployment of the SAPS. SAPS were set to pump for 120 min as a result pumping between 1000-2000 L of seawater. When recovered, particles were rinsed off each of the mesh with exactly 1 L of filtered seawater (0.4 µm Polycarbonate filters). Resulting solution was split into four equal parts with Folsom splitter. Four splits were then filtered onto pre-combusted and pre-weighed 25 mm GF/F (Whatman) filters for particulate organic carbon and nitrogen (POC(PON)) analysis, onto pre-combusted 25 mm QMA (Whatman) filter for Th-234 analysis, and onto 25 mm membrane polycarbonate filters for particulate inorganic carbon (PIC) and biogenic silica (BSi, opal) analyses. SAPS were deployed once and were pumping at 50, 150 and 400 m depth for 120 min with a delay time of 60 min. Due to a low charge of the batteries, only SAPS at 400 m succeeded.

Future chemical analyses

After completing the background radiation count of Th-234 samples, they together with POC (PON) and PIC samples will be fumed with HCl for 24 h, dried for 24 h and then analyzed for organic carbon and nitrogen with CHN analyzer, and for Ca (as PIC) with inductively-coupled plasma atomic emission spectroscopy (ICP-AES) after a 24 h leach with 1 M acetic acid. BSi sample will be digested in 0.2 M NaOH for 3 h at 90°C and measured as Si with an autoanalyzer.

Dissolved and particulate metal concentration will be determined with ICP-MS analysis.

Calculation of daily particle fluxes will be based on known duration of the sample collection, area of the sediment trap funnel opening, and a split size of the sample as shown below (example of POC flux calculations):

$$POC\ flux\ (mgm^{-1}d^{-1} = POC(in\ split) * \frac{1}{days} * \frac{1}{area} * \frac{1}{split\ size}$$

The complete analysis of all the samples is expected by October 2012.

Samples collected

In total 13 CTD casts and 5 PELAGRA deployments were performed to examine particle fluxes from the surface waters to the depth of 500 and 1000 m. Summary of the Stainless CTD casts sampled for Th-234 is given in Table 28. A detailed description of PELAGRA trap deployment and recovery is given by Sam Ward in the 'Deploying PELAGRA Drifting Sediment Traps' section from page 141 onwards. A summary of samples collected for which parameters of interest, is given in Table 29.

Table 28: Summary of CTD casts showing their location and the depths where water was taken for Th-234 analysis

Cruise	**Cast**	**Lat N**	**Long W**	**Depths sampled [m]**
LINK_D369	CTD_1	28.7316	23.5586	5, 10, 20, 30, 60, 75, 80, 100, 250
LINK_D369	CTD_5	25.6097	30.2887	5, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 250
LINK_D369	CTD_8	26.0013	30.2833	5, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 150, 500
LINK_D369	CTD_13	26.8198	30.8036	5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 250, 500
LINK_D369	CTD_16	25.6649	30.3973	5, 20, 50, 80, 90, 110, 120, 300, 500
LINK_D369	CTD_36	26.1470	31.4435	5, 20, 50, 86, 90, 110, 114, 150, 300, 500
LINK_D369	CTD_42	26.7007	31.7047	4.8, 20, 35, 40, 50, 60, 65, 80, 90, 123, 130, 160, 300, 500
LINK_D369	CTD_46	26.5952	30.7243	5, 10, 20, 30, 40, 50, 60, 70, 80, 89, 100, 130, 160, 500
LINK_D369	CTD_54	26.5206	31.4956	5, 10, 30, 40, 50, 60, 70, 76, 90, 100, 144, 200, 500
LINK_D369	CTD_62	26.5024	31.5060	5, 30, 40, 50, 60, 70, 100, 110, 136, 150, 500
LINK_D369	CTD_73	26.5678	31.3926	5, 10, 20, 40, 50, 60, 70, 87, 100, 300, 500
LINK_D369	CTD_77	26.4798	30.7359	5, 21, 30, 40, 50, 60, 71, 80, 90, 110, 151, 200, 501

Table 29: Summary of processed (filtered) PELAGRA sediment trap samples

PELAGRA ID	Deployment	Depth (m)	Parameter of interest	# Cups pooled	Split size	Swimmers (<350 m) picked	Date
P4	Test Deplt 1	350	Th-234	3	1/4	no	20.08.2011
P6	Test Deplt 1	150	Th-234	3	1/4	no	20.08.2011
P4	Test Deplt 1	350	POC/PON/Mass	3	1/4	yes	22.08.2011
P6	Test Deplt 1	150	POC/PON/Mass	3	1/8	yes	22.08.2011
P4	Test Deplt 1	350	PIC	3	1/8	yes	22.08.2011
P6	Test Deplt 1	150	PIC	3	1/8	yes	22.08.2011
P4	Test Deplt 1	350	BSi	3	1/8	yes	22.08.2011
P6	Test Deplt 1	150	BSi	3	1/8	yes	22.08.2011
P5	Test Deplt 2	750	Th-234	3	1/4	no	25.08.2011
P5	Test Deplt 2	750	POC/PON/Mass	3	1/4	yes	25.08.2012
P5	Test Deplt 2	750	PIC	3	1/8	yes	25.08.2013
P5	Test Deplt 2	750	BSi	3	1/8	yes	25.08.2014
P6	Deplt 3	150	Th-234 (1)	3	1/8	no	27.08.2011
P4	Deplt 3	350	Th-234 (1)	3	1/8	no	27.08.2011
P2	Deplt 3	50	Th-234 (1)	1	1/32	no	29.08.2011
P2	Deplt 3	50	Th-234 (2)	1	1/32	no	29.08.2011
P2	Deplt 3	50	Th-234 (3)	1	1/32	no	29.08.2011
P6	Deplt 3	150	Th-234 (2)	3	1/8	no	29.08.2011
P4	Deplt 3	350	Th-234 (2)	3	1/8	no	29.08.2011
P6	Deplt 3	150	POC/PON/Mass	3	1/8	yes	30.08.2011
P4	Deplt 3	350	POC/PON/Mass	3	1/8	yes	30.08.2011
P2	Deplt 3	50	POC/PON/Mass (1)	1	1/32	yes	30.08.2011
P2	Deplt 3	50	POC/PON/Mass (2)	1	1/32	yes	31.08.2011
P6	Deplt 3	150	PIC	3	1/32	yes	31.08.2011
P6	Deplt 3	350	BSi	3	1/32	yes	31.08.2011
P2	Deplt 3	50	PIC (1)	1	1/32	yes	31.08.2011
P4	Deplt 3	350	PIC	3	1/8	yes	01.09.2011

PELAGRA ID	Deployment	Depth (m)	Parameter of interest	# Cups pooled	Split size	Swimmers (<350 m) picked	Date
P4	Deplt 3	350	BSi	3	1/8	yes	01.09.2011
P2	Deplt 3	50	PIC (2)	1	1/64	yes	02.09.2011
P2	Deplt 3	50	BSi (2)	1	1/64	yes	02.09.2011
P4	Deplt 4	350	Th-234	2	1/8	no	03.09.2011
P6	Deplt 4	150	Th-234	2	1/8	no	03.09.2011
P4	Deplt 4	350	POC/PON/Mass (1)	2	1/8	yes	06.09.2011
P4	Deplt 4	350	POC/PON/Mass (2)	2	1/8	yes	06.09.2011
P6	Deplt 4	150	POC/PON/Mass (1)	2	1/8	yes	06.09.2011
P6	Deplt 4	150	POC/PON/Mass (2)	2	1/8	yes	06.09.2011
P6	Deplt 4	150	Metals	2	1/8	no	07.09.2011
P4	Deplt 4	350	Metals	2	1/8	no	07.09.2011
P6	Deplt 4	150	PIC	2	1/8	yes	07.09.2011
P4	Deplt 4	350	Metals	2	1/8	no	08.09.2011
P4	Deplt 4	350	PIC	2	1/8	yes	08.09.2011
P4	Deplt 4	350	BSi	2	1/8	yes	08.09.2011
P6	Deplt 4	150	Metals	2	1/8	no	08.09.2011
P6	Deplt 4	150	BSi	2	1/16	yes	08.09.2011
P6	Deplt 5	150	Th-234 (1)	2	1/8	no	09.09.2011
P6	Deplt 5	150	Th-234 (2)	2	1/8	no	09.09.2011
P4	Deplt 5	350	Th-234	2	1/4	no	09.09.2011
P4	Deplt 5	350	Metals	2	1/8	no	09.09.2011
P4	Deplt 5	350	Metals	2	1/8	no	09.09.2011
P6	Deplt 5	150	Metals (1)	2	1/32	no	10.09.2011
P6	Deplt 5	150	Metals (2)	2	1/32	no	10.09.2011
P6	Deplt 5	150	Metals (3)	2	1/32	no	10.09.2011
P4	Deplt 5	350	PIC	2	1/8	yes	11.09.2011

PELAGRA ID	Deployment	Depth (m)	Parameter of interest	# Cups pooled	Split size	Swimmers (<350 m) picked	Date
P4	Deplt 5	350	BSi	2	1/8	yes	11.09.2011
P4	Deplt 5	350	POC/PON/Mass (1)	2	1/8	yes	11.09.2011
P4	Deplt 5	350	POC/PON/Mass (2)	2	1/8	yes	11.09.2011
P6	Deplt 5	150	POC/PON/Mass	2	1/8	yes	11.09.2011
P6	Deplt 5	150	POC/PON/Mass (1)	2	1/32	yes	12.09.2011
P6	Deplt 5	150	POC/PON/Mass (2)	2	1/32	yes	12.09.2011
P6	Deplt 5	150	PIC	2	1/8	yes	12.09.2011
P6	Deplt 5	150	BSi	2	1/8	yes	12.09.2011

Microbial Respiration in the Deep Sea

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Introduction

The aim of this work was to measure microbial respiration in the deep sea. As metabolic rates in the deep sea are so slow, we proposed that large volume incubations with a sensitive tracer (e.g. ^{14}C-Glucose) would be required to measure respiration within 24 h. With this in mind, we developed the Deep *in situ* Incubator (DISI), which is a rosette of 24, 20 L Niskin bottles adapted for use as incubation vessels *in situ*. We currently do not have a licence to incubate samples with radioactive tracers *in situ*. Therefore, the aim for this cruise was to prove the proposed methods viable by measuring $^{14}CO_2$ production in deep sea samples incubated with ^{14}C-Glucose in Niskin bottles in the lab.

Methods

A standard rosette of 24, 20 L Niskin bottles on a stainless steel frame, has been adapted for the use as incubation vessels. Each Niskin bottle was fitted with two valved polypropylene connectors, one at the top and one at the bottom of the main cylinder. To these connectors, braided silicone ½" tubing is attached using the mating valved coupling insert, thereby allowing the incorporation of additional components, e.g. pumps, to the system. We used a titanium-housed submersible Sea-Bird pump to mix tracers into the incubation Niskin bottles. The pump has barbed attachments to which ½" braided silicone tubing is attached.

Six Niskin bottles were used per incubation, of which five were used for ^{14}C-Glucose respiration measurements ('Respiration Niskins'). The remaining Niskin bottle received no tracer addition and was used only to assess stability of the microbial community throughout the course of the experiment by analysing samples by flow cytometry ('AFC Niskin'). Seawater was collected directly into the 20 L Niskin bottles, which had been washed with 10% HCl. Seawater was collected from 1000 m for five incubations, and from 300 m for one other (Table 30). Once retrieved, six Niskin bottles were transferred to a cooled, trace metal clean laboratory and secured in purpose built racks for the duration of the incubations.

Table 30: Date, time, position and depth of sampling for deep sea respiration measurements.

Deployment	Date	Time	Lat, °N	Lon, °W	Depth, m
1	19.08.11	13:50	25°35.65	030°42.99	1000
2	24.08.11	11:15	26°43.89	031°42.12	300
3	29.08.11	11:50	26°36.16	031°20.47	1000
4	03.09.11	15:40	26°49.57	031°33.55	1000
5	08.09.11	13:00	25°23.95	031°48.52	1000
6	11.09.11	14:10	25°52.95	031°30.31	1000

Previous measurements of amino acid (Leucine) concentration in the deep sea indicated concentrations in the region of 10-70 pM (Mike Zubkov, personal communication); we assume glucose to be available at similar concentrations as this, as was shown for surface waters (Zubkov, *et al.*, 2008) . Thus, D-[^{14}C(U)]Glucose (specific activity 11.1 TBq/mmol; Hartmann Analytic, Germany) tracer was added to seawater samples at 40 pM final concentration; we predicted this to be the lowest concentration at which we would be able to detect $^{14}CO_2$ production assuming 1% respiration of added ^{14}C-Glucose. Seawater was circulated for 8 min using the submersible pump to mix the tracer homogeneously. Eight minutes was shown to be ample time to homogeneously mix dye throughout 20 L of seawater in Niskin bottles. Two samples of 1.6 mL were removed from the AFC Niskin and fixed with paraformaldehyde (PFA, 1% final concentration). At each incubation time point, the AFC Niskin was mixed before fixing two 1.6 mL flow cytometry samples.

The Respiration Niskins were incubated for a range of time periods, e.g., 6, 16, 20, 24, 28 h. At the end of each incubation period, seawater within the Respiration Niskin was mixed for 8 min then 3 L were decanted into three 1 L bottles containing glutaldehyde (1% final concentration) to fix cells. These samples were filtered in a range of volumes (0.3, 0.6, 0.9, 1.2 L) onto 47 mm, 0.2 µm PC filters to assess the proportion of ^{14}C that was incorporated into biomass.

To the Respiration Niskin, 80 mL of 25% HCl was added to acidify the sample to <pH 2, thereby driving any dissolved CO_2 out of solution. Braided ½" PVC tubing, with a gas diffuser at its end, was fed into a 1 L borosilicate bottle containing 700 mL of 1.4 M NaOH 'bubbler' and the other end connected to the upper valve of the Niskin. A rotary vein pump was connected to the lower valve of the Niskin bottle with braided ½" silicone tubing. Two plastic tubes were placed within the line, between pump and Niskin; the first contained glass wool to absorb any particles coming from the pump, the second contained soda lime which efficiently removed CO_2 from the air entering the Niskin, thereby increasing the efficiency of CO_2 trapping from the Respiration Niskin. Seawater

samples were bubbled for 2 h, then seawater aliquots (1, 2, 3, 4 mL) were removed to assess the remaining dissolved fraction of ^{14}C so that a budget could be produced for the tracer.

The 700 mL bubblers, now containing a mixture of Na_2CO_3 and $Na_2{}^{14}CO_3$, were divided into aliquots of 50, 100, 150 and 400 mL. Precipitates of $BaCO_3$ and $Ba^{14}CO_3$were produced in each aliquot by the addition of 40 mM (final concentration) Barium chloride dihydrate. The precipitates were left to settle for at least 3 h; the clear supernatant was poured off and the remaining slurry of precipitate filtered onto 47 mm glass fibre filters (Whatman GF/F). The precipitate filters were transferred to 20 mL scintillation vials, a slurry was produced with 3 mL Milli-Q water and suspended in 16 mL of StarGel scintillation cocktail (Meridian, UK). The activity within all biomass, dissolved and precipitated respiration samples was measured in an ultra-low-level liquid scintillation counter (1220 Quantulus, Wallac, Finland).

Flow cytometry samples were stained with SYBR Green I DNA stain (Sigma-Aldrich, Poole, UK) in the presence of Potassium citrate and analysed on a FACSort instrument (BD Biosciences, Oxford, UK). The instrument was triggered on FL1 (green) fluorescence for DNA and SSC (side scatter) for approximate cell size. Cells were enumerated from flow rate which was determined by the addition of a known concentration of latex beads. Data were acquired and analysed using CellQuest software (BD Biosciences, Oxford, UK). Cell concentration for High Nucleic Acid (HNA) and Low Nucleic Acid (LNA) containing groups were noted, in addition to total bacterioplankton community numbers.

Preliminary results

Due to low activity in samples and the insensitivity of the scintillation counter onboard RRS *Discovery*, we need to recount the samples using an ultra low level scintillation counter at NOCS. However, from the data we currently have we estimate glucose respiration at 15-110 fmol L^{-1} h^{-1}, with 2.7-6.4 times more ^{14}C being incorporated into biomass than is respired.

4. Conclusions

This cruise was designed to field test a prevailing hypothesis that open ocean ecosystems are in steady state. The cruise achieved its main goal of collecting data that will allow us to link the functioning of oligotrophic oceanic microbial communities with their fluidic environment, in particular with the propagation of eddies through strongly stratified photic and twilight waters. Advanced pre-cruise planning and close collaboration between physicists, biologists and chemists onboard, ensured cohesion in sampling efforts resulting in a rare collection of multidisciplinary scientific evidence, as outlined above. Further analyses of collected samples and data in conjunction with computer modelling in the coming months have a strong synthesis potential to qualitatively advance our knowledge of the pelagic ecosystem biogeochemistry in sub/tropical oceanic gyres – the most extensive regions on Earth (>40% of Earth's surface) of global climatic importance.

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References

Allen J, Dunning J, Cornell V, Moore CM & Crisp N (2002) Operational oceanography using the new SeaSoar undulator. *Sea Technology* **43**: 35-40.

Buesseler KO, Steinberg DK, Michaels AF, Johnson RJ, Andrews JE, Valdes JR & Price JF (2000) A comparison of the quantity and composition of material caught in a neutrally buoyant versus surface-tethered sediment trap. *Deep Sea Research I* **47**: 277-294.

Chen JH, Edwards RL & Wasserburg GJ (1986) 238U, 234U and 232Th in seawater. *Earth and Planetary Science Letters* **80**: 241-251.

Field KG, Gordon D, Wright T, Rappé M, Urbach E, Vergin K & Giovannoni SJ (1997) Diversity and depth-specific distribu- tion of SAR 11 cluster rRNA genes from marine planktonic bacteria. *Applied and Environmental Microbiology* **63**: 63-70.

Jardillier L, Zubkov MV, Pearman J & Scanlan DJ (2010) Significant CO2 fixation by small prymnesiophytes in the subtropical and tropical northeast Atlantic Ocean. *ISME Journal* **4**: 1180-1192.

Kim E, Harrison JW, Sudek S, Jones MDM, Wilcox HM, Richards TA, Worden AZ & Archibald JM (2010) Newly identified and diverse plastid-bearing branch on the eukaryotic tree of life. *Proceedings of the Academy of Natural Sciences USA* **108**: 1496-1500.

Kirkwood D (1996) Practical notes on their determinations in seawater. *ICES Techniques in marine environmental sciences* **17**: 1-25.

Lampitt RS, Boorman B, Brown L, Lucas M, Salter I, Sanders R, Saw K, Seeyave S, Thomalla SJ & Tumewitsch R (2008) Particle export from the euphotic zone: Estimates using a novel drifting sediment trap, 234Th and new production. *Deep Sea Research I* **55**.

Li QP & Hansell DA (2008) Intercomparison and coupling of magnesium-induced co-precipitation and long-path liquid-waveguide capillary cell techniques for trace analysis of phosphate in seawater. *Analytica Chimica Acta* **611**: 68-72.

Maiti K, Benitez-Nelson CR & Buesseler KO (2010) Insights into particle formation and remineralization using the short-lived radionuclide, Thoruim-234. *Geophysical Research Letters* **37**: L15608.

Mary I, Heywood JL, Fuchs BM, Amann R, Tarran GA, Burkill PH & Zubkov MV (2006) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquatic Microbial Ecology* **45**: 107-113.

Patey MD, Rijkenberg MJA, Statham PJ, Mowlem M, Stinchcombe MC & Achterberg EP (2008) Determination of nitrate and phosphate in seawater at nanomolar concentrations. *Trends in Analytical Chemistry* **27**: 169-182.

Pike SM, Buesseler KO, Andrews J & Savoye N (2005) Quantification of Th-234 recovery in small volume sea water samples by inductively coupled plasma-mass spectrometry. *Journal of Radioanalytical and Nuclear Chemistry* **263**: 355-360.

Rutgers van der Loeff MM & Moore WS (1999) Determination of natural radioactive tracers. *Methods of Seawater Analysis* (Grasshoff K, Ehrhardt M & Kremling K, eds.), pp. 365-397. Verlag Chemie, Weinheim.

Rutgers van der Loeff MM, Sarin MM, Baskaran M, Benitez-Nelson C, Buesseler KO, Charette M, Dai M, Gustafsson Ö, Masque P, Morris PJ, Orlandini K, Rodriguez y Baena A, Savoye N, Schmidt S, Turnewitsch R, Vöge I & Waples JT (2006) A review of present techniques and methodological advances in analyzing 234Th in aquatic systems. *Marine Chemistry* **100**: 190-212.

Santschi PH, Murray JW, Baskaran M, Benitez-Nelson CR, Guo LD, Hung CC, Lamborg C, Moran SB, Passow U & Roy-Barman M (2006) Thorium speciation in seawater. *Marine Chemistry* **100**: 250-268.

Schattenhofer M, Fuchs BM, Amann R, Zubkov MV, Tarran GA & Pernthaler J (2009) Latitudinal distribution of prokaryoplankton populations in the Atlantic Ocean. *Environmental Microbiology* **11**: 2078-2093.

Thierry V, Gilbert D & Kobayashi T (2011) Processing Argo oxygen data at the DAC level. Version 1.2. http://www.argodatamgt.org/content/download/2928/21973/file/ARGO_oxygen_proposition_v21971p21972.pdf.

Verdeny E, Masque P, Garcia-Orellana J, Hanfland C, Cochran JK & Stewart GM (2009) POC export from ocean surface waters by means of Th-234/U-238 and Po-210/Pb-210 disequilibria: A review of the use of two radiotracer pairs. *Deep Sea Research II* **56**: 1502-1518.

Zhang JZ & Chi J (2002) Automated analysis of nanomolar concentrations of phosphate in natural waters with liquid waveguide. *Environmental Science & Technology* **36**: 1048-1053.

Zubkov MV, Tarran GA & Fuchs BM (2004) Depth related amino acid uptake by *Prochlorococcus* cyanobacteria in the Southern Atlantic tropical gyre. *FEMS Microbiology Ecology* **50**: 153-161.

Zubkov MV, Mary I, Woodward EMS, Warwick PE, Fuchs BM, Scanlan DJ & Burkill PH (2007) Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre. *Environmental Microbiology* **9**: 2079-2089.

Zubkov MV, Tarran GA, Mary I & Fuchs BM (2008) Differential microbial uptake of dissolved amino acids and amino sugars in surface waters of the Atlantic Ocean. *Journal of Plankton Research* **30**: 211-220.