

Plymouth Marine Laboratory Core cruise 2004

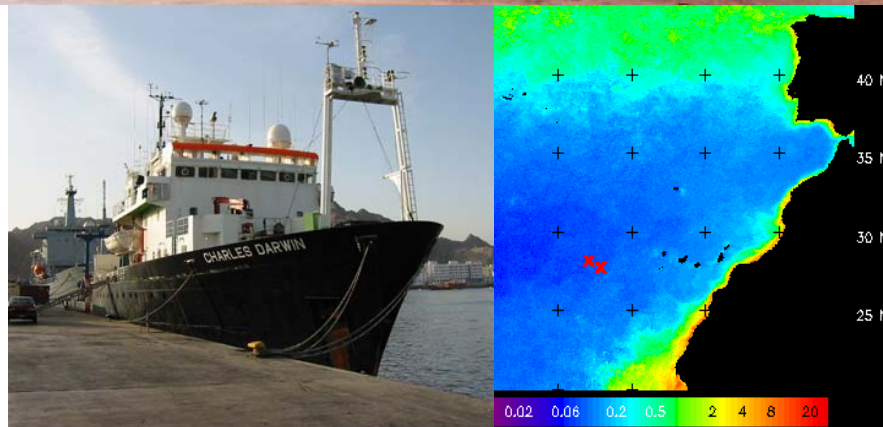
Phosphate & Iron Addition Experiment (*FeeP*)

RV Poseidon
PO311

29th April – 25th May 2004

Santa Cruz de Tenerife – Santa Cruz de Tenerife

Fahrtleiter : Andy Rees



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Acknowledgements

I would like to extend my personal thanks to all involved with this cruise from conception through to delivery. As my first attempt at fahrleiter I'm sure I made a few mistakes along the way (I even know what a few of them were!) and would like to apologise to anybody affected. This was a huge effort from all concerned and I would like to thank in particular Chris Wing - for bringing some order to the chaos that seems to surround me at the moment, Phil Nightingale - my partner in crime throughout this adventure, Cliff Law for initial conception with Nick Owens – principal scientist onboard RRS Charles Darwin.

The officers, engineers and crews of both ships were fantastic in their delivery of what was at times a testing operation. I am particularly indebted to Captain Michael Schneider for his flexible and accommodating attitude and would like to acknowledge first officer, Matthias Günther's contribution to maintaining my focus.














Background

Between the 24th April and 26th May 2004 Plymouth Marine Laboratory in collaboration with scientists from NIWA, New Zealand, Laboratoire Arago, France and University of East Anglia conducted a two-ship exercise using RRS Charles Darwin and RV Poseidon to test the hypothesis that: the supply of, and the interaction between, iron and phosphorous control biological activity and fluxes in the subtropical North Atlantic. An experimental area in international waters to the west of the Canary Islands was selected following an intense period of vertical and horizontal mapping by the Charles Darwin. Using SF₆ as a tracer for amended waters, two separate experiments were performed. The first (5th – 15th May) involved the addition of 20 tonnes of anhydrous monosodium phosphate at 10 m depth over an area of approximately 25 km², centered at 27.8°N 23.3°. The second experiment was conducted following a mid-cruise return by the Poseidon to Tenerife between 16th and 22nd May, at 27.5°N 22.5°W when 5 tonnes of acidified iron sulphate were added over the first 12 hours and following a brief recovery period, 20 tonnes of phosphate were added over the top of the iron. Measurements of nutrient chemistry, gas exchange and biological activity were monitored prior to and after deployment (IN stations) of the fertilised patches relative to several (OUT) control stations.



Scientific Personnel

Title	Fore Names	Surname		Affiliation	Role
Dr	Andrew Paul	Rees		Plymouth Marine Laboratory	Principal Scientist Phytoplankton production & nitrogen fixation
Dr	Delphine	Bonnet		Plymouth Marine Laboratory	Mesozooplankton productivity
Dr	Darren Raymond	Clark		Plymouth Marine Laboratory	Nitrogen cycling
Miss	Victoria Anne	Collins		PML, University of East Anglia	Nitrogen cycling & microzooplankton grazing
Dr	Joanna Lee	Dixon		Plymouth Marine Laboratory	Bacterial & phytoplankton production
Dr	Christopher Paul	Gallienne		Plymouth Marine Laboratory	CTD & instrumentation
Dr	Clifford Stephen	Law		NIWA, New Zealand Plymouth Marine Laboratory	SF6 analysis & nitrogen fixation
Dr	Carol	Robinson		Plymouth Marine Laboratory	Heterotrophic - Autotrophic balance. Dissolved inorganic carbon
Dr	Gary Randall	Smerdon		Plymouth Marine Laboratory	Molecular diversity of diazotrophs
Mr	John Anthony	Stephens		Plymouth Marine Laboratory	Nutrient Analysis
Miss	Hester Ruth	Willson		Plymouth Marine Laboratory	SF6 Analysis

Ship's Personnel

Captain	Michael Schneider
Chief Officer	Matthias Günther
2nd Officer	Theo Griese
Chief Engineer	Hans-Otto Stange
2nd Engineer	Frank Dohmann
Electrical Officer	Dietmar Klare
Bosun	Thomas Krüger
Seamen	Kurre Kroger Firmino Ferreira Ralf Meiling Pedro Barbosa
Cook	Stefan Potthoff
Cook/Steward	Karl-Heinz Lohe
Motorman	Rüdiger Engel
Cadet	Martin Kunad (29.04 – 13.05) Gent Wichmann (14.05 – 26.05)

Diary

Date	Poseidon	Charles Darwin
22 nd April		
23 rd		Full party onboard
24 th		
25 th		
26 th	JAS & CG join ship Gran Canaria	Sail
27 th	1224 CTD test	
28 th	Full party onboard Santa Cruz	
29 th	1500 Sail	
30 th		
1 st May	First CTD's	
2 nd	Ships rendez-vous & boat transfers 1100 onwards (27° 48'N, 23°20'W)	
3 rd	Background station patch#1 p.m. underway survey	
4 th	Background station	1900 Begin patch#1 deployment
5 th	In station – PO4 only	
6 th		
7 th	1830 Boat transfer	
8 th		
9 th		
10 th	0400 last station patch#1 1015 boat transfer Sail for Tenerife	
11 th	Begin nutrient addition expt#1	
12 th	Port Call Santa Cruz	
13 th		
14 th	a.m. CTD Survey p.m. Ships rendez-vous & boat transfers (27°41'N, 23°12'W)	
15 th	a.m. Experimental station on patch#1 0900 boat transfer 1300-1430 ADCP intercalibration	
16 th	Background station patch#2 (27°32'N, 22° 34'W)	~0900 Begin deployment patch#2 - Fe
17 th	In station - Fe only	~1700 Begin deployment patch#2 – PO4
18 th	In station – Fe & PO4	
19 th		
20 th	1600 Swimming party	
21 st	1300 boat transfer	
22 nd	0400 last station Sail for Tenerife	
23 rd		
24 th	Alongside Santa Cruz	
25 th	1300 Leave ship	

Narrative

29th April

After much wailing and gnashing of teeth we sailed at 1500 local time to the north and west of the island with blue skies and constant winds of 4 - 5. A number of boxes containing the FRRF and equipment for Cliff had been held up in Madrid by customs; hopefully we'd collect them in two weeks time when we returned for a port-call. After advice from the mate and bosun a last minute decision was made to switch from 24 to 12 bottle CTD rosette, the former then being located in the hold for the duration of the cruise. Soon after sailing it was noticed that a number of gas cylinders had not been transferred from the Charles Darwin (CD). Passed Mount Tiede at approx 2000. Requested by mate to post hazard labels and fire-fighting precautions on all lab doors. Darwin working at 26° 45'N 23°28W (0445z) surveying area

30th April

Heading south west towards Darwin (25° 05' N 24° 32' W at 0600). Boat Drill 1020. Blue skies, winds southerly 3 - 5, moderate seas, making about 8 – 8.5 knots. On going niggles with gear for a number of scientists, not helped by the large amount of equipment squeezed into the laboratories. Brief contact with Nick Owens on the Darwin late afternoon, Darwin headed for 28°N 25°W, Poseidon change heading at 1720 (27° 37'N 19° 56'W). Problems with nutrient chart recorders into night.

1st May

Weather change overnight, increase in winds 5 – 6, moderate to rough seas, some scientists suffering a little! CTD shakedown station at 0800z to 1000m, 2 bottles leaking slightly, water collected at 10 and 1000m, significant pycnocline at 20 m approx, then further 3 casts to assess variability in upper structure. Steam towards 27° 48.2'N 23° 20.2'W for early am rendezvous with CD.

2nd May

Charles Darwin in vicinity 0600. Spoke with Nick Owens on VHF approx 0700. Boat transfers planned for later in day weather permitting. Sea moderate, wind northerly 22 knots decreasing. 1100 boat from CD with Nick, Phil, James and Tim onboard, lunch first then meeting with above plus Cliff and Carol. Initial plan for patch deployment 3rd May 1600 start, phosphate only, ambient iron concentrations appear relatively high. CD to conduct CTD survey of immediate area to investigate homogeneity of mixed layer (40m approx) depth. Nick, James, Tim return approx 1300. Malcolm and Ricardo on next boat to assist with niggles concerning nutrients and ADCP. Set up incubation tanks in afternoon in preparation for next day's start of experimental work. Plans in place for 0200 start.

3rd May

0200 plankton nets at 27° 48.2'N 23° 20.2'W (nominal patch centre) reveal surprisingly rich population with relatively large animals. 4 CTDs at 0400 to 200m for experimental water for first productivity day. Following discussion with Nick plan to re-visit their grid of last night to give better adcp coverage, Poseidon surveying area 1750 – 2358 (Z).

4th May

Grid survey completed prior to plankton nets at 0200. Weather force 3 - 4 northeasterly pressure approx. 1020 stable or increasing, early cloud but good sun later. Few dolphins spotted. Poseidon now into regular pattern of station work. CD plan to deploy patch 1600 gmt was delayed, finally commenced approx 1850. Buoy software on Poseidon bridge receiving signal from drifter deployed by CD during patch laying.

5th May

0210 call from Darwin to give position of buoy (27° 47.9' N 23° 17.77' W) for our initial IN station, sampling program delayed by 1 hour due to re-positioning of ship. Aerosol sampler broken down – ships engineers attending to problem. Mate requests scientific watch-keeper to lookout for buoys during night manoeuvres at patch centre.

7th May

1830 exchange of gear with Darwin, lot of banter on walkie-talkies. Milli-Q broken due to fault in internal switch, fixed by Chris. Elevated phosphate in surface 40m and nitrate at 25-40 m. Cliffs gin, crisps and olives on deck for night-cap – good end to the day.

8th May

Normal sampling continues, weather overcast – no sunbathing today. Conversation with Nick on radio then telephone re exchange of personnel during trip to Santa Cruz. No phosphate at IN station though high SF6. Steamed 10 miles south for out station, confirmed no SF6 before CTD cast.

10th May

1015 exchange of staff with CD in preparation for port call. Hester to CD, Mimi and CD electrician onboard Poseidon for transfer to Tenerife. Station work completed at 1330, water collected for on-deck nutrient addition experiments during transit to and from Santa Cruz.

14th May

Return to previous patch and CTD survey towards the area (27°48'N 22°23'W – 27°25'N 23°02'W). Rendez-vous with CD at 1330 for transfer of Pascal to CD and return of Hester to Poseidon. Rees, Dixon and Law transferred to CD for afternoons meeting to discuss plans for patch#2. Plan to sample one more time at patch#1 and then survey to the east in search of new area for Fe and PO₄ patch.

15th May

SF6 concentrations at experimental station were very low, and so need to be examined alongside CD data for position relative to patch centre. 0900 James Fishwick briefly onboard to collect FRRF equipment. Steam to the east at 9 knots with ships doing parallel course in order to calibrate ADCP.

16th May

Position chosen for patch#2 (27°32'N, 23°12'W) and background station occupied before Fe deployment by CD at 0900 onwards, small number of small dorado around the ship for our early am casts. Station 47 then occupied approximately 2 hours after the addition of Fe to patch centre. Iron sulphate very obvious as green streaks across the sea surface. Iron deployment complete at 2330.

17th May

Full pre-dawn production station on Fe only patch. CD begin deposition of PO₄ at 1600 with the intention of laying a 20 km² layer over the top of the Fe patch which was originally 9 km². *Trichodesmium* found in Delphinus net sample for the first time and first green flash of the cruise.

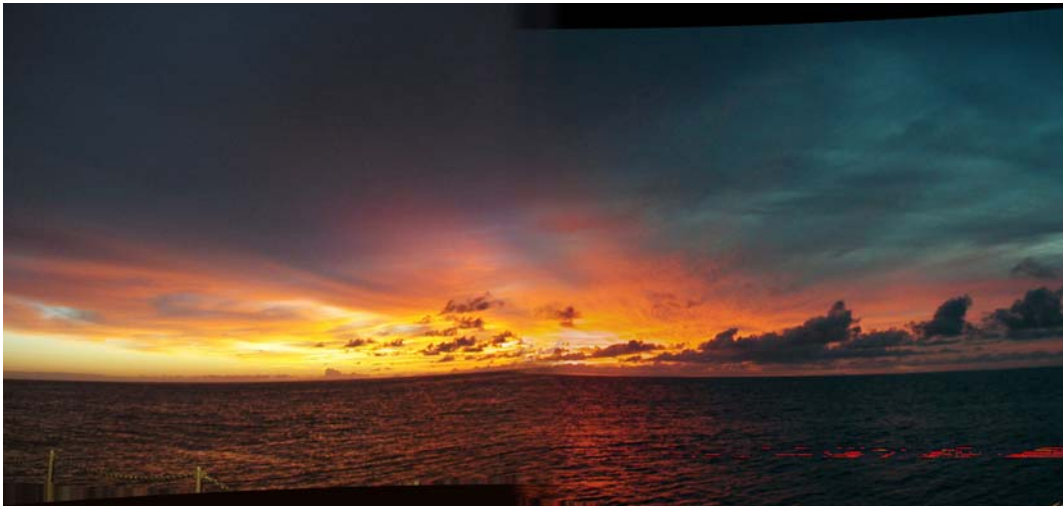
20th May

Best decision of the cruise so far – swimming party at 1600, crew set up boarding ladder and freshwater shower. Just the motivation everyone onboard needed to see the job through – was the whale that appeared shortly after all were back onboard attracted to anyone in particular?



22nd May

0442 (27°41'N, 22°06'W) completion of scientific works, commence voyage back to Santa Cruz de Tenerife.



ON-DECK NUTRIENT ADDITION EXPERIMENTS

ALL SCIENTISTS

Experiment 1 – 11th to 14th May

Approximately 100 L seawater was collected from 25m (approx. 33% light level) on 10th May 2004 from a representative OUT station (27°41'N, 22°56'W). 5 x 23 L cubitainers (previously cleaned with 5% DECON, then 10% HCL) were filled for the following nutrient addition experiment;

Cubitainer 1: Control

Cubitainer 2: +Fe (3 nM addition)

Cubitainer 3: + P (100 nM addition) + Fe (3 nM addition)

Cubitainer 4: + Fe (3 nM addition) + P (100 nM addition) + NH₄⁺ (1.7 μM addition)

Cubitainer 5: + P (100 nM)

Following nutrient addition cubitainers were transferred to on-deck incubators which were light screened to 33% of incident irradiation and temperature controlled using surface seawater. Samples were collected at approximately 24h intervals from each of the 5 cubitainers over a period of 3 days for determination of rates of carbon fixation (¹⁴C method), phosphate assimilation (³³P method), bacterial production, nitrification, nitrogen fixation, O₂ respiration and analysis of: nutrients, AFC cell counts, chlorophyll, nutrients, Fe, DON/DOC, phyto- and microzooplanton.

Experiment 2 – 18th to 22nd May

A similar experiment as above but to investigate the impact of PO₄ and Fe additions on patch waters incubated in-vitro. 4 x 23l cubitainers were filled with seawater as identified below and incubated under the same conditions as for experiment 1.

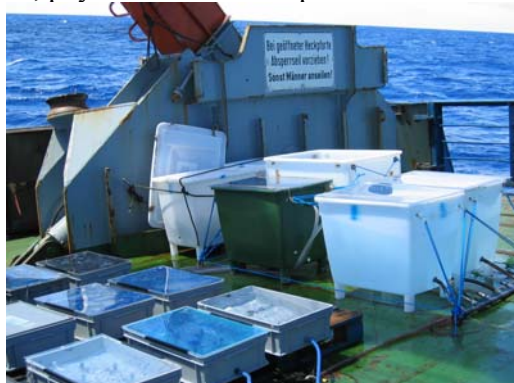
Cubitainer 2: Control (15m water from OUT station (27°33'N, 22°28'W) collected 1200 17/5/04)

Cubitainer 3: + P & Fe (15m water from Fe&P-fertilised patch collected 0530 18/05/04). Pre-experiment analysis ~160 nM PO₄

Cubitainer 4: + P (15m water from OUT station collected 1200 17/5/04) spiked with 400 μL PO₄ stock solution for target ~200 nM

Cubitainer 5: + Fe (15m water from Fe-fertilised patch collected 0530 16/5/04)

Samples were collected at approximately 24h intervals from each of the 5 cubitainers over a period of 3 days for determination of rates of carbon fixation (¹⁴C method), phosphate assimilation (³³P method), bacterial production, nitrification, nitrogen fixation, O₂ respiration and analysis of: nutrients, AFC cell counts, chlorophyll, nutrients, Fe, DON/DOC, phyto- and microzooplanton.



ZOOPLANKTON RESPONSE TO IRON AND PHOSPHATE ENRICHMENT IN THE SUB-TROPICAL ATLANTIC

DELPHINE BONNET

Objectives:

- To determine the mesozooplankton community structure and vertical distribution and migration in relation to phytoplankton patch development.
- To estimate an index of zooplankton secondary production with a new method (AARS-enzyme activity)
- To estimate mesozooplankton grazing – gut fluorescence technique, estimation of gut clearance rate.
- To estimate the contribution of micro- and phytoplankton to zooplankton diet (stable isotope) with a specific focus on *Pleuromamma* sp. diet (grazing experiments).

Method:

Zooplankton was collected three times per day at 6pm (day station), 12pm and 3am (night stations) with a vertical WP2 net (200µm mesh) to monitor several parameters.

Abundance and identification of mesozooplankton and vertical distribution:

Zooplankton collected from vertical tows (200m-surface) 3 times per day have been fixed in 4% formalin for identification and counts in the laboratory.

Closing nets were used twice a day (6pm and 12pm) to determine the vertical distribution of zooplankton between 200 and 50 m and 50 m and surface. Samples were fixed in 4% formalin for later counts and identification.

Biomass:

Three times a day, a cod end of a vertical net (200-0m) was subdivided in 4 size class (>2000µm, 2000-1000µm, 1000-500µm, 500-200µm) and each fraction filtered on pre combusted GF/F filters. Samples were then frozen at -20°C and will be processed using a Carbo Erba Carbon and Nitrogen analyser.

¹⁵N:

Twice a day (6pm and 12pm), a cod end of a vertical net (200-0m) was subdivided in 4 size class (>2000µm, 2000-1000µm, 1000-500µm, 500-200µm) and each fraction filtered on pre combusted GF/F filters. Samples were then frozen at -20°C and will be analysed for stable isotope measurement (¹⁵N).

Gut content/ gut evacuation experiment:

Gut content

Three times a day, a cod end of a vertical net (200-0m) was subdivided in 4 size class (>2000µm, 2000-1000µm, 1000-500µm, 500-200µm) and each fraction filtered on GF/F filters. Samples were then frozen at -20°C and will be processed using a fluorometer for gut content chlorophyll *a* determination.

Gut evacuation experiment

Occasionally on a day or night station, a gut evacuation rate experiment was carried out. The cod end of a vertical net (200-0m) was subdivided in 4 sizes class (>2000µm, 2000-1000µm, 1000-500µm, 500-200µm) and each of the fraction incubated in a 4 litre carboy filled with 0.2µm filtered sea water. Five, 10, 15, 20, 30 and 45 minutes after the start of the incubation, aliquots of 500ml were sampled for each size fraction and filtered on GF/F filters and frozen at -20°C. The samples will be processed using a fluorometer for gut content chlorophyll *a* measurement.

Enzyme activity:

Twice a day (6pm and 3am), a cod end of a vertical net (200-0m) was subdivided in 4 size class (>2000µm, 2000-1000µm, 1000-500µm, 500-200µm) and each fraction was placed in cryo-vials and frozen in liquid nitrogen. Samples will be analysed for protein content and AARS enzyme activity.

Grazing experiments:

From zooplankton collected at the 3am station, females of the most abundant large copepod species, *Pleuromamma* sp., were sorted and isolated in groups of three.

Twenty litres of water from the Chlorophyll maximum layer were collected from the 4am CTD cast. The water was pre-filtered on a 200µm mesh to avoid the presence of other large predators other than the ones introduced. Two T0 samples (200ml) of the water were fixed in Lugols iodine. Four controls bottles (1L without copepods) as well as 4 to 5 bottles containing each 3 *Pleuromamma* sp., were then incubated on a plankton wheel (with a low rotation: 1 rpm) at surface temperature (~21°C) in the dark. After 20h, the experiment was stopped, 200ml of each bottle fixed in 2% Lugols iodine and females of *Pleuromamma* sp., frozen in liquid nitrogen. Microzoo- and phytoplankton counts will be carried out in the laboratory.

Additional information:

The presence of the cyanobacteria *Trichodesmium* sp. was noticed in the plankton nets during the second enrichment experiment. *Trichodesmium* abundance was then determined from the vertical nets (0-50m) and colonies were sorted for nitrogen fixation experiments (Andy Rees and Cliff Law). Concentration of *Trichodesmium* during the first part of the cruise is going to be determined from the zooplankton formalin samples.

Samples Collected

Phosphate enrichment										
Date	Station	Lat	long	location from the patch	Formalin	gut	Gut clearance	CHN	Enzyme	stable isotopes
03/05/04	1	27°48.2	23°20.2		X	X		X	X	X
	2	27°48.6	23°20.2		X	X	X	X	X	X
04/05/04	3	27°48.6	23°20.2		X	X		X	X	X
	6	27°46.6	23°21.6		X	X	X	X	X	X
	7	27°46.4	23°21.6		X	X				
05/05/04	8	27°46.4	23°21.6	IN	X	X		X	X	X
	12	27°47.3	23°18.5	IN	X	X		X	X	X
	13	27°47.5	23°17.8	IN	X	X				
06/05/04	14	27°46.9	23°16.7	IN	X	X		X	X	X
	17	27°45.1	23°23.8	OUT	X	X		X	X	X
	18	27°45.1	23°23.7	OUT	X	X	X			
07/05/04	19	27°45.0	23°23.8	OUT	X	X		X	X	X
	22	27°44.8	23°13.8	IN	X	X	X	X	X	X
	23	27°41.9	23°16.3	IN	X	X				
08/05/04	24	27°41.9	23°15.8	IN	X	X		X	X	X
	27	27°29.7	23°13.3	OUT	X	X	X	X	X	X
	28	27°29.7	23°13.3	OUT	X	X				
09/05/04	29	27°30.4	23°13.1	OUT	X	X		X	X	X
	32	27°42.2	23°12.5	IN	X	X		X	X	X
	33	27°39.6	23°16.3	IN	X	X	X			
10/05/04	35	27°39.4	23°16.1	IN	X	X		X	X	X
14/05/04	40	27°37.0	23°16.3	IN?	X	X				X
15/05/04	41	27°37.0	23°16.2	IN?	X	X		X	X	X

Phosphate and Iron enrichment										
Date	Station	lat	long	location from the patch	Formalin	gut	Gut clearance	CHN	Enzy me	stable isotopes
15/05/04	44	27°32.3	22°34.4		X	X		X	X	X
	45	27°32.4	22°34.4		X	X				
16/05/04	46	27°31.8	22°34.1		X	X		X	X	X
	48	27°33.3	22°32.7	IN (Iron patch)	X	X		X	X	X
	49	27°33.4	22°30.9	IN	X	X	X			
17/05/04	50	27°33.7	22°30.2	IN	X	X		X	X	X
	53	27°34.4	22°27.0	IN (Iron + Phosphate patch)	X	X	X	X	X	X
	54	27°34.9	22°26.0	IN	X	X				
18/05/04	55	27°35.4	22°25.1	IN	X	X		X	X	X
	59	27°34.2	22°33.5	OUT	X	X		X	X	X
	60	27°34.4	22°33.5	OUT	X	X	X			
19/05/04	61	27°24.3	22°33.5	OUT	X	X		X	X	X
	64	27°29.7	22°13.3	IN	X	X		X	X	X
	65	27°29.7	22°13.3	IN?	X	X				
20/05/04	66	27°41.0	22°16.4	IN?	X	X		X	X	X
	70	27°39.0	22°23.6	OUT	X	X		X	X	X
	71	27°39.1	22°23.7	OUT	X	X	X	X	X	X
21/05/04	72	27°39.0	22°23.7	OUT	X	X		X	X	X
	76	27°41.4	22°10.6	IN	X	X		X	X	X
	77	27°41.5	22°10.1	IN	X	X		X	X	X
22/05/04	78	27°41.5	22°06.6	IN	X	X		X	X	X

AMMONIUM REGENERATION AND NITRIFICATION ESTIMATIONS DETERMINED BY GC-MS ANALYSIS

DARREN CLARK

Objectives:

- To determine the rate of ammonium regeneration at the 1 % and 55 % SPAR depths.
- To determine the rate of nitrification by measuring the rate of ammonium oxidation to nitrite, and the rate of nitrite oxidation to nitrate at the 1 % and 55 % sPAR depths.
- To determine rate of NO_3^- assimilation by the plankton assemblage and estimate the potential contribution that nitrification makes to this assimilation rate.

Method overview:

The method is based upon changes in isotopic enrichment of inorganic nitrogen resulting from regeneration/nitrification activity during deck incubations. Dissolved inorganic nitrogen in a sample is derivitised forming indophenol (in the case of ammonium) or the azo dye sudan-1 (in the case of nitrite/nitrate). Indophenol or sudan-1 is collected by solid phase extraction onto C18 cartridges. These cartridges are transported back to the lab for further processing and analysis by Gas Chromatography-Mass Spectrometry (GC-MS).

Method details:

The protocol was identical on a day to day basis. It was undertaken for two sets of eight days. 25 litres of seawater was collected from depths corresponding to 55 % and 1 % sPAR during each days pre-dawn cast. 5 litres of seawater was filtered using a GF/F filter and distributed into triplicate samples for the determination of ambient NH_4^+ , NO_2^- , NO_3^- concentrations as described below.

7 litres from each depth were spiked with 10 nM $^{15}\text{NH}_4\text{Cl}$ and used to fill a 2.2 L polycarbonate vessel which was placed in deck incubators with either 1 % or 55 % sPAR screens. The remaining volume was filtered and distributed between triplicate samples for the determination of pre-incubation enrichment of NH_4^+ . These incubations were identified as the 'Ammonium Oxidising Bacteria' incubations (AOB).

7 litres from each depth were spiked with 10 nM $\text{Na}^{15}\text{NO}_2$ and used to fill a 2.2 L polycarbonate vessel which was placed in deck incubators with either 1 % or 55 % sPAR screens. The remaining volume was filtered and distributed between triplicate samples for the determination of pre-incubation enrichment of NO_2^- . These incubations were identified as the 'Nitrite Oxidising Bacteria' incubations (NOB).

AOB and NOB vessels were incubated on deck for a period of approximately 24 hours. During this time, additional deck incubations were performed in which the rate of NO_3^- assimilation by phytoplankton was determined. 2.2 L of seawater from each depth was spiked with 10 nM $\text{Na}^{15}\text{NO}_3$ and placed in deck incubators for 6 hours. Incubations were terminated by the collection of PON onto GF/F filters. The filters were frozen until IRMS analysis in the lab could be performed.

Upon completion of AOB and NOB deck incubations the vessels were processed as follows. AOB vessels were filtered by GF/F. The filters were frozen and stored until IRMS analysis could be performed at the lab. The filtrate was split into two sets of triplicate samples for NH_4^+ and NO_2^- concentration and ^{15}N enrichment determinations. NOB vessels were filtered by GF/F and the filter was frozen and stored for IRMS

analysis at the lab. The filtrate was split into two sets of triplicate samples for NO_2^- and NO_3^- concentration and enrichment determinations.

Development of N-derivatives:

Samples and standards for the determination of dissolved inorganic ammonium was determined by the addition of 2 reagents. A phenolic/nitroprusside reagent followed by an oxidising reagent (sodium dichloroisocyanurate/NaOH) was added and indophenol allowed to develop. Once developed, indophenol was collected by solid phase extraction. The SPE cartridge was then stored at 4°C.

Samples and standards for dissolved inorganic nitrite analysis was determined by the addition of aniline sulphate in HCl followed by 2-naphthol in NaOH. Sudan-1 was allowed to develop and was collected by solid phase extraction. SPE cartridges were stored at 4°C. Samples for dissolved inorganic nitrate were first reduced to nitrite by the use of a cadmium column which operated at > 90 % efficiency for the entire cruise. Nitrite derived from nitrate was then derivitised as described above.

Summary of samples collected

	Sample taken	Measurement
Pre-incubation	NH_4^+ (SPE, GC-MS)	Ambient NH_4^+ concentration, natural abundance
Determinations	NO_2^- (SPE, GC-MS)	Ambient NO_2^- concentration, natural abundance
	NO_3^- (Cd reduction, SPE, GC-MS)	Ambient NO_3^- concentration, natural abundance
	PON (IRMS)	PON concentration and natural abundance
AOB incubations	Pre- and post incubation NH_4^+ (SPE, GC-MS)	Changes in NH_4^+ concentration and enrichment used to derive NH_4^+ regeneration rate.
	Post incubation NO_2^- (SPE, GC-MS)	Changes in NO_2^- concentration and enrichment used to derive NH_4^+ oxidation rate
	PON (IRMS)	24 hours averaged NH_4^+ assimilation rate
NOB incubations	Pre- and post incubation NO_2^- (SPE, GC-MS)	Changes in NO_2^- concentration and dilution used to derive NH_4^+ oxidation rate.
	Post incubation NO_3^- (SPE, GC-MS)	Changes in NO_3^- concentration and enrichment used to derive NO_2^- oxidation rate.
	PON (IRMS)	24 hours averaged NO_2^- assimilation rate
$^{15}\text{NO}_3^-$ incubations	PON (IRMS)	Used to estimate 6 hour averaged NO_3^- assimilation rates.



SIZE FRACTIONATED AMMONIUM REGENERATION AND RESPONSE OF MICROPLANKTON TO PHOSPHATE AND IRON ENRICHMENT

VICTORIA COLLINS

Objectives:

- Quantify the microphytoplankton, microzooplankton and heterotrophic nanoflagellates in terms of their abundance, biomass and community composition with depth, in the fertilized patches and out stations.
- Determine phytoplankton growth and mortality due to microzooplankton grazing, before and after the addition of phosphate and iron.
- Determine ammonium concentrations in the fertilized patches and out stations.
- Quantify how, by what mechanism and by whom, ammonium is being regenerated in and outside the fertilized patches.

Methodology:

Microplankton community structure

a) 500ml water samples were fixed with 2% (final conc.) acid lugol's solution and collected twice daily at the dawn CTD casts and afternoon casts, at the "in" and "out" station at 6 light depths: 97%, 55%, 33%, 20%, 7% and the Deep Chlorophyll Maximum (DCM). Samples will be analysed by Claire Widdicombe and Elaine Fileman at PML by settlement microscopy.

b) 250ml water samples were fixed in 0.5% glutaraldehyde, dual stained with DAPI and proflavine (at a final concentration of 5µg ml⁻¹), filtered onto 0.8µm black polycarbonate filters and mounted onto glass slides and frozen. These samples will be analysed by Elaine Fileman at PML using epifluorescence microscopy (EFM).

Microzooplankton grazing experiments (MZG)

The dilution technique of Landry and Hassett (Mar. Biol. 67:283-288, 1982) was used to quantify phytoplankton growth responses and mortality to the phosphate and iron fertilization, through grazing. Each herbivory experiment consisted of four dilutions levels (0.1, 0.4, 0.7 and 1.0 concentrations of ambient seawater), set up in triplicate and at 1.0 conc. of ambient seawater an extra duplicate set were spiked with 1mM ammonium chloride to monitor any possible nitrogen limitation.

Experimental water was collected at pre-dawn from the depth corresponding to the 33% light level on alternate days in the phosphate and iron patch. Approximately 30 litres were filtered through a 0.2µm capsule filter (acid-washed and pre-washed in milliQ) and combined, in three 10 litre and one 20 litre carboy, with the unfiltered seawater (sieved through a 200µm mesh bag to remove mesozooplankton) to make the four dilutions. These dilutions were transferred into 2.3 litre polycarbonate bottles and incubated on-deck in ambient temperature and 33 % light level for 24 hours. Sub-samples collected at T₀ and T₂₄ hours for chlorophyll *a*, phyto- and microzooplankton community composition (as described above) and flow cytometry (AFC). Chlorophyll *a* samples were filtered onto 0.2µm, 47mm polycarbonate filters and frozen for subsequent extraction with 90% acetone and analysis by fluorometry at PML by Elaine Fileman. The preserved samples were stored under cool, dark conditions until analysis in the laboratory by Elaine Fileman. For flow cytometry analysis at PML, 1.8ml of sub-sample was fixed in 90µl of 20% paraformaldehyde in cryovials and refrigerated for 24 hours and then placed in the -20 freezer (ideally -80).

Ammonium analysis

Water samples were collected at the dawn CTD cast, mid-morning cast and the afternoon cast at the following light level depths: 97%, 55%, 33%, 20%, 7%, 1%, Deep Chlorophyll Maximum (DCM) and 20m below the Deep Chlorophyll Maximum (BDCM). Ammonium samples were collected at both stations in and outside the phosphate patch. These samples were filtered through a 0.2µm capsule filter upon collection and analysed onboard using Holmes *et. al.* 1999 manual fluorescence analysis method for ammonium.

Size fractionated ammonium regeneration (SFAR)

The community was size fractionated to separate trophic levels and to determine who was regenerating ammonium.

Approximately 20 litres of experimental water was collected at pre-dawn from the depth corresponding to the 33% light level on alternate days in the phosphate and iron patch. There were four size fractions: >200µm, <200µm, <20µm, <1µm. For each size fraction 4.5 litres of experimental water was filtered using the following: >200 and <200µm were filtered through a 200µm mesh bag; <20µm was filtered through a 20µm mesh and <1µm were filtered through a 1µm capsule filter. Sub-samples were taken for chlorophyll *a*, microplankton, heterotrophic nanoflagellates and flow cytometry from each size fraction to determine the collected community in each fraction (as described above). ¹⁵N-NH₄ was added at ~10% of ambient concentration to each 4.5 litre polycarbonate bottle and then each fraction was siphoned into triplicate 0.64 litre polycarbonate bottles. For the >200µm size fraction, 10 medium size mixed species copepods (picked out by Delphine Bonnet) were added to each >200µm 0.64 litre bottle, to create a controlled total fraction. These size fractions were incubated on-deck at ambient temperature and 33% light level for 10 hours. From the remainder of the T₀ experimental water from each size fraction, triplicate 500ml samples were filtered through a 0.7µm GFF 47mm filter into 500ml clean amber bottles for T₀ NH₄ analysis using solid phase extraction and the GFF filters were frozen for PON analysis. After 10 hours the sub-samples for flow cytometry were collected from the <20µm and <1µm size fractions. For the >200µm size fraction, each replicate sample was sieved through a 200µm mesh to collect the copepods (which were frozen for analysis later in the laboratory); prior to filtration on 0.7µm GFF filters. Also at T₁₀ each replicate for each size fraction was filtered through 0.7µm GFF filter into a clean 500ml medicine bottle for T₁₀ NH₄ analysis by solid phase extraction and each filtered was frozen for PON analysis. All of the samples collected during this experiment will be analysed at PML.

Deck incubations:

Deck incubations 1

Experimental water for the deck incubation was collected from an out station at 25m (33% light level), where samples for ammonium analysis and microplankton were also collected. Experimental water was collected for five different enrichment experiments: control; addition of iron only; phosphate and iron; phosphate, iron and ammonium and phosphate only. One cube container was used for each enrichment and incubated on-deck at ambient temperature and at 33% light level. Sub-samples from each container were taken at T₀, T₁₂, T₃₆, and T₆₀ for 3 * 50ml per container for ammonium concentrations, which were analysed onboard and 2* 250ml fixed in 2% acid lugol's solution for microplankton, which will be analysed at PML. Other variables were collected at these time intervals (refer to Andy Rees for more details of the incubation experiments).

Deck incubations 2

Another set of similar deck incubation were set up by Cliff Law, with 4 enrichments: control, iron, phosphate and iron and phosphate. 1*500ml sample for microplankton was collected from each enrichment and fixed as described above. These samples will be analysed at PML. For further experimental details refer to Cliff Law.

Sampling log

Sampling event				Light depths sampled	
Patch 1+ PO ₄	Date	CTD	Microplankton	EFM	NH ₄
IN @ T0	03.05.04	4	55	55	
IN @ T0	03.05.04	5	97, 33	97, 33	
IN @ T0	03.05.04	6	20, 7	20, 7	
IN @ T0	03.05.04	7	DCM	DCM	
IN @ T0	04.05.04	9	55	55	55
IN @ T0	04.05.04	10	97, 33	97, 33	97, 33
IN @ T0	04.05.04	11	20, 7	20, 7	20, 7
IN @ T0	04.05.04	12	DCM	DCM	1, DCM, BDCM
IN	04.05.04	14			97, 55, 33, 20, 7, 1, DCM, BDCM
IN	05.05.04	15	55	55	55
IN	05.05.04	16	97, 33	97, 33	97, 33
IN	05.05.04	17	20, 7	20, 7	20, 7
IN	05.05.04	18	DCM	DCM	1, DCM, BDCM
IN	05.05.04	19	97, 55, 33, 20, 7, DCM	97, 55, 33, 20, 7, DCM	97, 55, 33, 20, 7, 1, DCM, BDCM
IN	06.05.04	21	55	55	55
IN	06.05.04	22	97, 33	97, 33	97, 33
IN	06.05.04	23	20, 7	20, 7	20, 7
IN	06.05.04	24	DCM	DCM	1, DCM, BDCM
IN	06.05.04	26			97, 33, 7, BDCM
OUT	06.05.04	27	97, 55, 33, 20, 7, DCM	97, 55, 33, 20, 7, DCM	97, 33, 7, BDCM
OUT	07.05.04	33	97, 55, 33, 20, 7, DCM		
IN	07.05.04	35	97, 55, 33, 20, 7, DCM	97, 55, 33, 20, 7, DCM	97, 55, 33, 7, BDCM
IN	08.05.04	37	55	55	
IN	08.05.04	38	33	33	
IN	08.05.04	39	20, 7	20, 7	
IN	08.05.04	40	DCM, 97	DCM, 97	
OUT	08.05.04	43	97, 55, 33, 20, 7, DCM		
OUT	09.05.04	45	55	55	55
OUT	09.05.04	46	97, 33	97, 33	97, 33
OUT	09.05.04	47	20, 7	20, 7	20, 7
OUT	09.05.04	48	DCM	DCM	1, DCM, BDCM
OUT	09.05.04	49			97, 55, 33, 7, BDCM

Sampling event				Light depths sampled	
Patch 1+ PO₄	Date	CTD	Microplankton	EFM	NH₄
IN	09.05.04	51	97, 55, 33, 20, 7, DCM	97, 55, 33, 20, 7, DCM	97, 55, 33, 7, BDCM
IN	10.05.04	54	55	55	55
IN	10.05.04	55	97, 33	97, 33	97, 33
IN	10.05.04	56	20, 7	20, 7	20, 7
IN	10.05.04	57	DCM	DCM	1, DCM, BDCM
IN	15.05.04	63	55	55	55
IN	15.05.04	64	97, 33	97, 33	97, 33
IN	15.05.04	65	20, 7	20, 7	20, 7
IN	15.05.04	66	DCM	DCM	1, DCM, BDCM
IN	15.05.04	67			97, 55, 33, 20, 7, 1, DCM, BDCM
OUT	15.05.04	69			97, 55, 33, 7, BDCM

Sampling event			Light depths sampled	Incubation experiments	
Patch 2 + Fe, PO₄	Date	CTD	Microplankton	MZG	SFAR
IN @ To	16.05.04	70		33	
IN @ To	16.05.04	71	55		
IN @ To	16.05.04	72	97, 33		33
IN @ To	16.05.04	73	20, 7		
IN @ To	16.05.04	74	DCM		
IN + Fe	16.05.04	75	97, 55, 33, 20, 7, DCM		
IN + Fe	17.05.04	76	55		
IN + Fe	17.05.04	77	97, 33		
IN + Fe	17.05.04	78	20, 7		
IN + Fe	17.05.04	79	DCM		
OUT	17.05.04	?	97, 55, 33, 20, 7, DCM		
IN + PO ₄	18.05.04	87		33	
IN + PO ₄	18.05.04	88	55		
IN + PO ₄	18.05.04	89	97, 33		33
IN + PO ₄	18.05.04	90	20, 7		
IN + PO ₄	18.05.04	91	DCM		
OUT	18.05.04	94	97, 55, 33, 20, 7, DCM		
OUT	19.05.04	96	55		
OUT	19.05.04	97	97, 33		
OUT	19.05.04	98	20, 7		
OUT	19.05.04	99	DCM		
IN	19.05.04	102	97, 55, 33, 20, 7, DCM		
IN	20.05.04	?		33	
IN	20.05.04	104	55		
IN	20.05.04	105	97, 33		33
IN	20.05.04	106	20, 7		
IN	20.05.04	107	DCM		
OUT	20.05.04	111	97, 55, 33, 20, 7, DCM		
OUT	21.05.04	113	55		
OUT	21.05.04	114	97, 33		
OUT	21.05.04	115	20, 7		
OUT	21.05.04	116	DCM		
IN	21.05.04	120	97, 55, 33, 20, 7, DCM		

Sampling event			Light depths sampled	Incubation experiments	
Patch 2 + Fe, PO₄	Date	CTD	Microplankton	MZG	SFAR
IN	22.05.04	122		33	
IN	22.05.04	123	55		
IN	22.05.04	124	97, 33		33
IN	22.05.04	125	20, 7		
IN	22.05.04	126	DCM		



CARBON FIXATION, PHOSPHATE ASSIMILATION AND BACTERIAL PRODUCTION: RESPONSE TO IRON AND PHOSPHATE LARGE SCALE ENRICHMENTS IN THE OLIGOTROPHIC SUB-TROPICAL NORTH ATLANTIC

JOANNA DIXON, ANDREW REES

Objectives:

- To determine the impact of phosphate and iron limitation on rates of carbon fixation
- To determine the impact of phosphate and iron limitation on rates of phosphate assimilation
- To determine the impact of phosphate and iron limitation on rates of bacterial production

Relevance to PML core aims:

These objectives contribute to the following PML Core Strategic Research Programme:

- Aim 5.1 To determine how microbial community structure influences the photosynthesis/respiration ratio
- Aim 6.1 To quantify key nitrogen fluxes within the plankton community in order to refine estimates of oceanic carbon uptake.
- Aim 6.2 To investigate how macro- and micro-nutrient limitation (e.g. iron, nitrogen, phosphorus and silicate) influences the ratio of new to total production.

Methods:

The daily sampling routine on Poseidon essentially consisted of CTD casts at 04:00 local time (pre-dawn), - 10:00 am local time and - 14:00 pm local time. Everyday we aimed to sample both in and out of the 'fertilised' patch e.g. if the pre-dawn and 10:00 am casts were in the patch, then the 14:00 cast would tend to be outside the patch etc. Water was collected from the pre-dawn casts from depths equivalent to 97%, 55%, 33%, 20%, 7% and 1% of surface irradiance (c. 1m, 15m, 26m, 39m, 64m & 110m) and incubated in surface water cooled deck incubators for 24 hours.

Carbon fixation

Rates of carbon fixation were estimated from the incorporation of ^{14}C -bicarbonate. Approximately 60 ml aliquots of seawater samples in polycarbonate bottles (3 x colourless & 1 x black) were spiked with 10 μCi $\text{NaH}^{14}\text{CO}_3$ and incubated in the on deck system. Incubations were terminated after 24 hr by sequential filtration through 2.0 and 0.2 μm polycarbonate filters. The filters were subsequently placed in a desiccator with fuming HCl for 10 minutes before being dried and stored in a desiccator overnight prior to measurement in a liquid scintillation counter.

Phosphate assimilation

Incorporation of ^{33}P labelled orthophosphate was determined following procedures similar to those used for ^{14}C uptake measurements. As above 60 ml water samples were inoculated with 1 μCi ^{33}P orthophosphate and incubated on deck for 24 hr. The samples were then sequentially filtered through 2.0 and 0.2 μm polycarbonate filters that had been boiled in 0.5 M lithium chloride (made up in 1 mM phosphate buffer, pH 9.0). After filtration the filters were washed twice with 10 ml lithium chloride/phosphate buffer to minimise any surface adsorption of phosphate. The filters were dried and stored in a desiccator before counting in a liquid scintillation counter.

Bacterial production

Incorporation of L-[4,5-³H]Leucine into bacterial protein in seawater samples was determined following the method of Smith and Azam 1992.¹ 1.7 ml seawater samples were inoculated with 25 nM ³H Leucine (7 µl) (as determined by a Vmax experiment carried out on 01/05/04) and incubated in the dark at in situ temperature for 1 hr. Samples were terminated with 100 µl TCA (5% final concentration) and incorporated ³H extracted following procedures outlined in Smith & Azam 1992 before being measured by liquid scintillation counting.

Bacterial Numbers

Between 5-10 ml seawater was fixed with Glutaraldehyde (2.5% final concentration) and subsequently DAPI stained and filtered through 0.2 µm black polycarbonate filters. The filters were mounted onto slides and frozen for epifluorescent counting back in the laboratory.

Chlorophyll a

Between 100-200 ml seawater was filtered through 0.2 µm polycarbonate filters and placed in acetone resistant centrifuge tubes. The filters were subsequently frozen for later analysis back in the laboratory.

On a limited number of occasions, samples were also collected from the depth of the chlorophyll maximum and from 20m below the chlorophyll maximum for bacterial production. However these samples were incubated for 1 hr at sea surface temperature (as only 1 incubator) which was often between 2-4°C higher than the in situ temperature therefore I suggest caution when interpreting these results!

Water from light equivalent depths of 55% and 33% was collected from the 10:00 am and 14:00 pm casts and incubated at sea surface temperature at approximately *in situ* light conditions (in an incubator fitted with a calibrated light source) for c. 2-4 hrs for determination of rates of carbon fixation (¹⁴C method) and phosphate assimilation (³³P method). Rates of bacterial production were also determined for these samples.

Full list of samples collected

Date	Time	Station	Samples collected
Mon 3 May 04	04:00	CTDs 04-07	97%, 55%, 33%, 20%, 7%, 1% (Prod #1) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production Bacterial numbers*
3 May 04	12:30	CTD 08	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Tue 4 May 04	04:00	CTDs 09-12	97%, 55%, 33%, 20%, 7%, 1% (Prod #2) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production Bacterial numbers*
4 May 04	12:00	CTD 14	55%, 33% Carbon fixation (+/- fuming HCl test) Phosphate assimilation

¹ Smith, D.C and Azam, F. 1992 Marine Microbial Food webs 6(2): 107-114.

Date	Time	Station	Samples collected
4 May 04	12:00	CTD 14	Bacterial production 1000 m bacterial production ¹
Wed 5 May 04	04:00	CTD 15-18 IN PO ₄ patch ~ 1 hr late in getting to patch centre	97%, 55%, 33%, 20%, 7%, 1% (Prod #3) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production (+ Chl max & -20 m below chl max) Bacterial numbers*
5 May 04	12:00	CTD 20 IN PO ₄ patch Not enough water from mid day cast (CTD 19)	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Thur 6 May 04	04:00	CTDs 21-24 IN PO ₄ patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #4) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production (+ Chl max & -20 m below chl max) Bacterial numbers*
6 May 04	10:30	CTD 26 IN PO ₄ patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
6 May 04	14:30	CTD 27 OUT patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Fri 7 May 04	04:00	CTDs 29-33 OUT patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #5) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production (+ Chl max & -20 m below chl max) Bacterial numbers*
7 May 04	10:30	CTD 35 OUT patch Took water late!	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
7 May 04	15:30	CTD 36 IN PO ₄ patch	97%, 55%, 33%, 20%, 1% Carbon fixation Phosphate assimilation Bacterial production
Sat 8 May 04	04:00	CTDs 37-40 IN PO ₄ patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #6) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production (+ Chl max & -20 m below chl max)
8 May 04	12:00	CTD 42 IN PO ₄ patch	55%, 33% Carbon fixation

Date	Time	Station	Samples collected
8 May 04	12:00	CTD 42 IN PO ₄ patch	Phosphate assimilation Bacterial production
8 May 04	15:00	CTD 43 OUT patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Sun 9 May 04	04:00	CTDs 45-48 OUT patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #7) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production (+ Chl max & -20 m below chl max)
9 May 04	10:00	CTD 50 OUT patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
9 May 04	14:00	CTD 51 IN patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Mon 10 May 04	04:00	CTDs 54-57 IN PO ₄ patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #8) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
10 May 04		Steam back to Santa Cruz	Collected OUT water for large volume nutrient addition experiments
Tue 11 May 04	19:30	Cubitainer Exp 1 (T ₀) (5 treatments)	Carbon fixation Phosphate assimilation Bacterial production AFC, chlorophyll a & Total Fe (cubitainers 1-4)
Wed 12 May 04	09:00	Cubitainer Exp 1 (T ₁) (5 treatments)	Carbon fixation Phosphate assimilation Bacterial production AFC & chlorophyll a
Thur 13 May 04	10:00	Cubitainer Exp 1 (T ₂) (5 treatments)	Carbon fixation Phosphate assimilation Bacterial production AFC, chlorophyll a & Total Fe (cubitainers 1-4)
Fri 14 May 04	10:00	Cubitainer Exp 1 (T ₃) (5 treatments)	Carbon fixation Phosphate assimilation Bacterial production AFC, chlorophyll a & Total Fe (cubitainers 1-4)
Sat 15 May 04	04:00	CTDs 63-66 ALMOST IN OLD PO ₄ patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #9) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
15 May 04	10:00	CTD 67 IN patch	55%, 33% Carbon fixation

Date	Time	Station	Samples collected
15 May 04	10:00	CTD 67 IN patch	Phosphate assimilation Bacterial production
15 May 04	15:00	CTD 68 OUT patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Sun 16 May 04	04:00	CTDs 71-74 OUT patch Before 2 nd patch start	97%, 55%, 33%, 20%, 7%, 1% (Prod #10) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
16 May 04	16:30	CTD 75 IN Fe patch 1-2 hrs after Fe start	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Mon 17 May 04	04:00	CTDs 77-80 IN Fe patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #11) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
17 May 04	10:00	CTD 83 OUT patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
17 May 04	14:00	CTD 85 IN Fe patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
17 May 04	16:00		Darwin started to deploy PO ₄
Tue 18 May 04	04:00	CTDs 88-91 IN Fe+PO ₄ patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #12) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
18 May 04	10:00	CTD 92 IN Fe+PO ₄ patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
18 May 04	12:00	Cubitanor Exp 2 (T ₁) (4 treatments)	Carbon fixation (Light reps only) Bacterial production AFC & chlorophyll a
18 May 04	14:00	CTD 94 OUT patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Wed 19 May 04	04:00	CTDs 96-99 OUT patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #13) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
19 May 04	10:00	CTD 100 OUT patch	55%, 33% Carbon fixation Phosphate assimilation

Date	Time	Station	Samples collected
19 May 04	10:00	CTD 100 OUT patch	Bacterial production
19 May 04	12:00	Cubitanor Exp 2 (T ₂) (4 treatments)	Carbon fixation (Light reps only) Bacterial production AFC & chlorophyll a
19 May 04	14:00	CTD 102 IN Fe+PO ₄ patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Thur 20 May 04	04:00	CTDs 105-108 IN Fe+PO ₄ patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #14) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
20 May 04	12:00	Cubitanor Exp 2 (T ₃) (4 treatments)	Carbon fixation (Light reps only) Bacterial production AFC & chlorophyll a
Fri 21 May 04	04:00	CTDs 113-116 OUT patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #15) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
21 May 04	10:00	CTD 118 OUT	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
21 May 04	12:00	Cubitanor Exp 2 (T ₄) (4 treatments)	Carbon fixation (Light reps only) Bacterial production AFC & chlorophyll a
21 May 04	16:45	CTD 120 IN Fe+PO ₄ patch	55%, 33% Carbon fixation Phosphate assimilation Bacterial production
Sat 22 May 04	04:00	CTDs 123-126 IN Fe+PO ₄ patch	97%, 55%, 33%, 20%, 7%, 1% (Prod #16) Carbon fixation (+T ₀ & Carbosorb samples) Phosphate assimilation (+T ₀ & spike samples) Bacterial production
22 May 04	12:00	Cubitanor Exp 2 (T ₅) (4 treatments)	Carbon fixation (Light reps only) Bacterial production AFC & chlorophyll a

*DAPI stained slide preparations were frozen for bacterial enumeration. These were unfortunately only completed for the first couple of stations due to lack of time! ¹*In situ* temperature 8°C (SST 21°C). Nearest I could get to *in situ* was fridge incubation temperature of 4°C!

INFLUENCE OF ZINC AND COBALT ENRICHMENTS ON PHYTOPLANKTON GROWTH AND BACTERIOPLANKTON ECOLOGY

JOANNA DIXON

Objectives:

- To determine the influence of zinc enrichment, both in and out of the iron and phosphate enriched patches, on phytoplankton growth and bacterioplankton dynamics
- To determine the influence of cobalt enrichment, both in and out of the iron and phosphate enriched patches, on phytoplankton growth and bacterioplankton dynamics; including numbers of cyanobacteria

Relevance to PML core aims:

- Aim 6.1 To quantify key nitrogen fluxes within the plankton community in order to refine estimates of oceanic carbon uptake.
- Aim 6.2 To investigate how macro- and micro-nutrient limitation (e.g. iron, nitrogen, phosphorus and silicate) influences the ratio of new to total production
- Aim 6.3 To determine the fate of phytoplankton production through respiration, re-mineralisation and nutrient regeneration in the water column and within aggregated particles.

Methods:

Water samples were taken from the pre-dawn casts from depths equivalent to 97%, 55%, 33%, 20%, 7% and 1% of surface irradiance (c. 1m, 15m, 26m, 39m, 64m & 110m) and incubated in surface water cooled deck incubators for 4 days. For each depth 3 x 500 ml acid washed polycarbonate bottles were filled with seawater and treated as follows:

1. Control
2. + Cobalt(II) (added as Cobalt(II) chloride hexahydrate 99.999%) (5nM final concentration)
3. + Zinc(II) (added as Zinc(II) nitrate c(HNO₃=0.5M) SpectrosoL standard) (10 nM final concentration). Because of space limitations in the on deck incubators Zn additions were only carried out at selected depths.

before being placed in the incubators. After 4 days incubation the bottles were removed from the incubators and sub-samples were taken for the determination of the following parameters:

1. Rates of carbon fixation (¹⁴C method)
2. Rates of bacterial production (L-[4,5-³H]Leucine method)
3. Concentration of chlorophyll a
4. AFC samples
5. Nutrient concentration
6. Cyanobacteria biomass (via slide preparations and freezing)

For detailed descriptions of the methods used please refer to cruise report by Dixon and Rees titled 'Carbon fixation, phosphate assimilations & bacterial production...'

Cobalt and zinc addition experiments were carried out **IN** the **PO₄** only patch, **OUT** of any patches and **IN** the **Fe + PO₄** patch in order to examine any co-limitation between Fe and PO₄ with cobalt and zinc. During metal incubation #3 (**IN** the Fe + PO₄) water

samples were only incubated at four depths (97%, 55%, 33% and 20%) for cobalt, control and zinc treatments.

Event Log

Date	Time	Station	Samples collected
Wed 5 May 04	04:00	CTDs 15-18 IN PO₄ patch Metal inc#1	97%, 55%, 33%, 20%, 7%, 1% (Prod #1 for T₀ data) Control, cobalt and zinc (55% & 33% only) incubations started
Sat 8 May 04	21:00 (dusk)	Metal inc#1	Metal inc#1 bottles removed from on deck incubators and placed in laboratory incubator (in the dark at sea surface temperature) ready for sub-sampling pre dawn.
Sun 9 May 04	06:00	Metal inc#1	97%, 55%, 33%, 20%, 7%, 1% Carbon fixation (¹⁴ C 60 ml samples back in the on deck incubators) Bacterial production, nutrients, chlorophyll a, AFC samples & cyanobacterial slide preparations.
Sun 16 May 04	04:00	CTDs 71-74 OUT patch (just before Fe deployment started) Metal inc#2	97%, 55%, 33%, 20%, 7%, 1% (Prod #10 for T₀ data) Control, cobalt and zinc (55% & 33% only) incubations started
Tue 18 May 04	04:00	CTDs 88-91 IN Fe+PO₄ patch Metal inc#3	97%, 55%, 33%, 20% (Prod #12 for T₀ data) Control, cobalt and zinc incubations started
Wed 19 May 04	21:00 (dusk)	Metal inc#2	Metal inc#2 bottles removed from on deck incubators and placed in laboratory incubator (in the dark at sea surface temperature) ready for sub-sampling pre dawn.
Thu 20 May 04	06:00	Metal inc#2	97%, 55%, 33%, 20%, 7%, 1% Carbon fixation (¹⁴ C 60 ml samples back in the on deck incubators) Bacterial production, nutrients, chlorophyll a, AFC samples & cyanobacterial slide preparations.
Fri 21 May 04	21:00 (dusk)	Metal inc#3	Metal inc#3 bottles removed from on deck incubators and placed in laboratory incubator (in the dark at sea surface temperature) ready from sub-sampling pre dawn.
Sat 22 May 04	06:00	Metal inc#3	97%, 55%, 33%, 20% Carbon fixation (¹⁴ C 60 ml samples back in the on deck incubators) Bacterial production, nutrients, chlorophyll a, AFC samples & cyanobacterial slide preparations.

COLLECTION OF AEROSOL SAMPLES FOR PARTICULATE Fe

JOANNA DIXON

Objective:

Aerosol samples were collected by a high volume air sampler provided by the University of East Anglia. These particulate samples were collected to determine and monitor the input of principally particulate aerosol deposition of Fe; particularly in case of a major input of Sahara dust.

Unfortunately due to a number of recurring problems with the aerosol sampler only 7 filter samples (& 1 exposure blank) were collected (each with a collection time of ~24 hrs). The filters were unloaded from the cassettes in a clean large volume plastic bag, using only plastic forceps, and placed folded into a pre-labelled plastic bag before being frozen (ensuring that each filter was at least double bagged in the freezer to try and minimise contamination).

Date On	Time On	Location start	Location end	Filter No	Comments
30/04/04	24.3h	28:00.218 -18:49.865	27:47.734 -22:33.58	Exposure blank Pos04TM.EB	Counter off for exposure blank. Calm, blue skies. Light rain at 14:30, weather deteriorating.
01/05/04	24.2	27:47.900 -22:37.300	27:54.917 -23:30.31	FEEP2 Pos04TM.01	Grid survey, sampler situated well forward of stack. No visual evidence of contamination. Filter removed when ship hove to. Brief spell of heavy rain.
02/05/04	21.7	27:48.633 -23:20.517	27:48.824 -23:20.583	FEEP3 Pos04TM.02	On station a lot of time with steaming in a box survey.
03/05/04	23.75	27:49.573 -21:27.855	27:47.181 -23:22.200	FEEP4 Pos04TM.03	Started to steam until on station at ~02:00 hrs. Blue sky, patchy cloud. Slight ocean swell. Darwin started deploying PO ₄ at 21:00 hrs (powder mixed on deck!).
04/05/04	23.0	27:47.375 -23:22.23	27:47.475 -23:18.724	FEEP5 Pos04TM.04	Sampler stopped working during collection.
<p>Ships electrician worked on the aerosol sampler and managed to get the aerosol sampler to work again but he had to by pass the on/off switch so now sampler is permanently on & slightly modified!</p> <p>Sampler working again on 07/05/04 but left it running without filter or cassette for ~ 24hr to try and flush through any workshop contamination. Unit thoroughly brushed off and checked for visual dirt etc. The 'flow rate' now seems greatly increased (as does the noise the unit is making!) so in retrospect pump sounded at best intermittent and query whether it had previously been working correctly!</p>					
08/05/04	45.0	27:36.660 -23:13.772	27:39.208 -23:13.970	FEEP6 Pos04TM.05	Sampler can only be turned on/off via the mains switch. Not turned off to change filter.
10/05/04	26.5	27:40.366 -23:07.091	28:05.347 -19:48.109	FEEP7 Pos04TM.06	On passage back to Santa Cruz Tenerife after PO ₄ release.

Date On	Time On	Location start	Location End	Filter No	Comments
Did not put a filter back on for ~48 hours as too close to Canary Islands and too far from sampling region.					
13/05/04	43.0	28:05.569 -19:46.511	27:36.945 -23:06.277	FEEP8 PosTM.07	On passage back to sampling region to rendez vous with Darwin. Working close to Darwin; filter very discoloured (black) therefore query contamination from either our stack or from Darwin!
15/05/04	18.92	27:36.918 -23:02.217	27:32.807 -22:28.316	FEEP9 Pos04TM.08	Aerosol sampler not working when went to change filter – not sure when it stopped! However, another highly discoloured (black) filter; not sure reason? Brushes totally worn – no spares
The end of the aerosol sampling days!					

CTD OPERATIONS & SHIP'S INSTRUMENT SYSTEMS

CHRIS GALLIENNE

Introduction:

Due to operational requirements, no instrument technician was aboard the Poseidon311 cruise. Chris Gallienne therefore joined the vessel two days early in order for the ship's instrument technician to familiarise him with CTD systems before leaving the vessel. This familiarisation was less than adequate, given that the ship's computer networking systems were down, and the technicians had to devote a considerable proportion of these two days to trying to solve this problem. Some rudimentary training was obtained, but the technicians were forced to leave the ship without a working network system.

CTD Equipment:

We originally specified 24-bottle CTD rosette, which was commissioned by the ship's instrument technician before he left the vessel. We were then informed that it would not be deployable in all but the calmest weather. We therefore made a last-minute switch to the 12-bottle rosette, necessitating the exchange of CTD assemblies between rosettes in order to have the right combination of instruments. Fortunately, this exchange was completed without significant problems.

CTD operations during Poseidon311 were therefore conducted using the ship's 12-bottle rosette fitted with a Seabird 911 CTD unit, including a Dr Haardt fluorimeter and Beckman/YSI dissolved oxygen probe. Salinity and chlorophyll 'a' calibrations were to be performed after the cruise using salinity and HPLC samples taken periodically from CTD casts for this purpose.

Ship's navigation & underway data:

The ship's technician set up the ship's logging system before leaving the vessel. This system was not touched throughout the cruise, navigation and temperature/salinity data being read from the monitor as required.

Ship's ADCP data:

The hull-mounted ADCP was set up by the ship's technician, and was left to log as set up throughout the first part of the cruise. Upon meeting with RRS Charles Darwin, R. Torres transferred across and set up the ADCP parameters to match those on board Darwin. Subsequently the ADCP was left to run and log data automatically, with a daily visual check that data was available and being logged.

Drifting marker buoy:

Drifting marker buoys were deployed from RRS Charles Darwin as required to mark the fertilised patch. Receiving equipment to enable tracking of these buoys using the transmitted GPS signal was set up aboard FS Poseidon, and during periods when Darwin was out of range of the signal, and at the request of Darwin, data from these buoys was logged on board Poseidon.

Data Archiving:

All data was recovered from the above systems at the end of the cruise. This proved to be problematic, as networking was not working aboard the ship during Poseidon311, except on the bridge. Given the volume of data involved, none of the host systems had suitable means of downloading data. It was therefore necessary to connect a parallel-port zip drive to each system in turn to download data piecemeal, and store it on a laptop computer. Given language difficulties, and unfamiliarity with the ship's systems,

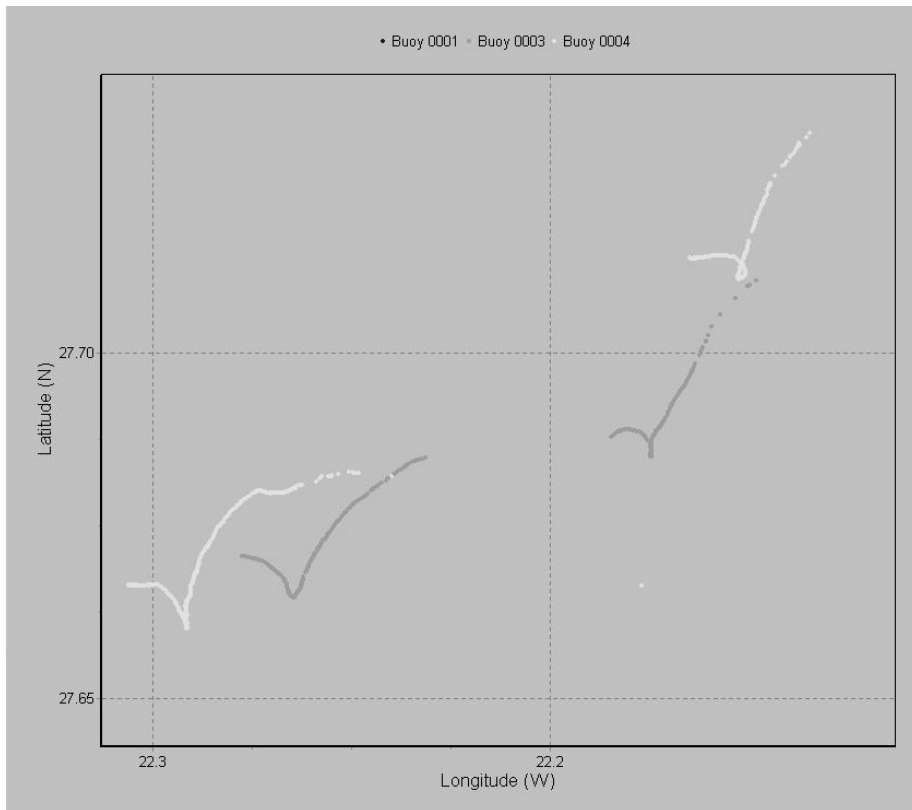
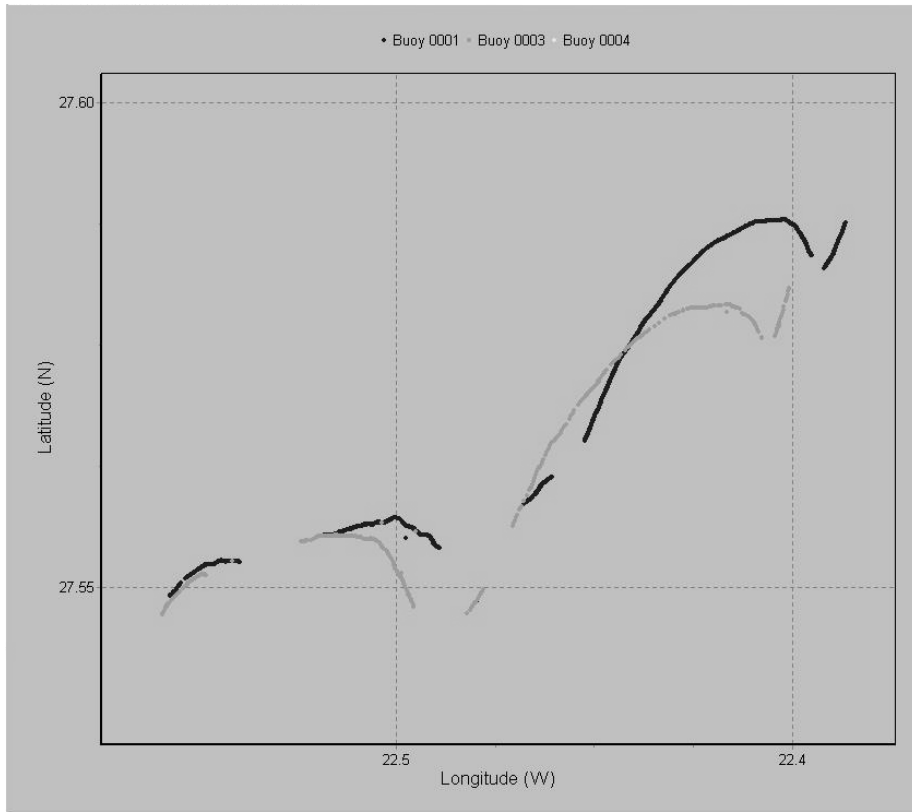
it was not always straightforward to find the relevant data archives, which has caused some problems in subsequent data retrieval.

- **CTD data** - all CTD data were recovered from the host system aboard Poseidon and subsequently archived by C. Gallienne. Raw data, converted data and ascii data for each of casts 1 to 126 were archived at PML, and have been sent to BODC for further processing and archiving.
- **Ship's underway data** – all underway data that could be located on the host system were downloaded and archived. These were raw data as produced by the ship's logging system. The program files necessary to process these files were also therefore downloaded with the data.

Subsequently it was found that these programs would not run. Some rather painful translation of the German help files, together with some internet research, indicated that the main processing program, 'xlog.exe' was a DOS program which required 'true DOS' to run (i.e. not just a command line from within Windows. Furthermore a bug in the program meant that it would not run on any processor faster than about 250MHz – i.e. no computer at PML. A patch was found to be available for this problem, and applied to the program. For these reasons, however, it was decided to process these files at PML and send to BODC only the ascii output from this processing.

- **Ship's ADCP data** – It was found that no data were available from the ADCP between 2nd And 18th May 2004. Whether this was due to equipment failure, or to intervention in the parameter setup on the 2nd, leading to storage of this data somewhere else, is not known. Enquiries have been made to the ship's technicians to attempt to find the data somewhere else on the ADCP host computer, but no answer has yet been received. Existing ADCP data from outside this period has been handed to R. Torres for archiving and processing.
- **Marker buoy data** – marker buoys were monitored on board FS Poseidon for three periods: 6th May, 22:46 to 11th May, 11:02 hrs; 10th May, 08:25 to 18th May 13:14 hrs; and 16th May, 13:17 to 18th May, 10:38 and 18th May, 12:07 to 20th May, 04:58 hrs. These data have been sent to archived at PML and sent to BODC.

Drifter Buoy Data for 16th to 18th May & 18th – 20th May



THE IMPACT OF PHOSPHATE AND IRON FERTILISATION ON RATES OF NITROGEN FIXATION IN SURFACE WATERS OF THE SUB-TROPICAL NORTH EASTERN ATLANTIC OCEAN

CLIFF LAW, ANDY REES

Primary production in oligotrophic sub-tropical North Atlantic is considered to be limited by nitrogen availability. Nitrogen fixation, in which dinitrogen gas (N_2) is converted by diazotrophic microorganisms to ammonium (NH_4^+), represents a potentially important source of nitrogen in this region. Although the surface waters of the sub-tropical Atlantic are characterised by the diazotroph *Trichodesmium*, which has the capacity to fix nitrogen, there is evidence that this process may be limited at a cellular level by iron and/or phosphate availability. Consequently although nitrogen may be the ultimate limiting nutrient, either phosphate or iron may represent the proximal limiting nutrient over short timescales.

Objectives:

- To quantify nitrogen fixation rates in surface waters of the sub-tropical Atlantic
- To examine the impact of *in situ* and *in vivo* phosphate and phosphate/iron enrichment on nitrogen fixation rate
- To examine the impact of light, and so diel and depth variability, on nitrogen fixation To quantify nitrogen fixation rates by *Trichodesmium* in concentrated samples

Methodology:

Two methods were used to determine nitrogen fixation rate. The first, the Acetylene Reduction Technique (ART) utilises the fact that the diazotrophy relies upon the enzymatic cleavage of the triple bond in a N_2 molecule. Addition of acetylene (C_2H_2) at saturating levels causes preferential cleavage of the triple bond in the organic molecule, resulting in the production of ethylene (C_2H_4) from which nitrogen fixation rate is estimated using a theoretical $C_2H_4:N_2$ ratio of 3:1. The second involves determining the incorporation of ^{15}N -labelled N_2 into particulate organic nitrogen. The two methods have advantages and disadvantages; although the ART technique is relatively more sensitive and cheap, the $C_2H_4:N_2$ ratio may vary and the addition of C_2H_2 may influence and potentially inhibit microbial processes. The $^{15}N-N_2$ technique is more direct and less likely to alter ambient conditions, but is more expensive and some of the fixed ^{15}N may be lost via DON excretion.

Acetylene Reduction Technique (ART)

Water from 4 depths was sub-sampled from the Hydrocast Niskin bottles and decanted into 300 ml bottles in triplicate for each depth. Various approaches were tested for adding C_2H_2 at a ratio of ~10% (vol/vol) before settling on a technique in which 60 mls of sample were replaced with an equivalent volume of C_2H_2 -saturated seawater. T_0 samples were immediately analysed in triplicate to determine the initial concentrations of ethylene, whereas the remaining samples were incubated for 12 hours at respective light levels in deck incubation units. Control samples, poisoned by addition of 300 μ l mercuric chloride, were also incubated for 12 hours. Analysis involved the withdrawal of 80 ml of water in a 100 ml glass syringe and equilibration with 20 ml air. 2 ml of the dried equilibrated gas were injected onto chromatographic column that separated methane, C_2H_2 and C_2H_4 . C_2H_4 peaks were calibrated against 5 and 10 ppmv standard with the N fixation rate estimated from the change in C_2H_4 peak height over the 12 hr incubation period.

Different aspects of the methodology were tested. Different approaches and amendment levels were tested for the C_2H_2 addition, and a purification stage was added to reduce the level of impurities in the C_2H_2 - saturated water. In addition to the standard incubations, samples were also run from two deckboard nutrient amendment experiments and with pre-concentrated *Trichodesmium* colonies (see sample table).

¹⁵N-N₂ technique

Seawater was collected into 5 x 4l clear polycarbonate bottles at each of three depths selected to represent 55%, 33% and 20% of surface irradiance. Each bottle was fitted with a cap with small drilled hole and teflon backed butyl rubber septa. ¹⁵N-N₂ or ¹⁴N-N₂ (air) was added to each bottle by injection through the septa at 1.25 ml l⁻¹ of seawater. Following inoculation bottles were transferred to the on-deck incubators which were maintained at surface seawater temperature and fitted with appropriate light filters. At each daily production station (pre-dawn), experiments were performed to investigate:

- Daily (24 h) nitrogen fixation rate
- Size fractionated (total, <1.0µm, <0.2µm) daily (24 h) nitrogen fixation rate
- Time series of nitrogen fixation rate (6h, 12h, 24h)

Relative to control experiments of either ¹⁴N-N₂ addition or ¹⁵N-N₂ poisoned with HgCl. Incubations were terminated by filtration onto 25mm GF/F filters and frozen at -20°C until return to the laboratory. ¹⁵N enrichment will be determined using continuous flow stable isotope mass spectrometry.

¹⁵N- Natural abundance

In support of the above rate measurements, 2l of seawater was collected at 5 depths from within the euphotic zone and filtered onto 25 mm GF/F filters from each of the stations occupied. Filters were frozen and on return to the laboratory ¹⁵N- natural abundance will be determined in order to provide a qualitative assessment of the nitrogen fixation potential in amended and control waters.

Sample Table

DATE	TIME	STN No.	CTD No.	ART	¹⁵ N-N ₂
03/05	0320	1	4-7		55%, 33%,20%
03/05	1130	2	8	ACE1 – 55%, 33%	
04/05	0304	4	9-12	ACE2 – 55%, 33%, 20%, 1%	55%, 33%,20%
04/05	1137	5	13,14		¹⁵ NAB
05/05	0420	9	15-18	ACE3 – 55%, 33%, 20%, CM	55%, 33%,20%
05/05	1136	10	19	ACE4 – 55%, 33%	¹⁵ NAB
06/05	0256	14	21-24	ACE5 – 55%, 33%, 20%, CM	55%, 33%,20%
06/05	0939	15	25,26		¹⁵ NAB
06/05	1425	16	27,28	ACE6 – 55%, 33%	¹⁵ NAB
07/05	0309	19	29-32	ACE7 – 55%, 20%	55%, 33%,20%
07/05	0938	20	33,34		¹⁵ NAB
07/05	1340	21	35,36	ACE8 – 55%, 20%, CM	¹⁵ NAB
08/05	0305	24	37-40	ACE9 – 55%, 33%, 20%, CM	55%, 33%,20%
08/05	0932	25	41,42		¹⁵ NAB
08/05	1331	26	43,44		¹⁵ NAB
09/05	0302	29	45-48	ACE10 - 55%, 33%, 20%, CM	55%, 33%,20%
09/05	0913	30	49,50		¹⁵ NAB
09/05	1303	31	51,52		¹⁵ NAB
10/05	0308	35	54-57	ACE11 - 55%, 33%, 20%, CM	55%, 33%
11/05				1 st Nutrient addition experiment (Control, Fe, FE&P&N, Fe&P, P)	
11/05				ACE12 – Day 1 1 st NAE	
12/05				ACE13 – OUT station water from 11/05 – method test	
				ACE14 – Day 2 1 st NAE	
13/05				ACE15 – Day 3 – 1 st NAE	
14/05				ACE16 - 55%, 33%, 20%, CM	
15/05	0300	41	63-65	ACE17 - 55%, 33%, 20%, CM	55%, 33%,20%
15/05	0952	42	67		¹⁵ NAB
15/05	1335	43	68		¹⁵ NAB
16/05	0138	46	71-74	ACE18 - 55%, 33%, 20%, CM	55%, 33%,20%
16/05	1509	47	75	ACE19 - 55%, 33%, 20%,	¹⁵ NAB
17/05	0257	50	77-81	ACE20 - 55%, 33%, 20%, CM	55%, 33%,20%
17/05				<i>Trichodesmium</i> incubation 1	<i>Trichodesmium</i> incubation 1
17/05	0900	51	82-84		¹⁵ NAB
17/05	1305	52	85,86		¹⁵ NAB
18/5				2nd Nutrient addition experiment (Control, Fe, Fe&P, P)	
18/05	0304	56	88-91	ACE21 – Surface, 55%, 33%, 20%	55%, 33%,20%
18/05	0912	57	92,93	ACE22 - Day 1 2 nd NAE	¹⁵ NAB

DATE	TIME	STN No.	CTD No.	ART	¹⁵ N-N ₂
19/05	0310	61	96-99	ACE23 - 55%, 33%, 20%, CM	55%, 33%,20%
19/05	0902	62	100,101		¹⁵ NAB
19/05	1320	63	102,103	ACE24 - Surface, 55%, 33%, 20%	¹⁵ NAB
20/05	0342	67	105,108	ACE25 - Surface, 55%, 33%, 20%	55%, 33%,20% & <i>Tricho</i>
20/05	0901	68	109,110		¹⁵ NAB
20/05	1305	69	111,112	ACE26 - Surface, 55%, 33%, 20%	¹⁵ NAB
20/05				Day 2 2 nd NAE	
21/05	0303	72	113-116	ACE27 - Surface, 55%, 33%, 20% & <i>Tricho</i>	55%, 33%,20%
21/05	0900	73	117,118		¹⁵ NAB
21/05	1540	75	120,121	ACE28 - Surface, 55%, 33%, 20%	¹⁵ NAB
22/05	0317	78	123-126	ACE29 - Surface, 55%, 33%, 20%	55%, 33%,20% & <i>Tricho</i>
22/05	0348			Day 4 2 nd NAE	

Preliminary results:

Preliminary comparison of C₂H₄ peak height at the start and end of the incubations suggests that nitrogen fixation was initially low to negligible at the start, but increased slightly towards the end of the first patch experiment. A similar pattern was apparent during the second experiment, although low level N fixation was apparent throughout. Highest N fixation rates were observed with the concentrated *Trichodesmium* samples. Data analysis and comparison of results from the two techniques will confirm the impact of the iron and phosphate additions on N fixation rate.

MICRO-PLANKTON GROSS PRODUCTION, NET COMMUNITY PRODUCTION AND DARK COMMUNITY RESPIRATION

CAROL ROBINSON

Objectives:

- To determine the impact of phosphate and iron limitation on plankton respiration
- To determine the impact of phosphate and iron limitation on the balance between plankton production (P) and respiration (R), and hence the capacity for export
- To determine the impact of phosphate and iron limitation on surface water dissolved oxygen and dissolved inorganic carbon concentrations
- To determine the balance between photochemical oxygen consumption and bacterio- and micro-plankton oxygen consumption

Relevance to cruise and PML aims:

These objectives contribute to the testing of two of the cruise hypotheses:

- 1) Primary production is limited by iron and / or phosphate availability
- 2) Carbon export is limited by iron and / or phosphate availability

And to the achievement of three of the aims of the PML Core Strategic Research Programme:

- Aim 5.1 To determine how microbial community structure influences the photosynthesis/respiration ratio
- Aim 6.3 To determine the fate of phytoplankton production through respiration, remineralisation and nutrient regeneration in the water column and within aggregated particles
- Aim 7.3 To investigate the impact of increasing incident UV-radiation and the role of photochemical pathways in carbon, nutrient and trace gas cycling

Methods:

The daily sampling schedule adopted on RV Poseidon consisted of CTD casts at 04:00 local time (pre-dawn), - 10:00 am local time and - 14:00 pm local time. Within any one day, we aimed to sample both within and outwith the fertilised patch. Hence if the pre-dawn and 10:00 am casts were from within the patch, then the 14:00 cast would tend to be outside the patch and *vice versa*.

Water samples were collected from a subset of the CTD profiles to calibrate the oxygen electrode on the CTD and determine the concentration of dissolved inorganic carbon. Dissolved oxygen was measured by automated Winkler titration, and dissolved inorganic carbon by automated coulometry.

Water was collected from the pre-dawn casts each morning from depths equivalent to 55%, 33%, 20%, and 1% of surface irradiance and incubated in 60 ml borosilicate glass bottles in surface water cooled deck incubators for 24 hours. On a limited number of occasions, samples were also collected from 97% surface irradiance, the depth of the chlorophyll maximum and from a depth 20m below the chlorophyll maximum. From each depth, six replicates were incubated in the light, six in the dark and six fixed for determination of zero time dissolved oxygen concentration. Dark community respiration, net community production and gross production were determined from the difference between the mean dissolved oxygen concentration of the zero, light and dark incubated replicates.

Surface samples from the pre-dawn casts were gently filtered through 0.8 µm polycarbonate filters and decanted into 12 x 60 ml borosilicate glass bottles. Six were fixed for zero time concentrations and six were incubated in the dark for 24 hours. Bacterio-plankton respiration was estimated from the difference between the means of the dissolved oxygen concentrations of the zero time and dark incubated replicates. A further surface sample was filtered through a 0.2 µm criticap cartridge and decanted into 12 x 120 ml borosilicate glass bottles and 6 x 120 ml quartz bottles. Six glass bottles were fixed for zero time concentrations, six were incubated in the dark and the six quartz bottles were incubated in a surface water cooled deck incubator for

the duration of daylight (normally 10 – 12 hours). The photochemical consumption of oxygen was estimated from the difference between the zero time and light incubated samples. The dark incubated samples acted as a control to assess any bacterial contamination of the 0.2 µm filtrate.

Surface water was collected from the 10:00 am and 14:00 pm casts and incubated in the dark in surface water cooled deck incubators for the determination of micro-plankton respiration.

On two experimental occasions, samples were collected at 12h or 24h intervals from each of 5 nutrient amended (control, Fe, PO₄, Fe+PO₄, and Fe+PO₄+NH₄) 20 litre cubitainers over a period of 3-5 days for determination of microplankton respiration and dissolved oxygen concentration.

Full list of samples collected

Date	Time	Station	Samples collected
3 May 04	04:00	CTDs 04-07	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration
3 May 04	12:30	CTD 08	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Surface respiration
4 May 04	04:00	CTDs 09-12	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface photo-oxidation
4 May 04	12:00	CTD 14	Depth profile of dissolved oxygen Surface respiration
5 May 04	04:00	CTD2 15-18 IN PO ₄ patch	Depth profile of dissolved oxygen Depth profile of gross production, net community production and respiration
5 May 04	12:00	CTD 19 IN PO ₄ patch	Depth profile of dissolved oxygen Surface respiration
6 May 04	04:00	CTDs 21-24 IN PO ₄ patch	Depth profile of dissolved oxygen Depth profile of gross production, net community production and respiration Surface photo-oxidation
6 May 04	10:30	CTD 25 IN PO ₄ patch	Depth profile of dissolved oxygen Surface respiration
6 May 04	14:30	CTD 27 OUT patch	Depth profile of dissolved oxygen Surface respiration
7 May 04	04:00	CTDs 29-32 OUT patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration
7 May 04	10:30	CTD 33 OUT patch	Depth profile of dissolved oxygen Surface respiration
7 May 04	15:30	CTD 35 IN PO ₄ patch	Depth profile of dissolved oxygen Surface respiration
8 May 04	04:00	CTDs 37-40 IN PO ₄ patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface photo-oxidation

Date	Time	Station	Samples collected
8 May 04	12:00	CTD 41 IN PO ₄ patch	Depth profile of dissolved oxygen
8 May 04	15:00	CTD 43 OUT patch	Depth profile of dissolved oxygen
9 May 04	04:00	CTDs 45-48 OUT patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration
9 May 04	10:00	CTD 49 OUT patch	Depth profile of dissolved oxygen
9 May 04	14:00	CTD 51 OUT patch	Depth profile of dissolved oxygen
10 May 04	04:00	CTDs 54-57 IN PO ₄ patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface photo-oxidation
10 May 04			Collected OUT water for large volume nutrient addition experiments
11 May 04	19:30	5 cubitainers	Dissolved oxygen
12 May 04	09:00	5 cubitainers	Dissolved oxygen
12 May 04	21:00	5 cubitainers	Dissolved oxygen
13 May 04	10:00	5 cubitainers	Dissolved oxygen
13 May 04	21:00	5 cubitainers	Dissolved oxygen
14 May 04	10:00	5 cubitainers	Dissolved oxygen
15 May 04	04:00	CTDs 63-66 ALMOST IN PO ₄ patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface photo-oxidation
15 May 04	15:00	CTD 68 OUT patch	Surface respiration
16 May 04	04:00	CTDs 71-74 OUT patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface photo-oxidation
16 May 04	16:30	CTD 75 IN Fe patch	Surface respiration Surface respiration in 0.8 μm fraction
17 May 04	04:00	CTDs 77-80 IN Fe patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface respiration in 0.8 μm fraction Surface photo-oxidation
17 May 04	10:00	CTD 82 OUT patch	Surface respiration
17 May 04	14:00	CTD 85 IN Fe patch	Surface respiration
18 May 04	04:00	CTDs 88-91 IN Fe+PO ₄ patch	Depth profile of gross production, net community production and respiration Surface respiration in 0.8 μm fraction 500m photo-oxidation
18 May 04	10:00	CTD 92 IN Fe+PO ₄ patch	Surface respiration Respiration in 4 large volume nutrient addition expts

Date	Time	Station	Samples collected
18 May 04	14:00	CTD 94 OUT patch	Surface respiration
19 May 04	04:00	CTDs 96-99 OUT patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration
19 May 04	10:00	CTD 100 OUT patch	Surface respiration
19 May 04	14:00	CTD 102 IN Fe+PO ₄ patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Surface respiration
20 May 04	04:00	CTDs 105-108 IN Fe+PO ₄ patch	Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface photo-oxidation
20 May 04	10:00	CTD 109 IN Fe+PO ₄ patch	Surface respiration
20 May 04	14:00	CTD 111 OUT patch	Surface respiration
21 May 04	04:00	CTDs 113-116 OUT patch	Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface respiration in 0.8 μm fraction
21 May 04	16:45	CTD 120 IN Fe+PO ₄ patch	Surface respiration
22 May 04	04:00	CTDs 123-126 IN Fe+PO ₄ patch	Depth profile of dissolved oxygen Depth profile of dissolved inorganic carbon Depth profile of gross production, net community production and respiration Surface respiration in 0.8 μm fraction

Preliminary Results:

The magnitude of dark community respiration (R) was consistently greater than the magnitude of gross production (P) i.e. the water column was net heterotrophic ($P < R$), with surface rates of R of approximately $0.8 \text{ mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$ and rates of P of about $0.4 \text{ mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$. An analysis of whether iron or phosphate availability influenced the *in situ* concentrations of dissolved oxygen, dissolved inorganic carbon, the magnitude of P or R or the balance between the two, awaits concurrent data on SF₆, phosphate and iron concentrations. Surface respiration samples collected at 04:00 am, 10:00 am and 14:00 pm showed no evidence of a diel trend. The respiration rate of the $0.8 \mu\text{m}$ filtered samples accounted for the majority of the respiration rate of the unfractionated samples. The photochemical oxygen demand of $0.2 \mu\text{m}$ filtered surface samples was always less than twice the standard error on the dissolved oxygen determinations i.e. $< 0.1 \text{ mmol O}_2 \text{ m}^{-3} \text{ 12h}^{-1}$. The photochemical oxygen demand of a sample collected from 500m was $0.7 \pm 0.09 \text{ mmol O}_2 \text{ m}^{-3} \text{ 12h}^{-1}$ i.e. similar to the daily surface microplankton respiratory oxygen demand. All data should be deposited at BODC within 6 months of the end of the cruise.

Acknowledgements:

Many thanks to the Officers and Crew of RV Poseidon for their invaluable help and support (and for allowing us to go swimming ☺). Special thanks to the Principal Scientist, Andy Rees for his excellent co-ordination and liaison with the ship's staff. Thanks to all the scientific personnel for their amazing teamwork, without which these measurements could not have been obtained. Particular thanks to John Stephens for man-handling the CTD every day, to Chris Galliene for co-ordinating the deployment of the CTD, to Gary Smerdon for $0.2 \mu\text{m}$ filtrates and co-ordination of CTD sampling, and to Cliff Law who kept me awake and alert with endless questions about PML life, the universe and everything. Thanks to Niki Gist (PML) for help with preparation for the cruise, to Dominique Lefevre (University of Marseille) for advice on visual basic programming and to Peter J. le B. Williams (University of Wales; Bangor) for loan of analytical equipment.

SAMPLE COLLECTION FOR *nifH* SEQUENCE HETEROGENEITY AND BACTERIAL DIVERSITY

GARY SMERDON, ANDREW REES

Background:

Microorganisms catalyse biological nitrogen fixation with the enzyme nitrogenase, which has been highly conserved throughout evolution. Cloning and sequencing of one of the nitrogenase structural genes, *nifH*, has provided a large (and still rapidly expanding) database of sequences from diverse terrestrial and aquatic environments. Analysis of the distribution of *nifH* phylotypes among habitats indicates characteristic patterns of nitrogen fixing microorganisms in habitats ranging from termite guts to oligotrophic oceans. The ability to assay for gene presence, and the extension of the methodology to potentially include both patterns of gene expression and the range of active phylotypes, provides a new range of tools for interrogating natural populations of diazotrophs. The analysis of nitrogenase genes thus provides a basis for the development of molecular assays and bioinformatics approaches for the study of nitrogen fixation in the environment, and it is these tools that we propose to develop and deploy within the context of the 2004 PML cruise. Using these tools we will determine the diversity of nitrogen fixers present, and determine the patterns of *nifH* expression associated with nutrient enrichments on temporal and spatial scales.

Whilst much of the analysis on *nifH* genes in marine environments has concentrated in regions of intense nitrogen fixation, often involving *Trichodesmium* blooms, the technology for *nifH* detection now affords analysis of the diazotroph community in regions with significantly lower levels of nitrogen fixation. Initial systems depended on the use of two degenerate oligonucleotides to amplify *nifH* sequences, but the change into environments where the proportion of N-fixers can be extremely low has led to the development of a nested PCR protocol which significantly increases the sensitivity of the system. Both systems can be deployed depending on the level of diazotrophs present.

Sample collection and analysis:

Bacteria were collected using 0.2µL Sterivex filters (Millipore). For the majority of samples no pre-filtration was performed but for some samples a 5 µL Durapore prefilter was used. Water sampled using the CTD was collected in 9 litre carboys and transferred to the laboratory. Filtration was performed using a 4-headed peristaltic pump (Figure 1)



Figure 1 – Watson Marlow peristaltic pump with 4 pump heads. This set up shows an in-line prefilter housing (containing a 5µm Durapore filter followed by a single 0.2µm Sterivex filter for each channel).

Using this system, 9 litres of seawater were generally filtered per channel. After filtration (generally 5-7 hours to pass 9L seawater) and removal of all residual seawater from the housing, the filters were blocked at one end with Blu-Tack and 1.8 mL lysis buffer (50mM Tris-Cl pH 8.3, 40mM EDTA, 0.75M sucrose) added (for detailed protocol see Appendix I). The open end of the filter housing was then sealed with Blu-Tack and the filters frozen at -20°C (freezer).

Using the system described briefly above 195 sterivex filters were stored for future DNA extraction and further analysis. Details of each of the samples collected are given in Appendix II.

DNA extraction

DNA was extracted from filters (No. 70, 71, 72 and 73 – see appendix II) according to the method given in appendix II. To check the quality and quantity of the DNA 5µL from 100 µL concentrate were analysed by agarose gel electrophoresis (Figure 2) using precast bufferless e-gels (Invitrogen Ltd).

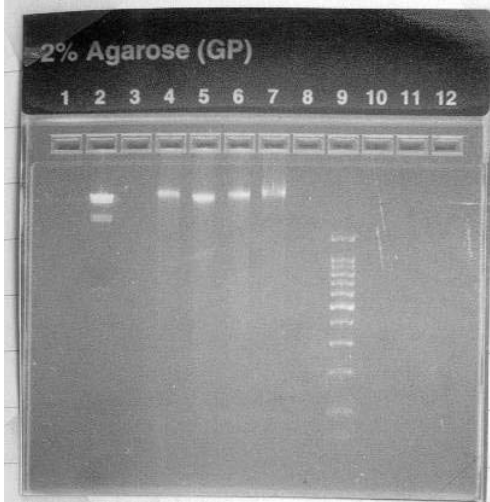


Figure 2 – Agarose gel electrophoresis (E-gel) of total DNA extracted from four water samples. Lane 2 = Lambda HindIII markers, Lanes 4,5,6 and 7 show DNA extracted from samples 70, 71, 72 and 73 respectively. Lane 9 = 100bp ladder (Promega).

From figure 2 it can be seen that high quality DNA was extracted from each of the four samples, the DNA being an apparently suitable template for the amplification of *nifH* using the polymerase chain reaction.

nifH amplification using the Polymerase Chain Reaction (PCR)

For amplifications the primers nifH1 and nifH2 were used:

nifH1(for) 5' TGYGAYCCNAARGCNGA 3'

nifH2(rev) 5' ADNGCCATCATYTCNCC 3'

Amplifications were performed on reactions containing a final concentration of 4mM MgCl₂ for 30 cycles of 94°C, 1 min; 57°C, 1 min; 72°C, 1 min. The products of amplification 5µL from a 50 µL reaction are shown in figure 3.



Figure 3 – Agarose gel electrophoresis of the PCR products of *nifH* amplification. Lane 1 = 100bp ladder (Promega); Lanes 2-5 = amplicons from samples 70-73 respectively. Amplification products of the expected size (can be clearly seen in lanes 4 and 5 (samples 72 and 73 from depths 25m and 40m respectively)).

Date	Statn	CTD	Depth (m)	Time (GMT)	In / Out	Prefilter Sum	Vol (l)	Tube No
3.5.04	1	7	surf	04:55:00	Pre	Y	18	2
3.5.04	1	4	15	03:20:00	Pre	Y	17	3
3.5.04	1	5	25	00:00:00	Pre	Y	13	4
3.5.04	1	5	40	00:00:00	Pre	Y	18	5
4.5.04	4	12	surf	04:41:00	Pre	N	15	6
4.5.04	4	9	15	03:04:00	Pre	N	15	7
4.5.04	4	10	25	03:04:00	Pre	N	18	8
4.5.04	4	11	40	04:07:00	Pre	N	15	9
5.5.04	9	18	surf	05:59:00	Being laid	N	14	10
5.5.04	9	15	15	04:20:00	Being laid	N	15	11
5.5.04	9	16	25	04:51:00	Being laid	N	15	12
5.5.04	9	17	40	05:03:00	Being laid	N	14	13
5.5.04	10	19	15	11:36:00		N	9	14
5.5.04	10	19	15	11:36:00		N	9	15
5.5.04	10	19	25	11:36:00		N	9	16
5.5.04	10	19	25	11:36:00		N	9	17
6.5.04	14	24	surf	04:29:00	In	N	9	18
6.5.04	14	21	15	02:56:00	In	N	9	19
6.5.04	14	22	25	03:26:00	In	N	9	20
6.5.04	14	23	40	03:50:00	In	N	9	21
6.5.04	14	24	surf	04:29:00	In	N	9	22
6.5.04	14	21	15	02:56:00	In	N	9	23
6.5.04	14	22	25	03:26:00	In	N	9	24
6.5.04	14	23	40	03:50:00	In	N	9	25
6.5.04	15	26	surf	10:22:00	In	N	9	26
6.5.04	15	26	15	10:22:00	In	N	9	27
6.5.04	15	26	25	10:22:00	In	N	9	28
6.5.04	15	26	40	10:22:00	In	N	9	29
6.5.04	16	28	surf	15:12:00	Out	N	9	30
6.5.04	16	28	15	15:12:00	Out	N	9	31
6.5.04	16	28	25	15:12:00	Out	N	9	32
6.5.04	16	28	40	15:12:00	Out	N	9	33
7.5.04	19	32	surf	04:40:00	Out	N	9	34
7.5.04	19	29	15	03:09:00	Out	N	9	35
7.5.04	19	30	25	03:38:00	Out	N	9	36
7.5.04	19	31	40	04:09:00	Out	N	9	37
7.5.04	19	32	surf	04:40:00	Out	N	9	38
7.5.04	19	29	15	03:09:00	Out	N	9	39
7.5.04	19	30	25	03:38:00	Out	N	9	40
7.5.04	19	31	40	04:09:00	Out	N	9	41
7.5.04	20	34	surf	10:26:00	Out	N	9	42
7.5.04	20	34	15	10:26:00	Out	N	9	43
7.5.04	20	34	25	10:26:00	Out	N	9	44
7.5.04	20	34	40	10:26:00	Out	N	9	45
7.5.04	21	36	surf	14:35:00	In	N	9	46
7.5.04	21	36	15	14:35:00	In	N	9	47
7.5.04	21	36	25	14:35:00	In	N	9	48
7.5.04	21	36	40	14:35:00	In	N	9	49
8.5.04	24	40	surf	04:22:00	In	N	9	50
8.5.04	24	37	15	03:05:00	In	N	9	51
8.5.04	24	38	25	03:37:00	In	N	9	52

Date	Statn	CTD	Depth (m)	Time (GMT)	In / Out	Prefilter 5µm	Vol (l)	Tube No
8.5.04	24	40	surf	04:22:00	In	N	9	54
8.5.04	24	37	15	03:05:00	In	N	9	55
8.5.04	24	38	25	03:37:00	In	N	9	56
8.5.04	24	39	40	04:05:00	In	N	9	57
8.5.04	25	42	surf	10:13:00	In	N	9	58
8.5.04	25	42	15	10:13:00	In	N	9	59
8.5.04	25	42	25	10:13:00	In	N	9	60
8.5.04	25	42	40	10:13:00	In	N	9	61
8.5.04	26	43	surf	13:31:00	Out	N	9	62
8.5.04	26	43	15	13:31:00	Out	N	9	63
8.5.04	26	43	25	13:31:00	Out	N	9	64
8.5.04	26	43	40	13:31:00	Out	N	9	65
9.5.04	29	48	surf	04:33:00	Out	N	9	66
9.5.04	29	45	15	03:02:00	Out	N	9	67
9.5.04	29	46	25	03:35:00	Out	N	9	68
9.5.04	29	47	40	04:08:00	Out	N	9	69
9.5.04	29	48	surf	04:33:00	Out	N	9	70
9.5.04	29	45	15	03:02:00	Out	N	9	71
9.5.04	29	46	25	03:35:00	Out	N	9	72
9.5.04	29	47	40	04:08:00	Out	N	9	73
9.5.04	30	50	surf	09:51:00	Out	N	9	74
9.5.04	30	50	15	09:51:00	Out	N	9	75
9.5.04	30	50	25	09:51:00	Out	N	9	76
9.5.04	30	50	40	09:51:00	Out	N	9	77
9.5.04	31	52	surf	13:35:00	In	N	9	78
9.5.04	31	52	15	13:35:00	In	N	9	79
9.5.04	31	52	25	13:35:00	In	N	9	80
9.5.04	31	52	40	13:35:00	In	N	9	81
10.5.04	35	58	surf	04:40:00	In	N	9	82
10.5.04	35	55	15	03:08:00	In	N	9	83
10.5.04	35	56	25	03:36:00	In	N	9	84
10.5.04	35	57	40	04:11:00	In	N	9	85
10.5.04	35	58	surf	04:40:00	In	N	9	86
10.5.04	35	55	15	03:08:00	In	N	9	87
10.5.04	35	56	25	03:36:00	In	N	9	88
10.5.04	35	57	40	04:11:00	In	N	9	89
10.5.04			25	<i>Deck Incs</i>	<i>Tzero</i>	N	9	90
			25	<i>Deck Incs</i>	<i>Tzero</i>	N	9	91
			25	<i>Deck Incs</i>	<i>Tend</i>	N	9	93
15.5.04	41	66	surf	04:19:00	In ?	N	9	94
15.5.04	41	63	15	00:00:00	In ?	N	9	95
15.5.04	41	64	25	03:29:00	In ?	N	9	96
15.5.04	41	65	40	03:53:00	In ?	N	9	97
15.5.04	41	66	surf	04:19:00	In ?	N	9	98
15.5.04	41	63	15	04:19:00	In ?	N	9	99
15.5.04	41	64	25	03:29:00	In ?	N	9	100
15.5.04	41	65	40	03:53:00	In ?	N	9	101
15.5.04	43	69	surf	14:46:00	Out	N	9	102
15.5.04	43	69	15	14:46:00	Out	N	9	103
15.5.04	43	69	25	14:46:00	Out	N	9	104
15.5.04	43	69	40	14:46:00	Out	N	9	105
16.5.04	46	74	surf	04:17:00	Pre	N	9	106
16.5.04	46	71	15	00:00:00	Pre	N	9	107
16.5.04	46	72	25	03:31:00	Pre	N	9	108
16.5.04	46	73	40	03:55:00	Pre	N	9	109
16.5.04	46	74	surf	04:17:00	Pre	N	9	110

Date	Statn	CTD	Depth (m)	Time (GMT)	In / Out	Prefilter 5µm	Vol (l)	Tube No
16.5.04	46	72	25	03:31:00	Pre	N	9	112
16.5.04	46	73	40	03:55:00	Pre	N	9	113
16.5.04	47	76	surf	15:48:00	Fe being laid	N	9	114
16.5.04	47	76	15	15:48:00	Fe being laid	N	9	115
16.5.04	47	76	25	15:48:00	Fe being laid	N	9	116
16.5.04	47	76	40	15:48:00	Fe being laid	N	9	117
17.5.04	50	80	surf	04:24:00	In Fe	N	9	118
17.5.04	50	77	15	02:57:00	In Fe	N	9	119
17.5.04	50	78	25	03:31:00	In Fe	N	9	120
17.5.04	50	79	40	03:55:00	In Fe	N	9	121
17.5.04	50	80	surf	04:24:00	In Fe	Y	9	122
17.5.04	50	77	15	02:57:00	In Fe	Y	9	123
17.5.04	50	78	25	03:31:00	In Fe	Y	9	124
17.5.04	50	79	40	03:55:00	In Fe	Y	9	125
17.5.04	51	83	surf	09:58:00	Out	N	9	126
17.5.04	51	83	15	09:58:00	Out	N	9	127
17.5.04	51	83	25	09:58:00	Out	N	9	128
17.5.04	51	83	40	09:58:00	Out	N	9	129
17.5.04	52	86	surf	13:38:00	In Fe	N	9	130
17.5.04	52	86	15	13:38:00	In Fe	N	9	131
17.5.04	52	86	25	13:38:00	In Fe	N	9	132
17.5.04	52	86	40	13:38:00	In Fe	N	9	133
18.5.04	56	91	surf	04:29:00	In Fe + P	N	9	134
18.5.04	56	88	15	03:04:00	In Fe + P	N	9	135
18.5.04	56	89	25	03:40:00	In Fe + P	N	9	136
18.5.04	56	90	40	04:03:00	In Fe + P	N	9	137
18.5.04	56	91	surf	04:29:00	In Fe + P	Y	9	138
18.5.04	56	89	25	03:40:00	In Fe + P	Y	9	140
18.5.04	56	90	40	04:03:00	In Fe + P	Y	9	141
18.5.04	58	95	surf	13:34:00	Out	N	9	142
18.5.04	58	95	15	13:34:00	Out	N	9	143
18.5.04	58	95	25	13:34:00	Out	N	9	144
18.5.04	58	95	40	13:34:00	Out	N	9	145
19.5.04	61	99	surf	04:27:00	Out	N	9	146
19.5.04	61	96	15	03:10:00	Out	N	9	147
19.5.04	61	97	25	03:41:00	Out	N	9	148
19.5.04	61	98	40	04:03:00	Out	N	9	149
19.5.04	61	99	surf	04:27:00	Out	Y	9	150
19.5.04	61	96	15	03:10:00	Out	Y	9	151
19.5.04	61	97	25	03:41:00	Out	Y	9	152
19.5.04	61	98	40	04:03:00	Out	Y	9	153

Date	Statn	CTD	Depth (m)	Time (GMT)	In / Out	Prefilter 5µm	Vol (l)	Tube No
19.5.04	63	103	15	14:06:00	In Fe + P	N	9	155
19.5.04	63	103	25	14:06:00	In Fe + P	N	9	156
19.5.04	63	103	40	14:06:00	In Fe + P	N	9	157
20.5.04	67	108	surf	04:57:00	In Fe + P	N	9	158
20.5.04	67	105	15	03:42:00	In Fe + P	N	9	159
20.5.04	67	106	25	04:11:00	In Fe + P	N	9	160
20.5.04	67	107	40	04:35:00	In Fe + P	N	9	161
20.5.04	67	108	surf	04:57:00	In Fe + P	N	9	162
20.5.04	67	105	15	03:42:00	In Fe + P	N	9	163
20.5.04	67	106	25	04:11:00	In Fe + P	N	9	164
20.5.04	67	107	40	04:35:00	In Fe + P	N	9	165
20.5.04	68	110	surf	09:41:00	In Fe + P	N	9	166
20.5.04	68	110	15	09:41:00	In Fe + P	N	9	167
20.5.04	68	110	25	09:41:00	In Fe + P	N	9	168
20.5.04	68	110	40	09:41:00	In Fe + P	N	9	169
20.5.04	68	110	65	09:41:00	In Fe + P	N	9	170
20.5.04	68	110	110	09:41:00	In Fe + P	N	9	171
21.5.04	72	116	surf	04:17:00	Out	N	9	172
21.5.04	72	113	15	03:03:00	Out	N	9	173
21.5.04	72	114	25	03:34:00	Out	N	9	174
21.5.04	72	115	40	03:55:00	Out	N	9	175
21.5.04	72	116	surf	04:17:00	Out	N	9	176
21.5.04	72	113	15	03:03:00	Out	N	9	177
21.5.04	72	114	25	03:34:00	Out	N	9	178
21.5.04	72	115	40	03:55:00	Out	N	9	179
21.5.04	73	118	surf	09:30:00	Out	N	9	180
21.5.04	73	118	15	09:30:00	Out	N	9	181
21.5.04	73	118	25	09:30:00	Out	N	9	182
21.5.04	73	118	40	09:30:00	Out	N	9	183
21.5.04	75	121	surf	16:20:00	In Fe + P	N	9	184
21.5.04	75	121	15	16:20:00	In Fe + P	N	9	185
21.5.04	75	121	25	16:20:00	In Fe + P	N	9	186
21.5.04	75	121	40	16:20:00	In Fe + P	N	9	187
22.5.04	78	126	surf	04:29:00	In Fe + P	N	9	188

Date	Statn	CTD	Depth (m)	Time (GMT)	In / Out	Prefilter 5µm	Vol (l)	Tube No
22.5.04	78	124	25	03:48:00	In Fe + P	N	9	190
22.5.04	78	125	40	04:08:00	In Fe + P	N	9	191
22.5.04	78	126	surf	04:29:00	In Fe + P	N	9	192
22.5.04	78	123	15	03:17:00	In Fe + P	N	9	193
22.5.04	78	124	25	03:48:00	In Fe + P	N	9	194
22.5.04	78	125	40	04:08:00	In Fe + P	N	9	195

Sample collection for general nucleic acid extraction

Water was filtered (9 litres per Sterivex filter) using the same system as shown in Figure 1. On completion of the filtration all remaining seawater was removed from the system and the filters frozen at -40°C after sealing the ends of the filter with Blu-Tack. Samples were transported back to the UK at -70°C and stored in a -70°C freezer until required. Details of the samples collected are given in appendix III.

Sample collection for Analytical Flow Cytometry (AFC)

100µL aliquots 4% paraformaldehyde were pipetted into individual 2mL cryovials. On retrieving water samples using the CTD 1.8mL seawater was pipetted into each of the tubes and the screw cap fitted. The samples were then incubated at 4°C for 24 hours before being frozen at -20°C for storage and transport back to the laboratory. Details of the samples taken for AFC analysis are given in appendix IV.

Sample collection for pigment analysis

For each depth at each station approximately 2 litres of water were filtered under vacuum onto GF/F filters and stored in liquid nitrogen for analysis at PML. The details of the samples taken including actual volumes filtered are given in appendix IV.

Sample collection for virus amplification

At various stations and depths (see appendix V) 5mL water samples were collected from the CTD in 5mL cryovial tubes (Nalgene) and stored at 4°C.

NUTRIENT ANALYSIS

JOHN STEPHENS

A total of 41 CTD casts were analysed for nutrients: nitrate, nitrite, phosphate and silicate using a five channel segmented flow autoanalyser within two hours of collection. Phosphate and silicate were determined according to Kirkwood (1989), nitrate and nitrite according to Brewer & Riley (1965) and Grasshoff (1976) respectively.

Casts generally comprised 8 standard depths determined by light levels appropriate to production rate experiments. Additional analyses were undertaken when required for standards concentration confirmation and in-situ deck incubations.

Date	Station	CTD(s)	Event	Time (BST)	No. Depths	Depth Range (m)
03/05/2004	1	4-7	105-108	04:00	8	0-160
	2	8	109	12:30	8	0-160
04/05/2004	4	9-12	116-119	04:00	8	0-140
	5	14-15	121	13:00	8	0-155
05/05/2004	9	15-18	132-135	04:00	8	0-145
	10	19	136	12:30	8	0-148
06/05/2004	14	21-24	149-152	04:00	8	0-158
	15	25-26	153-154	10:30	8	0-157
	16	27-28	155-156	15:30	8	0-145
07/05/2004	19	29-32	163-166	04:00	8	0-161
	20	33-34	167-168	10:30	8	0-138
	21	35-36	169-170	14:30	8	0-132
08/05/2004	24	37-40	177-180	04:00	10	0-150
	25	41-42	181-182	10:30	11	0-145
	26	43-44	183-184	14:30	8	0-146
09/05/2004	29	45-48	191-194	04:00	8	0-150
	30	49-50	195-196	10:00	8	0-156
	31	51-52	197-198	14:00	11	0-125
10/05/2004	35	54-57	206-209	04:00	12	0-136
	36	58-59	210-211	13:00	9	0-145
15/05/2004	41	63-66	218-221	04:00	8	0-150
	42	67	222	10:45	8	0-155
	43	68/69	223-224	14:40	8	0-125

Date	Station	CTD(s)	Event	Time (BST)	No. Depths	Depth Range (m)
	47	75-76	239-240	16:00	8	0-142
17/05/2004	50	77-80	249-252	04:00	8	0-143
17/05/2004	51	82-84	254-256	10:00	8	0-135
	52	85-86	257-258	14:00	7	0-143
18/05/2004	56	88-91	268-271	04:00	8	0-142
	57	92-93	272-273	10:00	8	0-140
	58	94-95	274-275	14:00	8	0-143
19/05/2004	61	96-99	284-287	04:30	8	0-150
	62	100-101	288-289	10:00	8	0-135
	63	102-103	290-291	14:00	8	0-150
20/05/2004	67	105-118	300-303	04:00	8	0-137
	68	109-110	304-305	10:00	8	0-145
	69	111-112	306-307	14:00	8	0-140
21/05/2004	72	113-116	316-319	04:00	8	0-127
	73	117-118	320-321	10:00	8	0-150
	74	119	322	12:30	12	0-160
22/05/2004	78	123/126	333-336	04:00	8	0-150

SULPHUR HEXAFLOURIDE MEASUREMENTS

HESTER WILLSON, CLIFF LAW

Objectives:

The aims of the SF6 measurements during FEEP were:

- To provide a framework for the 1) phosphate and 2) iron and phosphate addition experiments by the release of SF6 simultaneously with the phosphate in the first experiment and with the iron in the subsequent experiment. The tracer could then be used to re-locate the region of water that was influenced by the added phosphate and iron and phosphate, and provide a proxy for the nutrients when no longer detectable.
- To enable the rates of dilution and spreading affecting the mixed layer water to be measured in both horizontal and vertical directions. These measurements will be related to the physical processes forcing vertical mixing.

Methodology:

Discrete SF6 samples were sub-sampled into 500ml glass stoppered bottles from the sampling rosette. The SF6 was removed by sparge-cryotrapping, isolated chromatographically and detected by electron capture dectector, by the method of Law et al, 1994. Measurements were calibrated against standards prepared by the Biogas and Tracer group at PML and an inter-calibration of samples between the systems on Darwin and Poseidon was undertaken.

CTD samples analysed

DATE	TIME	STN No.	CTD No.	Lat	Long	Depths
05.05	1136	10	19	27:46.5	23:18.4	90,70,40,25,15,Surf
	1305	11	20	27:46.8	23:18.4	40,Surf
06.05	0256		21	27:46.9	23:16.6	15,Surf
	0326		22	27:46.3	23:16.3	26
	0356		23	27:46.0	23:16.1	39
	0939	15	25	27:45.3	23:16.9	110,70,55,50,45,40,25,15, Surf
07.05	0309		29	27:45.0	23:23.7	15,Surf
	0338		30	27:44.8	23:23.3	26
	0409		31	27:44.8	23:23.3	39
	0440		32	27:44.5	23:23.8	110
	0938	20	33	27:44.9	23:23.7	110,70,55,50,45,40,25,15, Surf
	1340	21	35	27:44.6	23:13.7	110,70,60,50,45,40,25,15, Surf
08.05	0305		37	27:42.2	23:15.8	15,Surf
	0337		38	27:42.3	23:15.6	25
	0405		39	27:42.3	23:15.6	45
	0442		40	27:42.3	23:15.5	55,50
	0932	25	41	27:40.0	23:14.0	110,70,60,55,50,40,25,15, Surf
	1331	26	43	27:30.1	23:13.6	40,25,15,Surf
09.05	0302		45	27:30.7	23:12.9	15,Surf
	0335		46	27:30.5	23:12.5	25
	0408		47	27:30.3	23:12.0	64,40
	0433		48	27:30.1	23:11.5	110
	0913	30	49	27:29.5	23:12.7	110,70,40,25,15,Surf
	1303	31	51	27:42.0	23:12.3	75,70,65,60,50,40,25,15, Surf
	1335		52	27:42.2	23:12.3	110
10-05	0308	35	54	27:39.2	23:15.0	15,Surf
	0336		55	27:39.1	23:14.6	25
	0411		56	27:39.1	23:14.2	65,39
	0440		57	27:39.0	23:14.0	110,70,65,60,50
15-05	0300	41	63	27:37.1	23:16.4	25
	0329		64	27:37.0	23:16.5	65,39
	0353		65	27:36.8	23:16.4	110,70,65,60,50
	0952	42	67	27:36.9	23:16.4	85,70,65,39,25
	1335	43	68	27:36.9	22:50.3	Surf
	1446		69	27:36.7	22:49.7	Surf
16.05	0300		71	27:32.4	22:34.3	15,Surf
	0331		72	27:32.3	22:33.9	25
	0355		73	27:32.1	22:33.7	65,39
	0417		74	27:32.1	22:33.4	110
	1548		76	27:33.0	22:32.7	110,65,39,25,15,Surf
17.05	0331		78	27:33.6	22:29.6	15,Surf
	0355		79	27:33.4	22:29.4	25
	0424		80	27:33.5	22:29.5	65,39
	0451		81	27:33.4	22:29.3	110
	1305	52	85	27:33.3	22:28.0	110,85,80,65,39,30,25,15, Surf

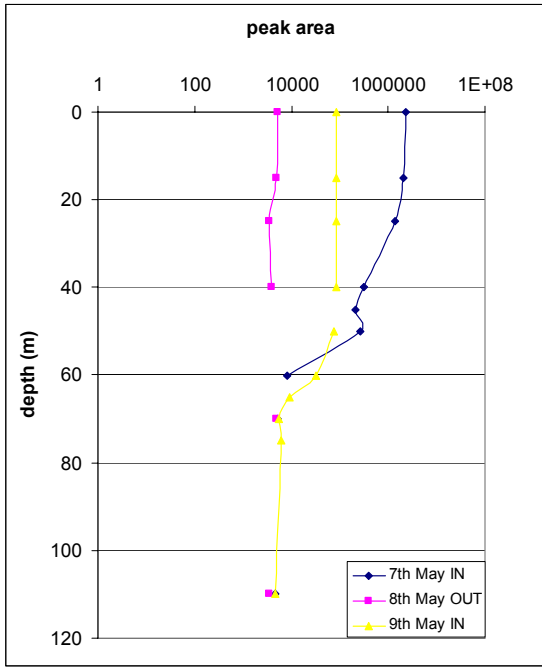
DATE	TIME	STN No.	CTD No.	Lat	Long	Depths
	0340		89	27:35.5	22:23.6	25
	0403		90	27:35.4	22:23.4	65,39
	0428		91	27:35.3	22:23.2	110
	0912	57	92	27:35.0	22:23.3	110,65,39,30,25,20,15, Surf
19.05	0310		96	27:34.3	22:32.7	Surf
	0902	62	100	27:34.3	22:33.6	Surf
	1320	63	102	27:40.7	22:16.4	110,65,45,39,35,30,25,20, 15, Surf
20.05	0342	67	105	27:39.2	22:16.8	15, Surf
	0411		106	27:39.3	22:16.6	25
20.05	0435	67	107	27:39.5	22:16.4	65,39
	0457		108	27:39.7	22:16.3	110,45,35,30
	0901	68	109	27:39.0	22:16.8	110,65,45,39,35,30,25,15, Surf
21.05	1127	74	119	27:40.5	22:12.9	65,39,35,30,27,25,15, Surf
22.05	0317		123	27:41.9	22:06.5	15, Surf
	0348		124	27:41.6	22:06.5	25
	0408		125	27:41.8	22:06.6	65,39
	0429		126	27:41.9	22:06.7	110,36,32,28

Preliminary results:

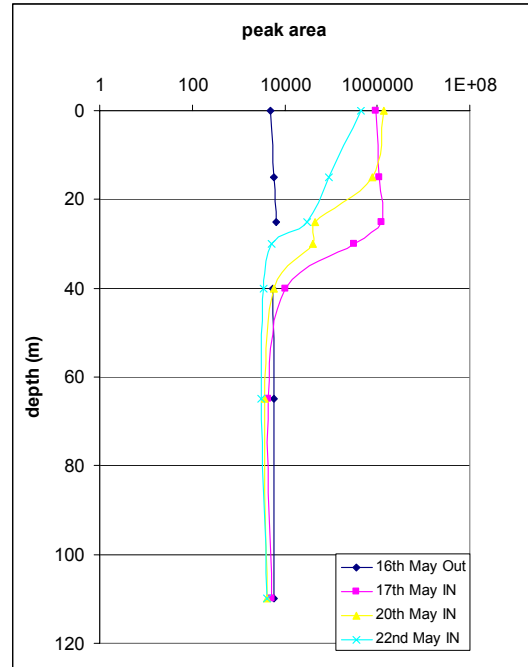
The SF6 provided an effective label for the enriched water, providing clear confirmation of whether a Station was IN or OUT. During the first experiment the SF6 distribution deepened from 40m to 80 m consistent with surface mixed layer, whereas in the 2nd experiment both SF6 distribution and mixed layer depth remained relatively constant to 28-35m. In both experiments additional depths were sampled at the pycnocline to improve spatial resolution of the SF6 distribution for greater accuracy in vertical diffusion (K_z) estimates. The SF6 measurements from Poseidon will be combined with the measurements taken on the Charles Darwin and used to determine Vertical diffusivity and mixing of the water column and horizontal spreading. SF6 will also be used to provide volume and dilution estimates for the patch, and so contributes to the generation of nutrient budgets.

SF6 profiles, depth against peak area, measured on Poseidon are shown below

SF6 profiles from the phosphate release experiment



SF6 profiles from the iron and phosphate release experiment



APPENDIX I

POSEIDON 311
PML CORE CRUISE APRIL-MAY 2004

STATION LOG

Station No. – consecutive from 01 for each station occupied; Event No. – ships station number; Event – CTD, Go-Flo, Net, Optics etc.; Comments – any relevant information.

DATE	TIME (gmt)	STN No.	EVENT NO.	EVENT	Lat	Long	COMMENTS
01.05	0800	A	98	CTD	27:47.5	22:31.4	CTD00 Shakedown
	1000	B	99	CTD	27:47.9	22:37.3	CTD01 Survey
	1220	C	100	CTD	27:49.3	22:53.2	CTD02 Survey (150m)
	“	“	“	“	“	“	CTD02a Re-drop (50m)
	1500		101	CTD	27:50.7	23:09.7	CTD03
02.05	1600	D	102	NET	27:52.9	23:30.4	Net trial
03.05	0200	01	103	NET	27:48.2	23:20.2	Patch Background
	0228		104	NET	27:48.0	23:20.2	
	0320		105	CTD	27:47.9	23:20.7	CTD04
	0400		106	CTD	27:47.9	23:20.7	CTD05
	0425		107	CTD	27:47.8	23:20.6	CTD06
	0455		108	CTD	27:47.8	23:20.7	CTD07
	1134	02	109	CTD	27:48.6	23:20.2	CTD08
	1800	03	111	NET	27:48.6	23:20.2	WP2 Triple
	1830		112	NET	27:48.6	23:20.2	WP2 Triple
04.05	0200	04	113	NET	23:48.3	23:20.5	WP2 Triple
	0230		114	NET	23:48.3	23:20.5	WP2 Triple
	0304	04	116	CTD	27:48.2	23:20.1	CTD09
	0340		117	CTD	27:47.9	23:19.8	CTD10
	0407		118	CTD	27:47.7	23:19.7	CTD11
	0441		119	CTD	27:48.2	23:20.1	CTD12
	1137	05	120	CTD	27:48.1	23:19.9	CTD13 1000m
	1230		121	CTD	27:48.1	23:19.9	CTD14
	1704	06	123	NET	27:46.6	23:21.6	WP2 Triple
	1725		124	NET	27:46.6	23:21.6	WP2 Triple
	1751		125	NET	27:46.9	23:22.0	Closing Net 200-50
	1825		126	NET	27:46.9	23:22.0	Closing Net 50-0
	2305	07	127	NET	27:46.4	23:21.6	WP2 Triple
	2333		128	NET	27:46.4	23:21.6	Closing Net 200-50
05.05	0003		129	NET	27:46.4	23:22.6	Closing Net 50-0
	0313	08	130	NET	27:48.2	23:17.8	WP2 Triple
	0337		131	NET	27:48.2	23:17.8	WP2 Triple
05.05	0420	09	132	CTD	27:48.0	23:17.5	CTD15
	0451		133	CTD	27:47.7	23:17.3	CTD16

DATE	TIME	STN No.	EVENT NO.	EVENT	Lat	Long	COMMENTS
	0530		134	CTD	27:46.7	23:17.6	CTD17
	0559		135	CTD	27:46.3	23:17.5	CTD18
	1136	10	136	CTD	27:46.5	23:18.4	CTD19
	1305	11	137	CTD	27:46.8	23:18.4	CTD20 (extra water)
	1701	12	138	NET	27:47.8	23:18.5	WP2 Triple
	1721		139	NET	27:47.8	23:18.5	WP2 Triple
	1746		140	NET	27:47.8	23:18.5	Closing Net 200-50m
	1815		141	NET	27:47.8	23:18.5	Closing Net 50-0m
	1837		142	NET	27:47.8	23:18.5	Closing Net 50-0m
	2309	13	143	NET	27:47.5	23:17.8	WP2 Triple
	2335		144	NET	27:47.5	23:17.8	Closing Net 200-50
	2354		145	NET	27:47.5	23:17.8	Closing Net 50-0
06-05	0014		146	NET	27:47.5	23:17.8	Closing Net 50-0
	0159	14	147	NET	27:46.0	23:16.7	WP2 Triple
	0222		148	NET	27:46.0	23:16.7	WP2 Triple
	0256		149	CTD	27:46.9	23:16.6	CTD21
	0326		150	CTD	27:46.3	23:16.3	CTD22
	0356		151	CTD	27:46.0	23:16.1	CTD23
	0429		152	CTD	27:45.8	23:16.0	CTD24
	0939	15	153	CTD	27:45.3	23:16.9	CTD25
	1022		154	CTD	27:45.0	23:16.8	CTD26
	1425	16	155	CTD	27:45.0	23:23.7	CTD27
	1512		156	CTD	27:44.9	23:23.8	CTD28
	1703	17	157	NET	27:45.1	23:23.8	WP2 Triple
	1725		158	NET	27:45.3	23:23.9	WP2 Triple
	2302	18	159	NET	27:45.1	23:23.7	WP2 Triple
	2323		160	NET	27:45.1	23:23.7	WP2 Triple
07-05	0207	19	161	NET	27:45.0	23:23.8	WP2 Triple
	0233		162	NET	27:45.0	23:23.8	WP2 Triple
	0309		163	CTD	27:45.0	23:23.7	CTD29
	0338		164	CTD	27:44.8	23:23.3	CTD30
	0409		165	CTD	27:44.8	23:23.3	CTD31
	0440		166	CTD	27:44.5	23:23.8	CTD32
	0938	20	167	CTD	27:44.9	23:23.7	CTD33
	1026		168	CTD	27:45.0	23:23.8	CTD34
	1340	21	169	CTD	27:44.6	23:13.7	CTD35
	1435		170	CTD	27:44.8	23:13.8	CTD36
	1635	22	171	NET	27:44.8	23:13.8	WP2 Triple
	1658		172	NET	27:44.8	23:13.8	WP2 Triple
	1725		173	NET	27:44.8	23:13.8	WP2 Triple
	2302	23	174	NET	27:41.9	23:16.3	WP2 Triple
08-05	0159	24	175	NET	27:41.9	23:16.3	WP2 Triple (0-200m)
08-05	0216		176	NET	27:41.9	23:15.8	WP2 Triple (0-200m)
	0305		177	CTD	27:42.2	23:15.8	CTD37
	0337		178	CTD	27:42.3	23:15.6	CTD38
	0405		179	CTD	27:42.3	23:15.6	CTD39

DATE	TIME	STN No.	EVENT NO.	EVENT	Lat	Long	COMMENTS
	0442		180	CTD	27:42.3	23:15.5	CTD40
	0932	25	181	CTD	27:40.0	23:14.0	CTD41
	1013		182	CTD	27:40.1	23:13.9	CTD42
	1331	26	183	CTD	27:30.1	23:13.6	CTD43
	1423		184	CTD	27:29.9	23:13.3	CTD44
	1657	27	185	NET	27:29.7	23:13.3	WP2 Triple (0-200m)
	1713		186	NET	27:29.7	23:13.5	WP2 Triple (0-200m)
	1729		187	NET	27:29.7	23:13.6	WP2 Triple (0-200m)
	2254	28	188	NET	27:29.7	23:13.3	WP2 Triple (0-200m)
09-05	0153	29	189	NET	27:30.4	23:13.1	WP2 Triple (0-200m)
	0215		190	NET	27:30.6	23:13.1	WP2 Triple (0-200m)
	0302		191	CTD	27:30.7	23:12.9	CTD45
	0335		192	CTD	27:30.5	23:12.5	CTD46
	0408		193	CTD	27:30.3	23:12.0	CTD47
	0433		194	CTD	27:30.1	23:11.5	CTD48
	0913	30	195	CTD	27:29.5	23:12.7	CTD49
	0951		196	CTD	27:29.2	23:12.4	CTD50
	1303	31	197	CTD	27:42.0	23:12.3	CTD51
	1335		198	CTD	27:42.2	23:12.3	CTD52 (6,7,10 taps op)
	1703	32	199	NET	27:42.2	23:12.5	WP2 Triple 0-200m
	1721		200	NET	27:42.3	23:12.9	WP2 Triple 0-200m
	2316	33	201	NET	27:39.6	23:16.3	WP2 Triple 0-200m
	2339		202	NET	27:39.7	23:16.5	WP2 Triple 0-200m
10-05	0133	34	203	CTD	27:39.3	23:16.0	CTD53
	0202	35	204	NET	27:39.4	23:16.1	WP2 Triple 0-200m
	0225		205	NET	27:39.5	23:16.3	WP2 Triple 0-200m
	0308		206	CTD	27:39.2	23:15.0	CTD54
	0336		207	CTD	27:39.1	23:14.6	CTD55
	0411		208	CTD	27:39.1	23:14.2	CTD56
	0440		209	CTD	27:39.0	23:14.0	CTD57
	1230	36	210	CTD	27:41.5	22:59.3	CTD58
	1320		211	CTD	27:41.0	22:56.7	CTD59
14-05	0700	37	212	CTD	27:48.8	22:23.2	CTD60 Survey
	0943	38	213	CTD	27:46.8	22:43.8	CTD61 Survey
	1206	39	214	CTD	27:45.0	23:02.8	CTD62 Survey
	22:59	40	215	NET	27:37.0	23:16.3	WP2 Triple 0-200m
15-04	01:58	41	216	NET	27:37.0	23:16.2	WP2 Triple 0-200m
	02:19		217	NET	27:37.1	23:16.3	WP2 Triple 0-200m
15-05	0300	41	218	CTD	27:37.1	23:16.4	CTD63 Old Patch
	0329		219	CTD	27:37.0	23:16.5	CTD64
	0353		220	CTD	27:36.8	23:16.4	CTD65
	0419		221	CTD	27:36.5	23:16.4	CTD66
	0952	42	222	CTD	27:36.9	23:16.4	CTD67 Old Patch
	1335	43	223	CTD	27:36.9	22:50.3	CTD68 Out
	1446		224	CTD	27:36.7	22:49.7	CTD69
	1659	44	225	NET	27:32.3	22:34.4	WP2 Triple 0-200

DATE	TIME	STN No.	EVENT NO.	EVENT	Lat	Long	COMMENTS
	1716		226	NET	27:32.4	22:34.5	WP2 Triple 0-200
	1743		227	NET	27:32.5	22:34.6	Closing Net 200-50
	1815		228	NET	27:32.6	22:34.8	Closing Net 50-0
	1834		229	NET	27:32.7	22:34.8	Closing Net 200-50
	2302	45	230	NET	27:32.4	22:34.3	WP2 Triple 0-200
	2322		231	NET	27:32.4	22:34.4	WP2 Triple 0-50
16-05	0138	46	232	CTD	27:32.1	22:34.2	CTD70 25m
	0200		233	NET	27:31.8	22:33.8	WP2 Triple 0-200
	0233		234	NET	27:31.8	22:33.8	WP2 Triple 0-200
	0300		235	CTD	27:32.4	22:34.3	CTD71
	0331		236	CTD	27:32.3	22:33.9	CTD72
	0355		237	CTD	27:32.1	22:33.7	CTD73
	0417		238	CTD	27:32.1	22:33.4	CTD74
	1509	47	239	CTD	27:33.1	22:33.0	CTD75 2 nd Patch
	1548		240	CTD	27:33.0	22:32.7	CTD76
	1656	48	241	NET	27:33.3	22:32.7	WP2 Triple 0-200
	1716		242	NET	27:33.3	22:32.7	WP2 Triple 0-200
	1731		243	NET	27:33.4	22:32.7	WP2 Triple 0-50
	2330	49	244	NET	27:33.4	22:30.9	WP2 Triple 0-200
	2346		245	NET	27:33.6	22:30.9	WP2 Triple 0-200
17-05	0004		246	NET	27:33.7	22:30.8	WP2 Triple 0-50
	0201	50	247	NET	27:33.7	22:30.2	WP2 Triple 0-200
	0220		248	NET	27:33.8	22:30.1	WP2 Triple 0-200
	0257		249	CTD	27:33.7	22:29.8	CTD77
	0331		250	CTD	27:33.6	22:29.6	CTD78
	0355		251	CTD	27:33.4	22:29.4	CTD79
	0424		252	CTD	27:33.5	22:29.5	CTD80
	0451		253	CTD	27:33.4	22:29.3	CTD81
	0900	51	254	CTD	27:37.9	22:28.0	CTD82
	0958		255	CTD	27:38.0	22:27.9	CTD83
	1029		256	CTD	27:38.0	22:27.7	CTD84 1000m
	1305	52	257	CTD	27:33.3	22:28.0	CTD85
	1338		258	CTD	27:33.2	22:27.8	CTD86
	1919	53	259	NET	27:34.4	22:27.0	WP2 Triple 0-200
17-05	1936	53	260	NET	27:34.5	22:26.9	WP2 Triple 0-200
	1950		261	NET	27:34.5	22:26.8	WP2 Triple 0-200
	2020		262	NET	27:34.6	22:26.8	WP2 Triple 0-50
	2302	54	263	NET	27:34.9	22:26.0	WP2 Triple 0-200
	2324		264	NET	27:35.0	22:25.7	WP2 Triple 0-50
18-05	0130	55	265	CTD	27:35.5	22:25.7	CTD87 (25m)
	0148		266	NET	27:35.4	22:25.1	WP2 Triple 0-200
	0207		267	NET	27:35.4	22:25.1	WP2 Triple 0-200
	0304	56	268	CTD	27:35.6	22:23.9	CTD88
	0340		269	CTD	27:35.5	22:23.6	CTD89
	0403		270	CTD	27:35.4	22:23.4	CTD90
	0428		271	CTD	27:35.3	22:23.2	CTD91

DATE	TIME	STN No.	EVENT NO.	EVENT	Lat	Long	COMMENTS
	0912	57	272	CTD	27:35.0	22:23.3	CTD92
	0958		273	CTD	27:34.9	22:23.3	CTD93
	1302	58	274	CTD	27:34.0	22:32.5	CTD94
	1334		275	CTD	27:33.8	22:32.6	CTD95
	1703	59	276	NET	27:34.2	22:33.5	WP2 Triple 0-200
	1719		277	NET	27:34.3	22:33.6	WP2 Triple 0-200
	1739		278	NET	27:34.4	22:33.6	WP2 Triple 0-50
	2306	60	279	NET	27:34.4	22:33.5	WP2 Triple 0-200
	2324		280	NET	27:34.5	22:33.2	WP2 Triple 0-200
	2341		281	NET	27:34.5	22:32.9	WP2 Triple 0-50
19-05	0206	61	282	NET	27:34.3	22:33.5	WP2 Triple 0-200
	0224		283	NET	27:34.3	22:33.1	WP2 Triple 0-200
	0310		284	CTD	27:34.3	22:32.7	CTD96
	0341		285	CTD	27:34.6	22:32.6	CTD97
	0403		286	CTD	27:34.8	22:32.6	CTD98
	0427		287	CTD	27:34.8	22:32.6	CTD99
	0902	62	288	CTD	27:34.3	22:33.6	CTD100
	0937		289	CTD	27:34.4	22:33.6	CTD101
	1320	63	290	CTD	27:40.7	22:16.4	CTD102
	1406		291	CTD	27:40.9	22:16.1	CTD103
	1659	64	292	NET	27:40.5	22:15.8	WP2 Triple 0-200
	1717		293	NET	27:40.4	22:15.9	WP2 Triple 0-200
	1758		294	NET	27:40.2	22:16.1	WP2 Triple 0-50
	2301	65	295	NET	27:40.8	22:15.3	WP2 Triple 0-200
	2326		296	NET	27:40.9	22:15.4	WP2 Triple 0-50
20-05	0137	66	297	CTD	27:40.9	22:16.4	CTD104 (25m)
	0157		298	NET	27:41.0	22:16.4	WP2 Triple 0-200
	0214		299	NET	27:41.1	22:16.4	WP2 Triple 0-200
	0342	67	300	CTD	27:39.2	22:16.8	CTD105
	0411		301	CTD	27:39.3	22:16.6	CTD106
20-05	0435	67	302	CTD	27:39.5	22:16.4	CTD107
	0457		303	CTD	27:39.7	22:16.3	CTD108
	0901	68	304	CTD	27:39.0	22:16.8	CTD109
	0941		305	CTD	27:39.0	22:16.7	CTD110
	1305	69	306	CTD	27:38.5	22:22.1	CTD111
	1336		307	CTD	27:38.5	22:21.9	CTD112
	1701	70	308	NET	27:39.0	22:23.6	WP2 Triple 0-200
	1716		309	NET	27:39.0	22:23.9	WP2 Triple 0-200
	1731		310	NET	27:38.9	22:23.7	WP2 Triple 0-50
	2259	71	311	NET	27:39.1	22:23.7	WP2 Triple 0-200
	2319		312	NET	27:39.0	22:23.6	WP2 Triple 0-200
	2336		313	NET	27:39.0	22:23.6	WP2 Triple 0-50
21-05	0201	72	314	NET	27:39.0	22:23.7	WP2 Triple 0-200
	0218		315	NET	27:38.9	22:23.7	WP2 Triple 0-200
	0303		316	CTD	27:38.9	22:23.6	CTD113
	0334		317	CTD	27:38.9	22:23.5	CTD114

DATE	TIME	STN No.	EVENT NO.	EVENT	Lat	Long	COMMENTS
	0355		318	CTD	27:39.0	22:23.4	CTD115
	0417		319	CTD	27:39.0	22:23.3	CTD116
	0900	73	320	CTD	27:39.0	22:23.6	CTD117
	0930		321	CTD	27:39.0	22:23.6	CTD118
	1127	74	322	CTD	27:40.5	22:12.9	CTD119 Intercalibrate
	1540	75	323	CTD	27:41.5	22:11.0	CTD120
	1620		324	CTD	27:41.6	22:10.8	CTD121
	1656	76	325	NET	27:41.4	22:10.6	WP2 Triple 0-200
	1711		326	NET	27:41.3	22:10.4	WP2 Triple 0-200
	1728		327	NET	27:41.3	22:10.3	WP2 Triple 0-50
	2306	77	328	NET	27:41.5	22:10.1	WP2 Triple 0-200
	2322		329	NET	27:41.5	22:10.0	WP2 Triple 0-50
22-05	0155	78	330	CTD	27:41.5	22:06.6	CTD122 (25m)
	0208		331	NET	27:41.6	22:06.6	WP2 Triple 0-200
	0225		332	NET	27:41.6	22:06.5	WP2 Triple 0-200
	0317		333	CTD	27:41.9	22:06.5	CTD123
	0348		334	CTD	27:41.6	22:06.5	CTD124
	0408		335	CTD	27:41.8	22:06.6	CDT125
	0429		336	CTD	27:41.9	22:06.7	CTD126

APPENDIX II

DNA extraction from seawater – Sterivex filters

1. Prefilter seawater² using a 5µm or 2µm Durapore filter and collect bacteria in a 0.2µm Sterivex filter – normally 10-20 litres using a peristaltic pump system.
2. Push all remaining liquid through the filter and add 1.8mL (50mM Tris-Cl pH 8.3, 40mM EDTA, 0.75M sucrose) to the filter with a pipette. Store filters at -20°C or -80°C until extraction.
3. Add 50µL Lysozyme (2mg in 50µL lysis buffer → final conc. 1mg.mL⁻¹ [prepare fresh]). Incubate at 37°C for 45 min with gentle agitation.
4. Add 50µL Proteinase K (0.4mg in 50µL lysis buffer → final conc. 0.2 mg.ml⁻¹ prepare fresh]) and 200µL 10% SDS. Incubate for 1 hour at 55°C with gentle agitation.
5. Recover lysate from Sterivex filter using a syringe and expel into a 15mL Falcon tube. Add 700µL lysis buffer to the Sterivex and incubate at 55°C for 15 minutes. Recover lysate and combine with previous lysate.
6. Extract **twice** with phenol / chloroform / isoamyl alcohol – add an equal volume (3mL) of phenol / chloroform / isoamyl alcohol, mix gently and centrifuge for 10 minutes (RCF=3000). Remove aqueous phase to a clean tube and repeat.
7. Extract once with chloroform / isoamyl alcohol - add an equal volume (3mL) chloroform / isoamyl alcohol, mix gently and centrifuge for 10 minutes (RCF=3000). Remove aqueous phase to a clean tube.
8. Concentrate aqueous phase using a Centricon concentrator (reduce volume from 3mL to 200µL (RCF = 1000). Add 1mL MilliQ water and reduce volume to 100µL. Add a further 1mL MilliQ, reduce to 100µL and repeat.
9. Collect concentrated nucleic acids and store at -20°C.

² May not be required in oligotrophic waters

