

## 12. Phytoplankton measurements

### 12.1 Phytoplankton Community Structure, Productivity and New & Regenerated Production

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#### 12.1.1 Overview

We had planned to make primary production (PP) measurements every two days, resulting in 12 PP stations on each leg of the cruise. However, we managed 10 PP stations on each of the D285 and D286 legs; losing two on each leg either to poor weather or to alternate activities. Size-fractionated (total,  $>20\mu\text{m}$ ,  $>2\mu\text{m}$ ) primary productivity ( $^{14}\text{C}$ ) plus “new” and “regenerated” production ( $^{15}\text{N-NO}_3$ ,  $^{15}\text{N-NH}_4$ ,  $^{15}\text{N-urea}$ ) simulated *in situ* incubations were undertaken at six light depths (97, 55, 33, 14, 4.5, 1%) on seawater samples collected in an Fe free manner from night-time Ti-CTD casts between 11th Nov and 3rd December 2004 (D285) and between 18<sup>th</sup> December and 13<sup>th</sup> January (D286). For the second leg, we introduced an additional  $10\mu\text{m}$  size-fraction for chlorophyll analyses and for the  $^{15}\text{N}$  size-fractionated uptake experiments, but not for the PP work.

Seawater was collected directly into 3 x 2L Fe clean Nalgene polycarbonate bottles that were then transferred in dark cool boxes to the laminar flow hood in the RN container. Here the water was split into sub-samples required for  $^{14}\text{C}$  and  $^{15}\text{N}$  uptake and  $\text{NH}_4$  regeneration experiments as well as water for nutrient and size-fractionated chl-a measurements. Incubations were run for a 24 hour period in Perspex incubation tubes cooled with surface seawater and covered with neutral density (Lee: Misty Blue [061] & Neutral Density Grey [210 ND]) filters to re-construct water column light attenuation spectrally corrected to remove red light.



Wherever possible, all incubation bottles were placed in the incubators before dawn. In addition, some Ti-CTD water samples (“surface” & “deep”) were used to construct  $^{14}\text{C}$  P vs E curves that were size-fractionated on some occasions.

Additionally, a water sample (~20L) was collected from the underway Fe-fish (~10m) on approaching the Ti-CTD (PP) station for size-fractionated (total,  $<20\mu\text{m}$ ,

Fig. 12.1 Incubator tubes set up on a steel frame so that waves would not wash them overboard! The RN container is behind and the SeaSoar winch is on the right.

<2 $\mu$ m)  $^{15}\text{N}$  uptake experiments ( $\text{NO}_3$ ,  $\text{NH}_4$ , urea). However, from 27 Nov. onwards (Stn. 15524), water was collected from the Ti-CTD because of Fe contamination problems with the Fe fish.

Size-fractionated chl-a measurements (total, >20 $\mu$ m, >2 $\mu$ m) and nutrient determinations ( $\text{NO}_3$ , Si,  $\text{PO}_4$ ,  $\text{NH}_4$  & urea) were also made from the Ti-CTD PP water samples as well as from the Fe fish water samples when this was used.

The PP Station Logs are shown in Tables 12.1 and 12.2. A summary of the PP experiments performed is given in Tables 12.3 and 12.4.

### 12.1.2 Primary Productivity ( $^{14}\text{C}$ )

#### 12.1.2.1 Size-fractionated on-deck incubations

Water for the  $^{14}\text{C}$  incubation experiments was withdrawn from the “ $\text{NO}_3$ ” labelled 2L polycarbonate Fe clean bottle into which water from the Ti-CTD had been decanted directly (and prior to  $^{15}\text{N}$  spiking). For each light depth, 4 seawater samples (3 replicates at each depth and 1 dark bottle) in 60ml acid-rinsed polycarbonate bottles were inoculated with  $\sim 10 \mu\text{Ci NaOH}^{14}\text{CO}_3$  (100 $\mu\text{l}$  stock solution) in the laminar flow hood. The same procedure was carried out for size-fractionated primary productivity. These bottles were placed in the on-deck incubators together with the  $^{15}\text{N}$  experiment bottles for a 24 hour period before recovering the incubations. Five total activity standards were made up in 7ml polycarbonate vials by adding 10ml Packard “Carbosorb” ( $\text{CO}_2$  trapping agent) to 100 $\mu\text{l}$   $^{14}\text{C}$  working stock then dispensing 100 $\mu\text{l}$  of this solution into the pony vials before adding 5ml Packard “Permafluor” scintillation cocktail. At the end of the experiment, samples were filtered under vacuum onto 25mm diameter, 0.2 $\mu\text{m}$  (Whatman), 2 $\mu\text{m}$  and 20 $\mu\text{m}$  (Osmonics) polycarbonate filters.

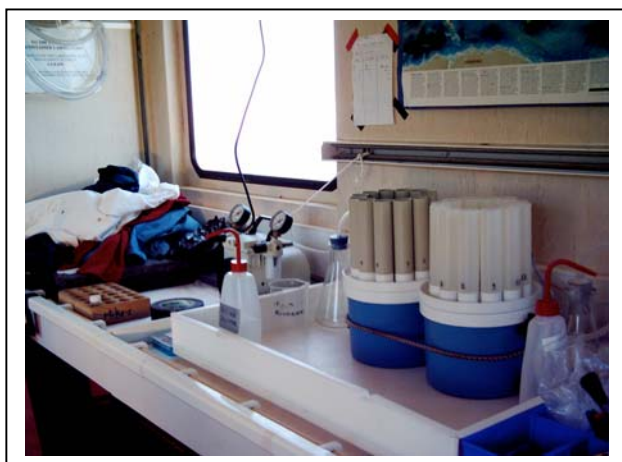


Fig. 12.2 Two x 12 port filtration rigs for  $^{14}\text{C}$  filtering in the RN container

Filters were rinsed with filtered seawater and 0.5% HCL solution made up in artificial seawater before being acid-fumed under a fume hood for several hours to expel any unfixed inorganic  $^{14}\text{C}$ . The filters were then placed in 7ml polyethylene Pony vials and 5ml Packard “Hi-Safe 3” scintillation cocktail was added before samples were counted on-board on a Tri-Carb 3100 TR liquid scintillation counter.

#### 12.1.2.2 P vs E experiments

PvsE incubations were carried out at 8 out of the 11 PP stations on leg 1 and at 9 of the 10 PP stations on leg 2 (see Tables 12.3 & 12.4 for details on depth and size fractions). For these, water was collected into separate 2L polycarbonate bottles (generally 2 x 2L bottles at each depth [surface and 4.5% light depth]) and transferred into 15 light and 3 dark 60mL polycarbonate bottles, under the laminar flow hood, and spiked with 10 $\mu\text{Ci}$   $^{14}\text{C}$  stock. These were then placed in racks in the PvsE incubators, where they were subjected to a light gradient ( $\sim 3\text{-}900 \mu\text{E m}^{-2} \text{s}^{-1}$ ) for 2h. The incubator channels were chilled by a flow of surface water from the non-toxic supply; in addition, glass water jackets with circulating surface water were placed in between the light sources (halogen lamps) and the incubators, in order to reduce the heat emitted by the lamps. Blue

filters (Lee “misty blue” [061]) were placed on the perspex front-ends of the incubators to screen out light from the red end of the spectrum. Filters (neutral density grey [0.3 ND]) were also placed before and after the 14<sup>th</sup> bottle in the sequence, to achieve a near-zero irradiance at the far end of the channel. At the end of the incubation, the same filtering protocol was followed as for the primary production samples.

#### 12.1.2.3 P vs E experiments in association with Fe enrichment experiments

To test the theory of Fe limited growth rates by phytoplankton, a total of eight of 5-6 day bottle incubations (+ Fe additions) were carried out in “high light” and “low light” deck incubators. At time zero and at the end of the experiment, P vs E incubations (and FRRf measurements) were carried out to test phytoplankton physiological responses to light with and without Fe supplements. The P vs E protocols for these experiments were identical to those described above. For each experiment, there were four treatments and therefore 4 x end of experiment P vs E curves; viz. one each for high light control (HLC), high light + Fe (HLFe), low light control (LLC) and low light + Fe (LLFe). In addition to the Pvs E curves, chl-a determinations and HPLC, POC and Lugol’s samples were taken from each treatment bottle. The FRRf experiments are described elsewhere in cruise reports by M. Moore and A. Hickman.

#### **12.1.3 New and regenerated production (<sup>15</sup>N)**

##### 12.1.3.1 Standard <sup>15</sup>N Uptakes

Three water sub-samples were taken for analyses of “new” and “regenerated” production; one each for NO<sub>3</sub>, NH<sub>4</sub> and urea uptake. Approx. 1L samples (but ~2 L for NH<sub>4</sub>) decanted directly from the Ti-CTD into 3 x acid rinsed Fe clean 2.0L polycarbonate bottles for each light depth were inoculated (in the RN container Laminar Flow Hood) with 200µl stock solutions of K<sup>15</sup>NO<sub>3</sub> (1µmol / 100µl) and <sup>15</sup>NH<sub>4</sub>Cl (0.1µmol / 100µl) and 100µl of CO(<sup>15</sup>NH<sub>2</sub>)<sub>2</sub> (0.1µmol / 100µl) respectively. The volume of <sup>15</sup>N spike in each case was adjusted to approximately 10% of the ambient nutrient concentration – typically ~ 20µmol NO<sub>3</sub> and <1µmol for NH<sub>4</sub> and urea respectively. To avoid the risk of Fe contamination, the initial incubation volumes were not measured out, as is usual. Instead, the incubation volume was measured at the end of the experiment. However, immediately after spiking the NH<sub>4</sub> incubation bottle (~2L), exactly 1.0 L was withdrawn into a separate 2.0L polycarbonate bottle to measure time zero (To) ammonium concentrations (see ammonium regeneration, below). The remaining unknown volume (but ~1L) was used for the NH<sub>4</sub> uptake incubation.

At the end of the incubation period (+24hrs), all <sup>15</sup>N incubations were terminated by filtering the incubation volumes (measured at this point) onto 25 mm ashed GF/F filters. After filtration, the filters were stored at –20 °C to await analysis by stable isotope mass spectrometry back at SOC. To monitor changes to the chl-a and nutrient concentrations during the incubation, post-incubation samples were recovered from each light depth and treatment for chl-a and nutrient (NO<sub>3</sub>, Si, P, NH<sub>4</sub> and urea) measurements. All urea samples were frozen at –20°C in ~80ml acid-cleaned glass bottles for later analysis at the University of Cape Town.

##### 12.1.3.2 Ammonium regeneration

Isotopic dilution (<sup>15</sup>N-NH<sub>4</sub> by excreted <sup>14</sup>N-NH<sub>4</sub>) ammonium regeneration experiments were conducted simultaneously with the ammonium uptake experiments. This is essential to correct the NH<sub>4</sub> uptakes for NH<sub>4</sub> re-cycling in the incubation bottles. 1L recovered from each of the 6 depths (time zero) NH<sub>4</sub> uptake incubation bottles (above) was immediately filtered through a 25mm

(ashed) Whatman GF/F filter to collect 900ml filtrate for transfer into 6 x 1.0L glass Schotte bottles. Exactly 600µl NH<sub>4</sub>Cl solution (10µmol / ml) was added to each bottle as a “carrier” prior to freezing the samples at –20°C. This sample provides the time zero NH<sub>4</sub> regeneration concentration (R<sub>0</sub>). The GF/F filter (PN) from this sample was retained for HPLC analyses. (See below). At the end of the 24 hr incubation period, a further 900ml of filtrate was recovered from the NH<sub>4</sub> uptake filtration to measure <sup>15</sup>N isotopic dilution by excreted NH<sub>4</sub>, Carrier (600µl) was added as before and the sample (R<sub>t</sub>) was also frozen as before. Back in Cape Town, the aqueous NH<sub>4</sub> will be recovered onto GF/F filters by diffusion and the isotopic composition measured by mass spectrometry back at SOC to provide a measure of NH<sub>4</sub> regeneration.

#### 12.1.3.3 Size-fractionated <sup>15</sup>N uptakes

Water from the Fe-fish @ ~10m, or later (after Nov. 27<sup>th</sup>) from the Ti-CTD (usually ~25m), was decanted directly into 3 x 6.0L volume Perspex bottles for size-fractionated NO<sub>3</sub>, NH<sub>4</sub> and urea uptake experiments. Spikes (<sup>15</sup>N) to ~5-6 L water were added at the same concentrations as for the standard un-fractionated incubations and the incubation bottles were incubated in the 55% light tube for 24 hours. At the end of the experiment, each bottle treatment was fractionated into “total” (i.e. un-fractionated), < 20µm (plankton mesh screened) and < 2µm (membrane filters) sub-samples of approx. 1-2 L depending on the chl-a concentration and filtered onto 25mm ashed Whatman GF/F filters. As before, these filters were frozen at –20°C for later mass spectrometry analyses at SOC.

### **12.1.4 Chlorophyll, nutrients and preserved phytoplankton samples**

#### 12.1.4.1 Chlorophyll

Approximately 1L of sample water at each depth was withdrawn from the “urea” labelled Fe clean 2.0L polycarbonate (<sup>15</sup>N) incubation bottle into separate 1 L polycarbonate bottles to make immediate measurements of size-fractionated chl-a (total, >20µm, >2µm). Between 100-200ml sample was filtered separately onto a GF/F (total) and onto 20µm & 2µm filters respectively. On the second cruise (D286), an additional 10µm fraction was included for chl-a analyses. The filters were placed in 20ml glass scintillation vials and 10mls 90% HPLC grade Acetone was added for pigment extraction over 24 hours in a fridge. Pigment was measured fluorometrically on a Turner fluorometer following the Welschmeyer protocol. The Fluorometer was calibrated with a chl-a standard (Sigma) read on a Spectrophotometer.

#### 12.1.4.2 Nutrients

Approx 20mls of the water sample from each depth (above) was placed in diluvials for immediate analysis of NO<sub>3</sub>, Si & PO<sub>4</sub> on a Skalar autoanalyser on-board. Ammonium measurements were made on fresh samples (T<sub>0</sub>) and at T<sub>24</sub> from the incubation bottles using a modified (Probyn) Indo-Phenol Blue protocol (Grasshof) for small 5ml samples. The developed colour (after 8 hours) was measured on a Unicam Spectrophotometer at 630nm and NH<sub>4</sub> concentrations were calculated from a calibration curve constructed from NH<sub>4</sub>Cl “carrier” solution (10µmol / ml) additions to artificial sea-water. Urea samples at T<sub>0</sub> and at T<sub>24</sub> were collected and frozen for later analysis as described above.

#### 12.1.4.3 HPLC

Apart from HPLC samples taken from the Ti-CTD “associated” stainless steel CTD casts, the GF/F filter sample (usually 1.0L) from the time-zero filtration of the ammonium regeneration bottle at each of the six light depths was frozen at –80°C for later HPLC analysis.

#### 12.1.4.4 Lugols and Formalin preserved phytoplankton & microzooplankton samples

There was insufficient water from the Ti-CTD casts to preserve phytoplankton samples for later taxonomy. However, for each stainless steel CTD sampled, preserved Lugol's and Formalin samples were typically taken at 10m, at the chl-a max (40-80m) and sometimes within the thermocline (~100-125m). For each, 200mls of sample was preserved in a brown glass medicine bottle containing 4mls Lugols Iodine, and in a separate bottle, 4mls buffered Formalin to yield a final concentration of 2% for each.

#### **12.1.5 Progress & Plans for Analysing Frozen or Preserved Samples**

##### Phytoplankton (<sup>14</sup>C) Production

All samples have been successfully counted at sea using the on-board scintillation counter. Simulated *in situ* productivity rates have been calculated and P vs E curves and parameters have been calculated also. Integrated water column production rates have been calculated but these calculations will require some revision based on accurately determined light depths from the limited PAR profiles taken on daylight Thorium CTD casts and also from the PAR sensor on SeaSoar. Given that the <sup>14</sup>C results are at an advanced stage of analysis, a manuscript can be prepared for publication as soon as some preliminary analysis of phytoplankton community structure (HPLC & taxonomy) has been done, as well as a hydrographic and ocean colour (satellite imagery) framework to place the results in context.

##### New (<sup>15</sup>N) Production

Although the incubations have been successfully completed, the frozen (@ -20°C) filter samples (840; including regeneration samples, below) need to be dried, punched & pelleted before the samples can be run on the Mass Spec back at SOC. Some sample preparation has been carried out at sea. The remaining <sup>15</sup>N sample preparation will be carried out at the University of Cape Town (UCT) prior to transporting the dried and pelleted samples back to SOC for running on the Mass Spec. To calculate <sup>15</sup>N uptake rates, we need to know ambient nutrient concentrations of nitrate, ammonium and urea. Both the nitrate and ammonium values have been measured at sea but the frozen urea samples (~180) will need to be measured back at the University of Cape Town.

##### Ammonium Regeneration (Ro + Rt)

Approximately 240 Schotte Bottles (1.0L) containing 900mls of frozen filtrate from which the regenerated ammonium will be transported to Cape Town (UCT) so that regenerated ammonium can be recovered by diffusion. The process is simple but time-consuming. After each bottle has been thawed, a teaspoon of MgO is added to the bottle which raises the Ph to ~9. This drives the ammonium into the head-space of the bottle and the ammonium is recovered as ammonium chloride on an HCL acidified 25mm Whatman GFF filter placed in the bottle cap. Diffusion recovery of the ammonium takes approx. 3 weeks at ambient room temperature. Once this has been completed, the filters are dried, punched and pelleted as before prior to running the samples on the Mass Spec. Assuming the ammonium recovery procedure is completed within February, the regeneration samples will be ready for the Mass Spec in March.

##### Chlorophyll

All samples – some 2500! - have been measured on the Turner fluorometer and calculated. On the first leg, all the PP stations were fractionated for the six light depths into total, >20µm & >2µm fractions. However, on the second leg, we included a >10µm fraction to assess the contribution of

small diatoms, if any, in the absence of the large colonies of the prymnesiophyte flagellate, *Phaeocystis* sp. that characterised the high chlorophyll stations during Leg 1. Repeat visits to station M3 at the end of Leg 2 were rewarded with this policy where total chl-a concentrations reached  $\sim 6\text{mg m}^{-3}$  in a shallow (<40m) surface layer, dominated by small diatoms mostly ( $\sim 60\%$ ) in the 10-20 $\mu\text{m}$  fraction.

### HPLC

At each PP station for both legs of the cruise, HPLC samples are available for each of the six light depths. They were frozen at  $-80^\circ\text{C}$  and will be shipped in a dry (liquid N) shipper to UCT. One option is to analyse the HPLC samples ( $\sim 120$  samples) in Cape Town, or to ship them back to SOC to run on the SOC HPLC instrument. The problem with the latter option is that there is currently only one HPLC instrument in operation at SOC that is fully committed to AMT samples. Permission to use the AMT instrument is required.

### SeaSoar and Underway (NT supply) FRRf (see Report by Moore & Hickman)

Although the experimental FRRf work on the Fe incubation experiments is of paramount importance, there are useful analyses and results to be obtained from the SeaSoar and underway NT FRRf measurements; particularly in terms of 3-D mapping of phytoplankton distribution and physiological “fitness”.

### Preserved Phytoplankton and Micro-zooplankton samples (Formalin & Lugols)

There are approximately 140 phytoplankton samples preserved in Lugols, and approx. 100 samples preserved in buffered Formalin. In addition there are approx. 100 samples preserved in Formalin for micro-zooplankton enumeration. These samples have been carefully numbered and crated for transport back to SOC. It is not yet clear who will undertake the phytoplankton counting; this task alone requires approximately 3-6 months to complete, including the calculation of bio-volume and cellular carbon for the different taxa.

### Nutrients

All the nutrients required for the  $^{15}\text{N}$  uptake and ammonium regeneration calculations have been analysed on-board, with the exception of the frozen urea samples. These will be analysed at UCT (see earlier commentary).

### POC, PIC & BSi

These primary “state variables” were obtained from the stainless steel CTD casts and are linked at the PP stations with the appropriate TI CTD. For each CTD deployment, 12 standard depths to 500m were sampled. At two depths within the euphotic layer (surface & chl-a max.), the state variables (except for PIC) were size-fractionated into total, 20 & 2 $\mu\text{m}$  fractions, with a 10 $\mu\text{m}$  fraction being added on the second leg. Commentary on the chl-a samples has already been provided above so will be ignored here.

The POC samples ( $\sim 800$ ) were frozen on-board at  $-20^\circ\text{C}$  and will be transported back to UCT to be acid-fumed (to remove PIC), dried and pelleted in preparation for running on a CHN analyser. Most of the POC samples originated from the “standard” stainless steel CTD casts but on the second leg in particular, Thorium CTD casts generated their own chl-a and POC samples. There is some doubt about running the POC samples at SOC because the SOC instrument is set up to run geochemistry (rock) samples so the precision for low value phytoplankton POC samples is

poor. The PML instrument is probably the more appropriate instrument to run the samples on. Once again, Ms Seeyave emerges as the most likely person to process the POC samples.

The PIC samples (~600) have been stored semi-dried at room temperature in 20ml plastic scintillation vials and can be readily transported back to SOC for analysis. Calcite needs to be extracted by acidification and the samples run on the IPC Atomic Emission Spectrometer (AES) at SOC. Who will do this is unclear, but the PIC sample analysis is a low priority.

The BSi samples (~700) have been digested and run on-board ship by Ms Megan French (UEA). The results look extremely interesting indeed and one obvious question is whether we can match inorganic silicate draw-down with BSi production. Although preliminary analyses have therefore been completed, further interpretation remains to be done

### **12.1.6 Conclusion & Highlights**

The phytoplankton research undertaken during CROZEX has gone exceptionally smoothly and results we have to date are exciting. All phytoplankton communities demonstrate varying degrees of Fe stress and Si limitation with N being abundant everywhere. Photo-physiological responses to Fe and light are complex and require careful interpretation and indeed we don't yet have a clear idea of the processes at work. Productivity rates varied from as low as 100mg m<sup>-2</sup> d<sup>-1</sup> (M2) to as high as 3000 mg m<sup>-2</sup> d<sup>-1</sup> in the diatom-dominated communities we encountered at M3 late in the second leg of the cruise. *Phaeocystis* were prolific almost everywhere on the first leg of the cruise wherever chlorophyll concentrations exceeded ~ 1mg m<sup>-3</sup>. They were insensitive to Si limitation but were also clearly limited by Fe availability which appears to be a strong determinant of colony size. At M8W & M8E we were fortunate enough to be able to relate primary production to "export" captured by "Pelagra". Other measurements of export are yet to be determined from the f-ratio and from <sup>234</sup>Thorium measurements, although early evidence indicates a good positive correlation between chlorophyll concentration and <sup>234</sup>Th activity, suggesting higher export associated with high chlorophyll concentrations.

**Table 12.1 Log of Primary Production Ti-CTD casts on D285**

<b>Ti-CTD Date (GMT)</b>	<b>Time CTD in water (GMT)</b>	<b>Time CTD inboard (GMT)</b>	<b>JD (GMT)</b>	<b>Stat</b>	<b>Loc</b>
11-Nov	1410	1916	316	15491	M1
13-Nov	1502	1706	318	15496	M3
18-Nov	2058	2144	323	15499	M3
19-Nov	2121	0009	324	15502	M2
22-Nov	2203	0141	327	15511	M6
25-Nov	0039	0120	330	15516	M3
27-Nov	1834	1926	332	15524	M7
29-Nov	2351	0018	334	15531	M8 E
01-Dec	2230	0050	336	15537	M8 W
03-Dec	2240	0119	338	15543	M9

**Table 12.2 Log of Primary Production Ti-CTD casts on D286**

<b>Ti-CTD Date (GMT)</b>	<b>Time CTD in water (GMT)</b>	<b>Time CTD inboard (GMT)</b>	<b>JD (GMT)</b>	<b>Stat</b>	<b>Loc</b>
18-Dec	1805	2100	353	15552	M9
20-Dec	1846	1935	355	15561	M10
22-Dec	2125	2217	357	15572	M3
27-Dec	1845	2217	362	15581	M5
31-Dec	1410	1438	366	15592	M3
04-Jan	1852	2152	004	15598	M6
06-Jan	1617	1845	006	15602	M2
08-Jan	2248	2335	008	15612	M3
10-Jan	1813	1853	010	15621	M3
13-Jan	0118	0204	013	15629	M3



**Table 12.3 Log of Primary Production, P.vs.E & <sup>15</sup>N stations, 1<sup>st</sup> Leg (D285)**

CTD Date (GMT)	JD	CTD	Stat	Primary production		P vs E		<sup>15</sup> N			
				Depth	Size fraction	Depth	Size fraction	Depth	NO <sub>3</sub> , NH <sub>4</sub> , urea	NH <sub>4</sub> regen	Size fraction
11-Nov	316	15491	M1	5	T, >2µm, >20µm	5	T, >20µm	5	T		
				10	T, >2µm, >20µm			10	T		
				20	T, >2µm, >20µm			20	T		
				40	T, >2µm >20µm			40	T		
				60	T, >2µm, >20µm	60	T, >20µm	60	T		
				70	T, >2µm, >20µm			70	T		
13-Nov	318	15496	M3	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <2µm, <20µm
				15	T, >2µm, >20µm			15	T	Ro, Rt	
				27	T, >2µm, >20µm			27	T	Ro, Rt	
				42	T, >2µm, >20µm	42	T	42	T	Ro, Rt	
				63	T, >2µm, >20µm			63	T	Ro, Rt	
18-Nov	323	15499	M3	5	T						
				5	T						
				10	T						
				15	T						
				40	T						
				60	T						
19-Nov	324	15502	M2	5	T, >2µm, >20µm	5	T, >2µm, >20µm	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <2µm, <20µm
				20	T, >2µm, >20µm			20	T	Ro, Rt	

				40	T, >2µm, >20µm			40	T	Ro, Rt	
				60	T, >2µm, >20µm			60	T	Ro, Rt	
				80	T, >2µm, >20µm			80	T	Ro, Rt	
22-Nov	327	15511	M6	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <2µm, <20µm
				20	T, >2µm, >20µm			20	T	Ro, Rt	
				40	T, >2µm, >20µm			40	T	Ro, Rt	
				60	T, >2µm, >20µm	60	T	60	T	Ro, Rt	
				80	T, >2µm, >20µm			80	T	Ro, Rt	
25-Nov	330	15516	M3	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	
				20	T, >2µm, >20µm			20	T	Ro, Rt	
				40	T, >2µm, >20µm			40	T	Ro, Rt	
				60	T, >2µm, >20µm	60	T	60	T	Ro, Rt	
				80	T, >2µm, >20µm			80	T	Ro, Rt	
27-Nov	332	15524	M7	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <2µm, <20µm
				15	T, >2µm, >20µm			15	T	Ro, Rt	
				25	T, >2µm, >20µm			25	T	Ro, Rt	
				35	T, >2µm, >20µm	35	T	35	T	Ro, Rt	
				55	T, >2µm, >20µm			55	T	Ro, Rt	
29-Nov	334	15531	M8 E	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <2µm, <20µm
				15	T, >2µm, >20µm			15	T	Ro, Rt	
				25	T, >2µm, >20µm			25	T	Ro, Rt	
				35	T, >2µm, >20µm			35	T	Ro, Rt	
				55	T, >2µm, >20µm			55	T	Ro, Rt	

01-Dec	336	15537	M8 W	5	T, >2µm, >20µm			5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <2µm, <20µm
				15	T, >2µm, >20µm			15	T	Ro, Rt	
				25	T, >2µm, >20µm			25	T	Ro, Rt	
				35	T, >2µm, >20µm			35	T	Ro, Rt	
				55	T, >2µm, >20µm			55	T	Ro, Rt	
03-Dec	338	15543	M9	5	T			5	T	Ro, Rt	
				10	T			10	T	Ro, Rt	
				15	T			15	T	Ro, Rt	
				25	T			25	T	Ro, Rt	
				35	T			35	T	Ro, Rt	
				55	T			55	T	Ro, Rt	

**Table 12.4 Log of Primary Production, P.vs.E & <sup>15</sup>N stations on D286**

CTD Date (GMT)	JD	CTD	Stat	Primary production		P vs E		<sup>15</sup> N			
				Depth	Size fraction	Depth	Size fraction	Depth	NO <sub>3</sub> , NH <sub>4</sub> , urea	NH <sub>4</sub> regen	Size fraction
18-Dec	353	15552	M9	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <2µm, <20µm
				15	T, >2µm, >20µm			15	T	Ro, Rt	
				25	T, >2µm, >20µm			25	T	Ro, Rt	
				35	T, >2µm, >20µm	35	T	35	T	Ro, Rt	
				55	T, >2µm, >20µm			55	T	Ro, Rt	
20-Dec	355	15561	M10	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	
				15	T, >2µm, >20µm			15	T	Ro, Rt	

				25	T, >2µm, >20µm	25	T	27	T	Ro, Rt	
				35	T, >2µm, >20µm			42	T	Ro, Rt	
				55	T, >2µm, >20µm			63	T	Ro, Rt	
23-Dec	357	15572	<b>M3</b>	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <20µm <10µm, <2µm
				15	T, >2µm, >20µm			15	T	Ro, Rt	
				25	T, >2µm, >20µm						
				35	T, >2µm, >20µm	35	T				
				55	T, >2µm, >20µm						
27-Dec	362	15581	<b>M5</b>	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <20µm <10µm, <2µm
				20	T, >2µm, >20µm			20	T	Ro, Rt	
				40	T, >2µm, >20µm			40	T	Ro, Rt	
				60	T, >2µm, >20µm	60	T	60	T	Ro, Rt	
				80	T, >2µm, >20µm			80	T	Ro, Rt	
31-Dec	366	15592	<b>M3</b>	5	T, >2µm, >20µm			5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <20µm <10µm, <2µm
				15	T, >2µm, >20µm			15	T	Ro, Rt	
				25	T, >2µm, >20µm			25	T	Ro, Rt	
				35	T, >2µm, >20µm	35	T	35	T	Ro, Rt	
				55	T, >2µm, >20µm			55	T	Ro, Rt	
4-Jan	005	15598	<b>M6</b>	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
				10	T, >2µm, >20µm			10	T	Ro, Rt	T, <20µm <10µm,

											<2µm
				20	T, >2µm, >20µm			20	T	Ro, Rt	
				40	T, >2µm, >20µm			40	T	Ro, Rt	
				60	T, >2µm, >20µm	60	T	60	T	Ro, Rt	
				80	T, >2µm, >20µm			80	T	Ro, Rt	
06-Jan	006	15602	M2	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
											T, <20µm
				10	T, >2µm, >20µm			10	T	Ro, Rt	<10µm, <2µm
				20	T, >2µm, >20µm			20	T	Ro, Rt	
				40	T, >2µm, >20µm			40	T	Ro, Rt	
				60	T, >2µm, >20µm	60	T	60	T	Ro, Rt	
				80	T, >2µm, >20µm			80	T	Ro, Rt	
9-Jan	009	15612	M3	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
											T, <20µm
				10	T, >2µm, >20µm			10	T	Ro, Rt	<10µm, <2µm
				20	T, >2µm, >20µm			20	T	Ro, Rt	
				30	T, >2µm, >20µm			30	T	Ro, Rt	
				40	T, >2µm, >20µm			40	T	Ro, Rt	
				60	T, >2µm, >20µm			60	T	Ro, Rt	
10-Jan	010	15621	M3	5	T, >2µm, >20µm	5	T	5	T	Ro, Rt	
											T, <20µm
				8	T, >2µm, >20µm			8	T	Ro, Rt	<10µm, <2µm
				12	T, >2µm, >20µm			12	T	Ro, Rt	
				20	T, >2µm, >20µm	20	T	20	T	Ro, Rt	
				30	T, >2µm, >20µm			30	T	Ro, Rt	
				45	T, >2µm, >20µm			45	T	Ro, Rt	
13-Jan	013	15629	M3	5	T			5	T	Ro, Rt	
				8	T			8	T	Ro, Rt	T, <20µm

											<10µm, <2µm
				12	T			12	T	Ro, Rt	
				20	T			20	T	Ro, Rt	
				35	T			35	T	Ro, Rt	
				55	T			55	T	Ro, Rt	

**Note:** Station locations in bold (eg **M3**) denote stations where discrete FRRf measurements were made on the bottle water samples. At station 15629, there was insufficient <sup>14</sup>C spike remaining to do size-fractionated experiments or P vs E curves.

**Table 12.5 Summary of Fe addition experiments**

(PvsE incubations were carried out at the start and finish of each experiment).

	Date Start	Date Finish	Location	Depth
<b>LEG 1</b>				
Expt 1	11/11/04	16/11/04	M1	5m
Expt 2	22/11/04	26/11/04	M6	5m
Expt 3	26/11/04	30/11/04	M3	5m
Expt 4	30/11/04	04/12/04	M8E	5m
<b>LEG 2</b>				
Expt 5	20/12/04	26/12/04	M10	25m
Expt 6	31/12/04	05/01/05	M3	35m
Expt 7	06/01/05	12/01/05	M2	60m
Expt 8	10/01/05	15/01/05	M3	20m