Contents

Compiled by Jerry Blackford
Pictures by Gary Smerdon

1 Introduction

Carol Turley, Plymouth Marine Laboratory

1.1 Cruise Rationale

This, the first cruise of the 5 year (2001-2006) NERC PML Core Strategic Research Project on Microbially Driven Biogeochemistry (MDB), focused on changes in the microbial community structure, dynamics and processes in key biogeomes from the English Channel to off the shelf break in the North East Atlantic.

Community structure, dynamics and processes were studied using SRS, bioptics, microscopy, flow cytometry, rate process measurements (primary and bacterial production, grazing rates, respiration), autoanalysers, mass spectrometry, HPLC pigment characterisation, molecular biology and novel underway measurements including CytoSub (underway analytical flow cytometry), Fast Repetition Rate Fluorometer (FRRF) and Optic Plankton Recorder (OPC). This was also an opportunity for method development and testing (e.g. bioptics, bacterial pigments, zooplankton developmental gene expression, in vitro production of ^{18}O) and algorithm development and 'sea-truething' of satellite derived data (SeaWiFS and MERIS).

We sampled an underway gradient from Southampton to off the Atlantic shelf edge and 2-3 comparative stations that covered the winter baseline and the onset of the spring bloom. We also sampled the PML/MBA's long term time series stations L4 and E1 off Plymouth in order to link into the weekly data set.

With early April (1-14 April) as the cruise period, the study of the onset of the spring bloom was the target for the main body of work for Lagrangian study using a floating drogued buoy to mark the water mass in order that we could follow the development of the spring bloom, its community structure and dynamics. A study prior to the cruise of satellite ocean colour (chlorophyll) images from previous years indicated that the area in the North Western Celtic Sea tends to increase in sea surface chl a concentrations before other areas at this time of year. This general area looked the best location for this Lagrangian Spring Bloom station. During the cruise concurrent satellite remote sensed images of ocean colour, transmitted to the ship on a daily basis, in addition to the underway measurements enabled the exact selection of the location of both the Spring Bloom station and the two comparative pre-bloom stations (one on the shelf and one off the shelf).

1.2 Aims and Objectives

This cruise met aspects of the following MDB Aims and Objectives:

Aim 5: How does the composition of the autotrophic and heterotrophic community affect carbon cycling and ecosystem stability?

Objectives:

5.1 To determine how microbial community structure influences the photosynthesis/respiration ratio.

5.2 To investigate the factors that determine zooplankton community structure and the role of grazing in linking microbial communities and higher trophic levels.

Aim 6: How does nutrient supply influence carbon flux and trace gas production?

Objectives:

6.1 To quantify key nitrogen fluxes within the plankton community in order to refine estimates of oceanic carbon uptake.

6.2 To investigate how macro- and micro-nutrient limitation influences the ratio of new to total production.

Aim 7: What factors determine the air-sea flux of climate-active gases?

Objective:

7.1 To investigate the sites and mechanisms (anaerobic micro-zones, community structure/ functionality, trophic interactions) that maintain the atmospheric flux of reduced gases from the oxic upper ocean.

Aim 8: Integration of regional studies and extrapolation to larger space and time scales.

Objectives:

8.2 To simulate seasonal variability in northern European shelf seas and the North Atlantic using existing 1D and 3D coupled biogeochemical-hydrodynamical representations and remote sensing.

8.3 To investigate inter-annual changes in microbial community structure interelation to the onset of spring.

8.4 To integrate biogeochemical data, multi parameter remote sensing and bio-optical and numerical modelling from a range of biogeomes to extrapolate region and basin scale variability.

2 Personnel

Carol Turley & Jerry Blackford, Plymouth Marine Laboratory

2.1 Scientific personnel at sea

NAME	MEASUREMENT
Jo Dixon Rachel Lamb Carol Turley **(PSO)**	Effect of metals on bacterial production, diversity and enzyme activity
Gavin Tilstone James Fishwick Tim Smyth	Bioptics CDOM SPM Download SRS images, interpretation, etc. P vs I FRRF Radiometry POC/DOC Physical Oceanography Met package RS surface roughness
Andy Rees John Stephens Susana Barquero-Molina	Nutrients N uptake Primary Production (^{14}C 24 hr)
Gary Smerdon Pennie Lindeque	Bacterial diversity/ gene sequencing Zooplankton
Glen Tarran	AFC
Tania Smith Chris Gallienne	Copepod abundance, egg production, excretion OPC and LiZa
Denise Cummings	Pigment analysis by HPLC link meso zoo/micro zoo
Claire Widdicombe	Microzoo Lugols Het nan biomass enzyme assay
Claire Hughes *(UEA/PML)*	Halocarbons
Carol Robinson Efrat David *(The Inter-University, Eilat)*	P → 0_2 [^{18}0] R → 0_2 ETS
Jerry Blackford	Post Cruise modelling Cruise dogsbody
Brendan Keely *(Uni. York)*	Bacterial chlorophyll products
UKORS Phil Taylor Rhys Roberts Darren Young Paul Duncan	 (electronic engineer) (TLO) CTD (mechanical engineer) Winches (mechanical engineer) Winches (computer operator) Cruise/station data

The cruise was supported from PML by Chris Wing and Malcolm Woodward who assisted with pre-cruise logisitics and Pete Miller and Kate Evans-Jones who provided satellite imagery to the cruise.

2.2 Ships personnel

Robin Plumley, Master

R. C. Plumley	Master
R. A. Warner	Chief Officer
J. W. Mitchell	2nd Officer
P. C. Reynolds	3rd Officer
D. Stewart	ETO
K. Jethwa	Chief Engineer
I. M. Slater	2nd Engineer
J. R. Harnett	3rd Engineer
S. J. Bell	3rd Engineer
P. R. Bennett	CPO (D)
I. N. M. Thomson	PO (D)
D. G. Buffery	Seaman
N. P. Tuppenny	Seaman
S. P. Day	Seaman
G. Cooper	Seaman
P. Blacker	Cadet
E. Staite	SCM
P. A. Lynch	Chef
W. C. Isby	Mess Steward
J. A. Osborn	Steward
J. G. Smyth	ERPO Motorman

3 Program

Carol Turley, Plymouth Marine Laboratory

3.1 Cruise program

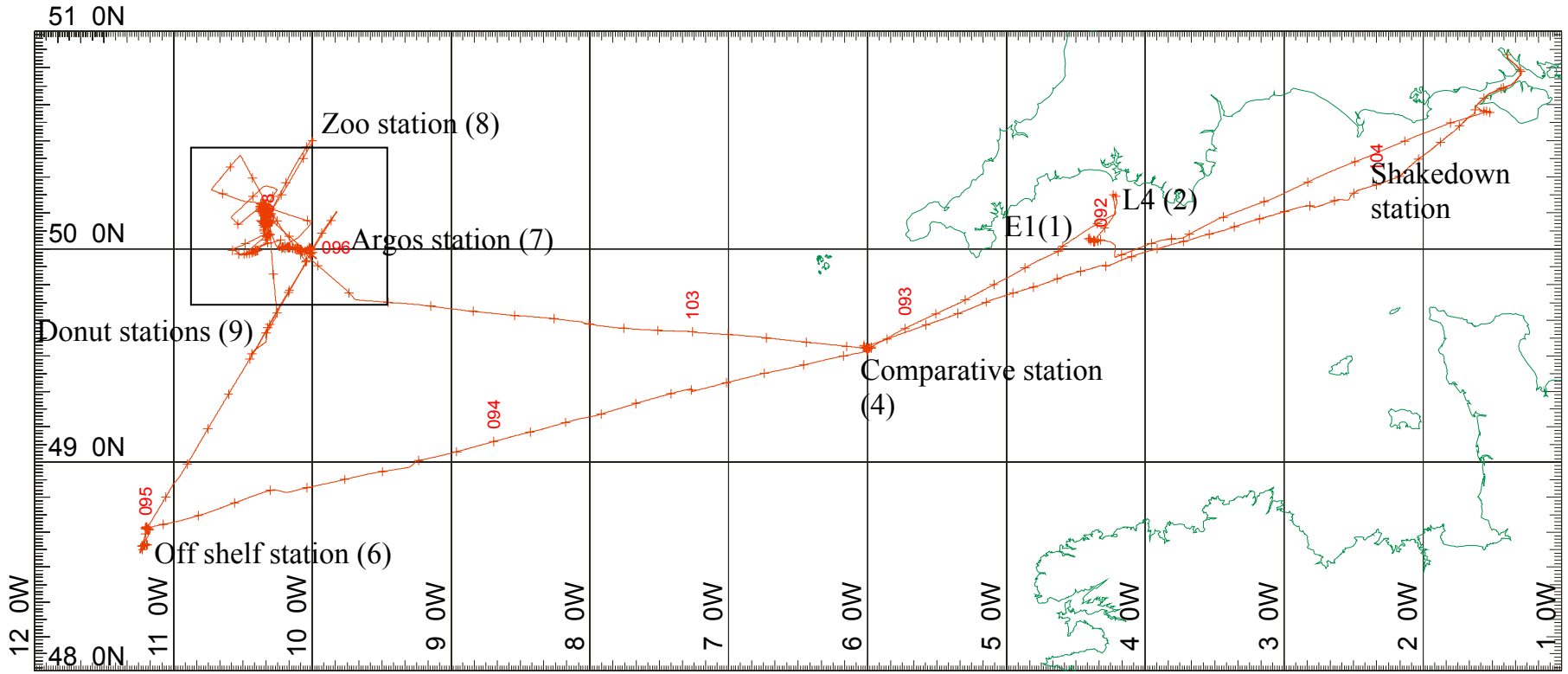
Date	Time	Description	Event #
	0800	Depart Southampton	
		Shakedown station Freshwater Bay	
		CTD	
		Free-fall optics	
		Free-fall optics	
		Niskin samples	
		Zooplankton OPC	
		Zooplankton net	
		Station E1 50° 02'N 04° 22'W	**1**
Tue 2 Apr	0200	Level 1 CTD cast	1.1
	0300	Productivity CTD cast	1.2
	0330	Zooplankton vertical net haul	1.3
	0400	Zooplankton tow	1.4
	0430	Zooplankton OPC profiler	1.5
	0500	FRRF rig	1.6
	0830	Zooplankton net haul	1.7
	0900	Free-fall optics	1.8
	0915	FRRF rig	1.9
	1100	Free-fall optics	1.10
	1115	FRRF rig	1.11
	Midday	CTD cast	1.12
	1230	TM Niskin cast	1.13
	1315	Free-fall optics	1.14
	1330	FRRF cast	1.15
	1350	Zooplankton OPC profiler	1.16
		Station L4 50° 15'N 04° 13'W	**2**
	1545	Free-fall optics	2.1
	1600	FRRF rig	2.2
		Zooplankton Station	**3**
	1900 on	Zooplankton net haul / tow	3.1
		Comparative Station 49° 32'N 06° 00'W	**4**
Wed 3 Apr	0200	Level 1 CTD cast	4.1
	0300	Productivity CTD cast	4.2
	0330	2 x Zooplankton vertical 50um net haul	4.3a-b
	0400	3 x Zooplankton 200um net haul	4.3c-e
	0430	Zooplankton OPC profiler	4.4
	0900	Free-fall optics	4.5
	0915	FRRF rig	4.6
	0935	SAP deployment (CTD wire)	4.7
	1100	Free-fall optics	4.8
	1115	FRRF rig	4.9
	Midday	CTD cast	4.10
	1315	Free-fall optics	4.11
	1330	FRRF cast	4.12
	1350	Zooplankton OPC profiler	4.13
		Off Shelf Station	**6**

		48° 41'N 11° 12'W	
Thu 4 Apr	1115	FRRF rig	6.1
	1135	Midday CTD cast to 1000m	6.2
	1300	TM Niskin cast	6.3
	1400	FRRF cast	6.4
	1420	Zooplankton OPC profiler	6.5
	1510 on	SAPS (1500, 1000, 500, c.25-50m)	6.6
Fri 5 Apr	0200	Level 1 CTD cast	6.7
	0300	Productivity CTD cast	6.8
	0330	2 x Zooplankton 50um net haul	6.9 a-b
	0400	3 x Zooplankton 200um net haul	6.10 a-c
	0430	Zooplankton OPC profiler	6.11
		Underway transect across Spring Bloom	
		Great Sole Bank **Spring Bloom Lagrangian Station** 49° 37'N 10° 20'W	**7**
	1205	Midday CTD	7.1
	1235	Free-fall optics	7.2
	1250	FRRF cast	7.3
	1440	Regain Position and Deploy Argos Drifter	7.4
	2015	Zooplankton net tow	7.5
Sat 6 Apr	0100	Reposition to Argos	
	0200	Level 1 CTD cast	7.6
	0300	Productivity CTD cast	7.7
	0330	2 x Zooplankton 50um net haul	7.8a-b
	0400	3 x Zooplankton 200um net haul	7.9a-c
	0430	Zooplankton OPC profiler	7.10
	0500	FRRF rig	7.11
	0630	Deploy primary production Rig	7.12
	0930	FRRF cast	7.13
	1205	Midday CTD	7.14
	1310	Zooplankton OPC profiler	7.15
Sun 7 Apr	0100	Reposition to Argos Drifter	
	0200	Level 1 CTD cast	7.16
		Remainder of 7th lost to weather	
Mon 8 Apr	0100	Reposition to Argos Drifter	
	0200	Zooplankton OPC profiler	7.17
	0230	Level 1 CTD cast	7.18
	0300	2 x Zooplankton 50um net haul	7.19
	0330	3 x Zooplankton 200um net haul	7.20
	0400	Productivity CTD cast	7.21
	0630	Deploy primary production Rig	
	0830	Reposition to Argos Drifter	
	0900	FRRF cast	7.22
	1000	CTD	7.23
	1130	Free-fall optics	7.24
	1145	FRRF rig	7.25
	1205	Midday CTD	7.26
	1235	Free-fall optics	7.27
	1250	FRRF cast	7.28
	1310	Zooplankton OPC profiler	7.29
	1340	CTD	7.30
	1420	FRRF cast	7.31
	2000	Reposition to Argos Drifter	
	2015	Zooplankton net tow	7.32
Tue 9 Apr	0100	Reposition to Argos Drifter	
	0200	Zooplankton OPC profiler cast	7.33
	0230	Level 1 CTD	7.34

	0300	2 x Zooplankton 50um net haul	7.35 a-b
	0330	3 x Zooplankton 200um net haul	7.36 a-c
	0400	Productivity CTD cast	7.37
	0500	FRRF rig	7.38
	0900	FRRF cast	7.39
	1000	CTD cast	7.40
	1130	Free-fall optics	7.41
	1145	FRRF rig	7.42
	1205	Midday CTD	7.43
	1235	Niskin samplers for trace metals	7.44
	1305	Free-fall optics	7.45
	1320	FRRF cast	7.46
	1340	Zooplankton OPC profiler	7.47
	1400	FRRF cast	7.48
	1430	CTD	7.49
	1630	FRRF cast	7.50
		Non-bloom station *50°30'N 10°W*	**8**
	2000	Niskin sample at 20m	8.1
	2015	Zooplankton net tow	8.2
Wed 10 Apr	0100	*Reposition to Lagrangian Station*	**7**
	0200	Zooplankton OPC profiler cast	7.51
	0230	Level 1 CTD	7.52
	0300	2 x Zooplankton 50um net haul	7.53a-b
	0330	3 x Zooplankton 200um net haul	7.54a-d
	0400	Productivity CTD cast	7.55
	0500	FRRF rig	7.56
	0900	FRRF cast	7.57
	1000	CTD cast	7.58
	1130	Free-fall optics	7.59
	1145	FRRF rig	7.60
	1205	Midday CTD	7.61
	1305	Free-fall optics	7.62
	1320	FRRF cast	7.63
	1340	Zooplankton OPC profiler	7.64
	1400	FRRF cast	7.65
	1430	CTD	7.66
	1630	FRRF cast	7.67
		'Bow-Tie???' underway programme	**9**
	1620	Depart for 49°56.34N 10°02.63W and then follow track towards 49°29.31N 10°26.89W	
	1630 on	Underway measurements	
	2020	Zooplankton net tow	9.1
	2040	Return along track to 49°56.34N 10°02.63W and then to Argos – *Doughnut edge transect*	
	2040 on	Underway measurements	
Thu 11 Apr	0100	*Reposition to Lagrangian Station*	**7**
	0200	Zooplankton OPC profiler cast	7.68
	0230	Level 1 CTD	7.69
	0300	2 x Zooplankton 50um net haul	7.70
	0330	3 x Zooplankton 200um net haul	7.71
	0400	Productivity CTD cast	7.72
	0500	FRRF rig	7.73
	0900	FRRF cast	7.74
	1000	CTD cast	7.75
	1130	Free-fall optics	7.76
	1145	FRRF rig	7.77
	1205	Midday CTD	7.78

	1235	Free-fall optics	7.79
	1250	FRRF cast	7.80
	1310	Zooplankton OPC profiler	7.81
	1340	Reposition to Argos	
	1420	FRRF cast	7.82
	1530	CTD	7.83
	1600	FRRF cast	7.84
	2000	Reposition to Argos Drifter	
	2015	Zooplankton net tow	7.85
Fri 12 Apr	0100	Reposition to Argos Drifter	
	0200	Zooplankton OPC profiler cast	7.86
	0230	Level 1 CTD	7.87
	0300	2 x Zooplankton 50um net haul	7.88
	0330	3 x Zooplankton 200um net haul	7.89
	0400	Productivity CTD cast	7.90
	0500	FRRF rig	7.91
	0900	FRRF cast	7.92
	1000	CTD cast	7.93
	1130	Free-fall optics	7.94
	1145	FRRF rig	7.95
	1205	Midday CTD	7.96
	1630	Recover Argos drifter	
	1800	Head for home with underway tracking	
Sun 14 Apr	0900	Arrive Southampton	

1.2 Cruise.track



MERCATOR PROJECTION

SCALE 1 TO 5000000 (NATURAL SCALE AT LAT. 0)

INTERNATIONAL SPHEROID PROJECTED AT LATITUDE 0

GRID NO. 1

Track plotted from gps_g12

RRS Discovery cruise 261

4 Satellite imagery

Kate Evans-Jones and Pete Miller (PML)

SeaWiFS chlorophyll-a composite images for week one and week two of the cruise with the approximate position of the lagrangian drift station circled.

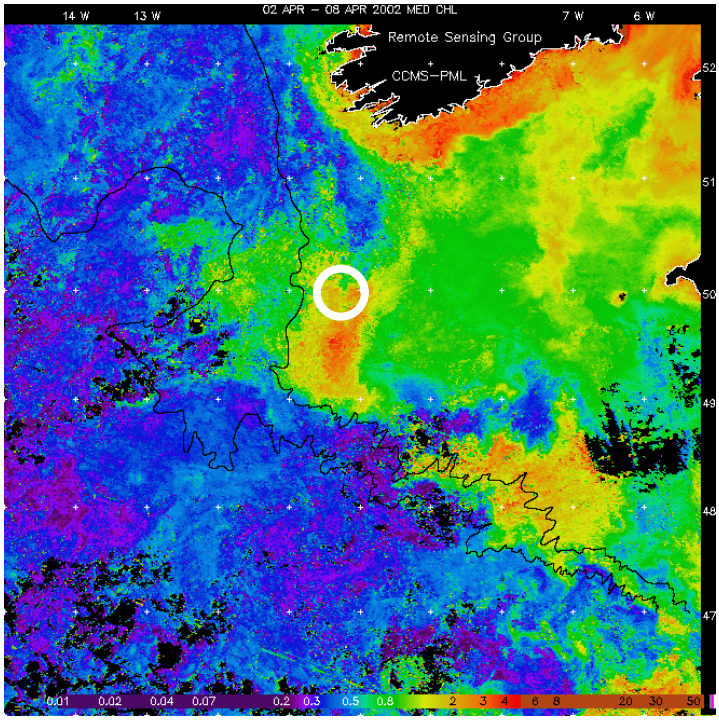


Fig 4.1 SeaWiFS chlorophyll-a composite ocean colour image for 02-08 April 2002

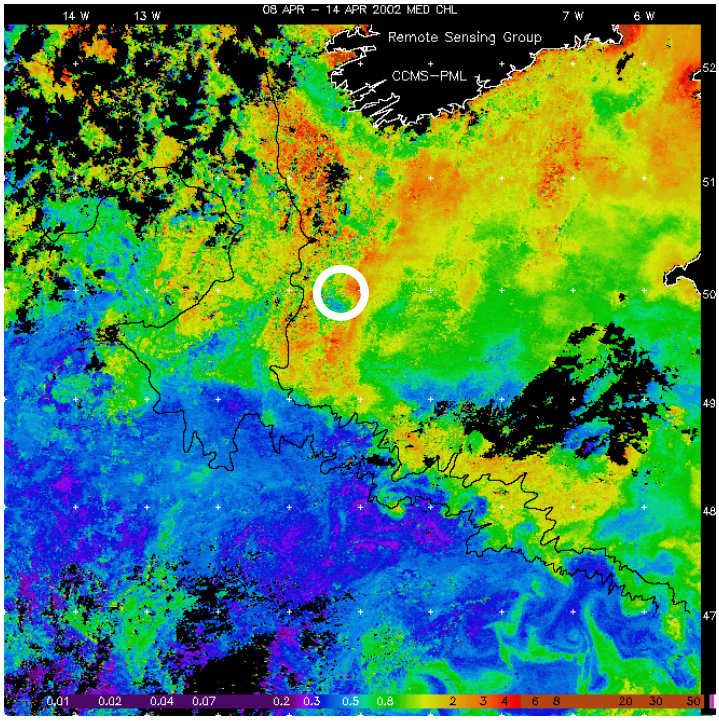


Fig 4.2 SeaWiFS chlorophyll-a composite ocean colour image for 08-14 April 2002

Further images used during the cruise can be found at
http://www.npm.ac.uk/rsdas/projects/mdb/d261_apr02/

5 Underway operations

5.1 Underway data

5.2 Selection of Location for Argos Lagrangian Station

Carol Turley, Plymouth Marine Laboratory

Rationale and aim

The aim was to deploy the Argos drifting buoy (Fig 5.2.1) in water about to or just developing a spring bloom. To do this we had to have an insight into the position and stage of development of different parts of the bloom.

Method

The comparative and off shelf station had given us an insight of non- bloom conditions and characteristics and the SRS ocean colour images, which gave us a good indication of the position of the bloom (see section 4), indicated that the centre of the bloom in the Great Sole Bank had been developing for a number of days, that is the centre may be too far developed for our needs. We therefore decided to traverse the bloom from the off-shelf station from south west to north east, monitoring it in real time through the underway non-toxic seawater supply for fluorescence, nutrients and zooplankton using the OPC. We also took 53um mesh samples of plankton to examine them microscopically. Other underway data was also collected – using the FRRF and CytoSub – but not in real time. Chlorophyll samples were collected in order to calibrate the underway fluorimeter.

Preliminary Results

Underway fluorescence revealed an extensive bloom on the shelf along the track (Fig 5.2.2). The phytoplankton towards the southern edge of the track were of mixed diatoms, looked very healthy, early stages of nauplii were also present, there was medium fluorescence levels (0.4-0.5v), few chlorophyll breakdown products were present and nitrate values of c. 1.5 uM indicated that these waters were not yet depleted.

In the centre of the bloom, phytoplankton cells looked very unhealthy, zooplankton stages were more developed, fluorescence was high (0.7v), chl a breakdown products were high, nitrate was low (0.5uM) as was silicate. It very much looked like the centre of the bloom was being grazed out or sedimenting and getting close to nutrient depletion. On the far side of the bloom we observed an area where the fluorescence was medium (0.4-0.5v), the nitrate around 4-5 uM and silicate was present in concentrations just below typical winter concentrations, phytoplankton were healthy looking diatoms (mainly Rhizosolenia and Chaetoceros species with some Cosinodiscus and Ceratium. This position was chosen for the Argos drifter station after a further survey along the track to ensure that we were not obviously close to a feature, such as the edge of the bloom or a front, which might result in the drift of Argos into waters of differenr characteristic. We tracked through the edge of the bloom into pre-bloom waters for a period and then retraced our track back to the station and deployed Argos drifter at 1900 5 April 2002 at c. 49^{o} 48.52'N 10^{o} 00.80'W in order to start the lagrangian experiment.

Underway FRRF analysis along the track (Fig 6.5.2) later confirmed that the centre of the bloom had low potential photosynthetic efficiency and high fluorescence and the Argos station had medium fluorescence but high potential photosynthetic efficiency – further evidence that the station chosen was just beginning to bloom.

The Lagrangian experiment ran from 5 -12 April 2002, although there was a sampling break of c 36hrs (give time and date) due to high winds and rough seas with a daily sampling scheme outlined in 3.1. The storm itself was an interesting feature of the experiment as the

sky was mostly clear and sunny. Therefore we were able to observe a strong mixing process and high radiation at the same time. After the storm the waters were well mixed but soon stratified so that we were able to observe the temporal development of the spring bloom and its associated biogeochemistry. We also believe that we observed the decline of the bloom through loss processes such as grazing or sinking during the last 2-3 days on station. That is growth and grazing occurred rapidly. This observation was later supported by an underway survey and SRS which showed that the chla in the centre of the bloom was declining.

As most of the overside activity was between 0200 and 1500 we were able to use ship time to investigate other areas using underway measurements. We did this on 3 occasions when working at the Argos lagrangian station:

2. A transect north to delineate the bloom edge and to sample pre-bloom waters for Calanus females to study egg development genes.
3. A transect into the centre of the bloom, the doughnut transect, to carry out a multidisciplinary study along a transect of post bloom to bloom conditions and to collect more Calanus females to study egg development genes under post bloom conditions.
4. A bowtie and box survey around the Argos position to confirm shape of bloom around us.

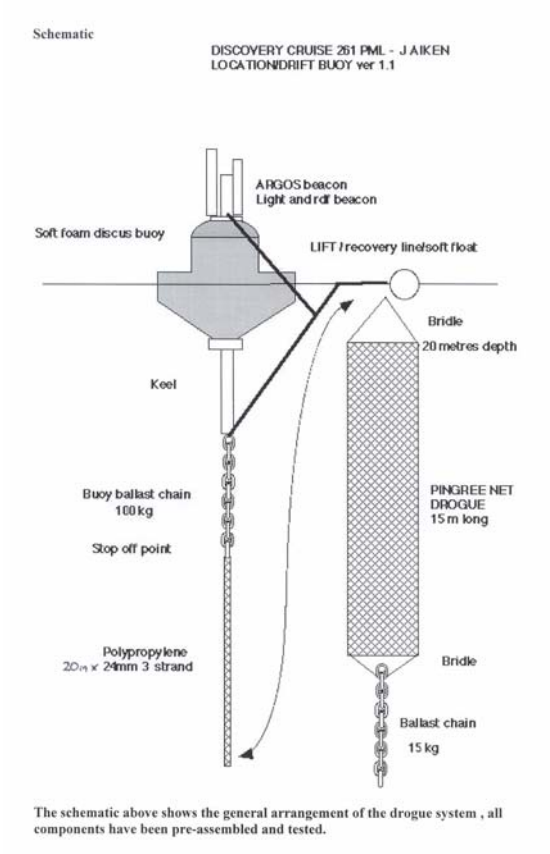


Fig 5.2.1. Schematic of Argos drifting buoy

NE Edge of Bloom:
$N0_3$ = 3.8uM
Si = 2uM
Fl =0.36v
Phyto = happy & healthy
Zoop = early stages

Centre of Bloom:
$N0_3$ = 1.5uM
Si = 0.5uM
Fl =0.72v
Phyto = unhappy & unhealthy, breakdown pigments - grazed
Zoop = more developed stages

SW Edge of Bloom:
$N0_3$ = 3.8uM
Si = 2uM
Fl =0.45v
Phyto = happy & healthy, few breakdown pigments
Zoop = early stages of nauplii

Off Shelf:
$N0_3$ = 7uM
Si = 5uM
Fl =0.12v
Pre-bloom

The other side of the Bloom:
$N0_3$ = 5uM
Si = 4uM
Fl =0.14v

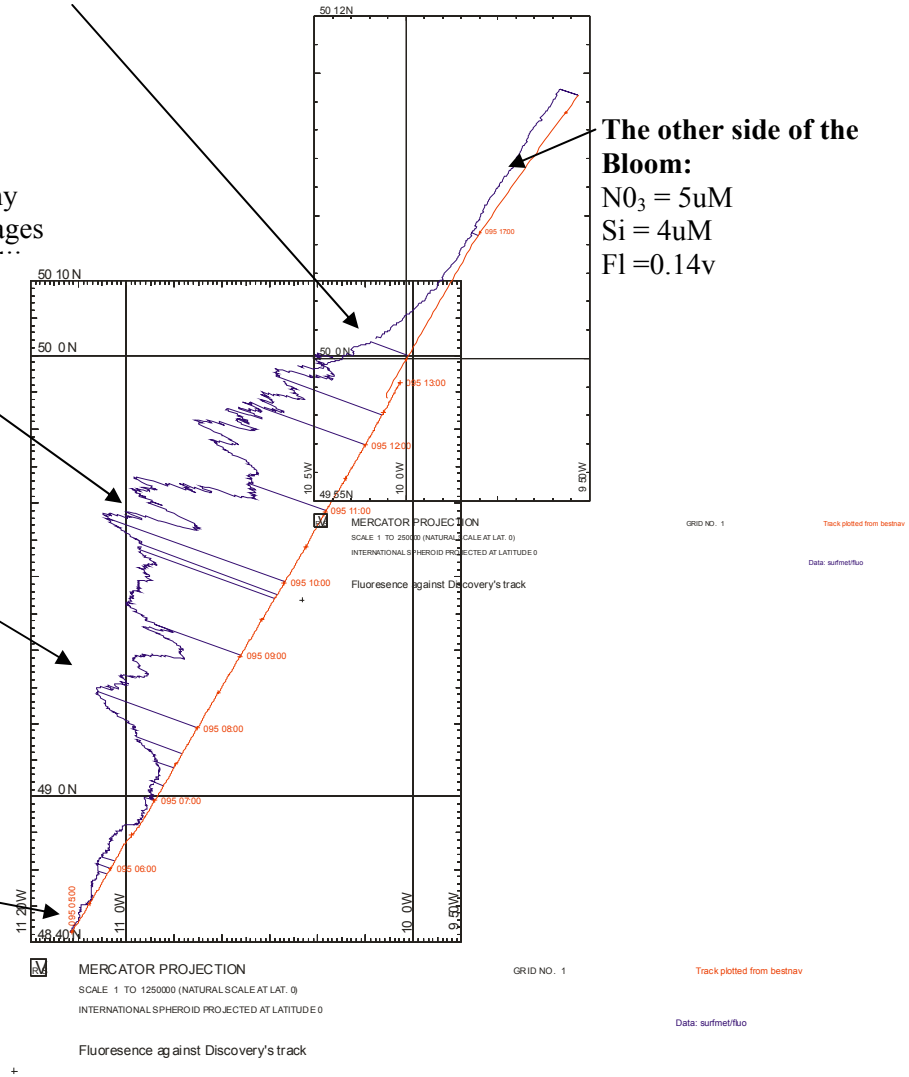


Fig 5.2.2. Underway fluorescence along cruise track from off shelf station through the spring bloom

5.3 From bloom interior to edge – the doughnut transect.

Carol Turley, Plymouth Marine Laboratory

Rationale & Aim

Satellite remote sensing from 8 April 20002 revealed that chlorophyll in the centre of the bloom on the Great Sole Bank was decreasing, revealing a doughnut type structure. Our previous transect across the bloom relieved that the phytoplankton in the centre of the bloom had low efficiency (FRRF) despite having relatively high Chl fluorescence, microscopic observations showed that they were not in a healthy state, pigment analysis indicated a number of chlorophyll a breakdown products. Our hypothesis was that the phytoplankton in the centre of the doughnut ring were reduced due to extensive grazing or sinking and were essentially post-bloom waters whereas the doughnut represent bloom conditions. A transect across would represent a time sequence from bloom to post bloom conditions. Our aim was to test this hypothesis.

Method

On Wednesday 10 April 2002 we left the Argos Drifter Buoy position and carried out a transect in towards the centre of the bloom with underway non-toxic measurements, nutrients, OPC, CytoSub and FRRF measurements enabling tracking of the bloom characteristics. There was a very steep delineation between the high fluorescence & low NO_3 (0.5uM) doughnut and lower fluorescence & medium NO_3 (1.5uM) hole, (fig. 5.3.1). On entering the centre (station 9.1) a zooplankton net haul was carried out as were the measurements outlined in table 5.3.1. The zooplankton were numerous and with many late stage carnivores and small fish (sample preserved). Seine trawlers were also very active in this area, that is, there was high fishing activity. Other samples were taken back along the steep fluorescence gradient between hole and doughnut, a distance of only 2.5nml over a period of 15 min. 10-20 litre samples were taken from the underway non-toxic seawater supply and then sub-samples taken for the measurements in table 5.3.1.

Results

Early indications indicate that the hypothesis may hold. Flow cytometry indicates little change in the small photosynthetic cells such that the substantial gradient seen was probably due to large diatoms. This is substantiated by the changes seen in the nitrate and silicate. We will have to await the full data set in order verify this.

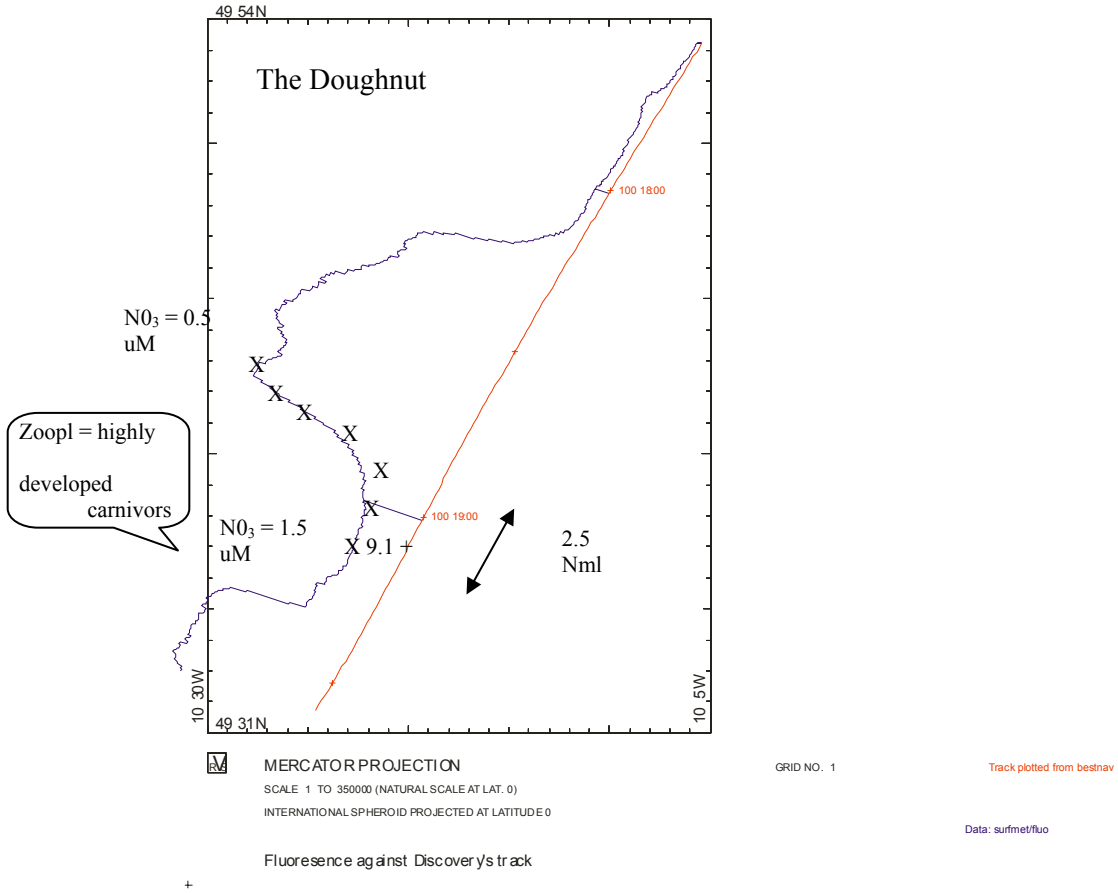


Fig 5.3.1. Underway fluorescence against cruise track during the Doughnut transect

Aspirator No. & vol	Station No.	Time BST	Lat (N) Long (W)	Fluor (v)	chl	HPLC	HaloC	ETS	Leuc	Lugols	Formalin	AFC	Nuts	Resp
97% 20L	9.1	2155	49°36.35 10°19.84	0.29	√	√	1	√	1	√	1408	13	1	√
20% 10L	9.2	2225	49°37.31 10°18.98	0.39	√	√	2		2	√	1409	14	2	
55% 20L	9.3	2230	49°37.67 10°18.63	0.429	√	√	3	√	3	√	1410	15	3	√
E-4 10L	9.4	2232	49°38.09 10°18.25	0.56	√	√	4		4	√	1411	16		
33% 20L	9.5	2234	49°38.33 10°18.04	0.61	√	√	5	√	7	√	1412	17	4	√
3% 10L	9.6	2236	49°38.61 10°17.77	0.64	√	√	6		6	√	1413	21		
1% 20L	9.7	2240	49°39.15 10°17.28	0.76	√	√	7	√	5	√	1414	18	5	√
					2l	4l	100ml	5l	25ml	250ml	250ml	150ml	60ml	3l

Table 5.3.1 Summary of samples taken during the Doughnut transect

5.4 Underway bowtie survey around Argos drifting station

Carol Turley, Plymouth Marine Laboratory

Rationale

As we had had no SRS ocean colour images for several days and preliminary results indicated that the Argos Drifting station was developing post-bloom characteristics similar to those seen in the centre of the Great Sole Bank doughnut we aimed to characterize the local area around the Argos drifting station.

Method

An underway survey of the Argos station area was carried out on 11 & 12 April using the underway non toxic parameters, FRRF, CytoSub and OPC. The survey pattern was bowtie shape with a further box survey centred around the current Argos drifter position and then a final transect through the Argos drift track since deployment towards the southeast (Fig 5.4.1).

Preliminary results

Underway fluorescence indicated that the Argos drifter station was now in the centre of a bloom which exhibited post bloom characteristic and that on three sides the bloom was still at an early bloom stage (Fig 5.4.2). SRS images on 12 April confirmed our perception of the bloom development (Fig 5.4.3).

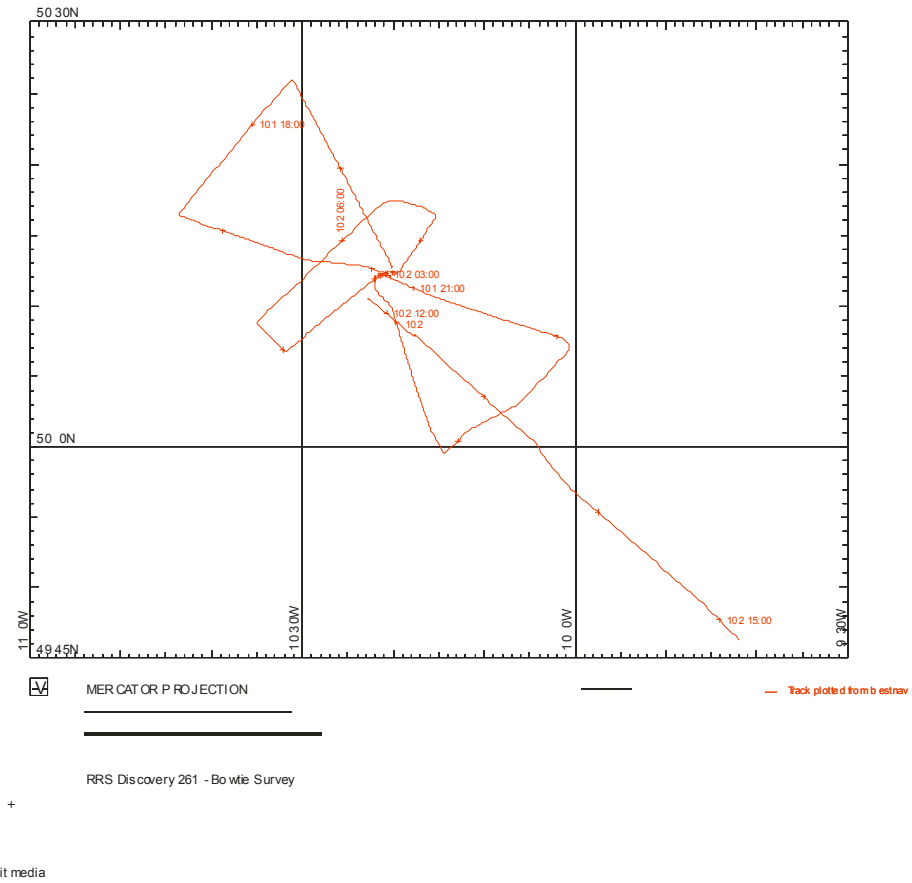


Fig 5.4.1. Track of underway Bowtie and Box survey around Argos drifter

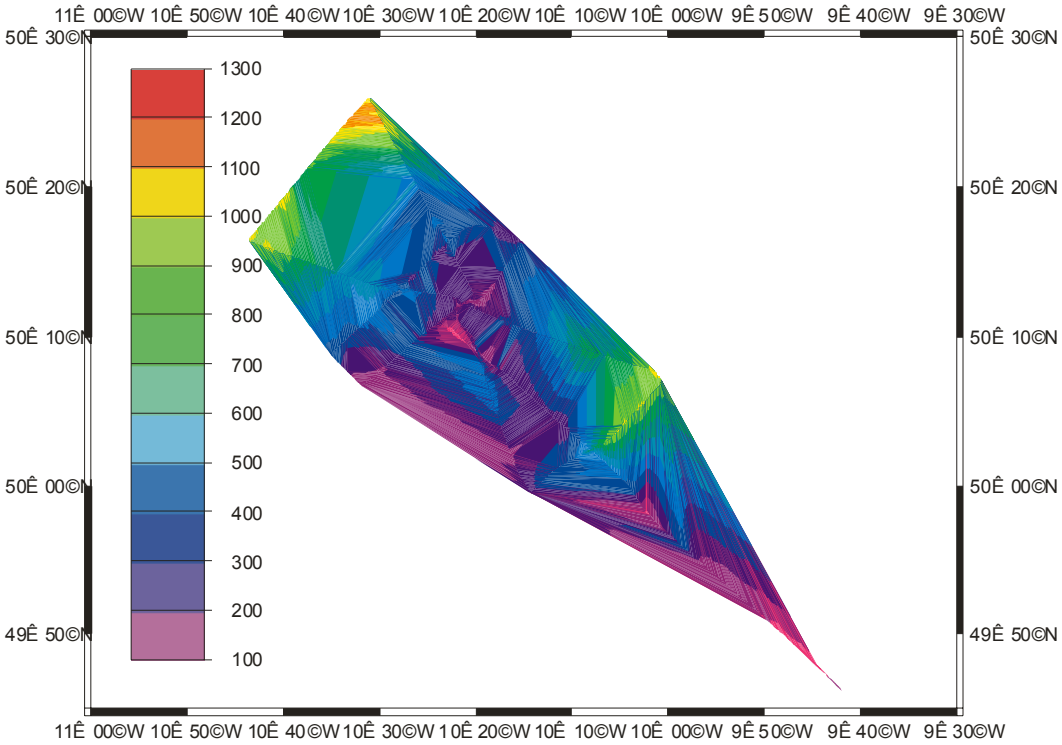


Fig 5.4.2. Contour plot of fluorescence in Lat/Long box of Bowtie and box survey

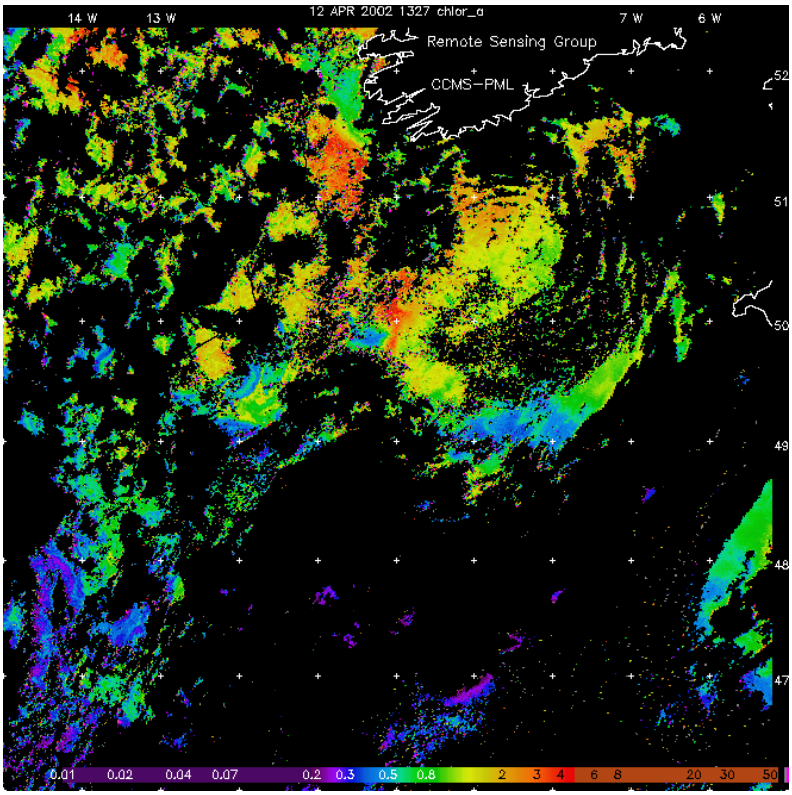


Fig 5.4.3. SRS image of Fri 12 April showing the low chl (blue) which had developed in the centre of the bloom.

6 Event sampling program

6.1 CTD Operations

6.2 Phytoplankton community structure, abundance and dynamics by flow cytometry and microscopy

Glen Tarran, Plymouth Marine Laboratory

Studies undertaken

1) Analysis of fresh seawater samples to determine the distribution, abundance and community structure of nano and picoplankton in surface waters by flow cytometry.

2) Collection of preserved seawater samples (formalin) for post-cruise analysis of heterotrophic bacteria and virus distribution and abundance in surface waters by flow cytometry.

3) Automated underway analysis of fresh seawater samples to test the capability of the CytoSub flow cytometer and to determine the distribution, abundance and community structure of phytoplankton (approx. 1 – 1000µm) in surface waters.

4) Size fractionation of pico- and nanophytoplankton communities from the mixed layer to determine median cell diameters for pico-eucaryotes, nanoeucaryotes, coccolithophores, cryptophytes and cyanobacteria.

5) Analysis of phytoplankton community structure at major stations from live concentrated seawater samples by microscopy and digital photography.

6) Determine the grazing-mediated mortality of pico-eucaryotes, nanoeucaryotes, coccolithophores, cryptophytes and cyanobacteria by flow cytometry from dilution experiments conducted in collaboration with Claire Widdicombe

1) Phytoplankton community structure and abundance

Fresh seawater samples were collected in clean 125 mL polycarbonate bottles from a Seabird CTD system containing 24 x 20 L Niskin bottles from productivity CTD casts. Samples were stored in a refrigerator until analysed (less than 1 hour). 2. mL samples were used for immediate flow cytometric analysis to characterise and enumerate *Synechococcus* sp. (cyanobacteria), pico-eucaryotes, cryptophytes, coccolithophores and other nanophytoplankton based on their light scattering and fluorescence properties. The flow cytometer used was a Becton Dickinson FACSort instrument. Of the 2 mL, approx 300µl of sample was actually analysed to provide vertical profiles of phytoplankton abundance per millilitre. Table 6.2.1 summarises the CTD casts sampled and analysed during the cruise with phytoplankton abundance integrated to 60m.

DATE	CTD	RSU cast no.	TIME (GMT)	LAT. N	LONG. W	Depth range sampled (m)	Phytoplankton abundance integrated to 60 m (cells cm^2)				
							Synecho	Crypto	Cocco	Peuk	Neuk
2-Apr	01/02	3	3:21	50.03	4.37	2-66	701104	22387	34117	468958	144470
3-Apr	04/02	6	3:54	49.54	6.00	2-94	365327	11058	7235	206260	117477
5-Apr	06/11	10	4:04	48.69	11.20	2-500	2260469	4968	7973	1302021	108183
5-Apr	07/01	11	13:46	49.94	10.04	5-90	189815	4054	6655	1721148	93397
6-Apr	07/07	13	2:45	50.00	10.01	2-120	176940	6289	8453	2807268	94684
7-Apr	07/16	15	2:25	50.01	10.18	11-122	168147	5748	10525	1795493	64571
8-Apr	07/20	17	3:46	50.07	10.31	2-120	123251	2621	4182	1014575	56876
9-Apr	07/36	22	3:40	50.13	10.32	2-120	43426	1163	1678	306612	24896
10-Apr	07/54	27	3:48	50.17	10.30	2-120	35252	486	2283	315393	28946
11-Apr	07/71	32	3:35	50.20	10.34	2-120	47819	1683	3552	533076	32188
12-Apr	07/90	37	3:27	50.21	10.35	2-120	36699	1653	4234	307110	30031

Table 6.2.1: CTD casts sampled for phytoplankton community structure, bacteria and virus abundance

2) Collection of preserved samples for heterotrophic bacteria and viruses
The fresh seawater samples collected in **1)** above were also used for preserved samples Duplicate 1.8 mL samples from all depths were preserved with 50 µL 0.22 µm filtered formaldehyde (37%) in 2 mL cryovials, stored in a refrigerator for 24 h and then frozen at –20°C. Samples will be stained with Sybr Green-I back in the laboratory to enumerate heterotrophic bacteria and viruses.

3) Underway sampling with the CytoSub flow cytometer
CytoSub is a unique submersible flow cytometer, capable of autonomously quantifying phytoplankton from approx. 1-1000-µm. It has data storage capacity for approx. 200 sample files and can be pre-programmed to sample at set time intervals from 10 minutes to days and if, attached to a mains power supply should be able to operate indefinitely. During the cruise there were two main objectives; a) to test the capability/reliability of CytoSub to take samples over prolonged periods of time and b) to analyse phytoplankton distributions and abundance i) at regular (1 hour) intervals, ii) over fine spatial scales (drift station) and iii) with minimum effort. CytoSub was housed in the water bottle annex close to the primary inlet of the non-toxic seawater supply (pumped from a depth of 6 m). Three trials were conducted during the cruise, all of which were successful, the longest being of 5 days duration. Details of the trials are detailed in Table 6.2.2. To verify samples analysed by CytoSub, 100mL seawater samples were collected periodically from the non-toxic supply and preserved with approx.1% formalin (final conc.) for post-cruise analysis by flow cytometry and microscopy. Interpretation of the CytoSub data will be carried out back in the lab.

Trial	Start (BST)	End (BST)	No. samples	No. formalin samples
1	2 April 1415	3 April 1425	25	3
2	3 April 2030	8 April 0940	110	22
3	8 April 1300	13 April 1210	120	20

Table 6.2.2: Details of CytoSub trials

4) Phytoplankton size structure
Three size fractionation experiments were carried out during the drift study (Station 7). Water samples from the mixed layer were gravity filtered through 12, 10, 5, 3, 2, 1, 0.8, 0.6, 0.4 and 0.2µm Nuclepore filters and the filtrate analysed by flow cytometry to enumerate the phytoplankton. The cell counts were compared to unfiltered seawater cell numbers by plotting % cells remaining against filter pore size. The median cell diameters were then read off the X axis where they intersected with the 50% line on the Y axis. The results are summarised in Table 6.2.3.

	Median cell diameter (µm)				
Experiment	Synechococcus	Cryptophytes	Coccolithophore	Picouecaryotes	Nanoeucaryotes
1	0.95	4.8	6.73	0.69	2.75
2	1.25	7.5	7.8	0.72	4.75
3	1.27	4.18	8.5	0.68	5.4
Overall average (µm)	1.16	5.49	7.68	0.70	4.30
Standard deviation	0.18	1.77	0.89	0.02	1.38

Table 6.2.3: Median cell diameters of flow cytometrically derived phytoplankton groups from size fractionation experiments

5). Microscopic studies of phytoplankton community structure from live samples.
Approx 37 L of seawater were collected from 2 depths in the mixed layer from each station and were concentrated down to approx 60-75 mL by gravity filtering through a 0.22µm Gelman Criticap 100 cartridge filter. Filtering took approx. 1-1.5 hours. Samples were then taken to the dark room and a 3 mL sample pipetted into a Hydrobios settling chamber. Samples were analysed using a Leica DM-IRB

inverted microscope, with a Canon EOS D30 colour digital stills camera. All samples were scanned with a x63 objective to take pictures of representative nanoplankton and, where appropriate, with x4, x10, x20 and x40 objectives to record larger plankton. Analysis of the pictures will be conducted back in the lab. to characterise each of the stations in terms of their planktonic communities.

6). Group-specific grazing experiments analysed by flow cytometry

Details of the grazing experiment setup are described elsewhere in this cruise report by Claire Widdicombe.

Samples for flow cytometirc analyses were collected from experimental bottles in rinsed 15 mL polypropylene conical centrifuge tubes and stored in a refrigerator until analysed. Samples were analysed to provide abundance estimates for *Synechococcus* sp., coccolithophores, picoeucaryotes and nanoeucaryotes with the FACSort flow cytometer using the same settings as for CTD bottle samples. Analysis times varied with bottle dilution as follows: 100% - 8 mins, 70% - 13 mins, 40% - 17.42 mins, 20% - 23.5 mins. The variations were made to provide significant cell counts for experimental calculations. By comparing the abundance of the phytoplankton groups at T0 and after 24 hours it was possible to obtain estimates of phytoplankton growth rates and microzooplankton grazing rates, expressed as % turnover of the particular phytoplankton group. The results are summarised in Table 6.2.4 below.

Expt.	Date April	Lat. N	Long. W	Station	Phytoplankton growth rate				Microzooplankton grazing rate (Turnover %)			
					Syn	Cocco	Peuk	Neuk	Syn	Cocco	Peuk	Neuk
1	2	50.03	4.37	1	0.43	0.11	0.80	0.63	15.29	34.84	37.05	25.04
2	3	49.54	6.00	4	0.36	NS	NS	0.41	16.80	NS	NS	16.14
3	5	48.69	11.20	6	0.51	0.83	0.73	0.41	34.95	75.17	39.37	45.51
4	6	50.00	10.00	7	0.43	NS	0.69	-0.08	6.12	NS	12.51	-44.18
5	8	50.07	10.31	7	0.33	NS	0.77	0.35	14.98	NS	28.00	37.54
6	9	50.13	10.32	7	NS	NS	NS	NS	NS	NS	NS	NS
7	10	50.17	10.30	7	-0.17	-0.68	0.82	1.11	-7.97	-204.3	21.27	71.21
8	11	50.12	10.34	7	0.32	0.54	0.87	0.68	-9.06	54.95	44.97	38.37

Table 6.2.4: Dilution grazing experiment results. Phytoplankton growth rates and Microzooplankton grazing rates. Station 1, south of Plymouth, Station 4, SSE of Scilly, Station 6, off the continental shelf, Station 7, shelf drift study. NS = Not significant

Grazing on and growth of the phytoplankton groups measured by flow cytometry was generally significant at all the low chlorophyll stations (1, 4, 6) and was highest off the continental shelf. During the drift study, *Synechococcus* growth and grazing generally decreased. Coccolithophores were inactive and were not grazed until the end of the drift study. Picoeucaryotes showed high levels of growth throughout the drift study and were actively grazed. Finally, the nanoeucaryotes were inactive and ungrazed at the beginning of the drift study but soon grew actively, resulting in significant grazing by the microzooplankton.

6.3 Pigments

Denise G. Cummings, Plymouth Marine Laboratory

Chemotaxonomic assessment of phytoplankton distribution: Background

The photosynthetic pigments, particularly chlorophyll a (*Chla*) are recognised as molecular markers of phytoplankton biomass. Whilst the distribution of *Chla* has typically been studied by spectrophotometry or fluorimetry, these methods suffer from inaccuracies associated with spectral interferences from chlorophyll b (*Chlb*), carotenoids and *Chla* degradation products. These degradation products include chlorophyllides, phaeophytins and phaeophorbides which may occur during senescence, grazing, sedimentation, and re-suspension of phytoplankton. The use of high performance liquid chromatography (HPLC) allows a more accurate estimate of *Chla* to be obtained and also the rapid separation and quantification of additional chloropigments and carotenoids in extracts of marine plankton.

Many of these pigments exhibit strong chemotaxonomic associations which may be used to characterise the distribution and composition of phytoplankton assemblages. For example:

Pigment	**Key marker for:**
Peridinin (*per*)	Dinoflagellates
19'-Butanoyloxyfucoxanthin (*but*)	Chrysophytes/Prymnesiophytes
Fucoxanthin (*fuc*)	Diatoms/Prymnesiophytes
19'-Hexanoyloxyfucoxanthin (*hex*)	Prymnesiophytes
Alloxanthin (*allo*)	Crypyophytes
Zeaxanthin (*zea*)	Cyanobacteria/Prochlorophytes
Lutein (*lut*)	Green Algae
Divinyl chlorophyll a (*dv chla*)	Prochlorophytes

Methodology

Samples were collected on-board by vacuum filtering of seawater through 25mm GF/F. The filters were preserved in liquid nitrogen. Back at the lab pigments will be extracted from the filters using 90% acetone with apo-carotenal as an internal standard. Pigment extracts will be analysed by reverse-phase HPLC using: a Shandon Hypersil MOS-2 C8 column (100 x 4.6mm, 3micron); Thermoseparations UV6000 photodiode array detector (300-700nm) ; and a binary mobile phase (70:30% methanol:1M ammonium acetate and 100% methanol). Pigment identities are secured through co-elution with authentic pigment standards (VKI, Denmark and Sigma Chemical Co.)

Summary of samples collected for pigments:

Date	Station	Pig Depths (m)
02/4/02	PP 1-2	3,5,10,13,16,21,25,30,45,66
02/4/02	M 1-12	2,5,10,25,35,50,66
03/4/02	PP 4-2	2,5,7,10,15,17,20,30,60,95
03/4/02	M 4-10	2,5,10,15,20,30,40,80,95
04/4/02	M 6-2	5,10,20,30,40,55,80,100,200
05/4/02	PP 6-11	2,7,13,19,24,32,42,55,80,150
05/4/02	M 7-1	5,15,30,45,60,90
06/4/02	PP 7-7	2,5,10,14,17,23,30,40,60,120
06/4/02	M 7-15	5,10,20,30,39,50,75,90,105,120
08/4/02	PP 7-20	2,5,10,14,17,23,30,40,60,120
08/4/02	M 7-26	2,5,15,30,45,60,75,91,105,125
09/4/02	PP 7-36	2,5,10,17,30,40,50,60,80,120
09/4/02	M 7-43	5,10,20,30,40,60,75,90,105,120
10/4/02	PP 7-54	2,5,8,12,15,20,27,35,60,120
10/4/02	M 7-58	5,15,30,45,60,75,105,120
11/4/02	PP 7-71	2,5,15,20,27,35,45,60,80,120
11/4/02	M 7-78	5,15,25,35,45,75,105,120
12/4/02	PP 7-90	2,5,8,14,20,27,35,45,60,80,120
12/4/02	M 7-93	5,10,20,35,60,90,120

~30 underways for fluorometer calibration

PP = Primary Productivity
M = Midday

6.4 The contribution of microzooplankton to the diet of copepods

Tanya Smith, Plymouth Marine Laboratory

The experiments aim is to help quantify the role of microzooplankton in the diet of small and large copepods. This was carried out using an on deck exclusion/addition incubation method. The most abundant species at each station determined the copepod species used . The stations sampled with incubations set up are in the list below.

Station 1 – 2/04/02 (E1)
Station 4 – 3/04/02 (Comparative station)
Station 6 - 5/04/02 (Offshelf)
Station 7 – 6/04/02 (lagrangian)
Station 7 – cancelled due to bad weather
Station 7 - 08/04/02
Station 7 – 9/04/02
Station 7 – 10/04/02
Station 7 – 11/04/02

Water from the 33% (10m) light depth was collected at approx 2am on the dates above. This water was used as the natural food source for the animals, after being gently siphoned through a 200um mesh (to remove mesozooplankton). Incubations were also set up with water siphoned through a 50um mesh to exclude nauplii (and some larger microzooplankton), thus showing their possible effect on grazing.

The samples taken/set up;

T zero 200um
Tzero 50um

50um control x3
200um control x3
Large copepod x3
Small copepod x3

The copepods for the experiment were picked out of a plankton sample collected with a 200um WP-2 net. Female *Calanus* (large copepod) and *psudo/paracalanus* (small copepod) were the most abundant copepods at each station and only healthy specimins were used. Stocking densities were at 5 *Calanus* per litre and 25 per litre for *psudo/paracalanus.* Time zero samples were fixed in lugols iodine and 3x 100ml aliquots were filtered for chlorophyll analysis. The control (without copepods) and the experimental bottles were transferred to the on deck incubator and left for 24 hours. After the incubation period samples were treated as for the time zero samples. The copepods were removed and kept for Carbon analysis.

Chlorophyll samples and the microzooplankton counts will be carried out back at the laboratory.

6.5 Optics

Tim Smyth & James Fishwick, Plymouth Marine Laboratory

Aims

- Take FRRF measurements for the determination of primary productivity parameters.
- Take Inherent Optical Property (IOP) measurements for use within primary production models.
- Take "sea-truth" optical measurements for SeaWiFS and MERIS ocean colour validation and algorithm development.
- Take measurements of marine aerosols to improve SeaWiFS and MERIS atmospheric correction algorithms.
- Provide remote sensing data analysis.

Method

A rig containing a Fast Repetition Rate Fluorometer (FRRF), ac-9, Volume Scattering Function (VSF) meter, CTD and optical radiometers was deployed at approximately the same times throughout the day. This was generally at 05:00, 09:00, 12:00, 13:30, 14:30 and 16:00 (all times BST) during the Lagrangian phase of the cruise when a drogued drifter buoy was deployed. The rationale behind this was to sample the water column at sufficiently high temporal resolution to resolve the diurnal variability in photosynthetic parameters. The rig was deployed from the aft deck via a crane to minimise ship shading, which would have occurred if the rig had been deployed midships. The rig was lowered quickly to 50 m before being raised slowly to the surface to provide sufficient interrogation of the phytoplankton within the water column.

During transects an FRRF was put into a container filled by a continuous flow from the non-toxic supply. This allowed 10 minute resolution of the transect in terms of the various photosynthetic parameters measured using the FRRF.

A Satlantic freefall optical profiler was also deployed from the aft of the ship around solar noon. This operated at eight wavelengths (412, 443, 490, 510, 555, 670, 715 and 870 nm) measuring upwelling radiance and downwelling irradiance. This was deployed by hand and allowed to drift away from the ship before allowing the freefall stage to commence. The data was logged in real-time down the wire.

A SIMBADA radiometer (on loan from Laboratoire d'Optique Atmospherique) was used to derive properties of marine atmospheric aerosols. The instrument has eleven wavelengths in the optical and near infra-red and is operated by pointing the instrument directly at the sun when the sun's disk is unobscured by clouds. SIMBAD measures direct sunlight intensity and, by knowing an accurate calibration for the instrument and using algorithms found in the literature, the contribution to the total atmospheric absorption at the eleven wavelengths by aerosols can be calculated.

Ocean Colour satellite imagery from SeaWiFS was sent via email from the Plymouth Marine Laboratory Remote Sensing Group (RSG). The RSG possesses the capability of receiving and processing satellite data in near-real time; the imagery being ready for dissemination to the RRS Discovery about 1 hour after the satellite overpass time. The chlorophyll imagery was invaluable at determining the position and evolution of the spring bloom

Results

a) FRRF

Figure 6.5.1 shows the variation of various parameters throughout the day measured using the FRRF. The top panels show how PAR varies throughout the water column – higher values penetrating to greater depths toward noon. The middle panels show how that around the middle of the day Fv/Fm is

dramatically reduced by the action of photo-chemical quenching. There is a recovery during the afternoon. The bottom panel shows the variation of primary production with depth calculated using the equation:

P(z) = factor * PAR(z) * chl(z) * sigma * Fv/FmL(z)

The interesting feature to note is the reduction in primary production when the sun is at its zenith followed by a recovery in the middle of the afternoon. This shows that the production does not follow a simple sinusoid but rather that there are secondary peaks in production flanking the noon time period. This is entirely to do with photo-chemical quenching.

In addition to this the photosynthetic parameters of E_k, P_m and α were calculated using exponential and hyperbolic tangent formulations. These will be used in a direct comparison with the simultaneous P-E measurements taken using the C14 method.

Figure 6.5.2 shows the variation of F_v/F_m and F_m through a transect made on 05/04/2002. The aim was to find where F_v/F_m was high (i.e. the phytoplankton are in a healthy condition) and F_m is low (i.e. where the biomass / chlorophyll is low). The rationale behind this was to determine the best place to set up the Lagrangian experiment the requirement being where the bloom was about to occur. This data was taken to complement the underway nutrient, optical plankton counter and fluorometry measurements

Note:fv is the variable fluorescence; fm is the maximum fluorescence; fv/fm is the ratio of the variable to maximum fluorescence and gives a measure of the health of the photoplankton (theoretical maximum is 0.65); fmL is the maximum fluorescence in the light chamber.

b) Free-fall optics
There were a total of 20 free-fall optics casts made (table 6.5.1). As the operational protocols state that the instrument should be deployed outside the whitecap zone (i.e. below winds of 20 mph) this limited the total number of casts that could be made. The free-fall optics processing software is currently under development and the raw data will be processed back at PML.

c) Aerosols
A total number of 65 direct sun measurements were taken with the SIMBADA sunphotometer. As the SIMBADA is a relatively new instrument there is no higher level processing software. The data will be used to validate MERIS satellite imagery and will be processed back at the LOA.

d) Remote Sensing
The images sent to the RRS Discovery by the PML Remote Sensing Group were vital in determining the location of the spring bloom (centred on approximately 50 N, 10 W). The images showed the complex structure of the bloom and allowed several transects to be made in and around the bloom environs.

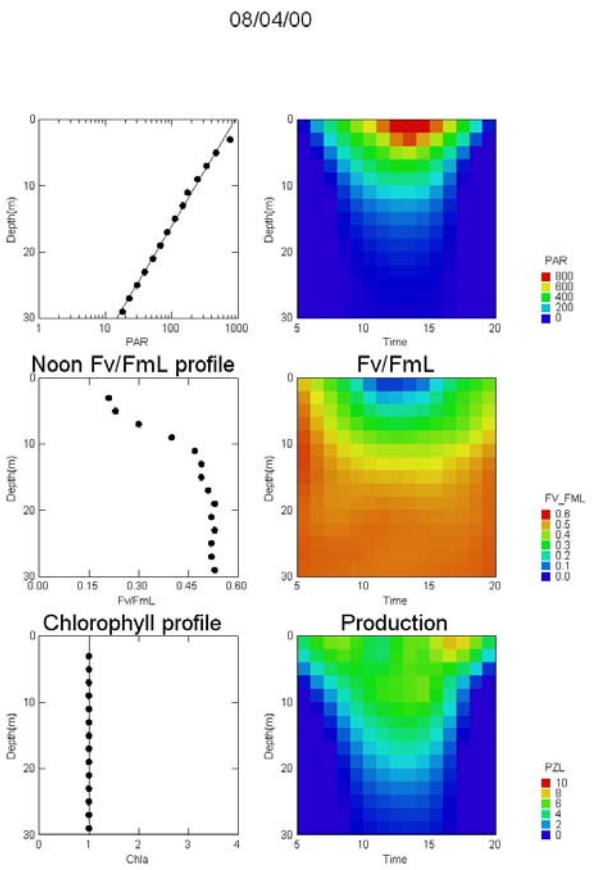


Figure 6.5.1: Top panels: Left; noon PAR profile, Right; temporal variation of PAR throughout the water column. Middle: Left; noon Fv/FmL profile, Right; temporal variation of Fv/FmL throughout the water column. Bottom: Left; vertical profile of chlorophyll (normalised to 1), Right; temporal variation of primary production throughout the water column.

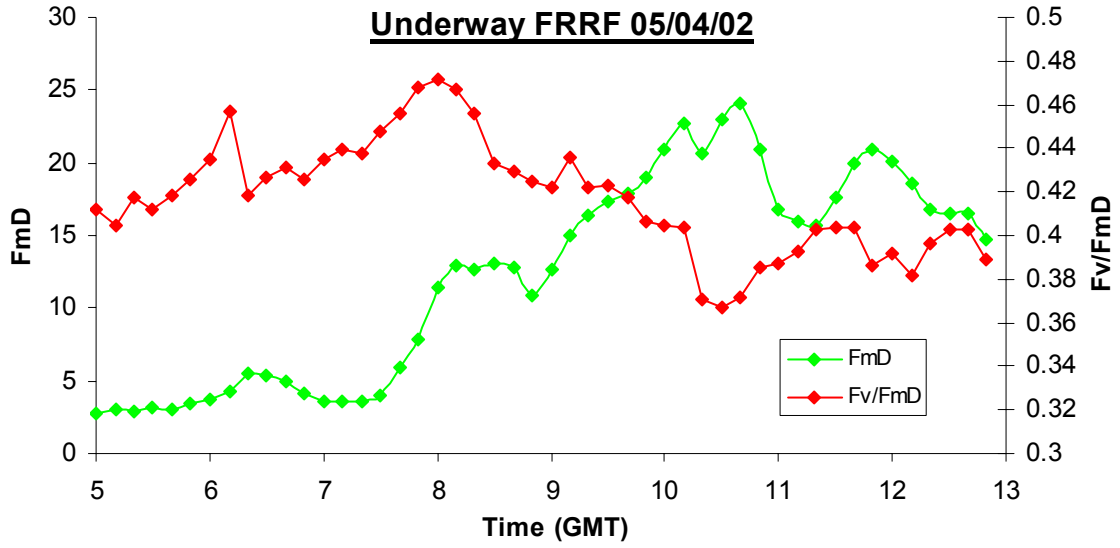


Figure 6.5.2: Underway transect taken using the FRRF across the centre of the bloom

Cast I.D	Rig Deployed	File I.D	Lat	Lon	Time in (BST)	Time out (BST)
	FRRF	010402a			12:00	
	FRRF	020402a			05:55	06:12
	Freefall	020402b			09:00	09:10
	FRRF	020402c			09:47	10:02
	Freefall	020402d			11:08	
	FRRF	020402e			11:28	11:43
	Freefall	020402f			13:22	
	FRRF	020402g			13:45	13:59
	Freefall	020402h			16:09	16:20
	FRRF	020402i			16:32	16:40
	Freefall	030402a			08:58	
	FRRF	030402b			09:21	09:37
	Freefall	030402c			11:41	
	FRRF	030402d			12:08	12:23
	Freefall	030402e			13:17	
	FRRF	030402f			13:36	13:46
6.2	FRRF	040402a			10:49	11:06
6.6	FRRF	040402b			14:19	14:43
7.2	Freefall	050402a			11:57	
7.3	FRRF	050402b			15:28	15:38
Underway FRRF transect 06:00-13:50						
	FRRF	060402a			05:21	05:30
	FRRF	060402b			09:47	
070402 No casts could be attempted on this day due to bad weather						
	FRRF	080402a			09:08	09:47
	Freefall*2	080402b			11:34	11:55
	FRRF	080402c			12:04	12:15
	Freefall*2	080402d			13:13	13:30
	FRRF	080402e			13:37	13:54
	FRRF	080402f			15:05	15:20
	FRRF	080402g			16:35	16:58
Underway FRRF transect 17:30-00:30						
	FRRF	090402a			05:10	05:27
	FRRF	090402b			09:31	09:52
	Freefall	090402c			11:35	
	FRRF	090402d			11:55	12:16
	Freefall	090402e			13:35	
	FRRF	090402f			13:56	14:09
	FRRF	090402g			15:06	15:23
	FRRF	090402h			16:59	17:17
	FRRF	100402a			05:06	05:23
	FRRF	100402b			09:34	09:57
	Freefall	100402c			11:35	
	FRRF	100402d			11:53	12:11
	FRRF	100402e			13:11	13:25
	Freefall*2	100402f			13:50	
	FRRF	100402g			14:28	14:42
	FRRF	100402h			16:06	16:24
	FRRF	110402a			05:00	05:21
	FRRF	110402b			09:09	09:36
	Freefall	110402c			11:38	
	FRRF	110402d			11:50	12:14
	Freefall	110402e			13:03	
	FRRF	110402f			13:18	13:34
	FRRF	110402g			14:33	14:49
	FRRF	110402h			16:08	16:23
Underway FRRF transect 17:00-00:30						
	FRRF	120402a			05:00	
	FRRF	120402b			09:41	

Table 6.5.1: Summary of FRRF and freefall optics casts taken during D261

6.6 Analysis of Photosynthetic Pigments

Brendan J Keely , University of York

Introduction

Photosynthetic pigments (chlorophylls and carotenoids) can be utilised as indicators of the primary producer community both in relation to photosynthetic activity (pigment abundance) and to the diversity (range of pigments present) and health (pigment transformation products) of the population. Recent literature reports have suggested that purple phototrophic prokaryotes occur in the open marine environment and it was estimated that their contribution to primary production might represent as much as 10%.

The aims of this work were to sample from the pre-dawn CTD cast to enable vertical profiles of the water column pigment distribution to be constructed. The specific objectives were to look for chlorophylls of purple phototrophic prokaryotes and to examine the abundance profiles of chlorophyll allomers, products of the autoxidation of chlorophyll.

Experimental Approach

Sampling

Water samples were collected by CTD to examine depth profiles, from the non-toxic supply to examine changes in the surface waters during transit, and by Niskin at a single depth to examine the pigment composition at an oligotropic water mass.

During the initial transect across the bloom plankton concentrates were prepared from the non-toxic supply by filtration through a mesh (Chris Gallienne and Carol Turley). Two samples were collected pre-dawn in zooplankton nets to determine if signatures of the grazers could be detected.

Pigments were analysed during a study of phytoplankton degradation (with Carol Turley and Claire Hughes – for details see report by Claire Hughes).

Sample Work-up

Water samples were filtered through 25 mm GF/F filters, the filters were extracted into acetone (3 mL) by sonication (VC 130 PB ultrasonic processor equipped with a 3 mm diameter probe [TI-6AL-4V]; Sonics & Materials Inc, Connecticut, USA). The extracts were filtered through defatted cotton wool packed tightly in a pasteur pipette and volumes were reduced under a stream of nitrogen. Extracts were diluted to volumes between 0.5 and 1 mL immediately prior to high performance liquid chromatography (HPLC) analysis.

Pigment analysis was performed during the cruise using a Thermo Separations (Hemel Hempstead, UK) system comprising a P4000 pump and vacuum degasser, AS3000 autosampler, UV6000 diode array detector and FL2000 fluorescence detector controlled through ChromQuest software (version 3.0 build 1073) operated under WindowsNT4 (service pack 5). The UV6000 detector was operated over the range 350 – 798 nm with a step of 1 nm, bandwidth of 5 nm and sampling frequency of 2 Hz. The fluorescence detector was operated for excitation at 650 nm and emission above 760 nm, using a flash rate of 100 Hz, data rate 10 Hz, rise time 2.0 s and photomultiplier voltage of 600 V. The autosampler was chilled to 4°C and sample injection (100 μL) was carried out using the Push loop method and with a flush volume of 400 μL. The separation method used was Method A as described by Airs *et al.,* (2001) with the ammonium acetate at a concentration of 0.5M.

A calibration was established for chlorophyll *a* HPLC measurements by serial dilution of a stock solution to three other concentrations. The concentration of the stock solution was measured, by UV/vis spectrophotometry, at 7.7 x 10^{-6} g L^{-1}. The stock solution and each of the dilutions were measured and a linear calibration was established over two orders of magnitude. Data for phaeopigments were calculated using a conversion factor from the extinction coefficient of chlorophyll *a* and will need to be re-evaluated post-cruise.

Duplicate samples were collected for more detailed examination of the pigment distributions in York and for analysis by liquid chromatography–mass spectrometry to confirm assignments and identify a greater range of components.

Preliminary Results

Water Samples

The water samples showed significant variation in chlorophyll concentration across the bloom (recorded in both transects – see Figure 6.6.1 for example) and with depth in the water column. Good correspondence to the fluorescence responses determined from the underway samples and from the CTD was apparent. The pigment distributions exhibited a high degree of similarity throughout the cruise, being dominated by chlorophyll *a* but with a substantial contribution from chlorophylls *c.* Changes in the relative proportions of chlorophyll to phaeopigments (phaeophytin *a* and pyrophaeophytin *a*) were apparent with the latter present in increased relative proportion as the bloom developed. Distinct changes with depth were also evident, particularly at the sampling stations later in the cruise. Further studies will attempt to establish if this change relates to grazing or senescence. Example depth profiles for three Stations are given in Figure 6.6.2.

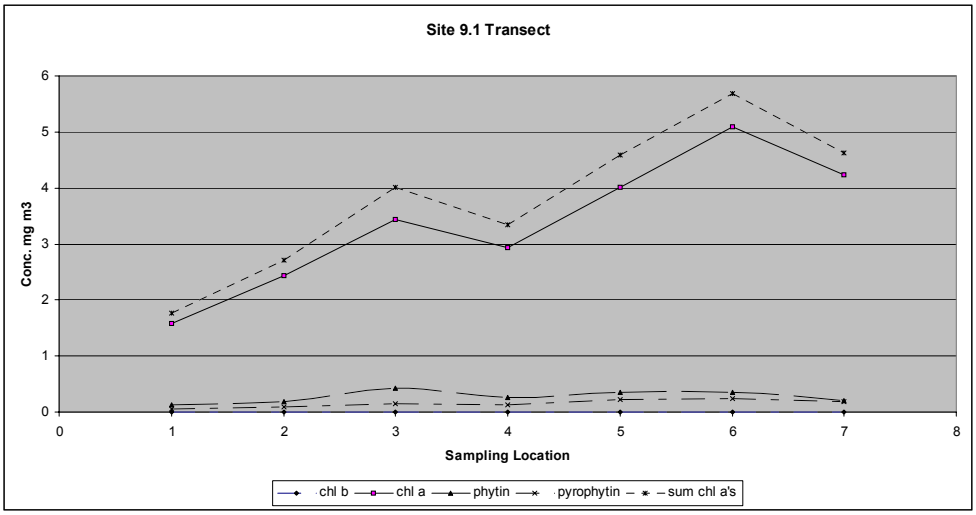


Figure 6.6.1: Pigment concentrations recorded from analysis of water samples collected from the non-toxic supply during the doughnut transect across the bloom.

The presence of oxidation products of chlorophylls was noted throughout the programme, with the greatest relative abundance being observed in the oligotrophic waters at Station 8.1. Detailed analysis by LC-MS is required to ascertain the identities of the derivatives and for the purposes of quantification. Throughout the duration of the monitoring programme there was no evidence for the presence of bacteriochlorophylls.

Plankton Concentrates

During the initial transect across the bloom that had been identified from the satellite images the pigments were analysed in the SW extent, the centre, and twice in the NE. The analysis showed the lowest chlorophyll levels in the centre of the bloom. The highest chlorophyll levels were present in the NE extent of the bloom, accompanied by high levels of phaeopigments. The phaeopigments are indicative either of active grazing of the phytoplankton or of senescence.

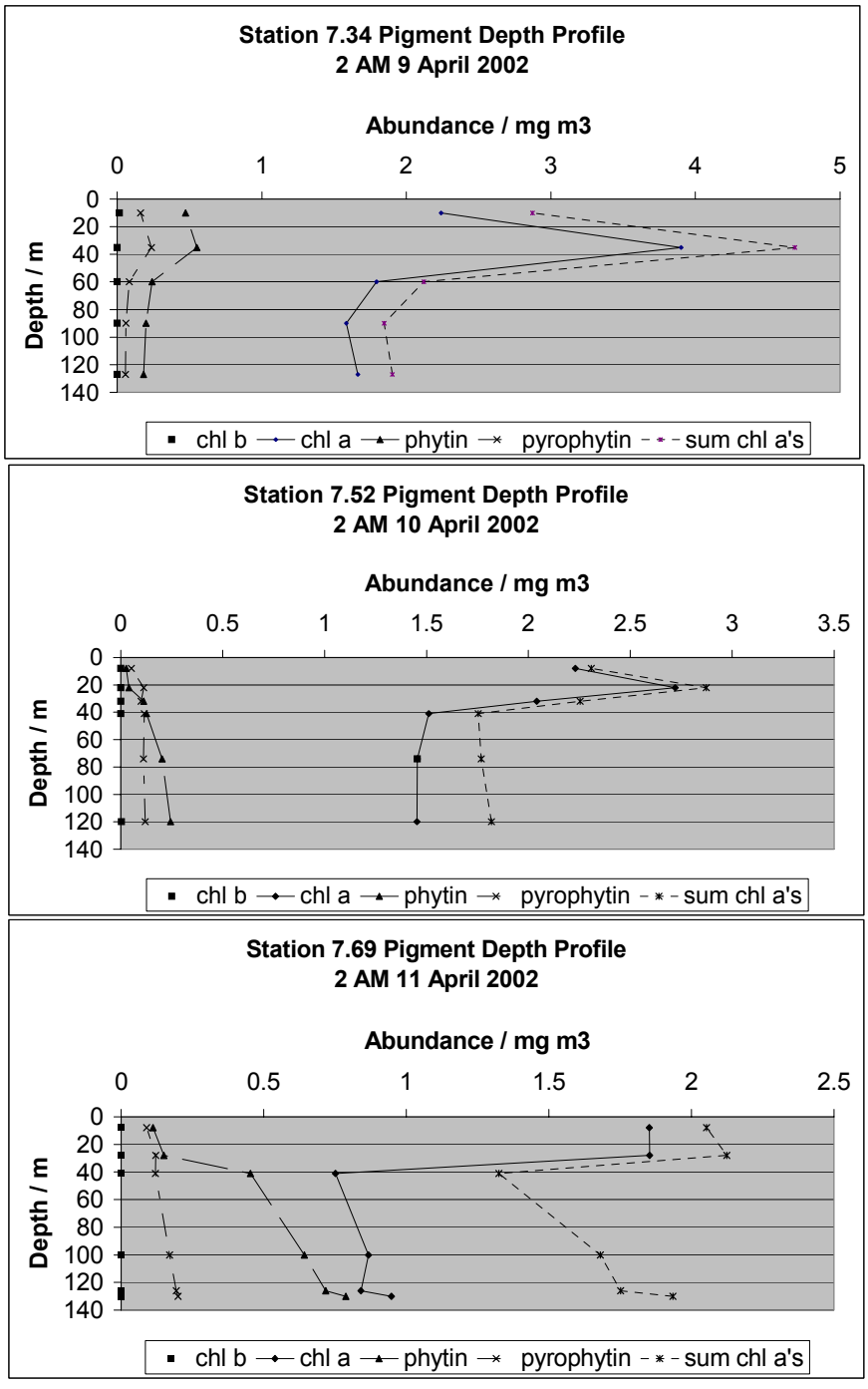


Figure 6.6.2: Pigment depth profiles recorded at three Stations.

Plankton Net Samples

The net sample from Station 5 contained both algal and zooplankton material. The algae was mostly removed by pipette to allow the animal concentrate to be examined. The HPLC analysis revealed that the extract contained only very low amounts of chlorophyll derivatives and was dominated by carotenoids. The net sample from Station 7.89 also contained both algae and zooplankton. Given that many of the zooplankton were intimately associated with the algae, the sample was filtered and analysed without any attempt to separate the animals from the two. The HPLC analysis revealed a high proportion of phaeophytin *a*. Detailed comparison with the data from the water column samples is required to determine if the signal relates to the algal material that was recovered in the net or to the grazers.

Phytoplankton Degradation Study

The data from the phytoplankton degradation study showed the onset of degradation of the pigments after 24 h. The results from some analyses showed a compete absence of pigments and in all samples the pigments were extensively degraded, mostly with oxidation products of chlorophyll dominating the remaining pigment distribution. The results indicate that degradation ensued rapidly following the commencement of the experiment and that the algae were in a poor condition. Further analysis of the duplicate samples is required to establish if the null responses were due to the extent of variability in preparation of the samples for the experiment or to differences in activity.

Reference

Airs R.L. Atkinson J.E. & Keely B.J. (2001). Development and application of a high resolution liquid chromatographic method for the analysis of complex pigment distributions. *Journal of Chromatography A,* **917,** 167-177.

6.7 Oxygen isotopes

Efrat David, The Inter-University Institute for Marine Sciences, Eilat

Samples for $\delta^{17}O$, $\delta^{18}O$ and δO_2 /Ar analysis were collected during cruise D261 from the productivity casts. Seawater was sampled directly from a full niskin into vacuumed glass bottles containing mercuric chloride. Additional 24 hours bottle incubation experiments were undertaken after every sampling and incubation water was sampled at the end of each experiment.

Station	Samples (Light %)	Samples for Incubation (Light %)
4.2	1, 7, 14, 33, 55, 97	1, 7, 14, 33, 55, 97
6.11	1, 7, 14, 33, 55, 97	1, 7, 14, 33, 55, 97
7.7	1, 7, 14, 33, 55, 97	1, 14
7.20	1, 7, 14, 33, 55, 97	1, 7, 14, 33, 55, 97
7.36	1, 7, 14, 33, 55, 97	1, 7, 14, 33, 55, 97
7.52	1, 7, 14, 33, 55, 97	1, 7, 33, 97
7.69	1, 7, 14, 33, 55, 97	

All sample analyses will be done in the Hebrew University and will be available in about three months.

6.8 Plankton dark community respiration, gross production and net community production

Carol Robinson[1] & Efrat David[2] , [1]Plymouth Marine Laboratory &[2]The Inter-University Institute for Marine Sciences, Eilat

Objectives

1. To compare the magnitude and time progression of autotrophic production and heterotrophic respiration during a phytoplankton bloom

2. To determine the depth distribution of respiration above and below the euphotic zone

3. To relate the magnitude and variability of community respiration to bacterial, algal and microzooplankton respiration

4. To compare two methods of determining plankton respiration : *in vitro* dissolved oxygen flux and an instantaneous measure of the electron transport system activity (ETS)

5. To compare two methods of determining plankton gross production : *in vitro* dissolved oxygen flux and *in vitro* production of $^{18}O_2$.

6. To determine the depth distribution of dissolved oxygen in order to calibrate the oxygen sensor on the CTD

Methods

Dark community respiration, net community production and gross production were determined from *in vitro* changes in dissolved oxygen. Dissolved oxygen was measured by automated Winkler titration. Water was collected from the productivity casts each morning from depths equivalent to 97%, 55%, 33%, 14%, 3% and 1% of surface irradiance plus up to three depths below the euphotic zone and incubated in 60 ml glass bottles in surface water cooled deck incubators for 24 hours. Light depths were calculated from PAR measurements made the previous day (T.Smythe & J.Fishwick). Normally four replicates were incubated in the light, four in the dark and four fixed for determination of zero time concentrations. Samples were collected, filtered and stored in liquid nitrogen for later analysis of plankton electron transport system activity (ETS). Water was collected for ΔO_2 and ETS from the same depths as that analysed for phytoplankton assimilation of ^{14}C (S.Barquero-Molina) and ^{15}N (A.Rees), production of $^{18}O_2$ from ^{18}O labelled H_2O (E.David), microzooplankton grazing (C.Widdecombe) and bacterial production (R.Lamb).

Samples Collected

Date	CTD / Non toxic	Measurements
2 April	CTD 1.2	Dissolved oxygen 11 depths, ETS 8 depths, GP, NCP & DCR 8 depths
4 April	CTD 4.2	Dissolved oxygen 10 depths, ETS 9 depths, GP, NCP & DCR 9 depths
4 April	CTD 6.2	Dissolved oxygen 11 depths
5 April	CTD 6.11	Dissolved oxygen 12 depths, ETS 10 depths, GP, NCP & DCR 8 depths
6 April	CTD 7.7	Dissolved oxygen 12 depths, ETS 10 depths, GP, NCP & DCR 9 depths
8 April	CTD 7.20	Dissolved oxygen 12 depths, ETS 10 depths, GP, NCP & DCR 9 depths

9 April	CTD 7.36	Dissolved oxygen 12 depths, ETS 10 depths, GP, NCP & DCR 7 depths
10 April	CTD 7.54	Dissolved oxygen 12 depths, ETS 10 depths, GP, NCP & DCR 9 depths
10 April	Non-toxic	4 stations along transect for ETS and DCR
11 April	CTD 7.71	Dissolved oxygen 12 depths, ETS 10 depths, GP, NCP & DCR 7 depths
12 April	CTD 7.90	Dissolved oxygen 12 depths, ETS 10 depths

Preliminary Results

The temporal change in gross production, net community production and dark community respiration during the Lagrangian study is shown in figure 6.8.1. Dissolved oxygen and oxygen flux measurements will be checked and ready for distribution within 3 months.

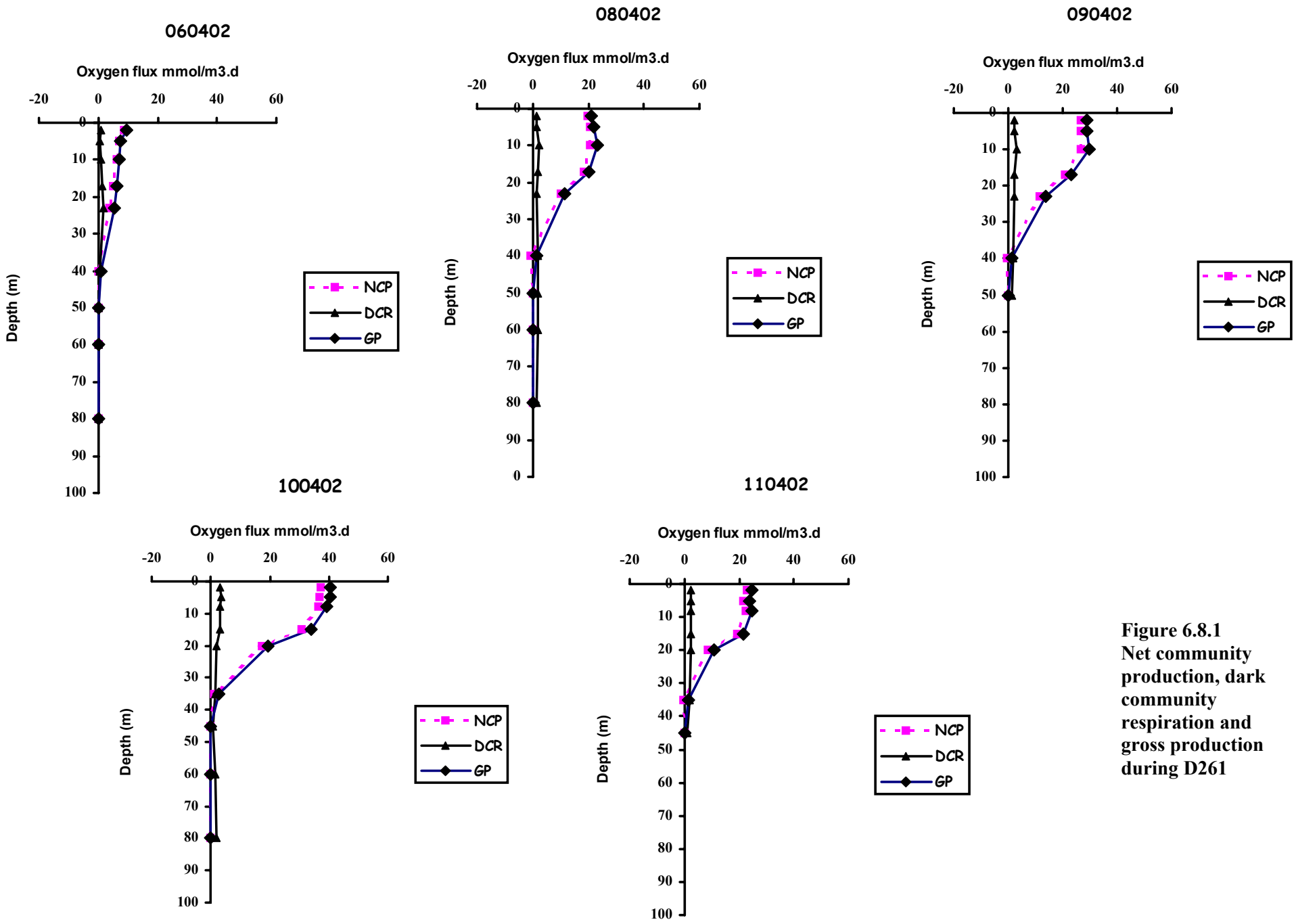


Figure 6.8.1
Net community production, dark community respiration and gross production during D261

6.9 Halocarbon Studies

Claire Hughes, University of East Anglia & Plymouth Marine Laboratory

Measurements of the halogenated organic compounds listed in table 6.9.1 were made during cruise D261 using a purge-and-trap preparation system and Hewlett Packard gas chromatograph - mass spectrometer (GC-MS). The aim of these studies was to gain some understanding of the processes controlling the production of halocarbons in seawater.

Table 6.9.1 - Halocarbons analysed by GC-MS during Discovery Cruise D261

Compound	Molecular Formula
Methyl chlroide	CH_3Cl
Methyl bromide	CH_3Br
Methyl iodide	CH_3I
Bromoethane	CH_3CH_2Br
Iodoethane	CH_3CH_2I
Carbon tetrachloride	CCl_4
1-iodopropane	$CH_3CH_2CH_2I$
Dibromomethane	CH_2Br_2
2-iodopropane	CH_3CHICH_3
Chloroiodomethane	CH_2ClI
Dibromochloromethane	$CHBr_2Cl$
Tetrachloroethylene	CH_2Cl_4
Bromoiodomethane	CH_2BrI
Bromoform	$CHBr_3$
Diiodomethane	CH_2I_2

Data Quality

Various factors (i.e. column humidity, repeller coating) can cause the GC-MS response to fluctuate. To correct for such variations, the stability of the GC-MS response was determined throughout the cruise using deuterated internal standards of methyl iodide and 2-iodopropane diluted in methanol. The instrument was also calibrated at the start and end of the cruise.

Data Collected

In all, approximately 200 samples were analysed for halocarbons during cruise D261. All revealed that halocarbon concentrations were low in the sampled waters. Samples taken from on-deck incubations and grazing experiments carried out by both Claire Widdicome and Tania Smith suggested that halocarbon production was not occurring. A transect of the bloom (described by Carol Turley) carried out on 10th April showed that there were no significant differences in halocarbon concentrations from the centre (senescing) to the outer reaches of the bloom. Halocarbon production was only observed in the two experiments detailed below.

1. Plankton degradation

For this experiment, plankton (>53um) was concentrated from 1000 litres of seawater taken from the non-toxic seawater supply (from a depth of 6m at 50^{o}N, 10^{o}W on 6th April) and resuspended in 1 litre of filtered seawater. This 1 litre of plankton concentrate was then distributed between 10 100ml glass syringes and incubated in the dark in the constant temperature room at 12 ^{o}C. During the course of this experiment samples were taken for halocarbon analyses, bacterial production, diversity and numbers (Carol Turley) and the HPLC analyses of pigments (Brendan Keely). To accompany this data, photographs were also taken at various intervals to illustrate the degradation of the plankton. Figures 6.9.1a and b are photographs of the incubated plankton community taken at t=0 and t=72 respectively. Preliminary results revealed that there were significant increases in methyl iodide,

methyl bromide, iodoethane, bromoethane and both 1- and 2-iodopropane during the course of this experiment.

2. Diatom mat incubation

A zooplankton net tow carried out on 5th April at 49° 37' N, 10° 20' W at 25m for 15 minutes and 40m for a further 15 minutes brought a mucous mat to the surface. On microscopic examination this mucous was found to be a diatom mat with cells of *Thalassiosira* embedded within. To investigate the ability of this mat to produce halocarbons, a portion of it was placed in a glass syringe and incubated for 14.5 hours (samples taken at 0, 2.5, 7.5 and 14.5 hours). As a control, the seawater in which the mat was placed when it was brought to the surface was filtered and also placed in a syringe. The mat was found to be a prolific producer of a number of halocarbons.

Fig 6.9.1a. Phytoplankton cells at the start of the decomposition experiment

Fig 6.9.1b. Phytoplankton cells towards the end of the decomposition experiment

6.10 Changes in embryonic gene expression and the genetic control of diapause

Pennie Lindeque & Gary Smerdon, Plymouth Marine Laboratory

Rationale:
The study of developmental genes is critical for the understanding of how an organism makes itself and the control mechanisms which operate during the development process. Study of such genes in terrestrial organisms, both vertebrate and invertebrate, has been both intense and fruitful. We have been extending this understanding to the ecologically important marine copepod *Calanus*.

To date our studies have been laboratory based and involved animal culture. During the cruise our aim was to use gene expression studies to determine the developmental status of early *Calanus* embryos in their natural environment, to allow a comparison of changes in development/expression rates under different environmental conditions. In particular, this cruise has focused on the physical and biological changes associated with the development of a spring bloom, and our interest here lies in how changes in nutritional status associated with the phytoplankton succession affects gene expression during early *Calanus* development. We aimed to specifically assess, at the basic biological level, the effect of a diatom rich phytoplankton composition on development and hence viability - currently an important topic of debate.

In addition to the above, we hoped to gain a better understanding of regulation of overwintering (diapause?) in *Calanus* by investigating differential gene expression in animals at varying depths off the continental shelf compared to those at the surface.

Changes in embryonic gene expression

Sampling
Calanus were collected with a WP2 net (500 μm) either with a vertical net haul or a tow. The net was washed thoroughly with the non-toxic supply and the animals washed from the cod end into a bucket. Animals were transferred immediately to the constant temperature room for sorting.

***Calanus* culturing and egg production**
Adult female *Calanus* were identified by microscopy and incubated in 200 μm mesh bottomed plexiglass tubes in 5L beakers of filtered (53μm) seawater. Females were removed from the cultures and eggs were further incubated at 12^{o}C. Developing eggs were processed at 4 hour time intervals from 0h to 24h.

Preparation for Molecular Analysis
Eggs were processed for molecular analysis of embryonic gene expression by homogenization in suitable lysis buffer and storage of the RNA lysate at -70^{o}C. Total RNA extractions were performed for some of the samples; the remainder will be completed at the laboratory. When sufficient numbers of eggs allowed, preparation for both total RNA extractions and solid phase cDNA library constructions were performed.

RNA extractions will be processed further for the quantitative analysis of embryonic gene expression with real-time PCR. The expression of developmental genes by real-time PCR will be compared to gene analysis ascertained by solid-phase cDNA libraries.

Genetic control of diapause

Sampling Date and Time and location: 04.04.02 15.10 6.6 48^{o}36.07'N 011^{o}13.33'W
Stand Alone Pumps (SAPs) were used to collect *Calanus* from 4 separate depths. The SAPs were started at 14.15 with a programmed delay time of 1.5h and a pump time of 3.0h. Stand Alone Pumps

were deployed to the following depths, litres pumped at each depth are indicated in brackets: -20m (3,453); -470m (3,623); -970m (3427); -1,470m (3,738). SAPs were lowered 15m every hour.

Analysis

The holding tank of each SAP was emptied into a bucket and animals were washed from the 300μm mesh socks with non-toxic water. At each depth the samples were sorted under the microscope for *Calanus* stage V juveniles. In order to investigate diapause it was hoped to obtain at least 5 animals from each depth for RNA extraction and subsequent cDNA subtractive hybridization library construction. From the constructed libraries it should be possible to determine the differential gene expression (upregulation or downregulation) between *Calanus* at different depths.

Results

An insufficient number of animals were collected at each depth for an RNA extraction and subsequent molecular analysis. When the SAPs were returned to deck the battery power had been exhausted, therefore actual pump time is uncertain, although an average of 3,300 litres had been pumped. It is also possible that without a positive pressure on the holding tanks animals were able to 'swim free'. Future deployments will incorporate a non-return device on the inlet aperture, and pumping will be limited to two hours.

Date	Station	Time	WP2 Nets[1]	Comments	No. of Calanus females picked (# of eggs produced)
02.04	S/N 3.1 050°N 04°36' W	19.30	Haul 20M → surface Tow 5M for 20 min	Tow limited to surface waters due to rough conditions.	0 (0)
03.04	S/N Zoostation 044°20 N 007°18'W	19.00	Haul 100M → surface	Too rough for a tow, haul abandoned @ 20 m due to rough conditions.	200 (≈454 in 14 h)
05.04	Great Sole Bank S/N 7.5 049°37'N 010°20/W	20.15	30 min tow 25m for 15 min 40m for 15 min	Good tow, plenty of animals. Nets clogged with *Thalassiosira spp*.	400 (≈ 8000 in 12 h)
08.04	S/N 7.32 050°N 010°W	20.15	30 min tow 20m for 15 min 35m for 15 min	Good tow, plenty of animals. Nets still clogged with phytoplankton.	500 (≈11,400 in 12 h)
09.04	S/N Non-bloom 8.1 050° 30.45'N 09°59.79'W	20.00	Niskin bottle on Kevlar 25m	Water taken for nutrients and phytoplankton community structure (398)	
09.04	S/N Non-bloom 8.2 050° 30.45'N 09°59.79'W	20.15	30 min tow 25m for 15 min 40m for 15 min	Oligotrophic comparative station	700 (≈9,000 in 12 h)
10.04	S/N 9.1 049°36.41'N 010°19.90'W	22.00	13 min tow 15m for 8 min 30m for 5 min	Possible Postbloom sn Net dominated by larger zooplankton	150 (≈ 3,000 in 12h)
11.04	S/N 7.85 050° 12.6'N 010° 21.03'W	21.30	20 min tow	Argos buoy in middle of bow tie. Zooplankton dominated by late juvenile stages.	170 (4,000 in 12h)

[1] tows performed at approx 30^0 angle. Depth below refers to line payed out.

Table 6.10.1 Sampling regime - zooplankton collection for molecular analyses

6.11 Bacterial production.

Rachel M. Lamb, **Plymouth Marine Laboratory & Southampton Oceanographic Centre**

Bacterial production:

Level 1 production CTD.
Samples were taken at 97%, 55%, 33%, 20%, 14%, 7%, 3% and 1% light intensities and also at four further variable depths (see table 6.11.1) from the 3 (or 4) am CTD cast.
Four replicate live samples and two TCA killed controls were spiked with tritiated leucine for each depth sampled and incubated for approximately one hour. The samples were then terminated and extracted for scintillation counting at PML.

Deep water CTD cast.
Samples were taken at 200M, 500M and 1000M, processing as previous.

Underway doughnut transect.
Samples were taken from the non-toxic water supply (details of track elsewhere) and processed as described above.

Table 6.11.1: Summary of samples taken for Bacterial Production:

Date	Activity Number (Station + Deployment).	Depths sampled (M)
02/04/02	1.2	2,5,10,12.5,15,25,30,35,45,65
03/04/02	4.2	2,5,7,10,15,17,20,30,60,95
04/04/02	6.7	2,7,13,19,24,32,42,55,80,150,200,500
06/04/02	7.6	2,5,10,14,17,23,30,40,50,60,80,120
08/04/02	7.18	2,5,10,14,17,23,30,40,50,60,80,120
09/04/02	7.49	2,5,10,14,17,23,30,40,50,60,80,120
10/04/02	7.55	2,5,8,12,15,20,27,35,60,120
11/04/02	7.69	2,5,15,20,27,35,45,60,80,120
12/04/02	7.90	2,5,8,15,20,35,45,60,80,120
04/04/02	6.2 deep water CTD	200,500,1000
10/04/02	Underway doughnut transect.	non-tox. supply.

6. 12 Determination of dissolved nutrients and euphotic zone nitrogen cycling

Andy Rees, John Stephens & Denise Cummings, Plymouth Marine Laboratory

Aims

During a series of contrasting oceanographic stations and a Lagrangian study of a developing spring bloom:

1) To determine the concentrations of dissolved nitrate, nitrite, ammonium, urea and silicate.
2) To determine the rates and relative proportions of nitrate, ammonium and urea uptake by size fractionated marine phytoplankton.
3) To estimate the rate of ammonium regeneration in parallel with nitrogen uptake and micro- and meso-zooplankton grazing experiments.
4) To investigate the production of urea in parallel with nitrogen uptake and micro- and meso-zooplankton grazing experiments.

Methodology- Nutrient analysis

Dissolved inorganic nutrient concentrations were analysed using a segmented flow autoanalyser within two hours of collection. Silicate was determined according to Kirkwood (1989), Nitrate by Brewer & Riley (1965) and Nitrite, Grasshoff (1976). Ammonium concentrations were generally measured using a fluorescence analysis method developed from Jones (1991), although on a number of occasions manual determinations based on the formation of indophenol were necessary. Urea concentration was determined manually on filtered (0.2µm) samples, according to Goeyens et al. (1998), the majority of which were performed onboard, though owing to a 70 hour development period, samples from the last few days were frozen and transported to the laboratory for analysis.

Nitrogen uptake

At eight depths within the euphotic zone uptake rates for nitrate, ammonium and urea were determined following the incorporation of the stable isotope ^{15}N. Duplicate samples of water from each depth were distributed into 620 ml clear polycarbonate bottles and $^{15}N\text{-}NO_3$, $^{15}N\text{-}NH_4$ and $^{15}N\text{-}CO(NH_2)_2$ were added at a final concentration of 10% ambient nitrate or ammonium concentration. Incubations were in an on-deck incubator which consisted of a series of 8 tanks with spectrally corrected light screens, which permitted transmission of ambient irradiance in the range 97 – 1% and was maintained at surface seawater temperature. Incubations were terminated after 24 hours by size fractionated (<5µm and total community) filtration (< 40 cm Hg vacuum) onto ashed Whatman GF/F filters, which were frozen until return to the laboratory, where they will be analysed by continuous flow nitrogen analysis-mass spectrometry.

Ammonium regeneration

From each of microzooplankton (Claire Widdicombe) and mesozooplankton (Tania Smith) grazing experiments and the base of euphotic zone: A single 2.4l polycarbonate bottle was filled with seawater and $^{15}N\text{-}NH_4$ was added at ~10% ambient concentration. 2 x 50 ml was removed and filtered through 0.2µm puridisc to clean medicine bottle for T_0 NH_4 analysis by solid phase extraction and either manual or autoanalyser analysis. The remainder of the sample was then incubated at an appropriate light level for 24 hours, after which 2 x 50 ml was removed and filtered through 0.2µm puridisc to clean medicine bottle for T_{24} NH_4 analysis by solid phase extraction and either manual or autoanalyser analysis. The remaining sample was then filtered through 25mm gf/f which was then stored frozen prior to analysis in the laboratory.

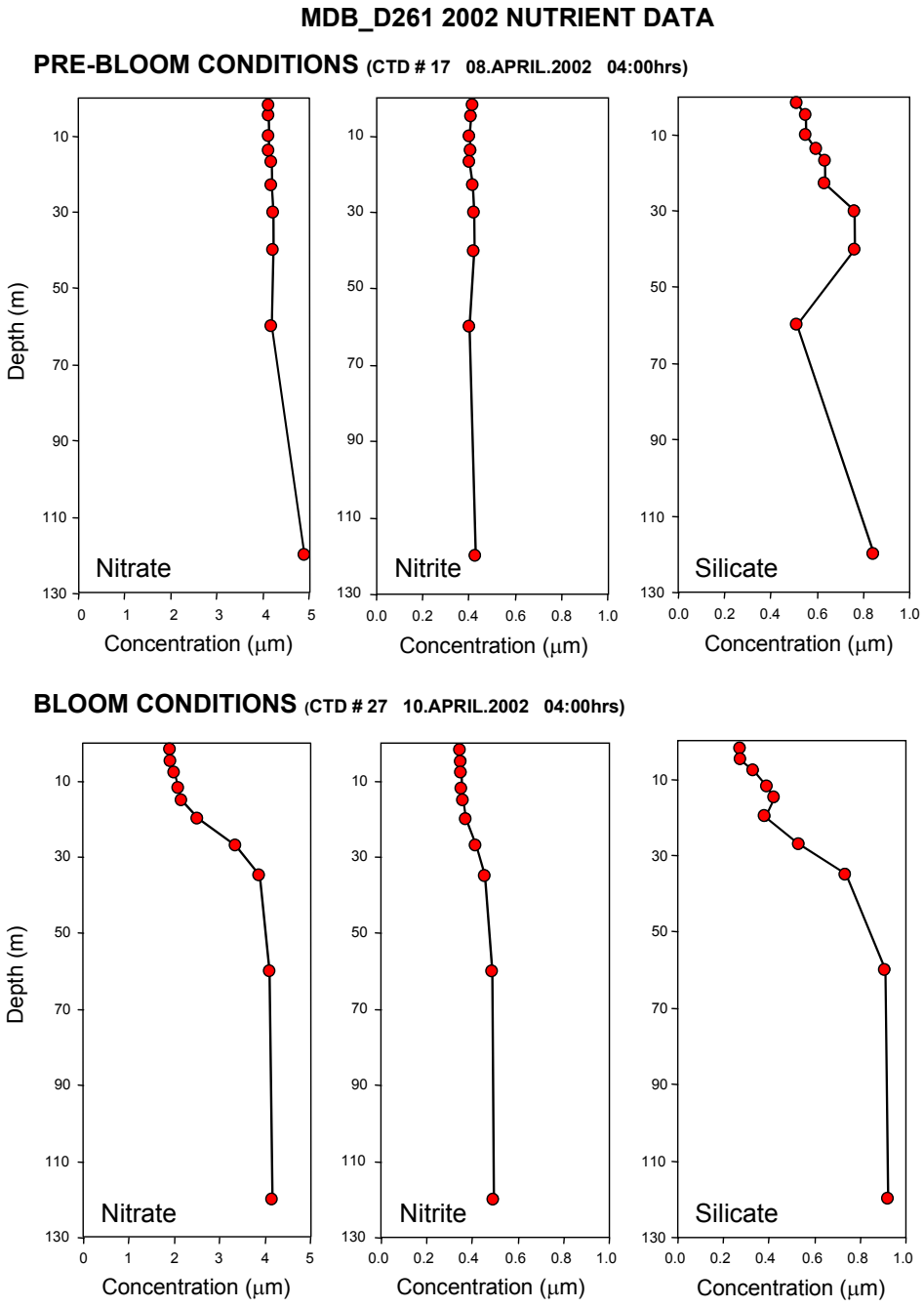
Urea production

From each of microzooplankton and mesozooplankton grazing experiments and the base of the euphotic zone: A single 0.6l polycarbonate bottle was filled with seawater. 40ml was removed and filtered ino a clean medicine bottle for T_0 urea analysis. The remaining sample was then incubated in

the dark (to minimise phytoplankton uptake) for 24 hours after which a further 40ml was removed and filtered into a clean medicine bottle for T_{24} urea analysis.

Results

Very little information is available at this stage and a period of intense laboratory analyses is required in forthcoming months, so that most data should be available within six months. Preliminary analysis of nutrient data is complete and available on request.



DATE-TIME (Z)	POSITION	No. DEPTHS	DEPTH RANGE (m)	VARIABLE
2nd April	50°.01'N 04°22'W	9	0 – 65	NO_3, NO_2, SiO_4
0253		6	0 - 65	urea
		7	0 – 30	^{15}N uptake – 24h, size fractionated
		2	10, 30	Urea prodn.
3rd April	49° 32'N 06° 00'W	10	0 – 95	NO_3, NO_2, SiO_4, NH_4, urea
0227		8	0 – 30	^{15}N uptake – 24h, size fractionated
		1	10, 30	^{15}N – NH_4 regeneration
		6	0 – 46	Urea prodn.
1134	49°32'N 06°00'W	9	2 - 95	NO_3, NO_2, SiO_4, NH_4
1548-2400	49°26'N 06°41'W - 49°10'N 8°30'W	S		NO_3, NO_2, SiO_4 - UNDERWAY
4th April 0001-0940	49°10'N 8°30'W - 48°43'N 10°59'W	S		NO_3, NO_2, SiO_4 - UNDERWAY
1034	48°41'N 11°12'W	12	5 - 1000	NO_3, NO_2, SiO_4, NH_4
5th April	48°41'N 11°12'W	10	2 - 150	NO_3, NO_2, SiO_4, NH_4, urea
0214		8	2 - 55	^{15}N uptake – 24h, size fractionated
		2	13, 55	^{15}N – NH_4 regeneration
		2	13, 55	Urea prodn.
0647-1840	48°53'N 11°01'W - 49°59'N 10°00'W	S		NO_3, NO_2, SiO_4 - UNDERWAY
6th April	49°59'N 10°00'W	10	2 - 120	NO_3, NO_2, SiO_4, NH_4, urea
0209		8**	2-40	^{15}N uptake – 24h, size fractionated
		2	10, 40	^{15}N – NH_4 regeneration
		2	10, 40	Urea prodn.
				** In-situ rig lost during bad weather – no ^{15}N uptake.
1105		10	5 - 120	NO_3, NO_2, SiO_4
8th April	50°04'N 10°19'W	10	2 - 120	NO_3, NO_2, SiO_4, NH_4, urea
0312		8	2 - 40	^{15}N uptake – 24h, size fractionated
		2	10, 40	^{15}N – NH_4 regeneration
		2	10, 40	Urea prodn.
1130	50°06'N 10°21'W	10	2 - 125	NO_3, NO_2, SiO_4
9th April	50°08'N 10°19'W	10	2 - 120	NO_3, NO_2, SiO_4, NH_4, urea
0308		8	2 - 40	^{15}N uptake – 24h, size fractionated
		2	10, 40	^{15}N – NH_4 regeneration
		2	10, 40	Urea prodn.
1129	50°08'N 10°22'W	10	5 - 120	NO_3, NO_2, SiO_4
1550-1840	50°10'N 10°19'W - 50°30'N 9°59'W	S		NO_3, NO_2, SiO_4 - UNDERWAY
10th April	50°10'N 10°18'W	10	2 - 120	NO_3, NO_2, SiO_4, NH_4, urea
0317		8	2 - 40	^{15}N uptake – 24h, size fractionated
		2	8, 35	^{15}N – NH_4 regeneration
10th April 0909	50°09'N 10°21'W	9	5 - 120	NO_3, NO_2, SiO_4
1617-2100	50°05'N 10°13'W	S		NO_3, NO_2, SiO_4 - UNDERWAY

	- 49°31'N 10°26'W			
11th April 0306	50°12'N 10°20'W	10 8 2	2 - 120 2 - 35 8, 35	NO_3, NO_2, SiO_4, NH_4, urea ^{15}N uptake – 24h, size fractionated ^{15}N – NH_4 regeneration
1133	50°11'N 10°22'W	10	5 – 120	NO_3, NO_2, SiO_4
1638-0152	50°16'N 10°23'W - 50°12'N 10°22'W	S	BOW-TIE SURVEY	NO_3, NO_2, SiO_4 - UNDERWAY
12th April 0256	50°12'N 10°21'W	10	2 – 120	NO_3, NO_2, SiO_4, NH_4, urea
0550-0912	50°15'N 10°24'W - 50°12'N 10°21'W	S	BOX SURVEY	NO_3, NO_2, SiO_4 – UNDERWAY
1117	50°11'N 10°23'W	10	5 - 120	NO_3, NO_2, SiO_4

S indicates surface measurement

Table 6.12.1. Sampling log for Rees, Stephens & Cummings (Di261/02).

6.13 Primary Production

Susana Barquero-Molina, Plymouth Marine Laboratory

Introduction

Primary production is a central process of the phytoplankton community where dissolved carbon dioxide is fixed by photosynthesis into the cell particles. Total phytoplankton production, i.e. the production based on all new and regenerated nutrients, can be measured with ^{14}C incubations. Net total production (gross production minus respiration) can be estimated in 24-h incubations from dawn to dawn. These measurements will be related to new production rates and macro- and micro-nutrient concentrations to investigate how the nutrient supply influences carbon flux and trace gas production.

A productivity gradient transect was covered from the English Channel to the off-shelf edge in the Celtic Sea, and a Lagrangian experiment was set up from 8-11 April 2002 to study the development and decline of a spring bloom of phytoplankton in Great Sole Bank.

Methods

Sampling

At the selected stations (Table 6.13.1), water was collected with Niskin bottles during a pre-dawn (0300-0400 am) productivity CTD cast. A maximum of 8, 1-L seawater samples were taken in polycarbonate bottles from depths corresponding to the incident light percentages 97% (surface), 55%, 33%, 20%, 14%, 7%, 3% and 1% (critical depth).

From each seawater sample, four 60-ml transparent polycarbonate bottles were filled to the rim, one of them wrapped in tin foil (control dark bottle). The experimental bottles were kept dark at surface temperature until dawn.

Station.Cast	Date	Depths (equivalent % I_o) sampled
1.2 (station E1)	2 Apr	surface (97%), 5 (55%), 10 (33%), 12.5 (20%), 15 (7%), 25 (3%), 30 m (1%)
4.2 (comparative station)	3 Apr	2 (97%), 5 (55%), 7 (33%), 10 (20%), 15 (14%), 17 (7%), 20 (3%), 30 m (1%)
6.8 (off-shelf station)	5 Apr	2 (97%), 7 (55%), 13 (33%), 19 (20%), 24 (14%), 32 (7%), 42 (3%), 55 m (1%)
*7.7 (Lagrangian sta.)	6 Apr	5 (97%), 7 (55%), 9 (33%), 11 (20%), 13 (14%), 15 (7%), 21 (3%), 23 m (1%)
7.21 (Lagrangian sta.)	8 Apr	2 (97%), 5 (55%), 10 (33%), 14 (20%), 17 (14%), 23 (7%), 30 (3%), 40 m (1%)
7.37 (Lagrangian sta.)	9 Apr	2 (97%), 5 (55%), 10 (33%), 14 (20%), 17 (14%), 23 (7%), 30 (3%), 40 m (1%)
7.55 (Lagrangian sta.)	10 Apr	2 (97%), 5 (55%), 8 (33%), 12 (20%), 15 (14%), 20 (7%), 27 (3%), 35 m (1%)
7.72 (Lagrangian sta.)	11 Apr	2 (97%), 5 (55%), 8 (33%), 12 (20%), 15 (14%), 20 (7%), 27 (3%), 35 m (1%)

*only chl a data, no production available

Table 6.13.1. Samples taken for primary production experiments during the cruise.

On deck 24-h incubations

Primary production experiments followed the standard JGOFS protocol.

Just before dawn (0600-0630 am), co-ordinated with ^{15}N and oxygen experiments, the experimental bottles were spiked with the ^{14}C solution (final specific activity per bottle 10 μCi approx.) and placed in on-deck incubators. The incubators were supplied with running surface seawater and covered with appropriate screens to simulate the in situ light percentages. The bottles were incubated for 24 hours until next day dawn and removed from the incubators just before replacing with a new set of bottles. At nightfall the incubators were covered with lids to prevent the deck lights affecting the experiment.

Filtration: size fractions

The filtration of the incubated ^{14}C samples started immediately after dawn. During the filtration, the bottles were kept in the dark. Each sample was size-fractionated through 5.0 μm and 0.2 μm polycarbonate membrane filters (47-mm diameter) at low vacuum. Filters were fumed with HCl and desiccated for ~12 h before adding the scintillation cocktail. After impregnated with the cocktail for at least 6 hours, DPMs were counted in a scintillation counter.

Complementary chlorophyll measurements

Chlorophyll analysis followed the standard JGOFS protocol.

Chlorophyll a was determined in the remaining seawater from the 1-L sampled at every depth. On average, 250 ml of seawater were filtered and fractionated in the same fashion as the primary production samples. Because the filtration of chlorophyll samples took place at midday (after the primary production filtration), water was kept refrigerated in the dark until then. Filters were placed in centrifuge tubes with 10 ml of 90% acetone overnight at –20°C to extract the pigments. Finally, chlorophyll a and phaeopigments were determined in a Turner fluorometer, reading the fluorescence before and after adding 100 μl 10% HCl. The fluorometer was calibrated onboard with a chlorophyll a standard (Sigma) and the resultant door factors and constants were used to convert fluorescence into chlorophyll concentration. The concentration of the chlorophyll stock was checked in a spectrofluorometer.

Preliminary Results

Station E1

The chlorophyll a profile showed a vertically mixed distribution typical of a winter situation in this coastal station (Fig. 6.13.1). Chlorophyll concentration was higher in the >0.2 μm fraction, which indicates dominance by the pico-phytoplankton community. The distribution of chlorophyll did not show any clear maximum.

Primary production rates were low throughout, though slightly higher in the small size fraction. Total primary production rates estimated in the photic layer were 289 and 349 mg C m^{-2} d^{-1} for large and small phytoplankton, respectively.

Comparative station

Chlorophyll concentration was lower than that at E1 (Fig. 6.13.2). Ranges of chlorophyll were 0.08-0.13 mg m^{-3} in >5 μm fraction and 0.19-0.25 mg m^{-3} in >0.2 μm fraction. The prevailing fraction in terms of chlorophyll concentration is still the >0.2 μm phytoplankton. No vertical maximum was observed in the distribution of any size fraction.

However, primary production rates doubled compared to station E1 (635 and 633 mg C m^{-2} d^{-1} for >5 μm and >0.2 μm fractions, respectively). This increase of production accompanied by low chlorophyll concentration suggests that the comparative station was relatively more productive than E1.

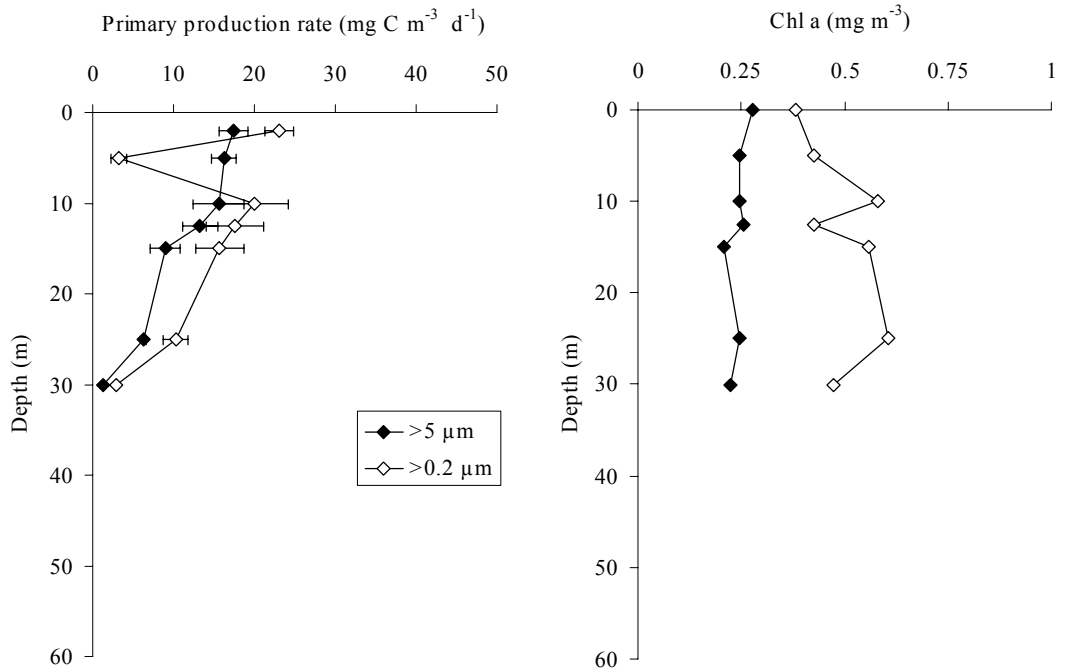


Fig. 6.13.1. Primary production rate and chlorophyll a of phytoplankton size fractions >5 µm and 0.2-5.0 µm in the photic layer of station E1.

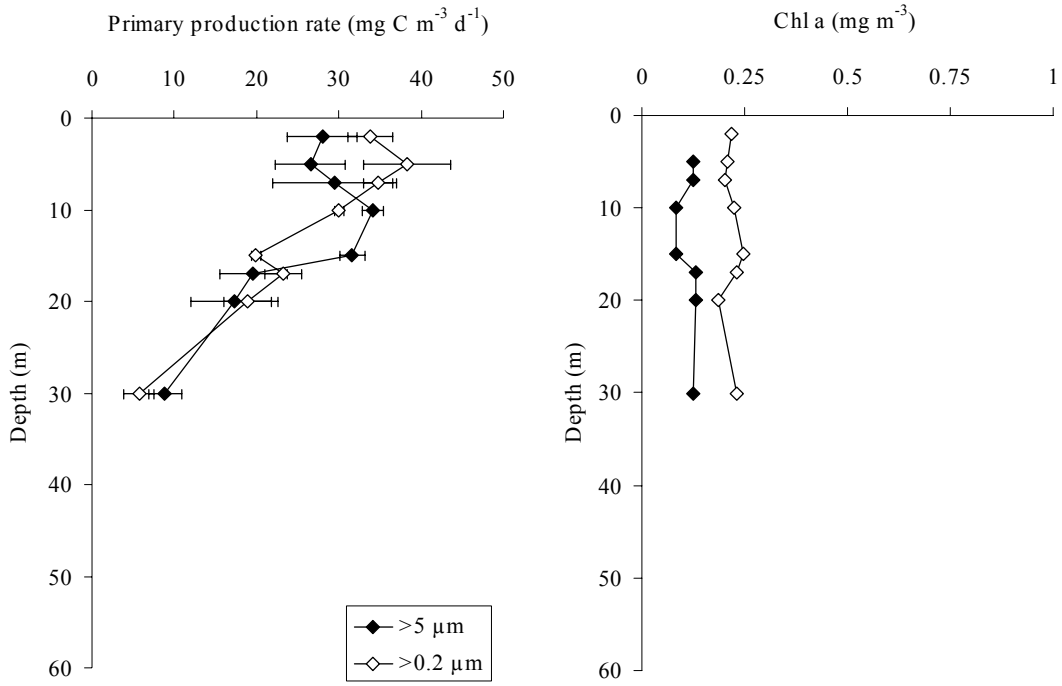


Fig. 6.13.2. Primary production rate and chlorophyll a of phytoplankton size fractions >5 µm and 0.2-5.0 µm in the photic layer of the comparative station.

Off-shelf station

The values of phytoplankton found in this area were very similar to those measured in the previous Comparative station (Fig. 6.13.3). Chlorophyll concentration ranged 0.06-0.13 mg m^{-3} in >5 μm fraction and 0.16-0.25 mg m^{-3} in >0.2 μm fraction. Total primary production rate by large phytoplankton was also very close to the Comparative station estimation (641 mg C m^{-2} d^{-1}). The small fraction, however, increased their production rates over 1700 mg C m^{-2} d^{-1}.

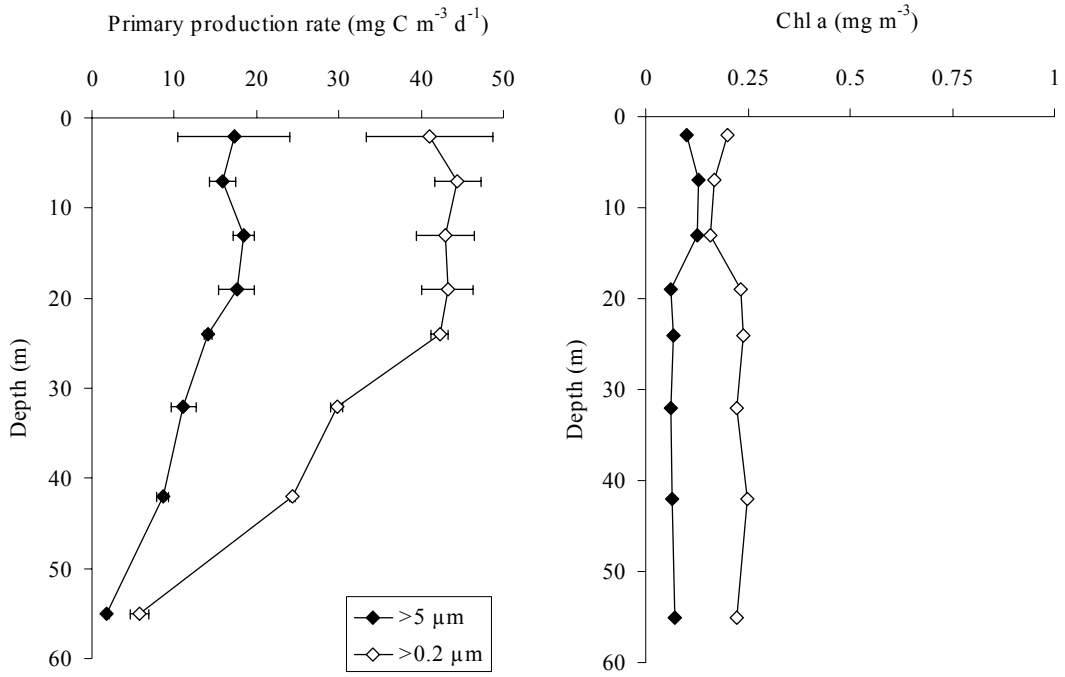


Fig. 6.13.3. Primary production rate and chlorophyll a of phytoplankton size fractions >5 μm and 0.2-5.0 μm in the photic layer of the off-shelf station.

Lagrangian experiment, Great Sole Bank bloom

During the Lagrangian experiment, a water body in the periphery of a dense patch of phytoplankton on Great Sole Bank was followed with an Argos drogue for several days to study the developing and the decline of the bloom.

The data of chlorophyll concentration and primary production rates collected during 8-11 April precisely depicted the complete sequence of the bloom (Fig. 6.13.4). As indicated by the chlorophyll fractions, large phytoplankton dominated the bloom waters and reached notably high values of biomass and production during the "peak" of the bloom on 9-10 April. A weak maximum of chlorophyll was only visible around 10-m depth during that peak; the production maximum however was shallower at 5 m (Fig 6.13.4b,c). The resultant primary production rates were notably high (Table 6.13.2). Particularly, the biomass and production of the large phytoplankton thrived in just 24-48 hours. Chlorophyll data available from April 6 confirmed the rapid growth of the large phytoplankton and the recession of the pico-cells at the onset of the bloom, which continued until the end of the experiment. This situation contrasted sharply with the observations at the shelf and off-shelf stations previously visited during the cruise track, which showed typically winter characteristics (vertical mixing, small cell sizes and low biomass and production).

Data suggest that in the Lagrangian station the bloom completed its rise and fall in a few days. Likely, storm events at the start and at the end of the experiment would have contributed to the initial mixing

and to the final dispersion of the bloom. A stable and bright weather in between enhanced the observed high values of phytoplankton.

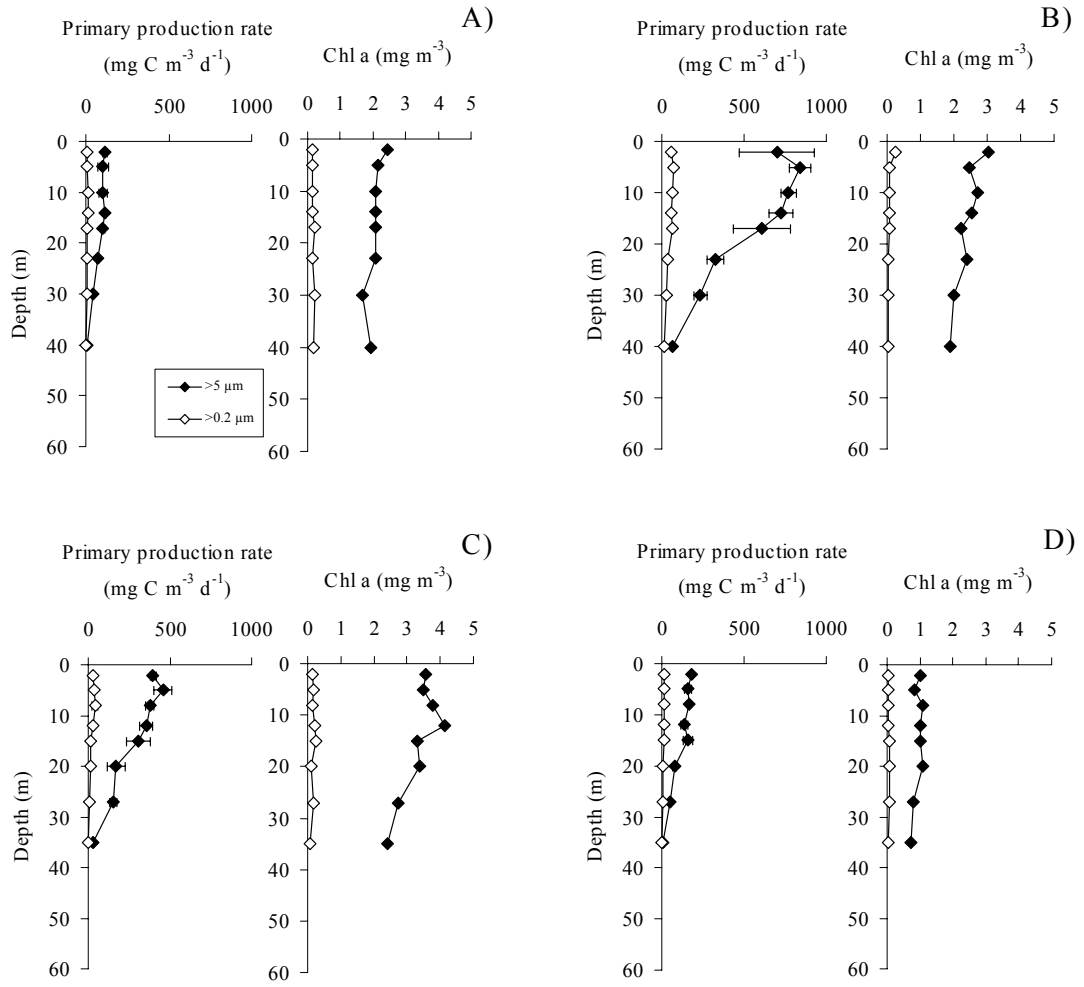


Fig. 6.13.4. Primary production rate and chlorophyll a of phytoplankton size fractions >5 µm and 0.2-5.0 µm in the photic layer during the Lagrangian experiment (5-12 April 2002): A) day 8, B) day 9, C) day 10, D) day 11.

Date	>5 µm		>0.2 µm	
	Primary production (mg C m^{-2} d^{-1})	Biomass (mg Chla m^{-2})	Primary production (mg C m^{-2} d^{-1})	Biomass (mg Chla m^{-2})
*6 Apr		28.6		14.69
8 Apr	2749	75.8	316	6.50
9 Apr	17592	87.6	1696	2.41
10 Apr	8135	107.1	626	5.24
11 Apr	3336	30.8	305	1.69

*only chl a data, no production available

Table 6.13.2. Integrated values (0 – 35 or 40m) of phytoplankton biomass and production during the Lagrangian experiment.

6.14 Microzooplankton grazing and plankton community structure

Claire Widdicombe, Plymouth Marine Laboratory

Introduction

A significant proportion of total zooplankton biomass in many oceanic environments is made up of the microzooplankton (<200 µm in size) and measurements of their grazing on phytoplankton show that they are important mediators of energy, carbon and nutrients through the food chain, either directly by controlling phytoplankton standing stocks or indirectly through predation by larger zooplankton.

Objectives

- Quantify the microphytoplankton, microzooplankton and heterotrophic nanoflagellates in terms of their community structure and biomass
- Determine phytoplankton growth and mortality, due to microzooplankton grazing
- Quantify the biomass of the heterotrophic nanoflagellates using an enzyme assay technique

Methodology

Phyto- and microzooplankton community structure

Water samples were collected daily from the pre-dawn CTD casts and preserved as follows:

1. 250ml samples were fixed with 2% (final conc.) acid lugol's solution and 2% hexamine-buffered formaldehyde for the enumeration of both the phyto- and microzooplankton. Samples will be analysed at PML by settlement microscopy.
2. 100ml samples were fixed in 1% glutaraldehyde, dual stained with DAPI and proflavine (at a final concentration of 5 µg ml^{-1}), filtered onto 0.8µm black polycarbonate filters and mounted onto glass slides. These samples will be analysed in the laboratory using epifluoresence microscopy.

Microzooplankton grazing experiments

The dilution technique of Landry and Hassett (*Mar. Biol.* 67:283-288, 1982) was used to quantify phytoplankton growth and mortality through grazing. Each experiment consisted of a series of four dilution levels, set up in quadruplicate, which were nominally at concentrations of 0.2, 0.4, 0.7 and 1.0 that of the ambient seawater. Experimental water was collected pre-dawn from the depth corresponding to the 33% light level and approximately 40 litres was filtered through a 0.2µm capsule filter (acid-clean and pre-washed in using deionised water) and combined, in four 20 litre carboys, with the unfiltered water (screened through a 200 µm mesh bag to remove mesozooplankton) to make the four dilutions. The dilution water was transferred into 2.3 litre polycarbonate bottles and incubated on-deck in ambient temperature and light levels for 24 hours. Sub-samples were collected at T0 and T24 hours for chlorophyll *a* and phyto- and microzooplankton community structure (as described above). Chlorophyll *a* samples were extracted using 90% acetone and analysed onboard by fluorometry, whilst the preserved samples were stored in cool, dark conditions until analysis in the laboratory by settlement microscopy. Sub-samples were also analysed by flow cytometry in order to determine the grazing pressure on specific phytoplankton taxa (see Glen Tarran's report for details).

Glucosaminidase enzyme assay

One litre seawater samples were collected pre-dawn from the same depths as the grazing experiments, i.e. the depth corresponding to the 33% light level, and passed through a 60µm mesh to remove large heterotrophs. For each assay, 0ml, 30ml, 60ml and 90ml sub-samples were gravity filtered, in duplicate, through GF/A filters and immersed in 50ml centrifuge tubes containing 5ml of 0.1M acetate buffer (pH 4.6), 40µl of Brij 35, 5ml of experimental water and 100µl of 4mM 4-methylumbelliferyl-n-acetyl-ß-D-glucosaminidase. Incubations were conducted in the dark at in-situ temperature, for 36

hours. 2ml sub-samples were fixed at the beginning of the assay and subsequently at 12 hourly intervals by mixing with 1ml of 0.4M glycine-sodium hydroxide buffer (pH 11.2). Samples were stored at 4°C until analysis can be carried out at PML. This will be done using a fluorescence spectrophotometer at 370nm excitation and 460nm emission wavelengths. Fluorescence will be calibrated against the concentration of fluorescent 4-methylumbelliferone (MUF) and the rate of enzyme activity in the experimental assays will be calculated as the slope of a linear regression of MUF concentration against time. Biomass estimates from the enzyme assay will then be correlated against the epifluoresence microscopy estimates of heterotrophic nanoflagellate biomass described above.

Preliminary results

An opportunistic 'look' at material from a 50µm mesh zooplankton net haul (0-50m) on the 10th April, showed that the phytoplankton during the lagrangian study were dominated by a diverse abundance of diatoms. Some of the diatom species included *Corethron hystrix, Thalassiosira rotula, T. fallax, Rhizosolenia delicatula, R. stolterfothii, Chaetoceros densus, C. decipiens, C. borealis,* and *Thalassionema nitzschioides*. Large dinoflagellates were also present, such as the mixotrophic *Ceratium tripos, C. furca* and *C. fusus* and the heterotrophic *Protoperidinium depressum*. A full detailed analysis of the preserved sample material will take place over the next 8 months or so.

Figure 6.14.1. Photomicrograph of the phytoplankton collected on the 10th April using a vertical net haul fitted with a 50µm mesh. The image clearly shows the abundance of several species of diatoms.

The preliminary grazing data suggest that the microzooplankton were grazing between 6% and 24% of the phytoplankton population per day. Lowest grazing was observed at the Offshelf station (5th April) where chlorophyll concentrations were still low, 0.3 mg m^{-3}. Highest grazing was recorded during the lagrangian study, just after the storm on the 8th April (Figure 6.14.2). Thereafter the phytoplankton (<200µm) steadily grew and grazing pressure steadily decreased. One explanation for this appears to be the size of the phytoplankton, since the diatoms were actively growing and dominating the biomass it is likely that the larger microzooplankton had not yet been able to respond to the sudden increase in phytoplankton biomass. Detailed microscopical analysis of the preserved samples will provide a better insight into the structure of the food web during our study.

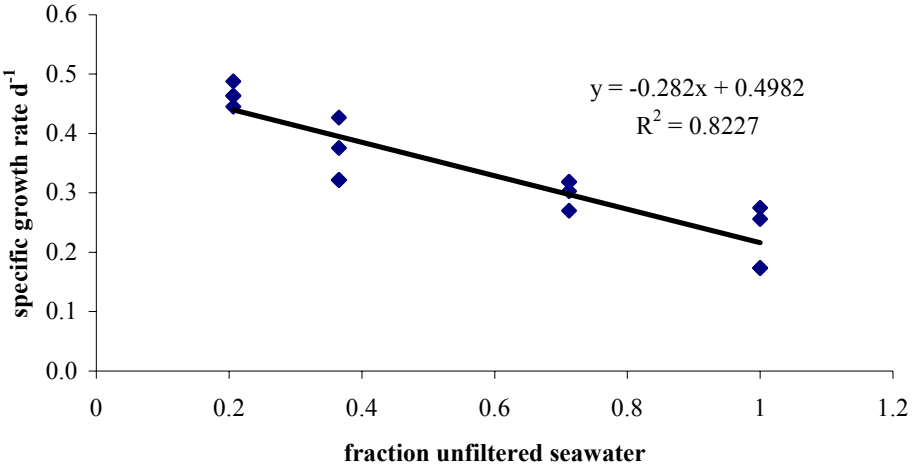


Figure 6.13.2. Results from the dilution experiment conducted on the 8th April. The growth and grazing rates are calculated from the net change in chlorophyll a over the 24 hour incubation. The results indicate a specific growth rate of 0.5 d^{-1}, equivalent to 0.7 doublings per day, and a daily turnover rate of 24% of the chlorophyll standing stock through grazing by the microzooplankton.

Date	Station	Lugol's, Formaldehyde & Glutaraldehyde	Dilution experiment	Enzyme assay
2 April	1.2	2, 5, 10, 15, 20, 30, 45 & 65m	10m	10m
3 April	4.2	2, 5, 10, 23, 30, 40, 60 & 80m	7m	7m
5 April	6.12	2, 5, 10, 23, 30, 40, 60 & 80 m	13m	13 & 55m
6 April	7.7	2, 5, 10, 23, 30, 40, 60 & 80m	10m	10m
6 April	7.8	50µm net	-	-
8 April	7.20	2, 5, 10, 23, 30, 40, 60 & 80m	10m	10m
9 April	7.36	2, 5, 10, 23, 30, 40, 60 & 80m	10m	10m
9 April	8.2	25m	-	-
10 April	7.54	2, 5, 8, 20, 27, 35, 60 & 80m	8m	8 & 35m
10 April		Donut transect – non-toxic	-	-
11 April	7.71	2, 5, 8, 20, 27, 35, 60 & 80m	8m	8m
12 April	7.90	2, 5, 8, 20, 27, 35, 60 & 80m	-	-

Table 6.14.1. Details of sampling schedule

6.15 Photo-physiological and bio-optical characteristics of phytoplankton for the development of remote sensing algorithms for primary production

Gavin Tilstone, Plymouth Marine Laboratory

Introduction

Accurate ship borne and satellite determinations of primary production are needed for quantitative estimation of the global CO_2 flux at the air sea interface and for modelling carbon transfer through the pelagic food web (Sarmiento et al. 1998). In situ primary production measurements are limited spatially and temporally and, hence, a concerted effort is required to derive accurate estimates of primary production from space (e.g. Platt and Sathyendranath 1988). Understanding the variations in photo-physiological and bio-optical properties of phytoplankton in relation to changes in environmental conditions is fundamental to modelling carbon fixation in specific bio-geochemical provinces and to improving estimates of primary production from remotely sensed data (Sathyendranath et al. 1995, Beherenfield & Falkowski 1997).

Objectives

- To collect bio-optical data from Case I & II locations in the English Channel and Celtic sea to validate Medium Resolution Imaging Spectrometer (MERIS) satellite observations.

- To test satellite primary production algorithms using measurements of gross primary production based on short term photosynthesis-irradiance parameters.

- To study variations in photo-physiology and inherent optical properties in the water column in relation to changes in nutrients, light, phytoplankton species succession and diel variability.

Methodology

A total of 22 stations were sampled (Table 6.15.1). A preliminary downwelling CTD cast was used to survey the water column using a Seabird 911 plus. Between three and six depths were selected for a more detailed analysis of the water column based on percentage irradiance levels and fluorescence levels.

Photosynthesis - Irradiance (P-E) curves

P-E experiments were conducted at 3 to 6 depths in the water column three times a day. Fifteen 75 ml sub-samples (14 samples plus one dark bottle) were inoculated with 5 or 10μ Ci $NaH^{14}CO_3$ and illuminated with 100 watt halogen tungsten in linear incubators for 1 to 2 hrs. The samples were then filtered onto GF/F's, exposed to HCL acid fumes for 8 hrs to remove inorganic ^{14}C and then suspended in 3.0 ml scintillation liquid. DPM's were then counted on a Beckman LS600 scintillation counter using internal quenched corrected curves.

Phytoplankton and suspended particulate absorption coefficients

Samples were collected from 3 to 6 depths at every station for the determination of absorption coefficients of total particulate and detrital material. Between 500 and 2000 ml of sea water was filtered onto GF/F filters which were then flash frozen in liquid nitrogen. Absorption coefficients will be measured at the laboratory on a Perkin Elmer Lambda 800 spectrophotometer retro-fitted with an integrating sphere using the methods of Tassan & Ferrari (1995).

Station	Lat	Long	Date	Time GMT	Measurement

1A	50º37.59	01º32.25	01.04	11:45	5 mts PE, Pabs, CDOM, SPM & POC 15 mts PE & Pabs
1.12	50º02.02	04º21.15	02.04	11:08	5 mts PE, Pabs, CDOM, SPM & POC 15, 25 mts PE & Pabs
4.10	49º31.92	06º00.45	03.04	11:48	5 mts PE, Pabs, CDOM, SPM & POC 10, 20, 30 mts PE & Pabs
6.2	48º41.03	11º11.68	04.04		5 mts PE, Pabs, CDOM, SPM & POC 15, 30, 55 mts PE & Pabs
7.1	49º56.50	10º02.65	05.04	13:24	5 mts PE, Pabs, CDOM, SPM & POC 15, 30, 45 mts PE & Pabs
7.7	49º59.83	10º01.56	06.04	4:23	Underway PE & Pabs
7.14			06.04	10:00	Underway PE & Pabs
7.15	49º59.88	10º05.44	06.04	11:00	5 mts PE, Pabs, CDOM, SPM & POC 20, 40, 50 mts PE & Pabs
UW 11.08	50º02.04	10º15.75	07.04	11:08	Underway PE & Pabs
7.22	50º04.95	10º20.93	08.04	9:03	5 mts PE, Pabs, CDOM, SPM & POC 20, 40 mts PE & Pabs
7.26	50º06.01	10º20.72	08.04	11:36	5 mts PE, Pabs, CDOM, SPM & POC 20, 30, 45 mts PE & Pabs
7.29	??	??	08.04	??	5 mts PE, Pabs, CDOM, SPM & POC 15, 30 mts PE & Pabs
7.40	50º07.04	10º21.00	09.04	8:57	5 mts PE, Pabs, CDOM, SPM & POC 15, 45 mts PE & Pabs
7.43	50º07.57	10º21.74	09.04	11:32	5 mts PE, Pabs, CDOM, SPM & POC 20, 30, 40 mts PE & Pabs
7.49	50º09.28	10º20.04	09.04	14:48	5 mts PE, Pabs, CDOM, SPM & POC 23, 35 mts PE & Pabs
7.58	50º08.95	10º20.37	10.04	8:58	5 mts PE, Pabs, CDOM, SPM & POC 15, 25 mts PE & Pabs
7.61	50º09.89	10º21.67	10.04	11:22	5 mts PE, Pabs, CDOM, SPM & POC 15, 30 mts PE & Pabs
7.66	50º11.61	10º20.25	10.04	14:29	5 mts PE, Pabs, CDOM, SPM & POC 15, 38 mts PE & Pabs
7.75	50º10.73	10º20.91	11.04	8:50	5 mts PE, Pabs, CDOM, SPM & POC 18, 35 mts PE & Pabs
7.78	50º11.26	10º22.49	11.04	11:30	5 mts PE, Pabs, CDOM, SPM & POC 15, 25, 35 mts PE & Pabs
7.84	50º12.40	10º21.61	11.04	14:41	5 mts PE, Pabs, CDOM, SPM & POC 20, 35 mts PE & Pabs
7.92	50º10.28	10º21.62	12.04	09:21	5 mts PE, Pabs, CDOM, SPM & POC 20, 40 mts PE & Pabs
7.93	50º10.60	10º22.88	12.04	11:30	5 mts PE, Pabs, CDOM, SPM & POC 20, 35 mts PE & Pabs

Table 6.15.1. Stations sampled and measurements taken.

Chromophoric dissolved organic material

Surface seawater was filtered through 0.2 μm filters using pre-ashed glassware. The first and second 150 ml of filtered water was discarded. The absorption properties of the third sample were determined spectrophotometricaly in 10 cm quartz cuvettes from 350 to 800 nm. Bi-distilled seawater was used as system blank.

Suspended particulate material

Between 1.5 & 4 litres of seawater was filtered through pre-washed, pre-ashed, pre-weighed 0.7 μm filters in triplicate. After filtration the filters were washed with distilled water on the ground glass filtration frits. The filters will be oven dried at 65°C for 24 hrs after which they are stored in a dessicator before weighing on an electrobalance to determine the total suspended particulate material concentration. A blank filter was also included to calculate the handling error.

Particulate organic carbon

Between 1.5 & 4 litres of seawater was filtered through pre-ashed and pre-weighed 47mm GF/F filters in triplicate. The filters were then washed with distilled water and stored at -20 ºC and particulate organic carbon content was ascertained back at the laboratory.

Preliminary Results: Eulerian sampling

Fig 6.15.1 presents photosynthesis – irradiance curves from comparative stations along the cruise track. Maximum photosynthetic rates (P_m^B) were lower at station E1 compared with other stations possibly due to the influence of coloured dissolved organic matter from the coast. Light limited slope of photosynthesis (α^B) was relatively low for this time of year indicating low light adaptation of phytoplankton. Highest values were recorded in the blue waters of the off shelf station.

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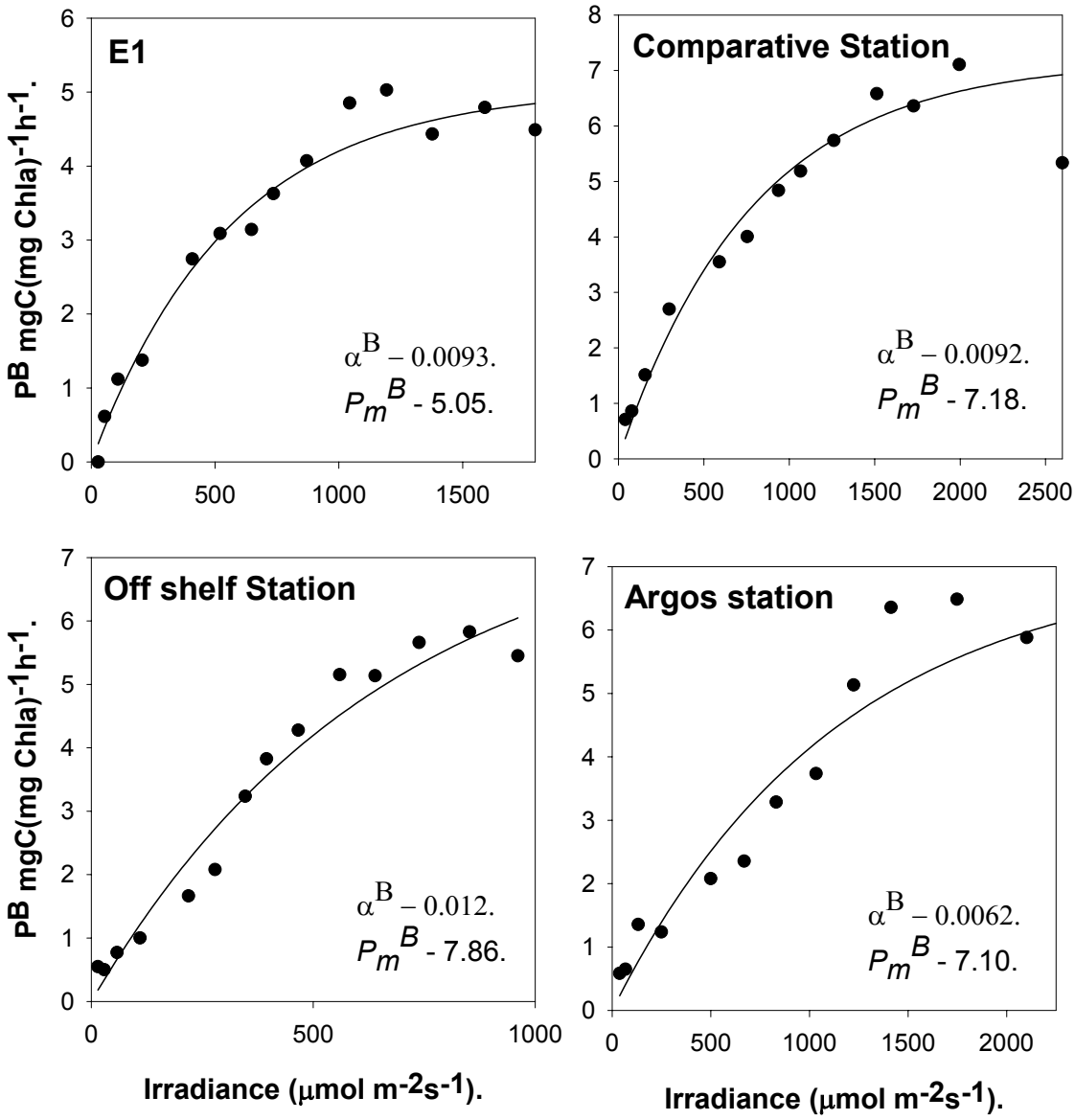

Fig 6.15.1. Photosynthesis – irradiance curves from E1, comparative station, off shelf station and Lagrangian station. Corresponding light limited slope of photosynthesis (α^B, mg C(mg chl) $^{-1}$ h^{-1} μmol m^{-2} s^{-1}) $^{-1}$) and maximum photosynthetic rate values (P_m^B, mg C(mg Chl)$^{-1}$ h^{-1}) for each station are given in the figure legend of each graph.

6.16 Trace Metals

Joanna Dixon & Rachel Lamb, Plymouth Marine Laboratory

Aims

1. To investigate the extent to which phytoplankton influence the biogeochemical cycling of selected trace metals (cadmium, manganese and zinc) in the photic zone.
2. To analyse the diversity and activity of naturally occurring bacteria from selected marine environments with low to high trace metal concentrations, and to follow their evolution with zinc enrichment.
3. To determine the extent of uptake and changes in speciation of dissolved zinc by the bacterial consortia.

Methods and Summary of Experiments

1. Primary Productivity (Aim 1)

Water samples were taken from each light level (1-97%) of the pre dawn CTD cast and 250 ml aliquots (3 replicates per light level), that had been spiked with a gamma cocktail containing ^{109}Cd, ^{54}Mn and ^{65}Zn, were placed in the on-deck incubator for 24 hours. Control samples (2 replicates at 1 and 97% light levels) that had been killed with mercuric chloride were similarly incubated. After the incubation period the samples were size fractionated by filtering sequentially through 5 and 0.2 µm pore-size (47 mm diameter) Nuclepore polycarbonate filters in a Sartorius cascade filtration apparatus. The filters were stored at room temperature and the filtrate was acidified to c. pH1 by the addition of 50 µl of 1:1 Hydrochloric acid (AristaR grade) and stored at room temperature. The samples will be counted on return to the laboratory by a Wallac 1480 NaI gamma detector.

Stations sampled

Sample No	Date sampled	Description	Station No
Prod #1	2/4/2002	E1	1
Prod #2	3/4/2002	Comparative (non bloom conditions)	4
Prod #3	5/4/2002	Offshelf	6
Prod #4	8/4/2002	Bloom station	7
Prod #5	11/4/2002	Bloom station	7

2. Long term Zinc Incubations (Aims 2 & 3)

Water samples were obtained from the stations outlined below with clean externally closed Niskin bottles (with teflon taps) on a pre marked Kevlar line. After deployment the Niskin bottles were taken into a clean containerised laboratory for all further manipulations. All water samples were subsequently manipulated with clean laboratory techniques. Three 2 litre pre acid cleaned polycarbonate bottles were filled with seawater straight from the Niskin taps from each depth sampled. The pH of the seawater was recorded in each bottle prior to and after the addition of dissolved zinc (Spectrosol [Zn] standard = 1000 ppm = 1000 mg/l $\{Zn(NO_3)_2.3H_2O$) to 2 of the bottles and subsequently adjusted back to ambient levels, if necessary, with 0.1M sodium hydroxide (AristaR) (see Table below).

Summary of dissolved zinc additions

M_r = 65.39g	[Zn] final	
	Low (~6.54 µg/l)	High (~65.4 µg/l)
Final conc	100 nM (typical of Tamar concentrations)	1000 nM (typical of Fal concentrations)
No moles in 2 litres	200 nmoles	2000 nmoles
No grams in 2 litres	13.1 µg	131 µg
Vol of Zn standard	13.1 µl	131 µl

The bottles were then incubated long term in the dark on stirrers (in Thermotote portable incubators at in-situ temperatures of 11-12°C). At each time point aliquots were taken for bacterial production, bacterial numbers, bacterial biodiversity (DGGE techniques), total and labile dissolved zinc concentrations and radioisotope metal uptake (^{109}Cd, ^{54}Mn and ^{65}Zn). In addition at T_0 and T_{end}, samples were taken for complexing capacity titrations in order to determine the extent to which dissolved zinc is complexed. The experiments were ongoing at the end of the cruise and were subsequently transferred to the laboratory maintaining *in situ* conditions. Samples for the above variables were taken at T_0, 1, 4, 8, 20, 27 and 35 (T_{end}) days for E1 and the offshelf station, and at T_0, 1, 14, 21, 28 and 35 (T_{end}) for the bloom station.

Stations sampled

No.	Date	Description	Sample depths (m)	Station No	Comment
Inc #1	2/4/2002	E1	50	1	well mixed water column
Inc #2	4/4/2002	Offshelf	30 & 150	6	deep water station -offshelf
Inc #3	9/4/2002	Bloom	20 & 100	7	surface fluorescence peak

Measurements taken:

At each time point samples were taken for metals and metal uptake (see above).
Bacterial numbers; Triplicate 1mL samples were fixed with Glut. for analysis at PML and bacterial activity; as described in section 6.11.

Bacterial Diversity; triplicate 3mL samples were centrifuged to pellet the bacteria present. These pellets were washed and resuspended in MQ then subjected to three freeze (-70°C for 2 hours) / thaw (100°C for 5 minutes) cycles to release genomic DNA. Following the freeze/thaw extraction process 10µL aliquots of the DNA suspension were amplified using primers designed for bacterial 16S rDNA in a polymerase chain reaction (PCR). The resulting products were then run down an E-gel to establish the success of the extraction procedure. The PCR products will be analysed for diversity using DGGE gels and possibly sequencing at PML.

6.17 Bacterial Diversity

Gary Smerdon, Plymouth Marine Laboratory

Aims:-

To determine profiles of bacterial community diversity throughout the water column at selected stations.

Methodology:-

Samples were collected by (a) centrifugation and/or (b) filtration and stored at –20°C for analysis back at PML.

(a) *Centrifugation*: Seawater samples were collected from the Seabird CTD system containing 24x20L Niskin bottles. 1.5mL subsamples were taken in triplicate from various depths (see Table 6.17.1) and centrifuged (RCF=16000) for 15 minutes. The supernatant was discarded, and each tube refilled with a further 1.5 mL seawater from the same depth and centrifugation repeated (RCF=16000) for a further 15 minutes. The supernatant was again discarded and the pellet (from a total of 3mL seawater) was stored at –20°C for later analysis.

(b) *Filtration*: 0.5L seawater samples collected from the Seabird CTD system as above were filtered using 0.2µm Nucleopore filters. The filtrate was discarded and the filters cut into two equal halves with each half being stored in a separate microcentrifuge tube at –20°C for later analysis.

Date	*Station*	**Location**	*Sample**	**Depths (m)**
03-04-02	4.2	Comparative	C	5,10,20,30,40,60,80
04-04-02	6.3	Off shelf	C	5,20,40,80,100,200,500,1000
05-04-02	6.12	Off shelf	C + F	2,7,13,19,24,32,42,55,80,150,200,500
06-04-02	7.15	Lagrangian / bloom	C	5,10,20,30,39,50,75,90,105,120
09-04-02	7.36	Lagrangian / bloom	C + F	2,10,23,30,40,60,80,120
11-04-02	7.71	Lagrangian / bloom	C	2,5,8,12,15,20,27,35,45,60,80,120
12-04-02	7.90	Lagrangian / bloom	C + F	2,12,20,35,45,60,80,120

* C = samples collected by centrifugation (3 mL) F = samples collected by filtration (500 mL)

Table 6.17.1

Laboratory analysis of the above samples will include cell lysis, DNA extraction (for filters only) and polymerase chain reaction (PCR) amplification of a section of the 16S rRNA gene. Amplified fragments will be fractionated by Denaturing Gradient Gel Electrophoresis (DGGE) to determine community profiles. Any changes in such profiles may be further analysed by DNA sequencing of significant bands to suggest probable identities.

6.18 Size-fractionated mesozooplankton

Chris Gallienne, Plymouth Marine Laboratory

6.18.1 Introduction

The primary aim of the zooplankton work on this cruise is (i) to characterise vertically integrated net samples and inline underway samples of zooplankton in terms of taxonomy and size-distributed biomass and (ii) to compare these to optical characterisation in terms of biovolume and size.

The optical characterisation of mesozooplankton was carried out using the laboratory Optical Plankton Counter (OPC; Herman, 1992) in two modes: (i) in continuous flow through mode using the ship's uncontaminated supply, drawn from approximately 6 metres below the surface; (ii) in pump through mode using samples collected from WP-2 net casts integrated over 0-120m depth at daily sampling stations. The data from (i) and (ii) above were used to generate near real time estimates of size structure and carbon content.

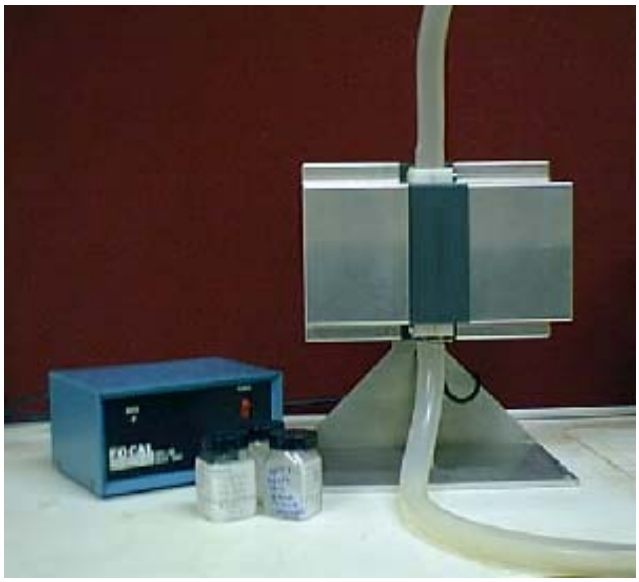

Fig 6.18.1 Laboratory OPC and Deck Unit for use in pump-through mode for continuous underway or discrete net samples.

The OPC is capable of large-scale, rapid and continuous characterisation of zooplankton. It can produce reliable abundance and size distribution indicators for zooplankton between 0.25 and 16mm in equivalent spherical diameter (ESD, Herman, 1992) in up to 4096 size classes, and at data rates up to 200 events sec^{-1}. The size range of 200µm to 20mm is often given as the range for mesozooplankton, to which the OPC is well suited. The OPC measures cross sectional area as digital size, which is converted to equivalent spherical diameter (ESD) using a semi-empirical formula. This is the diameter of a sphere that would present the same cross sectional area as the particle being measured. We calculate volume from the cross sectional area using an ellipsoidal model more appropriate to mesozooplankton (Gallienne & Robins, 2001) to give further information about the particles being analysed. This is presented in the results as biovolume concentration in mm^3 m^{-3} (equivalent to mg m^{-3}).

6.18.2. Methods

Vertical net samples for size fractionated biomass and OPC-1L discrete samples

Mesozooplankton samples were taken at each station for taxonomic identification and for determining size fractionated biomass. Triple WP-2 nets with a mouth diameter of 0.57m were used. Two nets used 200µm mesh (one acting as a concentrator for the profiling OPC) and the third used 125µm mesh, ideally suited to the laboratory OPC-1L (Gallienne & Robins, 2001), through which these samples would be passed post-cruise for vertically integrated size-distributed biomass measurements. This data could then be compared to vertically integrated data from the profiling OPC. Net samples for microscopic analysis (for total counts and identification of major taxonomic groups) and for the laboratory OPC analysis were preserved in 4% borax buffered formaldehyde.

The OPC-1L in surface underway mode

The OPC-1L was operated in continuous underway mode between stations throughout the cruise from station 1 (E1) to the return to the Channel on the homeward leg, sampling surface water from the ship's uncontaminated sea water supply. Seawater is pumped from beneath the ship through a 6mm

steel mesh filter, and distributed to the laboratories. The OPC was connected to this supply, continuously sampling surface (6m) seawater at ~20 litres min^{-1} via a de-bubbling device to prevent spurious counts. The OPC-1L has a 20mm square section glass flow cell, through which water containing the sample was pumped. An in-line flow meter was installed to give a record of volume of water passing through the OPC.

Validation of OPC data

Laboratory calibration against spherical glass beads of known size provides the initial calibration. Size calibration exercises were performed during the cruise using calibration beads of 501 ± 10, 1004 ± 20, and 2022 ± 40µm ESD (Duke Scientific Corporation, Inc.). These were each mixed with filtered seawater and recirculated through the system. Continuous OPC underway counts are compared to microscope counts of zooplankton from in-line samples taken from the outlet of the OPC once a day. There is good agreement between the microscope and real time OPC continuous underway counts, and between OPC biovolume and carbon analysis of preserved samples (Gallienne & Robins, 1998; Gallienne *et al.*, 2001). It should be acknowledged that avoidance of the pump intake might result in under-sampling of some of the larger organisms within the size range of the system.

6.18.3. Preliminary results

A summary of the profiling OPC data for the Lagrangian drift station is given in Figure 6.18.2. Data represent three size fractions for each station integrated over the water column (0-120m depth). Note that the smallest size fraction (less than 800µm ESD) is consistently low. Although the manufacturer claims 250µm ESD as the lower size limit for the OPC, it is the experience of most users that the towed OPC (OPC-1T) used in the profiler has a realistic lower limit of around 700µm ESD (e.g. Heath (1995). The OPC-1L, having a much shorter path length, is more sensitive and is reliable down to 250µm ESD (Gallienne & Robins, 1998; Gallienne *et al,* 2001).

The data for the two larger fractions (800-1600µm and >1600µm ESD) show a trend to decreasing biomass after the storm event, followed by a steady increase from 2 days after the storm. No diurnal variation is evident from these data, indicating that diel vertical migration was not significant during the Lagrangian study.

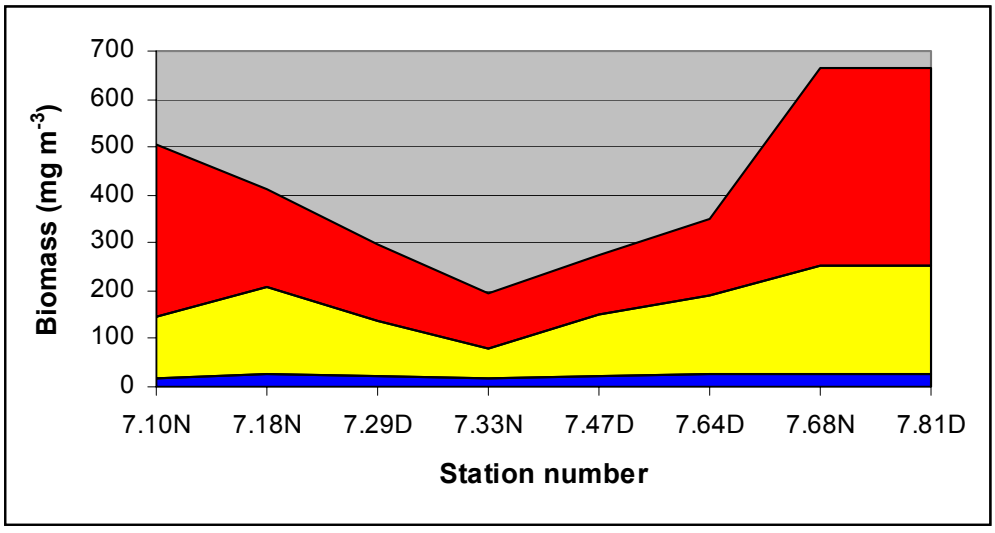

Fig. 6.18.2. Integrated OPC Profile 0-120m for Drift Station 7.10 (6th April 04:30) to 7.81 (11th April 13:10). Key: Blue - <800µm ESD; Yellow – 800 – 1600µm ESD; Red - >1600µm ESD. D: daytime; N: nighttime

The continuous surface underway OPC data for the transect between station 1 (E1) and the off-shelf station are presented in figure 6.18.3, below. Biomass is seen to increase from a low value at station 1 (<100mg m^{-3}, 20:20 hrs, 2nd April) to a peak around midnight the following day (3rd April, c. 900mg m^{-3}). This peak is associated with the transect through a bloom centered to the south of the transect in French territorial waters. Its occurrence around midnight, together with an increase in the relative contribution of the larger size fractions, suggests that diel vertical migration of larger mesozooplankton may be responsible for some if this increase. The comparative station (station 2) occurred during this transect from 0200 to 1400hrs. During the first 11 hours of the 4th April, continuing across the shelf break to the off-shelf station, biomass declines to a minimum of 33mg m^{-3}.

Figure 6.18.4 shows similar continuous data for the transect during 5th April from the off-shelf station, through the bloom and the Argos deployment position, to the 'pre-bloom' waters to the north of the bloom. The data in figure 6.18.4 are contaminated by very abundant large phytoplankton cells, as may be seen from the unusually high biomass values as we pass through the bloom and the complete dominance by the smaller size fractions (250-500µm ESD). This was assumed be a combination of plant cells larger than 250µm and somewhat smaller plant cells passing through the OPC in sufficient numbers to cause coincidence (several particles smaller than 250µm ESD being present in the beam at the same time, and being registered as a single larger particle). Examination of samples filtered from the OPC outlet at these times using a 53µm mesh and examined under a microscope confirmed this assumption. Although this makes the data less than useful in terms of characterisation of mesozooplankton, the instrument used in this mode was very useful as an aid in responsive-mode sampling, indicating areas of ecological change and aiding in the location of the edge of the bloom, where we wished to deploy the Argos buoy.

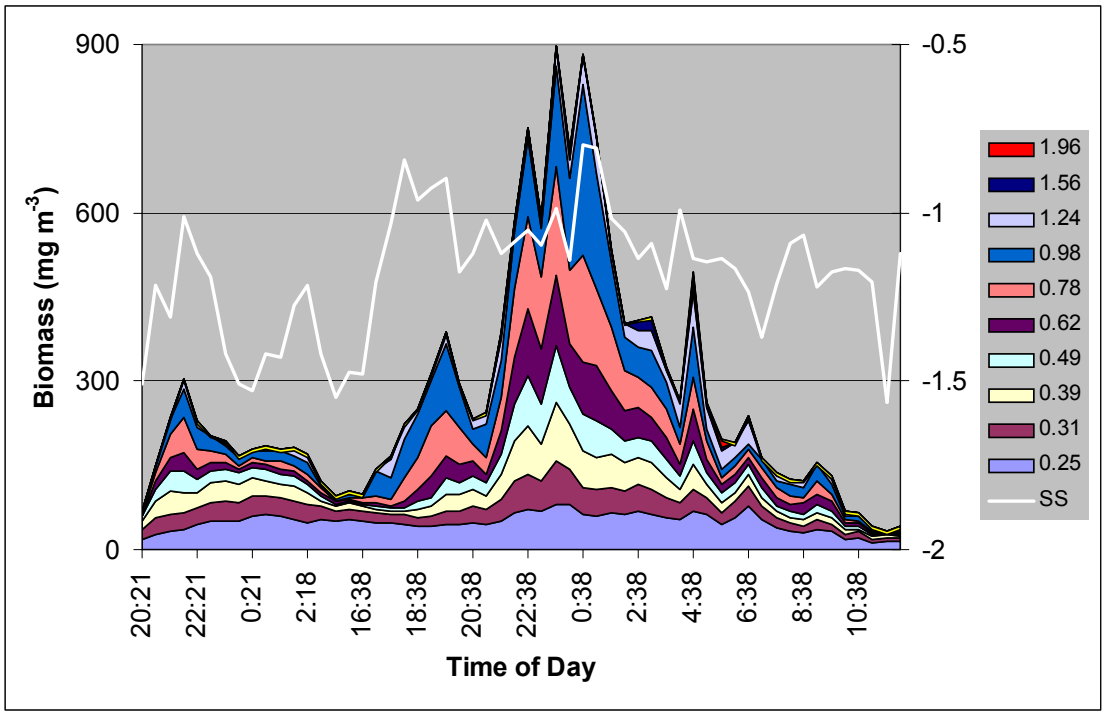

Fig. 6.18.3. Size fractionated OPC biomass data from continuous surface underway sampling between Station 1 (E1) and the off-shelf station. Key: ESD in mm. SS = biomass spectral slope.

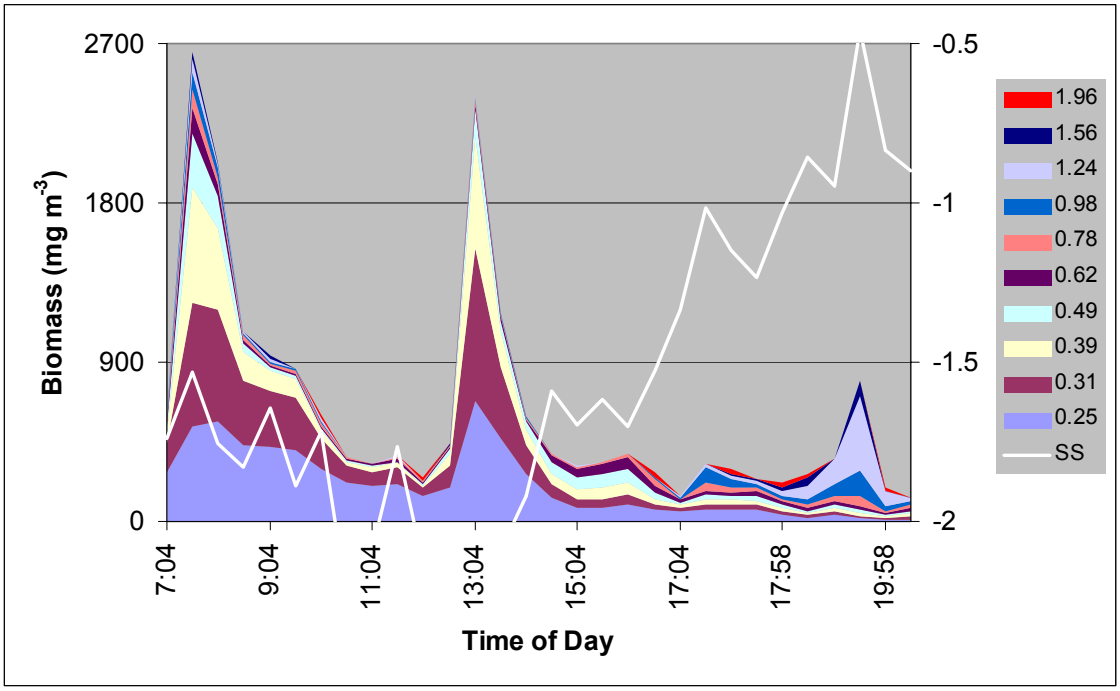

Fig. 6.18.4. Size fractionated OPC biomass data from continuous surface underway sampling between off-shelf station 6 (5th April) and the 'pre-bloom' waters to the north of the Argos deployment site (5th April). Key: ESD in mm. SS = biomass spectral slope.

6.18.4. Further analysis

Net samples from the 125 and 200µm mesh nets have been preserved and returned to the laboratory. During the weeks following the cruise the 125µm mesh samples will be passed through the OPC-1L system to produce rather more precise data on vertically integrated mesozooplankton biomass, and for validation of the profiling OPC data. This validation will be further aided by microscopic analysis of the 200µm mesh samples, which will also give valuable information on taxonomic distribution of zooplankton, and the developmental stage of the zooplankton at the various stations during the Lagrangian drift study.

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7. Acknowledgements

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