

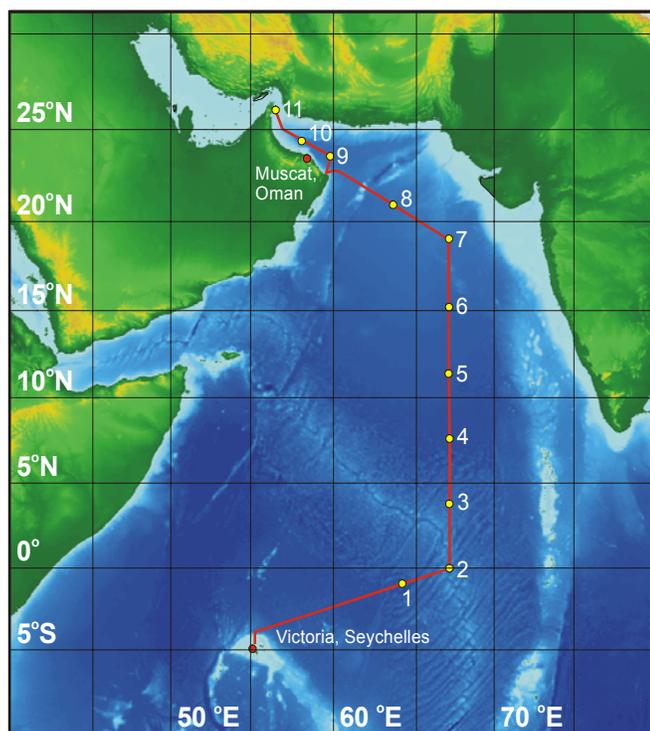
RRS CHARLES DARWIN 132 Cruise Report

Analysing the Microbial Biodiversity of the Indian Ocean

AMBITION

30 August – 29 September 2001

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This cruise is a component of the NERC Marine & Freshwater
Microbial Biodiversity (M&FMB) thematic programme

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Document Data Sheet

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<p>ABSTRACT</p> <p>The AMBITION research cruise provided the opportunity for coordinated, multi-institute marine fieldwork by five projects supported by the NERC Marine & Freshwater Microbial Biodiversity thematic programme. Sampling and experimental work were carried out at 11 stations along a 5500 km transect in the north west Indian Ocean, mostly following the 67°E meridian, from Victoria, Seychelles to Muscat, Oman. As anticipated, a wide range of environmental conditions was encountered, with high variability (of an order of magnitude or more, laterally and/or vertically) in nitrate, oxygen, chlorophyll levels and productivity. Information was collected on the abundances and functionality of bacteria and phytoplankton (including picoeukaryotes and <i>Trichodesmium</i> spp), and their relationship to upper ocean nutrients, physical conditions, photosynthetic pigments and new production. Additional post-cruise studies will be carried out on the material collected.</p>	
<p>KEYWORDS</p> <p>INDIAN OCEAN, ARABIAN SEA, MONSOON MIXING, NUTRIENTS, OLIGOTROPHY, MICROBIAL BIODIVERSITY, DIATOMS, BACTERIOPLANKTON, PICOEUKARYOTES, PHOTOSYNTHETIC PROKARYOTES, TRICHODESMIUM, PROCHLOROCOCCUS, SYNECHOCOCCUS, NEW PRODUCTION, DOC MEASUREMENTS, NITROGEN FIXATION, NITRIFICATION, METHYL BROMIDE METABOLISM, OXYGEN DEPLETION, SEA-BIRD CTD SYSTEM, FLOW CYTOMETRY, NANONUTRIENT ANALYSES, HPLC PIGMENT ANALYSES, CHEMOTAXONOMY, DNA AND RNA ANALYSES, FAST REPETITION RATE FLUOROMETER, MOVING VESSEL PROFILER.</p>	
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Executive summary

1. The aim of the AMBITION research cruise was “to investigate microbial diversity in the wide range of oceanographic conditions found in the NW Indian Ocean, for integrated implementation of five projects supported by the NERC Marine and Freshwater Microbial Biodiversity thematic and the M&FMB programme as a whole”.
2. The research was carried out on *RRS Charles Darwin* (CD 132) by 17 scientists and technicians from the Universities of Cardiff, Stirling, Warwick, the Plymouth Marine Laboratory (PML) and the UK Oceanographic Research Service. Science logistics and administration were coordinated by PML. An observer from the Marine Fisheries Centre in Muscat, Oman joined the ship for the last few days of the cruise.
3. A total of 11 oceanographic stations were sampled along a transect of 5500 km. Six stations lay along the 67°E meridian, for which previous oceanographic data were available. The sample sites reflected a wide range of surface water trophic states with surface water nitrate values that ranged from <1 nmol litre⁻¹ to around 3 µmol litre⁻¹.
4. Vertical conditions at the stations also varied considerably with concentrations of nitrate that increased to around 20 µmol litre⁻¹ at depth. Oxygen depleted waters (ODW) were present from depths of 100 - 2500 metres at open ocean stations. Oxygen levels ranged from 1% to 4% saturation (provisional uncalibrated data) in the ODW.
5. A wide range of samples was collected to investigate the molecular characterisation and quantities of bacteria and phytoplankton (including picoeukaryotes and *Trichodesmium* spp) and for shipboard experiments on their functionality. To underpin the data gained from these samples and experiments, compatible information on nutrients, phytoplankton (microscopy and flow cytometry), photosynthetic pigments (by HPLC), primary and new production (¹⁴C and ¹⁵N) will be generated.
6. The 67°E meridian provided a strong gradient of microbial diversity and concentrations. Surface chlorophyll *a* concentrations ranged from 19ng litre⁻¹ at 1°S to 180 ng litre⁻¹ at 15°N. South of around 12°N, photosynthetic prokaryote populations were dominated by *Prochlorococcus*, while north of around 8°N, the community was dominated by *Synechococcus*. Between 8°N and 12°N, a transition zone with mixed populations was found. This boundary corresponded to a shift up in primary production from around 20 mg C m⁻² h⁻¹ to 30-60 mg C m⁻² h⁻¹ north of it. Interestingly no clear division of eukaryote algae seemed to occur, suggesting that prokaryote and eukaryote algae respond differently. It will be interesting to see whether there is evidence of changes in the heterotrophic prokaryotes or eukaryote populations.
7. Scientific capability was very high. One day was lost due to late sailing from the Seychelles as scientific gear was not secure and because the shipboard scientific fridge required new parts. A further three hours were lost for compassionate landing one of the ship’s crew at Sur. No significant scientific equipment failures, bad weather or medical problems were suffered. A Dahn buoy was the only item missing from the UKORS equipment list requested – a substitute was made up from a pellet float and a whip aerial.
8. The Deputy High Commissioner, Ms Jacqui Currie, was visited in the Seychelles on 29 August to appraise her of AMBITION. On 30 September, a half-day workshop was held at the Ministry of Fisheries in Muscat. It was hosted by senior Omani scientists and policy administrators in order to discuss common research interests and the capabilities of *RRS Charles Darwin*.

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1. BACKGROUND

1.1 Programme context

The AMBITION cruise was the main oceanographic field activity for NERC'S Marine & Freshwater Microbial Biodiversity (M&FMB) thematic programme. The overall goal of M&FMB is to improve our understanding of marine and freshwater microbial biodiversity, with emphasis on community interactions, ecosystem function, and the potential for biotechnological exploitation.

The Arabian Sea, situated in the NW Indian Ocean, provided an exceptionally wide range of environmental conditions for M&FMB fieldwork, as discussed below. It was identified as a suitable region for upper ocean studies by five M&FMB projects, involving 10 Principal Investigators and Co-Investigators, as detailed under Section 2 below. Data and material collected on the cruise will not only be used for later laboratory studies by these groups, but are also expected to be made available to other researchers within the thematic programme - and subsequently, through collaborative arrangements and agreements, to the wider national and international scientific community.

1.2 Science context

The Arabian Sea is strongly influenced by the bi-annual monsoons that blow alternately from the south-west (SWM) in May-September and from the north-east (NEM) between November and March. The SWM is vigorous, resulting in winds of Beaufort 8-10 that generate upwelling along the southern Oman margin. This upwelling allows a luxuriant proliferation of phytoplankton, particularly diatoms, along this coast and in the Gulf of Oman once the winds cease in September and the surface waters stabilise.

In contrast to the highly productive waters of the N and NW margins, the central and more southerly waters of the Arabian Sea are little forced by the SWM. As a result, highly oligotrophic conditions (with nano-molar nitrate concentrations in surface waters) are normal in this region, particularly in the inter-monsoon periods when the algae are dominated by photosynthetic prokaryotes.

The southern boundary of the Arabian Sea is generally considered to be the equator where there is a complex but relatively stable pattern of upwelling and downwelling, caused by local wind conditions.

In addition to the oligotrophy/eutrophy gradients in the northern basin and the downwelling and upwelling features of the southern basin, there are also marked vertical changes in oxygenation. Thus a slab of oxygen-depleted ($2-10 \mu\text{M O}_2$) water occurs, typically between 200 - 2500m depth. While there is a general North to South (low to high) concentration gradient in oxygen, the most pronounced oxygen deficiency is found to the east of the basin where it coincides with the zone of maximum denitrification. This condition is maintained by a combination of factors including midwater respiration fuelled by surface water primary production.

During the wane of the SWM in September/October, the Arabian Sea provides a mosaic of contrasting oceanographic conditions - offering probably the best location in the world for comparative marine environmental studies. Previous studies had shown that phytoplankton respond strongly to this spatial variability, with many different types of communities in the upper ocean. These range from large phytoplankton (such as diatoms) in upwelling and eutrophic waters, to picophytoplankton where oligotrophy prevails. However, we do not know whether other microbial communities respond in the same way as phytoplankton in surface waters, nor do we know how the microbial populations of the oxygen minimum respond to the signals from the phytoplankton in the overlying surface waters. These were major shared scientific goals for the cruise, involving all participants.

2. AIM AND OBJECTIVES

The aim of the cruise was *to investigate microbial diversity in the wide range of oceanographic conditions found in the NW Indian Ocean, for integrated implementation of M&FMB projects and the programme as a whole.*

The objectives of the five participating M&FMB projects were:

- i) To collect material (preserved filtrates and frozen samples) for DNA extraction and analysis on return to Cardiff, particularly in more eutrophic waters [Fry & Weightman (Cardiff): *Isolation and characterisation of 'unculturable' heterotrophic marine bacteria*].
- ii) To sample the upper-ocean for methyl bromide (MeBr) and isolation of MeBr-utilising bacteria. Carry out onboard experiments using gas chromatographs, liquid nitrogen, gas bottles and incubators. [Murrell & McDonald (Warwick), Nightingale (PML): *Methyl bromide metabolism in the marine environment*]
- iii) To sample the horizontal and vertical distributions of picoeukaryotes in relation to environmental gradients. Also investigate temporal responses (diel changes *in situ* and via deck incubations) and carry out DNA and RNA analyses. [Scanlan (Warwick): *In situ community structure of marine photosynthetic picoeukaryotes*]
- iv) To generate a range of size-fractionated samples for analysis of *nif* gene diversity, together with information on environmental conditions (to determine factors affecting gene expression). [Wyman (Stirling) & O'Donnell (Newcastle): *Nitrogen fixing microbes in the sea - a molecular approach linking phenotype to 16S rRNA phylotype*]
- v) To characterise bacterioplankton community and to investigate their links to biogeochemical functions. [Zubkov & Burkill (PML): *Bacterial biodiversity; dominance, competitiveness and functionality within communities*].

These projects were supported by a suite of underpinning measurements carried out by PML researchers, providing the information on biological and physico-chemical conditions needed for data interpretations and comparisons with previous studies.

3. APPROACH

To meet the aim and objectives, we occupied eleven stations and carried out over 100 gear deployments along a south-to-north transect. This transect was designed to follow an ecological gradient generated by ambient nutrient levels (NO₃ in particular) and reflected in primary production.

On each station, a regular pattern of sampling and experiments was adopted following considerable discussion between all cruise participants. This pattern involved arriving on station prior to dawn when the initial samples were taken with a CTD water bottle system. These provided samples for the wide range of variables required for analyses or experiments over the day. Intermittently during the day further specialised samples were obtained using CTD, stand-alone pumps (SAPs) or plankton nets. Implementation of the new working/rest hours regulations for science personnel, including UKORS, meant that sampling finished at 16:00 (local time) and began again at 04:00 the next day. Further sampling continued until around midday when the ship left to steam for the next station.

Background oceanographic data were provided by PML scientists who quantified nutrient levels (Woodward), phytoplankton composition (Tarran, with post-cruise analyses by Claire Widdicombe), photopigments (Cummings), and primary and new production (Morgan and Rees). Satellite imagery relevant to the cruise was processed by the PML remote sensing group and provided to the ship in near-real time. On-board molecular biology investigations were carried out by the M&FMB-funded teams. Their investigations included community structure and function of communities of bacteria (Zubkov, PML), eukaryote picoplankton (Fuller and Orcutt, Warwick), nitrogen fixers (Wyman and Bird, Stirling), microbes that consume and produce methyl bromide (Cox, Warwick/PML) and bacteria that could not normally be cultured (Ashelford, Cardiff).

4. MAIN SHIPBOARD ACHIEVEMENTS

Eleven stations were occupied along an oceanographic transect some 5500 km in length in the Arabian Sea. Each station was occupied for about 30 hours to allow 2 cycles of predawn sampling to be achieved. A total of 65 CTD deployments were made. While most of these were in the upper 300 metres, a few were made to depth (max depth sample 3011 metres). Shipboard equipment worked well.

The transect showed a considerable range of environmental conditions. Surface water nitrate values ranged from $< 1 \text{ nmol litre}^{-1}$ to a few $\mu\text{mol litre}^{-1}$. Beneath the mixed layer, concentrations of nitrate increased to ca $20 \mu\text{mol litre}^{-1}$ at depth. Oxygen depleted waters (ODW) were present from depths of 100 to 2500 metres at open ocean stations. Oxygen levels were routinely a few percent saturation in the ODW.

Biological communities varied following those of nutrients. Along the 67°E meridian strong gradients of microbial diversity were found. To the south of 12°N , photosynthetic prokaryote populations were dominated by *Prochlorococcus*. To the north of 8°N , *Synechococcus* dominated. Between 8°N and 12°N , mixed populations co-existed. This boundary corresponded to a step up in primary production from around $20 \text{ mg C m}^{-2} \text{ h}^{-1}$ south of this boundary to $30\text{-}60 \text{ mg C m}^{-2} \text{ h}^{-1}$ north of it. Interestingly no clear division of eukaryote algae seemed to occur. It will be interesting to see whether there is evidence of changes in the heterotrophic prokaryotes.

All objectives were met in full and AMBITION was a superb cruise despite the problems created by 11 September. The ship's complement, UKORS and RSU, can take great pride in providing an excellent professional service to the scientists onboard.

5. PERSONNEL**5.1 Scientists and technical**

<i>Name</i>		<i>Role</i>	<i>Institution</i>	<i>M&FMB Project</i>
Peter Burkill	Co-I	Principal Scientist	PML*	All
Kevin Ashelford	PDRA	Sample collection & processing	Cardiff	Fry & Weightman
Mike Cox	PhD student	Sample collection & processing	Warwick	Murrell et al
Nick Fuller	PDRA	DNA/protein extraction & analysis	Warwick	Scanlan
Karen Orcutt	PDRA	DNA/protein extraction & analysis	Warwick*	Scanlan
Mike Wyman	PI	Collection of DNA/RNA	Stirling	Wyman & O'Donnell
Clare Bird	PDRA	DNA/RNA processing/purification	Stirling	Wyman & O'Donnell
Mike Zubkov	PI	Bacterial rate studies; radioisotope officer	PML*	Zubkov & Burkill
Gwyn Morgan	PGRA	Bacterial analysis & primary production	PML*	Zubkov & Burkill
Glen Tarran		AFC phytoplankton analysis	PML	All
Malcolm Woodward		Logistics / Technicon AA nutrients	PML	All
Andy Rees		Nanonutrients & new production	PML	All
Denise Cummings		HPLC Pigments	PML	All
Jeff Benson		TLO & CTD Engineer	UKORS	All
Darren Young		Mechanical Engineer	UKORS	All
Alan Sherring		Mechanical Engineer	UKORS	All
Paul Duncan		Computing	UKORS	All
Hilal Al-Shaqsi		Scientific Observer	MSFC	All

* See [Appendix 7](#) for updated contact information. MSFC, Marine Science & Fisheries Centre, Muscat; PML, Plymouth Marine Laboratory; UKORS, UK Oceanographic Research Service, Southampton.

5.2 Ship's Officers and crew

<i>Name</i>	<i>Rank</i>
Keith Avery	Master
Philip Gauld	Chief Officer
Andrew Cope	Second Officer
Peter Reynolds	Third Officer
Andrew Adams	Chief Engineer
Bernie McDonald	Second Engineer
Keith Connor	Third Engineer
Tony Healey	Third Engineer
Philip Parker	Electrical Engineer
Kevin Luckhurst	Bosun
Bob Johnson	Bosun's Mate
Mark Moore	Seaman
Timmy Edwards	Seaman
Gerry Cooper	Seaman
Stu Cook	Seaman
Max Ottesen	Motorman
Ray Bell	Catering Manager
Patrick Fahey	Chef
Chris Cullen	Chef Steward
Jeff Osborn	Steward
Peter Robinson	Steward

6. SCIENTIFIC LOG

6.1 Log of science activities for CD132

Table 1. Log of activities, 21 August - 29 September 2001 (including pre-cruise passage)

Date (Julian day)	Local time (GMT + 4 hr)	Event	Position	Station/ deployment
Tues 21 Aug (233)	1000	Sailed Durban		
Wed 22 Aug (234) - Tues 28 Aug (240)		Pre-cruise passage leg. Best possible speed		
Wed 29 Aug (241)	1536	Secure alongside Victoria, Seychelles. 1800 to 2000 scientists unpacking container. Electric motor landed for rewinding.		
Thurs 30 Aug (242)		Scientists join ship and set up equipment. Electric motor refitted.		
Fri 31 Aug (243)	0954	All clear fore and aft		
	1024	Full away on passage		
Sat 1 Sep (244)	1410 to 1551	Hove to for equipment tests, incl test CTD (CD132 000)	02°26.7'S 59° 22.3'E	test
Sun 2 Sep (245)	1057 to 1120 1318 to 1528	Hove to for equipment tests Hove to for equipment tests		
Mon 3 Sep (246)	0510 to 0552	CTD (CD132 001)	00°54.8'S 64° 08.5'E	01/01
	0734	Productivity rig deployed		01/02
	0806 to 0842	CTD (CD132 002)	00°54.3'S 64°08.2'E	01/03
	0935 to 1003	CTD (CD132 003)	00°54.0'S 64°01.8'E	01/04
	1037 to 1120	CTD (CD132 004)	00°54.4'S 64°08.4'E	01/05
	1159	Productivity rig recovered	00°53.0'S 64°08.6'E	(01/02)
	1235 to 1319	CTD (CD132 005)	00°54.9'S 64°08.3'E	01/06
	1339 to 1448	SAPs	00°54.9'S 64°08.4'E	01/07
	1508 to 1524	Plankton net	00°54.9'S 64°08.4'E	01/08
	1534 to 1547	Plankton net	00°55.0'S 64°08.5'E	01/08
	1552 to 1640	GoFlo	00°55.2'S 64°08.7'E	01/09
Tues 4 Sep (247)	0658 to 0759	CTD (CD132 006)	00°53.7'S 64°05.1'E	01/10
	0859 to 0942	CTD (CD132 007)	00°54.4'S 64°08.3'E	01/11
	0945	All secure: sail for Station 2		
Wed 5 Sep (248)	0402 to 0450	CTD (CD132 008)	00°00.9'S 67°00.0'E	02/01
	0613	Productivity rig deployed	00°00.2'S 67°00.0'E	02/02
	0629 to 0709	CTD (CD132 009)	00°00.3'S 66°59.8'E	02/03
	0814 to 0934	CTD (CD132 010)	00°01.5'S 66°59.6'E	02/04
	0935	CTD (CD132 011)	00°01.2'S 66°59.5'E	02/05
	1155	Productivity rig recovered	00°01.0'S 67°00.2'E	(02/02)
	1220 to 1300	CTD (CD132 012)	00°00.1'S 67°00.0'E	02/06
	1341 to 1500	SAPs	00°00.1'S 67°00.1'E	02/07
	1505 to 1526	Plankton net	00°00.9'S 67°00.0'E	02/08a
	1548 to 1600	Plankton net	00°00.6'S 66°59.8'E	02/08b
	1607 to 1720	GoFlo	00°01.9'S 66°59.6'E	02/09
Thurs 6 Sep (249)	0555 to 0700	CTD (CD132 013)	00°00.0'S 66°59.3'E	02/10
	0758 to 0833	CTD (CD132 014)	00°00.1'S 67°00.0'E	02/11
	0836	All secure: sail for Station 3		
Fri 7 Sep (250)	1205 to 1309	CTD (CD132 015)	03°48.0'N 67°00.0'E	03/01
	1803 to 1903	CTD (CD132 016)	03°48.0'N 67°00.1'E	03/02
	2105	CTD (CD132 017)	03°48.0'N 67°00.2'E	03/03
Sat 8 Sep (251)	0004	CTD (CD132 018)	03°47.8'N 67°00.0'E	03/04

	0557 to 0650	CTD (CD132 019)	03°48.0'N 67°00.1'E	03/05
	1200 to 1255	CTD (CD132 020)	03°47.9'N 67°00.0'E	03/06
	1405 to 1548	SAPs	03°48.0'N 66°59.8'E	03/07
	1556 to 1638	Plankton net	03°47.7'N 66°59.6'E	03/08
Sun 9 Sep (252)	0400 to 0454	CTD (CD132 021)	03°47.8'N 67°00.0'E	03/09
	0611	Productivity rig deployed	03°48.7'N 67°00.0'E	03/10
	0627 to 0702	CTD (CD132 022)	03°47.8'N 66°59.7'E	03/11
	0829 to 0939	SAPs	03°46.6'N 66°59.3'E	03/12
	0953 to 1108	CTD (CD132 023)	03°46.3'N 66°59.2'E	03/13
	1143	Productivity rig recovered	03°45.6'N 66°59.2'E	(03/10)
	1148	All secure: sail for Station 4		
Mon 10 Sep (253)	0958 to 1106	MVP test station	07°35.6'N 67°00.2'E	04/01
	1200 to 1300	CTD (CD132 024)	07°36.0'N 67°00.1'E	04/02
	1400 to 1508	SAPs	07°36.0'N 67°00.0'E	04/03
	1510 to 1555	Nets	07°35.9'N 67°00.0'E	04/04
	1600 to 1705	GoFlo	07°36.1'N 67°00.0'E	04/05
Tues 11 Sep (254)	0359 to 0449	CTD (CD132 025)	07°36.0'N 67°00.0'E	04/06
	0557	Productivity rig deployed	07°36.0'N 67°00.0'E	04/07
	0635 to 0701	CTD (CD132 026)	07°35.9'N 67°00.3'E	04/08
	0811 to 0846	CTD (CD132 027)	07°36.1'N 67°01.3'E	04/09
	0924 to 1024	CTD (CD132 028)	07°36.1'N 67°02.4'E	04/10
	1050 to 1108	Net	07°36.2'N 67°03.2'E	04/11
	1145	Productivity rig recovered. Set course for Station 5	07°36.4'N 67°03.3'E	(04/07)
	1227	MVP deployed	07°41.4'N 67°03.4'E	04/08
	1656	MVP recovered	08°25.4'N 67°00.8'E	(04/08)
Wed 12 Sep (255)	0915	MVP deployed	11°06.2'N 66°59.6'E	05/01
	1104	MVP recovered	11°22.5'N 66°59.7'E	(05/01)
	1152 to 1250	CTD (CD132 029)	11°23.9'N 66°59.9'E	05/02
	1345 to 1445	SAPs	11°23.9'N 66°59.8'E	05/03
	1506 to 1545	Nets deployed	11°23.3'N 66°59.4'E	05/04
	1545 to 1648	SAPs	11°22.8'N 66°58.6'E	05/05
Thurs 13 Sep (256)	0358 to 0452	CTD (CD132 030)	11°24.0'N 67°00.0'E	05/06
	0610	Productivity rig deployed	11°24.0'N 67°00.0'E	05/07
	0626 to 0706	CTD (CD132 031)	11°23.9'N 67°00.0'E	05/08
	0807 to 1055	CTD (CD132 032)	11°23.3'N 67°00.0'E	05/09
	1137	Productivity rig recovered. Set course for Station 6	11°21.4'N 67°00.3'E	(05/07)
	1407	MVP deployed	11°43.4'N 67°00.2'E	05/10
	1623	MVP recovered	12°04.6'N 66°59.9'E	(05/10)
Fri 14 Sep (257)	1004 to 1018	CTD (CD132 033)	15°12.0'N 67°00.0'E	06/01
	1027 to 1300	CTD (CD132 034)	15°12.0'N 67°00.0'E	06/02
	1418 to 1435	CTD (CD132 035)	15°12.0'N 67°00.0'E	06/03
	1509 to 1603	SAPs	15°11.9'N 67°00.0'E	06/04
	1613 to 1650	Net	15°11.6'N 66°59.8'E	06/05
	1702 to 1722	GoFlo	15°11.9'N 67°00.0'E	06/06
Sat 15 Sep (258)	0357 to 0453	CTD (CD132 036)	15°12.0'N 67°00.0'E	06/07
	0642	Productivity rig deployed	15°11.8'N 67°00.1'E	06/08
	0652 to 0732	CTD (CD132 037)	15°11.6'N 67°00.0'E	06/09
	0856 to 0920	CTD (CD132 038)	15°11.3'N 67°00.3'E	06/10
	0930 to 1030	FRRF	15°11.2'N 67°00.2'E	06/11

	1119	Productivity rig recovered. Set course for Station 7	15°10.6'N 67°00.6'E	(06/08)
	1218	MVP deployed	15°18.7'N 67°00.8'E	06/12
	1512	MVP recovered	15°46.4'N 67°00.4'E	(06/12)
Sun 16 Sep (259)	0936 to 1045	FRRF	18°59.8'N 67°00.0'E	07/01
	1057 to 1110	MVP	18°59.7'N 66°59.7'E	07/02
	1200 to 1300	CTD (CD132 039)	19°00.0'N 67°00.0'E	07/03
	1801 to 1857	CTD (CD132 040)	19°00.0'N 67°00.1'E	07/04
Mon 17 Sep (260)	0000 to 0100	CTD (CD132 041)	19°00.0'N 67°00.0'E	07/05
	0557 to 0656	CTD (CD132 042)	19°00.0'N 67°00.0'E	07/06
	1200 to 1242	CTD (CD132 043)	19°00.0'N 67°00.0'E	07/07
	1400 to 1530	SAPs	19°00.0'N 67°00.0'E	07/08
	1536 to 1554	Nets deployment	19°00.0'N 67°00.0'E	07/09
	1558 to 1621	GoFlo deployment	19°00.0'N 66°59.9'E	07/10
Tues 18 Sep (261)	0356 to 0500	CTD (CD132 044)	19°00.0'N 67°00.0'E	07/11
	0559	Productivity rig deployed	19°00.1'N 66°59.9'E	07/12
	0626 to 0704	CTD (CD132 045)	19°00.2'N 67°00.1'E	07/13
	0826 to 1033	CTD (CD132 046)	18°59.5'N 67°00.3'E	07/14
	1126	Productivity rig recovered. Set course for Station 8	18°59.1'N 67°00.2'E	(07/12)
Wed 19 Sep (262)	0958 to 1106	FRRF deployed	20°55.0'N 63°39.9'E	08/01
	1158 to 1255	CTD (CD132 047)	20°54.9'N 63°39.9'E	08/02
	1416 to 1535	SAPs	20°54.8'N 63°40.0'E	08/03
	1537 to 1600	Nets deployment	20°54.9'N 63°39.9'E	08/04
Thurs 20 Sep (263)	0356 to 0458	CTD (CD132 048)	20°55.0'N 63°40.0'E	08/05
	0606	Productivity rig deployed	20°55.0'N 63°39.9'E	08/06
	0626 to 0701	CTD (CD132 049)	20°55.3'N 63°39.8'E	08/07
	0757 to 0900	SAPS	20°55.5'N 63°39.5'E	08/08
	0906 to 1052	CTD (CD132 050)	20°55.2'N 63°38.8'E	08/09
	1122	Productivity rig recovered. Set course for Sur	20°55.2'N 63°38.8'E	(08/06)
	1130	MVP deployed	20°55.4'N 63°38.4'E	08/10
Fri 21 Sep (264)	0012	MVP recovered	22°06.2'N 61°39.8'E	(08/10)
	1317	Omani observer on board	22°35.5'N 59° 4.5'E	
	1730	Ship's boat returns after landing crew member. Set course for station 9		
Sat 22 Sep (265)	0106	Commenced bow tie survey in the region of 23°49.8'N 60°00.0'E		
	0749	Completed survey	23°50.2'N 60°00.2'E	
	1030 to 1132	FRRF	23°33.4'N 59°54.0'E	09/01
	1158 to 1303	CTD (CD132 051)	23°33.3'N 59°53.9'E	09/02
	1351 to 1508	SAPs	23°34.8'N 59°53.8'E	09/03
	1513 to 1534	Nets deployment	23°33.7'N 59°54.3'E	09/04
Sun 23 Sep (266)	0359 to 0502	CTD (CD132 052)	23°33.7'N 59°54.2'E	09/05
	0615	Productivity rig deployed	23°33.7'N 59°54.0'E	09/06
	0625 to 0708	CTD (CD132 053)	23°33.8'N 59°54.1'E	09/07
	0822 to 0852	CTD (CD132 054)	23°32.8'N 59°53.8'E	09/08
	0944	CTD (CD132 055)	23°32.0'N 59°53.6'E	09/09
	1200	Productivity rig recovered	23°31.4'N 59°53.3'E	(09/06)
	1255 to 1352	SAPs. Set course for station 10	23°33.7'N 59°53.9'E	09/10
Mon 24 Sep (267)	0949 to 1045	FRRF	24°19.9'N 58°10.1'E	10/01
	1158 to 1306	CTD (CD132 056)	24°20.0'N 58°10.0'E	10/02
	1353 to 1455	SAPs	24°20.0'N 58°09.9'E	10/03
	1457 to 1535	Nets	24°19.8'N 58°09.8'E	10/04

Tues 25 Sep (268)	1540 to 1604	GoFlo bottle deployment	24°19.6'N 58°09.5'E	10/05
	0356 to 0502	CTD (CD132 057)	24°20.0'N 58°10.0'E	10/06
	0618	Productivity rig deployed	24°20.0'N 58°10.7'E	10/07
	0632 to 0713	CTD (CD132 058)	24°19.9'N 58°10.4'E	10/08
	0829 to 0901	CTD (CD132 059)	24°19.5'N 58°10.2'E	10/09
	0938 to 1138	CTD (CD132 060)	24°19.0'N 58°10.5'E	10/10
	1204	Productivity rig recovered	24°18.3'N 58°10.4'E	(10/07)
Wed 26 Sep (269)	1308 to 1414	SAPs. Set course for station 11	24°20.4'N 58°10.1'E	10/11
	0959 to 1046	FRRF	26°00.4'N 56°35.0'E	11/01
	1200 to 1252	CTD (CD132 061)	26°00.1'N 56°35.0'E	11/02
	1355 to 1454	SAPs	26°00.0'N 56°35.0'E	11/03
	1457 to 1533	Nets	26°00.0'N 56°35.0'E	11/04
Thurs 27 Sep (270)	1538 to 1556	GoFlo bottle deployment	26°00.0'N 56°35.4'E	11/05
	0355 to 0445	CTD (CD132 062)	26°00.0'N 56°35.1'E	11/06
	0611	Productivity rig deployed	26°00.4'N 56°35.0'E	11/07
	0630 to 0701	CTD (CD132 063)	26°00.3'N 56°35.2'E	11/08
	0802 to 0826	CTD (CD132 064)	26°01.2'N 56°35.1'E	11/09
	1140	Productivity rig recovered	26°03.1'N 56°34.8'E	(11/07)
Fri 28 Sep (271)	1000	End of science. Steam to Muscat	26°00.5'N 56°35.1'E	
Sat 29 Sep (272)	0900	Tied up alongside Port Sultan Qaboos, Muscat.	23°40.0'N 58°30.0'E	

6.2 Positions of the principal stations

The nominal positions and dates of occupancy of the principal stations are given below.

Table 2. Positions of the principal stations

<i>Station</i>	<i>Lat</i>	<i>Long</i>	<i>Distance to next station (km)</i>	<i>Start</i>	<i>Finish</i>	<i>Total distance (km)</i>
Victoria	04° 35' S	55° 30' E	1040		30 Aug 01	
1	00° 55' S	64° 08' E	335	3 Sep	4 Sep 01	1040
2	00° 00' N	67° 00' E	420	5 Sep	6 Sep 01	1375
3	03° 48' N	67° 00' E	420	7 Sep	9 Sep 01	1795
4	07° 36' N	67° 00' E	420	10 Sep	11 Sep 01	2215
5	11° 24' N	67° 00' E	420	12 Sep	13 Sep 01	2635
6	15° 12' N	67° 00' E	420	14 Sep	15 Sep 01	3055
7	19° 00' N	67° 00' E	420	16 Sep	17 Sep 01	3475
8	20°55' N	64° 00' E	379	18 Sep	19 Sep 01	3895
Sur				20 Sep		
9	22° 40' N	60° 41' E	201	21 Sep	22 Sep 01	4274
10	23° 55' N	59° 15' E	354	23 Sep	24 Sep 01	4475
11	26° 00' N	56° 35' E	323	25 Sep	26 Sep 01	4829
Muscat	23° 40' N	58° 30' E		29 Sep		5152

6.3 Ship's cruise track

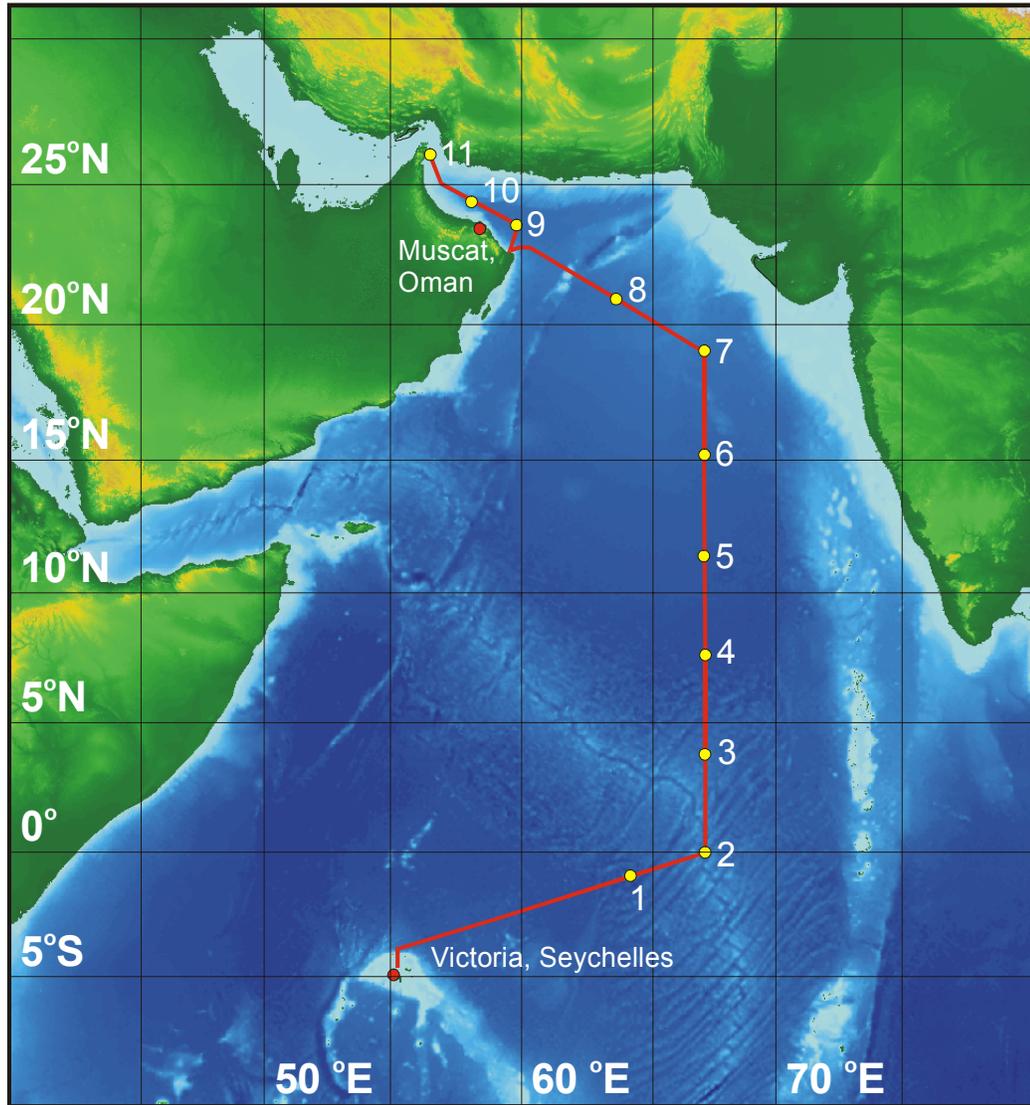


Fig 1. Ship's track and positions of the principal stations

7. SCIENTIFIC AND TECHNICAL REPORTS

7.1 Satellite derived fields (Peter Burkill)

Relevant satellite images processed at PML were routinely sent to the ship. These were used to plan station work, and to ensure we could work in an algal bloom for the final part of the cruise. Examples of composite images for sea surface temperature and estimated chlorophyll are shown below in [Figs 2 and 3](#) respectively.

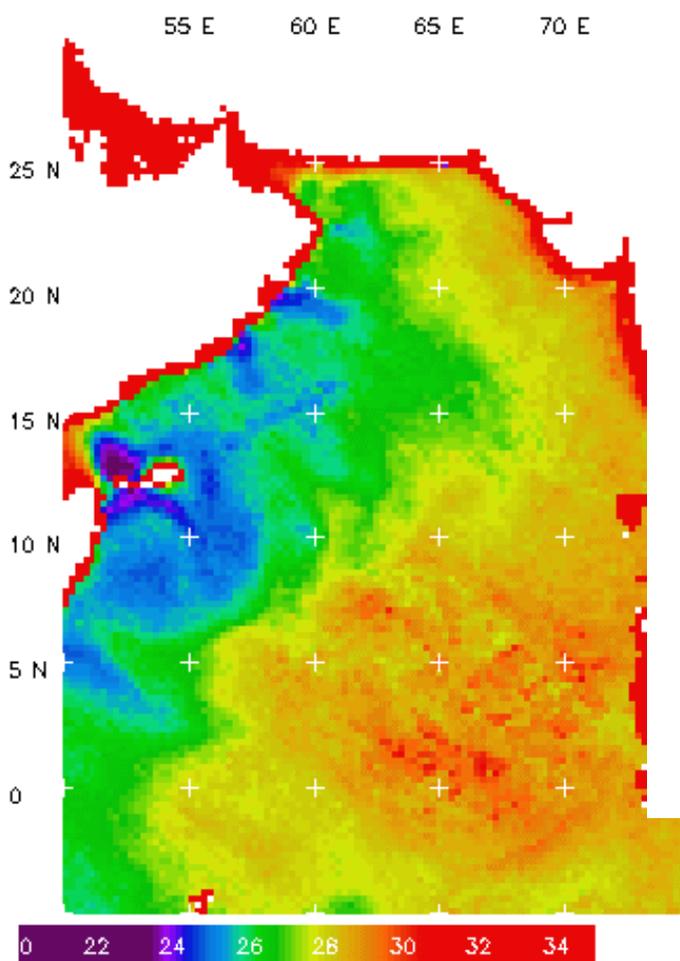


Fig 2. Sea surface temperature (°C) composite for 13 -19 September 2001

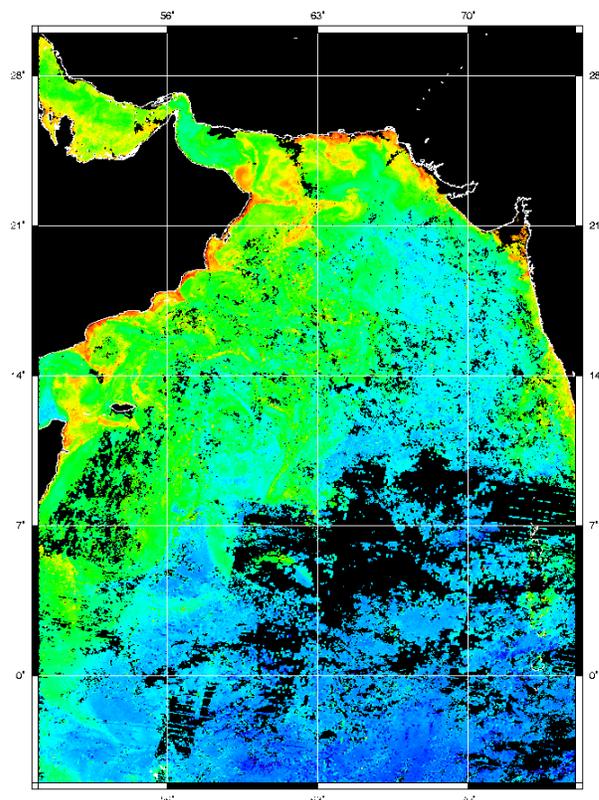


Fig 3. Estimated chlorophyll composite for 11-25 September 2001. Blue signifies low concentrations while red indicates high levels.

Sea surface temperature and chlorophyll fields showed the typical post-SW monsoon condition, with (i) warmer water and lower chlorophyll fields found to the south and (ii) cooler water and higher chlorophyll concentrations to the north west of the basin.

7.2 Nutrients

(Malcolm Woodward and Andy Rees)

Objectives

To study the spatial and temporal variations of nitrate, nitrite, phosphate, silicate and ammonium, using a classical colorimetric nutrient autoanalyser through contrasting oceanic regions along the cruise track. Where ambient concentrations were below the detection limits of the colorimetric systems, we used a nanomolar ammonium analysis system, and for nitrate, nitrite and phosphate, a unique nanomolar analysis system. The latter system was trialed for the first time as a multi-channel analyser.

Methodology

The nutrient analyser was the five-channel Technicon AAll, segmented flow autoanalyser. The chemical methodologies used were nitrate (PG Brewer & JP Riley, 1965; *Deep Sea Res* 12, 765-72), nitrite (K Grasshoff, 1976; *Verlag Chemie, Wehiem*, 317pp), phosphate and silicate (D Kirkwood, 1989; *ICES CM 1989/C*; 29), and ammonium (RFC Mantoura & EMS Woodward, 1983; *Est Coastal & Shelf Sci* 17, 219-24).

The nanomolar ammonium system is an adaptation from RD Jones, 1991 (*Limnol & Oceanogr* 36, 814-9) which uses a fluorescence analysis technique following ammonia gas diffusion out of the samples, passing across a hydrophobic teflon membrane, due to pH differential chemistry.

This cruise was the first deployment of a new unique multi-channel nanomolar analyser combining the segmented flow colorimetric analytical techniques with a Liquid Waveguide Capillary Cell (LWCC). This system is only in use in one other laboratory in the world.

Water samples were taken from the 30 litre CTD/Rosette system (SeaBird), sub-sampled into acid cleaned 60 ml HDPE (Nalgene) sample bottles. Analysis for the nutrient samples was in every case complete within 3 hours of sampling. Clean handling techniques were employed to avoid any contamination of the samples, particularly by ammonium. No samples were stored.

Analytical equipment operation

All CTD samples were analysed with a negligible sample loss rate. The ageing Technicon 5-channel system showed its reliability and reproducibility in the extreme environment of tropical, on-board deployment. However, additional investment in this system would be desirable for future work.

The ammonium fluorescence system performed well early in the cruise but there developed a severe loss of sensitivity which was finally diagnosed as a fluorometer problem. The system was inoperable for the second half of the cruise and we had to rely on the colorimetric system on the Technicon, which performed as well as could be expected.

The new nanomolar LWCC system is still essentially a developmental analyser but the nitrate and nitrite channels both were shown to operate at a sensitivity of about 1 nanomole or better. The nitrate channel was successfully operated and performed well for the majority of the cruise. Due to lack of suitable equipment (and having to use old detectors and light sources) it was not possible to make the phosphate channel operate to any sort of acceptable level, despite many attempts. As there are only enough detectors for two channels, the nitrite was sacrificed early on in the cruise in order to try to develop the phosphate system into an operational unit. Sadly this failed due to time and lack of modern high-precision equipment.

CTD samples analysed

There were essentially three types of CTDs during the two day stations that we occupied. The pre-dawn sampling was carried out as a biogeochemistry cast as well as providing the water for the primary production and nitrogen uptake *in situ* determinations. There were also some deep CTDs down to 2500 metres. The second day was a mid-day biogeochemical high precision CTD. The advent of the 24 bottle, 20 litre CTD (obtained via JIF funding) enabled a high detail profile for the sampling and sufficient water to satisfy most needs.

Sampling depths for nutrient analyses are given below, from which it can be seen that the greatest sampling depth was usually 300 metres.

Table 3. CTD samples analysed for nutrients

Station/ deploy ment	CTD cast [all CD132..]	Date	Depths (m)
Test	Test	1 Sep 01	1,10, 20, 30, 40, 50, 60, 70, 100, 200, 300, 500
01/01	001	3 Sep 01	1, 5, 10, 25, 50, 60, 74, 100, 120, 150, 200, 300
01/05	004		40, 50, 60, 73, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300
01/10	006	4 Sep 01	1, 5, 10, 25, 40, 55, 65, 75, 85, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300
02/01	008	5 Sep 01	1, 5, 10, 25, 50, 62, 80, 100, 120, 150, 160, 200, 300
02/05	011		1, 5, 10, 25, 35, 48, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300
02/10	013	6 Sep 01	1, 5, 10, 25, 32, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 170, 200, 230, 260, 300
03/01	015	7 Sep 01	1, 5, 10, 25, 35, 45, 55, 65, 75, 85, 110, 140, 170, 215, 250, 300
03/02	016		1, 5, 10, 25, 35, 45, 55, 65, 79, 85, 120, 140, 170, 215, 250, 300
03/03	017		1, 10, 25, 35, 45, 55, 65, 70, 120, 140, 170, 215, 250, 300
03/04	018	8 Sep 01	1, 5, 10, 25, 40, 55, 65, 75, 85, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300
03/05	019		1, 10, 25, 45, 55, 58, 70, 85, 115, 140, 170, 215, 250, 300
03/06	020		10, 25, 60
03/09	021	9 Sep 01	1, 5, 10, 25, 50, 63, 74, 80, 100, 120, 150, 200, 300
03/13	023		1, 5, 10, 25, 35, 44, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300
04/02	024	10 Sep 01	1, 5, 10, 25, 35, 40, 50, 57, 66, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 270, 300
04/06	025	11 Sep 01	1, 5, 10, 25, 50, 60, 65, 77, 90, 100, 120, 150, 200, 300
04/10	028		1, 10, 20, 35, 50, 55, 60, 66, 67, 75, 85, 90, 95, 100, 110, 120, 130, 150, 175, 200, 225, 250, 280, 300
05/02	029	12 Sep 01	1, 5, 10, 25, 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 110, 120, 130, 150, 200, 274, 300
05/06	030	13 Sep 01	1, 5, 10, 25, 31, 36, 50, 60, 70, 80, 90, 100, 120, 150, 200, 282, 300
05/09	032		601, 999, 1398, 1804, 2400, 3007
06/02	034	14 Sep 01	1,5,10, 25, 29, 35, 40, 50, 60, 80, 100, 120, 150, 200, 250, 300, 500, 700, 1000, 1300, 1600, 1900, 2200, 2500
06/07	036	15 Sep 01	1, 5, 10, 20, 30, 40, 45, 50, 60, 75, 100, 110, 120, 150, 200, 250, 300
07/03	039	16 Sep 01	1, 5, 10, 25, 35, 39, 47, 62, 80, 100, 110, 130, 150, 200, 250, 300
07/04	040		1, 5, 10, 25, 38, 44, 50, 65, 80, 100, 110, 130, 150, 200, 250, 300
07/05	041	17 Sep 01	1, 5, 10, 25, 35, 41, 50, 61, 80, 100, 110, 130, 150, 200, 250, 300
07/06	042		1, 5, 10, 25, 45, 52, 60, 80, 100, 110, 130, 150, 200, 250, 300

Table 3. CTD samples analysed for nutrients - continued

Station/ deployment	CTD cast [all CD132..]	Date	Depths (m)
07/11	044	18 Sep 01	1, 5, 10, 20, 30, 35, 40, 44, 49, 60, 70, 80, 90, 100, 110, 120, 130, 150, 200, 250, 300
08/02	047	19 Sep 01	1, 5, 10, 13, 16, 18, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 125, 150, 200, 250, 300
08/05	048	20 Sep 01	1, 5, 10, 15, 20, 25, 30, 46, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150, 200, 250, 300
09/02	051	22 Sep 01	1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 90, 100, 120, 150, 180, 190, 200, 210, 230, 250, 300
09/15	052	23 Sep 01	1, 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 125, 150, 200, 225, 250, 300
10/02	056	24 Sep 01	1, 5, 10, 15, 20, 22, 24, 29, 40, 50, 55, 60, 70, 80, 90, 100, 110, 120, 150, 200, 250, 300
10/06	057	25 Sep 01	1, 5, 10, 15, 20, 24, 28, 31, 35, 40, 55, 60, 65, 80, 90, 100, 110, 120, 150, 200, 250, 300
11/02	061	26 Sep 01	1, 5, 10, 15, 21, 24, 28, 31, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 92

Preliminary results

The thermocline region was in most stations at about 100 to 150 metres. However, rather than the expected oligotrophic situation throughout this depth (as found by previous studies) the chlorophyll maximum was normally between 25 and 50 metres, and it was at this maximum that the nutricline was observed.

With the excellent performance of the nanomolar nitrate system the fine scale structure of the profiles could be accurately observed.

Surface nitrate was between 0.5 and 2 nanomoles per litre, with the increase at the nutricline at over 2 orders of magnitude in a depth increase of less than 3 metres. These figures are in good agreement to those previously found - but may be more accurate, due to a greater sensitivity of this waveguide system to that of the old chemiluminescence technique.

Nitrite profiles always showed the nitrite maximum associated with the chlorophyll maximum but in the central region at stations 5 to 7 we observed the deeper secondary nitrite maximum, below the oxycline, with concentrations over 3 micromolar, from around 200 metres to below the bottom sampling depth of 300 metres. This was the area in the Indian Ocean where nitrification is known to occur. Generally the ammonia concentrations were around 30 to 50 nanomoles in the surface and deeper waters and at a few stations the ammonia maximum was observed, up to 300 nanomoles, for the sample depths above the nitrite maximum. Ammonium ion is produced due to the 'sloppy-feeding' of zooplankton on phytoplankton, and the nitrite is produced as an intermediate product of nitrification, the conversion of ammonium to nitrite, by nitrifying bacteria.

Generally the nutrients showed that the oligotrophic regions had a surprisingly shallow mixed, nutrient deplete surface layer, maybe only to 50 metres at most – unlike the oligotrophic gyres of the north and south Atlantic where it is generally over 150 metres. This implies that we have studied this oceanic system soon after the monsoon season and the water column had not yet stabilised from the monsoon with the shallowing of the thermocline and the greater availability of nutrients to the surface waters at this time.

7.3 Pigments: chemotaxonomic assessment of phytoplankton distribution (Denise G. Cummings)

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:

Table 4. Chemotaxonomic associations for phytoplankton

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

Methods

Samples were obtained on-board by vacuum filtering of CTD-collected seawater through 25mm GF/F filters. Pigments were extracted from the filters using 90% acetone with apo-carotenal as an internal standard. Pigment extracts were 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 were secured through co-elution with authentic pigment standards (VKI, Denmark and Sigma Chemical Co). Not all samples were analysed on-board as the HPLC broke down around 200 mile off Oman (station 8). From station 8 onwards the filters were preserved in liquid nitrogen and will be analysed back at the laboratory.

Table 5. Summary of CTD samples collected for pigments

Date	Station/deployment	Depths sampled for pigments (m)
1 Sep 01	test	1,10,20,30,40,50,60,70,100,200
3 Sep 01	01/01	1,5,10,25,50,60,74,100,120,150,200
	01/06	1,5,10,30,40,50,60,70,90,100,140,170
4 Sep 01	01/10	1,10,25,40,75,85,100,120,140,180
5 Sep 01	02/01	1,5,10,25,50,62,80,100,120,150
	02/06	1,10,20,30,40,50,60,80,100,150
6 Sep 01	02/10	1,5,11,26,41,50,60,70,100,150
7 Sep 01	03/01	1,10,25,35,45,55,65,75,85,110,140,170
	03/02	1,10,25,55,79,85,110,170
	03/04	1,10,25,55,71,115,215
8 Sep 01	03/05	1,10,25,55,70,115,210
	03/06	1,10,25,60,80,105,140
	03/09	1,5,10,25,50,60,80,100,150
9 Sep 01	03/09	1,5,10,25,50,60,80,100,150
10 Sep 01	04/01	1,5,10,25,40,57,66,80,100,140
11 Sep 01	04/06	1,5,10,25,50,65,77,90,100,120
12 Sep 01	05/02	1,5,10,25,40,50,60,80,100,120
13 Sep 01	05/06	1,5,10,25,36,50,60,90,120,150
14 Sep 01	06/02	2,5,10,25,35,40,60,80,99,153
15 Sep 01	06/07	1,5,10,20,30,40,50,75,100,150
16 Sep 01	07/03	1,11,25,47,80,100,130
	07/04	1,10,25,50,80,100,130
17 Sep 01	07/07	10,25,50,80,130
18 Sep 01	07/11	1,5,10,20,40,49,60,70,90,120
19 Sep 01	08/02	1,5,10,16,21,40,60,80,100,125
20 Sep 01	08/05	1,5,10,21,29,45,60,70,90,120
22 Sep 01	09/02	1,5,10,15,20,30,50,80,100,150
23 Sep 01	09/05	1,2.5,5,7.5,10,20,30,50,70,125
24 Sep 01	10/02	1,5,10,20,25,29,40,55,70,120
25 Sep 01	10/06	1,5,10,15,20,25,27,40,60,110
26 Sep 01	11/02	1,5,10,21,31,40,50,60,70,92
27 Sep 01	11/06	1,5,10,15,19,26,40,55,70,92

Table 6. On-board pigment analyses

Station	Pigment in order of highest concentration
Shakedown	<i>chla</i> , <i>hex</i> , <i>fuc</i> , <i>but</i> , <i>zea</i> , <i>dv chla</i>
1	<i>chla</i> , <i>dv chla</i> , <i>hex</i> , <i>but</i> , <i>zea</i>
2	<i>chla</i> , <i>hex</i> , <i>dv chla</i> , <i>but</i> , <i>zea</i>
3	<i>chla</i> , <i>dv chla</i> , <i>hex</i> , <i>zea</i> , <i>but</i>
4	<i>chla</i> , <i>hex</i> , <i>dv chla</i> , <i>zea</i> , <i>but</i>
5	<i>chla</i> , <i>hex</i> , <i>but</i>
6	<i>chla</i> , <i>hex</i> , <i>but</i> , <i>zea</i>
7	<i>chla</i> , <i>hex</i> , <i>but</i> , <i>fuc</i> , <i>zea</i> , <i>dv chla</i>

Highest *chla* values were at station 06/02 (931ng/l at 35m). This station also had the highest *hex* values (457ng/l at 35m). Highest *zea* concentrations were at station 04/01 (137ng/l at 57m). Highest *dv chla* values were at station 01/01 (246ng/l at 74m). Surface *chla* concentrations ranged from 19ng/l (stn 01/01) to 179ng/l (06/02).

7.4 Primary production (Gwyn Morgan)

Aims

- To estimate primary production at 11 stations along the AMBITION transect, by comparison of *in situ* and on-deck incubations.
- To investigate dissolved organic carbon production (DOC).

Methods

Water from the appropriate depths was sampled from CTD casts, and 60ml square polycarbonate experimental bottles were filled to the brim. Approximately 0.01mCi ¹⁴C bicarbonate was added to each bottle and incubated for the appropriate amount of time either *in situ* at the depth sampled or in an on-deck incubator equipped with an appropriate light filter. For measurement of primary production, experimental bottles were filtered across a 0.2µm pore polycarbonate filter washed two times with 10ml of filtered sea-water (the first wash was used to rinse the experimental bottle). Radioactivity was assayed by liquid scintillation counting. For estimation of DOC production, 7.5ml was taken from an experimental bottle, spiked as described above, and filtered through a 0.2µm acrodisc syringe filter into a 20ml glass scintillation vial. 100µl 5M HCl was added and the vial was shaken from 12 to 24 hours to drive out any radioactive inorganic carbon. Production was calculated by multiplying the proportion of measured dpm over total dpm by the concentration of CO₂ dissolved in the water (estimated from salinity).

Sampling

For *in situ* measurements, water was sampled in triplicate from each of eight depths. In addition, a fourth bottle at each depth, covered in aluminium foil, was sampled to determine carbon fixation in the dark as a control. At four of these depths, eight additional bottles were sampled so that on-deck incubator measurements could be made: half with a similar incubation period to the *in situ* experiment; half were 24 hr incubations (to compare net and gross productivity). An additional bottle at three depths was incubated on deck for 24 hr for DOC measurement together with a blank of filtered seawater as a control.

Table 7. Incubation experiments for primary production and DOC production

Deployment	<i>In situ</i> (8 depths)	On deck (4)	24h (4)	DOC (3)
01/01	✓	✓	✓	
02/01	✓	✓	✓	
03/09	✓	✓	✓	✓
04/06	✓	✓	✓	✓
05/06	✓	✓	✓	✓
06/07	✓	✓	✓	✓
07/11	✓	✓	✓	✓
08/06	✓	✓	✓	✓
09/05	✓	✓	✓	✓
10/06	✓	✓	✓	✓
11/06	✓	✓		

Results

In general, production was at its lowest at the southern end of the transect. Hourly production was mostly similar whether measured *in situ* or on-deck, though on deck measurements seem to be characteristically slightly higher. In a few cases samples from the deeper depths exhibited a significantly higher rate of primary production when measured using the on deck incubator (at greater than *in situ* temperature). The much higher production at station 9, which had very green water, when integrated for the water column, does not have vastly greater carbon fixation per square metre of sea water; production is concentrated to the top few metres. Highest production tends to correlate with the maximum fluorescence, but is often nearly as high at the surface.

Table 8. Depth integrated primary production at all stations

Station	Depth integrated PP (mg C m ⁻² h ⁻¹)
1	19.1
2	20.7
3	19.4
4	22.1
5	37.7
6	43.7
7	28.6
8	36.6
9	62.8
10	49.4
11	26.4

Conclusions

The initial analysis of these data seems reasonable, agreeing with what one might predict. It will be interesting to see how these results compare to data collected by other cruise participants. This work was greatly assisted by help and advice from Mike Zubkov and Andy Rees, and UKORS engineers for rig deployment.

7.5 Microbial uptake and flux of inorganic nitrogen (Andy Rees)

Aims

- To determine the phytoplankton uptake characteristics of nitrate and ammonium at a series of stations in the Indian Ocean.
- To estimate rates of ammonium regeneration and nitrification within the euphotic zone.
- To investigate the incorporation of ¹⁵N₂ into the DNA of marine nitrogen fixers.
- To investigate the diel variation in nitrification at selected stations.
- To compare the use of stable- (¹³C) and radio- (¹⁴C) isotopes in estimating carbon fixation rates.

Methods

Eleven oceanographic stations were occupied and at each a series of experiments were performed to meet the above aims.

Phytoplankton NO₃ and NH₄ uptake

Two separate approaches were taken. Firstly, an experiment was performed at each station to determine the kinetic uptake parameters V_{max} and K_s . Water was collected into a series of 0.6 litre polycarbonate bottles from the depth equivalent to the 33% light level (10 – 25m), chosen as representative of the upper mixed layer without the photo-inhibitory effects potentially associated with surface waters. ¹⁵N-NO₃ and ¹⁵N-NH₄ were added to replicates (x2 or x3) at 8 concentrations in the range 5 – 1500 nmol l⁻¹ and incubated in on-deck incubators. Temperature control was maintained by the continuous flow of surface seawater whilst light levels were at 33% of incident irradiation using blue filter. Incubations were terminated after approximately 1 – 2 hours by filtration onto ashed GF/F filters which were dried onboard and stored over silica gel prior to analysis in the laboratory using continuous flow isotope-ratio mass spectrometry (IRMS).

Secondly, NO₃ and NH₄ uptakes were determined at 8 depths throughout the euphotic zone (defined as the depth of the 0.1% irradiance level) to allow determination of integrated rates of nitrogen uptake. Water was collected pre-dawn into 2 x triplicate 0.6 litre polycarbonate bottles and inoculated with ¹⁵N-NO₃ and ¹⁵N-NH₄ to 10% of the ambient concentration to a minimum of 5 nmol l⁻¹. Bottles were attached to a free floating incubation rig at the depth from which they were collected for approximately 5 hours, after which they were size fractionated by filtration into the total and <2.0 µm communities before drying and storing as above.

NH₄ regeneration and nitrification

An isotope-dilution approach was taken to estimate the rates of NH₄ regeneration and nitrification on filtrate collected from a number of the in-situ incubation bottles. Approximately 300ml was collected into clean pyrex bottles, into which was added a magnetic stirrer bar and 300 mg magnesium oxide. Ammonia evolved was collected onto an acidified filter paper suspended within the bottle over a period of 7 days at ~50°C. Following this Devarda's alloy and magnesium oxide were added to convert NO₃ into NH₄ which was collected as above. Filters were dried overnight at 50°C and stored over silica gel prior to laboratory analysis using IRMS.

Nitrogen fixation

A novel approach was taken to investigate the presence and potential identification of nitrogen fixing cyanobacteria. 24 l of seawater from 25m was collected onto a single 0.2 µm filter, and re-suspended into 2.4 l of seawater from the same depth i.e. a 10 x dilution. This was then distributed into a series of 100ml aliquots in universal bottles which were sealed with gas tight crimps. Three treatments were then set up as follows; 1) + 20ml ¹⁵N₂, 2) + 20ml ¹⁵N₂ + 4ml glutaraldehyde and, 3) + 20ml air. Samples were incubated at surface seawater temperature under 20% of incident irradiance and terminated by filtration after 0, 24, 48 and 90 hours.

Diel variability in nitrification

Bacterial nitrification is known to be inhibited by light, although recently a number of reports have identified nitrifying activity in surface waters. At two depths, 25m and depth of the fluorescence maximum, nitrification rates were determined 4 times at 6 hourly intervals. 6 x 50 ml culture bottles were filled from each depth, to each bottle was added 10 µCi ¹⁴C-bicarbonate and to 3 bottles, the nitrification inhibitor allylthiourea (ATU) was also added. Bottles were incubated in the dark at sea surface temperature for approximately 8 hours. Incubation was terminated by

filtration onto 0.2µm polycarbonate filters, ^{14}C was counted on board by liquid scintillation counter, and nitrification rate considered to be the difference between the inhibited and uninhibited samples.

^{13}C bicarbonate fixation

At the last three stations occupied, a number of 0.6 l bottles were inoculated with ^{13}C bicarbonate in parallel with ^{14}C bicarbonate carbon fixation experiments (by Gwyn Morgan) and incubated under the same conditions of light and temperature. Incubations were terminated by filtration and dried at 50°C overnight, and stored over silica gel prior to IRMS analysis in the laboratory.

Table 9. Summary of nitrogen flux experiments

Station/ deployment	CTD cast no [all CD132..]	Variable determined	No. of depths
01/01	001	N uptake – in situ rig	8 (1 – 100m)
01/10	006	N uptake – kinetic NH ₄ regen./nitrification	1 (25m) 1 (25m)
02/01	008	N uptake – <i>in situ</i> rig NH ₄ regen./nitrification	8 (1 – 100m) 4 (10 – 62m)
02/10	013	N uptake – kinetic	1 (25m)
03/01 to 03/05	015 to 019	Diel nitrification	4 x [2 (25m & Chl. max.)]
03/06	020	N uptake – kinetic	1 (25m)
03/09	021	N uptake – <i>in situ</i> rig NH ₄ regen./nitrification	8 (1 – 100m) 4 (10 – 63m)
04/02	024	N uptake - kinetic	1 (25m)
04/06	025	N uptake – in situ rig NH ₄ regen./nitrification	8 (1 – 100m) 4 (25 – 77m)
05/02	029	N uptake - kinetic	1 (25m)
05 -	030	N fixation	1 (25m)
05/06		N uptake – <i>in situ</i> rig	8 (1 – 90m)
06/01		N uptake - kinetic NH ₄ regen./nitrification	1 (25m) 1 (25m)
06/07	036	N uptake – <i>in situ</i> rig	8 (1 – 75m)

Table 9. Summary of nitrogen flux experiments - continued

Station/ deployment	CTD cast no [all CD132..]	Variable determined	No. of depths
07/03 to 07/06	039 to 042	Diel nitrification	4 x [2 (25m & Chl. max.)]
07/07	043	N uptake – kinetic	1 (25m)
07/11	044	N uptake – in situ rig NH ₄ regen./nitrification	8 (1 – 90m) 3 (10 – 49m)
08/02	047	N uptake - kinetic	1 (10m)
08/05	048	N uptake – <i>in situ</i> rig NH ₄ regen./nitrification	8 (1 – 60m) 4 (5 – 30m)
09/02	051	N uptake - kinetic	1 (non-toxic supply)
09/05	052	N uptake – <i>in situ</i> rig	8 (1 – 30m)
10/02	056	N uptake - kinetic	1 (20m)
10/06	057	N uptake – <i>in situ</i> rig	8 (1 – 55m)
11/02	061	N uptake - kinetic	1 (10m)
11/06	062	N uptake – <i>in situ</i> rig	8 (1 – 55m)

7.6 Phytoplankton community structure and abundance by flow cytometry and microscopy (Glen Tarran)

Introduction

In order to provide important underpinning information on phytoplankton communities, flow cytometry was used to quantify pico and nanophytoplankton through the water column. This approach enabled basic distribution data to be generated within a few hours of arriving on each station, assisting others in formulating their sampling strategies. In addition, samples were collected for post-cruise analysis of nano and microplankton by microscopy and data collected to determine the size structure of different picophytoplankton groups – picoeukaryotes (size, <2µm); prochlorophytes (0.6µm) and cyanobacteria (0.8-1µm).

Studies undertaken

- Analysis of fresh seawater samples to determine the distribution, abundance and community structure of nano and picoplankton in surface waters at CTD stations by flow cytometry.
- Collection of preserved seawater samples (formalin and Lugol's iodine) to determine the distribution, abundance and community structure of nano and microplankton in surface waters at CTD stations by microscopy.
- Collection of preserved seawater samples (Lugol's iodine) for analysis of microplankton abundance and community structure in the oxygen depleted zone (Generally below 100 m).

- Size fractionation of picophytoplankton communities from the mixed layer to determine median cell diameters for pico-eucaryotes, prochlorophytes, and cyanobacteria.
- Analysis of phytoplankton community structure from live concentrated seawater samples by microscopy and video recording.

Phytoplankton community structure and abundance

Fresh seawater samples were collected in clean 250 ml polycarbonate bottles from a Seabird CTD system containing 24 x 20 litre Niskin bottles from biogeochemistry CTD casts. Samples were stored in a refrigerator until analysed (less than 1.5 hours). Additional samples from molecular biology casts were also collected by Nick Fuller and Karen Orcutt (University of Warwick). 2.2 ml samples were used for immediate flow cytometric analysis, to characterise and enumerate prochlorophytes, cyanobacteria, pico-eukaryotes and nanophytoplankton (BGC casts only) based on their light scattering and fluorescence properties. The flow cytometer used was a Becton Dickinson FACSort instrument. Of the 2.2 ml, approx 300µl of sample was actually analysed to provide vertical profiles of phytoplankton abundance per millilitre. [Table 10](#) summarises the CTD casts sampled and analysed during the AMBITION cruise and [Fig 4](#) shows contour plots of phytoplankton abundance along the cruise track down to 150 m.

Collection of preserved samples for nano and microplankton analysis from biogeochemistry casts

The fresh seawater samples collected as described above were also used for preserved samples. While samples were being analysed on the flow cytometer, twin 100 ml sample bottles, one containing 1 ml hexamine buffered formaldehyde and the other containing 2 ml Lugol's iodine were filled with seawater from 12 depths from the cast. All of the biogeochemistry CTD casts were sampled (see [Table 10](#)) in this way and a subset of the samples will be chosen for microscopic analysis after the cruise.

Table 10. CTD samples analysed for phytoplankton community structure

Date	Station/ deployment	CTD cast no. [CD132...]	Time (local)	Lat	Long	Depth range sampled (m)
3 Sep 01	01/01	001	05:52	00°54.7'S	64°08.5'E	1 - 300
	01/03	002	08:43	00°54.3'S	64°08.2'E	10 - 150
4 Sep 01	01/10	006	08:00	00°54.9'S	64°08.4'E	1 - 140
5 Sep 01	02/01	008	04:49	00°00.2'S	66°59.9'E	1 - 300
	02/03	009	07:08	00°00.1'S	66°59.7'E	10 - 150
6 Sep 01	02/10	013	07:00	00°01.2'S	67°01.4'E	1 - 100
7 Sep 01	03/01	015	13:10	03°48.0'N	67°00.0'E	1 - 170
	03/02	016	19:01	03°48.0'N	67°00.1'E	1 - 170
8 Sep 01	03/04	018	00:50	03°47.8'N	67°00.0'E	1 - 170
	03/05	019	06:50	03°48.0'N	67°00.1'E	1 - 170
	03/06	020	06:50	03°47.6'N	67°00.1'E	10 - 210
9 Sep 01	03/09	021	04:54	03°47.4'N	67°00.0'E	1 - 170
	03/13	023	10:58	03°46.0'N	66°59.1'E	1 - 90
10 Sep 01	04/02	024	13:05	07°36.0'N	67°00.3'E	1 - 160
11 Sep 01	04/06	025	04:49	07°36.1'N	67°00.3'E	1 - 150
	04/08	026	07:04	07°36.0'N	67°00.5'E	10 - 150
12 Sep 01	05/02	029	12:51	11°23.7'N	66°59.8'E	1 - 80

Table 10. CTD samples analysed for phytoplankton community structure - continued

Date	Station/ deployment	CTD cast no. [CD132...]	Time (local)	Lat	Long	Depth range sampled (m)
13 Sep 01	05/06	030	04:53	11°23.7'N	66°59.8'E	1 - 100
	05/08	031	07:05	11°23.9'N	66°59.9'E	10 - 200
14 Sep 01	06/02	034	13:02	15°11.9'N	67°00.0'E	1 - 153
15 Sep 01	06/07	036	04:55	15°11.9'N	67°00.0'E	1 - 110
	06/09	037	07:30	15°11.6'N	67°00.0'E	10 - 200
16 Sep 01	07/03	039	12:58	19°00.0'N	67°00.1'E	1 - 150
	07/04	040	18:54	19°00.1'N	67°00.0'E	1 - 130
17 Sep 01	07/05	041	12:58	19°00.0'N	67°00.2'E	1 - 130
	07/06	042	06:56	19°00.1'N	67°00.0'E	1 - 80
	07/07	043	12:44	19°00.0'N	67°00.0'E	10 - 130
18 Sep 01	07/11	044	04:57	18°59.9'N	66°59.9'E	1 - 150
19 Sep 01	08/02	047	12:56	20°54.8'N	63°39.9'E	1 - 150
20 Sep 01	08/05	048	04:58	20°54.8'N	63°39.9'E	1 - 150
22 Sep 01	09/02	051	13:03	23°33.2'N	59°53.8'E	1 - 150
23 Sep 01	09/05	052	05:00	23°33.3'N	59°54.0'E	1 - 150
24 Sep 01	10/02	056	12:59	24°19.9'N	58°10.1'E	1 - 150
25 Sep 01	10/06	057	05:01	24°19.9'N	58°10.1'E	1 - 120
26 Sep 01	11/02	061	12:52	26°00.1'N	56°34.9'E	1 - 92
27 Sep 01	11/06	062	04:46	26°00.1'N	56°35.1'E	1 - 92
	11/07	063	07:01	26°00.4'N	56°35.2'E	1 - 95

Table 11. Samples taken for microplankton analysis from oxycline CTD casts

Date	Deployment	Lat	Long	Depths sampled (m)
6 Sep 01	02/10	00°01.2'S	67°01.4'E	110, 120, 130, 140, 150, 170
9 Sep 01	03/13	03°46.0'N	66°59.1'E	100, 120, 140, 160, 180, 200
18 Sep 01	07/11	18°59.9'N	66°59.9'E	120, 130, 150, 200, 250, 300
20 Sep 01	08/09	20°55.1'N	63°38.9'E	100, 140, 200, 240, 300, 400
23 Sep 01	09/09	23°32.0'N	59°53.6'E	80, 100, 140, 180, 220, 300
25 Sep 01	10/10	24°18.8'N	58°10.7'E	50, 60, 80, 100, 160, 269

Collection of preserved samples from oxycline casts

Six stations were sampled to analyse microplankton community structure in the oxygen depleted zone, generally below 100 m. At each station sampled, 500 ml jars containing 10 ml Lugol's iodine were filled from six depths. A summary of samples taken is given in [Table 11](#) above.

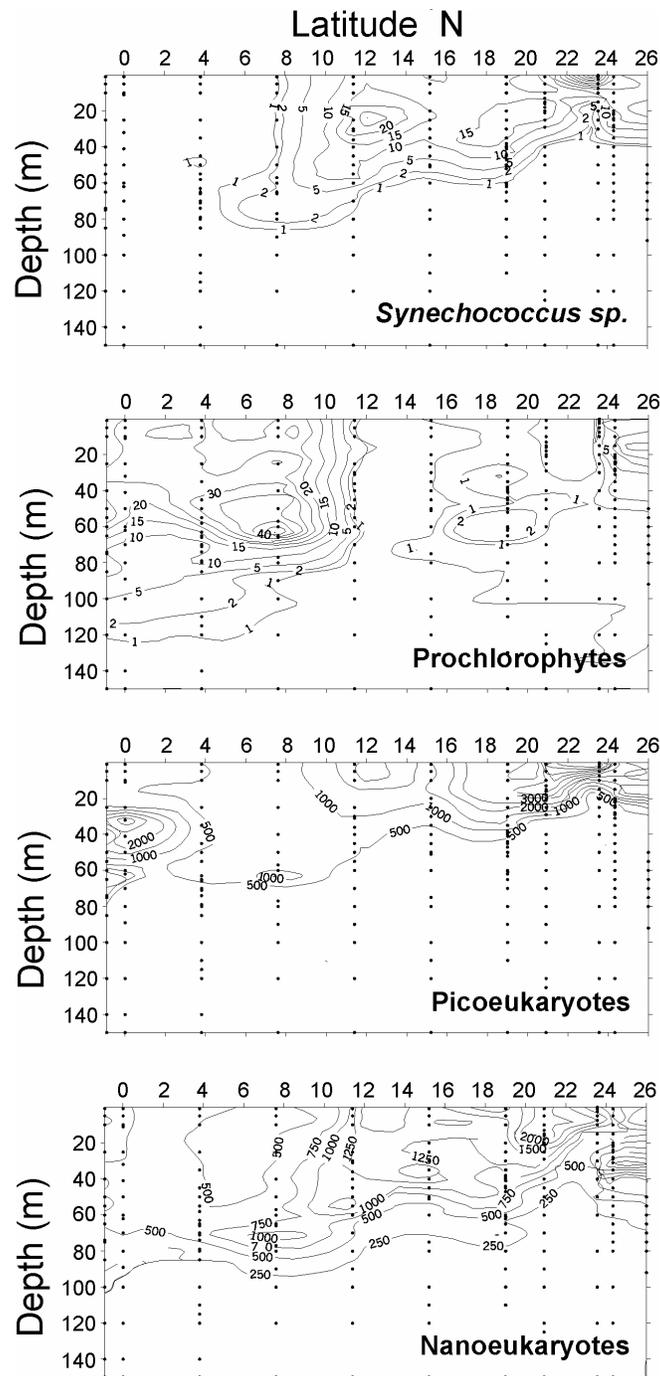


Fig 4. Contour plots of phytoplankton abundance along the AMBITION cruise track. *Synechococcus sp.* and *Prochlorophyte* data are $\times 10^4$ cells mL^{-1} , pico- and nanoeukaryote contours are cells mL^{-1}

Picophytoplankton size structure

Water samples from the mixed layer were gravity filtered through 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. After the cruise the cell counts will be compared to unfiltered seawater cell numbers by plotting % cells remaining against filter pore size. The median cell diameters will then be read off the X axis where they intersect with the 50% line on the Y axis.

Video micrography studies of phytoplankton community structure from live samples

Approx 37 litres of seawater were collected from 2 depths in the mixed layer from each station and were concentrated down to around 70 -100 ml by gravity filtering through a 0.22µm Gelman Criticap 100 cartridge filter. Filtering took around 1 hour. Samples were then taken to the dark room and a 3 ml sample pipetted into a Hydrobios settling chamber. Samples were analysed using an Olympus IMT-2 inverted microscope, with colour CCD camera and SVHS video recording capability. All samples were videoed with a x60 objective to record nanoflagellates and, where appropriate, with x4, x10, x20 and x40 objectives to record larger plankton. Analysis of the 8 hr of video material will be carried out back in the laboratory to characterise each of the stations in terms of their planktonic communities.

7.7 Bacterial collection (Kevin Ashelford)Aim

To collect bacterial samples from a range of sites for subsequent DNA extraction and culturing at Cardiff University.

Outcome

Water samples were collected and filtered from all 11 stations visited. At least three depths were sampled from each station, usually at 10 m, at the chlorophyll maximum, and around the oxygen minimum. Where possible, additional water samples were taken, either whilst at station, or in transit between stations from the non-toxic water supply. Water samples were either filtered through Millipore Sterivex filter units (pore size, 0.22 µm), or 142 mm or 47 mm diameter, 0.22 µm pore, membrane filters. Typically, at least 40 litres were collected and filtered per sampling depth per sampling occasion. Filters and filter units were stored at -80°C pending transport back to Cardiff University, with Sterivex units being flooded with Lysis buffer for subsequent DNA extraction and the membrane filters being stored in 50% glycerol for culturing purposes. All filtration included a pre-filtration step and pre-filters were also stored at -80°C for possible future use. In total, over the course of the cruise, 143 filters and filter units were amassed, representing a total of 2187.5 litres of seawater filtered.

7.8 *In situ* community structure of photosynthetic picoeukaryotes
(Nick Fuller and Karen Orcutt)Aims

- To investigate the community structure of marine photosynthetic picoeukaryotes (<3 µm), along the AMBITION transect, particularly their horizontal and vertical distribution
- To determine how the distribution of these picoeukaryotes is affected by the *in situ* environmental gradients of light and nutrients

- To correlate the distribution of specific picoeukaryote classes with the prokaryotic cyanobacterial picophytoplankton, *Synechococcus* and *Prochlorococcus*.

Post-cruise analysis of photosynthetic picoeukaryote diversity will be based on the 16S rRNA gene present in their chloroplasts. Samples were therefore taken for nucleic acid extraction. Extracted DNA will be amplified by the polymerase chain reaction (PCR) for diversity to be assessed through denaturing gradient gel electrophoresis analysis and dot blot hybridisation technology, using oligonucleotide probes specific for different photosynthetic picoeukaryote groups. These probes have first to be designed from existing sequence data in the database, and from data to be obtained from current isolates, and from any isolates resulting from this cruise. Extracted RNA will also be amplified by reverse transcriptase-PCR and screened similarly to DNA to determine the active members of the community, since it is these which are clearly more relevant to carbon fixation in the water column. The active component of the community was also investigated through on-deck incubations with ^{13}C -labelled bicarbonate. DNA which has incorporated ^{13}C via photosynthesis can be separated from 'normal' ^{12}C -DNA by caesium chloride density gradient centrifugation.

The phosphate status of *Prochlorococcus* and *Synechococcus* will also be determined, using antibodies to the phosphate transport protein PstS, expressed only under conditions of phosphate stress. Large volumes of seawater were concentrated in order to supply sufficient cellular material for subsequent western analysis. *Prochlorococcus* and *Synechococcus* diversity will also be assessed using fluorescent *in situ* hybridisation (FISH).

Main Activities

- Filtration of 5 or 10 litres of seawater onto 47 mm diameter, 0.45 μm pore-size membranes, separately for DNA extraction and for RNA extraction (prefiltered through 3 μm).
- Fixation of 2 x 50 ml of seawater for analysis of picophytoplankton by FISH.
- On-deck incubation of 12 litre samples of seawater with ^{13}C -sodium bicarbonate, for 3 to 14 days, before filtering for DNA extraction.
- Filtration of 50 ml of seawater, enrichment and incubation in light for isolation of photosynthetic picoeukaryotes.
- Concentration of 100 litres of seawater to 100 μl by tangential flow filtration and centrifugation of the subsequent concentrate, for PstS assays.
- Filtration of 15 ml of seawater through 0.2 μm for isolation of *Synechococcus* viruses (a separate Warwick project).

Plankton tows

Surface plankton tows were conducted along the transect to examine the species distribution of *Trichodesmium* colonies along the oligotrophic to eutrophic gradient. *Trichodesmium* colonies representing several species were collected and frozen for sequence analysis of the intergenic transcribed spacer region (ITS) between the 16S/23S rRNA genes and for DNA fingerprinting to examine the genetic diversity of the colonies from different geographical areas. *Trichodesmium* colonies were found in the oligotrophic stations 1-7 and were dominated by *T. thiebautii* with *T. erythraeum* being observed at stations 3 and 7 only. Morphological differences in *T. thiebautii* colonies were also observed at stations 1, 3 and 4 where both puffs (colonies with trichomes arranged radially) and tufts (colonies with trichomes arranged in parallel) were observed. No *Trichodesmium* colonies were observed when the transect headed to the northwest toward

Oman. However, the plankton tows showed a dramatic change as we entered more eutrophic stations (8-11). The plankton tow at Station 8 was dominated by a colonial salp swarm and Station 9, the most eutrophic, was very dense brown and dominated by diatoms. Stations 10 and 11 had lots of *Noctiluca* cells that were characterized by a green color which was not seen in the *Noctiluca* cells at the other stations. On close examination using the microscope and video recorder, it was observed that the cells were full of gametes which had a green pigment.

7.9 Nitrogen-fixing microbes in the ocean: linking phenotype to 16S rRNA phylotype (Mike Wyman and Clare Bird)

Aims

- To examine the molecular diversity of nitrogen fixing bacteria at contrasting open ocean sites along the AMBITION cruise track.
- To determine whether diazotrophic bacteria are actively fixing nitrogen at these sites by means of RT-PCR based technologies and *in situ* hybridization
- To develop finer resolution techniques to examine the taxonomic affiliations of marine diazotrophs by phylogenetic analysis of *nif* and *rrn* gene sequences.

Methods

Eleven stations were sampled covering a broad range of oceanic habitats from highly oligotrophic surface waters to nutrient-rich upwelling waters off the Omani coast. Water samples were collected with 20 litre Niskin bottles and/or 30 litre GoFlos at selected depths ranging from near surface to 250m. Samples were filtered through 90 mm, 0.2µm pore size polycarbonate filters and taken up in DNA extraction buffer (100 mM Tris-HCl [pH 8.0], 100 mM EDTA, 250 mM NaCl, 0.1% [w:v] lithium dodecyl sulphate) or RNAlater™ (Ambion Inc.) prior to storage at -20 °C or 4°C respectively. At each station, SAPs (Stand Alone Pumps) were deployed equipped with 293 mm polycarbonate filters to collect the >0.2 µm fraction from the upper mixed layer and from selected depths within the oxygen depleted zone. Pumping times were set at 18 – 30 minutes and the collected material was washed from the filters and re-suspended in RNAlater for RNA/DNA extraction ashore.

Five diel experiments were performed at stations 3, 6, 7, 8, and 9. At stations 3 and 7, samples from 4-6 depths within the upper water column were obtained every six hours and the >0.2 µm fraction collected on 90 mm polycarbonate filters and stored in DNA extraction buffer. At stations 3, 6, 8, and 9, water samples were collected every 2-3 hours and fractionated through 2.0 and 0.6 µm pore size polycarbonate filters to enrich for *Synechococcus* spp. in the 0.6 - 2.0 µm size fraction. The material collected was stored in RNAlater for RNA extraction ashore. In addition, an aliquot of the concentrated suspension or original water sample was fixed in paraformaldehyde at 4 °C for cell cycle analysis by flow cytometry.

20µm horizontal net tows of 15 minutes duration were performed at the majority of stations at depths ranging from 5 – 15 m. *Trichodesmium* spp. were sorted into RNAlater when present and stored at 4°C for DGGE analysis of *nif* diversity.

General comments and future work

A comprehensive set of samples was collected for nucleic acid analysis (see [Appendix 1](#)) that will be processed over the coming months at the University of Stirling. The choice of the Arabian Sea for this cruise was highly suitable and ensured that the widest possible range of oceanic

conditions were sampled. Those organising the cruise are to be thanked for the long hours spent in planning and managing the complex logistics to ensure the successful outcome of the sampling programme. The level of performance and standard of maintenance of the sampling gear required for this study (CTD, GoFlos, SAPs) was quite exceptional. The technical staff are to be complimented on their cheerful and highly professional performance.

7.10 Biodiversity of methyl bromide utilising bacteria and other methylotrophs

(Mike Cox)

Aims

- To set up enrichments of bacterial samples concentrated from sea-water on methyl bromide (MeBr) and various other substrates
- To collect bacterial samples for further molecular analyses including PCR of the *cmuA*, *mxnF* and *rnnA* genes
- To detect the presence of and measure the abundance of MeBr in sea-water using gas chromatography with electron capture detection.

Methods

Bacterial samples were concentrated from seawater using filtration onto 0.2 μm membrane filters and resuspended into 2 ml of the original sample. This was inoculated into pre-prepared stoppered and crimp-sealed vials containing 0.1 X Ammonium nitrate mineral salts media (ANMS) with 3.5 % NaCl, plus vitamins and trace elements. The vials also contained one or more of the growth methylotrophic growth substrates outlined in [Table 12](#).

Table 12. Methylotrophic growth substrates used for enrichment experiments

Vial No	Substrate	Concentration
1	Methyl bromide	0.1 %*
2	Methyl bromide	0.5 %*
3	Methyl bromide	1.0 %*
4	Methanol	10 mM
5	Methyl bromide and methanol	0.5 %* and 10 mM
6	Methylamine	10 mM
7	Methyl bromide and methylamine	0.5 %* and 10 mM
8	Formate	10 mM
9	Methyl bromide and formate	0.5 %* and 10 mM
10	Methane	20 %*
11	Methyl chloride	2 %*
12	Methyl bromide and L-methionine	0.5 %* and 10 mM

*percentage of headspace by volume at room temperature and pressure

The enrichments were stored in the dark in the constant temperature room (~24 °C) for the duration of the cruise. On return to the laboratory, filters were collected for DNA extraction. Various volumes were passed through a 0.2 μm polyethersulfone Supor filter (Pall Gellman) which have the advantage of being phenol soluble, or 0.2 μm Sterivex cartridge filters (Millipore) and fast frozen in the -80 °C freezer.

It had originally been planned to use a GC-ECD for on-board analyses; however, this equipment was non-operational at the deadline for pre-cruise gear transport. It was therefore not taken,

and Phil Nightingale did not participate in the cruise, since there was only enough work for one. Although this was disappointing, extra effort was expended on other aspects of the work.

Results

A total of 169 samples for DNA extraction and 298 enrichments were obtained. These encompassed all stations and two diel cycles at stations 3 and 7. Preliminary results are that a number of the enrichments are actively oxidising MeBr and that our molecular marker for methyl halide degradation, *cmuA* is present in these waters.

Acknowledgements

This was my first research cruise and it occurred very early in my PhD. In light of this I would like to thank all my fellow participants for their assistance, advice and support. My thanks also go to Colin Murrell, Ian McDonald, Phil Nightingale and Hendrik Schäfer for setting up equipment and filling a large number of vials with media.

7.11 Bacterioplankton dominance and functional diversity within communities in contrasting regions (Mike Zubkov)

Aim

To compare abundance and metabolic activities of dominant groups of bacterioplankton in widely different planktonic communities of the Arabian Sea.

Methods

Water samples were collected and preserved for determination of bacterioplankton concentration, biomass and composition using flow cytometry and molecular methods including *in situ* hybridisation. Bacterioplankton metabolic activities and production were determined on board by incubating samples with isotopically labelled precursor molecules: ³H-leucine, ¹⁴C-leucine, ³H-glucose, ¹⁴C-glucose, ³³P-phosphate and ³⁵S-methionine.

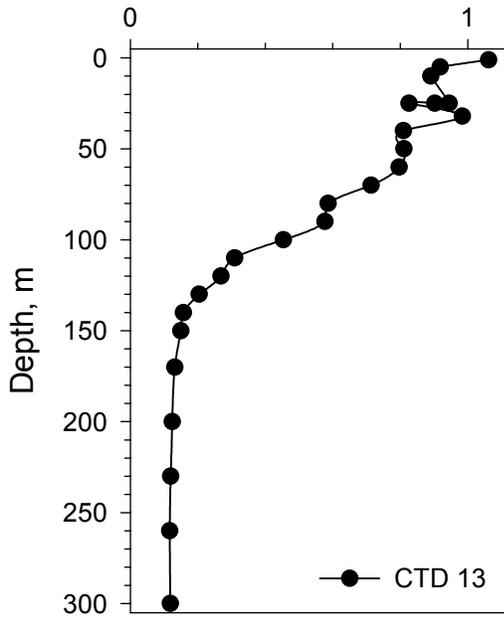
Results

Bacterioplankton concentration was determined by flow cytometry on board the ship in all collected samples. Examples of vertical distribution of bacterioplankton are presented on Figs. 5 and 6. Bacterioplankton concentration varied 500 fold, from 20×10^6 cells l⁻¹ at 3000m depth to 10×10^9 cells l⁻¹ in the surface up-welled waters. Preliminary scintillation counts were done on board the ship and a wide range of rates of bacterial activity was observed. Accurate counts will be done at the laboratory after adequate extraction of labelled material from filters. The molecular analysis will be also be done after the cruise. The dataset will allow us to estimate rates of bacterioplankton metabolic activity and production and to link bacterial function and composition with the hydro-physical structure of the water column.

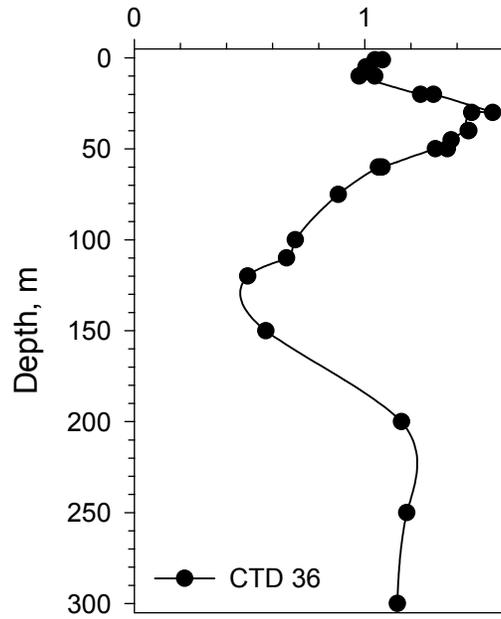
Table 13. Summary of samples collected for bacterioplankton analyses

Station	Date	Julian Day	CTD cast no. [all CD132..]	Depth range (m)	Abundance	Activity
1	3 Sep 01	246	001	1-300	24 depths	6 depths
			004	40-300	16 depths	-
2	4 Sep 01	247	006	1-300	21 depths	6 depths
	5 Sep 01	248	008	1-300	24 depths	6 depths
3	6 Sep 01	249	011	1-300	19 depths	-
			013	1-300	24 depths	2 depths
			015	1-300	24 depths	-
			016	1-300	24 depths	-
	7 Sep 01	250	017	1-300	24 depths	4 depths
			018	1-300	24 depths	-
8 Sep 01	251	019	1-170	12 depths	-	
4	9 Sep 01	252	021	1-300	24 depths	6 depths
			023	1-300	24 depths	-
			024	1-300	24 depths	6 depths
5	10 Sep 01	253	025	1-300	24 depths	6 depths
			028	1-300	24 depths	-
			029	1-300	24 depths	6 depths
6	12 Sep 01	255	030	1-300	24 depths	6 depths
			032	200-3000	14 depths	-
			034	1-2500	24 depths	6 depths
7	14 Sep 01	257	036	1-300	24 depths	6 depths
			039	1-300	16 depths	-
8	15 Sep 01	258	040	1-300	16 depths	5 depths
			041	1-300	16 depths	-
			042	1-130	12 depths	-
	17 Sep 01	260	044	1-300	24 depths	8 depths
			046	1-2000	23 depths	-
	19 Sep 01	262	047	1-300	24 depths	8 depths
9	20 Sep 01	263	048	1-300	24 depths	-
			050	90-1200	24 depths	10 depths
			051	1-300	24 depths	8 depths
10	22 Sep 01	265	052	1-300	24 depths	8 depths
			055	80-2000	24 depths	-
			056	1-300	24 depths	8 depths
11	23 Sep 01	266	057	1-300	24 depths	8 depths
			060	40-2000	24 depths	-
			061	1-92	21 depths	8 depths
26 Sep 01	269	062	1-92	22 depths	-	

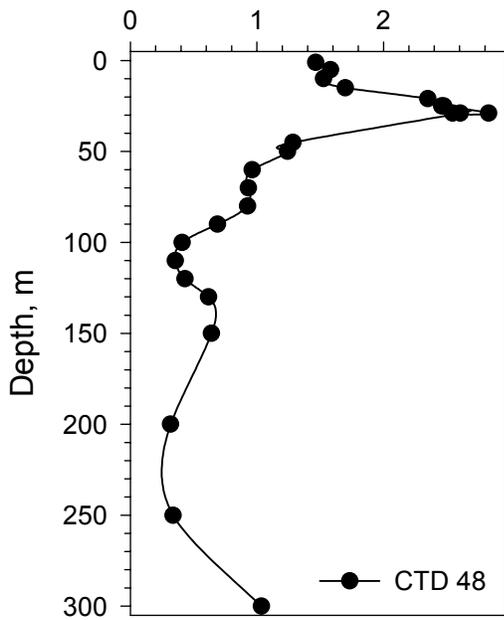
St. 2 Total bacteria, $\times 10^9 \text{ cells l}^{-1}$



St. 6 Total bacteria, $\times 10^9 \text{ cells l}^{-1}$



St. 8 Total bacteria, $\times 10^9 \text{ cells l}^{-1}$



St. 9 Total bacteria, $\times 10^9 \text{ cells l}^{-1}$

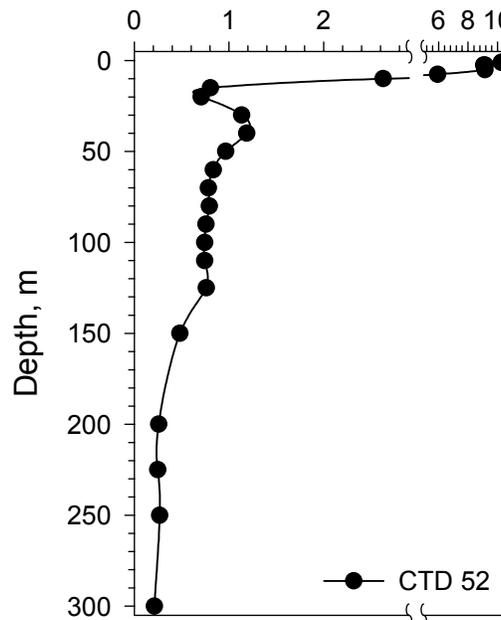
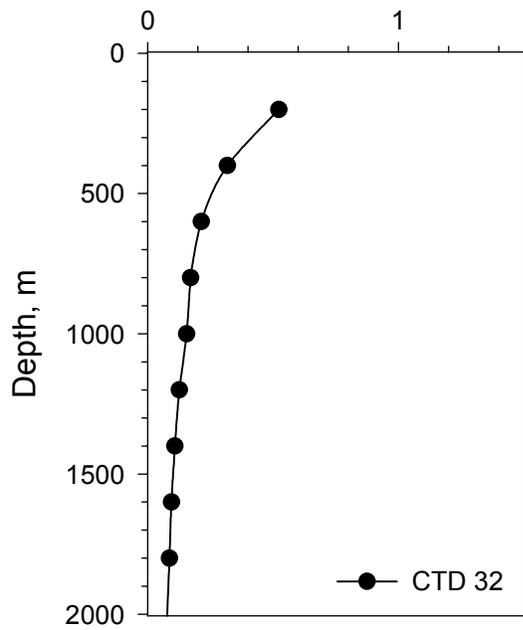
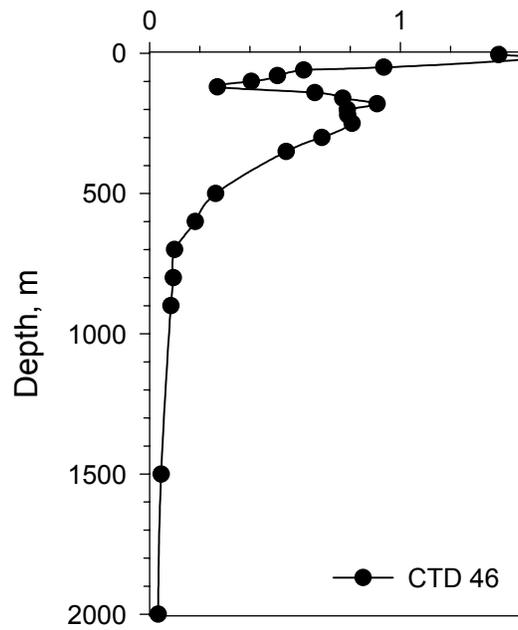


Fig 5. Vertical distribution of bacterioplankton in the top 300 m at contrasting stations.

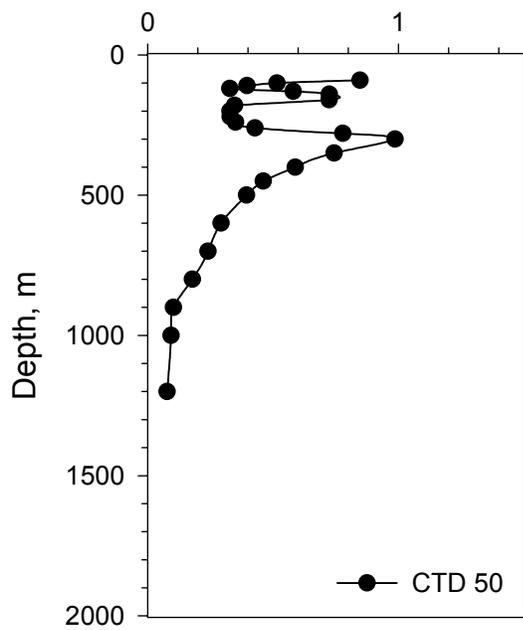
St. 5 Total bacteria, $\times 10^9 \text{ cells l}^{-1}$



St. 7 Total bacteria, $\times 10^9 \text{ cells l}^{-1}$



St. 8 Total bacteria, $\times 10^9 \text{ cells l}^{-1}$



St. 10 Total bacteria, $\times 10^9 \text{ cells l}^{-1}$

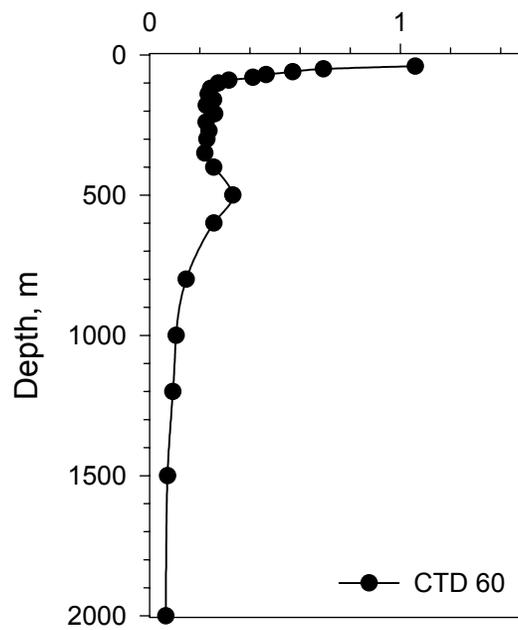


Fig 6. Vertical distribution of bacterioplankton to 2000 m at four stations.

7.12 CTD and other sensor packages (Jeff Benson)

CTD operations

A total of 65 CTD casts (including an initial test) were undertaken on the cruise. The general shallow (less than 500 m) cast configuration was as follows:

- Sea-Bird 9/11+ CTD system
- 24 by 20 litre Ocean Test Equipment water samplers
- OED Breakout Box
- Sea-Bird 43B Oxygen sensor
- Chelsea MKIII Aquatracka Fluorometer

- Benthos PSA-916T Altimeter
- Chelsea 2 pi PAR Irradiance sensors (Upwelling and Downwelling)
- SeaTech Light Scattering sensor
- SeaTech 20cm path Transmissometer

Deep cast configuration was the same with the exception of the removal of the irradiance sensors. The Sea-Bird CTD configuration was as follows:

- SBE 9+ Underwater unit s/n 09P-24680-0635
- Frequency 0 — SBE 3P Temperature sensor s/n 03P-4103 (primary)
- Frequency 1 — SBE 4C Conductivity sensor s/n 03P-2570 (primary)
- Frequency 2 — Digiquartz temperature compensated pressure sensor s/n 83007
- Frequency 3 — SBE 3P Temperature sensor s/n 03P-4116 (secondary)
- Frequency 4 — SBE 4C Conductivity sensor s/n 03P-2580 (secondary)
- SBE 5T submersible pump s/n 05T-3086
- SBE 5T submersible pump s/n 05T-3088
- SBE 32 Carousel 24 position pylon s/n 32-24680-0344
- SBE 11+ deck unit s/n 11P-19817-0495

The auxiliary A/D output channels were configured as below, for casts 000 through 021

- V0 SBE 43B Oxygen s/n 43B-0013
- V2 Benthos PSA-916T Altimeter s/n 875
- V3 Chelsea MKIII Aquatracka Fluorometer s/n 088243
- V4 Chelsea 2 pi PAR Irradiance sensor s/n 10 (UWIRR)
- V5 Chelsea 2 pi PAR Irradiance sensor s/n 5 (DWIRR)
- V6 SeaTech Light Scattering sensor s/n 339
- V7 SeaTech 20cm path Transmissometer s/n T-1017D

After cast 002, the cable for the transmissometer was changed from High Gain (0 to 100% range) to Low Gain (80 to 100% range) in order to reduce the temperature hysteresis exhibited by the instrument in waters 25C and higher. The SeaTech LSS gain used throughout the cruise was Full Range (0 to 33mg/l), as this instrument is not subject to temperature hysteresis.

For cast number 022 and onwards, V4 sensor UWIRR s/n 10 was replaced by sensor s/n 7, as the original sensor was exhibiting noise in the 0-5V output at depths of 300 to 400 metres.

Cast number 023 was deployed with a Chelsea Fast Repetition Rate Fluorometer (FRRF) added to the frame to observe if the FRRF analog outputs could be successfully integrated with the SBE CTD system. The one-off configuration was as below:

- V0 SBE 43B Oxygen s/n 43B-0013
- V1 Chelsea MKIII Aquatracka Fluorometer s/n 088243
- V2 Chelsea FRRF (F0) s/n 182042
- V3 Chelsea FRRF (Fm) s/n 182042
- V4 Chelsea 2 pi PAR Irradiance sensor s/n 7 (UWIRR)
- V5 Chelsea 2 pi PAR Irradiance sensor s/n 5 (DWIRR)
- V6 SeaTech Light Scattering sensor s/n 339
- V7 SeaTech 20cm path Transmissometer s/n T-1017D

Whilst the FRRF data was logged successfully, hauling/veering speeds for the winch were required to be 6 m/min or less through the chlorophyll distribution depths, and this adversely affects the quality of the SBE CTD data. (Ideal winch speed is near 60 m/min).

Casts 024 through 032 utilised either the shallow or deep configurations as in cast 022; from cast 033 for the duration of the cruise the Chelsea fluorometer was shifted from V3 to V1, as it was discovered that the Benthos altimeter had been introducing “spiking” into the data when the altimeter range shifted from full scale to a lesser value. The cause of this has not been determined, but it appears initially that the Chelsea fluorometer requires its own separate AUX output on the SBE when being used in high chlorophyll regions.

SurfMet

The SurfMet instrument configuration for the cruise was as follows:

- TSG: FSI OTM (housing) s/n 1401
- FSI OTM (remote) s/n 1334
- FSI OCM s/n 1353
- WETLabs Fluorometer s/n WS3S-246
- SeaTech 20cm path Transmissometer s/n T-1019D
- Met: Vaisala PTB100A barometric pressure s/n S3440012
- Vaisala HMP44L temperature/humidity s/n 018500L3
- Vaisala WAA anemometer s/n S45517
- Vaisala WAV wind vane s/n R05426
- Vaisala QLI50 sensor collector s/n R381006
- Didcot/ELE DRP-5 PAR sensor (port) s/n 5141
- Didcot/ELE DRP-5 PAR sensor (starboard) s/n 5145
- Kipp & Zonen TIR Pyranometer (port) s/n 973134
- Kipp & Zonen TIR Pyranometer (starboard) s/n 962276

All sensors performed without fault for the duration of the cruise; the Sea-Tech transmissometer and the WETLabs fluorometer required cleaning approximately every third day because of the build up of growth on the light sources, from the high productivity and warm temperatures of the sampled water. This was accomplished by back-flushing with fresh water and a dilute solution of Triton-X 100 biological growth inhibitor, thereby reducing the downtime of TSG system and eliminating the need for removal/reinstallation of sensors.

Fast Repetition Rate Fluorometer (FRRF)

A total of 6 casts were performed on the cruise, not including the above mentioned single CTD integrated cast. All profiles were conducted on the starboard side gantry using the Rexroth winch, spooled with 250 metres of 6mm diameter Kevlar rope. Both up and down cast data were recorded, lowering to 150 metres maximum depth and recovering to the surface at 6 metres per minute wire rate. The vessel was positioned starboard side to the sun, and if possible during the

high daylight hours of midday. All casts were internally recorded to Flash disk and then offloaded via binary transfer to a host computer. The default configuration file ([Appendix 2](#)) as provided by PML was used for all casts. Sensor configuration was as follows:

Chelsea FRRF s/n 182042
Chelsea 2pi PAR Irradiance sensor s/n 046060
Druck pressure sensor s/n 1265910 with PDM cable s/n 001

Moving Vessel Profiler (MVP)

A total number of 62 profiles were conducted during the cruise; this was the initial use of the instrument and consequently the trials cruise for the MVP. The sensor configuration was as below:

MVP300-1700 s/n 0101
AML Micro Sensor CTD s/n 7027
WETLabs Flash Lamp Fluorometer s/n FLF-362D
Sea-Bird/YSI 23-01Y Dissolved Oxygen sensor s/n 23-0960

A report on the deployment procedures and performance of the MVP is provided in [Appendix 3](#).

Miscellaneous

Salinometer. A Portasal 8410 salinometer was used on this cruise to process 48 TSG surface samples and 36 CTD water bottle samples. No problems with the salinometer or its performance were noted. The Portasal was located in the Constant Temperature laboratory and operated at 24C ambient temperature. All samples were processed according to WOCE standards and protocols.

Chernikoeff EM Log. During the first few days of the cruise, the EM Log repeatedly lost its calibration constants and failed to produce RS-232 data to the ship's logging system and to the bridge repeater display. Subsequent cycling of the power and re-entering of the calibration fields provided only temporary repairs on numerous occasions; the system was then cleared of all old calibration records and re-started successfully, and has had no reported operating problems since then. However, there is some question of the accuracy of the last recorded calibrations and it is recommended that a calibration exercise is conducted at the earliest convenient time.

EA500 Echo Sounder. The hull mounted and PES fish were both operated during the cruise; after the initial deployment of the PES fish it was discovered that a considerable length of fibre rope had fouled the inside and outside of the fish. There was no observable damage to any of the transducers, but a later measurement of the cable resistance provided less than satisfactory results. As a consequence only the hull transducers have been used since, with no reported problems. Replacement parts for the transducers on the PES fish have been arranged and will be installed at a later date.

The VMADCP and XBT system were not operated during this cruise.

7.13 Ocean Engineering Group report (Darren Young and Alan Sherring)

Mobilisation/demobilisation

Equipment and supplies were taken on board at Durban, South Africa over the period 17-20 August 2001. This mobilisation was reasonably straight forward, with various items of equipment being taken off the ship and put into shipping containers, and vice versa. The MVP was not

delivered until the afternoon of the 20 August, which resulted in a rather last minute rush to complete the build before sailing.

The main scientific party boarded at Victoria, Seychelles, on 29 August 2001.

All scientific staff and technicians disembarked on 29 September in Port Sultan Qaboos, Oman.

Scientific starboard gantry frame

Used continuously for the duration of the cruise. The cover plate on the main block had over time become more and more bent out of shape. At the beginning of the cruise the cover plate kept fouling the CTD wire. The Block was lashed out of the way for the remainder of the cruise. Wear was noted in the main pivot pins/bushes allowing the gantry to "rock" at its lower mounting points when in the stowed position.

Starboard gantry and aft AA@ Frame Power Pack

The Power Pack was used continuously throughout the cruise without problems.

CTD winch and wire

Used continuously during the cruise for the deployment of the CTD system. The existing termination was load tested at the beginning of the cruise. It was noted that during the deeper deployments a mechanical knocking noise was coming from the winch. This would appear to be caused by backlash between the scroll carriage and lead screw allowing the carriage to 'jump' occasionally.

Total number of CTD deployments:	64 + 1 test
Maximum depth:	3011 metres.
Maximum tension observed:	Unknown due to load cell fault.

Hydrographic winch and wire

Not used during the cruise. The winch was run up and greased at the end of the cruise.

Main winch Power Pack

Used extensively for the duration of the cruise without problems. A suction filter gauge was indicating two of the filters required replacing so all hydraulic oil filters on the powerpack were replaced during the cruise. Oil level was topped up at the end of the cruise.

Main winch controls, associated electrics and Clam monitoring system

Only the starboard winch control console was used during the cruise. During CTD cast 016, the wire tension readout failed jumping from 1T to 7T. This was traced to a fault in the gantry sheave load cell which showed different electrical resistance readings across the connecting wires compared to a new unit. As all CTD deployments were shallow and the sea state calm, it was known the loads imposed on the wire would be well within the maximum allowable levels. It was therefore decided not to attempt to remove the load cell during the cruise with the risk of interrupting the science program.

It is understood the CTD winch is not required until after the refit. It is therefore intended to replace the load cell during refit.

The alarm buzzer failed midway through the cruise. This would appear to be a fault in the buzzer itself as the alarm within the main lab is still functioning. No spare was found on board.

Rexroth winch starboard gantry

Fitted with a new 250 m Vectron rope at the beginning of the cruise. Used for the deployment of SAPs, Go Flos, plankton nets & a fluorescence meter. No problems were encountered with the operation of the winch. Depth of deployment was gauged by placing tape marks on the rope. This was OK for depths of up to 50 m but for deeper deployments, particularly with the fluorescence meter, a "lightweight" electronic counting sheave would have made the job much easier. Slight leak on the winch main block, pressure setting valve, 'O' ring changed.

Effer cranes and Power Packs

Used only for mobilisation/demobilisation. No problems encountered.

PES davit and Power Pack

Deployed for a period at the start of the cruise. No problems encountered.

Non-toxic sea water system

Used extensively throughout the cruise for surface water sampling and to provide cooling water for incubation tanks, the two container A/Cs and MVP. Two pumps were run due to the high water usage. No problems encountered.

Scientific workshop and equipment

The workshop A/C failed several times during the cruise. This was attended to by the ship's engineers.

OED pool scientific equipment

Equipment used comprised: -

- Clean Chemistry Container
- Radio Nuclide Container
- Millipore water purifiers (2 No)
- Sensair 20 Fume Cupboard
- CTD
- SAPs (2 No)
- Go Flo Bottles
- Moving Vehicle profiler. See Instrumentation group report, [Appendix 3](#).

Non-OED scientific equipment

Plankton nets.

Items for repair/replacement/defects reported

Repair to the cover plate on the starboard gantry main block. To be done during the refit.

Secure materials storage locker hinges require replacing. To be done during refit.

The CTD load cell to be replaced and calibrated during refit.

The starboard winch station CLAM monitoring system alarm buzzer requires replacing.

Starboard gantry main pivot pins/bushes to be inspected/replaced during refit.

Spares and Stores required/used

Spare replacement main winch p/pack hydraulic oil filters :

4 No Ikron Pt No HEK 45-30.155-AS-SP010
1 No Filtrec Pt No A-1-10-C10

7.14 Computing (Paul Duncan)

During the cruise the ABC computer system was run to collect data from the following instruments/devices:

Chernikeeff log
Ship's gyro
Simrad EA-500 Echo sounder
Ashtech GG-24 combined GPS and GLONASS receiver
Ashtech ADU attitude GPS
Trimble 4000DL GPS receiver
OED Surfmet system
OED Winch system (aka "CLAM")

The gyro and log were processed into relative motion, so that this would be available for dead reckoning, should there be periods when GPS was not available (this has been observed in high northerly latitudes due to auroras). No such periods were observed during this cruise.

The bathymetry data from the echo sounder was corrected for Carter Area, and the temperature and conductivity data from the Surfmet system were combined to derive salinity data.

In addition to the ABC system logging data, two other systems produced data. The Seabird CTD system had its data "walknetted" over to the translation PC where processing and production of plots and bottle data took place. The MVP towed CTD system also logged data, but the PC was connected to the ship's network at the beginning of the cruise, and some disk space on the data server "darwin3" was made available for storage of the data. The open source "Samba" package is used in order to make the "darwin2" system look like a Windows NT server to other systems on the network, and this was used to export the data area so that the MVP system could log data directly to the Unix system's disk. The MVP manufacturers (Brooke Ocean Technology) e-mailed out a post processing package for the MVP data (in fact two different versions were eventually mailed out), and this was installed and run on the computer room translation PC, which could also access the MVP data over the network. Once processed, the data was used by Malcolm Woodward in the "Surfer" contouring package.

Nightly backups were made of the Level C, Seabird and MVP data onto 70GB DLT tapes.

Numerous plots of CTD profiles were produced for scientists and data was also made available to them, on floppy disk, Zip disk and towards the end of the cruise on CD-ROM.

Although not a requirement of the cruise, several satellite images received by the Dartcom system were processed, and some were displayed in the laboratory.

8. ACKNOWLEDGEMENTS

AMBITION was a fun cruise, doing fun science in a fun ocean - but at a difficult time after 11 September. It is a pleasure to thank everyone on board who made the cruise possible, and for their professional commitment to getting the job done. This was achieved through the collective efforts of the scientific party, technical support (very ably led by Jeff Benson), Captain Keith Avery, and other members of the ship's complement, who all rose to the occasion and ensured that the research was able to continue.

I also wish to recognise the role of many other people working "behind the scenes". In particular to Julia Crocker, who (together with logistics supremo Malcolm Woodward) did a superb job in managing pre-cruise and post-cruise administration - to satisfy many new H&S requirements - and to Andy Louch and his RSU team. Thanks also to Nick Latta (British Embassy, Muscat), Dr Al Mazrooei and Dr Phil Williamson, who were all able to welcome us back to shore. The message from Professor John Lawton delivered to the workshop on 30 September in Muscat showed the importance attached to our work, and our safety, by NERC. Finally, my thanks go to the M&FMB Steering Committee who provided us with the funds to do some great and inspirational science.

Peter Burkill

Appendix 1: Samples collected for nucleic acid analysis to detect nitrogen-fixation

(Mike Wyman and Clare Bird)

Station/ deployment	Date	Local time	Sampling gear	Position	Depth (m)	Vol. (litre)	Pore size (µm)	Storage
00/01	1 Sep 01	1100	test CTD	02 26' S 59 22' E	100	5	0.2	DIB
	1	4.5		
00/02	2 Sep 01	1500	SAP	01 25' S 62 30' E	20	96	0.2	DIB
01/03	3 Sep 01	0810	CTD 002	00 54' S 64 98' E	150	4-6	0.2	RNAlater
	74	4-6	0.2	
	50	4-6	0.2	
	25	4-6	0.2	
01/04	3 Sep 01	0940	CTD 003	00 55' S 64 07' E	35	4-6	0.2	RNAlater
	10	4-6	0.2	
01/08	3 Sep 01	1354	SAP	00 54' S 64 08' E	30	96	0.2	RNAlater
	..	1500	Net tow		30			RNAlater
01/09	3 Sep 01	1530	Go Flo	00 55' S 64 08' E	10	13	0.6	
	70	20	0.6	
01/11	4 Sep 01	0902	CTD 007	00 54'S 64 88'E	300	5	0.2	DIB
	150	5	0.2	
	75	5	0.2	
	50	5	0.2	
	35	5	0.2	
	25	5	0.2	
	10	5	0.2	
	5	5	0.2	
02/03	5 Sep 01	0632	CTD 009	00 00'N 66 59' E	150	5	0.2	DIB
	100	5	0.2	
	80	5	0.2	
	50	5	0.2	
02/04	5 Sep 01	0815	CTD 010	00 00'S 66 59' E	70	5	0.2	DIB
	40	5	0.2	
	25	5	0.2	
	10	5	0.2	
02/07	5 Sep 01		SAP	00 00' S 67 08' E	45	177	0.2	RNAlater
02/08	5 Sep 01		Net tow	00 01'S 67 00'E	10		20	RNAlater
02/09	5 Sep 01		Go Flo		20	15	2 and 0.6	
02/11	6 Sep 01	0801	CTD 014	00 00' S 66 59' E	310	5	0.2	
	150	5	0.2	
	70	5	0.2	
	50	5	0.2	
	35	5	0.2	
	10	5	0.2	
DIEL St. 3	7 Sep 01	1500	Non-toxic	16 03' N 66 59' E		9	2 and 0.6	RNAlater
	..	1630		9	2 and 0.6	
	..	2220		9	2 and 0.6	
	..	2015		9	2 and 0.6	
	..	2205		9	2 and 0.6	
	(8 Sep 01)	0005		9	2 and 0.6	
	..	0200		9	2 and 0.6	

Appendix 1 - continued

Station/ deployment	Date	Local time	Sampling gear	Position	Depth (m)	Vol. (litre)	Pore size (μm)	Storage
	..	0600		9	2 and 0.6	
	..	0845		9	2 and 0.6	
	..	1045		9	2 and 0.6	
	..	1300		9	2 and 0.6	
03/01	7 Sep 01	1210	CTD 015	03 48' N 67 80' E	140	5	0.2	DIB
	110	5	0.2	
	70	5	0.2	
	45	5	0.2	
	25	5	0.2	
	10	5	0.2	
03/02	7 Sep 01	1805	CTD 016	3 48' N 67 00' E	140	5	0.2	DIB
	120	5	0.2	
	79	5	0.2	
	45	5	0.2	
	25	5	0.2	
	10	5	0.2	
03/04	8 Sep 01	0004	CTD 018	3 57' N 67 00' E	115	5	0.2	DIB
	71	5	0.2	
	45	5	0.2	
	25	5	0.2	
03/05	8 Sep 01	0600	CTD 019	03 48' N 67 00' E	115	5	0.2	DIB
	70	5	0.2	
	45	5	0.2	
	25	5	0.2	
03/07	8 Sep 01		SAP	03 47' N 66 59' E	20	85	0.2	RNAlater
	..		Net tow		5		2	RNAlater
03/11	9 Sep 01	0630	CTD 022	3 47' N 66 59' E	120	5	0.2	RNAlater
	74	5	0.2	
	40	5	0.2	
	10	5	0.2	
03/12	9 Sep 01	0845	SAP	03 46' N 66 59' E	120	118	0.2	RNAlater
04/02	10 Sep 01	1612	SAP	07 35' N 67 00' E	20	200	0.2	RNAlater
04/03	10 Sep 01	1510	Net tow	07 36' N 67 00' E	5		2	RNAlater
04/04	10 Sep 01		Go Flo	07 36' N 67 00' E	60		2x 0.2	DIB
04/08	11 Sep 01	0629	CTD 026	07 36' N 67 00' E	150	5	0.2	DIB
	76	5	0.2	
	25	5	0.2	
	10	5	0.2	
04/09	11 Sep 01	0811	CTD 027	07 35' N 67 01' E	219	5	0.2	DIB
	100	5	0.2	
	40	5	0.2	
	..	1050	Net tow	07 36' N 67 03' E	10		2	RNAlater
05/02	12 Sep 01	1505	SAP	11 23' N 66 59' E	15	59	0.2	RNAlater
05/03	12 Sep 01		Net tow	11 22' N 66 58' E	10		2	No Tricho.
05/04	12 Sep 01		SAP for AR					

Appendix 1 - continued

Station/ deployment	Date	Local time	Sampling gear	Position	Depth (m)	Vol. (litre)	Pore size (µm)	Storage
05/08	13 Sep 01	0630	CTD 031	11 24' N 67 00' E	150	5	0.2	DIB
	75	5	0.2	
	50	5	0.2	
05/09	13 Sep 01	0810	CTD 032	11 27' N 67 00' E	35	5	0.2	DIB
	25	5	0.2	
	10	5	0.2	
DIEL St. 6	14 Sep 01	1238	non-toxic	15 12' N 67 00' E	5	8	2 and 0.6	RNAlater
	..	1420	5	8	2 and 0.6	
	..	1638	5	8	2 and 0.6	
	..	1835	5	8	2 and 0.6	
	..	2030	5	8	2 and 0.6	
	..	2245	5	8	2 and 0.6	
	(15 Sep 01)	0229	5	8	2 and 0.6	
	..	0446	5	8	2 and 0.6	
	..	0600	5	8	2 and 0.6	
	..	0838	5	8	2 and 0.6	
	..	0705	5	8	2 and 0.6	
06/03	14 Sep 01	1515	SAP	15 11' N 66 59' E	15	100	0.2	RNAlater
06/04	14 Sep 01	1625	Net tow		20		2	No Tricho.
06/05	14 Sep 01	1713	Go Flo	15 11' N 66 59' E	35	15		RNAlater
06/09	15 Sep 01	0655	CTD 037	15 11' N 66 59' E	200	5	0.2	DIB
	140	5	0.2	
	44	5	0.2	
	25	5	0.2	
06/10	15 Sep 01	0848	CTD 038	15 11' N 67 00' E	67	5	0.2	
	10	5	0.2	
07/03	16 Sep 01	1203	CTD 039	19 00' N 67 00' E	130	5	0.2	RNAlater
	80	5	0.2	
	47	5	0.2	
	25	5	0.2	
07/04	16 Sep 01	1804	CTD 040	19 00' N 67 00' E	130	5	0.2	RNAlater
	80	5	0.2	
	50	5	0.2	
	25	5	0.2	
07/05	17 Sep 01	0004	CTD 041	19 00' N 67 00' E	130	5	0.2	RNAlater
	80	5	0.2	
	50	5	0.2	
	25	5	0.2	
07/06	17 Sep 01	0601	CTD 042	18 59' N 67 00' E	130	5	0.2	RNAlater
	80	5	0.2	
	52	5	0.2	
	25	5	0.2	
07/07	17 Sep 01	1205	CTD 043	18 59' N 64 59' E	130	5	0.2	RNAlater
	80	5	0.2	
	50	5	0.2	
	25	5	0.2	

Appendix 1 - continued

Station/ deployment	Date	Local time	Sampling gear	Position	Depth (m)	Vol. (litre)	Pore size (μm)	Storage
	10	5	0.2	
07/08	17 Sep 01	1410	SAP	19 00' N 66 59' E	220	106	0.2	RNAlater
07/10	17 Sep 01	1605	Go Flo	19 00' N 66 59' E	50		2 and 0.6	RNAlater
07/12	18 Sep 01	0628	CTD 045	19 00' N 67 00' E	60	5	2 and 0.6	RNAlater
	49	5	2 and 0.6	
	35	5	2 and 0.6	
	25	5	2 and 0.6	
	10	5	2 and 0.6	
	5	5	2 and 0.6	
DIEL St. 8	19 Sep 01	1015	non-toxic	20 54' N 63 39' E		8	2 and 0.6	RNAlater
	..	1225		8	2 and 0.6	
	..	1455		8	2 and 0.6	
	..	1645		8	2 and 0.6	
	..	1830		8	2 and 0.6	
	..	2050		8	2 and 0.6	
	..	2300		8	2 and 0.6	
	(20 Sep 01)	0303		8	2 and 0.6	
	..	0125		8	2 and 0.6	
	..	0300		8	2 and 0.6	
	..	0545		8	2 and 0.6	
	..	0730		8	2 and 0.6	
08/02	19 Sep 01	1420	SAP (Old)		20	797	0.2	RNAlater
	..		SAP (new)		5	47	0.2	RNAlater
08/08	20 Sep 01	0818	SAP	20 55' N 63 39' E	28	36	0.2	RNAlater
09/03	22 Sep 01	1602	SAP (new)	23 34 N 59 53' E	5		0.2	RNAlater
09/03	22 Sep 01	1602	SAP (old)	23 34 N 59 53' E	20	286	0.2	RNAlater
DIEL St. 9	22 Sep 01	1445	Non toxic	23 33' N 59 54' E			2 and 0.6	RNAlater
	..	1620		1	2 and 0.6	
	..	1710		1	2 and 0.6	
	..	1840		1	2 and 0.6	
	..	2015		1	2 and 0.6	
	..	2220		1	2 and 0.6	
	..	2400		1	2 and 0.6	
	(23 Sep 01)	0200		1	2 and 0.6	
	..	0400		1	2 and 0.6	
	..	0615		1	2 and 0.6	
	..	0805		1	2 and 0.6	
	..	1015		1	2 and 0.6	
	..	1137		1	2 and 0.6	
	..	1315		1	2 and 0.6	
	..	1415		1	2 and 0.6	
09/07	23 Sep 01	0629	CTD 053	23 37' N 59 54' E	150	5	0.2	DIB
	30	5	0.2	
	1	5	0.2	
09/08	23 Sep 01	0825	CTD 054	23 32' N 59 53' E	200	5	0.2	
09/10	23 Sep 01	1300	SAP	23 33' N 59 54' E	2	513	2	RNAlater
10/03	24 Sep 01	1414	SAP	2419' N 58 09' E	5	740	0.2	sample not retained
10/05	24 Sep 01	..	Net tow	24 19' N 58 09' E	5		2	No Tricho.

Appendix 1 - continued

Station/ deployment	Date	Local time	Sampling gear	Position	Depth (m)	Vol. (litre)	Pore size (μm)	Storage
10/08	25 Sep 01	0636	CTD 058	24 19' N 58 10' E	120	5	0.2	DIB
	74	5	0.2	
	27	5	0.2	
	10	5	0.2	
10/09	25 Sep 01	0831	CTD 059	24 19' N 58 70' E	1	4.5	0.2	
10/10	25 Sep 01	1331	SAP	24 20' N 58 10' E	5	181	0.2	RNAlater
11/05	26 Sep 01		Net tow	26 00' N 56 35' E	5		20	No Tricho.
11/06	..		Go Flo	26 00' N 56 35' E	2		2 and 0.6	RNAlater
11/08	27 Sep 01	0633	CTD 063	24 00' N 56 31' E	27	4	0.2	DIB
	57	4	0.2	
	70	4	0.2	
11/09	27 Sep 01	0805	CTD 064	26 01' N 56 35' E	10	4	0.2	DIB
11/10	27 Sep 01	1004	SAP	26 02' N 56 34' E	30		2	RNAlater

Appendix 2: Fast Repetition Rate Fluorometer (FRRF) deployment log

1. File 252055157 (9 Sept at 05:51:57 GMT)
On CTD frame, integrated with Sea-Bird
Cast number CD132_023
Station number 03/13
Offloaded as file CD132252.FRF

2. File 253124543 (10 Sept at 12:45:43 GMT)
On Kevlar line
Station number 04/05a (not logged on bridge as separate event)
Offloaded as file CD132253.FRF

3. File 259053644 (16 Sept at 05:36:44 GMT)
On Kevlar line
Station number 07/01
Offloaded as file CD132259.FRF

4. File 262055905 (19 Sept at 05:59:05 GMT)
On Kevlar line
Station number 08/02
Offloaded as file CD132262.FRF

5. File 265062843 (22 Sept at 06:28:43 GMT)
On Kevlar line
Station number 09/01
Offloaded as file CD132265.FRF

6. File 267054839 (24 Sept at 05:48:39 GMT)
On Kevlar line
Station number 10/01
Offloaded as file CD132267.FRF

7. File 269055932 (26 Sept at 05:59:33 GMT)
On Kevlar line
Station number 11/01
Offloaded as file CD132269.FRF

Appendix 3: Moving Vessel Profiler (MVP) deployment and performance

(Jeff Benson, Darren Young, Alan Sherring & Malcolm Woodward)

Deployment/recovery and operations on CD132

Most profiles were done once the ship departed station, during daylight hours, in calm sea conditions (Force 1 or 2), lasting 3 to 4 hours, at maximum ship speed. We completed one overnight tow, consisting of 24 profiles. If tows are at the maximum speed of the Darwin of 12 knots, then total depth of the profile is limited to 300 m; deeper depths of up to 1700 m can be reached by slowing the ship down to 1 knot, with various depths in between depending upon vessel speed. The Auto Deployment works well, in that profiles can be done on a timed basis, freeing up personnel to do other tasks. We used the Auto mode for anywhere from 5 to 20 minute intervals between profiles.

The fish itself is easy to handle physically, and the winch is not particularly sensitive nor difficult to operate by inexperienced personnel. In reasonable sea states (Force 3 to 5), deployments should be able to proceed with the vessel moving ahead at 4 plus knots, although without having those actual weather conditions on CD132 this has not been tested. The timing of the Auto Deploy profiles can be arranged around the schedule, weather conditions, cruise track and personnel available very easily.

The faster the vessel speed, the more cable the winch pays out on the downcast, and coupled with the deeper cast profiles, the longer it takes to complete a full up/down profile. For example, at 10 knots attempting a 300 m cast, approximately 650 m of cable is paid out at 7 to 9 m per second; hauling in at 1.5 m per second on average means that the entire profile lasts 15 minutes. The downcast data only can be recorded to minimise data logging, or as many as 9 out of 10 scans can be “skipped” on the upcast (25 Hz raw data rate). If only one profile is required every 10 nautical miles, then the Auto Deploy can be set to profile once per hour, and personnel can be performing other duties.

The fish is towed at a pre-determined depth at the surface between casts; usually at 1 to 2 m in calm sea states. (The surface fish depth is set by the location of the Docking Messenger prior to deployment in profiling mode.) The Auto Deploy feature will not begin until the operator has confirmed the correct Log File at the end of the set time period between profiles, thus the operator has to check on the system prior to each cast, or the fish will simply tow along at the surface. If profiling is over an area of shallow water, then the MVP system must be receiving good quality bottom data, to prevent the profiles from aborting and the fish being brought to the surface unbeknownst to the operator. (The software does provide the facility to increase the time period without an update from the EA500 to practically any amount of time; however we recommend the “timeout” be no longer than 10 seconds to avoid crashing into the seabed.) Similar time periods without updates can be applied to the GPS data as well, again to prevent unneeded cast aborts.

Recovery has been done in calm sea states at 4 to 5 knots ahead without problems, as the fish is very hydrodynamic and does not “plough” the water surface with the subsequent drag that other undulating profilers have.

Personnel required

As noted above, much depends upon the frequency of deployments once the fish is in the water. In calm weather, two people can easily get the fish into the water and/or back onboard, one for the winch and one to handle the fish. In rougher sea states, an additional two people to help with tag lines would be advisable; one OED person can successfully deploy the instrument with help from the scientific party and/or deck crew. An additional OED mechanical technician would be recommended for heavy use, to do the daily and weekly maintenance and checks of the winch system, otherwise the operator can check the hydraulic oil reservoir, cooling water flow, temperature, leakage, etc. whilst the fish is profiling.

Periodic checks of the cable for wear, fouling and damage, paying especial attention to the messengers and limit switches, is a normal part of any profiling schedule, and can be done whilst the fish is in any part of the deployment/recovery cycle; being “glued” to the MVP Controller monitor is not necessary. The various fasteners, cable termination, towing bridle and shackle should be inspected upon each recovery for wear, vibration induced loosening and damage. We recommend that during profiling, a minimum of one person be designated to handle the Auto Deployments, and monitor the physical state of the system; whenever the fish is in the water it is not a walk-away-and-leave-it-alone instrument.

Software

The manufacturer supplied software is very friendly, although some knowledge of Labview is helpful. The Configuration files are simple and straightforward to set up for any deployment criteria, and the Manual Control program for diagnostics is quite useful also. Technical and software support is easily and promptly obtained via e-mail, and assistance for troubleshooting is readily available. Direct logging of raw data to the vessel’s computer system has been trouble free, and the software produces printable graphs at the end of each profile. Post-processing software is in development; although the beta version has been successfully used with Surfer to obtain contour plots of five parameters over depth and distance covered in nautical miles.

Spares and miscellaneous items

Several needs come to mind; firstly the instrumentation and all small spares were shipped in wooden or cardboard boxes, so aluminium boxes for shipping will be requested for future cruises. A bolt-down metal cradle for securing the fish next to the winch on deck is needed to prevent damage in rough seas. A shock resistant electronics rack would be helpful for the Controller system and monitor. As the system was manufactured in Canada, a small set of Imperial tools, as well as a complete set of Imperial spare fasteners, is requested. Spare cables, replacement temperature probes and conductivity cells, an oxygen sensor and a fluorometer should be considered in the future. Calibration costs need to be taken into account also. The option of installing different sensors, such as irradiance, transmissometer, etc. should be included in any plans for future purchases. Lastly, a remote display for the winch controls on deck is an item that would be helpful on larger vessels where the distance from the lab and Controller to the winch can be considerable.

Observations and problems

The system is still in the shakedown stage, as several problems came to light during the overnight tow. One item is the loss of communication to the fish and/or Controller, which occurred frequently enough for us to question the termination. Upon further inspection, it was discovered that the Impulse cable splice that joins the Kevlar EM cable to the fish has an open circuit. This could have been caused by incorrectly routing the cable splice into the fish, or vibration/strumming of the tow cable. We have asked BOT for advice on preventing what is seen as premature loss of the termination integrity; their recommendation is to tape the splice and termination to the entire length of the towing bridle. This allows just enough freedom of movement for the bridle to pivot, and enough for the towing shackle to handle slack cable. The MVP was used for approximately 16 hours only prior to the overnight tow; this should not be deemed “heavy” use and one would think the termination would survive longer, especially in the calm sea states we have had. Also, the Docking Messenger seems to need a lot of maintenance; we have used the electrical tape recommended by BOT for securing the Messenger but the tape still unwinds. Additionally, the Outer Boom Switch does not always detect the Docking Messenger, or gives “false” Docking signals. The switch itself will need further attention.

Appendix 4: "AMBITION in the front line" – message to the Challenger Society

Cruises are exciting, none more so than when you start by spending a few days relaxing in the Seychelles before the cruise. Planning the cruise had started more than a year earlier with a myriad of forms, correspondence and meetings addressing who wanted to do what, where, when and how. Our cruise, which we termed AMBITION (Analysing the Microbial Biodiversity of the Indian Ocean), gradually took shape and the requirement for 28 berths (requested) was whittled down to 18 (provided). AMBITION serves as the major field component of the NERC Marine & Freshwater Microbial Biodiversity programme. So AMBITION evolved as a federated set of five research teams funded by M&FMB and supported by the Plymouth Marine Laboratory (PML). A common requirement of these teams was to work in a wide range of different marine environments. Nowhere offers this better than the Arabian Sea, after the southwest monsoon has blown itself out in September. At that time, the strong coastal upwelling forced by the monsoon blowing off Oman has injected massive quantities of nutrients into surface waters. These nutrients fertilise the ocean, which responds with high levels of biological production. In contrast, some 800 km away in the central Arabian Sea, the surface waters are unaffected by the monsoon and remain calm throughout the year, with nutrient levels 3 orders of magnitude lower and nitrate present at the nanomolar level. From previous experience in this basin, we knew that the phytoplankton communities differ radically in these two environments. Large diatoms and dinoflagellates dominate the high nutrient waters while sub-micron sized cyanobacteria dominate waters that are nutrient sparse. This allowed us to set up the cruise to address two questions: What mechanisms cause the phytoplankton community to vary so radically? Does the remainder of the microbial community of bacteria, viruses and protists vary in tune with the phytoplankton? As well as the gradients of nutrients and biological production, other gradients exist in this ocean. Much of the phytoplankton production sinks out of the surface waters and the organic matter is degraded by microbial respiration. This is so intense that the water at depths of between 100 and 2000 metres has oxygen content only a few percent saturated. Thus a gradient exists between the fully oxygenated surface waters and the underlying deoxygenated waters.

To investigate the microbial diversity on these gradients, we are occupying a series of stations along the main axis of low/high production. At each station, colleagues from PML quantify nutrient levels (Malcolm Woodward), phytoplankton composition (Glen Tarran), photopigments (Denise Cummings), and primary and new production (Gwyn Morgan and Andy Rees). This characterisation involves obtaining water before dawn (typically 04:00) with a CTD water bottle system and running a series of analyses or experiments over the day. Microbial biodiversity work involves molecular biology studies carried out by the M&FMB-funded teams. Their investigations focus on bacterial community structure and function (Mike Zubkov, PML), eukaryote picoplankton (Nick Fuller & Karen Orcutt, Warwick), nitrogen fixers (Mike Wyman & Clare Bird, Stirling), microbes that metabolise methyl bromide (Mike Cox, Warwick/PML) and bacteria that cannot normally be cultured (Kevin Ashelford, Cardiff). Further samples are obtained during the day to fuel our research until we wind down around 17:00. It's a long day, but we normally have time for some fishing before we turn in. On one station, we caught a dozen tuna each weighing over 10 lbs. Fresh fish was on the menu the next day!

In the middle of the cruise, we listened not really believing the rapidly evolving news reports on the tragic happenings of planes crashing into the World Trade Centre in New York. We normally feel cushioned onboard, insulated from the worlds' affairs. However, over the last few weeks, the ramping up of US military might has become all too familiar to us. Battle grey frigates, supply ships and the occasional aircraft-carrier all sporting the 'stars and stripes' occupy the same waters as we work in the northern Arabian Sea. Our concerns, and those of our friends and families, with whom we have regular email contact, are whether we'll be able to finish our work as planned in Oman. Or whether we'll be advised to leave what is rapidly becoming a front line in this escalating international situation. We're told that the escape route is to high tail it back to the Seychelles. Cruises are exciting - for all sorts of different reasons.

Peter Burkill (Principal Scientist) aboard *RRS Charles Darwin* in the Arabian Sea, 27 Sept 2001

Appendix 5: Programme for post-cruise Workshop, Muscat

Workshop hosted by Dr Al Mazrooei (Director, Marine Fisheries Centre) and held at the Ministry of Fisheries, Muscat, 30 September 2001. It was attended by cruise participants and around 50 Omani researchers, policy administrators and other individuals.

Time	Title	Speaker
<i>Background and Introductions</i>		
10:00	Marine Science in Oman	Ahmed Al-Mazrooei
10:15	Ecological Studies in Oman Waters	Dr. Thangaraja
10:30	Marine Science in UK and the Marine & Freshwater Microbial Biodiversity Programme	Phil Williamson
10:45	Cruise CD 132 (AMBITION)	Peter Burkill
<i>Studies of microbial biogeochemistry in the Gulf of Oman and the Arabian Sea</i>		
11:00	Nutrient biogeochemistry	Malcolm Woodward
11:15	Phytoplankton production	Andy Rees
11:30	Nitrogen fixation	Mike Wyman
12:15	Phytoplankton Communities	Glen Tarran
12:30	Bacterioplankton Communities	Mike Zubkov
<i>Enabling platforms</i>		
12:45	Marine Technology	Captain Keith Avery
13:00	RRS Charles Darwin	Captain Keith Avery
13:15	General Discussion	All
13:30	Conclusion	

Appendix 6: Photo gallery of AMBITION scientists at work



Peter Burkill
Principal Scientist



Kevin Ashelford



Clare Bird



Mike Cox



Denise Cummings



Nick Fuller



Gwyn Morgan



Karen Orcutt



Andy Rees



Hilal Al-Shaqsi
Omani Observer



Glen Tarran



Malcolm Woodward



Mike Wyman



Mike Zubkov



Jeff Benson



Darren Young



Alan Sherring



Paul Duncan

Appendix 7: Updated contact information for scientific and technical cruise participants (Oct 2002)

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Gwyn Morgan	<i>Contact on science issues via Mike Zubkov</i>	
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