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NATURAL ENVIRONMENT RESEARCH COUNCIL
INSTITUTE FOR MARINE ENVIRONMENTAL RESEARCH

CHARLES DARWIN 13/86

APPENDIX TO ROSCOP FORM

1) A chart is attached which shows the position of the station occupied.

2) A cruise report will be prepared within the next two weeks which will list the experiments done on the cruise. Detailed data from these experiments should be available in early 1987.

3) All cruise objectives were achieved. A single station was occupied from 17 May until 8 June and the changes in the populations of picoplankton, nanoplankton, and microplankton were followed. The development of a stabilised water column was followed by CTD profiling at least 3 times each day; in addition to CTD data, measurements were made with in vivo fluorometers, transmissometers and quantum sensors. The following experiments were done throughout the cruise; primary production by in situ incubations, determination of photosynthetic characteristics of different size fractions of phytoplankton; the biomass and production of heterotrophic bacteria; biomass and production of microzooplankton; precision oxygen determinations of photosynthesis and respiration of different size fractions of natural populations, novel methods were developed to distinguish marine cyanobacteria from other phytoplankton, and the NERC Analytical Flow Cytometer was used successfully for the first time at sea.

Indexed

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NATURAL ENVIRONMENT RESEARCH COUNCIL

INSTITUTE FOR MARINE ENVIRONMENTAL RESEARCH

CRUISE REPORT

File No VES 10.1
RVS Ref CD 13/86

VESSEL RRS CHARLES DARWIN

PERIOD 17 May to 9 June 1986

PERSONNEL

Whole cruise period

Dr Ian Joint	IMER (UK)
Dr Mike Fasham	IOS (UK)
Dr Peter Burkill	IMER (UK)
Dr Richard Geider	Birmingham (US)
Dr Duncan Purdie	Southampton (UK)
Dr Mike Wyman	Warwick (UK)
Brian Irwin	Bedford Institute (Canada)
Carla Caverhill	Bedford Institute (Canada)
John Taylor	RVS (UK)
Richard Phipps	RVS (UK)
Robin Powell	RVS (UK)
Tony Robinson	RVS (UK)

Leg I: 17 May to 28 May 1986

Dr Barry Leadbeater	Birmingham (UK)
Dr Trevor Platt	Bedford Institute (Canada)
Dr Roy Ricketts	Nottingham (UK)
Prof. Michael Sleigh	Southampton (UK)
Philip Boyd	Belfast (UK)
Omar Calvario-Martinez	UCNW (Mexico)

Leg II: 28 May to 9 June 1986

Graham Dixon	Swansea/MBA (UK)
Alison Edwards	Birmingham/MBA (UK)
Katharine Howard	Warwick/IMER (UK)
Ray Leakey	Southampton/IMER (UK)
Dr Alain Vezina	Bedford Institute (Canada)
Vanessa Walls	Swansea (UK)

ITINERARY

Sat 17 May Sailed Falmouth 1500.

Sun 18 May On station CS2 (50° 30'N: 07° 00'W) at 0440. Began sampling programme at 0830 (See Appendix for times of sampling)

Wed 21 May Left CS2, set course for Falmouth at 1700.

Thurs 22 May 0900 Anchored off Falmouth: picked up Coulter

Electronics engineer to repair Analytical Flow Cytometer (AFC). 1700 test AFC whilst ship steaming; 2045 engineer transferred to launch and set course for station CS2.

Fri 23 May 0800 continued sampling programme at station CS2.

Tues 27 May 1100 sampling halted because of deteriorating weather conditions. 1400 set course for Falmouth.

Wed 28 May Dock Falmouth 0800. End of Leg I. Exchanged personnel and equipment. Sailed Falmouth at 2000 and set course for station CS2.

Thurs 29 May 0615 recommenced sampling programme.

Mon 2 June 1500 left CS2, set course for St Ives Bay to pick up Decca engineer at 2000.

Tues 3 June 0330 on station CS2 and continued sampling programme. 1330 left station CS2, set course for Penzance to drop off Decca engineer at 1930.

Wed 4 June 0530 on station CS2 and recommenced sampling programme.

Sun 8 June 0100 completed sampling programme; set course for Falmouth, docking at 1430.

Mon 9 June Equipment unloaded by 1500.

CRUISE BACKGROUND

In May 1984, Preparatory Group C held a review meeting on pelagic processes on the shelf. At that meeting, participants from universities and institutes expressed a desire for a research cruise to study picophytoplankton, bacteria and protozoa. Preparatory Group C recognised the considerable world wide interest in marine microbiology and, particularly, the importance of protozoa and picoplankton to the pelagic food web; this cruise is a result of that Preparatory Group C review meeting.

OBJECTIVES

To study the trophic interactions occurring between phytoplankton (particularly pico- and nanophytoplankton), bacteria and protozoa in a shelf-sea pelagic ecosystem. The emphasis of the experimental work was on quantifying the flow of energy through the microbial components of the food web, the so-called "microbial loop". The individual specialists participating in the cruise determined specific aspects of the physiology of natural populations of picoplankton and protozoa; in addition, attempts were made to bring ecologically significant picoplankton and protozoa into axenic culture. The NERC Analytical Flow Cytometer was deployed successfully on this cruise for the first time at sea.

SHIP AND EQUIPMENT PERFORMANCE

The planned sampling programme was interrupted on two occasions. Firstly, on 22 May, the ship was at anchor off Falmouth so that an engineer from Coulter Electronics could repair the Analytical Flow Cytometer. No sampling was possible on that day, although work continued on samples and material collected earlier in the cruise. Secondly, on 2 and 3 June, sampling was curtailed so that an engineer from the Decca Instrument Company could spend time on board to correct problems with the automatic steering system on the ship. Bad weather limited sampling on only one occasion (27 May). All other aspects of the sampling programme were completed successfully.

The equipment failures were as follows. The Analytical Flow Cytometer did not function well on the first two days at sea and then ceased to operate at all. Temporary repairs were done when anchored off Falmouth on 22 May and the instrument was made fully operational during the port call on 28 May. On the second half of the cruise, the AFC worked perfectly.

The second instrument failure was of the Neil Brown CTD system. Migrating swallows took shelter on the ship during the first half of the cruise. One pair decided to roost on the CTD power supply one night and their excreta resulted in the power supply circuit boards burning out. The boards could not be repaired but a replacement was rigged up by connecting three other power sources in series. The CTD system then performed well for the rest of the cruise and a total of 71 CTD dips were made. There was one break in the conductive CTD wire and the connection with the CTD had to be respliced; the conductivity cell started to drift on dip 7 and this was eventually found to be caused by a leaking end cap. However, before this was realised, a new cell was fitted and used from dips 12-71. The radiometer broke down on dip 12 and was not repaired until dip 28.

All other equipment performed well.

RESULTS

The area in the Celtic Sea chosen for this study is fairly homogeneous; lateral variability around CS2 has been found to be low in the past and on this cruise a similar situation was found. An XBT survey done on the track into St Ives on 2 June showed an almost identical temperature structure from 07°W to 06° 15'W. Similarly, XBT measurements on a 5 nautical mile grid around station CS2 on 4 June showed the same features of temperature structure, *viz* a thermocline of *ca* 1.7° C at 50 m and a secondary thermocline of *ca* 0.7° C at 25 m. Station CS2 was, therefore, representative of a large area of the Celtic Sea.

Mike Fasham (IOS)

The CTD rig consisted of a Neil-Brown CTD, a Chelsea Instruments *in situ* fluorometer, a Seatek 1m path-length transmissometer, a

Plessey underwater irradiometer and a General Oceanics multisampler. A total of 71 CTD dips were made (see Appendix).

At the beginning of the cruise the water column was mixed down to 45m with chlorophyll *a* concentrations in the mixed layer of ~3mg m^{-3}. Between 18 and 20 May, a shallow thermocline developed at around 10m and the chlorophyll *a* concentration just below this depth increased to 7-8mg m^{-3}. Stormy weather between 20 and 23 May eroded the shallow thermocline and chlorophyll *a* concentrations declined to 3-4mg m^{-3}. Over the next three days, up to 26 May, the chlorophyll *a* levels in the surface 40m declined to 1.5mg m^{-3}; a second shallower thermocline now began to form at ~20m and became stronger throughout the cruise due to increased insolation. The surface chlorophyll concentrations declined rapidly from this date but a strong subsurface chlorophyll maximum began to develop between 20 and 40m. Between 26 May and 7 June, this subsurface maximum was always observed, increasing in magnitude from initial values of 1mg m^{-3} chlorophyll *a* to maximum observed values of 4.5mg m^{-3} (dip 66, 6 June). This presumed temporal change was however complicated by horizontal spatial changes of equal magnitude observed on the drifting buoy and CTD section sequence of observations (Dips 54-66).

The multisampler was used on many dips to obtain samples for nutrient, size-fractionated chlorophyll and occasionally phycoerythrin analyses. Also, a few samples were taken for phytoplankton, protozoa and gravimetric analyses.

Ian Joint (IMER)

A total of 13 *in situ* determinations of primary production were done during the cruise. Water samples taken from 10 depths in the surface 40m were inoculated with ^{14}C and incubated at the depths from which they were taken for 24h. At the end of the incubation period, the samples were fractionated through 5, 1 and 0.2µm pore-size Nuclepore filters and the amount of ^{14}C fixed was determined by liquid scintillation counting. On some days, simultaneous incubations were done by Duncan Purdie to measure primary production by oxygen production, and other incubations were done by Brian Irwin using ^{15}N nitrate and ammonia to determine rates of nitrogen assimilation. Samples were preserved for subsequent analysis of phytoplankton species, microflagellate and bacterial numbers. Samples were also taken for the subsequent determination of photosynthetic pigments by spectroscopy and high performance liquid chromatography. The activity of heterotrophic bacteria throughout the 100m water column was determined by following the incorporation of [^{3}H]-thymidine. Some of these measurements were done on the same samples as Duncan Purdie used for measurements of total community respiration.

Peter Burkill (IMER)

Grazing rate experiments to determine quantities of bacteria, cyanobacteria and phytoplankton ingested by microzooplankton

(<200μm). These experiments involved the dilution concept where the natural community of microplankton are diluted by filtered seawater of identical chemical composition and the growth of the food (bacteria, algae, cyanobacteria) measured at each dilution. Bacterial numbers and production were found to be low (< 10^6 ml^{-1} and < 5000 bacterial ml^{-1} h^{-1}) and grazing by microzooplankton was correspondingly low < 4% bacterial production h^{-1}). Grazing on cyanobacteria was ambiguous suggesting low flux. Grazing rates on algae were higher with up to 20% phytoplankton production being consumed by microzooplankton.

The cruise provided the first opportunity to carry out sea trials on the flow cytometer. This instrument, which is essentially a laser power optical bench capable of discriminating and sorting microbial size particles, blew a power supply board on the first leg of the cruise rendering the instrument inoperative. During the second leg, the instrument performed well giving precision typically found in the laboratory. Two detailed vertical profiles at CS2 were carried out where small algae (< ca 10 μm) and cyanobacteria were analysed and enumerated. In addition, on the second profile, the cytometer was reconfigured to analyse Type 1 and Type 2 cyanobacteria and a long overnight sort of these two populations was carried out. Distinct trends can be seen in the data from the profiles, indicating, among other things, a considerable increase in cellular pigment content in algae deeper in the euphotic zone.

Collaborative exeriments were done with Mike Wyman (University of Warwick) with cyanobacterial grazing studies and analytical flow cytometry and with Ray Leakey (University of Southampton) on supervision of his doctoral work on grazing rate estimates of oligotrichciliates using fluorospheres, and on microzooplankton identification and enumeration at sea.

Barry Leadbeater, Richard Geider and Alison Edwards. (Botany Department, University of Birmingham)

Estimates of microzooplankton (< 30μm nominal size class) grazing rates on phytoplankton and bacterioplankton were made by combining $^{14}CO_2$ and ^{3}H-glucose uptake rate measurements with the dilution-grazing methodology. Early experimental results indicate low growth rates of both phytoplankton and bacterioplankton, and low grazing rates on these organisms by microzooplankton. Subsequent experiments utilized time series observations of $^{14}CO_2$ uptake to further examine growth of phytoplankton. Our preliminary results lead to the paradoxical conclusion of high absolute rates of $^{14}CO_2$ uptake, but little evidence for either growth of phytoplankton or microzooplankton grazing in our experiments.

Duncan Purdie (Oceanography Department, University of Southampton)

The photosynthetic and respiration activity of microbial planktonic populations at station CS2 was investigated by determining rates of *in vitro* incubated oxygen flux. Water samples were collected from various depths using either 30 litre Niskin or 7 litre NIO bottle samplers. Samples were incubated and subsequently analysed for dissolved oxygen in 125 ml Pyrex glass bottles, using an automated Winkler titration procedure.

Rates of primary production throughout the water column were determined on a number of dates during the cruise. Water samples were incubated *in situ* in bottles suspended at several depths, from a free floating buoy and measurements of oxygen production determined after approximately 12 hours. Samples from each depth were also incubated in the dark on deck in a running sea water supply. From the oxygen results, rates of net and gross production over 12 and 24 hour periods were obtained. These data will later be compared to $^{14}CO_2$ data obtained by Ian Joint.

The respiration activity of the planktonic community throughout the water column was determined on two dates during the cruise. Samples were collected from 10 depths between surface and 80 metres then incubated in the dark at close to *in situ* temperatures. Bacterial biomass and production rates were determined by Ian Joint on samples taken from the same profile.

A total of ten experiments were conducted to estimate both the respiration and photosynthesis activity of size fractionated water samples. A simple reverse filtration procedure using large diameter Nuclepore filters was successfully employed to produce large volumes (several litres) of fractionated filtered seawater. The fractions (<10, <3, and <1 μm) were sequentially produced from a 'total' water sample which was normally collected from the chlorophyll maxima. Bottles containing samples from each fraction were subsequently incubated in the light and dark at near to *in situ* conditions in a laboratory incubator. Changes in oxygen concentration were determined over periods of between 6 and 12 hours thus enabling the size distribution of photosynthesis and respiration of the planktonic community to be compared. Fractionated seawater samples were supplied to Vanessa Walls and Katharine Howard for tracer/inhibitor studies and to Philip Boyd for comparison of $^{14}CO_2$ uptake rates in the light with those of oxygen production. The relative effectiveness of the fractionation procedure was monitored by storing samples of each fraction for later chlorophyll, ATP and PCN assay. Samples from each fraction were also observed under the epifluorescent microscope.

Several oxygen profiles were determined throughout the cruise using the CTD rosette sampler. On the 5/6 June, measurements of oxygen concentration were made at four depths in the water column every three to four hours in the vicinity of a drogue buoy. The aim of this experiment was to attempt to follow the diurnal changes in oxygen concentration and thus obtain an estimate of net water column production. Samples were also taken from the rosette

sampler for determinations of chlorophyll and cyanobacteria.

Mike Wyman (Department of Biological Sciences, University of Warwick)

A rapid and sensitive method for the quantitative estimation of the phycoerythrin content of natural populations of picoplanktonic cyanobacteria has been developed.

Depth dependent changes in the measured phycoerythrin (PE) concentration per litre were correlated with (i) the numbers of cyanobacteria present and (ii) the physico-chemical structure of the water column. A number of points emerged from this analysis:

(i) Little correlation between cell PE content, nitrogen availability and incident irradiance was apparent when the water column was well-mixed.

(ii) Following the development of calmer conditions during the second leg of the cruise, a sub-surface bloom of cyanobacteria developed at a depth of 20-25 m. The sub-surface maximum was distinct from the deeper (30-35 m) chlorophyll maximum. Cyanobacteria present in surface waters became progressively depleted in PE as the bloom developed; presumably in response to the decline in available nitrogen. At the population maximum little evidence of nitrogen deficiency was apparent; the implication being that the demand for nitrogen was met by upwelling of nitrate from below the thermocline and/or regeneration of N *in situ*.

(iii) Temporal changes in cyanobacterial cell numbers, and cellular PE content were monitored over the course of 24 hours on two occasions. On each occasion cell numbers declined somewhat during the day (indicative of grazing?) but showed a sharp increase immediately after sunset. These data indicate that cell division was synchronized to the diel cycle of light and dark periods. In confirmation of these observations, cellular PE content increased during the hours of sunlight but declined markedly during the early night-time hours. During the period of cell division PE concentration per litre showed little variation. Cell PE content declined progressively during the hours of darkness although following the initial period of division cell numbers remained more or less constant. Mobilization of PE in support of the N requirement for the *de novo* synthesis of other cell proteins would appear a plausible explanation of this observation. In collaboration with Duncan Purdie temporal changes in oxygen production were monitored in parallel with determinations of cyanobacterial production during the second 24 hour series. These data are not yet available.

(iv) Microscopic examination revealed the presence of at least two distinct 'pigment types' of *Synechococcus*; a larger cell type with characteristically bright orange fluorescence and a smaller cell type distinguished by a red shift in fluorescence emission. In collaboration with Peter Burkill the 0.6-1.0 μ fraction obtained

from eight depths was analysed by flow cytometry. Preliminary data suggest that surface waters were dominated by Synechococcus producing a C-type PE (in vivo emission maximum ~ 580 nm) whereas at greater depths this population coexisted with a distinct cell type producing a PE with both phycoerythrobilin and phycourobilin chromophores (in vivo emission max. 565-570 nm). Using instrumentation settings and cell sorting procedures originally developed for the separation of mixed cultures of Synechococcus, attempts were made to sort the mixed population present at 40 m. The sorted cells will be further analysed biochemically and, in addition, characterized by immunological techniques (in collaboration with Lisa Campbell, University of Hawaii).

Two further collaborative projects were undertaken:

(i) In collaboration with Peter Burkill the impact of grazing on the 0.6-1.0μm cyanobacterial fraction taken from the 25m population maximum was assessed by the dilution technique. Analysis of the change in cell numbers and PE content of the incubated samples at 100, 50 and 10 per cent dilution revealed little evidence of significant grazing pressure on the cyanobacterial population.

(ii) In collaboration with Mike Fasham and Trevor Platt temporal changes in chlorophyll fluorescence were followed by intensive sampling of surface waters over the course of two 24 hour periods. Little evidence for the occurrence of diurnal migration of the dinoflagellate population was obtained.

Trevor Platt, Carla Caverhill, Brian Irwin and Alain Vezina (Bedford Institute of Oceanography, Dartmouth, Canada.

Leg 1 May 17 - May 28

Primary Production. Water samples for size fractioned PI experiments were collected with 30 ℓ Niskin bottles on 13 occasions. All the samples were from the upper 40 m of the water column. Three fractions were examined in this phase of the cruise - whole, >1μm and <1μm. Samples for inorganic nutrients, chlorophyll, POC and PON were collected at the same time.

Nitrogen assimilation. Two in situ ^{15}N incubations were carried out. Samples were collected from 6 depths in the upper 40 m and $^{15}NO_3$ and $^{15}NH_3$ was added before returning the samples to their respective depths for incubation. Incubation times were twenty-four hours. Inorganic nutrient, chlorophyll, POC and PON samples were also collected.

Chlorophyll and Nutrient profiles. Samples for chlorophyll and inorganic nutrients were collected from the CTD rosette each time a CTD profile was completed. A total of 15 profiles were sampled, with 8 depths on each profile. Nutrients and whole chlorophylls were collected on all 15 profiles and >1μm and <1μm chlorophylls on 10 profiles.

Leg 2 May 28 - June 9

Primary Production. A total of 17 water samples were collected for PI experiments. 14 were collected with 30 ℓ niskins and 3 with 7ℓ N10 water bottles. All the 30 ℓ samples were collected in the upper 40 m and the remainder from 50, 60 and 80 m. The first 6 samples collected were fractioned into whole, >5µm and <5µm; the next 6 into whole, >1µm and <1µm. 4 samples were fractioned into whole, >1µm, <1µm, >3µm, <3µm and the three samples from below the euphotic zone were whole fractions only. Inorganic nutrients, chlorophyll, POC and PON were collected from each sample.

Nitrogen assimilation. A further two profiles were successfully completed. A third profile was lost when the in situ wire was cut off by an unidentified vessel with the loss of samples, bottles and frames.

Chlorophyll and nutrient profiles. A total of 28 CTD profiles were sampled for inorganic nutrients and whole chlorophylls. Eight depths were sampled on 22 profiles and four depths on the remainder. <1µm and >1µm fractions were measured on three profiles, <3µm, >3µm on 4 profiles and <5µm, >5µm on three profiles 8 depths sampled on all fractionated profiles.

Michael Sleigh (Biology Department, University of Southampton)

My aim on this cruise was to study protozoans, especially ciliates, which might play a significant part in the ecology of pico- and nano-plankton. There was a strong overlap of interest between Roy Ricketts and myself and we collaborated extensively. The number of ciliates in surface waters were studied in live counts and after fixation with Lugol's Iodine, and we concluded that this method of fixation faithfully preserved the ciliates present. Numbers of oligotrich ciliates were small (about 3/ml) in the surface waters, decreasing to about 1/ml at 20 m, and none were found from 40 m and below (there are however some fixed samples yet to be analysed). There were very few tintinnid ciliates at this time. The same collections and samples passed on by Duncan Purdie contained two species of flagellate, both about 10 µm long, present in similar numbers (100-300/ml) in the surface waters. These dinoflagellates and cryptomonads showed diurnal fluctuations in numbers in the surface waters which were followed because it was felt they may correlate with fluctuations in chlorophyll levels recorded using fluorometry by Mike Fasham. Samples of these flagellates have been collected for scanning electron microscopy, and examination of these samples revealed considerable numbers of small centric diatoms that had been overlooked in the living samples. It appears that copepods were grazing on the 10 µm flagellates, and some counts of copepods were also made. A brief study was made of protists from a core of sand from the sea floor at about 120 m; this contained interesting interstitial ciliates, live, pigmented, pennate diatoms, flagellates and a range of foraminiferan shells that will provide useful teaching material.

Roy Ricketts (Botany Department, University of Nottingham)

My work on the cruise was largely carried out in collaboration with Michael Sleigh. In addition to this we also collaborated with the other scientists present, for example, in the enumeration of copepods at various depths in the water column and in the identification of flagellates.

Our programme involved fractionating water from various depths into various size fractions for later study. We had constructed an apparatus for producing the differing sized fractions:- >200 µm, 50-200 µm, 20-50 µm, 10-20 µm and <10 µm. Unfortunately the vast majority of the natural population in the water column (apart from the copepods etc.) fell into the <20 µm size range, and most were below 10 µm. We therefore had to resort to the other methods. The incidence of ciliates in the water column was very low (<1 ciliate per ml) and necessitated the development of adequate methods of enumeration. Calculations making various assumptions, about clearance rates indicated that, for this water column and at this time of year, ciliates were not important grazers. Only one type of ciliate, tentatively identified as a Strombidium spp., was common; an occasional tintinnid was also seen. The predominant larger organisms in the water column were dinoflagellates and a (?) cryptophyte. There were also many smaller flagellates. We also attempted to determine feeding rates of size fractionated cell populations by addition of marine bacterial cultures or Synechococcus culture to these in a sea water-circulated cooled deck water bath. Unfortunately the ciliate populations were reduced below the starting level after 4 days "growth".

We also examined one core sample (bad weather precluded further collections). This proved much more interesting and contained numbers of different types of ciliates, some of which we think may be previously undescribed. It was noticeable that the core material contained a wide and varied flora of motile pennate diatoms and it was felt that these must be living heterotrophically on the seabed (c 115 m, and in the aphotic zone).

In addition to this joint work I inoculated about 150 assorted culture media with various materials in an attempt to grow both algal and ciliate species for later purification and study in my laboratory. The majority of these cultures have produced growth of various sorts and it will be a major task to isolate, purify and study the appropriate organisms. Fixed samples were also taken back for electron-microscope examination. The cruise was very valuable for a first time attender as a means of gaining experience and assessing the possibilities for future work.

Philip Boyd (Marine Station, Queens University of Belfast)

A series of experiments were carried out on samples from selected depths at station CS2 in order to investigate carbon fluxes between

components of the microbial ecosystem. Samples were taken from depths of 5, 10, 25 and 45 m, size fractionated using 3.0, 1.0, 0.4 and 0.2 µm Nuclepore filters and incubated under simulated in situ light conditions in a temperature controlled bath.

In the first set of experiments, ^{14}C labelled D.O.C. preparations were derived from the incubation of natural phytoplankton cultures. These preparations were used to estimate the rate of assimilation of D.O.C. from this source by free-living bacterial populations in the size range 0.2 - 0.4 µm. The results from these experiments will be interpreted in relation to other participants' concurrent measurements of the rates of excretion of D.O.C. by the various phytoplankton fractions.

The core of the experimental programme was an investigation of the pathways of carbon assimilation by the main components of the microbial plankton. These components were defined by filtration techniques employing the full range of filter pore sizes 3.0 - 0.2 µm in combination with pre- and post-filtration. Close spacing of filter pore sizes was employed in order to resolve more effectively the various fractions. Thus autotrophic carbon fixation by the 0.4 - 1.0 and 1.0 - 3.0 µm planktonic populations was recorded together with bacterial heterotrophic uptake of carbon by populations in the 0.2 - 0.4 and 0.4 - 1.0 µm size ranges. In addition concurrent estimates of carbon transfer by microheterotrophic grazing were attempted. An important component in this experimental set was an assessment of the time course of carbon transfer between the various components.

This principal experiment set will utilize biomass data obtained by other participants on the phyto-, bacteria- and microheterotrophic populations represented by the various size fractions employed.

The final aspect of the programme consisted of a series of experiments designed to estimate the contribution of each size fraction to community primary production. These experiments also permitted an assessment of the carbon transfer associated with grazing by the macro and micro-zooplankton and microheterotrophs. These experiments were run in parallel with oxygen flux measurements by Duncan Purdie using similar size fractions. Initial comparison of the data sets has provided considerable insight into the problems associated with the two approaches and will allow a fuller understanding of the methodology employed in microbial carbon flow studies and the trophic relationships between the various fractions.

Omar Calvario-Martinez (Department of Marine Biology, UCNW)

Cellular ^{14}C incorporation and extracellular organic ^{14}C release was determined simultaneously by means of the radioactive carbon technique at station CS2.

Water samples were collected from 100, 50, 30, and 20% of ambient light intensity. Four 295 ml "light" and one "dark" bottle were

filled with water from each of those depths, inoculated with ^{14}C and incubated for three hours in an on-deck incubator.

At the end of the incubation time parallel filtration, using 3, 2, 1 and 0.2 µm Nuclepore filters, was used to assess the amount of organic carbon produced by the phytoplankton fractions between the above filters.

The results obtained were evaluated in conjunction with physical data provided by Mike Fasham. In addition similar work involving longer incubation times was carried out by Ian Joint and comparison of the two data sets could provide useful information on the ecology of marine picoplankton.

Graham Dixon (School of Biological Sciences, University College Swansea and MBA)

Attempts were made to determine the nitrogen-status of the phytoplankton at two depths in the water columns using the ammonium enhancement of dark $^{14}CO_2$ fixation. Samples were taken at two depths, one in the nutrient-rich bottom water (40-50 m) and the second in the nutrient-impoverished surface water. Nutrient profiles were carried out daily in conjunction with the experiments.

Other experiments were carried out, using size-fractioned water supplied by Duncan Purdie, to estimate N-assimilation rates of the different size fractions by the amount of total ^{14}C fixed being incorporated into protein. These experiments, carried out over 24 hour incubations can be compared with Duncan Purdie's production figures (measured by oxygen production) to compare the two methods for measurement of primary production.

Katharine Howard (Department of Biological Sciences, University of Warwick and IMER)

Time course incubations of the 1-0.6 µm seawater fraction with ^{14}C bicarbonate in the dark were carried out and the effect of the two inhibitors, fluoracetate (0.83 mM) and malonate (1.6 mM) was studied.

Fluoracetate and malonate are inhibitors of the citric acid cycle. As cyanobacteria have an incomplete TCA cycle, only the heterotrophs in the 1-0.6 µm fraction should be effected. Fluoracetate was found to reduce ^{14}C bicarbonate uptake by approximately half the control value, whereas malonate only reduced the uptake marginally.

^{14}C bicarbonate uptake (under low light conditions) was found to be greatest in <5 - >1 µm fraction and least in the < 1.0 - > 0.2 µm fraction, with >5 µm fraction in between, over a period of 8 hours. Malonate showed some inhibition in the <1.0 - >0.2 µm fraction and virtually none in the other fractions. Filters, from samples under

the same conditions, were kept at -20° C in 5 ml of ethanol for measurement of the assimilation of ^{14}C into low molecular weight compounds, carbohydrate and protein.

Pre-fractionated samples were incubated for 6 hours and then filtered through 0.2 µm Nuclepore filters. The filtrate was acidified with concentrated sulphuric acid and returned to the lab to be counted for any extracellular ^{14}C.

Ray Leakey (Biology Department, University of Southampton and IMER)

Depth profiles of the major components of the microzooplankton (< 200 µ) at CS2 were determined, in conjunction with C.T.D. recorder profiles, on 31 May, 3 and 6 June, with particular attention paid to ciliate abundance and biomass. Grazing on algal and bacterial populations by the natural microzooplankton communities from surface, 10 m and 20 m waters was estimated on 1.6.86. Grazing activity was determined experimentally using the "dilution" technique of Landry and Hassett (1982), with algal and bacterial components enumerated by fluorometric determination (Parsons et al., 1984) and epifluorescence microscopy (Coleman, 1980) respectively. More specifically grazing activity by Oligotrich ciliates was determined using the ingestion of inert fluorescent microsperes as markers of food particle clearance rate. These experiments ran in sequence with those undertaken by Peter Burkill during the period 17 to 27 May.

References

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Parsons, T.R., Maita, Y. and Lalli, C.M. (1984). A manual of chemical and biological methods for seawater analysis. Pergamon Press, 173p.

Vanessa Walls (School of Biological Sciences, University College of Swansea)

My research was based on the use of inhibitors that would distinguish between prokaryotic and eukaryotic marine algae.

My main interests were in the smaller size fractions produced at depths from the chlorophyll maximum, these being of <3 and <1 µm in size, though some work was done for comparison on the larger size fractions. I worked with Duncan Purdie using his method of reverse filtration to obtain the fractions and then studied the inhibition of $^{14}CO_2$ fixation on these using chloramphenicol to inhibit prokaryotic protein synthesis and cycloheximide to inhibit eukaryotic protein synthesis. In addition I investigated the

effect of ammonium ions on these inhibitions. I found that inhibition of photosynthesis gave information about the contributions of prokaryotic and eukaryotic picoplankton in the sea that agreed with other worker's results.

PREPARED BY: I.R Joint

APPROVED BY: B.L. Bayne

DATE: 26 August 1986

CIRCULATION: Internal:-

B L Bayne	P Williamson
I R Joint	Notice Board
P H Burkill	File VES 10.1

External:- Cruise participants

NERC Swindon:	J D Woods
	P N Claridge
	S J White
IOS Wormley:	Mrs P Edwards (MIAS)
	Library
IOS Bidston	Library
DAFS:	McIntyre
RVS:	Skinner (2)
MBA:	Denton

APPENDIX

Station positions and times of overside operations

Date	Time (GMT)	Lat (N)	Long (W)	Operation
18 May	0739-0818	50° 29.7'	07° 02.2'	CTD 1
	0835-0915	"	"	Water bottles
	1308-1333	50° 30.0'	06° 00.6'	CTD 2
19 May	0538-0609	50° 29.6'	07° 00.7'	CTD 3
	0617-0739	"	"	Water bottles
	0710			In situ ^{14}C buoy deployed
	0742-0802	50° 28.9'	07° 00.9'	CTD 4
	0840-0950	"	"	Water bottles
	1002-1014	50° 28.7'	07° 00.8'	CTD 5
	1455-1515	50° 31.8'	06° 58.6'	CTD 6
	1520-1529	"	"	Water bottles
	1717-1727	50° 29.9'	07° 00.7'	CTD 7
	1840			In situ ^{14}C buoy recovered
	1914-1930	50° 29.7'	07° 01.5'	CTD 8
20 May	0450-0529	50° 30.0'	07° 00'	Water bottles
	0549	"	"	In situ ^{14}C buoy deployed
	0709-0732	50° 28.6'	07° 02.3'	CTD 9
	0742-0859	"	"	Water bottles
	1200-1218	50° 29.2'	07° 01.5'	Water bottles
	1415-1425	50° 31.0'	06° 58.0'	Water bottles
	1517-1527	50° 31.8'	06° 56.7'	CTD 10
	1844-1914	50° 32.2'	06° 59.4'	CTD 11
	2014			In situ ^{14}C buoy recovered
21 May	0845-0900	50° 29.8'	06° 59.1'	Water bottles
	1345-1500	50° 31.0'	07° 05.0'	Water bottles
	1603-1614			Water bottles
23 May	0656-0728	50° 29.9'	07° 00'	Water bottles
	0804			In situ ^{14}C buoy deployed
	0827-0849	50° 28.6'	07° 01.3'	CTD 12
	0926-0940	50° 28.3'	07° 03.3'	CTD 13
	0945-0955	"	"	Water bottles
	1144-1200	50° 28.8'	07° 05.2'	CTD 14
	1215-1238	50° 29.2'	07° 04.9'	Water bottles
	1536-1554	50° 29.3'	07° 01.1'	CTD 15
	1708-1919	50° 30.2'	07° 00.2'	CTD 16
	2004			In situ ^{14}C buoy recovered
24 May	0524-0554	50° 29.6'	07° 00.1'	Water bottles
	0630			In situ ^{14}C buoy deployed
	0718-0739	50° 29.5'	06° 58.6'	CTD 17
	0745-0807	"	"	Water bottles
	0852-0921	50° 28.7'	06° 59.5'	CTD 18
	0930-0945	"	"	Water bottles
	1157-1208	50° 28.4'	06° 59.1'	Fluorometer test

	1845			In situ ^{14}C buoy recovered
25 May	0705-0720	50° 28.2'	06° 59.4'	Water bottles
	0724-0743	50° 28.1'	06° 58.0'	CTD 19
	0800-0824	"	"	Water bottles
	1113-1131	50° 25.0'	07° 01.4'	CTD 20
	1132-1153	50° 26.4'	07° 02.1'	Water bottles
	1657-2245	50° 29.7'	07° 00'	CTD 20, Yo-Yo expt.
26 May	0534-0557	50° 30.1'	06° 59.4'	Water bottles
	0620			In situ ^{14}C buoy deployed
	0657-0730	50° 29.1'	06° 56.2'	Water bottles
	0752-0810	50° 30.2'	06° 57.1'	CTD 22
	0925-0945	50° 29.9'	06° 56.2'	Craib Corer
	0958-1018	50° 29.9'	06° 57.8'	CTD 23
	1206-1237	50° 29.3'	07° 00.3'	CTD 24
	1245-1251	50° 28.6'	07° 00.5'	Water bottles
	1702-1713	50° 28.7'	06° 56.4'	Water bottles
	1929			In situ ^{14}C buoy recovered
	2018-2140	50° 28.9'	06° 55.5'	CTD 25
27 May	0708-0718	50° 30.8'	06° 59.4'	Water bottles
	0955-1003	50° 30.4'	06° 57.0'	Water bottles
29 May	0515-0545	50° 29.8'	06° 59.5'	Water bottles
	0615			In situ ^{14}C buoy deployed
	0715-0732	50° 30.8'	06° 59.7'	CTD 26
	1132-1212	50° 32.8'	06° 57.4'	Spectroradiometer
	1222-1239	50° 31.2'	06° 58.0'	CTD 27
	1305-1325	50° 30.9'	06° 57.9'	Water bottles
	1607-1627	50° 28.8'	06° 55.8'	CTD 28
	1859			In situ ^{14}C buoy recovered
	2114-2122	50° 26.4'	06° 53.0'	CTD 29
30 May	0441-0511	50° 30.1'	07° 00.1'	Water bottles
	0535			In situ ^{14}C buoy deployed
	0548-0605	50° 29.3'	07° 00.9'	CTD 30
	0703-0735	50° 30.5'	07° 00.6'	Water bottles
	1055-1122	50° 29.9'	06° 57.4'	CTD 31
	1404-1412	50° 30.2'	06° 52.8'	Water bottles
	1545-1601	50° 28.8'	06° 51.2'	CTD 32
	1905			In situ ^{14}C buoy recovered
	1923-1942	50° 29.8'	06° 55.2'	CTD 33
31 May	0447-0524	50° 29.4'	06° 59.9'	Water bottles
	0540			In situ ^{14}C buoy deployed
	0709-0733	50° 28.6'	06° 59.4'	CTD 34
	0750-0818	50° 28.4'	06° 59.5'	Water bottles
	1111-1129	50° 29.5'	06° 56.0'	CTD 35
	1341-1351	50° 29.9'	06° 53.6'	Water bottles
	1659-1732	50° 28.3'	06° 49.9'	Water bottles
	1804-1819	50° 28.2'	06° 49.7'	CTD 36
	1931			In situ ^{14}C buoy recovered

1	June	0448-0510	50° 30.1'	07° 00.2'	Water bottles
		0530			In situ ^{14}C buoy deployed
		0704-0726	50° 29.0'	07° 00.1'	CTD 37
		0743-0754	50° 29.2'	06° 59.8'	Water bottles
		1105-1124	50° 29.1'	06° 54.3'	CTD 38
		1128-1141	50° 28.7'	06° 54.2'	Water bottles
		1143-1225	50° 28.3'	06° 53.3'	Spectroradiometer
		1335-1356	50° 28.7'	06° 50.8'	CTD 39
		1359-1414	50° 28.7'	06° 51.1'	Water bottles
		1700-1716	50° 28.6'	06° 56.7'	CTD 40
		1908			In situ ^{14}C buoy recovered
2	June	0657-0818	50° 29.4'	07° 00'	Water bottles
		0718-0730	50° 29.3'	07° 00.5'	CTD 41
		0820-0834	50° 28.9'	07° 00.8'	Water bottles
		0839-1011	50° 28.8'	07° 00.8'	CTD 42
		1110-1129	50° 29.2'	06° 58.9'	CTD 43
		1134-1200	50° 29.2'	06° 57.4'	Water bottles
		1315-1325	50° 29.3'	06° 54.9'	CTD 44
3	June	0250-0440	50° 28.9'	06° 58.1'	CTD 45, Yo-Yo expt
		0659-0748	50° 28.8'	07° 00.4'	Water bottles
		0750-0807	50° 28.7'	07° 01.3'	CTD 46
		1105-1122	50° 29.9'	06° 59.0'	CTD 47
		1129-1144	50° 29.8'	06° 59.1'	CTD 48
		1147-1225	50° 29.2'	06° 58.8'	Water bottles
4	June	0504-0525	50° 29.3'	06° 59.5'	Water bottles
		0550			In situ ^{14}C buoy deployed
		0650-0722	50° 27.6'	06° 58.6'	CTD 49
		0831-0850	50° 27.2'	06° 59.3'	Water bottles
		0845-0853	50° 26.9'	07° 00.5'	Hand plankton nets
		0915-0923	50° 26.9'	07° 00.4'	Hand plankton nets
		1106-1124	50° 26.2'	06° 59.9'	CTD 50
		1204-1246	50° 26.1'	06° 59.7'	Water bottles
		1706-1731	50° 26.1'	06° 50.1'	CTD 51
		1910			In situ ^{14}C buoy recovered
5	June	0445-0510	50° 29.8'	06° 59.0'	Water bottles
		0535			In situ ^{14}C buoy deployed
		0630			Drogue buoy deployed
		0700-0730	50° 27.9'	07° 02.8'	CTD 52
		0755-0824	50° 28.2'	07° 03.4'	CTD 53
		0830-0900	50° 28.3'	07° 04.0'	Water bottles
		0956-1012	50° 27.5'	07° 04.4'	CTD 54, Drifting buoy 1
		1206-1220	50° 27.5'	07° 05.0'	CTD 55, Drifting buoy 2
		1358-1416	50° 28.3'	07° 04.9'	CTD 56, Drifting buoy 3
		1705-1722	50° 28.4'	07° 02.7'	CTD 57, Drifting buoy 4
		1915			In situ ^{14}C buoy recovered
		2002-2018	50° 28.1	07° 03.3'	CTD 58, Drifting buoy 5
		2209-2224	50° 27.1'	07° 05.2'	CTD 59, Drifting buoy 6
6	June	0442-0513	50° 25.1'	07° 03.6'	Water bottles
		0530			In situ ^{14}C buoy deployed
		0712-0727	50° 26.2'	07° 03.3'	CTD 60

	0757-0818	50° 25.9'	07° 03.5'	CTD 61
	0825-0846	50° 26'	07° 03.5'	Water bottles
	0900			Drogue buoy recovered
	1207-1226	50° 30.3'	07° 00.2'	CTD 62
	1331-1348	50° 30.2'	06° 59.0'	CTD 63
	1414-1424	50° 28.4'	06° 59.7'	CTD 64
	1450-1458	50° 26.3'	07° 00.3'	CTD 65
	1526-1533	50° 24.9'	07° 01.6'	CTD 66
	1605-1630	50° 24.0'	07° 07.5'	CTD 67
	1710-1745	50° 23.9'	07° 02.8'	Water bottles
	1902			In situ ^{14}C buoy recovered
7 June	0507-0535	50° 30.1'	07° 00.1'	Water bottles
	0555			In situ ^{14}C buoy deployed
	0714-0742	50° 28.0'	06° 58.2'	CTD 68
	0824-0849	50° 26.5'	06° 55.9'	Water bottles
	1110-1130	50° 22.5'	06° 57.7'	CTD 69
	1133-1206	50° 23.5'	06° 58.1'	Water bottles
	1215-1320	50° 23.5'	07° 00.0'	1 metre plankton net hand
	1703-1723	50° 23.6'	06° 54.6'	CTD 70
	1904			In situ ^{14}C buoy recovered
	2347-0005	50° 30.2'	07° 01.4'	CTD 71