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Fluctuations in heterotrophic bacterial community structure, activity and production in response to development and decay of phytoplankton in a microcosm

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ABSTRACT: Recently upwelled water from the southern Benguela upwelling system was incubated in a 60 l microcosm for 43 d under simulated in situ conditions, to follow the development and activity of the heterotrophic microplanktonic community associated with phytoplankton growth and decay. The initial bacterial population (40 µg C l^{-1}), dominated by small rods ($\bar{v}$ = 0.198 µm^3) and large cocci ($\bar{v}$ = 0.142 µm^3), with *Vibrionaceae* as the dominant plateable strain, exhibited slow turnover times for added ^{14}C-labelled substrates ($\bar{x}$ = 5.7 h 10^6 cells^{-1}). Net bacterial growth was exponential (0.016 h^{-1}) during phytoplankton growth (12 µg C l^{-1} h^{-1}, Days 0 to 4). At maximum phytoplankton and bacterial biomass (1330 and 136 µg C l^{-1} respectively, Day 4) *Pseudomonadaceae* dominated the plateable isolates; bacterial turnover times for ^{14}C-substrates were rapid (glucose: 1.5 h 10^6 cells^{-1}, alanine: 0.49 h 10^6 cells^{-1}, glutamate: 0.29 h 10^6 cells^{-1}), suggesting a close coupling between phytoplankton growth and the ability of bacteria to utilise dissolved organic carbon (PDOC) substrates. Bacterial biomass was reduced to <15 µg C l^{-1} by Day 9, due to diminished availability of PDOC during phytoplankton senescence and predation by microflagellates which developed in the microcosm (<5 µg C l^{-1} up to Day 4, 96 µg C l^{-1} on Day 8). After phytoplankton senescence (Day 10) detrital carbon stimulated exponential growth (0.021 h^{-1}) of a second bacterial community (max. biomass: 231 µg C l^{-1} on Day 25) dominated by small cocci ($\bar{v}$ = 0.009 µm^3) and large rods ($\bar{v}$ = 0.672 µm^3), with *Flavobacteriaceae* as the dominant plateable bacteria. As this community exhibited no uptake of added ^{14}C-labelled substrates, we surmise that it was exploiting POC which dominated carbon resources at this time. Estimates of bacterial production calculated from net growth rates were ca 50 to 97 % higher than values based on [methyl-^{3}H] thymidine incorporation (TTI). These differences may be due to inadequate DNA extraction procedures, large numbers of bacteria without thymidine transport systems, or isotope dilution. Empirically determined conversion factors to correct for these differences fell within the range of 1.6 to 46 × 10^6 cells mol^{-1} TTI.

INTRODUCTION

Heterotrophic marine bacteria have recently been shown to exhibit highly complex morphological and physiological adaptations to diverse environments, and to temporal changes in substrate sources and availability (Davis & Robb 1985, Ducklow & Hill 1985a, b, Coffin & Sharp 1987). Estimates of bacterial production based on uptake or incorporation of radiolabelled substrates, such as adenine (Karl 1982) and thymidine (Fuhrman & Azam 1982, Moriarty 1986), do not always provide estimates of production comparable to other techniques (Newell & Fallon 1982, Ducklow & Hill 1985b). It seems probable that these difficulties may be explained partly by inadequacies of the techniques themselves (Moriarty 1986, Robarts & Wicks 1989) or by the non-uniform response of diverse bacterial populations to differing environmental influences. This has prompted a re-evaluation of methods for estimating bacterial activity, many of which are based on 2 principal assumptions: that bacterial populations respond to physical and biological events as a homogeneous group, and that natural populations of bacteria respond

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uniformly to added tracer substrates (see Riemann et al. 1982, van Es & Meyer-Reil 1982).

Before we can confidently construct carbon and nitrogen flux models for planktonic communities or properly evaluate the various techniques for measuring bacterial activity in aquatic environments, we need to gain a better understanding of the complex successions and adaptive responses of heterogeneous bacterial communities to changes in their environmental conditions. Upwelling environments provide ideal conditions for observing bacterial responses to the growth and decay of natural phytoplankton blooms. In the southern Benguela upwelling system, bacterial communities are subject to a relatively well-defined, rapid but extreme transition from cold (ca 10 °C) aphotic deep waters (100 to 200 m) into sun-warmed (up to ca 20 °C) surface waters characterised by the development of intense but transient phytoplankton blooms (up to ca 15 to 25 µg chl *a* l^{-1}) over 4 to 10 d periods (Andrews & Hutchings 1980, Brown & Hutchings 1987). Entrained bacterial communities are therefore advected from organically deficient deep waters where 'starvation-survival strategies' are appropriate (Novitsky & Morita 1977, Morita 1984) to surface waters where readily incorporated phytoplankton metabolites and, later, detrital particulates are likely to dominate the available substrates as a consequence of normal phytoplankton metabolism, growth and decay (Lancelot & Billen 1984, Lucas et al. 1986).

Recent ship-board studies in the southern Benguela upwelling system have suggested marked adaptive responses in bacterial community structure and activity to phytoplankton bloom development (Lucas et al. 1986, Verheye-Dua & Lucas 1988). However, dynamic physical processes preclude in situ investigation of the temporal relationships between phytoplankton and bacteria in the same body of upwelled water, or the use of large in situ enclosures such as those used during the CEPEX experiments (Grice & Reeve 1982). In this study we used a microcosm to simulate an upwelling event, allowing us to follow the development and interactions of natural phytoplankton and microbial communities at scales appropriate to the bacterial component. In doing so, we have been able to assess the sensitivity of bacterial uptake of ^{14}C-labelled substrates, incorporation of [methyl-^{3}H] thymidine, and population growth to temporal changes in substrate sources during phytoplankton growth and decay

MATERIALS AND METHODS

Experimental design. A 60 l pyramid-shaped aquarium was filled with recently upwelled surface seawater collected off the west coast of the Cape Peninsula, South Africa (33°59.1' S, 17°21.5' E) and incubated under a diurnal light regime at close to in situ temperatures (12 °C) for 43 d. The water was filtered (60 µm) prior to incubation, to remove mesozooplankton. Particulate material in the microcosm was prevented from sinking by agitation with a motorised teflon-coated stirring paddle. Growth or settlement of particulate material or micro-organisms on the sides of the aquarium was prevented by a fine 'bubble-curtain' of sterile air (<0.2 µm) gently forcing against the glass walls from porous air tubing fixed around the perimeter at the base of the aquarium. Before incubation the aquarium was filled with seawater for 8 wk, to decrease potential toxicity effects, sterilised with 10 % HCl, and well washed with filtered (0.2 µm) distilled water and sample water.

Recently upwelled water is characterised by relatively constant temperature and salinity conditions (ca 10 °C, <34.90 ‰), high concentrations of nutrients, particularly nitrate (15 to 20 µg-at l^{-1}) and silicate (10 to 15 µg-at l^{-1}), and negligible concentrations of chlorophyll *a* (Andrews & Hutchings 1980). Thus incubation of such water allows the development of a laboratory-based phytoplankton bloom and associated bacterioplankton community.

Sampling. To follow the development of the microplankton community, the abundance of micro-organisms in the microcosm was estimated every 12 h for the first 25 d, every 24 h for the next 5 d and approximately once a day for the remaining 13 d of incubation. At different stages of phytoplankton development and senescence the composition, activity and production of the heterotrophic bacterial community was assessed using plating techniques, ^{14}C-substrate uptake, and ^{3}H-thymidine incorporation. Glassware was presoaked in 10 % HCl for 3 wk, and distilled water for 1 wk. All equipment and materials used were sterilised by rinsing in 90 % alcohol and filtered (<0.2 µm) distilled water, autoclaving, muffling at 400 °C, or filtration (0.2 µm).

Phytoplankton development and production. Phytoplankton growth was monitored by relative u/v fluorescence of chlorophyll *a* in seawater using a Turner model III fluorometer. Changes in particulate carbon concentrations were measured by combustion analysis of particulates retained on pre-ashed Whatman GF/F filters using a Hereaus CHN (Rapid) analyser. Cyclohexanone (51.79 % C, 20.14 % N) was used as a standard. An estimate of primary production was obtained by fitting a linear regression ($Y = a + bX$) to the increase in particulate carbon concentration in the microcosm from Day 0 to 4 (96 h).

Microbial numbers and biomass. Water samples (20 ml) for microbial counts were preserved with 25 % Analar Glutaraldehyde (1.3 % final concentration) and stored in the dark at 4 °C prior to counting. Subsamples

were prepared and counted according to the AODC method of Hobbie et al. (1977). Bacteria were differentiated into 7 morphotype classes (Painting et al. 1985) and counted in at least 20 microscopic fields, or until 300 bacteria were counted. Heterotrophic flagellates were counted in 2 size-classes (see Lucas et al. 1987) in 100 microscopic fields. Bacterial and flagellate volumes were calculated for each size class using linear cell dimensions obtained from SEM micrographs (Linley et al. 1983) and from measurements during epifluorescence microscopy. The carbon biomass of bacteria and flagellates was calculated from total cell volume in all size classes and the conversion factor 1.21×10^{-13} g C μm^{-3} (Watson et al. 1977). Although bacterial cellular carbon content has recently been revised (Bratbak 1985) to be as high as 5.6×10^{-13} g C μm^{-3}, we have retained the more conservative values as the recent carbon content estimates were based on cultured bacteria (see also Norland et al. 1987).

Characterisation of bacterial community. Water samples (100 µl) were taken daily from the microcosm for the first 8 d and then at 2 to 3 d intervals, and plated in triplicate onto 0.1 % Peptone-seawater agar (1.0 g Bacto-Peptone, 0.1 g yeast extract, 0.01 g Fe_2PO_4, 750 ml 0.2 µm filtered seawater, 250 ml distilled water, 15 g agar) to produce ca 200 colonies per plate. Plates were incubated at 22 °C for 10 d and then counted using a Nikon 6 CTC Profile Projector (10 × mag.) which permitted the inclusion of very small colonies (0.1 to 0.5 µm) in the total viable count. The viable count expressed as a percentage of the total AODC count gave a measure of the percentage plateability. A generic classification of the plateable populations was determined by re-streaking 40 randomly picked isolates to purity on 0.5 % Peptone-seawater agar and classifying the bacteria according to the schedule proposed by Oliver (1982). Oxidative and fermentative strains were characterised sensu Cowan & Steel (1970). Oxidative strains were considered to be obligate aerobes which may produce acid from carbohydrates under aerobic conditions only. Fermentative isolates were those producing acid from carbohydrates under anaerobic conditions. The following classification lists the generic groups recognised on the basis of 21 separate tests.

Vibrionaceae: motile, fermentative isolates, generally oxidase-positive. *Enterobacteriaceae:* non-motile, fermentative and generally oxidase-negative. *Pseudomonadaceae:* motile, oxidative and generally oxidase-positive. *Neissereaceae:* non-motile, oxidative and generally oxidase-negative. *Cytophaga/Flavobacteriaceae:* chromogenic (yellow, orange or red) isolates, non-motile or showing gliding motility, oxidative but oxidase-positive (for details of tests and isolate characteristics see Muir 1986).

Uptake of ^{14}C-labelled substrates by bacteria. Samples (25 ml) of microcosm water were 4 µm filtered into 50 ml sterile bottles and inoculated separately with high specific activity universally-labelled mannitol (10 n*M*), glucose (10 n*M*), alanine (12 n*M*), aspartate (10 n*M*) and glutamate (10 n*M*). All radionuclides were supplied by Amersham International p/c.

Uptake of ^{14}C-labelled substrates by bacteria >0.2 µm was measured during 3 or 6 h dark incubations at 12 °C. At 60 or 90 min intervals, 2 ml subsamples were retained on 0.22 µm membrane filters which were washed twice with 0.1 µm filtered seawater containing 1 m*M* cold substrate, to remove aqueous label and minimise non-specific adsorption of the label. The filters were dried, placed in 5 ml Beckman HP/B scintillation cocktail, dark-adapted for 2 h, and counted in a Packard Tricarb 460-C liquid scintillation counter. The counts (DPM) were adjusted to reflect uptake by 1×10^6 cells ml^{-1} using the acridine orange direct counts.

Bacterial production. ***Incorporation of [methyl-^3H] thymidine:*** To estimate bacterial production in the microcosm during different stages of phytoplankton growth, 50 ml subsamples of water were transferred from the microcosm to sterile 150 ml conical flasks. Each flask was inoculated with 5 n*M* [methyl-^3H] thymidine (46 Ci $mmol^{-1}$) and incubated for 6 h on an orbital shaker in the dark at 12 °C. Incorporation of thymidine into bacterial DNA was measured after 0, 1, 2, 3 and 6 h, following the method of Fuhrman & Azam (1980, 1982). Bacterial cell production was calculated from the rate of incorporation of thymidine into TCA-insoluble macromolecules (h^{-1}), and from the conversion factor for nearshore bacteria of 1.7×10^{18} cells produced per mole of thymidine incorporated (Fuhrman & Azam 1982). To obtain an estimate of bacterial carbon production we used the mean cell volume of the appropriate population in the microcosm (from AODC) and the conversion factor of 1.21×10^{-13} g C μm^{-3} (Watson et al. 1977).

Exponential population growth of bacteria: Bacterial population growth was estimated from exponential curves ($Y = ae^{bx}$) of best fit to the AODC data from 0 to 96 h (Curve 1), 108 to 132 h (Curve 2), 168 to 312 h (Curve 3) and from 228 to 492 h (Curve 4) as shown in Fig. 1b. The curves were fitted by least sum of squares and the significance of each curve was tested using critical values of the *F*-test (ANOVA). The slope of each curve, *b*, was assumed to equal the net growth rate of bacteria, also symbolised here as b (h^{-1}). The net growth rate was assumed to represent the balance between the specific growth rate, μ, and losses due to grazing (Ducklow & Hill 1985a). Daily bacterial production rates were calculated from bacterial abundance in the microcosm (Table 1) and the net growth rate, b (h^{-1}). Data from the morning samples were used, to correspond with incubations for thymidine incorporation which were initiated at the same time.

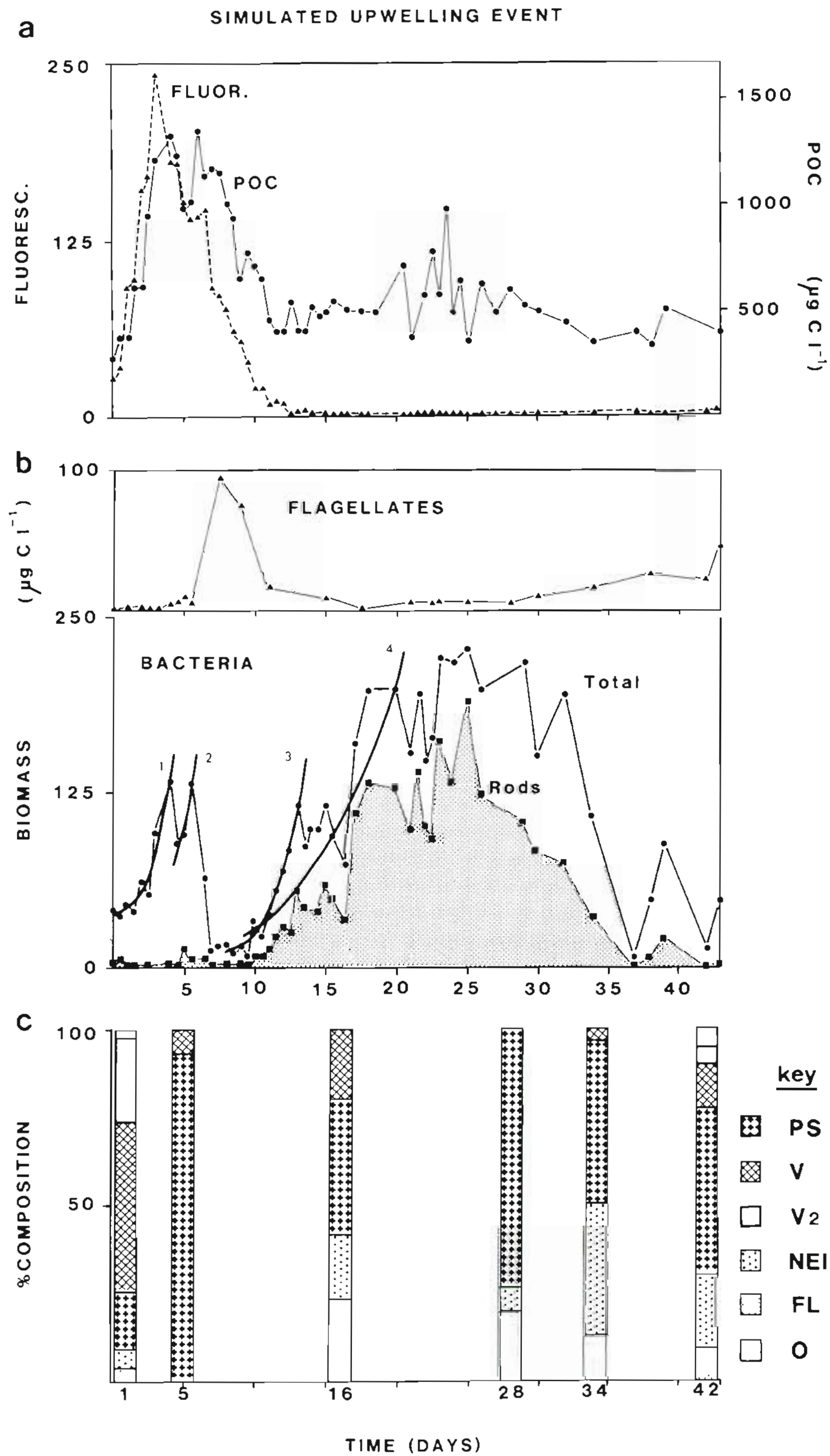

Fig. 1. Temporal relationship between phytoplankton, bacteria and flagellates after upwelling. (a) Changes in relative fluorescence and POC concentration during growth and senescence of the phytoplankton assemblage. (b) Biomass of bacteria and flagellates. The exponential curves marked 1 to 4 were fitted to the bacterial biomass data to calculate bacterial production (Fig. 2b, Tables 2 and 3). (c) Diversity and succession of the dominant plateable isolates in response to phytoplankton growth and senescence. Ps: *Pseudomonadaceae*, V, V_2: two *Vibrionaceae* genera; Nei: *Neissereaceae*; Fl: *Flavobacteriaceae*; O: other

Table 1 Changes in community structure in the microcosm up to Day 21 and including Days 22, 23, 25 and 28

Time (Day)	(h)	Bacteria Biomass (µg C l⁻¹)	Bacteria Numbers (×10⁶ ml⁻¹)	Flagellates[a] Biomass (µg C l⁻¹)	Flagellates[a] Numbers (×10³ ml⁻¹) A	Flagellates[a] Numbers (×10³ ml⁻¹) B	Particulate carbon (µg C l⁻¹)
0	0	40.08	2.118	0.392	0.058	0.000	290
1	12	38.24	1.952				380
1	24	46.19	2.819	1.480	2.199	0.000	370
2	36	41.61	2.734				630
2	48	60.84	3.352	2.077	2.696	0.084	630
3	60	53.31	3.994	2.400	1.303	0.489	970
3	72	98.66	5.378	0.617	0.916	0.000	1230
4	96	136.05	8.071	5.120	0.069	1.629	1330
5	108	89.16	6.403	6.165	1.040	1.755	1240
5	120	96.23	5.982	11.413	2.094	3.211	990
6	132	133.08	7.839				1060
6	144						1350
7	156	67.46	5.079				1150
7	168	13.44	1.550				1180
8	180	16.45	1.905	95.723	0.153	30.697	1170
8	192	16.99	1.652				1030
9	204	11.44	3.373				940
9	216	14.77	1.947	74.530	0.000	23.926	660
10	228	8.10	1.261				780
10	240	33.35	2.385				730
11	252	24.40	2.672				660
11	264	40.87	2.907	16.963	0.000	5.446	470
12	276	52.80	4.722				420
12	288	71.49	4.728				410
13	300	86.3	6.519				550
13	312	117.03	6.350				420
14	324	89.6	5.197				420
14	336	104.07	6.753				530
15	348	108.0	6.935				480
15	360	118.80	6.371	10.246	0.000	3.289	500
16	372	94.96	5.450				550
17	396	75.2	5.896				520
17	408	163.28	7.294				407
18	432	204.31	8.233				730
20	492	205.90	8.665				720
21	504	156.95	7.975	7.388	0.000	2.372	380
22	528	147.93	7.307				430
23	552	216.95	8.439	7.612	0.000	2.444	580
25	600	230.47	9.260	6.232	0.244	1.948	370
28	672	115.52	3.508	4.186	0.122	1.344	610

[a] The spherical flagellates *Pseudobodo* sp. consisted of 2 types:
Type A – 1.92 µm × 2.31 µm, radius = 1.0525 µm, v = 4.88 μm^3
Type B – 3.85 µm × 3.85 µm, v = 29.88 μm^3

RESULTS AND DISCUSSION

Development of the planktonic community

Phytoplankton development

As a result of favourable inorganic nutrient and light conditions, phytoplankton growth was rapid during the first 4 d of the microcosm incubation. Maximum particulate organic carbon (POC) and chlorophyll *a* concentrations (1330 µg POC l^{-1}, and ca 19 µg chl l^{-1}, assuming C:chl = 70) were attained by Day 4 (Fig. 1a, Table 1). The increase in POC from Day 0 to 4 represents a net primary production rate of 291 µg C l^{-1} d^{-1} (24 h), based on the linear increase in particulate carbon (POC = 200.63 + 12.14 h, r^2 = 0.935, n = 8, p < 0.001).

After Day 4 the phytoplankton biomass (in terms of u/v fluorescence) declined and was near zero by Day 12, presumably in response to nutrient limitation as the nitrate concentration was <1 µg-at l^{-1} by Day 12. The particulate carbon concentration during this time (Days

4 to 12; Fig. 1a, Table 1) decreased by 105 µg C l^{-1} d^{-1} (POC = 1749.8−4.39 h, $r^2 = 0.792$, $n = 13$, $p < 0.05$). Thereafter (up to Day 43) POC concentrations fluctuated between ca 300 and 700 µg C l^{-1}. Regeneration of a significant secondary phytoplankton bloom was not evident from fluorescence measurements or particulate carbon concentrations. The increase in POC from Day 20 to 25 may be due to large bacterial rods (up to 200 µg C l^{-1}) retained by the Whatman GF/F filters (Table 1).

Microbial community development

Bacterial numbers and biomass in the recently upwelled water were relatively low (2.1×10^6 cells ml^{-1}, 40.1 µg C l^{-1}; Fig. 1b, Table 1), but increased exponentially during phytoplankton growth to a maximum of 8.1×10^6 cells ml^{-1} and 136.1 µg C l^{-1} on Day 4. Dissolved photosynthetically derived organic carbon (PDOC) exuded during phytoplankton growth probably formed an important nutrient source for these bacteria (Williams 1981, Cole et al. 1982). The bacterial community was dominated (numbers and biomass) by large cocci and small rods (approximate volume $\bar{v}$ = 0.142 and 0.198 µm^3 respectively).

Percentage plateability was consistently low for the first 5 d (0.07 %), increasing to 12 % (Day 7) after maximum phytoplankton growth. Characterisation of the dominant plateable isolates showed a high bacterial diversity in the recently upwelled water, with 2 fermentative *Vibrionaceae (V)* isolates being dominant. By Day 4 the diversity had decreased and oxidative *Pseudomonadaceae (Ps)* isolates dominated the plateable bacteria (Fig. 1c). Most significantly, characterisation of the plateable isolates showed that the range of catabolic and hydrolytic properties increased during this bacterial succession (see also Muir 1986).

Following phytoplankton senescence bacterial numbers and biomass fell to low levels (1.6×10^6 cells ml^{-1}, 13.4 µg C l^{-1}, Day 7; Table 1), possibly due to diminished availability of PDOC substrates, or due to grazing by a microflagellate community which developed in synchrony with the bacteria (Fig. 1b, discussed below). Plateable bacteria showed an increase in diversity, with the reappearance of fermentative isolates, and were characterised as *V, Ps, Neissereaceae (Nei), Flavobacteriaceae (Fl)* and *Enterobacteriaceae (E)* genera.

For Days 0 to 4 estimates of flagellate biomass (0.392 to 6.165 µg C l^{-1}; Table 1) and bacterivory were low (<0.04 to 0.29 µg bacterial C l^{-1} h^{-1}; Lucas et al. 1987), suggesting minimal grazing impact on the bacterial population during this time. From exponential curves fitted to the biomass data (see Curve 1, Table 2) the bacterial community associated with phytoplankton growth up to Day 4 was calculated to have a net growth rate of 0.016 h^{-1} and net production rates increasing from 0.62 µg C l^{-1} h^{-1} on Day 1 to 2.22 µg C l^{-1} h^{-1} on Day 4 (see 'Materials and methods'). From Day 4 flagellate numbers increased rapidly, reaching a maximum of 30.7×10^3 ml^{-1} (96 µg C l^{-1}; Table 1) by Day 8. The concomitant reduction in bacterial biomass (to <15 µg C l^{-1} by Day 9; Table 1) was attributable to flagellate bacterivory. After Day 8 flagellate numbers decreased again, probably due to prey limitation (Lucas et al. 1987). The flagellates were dominated by a species resembling *Pseudobodo* ($\bar{v}$ = 30 µm^3) similar to that described by Parslow et al. (1986). However, the initial flagellate population was dominated by a smaller form (ca 5 µm^3), possibly the starved 'swarmers' of *Pseudobodo* described by Fenchel (1982a, b), which characterise unstable environments.

A second bacterial community developed in the microcosm in association with phytoplankton decay, when the major substrate sources were likely to be the more refractory dissolved and particulate substrates derived from phytoplankton detritus (Fukami et al. 1985a, b, Lucas 1986). This community had a maximum density similar to that of the first community (9.3×10^6 cells ml^{-1} on Day 25; Table 1). It also showed an initial high diversity of plateable bacteria *(V, Ps, Nei, Fl, E)*, with a succession of bacterial isolates and morphotypes. However, community structure differed considerably, and the plateable isolates displayed a restricted range of catabolic properties. Numerically the second bacterial community was dominated by small cocci ($\bar{v}$ = 0.009 µm^3), possibly fragmented cells reverting to 'starvation survival' strategies with the decreased availability of low molecular weight dissolved substrates. The biomass was dominated by large rods ($\bar{v}$ = 0.672 µm^3), resulting in a maximum community biomass (230.5 µg C l^{-1} Day 25; Table 1) almost twice that of the first community. These bacteria did not appear to be heavily grazed by microflagellates, possibly because their larger size (mean length = 1.2 µm) or association with particulate material offered a predation-free refuge. The increase in bacterial biomass was exponential from Days 7 to 13, during which time bacteria were calculated to have a net growth rate (0.021 h^{-1}, Curve 3; Table 2) and maximum net production rate (2.47 µg C l^{-1} h^{-1}) similar to that of the first community (see above). Percentage plateability was low, decreasing from 12 % on Day 7 to 1.5 % by Day 20. From Day 10 to 26 oxidative *Fl* formed an increasingly large percentage of the plateable population (<1 % on Day 10 to 25 % on Day 26; see also Muir 1986). The bacterial biomass peak on Day 26 was, however, characterised by a low bacterial diversity with *Ps* as the dominant plateable family.

Only near the end of the experiment (after Day 30)

when large cocci and small rods returned to dominate the biomass and *Ps* was the dominant plateable isolate, did microflagellate numbers begin to increase significantly again. The quantitative significance of flagellate bacterivory in the microcosm is described by Lucas et al. (1987).

A more complete description of the bacterial isolates present in the microcosm is given by Muir (1986). Although generalisations about the changes in population structure from only a small fraction of the population are subject to the constraints inherent in plating techniques, plateable bacteria may form a significant proportion of the active population (Bölter 1977). Furthermore, plate counts may be a sensitive indicator of the overall structure and nutritional capability of the population (Laake et al. 1983, Muir 1986). Similar well-ordered successions of bacterial isolates associated with dissolved and particulate phytoplankton carbon substrates have previously been described by Fukami et al. (1985a, b). Ciliates were not observed in the microcosm so their influence on bacterial and flagellates numbers was likely to be minimal.

Bacterial activity and production

Turnover times of ^{14}C-labelled substrates

Bacterial turnover times for glucose, alanine, aspartate and glutamate (Fig. 2a) were initially slow, and similar ($\bar{x}$ = 5.7 h 10^6 cells^{-1}), when the plateable bacteria were dominated by *V* isolates. By Day 4, however, when phytoplankton growth was at its maximum, substrate turnover times were 4 times faster for glucose (ca 1.5 h 10^6 cells^{-1}), 10 times faster for alanine (0.49 h 10^6 cells^{-1}) and 20 times faster for glutamate (0.29 h 10^6 cells^{-1}). Turnover times for aspartate did not change significantly. Following the onset of phytoplankton senescence after Day 5 (Fig. 1a) all dissolved substrate turnover times slowed considerably and by Day 8 were less than the initial turnover times. By Day 11 there was no further uptake of glutamate, while uptake of glucose and aspartate ceased by Day 21.

Our results show that bacterial substrate uptake activity is variable, and that this coincides with changes in the physiological state of phytoplankton and the composition of the bacterial community. In the newly-upwelled organically-deficient water, with *V* as the dominant plateable isolate, slow substrate turnover times may be indicative of suppressed metabolic rates characteristic of bacteria from oligotrophic or deep waters (Novitsky & Morita 1977, Morita 1984, Davis & Robb 1985, Hanson & Lowery 1985). For example, Novitsky & Morita (1977) reported that a psychrophilic marine *Vibrio* (ANT 300) was able to reduce its endogenous respiration rate by 99 % in <7 d starvation, so reducing its substrate demand. Nevertheless, the high affinity transport system for glutamate in ANT 300 was retained during starvation at a reduced but constant uptake rate (Faquin & Oliver 1984). Such mechanisms may account for the slow initial ^{14}C turnover times exhibited by bacteria in our microcosm.

Faster substrate turnover rates in association with phytoplankton growth may well be related to the induction of specific bacterial uptake mechanisms (Morita 1984, Davis & Robb 1985) in response to the increasing availability of excreted phytoplanktonic metabolites (PDOC). These are known to be rapidly incorporated by many bacteria (Cole et al. 1982, Azam & Ammerman 1984, Lancelot & Billen 1984). It seems likely that inducible non-fermenters, dominated by *Ps*, were responsible for the faster substrate turnover times on Days 3 and 4 relative to the initial slower substrate

Table 2. Exponential growth curves (1 to 4) for bacteria in the microcosm (see also Figs. 1b and 2b). Curve 1 = 0 to 96 h, Curve 2 = 108 to 132 h, Curve 3 = 168 to 312 h, Curve 4 = 228 to 492 h (see Table 1). The significance (p) of the curves was tested using critical values of the F-test (ANOVA). The slope of each curve, b, was assumed to equal the net growth rate of bacteria (h^{-1})

Curve	$Y = ae^{bX} \pm$ SE	n	F, calculated	p	r^2	Net growth (h^{-1})
Bacterial numbers (× 10^6 cells ml^{-1})						
1	$Y = 1.81\ e^{0.0146X} \pm 0.117$	8	111.15	0.001	0.95	0.0146
2	$Y = 6.05\ e^{0.0085X} \pm 0.138$	3	1.08	n.s.	0.52	0.0085
3	$Y = 1.39\ e^{0.0098X} \pm 0.306$	13	26.82	0.001	0.71	0.0098
4	$Y = 2.69\ e^{0.0059X} \pm 0.310$	17	29.09	0.001	0.66	0.0059
Bacterial biomass (µg C l^{-1})						
1	$Y = 27.79\ e^{0.0163X} \pm 0.199$	8	31.73	0.001	0.84	0.0163
2	$Y = 84.05\ e^{0.0181X} \pm 0.103$	3	7.63	n.s.	0.88	0.0181
3	$Y = 5.59\ e^{0.0210X} \pm 0.423$	13	37.16	0.001	0.77	0.0210
4	$Y = 44.21\ e^{0.0065X} \pm 0.214$	17	35.44	0.001	0.70	0.0065

turnover times when 2 groups of fermentative *V* isolates dominated (see also Muir 1986).

Diminishing substrate uptake rates coincided closely with a change in the physiological state of the phytoplankton cells after Day 5 (Barlow 1982a) when availability of readily utilisable PDOC probably decreased, to be replaced by more refractory DOC and POC as the major carbon sources for bacterial metabolism (Fig. 1a). This also coincided with a change in the bacterial community structure from *Ps* to *Fl* as the dominant plateable isolates, discussed above. Despite slow ^{14}C-labelled substrate turnover times, bacterial population growth was high during this period (Curve 3, Fig. 1b). A number of hypotheses can be offered to explain these results. It is possible that bacterial isolates associated with POM utilisation do not or cannot take up added solutes, including ^{3}H-thymidine, that are unrepresentative of the hydrolysis products arising from extracellular decomposition of POM (see also Hoppe 1986). A further possibility is that the bacterial population may have previously saturated its internal pool for that substrate, thus activating a negative feed-back mechanism on the uptake system. Respiratory losses can nevertheless continue while biosynthesis proceeds slowly at the expense of internal pools, as Davis & Robb (1985) have demonstrated. Kirchman & Hodson (1984) have also shown that amino-acid transport and incorporation in some bacteria can be non-competitively inhibited by extracellular dipeptide concentrations originating from extracellular protease activity. Further, they noted that incorporation of labelled monomers into macromolecules was isotopically diluted by unlabelled amino acids originating from intracellular hydrolysis of the dipeptides taken up. These observations may in part explain why the ^{14}C-labelled substrates were turned over very slowly, if at all, in the later stages of our microcosm.

Bacterial production

Bacterial production in our microcosm was calculated from net bacterial growth, and from measurements of [methyl-^{3}H] thymidine incorporation (TTI) into TCA-insoluble macromolecules.

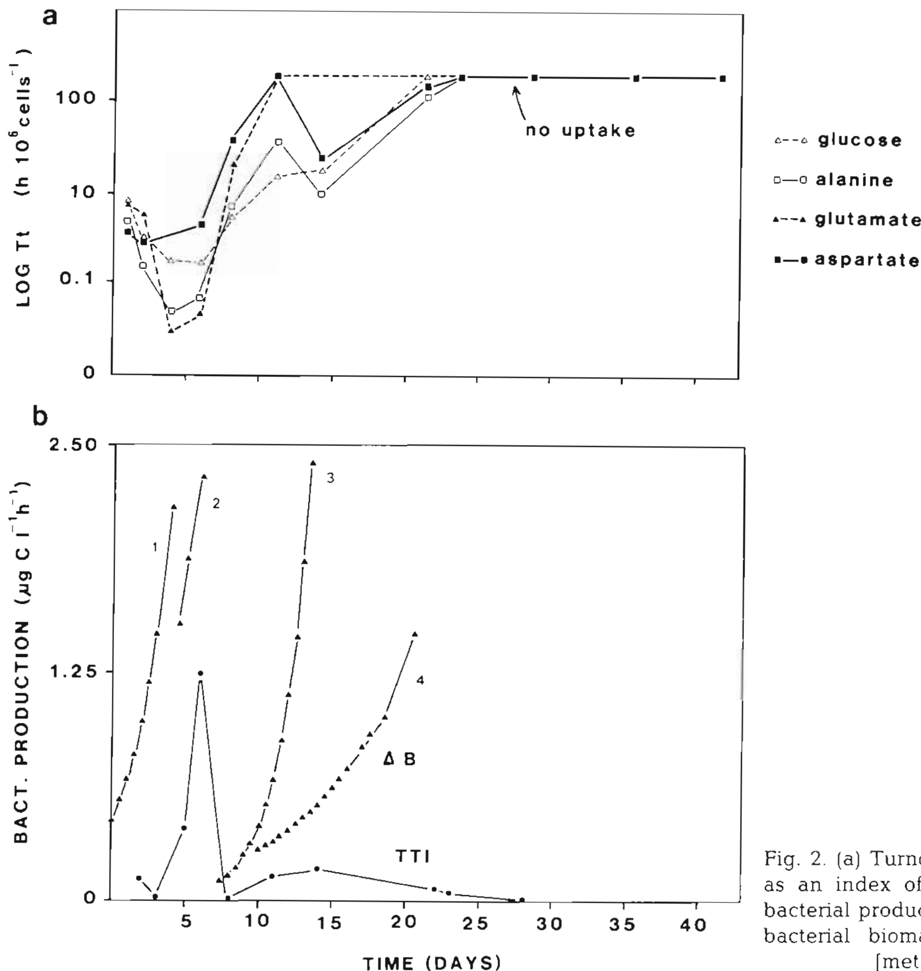

Fig. 2. (a) Turnover times of ^{14}C-labelled substrates, as an index of bacterial activity. (b) Estimates of bacterial production based on exponential growth of bacterial biomass (Δ B; ▲) and incorporation of [methyl-^{3}H] thymidine (TTI; ●)

Exponential curves ($Y = ae^{bx}$) fitted to the bacterial numbers and biomass data were highly significant ($p \ll 0.001$) for Curves 1, 3 and 4 (Table 2). Despite a good fit to the biomass data, Curve 2 was not significant for either linear or exponential curves, due to too few data points ($n = 3$). Incorporation of ^{3}H-thymidine was linear over the time-course incubations ($r > 0.9$) except for Days 23 and 28 ($r > 0.3$), when TTI was negligible and there was also no increase in bacterial abundance.

Table 3 and Fig. 2b show that estimates of bacterial production based on TTI were generally lower (50 to 97 %) than estimates based on net bacterial growth. Differences were particularly marked in newly upwelled water (oligotrophic), and during phytoplankton decay, when our previous data suggest that bacteria were utilising POM and more refractory DOM.

Our results may be subject to criticism because of extraction procedures for DNA that we adopted from Fuhrman & Azam (1980, 1982), who showed that DNA comprised 80 % of the TCA-insoluble material in their bacterial samples. Similar results were reported by Scavia & Laird (1987) but many authors have shown variable and sometimes significant distribution of the label in macromolecules other than DNA (Riemann et al. 1984, Moriarty 1986, Servais et al. 1987, Hollibaugh 1988, Robarts & Wicks 1989). However, Wicks (unpubl.) has recently shown in our laboratory that there is less than 2-fold variation in total macromolecular and DNA labelling of bacteria during phytoplankton growth and decay in the southern Benguela upwelling system. Specificity of DNA labelling may therefore account for a maximum of 50 % of the variability in our production estimates from the 2 independent techniques. Problems of isotope dilution and de novo synthesis were not addressed during this study as we used thymidine (Tdr) at the concentration (5 n*M*) expected to inhibit isotope dilution by de novo synthesis in marine bacteria (Fuhrman & Azam 1982). From the ratio of Tdr uptake to incorporation, Wicks (unpubl.) found evidence of isotope dilution only from measurements of TTI during phytoplankton decay in upwelled water. Moriarty (1986) recommended that a minimum concentration of 20 n*M* thymidine be used to inhibit isotope dilution. However, Davis (in press) found in our laboratory that high concentrations of Tdr (19 n*M*) inhibited TTI by 2 isolates (*Pseudomonas* and *Cytophaga*) from detritus-dominated seawater of southern Benguela origin, whereas low concentrations of Tdr (9.1 μ*M*) did not.

Variability between the 2 techniques used in this study may be partially due to inappropriate conversion of the number of cells produced per mole of thymidine incorporated (1.7×10^{18} cells mol^{-1} TTI, Fuhrman & Azam 1982). Conversion factors calculated from net growth rates in this study ($P_{\Delta N}/P_{TTI}$ from Table 3), which are likely to be underestimated due to grazing losses, suggest that 1.64 to 46×10^{18} cells were produced mol^{-1} TTI. This is similar to the range (1 to 60×10^{18} cells mol^{-1}) reported by Ducklow & Hill (1985b) for oligotrophic warm core rings. Furthermore, some of the variability we observed may be attributable to the succession of metabolically and phenotypically distinct bacteria associated with phytoplankton development and decay in upwelled water.

Table 3. Bacterial production estimates calculated from: (1) Exponential population growth (numbers and biomass) in the microcosm on the days on which thymidine incorporation was measured [net production = morning estimate of numbers and biomass (Table 1) × growth rates, b (h^{-1}), from exponential Curves 1 to 4 (Fig. 1b, Table 2)] and (2) incorporation of [methyl -^{3}H] thymidine (TTI) into cold TCA precipitates. Production was calculated from 1.7×10^{18} cells mol^{-1} TTI (Fuhrman & Azam 1980, 1982) and the weighted mean cell biomass (calculated from Table 1)

Time (Day)	(h)	Net bacterial production (× 10^6 cells l^{-1}h^{-1}) $P_{\Delta N}$	(μg C l^{-1}h^{-1}) $P_{\Delta B}$	(Curve)	Thymidine incorporated (pmol l^{-1}h^{-1}) TTI	Mean cell biomass (fg C cell^{-1})	Bacterial production from TTI (×10^6 Cells l^{-1}h^{-1})	(μg C l^{-1}h^{-1}) P_{TTI}	$P_{TTI}/P_{\Delta B}$ (%)
2	36	41.0	0.6782	(1)	5.19	15.21	8.82	0.134	19.1
3	60	60.0	0.8690	(1)	1.30	13.34	2.21	0.029	3.4
5	108	57.6	1.6138	(2)	17.10	13.90	29.10	0.404	24.8
6	132	70.6	2.4087	(2)	43.39	16.97	73.70	1.251	51.9
8	180	19.1	0.3455	(3)	0.63	8.63	1.07	0.009	2.9
11	264	29.1	0.8583	(3)	5.73	14.06	9.74	0.137	16.3
14	336	40.5	0.6765	(4)	6.65	15.41	11.30	0.174	25.0
22	528	–	–	–	2.24	20.25	3.81	0.077	–
23	552	–	–	–	1.19	25.71	2.02	0.052	–
28	672	–	–	–	0.23	32.93	0.39	0.013	–
Mean						17.64 ± 7.02			

Responses of bacteria to ^{3}H-thymidine and ^{14}C-substrate supply

Three distinct periods (Days 0 to 3, 4 to 6, 7 onwards) were apparent in our microcosm. In the first, the bacterial population was dominated by 2 small *V* isolates (Fig. 1c). Bacterial production estimates from TTI were on average only 12 % of the value obtained from net bacterial growth (Table 3). Apart from problems of specificity of DNA labelling it is possible that some bacteria in upwelled water do not take up exogeneous ^{3}H-thymidine, due to their physiological state.

Fermentative *V, Ps* and other 'starved' bacteria characteristic of newly upwelled or oligotrophic waters may be initially metabolically dormant, hence the slow initial ^{14}C-substrate turnover times (Fig. 2a). Characteristically also, DNA replication is not an immediate priority for such bacteria until more favourable nutritive conditions are encountered. Cell size may initially increase and substrate transport can take place although cell division does not immediately occur, resulting in biomass production but a lag phase in the requirements for precursor bases to DNA synthesis (see also Davis 1985). Apparent increases in cell numbers by AODC may be an artefact of microscope resolution as starved bacteria are small (<0.1 µm, Morita 1984) and not easily detected by epifluorescence microscopy. Using isolates from southern Benguela upwelled water, Davis (in press) found that not all strains took up and incorporated thymidine in the same ratio and that one *Vibrio* isolate totally lacked the ability to transport and incorporate thymidine.

Within the second period of the microcosm (Days 4 to 6), when phytoplankton growth was at its maximum, and ^{14}C-substrates were turned over rapidly, bacteria appeared to be metabolically active. Agreement between production estimates from TTI and net growth rates improved and there was little evidence to suggest that the bacterial community was incapable of using exogenously supplied ^{3}H-thymidine.

In the third period of the incubation agreement between the 2 techniques was again poor. Not only had the community structure become dominated by large rods *(Cytophaga/Flavobacteriaceae),* probably utilising phytoplankton detritus, but this community did not turn over the ^{14}C-labelled substrates offered. Davis (in press) found that during downwelling conditions in the southern Benguela, when detritus dominates the particulate carbon biomass, isolates of 3 genera *(Vibrio, Pseudomonas, Flavobacterium)* were unable to transport and incorporate thymidine presented at either high (19 n*M*) or low (9.1 µ*M*) concentrations. From data presented in this study we speculate that the poor incorporation rates of ^{3}H-thymidine at this time indicate that a large proportion of the total bacterial community in the marine environment cannot transport supplied thymidine (see also Moriarty 1986). Also, poor uptake and incorporation of ^{3}H-thymidine in water dominated by POM may be indicative of isotope dilution, as active bacteria may use exogenous thymine arising from the decomposition of phytoplanktonic DNA.

ECOLOGICAL SIGNIFICANCE

The extent to which our studies have a wider ecological significance will depend on the degree to which microcosm manipulations led to artefacts in our results. Recent studies have shown that particularly surface or wall effects in enclosures tend to increase bacterial growth and activity, and that these effects are more pronounced in small volumes (<3 l) than in larger enclosures (30 m^3; Kuiper et al. 1983, Ferguson et al. 1984, see also Grice & Reeve 1982). In addition, Ferguson et al. (1984) found that manipulation of natural seawater by filtration (3 µm) increased the growth rate of bacteria. To minimise these effects we used an enclosure of intermediate volume (63 l) and filtered water very slowly through a relatively coarse (60 µm) mesh. Furthermore, we limited adhesion of particulate material and bacteria to the wall by modifying the aeration apparatus to create a gentle 'bubble curtain'.

Comparisons of phytoplankton production and biomass between our microcosm and the Southern Benguela upwelling region show a remarkable similarity. Our data for primary production approximate to 12 µg C l^{-1} h^{-1} at a peak biomass of 19 µg chl *a* l^{-1} on Day 4 (hourly P/B = 0.63) and fall within the range of data characteristic of mature or aged upwelled water (Brown & Field 1986). Particulate carbon concentrations in the microcosm were characteristic of in situ values (Lucas et al. 1986, Muir 1986) and the development time of the bloom in the microcosm (4 to 5 d) was typical for the environmental situation (Barlow 1982b). Bacterial numbers and biomass were also typical of upwelled water (Muir 1986), and the percentage plateability fell within the commonly reported range (0.1 to 10 %; Buck 1979). The initial lag in increased plateability and the decline in % plateability after Day 7 suggest that changes in plateability were not an artefact of incubation, but reflective of the changing nutritional competence of bacteria as the availability of utilisable dissolved substrates changed.

Bacterial production rates (1.6 to 2.4 µg C l^{-1} h^{-1}; Table 3) associated with phytoplankton growth (Days 5 to 6) are similar to those recorded for the southern Benguela upwelling region (Lucas et al. 1986) and are

ca 17 % of the estimate of primary production within the microcosm. These values are not inconsistent with bacterial carbon requirements if their net growth efficiency is considered to be 60 to 80 % and PDOC production by phytoplankton is up to 30 % of gross primary production (Lucas 1986, Cole et al. 1988). Our production estimates are also comparable with estimates obtained in similar environments. For example, in the upwelling system off central Chile, McManus & Peterson (1988) estimated bacterial production to range from 0.13 to 1.4 μg C l^{-1} h^{-1}, using incorporation of ^{3}H-thymidine.

Our microcosm thus appears to be a realistic simulation of the temporal development of a phytoplankton bloom in recently upwelled water. Our results suggest that different bacteria have specific substrate preferences, possibly dependent upon their particular adaptive cell physiologies and substrate uptake mechanisms, which confer competitive advantages under differing environmental conditions. These preferences allow certain strains to exploit easily utilisable dissolved substrates, while enabling others to take advantage of recalcitrant substrates characterisitic of detrital phytoplankton. Non-uniform response of bacteria to added tracer substrates may result in underestimates of bacterial production by 2 to 34 times by TTI, particularly in deep and oligotrophic waters, or during phytoplankton decay. Our results indicate that estimates of bacterial production by the TTI technique require calibration against microscopic counts, and extraction and purification of DNA, to obtain reliable conversion factors. Some attention also needs to be paid to the taxa of bacteria involved as not all appear to use exogenously supplied thymidine. Furthermore, our data indicate that bacterial substrate specificities, possibly in conjunction with flagellate predation, may be a significant mechanism in the control of microbial successions in the marine environment.

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