BACTERIOPLANKTON DYNAMICS IN THE SOUTHERN BENGUELA UPWELLING REGION

by

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Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Zoology (Marine Biology Research Institute) at the University of Cape Town.

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September 1989

This thesis is dedicated to Gill and Tony, my parents.

DECLARATION

This thesis presents the results of original research which I carried out in the Department of Zoology at the University of Cape Town. This work has not been submitted for any other degree. Collaborative studies and assistance I have received in collecting, analysing and interpreting data are fully acknowledged. All publications arising from this study are referenced in the Appendix.

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ABSTRACT

Painting, Suzanne Jane. 1989. Bacterioplankton dynamics in the southern Benguela upwelling region. Ph.D. Thesis. Marine Biology Research Institute, University of Cape Town, Rondebosch, 7700, South Africa, (v) + 296 pp.

The role of heterotrophic bacteria in the carbon and nitrogen flux of the pelagic food web was investigated during laboratory and field-based studies of the temporal development of the planktonic community after upwelling. Bacterial community structure, activity and production were closely coupled to the upwelling cycle and to the dynamics of the phytoplankton community. The initial bacterial population (<1 x 10^6 cells ml^{-1}, 20 to 40 µg C l^{-1}) was metabolically dormant. Increased availability of phytosynthetically produced dissolved organic carbon (PDOC) stimulated bacterial growth (0.016 h^{-1}) and abundance (8 to 10 x 10^6 cells ml^{-1}, 140 to 200 µg C l^{-1}). Rapid successions in the dominant plateable strains were attributed to substrate preferences and substrate availability. Significant correlations of bacterial biomass with total standing stocks of phytoplankton and particulate carbon provided evidence of close coupling between bacteria and PDOC, and between bacteria and recalcitrant substrates available during phytoplankton decay. These relationships were best described by power functions, suggesting that bacterial biomass was relatively reduced at high levels by predation. A microcosm study indicated that zooflagellate predation could control bacterial biomass. Low net growth yields (34 to 36 %) of flagellates suggested inefficient transfer of carbon to higher trophic levels, but considerable nitrogen regeneration (ca 6 to 7 µg N mg dry weight^{-1} h^{-1}). Thymidine-measured bacterial production (TTI, <0.1 to 1.25 mg C m^{-3} h^{-1}) was linearly related to phytoplankton growth. 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 or oligotrophic

waters, or during phytoplankton decay. Close coupling of copepod (Calanoides carinatus) production to the upwelling cycle suggested co-existence of the microbial food web and the classical diatom-copepod food chain. Recently upwelled water was dominated by phytoplankton. Assuming that all phytoplankton carbon was available for utilisation, copepods and bacteria were calculated to consume approximately 12 and 22 % of primary production respectively. As the bloom declined the planktonic community was increasingly dominated by bacteria, detritus and mesozooplankton. On average, copepods consumed 60 % of primary production, while bacteria consumed 49 %. Carbon consumption requirements of both bacteria and copepods were satisfied by resource partitioning and carbon cycling. Under food-limiting conditions herbivorous copepods may switch to omnivory, ingesting microzooplankton of the microbial food web, and stimulating enhanced remineralisation to further sustain primary production. A generic size-based simulation model of the dynamics of the plankton community indicated that bacteria and the microbial food web increase the overall productivity of the planktonic food web, and that heterotroph predation in the smaller size classes (<200 μm) is an important mechanism in nutrient recycling.

ACKNOWLEDGEMENTS

I am extremely grateful to both the Benguela Ecology Program (BEP) of the South African National Committee for Oceanographic Research and to the Sea Fisheries Research Institute (SFRI) for generous financial and logistic support for this study, and similar work done by myself in Antarctica and the northern Benguela region.

Many people have played an important role in my research over the past few years, but I am particularly indebted to two people: To my supervisor, Mike Lucas, who was invaluable in the initiation, development and completion of this thesis, and who remained a good friend through all times good and bad; and to "my boss", Larry Hutchings, who made it financially possible for me to continue with research.

I have really appreciated the opportunity to have worked within a large co-operative marine research program (the BEP), and with the many other scientists involved. I would like to thank my friends and colleagues, Frieda Verheye-Dua, Trevor Probyn, Mike Lucas, Gay Perez and Dave Muir, with whom I shared a laboratory for many years, for the stimulating and happy atmosphere they created. My thanks are also extended to many other people who contributed their time and advice during the research and the writing of the various chapters of this thesis, including: Dot Armstrong, Penny Brown, Claire Davis, Jeanie Stenton-Dozey, Larry Hutchings, Annie Linley, Coleen Moloney, Bill Peterson, Venetia Stuart, Hans Verheye and Richard Wicks.

I am indebted to a large number of people who assisted me in many ways during my research. For their technical assistance and advice, I thank Deon Horstman, Piet van der Byl and Cecil Giddey of the SFRI, and Klaus Schultes and

Dane Gurneke (of the E.M. Unit, UCT). For advice, help and hands-on computer instruction I thank Dan Franco, Jane Pugh and Carlos Villacastin-Herrero of UCT, and John Roberts, Marius Coetzee, Gavin Olivier and Anastasia Polito of the SFRI. I would also like to thank Ian Humphreys and Wayne Smith for assistance with bacterial counts, Betty Mitchell-Innes for phytoplankton counts, Jill Burbidge, Diane Gianakouros, Susan Payne and Koos Williams for general research assistance, Lesley Elley and Meryl Painting for typing some of the text and tables, and Tony van Dalsen and his team for the final artwork.

The constant support and encouragement of my family and friends during the different stages of my research were invaluable. For this, I am deeply grateful to Gill and Tony, and Dennis and Meryl Painting. Of my friends, I would like to thank Wally Shave in particular. Also Elaine Rumbak and Mark Gibbons, and all those who had confidence in me.

Last but not least I would like to say a huge thankyou to those people who helped me so enthusiastically during the last days.....Susan Payne and Jenny Huggett who did whatever I didn't have time to do. And Gill, my mother, who gave up countless week-ends to type this thesis.

GENERAL INTRODUCTION

The role of heterotrophic marine bacteria in the carbon and nitrogen flux of pelagic food webs has been the subject of numerous studies during the last decade. There has been much controversy as to whether bacteria are a trophic link between phytoplankton and larger heterotrophs (Azam et al. 1983, Sherr et al. 1987) or a carbon sink (Ducklow et al. 1986). However, a revised concept of the pelagic food web appears to be emerging, in which the "link or sink" debate is discarded, and the microbial loop is regarded as an integral component of a larger microbial food web, which includes all autotrophic and heterotrophic unicellular organisms and is the ultimate food source for the larger zooplankton (Sherr and Sherr 1988, Hopkinson et al. 1989, Probyn et al. 1989).

Few studies have been done on the dynamics of pelagic heterotrophic bacterioplankton assemblages in the southern Benguela upwelling region, and their quantitative significance in the planktonic community of this system is uncertain. The objectives of the research presented in this dissertation were: firstly, to quantify temporal changes in the abundance, biomass and secondary production rates of natural bacterial communities in response to phytoplankton dynamics during the upwelling cycle. Secondly, to establish the functional relationship between bacteria, phytoplankton and particulate organic material in the southern Benguela and, thirdly, to determine the fundamental mechanisms which control bacterioplankton dynamics in this system.

The thesis is divided into four sections. The first section is introductory, including a chapter on the planktonic food web (Chapter 1) and a second chapter on the principal hydrographic and biological features of the southern Benguela upwelling system. The second section deals briefly with methods of estimating the abundance and production of heterotrophic pelagic bacteria, and descibes the details of bacterial methods used throughout this study (Chapter 3).

The third section, consisting of 2 chapters, presents the results of a laboratory-based microcosm simulation of an upwelling event. The objective of this study was to follow the development and interactions of natural phytoplankton and microbial communities during phytoplankton growth and decay, at scales appropriate to the bacterial component. In Chapter 4 the adaptive responses in bacterial community structure, activity and production to phytoplankton development are discussed. In addition, the sensitivity of bacterial uptake of exogenously supplied dissolved substrates (^{14}C-labelled substrates and [methyl-^{3}H] thymidine) and population growth to temporal changes in substrate sources during phytoplankton growth and decay is analysed. A descriptive Lotka-Volterra predator-prey model is used in Chapter 5, to analyse bacterial and flagellate interactions and to define the predation rate, growth efficiency and nitrogen-regeneration rates originating from flagellate bacterivory.

The fourth section deals with an in situ drogue study of the dynamics of heterotrophic bacterioplankton and zooplankton communities in response to the growth and decay of a natural phytoplankton assemblage in an upwelling plume in the southern Benguela. In Chapter 6, temporal changes in bacterial abundance and biomass during the development of phytoplankton and zooplankton communities in the plume are quantified, and temporal changes in planktonic community structure are examined. Estimates of in situ bacterial production during the active and quiescent phases of the upwelling cycle are presented in Chapter 7. In Chapter 8, the secondary production rate of the zooplankton community during the development of the plume is quantified. Carbon consumption requirements of the bacterioplankton and zooplankton communities are calculated from their respective secondary production rates, to obtain an estimate of the partitioning of autotrophically fixed carbon between these two heterotroph groups during the drogue study.

In the final section, the laboratory and field-based studies are combined with a theoretical simulation model to gain further insight into the fundamental processes governing the temporal changes in the biomass relationships and size-structure of the planktonic community, and to determine the trophic role of heterotrophic bacteria in the planktonic food web of the southern Benguela.

SECTION 1

INTRODUCTION

CHAPTER 1

The planktonic food web

Marine bacteria have traditionally been regarded as decomposers and remineralisers of organic matter (Steele 1974), responsible for recycling nutrients to primary producers. The potential importance of decomposers in the marine food web has therefore been recognised for many years. It is only during the last decade, however, that microheterotrophs have been incorporated into the classical concept of the trophic dynamics of the pelagic ecosystem (Pomeroy 1974, Williams 1981, Azam et al. 1983, Lucas 1986, Newell and Turley 1987, Hobbie 1988, Pomeroy and Wiebe 1988).

The traditional concept of pelagic food webs assumed that carbon flow from primary production was accounted for by grazing activities of zooplankton, which in turn formed the basis for pelagic fish stocks. An anomaly was that the ecological efficiency of this pathway was apparently insufficient to meet the known fish production in areas such as the North Sea (Steele 1974).

Eppley and Peterson (1979) recognised that extensive remineralisation occurred in surface waters of the ocean, and could account for 80 % of the nitrogen requirements of oceanic phytoplankton. The question posed was how sufficient nitrogen could be regenerated in the water column, if all the phytoplankton was grazed by herbivores, which themselves could not account for observed rates of nitrogen regeneration (Williams 1981). Accumulating evidence pointing to the significance of microheterotrophs was provided by Banoub and Williams (1973) who recognised that a large proportion of phytoplankton production was exuded as dissolved organic material which was unavailable to zooplankton. Organisms such as bacterioplankton which could utilise this material would thus become significant in the planktonic food web by making soluble carbon potentially available in particulate form. Furthermore, it became increasingly evident that a large fraction of primary production was by nanophytoplankton (<20 μm), and that micro-organisms were responsible for a

large fraction of total planktonic respiration (Pomeroy 1974). The emerging paradigm therefore clearly demonstrated that the smaller size-classes (<20 μm) were important both as a trophic link and in terms of nutrient recycling (Williams 1981).

Improved techniques for quantifying bacterial abundance and production (Hobbie et al. 1977, Meyer-Reil 1979, van Es and Meyer-Reil 1982, Ducklow 1983), protozoan predation (Fenchel 1982d, McManus and Fuhrman 1986, Coffin and Sharp 1986, Sherr et al. 1986a) and nutrient recycling (Glibert 1982, Goldman et al. 1985, Probyn 1985) have subsequently confirmed that the microbial food web may be an active component of pelagic food webs in the marine environment (for review see Azam et al. 1983).

Although bacterial densities have been shown to be relatively constant in the ocean (1×10^8 to 5×10^9 cells l^{-1}) estimates of bacterial production are variable, ranging from <1 to 200 mg C m^{-3} d^{-1} (Azam et al. 1983, Cole et al. 1988). In spite of methodological limitations, variability in production estimates is indicative of different levels of bacterial activity and, similarly, of different levels of activity of the microbial loop under different environmental conditions.

Recent studies have shown that bacterioplankton are an important component of the heterotroph biomass in the marine pelagial (eg. Linley et al. 1983, Azam and Fuhrman 1984). Correlations between bacterial biomass and chlorophyll concentrations (Bird and Kalff 1984, Verheye-Dua and Lucas 1988) and between bacteria and primary production (van Es and Meyer-Reil 1982, Cole et al. 1988) have suggested that bacteria are largely dependent upon phytoplankton production. Heterotrophic bacteria have been calculated to utilise up to 60 % of autotrophically fixed carbon, through the uptake of photosynthetically

produced dissolved organic carbon, PDOC (Hagström et al. 1979, Williams 1981, Cole et al. 1982, Fuhrman and Azam 1980, 1982, Lancelot and Billen 1984, Lochte and Turley 1985, Fuhrman 1987, Newell and Turley 1987), particulate organic carbon (Lucas 1986, Biddanda 1988, Painting et al. 1989, Pett 1989) or dissolved organic carbon released as a by-product of herbivorous feeding by zooplankton (Eppley et al. 1981, Pace et al. 1984, Pomeroy 1984, Cho and Azam 1988, Jumars et al. 1989).

Heterotrophic marine bacteria have recently been hypothesised as being either a significant pathway by which autotrophically fixed carbon is made available to larger heterotrophs (Azam et al. 1983, Ducklow 1983, Linley et al. 1983, Lochte and Turley 1985, Sherr et al. 1987) or as a pathway through which phytoplankton carbon is lost from the system through respiration (Ducklow et al. 1986). It is, however, becoming increasingly evident that the microbial loop forms an important component of a larger microplanktonic food web. Although a large proportion of carbon, and nitrogen, fixed by nanoplankton and picoplankton may be respired away or conserved within the microplankton community via excretion, the proportion of organic matter which would otherwise have been lost may be made available to larger heterotrophs via phagotrophic flagellates and ciliates (Sherr and Sherr 1988, Hopkinsen et al. 1989, Probyn et al. 1989). Evidence exists to suggest that heterotrophic nanoflagellates (Roman et al. 1988) and planktonic ciliates and rotifers (Stoecker and Egloff 1987, Gifford and Dagg 1988, Tiselius 1989) may be an important component of the copepod diet.

The traditional role of bacteria as direct mineralisers of organic matter has recently been questioned (Azam et al. 1983). Studies of protozoan bacterivory have suggested that active predation within the microbial food web is the principal mechanism by which nutrients are recycled (Eppley and Peterson

1979, Glibert 1982, Ducklow 1983, Davis and Sieburth 1984, Goldman et al. 1985, Sherr et al. 1986a, b, Probyn 1987, Probyn and Lucas 1987, Pomeroy and Wiebe 1988). Many of these studies have shown that interactions between autotrophs, microheterotrophs and other heterotrophs are complex, making quantification of the significance of the microbial food web in the overall trophic dynamics of the pelagic marine environment exceptionally difficult. Furthermore, recent evidence that a large fraction (>50 %) of total phytoplankton biomass may be due to picophytoplankton (<1 μm) and nanophytoplankton (<20 μm, Platt et al. 1983, Douglas 1984, Probyn 1985) has implications for the existence of a dynamic microbial food web (see Moloney 1988, Moloney and Field 1989c, Probyn et al. 1989).

The study of heterotrophic bacterioplankton dynamics in relation to substrate sources and predation has become of fundamental importance in marine ecology (see Hobbie and Williams 1984). Moreover, realistic models of the trophic dynamics of marine ecosystems require an estimate of the partitioning of phytoplankton production between bacteria and phytoplankton grazers, and the rate of microprotozoan (<200 μm) bacterivory (Sherr et al. 1987, Coffin and Sharp 1987).

Quantification of the proportion of primary production passed through pelagic bacteria demands an estimate of the bacterial consumption of phytoplankton carbon or nitrogen. This requires estimates, firstly, of bacterioplankton production (biosynthesis) and, secondly, of the efficiency with which phytoplankton biomass is converted to bacterial biomass (ie: the net

growth yield, NY_g, of bacteria). Where carbon is the common unit this may be represented as:

$$C_b = P_b/NY_g$$

where C_b = bacterial consumption of carbon, P_b = bacterial production and NY_g = bacterial net growth yield. The proportion of primary production (P_p) passing through bacteria may therefore be calculated as:

$$P_p = C_b/P_p \times 100$$

Very few studies have produced reliable estimates of the proportion of phytoplankton production utilised by bacteria on the basis of these apparently simple equations (for review see Lucas 1986). This is largely due to the sensitivity of the equations to estimates of bacterial production and net growth yield, both of which are extremely difficult to quantify.

Numerous methods are available for estimating bacterial production (see van Es and Meyer-Reil 1982, Moriarty 1986) but there is poor agreement between them. Many of the methods are based on the principal assumptions that marine bacterial populations respond to their physical and biological environments as a homogeneous group, and that natural populations of bacteria respond uniformly to added tracer substrates (see Riemann et al. 1982, van Es and Meyer-Reil 1982, Davis 1989, Painting et al. 1989). However, natural marine bacterial populations are complex aggregates of heterotrophic, phototrophic and chemotrophic organisms (Sieburth 1979, 1984). Furthermore, heterotrophic bacteria exhibit highly complex morphological and physiological adaptations to diverse environments and to temporal changes in substrate sources and availability (Pomeroy 1984, Wiebe 1984, Ducklow and Hill 1985a, b, Davis 1989).

Estimates of bacterial production based on uptake or incorporation of dissolved substrates, such as ^{3}H-adenine and ^{3}H-thymidine (Karl 1982, Fuhrman and Azam 1982, Moriarty 1986) do not always provide estimates of production comparable to other techniques, such as frequency of dividing cells and bacterial growth in seawater cultures (Newell and Fallon 1982, Ducklow and Hill 1985b). Difficulties experienced in obtaining comparable estimates of bacterial production by different methods may be partly explained by inadequacies of the techniques (Moriarty 1986, Robarts and Wicks 1988), or by the non-uniform response of heterogeneous bacterial populations to environmental conditions.

Estimates of the net growth yield of marine bacteria are similarly highly variable, depending on the nature of the substrate (Linley and Newell 1984, Newell and Linley 1984, Lucas 1986, Hopkinson et al. 1989). Bacteria in laboratory incubations have been shown to have a net growth yield as low as 10 % on detrital material and as high as 80 % on dissolved organic material, particularly when nitrogen is not limiting (Azam et al. 1983, Azam and Ammerman 1984, Newell and Linley 1984, Newell and Turley 1987). It appears that bacteria may consume carbon as a source of energy, and scavenge inorganic and organic nitrogen for protein synthesis (Azam et al. 1983). Estimates of bacterial net growth yield require simultaneous measurement of carbon incorporation and the loss of carbon or nitrogen in the substrate being utilised, which is often difficult due to uncertainties in the carbon content of bacteria, and rapid substrate turnover times and unknown substrate specificities of the bacteria. Methods currently available may result in an under-estimate of bacterial biomass by as much as 37 % (Fuhrman 1981, Verheye-Dua and Lucas 1988).

Fronts, eddies, upwelling regions, thermoclines and other boundaries as well as specific seasonal events or extreme environments have provided focal points for investigations of bacterial population dynamics (Holligan et al. 1984a, b,

Morita 1984, Davis and Robb 1985, Painting et al. 1985, Ducklow and Hill 1985a, b, Lochte and Turley 1985, Lucas et al. 1986, Lochte and Pfannkuche 1987, Verheye-Dua and Lucas 1988). What has emerged is that planktonic community structure and the temporal, morphological, physiological and metabolic adaptions exhibited by bacterial populations to such diverse environments and varied substrate resources is extremely complex. Examples include "starvation survival strategies" of bacterioplankton occupying nutrient deficient deep oceans (Novistky and Morita 1978, Morita 1984), the close coupling of bacterial activity in surface waters to the exudation of phytoplankton metabolites (Newell et al. 1981, Lancelot and Billen 1984, Billen and Fontigny 1987) and the slower decomposition of polymeric solutes or POM requiring hydrolysis to utilisable DOM (UDOM, see Azam and Ammerman 1984) by bacterial substrate-specific extracellular enzymes (Hoppe 1986, Meyer-Reil et al. 1980). Moreover, the competitive ability of natural diverse populations of heterotrophic bacteria to exploit particular resources (eg. mannitol) depends on the presence of the appropriate transport systems and whether the cells have constitutive or inducible enzyme systems (Davis and Robb 1985). Predator-prey relationships have also proven to be extremely complex and must involve many steps if we are to account for the observed nitrogen regeneration measured by ^{15}N labelling techniques (Goldman and Caron 1985, Goldman et al. 1985, Probyn 1987, Probyn and Lucas 1987).

Upwelling systems provide an ideal environment for observing the changes in bacterial activity, production and substrate uptake preferences in response to the growth and decay of natural phytoplankton assemblages. In the southern Benguela upwelling system, bacterial communities are subject to a relatively well defined, rapid and extreme transition from cold (ca 10 ^{o}C) aphotic deep waters (100 to 200 m) into sun-warmed (up to ca 20 ^{o}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 day periods (Andrews and Hutchings 1980). Entrained bacterial communities are therefore advected from organically deficient deep waters where "starvation-survival strategies" are appropriate (Novitsky and Morita 1978, 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 and Billen 1984, see review by Lucas 1986).

CHAPTER 2

The southern Benguela upwelling region.

The southern Benguela upwelling region, situated along the west coast of South Africa, is characterised by strong seasonal variations in its current and hydrological features which have been the subject of numerous physical and chemical oceanographic studies (Andrews and Hutchings 1980, Boyd 1982, Bailey 1985, Shannon 1985a [and authors therein], Waldron 1985, for review see Shannon 1985b, Chapman and Shannon 1985).

Episodic upwelling events are driven by coastal winds generated by synoptic weather systems. Strong southerly and south-easterly winds, occurring predominantly in summer, cause cold (<10 ^{o}C) South Atlantic Central Water (SACW) to be advected from the aphotic zone below the continental shelf (40 to 100 km offshore) to the surface coastal waters (Andrews and Hutchings 1980, Shannon 1985b). Localised increases in coastal wind stress, due to terrestrial topographic features such as mountains or headlands, and strong gradients in sea surface temperatures have resulted in major upwelling centres being located along the coasts of the Cape Peninsula, Cape Columbine and Hondeklip Bay (see Fig. 1, Nelson 1985, Jury 1985, Kamstra 1985). Active summer upwelling in the three major upwelling centres is maintained by the enhanced wind-stress of prolonged southerly long-shore and south-easterly off-shore winds (Taunton-Clark 1985), which often result in the development of off-shore plumes of upwelled water which form isolated tongues of high primary and secondary productivity within the older waters (Nelson and Hutchings 1983, Armstrong et al. 1987). The resultant boundary zones between the recently upwelled water and the older water are complex and thought to be characterised by intense biological activity similar to that found at other frontal areas (Hutchings et al. 1985, Armstrong et al. 1987, Mitchell-Innes and Winter 1987, Verheye-Dua and Lucas 1988).

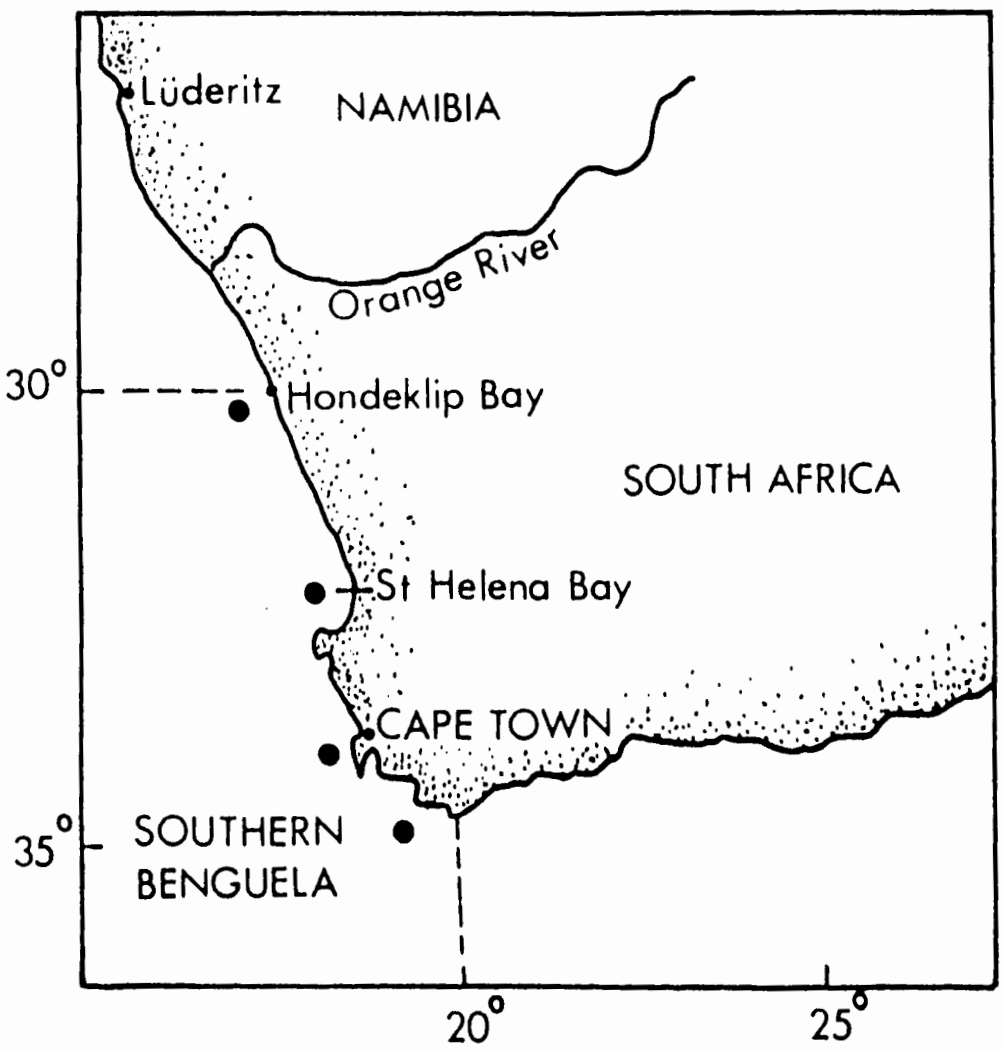


Fig. 1. The southern Benguela upwelling region, showing the location of major upwelling centres off Hondeklip Bay in the north, Cape Columbine (St. Helena Bay), the Cape Peninsula, and the Agulhas Bank in the south.

Maintenance of upwelling plumes over a number of days results in sun-warming of the surface layers. The subsequent thermal stratification of the water column is accompanied by a similar stratification in water column density, due to the temperature-dependence of density in this system. The resultant pycnocline is thought to prevent loss of sedimenting particles from the euphotic zone, and to serve as another boundary of increased biological activity.

Relaxation and reversals of the wind disturb the isolation of the upwelled water masses, causing mixing of the different surface waters and destratification of the water column. The details of water column structure are complex and will not be dealt with here (see Jury 1985, Waldron 1985).

Upwelling of South Atlantic Central Water is associated with a well defined sequence of physico-chemical and biological events, and for convenience they have been described in terms of the age of the upwelled water (Barlow 1982a, b, Brundrit 1986, Brown and Hutchings 1987b). Recently upwelled water characteristically has a high density (sigma-t >26.4) due to low temperatures (8 to 11 ^{o}C), a low salinity (<34.9 ‰), high nutrient concentrations (nitrate: 15 to 30 µg-at l^{-1}, silicate: >15 µg-at l^{-1}, phosphate: >20 µg-at l^{-1}), very little chlorophyll (<0.8 mg m^{-3}) and negligible primary production (Andrews and Hutchings 1980, Waldron 1985, Brown and Field 1986, Brown and Hutchings 1987a, b). In mature upwelled water (4 to 10 days old), increased temperatures (10 to 15 ^{o}C) stimulate increased primary production (mean value: 47 mg C m^{-3} h^{-1}), resulting in lower nitrates (2 to 15 µg-at l^{-1}) and increased standing stocks of chlorophyll (up to 6.4 mg m^{-3}) (Brown and Field 1986). Aged upwelled water (>10 d) may be as warm as 16 to 18 ^{o}C and the high rates of primary production (on average: 60 mg C m^{-3} h^{-1}) increase the chlorophyll concentrations to approximately 10 mg m^{-3}, but cause total depletion of "new"

nitrates (<2 μg at l^{-1}) (Brown and Field 1986). Maintenance of the bloom at this stage is largely dependent on regenerated nitrogen (Probyn 1985, 1987, 1988).

The partitioning of phytoplankton production between bacteria, phytoplankton grazers and fish is unknown. Zooplankton dynamics in the southern Benguela system appear to be sensitive to upwelling-induced phytoplankton dynamics. Highest biomasses of zooplankton are found when chlorophyll concentrations are typical of those in aged upwelling water, and may be 64 % of the phytoplankton biomass (Carter 1983, Shannon and Pillar 1986). However, recent studies have indicated that zooplankton may graze as little as 2 % of the phytoplankton production and biomass (Olivieri and Hutchings in prep., Peterson et al. 1989). It is important to realise that studies of the trophic dynamics of the system are complicated by the pulsed nature of upwelling, which may result in a temporal mismatch in the distribution of phytoplankton and zooplankton (Hutchings 1981, Hopson 1983). Field investigations at any particular point in time may thus give an unbalanced view of planktonic interactions.

The insularity of upwelling plumes provides an ideal environment in which to study temporal and spatial relationships between the different components of the plankton community. Similarly, such environments provide an ideal opportunity to gain insight into processes governing primary and secondary production, decomposition and nutrient regeneration.

In the field work presented in this study, a drogue was deployed into an upwelling plume off Cape Columbine, South Africa, and followed by ship for 8 days. The primary objectives of this cruise were to follow the temporal changes in the phytoplankton, bacterioplankton and zooplankton communities in the same patch of upwelled water, and to determine the relative proportions of autotrophically fixed carbon passing through the heterotrophic bacteria and

mesozooplankton. Further objectives of this study were to assess the trophic role of the pelagic heterotrophic bacterial community, and to construct a simple model of the carbon and nitrogen flux through different components of the planktonic community during phytoplankton growth and senescence in a developing plume of upwelled water.

SECTION 2

METHODS

CHAPTER 3

Details of methods used in this study to estimate bacterial abundance and production.

INTRODUCTION

Incorporation of the microbial component into the pelagic food web requires reliable estimates of the abundance and production of pelagic bacteria and their predators. During the past decade there have been vast improvements in the quantitative techniques for measuring the abundance and biomass of bacteria (see Hobbie et al. 1977, Porter and Feig 1980, van Es and Meyer-Reil 1982, Azam et al. 1983, Ducklow 1983, Bratbak 1985, Lee and Fuhrman 1987, Norland et al. 1987) and heterotrophic flagellates (Fenchel 1982a, b, Sherr and Sherr 1983a, b, Bloem et al. 1986, Borsheim and Bratbak 1987).

Similarly, there have been numerous recent developments in techniques for obtaining quantitative estimates of bacterial activity and production (for reviews see Wright 1978, Floodgate 1980, Fuhrman and Azam 1980, Riemann et al. 1984, Moriarty 1986). The most widely used techniques include the frequency of dividing cells (FDC, Hagström et al. 1989, Hagström and Larsson 1984), population growth in predator-reduced incubations (eg. Linley et al. 1983), bacterial growth in seawater cultures (Ducklow and Hill 1985a, Bjornsen 1986), respiration (Bauerfeind 1985, Hopkinsen et al. 1989) and bacterial uptake and/or incorporation of dissolved nucleic acid precursors (Fuhrman and Azam 1980, 1982, Karl 1982, Winn and Karl 1984, Fuhrman et al. 1986, Karl and Winn 1986, Moriarty 1986, Douglas et al. 1987, Chin-Leo and Kirchman 1988). Accurate measurements of heterotrophic bacterial production are, however, still problematic (Karl and Winn 1984, Fuhrman et al. 1986, Painting et al. 1989). Agreement between the different methods is poor (Newell and Fallon 1982, Bell et al. 1983, Riemann and Sondergaard 1984), possibly as a result of invalid assumptions on which the methods are based (eg. Riemann et al. 1984), inadequacies of the techniques themselves (Moriarty 1986, Robarts and Wicks 1989), inadequate conversion factors (Kirchman et al. 1982, Ducklow and Hill

1985b, Chin-Leo and Kirchman 1988) or because of non-uniform response of heterogeneous bacterial populations to different environmental conditions (Painting et al. 1989).

The objective of this chapter is to provide a detailed description of the methods for quantifying bacterial abundance, biomass and production throughout this study. Further discussion of the techniques and their limitations is incorporated into following chapters.

BACTERIAL NUMBERS AND BIOMASS

Bacterial samples (20 ml) were preserved with 25 % Analar Glutaraldehyde for Electron Microscopy (Merck, 1.3 % final concentration) in furnaced glass vials. Samples were stored at 4 ^{o}C until micro-organisms were counted or prepared for electron microscopy.

Estimation of bacterial numbers

Bacteria were counted according to the Acridine Orange Direct Counting technique of Hobbie et al. (1977). Samples were shaken vigorously for 1 minute to resuspend bacteria and particles which may have settled during storage. Sonication was avoided due to potential ruption of delicate cells. Subsamples (3 to 5 ml) were incubated with equal volumes of sterile-filtered (0.2 µm) Acridine Orange Dye (Merck, 10 mg l^{-1} artificial sea water) for 3 to 10 minutes at room temperature.

Nuclepore filters (0.2 µm pore size, 47 mm diameter) were stained over-night with Irgalan Black (2 g l^{-1} 2 % Acetic acid), rinsed 3 times in sterile-filtered (0.2 µm, Millipore disposable filters) distilled water, and stored in the final rinse water. Prior to sample filtration a stained Nuclepore filter was placed on an absorbent pad (Millipore AP10), used as a filter support. A surfactant (Photoflo, Kodak, 1 % final concentration) was rapidly filtered through the filter under high vacuum (>15 mm Hg), to promote even distribution of the sample.

The sample was filtered until dry under low vacuum (<5 mm Hg) to prevent damage to fragile cells. The filter paper was then transferred to a lightly oiled glass microscope slide, and a drop of Nikon non-fluorescent immersion oil

was slowly dispersed over the filter paper by gentle pressure on a clean coverslip.

Slides were viewed under oil-immersion at 1000 X magnification using a Leitz Laborlux Epifluorescence microscope fitted with an epifluorescence condensor, HBO 200W mercury burner, 455-500 band-pass excitation filter, 510 beam splitter and LP 520 barrier filter. The volume of sample filtered was selected such that that were approximately 15 bacteria per microscope field. The number of fields counted was variable, but at least 300 bacteria, or 30 fields, were counted to obtain a normal distribution of the sample (Linley et al. 1981, Scavia and Laird 1987). Differential counts were made for 7 easily distinguishable morphotype categories of bacteria (see Table 1).

Total bacterial numbers were calculated as follows, after subtraction of the total number of bacteria in the dye control from the total number in each sample:

$$N = \frac{n \times 10^{6} \times S}{s \times V}$$

where:

N = cells ml^{-1}

n = mean cell count per microscope field

S = working area of filter (mm^2)

s = area of microscope field (μm^2)

V = volume of sample filtered (ml)

Table 1. Calculation of bacterial biovolume from cell dimensions obtained during epifluorescence microscopy and from Scanning Electron Micrographs (SEM).

Category		Range in dimensions (μm)	Mean dimensions (μm)	Volume (μm^3)	Mean Volume (μm^3)	SEM Volume (μm^3)
Small cocci	S.C.	0.2 - 0.3	0.25	0.004-0.014	0.009	0.007
Large cocci	L.C.	0.31- 0.8	0.56	0.015-0.268	0.142	0.059
Small rods	S.R.	0.3 x 0.8 0.6 x 1.2	-	0.057-0.339	0.198	0.139
Large rods	L.R.	0.6 x 1.21 0.8 x 2.0	-	0.339-1.0	0.672	0.656
Small "u" shaped	S.U.	0.3 x 0.8 0.6 x 1.2	-	0.057-0.339	0.198	0.139
Large "u" shaped	L.U.	0.6 x 1.21 0.8 x 2.0	- -	0.339-1.0	0.672	0.656
"S" Shaped	"S"	0.3 x 0.8 0.6 x 1.2	-	0.057-0.339	0.198	0.139

Estimation of bacterial biomass

Bacterial biomass was calculated from the cell numbers and cell volumes as follows (Linley et al. 1981).:

$$B = \frac{N \times vol \times S.G}{10^6}$$

where:

B = bacterial biomass (mg l^{-1})

N = cells ml^{-1}

vol = cell volume (μm^3)

S.G.= specific gravity (1.1 g cm^{-3}) (Doetsch and Cook 1973)

Total bacterial biomass was calculated separately for each of the morphotype categories counted. Cell volumes (μm^3) were calculated from the cell dimensions estimated from epifluorescence microscopy and from scanning electron micrographs (SEM), assuming coccoid bacteria to be spheres and rod-shaped bacteria to be cylinders. Table 1 shows that these estimates were in close agreement for all categories except large cocci, and that SEM estimates were consistently lower than those from AODC. Cell volumes calculated from AODC may be over-estimated due to the "halo" created around the cells by the fluorescence (Fuhrman 1981, Linley 1983). However, estimates from scanning electron microscopy (SEM) are likely to be under-estimated due to shrinkage, which may be as high as 37 % (Fuhrman 1981). In addition, these estimates are influenced by the thickness of the gold-palladium coating used in preparation of the cells, which has been estimated to increase cell volumes by 20 to 32 nm (Linley 1983, Bratbak 1985), and may be variable depending on the size of the cells (Verheye-Dua and Lucas 1988). In this study the cell volumes estimated

from AODC were used to calculate bacterial biomass, as they are likely to be more accurate than the SEM estimates (Fuhrman 1981).

Bacterial carbon biomass ($\mu g\ C\ l^{-1}$) was calculated on the assumptions that bacterial cell density is 1.1 g cm^{-3}, that dry biomass is 22 % of the wet biomass, and that 50 % of the dry biomass is composed of carbon (Luria 1960, Newell et al. 1981). The equivalent conversion factor of 1.21×10^{-13} g C μm^{-3} (Watson et al. 1977, Fuhrman and Azam 1980) has recently been shown to be very conservative (Joint and Pomroy 1987), which has important implications for calculation of carbon flux through bacteria. However, estimates of 3.5×10^{-13} g C μm^{-3} (Bjornsen 1986) and 5.6×10^{-13} g C μm^{-3} (Bratbak 1985) were based on cultured cells, and may not be applicable to natural marine bacteria.

Dehydration and desalination technique used in the preparation of micro-organisms for Scanning Electron Microscopy (SEM)

The procedure described by Linley et al. (1981) was followed in the preparation of micro-organisms for SEM. Samples (20 ml) were filtered onto Nuclepore filter papers (0.2 µm pore-size, 25 mm diameter) under low vacuum (<5 mm Hg). Filtration was stopped just before the filter was dry, and the filter paper was covered with a second Nuclepore filter (0.2 µm pore-size). Both filter papers were placed between 2 ring magnets, trimmed to the approximate diameter of the magnets and transferred into sterile-filtered (<0.2 µm) artificial seawater. The samples were desalinated through a series of sterile-filtered solutions (artificial seawater and distilled water) with decreasing salinity (100, 90, 75, 50, 25, 10, and 0 %), spending 10 minutes in each. The samples were washed twice with distilled water, and dehydrated by holding for 10 minutes in each of an ascending ethanol series increasing with 10 %

increments. The filters were washed twice and stored in 100 % ethanol until they were critical point dried by the Electron Microscope Unit at the University of Cape Town. All dried filters were mounted on SEM stubs and sputter coated with 200 A^{o} gold palladium in a vacuum coater. The specimens were stored in a desiccator until viewed at 30 000 K on the Cambridge Stereoscope 200 Scanning Electron Microscope.

BACTERIAL PRODUCTION

Incorporation of [methyl-^{3}H] thymidine into bacterial DNA

Bacterial production estimates were obtained from measurements of tritiated thymidine incorporation (TTI) into bacterial DNA, following the method of Fuhrman and Azam (1980, 1982). Samples of natural seawater (50 ml) were transferred to sterile conical flasks (150 ml), inoculated with 5 nM [methyl-^{3}H] thymidine (46 to 52 Ci $mmol^{-1}$; Radiochemical Centre, Amersham International) and incubated at ambient temperature on an orbital shaker in the dark for at least 6 h. Subsamples (3 to 5 ml) taken at 0 h were transferred immediately to test tubes containing an equal volume of ice-cold 10 % Trichloracetic acid (TCA; Analar, Merck) to halt the rapid initial uptake of substrate, and stored on ice for 20 minutes. Subsamples (3 to 5 ml) taken after 1, 2 ,3 and 6 h were placed in separate test tubes and chilled for one minute before the addition of equal volumes of 10 % ice-cold TCA. All tubes were stored on ice for 20 minutes before filtration of TCA-insoluble macromolecules onto 0.2 µm nitro-cellulose Millipore filters. Filters were rinsed twice with 5 % TCA (3 to 5 ml) and once with 10 ml sterile-filtered (<0.2 µm) seawater to remove aqueous and adsorbed ^{3}H thymidine. Vacuum-dried rinsed filters were

stored frozen in plastic scintillation vials until returned to the laboratory, where they were dissolved in liquid scintillation cocktail (Filter Count, Packard Instrument Co.).

Bacteria in controls were killed before addition of [methyl-^{3}H] thymidine, by autoclaving or addition of an equal volume of 10 % TCA, and treated in the same way as the experimental flasks.

Counts per minute (CPM) were detected in a Packard Tri-Carb 460C Scintillation spectrometer, and corrected for quenching with an external standard to obtain disintegrations per minute (DPM). Blanks (un-used filter papers plus scintillation cocktail) and control counts were subtracted from experimental counts.

The following formulae were used to calculate bacterial production:

1. $\text{moles ml}^{-1}\ \text{h}^{-1} = \dfrac{\text{DPM} \times (4.5 \times 10^{-13})}{\text{SA}}$

2. $\text{cells l}^{-1}\ \text{h}^{-1} = \text{moles l}^{-1}\ \text{h}^{-1} \times (1.7 \times 10^{18}\ \text{cells mol}^{-1})$

3. $\mu\text{g C l}^{-1}\ \text{h}^{-1} = \text{cells l}^{-1}\ \text{h}^{-1} \times \text{average cell biomass}$

where:

DPM = disintegrations per minute on the filter paper

SA = specific activity of [methyl-^{3}H] thymidine (Ci mol^{-1})

4.5×10^{-13} = number of curies per DPM

To improve the total production estimate the average cell carbon biomass was calculated separately for each experiment, using AODC estimates of total numbers and biomass of bacteria in the initial population. All morphotypes were assumed to be equally active.

Bacterial population growth

The temporal increase in bacterial cell numbers and biomass in a predator-reduced incubation may be used as a measure of bacterial production (Newell and Fallon 1982, Riemann et al. 1984). In this study, water was gravity-filtered through 3 μm Nuclepore membrane filters and incubated in sterile conical flasks (150 ml) on an orbital shaker at ambient temperature in the dark. Manipulation of the water was done as carefully as possible to minimise modification of bacterial growth rates. Samples (5 ml) of incubation water were removed using a Gilson pipetteman after 0, 1, 2, 3 and sometimes 6 h for estimation of bacterial numbers and biomass by the AODC method. Growth curves were fitted to the time-course data using the curve of best fit, according to the least sum of squares method (Zar 1984).

SECTION 3

LABORATORY STUDIES

CHAPTER 4

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

ABSTRACT

Recently upwelled water from the southern Benguela upwelling system was incubated in a 60 l microcosm for 43 days 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 (mean volume = 0.198 μm^3) and large cocci (mean volume = 0.142 μm^3), with Vibrionaceae as the dominant plateable strain exhibited slow turnover times for added ^{14}C-labelled substrates (mean = 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 (mean volume = 0.009 μm^3) and large rods (mean volume = 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-^3H] 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 x 10^{18} 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 and Robb 1985, Ducklow and Hill 1985a, b, Coffin and Sharp 1987). Estimates of bacterial production based on uptake or incorporation of radiolabelled substrates, such as adenine (Karl 1982) and thymidine (Fuhrman and Azam 1982, Moriarty 1986), do not always provide estimates of production comparable to other techniques (Newell and Fallon 1982, Ducklow and Hill 1985b). It seems probable that these difficulties may be explained partly by inadequacies of the techniques themselves (Moriarty 1986, Robarts and Wicks 1989) or by the non-uniform response of diverse bacterial populatons 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 uniformly to added tracer substrates (see Riemann et al. 1982, van Es and 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 and Hutchings 1980, Brown and Hutchings 1987a). Entrained bacterial communities are therefore advected from organically deficient deep waters where "starvation-survival strategies" are appropriate (Novitsky and 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 and 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 and 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 and 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 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 °/₀₀), 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 and 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 pre-soaked 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_2P0_4, 750 ml 0.2 µm filtered sea-water, 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 x 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 and 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 nM), glucose (10 nM), alanine (12 nM), aspartate (10 nM) and glutamate (10 nM). 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 sea-water containing 1 mM 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 x 10^6 cells ml^{-1} using the acridine orange direct counts.

Bacterial production.

Incorporation of [methyl-^{3}H] 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 nM [methyl-^{3}H] 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 and 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 x 10^{18} cells produced per mole of thymidine incorporated (Fuhrman and 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 x 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 and 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.

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

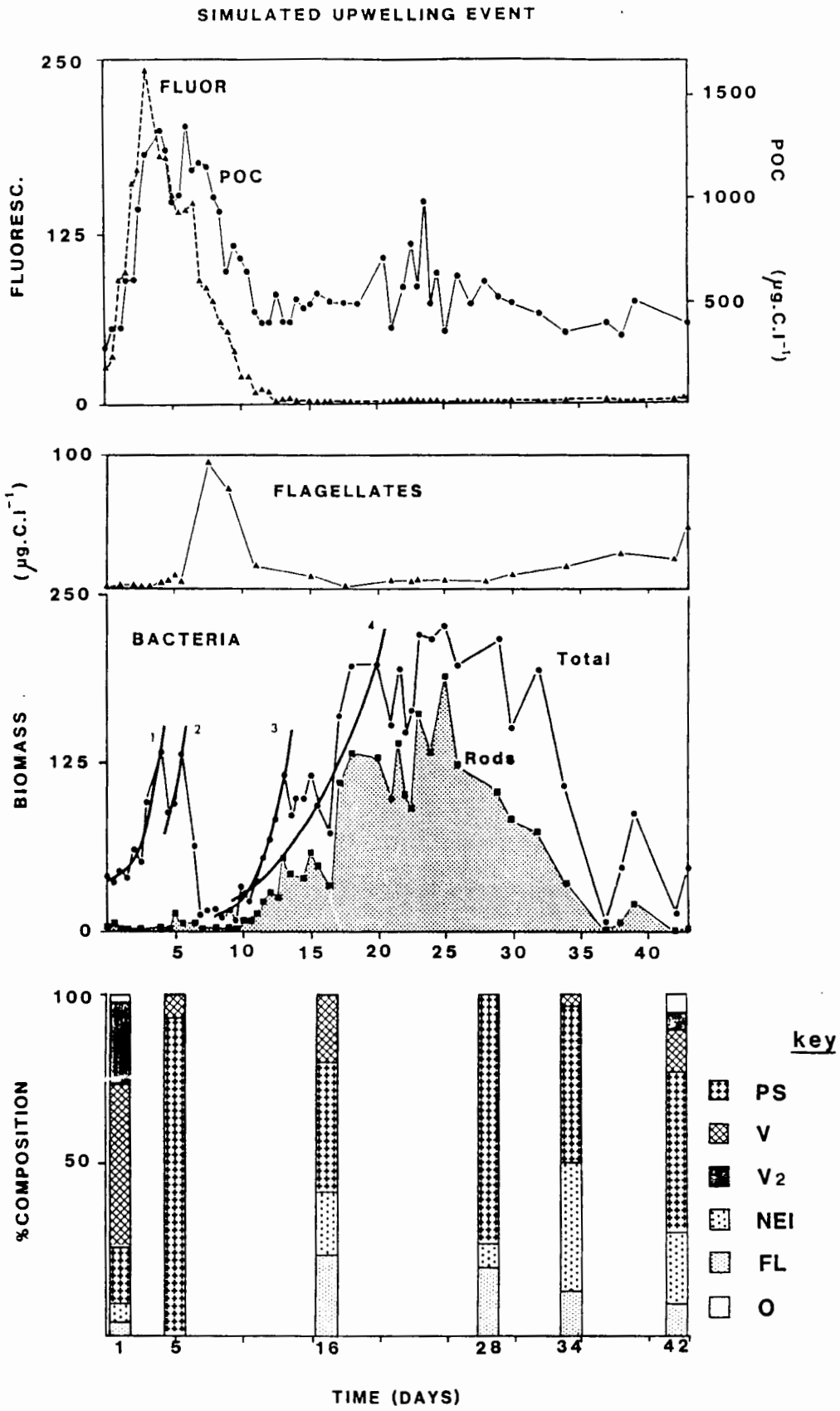


Fig. 1. Temporal relationship between phytoplankton, bacteria and flagellates after upwelling. (a) Changes in relative fluorescence and POC concentration during growth and senescence of the phytoplankton assemblage. (b) Biomass of bacteria and flagellates. The exponential curves marked 1 to 4 were fitted 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_1, 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 ($\mu g\ C\ l^{-1}$)	Bacteria numbers ($x10^6\ ml^{-1}$)	Flagellates biomass ($\mu g\ C\ l^{-1}$)	Flagellates numbers ($x10^3\ ml^{-1}$) A	Flagellates numbers ($x10^3\ ml^{-1}$) B	Particulate Carbon ($\mu g\ C\ l^{-1}$)
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

*The spherical flagellates Pseudobodo sp consisted of two types :

Type A - 1.92 μm x 2.31 μm, radius = 1.0525 μm, v = 4.88 μm^3
Type B - 3.85 μm x 3.85 μm, v = 29.88 μm^3

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 x 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 x 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 = 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 x 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 x 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 (mean vol = 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 x 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

Table 2. Exponential growth curves (1 to 4) for bacteria in the microcosm (see also Figs. 1b and 2b). Curve 1 = 0 to 96h, Curve 2 = 108 to 132h, Curve 3 = 168 to 312h, Curve 4 = 228 to 492h (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	$Y = ae^{bX} \pm$ S.E.	n	$F_{calculated}$	p	r^2	Net growth (h^{-1})
Bacterial numbers (x 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	$Y = 44.21\ e^{0.0210X} \pm 0.423$	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

displayed a restricted range of catabolic properties. Numerically the second bacterial community was dominated by small cocci (mean volume = 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 (mean vol = 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 in Chapter 5 (and 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 flagellate 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 (mean = 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 and

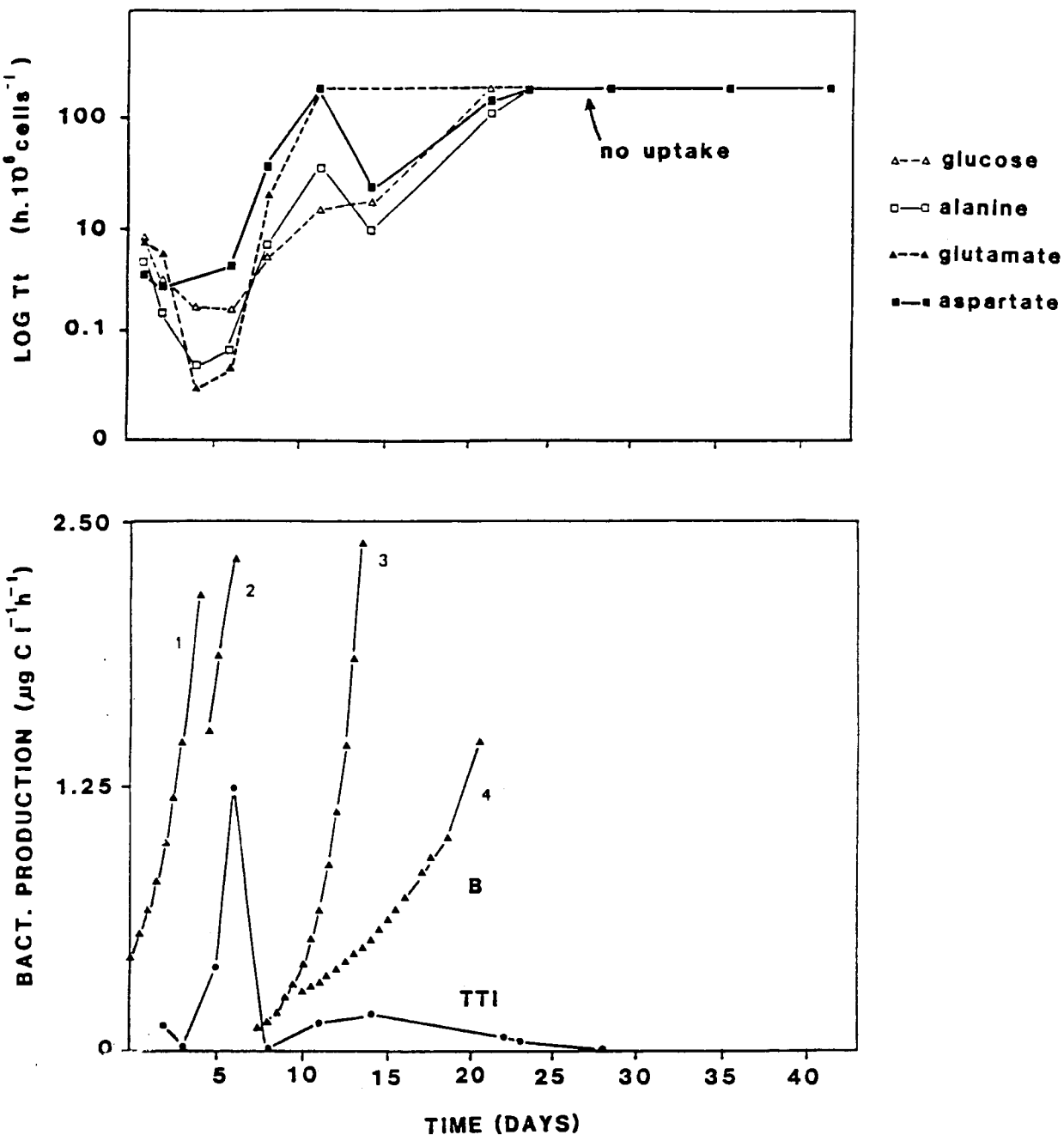


Fig. 2. (a) Turnover times of ^{14}C-labelled substrates, as an index of bacterial activity. (b) Estimates of bacterial production based on exponential growth of bacterial biomass (ΔB;▲) and incorporation of [methyl-^{3}H] thymidine (TTI;●).

Morita 1977, Morita 1984, Davis and Robb 1985, Hanson and Lowery 1985). For example, Novitsky and 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 and 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 and 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 and Ammerman 1984, Lancelot and 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 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 and Robb (1985) have demonstrated. Kirchman and 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.

Exponential curves ($Y = ae^{bx}$) fitted to the bacterial numbers and biomass data were highly significant ($p << 0.001$) 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 and Azam (1980, 1982), who showed that DNA comprised 80 % of the TCA-insoluble material in their bacterial samples. Similar results were reported by Scavia and 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 and 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 nM) expected to inhibit isotope dilution by de novo synthesis in marine bacteria (Fuhrman and 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 nM thymidine be used to inhibit isotope dilution. However, Davis (1989) found in our laboratory that high concentrations of Tdr (19 nM) inhibited TTI by 2 isolates (Pseudomonas and Cytophaga) from detritus dominated sea water of southern Benguela origin, whereas low concentrations of Tdr (9.1 µM) did not.

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) x 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 x 10^{18} cells mol^{-1} TTI (Fuhrman and Azam 1980, 1982) and the weighted mean cell biomass (calculated from Table 1).

Time (Day)	(h)	Net bacterial Production ($\times 10^6$ 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 (cells $l^{-1}h^{-1}$) ($\times 10^6$)	(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			

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 and 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 and 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.

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 (1989) 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 two 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 (1989) 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 nM) or low (9.1 μM) 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 and Reeve 1982). In addition, Ferguson et al. (1984) found that manipulation of natural sea water 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 and 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 1982c). 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 and 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.

Acknowledgements

I am grateful to Dr C.L. Davis (University of Cape Town) and Dr E.A.S. Linley (Plymouth Marine Laboratories) for many useful discussions on the work presented here, and to anonymous referees for constructive criticisms on an earlier draft of this manuscript. Dr D.G. Muir participated in this study, by doing the plating and characterisation of the bacterial community, and measurements of uptake of ^{14}C-labelled substrates. Dr M.I. Lucas provided valuable assistance in experimental design, sampling and during the writing of the final manuscript. Contributions by Drs Muir and Lucas are acknowledged by joint authorship on the published manuscript. Our thanks are extended to F. Verheye-Dua for assistance in sampling the microcosm. The financial support of the Benguela Ecology Programme (SANCOR) is gratefully acknowledged.

This chapter has been published in Marine Ecology Progress Series:

Painting, S.J., Lucas, M.I. and Muir, D.G. 1989. Fluctuations in heterotrophic bacterial community structure, activity and production in response to development and decay of phytoplankton in a micrososm. Mar. Ecol. Prog. Ser. 53: 129-141.

Chapter 5

An experimental study of microflagellate bacterivory: further evidence for the importance and complexity of microplanktonic interactions

ABSTRACT

By simulating an upwelling event in a laboratory microcosm it was possible to promote the development of a natural and diverse planktonic community. An initial bacterioplankton community which developed in response to phytoplankton growth was dominated by small coccoid forms (0.14 to 0.2 μm^3) of the families Vibrionaceae and Pseudomonadaceae. This group was heavily exploited by the heterotrophic microflagellate Pseudobodo sp. (30 μm^3). Later, the bacterioplankton community was dominated by large rods (0.7 μm^3) which the flagellates seemed unable to exploit. A Lotka-Volterra predator-prey model fitted to the observed data indicated that the flagellates consumed 2.4 times their carbon body mass per day or 19 bacteria flagellate^{-1} h^{-1} when prey were not limiting. Clearance rates were inversely proportional to prey density and ingestion rate, ranging from 2 x 10^{-3} to 20 x 10^{-3} μl flagellate^{-1} h^{-1}. At typical field densities of bacteria and heterotrophic flagellates in the southern Benguela region, between 5 and 30 % of the water column could be cleared per day. Specific growth rates of the flagellates were positively related to prey density, the maximal rate being 0.84 d^{-1}. Their initially faster growth rates allowed bacteria to increase in numbers despite predation. The net growth yield of the flagellates (34 to 36 %) was also positively related to food density. Such low values suggest inefficient transfer of carbon to higher trophic orders but considerable nitrogen regeneration. Nitrogen excretion rates were approximately 6 to 7 μg N mg dry weight^{-1} h^{-1}, comparable to other flagellates but faster than ciliates. These rates are comparable with in situ measures of NH_4-N excretion in pycnoclinal regions based on ^{15}N isotope studies but are only about 20 % of measured rates in surface waters. This is interpreted to mean that, in pycnoclinal regions where the relative input of "new" nitrogen is high, there are few regenerative steps and the model describes them satisfactorily. In surface waters, observed excretion rates can

only be accounted for by many regenerative steps in a highly complex food chain in which the cumulative total of nitrogen excretion at each step amounts to that based on ^{15}N labelling studies.

INTRODUCTION

"New" production in surface waters of productive upwelling and coastal systems is typically of the order of 50 to 70 %, indicating the dependence of primary production on nutrients advected from deeper waters into the euphotic zone (Harrison 1978, Eppley and Petersen 1979, Probyn 1985). Although the dependence of primary production on epipelagically produced nutrients is low, absolute rates of nutrient regeneration may be an order of magnitude higher in these productive systems relative to oceanic waters, where up to 80 % of the primary production is dependent upon low rates of regenerated nutrient supply (Harrison 1978, Probyn 1987). Evidently, high rates of primary productivity stimulate heterotrophic activity, which accounts for the high rates of "regenerated" nutrient supply. This raises the question: which organisms contribute most significantly to regenerative processes, particularly the reduced nitrogen species (ammonia and urea), because nitrogen has most frequently been demonstrated to be limiting with respect to other important nutrients (P and Si. See Falkowski et al. 1983, Holligan et al. 1984a, b, Probyn and Lucas 1987).

Recent evidence indicates the consistent and greater importance of small organisms (<150 to 200 μm) relative to meso- and macro-zooplankton in the regeneration of ammonia, urea and phosphorous in surface waters (Glibert 1982, Harrison et al. 1983, Holligan et al. 1984b, Goldman et al. 1985, Probyn 1987, Probyn and Lucas 1987). Indeed, uptake requirements of the entire autotroph community can frequently be met by regenerative processes in the <200 μm size class (Holligan et al. 1984b, Probyn 1987, Probyn and Lucas 1987). It is seldom that meso- and macro- zooplankton account for more than 30 % of phytoplankton nitrogen requirements except in stable or oceanic environments (Falkowski et al. 1983). Size-fractionated studies of nitrogen and phosphorous uptake and

regeneration have demonstrated that autotrophs and heterotrophs within the nanoplankton (<20 μm) and picoplankton (<1 μm) communities form the major pathways for nutrient fluxes (Glibert 1982, Harrison et al. 1983, Joint and Williams 1985, Probyn 1985, Joint et al. 1986, Probyn and Lucas 1987). However, it is not always clear what the relative roles of the microheterotrophs are in these processes.

Microheterotrophs (<200 μm) including bacteria, protozoans (e.g. microflagellates, loricate and aloricate ciliates) and juvenile zooplankton stages (e.g. nauplii and copepodites) may dominate the biomass of planktonic communities (Holligan et al. 1984a) but the interactions between these organisms are often complicated by an uncertain trophic status (e.g. autotrophy and/or heterotrophy) and relative sizes (Fenchel 1982a, b, Taylor 1982, Sherr and Sherr 1983a, b, Fuhrman and McManus 1984, McManus and Fuhrman 1986, Parslow et al. 1986, Sherr et al. 1986a, b). For example, the microflagellate Paraphysomonas imperforata may be both herbivorous and bacterivorous, consuming diatoms (Phaeodactylum tricornutum) almost as large as itself (approx 150 μm^3) and removing approximately 65 % of the POC (particulate organic carbon) during an 8-day incubation experiment. Furthermore, the microflagellate was responsible for remineralising the major fraction of PON (particulate organic nitrogen) and phosphorus. Such organisms may therefore be important consumers of both phytoplankton and bacterioplankton production (see Caron et al. 1985, Goldman and Caron 1985, Goldman et al. 1985). Another microflagellate, Ochromonas sp., contains a chloroplast, although it is doubtful if this flagellate can depend on autotrophy alone as it is probably an obligate bacterivore (Andersson et al. 1985). Nevertheless, it is clear that bacterioplankton may form up to 20 % of the living carbon biomass (Ducklow et al. 1986, Lucas 1986) and account for as much as 50 to 75 % of the total planktonic community respiration (Williams 1981). This implies that at least

50 % of the photosynthetically fixed primary production must pass through this community (Williams 1981, Fuhrman and McManus 1984, Newell and Linley 1984, Ducklow et al. 1986, Lucas et al. 1986). Notably, however, bacterial numbers and biomass in the world's oceans remain relatively constant despite bacterial production rates of 1 to 8 x 10^9 cells l^{-1} d^{-1} in many regions (Lucas 1986) and population doubling times in the order of 14 to 20 h. This implies removal of the bacterial production by predation or natural mortality. However, studies by Morita (1984) and others indicate that bacterioplankton can remain viable for up to 1 yr under starvation conditions, suggesting that predation rather than starvation-induced mortality is the major regulator of bacterial density.

Early work by Linley et al. (1983) provided evidence that bacterial densities in shelf waters were regulated by flagellate and ciliate predation. Fenchel (1982d) demonstrated that flagellates could be major consumers of bacteria, one small choanoflagellate (Monosiga sp.) consuming approximately 27 bacteria flagellate^{-1} h^{-1} or about 60 per cent of the flagellate body volume h^{-1}. More recently, Andersen and Fenchel (1985) estimated that, in situ, the small flagellate Pseudobodo tremulans (approx 3 μm diameter) consumed between 48 and 73 bacteria h^{-1}, thus removing the available bacterial production and clearing between 5 and 250 % of the water column per day. However, bacterial numbers below 10^6 ml^{-1} could not sustain flagellate growth. Ciliates (e.g. Euplotes and Uronema) may also be major predators of bacteria, particularly the larger rods of 1 to 2 μm length (Turley et al. 1986) although studies by Goldman and Caron (1985), Parslow et al.(1986) and Sherr et al. (1986b) indicate that many of these organisms cannot be allocated into exclusively autotrophic, heterotrophic or bacterivorous compartments. Indeed, some tintinnid and other ciliates may be capable of regulating phytoplankton standing stocks in the absence of predation (Verity 1985). Furthermore, some workers (Joint and Williams 1985) were unable to reconcile the high estimates

of flagellate predation obtained from laboratory cultures with field measurements, where their estimates of bacterial production (for the Celtic Sea) would have been unable to sustain flagellate predation at the high rates suggested.

The relative roles of nutrient regeneration ascribed to protozoa and bacteria have attracted considerable controversy. Recent evidence does, however, suggest that flagellate predation rather than bacterial mineralisation per se is responsible for the major proportion of nitrogen regeneration (Andersson et al. 1985, Goldman et al. 1985, Goldman and Caron 1985). In this context, the quality of the food, the assimilation efficiency of the organisms and the predation rate have a considerable bearing on nutrient cycling rates and on one's view of the microbial food chain. As Goldman et al. (1985) elaborated, the high assimilation efficiencies they noted for flagellate predation on bacteria necessitate a highly complex and long microbial food chain to account for the high estimates of regenerated nutrient supply in epipelagic waters. A further implication of this latter view is that the microbial food chain is likely to be a major sink for carbon, particularly if in excess of 50 % of the primary production passes to heterotrophic bacteria which utilise this resource with a growth efficiency of <30 % (see Linley and Newell 1984, Bjornsen 1986 and Lucas 1986). If this is indeed the case, little material can be transferred to higher trophic levels through the microbial food chain (see also Ducklow et al. 1986).

Central to an understanding of the role of bacteria and flagellate interactions is an understanding of their production rates, carbon requirements and more specifically the predation rate and assimilation efficiency of the flagellates. The relationship between predation rate and assimilation efficiency will determine the magnitude of regenerative processes due to

predation. In this paper the development of a microbial community in response to phytoplankton growth in a microcosm which simulated an upwelling event is described. Further, we have attempted to fit a predator-prey model to our data on bacterial and flagellate interactions in an attempt to define more precisely the predation rate, growth efficiency and nitrogen-regeneration rates originating from flagellate bacterivory. Finally, the predicted values for flagellate predation, growth efficiency and nitrogen regeneration have been used to determine whether the observed numbers of heterotrophic flagellates in the field could remove the bacterial production and simultaneously supply regenerated nitrogen at the rates measured by ^{15}N isotopic dilution studies.

METHODS

Development of the planktonic community

The objective was to promote the development of a natural and diverse planktonic community by simulating an upwelling event in a laboratory microcosm. In this way it was hoped to model microbial interactions in an environment that closely resembled the field.

It is important to realise that newly upwelled Southern Atlantic Central Water is distinctly characterised by a specific temperature and salinity (10 °C and 34.80 $^{o}/_{oo}$) and also by high nitrate (15 to 20 µg-at l^{-1}) and silicate (10 to 15 µg-at l^{-1}) concentrations, although chlorophyll *a* concentrations are negligible (see Waldron 1985). Incubation of this water at a near-ambient light and temperature regime promotes the development of a laboratory-based phytoplankton bloom and associated microbial community in a manner similar to that described for the natural environment (Barlow 1982c, Lucas et al. 1986, Painting et al. 1989).

Newly upwelled water from the Southern Benguela region was incubated in a 60 l aquarium at 12 °C for 43 days and sampled regularly. Details are given in Painting et al. (1989). Phytoplankton growth was detected by relative u/v fluorescence using a Turner model III fluorometer and by increments in particulate carbon retained on Whatman GF/F filters and analysed by combustion on a Hereaus CHN analyser. Bacteria (7 size categories) and flagellates (2 size categories) were counted by the AODC method of Hobbie et al. (1977) and Linley et al. (1981). Bacterial and flagellate biomass was calculated on the basis of estimates of biovolume (Linley et al. 1983, Painting et al. 1985), a cell density of 1.1 g cm^{-3} (Calkins and Summers 1941) and a carbon content of 0.1 x wet biomass. Dry biomass is equivalent to 0.2 x wet biomass (Luria 1960, Troitsky and Sorokin 1967).

Phytoplankton production

An estimate of primary production was obtained by fitting a linear regression ($Y = a + bX$) to the increase in POC concentration occurring in the microcosm between 0 and 96 hours (see Table 1 and Fig. 1).

Bacterial production

Bacterial population growth (production) was estimated by fitting an exponential curve ($Y = ae^{bx}$) to the biomass data up to Day 4 (96 h) as shown in Figure 2. From this curve-fit, bacterial biomass and production could be calculated up to and beyond 96 h (Table 2). This allows estimation of the projected biomass and production of bacteria in the absence of predators, this being important in the calculation of flagellate predation. Clearly, however, continued exponential growth would be restrained by substrate limitation. An

Table I: Numbers of bacteria and flagellates, biomass and total POC

Time (d)	Time (h)	Bacteria		Flagellates			Particulate carbon POC ($\mu gC \cdot l^{-1}$)
		Biomass ($\mu gC \cdot l^{-1}$)	Numbers ($\times 10^6 \cdot ml^{-1}$)	Total biomass ($\mu gC \cdot l^{-1}$)	Total numbers ($\times 10^3 \cdot ml^{-1}$) A*	Total numbers ($\times 10^3 \cdot ml^{-1}$) A + B*	
	0	40,08	2,118	0,392	0,582	0,582	290
	12	38,24	1,952				
1	24	46,19	2,819	1,480	2,199	2,199	500
	36	41,61	2,734				
2	48	60,84	3,352	2,077	2,696	2,780	800
	60	53,30	3,994	2,399	1,303	1,792	
3	72	98,66	5,378	0,617	0,916	0,916	1 230
4	96	136,05	8,071	5,120	0,068	1,697	1 330
	108	89,16	6,403	6,166	1,040	2,794	
5	120	96,22	5,982	11,413	2,094	5,305	1 025
	132	133,08	7,839				
6	144						1 250
	156	67,46	5,079				
7	168	13,43	1,550				1 175
	180	16,45	1,905	95,723	0,153	30,850	
8	192	16,99	1,652				985
9	216	14,77	1,946	74,529		23,926	720
11	264	40,87	2,907	16,963		5,445	
15	360	118,80	6,371	10,246		3,289	
21	504	156,95	7,975	7,388		2,372	
25	600	230,47	9,260	6,230		1,947	

* The spherical flagellates *Pseudobodo* sp. in the microcosm consisted of two types:
Type A — 1,92 $\mu m \times 2,31$ μm (radius 1,0525 μm); $v = 4,88$ μm^3
Type B — 3,85 $\mu m \times 3,85$ μm; $v = 29,88$ μm^3

S.G. of the flagellates = 1,0425 (Calkins and Summers 1941)

N.B. No samples were taken on Day 3,5

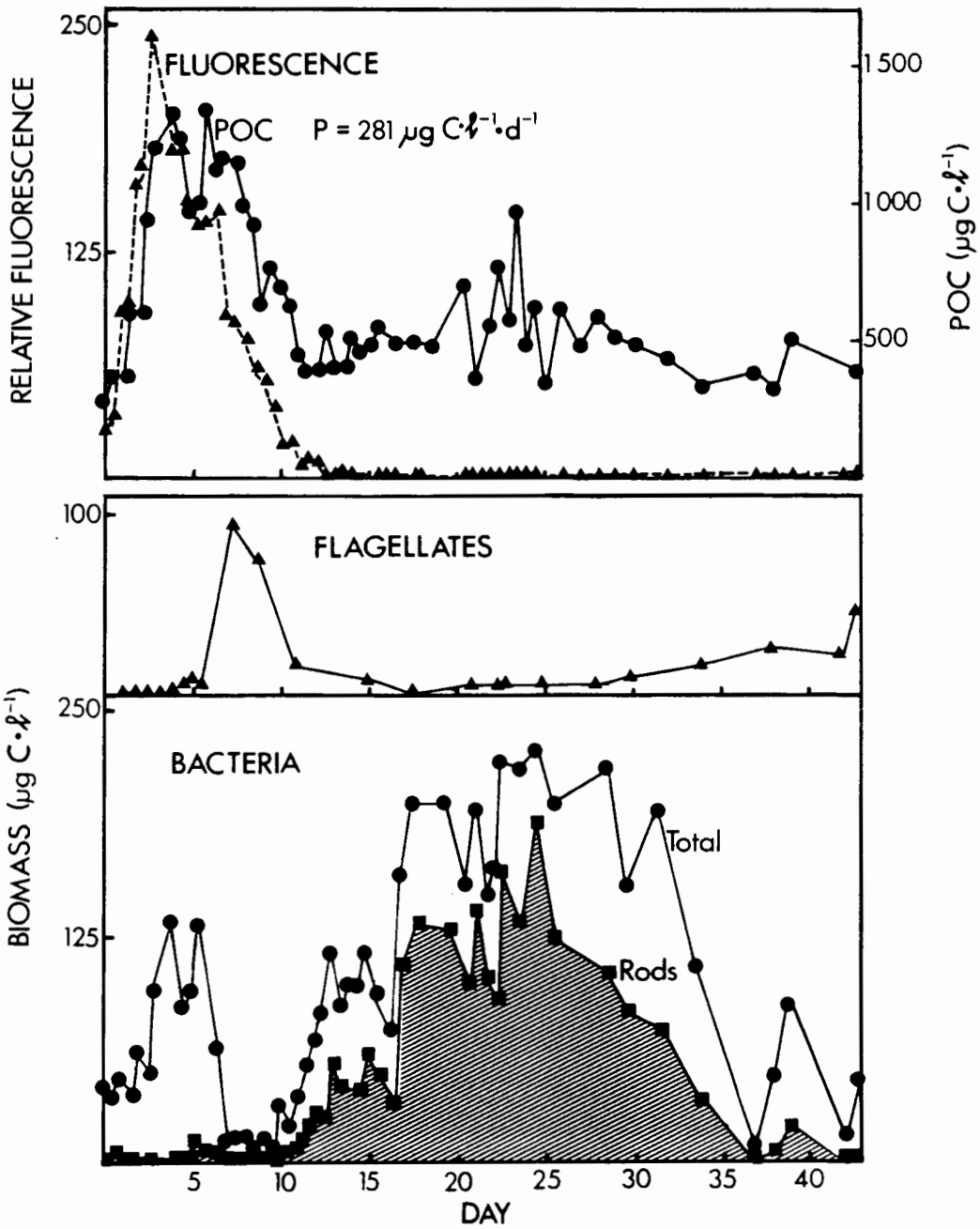


Fig. 1. Development of a natural planktonic community at 12 °C in a laboratory microcosm simulating an upwelling event. Change in phytoplankton abundance are expressed in terms of chlorophyll fluorescence and particulate organic carbon (POC).

Table 2 Bacterial growth and carbon requirements

Time		Biomass ($\mu gC \cdot l^{-1}$)		Production‡ ($\mu gC \cdot l^{-1} \cdot d^{-1}$)	Specific growth rate μ ($\cdot h^{-1}$)	Carbon consumption $C_c = P_c/0,21$** ($\mu gC \cdot l^{-1} \cdot d^{-1}$)	Cumulative carbon requirements*** ($\mu gC \cdot l^{-1}$)
Days	Hours (x)	Observed (y)	Predicted* (y)				
0	0	40,08	27,79	10,87	—	51,76	
0,5	12	38,24	33,79	13,22	—	62,95	
1	24	46,19	41,09	16,07	0,016	76,52	62,9
1,5	36	41,61	49,96	19,54	—	93,04	
2	48	60,84	60,75	23,76	0,032	113,14	155,9
2,5	60	53,30	73,87	28,90	—	137,62	
3	72	98,66	89,83	35,14	0,051	167,33	293,5
4	96	136,05	132,82	51,95	0,013	247,38	540,8
4,5	106	89,16	161,58	63,21	—	301,00	
5	120	96,22	196,48	76,86	0,006	366,00	841,8
5,5	132	133,08	238,95	93,48	0,027	445,14	

* From an exponential curve fit $y = ae^{(bx)}$ to the biomass data up to 96 h: $y = 27,787e^{(0,0163\ x)}$

‡ Production = predicted $y \times b$, where $b = \mu = 0,0163 \cdot h^{-1}$ ($0,40 \cdot d^{-1}$)

** Carbon consumption by bacteria C_c is calculated from bacterial carbon production P_c and their net growth efficiency, in this case 21%

*** Calculated from the median production rates for each day, 1–5

† μ is calculated over each time interval from the observed biomass data

N.B. No samples were taken on Day 3,5

Table 3 Flagellate growth parameters

Time (d)	Production ($\mu gC \cdot l^{-1} \cdot d^{-1}$)	Specific growth rate μ* ($\cdot h^{-1}$)	Generation time $1/\mu$ (h)	Doubling time $0,693/\mu$ (h)
2,5	0,324	0,0241	41,49	28,75
3,0	0,652	0,0300	33,33	23,10
3,5	1,268	0,0332	30,12	20,87
4,0	2,420	0,0347	28,81	19,97
4,5	4,564	0,0352	28,41	19,68
5,0	8,513	0,0352	28,41	19,68
5,5	15,596	0,0347	28,81	19,97
6,0	27,419	0,0333	30,03	20,81
6,5	42,284	0,0292	34,24	23,73
7,0	26,957	0,0123	81,30	56,34

* $\mu = (\log W_2 - \log W_1)/(t_2 - t_1)$

† Net growth yield = C production/C consumption ($\mu gC \cdot l^{-1} \cdot d^{-1}$).100

upper limit to the bacterial biomass (B_{max}) was therefore estimated on the basis of the available carbon and the carbon requirements of the bacteria (Tables 1 and 2).

Lotka-Volterra model parameters

Microbial dynamics in our microcosm were described by fitting a modified Lotka-Volterra model to the biomass data (always in terms of carbon) describing bacterial and flagellate populations (Fig. 2). Similar models have been used previously to describe bacterivory by microflagellates (see, for example, Andersen and Fenchel 1985, Parslow et al. 1986). Changes in bacterial biomass (B) with time (dB/dt) are a function of bacterial growth and predation pressure by flagellates, this being dependent on the flagellate biomass (F):

$$dB/dt = \mu.B(1 - B/K) - V_{max}FB/(K_S + B) \qquad (1)$$

where $\mu.B\ (1 - B/K)$ describes density-dependent growth of bacteria which is limited by the carrying-capacity (K) of the microcosm. The specific intrinsic growth rate ($\mu = 0.05\ h^{-1}$) for bacterial growth was calculated from the bacterial biomass data (B) over the time interval 60 - 72 h (Table 2). Prior to this, the bacterial community had not entered log-phase growth and, later, growth was influenced by predation. The carrying-capacity (K) is determined by the amount of available substrate (total carbon) and the carbon consumption requirements of the bacteria. Heterotrophic bacterial carbon requirements (C_C) are estimated as a function of bacterial carbon production (P_C) and their net growth efficiency (NY_g), now considered to be approximately 21 % for utilisation of photosynthetically produced dissolved organic carbon (PDOC) (Linley and Newell 1984, Bjornsen 1986, Lucas 1986). Thus bacterial carbon

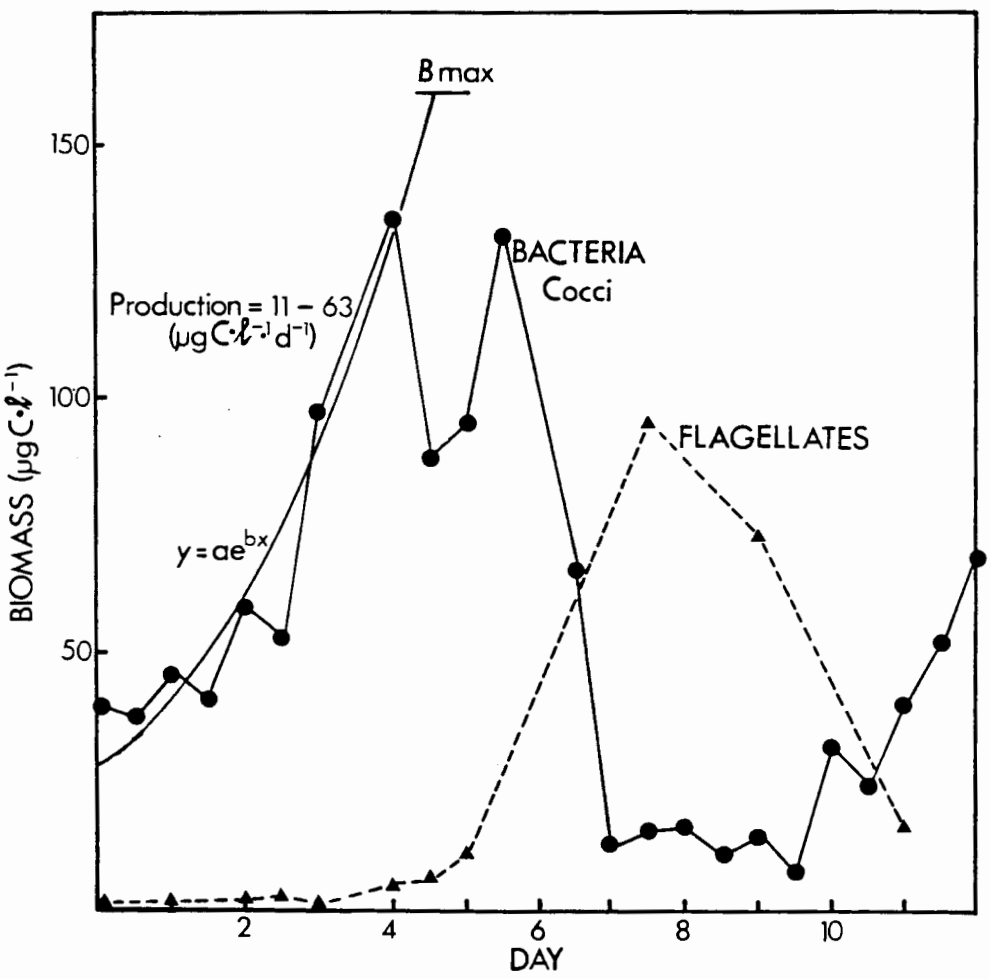


Fig. 2. Development of the initial bacterial community and flagellate consumers. An exponential curve was fitted to the period of exponential growth to determine the maximum potential bacterial biomass (B_{max}) in the absence of predation and limited only by substrate availability.

requirements to sustain production (see Table 2) can be calculated from:

$$C_C = P_C/NY_g \qquad (2)$$

The quantity of carbon available to bacteria (as PDOC) was estimated on the basis that approximately 30 % of gross primary production is secreted as PDOC (Lancelot 1979, 1984, Larsson and Hagström 1982, Lancelot and Billen 1984). Net primary production was calculated to be 281 μg C l^{-1} d^{-1} (see later). An approximate estimate of gross primary production (disregarding respiratory losses) would therefore be 365 μg C l^{-1} d^{-1} (net + 30 %), where PDOC secretion at 30 % of gross production represents 109.5 μg C l^{-1} d^{-1} or a cumulative total of 438 μg C l^{-1} by Day 4 at the peak of the phytoplankton bloom (Fig. 1 and Table 1). This quantity could meet bacterial carbon demands until about Days 3 to 4 (Table 2). Thereafter, bacterial carbon demands probably depended to some extent on POC (detritus) utilisation.

From Table 1 there is some evidence that particulate carbon declined by approximately 93 μg C l^{-1} d^{-1} ($Y = 1683.5-3.86\ X$, $r = 0.79$, $n = 6$, $p < 0.05$) so that, up to Day 5, the total available carbon (PDOC + POC) was very approximately 530 μg C l^{-1}. As bacterial carbon requirements up to Day 4 (Table 2) amounted to approximately 540 μg C l^{-1}, it is clear that carbon availability probably limited population growth soon after. It is unlikely, therefore, that bacterial growth could have proceeded exponentially beyond 96 h (Day 4) as predicted in Table 2. More likely, population growth could have been expected to approach an asymptote in a logistic fashion somewhere between 108 and 120 h, when bacterial biomass was unlikely to have attained a value exceeding 200 μg C l^{-1}, as predicted from the exponential equation for 120 h (Day 5). We, in fact, set a more conservative and somewhat arbitrary value for K of 165 μg C l^{-1} (B_{max}), which accords well with the predicted biomass at 106 h (see Table 2).

Changes in the bacterial population are also a function of density-dependent flagellate predation which is described by the Michaelis-Menten expression in Equation 1, V_{max} FB/(K_S + B). Here, F is the observed flagellate biomass (Table 1) and V_{max}, an unknown model parameter, is the maximum specific flagellate predation rate (units of bacterial carbon mass consumed per unit flagellate carbon mass per day). The half saturation constant, K_S (= 40 μg C l^{-1}), represents the bacterial density at 0.5 V_{max}. This value was estimated as the bacterial density (approximately 2 x 10^6 cells ml^{-1}) at the beginning of the experiment (Table 1) which corresponds also with literature estimates of K_S (6.4 to 120 μg C l^{-1}, approximate mean = 30 μg C l^{-1}, Andersen and Fenchel 1985, Parslow et al. 1986). This is close to the "threshold" prey density below which bacterial densities limit flagellate population growth.

Flagellate population growth (dF/dt) can be described by the predation rate, V_{max} FB/(K_S + B) and by the assimilation efficiency of the flagellates, α. In order to curb unrestricted flagellate population growth, a density-independent mortality factor (Z) is included. Changes in flagellate population growth can therefore be described by:

$$dF/dt = \alpha[V_{max}FB/(K_S + B)] - ZF \qquad (3)$$

The net assimilated bacterial biomass (i.e. that incorporated) is a function of the assimilation efficiency and predation of flagellates as described by $\alpha[V_{max}FB/(K_S + B)]$, where V_{max} and α are unknown. A constant density-independent mortality ($Z = 0.1\ d^{-1}$) was ascribed to the flagellate population for the whole period. Note that these parameters governing flagellate population growth also indirectly influence bacterial biomass (see Equation 1).

Nitrogen regeneration (r), associated with flagellate predation on bacteria can be estimated from the difference between predation and the assimilated biomass where the C:N ratio of bacteria and flagellates is considered to be 4:

$$r = V_{max}FB/(K_s + B) - \alpha[V_{max} FB/(K_s + B)] \quad (4)$$

Four of the six parameters in the model (μ, K, K_s, Z, V_{max} and α) were estimated from the observed microcosm data. Only V_{max} and α were estimated from the model. Initial values for bacterial biomass (B = 28.0 μg C l^{-1}) and flagellate biomass (F = 0.4 μg C l^{-1}) were assigned to the starting time of the model, these being obtained from the exponential curve fitted to the bacterial biomass data (Table 2) and from the initial observed flagellate biomass (Table 1).

To estimate V_{max} and α, the model was executed with every combination of V_{max} and α, where the tested range for α was 0.1 to 0.9 (increments of 0.1) and for V_{max} 1 to 4 (increments of 0.1). Values for V_{max} and α that provided predictions of bacterial and flagellate biomass changes which best fitted the observed data (Table 1 and Figure 2) were taken as the solutions. Approximately 300 estimates of dB/dt and dF/dt were produced on the basis of the different combinations of V_{max} and α. All predicted population data which varied by more than 15 % from the observed data for either bacteria or flagellates at their peak biomass (Table 2) were rejected. Successional timing of the peaks had also to be within 1 day for the data to be considered further. This reduced the combinations of V_{max} and α to 9, where V_{max} ranged from 2.9 to 3.1 and α from 0.4 to 0.6. To arrive at the best fit of predicted changes in bacterial and flagellate biomass for these combinations necessitates statistical treatment of the data (e.g. see Parslow 1986). However, as the ecological significance and

interpretation of the results is not influenced by the choice of V_{max} and α within the final range tested, this was not done.

RESULTS AND DISCUSSION

Development of the planktonic community

As a result of favourable nutrient and light conditions, a phytoplankton bloom developed rapidly within the first five days, attaining a maximum chlorophyll a concentration of approximately 19 $\mu g\ l^{-1}$ and a POC concentration of 1330 $\mu g\ C\ l^{-1}$ by Day 4 (see Fig. 1 and Table 1). The increase in POC represents a net primary production rate of 281 $\mu g\ C\ l^{-1}\ d^{-1}$ (24 h) based on the linear increase in particulate carbon ($Y = 268+11.71\ X$; $r = 0.99$, $n = 5$, $p < 0.001$). By Day 10 the chlorophyll a concentration had declined to near zero (in terms of u/v fluorescence), though POC concentrations (detritus) remained at approximately 500 $\mu g\ C\ l^{-1}$ for the remainder of the experiment. Comparisons of primary production and biomass between our microcosm and the southern Benguela upwelling region are not entirely satisfactory for many obvious reasons. Nevertheless, such a comparison is necessary in order to evaluate how closely our microcosm matches the natural environment. Our data for primary production approximates to 12 $\mu g\ C\ l^{-1}\ h^{-1}$ (12-h day) at a peak biomass of 19 μg Chl a l^{-1} on Day 4 (hourly P/B = 0.63) and falls within the range of biomass data and rates given by Brown and Field (1986) as characteristic of mature or aged upwelled water. Particulate carbon concentrations in the microcosm were characteristic of in situ concentrations (Lucas et al. 1986), and the development time of the bloom in the microcosm (4 to 5 days) was typical for the environmental situation (Barlow 1982c).

Primary production within the microcosm promoted the development of a physiologically and morphologically diverse bacterioplankton community which exhibited complex responses to the variable nature of the available carbon substrates (see Painting et al. 1989). The community biomass changes for both bacteria and flagellates are summarised in Figs. 1 and 2. The initial bacterial community of Vibrionaceae and Pseudomonadaceae genera was dominated (numbers and biomass) by large cocci and small rods (approximate volumes = 0.142 and 0.198 μm^3 respectively) which were replaced after Day 5 by bacterivorous flagellates dominated by what appeared to be Pseudobodo sp. (mean volume = 30 μm^3). The initial flagellate population was dominated by a smaller form (approximately 5 μm^3) of Pseudobodo, possibly the starved "swarmers" (25 % of the trophic form by volume) described by Fenchel (1982a, c) as characterising unstable environments. There does seem to be some ambiguity about the taxonomic status of Pseudobodo as the genus Fenchel (1982a) describes is considerably larger (90 μm^3) than that described by Parslow et al. (1986), which ranged in size from 4 to 33 μm^3 and also appeared to differ morphologically. In this study, the flagellates appeared to closely resemble the Pseudobodo sp. described by Parslow et al. (1986) and had a dry carbon mass of 3.115 pg C $cell^{-1}$ or 0.104 pg C μm^{-3}. The estimate of dry mass (approximately 0.21 pg μm^{-3}, 2 x carbon mass) is in close agreement with that of Sherr et al. (1983) for some ciliates. The flagellates were in turn largely replaced after Day 10 by a bacterial community dominated by large rods (mean volume = 0.67 μm^3), consisting of Flavobacteriaceae and Cytophaga/Neissereaceae amongst the plateable forms. Towards the end of the experiment (after Day 30), the bacterial population biomass declined rapidly, concurrent with a change in community structure as small coccoid forms re-emerged (see Painting et al. 1989). Well-ordered successions of bacterial strains associated with phytoplankton growth and decomposition processes have previously been described by Fukami et al. (1985b).

Complex successions of phagotrophic microflagellates and other protozoans (autotrophic and/or heterotrophic) usually characterise microzooplankton food chains (Goldman and Caron 1985, Parslow et al. 1986, Sherr et al. 1986a, b, Turley et al. 1986), although the rather simple succession of bacteria and phagotrophic micro-flagellates shown in Fig. 2 greatly facilitates predator-prey modelling.

At the maximum of bacterial biomass on Day 4, bacterial production (52 μg C l^{-1} d^{-1}) was approximately 20 % of our estimate of net primary production (281 μg C l^{-1} d^{-1}) and bacterial biomass (136 μg C l^{-1}) was approximately 10 % of total carbon. These values are typical of many environments (for reviews, see Linley and Newell 1984 and Lucas 1986) and indicate that the experimental system used in this study did indeed closely model microplankton communities in the southern Benguela upwelling environment. Microbial interactions within this system can therefore be expected to provide a realistic perception of these events in the field.

Model predictions of Vmax and α

The best "by-eye" fit of the model predictions for changes in bacterial and flagellate biomass (dB/dt and dF/dt) were achieved for values where the maximum specific flagellate predation rate, V_{max}, was 3.0 and flagellate assimilation efficiency, α, was 0.4, as shown in Fig. 3. Parslow et al. (1986) do, however, warn that parameters predicted by simple Lotka-Volterra Models may not remain consistently valid because of complex predator-prey responses to a non-steady-state environment.

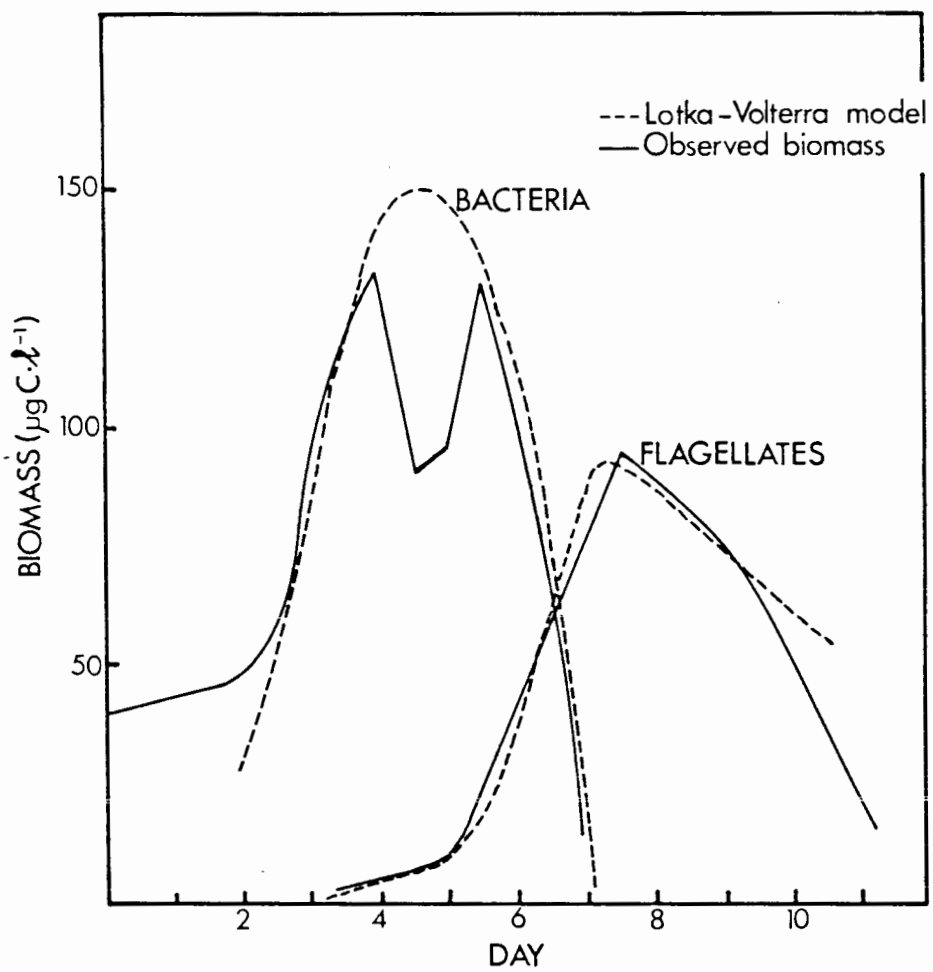


Fig. 3. The fit of the Lotka-Volterra model to the observed biomass data for bacteria and flagellates.

Flagellate growth

Flagellate growth rates in this study (see Table 3) showed a positive response to increasing prey densities, as has often been previously noted (e.g. Fenchel 1982d, Sherr et al. 1983, Caron et al. 1985). This implies a close coupling between bacterial production and flagellate predation, thus accounting for the observations of a relatively stable bacterial population (approximately 10^6 cells ml^{-1}) in the southern Benguela environment despite considerable fluctuations in bacterial production (Lucas 1986). Such close coupling is likely to confer adaptive predation advantages in unpredictable environments such as upwelling systems (see also Banse 1982).

Growth rates and doubling times were considerably slower than those frequently recorded (see Table 4), but this may be accounted for by the low and rapidly limiting prey density as well as the low temperature. For example, Verity (1985) demonstrated that the growth rates of two tintinnid species (Table 4) were positively dependent on the food concentration but also increased by a factor of 1.5 to 2.0 with a 10 °C temperature increase. In the present experiment, maximum growth rates for Pseudobodo sp. were 0.84 d^{-1} (doubling time = 20 h) at comparable prey densities (9 x 10^6 cells ml^{-1}) to those in Fenchel's (1982b) studies, but at a temperature almost 10 °C cooler. If growth rates in this study are corrected for the temperature differential on the basis of Verity's (1985) factors, then a growth rate of approximately 1.3 to 1.7 d^{-1} could be expected. This is within the range estimated by Fenchel (1982b) for Pseudobodo tremulans when feeding on a non-limited bacterial resource.

When food concentrations are limiting, lower growth rates can be expected. In a study in Kaneohe Bay, Hawaii, where heterotrophic microflagellates were

Table 4 : A summary of some values for growth and feeding in flagellates and ciliates

PREDATORS							PREY			
Flagellates	Vol. (μm^3)	Numbers ($\times 10^3 \cdot ml^{-1}$)	Growth ($\cdot d^{-1}$)	Growth efficiency (%)	Ingestion rate (cells $\cdot h^{-1} \cdot pred^{-1}$)	Clearance rate ($\mu l \times 10^{-3} \cdot h^{-1} \cdot pred^{-1}$)	Prey species	Vol. (μm^3)	Numbers ($\times 10^6 \cdot ml^{-1}$)	Source
Pseudobodo sp.	30	1-11	0,8	35-36	18-19	2-20	Large cocci & small rods	0,142 0,198	7 9	This study at 11-12°C
Monosiga sp.	20		1-3		27	2			5 35	Fenchel (1982b)
Paraphysomonas vestita	190	0,1-1,0	2-5		254	17			10-115	batch cultures at 20°C
Actinomonas mirabilis	75	up to	3-6		107	79	*Pseudomonas*	0,6	2-85	
Ochromonas sp.	200	approx.	0,5 3	34	190	10			5 170	
Pleuromonas jaculans	50	400		43	54	1,4				
Pseudobodo tremulans	90		1-3		84	10			5-50	
Monas	30		1,8-3,0	24-45	10-75	0,1-1,0	Bacteria	0,3-1,0	25-8 000	Sherr *et al.* (1983)
Pseudobodo tremulans	30					39				Davis and Sieburth (1984)
Bodo sp.	30				40-150**	19				Narragansett Bay, 21°C
Monas sp.						14	*Vibrio* sp.	*c.* 0,2	4-75	*excl. lorica
Paraphysomonas imperforata	33 113					28				**estimated from plots
Actinomonas mirabilis					224	203				
Acantheopsis unguiculata	33 65*				136	91			1 4	
Pseudobodo sp.	4-33		max. 2		0,12-0,42	0,7-1,6	*Micromonas*	*c.* 4,0		Parslow *et al.* (1986)
Paraphysomonas imperforata	180 900	1-30	2,1-2,5	44	1,7-4,6	3,9-10,4	*Phaeodactylum*		0,4-1,5	Caron *et al.* (1985)
			2,5	44	23 117	2,3-4,4	*Pseudomonas*	*c.* 0,6	20	Goldman *et al.* (1985)
							& mixed bacteria	0,3		(all max. rates)
Pseudobodo tremulans										Andersen and Fenchel (1985)
Pseudobo sp.		1 40 max	3,4		45 73	15 20	Mixed bacteria		2,4 4,8	natural populations, 15°C
Diaphanoeca grandis					(4-7)	(1-5)*			(*c.* 0,25)*	*Very low bacterial numbers
Ciliates	Length (μm)	Numbers ($\cdot ml^{-1}$)						Diameter (μm)	Numbers ($\times 10^3 \cdot ml^{-1}$)	
Favella sp.	65 75	4	1,0-1,2	21*-69*	15	990 1 140	*Heterocapsa*	16 × 22	1,4	Stoecker and Evans (1985)
	5 7				2,4	100 140	*triquertra*		2,4	*prey, 3 000 cells·ml^{-1}
										*prey, 500 cells·ml^{-1}
Uronema sp.	12 × 5	0-17,5	3,3	27			Mixed	0,014 to	5-35	Turley *et al.* (1986)
Euplotes sp.	20 × 10	0 2,5	1,3	19			bacteria	0,4 μm^3	2,5 35	
Tintinnopsis acuminata	45			33	29 80	1 700 2 600	*Isochrysis*	3,1-3,4*		Verity (1985) max. rates
Tintinnopsis vasculum	80			33	34 83	4 300 7 500	*Dicrateria*	9,9 10,5*		*pgC·cell^{-1}

food limited, flagellate growth rates were within the range 0.93 to 1.28 d^{-1} at a temperature presumably close to 25 to 30 °C (Landry et al. 1984). These studies appear to confirm that the slow growth rates of Pseudobodo in the present study are a function of low temperatures and prey limitation.

Gross growth efficiencies of Pseudobodo in this study (see Tables 3 and 4) were maximal (36 %) during early exponential growth of the flagellates when prey were not limiting. This estimate of growth efficiency is in general agreement with values reported for both flagellates and ciliates (see Table 4) when prey is not limiting. During late exponential growth of the flagellates (Day 7) when prey become limiting, our model suggests that growth efficiency falls to <30 %. In their study, Landry et al. (1984) showed that food-limited flagellates growing in Kaneohe Bay had a gross growth efficiency (increase in biomass/biomass ingested) of 22 %, confirming our model's predictions. The trend in the present data is for lowest growth efficiencies at low food concentrations and maximal rates when food concentration is highest. Verity (1985) found that growth efficiencies of two tintinnid ciliates were maximal at intermediate food concentrations and lowest at low and excessively high food concentrations. Low temperatures also increased growth efficiencies, presumably by minimising metabolic losses.

The growth efficiency of organisms has an important bearing on mineral cycling. Low growth efficiencies (in carbon terms) imply high respiration and excretion losses (as DOC), resulting in the inefficient transfer of carbon to higher trophic levels. However, DOC excretion associated with low flagellate growth efficiencies may stimulate bacterial productivity, therefore producing mutually enhanced predator-prey growth. Low growth efficiencies also imply high nitrogen-regeneration efficiencies, thus stimulating regenerated primary production, but the value for growth efficiency appears to be critical. Caron

et al. (1985) recorded generally higher growth efficiencies (31 to 62 %, mean = 44 %) for Paraphysomonas predation on bacteria and the diatom Phaeodactylum than are generally observed (Table 4). As Goldman and Caron (1985) pointed out, these higher growth efficiencies signify lower nitrogen regeneration efficiencies than have often been supposed, thus necessitating several trophic steps in order to supply the rates of epipelagic nitrogen regeneration that isotopic studies have suggested.

Flagellate predation

Population interactions between bacteria and flagellates can readily be accounted for by their relative rates of production and predation. These are summarised in Table 5. Prior to Day 4, flagellate predation (consumption) of bacteria did not exceed bacterial production, ensuring that the bacterial population continued to increase despite predation pressure. However, in an earlier argument it was shown that bacterial production would become substrate-limited after Day 4. Thus, by Day 4.5, when predation accounted for 13.3 μg C l^{-1} d^{-1}, bacterial population growth was close to zero (Table 5), indicating that predation pressure and bacterial production were approximately equal. This is contrary to the predicted bacterial production rate at this time of 63.2 μg C l^{-1} d^{-1} (Table 2) based on continued exponential growth. Bacterial growth is therefore better described by a logistic rather than exponential growth curve. Note that, when predation and bacterial production were approximately equal, flagellate ingestion rate (2.36 d^{-1} or 19 bacteria $flagellate^{-1}$ h^{-1}) and prey density (9.01 x 10^6 cells ml^{-1}) were highest when the predator density was still only 6 % of its maximum. It is also noteworthy that the maximum ingestion rate is lower than the value of V_{max} (=3), because the predation term in Equation (1) is dependent on the prey density. Later (Days 6 to 8) flagellate consumption increased dramatically (maximum 125 μg C l^{-1} d^{-1}) raising the

Table 5: Output from the Lotka-Volterra model

Time (days)	Bacteria		Flagellates		Ingestion*d^{-1} (per capita) e	Total flagellate consumption (μgbact.C·l^{-1}·d^{-1}) f	Consumption (bact·$flag^{-1}$·h^{-1}) g	Clearance rate ($\times 10^{-4}$ ml·$flag^{-1}$·h^{-1}) h	Nitrogen regeneration (μmole·l^{-1}·h^{-1}) i
	($\times 10^6$·ml^{-1}) a	(μgC·l^{-1}) b	($\times 10^3$·ml^{-1}) c	(μgC·l^{-1}) d					
0	1,69	28,00	0,13	0,40					
2,5	3,28	54,15	0,18	0,57	1,69	0,96	13	0,041	0,0004
3,0	5,38	88,86	0,30	0,94	2,05	1,93	16	0,030	0,0008
3,5	7,31	120,81	0,53	1,65	2,24	3,70	18	0,025	0,0016
4,0	8,52	140,87	0,97	3,02	2,33	7,04	18	0,021	0,0030
4,5	9,01	148,99	1,80	5,62	2,36	13,26	19	0,021	0,0057
5,0	8,96	148,18	3,37	10,50	2,36	24,78	19	0,021	0,0106
5,5	8,41	139,04	6,26	19,50	2,33	45,43	18	0,021	0,0195
6,0	7,15	118,14	11,44	35,64	2,25	80,19	18	0,025	0,0344
6,5	4,66	77,09	20,01	62,33	2,01	125,28	16	0,034	0,0539
7,0	0,98	16,23	29,67	92,45	1,00	92,45	8	0,082	0,0402
7,5	0,01	0,18	29,95	93,31	0,02	1,87	(0,16)	0,160	0,0008
8,0	(0,0001)	(0,001)	27,81	86,64	(0,0002)	0,18	(0,0016)	0,160	0,0001

1. To calculate carbon biomass from cell numbers (from Table I)
   Bacteria; $0{,}0605 \pm 0{,}0075 \times 10^6$ cells·ml^{-1} = 1 μg bacterial C·l^{-1} (n = 11 up to 132 h)
   Flagellates; $0{,}321 \times 10^3$ cells·ml^{-1} = 1 μg flagellate C·l^{-1} (Type B only)

2. To calculate flagellate consumption and clearance
   Ingestion*d^{-1} e = units of bacterial carbon mass consumed per unit flagellate carbon mass per day
   f = d.e
   g = $f.(0{,}0605 \times 10^6)/c.24$
   h = g/a

3. Nitrogen regeneration by flagellates is calculated from Equation 4 in the text

flagellate biomass to its maximum on Day 7.5 (93.3 μg C l^{-1}) when the prey biomass had been reduced to 0.18 μg C l^{-1}. As predation was dependent essentially on residual bacterial biomass at this time, the ingestion rate could not be sustained and rapidly fell to 0.02 d^{-1} or < 1 bacterium flagellate^{-1} h^{-1} (Day 7.5). This clearly could not support flagellate energy requirements and the flagellate population therefore collapsed. However, with consequently much-reduced predation pressure, the bacterial population began to grow again (see Table 1).

When bacterial prey are not limiting, our model proposes that flagellates of approximately 30 μm^3 (Pseudobodo) consume between 2.0 and 2.4 times their body mass (carbon) per day or approximately 16 to 19 bacteria flagellate^{-1} h^{-1}. These predation rates are generally more conservative than estimates reported in other studies (see Table 4). However, comparisons of the results from these studies are complicated by the densities and species of predator and prey, their relative sizes, mode of feeding, temperature and also by the degree and manner in which the experiments have been manipulated or analysed. Furthermore, physiological or morphological adaptive responses by micro-organisms to a changing environment can substantially modify microbial interactions.

Relative sizes of the organisms may be particularly important because of the well known allometric relationships relating body size with metabolic rate. Although a number of authors (e.g. Davis and Sieburth 1984, Sherr et al. 1986a, b) have summarised predation rates relative to predator and prey concentrations, few have focussed on the relative sizes of the organisms. Recent evidence confirms that the relative sizes of micro-organisms considerably influences predation, growth, metabolism and community structure (Verity 1985, Andersson et al. 1986, Turley et al. 1986, this study). From Fenchel's (1982b) data (see Table 4), it does appear that larger flagellates

have higher predation rates than smaller species and, furthermore, that the mode of predation influences predation rate. For example, Davis and Sieburth (1984) reported that filtering species (e.g. choanoflagellates) have higher predation rates (mean = 204 bacteria flagellate^{-1} h^{-1}) than predators dependent on directly encountering their prey (e.g. Monas and Pseudobodo).

However, despite considerable differences in the number of prey items captured per cell (Table 4), it is clear that weight-specific ingestion rates for predators covering 3 to 4 orders of magnitude may be similar at a given temperature and prey density, demonstrating the dependence of specific ingestion rates as a function of flagellate volume and prey size.

Flagellate clearance rates

From the estimate of predation rates and bacterial densities, it is possible to calculate the clearance rate of the flagellates. In the present study, flagellates cleared 0.021 to 0.160 x 10^{-4} ml flagellate^{-1} h^{-1} (Table 5). These values fall generally within the range of other recorded values (see Table 4). As Sherr et al. (1983) and Verity (1985) noted, clearance rates increased linearly with increasing prey density and are also inversely related to predator cell volume. As Verity (1985) demonstrated that ingestion rates increased with increasing temperature, it is not surprising that clearance rates also increased by a factor of 1.5 to 1.7 over a 10 °C range.

Heterotrophic flagellate densities in shelf waters of the southern Benguela region are normally between 1 and 3 x 10^3 cells ml^{-1} and bacterial densities are typically of the order 10^5 to 10^7 cells ml^{-1} (see Lucas et al. 1986, Probyn 1987). Based on clearance rates calculated in this study for the normal in situ range of bacterial and flagellate densities, 5 to 30 % of the water column

where such densities occur could be cleared per day by flagellates alone. Occasionally, much higher densities of flagellates have been recorded. For example, Probyn and Lucas (1987) measured densities as high as 5 x 10^4 cells ml^{-1} in the subsurface chlorophyll maximum of Agulhas Bank waters. Such densities would clear about 500 % of the water column per day in which they occur.

Particle-size selectivity by flagellates

The ability of filter-feeding organisms to preferentially select particles of a particular size range is well known and may contribute to resource partitioning between potential competitors (Stuart and Klump 1984). Within the marine pelagial, preferential particle size selection by the microflagellate Ochromonas sp. (Andersson et al. 1986) and by two ciliate species (Turley et al. 1986) has recently been demonstrated. The present study seems also to demonstrate a high degree of particle-size selectivity by Pseudobodo (see Fig. 1). The initial bacterial community, dominated by small coccoid Vibrionaceae and Pseudomonadaceae isolates (approximately 0.142 to 0.198 μm^3) was heavily exploited by the flagellates. Later, after Day 10, when the bacterioplankton were dominated by large rods (approximately 0.67 μm^3) such as Neissereaceae, and Flavobacteriaceae/Cytophaga (see Painting et al. 1989), the flagellates seemed unable to exploit this resource. Whether this represented a qualitative choice preference or a mechanistic limitation on the part of the flagellates is not known.

Interpretation of our findings are, however, complicated by conflicting reports. For example, Parslow et al. (1986) demonstrated that Pseudobodo sp. (although differing from Fenchel's [1982a] description) was able to capture particles as large as Micromonas (4.0 μm^3), at least an order of magnitude

larger than the bacteria consumed by Pseudobodo in this study. However, Ochromonas sp. (approximately 200 μm^3) preferentially consumed bacteria > 0.2 μm^3 (Andersson et al. 1986) whereas Pseudobodo in this study preferentially selected particles <0.2 μm^3, demonstrating that these two flagellates could non-competitively co-exist by partitioning the resource.

A study by Turley et al. (1986) has also demonstrated particle-size selectivity by two heterotrophic ciliates, Uronema sp. and Euplotes sp. They showed that these ciliates preferentially consumed the larger bacteria (0.12 to 0.4 μm^3) from a natural bacterioplankton community. In their study the smaller bacteria (0.014 to 0.113 μm^3) therefore occupied a predation refuge. In the present study, the large rods did. It has been argued that bacterioplankton can adapt, to escape predation pressure, by changing the size structure of the community (Andersson et al. 1986). More likely, such changes would be caused by predation on a particular size class rather than by a functional response of the bacteria. However, the in situ situation is likely to be far more complex where a number of heterotrophic flagellates and ciliates of differing sizes exploit different size components of the bacterioplankton community. Relative growth rates of predator and prey will also determine the structure of the microbial community and the success of the predator. Such resource partitioning reduces competition for the resource and provides an interesting parallel with similar resource partitioning that occurs in sponge, tunicate (Pyura) and mussel-dominated filter-feeding communities (Stuart and Klump 1984).

Nitrogen ingestion and regeneration

By calculating the difference between flagellate consumption of nitrogen and that assimilated as production (see Equation 4) it has been possible to obtain an estimate of nitrogen excretion (see Table 5). The maximal rate of nitrogen

ingestion was 1.8 pg N $cell^{-1}$ d^{-1} or 2.36 times the nitrogen mass of the flagellates per day (C:N = 4). For the much larger flagellate Paraphysomonas imperforata (180 to 900 μm^3), Goldman et al. (1985) recorded maximal nitrogen ingestion rates of 50 to 83 pg N $cell^{-1}$ d^{-1}, equivalent to 2 to 17 times the nitrogen body mass per day.

The regeneration rates calculated here range between 0.0004 and 0.054 µg-at N l^{-1} h^{-1}, equivalent to 0.75 to 0.91 pg N $cell^{-1}$ d^{-1} or about 56 to 58 % of the ingested nitrogen when bacterial prey are not limiting. Excretion occurred at the onset of flagellate predation and reached maximal rates during their early exponential growth when bacterial prey were not limiting (Fig. 4). During exponential flagellate growth, nitrogen-regeneration efficiencies nevertheless declined slightly, as indicated by the increasing carbon net growth efficiencies (Table 3), to which nitrogen excretion is coupled (C:N = 4). However, as the bacterial prey became limiting after Day 6, therefore also limiting nitrogen ingestion, excretion rates dropped rapidly (Fig. 4) despite a slight increase in the nitrogen-regeneration efficiency to approximately 60 %. The overall decrease in nitrogen excretion is therefore due to very limited nitrogen ingestion as bacterial prey become limiting. Goldman et al. (1985) describe a similar trend for nitrogen turnover and recycling by Paraphysomonas. They measured much lower regeneration efficiencies (34 %) during exponential growth of the flagellate when feeding on bacteria, although it subsequently increased towards the late stationary phase. Nevertheless, it never exceeded 49 % of the nitrogen ingested, a value close to the estimates given here at a similar stage of flagellate growth. Their data suggest that nitrogen excretion is not exclusively coupled to carbon flux as described by our model. Laboratory studies conducted by Goldman et al. (1985) indicate that flagellates fed on N-limited Phaeodactylum tricornutum are more efficient at nitrogen conservation than those fed on non N-limited prey.

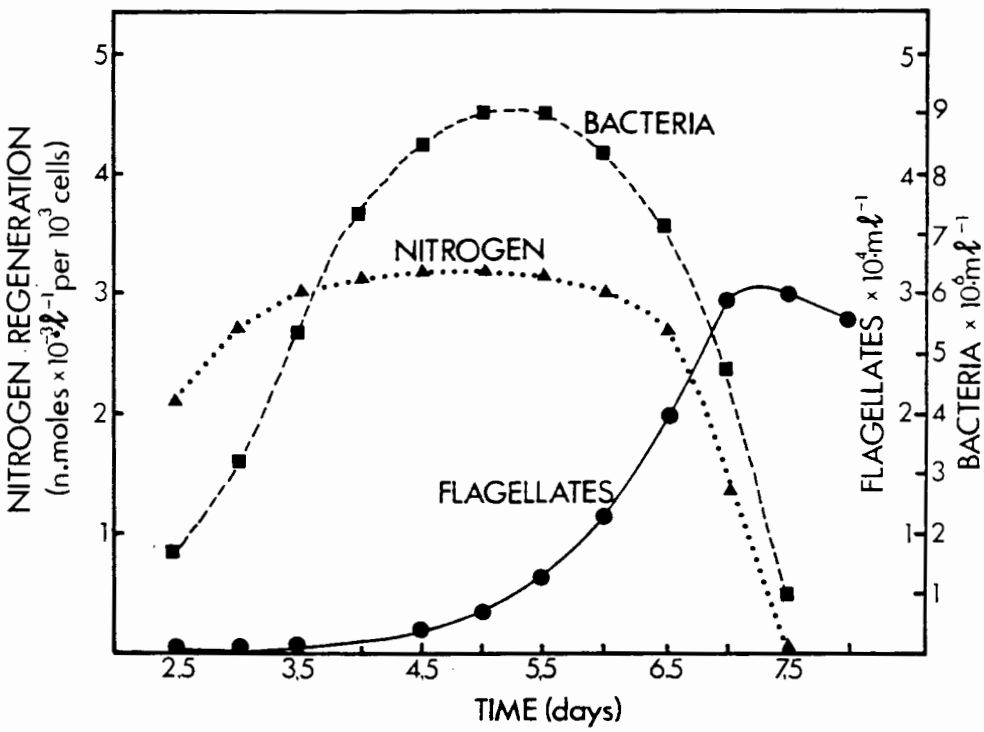


Fig. 4. The relationship between microbial population development and nitrogen excretion by the flagellates.

Weight-specific nitrogen-excretion rates calculated from this study fall within the range of other reported values, although conservatively so (see Table 6). This may be due to the relatively low prey densities used here. Characteristically, ingestion rates increase with increasing food concentration (e.g. Sherr et al. 1983, Davis and Sieburth 1984, Caron et al. 1985), but the growth efficiency is often lowest at high (and low) food concentrations (e.g. Stoecker and Evans 1985, Verity 1985), implying higher nitrogen-excretion rates at the higher food densities used in many of the other studies (see Table 6). Not surprisingly, ciliates have generally lower weight-specific excretion rates than flagellates because of their relatively reduced metabolic rates associated with larger cell volumes.

Although NH_4-N excretion by bacteria was not estimated in this study, NH_4-N excretion by bacterial-sized organisms in the field is usually small relative to nanoplanktonic size classes (Probyn 1987, Probyn and Lucas 1987). Goldman et al. (1985) did not find significant rates of NH_4-N excretion by bacteria, confirming the view that bacteria are more likely to immobilise soluble nitrogen pools. They noted also that urea excretion by flagellates represented about 15 % of the total and concluded also that there must have been low rates of amino-acid excretion, as Andersson et al. (1985) demonstrated.

ECOLOGICAL SIGNIFICANCE OF THE MODEL PREDICTIONS

Predation by flagellates in the sea

An intriguing question to ask is whether predicted predation rates from the model can be extrapolated to the field and account for the observed rates of removal of bacterial production in the marine environment. Estimates of bacterial production within the euphotic zone of the southern Benguela

Table 6: Comparison of nitrogen excretion rates for some microzooplankton

Species	Volume (μm^3)	Predator density ($\times 10^3 \cdot ml^{-1}$)	Prey density ($\times 10^6 \cdot ml^{-1}$)	NH_4^+ N excretion (μg N·mg dry wt^{-1}·h^{-1})	Comments and source
			Flagellates		
Pseudobodo	30	1 11	7 9	6,7 7,1	Days 2,5 3,5, prey not limiting.This study
Monas sp.	30	200 3 500	25 850	10,6 17,2	Bacterial prey, *Chlorobium phaeobacteroides*, *E. coli* and *S. typhimurium*; approx 1,0 μm^3
			80 3 000	12,5	Bacterium isolate 1, approx. 0,3μm^3
			900	6,4 50,8	Grown on isolate 1 at 3 30°C (Sherr *et al.* 1983)
Ochromonas	10	8	5	approx. 28	Fed ^{3}H-leucine-labelled mixed bacteria (mean vol. = 0,15 μm^3) and *E. coli*. Bacteria also excreted DFAA, notably serine. Andersson *et al.* (1985)
Paraphysomonas imperforata	180 900	1 30	4 25*	3‡ 10	*Mixed bacterial population
			0,01 1,0†		†*Phaeodactylum tricornutum* (alga)
					‡N-limited *P. tricornutum* (Goldman *et al.* 1985)
			Ciliates		
Euplotes vannus	100 × 10^3	0,10	0,01 0,05	1,5*	Prey, *Dunaliella tertiolecta* (alga)
		0,15	2 30	1,9*	Prey, *Vibrio* sp.
					*During log. growth of *Euplotes* Gast and Horstman (1983)
Tintinnopsis acuminata	14 × 10^3			3 7	Prey, *Isochrysis galbana* (phytoflagellate) Verity (1985)
Tintinnopsis vasculum	125 ×10^3	—	—	1 3	Prey, *Dicrateria inornata* (phytoflagellate) Tintinnid volumes are very approximate Verity (1985)

N.B. Cell densities given are initial densities or cover the approximate range used during the experiment
Dry weight of ciliates calculated from 0,2 pg·μm^{-3} (Sherr *et al.* 1983)

upwelling region are typically within the range 20 to 70 μg C l^{-1} d^{-1} but they may occasionally be as high as 140 μg C l^{-1} d^{-1} (Lucas et al. 1986). These rates are associated with primary production of 55 to 400 μg C l^{-1} d^{-1} (Lucas et al. 1986), and thus both primary and bacterial production are comparable with the rates obtained from this microcosm experiment.

For the west coast region of the southern Benguela upwelling system, the density of microflagellates within the euphotic zone is typically 1 to 3 x 10^3 cells ml^{-1} (Probyn 1987) where, on average, approximately 70 % of the flagellates are <5 μm in diameter (Probyn 1987). At a maximum predation rate of 19 bacteria $flagellate^{-1}$ h^{-1}, occurring when field and model flagellate densities were very similar (Tables 1, 5 and 7), the observed density of microflagellates in the environment could remove 0.46 to 1.4 x 10^6 bacteria ml^{-1} d^{-1}. Typical rates of bacterial production given above are equivalent to 1.21 to 4.23 x 10^6 cells ml^{-1} d^{-1} (0.0605 x 10^6 cells ml^{-1} = 1 μg bacterial C l^{-1}, see Table 5). Clearly, when flagellate numbers are reasonably high, they are quite capable of removing 30 to 100 % of the bacterial production, whereas at lower flagellate densities, only 10 to 40 % of the bacterial production would be removed. In Georgia coastal waters, Sherr et al. (1984) calculated that heterotrophic microflagellates would consume 30 to 50 % of daily bacterioplankton production.

However, some important points must be considered. We do not have flagellate densities and bacterial production values for exactly the same location or time. These estimates of in situ predation pressure are therefore very approximate. Furthermore, our estimates of bacterial production include all size classes of bacteria from 0.009 μm^3 micrococci to 0.672 μm^3 large rods. Approximately 60 % of the natural bacterioplankton are coccoid, between 0.009 and 0.142 μm^3 (i.e. up to 0.56 μm in diameter), and these represent the group

Table 7 : Nitrogen regeneration per 10^3 flagellates (nmoles $\times 10^{-3} \cdot h^{-1}$)

Size (μm)	Predator density ($\times 10^4 \cdot ml^{-1}$)	Prey density ($\times 10^6 \cdot ml^{-1}$)	Nitrogen regeneration		Number of values
			Mean ± % S.D.	Range	
			Model data		
(a) 4	0,2 - 0,5	3,3 7,3	2,64 ± 15	2,2 3,0	3
(b) 4	1,0 11,4	7,1 9,0	3,10 ± 2	3,0 3,2	5
(c) 4	29,67 29,95	1,0 0,01	1,35 ± 98	0,03 2,7	3
			Field data		
West Coast					
1-15	0,39 1,90	0,8 2,9	15,7 ± 99	1,98-30,9	4 (surface)
1 15	0,21 2,61	0,8 2,7	3,4 ± 93	0,96 - 2,99	4 (c.1% light)
Agulhas Bank					
1 15	4,1 24,6		0,58 ± 7	0,54 0,62	4 (surface)
5-15	0,3 - 1,7		8,65 ± 73	4,37-18,10	4 (surface)
1- 5	3,7 -22,9	—	0,13 ± 138	0 - 0,39	4 (surface)
1-15	3,8 - 9,1	—	1,17 ± 177	0,051 - 6,30	4 (Chl. max)
5-15	0,1 - 0,4	—	0,37 ± 140	0 - 0.74	2 (Chl. max)
1 - 5	3,7 - 8,8	—	0,20 ± 100	0 - 0,37	4 (Chl. max)

1. Model data calculated from Table 3. The model data are calculated for 3 periods:
(a) period of exponential bacterial growth, Days 2,5-3,5; (b) early exponential flagellate growth, Days 4,0-6,0, corresponding to bacterial biomass and production peak; (c) late exponential flagellate growth, Days 6,5-7,5, at the onset of prey limitation and flagellate starvation

2. Field data — Based on ^{15}N isotope dilution experiments. The West Coast data are from Probyn (1987) and the Agulhas Bank data from Probyn and Lucas (1987). Most flagellates were spherical and approx. 3 μm in diameter. Some flagellates in the Agulhas Bank Chl.max were probably autotrophic

exploited by the flagellate (Pseudobodo) in this study. Bacterial production available to flagellates of this size class (20 to 30 μm^3) may therefore be considerably lower than our estimate of total bacterial production, accounting for the apparently rather small fraction of the bacterial production exploited by these flagellates. Nevertheless, production of the larger size classes of bacteria is likely to be exploited by larger flagellates such as Ochromonas sp. (Andersson et al. 1986) and ciliates such as Uronema and Euplotes, as described by Turley et al. (1986).

In the Agulhas Bank region of the southern Benguela system, flagellate densities in surface waters were on average 1×10^4 ml^{-1} (Probyn and Lucas 1987) and, at a clearance rate of 20×10^{-3} $\mu l\ h^{-1}$ $flagellate^{-1}$, this density would filter approximately 200 $\mu l\ h^{-1}$. By contrast, ciliates (15 to 50 μm) in this region, at an average density of 1.5 ml^{-1} (Probyn and Lucas 1987), would clear only 1.5 to 11.25 $\mu l\ h^{-1}$ at clearance rates of 1 to 7.5×10^{-3} $\mu l\ h^{-1}$ $ciliate^{-1}$ (Table 4). This suggests that flagellates rather than ciliates are likely to be the major bacterial predators in this region.

Nitrogen regeneration by heterotrophic flagellates

Our model provides an estimate of nitrogen excretion based on a simple predator-prey relationship involving two well defined size classes and a single trophic step only. However, size-fractionated studies of nutrient cycling by ^{15}N isotopic methods do not discriminate between the importance of different organisms within each class, and nor does this technique offer insight into the complexity of the food chain within each size class. Can we, by comparing in situ estimates of nitrogen regeneration based on size-fractionated ^{15}N studies with nitrogen-regeneration estimates from our model, assess the importance of

the microflagellates alone and hypothesise about the trophic structure of the microzooplankton food chain?

Rates of nitrogen excretion normalised for 10^3 flagellates ml^{-1} for the present model and in two local regions are summarised in Table 7. As our microcosm simulates an upwelling event, comparisons with the West Coast data are the most environmentally compatible. Average estimates of nitrogen regeneration based on our model (2.9×10^{-3} nmole h^{-1} per 10^3 cells) fall within the range of NH_4-N excretion rates obtained by ^{15}N studies in West Coast waters (0.96 to 30.9×10^{-3} nmoles h^{-1} per 10^3 cells) but are in particular agreement with rates of excretion determined for the base of the euphotic zone (0.96 to 2.99×10^{-3} nmoles h^{-1} per 10^3 cells).

As measured regeneration rates in surface waters were generally higher (mean = 15.7 nmoles h^{-1} per 10^3 cells) than the estimate from our model, it is tempting to speculate that surface waters in this region are characterised by an extended microbial food chain of several trophic steps. If so, the cumulative total of regenerated nitrogen at each step would amount to that estimated by ^{15}N dilution where the size-class may be large (3 to 15 μm; equivalent to 9.4 to 235.6 μm^3) and encompass organisms within nearly three orders of magnitude in size.

At the base of the euphotic zone, where estimates of nitrogen regeneration based on experimental evidence and on our model are comparable, the implication points towards a short food chain with fewer regenerative steps, similar to that in our microcosm. The compelling feature of this hypothesis is that it fits the ideas of Goldman and Caron (1985) who proposed that, as the assimilation efficiencies of bacterivorous flagellates were relatively high (>50 %), many trophic steps were required in the microbial food chain to

account for observed rates of nitrogen regeneration. In the present model, the best curve-fit to the observed flagellate data occurred for an α (assimilation) value of 0.4, implying that no more than 60 % of the consumed nitrogen could be excreted. In further support of these ideas, as elaborated by Goldman and Caron (1985), is the concept that, where the relative input of "new" nitrogen is high (e.g. at the pycnocline or base of the euphotic zone), the food chain will be less complex than one associated largely with "regenerated" nutrients.

An interesting feature of the present model was the finding that, when flagellate densities were high (28 to 30 x 10^3 ml^{-1}) but bacterial densities were low (<0.001 to 0.18 x 10^6 ml^{-1}) on Days 7.5 and 8.0, excretion rates per 10^3 flagellates were <0.04 nmoles h^{-1}. We have noted previously that, when flagellate densities in situ were high (approx. 20 x 10^3 ml^{-1}), the expected high rates of ammonium regeneration did not always occur, much as the model predicts when prey is limiting (Probyn 1985, 1987, Probyn and Lucas 1987). Where bacteria and their predators develop in response to episodic phytoplankton blooms, it seems likely that oscillating rather than "steady-state" predator-prey relationships will initially exist. Thus, at momentary (rather than continuous) sampling periods, there is a likelihood of encountering peaks in biomass characterised by inactivity due to substrate limitation and starvation. Low specific excretion rates (per 10^3 cells) may therefore be due to starvation rather than high counts of autotrophic flagellates.

Acknowledgements

I would like to thank Dr Coleen Moloney for the extensive assistance with the computing of the Lotka-Volterra model, and Drs Mike Lucas and Trevor Probyn who were actively involved in the data interpretation and drafting of this manuscript. My thanks are also extended to Mr I. Humphrey and Mr W. Smith for assistance with counting of bacteria and flagellates, to Ms Frieda Verheye-Dua who assisted with sampling from the microcosm, and to Mrs L. Elley for typing the original manuscript. Funding provided by the SANCOR Benguela Ecology Program is gratefully acknowledged.

This chapter has been published in the South African Journal of Marine Science:

Lucas, M.I., Probyn, T.A. and Painting, S.J. 1987. An experimental study of microflagellate bacterivory: further evidence for the importance and complexity of microplanktonic interactions. S. Afr. J. mar. Sci. 5: 791-808

SECTION 4

FIELD STUDIES

CHAPTER 6

Temporal changes in bacterial abundance and biomass during the development of phytoplankton and zooplankton communities in an upwelling plume in the southern Benguela.

ABSTRACT

Temporal changes in bacterial numbers and biomass during the development of a phytoplankton and zooplankton community in a plume of maturing upwelled water (MUW) in the southern Benguela region were measured during 2 consecutive drogue studies from the R S Africana in the summer upwelling season in 1983 (13 to 20 March). Bacterial abundance and biomass were variable (1 to 10 x 10^6 cells ml^{-1}, 20 to 180 mg C m^{-3}). Bacteria were shown to constitute 11 to 41 % of the total microbial (phytoplankton and bacteria) biomass. Bacterial biomass was correlated with phytoplankton biomass (chlorophyll) and production, and with concentrations of particulate carbon (PC). These relationships were generally best described by power curves, suggesting that bacterial biomass is reduced at high levels of phytoplankton production and biomass, possibly due to predation. The mesozooplankton community was dominated by the copepod Calanoides carinatus. During the first drogue study (37 h), high nitrate concentrations (5 to 10 mg-at N m^{-3}) stimulated primary production (0.5 to 1 g C m^{-2} h^{-1}). The planktonic community was characterised by high biomass of bacteria (40 to 60 mg C m^{-3}), a diatom-dominated phytoplankton community (>5 mg Chl m^{-3}), and mesozooplankton (30 to 86 mg C m^{-3}). During the second study (94 h), a discrete mass of MUW was followed for 37 h. In this water nitrate concentrations (2 to 5 mg-at N m^{-3}), and phytoplankton production (ca 0.5 g C m^{-2} h^{-1}) and biomass (2 to 5 mg Chl m^{-3}) were lower. The phytoplankton community was dominated numerically by nanoplanktonic flagellates (2 to 20 μm). Estimates of bacterial biomass (up to 180 mg C m^{-3}) at the beginning of the second study were the highest recorded thus far in situ in the southern Benguela (bacterial carbon:chlorophyll carbon = 0.75), and then decreased to 40 mg C m^{-3}. High values for bacterial biomass may be due to predation on bactivores by Noctiluca which were unusually abundant (1 to 5 x 10^6 cells m^{-3}). After 37 h, salinity and biological fronts suggested mixing of MUW with older, more mature upwelled water. After mixing,

primary production was lowest (<0.2 g C m^{-2} h^{-1}) and the biomass of phytoplankton and bacteria decreased (<2 mg Chl m^{-3}, ca 20 mg bacterial C m^{-3}), possibly due to rapid senescence of the bloom, dilution or the effect of increased herbivory by a mesozooplankton population (C. carinatus) which increased from 26 mg C m^{-3} at the start of the second drogue to 58 mg C m^{-3} at 70 h and then declined. The ratio of bacterial biomass:phytoplankton biomass was lowest (mean value: 17 %) when primary production was high, increasing to approximately 50 % during the temporal decline of the bloom. Similarly, the ratio of copepod:phytoplankton biomass was low (mean value: 17 %) during high levels of primary production, increasing to 128 % during the phytoplankton decline. The results presented here suggest that the planktonic community of maturing upwelled water is increasingly dominated by bacteria and detritus, and that copepods become food food-limited during the quiescent phase of the upwelling cycle, possibly supplementing their dietary requirements by ingesting microzooplankton.

INTRODUCTION

The planktonic food web in the coastal southern Benguela upwelling system is strongly influenced by the spatial and seasonal variability in the physical environment (for reviews see Shannon 1985b, Chapman and Shannon 1985, Shannon and Pillar 1986; see also Moloney 1988). Upwelling of cold (8 to 10 ^{0}C) nutrient-rich water (nitrate: >20 mg-at NO_3-N m^{-3}) occurs frequently at localised centres in response to south-easterly wind stress during the austral summer. Under the influence of prevailing south-easterly winds, tongues of newly upwelled water move off-shore and northwards to mix with aged upwelled or oceanic water (Andrews and Hutchings 1980).

The discrete plumes of water which develop downstream of upwelling centres are ideal for studying temporal changes in plankton dynamics. Drogues have frequently been used to follow these water masses to investigate the development of phytoplankton communities, in the southern Benguela (Barlow 1982c, Olivieri 1983, Brown and Hutchings 1987a, b) and in other upwelling systems (Beers et al. 1971, Ryther et al. 1971, Herbland et al. 1973, Nelson and Goering 1978, Fuhrman et al. 1985).

Intensive studies of the dynamics of phytoplankton growth in upwelling plumes in the southern Benguela have shown that phytoplankton biomass and production are low in newly upwelled water (<1 mg chl *a* m^{-3}, <5 mg C m^{-3} h^{-1}). After stabilisation of the water column due to sun-warming of water in the euphotic zone phytoplankton primary production is high (100 to 200 mg C m^{-3} h^{-1}), elevating phytoplankton biomass to >10 mg chl *a* m^{-3} (Barlow 1982a, b, Brown 1984, Brown and Hutchings 1985, 1987a, Brown and Field 1986). However, because of thermal stratification and subsequent nitrate limitation, bloom senescence typically occurs within 6 to 8 days (Barlow 1982a, b, Brown and

1987a). In the absence of renewed upwelling of nitrate into the euphotic zone further primary production is sustained largely by regenerated nitrogen (Probyn 1985, 1987, 1988).

Few attempts have been made to quantify and study the dynamics of heterotrophic bacterio- and zoo- plankton communities in response to phytoplankton growth and decay in a plume of upwelled water. In this study a drogue was released downstream of an upwelling centre and followed for 8 days. The objective was to follow a patch of upwelled water within a plume and quantify temporal changes in the biomass of bacteria during the development of a phytoplankton and zooplankton community. In addition, changes in the structure of the planktonic community during phytoplankton growth and decay were examined. Bacterial and zooplankton production are discussed in Chapters 7 and 8. Estimates of the partitioning of primary production between zooplankton and micro-heterotrophs, and the significance of bacteria in the overall productivity of this highly pulsed marine system are discussed in following chapters.

SAMPLING AND ANALYTICAL METHODS

Cruise description

During the summer of 1983 a drogue was deployed from the R.S. Africana into upwelled water on the continental shelf north of Cape Columbine, one of the principal upwelling centres in the southern Benguela (Fig. 1). The 3 m biplanar tetrahedral drogue (see Brown and Hutchings 1987a) was deployed with its mid-point at 10 m depth, and followed for 8 days (13 to 20 March). The water column at the drogue was sampled on 19 occasions to determine the temporal changes in

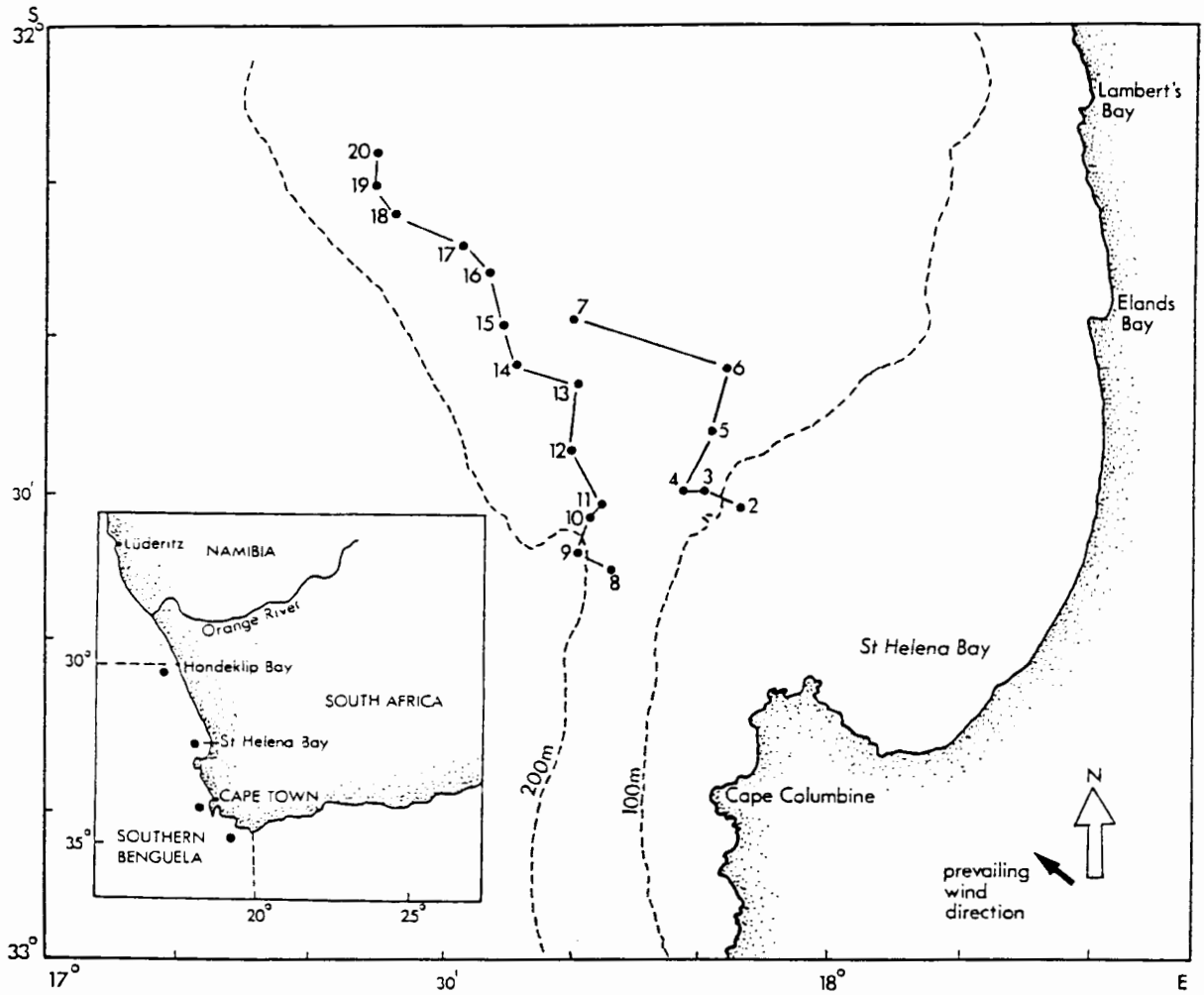


Fig. 1. A chart of the Cape Columbine region, showing drogue tracks and station positions. Insert shows major upwelling centres (•) in the southern Benguela.

the hydrography, phytoplankton production, and abundance and distribution of phytoplankton, zooplankton and bacterioplankton.

Hydrography

Continuous depth-profiles of salinity and temperature were obtained using a Neill-Brown (Mark III) submersible conductivity, temperature and depth (CTD) recorder. Light penetration depths were estimated by a LICOR quantum sensor lowered from a floating boom. Water samples were collected from the 100, 50, 10, 1 and 0.1 % light depths, and then at 10 or 20 m intervals to 100 m using a rosette of 10 5-litre Niskin sampling bottles. Water from each depth was analysed immediately for nutrient concentrations (nitrate, silicate, phosphate) on a Technicon Autoanalyzer (AAII) according to standard methods modified by Mostert (1983). For total particulate carbon and nitrogen analysis, subsamples (100 ml) were screened (180 µm) to remove mesozooplankton and filtered onto pre-ashed (6 h at 400 ^{o}C) Whatman GF/F filters. Filters were stored frozen until they were oven-dried (60 ^{o}C) and analysed by high temperature oxidation on a Hereaus (CHN Rapid) Analyser, with cyclohexanone (20.14 % N: 51.79 % C) as a standard.

Phytoplankton biomass and production

Niskin bottles were used to collect live plankton samples from the sampling depths described above. Chlorophyll *a*, used as an index of phytoplankton biomass, was concentrated from 1 litre of seawater from each depth onto Whatman GF/C filters and analysed spectrophotometrically by the procedure of the SCOR/UNESCO Working Group 17 (1966). Carbon values for phytoplankton were calculated using a carbon:chlorophyll *a* ratio of 60, which is considered to be the mean value for mature upwelled water in the southern Benguela. Surface

samples for microscopic examination (100 ml) were preserved with 5 % formalin and identified and counted using inverted microscopy (Utermöhl 1936, as described by Hasle 1978).

Primary production rates of phytoplankton were estimated from uptake of ^{14}C-bicarbonate during 4-h in situ incubations (Brown 1982, 1984).

Bacterial numbers and biomass

Water samples (20 ml) were preserved with 25 % Analar glutaraldehyde (1.3 % v/v) and stored at 4 ^{o}C. Bacteria were counted in 4 different size classes by the Acridine Orange Direct Counting (AODC) technique (Hobbie et al. 1977, Linley 1983, Painting et al. 1985). At least 20 random fields or 300 bacteria were counted, to obtain a normal distribution of cells (Linley et al. 1981, Scavia and Laird 1987). Bacterial biomass (mg C l^{-1}) was calculated from cell numbers, the total cell volume (μm^3) in all size classes, and the conversion factor of 0.121 pg C μm^{-3} (Watson et al. 1977).

Zooplankton abundance and biomass

Mesozooplankton were collected with a Bongo net (57 cm diameter, 200 µm mesh, McGowan and Brown 1966, Verheye 1989b) hauled vertically through the entire water column (100 to 200 m). Samples were preserved in 5 % buffered formalin. At selected stations, zooplankton samples were collected from discrete depths with the rosette sampler and 5-l Niskin bottles. A 4-litre sample of water was taken from each bottle, filtered through a 37 µm Nitex screen, and the material on the screen rinsed into a sample bottle.

In the laboratory, two 3.9 ml subsamples were taken from each Bongo net sample with a piston pipette and all the animals enumerated. Copepods were counted by species and developmental stage; most other zooplankton taxa were identified to genus. The Niskin bottle samples were enumerated without subsampling.

To calculate copepod biomass, the numbers of individuals of each species and developmental stage were multiplied by their respective dry weights, obtained from living material that had been oven-dried at 60 °C overnight and weighed on a Cahn Model 25 Electrobalance (Peterson et al. 1989). Total biomass was calculated by summing the biomass (B) of each species.

$$B \text{ (mg dry weight } m^{-3}) = \sum_{i=1}^{6} (n_i * w_i) * 10^{-3} \qquad (1)$$

where i = developmental stage (C1 to C6), n_i = total number of the $_i$th stage (m^{-3}) and w_i = individual weight of the $_i$th stage (µg dry weight $animal^{-1}$). Copepod carbon biomass was estimated to be 40 % of the dry weight (Andrews and Hutchings 1980, Parsons et al. 1984).

RESULTS

Upwelled water was distinguished from other water types on the basis of physical and chemical characteristics (Table 1, Andrews and Hutchings 1980, Waldron 1985, Brundrit 1986). The age of the upwelled water was determined from temperature and nitrate data: newly upwelled water (8 to 10 °C, 15 to 30 mg-at NO_3-N m^{-3}), mature upwelled water (10 to 15 °C, 2 to 15 mg-at NO_3-N m^{-3}), aged upwelled water (12 to 16 °C, 0 to 2 mg-at NO_3-N m^{-3}) (Barlow 1982a).

Table 1. Characterisation of water types in the southern Benguela upwelling region on the basis of the conservative property of salinity (from Waldron 1985).

Water type	Temperature (°C)	Salinity ($^{o}/_{oo}$)	Nitrate (μg-at N l^{-1})	Chlorophyll (mg m^{-3})
Offshore	>18	>35.00		
Shelf	<9.4	<34.70	12-20	
Upwelling*	9.4-11	34.70-34.90	12-20	<0.9
Maturing upwelled	10-18	34.78-34.90	1-10	2-13
inshore bottom mixed	9.8	34.75	12-28	1-4

* upwelling is water referred to as South Atlantic Central Water (SACW) in the text. Newly upwelled surface water has the same characterisitics.

A 36 h preliminary survey of surface temperatures, salinity and nutrients showed newly upwelled water to be absent in the study area. The drogue was consequently deployed into mature upwelled water about 10 nautical miles from the upwelling centre on 13 March (20h00), and the water column sampled (Station 2, Fig. 1). The drogue was followed for 37 h (for station sampling times see Table 2), until it was apparent from marked hydrological changes that the drogue had moved into a different body of water. The drogue was retrieved, mature upwelled water was relocated (1.5 days later), and the drogue was re-set. The second drogue was followed for 94 h (Stations 8 to 20, Fig. 1).

Hydrography

A section of water column temperatures (Fig. 2a) to 100 m shows a vertically stratified water column with warm surface water. From salinity data it was clear that water in the upper 30 m at all stations was of upwelling origin (<34.9 $^{o}/_{oo}$, Fig. 2b, Waldron 1985, Brundrit 1986), indicating upwelling and sun-warming of the surface layers prior to the study period. Furthermore, nitrate concentrations (1 to 10 µg-at N l^{-1}, Fig. 2c) in the warm surface waters (>13 ^{o}C) were typical of maturing upwelled water (Barlow 1982a, Waldron 1985). Variations in density (sigma-t) followed temperature variations and have not been shown here.

South Atlantic Central water (SACW, <11 ^{o}C, <34.9 $^{o}/_{oo}$, 15 to 20 µg-at NO_3 N l^{-1}) was present below 60 m along most of the drogue track, forming an uplifted ridge over the shallower coastal shelf region (Stations 2 to 5). Silicate and phosphate data (not presented) showed similar patterns of distribution to those of nitrate, being low in warm surface water (silicate: 2 to 10 µg-at l^{-1}, phosphate: 0 to 1.5 µg-at l^{-1}) and high in SACW (20 to 30 µg-at Si l^{-1} and 5 to 10 µg-at PO_4 l^{-1}) .

Table 2. Details of station sampling times.

Station	Date (1983)	Station time	drogue time (h)	
2	13/3	20h00	0	
3	14/3	8h30	13	
4	14/3	16h45	21	DROGUE 1
5	14/3	18h50	23	
6	15/3	8h30	37	
7	16/3	13h35		
8	16/3	19h20	0	
9	17/3	8h30	13	
10	17/3	13h00	18	
11	17/3	17h30	22	
12	18/3	8h30	37	
13	18/3	14h00	43	
14	18/3	18h00	47	DROGUE 2
15	19/3	9h40	62	
16	19/3	13h20	66	
17	19/3	17h00	70	
18	20/3	7h00	84	
19	20/3	11h00	88	
20	20/3	17h00	94	

Time of sun-rise: 06h45
Time of sun-set: 19h00

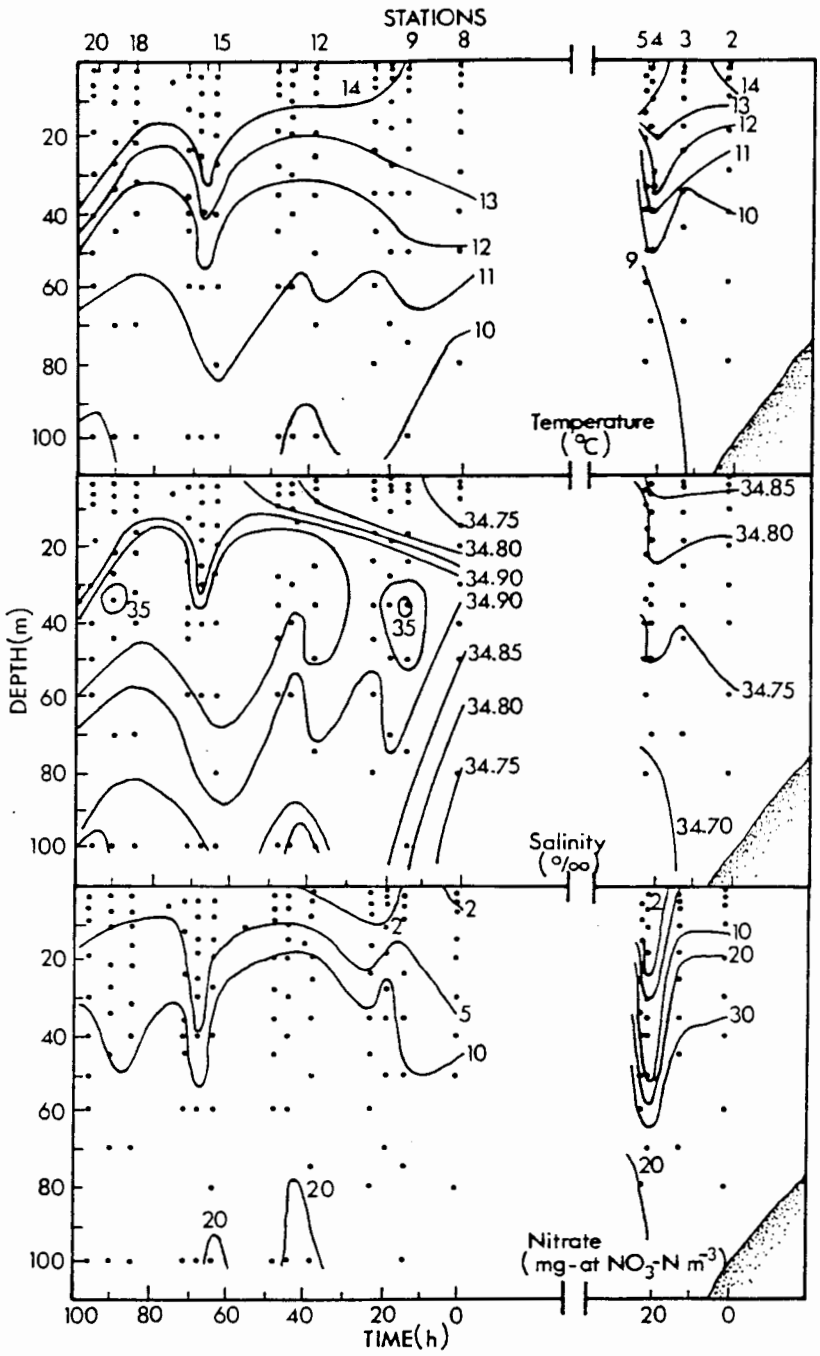


Fig. 2. Vertical sections of (a) Temperature (b) Salinity and (c) Nitrate.

The principal hydrographic features in the study area, identified from water column structure (Table 1), are shown schematically in Fig. 3. Vertical sections of temperature, salinity and nitrate (Figs. 2a, b, c) at Stations 2 to 5 indicate that maturing upwelled water (0 to 20 m, salinity 34.8 to 34.9 $^{0}/_{00}$) was overlying more recently upwelled water (11 to 13 ^{0}C, ca 20 to 30 m) and SACW (>ca 30 m, <11 ^{0}C, <34.9 $^{0}/_{00}$). Downturned isolines at Station 4, particularly for nitrate (Fig. 2c), indicate localised deep mixing. Inshore bottom mixed water (IBMW, 9.8 ^{0}C, 34.75 $^{0}/_{00}$) was identified below the SACW at Station 5. Station 6 could not be included in the interpretation of data as the CTD recorder malfunctioned during sampling.

During the second drogue, temperature, salinity and nitrate sections (Figs. 2a ,b, c) show that water in the upper 15 to 30 m was also MUW (Fig. 3). A salinity front was evident after 37 h (between Stations 12 and 13, Fig. 2b), suggesting mixing of the maturing upwelled water with more mature surface water (Stations 13 to 20), also of upwelling origin (salinity: 34.85 to 34.9 $^{0}/_{00}$, temperature: >14 ^{0}C), which is described as "MUW/mixed". Between approximately 30 and 60 m depth at Stations 8 to 20 salinities were high (>34.90 $^{0}/_{00}$) and representative of frontal water (11 to 14 ^{0}C, 34.90 to 34.95 $^{0}/_{00}$) formed by mixing of different masses of aged upwelled water (Waldron 1985).

Figure 3 also shows the extent of the euphotic zone (0.1 % light depth), which was shallow at Station 2 (5 m) and deepened to approximately 40 m at Station 5. At the start of the second drogue the 0.1 % depth was also shallower (Station 8: 20 m) than at later stations (ca 35 m at Stns 15 to 20).

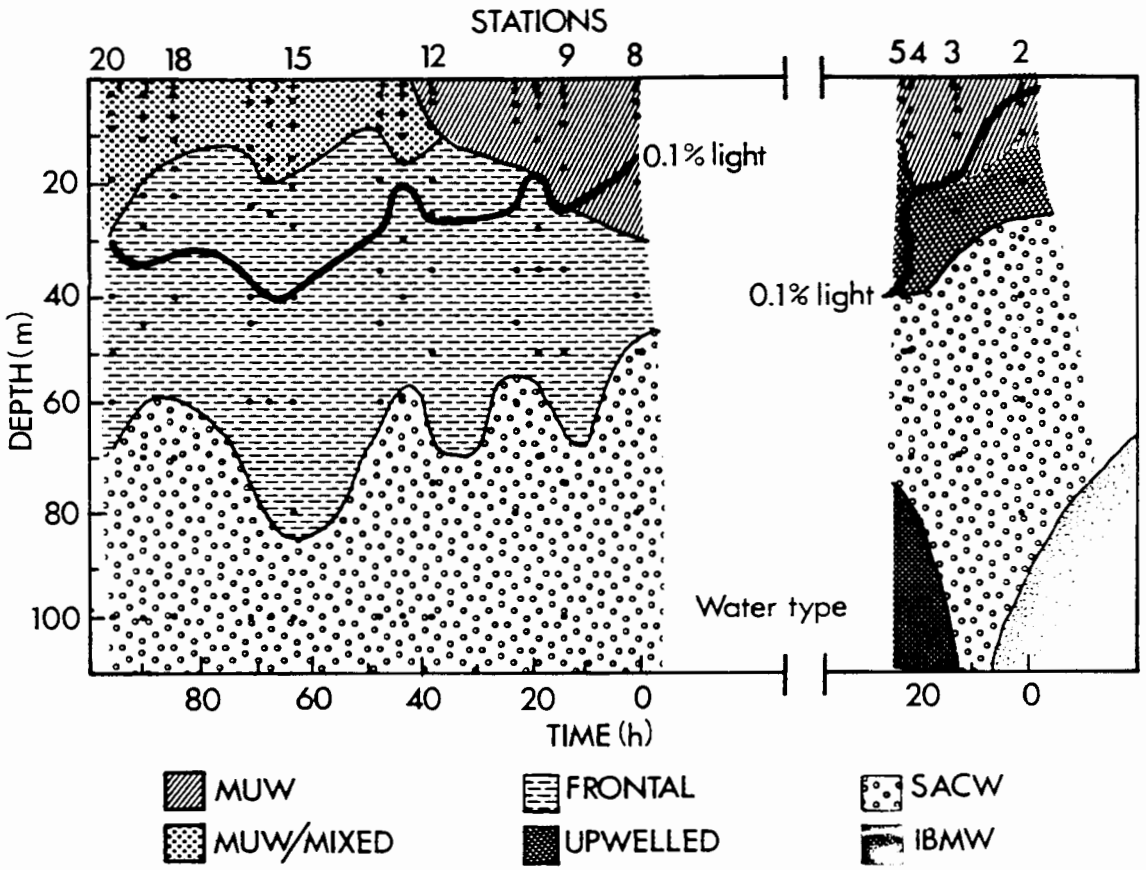


Fig. 3. Water types identified from water column temperature and salinity data. The 0.1 % light depth is indicated. MUW = maturing upwelled water. UPWELLED = more recently upwelled water. MUW/MIXED = maturing upwelled water mixing with older, more mature upwelled water. FRONTAL = mixing of different masses of aged upwelled water. SACW = South Atlantic Central Water. IBMW = Inshore Bottom Mixed Water.

Phytoplankton production

High rates of primary production were observed in MUW, where maximum rates were generally found in sub-surface layers (25 to 50 % light penetration depths, Table 3). Highest values for primary production were measured in the euphotic zone at Station 3 (mean = 108 mg C m^{-3} h^{-1}, Table 3). Total primary production was also highest here (1114.2 mg C m^{-2} h^{-1}). Primary production rates were considerably slower in the MUW sampled during drogue 2 (mean = 32 mg C m^{-3} h^{-1}), with total primary production increasing from Station 9 to 12 (465 to 539 mg C m^{-2} h^{-1}). In MUW/mixed water, phytoplankton production was reduced (overall mean = 6.3 mg C m^{-3} h^{-1}, 66 to 223 mg C m^{-2} h^{-1}).

Particulate carbon, phytoplankton and bacterial biomass

Vertical sections show a stratified distribution of particulate carbon (PC, Fig. 4a), chlorophyll a (Chl, Fig. 4b) and bacterial numbers and biomass (Figs. 5a, b). For each variable, largest concentrations were found in the upper 30 m at Stations 2 to 12. Station 4 was an exception, with high concentrations penetrating to about 60 m. When data were analysed relative to the water types (Table 4) largest values were found in the maturing upwelled water at Stations 2 to 5 and 8 to 12. In MUW/mixed water, frontal water and SACW, concentrations of PC, Chl a and bacteria were low.

Bacteria in all samples were observed to be chiefly free-living, probably as a result of the absence of large detrital aggregates. Small particles of detrital material (ca. 5 μm diameter) were common, often bounded by mucilaginous material, but were generally not characterised by high densities of attached bacteria.

Table 3: Estimates of phytoplankton production at selected light depths in the euphotic zone at stations sampled before mid-day. The biomass of phytoplankton (mg Chl m^{-3}) and bacteria (mg C m^{-3}) and functional regressions describing the relationships between phytoplankton biomass and production, and bacterial biomass and phytoplankton production, are also shown.

Station	Depth (m)	Light level (%)	Bacteria (mg C m^{-3})	Chl a (mg m^{-3})	Primary production (mg C m^{-3} h^{-1})	Integrated Chl (mg m^{-2})	Integrated Production (mg C m^{-2} h^{-1})
3	0	100	49.2	9.0	108.85	105.9	1114.2
	3	50	49.4	9.6	186.67		
	4	25	45.26	8.5	124.61		
	6	10	57.8	10.4	97.47		
	11	1	49.21	9.6	19.82		
6	0	100	31.88	3.0	14.15	65.9	522.2
	2	50	23.48	4.3	35.93		
	4	25	28.94	5.4	50.81		
	7	10	61.28	6.9	64.32		
	11	1	42.42	8.3	42.03		
9	0	100	63.4	4.4	38.24	77.3	465.3
	3	50	144.9	5.0	42.72		
	5	25	66.6	4.2	35.66		
	8	10	175.1	4.6	34.75		
	16	1	72.8	5.6	5.21		
12	0	100	49.60	4.9	42.78	86.5	539.3
	2	50	-	4.8	-		
	4	25	44.57	6.1	50.29		
	8	10	43.98	6.2	32.02		
	19	1	-	1.3	2.26		
15	0	100	25.7	-	1.12	-	66.1
	4	50	18.9	-	2.12		
	8	25	18.5	-	5.08		
	14	10	22.65	-	2.97		
	27	1	4.36	-	0.27		
18	0	100	13.0	0.7	7.56	11.5	223.0
	3	50	19.4	0.7	13.22		
	7	25	-	0.5	14.06		
	11	10	28.3	0.8	13.92		
	22	1	15.14	0	0.86		

Phytoplankton production vs. Chlorophyll: Chl = 0.20 $X^{1.53}$, n = 24, r = 0.775, $p < 0.001$
Phytoplankton production vs. Bacterial biomass (B): B = 2.74 $X^{0.46}$, n = 27, r = 0.694, $p < 0.001$

- no measurements made.

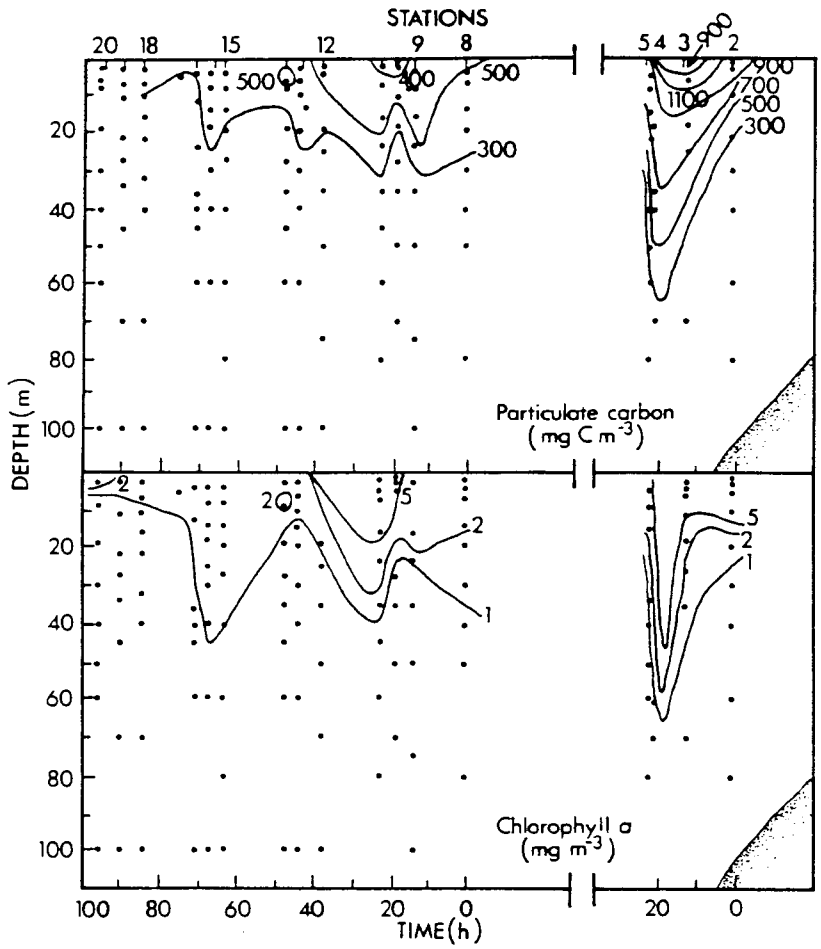


Fig. 4. Vertical sections of (a) Particulate carbon and (b) Chlorophyll a concentrations.

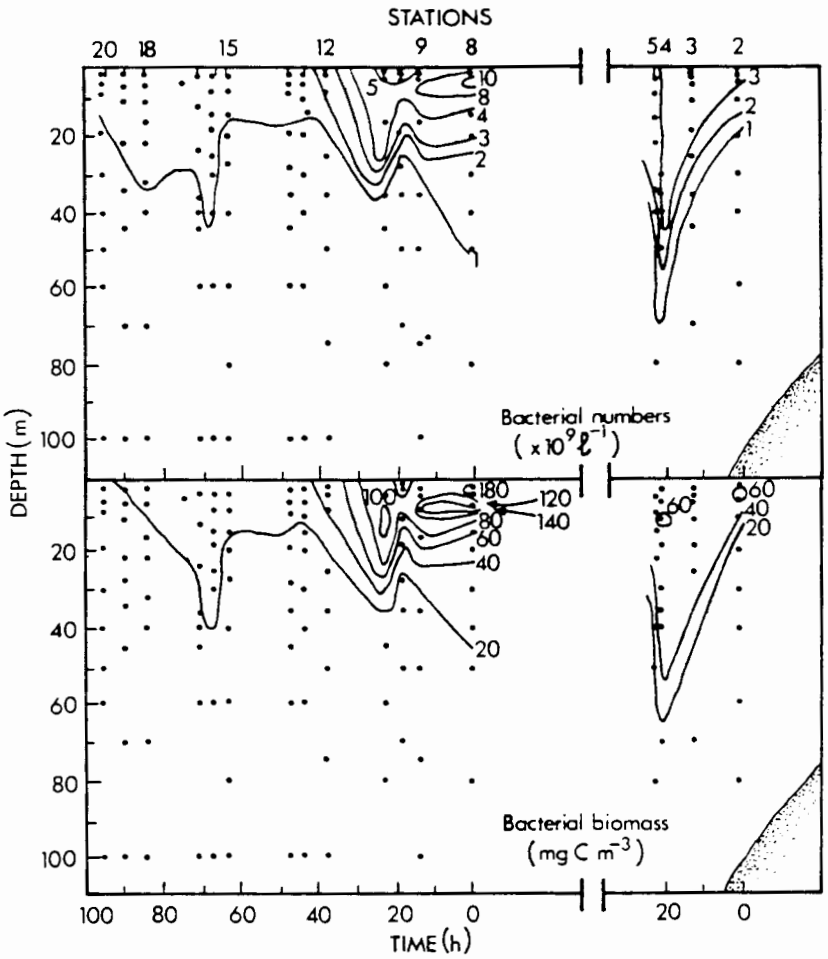


Fig. 5. Distribution of bacterial (a) Numbers and (b) Biomass.

Table 4. Ranges in concentrations of particulate carbon (mg C m^{-3}), Chl a (mg m^{-3}), bacterial numbers (x 10^9 l^{-1}) and bacterial biomass (mg C m^{-3}) in the different water types. MUW = mature upwelled water, SACW = South Atlantic Central Water.

Water type	Stns	Chl a (mg m^{-3})	Bacterial numbers (x 10^9 l^{-1})	Bacterial biomass (mg C m^{-3})	Particulate carbon (mg C m^{-3})
MUW					
	2-5 8-12	2-10	2-10	40-200	300-1200
MUW/mixed					
	13-20	<2	1-2	20-40	300-500
Frontal					
	8-20	<3.5	<2	<40	40-250
SACW					
	2-20	<2	<1	2-20	<250

Fig. 6 shows temporal changes in the concentration of particulate carbon, and the biomass of phytoplankton and bacteria in the upper 20 to 40 m of the water column (Note that time increases from right to left). Data were analysed in 4 different ways, each of which showed different trends. Fig. 6a shows changes in the mean concentration of PC, phytoplankton and bacterial biomass in MUW and MUW/mixed water. These values were calculated by integrating over the maximum depth of the MUW and MUW/mixed water for each station, and dividing by the depth. Mean concentrations were largest at Stations 2 to 5 and 8 to 12, and lowest at Stations 13 to 20 (see also Figs. 4 and 5 and Table 4). Data integrated to the maximum depth of the MUW and MUW/mixed water at each Station are shown on Fig. 6b. Although similar changes in the data were shown by this approach, it could be argued that observed differences are simply a function of variable integration depths (see Fig. 3). Integration to a constant light depth (1 %, Fig. 6c) reduced the differences in biomass between Stations 8 to 12 and 13 to 20 (cf Figs. 4, 5, 6a, b, Table 4). Integration to the 0.1 % light depth further reduced the differences (Fig. 6d). Although biomass concentrations decreased after Station 12 (Fig. 6a) the light depths deepened to include other water types (Fig. 3) so that integrals over greater depths compensated for low concentrations, thus accounting for the near-uniformity in integrated values for all stations. My conclusion is that concentration changes within a defined water mass provide the best measure of temporal patterns of planktonic community development.

Particulate carbon, phytoplankton and bacterial biomasses in MUW were largest during the first drogue study (Stations 2 to 5, Fig. 6a), when the phytoplankton community was dominated numerically by diatoms (<20 μm diameter, Table 5).

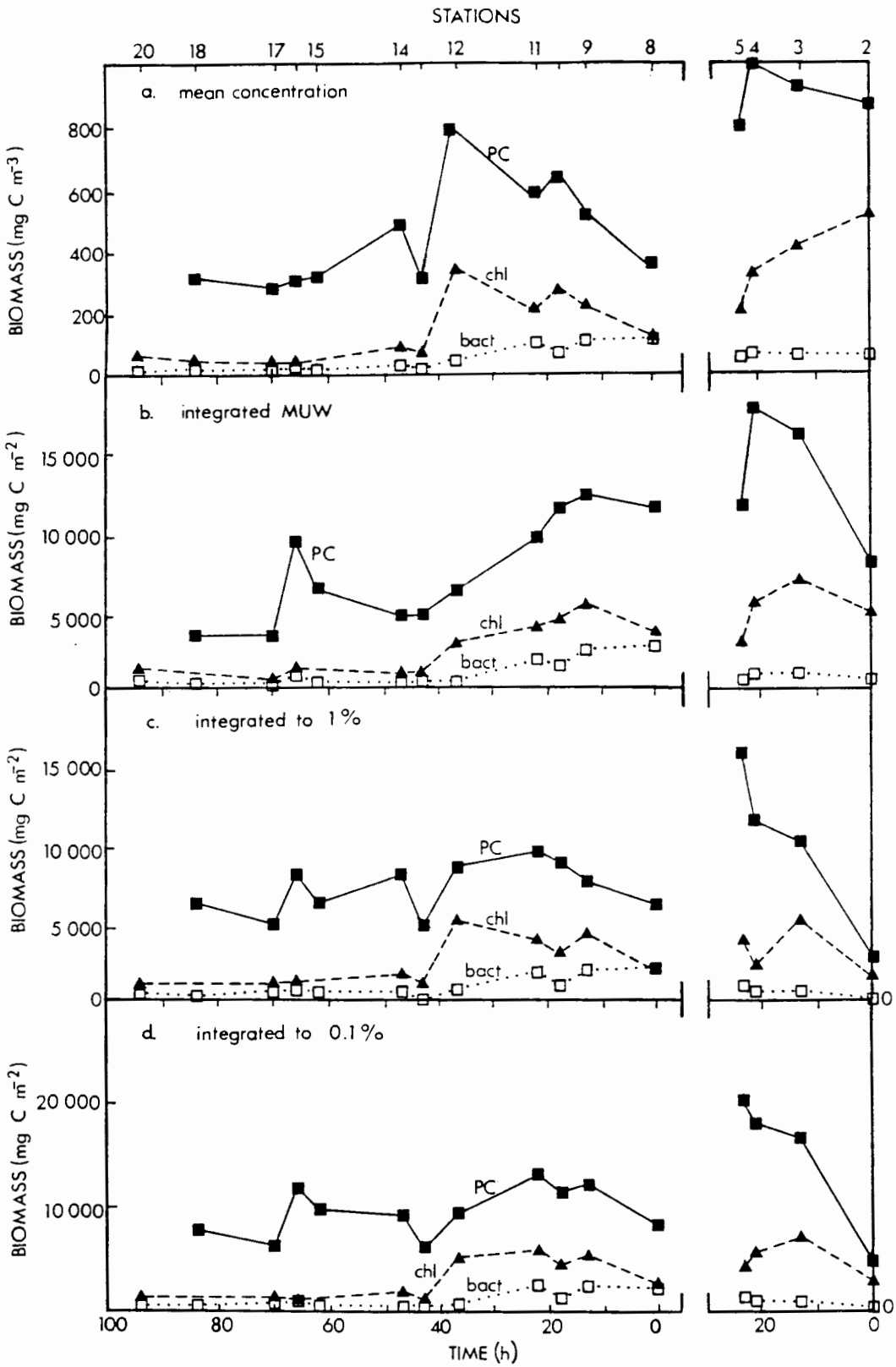


Fig. 6. Changes in the biomass of particulate carbon (PC), chlorophyll a (chl) and bacteria (bact) in the upper 20 to 40 m of the water column. Different methods of data analysis show different trends: (a) Average concentrations in mature upwelled water (MUW) and MUW/mixed water; (b) Biomass data integrated over the depth of the MUW and MUW/mixed water; (c and d) Biomass data integrated to a constant light depth: 1 % and 0.1 % respectively.

Table 5. Numerically dominant phytoplankton in surface waters. Cell concentrations ml^{-1}. Ciliates = Oligotrichs and Tintinnids. Flagellates include both autotrophs and heterotrophs.

Stn	Flagellates		Diatoms		Ciliates	Noctiluca	Dominant diatom
	2-20 μm	>20 μm	2-20 μm	>20 μm			
2	956	107	5240	386	64	0	Skeletonema
4	568	35	5212	342	48	0	"
8	4105	32	209	78	3	0.9	Chaetoceros
9	4092	20	312	225	6	1.6	and
10	3049	34	285	256	23	4.7	Cerataulina
11	4824	12	388	109	16	1.6	"
12	2566	33	972	178	20	0.6	"
13	1190	15	67	38	14	0.4	"
14	1659	9	311	45	10	0.3	"
15	1439	8	39	10	1	0	
16	1825	4	11	8	2	0	

Particulate carbon and phytoplankton biomass were relatively high in MUW during the second drogue, increasing from Station 8 to 12 (Fig. 6a). The numerically dominant phytoplankton here were small flagellates (2 to 20 μm diameter, Table 5). Bacterial biomass was largest at these stations, decreasing from Station 8 to 12. From Station 13 onwards the biomass of PC, Chl and bacteria was low.

Biomass data are compared in Table 6. Phytoplankton:PC ratios were similar at Stations 2 to 12, with chlorophyll carbon accounting for an average of 40 % (± 10 %, n=9) of the total particulate carbon. Smaller ratios were found at Stations 13 to 20 (mean = 16 ± 3 %, n=5). Bacterial carbon averaged 16 % (± 5 %, n=4) of chlorophyll carbon at Stations 2 to 5, 41 % (± 21 %, n=5) at Stations 8 to 12, and 43 % (± 14 %, n=6) at Stations 13 to 20. Bacterial:particulate carbon ratios were high (18 ± 4 %, n=3) at Stations 8 to 11, and low elsewhere (7 ± 1 %, n=11). Similar ratios, not shown here, were found for integrated data (MUW, 1 and 0.1 % light depths).

The relative contribution of different bacterial morphotypes to the total population differed with water type (and therefore also with depth). This was particularly true for small cocci (mean volume: 0.009 μm^3; Fig. 7a), which comprised a relatively small percentage of the total numbers in MUW and frontal water, but were one of the dominant morphotypes in SACW. Large cocci (mean volume: 0.142 μm^3) were numerically dominant in each water type. Small rods (mean volume: 0.198 μm^3) accounted for a relatively large proportion of the populations. Large rods (mean volume: 0.672 μm^3) were present in all water types, but numbers were low.

In terms of total bacterial biomass the contribution by small cocci was negligible in each of the different water types, while large cocci and small

Table 6. Average concentrations of particulate carbon (PC) and phytoplankton (P) and bacterial biomass (B) in mature upwelled water (MUW) and MUW/mixed water. A carbon:chlorophyll ratio of 60 was used to estimate phytoplankton carbon.

Station	Time (h)	Depth of MUW (m)	PC (mg C m^{-3})	Phyto (P) (mg C m^{-3})	Bact (B) (mg C m^{-3})	P:PC	B:P	B:PC
Mature upwelled water								
2	0	10	815.9	494.8	57.0	0.61	0.12	0.07
3	13	18	915.4	394.0	52.1	0.43	0.13	0.06
4	21	18	999.5	307.0	55.9	0.31	0.18	0.06
5	23	15	789.8	197.0	41.8	0.25	0.21	0.05
		mean:	880.0	348.2	51.7	0.40	0.16	0.06
8	0	30	383.1	115.5	86.5	0.30	0.75	0.23
9	13	24	512.8	222.8	97.7	0.43	0.44	0.19
10	18	18	640.6	243.3	74.9	0.38	0.31	0.12
11	22	16	597.6	236.6	104.6	0.40	0.44	0.18
12	37	8	778.8	357.8	44.2	0.46	0.12	0.06
		mean:	582.6	235.2	81.6	0.39	0.41	0.16
MUW/mixed water								
13	43	15	309.6	65.8	21.0	0.21	0.32	0.07
14	47	9	505.1	93.0	29.7	0.18	0.32	0.06
15	62	20	315.5	-	19.4	-	-	0.06
16	66	30	313.0	40.3	25.1	0.13	0.62	0.08
17	70	12	273.2	37.8	20.1	0.14	0.53	0.07
18	84	11	300.4	41.0	21.8	0.13	0.53	0.07
20	94	19	-	65.7	16.1	-	0.25	-
		mean:	336.1	57.3	21.9	0.16	0.43	0.07

- denotes no data available, or too few samples to calculate SE.

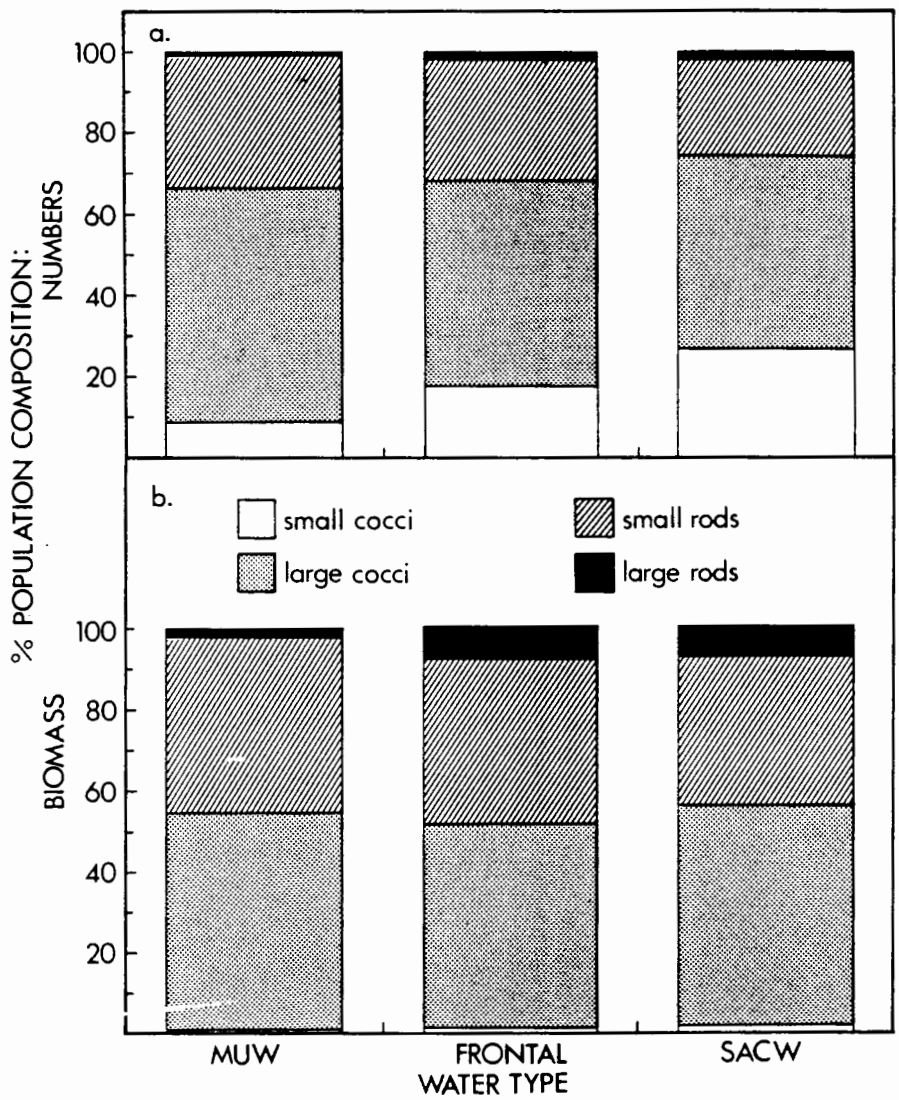


Fig. 7. Relative contribution of different bacterial morphotypes to the total population in each of the water types identified: mature upwelled water (MUW, including MUW/mixed water), frontal water and South Atlantic Central Water (SACW). (a) Numbers, (b) Biomass.

rods were clearly dominant (Fig. 7b). Due to their volume large rods were calculated to contribute about 10 % to the population in frontal and SACW. The weighted mean cell biomass, calculated from the total bacterial biomass and total cell numbers, was highest in MUW (18.33 fg C $cell^{-1}$), decreasing to 17.66 fg C $cell^{-1}$ in frontal water, and 15.69 fg C $cell^{-1}$ in SACW.

The relationships between bacteria and Chl a and PC during this study were examined in relation to the age of the surface water. All relationships were found to be significant (Table 7), and best described by power functions (see also Verheye-Dua and Lucas 1988). In general, bacterial biomass increased as chlorophyll and PC standing crops increased, but was reduced at high biomass values.

Zooplankton

The zooplankton was dominated by copepods, both in terms of numbers and biomass, with Calanoides carinatus as the dominant species. From counts of Niskin bottle samples it was evident that virtually all of the copepods were found in the upper 40 m of the water column (Fig. 8). It was therefore assumed that Bongo samples, although collected over the whole water column, actually represent the upper 50 m of the water column.

The total abundance of copepods was high (2000 to 17200 individuals m^{-3}, mean = 6400 m^{-3}), with C. carinatus accounting for an average of 30 % of total numbers (see Fig. 9a). Other important copepods in rank order of abundance were Oithona spp., Centropages brachiatus, and various small copepods (body weight <10 µg dry wt) including Ctenocalanus vanus, Paracalanus parvus and P. scotii. Copepods which occurred in five or more samples included Rhincalanus nasutus, Metridia lucens, Calanus australis and Oncaea spp..

Table 7: Regression equations describing the biomass relationships between bacteria (mg C m^{-3}) and the concentrations of chlorophyll (mg m^{-3}) and particulate carbon (mg C m^{-3}) in upwelled water during this study. Data from all depths were used, and were grouped according to the age of surface water. Maturing upwelled water (MUW) was observed at Stations 8 to 12; mature upwelled water (also MUW) was identified at Stations 2 to 5; mature upwelled water of different ages was mixing at Stations 13 to 20 (MUW/mixed).

Stations	Regression equation	n	r^2	p
Chlorophyll vs.Bacteria				
2-5	$Y = 1.69\ X^{0.60}$	43	0.655	0.001
8-12	$Y = 3.41\ X^{0.55}$	43	0.766	0.001
13-20	$Y = 2.98\ X^{0.33}$	52	0.450	0.001
Particulate carbon vs. Bacteria				
2-5	$Y = -3.81\ X^{1.14}$	34	0.816	0.001
8-12	$Y = -4.89\ X^{1.45}$	36	0.799	0.001
13-18	$Y = -6.51\ X^{1.66}$	35	0.704	0.001

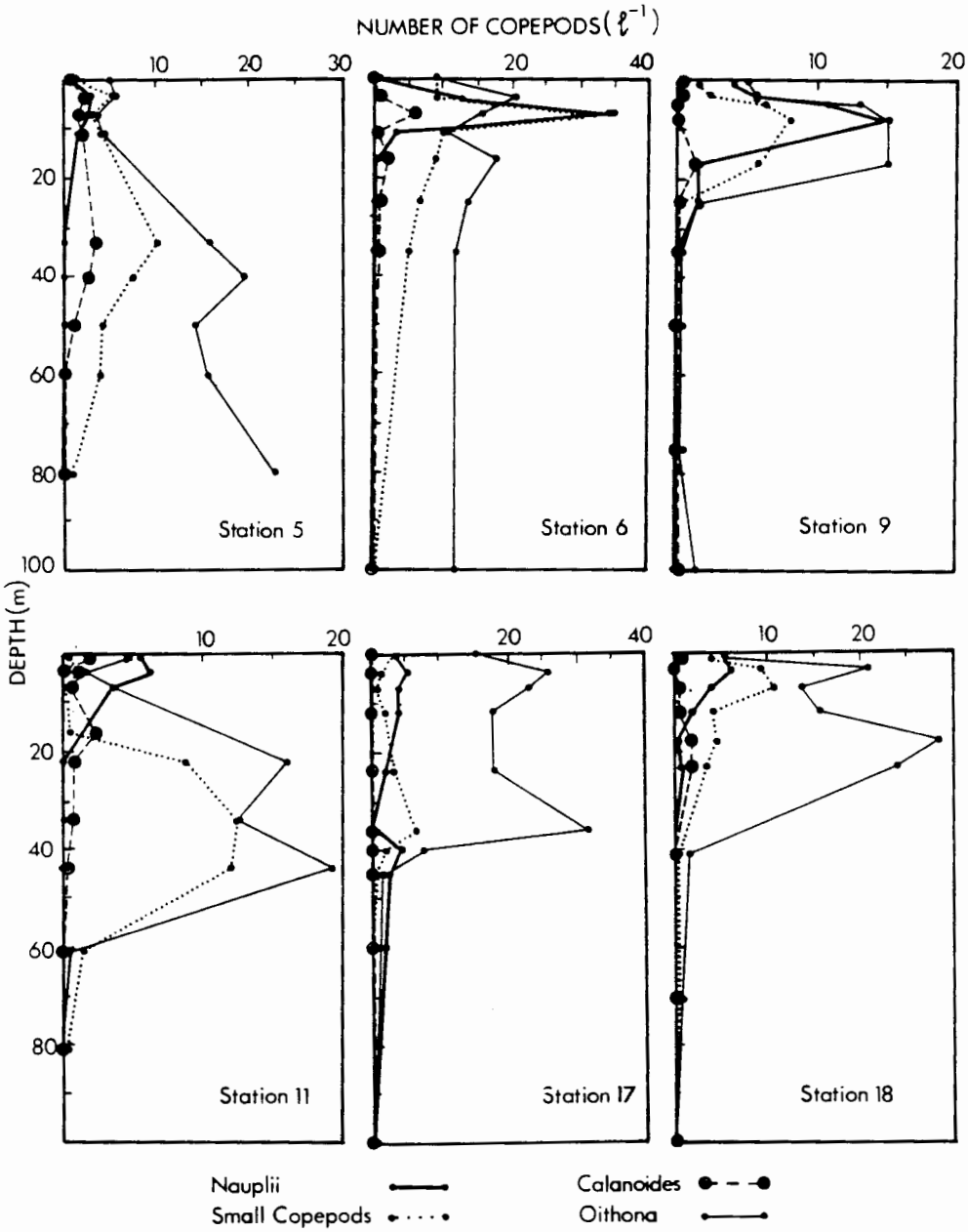


Fig. 8. Vertical distribution of copepods at 6 stations, determined from Niskin bottle samples. Small copepods include Paracalanus, Ctenocalanus, Centropages and Calanoides copepodites (C1 to C3).

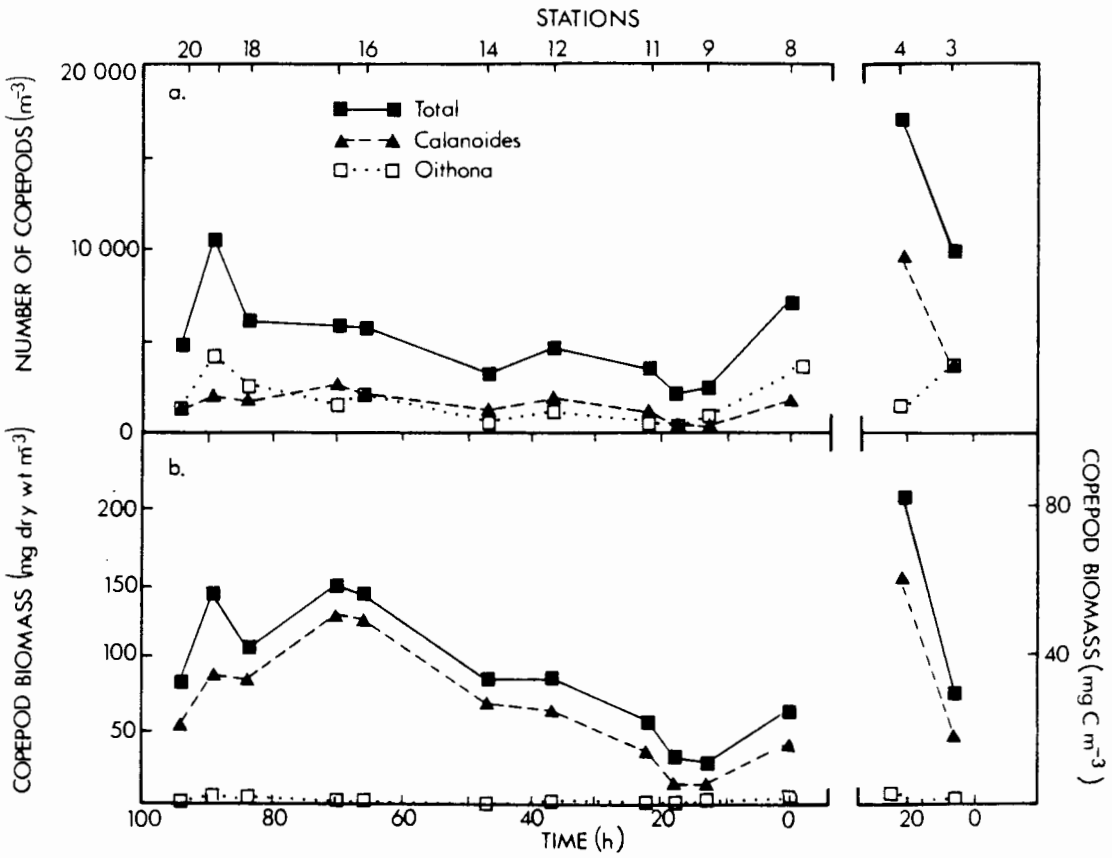


Fig. 9. (a) Number and (b) biomass of copepods in the upper 50 m of the water column.

Figure 9 (a, b) shows variations in copepod numbers and biomass. For Calanoides carinatus maximum abundances (≡ 60 mg C m^{-3}) were found at Station 4. Abundances were lowest at Stations 9 to 11 (mean value ≡ 8 mg C m^{-3}), increasing to a peak at Station 17 (50 mg C m^{-3}). Other copepods showed similar changes in abundance, being relatively high at the first few stations, low at Stations 8 through 17, with a secondary peak at Station 19.

Mean copepod biomass over the study period was 37 mg C m^{-3} (Fig 9b), with C. carinatus accounting on average for 67 % of this total. Copepod biomass data are compared with chlorophyll data in Table 8. Ratios of copepod:chlorophyll carbon calculated from the average concentrations in MUW and MUW/mixed water (mg C m^{-3}) showed that copepod biomass averaged 11 % of the phytoplankton biomass at Stations 3 to 12, and 96 % at Stations 14 to 20. If data were integrated over all sampling depths, the ratio of copepod carbon to phytoplankton carbon averaged 18 % for Stations 3 to 12, and 128 % for Stations 14 to 20 (Table 8).

Noctiluca were abundant during this study. Figure 10a shows changes in Noctiluca abundance estimated by the Bongo net. Numbers observed in the Niskin bottle samples (Fig. 10b) and from inverted microscope counts (Table 5) were 2 orders of magnitude larger than the Bongo samples, with highest numbers observed at Stations 8 to 12.

Table 8. A comparison of phytoplankton and copepod (Cope) biomass, using: (1) Average concentrations in mature upwelled and MUW/mixed water and (2) Biomass data integrated over the water column. Chlorophyll a (Chl) was used as an index of phytoplankton biomass.

	Concentration (mg C m^{-3})			Integrated (mg C m^{-2})		
Stn	Chl	Cope	Cope:Chl	Chl	Cope	Cope:Chl
3	394	30	0.08	9576	1490	0.16
4	307	86	0.28	26070	4310	0.17
6	-	33	-	8676	1630	0.19
8	116	26	0.22	5864	1290	0.22
9	223	11	0.05	5774	540	0.09
11	237	22	0.09	8282	1100	0.13
12	358	33	0.09	5269	1660	0.32
			0.11 ±0.06			0.18 ±0.07
14	93	33	0.35	1772	1660	0.94
16	40	56	1.40	3789	2800	0.74
17	38	58	1.53	1209	2920	2.42
18	41	42	1.02	-	2080	-
19	-	56	-	-	2820	-
20	66	33	0.50	1637	1650	1.01
			0.96 ±0.47			1.28 ±0.67

- denotes no data

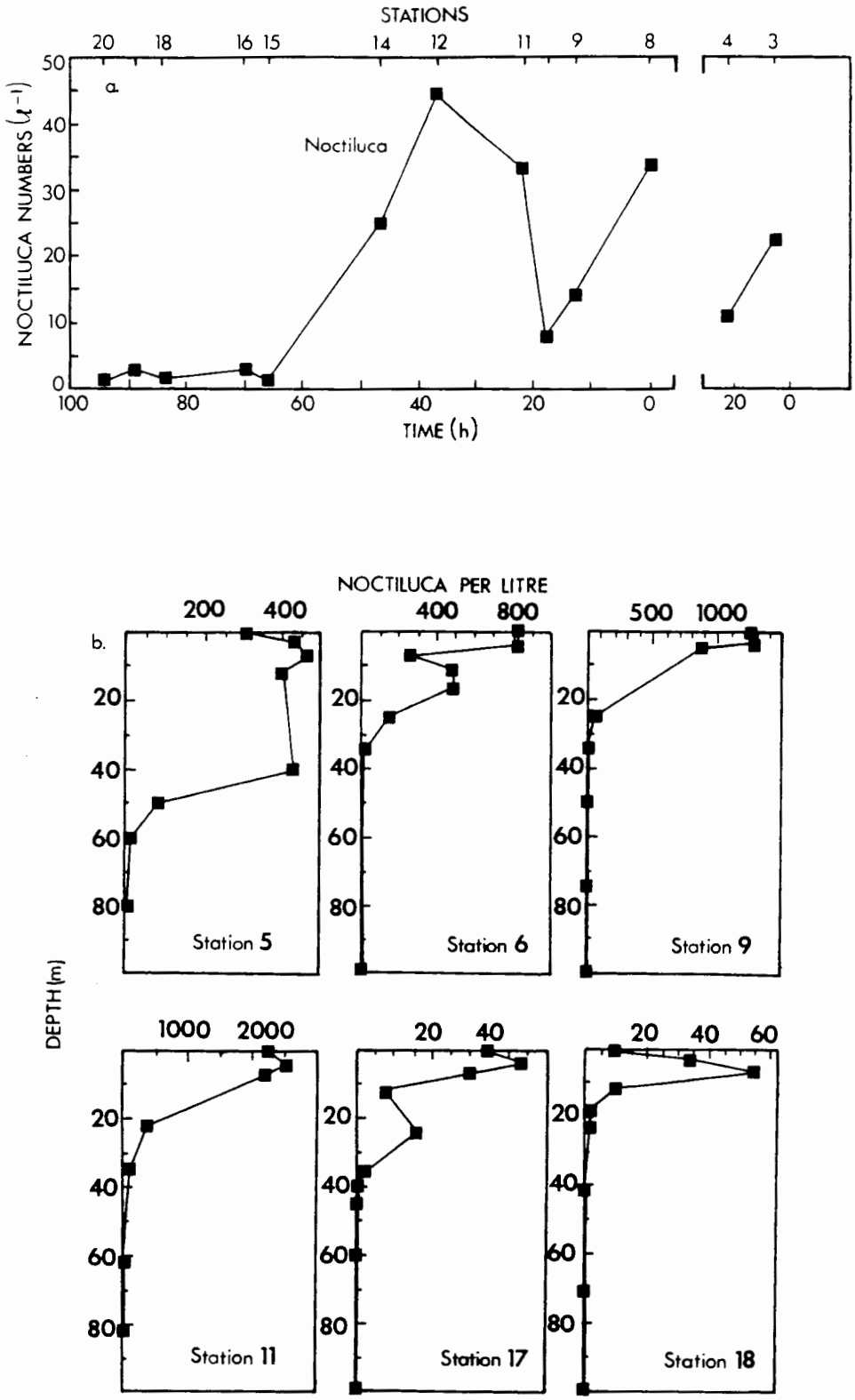


Fig. 10. Abundance of Noctiluca. (a) Changes estimated from Bongo net samples. (b) Vertical distribution determined from Niskin bottle samples.

DISCUSSION

Hydrography

Hydrographic features identified off Cape Columbine during this study are similar to those decribed by Waldron (1985). Quantitative data on concentrations of nitrate and chlorophyll within the different water types are consistent with those previously recorded for the southern Benguela (reviews by Shannon 1985b, Chapman and Shannon 1985, Shannon and Pillar 1986). It was evident that upwelling was not active in the study area. From temperature, salinity and nitrate sections it was clear that the water column was vertically stratified, and that water in the upper 20 to 30 m was maturing upwelled water (MUW). During the second drogue study, salinity and biological fronts between Stations 12 and 13 suggested that MUW was mixing with water which was somewhat older, although still of upwelling origin. As the Benguela current in this region flows northwards, older water will also have its origin to the south of Cape Columbine. Mixing of MUW masses of different ages effectively accelerated the biological development of the plume being studied. Major water masses in the aphotic zone were identified as frontal water, and South Atlantic Central water which is lifted onto the shelf during upwelling.

The Planktonic Community

Bacterial numbers and biomass were temporally and spatially variable. In surface waters, values were highest (2 to 10 x 10^6 cells ml^{-1}, 40 to 200 mg C m^{-3}) in maturing upwelled water, and lowest in MUW/mixed water (1 to 2 x 10^6 cells ml^{-1}, 20 to 40 mg C m^{-3}). In the aphotic zone, bacterial numbers and biomass were low. Spatial variability was observed in relation to the dominant water types. In frontal water, where concentrations of chlorophyll and

particulate carbon (PC) were relatively high (1 to 2 mg Chl m^{-3}, 40 to 250 mg PC m^{-3}) bacterial abundance and biomass values of up to 2 x 10^6 cells ml^{-1} and 40 mg C m^{-3} were measured. In SACW, chlorophyll and PC concentrations were low (<2 mg Chl m^{-3}, <250 mg PC m^{-3}). Bacterial numbers and biomass were similarly low (<1 x 10^6 cells ml^{-1}, 2 to 20 mg C m^{-3}).

Bacterial community structure, in terms of dominant morphotypes, was observed to vary spatially, in relation to the water types identified. Although large cocci (mean volume: 0.142 μm^3) and small rods (mean volume: 0.198 μm^3) dominated the community in each water type, the numerical contribution of small cocci (mean volume: 0.009 μm^3) increased as the trophic status of the water decreased. This is reflected in the decrease in the weighted mean cell biomass. In MUW the average cell biomass (Total biomass/Total number of cells, from AODC) was 18.33 fg C $cell^{-1}$. Approximately 25 % of the bacterial community in SACW was comprised of small cocci, probably representing the starved minicells characteristic of organically depauperate environments (Morita 1984, Davis and Robb 1985, Verheye and Lucas 1988, Painting et al. 1989), resulting in an average cell biomass of 15.69 fg C $cell^{-1}$. These values assume a constant carbon content per unit volume, and primarily reflect the decrease in average cell size in SACW.

Previous studies on the dynamics of phytoplankton after upwelling have shown that the initial phytoplankton community is generally dominated by chain-forming diatoms, such as Chaetoceros and Skeletonema (Olivieri 1983, Hutchings et al. 1984). Stratified conditions are not optimal for diatom growth and diatoms are succeeded by flagellates (<20 μm diameter) and pico-phytoplankton (<2 μm) (see Hutchings et al. 1984, Probyn 1987, Mitchell-Innes and Winter 1987). Although the drogue was released into two separate water masses, results from this study are consistent with earlier work. Large diatoms (>20 μm;

Skeletonema, Chaetoceros and Cerataulina) were present at relatively high abundances in MUW, decreasing from 386 to 178 cells ml^{-1}. Numerically, the phytoplankton community during the second drogue study was dominated by nano-phytoplankton (including diatoms and flagellates), showing a temporal decline in diatom abundance.

Copepods observed were typical of an upwelling community. Calanoides carinatus is common in the Benguela upwelling region, and is a dominant member of all upwelling systems on the African coast (Peterson and Painting 1989, Verheye 1989b). Estimates of copepod abundance and biomass in this study were larger than previously published estimates (see review by Shannon and Pillar 1986). The mean value of 2 g C m^{-2} for copepod biomass is more than double the average values reported by Borchers and Hutchings (1986), Shannon and Pillar (1986) and Verheye and Hutchings (1988). Furthermore, the values in this study are for copepods only, while the previous authors gave estimates of total zooplankton biomass. As a consequence of the high biomass measured, zooplankton grazing potential during this study (see below) was considerably greater than estimates previously recorded (Olivieri and Hutchings in prep., Peterson et al. 1989).

The relatively large temporal and spatial scales appropriate to the dynamics of copepod communities may make it unrealistic to interpret copepod dynamics only within the confines of the upwelling plume studied here. Development times and population maintenance mechanisms allow zooplankton to integrate their environment over time and space. The most plausible explanation for the high copepod biomass measured in this study, particularly as the drogue moved further downstream from the upwelling centre, is the hypothesised seasonal offshore accumulation of copepods over the summer upwelling season. In the northern Benguela, for example, studies on the distribution of phytoplankton

and zooplankton suggest that phytoplankton biomass is higher inshore, due to high primary production rates and a lag in the development of zooplankton, and that zooplankton dominates older offshore waters (see review by Branch et al. 1987). This hypothesis is supported by data on the population age structure of Calanoides carinatus (Chapter 8). MUW was dominated by early juveniles of C. carinatus, while MUW/mixed water was dominated by stage 5 copepodites and adults. Laboratory studies by Borchers and Hutchings (1986) have shown that older individuals are more tolerant of an intermittent food supply and starvation.

Abundances of the zooflagellate, Noctiluca, were also higher than average (Hutchings, pers. comm.). No quantitative studies have been done on this organism in the southern Benguela, but abundances observed in this study are ten times higher than estimates reported from the North Sea (Uhlig and Sahling 1982). It is likely that this heterotroph had a significant impact on the structure of the planktonic community. Apart from reports on Noctiluca predation on copepod eggs (Daan 1987), relatively little is known about the trophic ecology of Noctiluca. Unpublished data (Verheye, pers. comm.) show that Noctiluca are able to readily ingest large phytoplankton cells, such as Coscinodiscus gigas, as well as eggs of C. carinatus. Considering the range in organism size it seems likely that this zooflagellate could consume a wide range of planktonic size classes, including microzooplankton. High predation on bactivores by Noctiluca in MUW during drogue 2 may explain the large bacterial biomass measured at Stations 8 to 10.

Development of the Biological Community in MUW

Although phytoplankton and bacterial biomass were highest in MUW, copepods were distributed over the upper 50 m of the water column. Therefore, to discuss the dynamics of the biological community in upwelled water realistically, data were integrated over the water column (50 m for copepods, otherwise 100 m). The total biomass of phytoplankton, bacteria and copepods, and the general biomass relationships, are summarised in Table 9. All relationships are shown in carbon units, and are sensitive to the C:Chl ratio of 60 used throughout this study. Much of the previous work in the southern Benguela (see, for example, Brown and Hutchings 1987b) assumed a C:Chl ratio of 50. Recent microcosm studies by Pitcher (in prep.) have shown that the C:Chl ratio ranges from 38 to 146, with maximum values during phytoplankton senescence. Ideally, C:Chl ratios should be measured during each phytoplankton study, but this is often impractical. Total particulate carbon (PC) concentrations are also shown on Table 9. The ratio of total microbial biomass (phytoplankton and bacteria) to PC was used as a relative index of the proportions of living and detrital planktonic carbon.

Phytoplankton and bacterioplankton

Favourable nutrient and light conditions in the euphotic zone stimulated the development of the planktonic community in the plume of mature upwelled water. Phytoplankton production (Table 3) and biomass were high initially, and then decreased, presumably in response to nutrient limitation and herbivory. Total particulate carbon showed similar trends, decreasing throughout the study. In MUW the microbial biomass accounted for 45 % of the total PC. The remaining 55 % was assumed to be detrital carbon. This is, however, likely to be overestimated due to the possible incorporation of microzooplankton, which was not measured. In MUW/mixed water the low ratio of microbial biomass: PC

Table 9: A summary of the total biomass (PBK, g C m^{-2}) of phytoplankton (P), bacteria (B) and copepods (K) in the water column (100 m) at each station during the drogue study in the southern Benguela, and the general biomass relationships. Particulate carbon (PC) concentrations are also shown. The contribution of microbial biomass (phytoplankton and bacteria, PB) to total particulate carbon, PC, is shown by the ratio, PB:PC. The remaining PC was assumed to be largely detrital. Carbon values were calculated from a C:Chl ratio of 60 for phytoplankton, a factor of 0.121 pg C μm^{-3} for bacteria and a C:dry weight ratio of 40% for copepods.

	BIOMASS (g C m^{-2})					BIOMASS RELATIONSHIPS (%)						
Stn	P	B	K	Total PBK	PC	B/P	K/P	B/K	B/PB	B/PBK	K/PBK	PB/PC
MUW												
2	8.57	1.22	-	-	20.13	14	-	-	13	-	-	-
3	9.58	1.70	1.49	13	30.30	18	16	114	15	13	12	37
4	26.07	3.30	4.31	34	47.57	13	17	77	11	10	13	62
5	7.71	1.48	-	-	27.34	19	-	-	16	-	-	34
6	8.68	1.88	1.63	12	22.40	22	19	115	18	16	14	47
						mean: 17	17	102	15	13	13	45
8	5.86	3.36	1.29	11	22.20	57	22	261	36	31	12	42
9	5.77	2.92	0.54	9	21.10	51	9	541	34	32	6	41
10	5.19	1.69	-	-	15.80	33	-	-	25	-	-	44
11	8.28	3.88	1.10	13	19.21	47	13	353	32	30	9	63
12	5.27	0.99	1.66	8	18.55	19	32	60	16	12	21	34
						mean: 41	19	304	29	26	12	45
MUW/mixed												
13	1.37	0.77	-	-	28.22	56	-	-	36	-	-	8
14	1.77	0.85	1.66	4	20.35	48	94	51	32	21	42	13
15	-	0.66	-	-	14.37	-	-	-	-	-	-	-
16	3.79	1.39	2.80	8	19.72	37	74	50	27	17	35	26
17	1.21	0.83	2.92	5	14.67	69	242	28	41	17	58	14
18	-	0.88	2.08	-	13.41	-	-	42	-	-	-	-
19	-	-	2.82	-	-	-	-	-	-	-	-	-
20	1.64	0.69	1.65	4	-	42	101	42	30	17	41	-
						mean 50	128	43	33	18	44	15

suggested that the greater proportion (up to 85 %) of planktonic carbon was detrital.

Bacterial biomass was correlated with phytoplankton production (Table 3), providing evidence of close coupling between bacteria and phytoplankton growth. During high levels of phytoplankton production the ratio of bacterial biomass to phytoplankton biomass (assuming a C:chlorophyll ratio of 60) was small (mean value: 17 %, Table 9). Dissolved organic carbon substrates exuded during phytoplankton growth (PDOC) probably formed an important nutrient source for these bacteria (Cole et al. 1982). As primary production decreased, the ratio of bacterial:phytoplankton biomass increased, suggesting greater dependence of bacteria on the more refractory organic carbon substrates associated with phytoplankton senescence. Correlation of bacterial biomass with PC concentrations (Table 7) provided evidence for coupling between bacterioplankton and PC (see also Lucas 1986, Biddanda 1988, Verheye-Dua and Lucas 1988, Painting et al. 1989). In a study of bacteria-phytoplankton relationships across a frontal region in the southern Benguela, Verheye-Dua and Lucas (1988) observed that the ratio of bacterial biomass:chlorophyll _a_ increased with increased ageing of upwelled water. They proposed, as did Barlow (1982a), that older water may be dominated by bacteria and detritus, while younger water will be dominated by active phytoplankton with high chlorophyll concentrations.

Relationships between bacterial biomass and phytoplankton biomass and production, and between bacteria and PC were generally best described by power curves. This suggests that bacterial biomass is reduced at high levels of primary production, and at high chlorophyll and PC concentrations, possibly due to predation. Numerous recent studies have shown that flagellates and ciliates can control bacterial biomass (Andersen and Fenchel 1985, Sherr et al. 1986,

Lucas et al. 1987) and that there is a well-ordered succession of phytoplankton-bacteria-protozoa (Laake et al. 1983b, Hagström 1984, Linley and Newell 1984, Lucas et al. 1987, Painting et al. 1989). As predation is density-dependent, it is to be expected that at higher bacterial densities, predation will have a greater impact, so accounting for the non-linear relationship between bacteria and chlorophyll a or PC.

Zooplankton

A large zooplankton community, dominated by the copepod, Calanoides carinatus, was observed in the upper 50 m of the water column in the study area. In mature upwelled water, copepod biomass was equivalent to 16 to 32 % of phytoplankton biomass, or approximately 12 % of total planktonic biomass. Assuming a simple diatom-copepod food chain, a daily food-requirement of 30 % of their total biomass, and a purely herbivorous diet, copepods are calculated to consume 5 to 10 % of phytoplankton biomass. These grazing estimates may be underestimated if diurnal vertical migration of copepods occurs and if large numbers of copepods were present below the sampling depth. This seems unlikely, however, as evidence from Niskin bottles suggested that copepods were in the upper 50 m of the water column. Other studies in the Cape Columbine region (Pillar 1984) and elsewhere in the southern Benguela have shown that copepods occur in the upper 100 m of the water column and show little evidence of vertical migration to depths below 100 m (Verheye and Hutchings 1988, Peterson et al. 1989). In MUW/mixed water copepod biomass exceeded phytoplankton biomass (average ratio of copepod:phytoplankton biomass = 128 %, Table 9) and copepods are calculated to consume 38 % of phytoplankton biomass to meet their daily requirements.

The estimate of the grazing impact of copepods during this study exceeds estimates obtained from direct measurements in the southern Benguela, which have consistently shown that <10 % of phytoplankton biomass is grazed by copepods (Olivieri and Hutchings in prep., Peterson et al. 1989). In a study of the grazing impact of copepods in the southern Benguela, Peterson et al. (1989), calculated that the entire copepod assemblage in mid-shelf waters grazed only 5 % (or possibly up to 15 % due to calculation errors inherent in the gut fluorescence techniques used) of the phytoplankton standing stocks each day. Estimates of copepod grazing are complicated by evidence suggesting that not all phytoplankton biomass is available to these grazers. Bartram (1980) showed that many copepods feed inefficiently on particles <10 μm diameter. More recent studies have shown that larger copepod species prefer to feed on particles >10 μm (Harris 1982, Price et al. 1983). In this study, the phytoplankton community in surface waters was dominated by organisms in the 2 to 20 μm size-range. It is unlikely that all of the phytoplankton biomass and production was available to herbivorous mesozooplankton, particularly in MUW/mixed water where diatom abundances were low. This suggests that copepods in MUW/mixed water were food-limited and would face starvation and a reduction in fecundity (Attwood and Peterson 1989).

The estimates of copepod grazing impact reported here may be influenced by the ratio of 60 for C:chlorophyll, which was adopted as an average for the upwelling plume. Although this value may be appropriate for a healthy phytoplankton bloom, it is likely to be too low for a declining bloom (Pitcher, in prep.). A C:chlorophyll ratio of 120, for example, would halve the grazing impact of copepods in the upwelling plume.

Phytoplankton, bacterioplankton and copepods.

Bacteria constituted 11 to 41 % of the total microbial (phytoplankton and bacteria) biomass, and 10 to 32 % of the total planktonic biomass (microbial biomass plus copepod biomass). The biomass relationships of phytoplankton, bacteria and copepods during this study were complex, and suggest that dynamic microbial food webs may exist in the southern Benguela. Despite the high biomass of copepods and the high potential grazing impact of these heterotrophs on the phytoplankton community, bacterial biomass in MUW was generally higher than copepod biomass. Their combined requirements for photosynthetically fixed carbon are unlikely to be satisfied unless carbon is cycled through a planktonic community in which different size-classes of autotrophs and heterotrophs are present (see also Strayer 1988, Scavia 1988). For maturing upwelled water, it seems likely that the traditional diatom-copepod food chain and the microbial food web co-occur. On this basis, predation would follow a size-class basis rather than strict herbivory or carnivory (Moloney 1988). High concentrations of heterotrophically derived organic carbon (HDOC), and production by the nanophytoplankton community possibly supported large bacterial and microzooplankton communities both directly and indirectly, thereby providing further food sources for mesozooplankton (see also Jumars et al. 1989, Probyn et al. 1989).

ACKNOWLEDGEMENTS

I am indebted to Drs L. Hutchings and P. Brown, who organised this cruise as part of a series of drogue studies conducted by the Sea Fisheries Research Institute. I would like to thank the Captain, navigation officers and crew of the R.S. Africana, and technical assistants from the Sea Fisheries Research Institute, for their co-operation and assistance during the sampling procedures. Owing to my full-time occupation with bacterial data, I am extremely grateful for the generous contributions of raw data on "the rest" from Geoff Bailey (Physical and Chemical data), Dr P. Brown (Primary production), Dr L. Hutchings (Zooplankton samples). I would also like to thank my supervisor, Dr M.I. Lucas, for his guidance and active participation during the cruise. This study would have been less complete without the assistance of Dr W. T. Peterson who, during his consultancy to the Benguela Ecology Program, kindly offered to identify and count the zooplankton samples collected during this study. My thanks are also extended to Frieda Verheye-Dua for assistance with bacterial counts, to Dr Betty Mitchell-Innes for identifying and counting phytoplankton, to John Roberts and Marius Coetzee for computer assistance, and to Tony van Dalsen for the final figures. I am grateful to Mike Lucas, Larry Hutchings, Bill Peterson, Coleen Moloney, Dot Armstrong and Hans Verheye for constructive criticism of this chapter. CHN analysis was done by the Chemistry Department of the University of Cape Town. I am also indebted to the Benguela Ecology Program (BEP) of the South African National Committee for Oceanographic Research (SANCOR), and to the Sea Fisheries Research Institute, for financial contributions to this study.

CHAPTER 7

Bacterial production in the the southern and northern Benguela upwelling regions.

ABSTRACT

Heterotrophic bacterial production in the southern and northern Benguela upwelling regions, off the west coast of South Africa and Namibia, was measured from R.S. Africana during 2 oceanographic cruises: a drogue study in the southern Benguela (March 1983), and during the SNEC-85 cruise in the northern Benguela (October 1985). In the southern region, upwelling was quiescent, resulting in a stratified water column. The total biomass of bacteria, phytoplankton and particulate carbon (PC) was high (0.7 to 2.9 g C m^{-2}, 0.7 to 5.8 g C m^{-2} and 11 to 19 g C m^{-2} respectively). Bacterial production was measured during ship-based incubations of water collected from the euphotic zone, pycnocline and aphotic zone. Production rates, calculated from [methyl-^{3}H] thymidine incorporation (TTI) were variable (0.009 to 0.208 mg C m^{-3} h^{-1}) and highest in the euphotic zone. Bacterial production in the euphotic zone (2 to 3.3 mg C m^{-2} h^{-1}) was equivalent to 1 to 3 % of net phytoplankton production (66 to 465 mg C m^{-2} h^{-1}). Estimates from bacterial population growth (0.08 to 5.34 mg C m^{-3} h^{-1}) were ca 2 to 26 times higher (mean value = 10.5 times) than values based on TTI, and equivalent to 2 to 28 % of net phytoplankton production. Growth rates (d^{-1}) and doubling times (d) of bacteria calculated from population growth (0.55 to 1.14 d^{-1}, 0.6 to 2.3 d) were considerably higher than values calculated from TTI (0.04 to 0.2 d^{-1}, 4.1 to 17.3 d). In the northern Benguela the total biomass of bacteria, phytoplankton and PC was also high (1.3 to 2.6 g C m^{-2}, 9.5 to 10 g C m^{-2}, and 10 to 17 g C m^{-2}). Upwelling was active in this region, resulting in a well-mixed water column and relatively high standing stocks of bacteria, chlorophyll and PC in the aphotic zone. Phytoplankton production was low (41 to 102 mg C m^{-2} h^{-1}). From TTI, estimates of bacterial production (0 to 0.06 mg C m^{-3} h^{-1}), growth rates (0.02 to 0.06 d^{-1}) and doubling times (11.6 to 34.7 d) were similarly low, possibly due to a large proportion of inactive bacteria. Bacterial production

(1.7 to 3.5 mg C m^{-2} h^{-1}) was 2 to 5 % of phytoplankton production. In both regions, bacterial biomass contributed a large proportion (>10 %, maximum 55 % [southern] and 22 % [northern]) of the total microbial (phytoplankton and bacteria) biomass in the water column, and was correlated with chlorophyll standing stocks and primary production, indicating close coupling between bacterial and phytoplankton dynamics. Close coupling between bacteria and PC suggests bacterial utilisation of the more refractory organic carbon in both regions, and possible underestimation of bacterial production by TTI. Results of this study suggest that variability in bacterial production in the Benguela upwelling region is attributable to a combination of dynamic oceanographic processes and methodological limitations of the TTI procedure.

INTRODUCTION

The current concept of the trophic dynamics of pelagic ecosystems considers bacteria to play a potentially important role in the utilisation of phytoplankton production and in nutrient regeneration, via the microbial loop (Pomeroy 1974, Williams 1981, Azam et al. 1983, Ducklow 1983, Azam and Fenchel 1985, Sherr and Sherr 1988). Since the development of techniques for measuring bacterial biomass and production (for reviews see Floodgate 1980, van Es and Meyer-Reil 1982, Hobbie and Williams 1984, Moriarty 1986), considerable emphasis has been placed on obtaining quantitative estimates of these variables in a diversity of trophic environments. Recent studies have shown that bacteria are an important component of the heterotroph biomass in the marine environment (Linley et al. 1983, Azam and Fuhrman 1984, Chapter 7). That bacteria are largely dependent upon phytoplankton production is supported by numerous studies showing correlations between bacterial biomass and chlorophyll concentrations (Linley et al. 1983, Bird and Kalff 1984, Verheye-Dua and Lucas 1988, see also Jumars et al. 1989). Pelagic heterotrophic bacteria have been estimated to have high rates of production, and to utilise up to 60 % of phytoplankton production, primarily through the uptake of photosynthetically produced dissolved organic carbon, PDOC (Hagström et al. 1979, Williams 1981, Cole et al. 1982, Fuhrman and Azam 1980, 1982, Bell et al. 1983, Fuhrman 1984, 1987, Newell and Turley 1987).

Estimates of bacterial production in different marine environments are highly variable, ranging from <1 to 200 mg C m^{-3} d^{-1} (Cole et al. 1988). Similarly, growth rates may range from 0.2 to 2 d^{-1} (Ducklow 1983, McManus and Peterson 1988) and have been shown to vary diurnally and seasonally in response to phytoplankton growth (Lochte and Turley 1985, Turley and Lochte 1986a). Numerous techniques have been employed to measure bacterial growth and

production, including the frequency of dividing cells (Hagström and Larsson 1984), population growth in a predator-reduced environment (Linley et al. 1983) or in seawater cultures (Ducklow and Hill 1985a), respiration (Hopkinson et al. 1989) and bacterial uptake of dissolved substrates (see Fuhrman and Azam 1980, 1982, Fuhrman et al. 1986, Karl and Winn 1986, Moriarty 1986). None of these techniques is entirely satisfactory for quantifying the total production rate of natural heterogeneous bacterial populations (Newell and Fallon 1982, Riemann et al. 1984, Riemann and Sondergaard 1984, Moriarty 1986, Painting et al. 1989), and many field studies have been based on the method which involves minimum manipulation and simple assay techniques, viz: measurements of the incorporation of [methyl-^{3}H] thymidine into bacterial DNA (Fuhrman and Azam 1980, 1982).

Few measurements of bacterial production have been made in the Benguela upwelling region. Earlier work on the degradation of kelp detritus (Newell et al. 1980, Linley and Newell 1981, Stuart et al. 1981, 1982) and kelp exudates (Linley et al. 1981, Lucas et al. 1981) showed that heterotrophic bacteria and protozoa play an important role in decomposition and nutrient recycling in waters adjacent to the coast. The first quantitative work on phytoplankton and bacterial biomass in the pelagic environment of the southern Benguela (Linley et al. 1983) suggested that the microbial food web was also an important component of the pelagic food web. More recent studies have focussed on obtaining quantitative estimates of bacterial production in response to the development of phytoplankton communities. In a study of bacterial dynamics across an upwelling front approximately 20 nautical miles offshore, Armstrong et al. (1987) measured bacterial production rates of up to 5.5 mg C m^{-3} h^{-1} inshore of the front, using predator-reduced incubations. Bacterial production was calculated to be 11 to 67 % of phytoplankton production. The authors hypothesised that bacteria in offshore water, where the ratio of bacterial to

phytoplankton production was highest, were using PDOC and detrital particulate carbon as well as heterotrophically derived sources of dissolved carbon to meet their carbon requirements. During a microcosm study of the activity and production of heterotrophic bacteria in response to phytoplankton growth and decay in the southern Benguela, Painting et al. (1989) provided further evidence of high bacterial production on the more refractory carbon substrates associated with detrital carbon (see also Chapter 4).

The objective of this study was to obtain quantitative estimates of in situ bacterial production during the development of a phytoplankton community in a plume of upwelled water in the southern Benguela. Bacterial production was measured during a drogue study of the overall plankton dynamics in the summer upwelling season in March 1983 (see Chapter 6), using incorporation of [methyl-^{3}H] thymidine into bacterial DNA (Fuhrman and Azam 1980, 1982), and predator-reduced incubations. Due to quiescent upwelling conditions in the study area, bacterial production was measured during the declining phase of the phytoplankton bloom (Chapter 6).

Additional estimates of bacterial production during active upwelling conditions were obtained from measurements of bacterial incorporation of [methyl-^{3}H] thymidine during a collaborative oceanographic study between the Instituto de Ciencias del Mar of Barcelona and the Sea Fisheries Research Institute, in the northern Benguela upwelling region. Aspects of the physical, chemical and biological oceanography in this region have been reviewed by Nelson and Hutchings (1983), Chapman and Shannon (1985), Shannon (1985b) and Shannon and Pillar (1986). The northern Benguela supports an important pelagic fishery (Shelton et al. 1985), but few studies have been done on primary and secondary production (Shannon and Pillar 1986, Estrada and Marrasé 1987, Probyn

1988). To date, there are no published measurements of bacterial production in this region.

METHODS

Sampling and analytical methods

The production of heterotrophic bacteria in the Benguela upwelling region was measured during two oceanographic cruises on the R.S. Africana. The first cruise was in the southern Benguela during the summer of 1983 (13 to 20 March, Chapter 6). A drogue was released on two separate occasions into a plume of maturing upwelled water downstream of the upwelling centre at Cape Columbine (Fig. 1). The drogue was followed for 37 and 94 h respectively, to determine temporal changes in the hydrography and plankton dynamics during the development and offshore movement of the plume. The water column at the drogue was sampled at 19 Stations, Stations 2 to 6 during the first 37 h and Stations 8 to 20 during the second drogue track. Bacterial production measurements were made at Stations 9, 15 and 18 (Fig. 1).

The second cruise during which bacterial production was measured was in the northern Benguela in early spring 1985 (16 September to 6 October), during the Spanish/Namibian Environmental Cruise-85 (SNEC-85). Bacterial production estimates were obtained at random stations on sampling transects over the continental shelf and slope between Mowe Point (19^0 30' S) and Lüderitz (26^0 30' S) (Fig. 1, Gutiérrez et al. 1985, Estrada and Marrasé 1987).

At each station during both cruises temperature and salinity profiles were obtained using a CTD recorder (Neil Brown Mark III). Light penetration depths were measured using a LICOR (LI-192S) quantum sensor or, occasionally, a Secchi

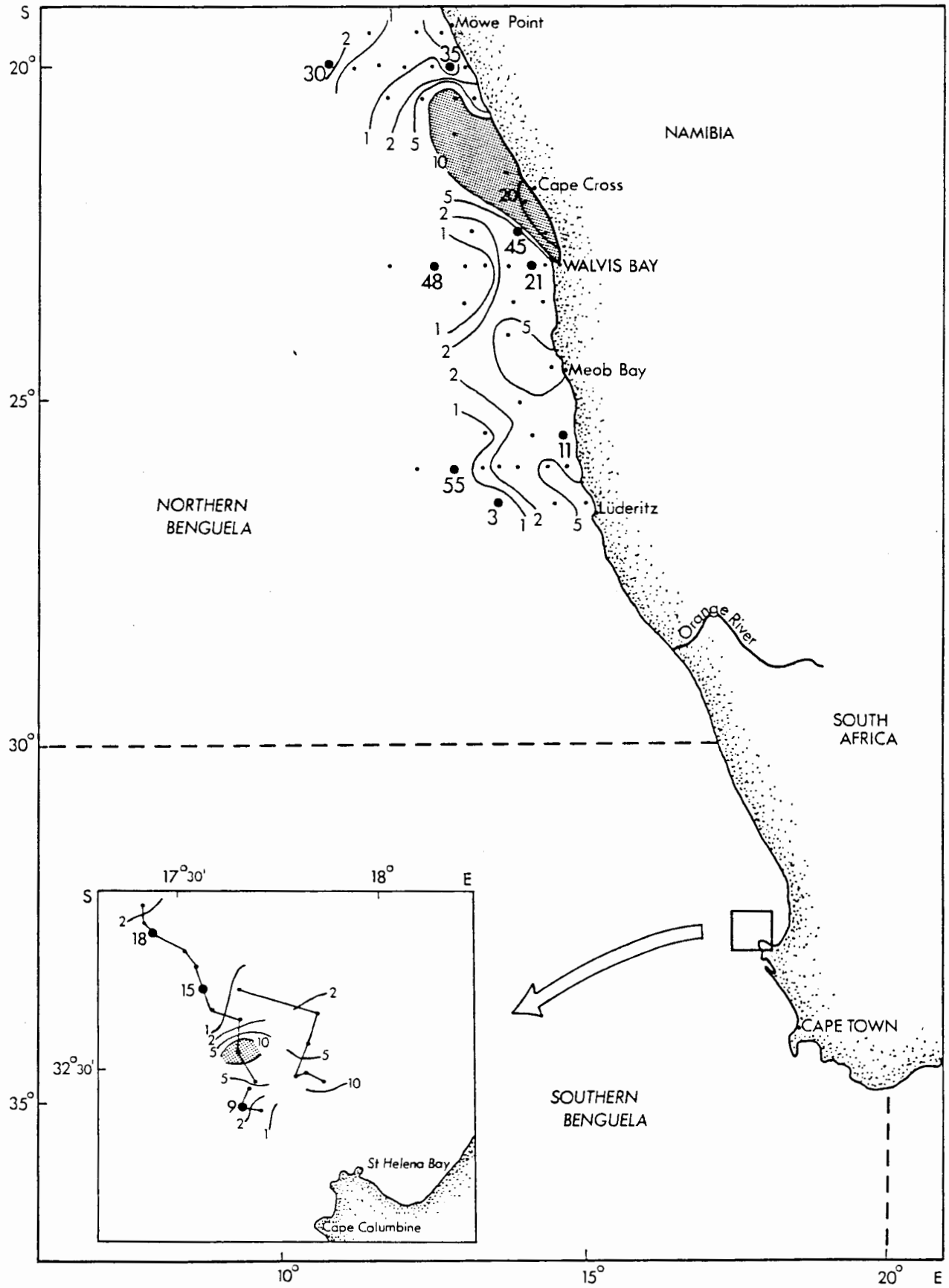


Fig. 1. A chart of the southern and northern regions of the Benguela upwelling system situated off the west coast of southern Africa, showing stations sampled during a drogue study north of Cape Columbine and during the SNEC-85 cruise off the Namibian coastline. Surface chlorophyll concentrations are shown, with shaded areas indicating high chlorophyll values (>10 mg Chl a m^{-3}). Bold dots and numbers indicate stations at which bacterial production was measured.

disc. Water samples were collected using a rosette of 10 5-litre Niskin sampling bottles from five depths in the euphotic zone (100 to 0.1 % light penetration depths). In the aphotic zone samples were collected at 10 or 20 m intervals to 100 m, and at 50 to 200 m intervals between 100 m and the bottom during SNEC-85.

Water from each depth was analysed immediately for nutrient concentrations on a Technicon Autoanalyser (Mostert 1983). Samples for chlorophyll a analysis were concentrated onto Whatman GF/C filters and analysed by spectrophotometry (SCOR/UNESCO Working Group 17 1966) in 1983, and by fluorometry (Strickland and Parsons 1972) in 1985. Particulate carbon was collected on Whatman GF/F filters, oven-dried at 60 °C and measured on a Hereaus CHN analyser. Samples for enumeration of bacteria were preserved with 25 % Analar Glutaraldehyde (1.3 % v/v) and stored at 4 °C prior to counting.

Bacterial numbers and biomass in the water column were estimated by epifluorescence microscopy, using the Acridine Orange Direct Count (AODC) technique of Hobbie et al. (1977). Bacteria were counted differentially in 7 morphotype classes, in at least 30 microscope fields or until 300 cells were counted. Bacterial volumes were calculated for each size-class using linear cell dimensions obtained from Scanning Electron Micrographs (Linley et al. 1983) and from measurements during epifluorescence microscopy (Painting et al. 1985). Bacterial biomass was calculated from total cell volume and a conversion factor of 121 fg C μm^{-3} (Watson et al. 1977).

Production measurements

Primary production rates in the euphotic zone were estimated from uptake of ^{14}C-bicarbonate by phytoplankton during 2 to 4 h in situ or ship-board incubations under simulated in situ conditions (Strickland and Parsons 1972), as described by Brown (1982, 1984) and Estrada and Marrasé (1987).

Estimates of heterotrophic bacterial production were obtained from ship-based incubations of seawater from stations sampled before mid-day (see Fig. 1). Water was collected from discrete depths within the euphotic zone, pycnocline and aphotic zone, inoculated with 5 nM [methyl-^{3}H] thymidine (49 to 52 Ci $mmol^{-1}$) and incubated on an orbital shaker in the dark at 12 ^{o}C. At four stations in the northern Benguela water was collected at 4 depths within the euphotic zone only. Incorporation of thymidine (Tdr) into bacterial DNA during time-course experiments was measured following the method of Fuhrman and Azam (1980, 1982; Painting et al. 1989 and Chapter 4). Bacterial cell production was calculated from the rate of incorporation of thymidine into TCA-insoluble macromolecules (h^{-1}) and a conversion factor for nearshore bacteria of 1.7 x 10^{18} cells mol^{-1} Tdr incorporated (Fuhrman and Azam 1982). To estimate carbon production the weighted mean cell biomass of the appropriate in situ bacterial population was used (fg C $cell^{-1}$ from AODC, Painting et al. 1989).

In the southern Benguela the estimates of bacterial production were compared with direct estimates of population growth in size-fractionated incubations maintained under similar incubation conditions. These incubations were done with bacteria in natural seawater which had been gravity filtered (<3 µm) to reduce the number of predators (Sorokin and Kadota 1972, Meyer-Reil 1977, Linley et al. 1983). Changes in bacterial biomass were measured from the AODC

method described above. Bacterial population growth was estimated from curves of best fit to the data (Chapter 4, Painting et al. 1989).

All sampling and incubation vessels were pre-rinsed with 10 % HCl, filtered distilled water (0.2 μm), and sample water. Bacteria in controls for measurements of thymidine incorporation were autoclaved before incubation.

RESULTS

Hydrographic features observed during the drogue studies in the southern Benguela showed that there was no active upwelling in the study area. From temperature, salinity and nitrate sections it was clear that the water column was vertically stratified, and that water in the upper 20 to 30 m depths at Stations 2 to 12 was maturing upwelled water (MUW). Salinity and biological fronts between Stations 12 and 13 suggested that the MUW was converging with, and possibly mixing with, older upwelled water (MUW/mixed water) thereby speeding up the age-dependent biological development of the water mass (see Chapter 6). Nitrate concentrations and primary production levels suggested that the study was conducted during the declining phase of phytoplankton growth.

Hydrographic data from SNEC-85 showed that upwelling was active during the study in the northern Benguela. Temperature and nitrate sections at transects along the 20^0, 23^0 and 26^0 S latitudes indicated more vigorous upwelling in the southern region off Luderitz, 26^0 S (Fig. 2, from Probyn 1988). Similarly, low surface temperatures (<12 ^{0}C) and high surface nitrate concentrations (15 to 20 μg-at N l^{-1}) around 26^0 S suggest more active upwelling in the southern regions (Fig. 3, from Estrada and Marrasé 1987). Vertical distribution of temperature, nitrate (Fig. 2) and salinity (Masó and Manríques 1986) were homogeneous throughout an upper mixed layer of 40 to 60 m depth, suggesting vertical mixing

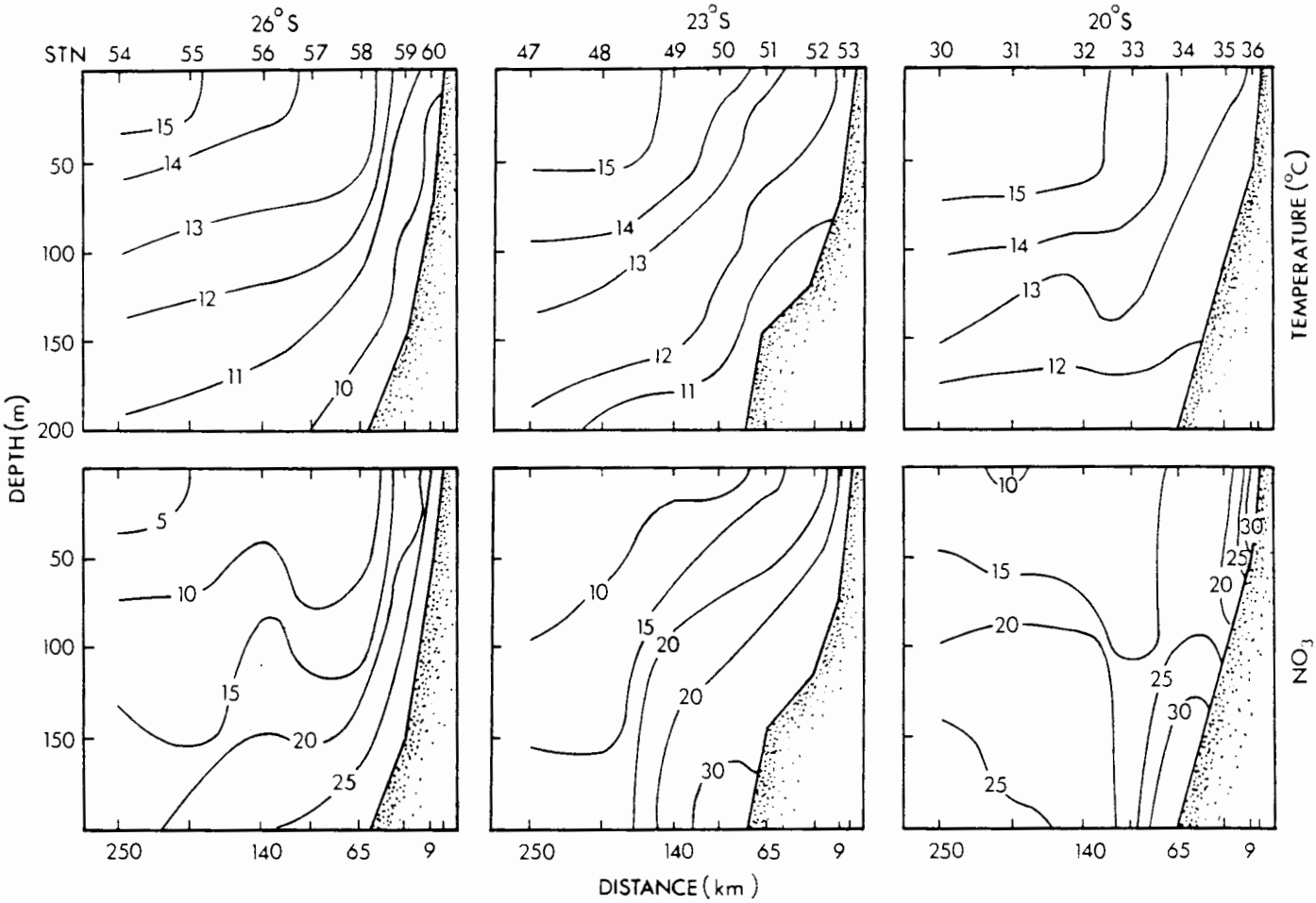


Fig. 2. Vertical sections of temperature (^{o}C) and nitrate (mg-at N m^{-3}) in the upper 200 m on transects at 26^{o}, 23^{o} and 20^{o} S during SNEC-85 (from Probyn 1988).

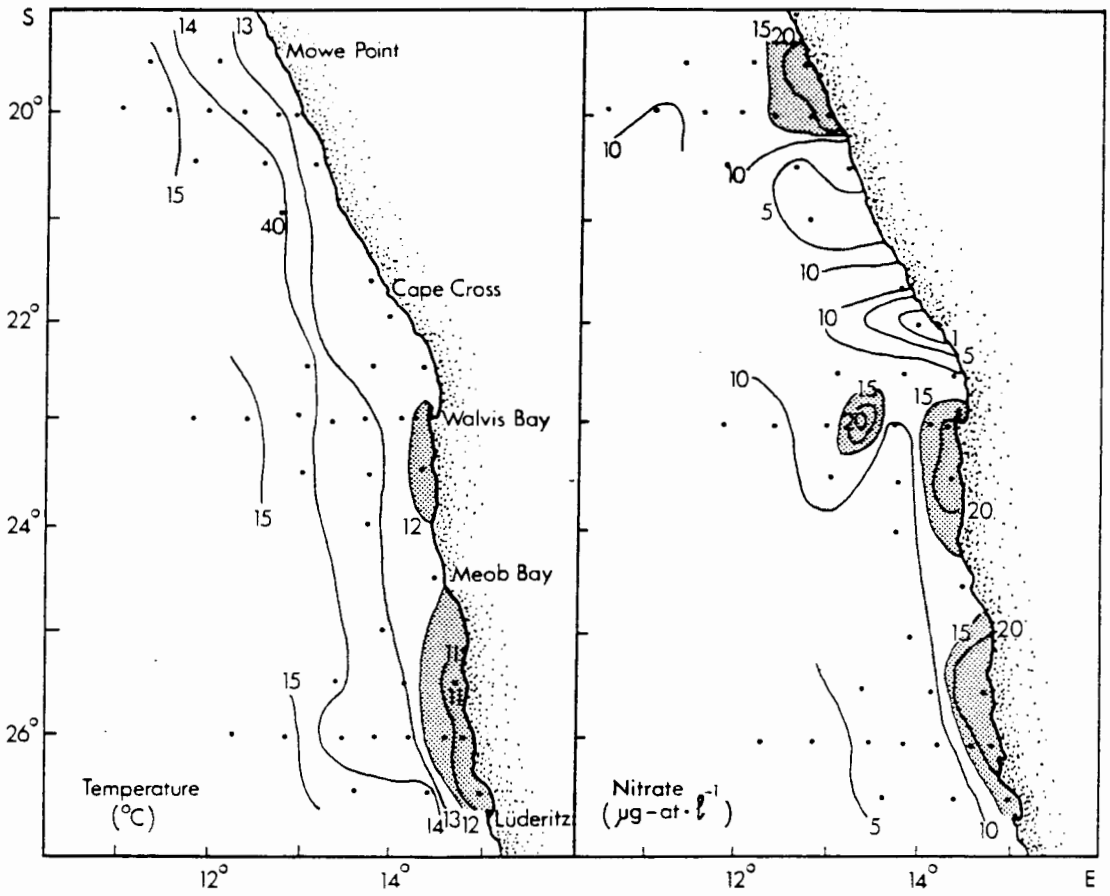


Fig. 3. Surface temperature (°C) and nitrate concentrations (mg-at N m^{-3}) during SNEC-85 (from Estrada and Marrasé 1987).

due to either convection processes or upwelling (Estrada and Marrasé 1987). Calculations of the proportion of light attenuation accounted for by phytoplankton in the euphotic zone suggested a large presence of detrital material (Estrada and Marrasé 1987).

Phytoplankton and bacterial biomass

Detailed data on phytoplankton biomass, determined from chlorophyll concentrations, and bacterial biomass in the southern Benguela are given in Chapter 6 (Table 3, Figs. 4b, 5b). For the northern Benguela details on chlorophyll distributions are presented by Guttieréz et al. (1985). Phytoplankton and bacterial biomass in the euphotic zone at the bacterial production stations are shown here in Table 1.

Surface chlorophyll a concentrations during both cruises are shown on Fig. 1. Values recorded at Stations 2 to 12 (2 to 10 mg chl a m^{-3}) in the southern Benguela were typical of MUW. After Station 13 phytoplankton biomass was low suggesting enhanced herbivory, rapid senescence of the bloom or dilution as a result of mixing with a chlorophyll-poor water mass. Surface chlorophyll a concentrations in the northern Benguela were generally low. Tongues of enhanced chlorophyll (>5 mg m^{-3}) were recorded off Lüderitz and Meob Bay, where upwelling was active (Estrada and Marrasé 1987). A tongue of chlorophyll-rich water (10 to 20 mg chl a m^{-3}, Fig. 1) and lower nitrate concentrations (1 to 10 µg-at N l^{-1}, Fig. 3) north of Walvis Bay indicate the development of a plume of maturing upwelled water in this region. Concentrations of chlorophyll a integrated over the euphotic zone for both cruises, not presented here, showed similar results (Chapter 6 and Estrada and Marrasé 1987).

Station	Depth (m)	Light level (%)	Bacteria (mg C m^{-3})	Chl a (mg m^{-3})	Primary production (mg C m^{-3} h^{-1})	Integrated Chl (mg m^{-2})	Integrated Production (mg C m^{-2} h^{-1})
3	0	100	30.8	0.75	1.59	42.2	53.5
	4	50	43.8	0.85	2.50		
	10	25	42.3	0.92	2.36		
	19	10	27.8	1.18	0.86		
	40	1	31.8	1.13	0.48		
11*	0	100	9.2	1.44	3.44	29.9	43.7
	4	50	-	1.43	3.79		
	7	25	10.6	1.60	3.41		
	10	10	16.9	1.74	1.74		
	19	1	13.1	1.50	0.64		
21	0	100	30.0	2.40	4.67	40.5	68.8
	4	50	-	2.40	5.22		
	8	25	32.3	2.47	4.29		
	12	10	32.7	2.69	4.47		
	16	1	23.3	2.72	1.75		
30	0	100	25.8	2.38	6.78	44.3	84.6
	4	50	-	-	7.60		
	8	25	28.0	-	6.31		
	10	10	26.3	2.44	3.45		
	18	1	27.7	2.61	1.12		
35	0	100	36.8	0.96	3.79	28.1	81.4
	5	50	-	1.02	4.52		
	10	25	35.3	1.04	3.80		
	16	10	43.5	0.97	2.57		
	31	1	17.4	0.62	0.19		
45	0	100	37.8	1.37	5.07	65.8	54.1
	5	50	-	2.33	4.73		
	9	25	24.4	2.11	1.84		
	15	10	25.9	2.47	1.08		
	29	1	18.7	2.38	0.41		
48	0	100	28.9	2.80	5.81	66.9	98.5
	3	50	-	2.80	6.40		
	6	25	23.9	3.20	8.01		
	11	10	26.8	3.23	3.83		
	20	1	36.8	4.20	1.05		
55	0	100	21.8	0.93	2.94	25.8	57.0
	4	50	-	0.90	3.55		
	10	25	17.0	0.90	3.56		
	13	10	17.6	0.92	1.24		
	30	1	16.6	0.73	0.33		

Phytoplankton production vs. Chlorophyll: Chl = 0.163 $e^{0.097X}$, n = 38, r = 0.368, $p < 0.05$

Phytoplankton production vs. Bacterial biomass (B):

(i) B = 10.64 $X^{0.41}$, n = 33, r = 0.261, p = n.s.

(ii)* B = 15.37 $X^{0.29}$, n = 29, r = 0.407, $p < 0.05$

*Data from station 11, where upwelling was most active and bacterial biomass was low, were omitted.

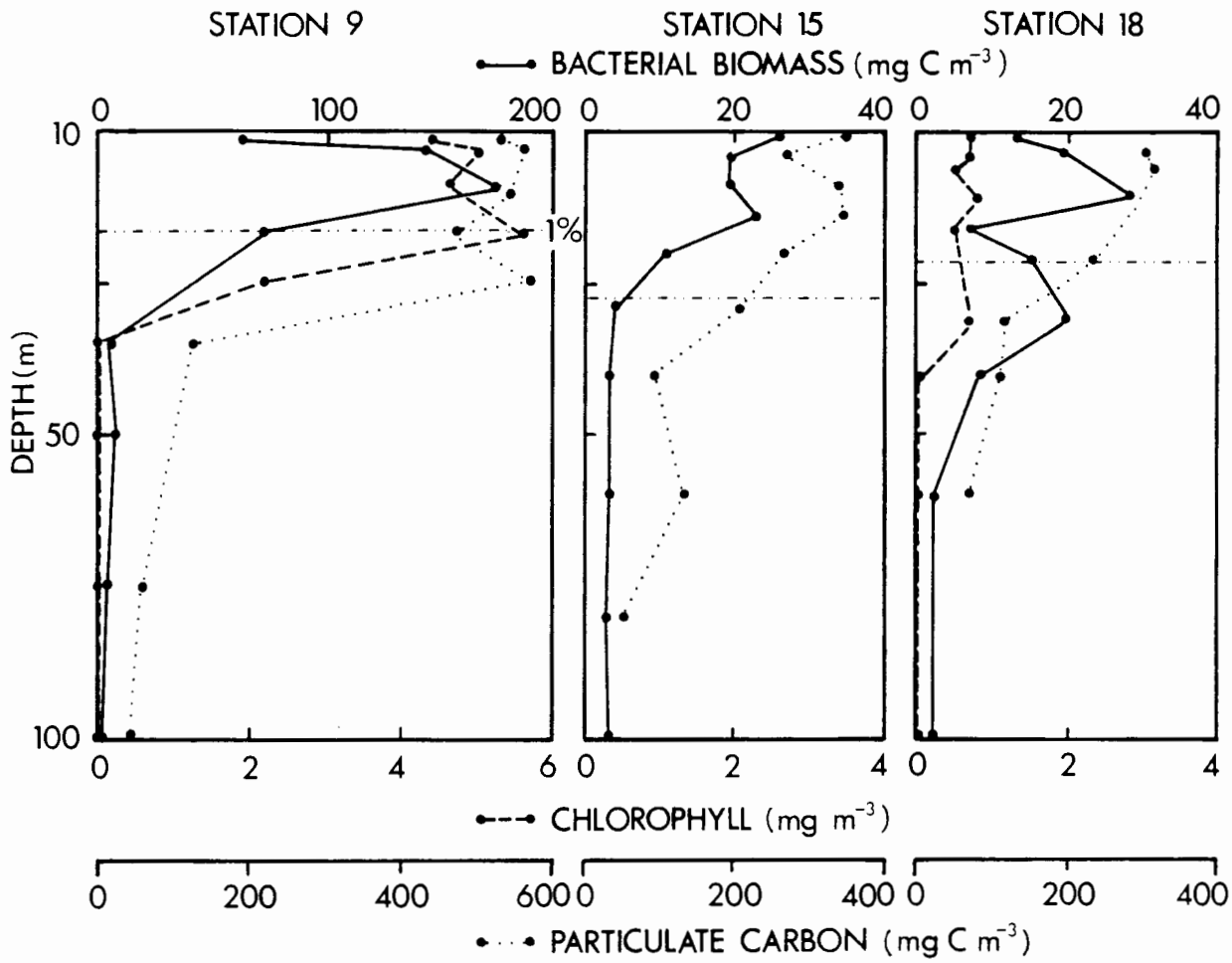


Fig. 4. Vertical profiles of phytoplankton biomass (mg Chl a m^{-3}), bacterial biomass (mg C m^{-3}) and particulate carbon concentrations (mg C m^{-3}) at the bacterial production stations in the southern Benguela. The 1 % light depth is indicated by - · - · - .

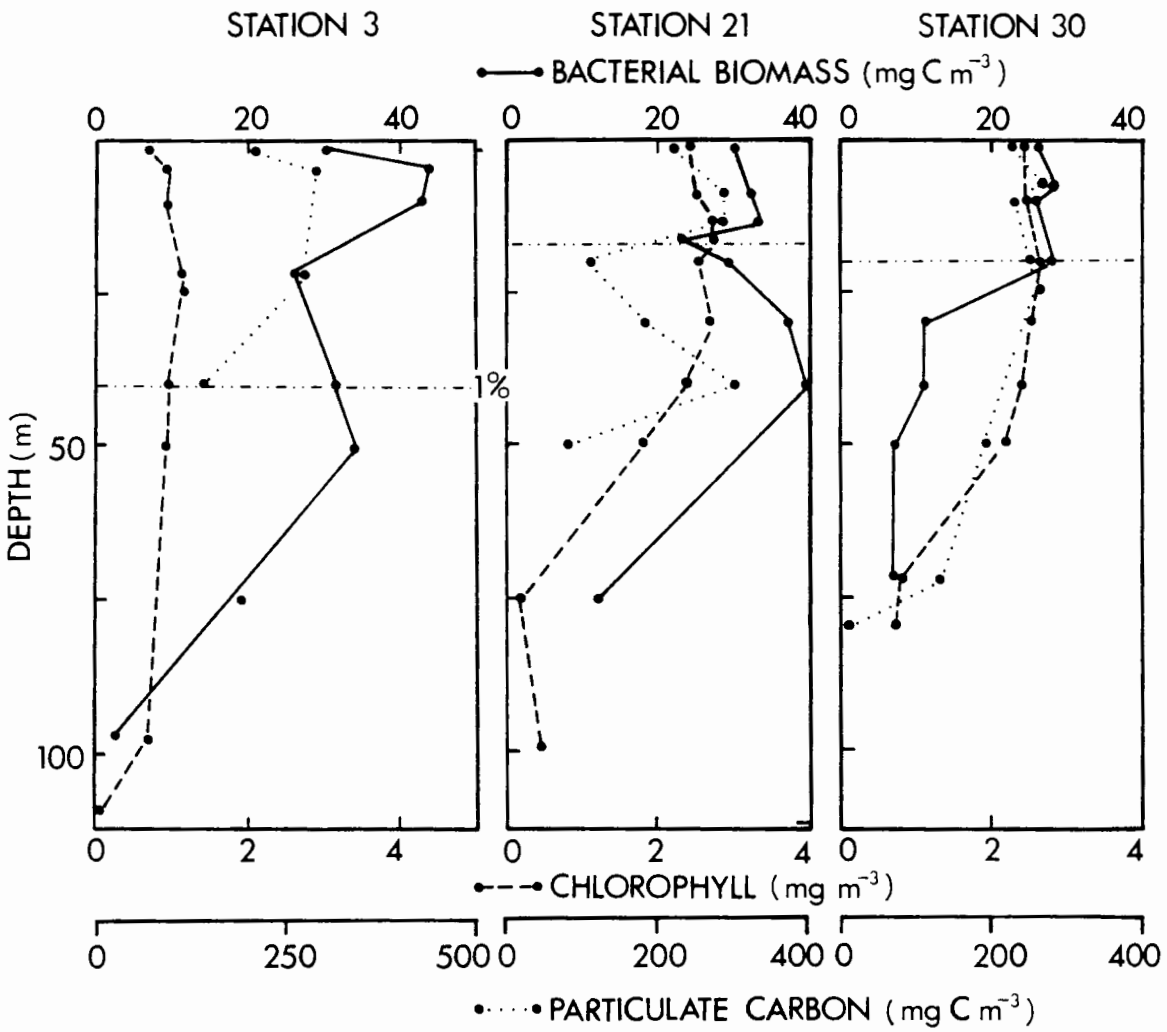


Fig. 5. Vertical profiles of phytoplankton biomass (mg Chl a m^{-3}), bacterial biomass (mg C m^{-3}) and particulate carbon concentrations (mg C m^{-3}) at 3 bacterial production stations in the northern Benguela. The depth of the euphotic zone is indicated by the 1 % light penetration depth (- · - · -).

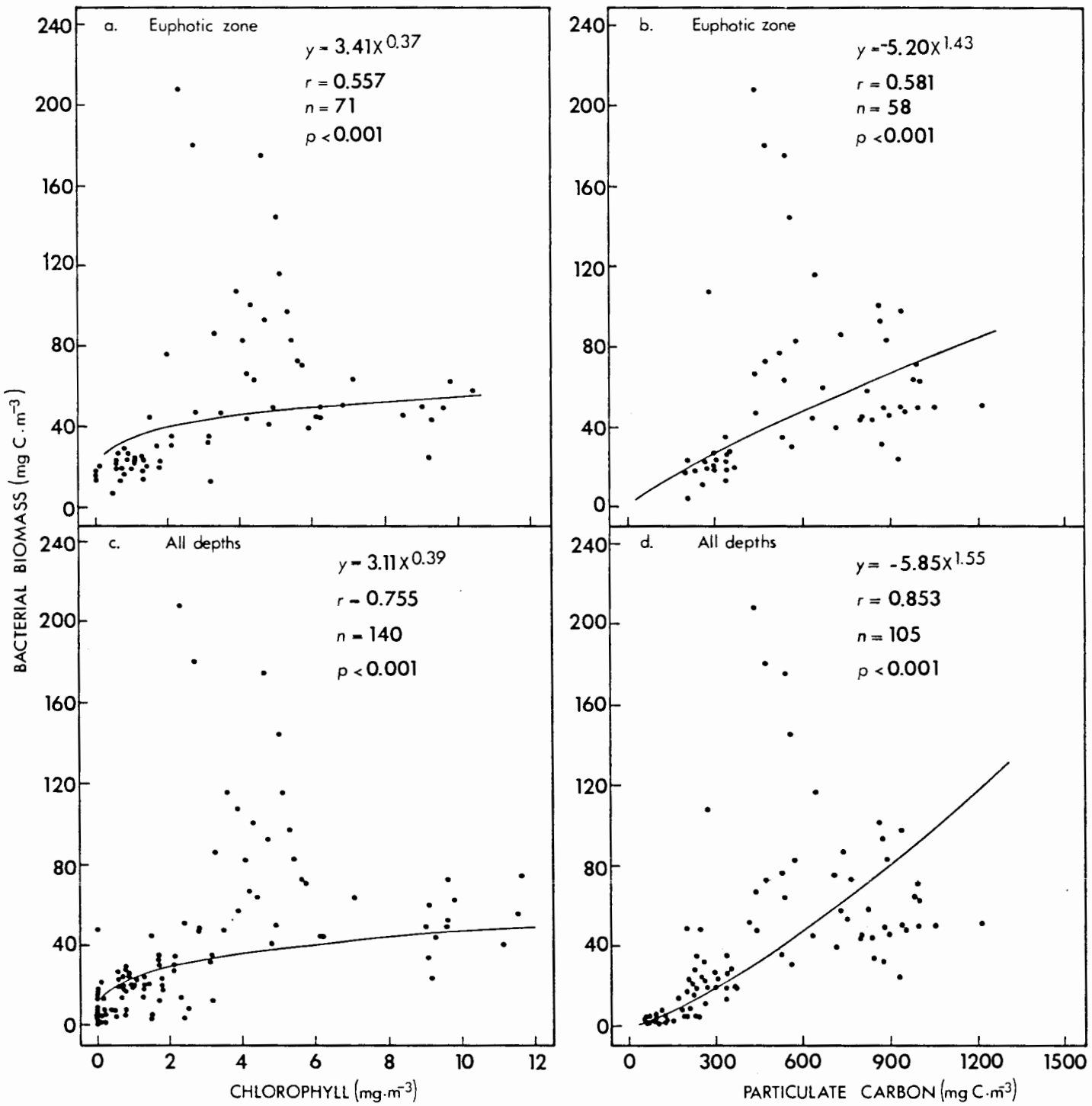


Fig. 6. Plots of the relationship between bacterial biomass (mg C m^{-3}) and concentrations of chlorophyll a (mg m^{-3}) and particulate carbon (mg C m^{-3}), in (a & b) the euphotic zone and (c & d) over all depths sampled in the southern Benguela. Results of functional regression analysis are shown.

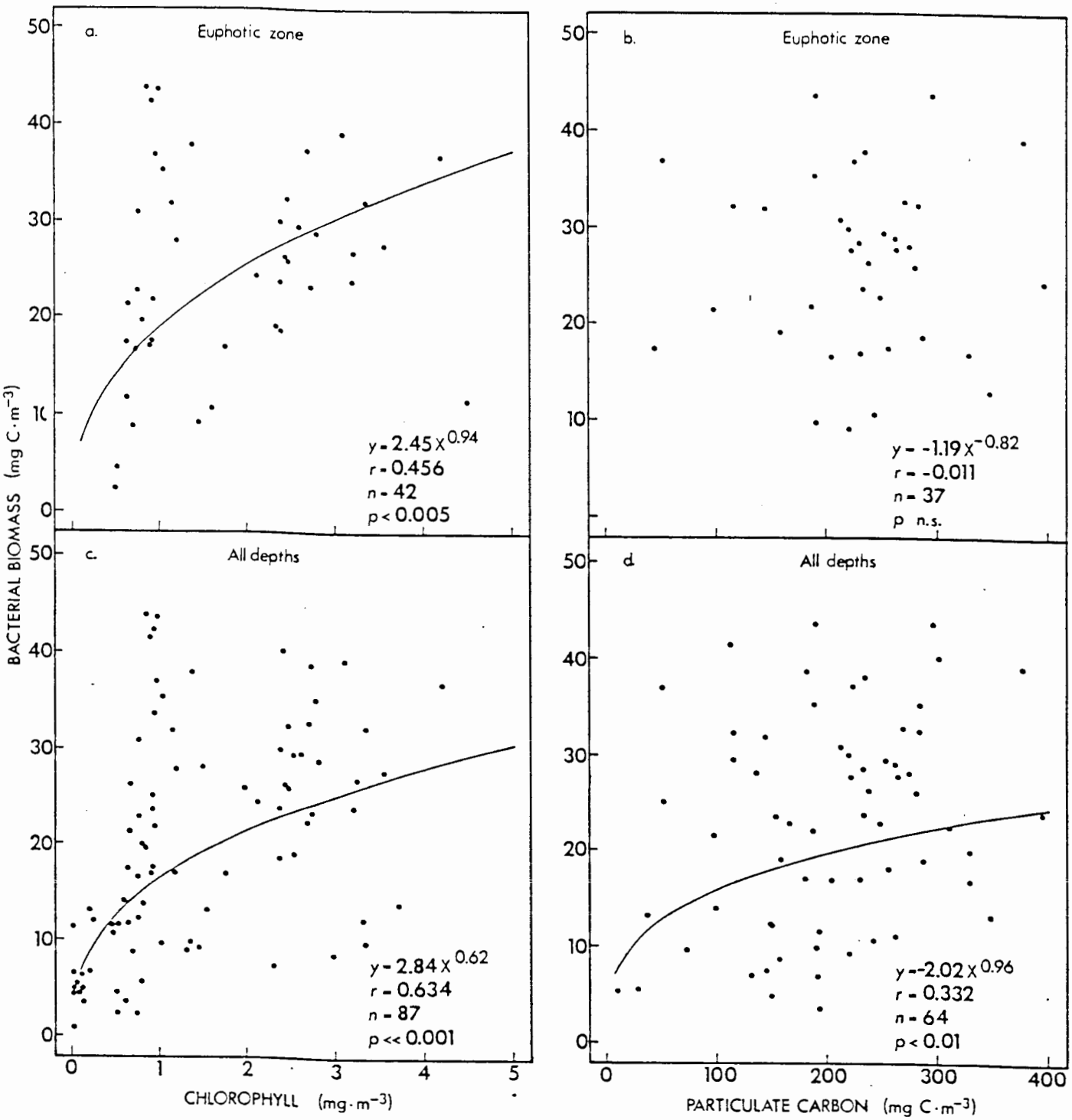


Fig. 7. The relationship between bacterial biomass (mg C m^{-3}) and concentrations of chlorophyll *a* (mg m^{-3}) and particulate carbon (mg C m^{-3}) in the northern Benguela. Data were analysed by functional regression analysis of samples collected (a & b) from the euphotic zone and (c & d) over all depths sampled.

Depth profiles of phytoplankton and bacterial biomass and concentrations of particulate carbon (PC) are shown for 3 production stations from each cruise on Figs. 4 and 5. In the southern Benguela bacterial biomass was highest in the euphotic zone, particularly in MUW at Station 9 (63 to 175 mg C m^{-3}, Fig. 4). Functional regression analysis, assuming both variables were subject to measurement errors (Laws and Archie 1981), showed that bacterial biomass was significantly correlated ($Y = aX^b$) with both chlorophyll a and PC concentrations in the euphotic zone and over all depths sampled (Fig. 6). Values for bacterial biomass in the northern Benguela were comparable to those in the southern Benguela, but not necessarily highest in the euphotic zone (see Fig. 5). Curves of best fit to the biomass data ($Y = aX^b$) showed a significant relationship between bacterial biomass and chlorophyll a and PC concentrations when data from all depths were used. If only the data from the euphotic zone were used curves of best fit showed a significant relationship between bacterial biomass and chlorophyll a, but not between bacteria and PC (Fig. 7).

Phytoplankton production

Southern Benguela

Primary production rates during the development of the upwelling plume in the southern Benguela were high in MUW (465 to 1114 mg C m^{-2} h^{-1}, Stations 3 to 12) and low in MUW/mixed water (66 to 223 mg C m^{-2} h^{-1}, Stations 13 to 20, Chapter 6). Both phytoplankton and bacterial biomass were correlated ($Y = aX^b$) with primary production (see Table 3; Chapter 6). Figure 8 shows production rates measured in the euphotic zone at Stations 9, 15 and 18, where bacterial production was measured. Temperature and salinity profiles are also shown, and indicate a stratified water column.

Northern Benguela

In the northern Benguela primary production was generally low (41 to 102 mg C m^{-2} h^{-1}), with lowest values measured in the southern regions (40 to 57 mg C m^{-2} h^{-1}, Estrada and Marrasé 1987). As in the southern Benguela, phytoplankton biomass in the euphotic zone was correlated ($Y = aX^b$) with primary production (see Table 1). Similarly, bacterial biomass was found to be correlated with primary production, and also best described by a power function (Table 1). This relationship was significant only if data from Station 11, where upwelling was most active and bacterial biomass was low, were omitted. Phytoplankton production rates in the euphotic zone at bacterial production stations are shown in Figs. 9 and 10. From temperature and salinity profiles it is evident that the upper 75 m of the water column was well mixed, and that there was no marked pycnocline.

Bacterial production

Southern Benguela

In the southern Benguela, estimates of bacterial production based on tritiated thymidine incorporation (TTI, Fig. 8) were highest in the euphotic zone. Average bacterial production rates (μg C l^{-1} h^{-1}) were calculated to be 0.181 in the euphotic zone, 0.126 at the pycnocline and 0.018 in the aphotic zone. Thymidine incorporation was linear over the time-course incubations ($r^2 > 0.67$, Table 2) except in the aphotic zone at Stations 15 and 18, where thymidine incorporation was minimal. Bacterial production expressed as a percentage of phytoplankton production is shown for each profile (Fig. 8). Lowest percentages (0.6 to 7 %) were found in the euphotic zone, where primary

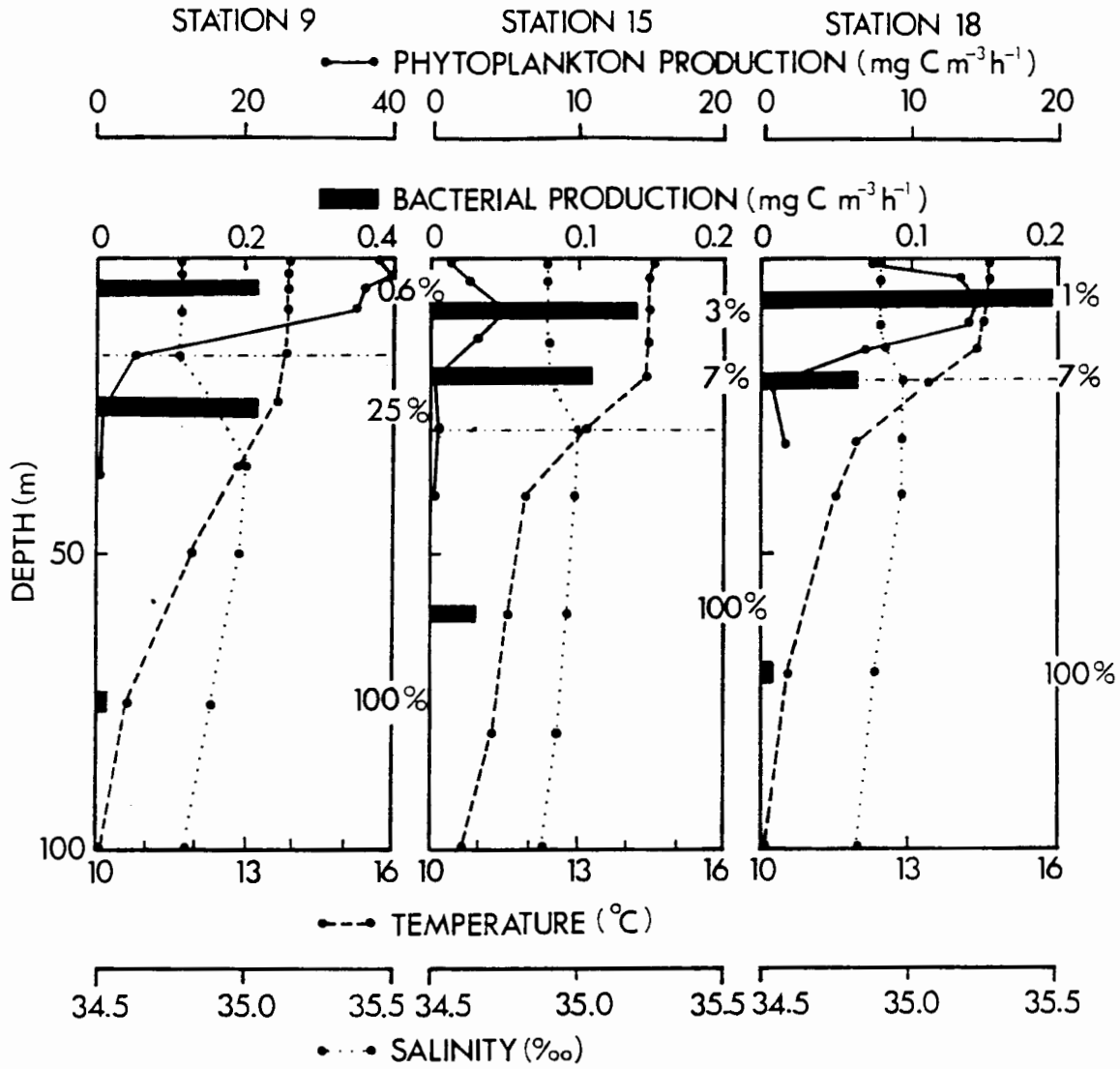


Fig. 8. Profiles of phytoplankton and bacterial production (mg C m^{-3} h^{-1}) in the southern Benguela. Production of bacteria was calculated from incorporation of [methyl-^{3}H] thymidine into bacterial DNA (TTI), a factor 1.7 x 10^{18} cells mol^{-1} TTI (Fuhrman and Azam 1982) and the weighted mean cell biomass (Table 2). Ratios of bacterial: phytoplankton production (%) are shown for depths at which bacterial production was measured. Temperature and salinity profiles are also shown. -·-·-·-represents the 1 % light depth.

Table 2. Bacterial production estimates at discrete depths within the euphotic zone, pycnocline and aphotic zone, calculated from incorporation of [methyl-^{3}H] thymidine (TTI) into bacterial DNA. Production rates were calculated from 1.7 x 10^{18} cells mol^{-1} TTI (Fuhrman and Azam 1982) and the weighted mean cell biomass (fg C $cell^{-1}$) estimated from AODC.

Stn	Depth (m)	DPM (ml^{-1} h^{-1})	r^2	n	TTI (pmoles l^{-1} h^{-1})	Cells (x 10^6 l^{-1} h^{-1})	Mean biomass (fg C $cell^{-1}$)	Production (µg C l^{-1} h^{-1})
Southern Benguela								
9	5	807	0.96	9	6.98	11.87	17.5	0.208
	24	778	0.91	9	6.73	11.45	18.2	0.208
	75	48	0.11	8	0.42	0.71	19.2	0.014
15	8	531	0.91	9	4.60	7.81	17.5	0.137
	21	416	0.69	9	3.60	6.12	17.4	0.107
	60	130	0.29	9	1.13	1.91	15.1	0.030
18	7	684	0.90	5	5.92	10.06	19.8	0.199
	22	233	0.80	5	2.02	3.43	18.6	0.064
	70	45	0.92	5	0.39	0.66	13.2	0.009
Northern Benguela								
3	0	125	0.67	6	1.148	1.95	12.9	0.025
	50	-	0.76	6	-	-	15.5	-
	100	29	0.44	6	0.27	0.45	12.6	0.006
	300	-	0.27	6	-	-	16.5	-
21	3	261	0.68	5	2.40	4.08	15.6	0.064
	30	231	0.70	5	2.12	3.61	17.7	0.064
	75	27	0.79	4	0.25	0.42	14.6	0.006
30	0	19	0.74	6	0.18	0.30	14.7	0.004
	75	235	0.94	6	2.16	3.67	12.5	0.046
	150	-	-	6	-	-	12.5	-

- denotes no uptake of [methyl-^{3}H] thymidine by bacteria.

production was highest. Highest percentages (100 %) were found in the aphotic zone, where there was no primary production.

Bacterial production rates calculated from net bacterial growth in size-fractionated incubations (Table 3) were 2 to 26 times higher (mean: 10.5 ± 8, n = 8) than estimates obtained from TTI. Curves of best fit to the biomass data were linear ($Y = a + bX$), and generally significant ($p < 0.05$). The slope of each line, b, was assumed to equal the net growth rate of bacteria ($\mu g\ C\ l^{-1}\ h^{-1}$). Values were highest in the euphotic zone at all stations (0.33 to 5.34 $\mu g\ C\ l^{-1}\ h^{-1}$, mean: 2.31 ± 2.17 $\mu g\ C\ l^{-1}\ h^{-1}$), particularly at Station 9. At the pycnocline and in the aphotic zone average estimates of bacterial production were 0.57 and 0.17 $\mu g\ C\ l^{-1}\ h^{-1}$ respectively. A loss of biomass was recorded in the incubation of water from the pycnocline at Station 9.

Northern Benguela

In the northern Benguela, bacterial production estimates from TTI (Table 2, Fig. 9) were an order of magnitude lower than estimates in the southern Benguela, and averaged 0.03 $\mu g\ C\ l^{-1}\ h^{-1}$ in both the euphotic and aphotic zones. Incorporation of thymidine was linear ($r^2 > 0.67$) in the euphotic zone at Stations 3, 21 and 30 (Table 2), and generally linear in the aphotic zone where there was some uptake of thymidine. Bacterial production was equivalent to <2 % of primary production in the euphotic zone, and 100 % in the aphotic zone.

Depth profiles of phytoplankton and bacterial biomass and production, PC distribution and water column structure at 2 of the stations where bacterial production was measured at the different light penetration depths (100, 25, 10 and 1 %) in the euphotic zone are shown in Fig. 10. It is clear that even within the euphotic zone of a well-mixed water column bacterial production

Table 3: Growth of bacteria in predator-reduced (<3 μm) incubations, calculated from increase in bacterial biomass. Growth curves of best fit were linear and significance (p) of the fits was tested using critical values of the F-test (ANOVA). The slope of each line, b, was assumed to be the net production rate of bacteria (μg C l^{-1} h^{-1}).

Stn	Depth (m)	Y = a + bX	n	$F_{calculated}$	p	r^2	Net Production (μg C l^{-1} h^{-1})
9	5	Y= 70.95 + 5.34X	5	10.55	<0.05	0.78	5.34
	24	Y= 83.37 - 2.49X	5	-	-	-	-
	75	Y= 1.65 + 0.08X	4	6.04	<0.25	0.75	0.08
15	8	Y= 26.10 + 1.27X	6	14.95	<0.025	0.79	1.27
	21	Y= 21.90 + 0.95X	5	34.41	<0.01	0.92	0.95
	60	Y= 3.95 + 0.26X	5	17.01	<0.025	0.85	0.26
18	7	Y= 29.35 + 0.33X	4	2.97	<0.25	0.60	0.33
	22	Y= 9.31 + 0.18X	4	4.26	<0.25	0.68	0.18
	70	Y= 1.84 + 0.18X	5	13.17	<0.05	0.82	0.18

Note: At 24 m at Station 9, bacterial biomass decreased during the incubation.

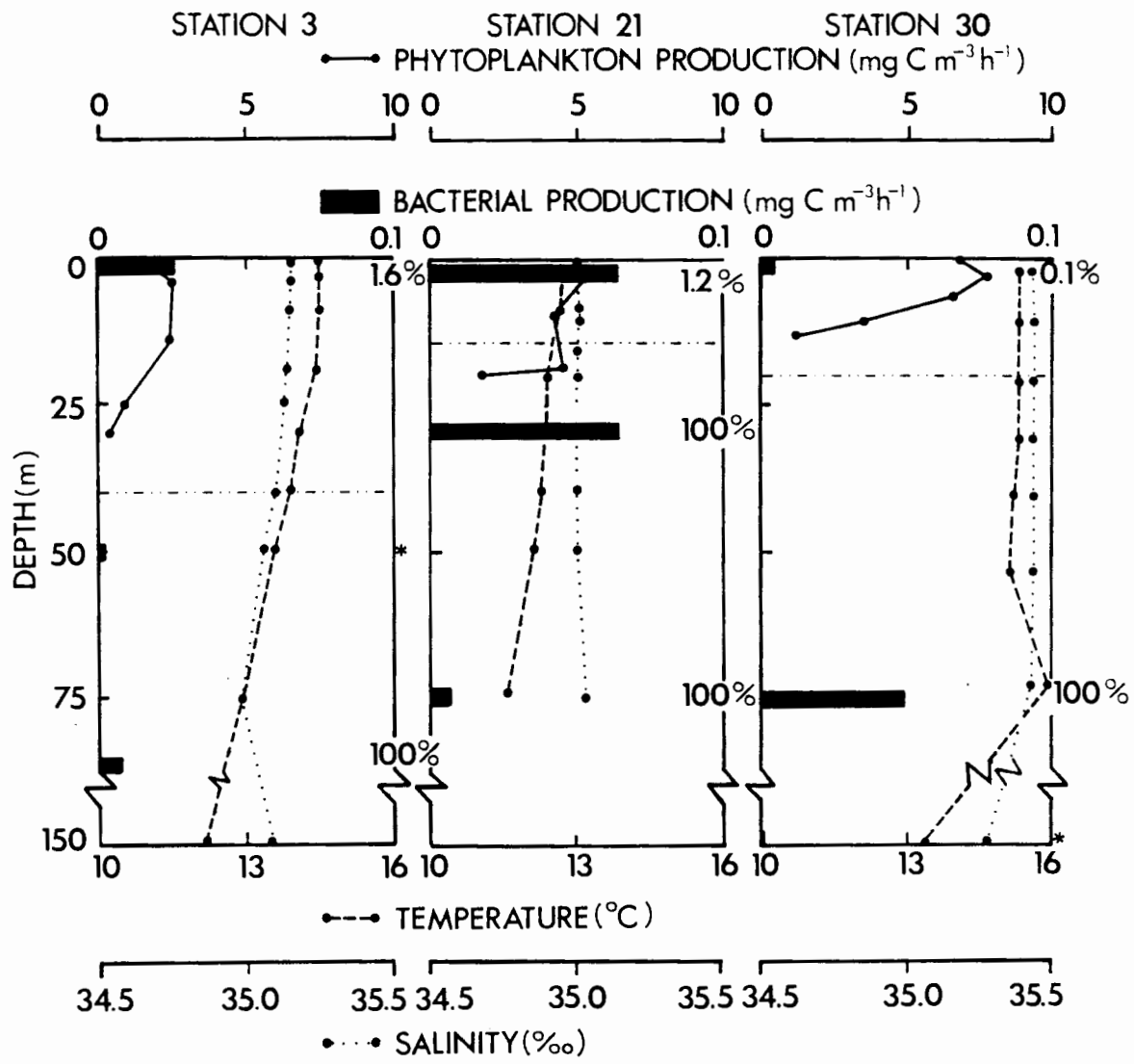


Fig. 9. Profiles of phytoplankton and bacterial production (mg C m^{-3} h^{-1}) in the euphotic and aphotic regions at 3 stations in the northern Benguela. Bacterial production was calculated from incorporation of [methyl-^{3}H] thymidine (see Fig. 8). An * indicates no uptake of thymidine by bacteria. Percentages (%) show the relationship between bacterial and phytoplankton production, -·-·-·- represents the 1 % light depth. Temperature and salinity profiles show a well-mixed water column.

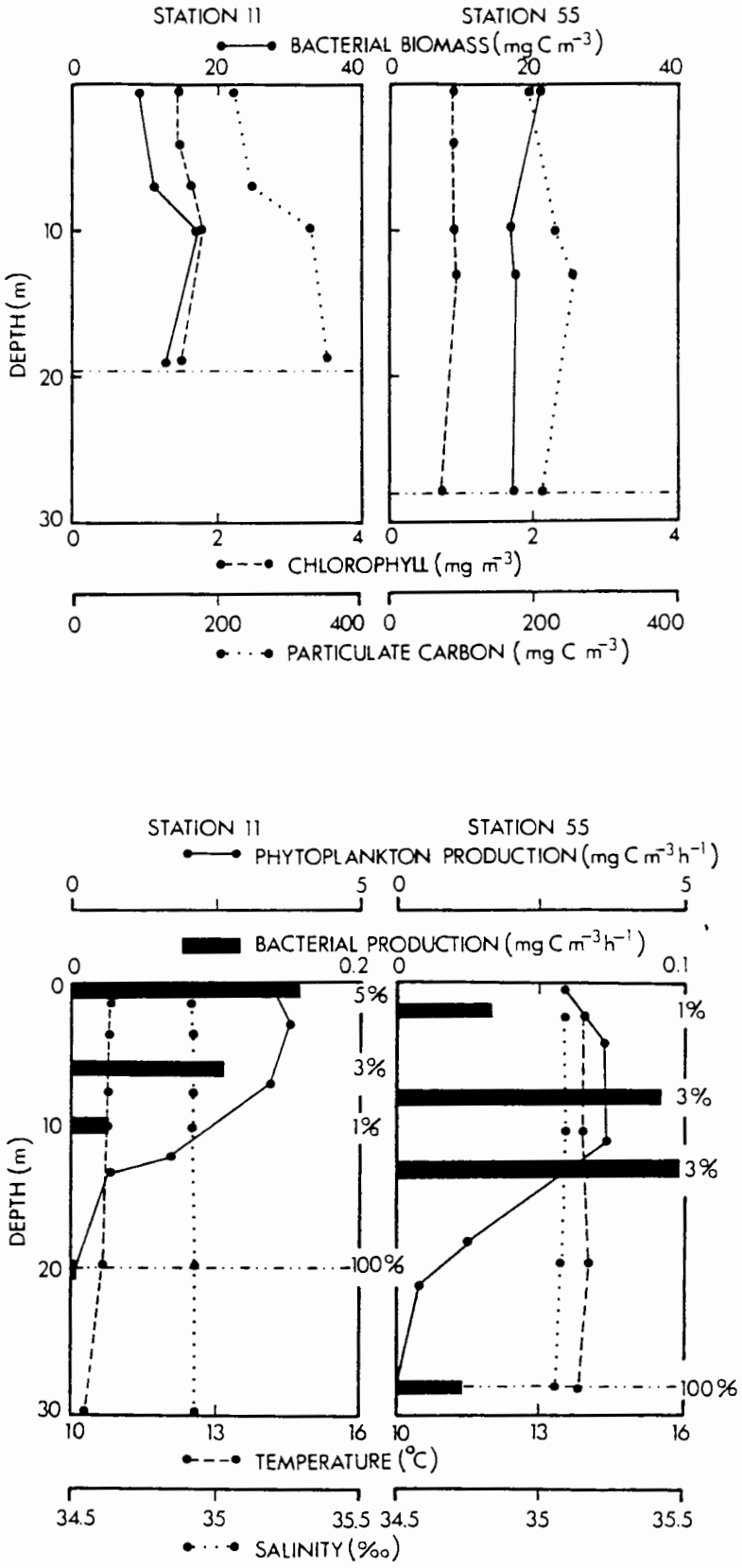


Fig. 10. Depth profiles of phytoplankton and bacterial (a) biomass and (b) production at different light penetration depths in the euphotic zone, at 2 stations in the northern Benguela region. Particulate carbon concentrations (mg C m^{-3}), temperature (°C) and salinity ($^{o}/_{oo}$) profiles are also shown.

estimates may be variable. Bacterial production was positively correlated ($Y = 0.009 + 0.027\ X$, $n = 8$, $r = 0.664$, $p < 0.05$) with primary production, showing a surface maximum at Station 11 and a subsurface maximum at Station 55, suggesting that factors affecting variability in primary production during active upwelling (eg. light, turbulence, nutrient supply) have a direct effect on bacterial production through the regulation of substrate availability.

Phytoplankton and bacterial relationships

Phytoplankton and bacterial biomass and production in the southern and northern Benguela upwelling regions are compared in Table 4. Data were integrated to the maximum depth of the euphotic zone (1 % light penetration depth) (Table 4a). Where only one bacterial production estimate was made in the euphotic zone, bacterial production was assumed to be constant over all depths in the euphotic zone. For three production stations from each cruise, data were integrated over all the sampling depths (Table 4b). Growth parameters in the two regions are compared in Table 5.

Euphotic zone

Integrated values of the total biomass of phytoplankton, bacteria and PC were of the same order of magnitude in both the southern and northern Benguela (Table 4a). In general, phytoplankton biomass was relatively low (mean value: 2594 ± 1194 mg C m^{-2}, $n = 10$) and bacterial biomass was high (mean: 740 ± 472 mg C m^{-2}, $n = 11$). As a result, bacteria contributed a relatively large proportion of the total microbial carbon (24 ± 10 %). The standing stock of PC in both regions was high (6.5 ± 2.1 g C m^{-2}), with bacteria contributing, on average, 12 ± 7 % of the total PC biomass.

Table 4: A comparison of phytoplankton and bacterial biomass (mg C m^{-2}) and production (mg C m^{-2} h^{-1}) in the southern and northern Benguela upwelling regions. (a) Data from all production stations integrated to the 1% light depth. (b) Data integrated over all depths sampled, for 3 production stations in each region (see Figs. 8 & 9). Chlorophyll was used as an index of phytoplankton biomass, using a C:Chl ratio of 60 (see Chapter 6). Primary production data for the southern Benguela are presented in Chapter 6. Bacterial production was estimated from ^{3}H-thymidine incorporation (P_{TTI}, Table 2), and from biosynthesis ($P_{\Delta B}$) in predator-reduced (<3 μm) incubations (Table 3) in the southern Benguela. PB = total microbial (phytoplankton plus bacteria) biomass. PC = particulate carbon.

		PHYTOPLANKTON			BACTERIA							
Stn	Integ. depth (m)	Chl (mg C m^{-2})	Prod (mg C m^{-2} h^{-1}) (P_P)	PC (g C m^{-2})	Biomass (mg C m^{-2}) (B)	Prod (mg C m^{-2} h^{-1}) P_{TTI}	$P_{\Delta B}$	$\frac{P_{TTI}}{P_{\Delta B}}$ (%)	$\frac{B}{PB}$ (%)	$\frac{B}{PC}$ (%)	$\frac{P_{TTI}}{P_P}$ (%)	$\frac{P_{\Delta B}}{P_P}$ (%)
a)												
Southern Benguela												
9	16	4638	465	8.2	1878	3.3	42.7	7.7	29	23	0.7	9.2
15	27	-	66	6.3	389	2.0	18.4	10.9	-	6	3.0	27.9
18	22	690	223	5.5	394	2.7	5.0	54.0	36	7	1.2	2.2
Northern Benguela												
3	40	2532	54	9.5	1349	1.0	-	-	35	14	1.9	-
11	19	1794	44	5.5	245	1.2	-	-	12	5	2.7	-
21	16	2429	69	4.1	491	1.0	-	-	17	12	1.5	-
30	18	2658	85	4.5	485	0.1	-	-	15	11	0.1	-
35	31	1684	81	4.1	1054	0.2	-	-	39	26	0.3	-
45	29	3949	54	8.1	743	0.9	-	-	16	9	1.7	-
48	20	4015	99	4.9	571	1.0	-	-	13	12	1.0	-
55	30	1549	57	9.7	537	2.0	-	-	26	6	3.5	-
b)												
Southern Benguela												
9	100	5772	465	19.3	2921	10.3	67.1	15.1	34	15	2.2	14.4
15	100	-	66	10.9	664	5.4	48.3	11.2	-	6	8.2	73.2
18	100	714	223	11.3	879	4.6	16.4	28.1	55	8	2.1	7.4
Northern Benguela												
3	150	9450	54	>9.5	2660	1.7	-	-	22	>28	1.7	-
21	110	9600	69	10.1	2214	3.5	-	-	19	22	5.1	-
30	150	9966	74	16.8	1348	3.3	-	-	12	8	4.5	-

Table 5: Growth parameters of phytoplankton and bacteria, calculated from total biomass (mg C m^{-2}) and production (mg C m^{-2} h^{-1}) in (a) the euphotic zone only and (b) over the entire water column (see Table 3). Specific growth rates, μ (d^{-1}), were calculated from the ratio of production (P, mg C m^{-2} h^{-1}) to biomass (B, mg C m^{-2}), assuming a day-length of 12 h for phytoplankton and 24 h for bacteria. Doubling times (d) = 0.693/μ.

Stn	Integration Depth	Phytoplankton		Bacteria			
		P/B	Doubling time	P/B		Doubling time	
	(m)	(d^{-1})	(d)	(d^{-1})		(d)	
				P_{TTI}	$P_{\Delta B}$	P_{TTI}	$P_{\Delta B}$
(a)							
Southern Benguela							
9	16	1.2	0.6	0.04	0.55	17.3	1.3
15	27	-	-	0.12	1.14	5.8	0.6
18	22	3.9	0.2	0.17	0.30	4.1	2.3
Northern Benguela							
3	40	0.3	3.2	0.02	-	34.7	-
11	19	0.3	3.2	0.12	-	5.8	-
21	16	0.4	1.7	0.05	-	13.9	-
30	18	0.4	1.7	0.005	-	138.6	-
35	31	0.6	1.2	0.005	-	138.6	-
45	29	0.1	6.9	0.03	-	23.1	-
48	20	0.3	3.2	0.04	-	17.3	-
55	30	0.5	1.4	0.09	-	7.7	-
b)							
Southern Benguela							
9	100	1.0	0.7	0.08	0.55	8.7	1.3
15	100	-	-	0.20	1.75	3.5	0.4
18	100	3.7	0.2	0.13	0.45	5.3	1.5
Northern Benguela							
3	150	0.1	6.9	0.02	-	34.7	-
21	110	0.1	6.9	0.04	-	17.3	-
30	150	0.1	6.9	0.06	-	11.6	-

Integrated values of phytoplankton production (P_P) and bacterial production (from TTI, P_{TTI}) were comparable in the two regions (Table 4a). Analysis of the relationship between P_P and P_{TTI} showed a significant linear correlation ($P_{TTI} = 0.712 + 0.006\ P_P$, $n = 11$, $r = 0.734$, $F_{calculated} = 10.49$, $p < 0.025$). The relationship between discrete measurements (mg C m^{-3} h^{-1}) of phytoplankton and bacterial production (from TTI) was also significant ($P_{TTI} = 0.042 + 0.005\ P_P$, $n = 22$, $r = 0.649$, $F_{calculated} = 14.25$, $p < 0.0025$). Bacterial production was calculated to be only 0.1 to 3.5 % of primary production (P_{TTI}/P_P, Table 4a).

In the southern Benguela, integrated bacterial production estimates obtained from direct measurement of bacterial growth in predator-reduced incubations ($P_{\Delta B}$ = 5 to 42.7 mg C m^{-2} h^{-1}) were 2 to 13 times higher than measurements from TTI. By this method, bacterial production was estimated to be 2 to 28 % of primary production ($P_{\Delta B}/P_P$, Table 4a).

Growth parameters of phytoplankton and bacteria in the southern and northern Benguela upwelling regions (Table 5) show that specific growth rates (calculated from production rate divided by biomass, Table 4) of phytoplankton were higher in the southern Benguela (1.2 to 3.9 d^{-1}) than in the northern Benguela (0.1 to 0.6 d^{-1}), resulting in shorter doubling times (0.2 to 0.6 d vs. 1.4 to 6.9 d). Specific growth rates of bacteria (P_{TTI}/Biomass) were 0.04 to 0.17 d^{-1} in the southern Benguela and 0.005 to 0.12 d^{-1} in the northern Benguela. Doubling times (0.693/growth rate) were calculated to be 4.1 to 17.3 d and 5.8 to 138.6 d in the two regions respectively. Bacterial growth rates and doubling times from bacterial growth ($P_{\Delta B}$/Biomass) in the southern Benguela were 0.3 to 1.14 d^{-1} and 0.6 to 2.3 d.

Water Column

The significant effects of integrating data over the entire water column (Table 4b) are most evident in phytoplankton and bacterial growth parameters (Table 5). Average growth rates and doubling times of phytoplankton in the southern Benguela were virtually unaffected. In the northern Benguela, integration of data over the whole water column resulted in decreased average specific growth rates of phytoplankton, and prolonged average doubling times.

For bacteria, integration of data over the water column resulted in increased values for bacterial production. The effects of this were firstly, a general increase in bacterial specific growth rates, with a concomitant decrease in doubling times (Table 5). More importantly, increased estimates of bacterial production from both the TTI and bacterial growth methods resulted in increased ratios of bacterial: phytoplankton production (2 to 8 % by TTI and 7 to 73 % by bacterial growth).

DISCUSSION

Phytoplankton and bacterioplankton production in the Benguela upwelling region during this study showed temporal and spatial variability, in relation to the upwelling cycle. Intensive studies of phytoplankton dynamics in the southern Benguela have shown that newly upwelled South Atlantic Central Water (SACW) is characterised by high inorganic nutrients and low biomass and production of planktonic organisms. Favourable light conditions in the euphotic zone stimulate increased primary production. Stratification and stabilisation of the water column due to sun-warming of the photic waters results initially in high primary production and high phytoplankton biomass. In the absence of new upwelling, phytoplankton growth becomes limited, largely due to decreased

availability of nitrate nitrogen which is utilised during growth, and the bloom declines (Barlow 1982a, c, Barlow 1984, Brown and Field 1986, Brown and Hutchings 1987a, b). Due to the coupling of phytoplankton and heterotroph dynamics, it is likely that bacterioplankton dynamics will also be related to the upwelling cycle. Evidence of this has been provided in other upwelling regions (Sorokin and Mikheev 1979, Sorokin and Kogelschatz 1979, Rheinheimer and Schmaljohann 1983, Herbland 1978, McManus and Peterson 1988) and in the southern Benguela (Lucas 1986, Armstrong et al. 1987, Painting et al. 1989).

Studies of bacterioplankton dynamics are complicated by the morphological and physiological adaptations of the bacterial populations to dynamic oceanographic processes. In organically deficient SACW, bacteria are likely to adopt various strategies for "starvation-survival" (Novitsky and Morita 1977, Wiebe 1984, van Gemerden and Kuenen 1984, Painting et al. 1989), which may be reversed during high levels of primary and secondary production in the euphotic zone, when dissolved and particulate organic carbon substrates become available.

Painting et al. (1989, Chapter 4) demonstrated marked fluctuations in activity and production of heterotrophic bacteria in response to phytoplankton growth and decay in a microcosm simulation of an upwelling event. The authors proposed that the bacterial community in upwelled water is initially metabolically dormant, and becomes active during phytoplankton growth, showing a succession in the dominant strains due to substrate preferences and substrate availability. Moreover, the authors proposed that not all metabolically active bacteria take up and/or incorporate exogeneous dissolved thymidine, due either to an absence of the transport system or because of a high intracellular thymidine pool leading to isotope dilution (see also Davis 1989). Apart from uncertainties in the conversion factors used to calculate bacterial production

from ^{3}H-thymidine incorporation (TTI, Kirchman et al. 1982, Ducklow and Hill 1985b), it appeared that bacterial responses to ^{3}H-thymidine supply were variable. During maximum phytoplankton growth, bacterial production estimates from TTI and net growth rates were comparable, suggesting that the thymidine method and conversion factors used were appropriate for bacterial production coupled to high rates of phytoplankton production. Poor agreement between production estimates obtained from these two methods in organically deficient recently upwelled water, and during phytoplankton decomposition, suggested that the TTI method may underestimate bacterial production under these conditions.

Phytoplankton and bacterioplankton relationships

In the southern Benguela upwelling region, vertical stratification of the water column suggests that phytoplankton-bacterioplankton relationships in the euphotic and aphotic zones are likely to be decoupled, and should be considered separately. Indeed, a study of the planktonic community showed that phytoplankton production and phytoplankton and bacterial biomass were highest in maturing upwelled water (MUW) in the euphotic zone, and lowest in other water types observed in the aphotic zone (Chapter 6). In the absence of vertical mixing processes, any microheterotroph activity in the aphotic zone is unlikely to be coupled directly to phytoplankton production and the excretion of PDOC. Alternative substrate sources available for utilisation by these organisms include detrital phytoplankton material, heterotrophically produced dissolved organic carbon (HDOC) and heterotrophically produced particulate organic material. Comparisons of phytoplankton and bacterial biomass and production in this region on the basis of data integrated over the whole water column (Table 4b) are therefore unrealistic, and only integrated values for the euphotic zone (Table 4a) should be considered.

In contrast, the water column over the continental shelf and slope in the northern Benguela was well mixed down to 40 to 60 m depth, probably due to active upwelling (Estrada and Marrasé 1987). The maximum depth of the euphotic zone (Ze, Table 1) was generally shallower than the mixed layer depth (Zm). A possible exception was Station 3 where Ze > Zm (Probyn 1988). Relatively homogeneous distributions of phytoplankton, bacteria and PC in this upper mixed layer indicate that phytoplankton-bacterioplankton relationships during this study were coupled over the Zm, including the euphotic and aphotic zones. On the basis of low primary production and low phytoplankton productivity indices (mg C mg Chl $\underline{a}^{-1}$ h^{-1}) Estrada and Marrasé (1987) hypothesised that phytoplankton production was inhibited by deep mixing. In addition, measurements of the utilisation of nitrogen by phytoplankton during the SNEC-85 cruise (Probyn 1988) showed that despite high nitrate concentrations in the upwelled water, nitrogen was taken up primarily in its reduced forms, as NH_4^+ and urea, in accordance with phytoplankton preference. From these somewhat unexpected results, Probyn (1988) proposed that phytoplankton production was inhibited by deep mixing of the water column, and that nitrogenous by-products (NH_4^+ and urea) of zooplankton grazing, possibly responsible for low measured chlorophyll biomass, resulted in inhibition of nitrate uptake. Inhibition of phytoplankton production probably had a direct effect on bacterial dynamics. Furthermore, the availability of reduced nitrogen in the environment, which suggests higher levels of heterotroph activity than is assumed to be typical of newly upwelled water, has implications for the existence of an active microbial food web during active upwelling conditions.

Phytoplankton biomass and production

Although spatial variations were observed, phytoplankton biomass and production in the northern Benguela were low (<5 mg Chl m^{-3}, 0.3 to 8 mg C m^{-3} h^{-1}). While such low estimates may have been expected at Station 11, where upwelling was most active, phytoplankton production was surprisingly low for other stations, where surface temperatures were warm (>13 ^{o}C) and nitrate concentrations were high (5 to 20 mg-at N m^{-3}, see Fig. 2). Furthermore, phytoplankton production and chlorophyll _a_ concentrations during the study period showed a significant exponential relationship (Table 1), indicating that specific growth rates of phytoplankton were low. These results support the hypothesis (Estrada and Marrasé 1987, Probyn 1988) that phytoplankton production in the northern Benguela was inhibited. Probyn (1988) showed that specific growth rates (μ) of phytoplankton were 0.2 d^{-1}.

Bacterial Biomass

Bacterial biomass was low (<40 mg C m^{-3}) during active upwelling conditions in the northern Benguela, particularly at Station 11 (9 to 13 mg C m^{-3}) where upwelling was most active. Correlations of bacterial biomass with phytoplankton biomass and production, and with concentrations of PC suggest a close coupling between bacteria and phytoplankton, and between bacteria and detrital material. Curves of best fit to the data indicate that even under active upwelling conditions bacteria may be grazed by predators when bacterial biomass increases. Similar phytoplankton-bacterioplankton relationships were described for the southern Benguela (Chapter 6).

The problems of scale in testing correlations of variables were discussed by McManus and Peterson (1988), who did not find significant correlation between

bacterial abundance and chlorophyll during successive upwelling cycles off Chile. From analysis of their results and those reported by Bird and Kalff (1984), Ducklow and Kirchman (1983) and Ducklow (1984), these authors concluded that data on bacterial abundance and chlorophyll which are collected on large spatial scales and span several orders of magnitude are more likely to be significantly correlated than observations on small spatial scales. Similar patterns were observed in this study. Generalised relationships were therefore described on the basis of the larger scale data. Relationships between bacteria and PC concentrations in the northern Benguela upwelling region support the hypothesis (McManus and Peterson 1988) that bacteria in newly upwelled water may utilise substrates other than PDOC.

Bacterial production

Bacterial production estimates obtained from TTI for both the northern and southern Benguela upwelling regions are comparable with estimates obtained by this method in other environments, including other upwelling areas (Table 6).

McManus and Peterson (1988) found that bacterial production estimates over 3 successive upwelling cycles off central Chile were within the range of 0.13 to 1.4 $\mu g\ C\ l^{-1}\ h^{-1}$, with lowest values occurring during active upwelling. Similar values (0.03 to 1.3 $\mu g\ C\ l^{-1}\ h^{-1}$) were measured over the first 6-d period in a simulated upwelling event in the southern Benguela (Painting et al. 1989). The highest bacterial production estimates were associated with the peak in the phytoplankton bloom. During phytoplankton senescence, bacterial production declined to 0.01 $\mu g\ C\ l^{-1}\ h^{-1}$ (Chapter 4).

Table 6. Comparison of bacterial production estimates obtained in different ecosystems by different methods. TTI = incorporation of [methyl-3H] thymidine into bacterial DNA, TAI = incorporation of tritiated adenine into bacterial RNA, FDC = frequency of dividing cells, <3 µm = predator-reduced incubations. Hourly production estimates were multiplied by 24.

Location	Production (mg C m^{-3} d^{-1})	Method	Authors
Antarctica	0.0 - 2.9	TTI	Fuhrman and Azam 1980
Baltic Sea	0.0 - 60.0	FDC	Larsson and Hagström 1982
- Coastal	2 - 16.0	FDC	Hagström et al. 1979
California	0.7 - 71.0	TTI	Fuhrman and Azam 1980
Caribbean Sea	1.9 - 5.5	TAI	Karl 1979
Chesapeake Bay Maryland	7.0 - 75.0	TTI	Ducklow 1982
Delaware Estuary	0.0 - 151.7	<3 µm	Coffin and Sharp 1987
Denmark Coastal	0.1 - 1.9		Riemann et al. 1984
Irish Sea	3.0 - 12.7	<3 µm	Turley and Lochte 1985
Nearshore Georgia Bight	19.2 - 178.0	FDC	Newell and Christian 1981
Nova Scotia	0.8 - 2.7	TTI	Douglas et al. 1987
Plymouth (UK)	10.0 - 149.0	Biomass	Newell et al. 1981
Sapelo Island	41.0 - 130.0	FDC	Newell and Fallon 1982
York River Estuary	1.0 - 132.0	TTI	Newell and Christian
York River, Virginia	7.0 - 75.0	TTI	Ducklow 1982
Upwelling Areas			
Chile	3.1 - 33.6	TTI	McManus and Peterson 1988
Portugal	0.3 - 12.3	TTI	Gocke et al. 1983
Southern Benguela	0.0 - 132.0	<3 µm	Armstrong et al. 1987
	0.2 - 5.0	TTI	This study
	6.2 - 127.9	<3 µm	This study
	0.3 - 30.0	TTI	Painting et al. 1989
	16.2 - 57.6	<3 µm	Painting et al. 1989
Northern Benguela	0.1 - 2.32	TTI	This study

Bacterial production estimates in the euphotic and pycnocline regions during the decline of the phytoplankton community in MUW in the southern Benguela, were initially 0.21 μg C l^{-1} h^{-1} (Table 2) and declined to ca 0.1 μg C l^{-1} h^{-1}. In the aphotic zone, bacterial production estimates were up to 20 times lower (0.009 to 0.014 μg C l^{-1} h^{-1}). The immediate conclusion to be drawn from these data is that bacterial production was low in the euphotic zone in response to decreased availability of PDOC during the declining phase of the bloom, and even lower in the aphotic zone where bacterial and phytoplankton relationships were decoupled. Bacterial production in MUW was calculated to be 0.7 to 3 % of primary production (Table 4). McManus and Peterson (1988) found that bacterial production was a smaller proportion of phytoplankton production during stratified conditions (6.8 %) than during active upwelling (23.9 %).

Estimates of bacterial production based on net bacterial growth suggest that phytoplankton-bacterioplankton relationships may in fact be more complex. Estimates are higher at all depths, possibly due to inappropriate conversion factors for the thymidine method, but more likely due to physiological properties of bacteria associated with more refractory carbon substrates available during the declining phase of the bloom (see Painting et al. 1989). Bacterial production estimates obtained from bacterial growth in predator-reduced incubations were calculated to be 2 to 30 % of primary production.

Bacterial production estimates in the upper mixed layer of the northern Benguela were generally lower (0.006 to 0.064 μg C l^{-1} h^{-1}) than the range reported by McManus and Peterson (1988) and Painting et al. (1989). These values were surprisingly low for recently upwelled water, and may possibly be attributed to poor response of bacteria to exogenously supplied thymidine. At Stations 3 and 30, for example, bacteria in the aphotic zone did not take up thymidine. The significant linear relationship between bacterial production and

phytoplankton production suggests that this hypothesis may not be applicable in the euphotic zone where phytoplankton are actively metabolising. Low bacterial production estimates may indicate inhibition of bacterial production in response to inhibited primary production (see above). McManus and Peterson (1988) found that under active upwelling conditions off Chile, bacterial production was 24 % of phytoplankton production. In this study, bacterial production was <5 % of primary production in the euphotic zone during active upwelling. (Figs. 9 and 10). Bacterial production was not measured by any other technique, but high PC concentrations and correlation between bacterial biomass and PC suggest that bacteria may also have been utilising PC substrates, and that bacterial production on these substrates was not reflected by measurements from the TTI method. Bacterial production estimates reported here are therefore likely to be conservative.

Bacterial growth parameters

Commonly reported growth rates for marine bacteria range between 0.2 and 2 d^{-1} (Ducklow 1983). Specific growth rates have been shown to be lowest (0.1 d^{-1}) during intense upwelling, and highest (0.5 d^{-1}) during stratification in an upwelling environment (McManus and Peterson 1988). From the microcosm simulation (Painting et al. 1989), calculation of specific growth rates from P_{TTI}/biomass shows similar results, with growth rates of 0.1 d^{-1} in newly upwelled water and 0.2 d^{-1} at the peak of the phytoplankton bloom. Calculations of growth rates from $P_{\Delta B}$ /biomass (Chapter 4) also show a similar trend, but specific growth rates are higher, increasing from 0.4 to 0.5 d^{-1}. During phytoplankton senescence (day 14; Painting et al. 1989) growth rates of 0.05 d^{-1} and 0.2 d^{-1} were calculated from P_{TTI}/biomass and $P_{\Delta B}$ /biomass respectively.

Growth rates of bacteria in the southern and northern Benguela upwelling regions measured during this study (Table 5) are comparable with the growth rates discussed above. During intense upwelling at Station 11 in the northern Benguela, the growth rate of bacteria in the euphotic zone (0.12 d^{-1}), calculated from thymidine-measured productivity, showed good agreement with previously calculated values (see above). At other stations in the northern, and southern, Benguela region observed growth rates (from P_{TTI}) were much lower than expected considering the environmental conditions. Growth rates calculated from $P_{\Delta B}$ in the southern Benguela show that bacterial growth rates in the euphotic zone in this region were considerably higher (max. 1.14 d^{-1}) than growth rates calculated from P_{TTI}. It is unfortunate that bacterial production in the northern Benguela was not measured by population growth, or by any other method independent of bacterial uptake of dissolved substrates. On the basis of earlier evidence it may be hypothesised that bacterial growth rates in both regions fall well within values reported for other regions, possibly approaching the upper limit of this range.

Bacterivorous protozoa were not quantified during either of the oceanographic cruises in the Benguela upwelling region. The abundance of heterotrophic microflagellates observed during the microcosm study (0.1 to 31 x 10^6 l^{-1}, Chapters 4 and 5) was similar to abundances reported during upwelling cycles off Chile (McManus and Peterson 1988). Power functions describing the relationships between bacterial biomass and concentrations of chlorophyll and PC during both cruises, suggest that bacterial biomass at high levels was removed by predation (see also Chapter 6). For the northern Benguela, Probyn (1988) hypothesised high grazing pressure on phytoplankton. It is likely that microzooplankton predators also grazed bacteria. Nutrient recycling by the microbial food web may account for high levels of reduced nitrogen taken up by the phytoplankton.

Diurnal variations in bacterial production were not addressed in this study. Bacterial production may continue, or even be enhanced, at night, resulting in higher ratios of bacterial production:phytoplankton production than calculated here.

CONCLUSIONS

Field measurements of bacterial production in the Benguela upwelling region are comparable with estimates obtained in other marine ecosystems, including those in upwelling areas off Chile and Portugal. Results of this study suggest that bacterial production is closely coupled to the upwelling cycle, in response to phytoplankton dynamics and substrate availability. Measured bacterial production rates (5.34 mg C m^{-3} h^{-1}, from net bacterial growth) associated with high rates of phytoplankton production are similar to those observed in a microcosm simulation of an upwelling event (Chapter 4) and are ca 10 % of net phytoplankton production. During the decline of the phytoplankton bloom, bacterial production in the euphotic zone is reduced (to ca 0.6 mg C m^{-3} h^{-1}) but may be up to 30 % of primary production. Estimates of bacterial production from TTI (0 to 0.208 mg C m^{-3} h^{-1}) during the quiescent phase of the upwelling cycle are, on average, 10 times lower than estimates from bacterial growth. The thymidine-incorporation method shows that bacterial production is low (0.004 to 0.064 mg C m^{-3} h^{-1}) during active upwelling conditions and higher (up to 0.208 mg C m^{-3} h^{-1}) during high levels of primary production after stratification of the water column. Evidence from a microcosm study (Chapter 4) suggests non-uniform response of bacteria to exogenously supplied thymidine, due to physiological adaptations to a variable environment and growth requirements. In particular, this method is likely to underestimate bacterial production during active upwelling as a result of a high proportion of

metabolically dormant cells, and during phytoplankton decay when bacteria are metabolising more refractory carbon substrates.

ACKNOWLEDGEMENTS

I am grateful to the Instituto de Ciencias del Mar of Barcelona and the Sea Fisheries Research Institute for my participation in the cruise in 1985 (the Spanish/Namibian Environmental Cruise, SNEC-85). I would like to thank Drs T. A. Probyn and E. Gutiérrez for contributing additional CHN data, and Dr. M. Estrada for providing data on primary production. Constructive comments on this chapter from Drs Mike Lucas and Trevor Probyn are gratefully acknowledged. I am indebted to the BEP and the Sea Fisheries Research Institute for financial contributions to this study.

CHAPTER 8

The relative significance of bacteria and copepods in the carbon flux of the southern Benguela.

ABSTRACT

Net production of the copepod Calanoides carinatus during the declining phase of a phytoplankton bloom in maturing upwelled water (MUW) in the southern Benguela was calculated from instantaneous production rates of the population at each station during two consecutive drogue studies in March 1983. Total abundances of C. carinatus in the upper 50 m of the water column were high (370 to 10 000 individuals m^{-3}, including all developmental stages), particularly during the first drogue. The average production rate was 4.84 mg C m^{-3} d^{-1} (range: 1.15 to 17.8 mg C m^{-3} d^{-1}) or 242 mg C m^{-2} d^{-1} over the upper 50 m depth. Highest values were found during the first drogue. During the temporal development of MUW over the period of the second drogue, C. carinatus production increased from about 60 to 292 mg C m^{-2} d^{-1}, and then declined to 102 mg C m^{-2} d^{-1}. C. carinatus production was, on average, 3.5 % (range: 0.5 to 12.6 %) of net primary production. Assuming that all the phytoplankton was available to copepods, and a growth efficiency of 30 %, copepods were calculated to graze 3 to 72 % (mean value: 22 %) of phytoplankton biomass and 4 to 100 % (mean value: 28 %) of total primary production. From bacterial production estimates based on population growth, bacteria were shown to have carbon consumption requirements equivalent to 14 to 171 % (mean value: 81 %) of autotrophically fixed carbon. These results suggest that the proportion of phytoplankton consumed by copepods may be higher than previously recorded in the southern Benguela. Furthermore, results suggest that a dynamic microbial food web co-exists with the metazoan food web during the quiescent phase of the upwelling cycle, and that carbon consumption requirements of both copepods and bacteria are satisfied by resource partitioning and cycling of autotrophically fixed carbon.

INTRODUCTION

The southern Benguela supports a large, commercially important pelagic fishery (Cushing 1971, Crawford et al. 1987). Consequently, much of the planktonic research in this ecosystem is directed towards increasing the understanding of trophic processes affecting pelagic fish. The dynamic physical oceanographic processes characteristic of upwelling ecosystems, and extreme temporal and spatial variability in upwelling processes (reviewed by Shannon 1985b), however, make this a difficult task. The biomass and distribution of phytoplankton and zooplankton are highly variable, and trophic processes are poorly understood (see Shannon and Pillar 1986). One of the fundamental questions is whether food webs are simple diatom-based food chains with short, efficient pathways making a large proportion of phytoplankton production available to fish, or whether the food web is dominated by a micro-planktonic community, with an active microbial food web but significant losses of carbon through respiration (Ducklow et al. 1986). Recent research suggests that both types of food webs may exist, either simultaneously, or on different time-scales governed by frequency, intensity and duration of upwelling cycles (Moloney 1988, Probyn et al. 1989).

Numerous studies have been directed at describing the distribution of zooplankton in the southern Benguela (Shannon and Pillar 1986, Verheye and Hutchings 1988). Although phytoplankton dynamics in response to the upwelling cycle are relatively well understood (Brown and Hutchings 1987a, b), little research has been done on zooplankton dynamics. Recent quantitative studies (Pillar and Stuart 1988, Stuart and Pillar 1988, Pillar et al. 1989) have shown that the population dynamics of the dominant euphausiid, Euphausia lucens, is coupled to the upwelling cycle and that this species adopts various strategies

to maintain itself within the coastal region of the southern Benguela upwelling system. Similarly, recent studies on copepod dynamics in this region have suggested that the population structure and reproductive biology of copepods, the dominant mesozooplankton, is strongly influenced by upwelling processes (Borchers and Hutchings 1986, Attwood and Peterson 1989, Peterson et al. 1989, Verheye 1989a, b). Studies in upwelling regions off Oregon (Peterson et al. 1979) and Peru (Smith et al. 1981) have also demonstrated close coupling between copepod dynamics and upwelling events. One of the common features of the upwelling regions studied to date is that total mesozooplankton biomass is lower than would be predicted from the high levels of primary production recorded (Borchers and Hutchings 1986, see also Nixon et al. 1986). A number of hypotheses have been proposed to explain the anomalously low biomass of copepods, including: A temporal mismatch between phytoplankton availability and zooplankton abundance (Olivieri and Hutchings in prep.); The size structure of the phytoplankton community (Price et al. 1983, Peterson and Bellantoni 1987, Peterson et al. 1989); The variable nature of the physical and food environment (Cushing 1969, Walsh 1976, Dagg 1977, Borchers and Hutchings 1986, Attwood and Peterson 1989); and predation by fish (Verheye and Hutchings 1988, Painting and Huggett in prep.).

One of the important questions in ecological studies of the southern Benguela is how much of the primary production is channelled into secondary production by copepods. A direct approach towards answering this question has been to determine the proportion of primary production removed by grazing. Olivieri and Hutchings (in prep.) and Peterson et al. (1989) have shown that the grazing impact of copepods is low (<11 %, see also Dagg and Turner 1982). A second approach has been to measure the secondary production rate of the dominant copepod species, Calanoides carinatus and Calanus australis (Borchers and Hutchings 1986, Attwood and Peterson 1989, Peterson and Painting 1989,

Peterson unpubl. data). This approach assumes that secondary production is a reliable indicator of feeding rate (Kiorboe et al. 1985b, Durbin et al. 1983, Peterson 1988). From laboratory and field studies it appears that in situ copepod production in the southern Benguela is also low (Borchers and Hutchings 1986, Peterson unpubl. data).

The total loss of phytoplankton from the southern Benguela due to export and sedimentation appears to be low (< 20 %, Bailey pers. comm., Pitcher et al. subm.). Work on the feeding ecology of anchovies (James 1987) suggests that, at most, 10 % of the phytoplankton standing crop is eaten by pelagic fish. A large proportion of phytoplankton primary production in the southern Benguela may therefore be channelled through the microbial food web via bacterioplankton and microzooplankton. Bacterial biomass in newly upwelled water is low (<40 mg C m^{-3}) but increases exponentially during phytoplankton growth, reaching approximately 10 % of the phytoplankton biomass at the peak of the bloom (Painting et al. 1989). It is now recognised that as much as 60 % of phytoplankton production may pass through heterotrophic bacteria as photosynthetically-produced dissolved organic carbon (PDOC) and particulate material (for review see Williams 1981, Lucas 1986). Sorokin and Mikheev (1979) calculated that 70 to 80 % of primary production was utilised by microheterotrophs in the Peruvian upwelling system, Laake et al. (1983a, b) found that 35 to 45 % of the total energy flux passed through bacteria in an enclosed planktonic system, and Linley et al. (1983) calculated that 20 to 60 % of primary production was consumed by bacteria in the English Channel. Furthermore, recent studies which have shown active predation within the microbial food web (Fenchel 1982d, Sherr et al. 1986a, Coffin and Sharp 1987, Lucas et al. 1987) have important implications for the role of bacteria in nutrient regeneration in the water column, and the maintenance of stable phytoplankton populations.

During a drogue study in a plume of maturing upwelled water in the southern Benguela in March 1983, temporal changes in the abundance and biomass of zooplankton and bacterioplankton were measured (Chapter 6). The total biomass of zooplankton, dominated by the copepod Calanoides carinatus, was relatively large (11 to 86 mg C m^{-3}). Assuming a daily food requirement of 30 % of their total biomass, copepods were calculated to consume 5 to 38 % of the phytoplankton standing crop (Chapter 6). The copepod grazing impact may have been overestimated by assuming a daily food ration of 30 % of the biomass, particularly if copepod dynamics are uncoupled from the upwelling cycle and the animals are starvation-adapted in response to variable or sub-optimal feeding conditions. Bacterial biomass was also high (20 to 200 mg C m^{-3}), and bacterial production estimates were 2 to 28 % of net phytoplankton production in the euphotic zone or 7 to 73 % of autotrophically fixed carbon available in the water column (Chapter 7).

The objective of this study was to obtain an estimate of the partitioning of phytoplankton biomass and production between the dominant copepod species (Calanoides carinatus) and bacterioplankton during the drogue study. The secondary production rate and carbon consumption requirements of copepods are calculated here, and compared with production estimates and calculated carbon requirements of bacterioplankton during the same period, to determine whether the available autotroph carbon could meet the consumption requirements of both copepods and bacteria .

METHODS

Sampling and analytical methods

Phytoplankton, bacterial and copepod production rates were measured from the R.S. Africana during the growth and decay of phytoplankton in a plume of upwelled water in the southern Benguela in summer 1983 (13 to 20 March). A drogue released into mature upwelled water downstream of the upwelling centre off Cape Columbine, South Africa, was followed for 37 h until it was apparent that the drogue had moved out of the water mass being studied. The drogue was retrieved, re-deployed into a similar patch of mature upwelled water and followed for another 94 h (Chapter 6).

The water column at the drogue was sampled at 19 stations, 5 during the first drogue (Stations 2 to 6) and 13 during the second drogue-study (Stations 8 to 20). Depth-profiles of temperature and salinity were obtained at each station using a Neill Brown CTD bathysonde, and penetration of incident light was measured using a LICOR quantum sensor. Water samples were collected with a rosette of 5-litre Niskin bottles from the 100, 50, 10, 1 and 0.1 % light depths, and at 10 or 20 m intervals to the bottom. Samples were analysed immediately for nutrient concentrations (Mostert 1983) and filtered and frozen for chlorophyll a (SCOR/UNESCO Working Group 17 1966) and particulate carbon analysis. Samples from the Niskin bottles were also preserved for microscope counts of the abundance, biomass and depth-distribution of bacteria and mesozooplankton (Chapter 6). Zooplankton in the whole water column (ca 100 m) were sampled with a vertical Bongo net (200 µm mesh, McGowan and Brown 1966, Verheye 1989b) and preserved in 5 % buffered formalin for estimation of mesozooplankton abundance and biomass.

Phytoplankton and bacterial production

Phytoplankton production in the euphotic zone was measured by uptake of ^{14}C-bicarbonate during in situ incubations (Strickland and Parsons 1972, Brown 1982, 1984). Heterotrophic bacterial production was measured at stations sampled before mid-day. Water samples (50 to 150 ml) from the euphotic zone, the pycnocline and the aphotic zone were incubated on an orbital shaker in the dark at 12 ^{o}C in the ship's laboratory. Net bacterial production rates were calculated from direct measurements of bacterial growth in predator-reduced incubations (<3 μm, Linley et al. 1983). These were compared with estimates of total bacterial production obtained from incorporation of [methyl-^{3}H] thymidine into bacterial DNA (Fuhrman and Azam 1980, 1982, Painting et al. 1989, Chapter 7).

Copepod production

Net production (P) of the copepod Calanoides carinatus was calculated from data on the age structure of the population at each station. Instantaneous rates of biosynthesis were calculated separately for each developmental stage, and summed (equation 1, Kimmerer 1987).

$$P \text{ (mg dry weight } m^{-3} d^{-1}) = \sum_{i=1}^{6} (b_i * g_i) \qquad (1)$$

where i = developmental stage (C1 to C6), b_i = total biomass of the $_i$th stage (mg dry wt m^{-3}) and g_i = growth rate of $_i$th stage (d^{-1}). The ratio for carbon biomass to dry weight (DW) was assumed to be 40 % (Andrews and Hutchings 1980).

Estimates of g_i for copepodite stages 1 to 5 were based on the temperature-dependent rates measured for Calanoides carinatus in the laboratory (Borchers

and Hutchings 1986, see also Verheye 1989b). Average ambient temperature was assumed to be 13 ^{o}C at Stations 3 to 9, and 14 ^{o}C at Stations 10 to 20.

The production rate of female Calanoides individuals (stage 6) was calculated from estimated egg production rates (eggs female^{-1} day^{-1}), using the egg ratio method (equation 2, Downing and Rigler 1984).

$$E \text{ (eggs female}^{-1} \text{ d}^{-1}) = \frac{N_t/C_t}{D} \qquad (2)$$

where N_t and C_t are the total numbers of eggs and females respectively in each sample, and D is the development time of eggs at the ambient temperature (^{o}C). Mean observed egg production rates (μg DW female^{-1} day^{-1}, assuming 0.45 μg DW egg^{-1}, Peterson, pers. comm.) were divided by the individual weight per female (120.8 μg DW female^{-1}) to obtain a growth rate (d^{-1}).

Production estimates were integrated over the upper 50 m of the water column as the copepods were shown to be concentrated within this depth range (Chapter 6).

RESULTS

The hydrography and data on the temporal changes in primary production and the abundance and distribution of phytoplankton, bacteria and zooplankton during this study are described in Chapter 6. It was clear that different water types were present in the study area. Water in the upper 20 to 30 m of the water column was maturing upwelled water (MUW). During the second drogue salinity and biological fronts between Stations 12 and 13 suggested that the MUW was converging with, and possibly mixing with, water which was somewhat

older although still of upwelling origin. Bacterial production is discussed in Chapter 7.

Zooplankton numbers were dominated by the copepods Calanoides carinatus and Oithona spp., which were found to be present largely in the upper 40 m of the water column from sunrise to sunset. The biomass at each station was dominated by the larger copepod, C. carinatus, for which estimates of secondary production were obtained.

From the population age structure of Calanoides carinatus no easily recognisable cohort could be followed throughout the study. Figure 1 suggests the presence of one recognisable cohort in MUW at Stations 3 to 6 where abundances were high (2600 to 9800 individuals m^{-3}) and most individuals (up to 50 %) were copepodite stage 3 or 4. A separate cohort was evident in MUW/mixed water (Station 14 onwards), where abundances were lower (1400 to 3000 animals m^{-3}) and most individuals were fifth stage copepodites (approx. 20 %) and adults (29 %). In MUW at Stations 8 to 12 abundances were lowest (500 to 2400 m^{-3}) and developmental stages appeared to be similarly distributed (9 to 17 % per stage) in the population, showing no presence of a cohort. In conjunction with a relatively high percentage of nauplii (mean = 22 %), this stable age distribution is indicative of a continuously reproducing population.

Population age structure for Oithona could not be established due to non-quantitative sampling of the young copepodite stages by the Bongo net (mesh size 200 μm).

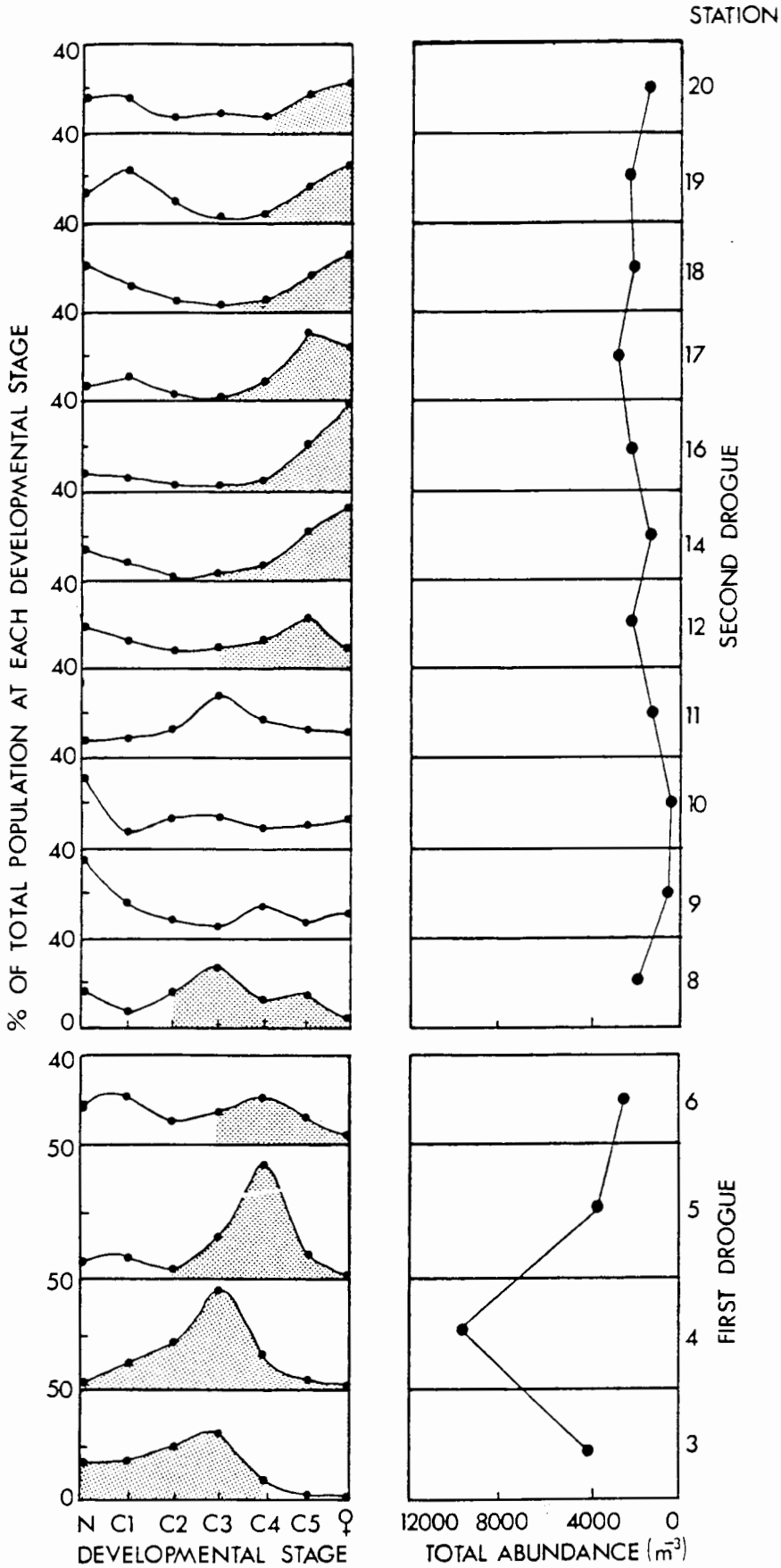


Fig. 1. Population age structure of Calanoides carinatus during the growth and decay of phytoplankton in a plume of upwelled water in the southern Benguela. The total abundance of C. carinatus at each sampling station is shown, and the percentage of the total population at each developmental stage from copepodite 1 to 6 (C1 to adult females). The percentage contributions of the 6 nauplius stages were combined (N). Shaded areas indicate cohorts.

Copepod Production

Production of Calanoides carinatus was estimated from calculations of the instantaneous production rate (Kimmerer 1987), a method commonly used to estimate production of steady-state zooplankton populations. An example of the calculation procedure, in which stage-specific data are used, is shown on Table 1.

Similar data were used to obtain estimates of the total production of Calanoides (mg dry wt m^{-3} d^{-1}) at all stations (Table 2). From the egg ratio data (Table 3) the egg production rate of Calanoides females was assumed to be constant at 60 eggs $female^{-1}$ day^{-1} from Station 3 to 12, decreasing exponentially to 3 eggs $female^{-1}$ d^{-1} until Stations 19 and 20. Production rates of females were therefore assumed to be constant initially (0.20 d^{-1}), decreasing to 0.01 d^{-1} by Stations 19 and 20.

The highest production rate of Calanoides was found in MUW at Station 4 (44.45 mg dry wt m^{-3} d^{-1}, Table 2). Large differences in the production rates between stations sampled during the first drogue, which was followed for only 1.5 days, support the earlier hypothesis that the drogue did not stay in the same water mass during this time.

During the second drogue, estimates of Calanoides production were low initially (2.87 mg dry wt m^{-3}, Station 9) and showed a temporal increase throughout the development of the MUW, reaching a maximum of 14.61 mg dry wt m^{-3} at Station 12. Despite a slight decrease in Calanoides production rates after the MUW converged with or mixed with the older upwelled water, the production rate was maintained at approximately this value for one day after the convergence and then declined to 5.11 mg dry wt m^{-3}. Depth-integrated estimates of instantaneous

Table 1. Stage-specific data used for calculation of instantaneous production of Calanoides carinatus (mg dry wt m^{-3} d^{-1}) at Station 4. DW = dry weight. Growth rates (d^{-1}) were based on laboratory measurements (Borchers and Hutchings 1986, Verheye 1989).

Copepodite Stage	Individ. Dry Weight (µg)	Total no.per stage (m^{-3})	Total Biomass (mg m^{-3})	Maximum Growth Rate (d^{-1})	Production (mg DW m^{-3} d^{-1})
C1	3.7	1098	4.1	0.35	1.44
C2	7.4	2194	16.2	0.29	4.70
C3	14.9	4361	65.0	0.37	24.05
C4	29.9	1521	45.5	0.24	10.92
C5	60.1	338	20.3	0.13	2.64
C6	120.8	29	3.5	0.20	0.70
					———
			Total production:		44.45

Table 2. Production of Calanoides carinatus (mg C m^{-2} d^{-1}) in the upper 50 m of the water column, calculated separately for each station from the instantaneous rate of production (see Table 1). Growth rates of females (d^{-1}) calculated for each station are shown in brackets. Measured biomass (mg DW m^{-3}) was used to calculate P:B values. Carbon biomass was assumed to be 40 % of the dry weight (Andrews and Hutchings 1980).

Station	Production (mg DW m^{-3} d^{-1})		Biomass (mg DW m^{-3})	P:B (d^{-1})	Total Production (mg C m^{-2} d^{-1})
Maturing upwelled water					
3	13.59	(0.2)	44.6	0.31	271.8
4	44.45	(0.2)	154.6	0.29	889.0
5	20.96	(0.2)	91.7	0.23	419.2
6	12.50	(0.2)	53.8	0.23	250.0
8	10.18	(0.2)	42.3	0.24	203.6
9	2.87	(0.2)	12.3	0.23	57.4
10	3.16	(0.2)	12.7	0.25	63.2
11	8.88	(0.2)	33.6	0.26	177.6
12	14.61	(0.2)	62.4	0.23	292.2
MUW/mixed water					
14	9.69	(0.2)	66.8	0.15	193.8
16	10.22	(0.1)	123.5	0.08	204.4
17	13.08	(0.05)	126.0	0.10	261.6
18	6.20	(0.03)	83.7	0.07	124.0
19	6.06	(0.01)	86.5	0.07	121.2
20	5.11	(0.01)	52.0	0.10	102.2
mean:	12.10		69.8	0.19	242.1

Table 3. Observed egg production rates of female Calanoides calculated by the egg ratio method. Development time of eggs was assumed to be 1.4 d at 13 °C and 1.2 d at 14 °C (Peterson, pers. comm.).

Station	No. eggs counted (m^{-3})	No. females counted (m^{-3})	Egg ratio (eggs fem^{-1})	Observed egg production (fem^{-1} d^{-1})
5	1500	30	50.0	35.71
6	5875	96	61.2	43.71
9	9063	62	146.2	104.43
11	7938	133	59.7	49.17
			mean:	58.26
17	861	707	1.2	1.00
18	1000	574	1.7	1.42
			mean:	1.21

production (over the upper 50 m) ranged from 57.4 to 889.0 mg C m^{-2} d^{-1} (Table 2).

Mean growth rates (d^{-1}) during this study, represented by the specific production rate (P:B), were clearly considerably higher (0.23 to 0.31) in MUW than in MUW/mixed water at Stations 14 to 20 (0.07 to 0.15). The average P:B calculated over the entire study period was 0.19 (Table 2).

Net production (mg C m^{-2} h^{-1}) by Calanoides and bacteria during this study are compared with hourly phytoplankton production measurements (from Chapter 6 and 7) in Table 4. On average, copepod production was 3.5 % of primary production. Bacterial production measured from biosynthesis ($P_{\Delta B}$) was a considerably higher percentage of phytoplankton production (13.1 %), while thymidine-measured production (P_{TTI}) was only 1.6 % of phytoplankton production.

Carbon consumption requirements

The carbon consumption requirements (mg C m^{-2} d^{-1}) of C. carinatus during the temporal development of the upwelling plume were calculated (Table 5) from copepod production rates integrated over the upper 50 m of the water column, assuming a net growth yield of 30 % (Conover 1956, Ikeda and Motoda 1978, in Omori and Ikeda 1984). The impact of copepod grazing on phytoplankton biomass and production was calculated independently, by dividing copepod consumption requirements by (1) total available phytoplankton biomass (integrated over the water column, from Chapter 6) and (2) by the phytoplankton production estimates (mg C m^{-2} d^{-1}, also from Chapter 6). It was assumed that all phytoplankton carbon was available for utilisation by copepods. In maturing upwelled water, phytoplankton biomass and production were relatively high. Copepods were shown to consume 3.3 to 18.5 % (mean value: 10.4 %) of available phytoplankton

Table 4. Comparison of phytoplankton (phyto), bacterial (bact) and copepod production, expressed in mg C m^{-2} h^{-1}. Bacterial production estimates were calculated from: population growth in predator-reduced incubations ($P_{\Delta B}$), and from incorporation of ^{3}H-thymidine into bacterial DNA (P_{TTI}) (Fuhrman and Azam 1982, Chapter 7). Copepod production estimates per day (Table 2) were divided by 24.

Station	Production (mg C m^{-2} h^{-1})						
	Phyto (P_P)	Bact ($P_{\Delta B}$)	Bact (P_{TTI})	Copepod (P_C)	$P_{\Delta B}/P_P$ (%)	P_{TTI}/P_P (%)	P_C/P_P (%)
3	1114.2	-	-	11.33	-	-	1.0
6	522.2	-	-	10.42	-	-	2.0
9	465.3	42.7	3.3	2.39	9.2	0.7	0.5
12	539.3	-	-	12.18	-	-	2.3
15	66.1	18.4	2.0	8.30	27.8	3.0	12.6
18	223.0	5.0	2.7	5.17	2.2	1.2	2.3
				mean:	13.1	1.6	3.5

Note: The copepod production estimate at Station 15 is the mean of the estimates at Stations 14 and 16.

Table 5. Calculations of daily consumption of phytoplankton standing crops and phytoplankton production by Calanoides carinatus. Total ingestion (mg C m^{-2} d^{-1}) was calculated from the net production rate (mg C m^{-2} d^{-1}), assuming a net growth yield of 30 %, and divided by available phytoplankton carbon (from Chapter 6). Hourly primary production estimates were converted to daily estimates assuming a 10-h day.

Station	Phytoplankton		Copepods			
	Biomass	Production	Production	Ingestion		
	(P_B) (mg C m^{-2})	(P_P) (mg C m^{-2} d^{-1})	(mg C m^{-2} d^{-1})	Total (mgC$m^{-2}d^{-1}$)	% P_B (d^{-1})	% P_P (d^{-1})
Maturing upwelled water (MUW)						
3	9576	11142	271.8	906.0	9.5	8.1
4	26070	-	889.0	2963.3	11.4	-
5	7710	-	419.2	1397.3	18.1	-
6	8676	5222	250.0	833.3	9.6	16.0
8	5864	-	203.6	678.7	11.6	-
9	5774	4653	57.4	191.3	3.3	4.1
10	5188	-	63.2	210.7	4.1	-
11	8282	-	177.6	592.0	7.2	-
12	5269	5393	292.2	974.0	18.5	18.1
Mean:	9157	6603	291.6	971.9	10.4	11.6
MUW/mixed water						
14	1722	-	193.8	646.0	36.5	-
15	-	661	199.1	663.7	-	100.4
16	3789	-	204.4	681.3	18.0	-
17	1209	-	261.6	872.0	72.1	-
18	690	2230	124.0	413.3	59.9	18.5
20	1637	-	102.2	340.7	20.8	-
Mean:	1819	1446	180.9	602.8	41.5	59.5

biomass, and 4.1 to 18.1 % (mean: 11.6 %) of phytoplankton production. In MUW/mixed water, phytoplankton biomass and production were considerably lower, but copepod consumption requirements were high (mean: 602.8 mg C m^{-2} d^{-1}). Average copepod consumption of phytoplankton biomass and production was 4 to 5 times higher than in MUW (41.5 % of phytoplankton biomass, and 59.5 % of phytoplankton production).

To determine the relative flux of autotrophically derived carbon to heterotrophic bacteria, the daily carbon consumption requirements (mg C m^{-2} d^{-1}) of the bacterial communities in the euphotic zone were similarly calculated (Table 6), assuming a bacterial net growth yield of 30 % (Lucas 1986). The daily carbon consumption requirements were expressed as a % of available autotroph carbon (biomass and production) and total particulate carbon (PC). Bacterial production estimates at 3 stations were obtained from measurements of bacterial growth in predator-reduced incubations ($P_{\triangle B}$), and from [methyl-^{3}H] thymidine incorporation (P_{TTI}, Chapter 7). In addition, to provide a more complete data set for comparison of copepod and bacterial carbon requirements during the temporal development of the plume, bacterial production in the euphotic zone was calculated ($P_{calculated}$) for all stations during this study by multiplying the measured bacterial biomass (Chapter 6) by a P:B ratio of 0.5 d^{-1}. This ratio was assumed to be realistic for bacterial populations which are likely to utilise both photosynthetically-produced dissolved organic carbon (PDOC) and more refractory carbon substrates during the declining phase of a phytoplankton bloom in a stratified water column (see Chapter 7). Phytoplankton biomass (mg C m^{-2}) was calculated by integrating chlorophyll concentrations over the euphotic zone depth, and assuming a C:Chlorophyll ratio of 60 (see Chapter 6). Phytoplankton production was corrected for losses due to excretion of PDOC, by assuming an excretion rate of 30 % d^{-1}.

Table 6. Daily carbon consumption requirements (mg C m^{-2} d^{-1}) of bacteria in the euphotic zone expressed as a % of available autotroph carbon and total particulate carbon (PC). Total consumption was calculated from the net production rate (mg C m^{-2} d^{-1}) assuming a net growth yield of 30 %. Bacterial production was calculated from (1) bacterial biomass and a P:B ratio of 0.5 d^{-1} ($P_{calculated}$), (2) [methyl-^{3}H] thymidine incorporation into bacterial DNA (P_{TTI}) and (3) bacterial growth ($P_{\Delta B}$) (see also Chapter 7). Phytoplankton biomass (P_B) was calculated by integrating chlorophyll data (mg m^{-3}, see Chapter 6) over the euphotic zone depth, and assuming a C:chlorophyll ratio of 60. Phytoplankton production per day (P_P) shown on Table 5 was corrected for losses due to excretion of metabolites assuming an excretion rate of 30 % d^{-1}.

Station	Phytoplankton			Bacteria					
	Biomass	Production	PC	Biomass	Production	Consumption			
	(P_B) (mg C m^{-2})	(P_P) (mg C m^{-2} d^{-1})	(g C m^{-2})	(mg C m^{-2})	(mg C m^{-2} d^{-1})	Total (mg C m^{-2} d^{-1})	% P_B (d^{-1})	% P_P (d^{-1})	% PC (d^{-1})
$P_{calculated}$									
3	6354	14485	10.2	566	283	943	14.8	6.5	9.3
4	3237	-	11.6	643	322	1073	33.2	-	9.3
5	5688	-	15.9	929	465	1550	27.3	-	9.8
6	3954	6789	6.4	451	226	753	19.0	11.1	11.8
8	2238	-	6.1	1982	991	3303	147.6	-	54.2
9	4638	6049	8.1	1878	939	3130	67.5	51.7	38.6
10	2992	-	8.8	892	446	1487	49.7	-	16.9
11	4335	-	9.6	1674	837	2790	64.4	-	29.1
12	5190	7011	9.8	794	397	1323	25.5	18.9	13.5
Mean:	4292	8584	9.6	1090	545	1817	49.9	22.1	21.4
14	1371	-	8.0	499	250	833	60.8	-	10.4
15	-	859	8.0	389	195	650	-	75.7	8.1
16	1149	-	8.0	627	314	1047	91.1	-	13.1
17	1209	-	6.1	518	259	863	71.4	-	14.2
18	690	2899	6.2	394	197	657	95.2	22.7	10.6
20	652	-	-	346	173	577	88.5	-	-
Mean:	1014	1879	7.3	462	231	772	81.4	49.2	11.3
Overall Means:	3121	6349	8.8	839	420	1399	60.8	31.1	17.6
P_{TTI}									
9	4638	6049	8.1	1878	79.2	264	5.7	4.4	3.3
15	-	859	8.0	389	48.0	160	-	18.6	2.0
18	690	2899	6.2	394	64.8	216	31.3	7.5	3.5
Mean:	2664	3269	7.4	887	64.0	213	18.5	10.2	2.9
$P_{\Delta B}$									
9	4638	6049	8.1	1878	1024.8	3416	73.7	56.5	42.2
15	-	859	8.0	389	441.6	1472	-	171.4	18.4
18	690	2899	6.2	394	120.0	400	58.0	13.8	6.5
Mean:	2664	3269	7.4	887	528.8	1763	65.9	80.6	22.4

In maturing upwelled water, the average percentages of phytoplankton biomass (P_B), phytoplankton production (P_P) and PC consumed by bacteria for which production was calculated from a P:B of 0.5 d^{-1}, were 49.9, 22.1 and 21.4 respectively (Table 6). In MUW/mixed water, average phytoplankton biomass and production were 4 to 8 times lower than in MUW, but bacterial carbon consumption requirements (from $P_{calculated}$) were only halved. Bacteria were therefore calculated to consume the equivalent of 81.4 % of P_B, 49.2 % of P_P and 11.3 % of PC.

Bacterial production was measured ($P_{\Delta B}$, P_{TTI}) at one station in MUW (Station 9) and two stations in MUW/mixed water (Stations 15 and 18). Average carbon consumption requirements calculated from $P_{\Delta B}$ (1763 mg C m^{-2} d^{-1}) closely approximated the requirements from $P_{calculated}$ (overall mean: 1399 ± 889 mg C m^{-2} d^{-1}). Similarly, the average percentages of P_B, P_P and PC consumed by bacteria (from $P_{\Delta B}$) were comparable with the overall means from $P_{calculated}$ ($P_{\Delta B}$ = 65.9 % P_B, 80.6 % P_P, 22.4 % PC. $P_{calculated}$ = 60.8 % P_B, 31.1 % P_P, 17.6 % PC).

Average carbon consumption requirements of bacteria calculated from P_{TTI} (213 mg C m^{-2} d^{-1}) were approximately 8 times lower than carbon requirements from P_B (1763 mg C m^{-2} d^{-1}) and $P_{calculated}$ (1399 mg C m^{-2} d^{-1}). Average percentages of B_B, P_P and PC consumed were consequently also lower (18.5 % P_B, 10.2 % P_P, 2.9 % PC).

DISCUSSION

The net production rates of Calanoides carinatus during the development of the plume of maturing upwelled water in the southern Benguela during this study were relatively high, ranging from 1.15 to 17.8 mg C m^{-3} d^{-1} (assuming a carbon:dry weight ratio of 40 %). The average value was 4.84 mg C m^{-3} d^{-1}, or

242.1 mg C m^{-2} d^{-1} in the upper 50 m of the water column. No comparable data appear to have been published for other upwelling areas, but these estimates compare well with copepod production measured in other marine environments, 0.04 to 23 mg C m^{-3} d^{-1} (Durbin and Durbin 1981, Uye 1982, Kimmerer and McKinnon 1987) supporting the hypothesis that secondary production in upwelling systems is not exceptionally high (Borchers and Hutchings 1986, Nixon et al. 1986). Results presented here are similar to previous estimates of average copepod production in the southern Benguela, 1.1 and 4.2 mg C m^{-3} d^{-1} (Verheye 1989b, Peterson unpubl. data). The calculated food chain efficiency (secondary production/primary production) of 3.5 % was comparable with values of 2 to 7 % and 5 % reported by these authors respectively.

Production rates of C. carinatus, calculated from instantaneous rates of biosynthesis by the population present at each station, are based on the assumptions (1) that the mean ambient temperatures affecting copepod production closely approximated temperatures in the upper 20 m (13 or 14 °C) (see Methods), (2) that copepodite growth rates were maximal, (3) that gravid females channelled energy into egg production rather than biosynthesis, and (4) that copepod biomass was not affected by advection processes or copepod migrations. The growth rates of copepodite stages used to calculate instantaneous production rates of each developmental stage were based on the temperature-dependent rates measured for C. carinatus under optimal food conditions. Copepod migration into cooler sub-surface waters and a sub-optimal in situ food environment are likely to result in an overestimate of secondary production. Alternatively, reduced day-time copepod abundances in the upper 50 m of the water column, due to diurnal migrations, may result in an underestimate of total copepod production. Although vertical migration was not studied here, evidence from Niskin bottle samples and previous studies in the southern Benguela (see Chapter 6) suggested that vertical migration was

unlikely to be an important factor influencing copepod production rates in this study.

Advection processes may have affected copepod distribution in the upwelling plume, resulting in overestimates of secondary production. In the nearshore water column off Central Chile, Peterson et al. (1988) observed that most copepod species were unaffected by the strong onshore-offshore advection associated with frequent upwelling, and proposed that copepod distributions in this ecosystem may be controlled by variables other than movement of the upper Ekman layer. Studies by Smith et al. (1981) in the Peruvian upwelling system and Verheye (1989a) in the southern Benguela upwelling region suggest that copepod distribution in these ecosystems is affected by onshore-offshore advection. From the results of four cruises in the southern Benguela, Verheye (1989a) hypothesised that the distribution of copepods is closely coupled to successive upwelling cycles. Ontogenetic layering, with concentration of juvenile copepods in the surface Ekman layers, may result in offshore transport of copepodites during upwelling-favourable winds. Development to the adult stage, which is able to migrate more strongly, may enable the copepods to move down into deeper waters where shoreward currents return them to the coast, thus maintaining the copepods within the coastal upwelling system (see also Hutchings et al. 1985). In this study, copepods were sampled during the quiescent phase of the upwelling cycle. Furthermore, the currents in the study area are generally sluggish (Hutchings, pers. comm.). It is therefore likely that copepod biomass was not affected by physical processes other than the offshore movement of the plume of upwelled water in which they were sampled. Direct evidence in support of this hypothesis was provided by comparing measured copepod biomass with predicted biomass (Fig. 2). For each station, the predicted biomass was calculated from the product of hourly copepod production rates and measured biomass at the previous station. A two-tailed paired-sample

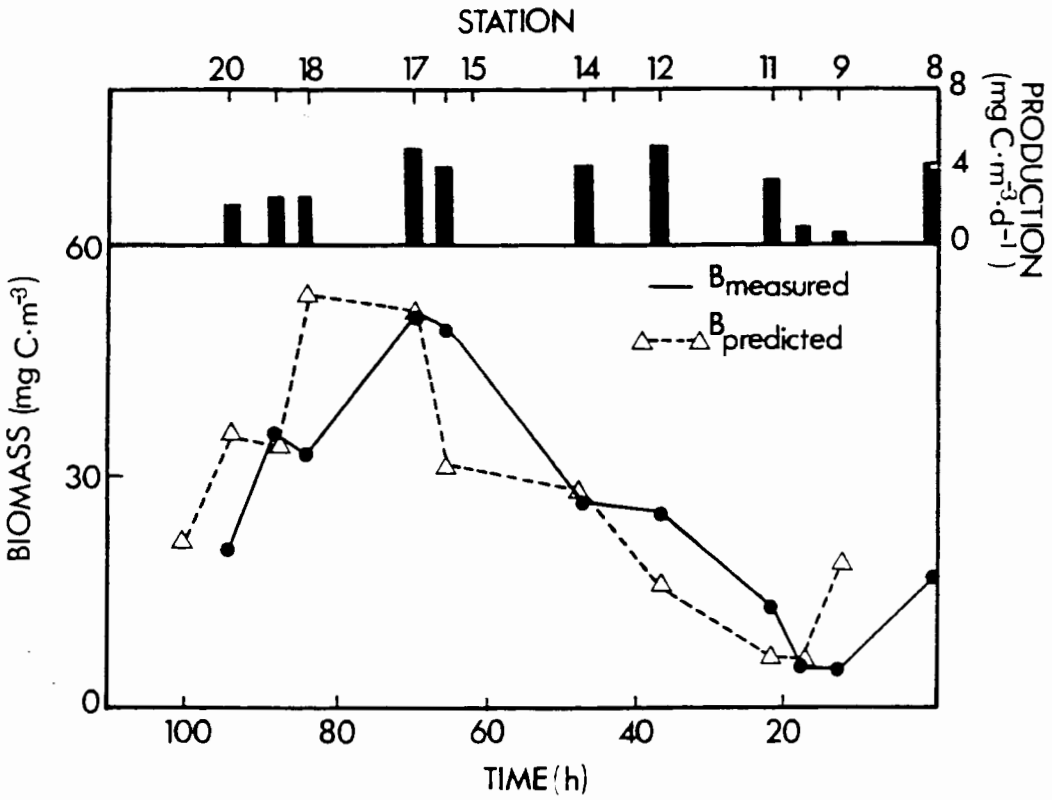


Fig. 2. Comparison of measured and predicted biomass of Calanoides carinatus. Predicted biomass at each station was calculated from the product of hourly copepod production rates and measured biomass at the previous station.

t-test (Zar 1984) showed no significant difference between measured and predicted biomass (n= 10, $|t_s| = 0.004$, $t_{0.05(2),9} = 2.226$), suggesting that observed changes in biomass were due to growth (biosynthesis), and were not significantly influenced by advection or migration of copepods into or out of the plume. Agreement between measured and predicted biomass indicates that the assumption of maximum growth rates of copepodites may be justified. Furthermore, observed egg production rates (eggs female^{-1} d^{-1}) in MUW were high. The mean value of 58.26 eggs female^{-1} d^{-1} approximates the average value (70 eggs female^{-1} day^{-1}) for individuals reared under optimal laboratory conditions (Borchers and Hutchings 1986). Variability in observed egg production rates may possibly be attributable to unmeasured factors such as cannibalism or predation. Noctiluca, for example, were abundant during this study, and have been shown to prey upon copepod eggs (Daan 1987, Chapter 6).

From the size structure of the phytoplankton community in surface waters (see Chapter 6) it appears that diatoms were sufficiently abundant (0.2 to 5.2 x 10^6 cells l^{-1}) to support the copepod populations in MUW if selective feeding occurs (Hutchings pers. comm.). Indeed, P:B ratios of 0.23 to 0.31 d^{-1} for C. carinatus in MUW suggest that the daily food requirements of copepods were satisfied, and that production rates were maximal. These P:B ratios were high, but comparable with values of 0.13 to 0.27 d^{-1} reported by Verheye (1989b), and Peterson (unpubl. data).

In a study of secondary production by the entire copepod community in the southern Benguela during the relaxation phase of the upwelling cycle in April 1988, Peterson (unpubl. data) showed that where phytoplankton cells were predominantly >10 μm the average P:B ratio of copepods was 0.2 d^{-1}. Under sub-optimal food conditions (cells predominantly <10 μm) P:B ratio's were 0.07 d^{-1}. Total copepod production was estimated to be 4.2 mg C m^{-3} d^{-1} where

phytoplankton cells were large, and 2.4 mg C m^{-3} d^{-1} where cells were small. The average P:B ratio for Calanoides carinatus during this study (0.19 d^{-1}) was also comparable with mean growth rates of 0.19 d^{-1} calculated for C. carinatus (C1 to Adult Stages) during an Anchor Station study in St. Helena Bay, north of Cape Columbine, in the upwelling season in 1987 (Verheye 1989b).

The stable-age distribution of C. carinatus in MUW during the second drogue indicates a continuously reproducing population, which also suggests that environmental conditions favoured maximal production by the copepods.

In MUW/mixed water (Stations 13 to 20) the P:B ratios of Calanoides carinatus were low (0.07 to 0.15 d^{-1}), suggesting a sub-optimal food environment for the copepods. The population was dominated by the late-copepodite stage (C5) and adults. From egg ratio data females were assumed to have slow production rates, but these rates may have been underestimated due to egg cannibalism or predation. However, close agreement between measured and predicted biomass (Fig. 2) suggests that copepod production was not underestimated, but that copepods were starved. This is also supported by data on phytoplankton composition, which showed that diatom abundances were low (Chapter 6). Changes in food quality were not addressed during this study, but evidence of a declining phytoplankton bloom and low nutrient concentrations suggest that the ratio of protein:carbohydrates was low (Barlow 1982a). One possibility is that in MUW/mixed water, where biological development of the water was accelerated (Chapter 6), copepods were storing or metabolising available lipids and carbohydrates rather than channelling protein into somatic growth and egg production.

Laboratory studies on C. carinatus have shown that C5's and females have a high tolerance to starvation, the success of which appears to be largely

dependent on the accumulation of substantial lipid reserves in the sub-adult (C5) stage (Borchers and Hutchings 1986). Within 24 h of the onset of food limiting conditions, egg production by gravid females of this lipidic species may be inhibited (see also Attwood and Peterson 1989), while lipid reserves are mobilised to meet basal metabolic requirements. In other upwelling areas, particularly off west Africa where the upwelling season is short (Binet and Suisse de Saint Claire 1975, see also Smith 1982) it has been shown that C. carinatus C5's are similarly starvation-adapted, and may persist in a resting phase in deep water for several months until the next upwelling period (Borchers and Hutchings 1986). Borchers and Hutchings observed C5's in samples collected from 600 m depth in the southern Benguela in 1983, possibly representing individuals which had migrated into deeper water where they would remain metabolically dormant until advected towards the coast during the next upwelling event.

From egg ratio data and the population age structure of C. carinatus in the upwelling plume it appears that, under quiescent upwelling conditions, copepod fecundity is high in maturing upwelled water where juveniles are not likely to be food-limited. As the ageing plume moves downstream from the upwelling centre, juveniles develop into the adult stage. Adults may experience food-limitation due to the decline of the phytoplankton bloom, and offshore mixing of the plume with plumes of older upwelled water. These animals may continue to be transported downstream by the mixed plume, eventually moving down into deeper currents to return to the nearshore environment. Results of this study suggest that copepod secondary production in the pelagic ecosystem of the southern Benguela is closely coupled to the upwelling cycle, and that copepod production responds rapidly to primary production and the availability of suitable phytoplankton food. During frequent pulses of upwelling this coupling is likely to be less evident, due to limitations of the sampling programme.

Grazing impact of Calanoides carinatus

A temporal gradient in the grazing impact of C. carinatus was observed during the ageing of the upwelling plume studied here. Under conditions of high phytoplankton biomass and production grazing impact was low. Copepods were calculated to consume approximately 10 % of the phytoplankton biomass and 12 % of the primary production. These results are similar to previously recorded estimates in the Peruvian upwelling system (<25 %, Beers et al. 1971) and the southern Benguela. Olivieri and Hutchings (in prep.) found that copepods removed <10 % of total available phytoplankton carbon (biomass and production) during active upwelling conditions in the southern Benguela. Peterson et al. (1989) calculated that the entire copepod assemblage grazed <10 % of phytoplankton biomass per day under stratified conditions. Verheye (1989a) showed that C. carinatus grazed 5 to 10 % of phytoplankton production over 2 successive upwelling cycles. Estimates presented here are, however, conservative as they do not include other copepod species which were present (Chapter 6). At the end of the bloom the grazing impact was high (42 % of phytoplankton biomass, and 60 % of production), suggesting control of total standing crops by grazers. These results are contrary to the general hypothesis that only a small proportion of primary production in upwelling areas is removed by herbivorous mesozooplankton. Furthermore, calculations of carbon consumption requirements are sensitive to the assumed net growth yield of 30 %. Variable growth efficiencies have been reported for copepods, ranging from 5 to 50 % (see Omori and Ikeda 1984). It is probable that copepod assimilation efficiencies and net growth yields are influenced by the availability and quality of preferred food sources, thus affecting their carbon consumption requirements. More efficient growth yields of copepods under optimum food conditions are likely to decrease their carbon requirements. Conversely, less efficient growth yields in a sub-optimal food environment will result in an

underestimate of calculated carbon requirements. In the absence of empirical measurements of the net growth yield of C. carinatus under varying food regimes, the value commonly assumed in studies of copepod dynamics, 30 %, was adopted here.

In this study of the grazing impact of copepods it was assumed that C. carinatus is herbivorous (see Mensah 1974, Hirche 1980), and that all phytoplankton carbon was available to copepods. The possible influence of particle-size selectivity (Peterson and Bellantoni 1987, Peterson et al. 1989) was discussed in Chapter 6 and, briefly, above. Few studies in the southern Benguela have considered copepod predation on microzooplankton. One of the implications of a revised concept of the structure of planktonic food webs since the emergence of the "new paradigm" (Pomeroy 1974, Williams 1981, Ducklow 1983) is that a relatively large microzooplankton community exists in the pelagic environment. Omnivorous copepods may therefore be able to supplement carbon demands by carnivory. Borchers and Hutchings (1986, Brownell, unpubl. data) reported observations of omnivory by C. carinatus. Conventional methods of measuring zooplankton grazing and carbon requirements which are based on the assumption that copepods are obligate herbivores (eg: electronic particle counters, HPLC, gut fluorescence) may therefore result in an underestimate of copepod carbon requirements. Possibly, methods such as those used in this study will provide more realistic estimates of the energy requirements of copepod species which may be omnivorous. However, these studies will need to be done in conjunction with research on the secondary productivity of the microbial food web in order to determine the relative proportions of autotroph and heterotroph carbon likely to be consumed by copepods. From somewhat preliminary evidence presented here it would seem that there is a temporal gradient in the nutritional mode adopted by copepods. Under conditions of favourable phytoplankton growth, and dominance of the assemblage by diatoms, copepods may

be largely herbivorous. As this trophic resource becomes depleted, copepods may switch to omnivory and sustain their metabolic requirements by feeding on the microzooplankton component of the microbial loop (see also Sherr et al. 1986, Frost 1987, Roman et al. 1988). In a recent study of size-fractionated nitrogen uptake in the southern Benguela, Probyn et al. (1989) constructed a model of the dissemination of nitrogen production in different size-classes to higher trophic levels and conservatively calculated that carnivory on microzooplankton contributed 14 % towards the production of an omnivorous mesozooplankton community in aged upwelled water.

Carbon flux to bacteria

Estimates of the carbon requirements of heterotrophic bacteria are sensitive to the production estimates and the efficiency with which carbon is converted to bacterial biomass (the net growth yield). From Chapter 7 it is clear that bacterial production during this study was spatially and temporally variable, with highest estimates recorded in the euphotic zone, and in MUW. Production estimates based on bacterial population growth ($P_{\Delta B}$) were 4 to 9 times higher than estimates based on [methyl-^{3}H] thymidine incorporation (P_{TTI}). Assuming that bacterial production measured by $P_{\Delta B}$ was more realistic for a bacterial population in a stratified water column, and that growth rates of 0.5 d^{-1} closely approximated the in situ growth rates, it is clear that the production rates and carbon consumption requirements of heterotrophic bacteria (from $P_{\Delta B}$ and $P_{calculated}$) during the development of the plume were approximately equal to or greater than the production rates and carbon requirements of C. carinatus (see Pace et al. 1984). However, these requirements may be overestimated as the net growth yield of bacteria may be as high as 60 to 80 % on dissolved carbon substrates exuded during phytoplankton growth (Linley and Newell 1984, Lucas 1986). Similarly, where regenerated sources of nitrogen are available, high

bacterial growth yields may be maintained during phytoplankton decay (see review by Lucas 1986).

During high levels of phytoplankton biomass and production in MUW, bacterial production and carbon requirements (from P_{calc}) were high (mean consumption = 1817 mg C m^{-2} d^{-1}). Assuming a net growth yield of 30 % and a PDOC excretion rate of 30 %, the dissolved organic carbon substrates exuded during phytoplankton growth were sufficient to meet the requirements for bacterial growth and respiration. On average, only 22 % of net primary production (including PDOC) was consumed by bacteria. This raises the interesting question of why bacterial production estimates from TTI were low at Station 9.

In MUW/mixed water, where phytoplankton production was limited, bacterial consumption remained relatively high (772 mg C m^{-2} d^{-1}). Consumption of carbon (mean value: 49.2 %) available as primary production exceeded the available PDOC, unless the excretion rates were higher than 30 % during the declining phase of the bloom. Alternative carbon substrate resources available to meet the carbon demand of bacteria included phytoplankton biomass, refractory carbon substrates associated with phytoplankton senescence, and detrital particulate carbon. Although bacterial consumption requirements were less (81.4 %) than the total phytoplankton biomass, only senescing cells would have been available for bacterial utilisation. Particulate carbon resources far exceeded bacterial demands. It is likely that the bacterial community in MUW/mixed water was utilising available carbon from all of the above nutrient pools. In addition, heterotrophically produced dissolved organic carbon (HDOC) derived from the large copepod population may have maintained the bacterial community (see also Eppley et al. 1981, Pace et al. 1984 and review by Pomeroy 1984). Strayer (1988) pointed out that total secondary production may at times exceed primary production and be sustained by cycling of autotrophically fixed carbon through

different heterotroph groups (see also Scavia 1988). In a recent study Jumars et al. (1989) argued that, from an energetic point of view, PDOC excretion rates are likely to be <10 % of total phytoplankton production. These authors proposed that 10 to 50 % of autotrophically fixed carbon may be made available to bacterioplankton through DOC released as a by-product of heterotroph feeding. From theoretical studies of mass balance for animal digestion Jumars et al. (1989) hypothesised that the dominant source of DOC supply to bacteria is through rapid diffusion of labile organic matter from zooplankton faeces into the surrounding water (see also Pomeroy et al. 1984).

CONCLUSIONS

Net production by Calanoides carinatus during this study ranged between 57 and 800 mg C m^{-2} d^{-1} (mean value: 242.1 mg C m^{-2} d^{-1}) and was generally less than or equivalent to bacterial production. Estimates of bacterial production ranged between 120 and 1025 mg C m^{-2} d^{-1} ($P_{\Delta B}$, mean: 529 mg C m^{-2} d^{-1}) or between 173 and 991 mg C m^{-2} d^{-1} ($P_{calculated}$, mean: 420 mg C m^{-2} d^{-1}).

In maturing upwelled water phytoplankton biomass and production were sufficiently high to maintain the developing copepod population. Assuming that all phytoplankton carbon was available as a food resource, C. carinatus was calculated to graze, on average, 10 % of the available phytoplankton biomass and 12 % of the particulate primary production per day. Bacterial carbon consumption requirements (from $P_{calculated}$ and $P_{\Delta B}$) were calculated to be of the same order of magnitude as the copepod requirements. On average 22 % of net primary production was consumed by heterotrophic bacteria, possibly largely as PDOC. Partitioning of carbon resources enabled high secondary production rates of both copepods and bacteria to be maintained by phytoplankton production.

During phytoplankton senescence in MUW/mixed water, the grazing impact of copepods was considerably higher: on average, 42 % of phytoplankton biomass and 60 % of primary production. Evidence of reduced growth rates (d^{-1}) and fecundity suggested food-limitation of copepod dynamics during the decline of the phytoplankton bloom. Bacterial consumption requirements showed a similar increase in relation to available autotroph carbon during phytoplankton senescence. Consumption requirements were on average 49 % of primary production, suggesting that alternative substrates were utilised to meet bacterial maintenance and growth requirements. Substrate sources likely to be utilised include the more refractory organic carbon substrates associated with phytoplankton senescence, detrital particulate carbon and HDOC released as a by-product of zooplankton feeding.

Results of this study suggest that primary and secondary production (by copepods and bacteria) in the southern Benguela are closely coupled. In the absence of frequent upwelling pulses a temporal mismatch of phytoplankton and copepod dynamics is not evident. High secondary production levels of herbivores and micro-heterotrophs may be maintained by resource partitioning and carbon cycling, which has implications for co-existence of the microbial food web and the classical diatom-herbivore food chain. Under stratified upwelling conditions there may be temporal changes in the structure and functioning of the planktonic food web. A diatom-dominated phytoplankton assemblage may be grazed preferentially by copepods. As this resource becomes limiting, copepods may adopt an omnivorous strategy, feeding on the microzooplankton of the microbial loop.

ACKNOWLEDGEMENTS

I would like to thank all members of the Sea Fisheries Research Institute who participated during this cruise, including the Captain, Officers and crew of the R.S. Africana. I am very grateful to Dr. W.T. Peterson for identifying and counting the zooplankton samples, for assistance with analysing the data, and for reviewing the various drafts of this manuscript and my interpretation of the results. I am also indebted to Dr. P. Brown for contributing data on primary production . My thanks are further extended to Dr. Mike Lucas, my supervisor, Dr. Laurence Hutchings, Dr. Coleen Moloney, Dr. Stan Pillar, Hans Verheye and Dot Armstrong for their comments and discussion. Financial support of the BEP and the Sea Fisheries Research Institute is gratefully acknowledged.

SECTION 5

SIZE-BASED SIMULATION MODEL

CHAPTER 9

Simulation and field measurements of phytoplankton - bacteria - zooplankton interactions in the southern Benguela upwelling region.

ABSTRACT

A sized-based model is used to simulate the pelagic food-web over a 25 day period in the euphotic zone of the southern Benguela upwelling region. The phytoplankton are divided into three size-categories, zooplankton are divided into four size-categories, and all parameters are determined using body-size relationships. A number of assumptions are made, including that nitrogen is the limiting nutrient in the system, that "new" nitrogen (nitrate) is supplied to the euphotic zone in a single initial pulse, and that recycled nutrients supply nitrogen requirements after nitrates are depleted. Bacteria are assumed to utilise inorganic nitrogen, and dissolved and particulate organic material. Limiting physical factors (eg. light, turbulence) are excluded. Simulation results are consistent with field and laboratory measurements of phytoplankton, bacterial, flagellate and meso-zooplankton biomass. The model predicts a small initial bloom of pico-phytoplankton and bacteria not observed in laboratory measurements. It is suggested that these blooms may have been overlooked in the field, or that metabolic rates of picophytoplankton and bacteria in newly upwelled water are overestimated by the model. The model simulation predicts a 3-d nanophytoplankton (diatoms and flagellates) bloom, which peaks on day 4. This is followed by pulses of picophytoplankton and a more stable netphytoplankton bloom, which persists for 10 days. A secondary nano-phytoplankton bloom is predicted. In the simulation output, heterotroph biomass in the <200 μm size-classes is pulsed as a result of the combined effect of food availability and predation. Variations in model execution suggest that bacteria increase the overall productivity of the planktonic food web.

INTRODUCTION

The potential importance of decomposers in the marine food chain has been recognised for many years, but only during the last decade have microheterotrophs been incorporated into the classical concept of the trophic dynamics of the pelagic ecosystem (Williams 1981, see reviews by Azam et al. 1983, Lucas 1986, Newell and Turley 1987, Hobbie 1988, Pomeroy and Wiebe 1988). Improved techniques for quantifying bacterial biomass and production (van Es and Meyer-Reil 1982, Ducklow 1983), protozoan predation (Fenchel 1982d, McManus and Fuhrman 1986, Coffin and Sharp 1987, Sherr et al. 1986a), and nutrient recycling (Glibert 1982, Goldman et al. 1985, Probyn 1987) have shown that the microbial food web may be very active in the pelagic marine environment. Although bacterial densities have been found to be relatively constant (1×10^5 to 5×10^6 ml^{-1}) in most parts of the ocean, estimates of bacterial production are highly variable (<0.1 to 200 $\mu g\ C\ l^{-1}\ d^{-1}$, Cole et al. 1988). These may be due, in part, to methodological limitations or they may be indicative of different levels of bacterial productivity (and bacterivory, by organisms such as flagellates, ciliates and pelagic tunicates) under different environmental conditions.

Bacteria have been calculated to utilise up to 60 % of the products of phytoplankton production (Williams 1981, Ducklow et al. 1986, Fuhrman 1987), and have been hypothesised as being either a significant pathway by which phytoplankton carbon is made available to larger heterotrophs (Linley et al. 1983, Lochte and Turley 1985), or a pathway through which carbon is lost from the system (Ducklow et al. 1986) Their traditional role as direct mineralisers of detrital organic matter has been questioned. Studies of microflagellate bacterivory suggest that active predation within the microbial food web is the mechanism by which nutrients are recycled (Eppley and Peterson 1979, Glibert

1982, Goldman et al. 1985, Probyn 1987, Lucas et al. 1987, Probyn and Lucas 1987). Many studies have shown that interactions between autotrophs, microheterotrophs and larger heterotrophs are very complex, making quantification of the significance of the microbial food web in the overall trophic dynamics of the marine ecosystem exceptionally difficult. In addition, recent studies which have shown that a large fraction (>50 %) of phytoplankton production may be due to picoplankton (<1 μm) and nanoplankton (<20 μm, Platt et al. 1983, Douglas 1984, Probyn 1985), have implications for the importance of the microbial food web not previously recognised.

Few studies in the southern Benguela upwelling region have been directed at determining the trophic role of bacteria and microprotozoa in the pelagic food web. Since the emergence of the new paradigm of the pelagic food web (Pomeroy 1974, Williams 1981), Ryther's (1969) hypothesis of high fish production in upwelling areas and the dominance of short, efficient diatom-based food chains has been a source of controversy. James (1987) has shown that copepods and euphausiids form the bulk of the diet of the commercially important fish species, Engraulis capensis (anchovy), in the southern Benguela. Furthermore, it appears that the productivity of copepods and, therefore, fish is not exceptionally high, possibly as a result of spatial and temporal variability in the physical environment (Shannon and Field 1985, Nixon et al. 1986, Branch et al. 1987, Peterson et al. in prep.)

Although phytoplankton production in plumes of upwelled water is high, bloom duration is shorter than the development time of the dominant copepod species entrained in the plume. Calanoides carinatus, for example, has a development time of approximately 25 d (Borchers and Hutchings 1986). Unless `allochthonous' zooplankton and fish communities locate and graze the dense aggregations of phytoplankton, the bulk of the phytoplankton bloom is lost to

detritivores. As a result of the slow sinking rates of senescent phytoplankton cells in the southern Benguela (<1 m d^{-1}, Pitcher et al. 1989), a significant proportion of phytoplankton biomass and production is likely to be channelled into the microheterotroph community (see reviews by Lucas 1986, Newell and Turley 1987).

Recent studies on pelagic microheterotrophs in the southern Benguela upwelling system have shown that bacterial densities and standing stocks may be high (up to 1 x 10^7 cells ml^{-1} and 230 mg C m^{-3}, Chapters 4 and 6). Variability in estimates is also high (see Muir 1986, Armstrong et al. 1987, Verheye-Dua and Lucas 1988, this study), and suggests adaptive responses in bacterial community structure and activity to phytoplankton bloom development. Studies directed at improving our knowledge of bacterioplankton dynamics in response to phytoplankton dynamics are presented in this thesis.

During a drogue study in the southern Benguela, temporal changes in the biomass and production of phytoplankton, bacteria and zooplankton during the hydrological and biological development of a plume of upwelled water were determined (Chapters 6, 7 and 8). In a laboratory-based microcosm study, Painting et al. (1989; see Chapters 4 and 5) followed the temporal development and interactions of natural phytoplankton, bacterial and flagellate communities in recently upwelled water on a scale more appropriate to the bacterial component. Results of these studies are used here to improve our understanding of the processes governing the temporal changes in the biomass relationships and size-structure of the planktonic community, and to determine the trophic role of bacterioplankton in the pelagic food web of the southern Benguela.

It is now recognised that the concept of trophic levels (Lindeman 1942) is no longer valid for the marine pelagic food web, and that conceptual models of

the functioning of the planktonic food web need to take cognisance of the diversity in sizes of planktonic organisms (Cousins 1985, Platt 1985, Moloney 1988, Moloney and Field 1989c). In this chapter a generic size-based model (Moloney 1988) is used to simulate temporal changes in the standing stocks of phytoplankton, bacteria, flagellates and mesozooplankton observed during laboratory and drogue studies in the southern Benguela. The model simulation is used to test the hypothesis that small autotrophs and heterotrophs dominate the planktonic community in the southern Benguela.

The dynamics of the simulated planktonic community are governed largely by substrate availability and predation. Model output may therefore be used to test the hypotheses that (1) rapid mineralisation by the microbial food web is an important process by which the productivity of phytoplankton populations is increased, and (2) that there is a transition from "new" to "regenerated" nitrogen in the control of plankton dynamics.

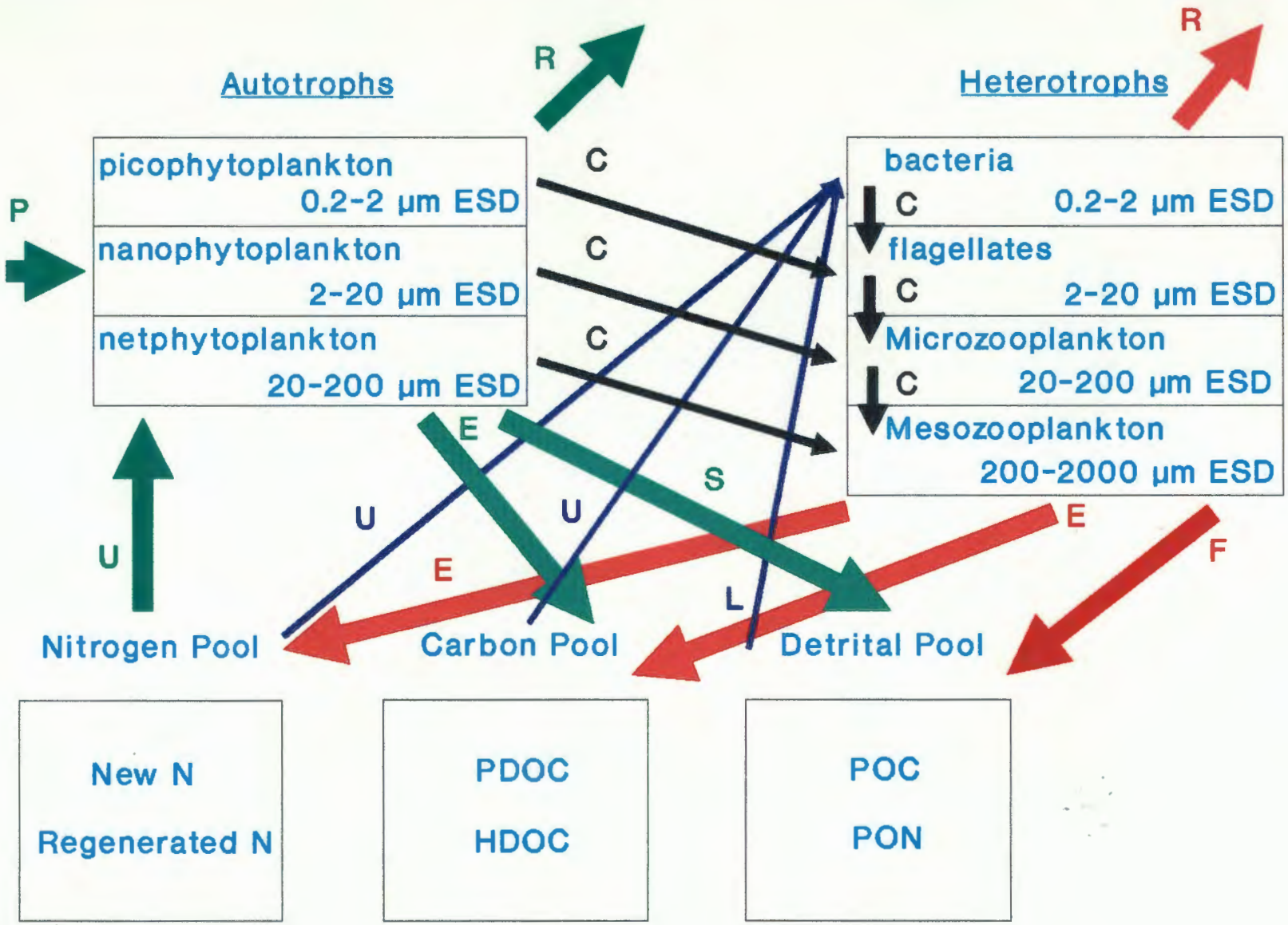
The model, based on the trophic continuum concept of Cousins (1985) and general ecological principles, was originally used to simulate hypothetical food webs in the southern Benguela (Moloney 1988). Moloney showed that the microbial food web was an inefficient pathway for the transfer of photosynthetically fixed carbon to large zooplankton and fish. In her work, however, bacteria were assumed to utilise only dissolved sources of C and N. Furthermore, bacteria had no refuge from predation by microprotozoa. Here, the model has been modified to simulate the bacterial component more realistically. Bacteria are assumed to utilise both dissolved and particulate (detrital) substrates, and are assumed to have a threshold biomass below which they are not grazed by larger microheterotrophs because of the density-dependence of encounter and grazing. Seven biotic compartments are considered on the basis of the equivalent spherical diameter, ESD, of organisms in different size

classes (0.2 to 2 μm, 2 to 20 μm, 20 to 200 μm and 200 to 2000 μm). Autotrophs are assigned to the three smallest compartments, while heterotrophs are present in all four size-classes. Three abiotic compartments are included in the model: dissolved nitrogen, dissolved organic carbon, and particulate organic carbon and nitrogen. All rate processes are determined objectively by the model on the basis of body-size relationships (see Moloney and Field 1989a), thus none of the field data that are presented here were used to estimate parameters in the model.

DESCRIPTION OF THE MODEL

Planktonic communities are divided into autotroph and heterotroph groups, which are then sub-divided into discrete size-classes based on equivalent spherical diameters. Cell volumes are calculated using the formula for a sphere. For autotrophs and bacteria cell volumes are converted to carbon using Strathmann's (1967) equations. A factor of 0.07 pg C μm^{-3} is used for other heterotrophs (Moloney 1988, Moloney and Field 1989b). The size-ranges of organisms in the autotroph and heterotroph continua and the conceptual model of the major carbon (C) and nitrogen (N) flows in the dynamic simulation model used in this chapter are shown on Fig. 1. Autotrophs fix C during photosynthesis and take up dissolved N. Losses of C are due to excretion of photosynthetically derived organic carbon (PDOC), respiration, grazing and senescence. Bacteria obtain C and N from the dissolved and detrital pools, whereas larger heterotrophs ingest particulate material (autotrophs and heterotrophs in the size-class smaller than their own). Loss of C from bacteria is due to respiration and predation. Losses of C from other heterotrophs are due to egestion, respiration and predation.

Fig. 1. Conceptual model of major carbon and nitrogen flows in the size-based simulation model (Moloney 1988), which is based on the trophic continuum concept (Cousins 1985). The model was modified to incorporate bacterial exploitation of the detrital pool. Green and red arrows indicate gross C and N flow between major compartments and total respiratory losses of C. Black arrows indicate C and N flow to and through heterotroph compartments. Blue arrows show pathways of C and N to bacteria. P = photosynthesis, U = uptake, R = respiration, E = excretion, F = egestion, S = senescence, C = consumption (grazing/predation), L = lysis.


Autotrophs
R
Heterotrophs
R
picophytoplankton
0.2-2 µm ESD
nanophytoplankton
2-20 µm ESD
netphytoplankton
20-200 µm ESD
P
C
C
C
bacteria
C
0.2-2 µm ESD
flagellates
C
2-20 µm ESD
Microzooplankton
C
20-200 µm ESD
Mesozooplankton
200-2000 µm ESD
E
S
U
U
U
E
E
L
F
Nitrogen Pool
Carbon Pool
Detrital Pool
New N
Regenerated N
PDOC
HDOC
POC
PON

"New" nitrogen (nitrate) is the only external input to the model (Fig. 1); regenerated nitrogen results from cycling of reduced nitrogen (urea, ammonia) by heterotrophs. Carbon and detrital pools receive input from both autotrophs (PDOC, senescence) and heterotrophs (egestion).

In the model rates of nutrient uptake, ingestion and respiration are calculated using the general allometric equations estimated from data covering a wide range of organism sizes (Moloney and Field 1989a). Other model parameters are described by empirical models derived from published data (Moloney 1988). These include half-saturation constants (K_s) for nitrogen uptake by phytoplankton and bacteria, K_s for ingestion of prey organisms, and sinking velocity of phytoplankton cells and faecal and detrital material.

Execution of the model allows temporal changes in standing stocks and the size structure of the planktonic community to be described. The model calculates carbon and nitrogen flows through the community. These can be used to analyse the dynamics of the model system. Numerous options are available for executing simulations, making it possible to simulate the dynamics of planktonic communities in a diversity of environments.

In this chapter parameter values and model assumptions were modified to produce an output consistent with measurements obtained during field studies in the southern Benguela (Chapter 6) and during a laboratory-based microcosm simulation of an upwelling event (Chapter 4). The simulation is executed over 25 days, with time increments of 0.05 d. Values of parameters used are shown in Table 1. Parameters dependent upon body-size were influenced only by the assumed ambient water temperature and Q_{10} value. Standing stocks of autotrophs were adopted from Moloney and Field (1989b).

Table 1. Values of parameters used to simulate carbon and nitrogen flows through the planktonic community. Ambient temperature was assumed to be 13 ^{o}C.

Parameter	0.2-2 μm	2-20 μm	20-200 μm	200-2000 μm
Autotrophs				
Maximum growth rate (d^{-1})	4.10	1.12	0.31	-
Respiration rate (d^{-1})	1.92	0.53	0.14	-
Half saturation constant for N uptake (mg N m^{-3})	0.007	1.19	54.54	-
PDOC production rate (d^{-1})	0.3	0.3	0.3	-
Initial standing stocks (mg C m^{-3})	0.5	5	50	-
Refuge size (mg C m^{-3})	10	1	0.1	-
Senescence rate (d^{-1})	0.44	0.12	0.03	-
Lysis rate (d^{-1})	0.1	0.1	0.1	-
C:N Ratio	6	6	6	-
Heterotrophs				
Maximum uptake rate (d^{-1})	4.10	-	-	-
Maximum ingestion rate (d^{-1})	-	22.22	3.95	0.70
Respiration rate (d^{-1})	1.92	4.94	0.88	0.16
Half saturation constant for N uptake (mg N m^{-3})	0.007	-	-	-
Half saturation constant for predation (mg C m^{-3})	37.13	64.53	112.14	194.88
Initial standing stocks (mg C m^{-3})	0.5	0.5	1	1
Refuge size (mg C m^{-3})	10	1	0.1	0.01
C:N ratio	4	4.5	4.5	4.5
Assimilation efficiency (d^{-1})	1	0.85	0.85	0.85

More than 50 simulations were executed in total to determine the size-classes of heterotrophs, refuge sizes, senescence rates of phytoplankton, lysis rates of bacteria, and the initial starting values of heterotroph standing stocks which would enable realistic simulation of the plankton communities studied in the southern Benguela. To keep the simulations as simple as possible, physical mixing of the water column, sinking of phytoplankton cells and growth of organisms into larger size-classes were not included. Organisms capable of adopting nutritional models apart from either photosynthesis or heterotrophy were excluded. Numerous assumptions, based on empirical measurements, were made:

1. Ambient water temperature is 13 ^{o}C, and the Q_{10} for all planktonic organisms is 2.
2. Light conditions in the euphotic zone are optimal.
3. Only nitrogen limits phytoplankton growth.
4. Phytoplankton growth in upwelled water is dependent upon a single initial pulse of nitrate into the euphotic zone. After depletion of "new" nitrate, phytoplankton growth is dependent upon recycled nutrients.
5. Excretion of photosynthetically-derived organic carbon (PDOC) by phytoplankton is constant at 30 % of gross production.
6. Bacteria are able to utilise dissolved inorganic nitrogen. They are not nitrogen limited when inorganic nitrogen concentrations are high.

In addition, it was assumed that:

7. Phytoplankton senescence occurs at a constant rate of 10 % of the gross production per day, contributing to the detrital carbon and nitrogen pool.

8. Bacteria break-down (lyse) the detrital pool at a rate of 10 % of the total detrital standing stocks per day.
9. Bacteria are nitrogen-limited and not carbon-limited at the start of the simulation. The dissolved carbon pool was therefore initialised at 10 mg-at C m^{-3}.

Laboratory and field measurements for comparison with simulation output

Measurements of temporal changes in phytoplankton, bacterial, flagellate and zooplankton biomass in laboratory studies in the southern Benguela presented in Chapters 4 and 6 are used here for comparison with simulation output. Additional data on the size-structure of the phytoplankton community during the drogue study in March 1983 are presented below.

Phytoplankton biomass in the 2 larger size-classes used in the model was calculated from cell counts (numbers ml^{-1}), and from the size distribution of particulate material obtained from an electronic particle counter (Coulter Counter, Model TAII) fitted with a 280 μm aperture tube. Limitations of microscope resolution and minimum particle size detected by the Coulter Counter preclude the calculation of phytoplankton biomass in the smallest size class (0.2 to 2 μm ESD). Cell numbers (ml^{-1}) were converted to carbon biomass using measured cell volumes (μm^3) and conversion factors (pg C $cell^{-1}$) calculated from Strathmann's (1967) equations for flagellates and diatoms. Cells were grouped into the nano- and net- plankton size-classes on the basis of equivalent spherical diameters. Thus small chain-forming diatoms were classified as nanoplankton. On the basis of individual cell surface to volume ratio's and hence metabolic rates this classification is likely to be valid. However, by forming chains and increasing their ESD's, these cells may effectively be

netplankton and are therefore likely to avoid predation by heterotrophs in the 20-200 μm ESD size-class.

Carbon biomass in the effective nanoplankton and netplankton size-classes was calculated from the distribution of particles in these size-classes as determined by Coulter Counts. Total particle volumes ($\mu m^3 \times 10^6$ ml^{-1}) in each size-class were converted to carbon biomass (μg C l^{-1}) using the conversion of 0.11×10^{-6} μg C μm^{-3} (Kiorboe et al. 1985a). The carbon biomass (mg C m^{-3}) in each size-class was integrated over the depth of the mature upwelled water for each station (mg C m^{-2}) and divided by the maximum depth of this water mass to estimate average concentrations (mg C m^{-3}). Large chains comprised of many individually small cells were counted as netplankton on the basis of their overall ESD's.

RESULTS AND DISCUSSION

The results presented here are discussed in terms of the size-classes used in the simulation. For autotrophs, categorisation as picophytoplankton (0.2-2 μm ESD), nanophytoplankton (2-20 μm ESD), and netphytoplankton (20-200 μm) follows the conventional nomenclature (see Sieburth 1984). It is important to emphasise that both diatoms and flagellates are present in each of the size-classes. For heterotrophs the compartments are named according to the familiar groups of animals in each size-class: bacteria (0.2-2 μm ESD), flagellates (2-20 μm ESD), microzooplankton (20-200 μm ESD) and mesozooplankton (200-2000 μm ESD, see also Azam et al. 1983). These categories are not, however, exclusive. Nor is the representative taxon necessarily dominant (Moloney 1988). The category names are adhered to largely for convenience.

Autotrophs

Pulses of high phytoplankton biomass developed in the model system (Fig. 2a). A small initial peak of short duration was observed on day 0.5, and was dominated by picophytoplankton. The maximum phytoplankton biomass (ca 1500 mg C m^{-3}) was attained during the second autotroph peak on day 4. This bloom, dominated by nanophytoplankton, declined rapidly on day 5 and was followed by a series of blooms of picophytoplankton. The first of these showed a high biomass (800 mg C m^{-3}), and lasted for 2 days. Thereafter, the magnitude and duration of picophytoplankton blooms became progressively smaller. The biomass of netphytoplankton (see also Fig. 3d) increased very slowly. This size-class, preyed upon by the slow-growing mesozooplankton, did not exhibit rapid cycling, and remained at a constant standing stock of approximately 200 mg C m^{-3} between days 4 and 15.

Phytoplankton biomass was lowest (ca 100 mg C m^{-3}) on day 20, when standing crops of all phytoplankton size-classes were low. After day 20 a second bloom of nanophytoplankton developed. This bloom was considerably smaller than the first nanophytoplankton bloom and lasted approximately 5 days.

Heterotrophs

Pulses of bacterial biomass were observed in the simulation (Fig. 2b). Bacterial biomass changes showed similar trends to the picophytoplankton, but with smaller magnitudes. A maximum bacterial biomass of 162 mg C m^{-3} developed on day 6. Flagellates and microzooplankton fluctuated in response to phytoplankton and heterotroph blooms in their optimal food size class (Figs. 2b, 3a, b, c).

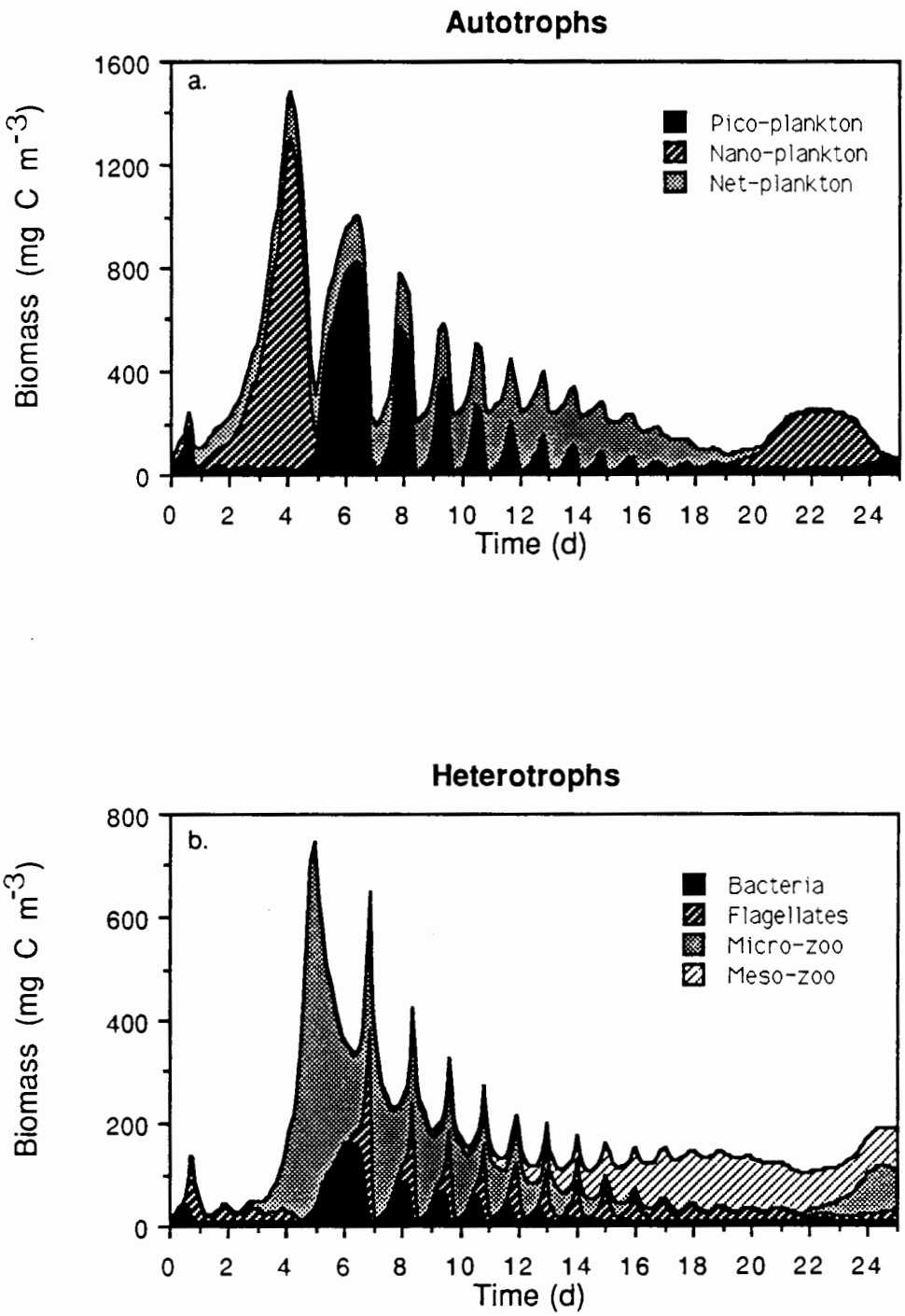


Fig. 2. Model output showing changes in biomass of (a) autotrophs and (b) heterotrophs in the planktonic community after upwelling. Results show total biomass changes, and the relative contribution of each size-class.

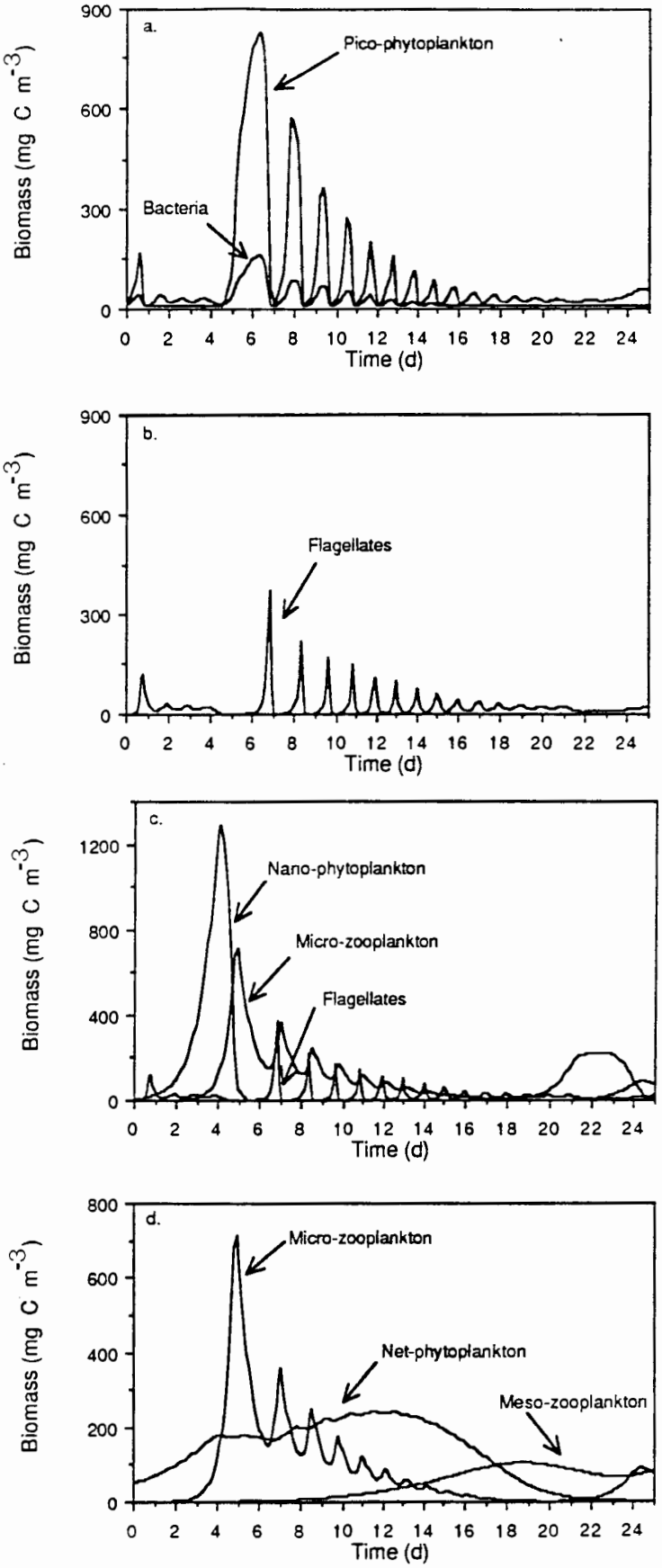


Fig. 3. Simulation results depicting biomass changes of the different size-classes and their predators. Pico-phytoplankton and bacteria (a) are shown independently of flagellates (b), which prey upon them. (c) Micro-zooplankton and their nano-phytoplankton and flagellate food sources. (d) Meso-zooplankton and their net-phytoplankton and micro-zooplankton prey.

Microzooplankton fluctuations after day 5, when their nanophytoplankton food source was absent, were in response to flagellate blooms (Fig. 3c). Mesozooplankton biomass was low initially, due to a low starting value. The slow growth rates of this size-class resulted in a very gradual increase in their biomass, up to a maximum of ca 113 mg C m^{-3} on day 19 (Fig. 3d). Rapid fluctuations in biomass were not observed in the model.

Nitrogen and carbon pools

Concentrations of nitrate were initially high (Fig. 4a) and decreased sharply during the picophytoplankton and bacterial blooms on day 1. This "new" nitrogen pool continued to decrease as a result of uptake by nanophytoplankton, and was depleted by day 4. The supply of regenerated nitrogen was pulsed, and cycled with the blooms of bacteria and other heterotrophs. The detrital pool of particulate organic nitrogen (PON) was zero initially, and increased continually due to phytoplankton senescence.

The dissolved carbon pool (PDOC, Fig. 4b) was relatively low initially and increased exponentially during the initial pico- and nano- plankton blooms to a concentration of 2000 mg C m^{-3} on day 5. The PDOC pool continued to increase during the remainder of the simulation due to further phytoplankton blooms. The PDOC pool is potentially available for bacterial utilisation, but is not used because bacteria become nitrogen-limited toward the end of the simulation and PDOC accumulates. The particulate carbon pool (POC) showed similar trends to the particulate nitrogen pool, due to the coupling of particulate carbon and nitrogen.

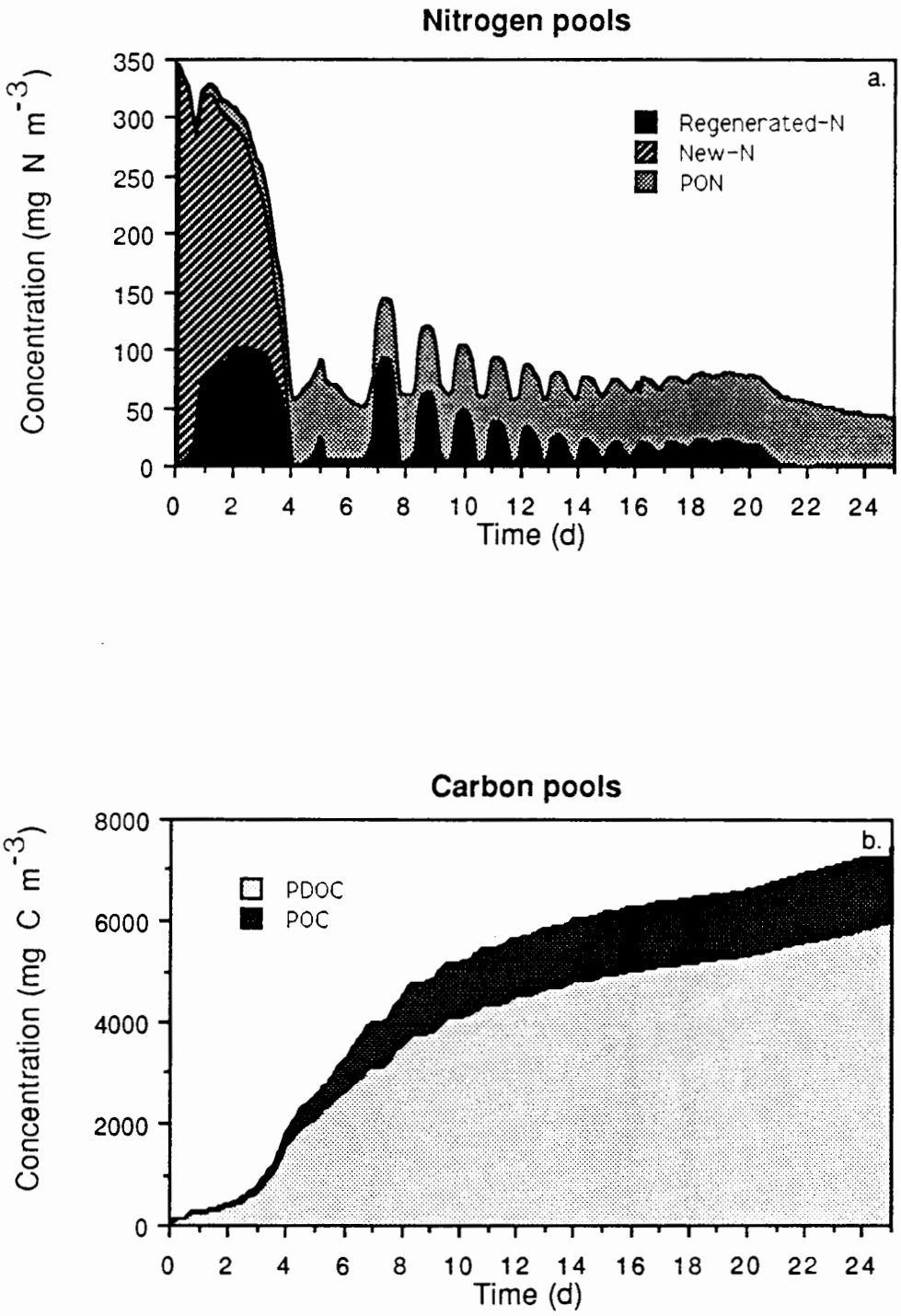


Fig. 4. Model results showing changes in the concentrations of (a) the nitrogen pools and (b) the carbon pools after upwelling.

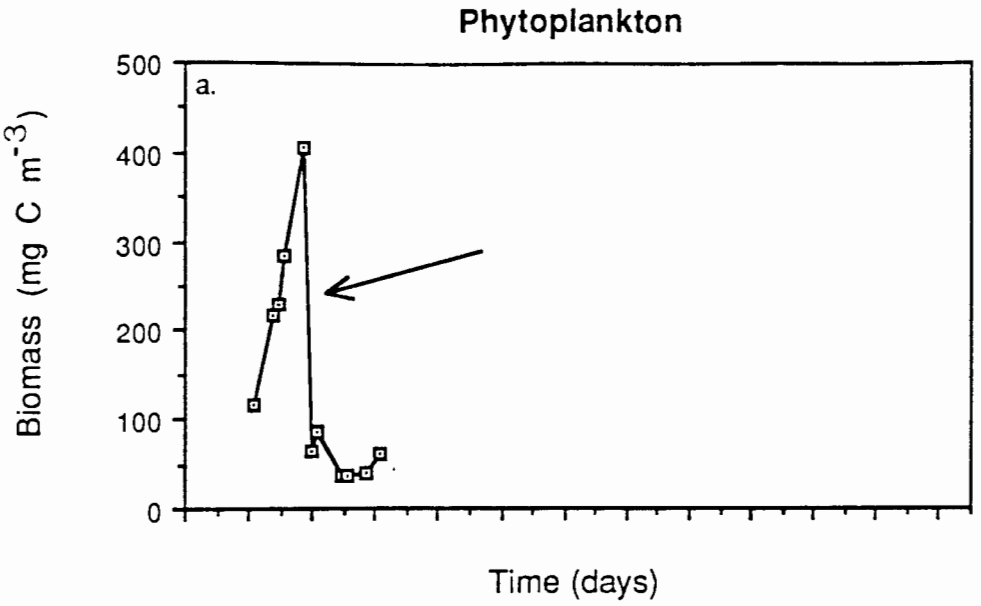
Comparison of simulation output with field data.

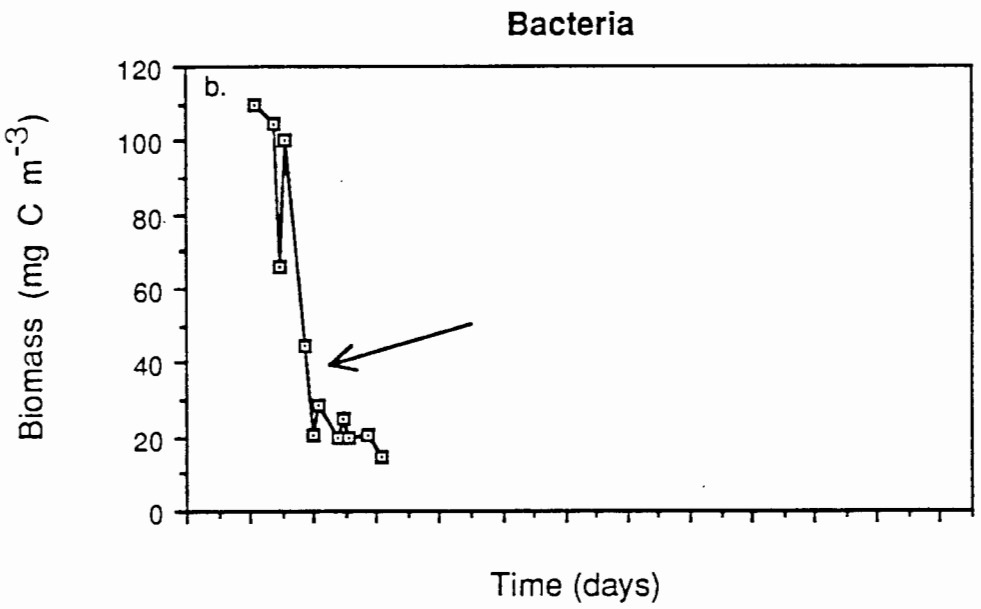
Temporal changes in biomass of phytoplankton, bacteria and zooplankton during the second of the two drogues followed during the field study in March 1983 (see Chapter 6) are shown in Figs. 5 a, b and c. The data are presented on a time-scale similar to that used in the simulation, but the time axis is unlabelled to allow the data to be moved along the time axis until peaks in biomass coincide with peaks in the simulation. This is done because it is not known when time-zero occurred in the field data since the time of initial upwelling was unknown. Marked fluctuations in the field data are clear.

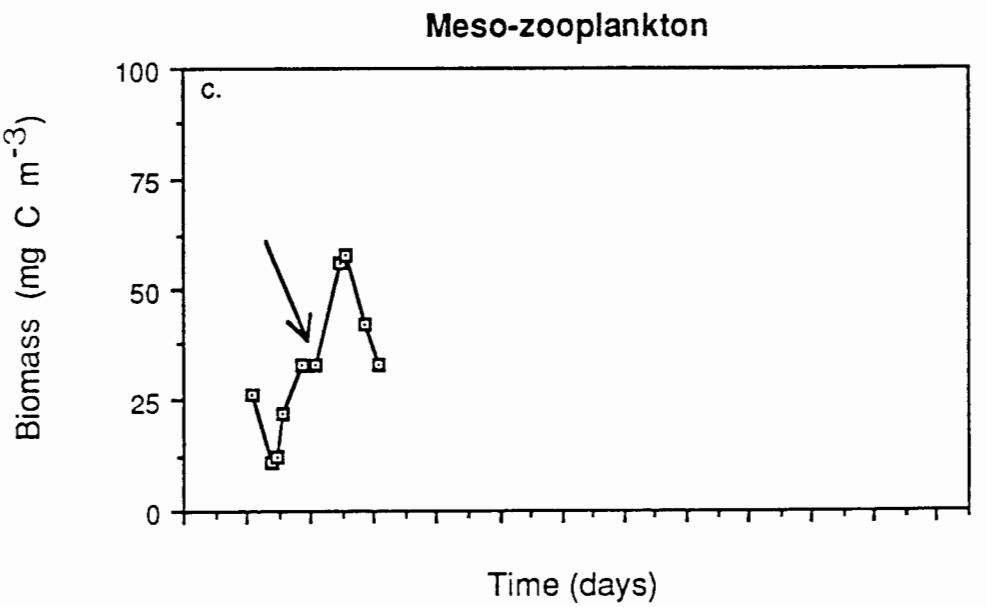
Phytoplankton biomass in the nano- and net- phytoplankton size-classes calculated from cell counts is presented in Fig. 5d. Each of the 2 size-classes contributed approximately 50 % of the total biomass. In contrast, Coulter Counter data (Fig. 5e) showed that the phytoplankton biomass was dominated by the effective netphytoplankton size class.

Comparison of field data with simulation output is complicated by evidence suggesting that the temporal biomass changes observed in the field were effectively accelerated due to movement of the drogue out of mature upwelled water (MUW) into MUW/mixed water (Chapter 6). The "break-point" in each data series is indicated by an arrow (Fig. 5a to e).The data are decoupled at this point and shifted independently along the time axis for comparisons with the simulation.

Changes in the total phytoplankton biomass calculated from chlorophyll measurements are shown superimposed on simulation results in Fig. 6a. The field data could be shifted such that the peak in phytoplankton biomass in MUW coincided with the peak on day 4. This is supported by field concentrations of


Phytoplankton
a.
500
400
300
200
100
0
Biomass (mg C m-3)
Time (days)


Bacteria
b.
120
100
80
60
40
20
0
Biomass (mg C m-3)
Time (days)


Meso-zooplankton
c.
100
75
50
25
0
Biomass (mg C m-3)
Time (days)

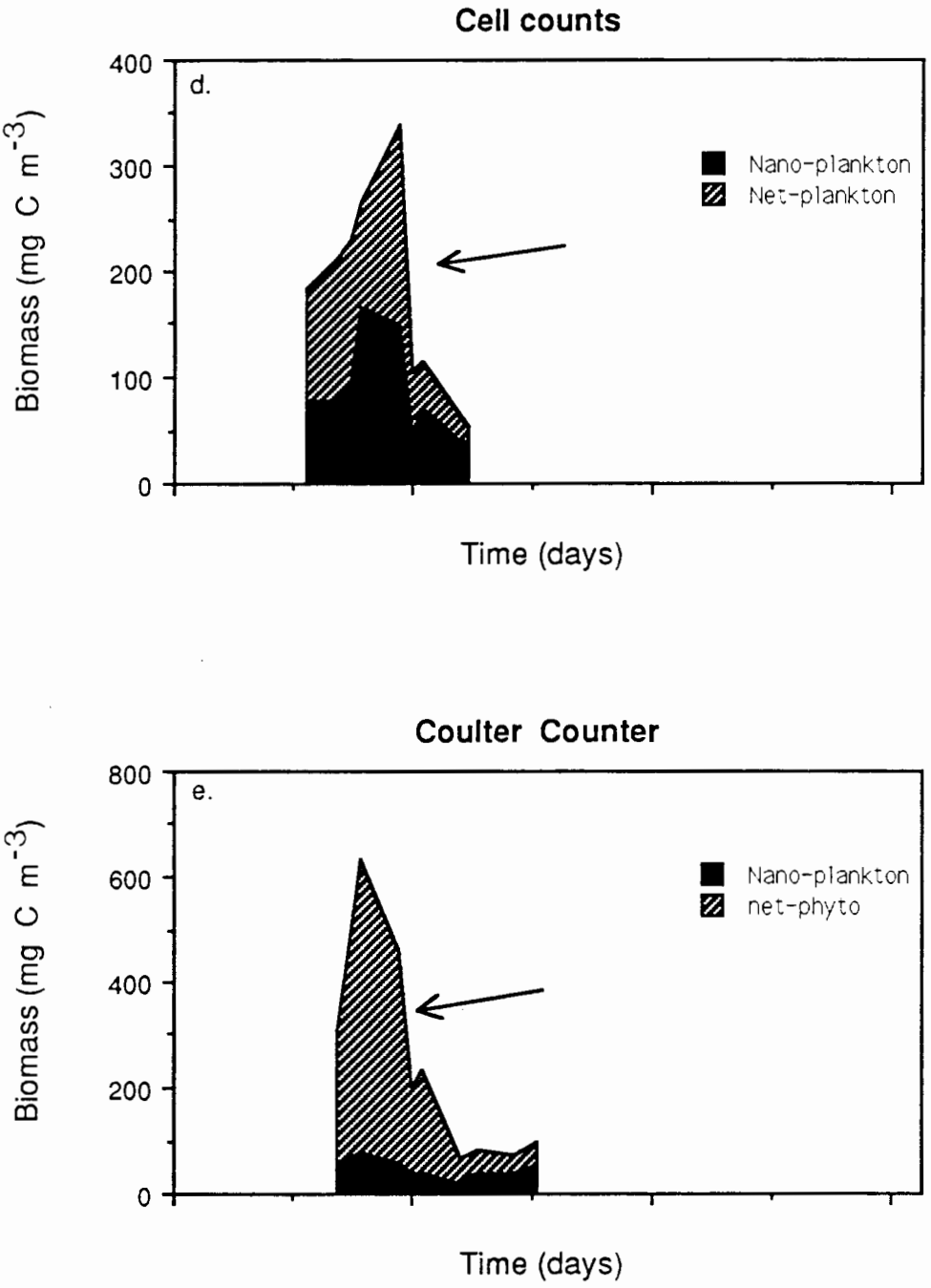
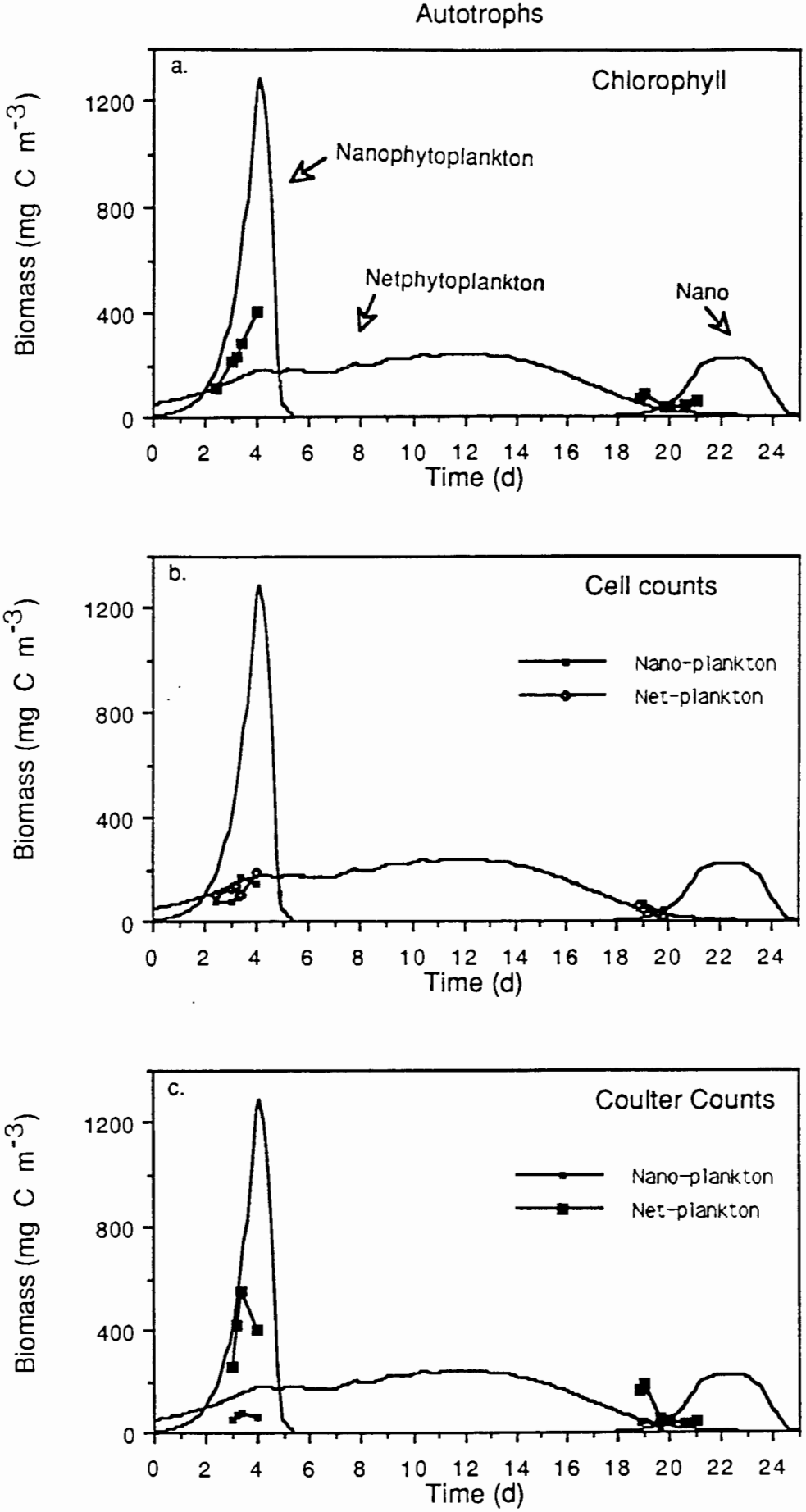


Fig. 5. Temporal changes in biomass of phytoplankton, bacteria and meso-zooplankton during the second drogue study in the southern Benguela upwelling region (see Chapter 6). (a) Phytoplankton biomass calculated from chlorophyll concentrations, using a C:Chlorophyll ratio of 60. (b) Bacterial biomass. (c) Meso-zooplankton. Additional data are presented, for phytoplankton biomass in the nano-plankton and net-plankton size-classes, calculated from (d) cell counts and pg C $cell^{-1}$ (Strathmann 1967) and (e) Coulter Counts and a factor of 0.11×10^{-6} μg C μm^{-3} (Kiorboe et al. 1985). Arrows indicate a change in the water mass. Note: Time-scales on (a), (b) and (c) are the same as for Fig. 2. The time-scale on (d) and (e) is magnified by 2 times.


Autotrophs
a.
Chlorophyll
Nanophytoplankton
Netphytoplankton
Nano
Biomass (mg C m-3)
Time (d)
b.
Cell counts
Nano-plankton
Net-plankton
Biomass (mg C m-3)
Time (d)
c.
Coulter Counts
Nano-plankton
Net-plankton
Biomass (mg C m-3)
Time (d)

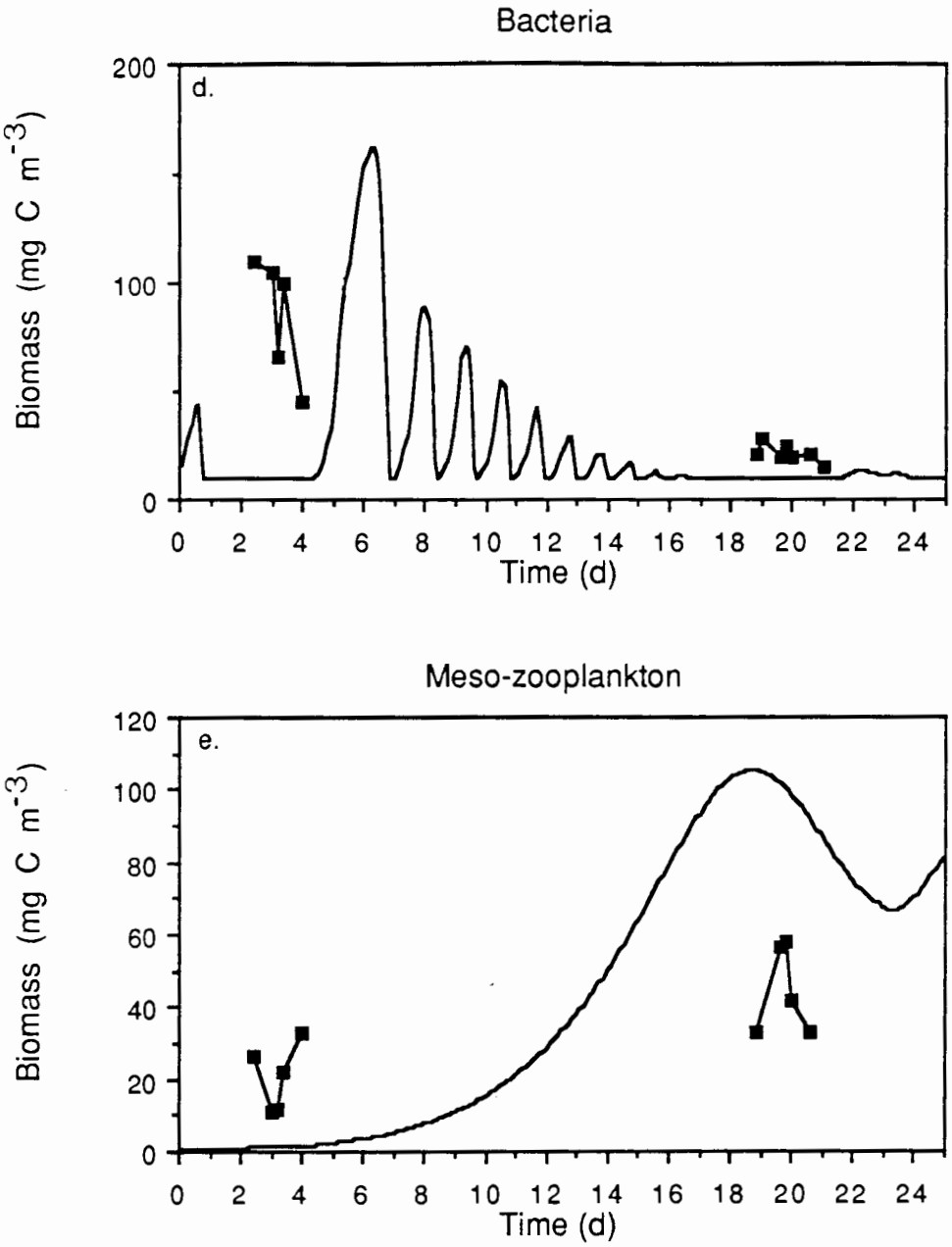


Fig. 6. Comparison of simulation output and field measurements (■-■). (a) to (c) Simulated changes in nano- and net-phytoplankton biomass, and field measurements obtained from (a) chlorophyll concentrations, (b) cell counts and (c) Coulter Counts. (d) Bacterial biomass changes. (e) Meso-zooplankton biomass. Note that cell counts and Coulter Counter data are plotted separately for the nano- and net- phytoplankton classes, not cumulatively (cf Fig. 5d, e).

nitrate-N of 28 to 70 mg N m^{-3} (Chapter 6), although there is a 3-fold difference between the 2 biomass estimates. The measured phytoplankton biomass is uncharacteristically low for maturing upwelled water in which "new" nitrogen is still present at low concentrations. Simulation results show that the phytoplankton bloom was dominated by nanoplankton on day 4. Cell counts show that nano- and net- phytoplankton were present in approximately equal proportions (Fig. 6b). It is, however, possible that phytoplankton biomass in the nanophytoplankton size-class was underestimated due to the difficulties of preserving and counting cells less that 15 μm (Bloem et al. 1986, Moloney 1988).

Coulter Counter results showed that the phytoplankton was dominated by the effective netphytoplankton size-class (Fig. 6c). This has important implications for the structure of the planktonic community, as it suggests a shift in the biomass spectrum. It is likely that the biomass of the microzooplankton size-class in the model will be suppressed, while the biomass of the netphytoplankton predators (mesozooplankton) will increase.

The phytoplankton biomass data in MUW/mixed water were shifted so that they coincided with the simulation data on days 19 to 21. Although fluctuations were observed in the field data, total biomass values were comparable. Furthermore both cell counts (Fig. 6b) and Coulter Counts (Fig. 6c) showed the simulated succession in the dominant phytoplankton, from netphytoplankton to nanoplankton.

Temporal development of phytoplankton in the simulation output presented here appears to conform well with other field observations on bloom development after upwelling in the southern Benguela. Drogue studies by Barlow (1982c) and Brown and Hutchings (1987a, b) have shown that the combination of light, high

nutrients and sun-warming after upwelling results in rapid development of phytoplankton blooms, dominated by chain-forming diatoms. These blooms typically take about 3 days to develop, reaching maximum standing stocks of up to 21 mg chl *a* m^{-3} (1300 mg C m^{-3}) and decline after 6 to 8 days due to thermal stratification and nitrate limitation. The cells either sink out of the water column or are grazed by herbivores. Any further production is sustained by regenerated nitrogen (Probyn 1985, 1987).

Rapid short-term fluctuations in autotroph biomass shown in the simulation output (Fig. 2a) have not been observed in field studies. These fluctuations are due to blooms of picophytoplankton, which may have been largely excluded from field measurements as a result of their small size. Development of phytoplankton in the nano- and net- phytoplankton size-classes (Fig. 6a) is comparable with field observations, if one recognises that the small chain-forming diatom cells, which dominate the initial phytoplankton bloom, fall into the category of nanoplankton as a result of the small size of individual cells.

Heterotrophs

The maximum value for bacterial biomass in the model output (162 mg C m^{-3}, Fig. 2b) was similar to the maximum biomass measured in the field (180 mg C m^{-3}, Chapter 6). For comparison of temporal changes the measurements of bacterial biomass were averaged over the maximum depth of the mature upwelled water (MUW) and MUW/mixed water, and shifted along the time axis of the simulation output (Fig. 6d) in synchrony with the autotroph measurements. As in the model, measured bacterial biomass showed fluctuations. Agreement between simulation and field results was good for MUW/mixed water, but poor in MUW. This may be due to complicating factors such as the cell physiologies and substrate uptake

activity of bacteria which operate in the field but are not included in the model, and which could delay bacterial growth.

The model assumes that bacteria (and picophytoplankton) actively take up dissolved nutrients: carbon and nitrogen are taken up at a maximum specific rate of 4.1 d^{-1}, although the actual uptake rates at each time step in the model are determined by ambient concentrations, and the K_s for nitrogen uptake (Table 1). In field and experimental measurements, however, it has been shown that bacteria in organically-deficient waters, such as newly upwelled water, may have suppressed metabolic rates due to adaptation to starvation conditions (Novitsky and Morita 1977, Davis 1985). In a microcosm study Painting et al. (1989) showed that bacterial substrate-uptake activity was low in recently upwelled water, and increased during phytoplankton growth. Thus the increase in bacterial biomass observed almost immediately in the model (day 0.5, Fig. 2b) may not occur in situ. Similarly, picophytoplankton growth rates in the field may be slower initially than the growth rate assumed in the model. Furthermore, initial flagellate growth may be slower in the field. If this is so, predator control of bacteria will be reduced and it is possible that bacterial biomass may peak during the simulated nanophytoplankton peak, as indicated by field results (Fig. 6d).

Measured changes in zooplankton biomass (Fig. 6e) compared favourably with the simulation results. The maximum measured biomass (56 mg C m^{-3}) was relatively close to the simulated biomass of approximately 100 mg C m^{-3}. The peak in measured mesozooplankton biomass (33 mg C m^{-3}, Fig. 6e) on day 4 may result from a combination of factors, notably a higher biomass in the seed population and herbivory on the effective netphytoplankton biomass (discussed above). This may explain the low phytoplankton biomass on day 4.

It is unrealistic to expect quantitative data to match simulation results precisely, particularly towards the end of the simulation. In the field it is unlikely that the water mass being studied is not influenced by factors such as the input of diffused nutrients, changes in the optical spectrum, and behaviour of migratory animals, particularly mesozooplankton and fish, which are free to move into and out of a particular water mass. In spite of this, and the de-coupling of the temporal sequence in the field data, the model used here appears to be a realistic simulation of the temporal changes in the biomass relationships and size-structure of the planktonic food web in the southern Benguela.

Comparison of microcosm and model simulation

The model was modified to simulate the results obtained in the microcosm study presented in Chapter 4, by excluding micro- and meso- zooplankton. Model output is shown on Fig. 7 for both autotrophs (Fig. 7a) and heterotrophs (Fig. 7b). A small picophytoplankton bloom developed on day 0.5, but decreased rapidly. This was followed by a phytoplankton peak on day 4 (ca 1800 mg C m^{-3}), which was dominated by nanophytoplankton (1600 mg C m^{-3}). Phytoplankton biomass declined gradually after day 4 due to nitrate depletion, and the absence of herbivores. The carbon biomass was approximately 1400 mg C m^{-3} on day 25.

Bacterial biomass showed a peak (ca 45 mg C m^{-3}) on day 0.5, which was followed by a peak in flagellate biomass (ca 95 mg C m^{-3}, Fig. 7b). The bacterial biomass decreased rapidly to the threshold value (10 mg C m^{-3}) on day 1, and was followed by a decrease in flagellate biomass on day 1.5. The model showed 3 pulses in flagellate biomass between days 1.5 and 4, when biomass was low (<10 mg C m^{-3}). Bacterial biomass recovered, reaching a second peak (ca 40 mg C m^{-3}) on day 4.5. Thereafter the model showed oscillations in bacterial and

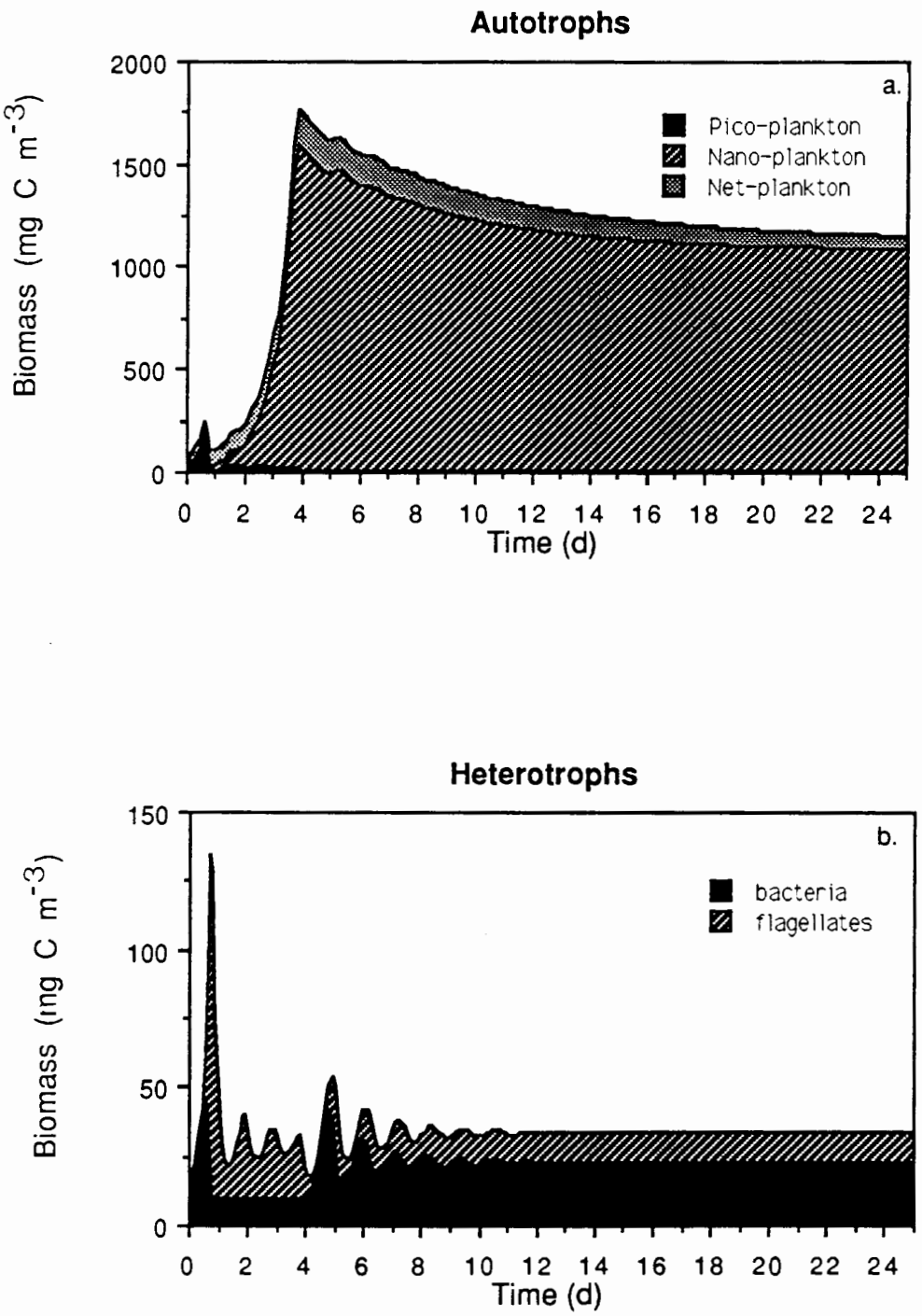


Fig. 7. Model results obtained from simulating the microcosm experiment (Chapter 4), showing changes in the biomass of (a) autotrophs and (b) heterotrophs.

flagellate biomass until day 11, when "steady-state" was reached. Bacterial biomass remained constant at 25 mg C m^{-3} and flagellate biomass remained at a steady 10 mg C m^{-3}.

Microcosm results showed approximate agreement with simulation output (Figs. 8 and 9, a, b), particularly for autotrophs. Changes in relative fluorescence and concentrations of particulate carbon (PC) did not show any evidence of the initial picophytoplankton bloom on day 0.5 (Fig. 8a), possibly due to inactivity of this group of organisms in recently upwelled water. The nanophytoplankton bloom on day 4 was reflected by both the fluorescence and PC (1330 mg C m^{-3}) measurements. The decline in fluorescence from days 4 to 13 suggested senescence of the phytoplankton bloom due to depletion of nitrates in the system (Chapter 4). The decline in PC concentrations during this time period suggested utilisation of senescent phytoplankton cells by bacteria. After day 12 PC concentrations fluctuated between 300 and 700 mg C m^{-3}, reflecting accumulation of the more refractory detrital phytoplankton in the absence of micro- and meso- zooplankton grazing.

For heterotrophs, agreement between microcosm and model simulations was poor, but the observed differences may be ascribed to species-specific parameters not catered for in the model. For example, the initial pulse of bacteria in the model may not occur due to suppressed metabolic rates of bacteria in newly upwelled water, as discussed above. Furthermore, bacteria in the model find refuge from predation only in their total biomass. Factors such as bacterial growth into a larger cell-size or the presence of micro-environments, which offer some predation refuges to bacteria, are not included. Microcosm results showed that the bacterial community which developed on the more refractory particulate substrates after day 10, was dominated by large rods. These bacteria did not appear to be grazed by flagellates, perhaps

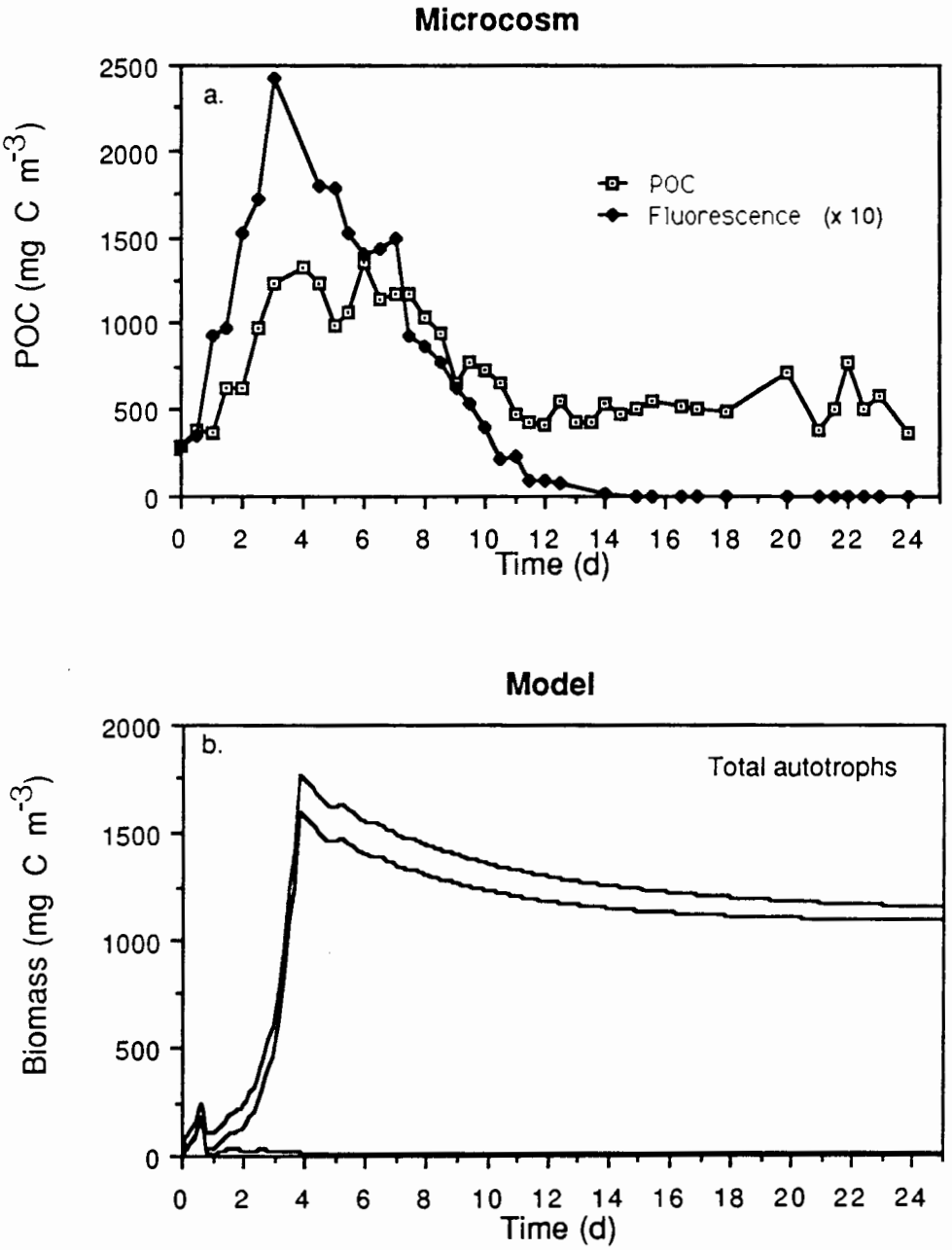


Fig. 8. Comparison of phytoplankton biomass from (a) the microcosm simulation and (b) model simulation. Fluorescence indicates living chlorophyll. Particulate carbon (PC) includes living and senescent phytoplankton.

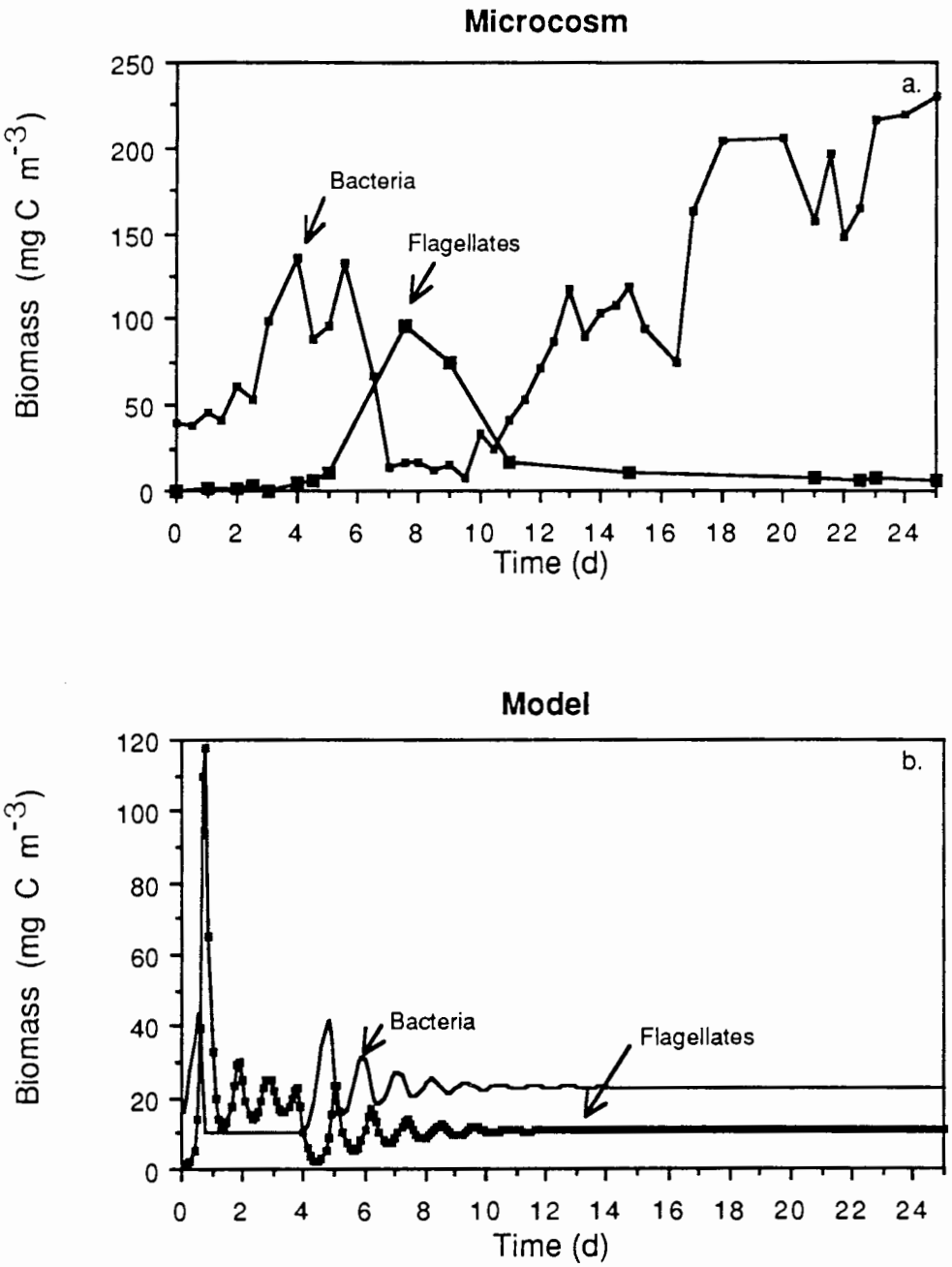


Fig. 9. Comparison of changes in bacterial and flagellate biomass in (a) the microcosm study and (b) the simulation model.

escaping predation due to their large cell-size, or by association with particulate material (Painting et al. 1989).

The maximum flagellate biomass in the microcosm and model simulations were similar (96 and 95 mg C m^{-3} respectively, Figs. 9a, b), but bacterial biomass in the model simulation (45 mg C m^{-3}, Fig. 9b) was considerably lower than measurements in the microcosm (up to 230 mg C m^{-3} on day 25, Fig. 9a). By increasing the senescence rate of phytoplankton in the model, comparable values for bacterial biomass were obtained. By doing so, however, the peak in phytoplankton biomass was reduced considerably. This exercise illustrated a limitation in the model, which assumes a constant rate for phytoplankton senescence. A more realistic simulation output would be achieved if phytoplankton senescence was low initially, but increased after nitrate depletion. Studies by Barlow (1982c) and Brown and Hutchings (1987a) have shown that phytoplankton senesce rapidly after the supply of "new" nitrogen is diminished.

The rate of break-down of detrital carbon and nitrogen by bacteria was increased in the model in an attempt to simulate the maximum bacterial biomass in the microcosm. The expected result (of increased bacterial biomass) was not obtained. Instead, flagellate biomass was increased due to increased availability of food (bacteria), and bacterial biomass was reduced as a result of flagellate predation. These results showed that flagellate predation is an important factor controlling bacterial biomass. By excluding micro- and meso-zooplankton from the simulation, flagellate growth was unrestricted, and increased in response to bacterial growth. The conservative assumptions of a 10 % senescence rate (phytoplankton) and a 10 % lysis rate (bacteria) were therefore retained in the model simulation.

Different options for the planktonic food web.

A number of hypothetical options were experimented with in the model simulation of the planktonic food web. In the first, bacteria were assumed to utilise only dissolved sources of C and N. Model output (Fig. 10a, b) showed that autotrophs were initially unaffected. Up to day 7 results were similar to those in Fig. 2a. Thereafter, autotroph biomass showed only 4 small pulses before declining to a low biomass of approximately 100 mg C m^{-3}. Pulses of regenerated nitrogen were reduced (Fig. 10c cf Fig. 2c), and a secondary nanophytoplankton bloom was absent. Similarly, heterotrophs were initially unaffected (Figs. 2b, 10b), but after day 6 total heterotroph biomass was reduced. These results show that if bacteria are assumed to utilise only dissolved sources of C and N excreted by autotrophs, the microbial loop is soon closed. Furthermore, remineralisation of nutrients by the microbial food web is reduced and phytoplankton production declines.

In the second manipulation (Figs. 11a, b) the initial biomass of mesozooplankton was increased from 1 to 150 mg C m^{-3}. The expected result was a much-reduced autotroph biomass. Instead, the magnitude of the initial nanophytoplankton bloom on day 4 (Fig. 2a, Fig. 11a) was increased, due to limitation of microzooplankton biomass by mesozooplankton predation. Netphytoplankton were absent, but picophytoplankton pulses were stimulated. The secondary nanophytoplankton bloom occurred earlier (day 16), and was of a much higher magnitude than in the original model. Maximum biomass values of all heterotrophs (Fig. 11b) were larger than in the drogue simulation, with 2 dominant pulses of peak bacterial and microzooplankton biomass (days 7 and 19). From these results it was evident that a large seeding stock of mesozooplankton had marked effects on the trophic structure of the planktonic food web through increased predatory control of heterotrophs (see also Riemann 1985).

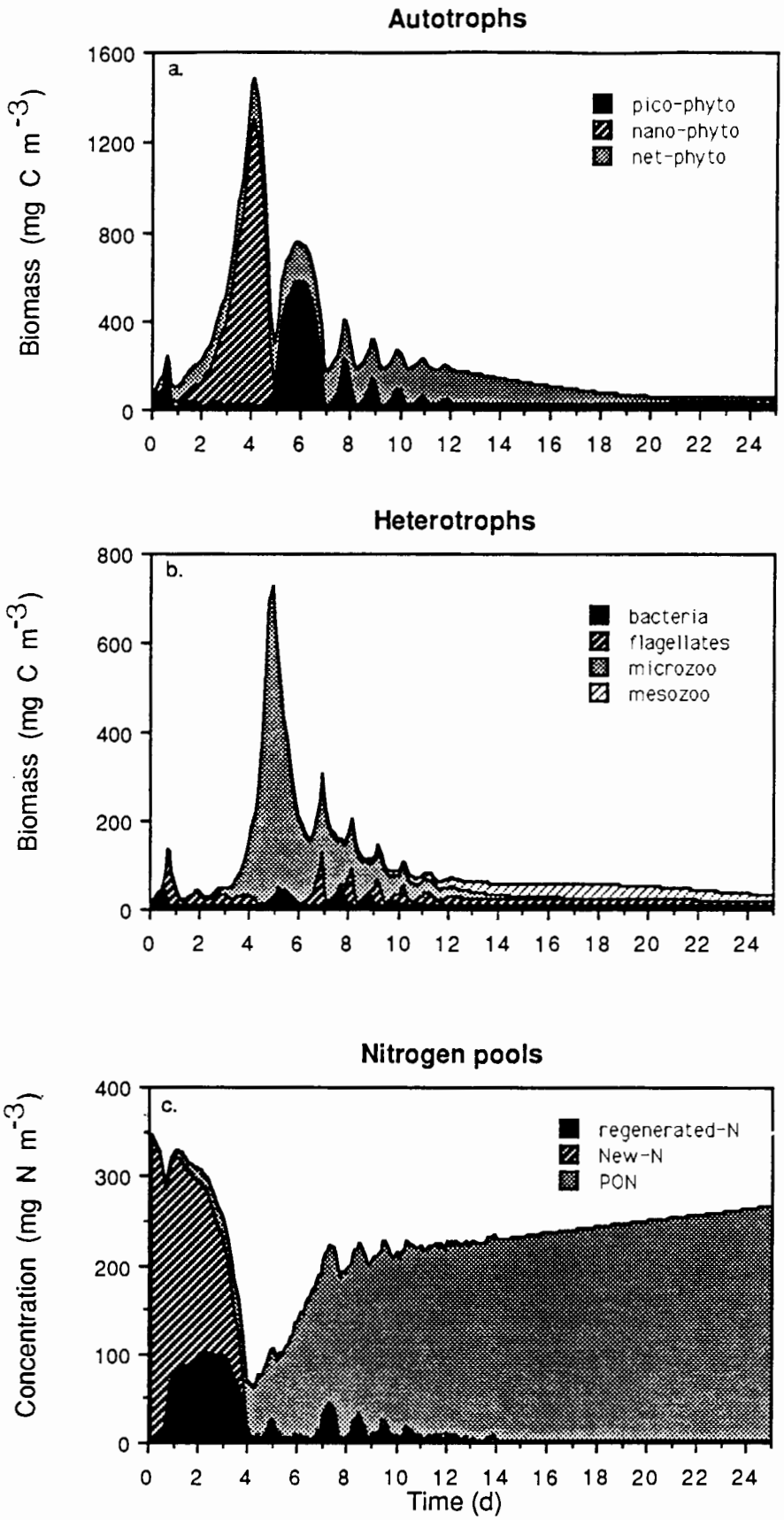


Fig. 10. Model output for simulation in which bacteria were assumed to utilise only dissolved sources of carbon. Results show changes in the biomass of (a) autotrophs, (b) heterotrophs, and (c) nitrogen pools.

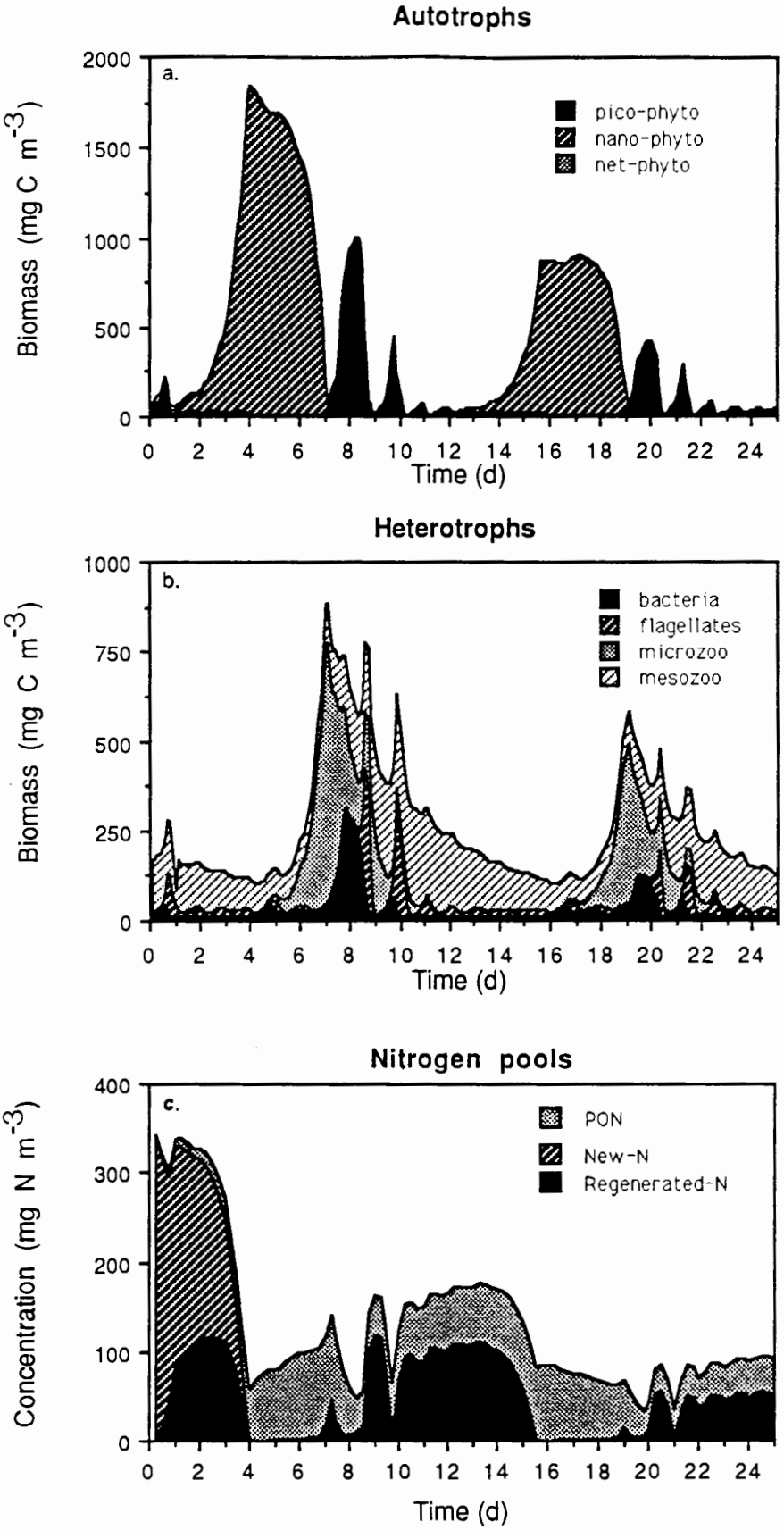


Fig. 11. Results of model simulation in which meso-zooplankton biomass was high (150 mg C m^{-3}) initially, showing output for (a) autotrophs, (b) heterotrophs and (c) nitrogen pools.

Results of the manipulated simulations are not inconsistent with field measurements, and show that numerous options are available for biomass relationships and size-structure of the planktonic food web. Furthermore, they increase confidence in the parameter values and assumptions used in the model.

The final modification to the simulation model was extreme. Bacteria were excluded, to determine if they are in fact an important component of the planktonic food web. Simulation results are shown on Fig. 12 (a, b). The initial peaks in autotrophs and heterotrophs prior to day 6 (Fig. 2a, b) are still present, and at similar magnitudes. After day 6 both autotrophs and heterotrophs show pulses in biomass for 4 days, but then decline to near zero. These results, in conjunction with those of the "drogue" simulation (Fig. 2), support the hypothesis that bacteria contribute to an increase in the overall productivity of the planktonic food web, and that mineralisation by the microbial food web is an important process by which the productivity of phytoplankton populations is increased.

ECOLOGICAL SIGNIFICANCE

The size-based model developed by Moloney (1988) appears to produce a realistic simulation of the dynamics of a plankton community in the southern Benguela upwelling region in terms of standing stocks and size composition. The model predicts rapid fluctuations in the biomass and structure of planktonic communities, which may explain the large spatial variations observed in the field. In the model an initial bloom of picophytoplankton occurs after new upwelling, prior to a larger bloom of nanophytoplankton (diatoms and flagellates), which lasts for approximately 3 days. Due to the chain-forming diatom species, this nanophytoplankton bloom is likely to be observed as a netphytoplankton bloom. After depletion of "new" nitrate the phytoplankton

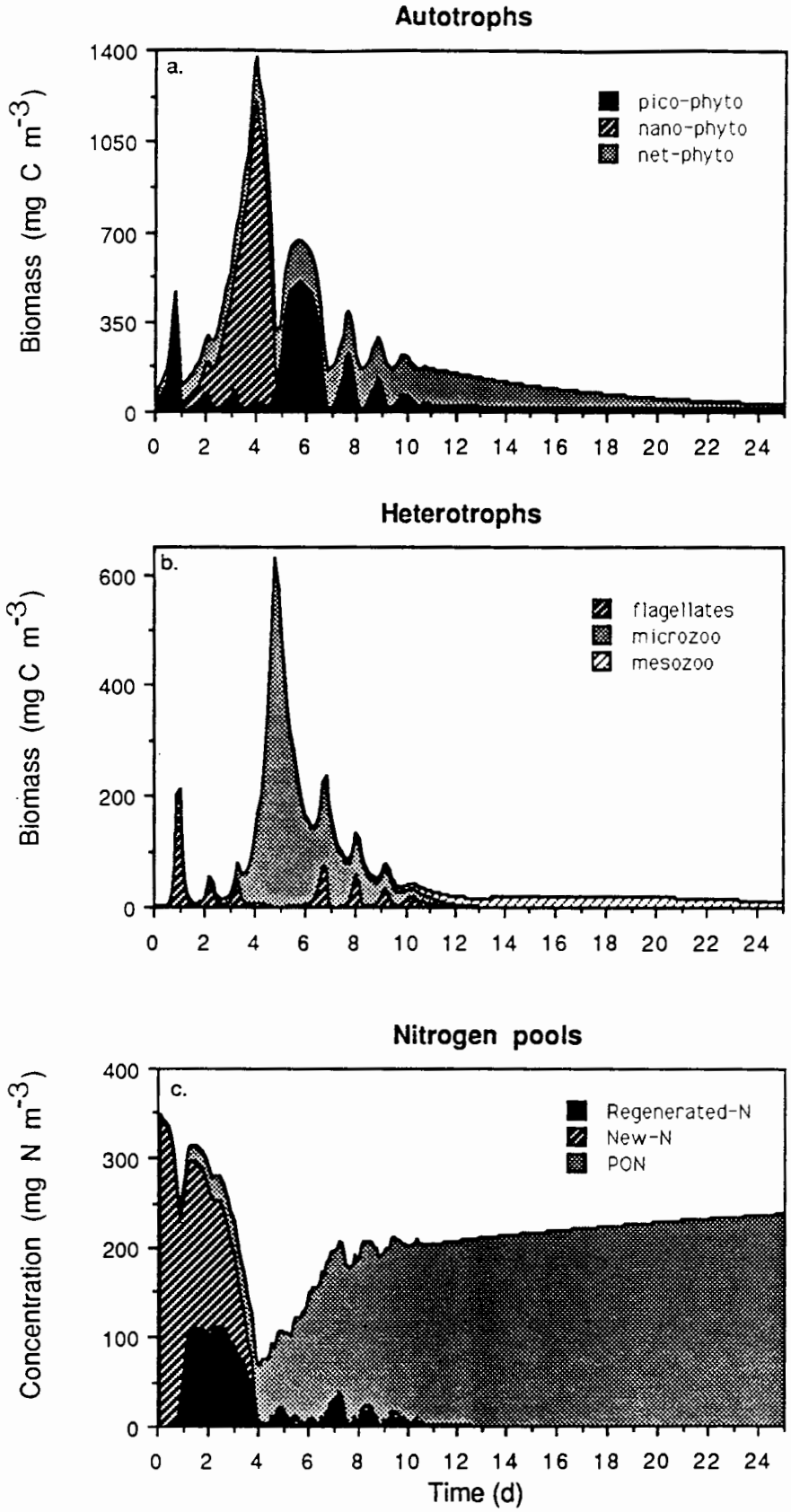


Fig. 12. Model results when bacteria were excluded. (a) Autotrophs, (b) heterotrophs, (c) nitrogen pools.

community is dominated by pulses of picophytoplankton, and a more stable netphytoplankton bloom which persists for about 10 days. A secondary peak of nanophytoplankton is observed towards the end of the 25 day simulation.

An initial bloom of bacteria and flagellates after new upwelling is also predicted by the model. During the nanophytoplankton bloom heterotroph biomass is low. Senescence of the bloom is accompanied by a peak in microzooplankton biomass, which declines rapidly. After day 5 alternating peaks are observed in bacterial, flagellate and microzooplankton biomass. Successive pulses are smaller in magnitude and duration. Mesozooplankton biomass increases gradually after day 10, and dominates the heterotroph biomass between days 15 and 24. Microzooplankton biomass increases again during the last 3 days of the simulation.

Biomass estimates and structure of the model planktonic community show temporal variability as a result of the combined effect of substrate/food availability and predation. Competition for nitrogen sets limits to the growth of phytoplankton and bacteria, and favours small organisms which are more efficient at utilising dissolved nitrogen at low concentrations (see also Bratbak and Thingstad 1985). Simulation results support the hypothesis that the planktonic community is dominated by small-sized organisms, notably pico- and nano- phytoplankton (<20 μm), bacteria (0.2-2 μm), flagellates (2-20 μm), and microzooplankton (20-200 μm). Model results obtained in the simulations executed in this study suggest that rapid mineralisation by the microbial food web is an important process by which the overall productivity of the planktonic community is increased, and that there is a successive dependency of "new" and "regenerated" nitrogen in the control of plankton dynamics.

One aspect of plankton dynamics not addressed in this study is the influence of the C:N (and C:N:P) ratio of phytoplankton and bacteria on C and N flux in the pelagic food web. It is generally accepted that the limiting nutrient in the marine environment is nitrogen. The relative proportion of nitrogen in autotrophs, bacteria and other heterotrophs is likely to have important effects on the net growth yield of heterotrophs, and on nitrogen recycling. For bacteria, it has been suggested that their net growth yields decrease as the C:N ratio of the substrate increases (Linley and Newell 1984, Goldman et al. 1987, Hopkinsen et al. 1989). The net result of high C:N ratios is an increase in the loss of carbon from the system, due to respiration, increased demand for substrate consumption in order to meet nitrogen requirements, and immobilisation of inorganic nitrogen. Conversely, when nitrogen demand is not limited by high C:N ratios, respiratory losses of carbon are likely to be less, while mineralisation and cycling of nitrogen are likely to be increased (see Hopkinsen et al. 1989).

This chapter clearly shows that size-dependent relationships and parameters may be used to model the planktonic community. Obviously, simulation results are dependent upon assumptions and size-classes used in the model. Sensitivity analyses have shown that the model is robust with respect to most parameters (Moloney 1988), although manipulations performed in this study indicate that changes in seeding conditions may have marked effects on simulation output, as would be expected.

Some of the simplifying assumptions made in the model may not be realistic. For example, it was assumed that temperature remains constant at 13 ^{o}C, whereas in practice it would probably increase during the simulated time period. Consequently, quantitative changes governed by rate processes, which are influenced by the assumed temperature and Q_{10} value, are likely to be

underestimated in the simulation output. Furthermore, estimates of the excretion rate of dissolved carbon substrates by phytoplankton are difficult to obtain, but appear to be highly variable, ranging from 10 to 80 % of the total carbon fixed during photosynthesis (Wolter 1982, Lancelot 1984, Turley and Lochte 1985, see also Jumars et al. 1989). PDOC excretion rates are influenced by a number of factors, and are likely to be higher when phytoplankton are nitrogen-limited (Lancelot 1983, see Jensen 1984). In the model PDOC production rates were assumed to be constant (30 %) at the lower end of this range. Nonetheless, bacteria were not carbon-limited and the PDOC pool was shown to accumulate carbon during the simulation, so the assumption probably does not affect model output substantially. Physical factors not included in the model are more likely to influence simulation results. For example, decreased light attenuation, turbulence, diffusion, and deep mixing below the euphotic zone. Incorporation of these factors would increase model complexity and obscure fundamental processes involved in determining plankton dynamics, although it could be argued that that physical processes are the most important controlling mechanisms in plankton dynamics.

In general, qualitative aspects of the model appear to produce an output consistent with laboratory and field results, making a valuable contribution towards our understanding of the dynamics of the plankton community. Observed differences in simulation results and experimental and field measurements are similarly constructive, as they provide insight into the effects of species-specific adaptations of planktonic organisms to a variable and unpredictable environment. For example, at times the allometrically determined growth rate of bacteria appears to be inconsistent with growth rates of these organisms in situ. This may be partly explained by bacterial substrate specificities and by the various strategies adopted by bacteria for surviving low-nutrient conditions.

Bacterial metabolic rates have been shown to be suppressed under starvation conditions. Adaptations in their enzyme systems enable them to survive a "feast and famine" existence (Davis 1985, 1989, see also Painting et al. 1989). In addition, morphological plasticity enables bacteria to moderate individual cell size. Under environmental conditions in which organic substrates are deficient, natural marine bacteria are able to form dormant "mini-cells" (Novitsky and Morita 1978, Amy and Morita 1983), which have lower maintenance requirements and a large surface to volume ratio which enables them to have a lower K_s value for preferred substrates. Similarly, Fenchel (1982c), working on 6 species of nanoplanktonic zooflagellates, found metabolic and morphological adaptation of these organisms to starvation conditions.

Further examples of planktonic adaptations to the stochastic environment of the southern Benguela are provided by diatoms, which fall into all the size-classes in the model. These organisms may be able to sustain themselves after available nutrients are depleted, by utilising reserve supplies accumulated in the cell vacuole (Malone 1980). Furthermore, growth of diatoms may not follow allometric rules. For example, Furnas (1982a, b) found that diatoms had faster growth rates than microflagellates and non-motile ultraplankton in diffusion cultures. Diatoms in the southern Benguela may form resting spores under nutrient or light-limiting conditions (Pitcher 1986) and sink out of the water column rapidly, thereby avoiding bacterial degradation and forming the seed stocks for subsequent upwelling cycles.

Mesozooplankton may also be adapted to variable temperature and food regimes. For example, one of the species often dominant in the southern Benguela, Centropages brachiatus, has a low Q_{10}, so that metabolic rates are not affected by large variations in temperature (Carter 1983). Another dominant species, Calanoides carinatus, has been shown to increase tolerance to

starvation in the older copepodite stages by utilising large stored lipid reserves (Borchers and Hutchings 1986). Lipid metabolism has similarly been suggested to maintain adults of Calanus australis during periods of starvation (Attwood and Peterson 1989). Behavioural adaptations of mesozooplankton may also influence the dynamics of the planktonic community. It has been hypothesised that species from upwelling environments may be able to enter diapause in a late copepodite stage (C5), and remain in the coastal aphotic zone until they are advected into surface waters during the upwelling process. During sun-warming and subsequent phytoplankton growth the diapause is reversed, and the zooplankton become metabolically active (see Borchers and Hutchings 1986). Thus large seeding stocks of mesozooplankton may be found in recently upwelled water.

In conclusion, results of this study show that the sized-based model (Moloney 1988) is useful for exploring relationships between different components of the planktonic community. As a heuristic model, its usefulness lies in providing further insight into bacterial dynamics than may be gleaned from field and laboratory observations. From the combined theoretical and field-based approach adopted in this study a number of important considerations emerged. Firstly, it was evident that bacteria are not necessarily carbon limited in an upwelling ecosystem, but that they do utilise carbon from both the dissolved and detrital pools contibuted to by autotrophs and heterotrophs. Secondly, senescence rates of phytoplankton (and not only primary production) may be important to bacterial growth. Thirdly, that flagellate predation, and not just primary production and substrate availability, is an important controlling factor for bacterial biomass and growth. Lastly, bacteria are important to plankton not as a link to fish, but as a food source for flagellates which regenerate nitrogen which sustains phytoplankton growth.

Model output emphasises the importance of improving estimates of plankton biomass (and production) in the smaller size-classes of the planktonic community, particularly picophytoplankton and heterotrophs in the 2 to 20 and 20 to 200 μm size-classes. In addition, we need to improve our understanding of the behavioural and other adaptations of dominant species to the physical environment, and to incorporate these adaptations in future models.

ACKNOWLEDGEMENTS

I am extremely grateful to Dr. Coleen Moloney for her contributions towards this study, particularly for her willingness to modify the program of her simulation model until I was satisfied that the bacterial component was being modelled more realistically. I also appreciate her criticisms, and those of Drs. M.I. Lucas and T.A. Probyn, on an earlier draft of this chapter. I am further indebted to many people who participated in the field and laboratory studies discussed here: to my co-authors, Drs. Mike Lucas and Dave Muir, on the microcosm study, to Geoff Bailey, Dr. P. Brown, Dr. L. Hutchings, Dr. Lucas, and others who participated in the drogue study, to Dr. Mitchell-Innes who identified and counted the phytoplankton, and to Dr. W.T. Peterson who counted the zooplankton samples. My thanks are further extended to Dr. E. Olivieri and Desireé Calder who operated the Coulter Counter during the cruise in March 1983, and to Dr. Hutchings who made the data available to me.

CONCLUSIONS

Studies of phytoplankton dynamics in the southern Benguela have demonstrated that the growth and decay of phytoplankton assemblages is closely coupled to the upwelling cycle. Due to the coupling of phytoplankton and heterotroph dynamics, it is not surprising that bacterioplankton dynamics are also related to the upwelling cycle. Availability of dissolved and particulate carbon and nitrogen substrates, derived from autotroph fixation, phytoplankton senescence and heterotroph feeding and metabolism; bacterial substrate specificites and uptake mechanisms; and protozoan predation are clearly the fundamental mechanisms governing the dynamics of the bacterioplankton community.

The heterotrophic bacterial community is heterogeneous. Small cocci (mean volume: 0.009 μm^3), representing the starved minicells characteristic of oligotrophic waters, are numerically dominant in South Atlantic Central Water, which is advected to coastal surface waters. During the development of the phytoplankton bloom, the bacterial community is dominated by large cocci (mean volume: 0.142 μm^3) and small rods (ca 0.198 μm^3). Dominant plateable bacteria include Vibrionaceae (V), Pseudomonadaceae (Ps), Enterobacteriaceae (E), Neissereaceae (Nei) and Flavobacteriaceae (Fl) isolates. A succession (V - Ps - Fl) is observed in the dominant plateable strains, reflecting different substrate-specific enzymes and uptake mechanisms in response to available substrates during phytoplankton growth and decay.

Bacterial abundance and biomass are variable (1 to 10 x 10^6 cells ml^{-1}, 20 to 200 mg C m^{-3}). Values are lowest in recently upwelled water, when phytoplankton biomass and production are negligible, and increase during the rapid phytoplankton growth characteristic of nutrient-rich upwelled water.

Tight coupling is observed between the biomass and activity of heterotrophic bacteria, and phytoplankton growth and decay. An exponential increase in

bacterial biomass during the phytoplankton bloom in the microcosm simulation of an upwelling event indicates utilisation of photosynthetically produced dissolved organic carbon (PDOC) by the bacterial community. In the field, the same simple relationship is less likely to be observed. Additional dissolved carbon and nitrogen substrates released into the water column as a result of zooplankton grazing provide further nutrient sources for primary producers. In particular, "sloppy feeding" (Eppley et al. 1981, Fuhrman 1987), excretion and the rapid diffusion of labile organic carbon substrates from faecal pellets are likely to contribute to enhanced availability of dissolved substrates to the heterotrophic bacterial community.

Relationships between bacteria and phytoplankton and particulate carbon in the southern Benguela are generally best described by power functions. Results of this study suggest that bacterial biomass is reduced at high concentrations of available substrates by predation. In the microcosm study (Chapters 4 and 5), for example, bacterial biomass at the height of the phytoplankton bloom, was rapidly reduced by bacterivorous flagellates which were calculated to consume 2.4 times their body mass per day, or 19 bacteria flagellate^{-1} h^{-1}, when prey were not limiting. Field measurements of bacterial biomass values of 180 mg C m^{-3} in an upwelling plume during a Noctiluca bloom are the highest recorded in situ in the southern Benguela. It is proposed that ingestion of bactivores by the zooflagellate, Noctiluca, accounted for the high bacterial biomass values.

Predation within the microbial food web is an important mechanism by which nutrients in the planktonic food web are recycled. Low net growth efficiencies (34 to 36 %) of flagellates suggest inefficient transfer of carbon to higher trophic levels, but considerable nitrogen regeneration. In pycnoclinal regions, where the relative input of "new" nitrogen is high, there are few regenerative

steps. Excretion rates observed from ^{15}N studies in surface waters can only be accounted for by many regeneration steps in a highly complex food web.

Temporal changes in planktonic community structure are observed during the development of an upwelling plume. In recently upwelled water the planktonic biomass is dominated by phytoplankton. As the water ages, the planktonic community is increasingly dominated by detritus, heterotrophic bacteria and zooplankton.

Measurements of bacterial activity and production are variable, and highest in the euphotic zone, particularly during phytoplankton growth. Thymidine-measured bacterial production (TTI, <0.1 to 1.25 mg C m^{-3} h^{-1}) is significantly correlated with primary production, and equivalent to 1 to 10 % of autotrophically fixed carbon. Estimates of bacterial production calculated from net growth rates are 2 to 34 times higher than values based on thymidine incorporation (TTI). It is possible that these differences are attributable to inadequate DNA extraction procedures, large numbers of bacteria without thymidine transport systems, or isotope dilution. Differences are particularly marked in newly upwelled water and during phytoplankton decay. Apart from uncertainties in the conversion factors used to calculate bacterial production from TTI, bacterial responses to exogenously supplied dissolved substrates (eg. ^{3}H-thymidine, ^{14}C-glucose) are variable.

Secondary production by copepods (Calanoides carinatus) is also coupled to the upwelling cycle. Net production during the quiescent stage of the upwelling cycle may be relatively high (57 to 800 mg C m^{-2} d^{-1}). Estimates of bacterial production (120 to 1025 mg C m^{-2} d^{-1}) are approximately equal to or greater than copepod production. During high levels of phytoplankton production, bacteria and copepods consume approximately 12 and 22 % of autotrophically fixed carbon

respectively. As the bloom declines, carbon consumption requirements of bacteria and copepods increase to about 49 and 60 % of the available primary production. The combined carbon consumption requirements of heterotrophic bacteria and Calanoides carinatus are satisfied by partitioning of autotrophically fixed carbon resources, and carbon cycling. During the decline of the phytoplankton assemblage herbivorous copepods become increasingly food-limited, and possibly supplement their dietary requirements through predation on microzooplankton. Low growth efficiencies of copepods will result in enhanced remineralisation.

Results of this study indicate that heterotrophic bacteria and the microbial loop are an important component of the pelagic food web of the southern Benguela. Trophic interactions, responsible for regeneration of nitrogen, contribute to an increase in the overall productivity of the planktonic food web. Furthermore, results presented here emphasise the importance of empirical and experimental studies directed at improving estimates of the biomass and production of the smaller size-classes of the planktonic community, particularly pico-phytoplankton and heterotrophs in the 2 to 20 and 20 to 200 µm size-classes. Improved quantification of microzooplankton abundance and production, microzooplankton feeding, mesozooplankton carnivory, assimilation efficiencies of micro- and meso-zooplankton and the availability of HDOC to bacteria is necessary to further improve our understanding of the trophic structure and functioning of the planktonic food web. In addition, we need to improve our understanding of the behavioural and other adaptations of dominant species to the physical environment, and to incorporate these adaptations in future models of carbon and nitrogen flows in plankton communities.

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APPENDIX I

Publications arising from this study

1. Lucas, M.I., Painting, S.J. and Muir, D.G. 1986. Estimates of carbon flow through bacterioplankton in the southern Benguela upwelling region based on ^{3}H-thymidine uptake and predator-free incubations. In: GERBAM - Second International Colloquium of Marine Bacteriology, CNRS, Brest, October 1984. IFREMER, Actes de Coll. 3: 375-383.

2. Lucas, M.I., Probyn, T.A. and Painting, S.J. 1987. An experimental study of microflagellate bacterivory: further evidence for the importance and complexity of micro-planktonic interactions. In: The Benguela and Comparable Ecosystems. Payne, A.I.L., Gulland, J.A. and Brink, K.H. (Eds.). S. Afr. J. mar. Sci. 5: 791-808.

3. Newell, R.C., Lucas, M.I., Painting, S.J. and Field, J.G. 1985. Some estimates of carbon and nitrogen flux through pelagic communities in coastal waters. Proceedings of the 19th European Marine Biological Symposium, 1984, Gibbs P.E. (Ed.) pp 51-59.

4. Painting, S.J., Lucas, M.I. and Muir, D.G. 1989. Fluctuations in heterotrophic bacterial community structure, activity and production in response to development and decay of phytoplankton in a microcosm. Mar. Ecol. Prog. Ser. 53: 129-141.

5. Painting, S.J., Lucas, M.I., Peterson, W.T., Brown, P.C and Hutchings, L. (in prep.). Temporal changes in bacterial abundance and biomass during the development of phytoplankton and zooplankton communities in an upwelling plume in the southern Benguela.

6. Painting, S.J. and Lucas, M.I. (in prep.). Bacterial production in the southern and northern Benguela upwelling regions.

7. Painting, S.J., Peterson, W.T. and Lucas, M.I. (in prep.). The relative significance of bacteria and copepods in the carbon flux of the southern Benguela.

8. Painting, S.J., Moloney, C.L. and Lucas, M.I. (in prep.). Simulation and field measurements of phytoplankton - bacteria - zooplankton interactions in the southern Benguela region.

9. Moloney, C.L. and Painting, S.J. (in prep.). Trophic structure of the planktonic food web in the southern Benguela: improved understanding of bacterioplankton in overall plankton dynamics, from network analysis.

APPENDIX II

Auxilliary publications during this study

1. Muir, D.G., Seiderer, L.J., Davis, C.L., Painting, S.J. and Robb, F.T. 1986. Filtration, lysis and absorption of bacteria by mussels Choromytilus meridionalis collected under upwelling and downwelling conditions. S. Afr. J. mar. Sci. 4: 169-179.

2. Painting, S.J., Lucas, M.I. and Stenton-Dozey, J.M.E. 1985. The South African SIBEX I cruise to the Prydz Bay region, 1984: 10. Biomass and production of bacterioplankton in Prydz Bay, Antarctica, and phytoplankton, detritus and bacterial relationships. S. Afr. J. Ant. Res. (15): 42-52.

3. Probyn, T.A. and Painting, S.J. 1985. Nitrogen uptake by size-fractionated phytoplankton populations in Antarctic surface waters. Limnol. Oceanogr. 30 (6): 1327-1332.

4. Peterson, W.T., Painting, S.J. and Hutchings, L. 1989. Diel variations in gut pigment content, diel vertical migration and estimates of grazing impact for copepods in the southern Benguela upwelling region, in October 1987. Mar. Ecol. Prog. Ser. (subm.).

5. Peterson, W.T. and Painting, S.J. 1989. Developmental rates of the copepods Calanus australis and Calanoides carinatus in the laboratory. Mar. Ecol. Prog. Ser. (subm.).

6. Painting, S.J. and Huggett, J.A. 1989. Zooplankton biomass and production in relation to anchovy recruits in the southern Benguela, July 1988. Rep. Benguela Ecology Program no. 17.

7. Painting, S.J., Huggett, J.A., Horstman, D., Van der Byl, P. and Verheye, H. (in prep.). Spatial variation in chlorophyll concentration, cell size, copepod egg production and zooplankton biomass in the southern Benguela upwelling system.

8. Peterson, W.T., Painting, S.J. and Barlow, R.G. (in prep.). Feeding rates of Calanoides carinatus: a comparison of five methods, including measurements of pigment digestion.