Library

3. Library loan and photocopying permit

Title of thesis THE PHYSIOLOGICAL ECOLOGY OF THE RED-WATER CILIATE MESODINIUM RUBRUM.

Candidate DAVID W. CRAWFORD

Three copies of the above thesis are now formally submitted for the degree of

Ph.D. Should the Senate of the University award me either this degree or another higher degree in respect of this thesis, I hereby agree that from the date of the award the thesis may be made available for inter-library loan or for photocopying and that the abstract may be made available for publication.

Signature [redacted] Date 24.6.92

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF OCEANOGRAPHY

THE PHYSIOLOGICAL ECOLOGY OF THE RED-WATER CILIATE *MESODINIUM RUBRUM*

David William Crawford B.Sc.(Hons), M.Sc.

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

June 1992

UNIVERSITY OF SOUTHAMPTON
ABSTRACT

FACULTY OF SCIENCE
OCEANOGRAPHY
Doctor of Philosophy

THE PHYSIOLOGICAL ECOLOGY OF THE RED-WATER CILIATE *MESODINIUM RUBRUM*

by David William Crawford

Red-tides caused by the phototrophic marine ciliate *Mesodinium rubrum* occur each summer in the Southampton Water estuary and were investigated in the spring/summer of 1985 and in June 1986. The initiation of these blooms appeared not to be light or nutrient mediated, but coincided with periods of water column stability in both years. Stability was promoted both by thermal and salinity gradients, and also by low mixing during neap tides. Blooms were characterized by chlorophyll *a* concentrations of over 100 $\mu g\ l^{-1}$, cell numbers of 2-3 x $10^6\ l^{-1}$ and near surface oxygen levels of over 150% saturation. Bloom initiation was accompanied by rapid removal of ammonia from the water column, followed by depletion of nitrate when ammonia had become exhausted; however, a considerable quantity of nitrate remained in the water column even at the peak of the bloom. The maximal biomass of *M. rubrum* appeared to be light limited in the estuary; self-shading by high cell densities of the ciliate imposed an upper limit of ca. 300 mg chl*a* m^{-2}. Irradiance, even at 1 m depth, was only just sufficient (20-30 $\mu E\ m^{-2}\ s^{-1}$) for photosynthesis to cover estimated respiratory demands. Bloom decline appeared to coincide with a decrease in water column stability, though with a lag period.

Vertical distribution of *M. rubrum* was shown to be strongly aggregated in the upper 3 m; however, a 12 h tidal survey revealed an avoidance of flushing from the estuary by means of swimming downwards during periods of surface turbulence, such as that generated during ebb tide. Vertical distribution examined under more stable conditions in a brackish lake, Inre Verkviken, on the Åland Islands (Finland) in May 1991 suggested that, in the absence of turbulence, multiple population peaks occurred between the surface and 15 m depth. Since nutrients were limiting at the surface, and light was limiting in the nutricline, these peaks could represent migrating groups of either light or nutrient deficient cells, though not necessarily migrating on a diel cycle.

Considerable morphological variation occurred in the Southampton Water population just prior to and during red-tides. Irregularly shaped cells and fragments were commonly observed during red-tides and could have been cells undergoing multiple fission. This may explain the wide size distribution of this species observed during blooms. Non-bloom populations examined from several locations tended to be divided into two groups, of both small and large cells, but also with co-occurring intermediate forms.

Few factors could be identified which might limit the growth of individuals or populations, however, theoretical considerations suggested that *M. rubrum* and other fast swimming ciliates and dinoflagellates expend a significant proportion of their energy budgets on locomotion. A technique is described which precisely determines respiration rates of planktonic protists, based upon trapping the release of $^{14}CO_2$ from prelabelled cells. Since *M. rubrum* could not be cultured or prelabelled with $^{14}CO_2$, the technique was utilised on two other planktonic ciliates, *Strombidium capitatum* and *Favella ehrenbergi*. Specific respiration rates of individual cells were in the range 0.5 - 2.0% cell C h^{-1}, although smaller cells had higher rates of up to 5% cell C h^{-1}.

Using evidence from this thesis and a review of the literature, a tentative hypothesis is presented that *M. rubrum* is respiration limited in well mixed environments; that is, the cost of swimming may consume much of the photosynthate produced by the endosymbiont. When the water column stabilises and swimming activity is reduced, then much of the saved energy is channelled into cell growth.

"Knowledge is proud that he has learnt so much;
wisdom is humble that he knows no more"

William Cowper (1731-1800)

To Candice:

this is where Daddy's been

ACKNOWLEDGEMENTS:

"All wish to know, but few the price will pay"

Juvenal (*circa* 60-140 AD)

Firstly and mostly I would like to thank Candice and Nancy who paid the price with me, and Nancy particularly for the moral support - your turn next perhaps? Thanks also to Mum and Dad who kept me going without asking why.

I particularly thank my supervisors Dr Duncan Purdie and Professor Peter Lockwood, for their continuous support and guidance, and also Professor Michael Sleigh for his valuable advice. I am also grateful to a number of others from my time in Southampton; Chris Inman, for assistance with the electron microscope, Don Hutchinson for steering the boat and supplying anecdotes, Drs Nick Taylor and Simon Boxall for providing raw physical data, Kate Saull for drawing (and redrawing) countless figures, Dr Sean Comber for kindly undertaking the nitrate analyses.

I am grateful to Dr Diane Stoecker for her invitation to work in her laboratory at Woods Hole Oceanographic Institution, and for her hospitality and advice during my stay. Anne Michaels, Linda Davis and Dr Mary Putt also gave valuable assistance in the laboratory. Dr Lynn Margulis and Greg Hinkle of the University of Amherst, Massachusetts made the visit possible by their administration of the NASA PBI award. Dr Tore Lindholm kindly invited me to work at his laboratory in Finland, the Åbo Akademi field station at Husö (Åland Islands) and has given me much advice on *Mesodinium* over the years. Maud Carlsson, also gave assistance with sample collection and laboratory analyses, and showed considerable patience with my pathetic attempts to row the boat. Thanks also to all the other staff and students at Husö for their very special hospitality. During my fellowship at Millport, Dr Andrew Rogerson has given me much moral support and has commented on several portions of the thesis.

Elements of financial support were provided from a number of sources; NASA 'Planetary Biology Program'; Royal Society 'John Murray Travelling Studentship in Limnology and Oceanography'; University of Southampton 'Richard Newitt Prize'; University Marine Biological Station Millport. I should also mention numerous loans from my Parents when food was low, and of course countless raids on my daughters piggy bank (I'll pay you back!). Because of the vagaries of the academic funding system, I would have achieved nothing without the financial support during my earlier Masters Degree, both from my parents (which they could ill afford), and from the kind loan given by Drs Dickinson, Waygood and Wheeler (medical practice).

Finally, I would like to acknowledge the moral support and countless conversations from a number of friends - Quentin and Tina, Franklin, Lawrence, Steve, and many others, and of course Duncan Purdie's 'class of 89' - Henry, Arantza, Virginia, Demek, Sandy and, last but not least, Giovanni, who knows only too well about completing a PhD the hard way.

TABLE OF CONTENTS:

CHAPTER ONE:
A BRIEF HISTORICAL EXCURSION

"Let knowledge grow from more to more,
But more of reverence in us dwell"

Tennyson (1809-1892)

One day in July 1676, on a visit to the Dutch seaside resort of Scheveningen, the so called 'father of protozoology', Anthony van Leeuwenhoek, recorded his observations in a letter[1]:

> "Finding myself upon the shore (the wind coming off the sea, with very warm sunshine), and observing the sea-water as well I could, I discovered in it divers living animalcules. I gave to a certain person, who went into the sea to bathe himself, a new glass phial (which I had bought on purpose) and besought him that, when he was in the sea, he would rinse it out twice or thrice and then fill it up with water. This having been carried out according to my orders, I tied the phial up tight with a clean bit of bladder: and on reaching home and examining the water, I perceived therein a little animal that was blackish, having a shape as if 'twere made of two globules. This little animal had a peculiar motion, after the manner of a very little flea, when seen, by the naked eye, jumping on a white paper; yet 'twas only displaced, at every jump, within the compass of a coarse sand-grain, or thereabouts. It might right well be called a water-flea; but 'twas not so big, by a long way, as the eye of that little animal which Swammerdam calls the water-flea[2]."

This appears to be the first documented reference to an observation of a marine protist by van Leeuwenhoek. Again in 1839, another eminent naturalist, Charles Darwin, described observing a similar organism, occurring in much greater

---

[1] See Dobell (1932) letter no. 18.

[2] i.e. *Daphnia* sp.

numbers off the coast of Chile. Darwin gave a particularly detailed description after passing through an area of discoloured water:

> "On the coast of Chile, a few leagues north of Concepcion, the Beagle one day passed through great bands of muddy water, exactly like that of a swollen river; and again, a degree south of Valparaiso, when fifty miles from the land, the same appearance was still more extensive. Some of the water placed in a glass was of a pale reddish tint; and, examined under a microscope, was seen to swarm with minute animalcula darting about, and often exploding. Their shape is oval, and contracted in the middle by a ring of vibrating curved ciliæ. It was, however, very difficult to examine them with care, for almost the instant motion ceased, even while crossing the field of vision, their bodies burst. Sometimes both ends burst at once, sometimes only one, and a quantity of coarse, brownish, granular matter was ejected. The animal an instant before bursting expanded to half again its natural size; and the explosion took place about fifteen seconds after the rapid progressive motion had ceased: in a few cases it was preceded for a short interval by a rotatory movement on the longer axis. About two minutes after any number were isolated in a drop of water, they thus perished. The animals move with the narrow apex forwards, by the aid of their vibratory ciliæ, and generally by rapid starts. They are exceedingly minute, and quite invisible to the naked eye, only covering a space equal to the square of the thousandth of an inch. Their numbers were infinite; for the smallest drop of water which I could remove contained very many. In one day we passed through two spaces of water thus stained, one of which alone must have extended over several square miles. What incalculable numbers of these microscopic animals! The colour of the water, as seen at some distance, was like that of a river which has flowed throuh a red clay district; but under the shade of the vessel's side it was quite as dark as chocolate. The line where the red and blue water joined was distinctly defined. The weather for some days previously had been calm, and the ocean abounded, to an unusual degree, with living creatures".

Although there is circumstantial evidence of earlier synonyms, Lohmann (1908) was the first author to name an organism of such a description; he called it *Halteria rubra*, placing it in the ciliate genus created by Claparède & Lachmann. His account described coloured 'platelets' contained within the ciliate which were extruded upon disintegration, and also free-living uniflagellates which he named

Erythromonas haltericola, and which both he, and Apstein (1908), considered to be a free-living stage of a symbiotic alga.

Paulsen (1909) described a water bloom in an Icelandic fjord which was attributed to *Mesodinium pulex*, but did in fact also state that this was probably the same organism as *Halteria rubra* Lohmann. Meunier (1910) also presented a rather incomplete description of a similar ciliate from the Barents Sea, this was based only upon fixed specimens and was tentatively ascribed to the genus *Cyclotrichium*, though without a specific name. *Cyclotrichium* is a genus characterised by an equatorial girdle of cilia, but without one of cirri.

Halteria rubra was subsequently placed by Hamburger & Buddenbrock (1911) in the genus *Mesodinium*, created by Stein (1862) for the taxon formerly known as *Halteria pulex* Claparède & Lachmann. Thus *Halteria rubra* Lohmann then became *Mesodinium rubrum* (Lohmann) Hamburger & Buddenbrock, within the family Didiniidae. Leegard (1920) distinguished apparently co-existing populations of large and small cells of *M. rubrum* in the Gulf of Bothnia, Baltic Sea, and referred to them as 'forma major' and 'forma minor' respectively. However, it was not clarified as to whether these represented separate taxa. Several other studies recorded *M. rubrum* in moderate numbers (e.g. Gran 1929*a,b*, Føyn 1929, Garder 1932), whereas Kahl (1930) mentioned the occurrence of *M. pulex* with a symbiont presumed to be a blue-green alga.

A red-tide was observed by Powers (1932) in the Gulf of Maine and the causative organism described as a new species *Cyclotrichium meunieri* Powers; the fixed material presumably resembled that of Meunier (1910). It was unfortunate however that neither author had observed the presence of cirri because of their disorganisation upon fixation. Hart (1934) and Clemens (1935) both attributed subsequent red-water events to *M. rubrum*, but it was Kahl (1935) who was the first to suggest the possibility of conspecificity between *C. meunieri* and *M. rubrum*.

Many accounts, either of blooms or simply species listings, then favoured the name *M. rubrum* (Braarud & Bursa 1939, Hart 1943, Grøntved 1952, Braarud & Hope 1952, Braarud *et al.* 1953, 1958, Grøntved & Nielsen 1957). However, Bary & Stuckey (1950) and Bary (1953) described red-tides in New Zealand waters, and identified their specimens *C. meunieri* on the basis of the similarity of their material with that of Powers (1932) despite observing the presence of cirri in their live material. Later in the 1950's a number of publications referred to red-water as being caused by *M. pulex* (Slobodkin 1953, Halme 1958, Margalef 1956) and these presumably were actually referring to *M. rubrum*.

A red-tide was observed by Hart & Currie (1960) off the coast of Angola, the causative organism was given as *C. meunieri*, but they repeated the speculation by Kahl (1935) of conspecificity with *M. rubrum*. Bakker (1966, 1967*a,b*) reverted to the name *M. pulex*, arguing that the association with the cryptophyte was in fact facultative, but Agamaliev (1967) and Fonds & Eisma (1967) were more tentative and adopted the name *M. pulex f. rubrum*. Interest grew through the 1960's, but a confusing dichotomy persisted with some authors referring to *C. meunieri* (e.g. Buchanan 1966, Ryther 1967, McAlice 1968, Barber *et al.* 1969) and others to *M. rubrum* (e.g. Lackey & Clendenning 1963, 1965, Lackey 1963, 1967, Lackey & Lackey 1963, Bursa 1961*a,b*, 1963, Michanek 1965, Parsons & Blackbourn 1968). Fenchel (1968*a*) described a red-tide caused by *M. rubrum* in Danish waters, but again emphasised the likelyhood of conspecificity with *C. meunieri*. The studies of Bakker (1967*a,b*), Ryther (1967), Parsons & Blackbourn (1968) and Barber *et al.* (1969) confirmed the cryptophyte identity of the endosymbiont by pigment analysis, and first demonstrated autotrophy during red-tides of both species by means of high chlorophyll, O_2 production, ^{14}C fixation and ^{32}P assimilation. This prompted much interest, and Taylor *et al.* (1969, 1971) finally clarified the taxonomic debate with ultrastructural studies and a thorough review. They suggested that *Mesodinium rubrum* (Lohmann) Hamburger & Buddenbrock and *Cyclotrichium meunieri* Powers should be considered conspecific as the former taxon, on the basis of the presence of the equatorial girdle of cirri. They also questioned Lohmann's observations of a free-living uniflagellate stage of the association, as well as Bakker's contention of a facultative endosymbiosis. This they argued on the basis that the exceptional thinness of the pellicle of *M. rubrum*, with lack of alveoli, seem major structural modifications not easily accomplished on a short-term basis. This study marked the beginning of a period of considerable interest in the taxonomy, ultrastructure, ecophysiology and distribution of this species.

Into the 1970's, the ultrastructure of the association was further examined; for a population from British Columbia, Taylor *et al.* (1969, 1971) had suggested that the cryptophyte chloroplasts and mitochondria were in fact isolated within small 'islands' of cytoplasm within ciliate cytoplasm, but which had no connections with a separate 'island' which contained the algal nucleus and some further mitochondria. However, Hibberd (1977) showed that in a separate population from the U.K., the algal parts were all located within the same algal cytoplasm. Oakley & Taylor (1978), on another population from British Columbia, later confirmed the earlier work of Taylor *et al.* (1971), although Grain *et al.* (1982) did observe traces of strands of connecting cytoplasm between the 'islands' containing cryptophyte chloroplasts and that containing the nucleus. This issue awaits clarification and it may be that different subspecies exist, or that the two conditions described represent developmental stages within growing individuals.

More recently, Small & Lynn (1985) formed a new family, Mesodiniidae, distinct from the Didiniidae, and created a new species and genus, *Myrionecta rubra* Jankowski, for what was formerly known as *Mesodinium rubrum* (Lohmann) Hamburger & Buddenbrock. This was based on the proposition, though only in the form of an abstract, of Jankowski (1976). The name change appears to have been rather premature, in that it is based upon the presumed absence of 'oral' tentacles in *M. rubrum* (*Myrionecta rubra*) which are present in other species of *Mesodinium*. However, these have more recently been shown by Lindholm *et al.* (1988) to be actually present in some populations of *M. rubrum*, and moreover to be a rather non-conservative character insofar as they are very easily lost in both live and fixed specimens. Because of these arguments many recent studies have persisted with the name *Mesodinium rubrum*.

With respect to physiological ecology, bloom studies in the 1970's & 80's provided ample evidence of the photosynthetic capacity of this organism; Packard *et al.* (1978), Setchell *et al.* (1979), Smith & Barber (1979), Hendrikson *et al.* (1982), Platt *et al.* (1980), Sellner (1981) and Cabeçadas *et al.* (1983) being notable examples. Many other red-tides caused by *M. rubrum* were also documented around the world from the 1970's onwards, particularly in South American Waters (e.g. Campodonico *et al.* 1975, Guzman & Campodonico 1978, Rodriguez 1978, Avaria 1979, Sorokin 1979, Sorokin & Kogelschatz 1979, Hernández-Becerril 1987, Jimenez & Intriago 1987, Dugdale *et al.* 1987). Other reports have come from Portuguese waters (Sampayo & Cabeçadas 1981), the Black Sea (Tumantseva 1985) and San Fransisco Bay (Cloern *et al.* 1985).

Water discoloration has also been noted to be caused by other unidentified ciliates (e.g. Holm-Hansen *et al.* 1970, Dale & Dahl 1987), but these appear to be rather rare occurrences.

The trophic position of *M. rubrum* as a photosynthetic ciliate caused much early confusion, resulting in its omittance from most phytoplankton counts until recently. This problem has been discussed in a recent critical review (Crawford 1989) which highlighted a serious underestimation of *M. rubrum*, at non-bloom densities, in coastal, estuarine and upwelling ecosystems. Many studies have pointed out its importance at non-bloom densities; for example in Baltic waters (Eriksson *et al.* 1977, Elmgren 1984, Mamaeva 1985, Andersen & Sørensen 1986, Kivi 1986), the North Sea (Gieskes & Kraay 1983, Baars & Franz 1984), the N.W. Atlantic (Blasco *et al.* 1980, 1981) the East Pacific (Beers *et al.* 1971, Chester 1978, Reid *et al.* 1978, Takahashi & Hoskins 1978, Cullen *et al.* 1982) and New Zealand waters (Mackenzie & Gillespie 1986, Mackenzie *et al.* 1986). In addition to trophic misinterpretation, other problems exacerbating such underestimation were highlighted; these were associated with sampling, enumeration and estimates of productivity (Crawford 1989).

Recent studies have shown that *M. rubrum* is not the only important photosynthetic ciliate. After earlier suspicions that marine planktonic ciliates harboured functional plastids (Blackbourn *et al.* 1973, McManus & Fuhrmann 1986), many have now been shown to photosynthesize; species of *Strombidium*, *Laboea* and *Tontonia* have been shown to be mixotrophic (Jonsson 1987, Stoecker *et al.* 1987, 1988, 1988/89, Laval-Peuto *et al.* 1986, Laval-Peuto & Rassoulzadegan 1988, Putt 1990*a*, Stoecker 1991). Moreover, some studies suggest that on average 40-50% of total planktonic ciliates in coastal waters are mixotrophic (Stoecker *et al.* 1987, Laval-Peuto & Rassoulzadegan 1988, Putt 1990*b*). Thus, given the now acknowledged critical role of ciliates in marine ecosystems (Beers & Stewart 1967, 1969*a,b*, 1970, 1971, Beers *et al.* 1975, 1980, Smetacek 1981, Burkill 1982, Sherr *et al.* 1986*a,b*, Sanders 1987, Montagnes & Lynn 1987, 1988*a,b*), such mixotrophic ciliates together with *M. rubrum* must make a highly significant contribution to overall primary productivity (e.g. Stoecker 1991). As such, great care should be taken with terminolgy and trophic classification of these photosynthetic forms (e.g. Sieburth *et al.* 1978, Sieburth & Estep 1985, Crawford 1989).

CHAPTER TWO:
RECURRENT RED-TIDES

> *"The disappearance of the plankton, although the components are microscopic, would probably in a short time eliminate every living thing in the sea and change the whole of man's life, if it did not through a seismic disturbance of balance eliminate all life on the globe. For these little animals, in their incalculable numbers, are probably the base food supply of the world. But the extinction of one of the rare animals, so avidly sought and caught and named, would probably go unnoticed in the cellular world"*
>
> John Steinbeck (Sea of Cortez)

2.1. INTRODUCTION

Brief reports of red-water caused by *M. rubrum* have appeared in the literature throughout the first half of this century (e.g. Paulsen 1909, 1934, Hart 1934, 1943, Clemens 1935, Margalef 1956, Hart & Currie 1960); some of these providing preliminary descriptions of the organism itself (Powers 1932, Bary & Stuckey 1950). However, it was not until the mid-1960's that mass occurrences of this species began to attract widespread attention and more detailed investigations (Bakker 1966, 1967*a*, Fonds & Eisma 1967, Ryther 1967, Fenchel 1968*a*, McAlice 1968, Parsons & Blackbourn 1968, Barber *et al.* 1969, Taylor *et al.* 1969, 1971, Avaria 1970). All of these studies either suspected or verified its photosynthetic activity. Since then Packard *et al.* (1978), Smith & Barber (1979), Platt *et al.* (1980) and Cabeçadas *et al.* (1983), have demonstrated it to be an exceptionally productive phototroph. For example, during a dense oceanic bloom in the upwelling area off the coast of Peru, Smith & Barber (1979) measured values for chlorophyll *a* as high as 1000 mg m^{-3}, an assimilation number of 16.8 mgC (mgChla)$^{-1}$ hr^{-1} and a productivity rate of over

2000 mgC m^{-3} hr^{-1}. Taylor (1982) considered this rate to be the highest aquatic micro-organismal primary productivity on record.

However, the majority of such studies have resulted from chance encounters with red-water and have thus tended to simply document their numerical occurrence and decline. Few researchers, if any, have had the opportunity to follow the development, maintenance and collapse of these events in a coherent time series. Consequently, very little information is available in any dynamic context on the requirements for initiation of blooms, and on those factors involved with their perpetuation and decline. Similarly, whilst these red-tides caused by *M. rubrum* appear not to be directly toxic (Lindholm 1985), the effects upon other organisms, and upon the chemical and physical properties of the water bodies in which they occur, are not known.

The biology of the organism itself is poorly described, both because of the above factors, and, due to the structural, behavioural and physiological peculiarities of this ciliate/alga association. *M. rubrum* is extremely fragile (Lindholm 1985) and thus does not respond well to laboratory containment and physiological studies (Crawford 1989); perhaps for the same reasons, all attempts at culture have repeatedly failed. *M. rubrum* swims extremely quickly (up to 8.5 mm sec^{-1}; Lindholm 1985; Jonsson & Tiselius 1990), but alternates this with periods of motionlessness; this presents a particular problem to microscopical investigation of living cells (Crawford 1989). Coupled with its phototactic and rheotactic behaviour (Lindholm 1985), such swimming also results in further difficulties in field sampling and laboratory sub-sampling (Crawford 1989). Moreover, the above factors have contributed, together with trophic misinterpretation, to a serious underestimation of its role as a regular member of the marine phytoplankton (Crawford 1989).

This study provided the first known opportunity for a detailed investigation throughout a red-water event caused by *M. rubrum*. The object of the study was firstly to provide a general descriptive account on the characteristics of the blooms, and to identify potential environmental 'triggers' which might be critical to red-water appearance, and limiting factors to its intensity and duration. It was anticipated that this information could then be combined with accumulating data from non-bloom studies to give insights into the mechanisms by which red-water is formed and maintained. Potential factors could then be tested in later chapters using a combination of laboratory methods, theoretical analysis and literature review. Implicit in the overall aims of the investigation is the understanding of the factors regulating the growth of regular, non-bloom populations.

2.2. SAMPLING AND SITE DESCRIPTION

Southampton Water is a partially stratified, shallow coastal plain estuary (Dyer, 1973). The estuary is an approximately linear body of water with dimensions about 16 km long and 2.5 km wide at its widest point, and has a central channel dredged to a depth of about 10 metres below chart datum. At its northern end it is fed by the Test and Itchen rivers which converge at Dock Head, with the Hamble river flowing into its eastern side. The maximum combined river flow into the estuary is about 28 m^3 s^{-1} in late winter, and about 14 m^3 s^{-1} during spring and summer (Dyer, 1973). It is an estuary characterised by a peculiar tidal regime consisting of a double high water, some two hours apart, followed by a short ebb-tide with near surface currents reaching 0.5-1.0 m s^{-1} (Dyer 1973). The tidal range varies between 1.5 and 5.0 metres.

Field sampling was conducted at 3 regular stations within the Southampton Water estuary, over a period of six months during the spring and summer of 1985. The positions of these stations are shown in Figure 2.1; Cracknore and North West Netley are buoys within the dredged channel of the estuary, profiles were taken as close to these as feasible. Princess Alexandra Dock is a small, semi-enclosed dock, and in which profiles were taken approximately from the centre. Sampling was undertaken as close as feasible to low water, on neap tides, at approximately 2 weekly intervals; this sampling strategy was adopted to allow integration of data with that of an earlier study of the estuary by Rees & Williams (1982). A more intensive

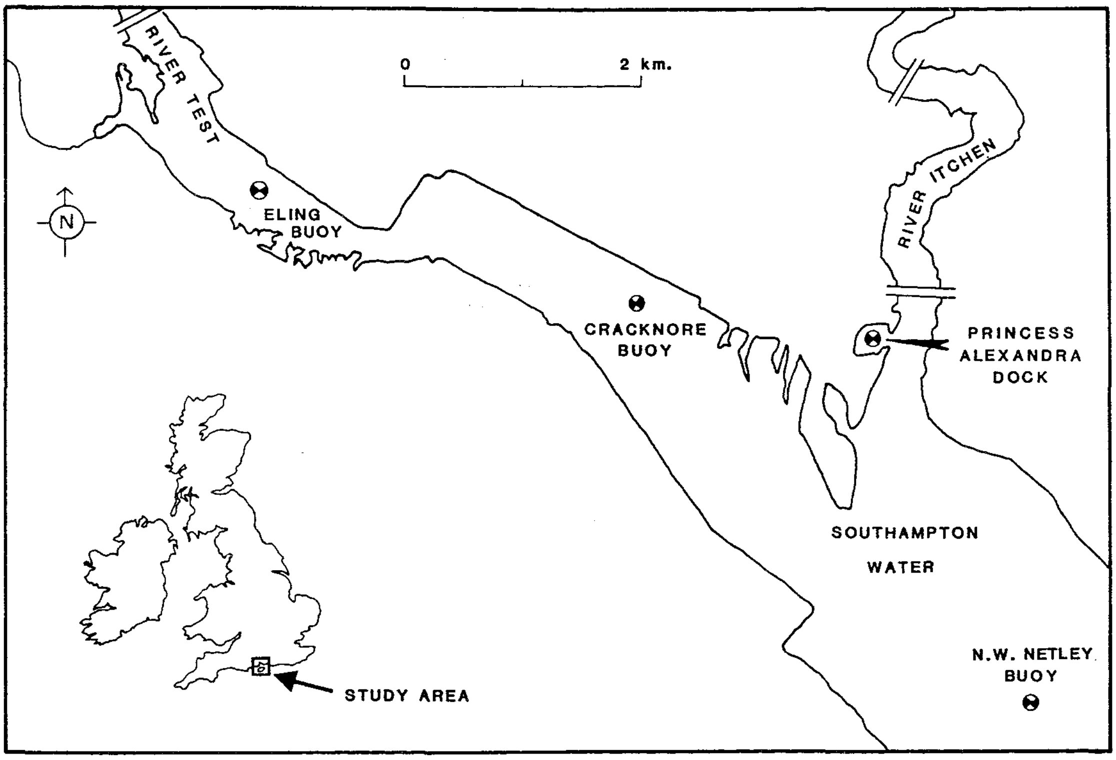


Figure 2.1.

Location of the sampling stations within the Southampton Water estuary.

programme of study was conducted during the onset of the bloom in Princess Alexandra Dock, in June 1986, over 6 sampling dates. During this study it was impossible to restrict sampling to a particular state of tide, since sampling was conducted approximately twice per week.

At each station, vertical profiles of temperature, salinity, oxygen and light intensity were recorded and water samples taken for cell counts, chlorophyll and nutrient analyses[1].

2.3. RESULTS

2.3.1. 1985 Study

Cell Numbers

The progress of the bloom is documented over the summer of 1985 by Figure 2.2; this shows total numbers of cells (m^{-2}) integrated from the surface to the bottom for the three stations. The general characteristics of the bloom are similar to those observed in other years (Rees & Williams 1982, Soulsby *et al.* 1984), though perhaps occurring slightly earlier. The bloom lasted between late May and late July, but with a temporary decline in mid-June coinciding with stormy weather. The use of total integrated numbers in Figure 2.2 demonstrates that red-water appearance is not simply a case of behavioural aggregation of cells at the surface, but a real increase throughout the water column

Variation of cell number with depth of *M. rubrum* is shown for each station in Figure 2.3; these plots are rather confused, although this reflects the degree of patchiness associated with blooms of this ciliate. Vertical profiles are given more graphically and with greater resolution for the 1986 study described later. It is interesting to note that the onset of the bloom at N.W. Netley was characterised by a deeper maximum (3-6m), whereas at P.A. Dock and Cracknore the peak was close

---

[1] Technical details of field sampling, laboratory analyses and data manipulation are given in appendices 1, 2 & 3.

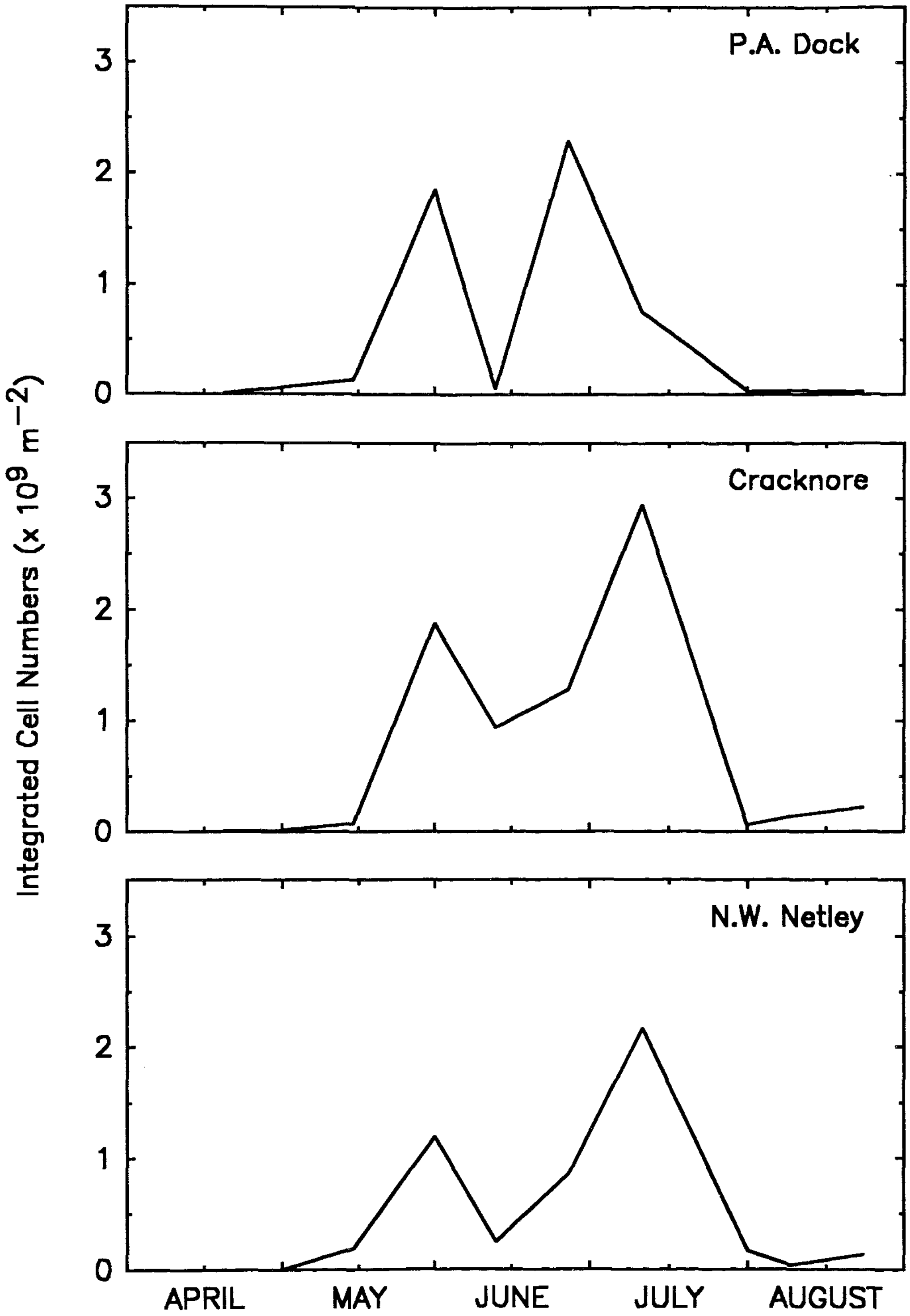


Figure 2.2.

Variation of total numbers of *Mesodinium rubrum* (integrated over depth) in the water column over spring/summer 1985.

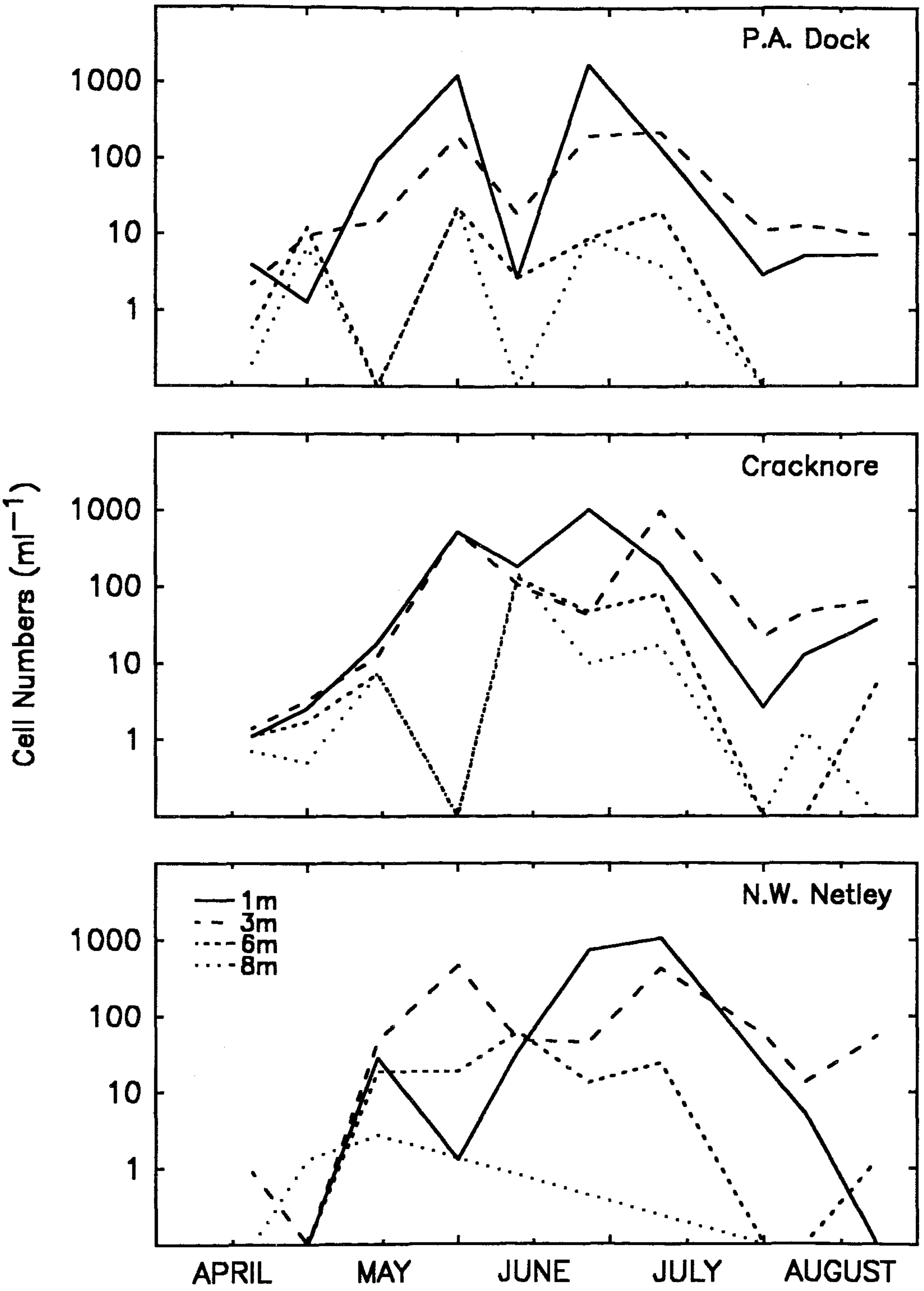


Figure 2.3.

Variation in abundance of *Mesodinium rubrum* (log scale) at 4 discrete depths over spring/summer 1985.

to the surface. This may result from the much greater degree of water column stability, as shown by temperature, salinity and density profiles (discussed later), at these latter two stations in late May. The bloom commenced in late May and continued until early July, with a temporary decrease in numbers apparently coincident with poor weather in mid-June. Red-water was visually apparent when cell numbers exceeded about 100-200 ml^{-1} (see also McAlice 1968), although this is, of course, a rather subjective estimate.

Chlorophyll

Figure 2.4 shows the strong association between numbers of *M. rubrum* and both chlorophyll concentration, and, % chlorophyll; the latter being defined as percentage chlorophyll *a* over chlorophyll + phaeopigments. This denotes whether a high level of phaeopigments is present, these being degradation products of chlorophyll. The results suggest that chlorophyll, in the form of *M. rubrum*, was photosynthetically in a highly active and healthy state during the bloom; even at low cell densities (1 - 10 ml^{-1}), phaeopigments did not increase above about 30-40% of total. However, at such low cell densities, the contribution of other phytoplankton to pigment concentrations will clearly be much more significant.

Because of the strong association existing between chlorophyll and numbers of *M. rubrum*, chlorophyll levels were only determined at one station, Cracknore, since chlorophyll gave little further information above that given by cell numbers. Figure 2.5 shows chlorophyll variation at 4 discrete depths over the bloom period at Cracknore; both concentration of chlorophyll *a* and % chlorophyll are shown. Chlorophyll clearly showed a similar seasonal pattern to that of cell numbers, although detectable chlorophyll in the deeper samples where cell numbers were very low - when the bloom commenced, and as the bloom declined - suggested the presence of other phytoplankton at these times. Higher levels of phaeopigments (lower % chlorophyll) were generally associated with deeper samples during the bloom, except during the stormy conditions in mid-June, when higher % chlorophyll values in deep samples reflected the mixing of chlorophyll throughout the water column (Figure 2.5).

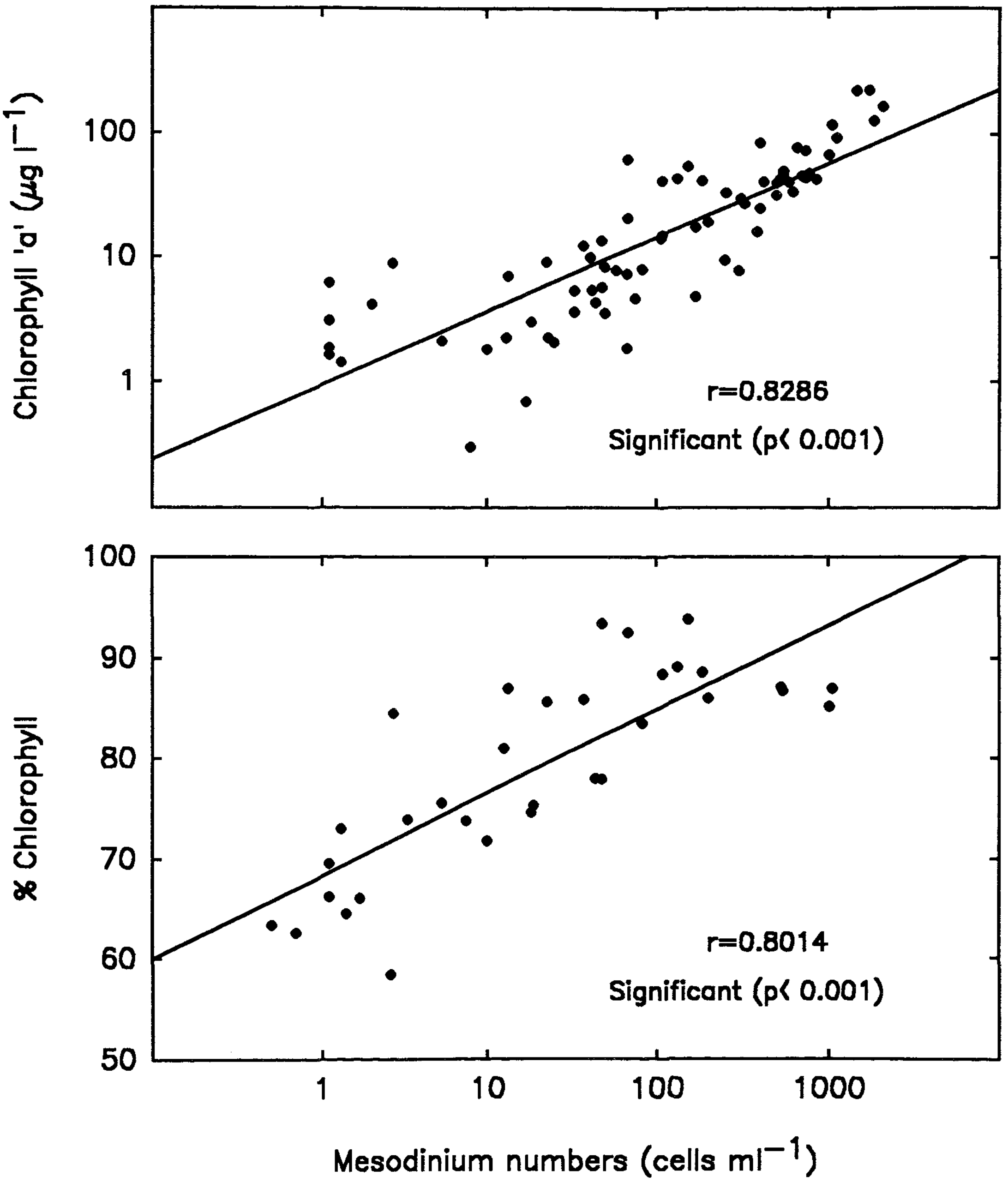


Figure 2.4.

Regressions of chlorophyll *a* concentration (log scale) and % chlorophyll (defined in text) on abundance of *Mesodinium rubrum*.

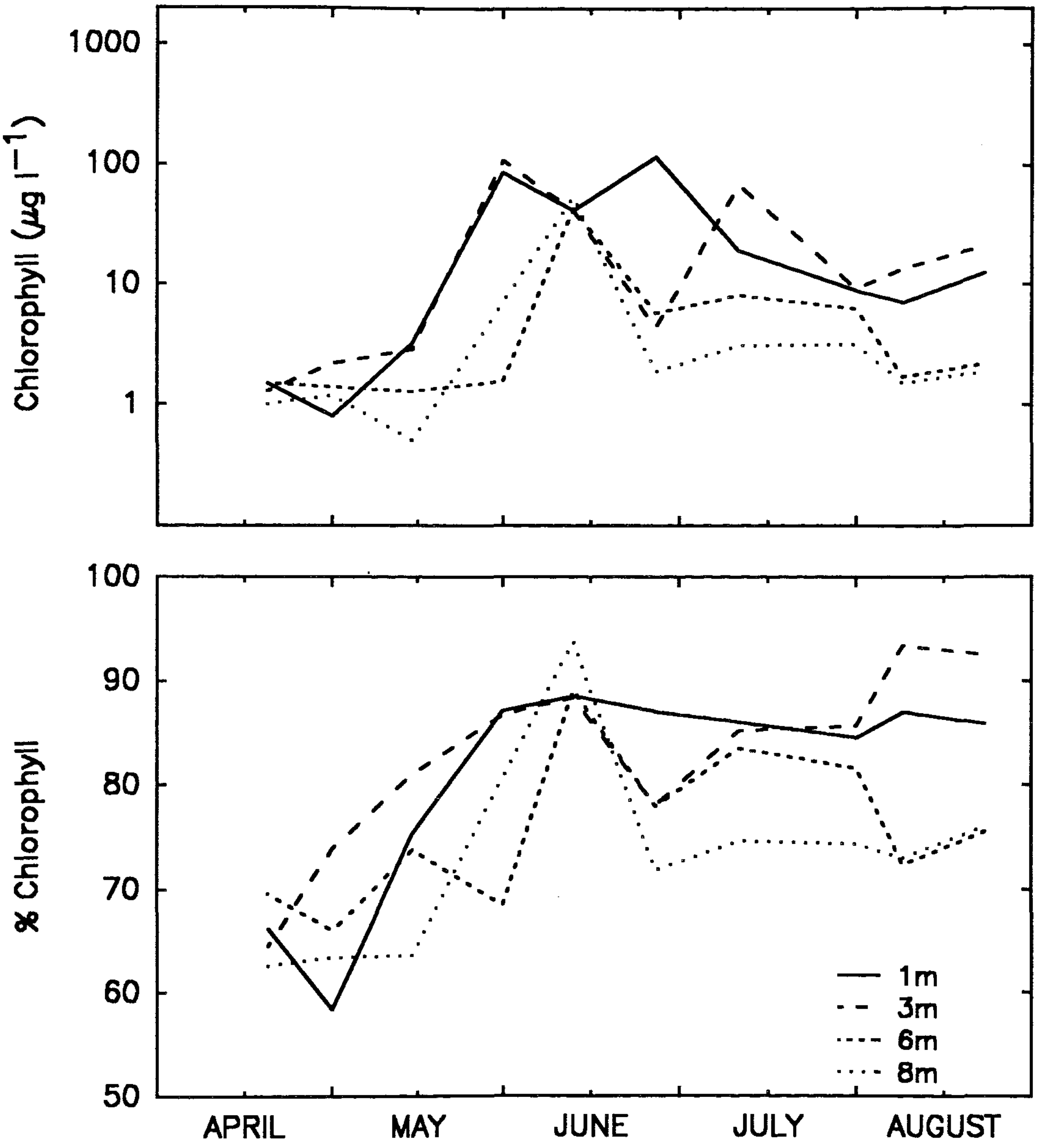


Figure 2.5.

Variation in chlorophyll *a* concentration (log scale), and % chlorophyll (defined in text) over spring/summer 1985 at Cracknore buoy.

Water Column Structure

The variation in temperature and salinity at the three stations over spring and summer 1985 is shown in Figures 2.6 & 2.7 respectively. Temperature profiles are very similar at the three stations, though with slightly greater stratification higher up the estuary at P.A. Dock and Cracknore than at N.W. Netley.

Stratification was greater at all stations during the period preceeding the appearance of the red-water, in late April and May. It is interesting to note that a maximum temperature stratification of 1.5°C (between 1m and 8m) occurred at P.A. Dock in late May when the bloom first appeared.

Salinity stratification is also shown to be greater higher up the estuary, at P.A. Dock and Cracknore; again, the most intense salinity stratification (4.5‰ between 1m and 8m) coincided with red-water appearance at P.A. Dock.

Temperature and salinity are only co-determinants influencing the actual stability of the water column; if an examination of true static stability of the water column is required, then temperature and salinity must be transformed into density profiles. The variation in density stratification over the study period is given in Figure 2.8. This shows a particularly marked density stratification at P.A. Dock over the whole summer, but particularly through June and July when the bloom was most intense. Less stratification was apparent at Cracknore and even less at N.W. Netley. The decline in the bloom through July was accompanied by a decline in density stratification at all three stations. The decline in the bloom in mid-June, during wet and windy weather, was not reflected in a breakdown in salinity or density profiles, but rather more so in those of temperature. This is probably due to wind and tidal mixing (spring tides), and lack of sunshine, disrupting the thermal gradient, with salinity and thus density stratification being maintained by freshwater input.

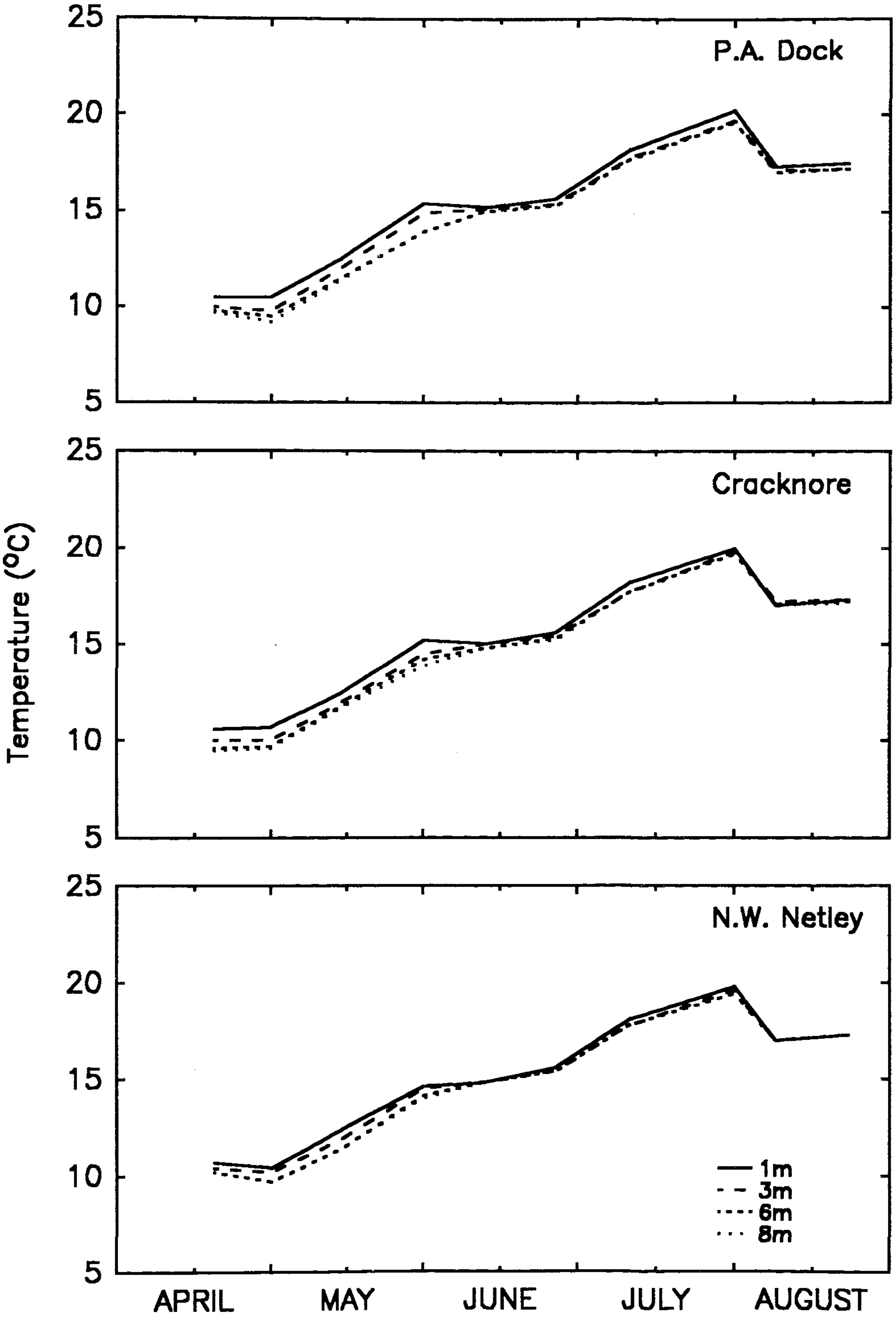


Figure 2.6.

Variation of temperature at 4 discrete depths over spring/summer 1985.

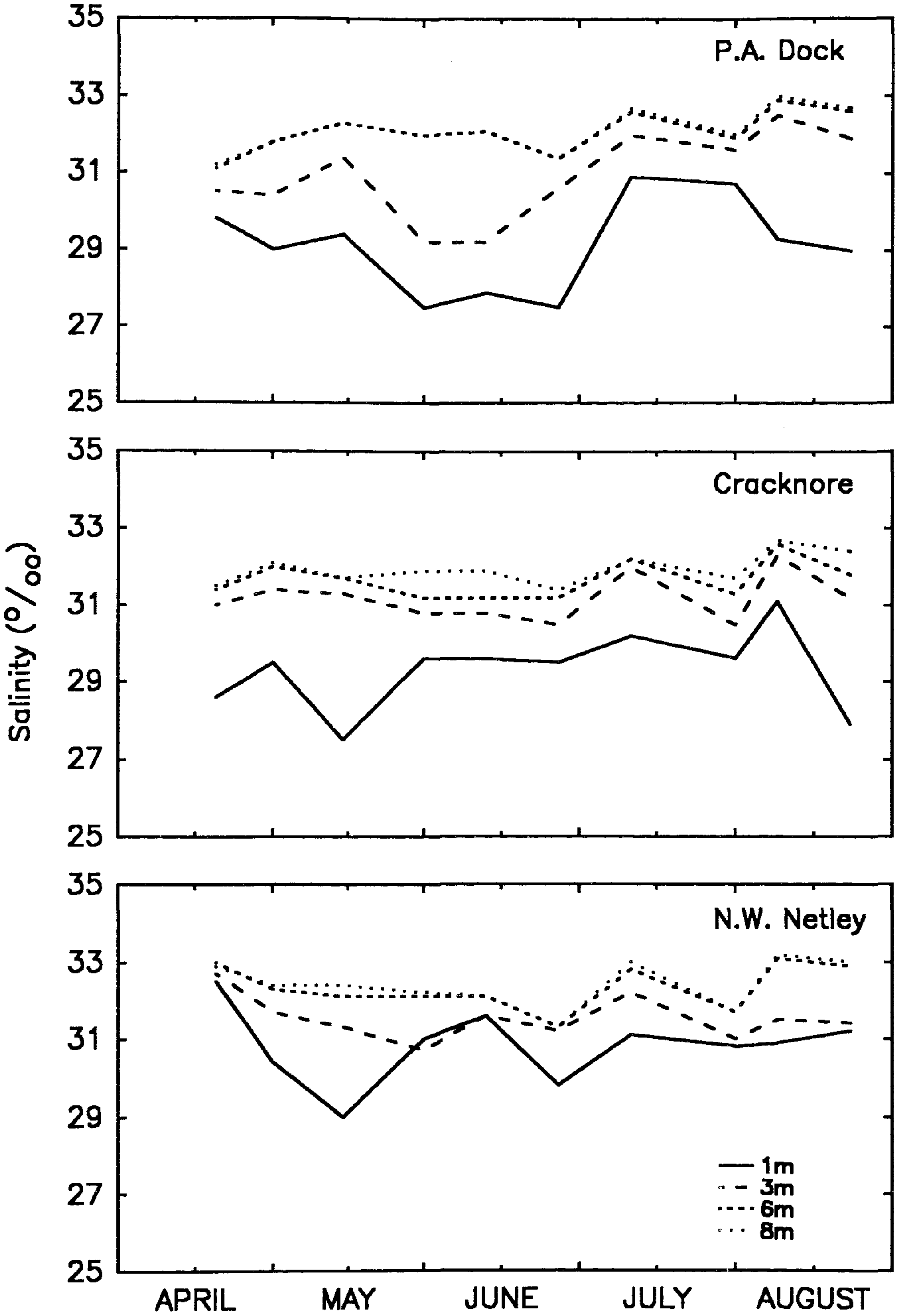


Figure 2.7.

Variation of salinity at 4 discrete depths over spring/summer 1985.

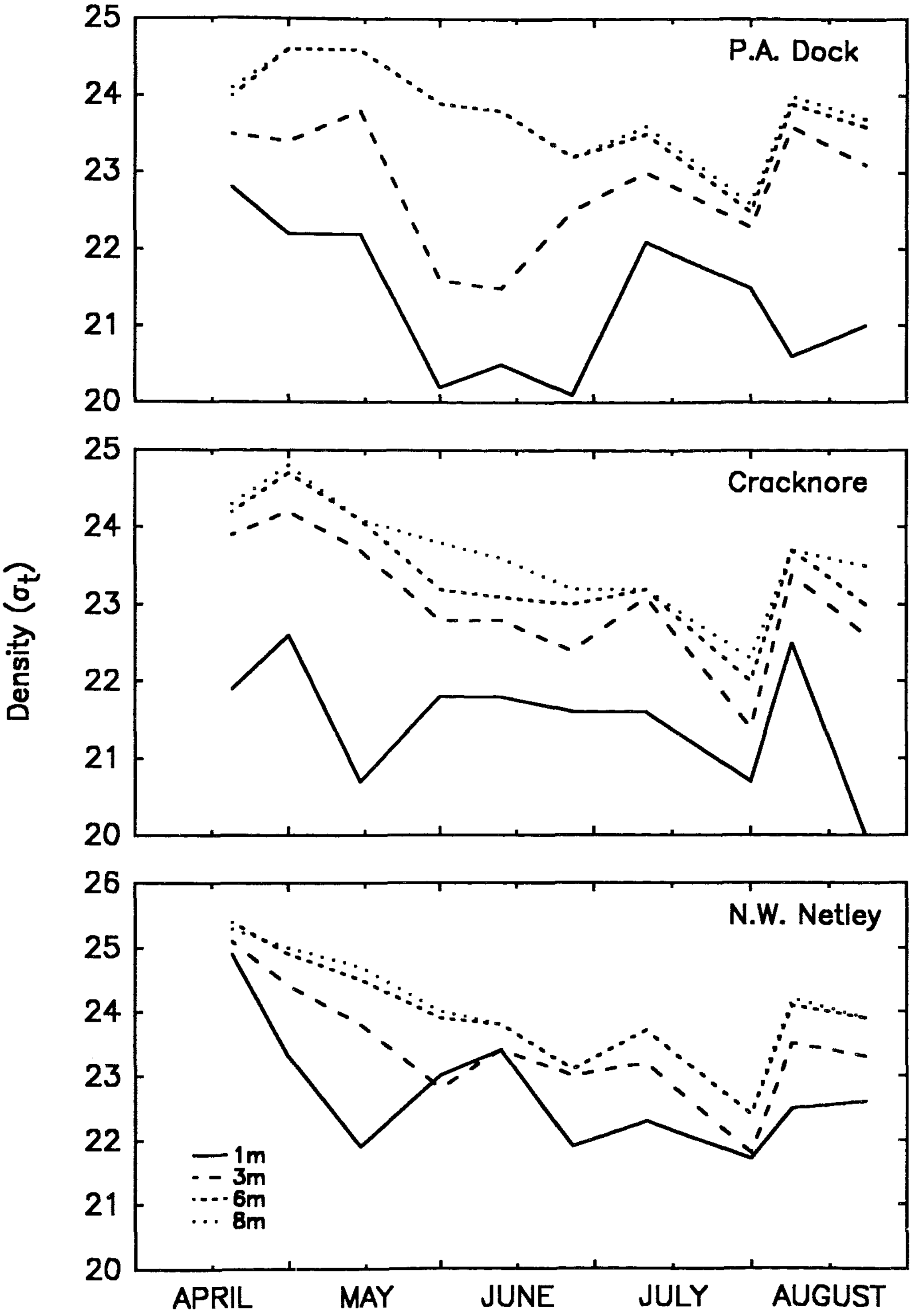


Figure 2.8.

Variation of density at 4 discrete depths over spring/summer 1985.

Light

Profiles of photosynthetically active radiation (PAR) are shown for each station through April and May in Figure 2.9. A downward trend is apparent in the PAR reaching subsurface waters over this period. This occurs despite an increase in surface PAR during development of the bloom in late May, and probably results from the self-shading effect of increasing cell numbers. This is particularly marked in late May when red-water first appeared; for example, PAR at 1m depth is only 3.4% that at the surface at P.A. Dock at this time. Also of note is the observation that the vast majority of the population during this period are residing at PAR levels significantly less than photosynthetic saturation values of I_k of 275 μE m^{-2} s^{-1} derived recently for isolated cells of *M. rubrum* (Stoecker *et al.* 1991).

Extinction coefficients (k) are given through April & May 1985 in Table 2.1; although showing considerable variation due to the presence of other phytoplankton and particulate matter prior to the bloom, k increases significantly in late May at P.A. Dock and Cracknore where the red-water bloom is most intense.

	N.W. Netley		Cracknore		P.A. Dock	
Date	k	r	k	r	k	r
19.4	0.54	0.9776	0.64	0.9986	0.65	0.9930
30.4	0.42	0.9955	0.74	0.9928	0.49	0.9968
14.5	0.49	0.9968	0.35	0.9968	0.53	0.9950
30.5	0.68	0.9232	0.98	0.9557	1.17	0.9656

Table 2.1. Water column extinction coefficients k (m^{-1}) for each station to May 1985, with coefficient (r) for the exponential regression of light extinction with depth.

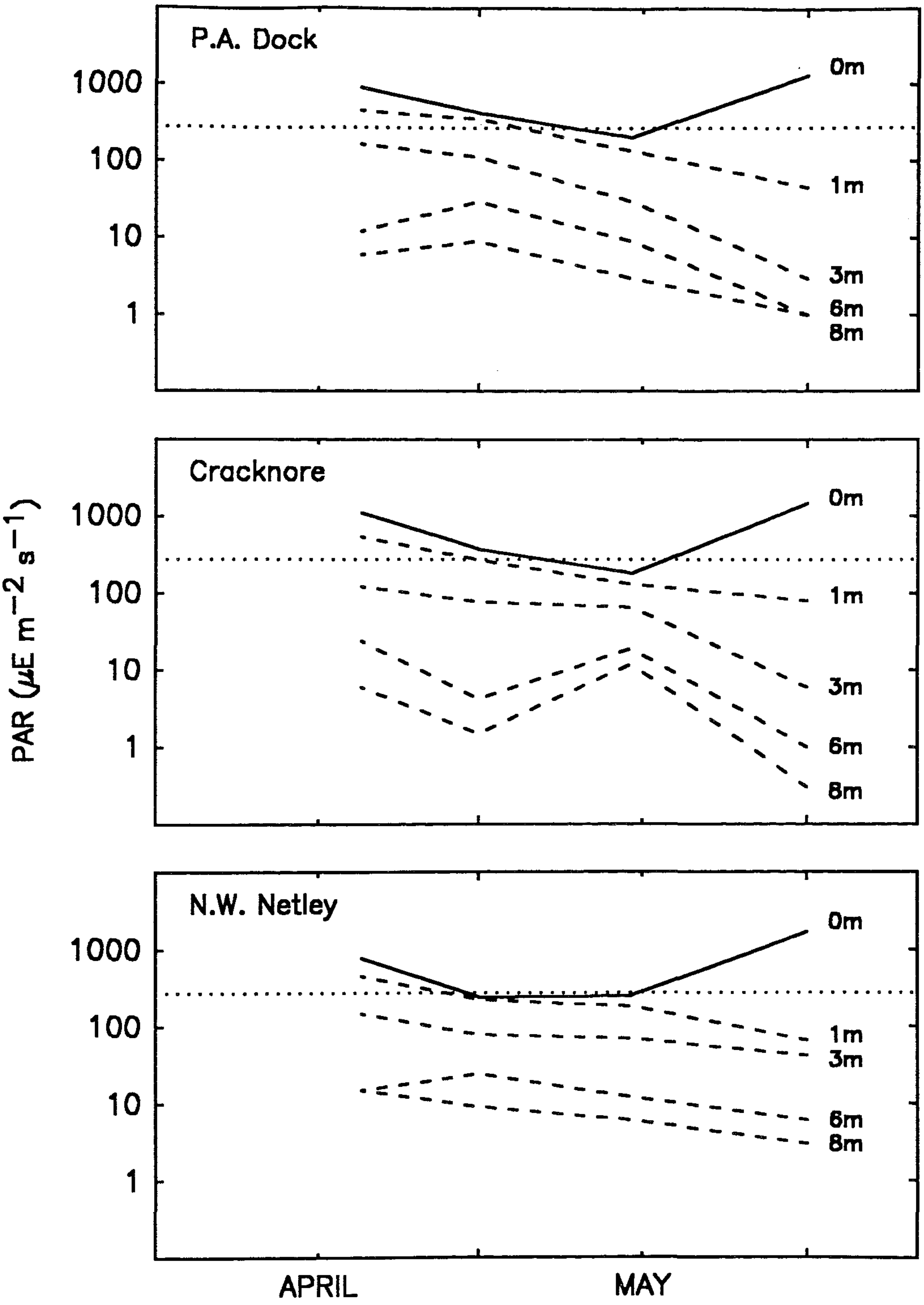


Figure 2.9.

Variation in photosynthetically active radiation (PAR) at the surface and 4 discrete depths over spring 1985; dotted line denotes I_k = 275 μE m^{-2} s^{-1}.

Oxygen

The oxygen distributions shown in Figure 2.10 principally reflect the photosynthetic activity of *M. rubrum* through the spring/summer; these show a strong peak at all stations, associated with the onset of the bloom in late May. The stratification of oxygen closely reflects the vertical distribution of *M. rubrum* shown in Figure 2.3, with superficial peaks at P.A. Dock and Cracknore, and a deeper maximum at N.W. Netley. The second large peak in cell numbers does not cause such substantial production of oxygen in late June and early July. It is interesting also to note an inversion of oxygen profiles, to some extent, after the bloom; oxygen levels in subsurface waters showing higher saturation than surface water. This is particularly apparent at P.A. Dock, where a similar phenomenon occurs immediately prior to the onset of the bloom. The explanation for this is not clear; profiles of abundance of *M. rubrum* showed mainly surface peaks and thus did not reflect these oxygen inversions. However, it should be stressed that because of the motility of *M. rubrum*, such coherence between abundance of cells and oxygen production will not necessarily follow; *M. rubrum* can vertically migrate into or out of zones of oxygen supersaturation or depletion (see Chapter 3 & 10).

Oxygen levels never fell below 60-70% saturation; lower oxygen levels have been recorded coinciding with bloom decline in the estuary by Rees & Williams (1982) and Soulsby *et al.* (1984). However, it should be emphasised that a two week sampling interval may not always be of sufficient resolution to record the lowest levels associated with the decline of the bloom.

Inorganic Nitrogen

The impact of the bloom on the distribution of inorganic nitrogenous nutrients is shown for 1985 in Figures 2.11 & 2.12. Sampling for nutrients commenced one month later than for other parameters, and thus appears to have missed some of the initial removal by *M. rubrum*, particularly for ammonia. Minimum levels of ammonia appear to have been reached earlier than those for nitrate, possibly suggesting preferential uptake of ammonia. Much of the ammonia in the water column was removed by late May, whilst nitrate did not reach a minimum until late June or early

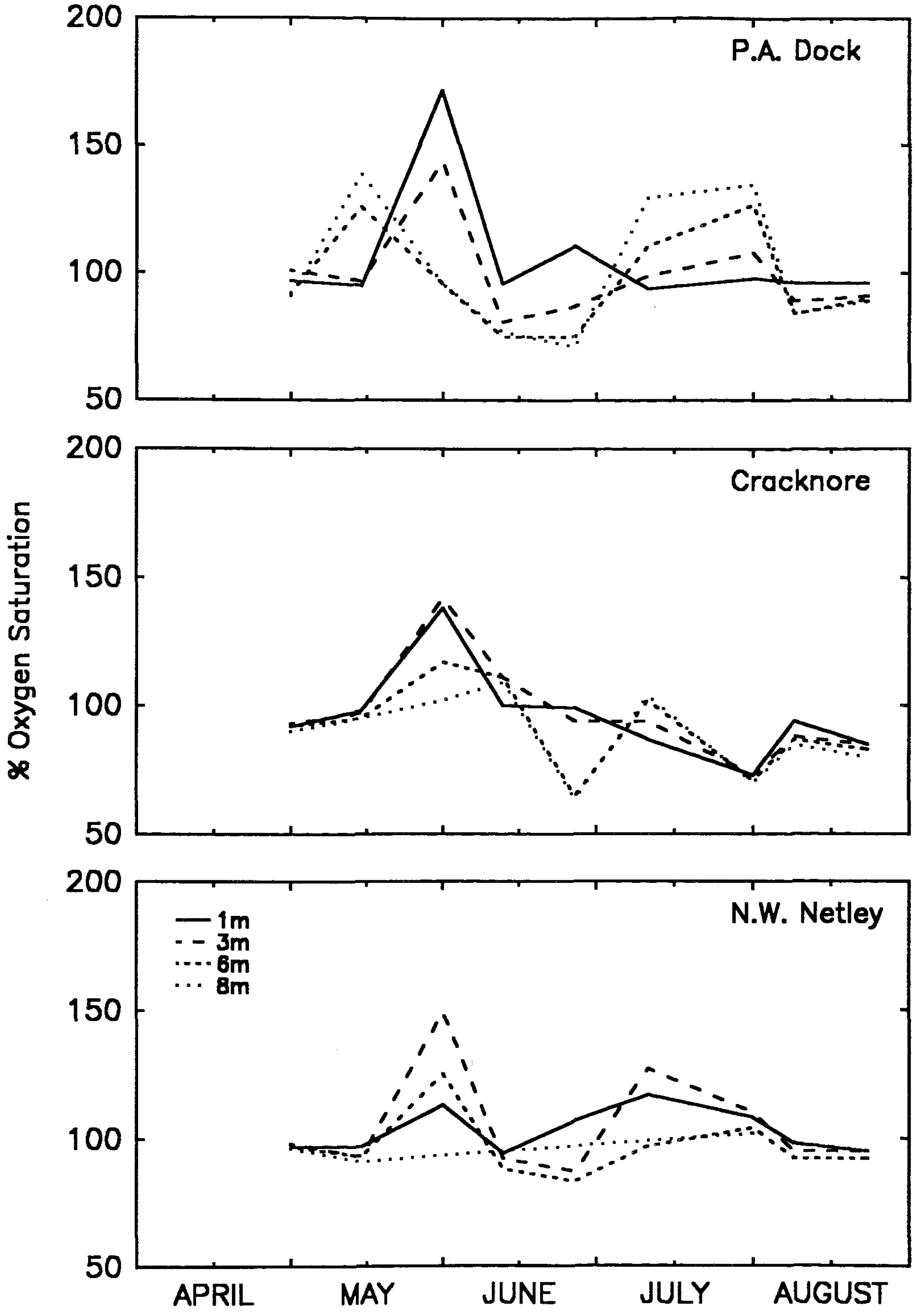


Figure 2.10.

Variation in dissolved oxygen (% saturation) at 4 discrete depths over spring/summer 1985.

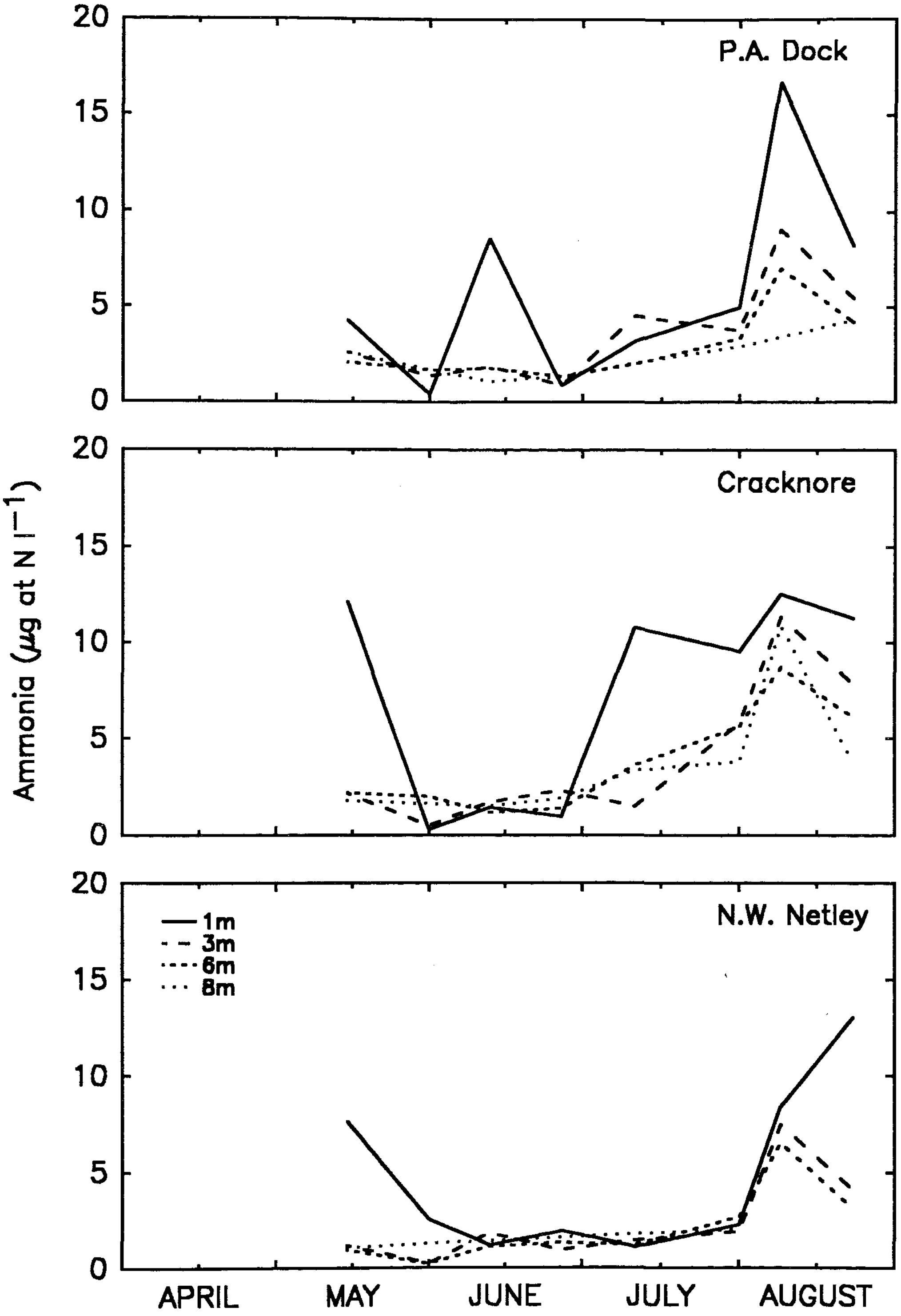


Figure 2.11.

Variation in concentration of ammonia-nitrogen at 4 discrete depths over spring/summer 1985.

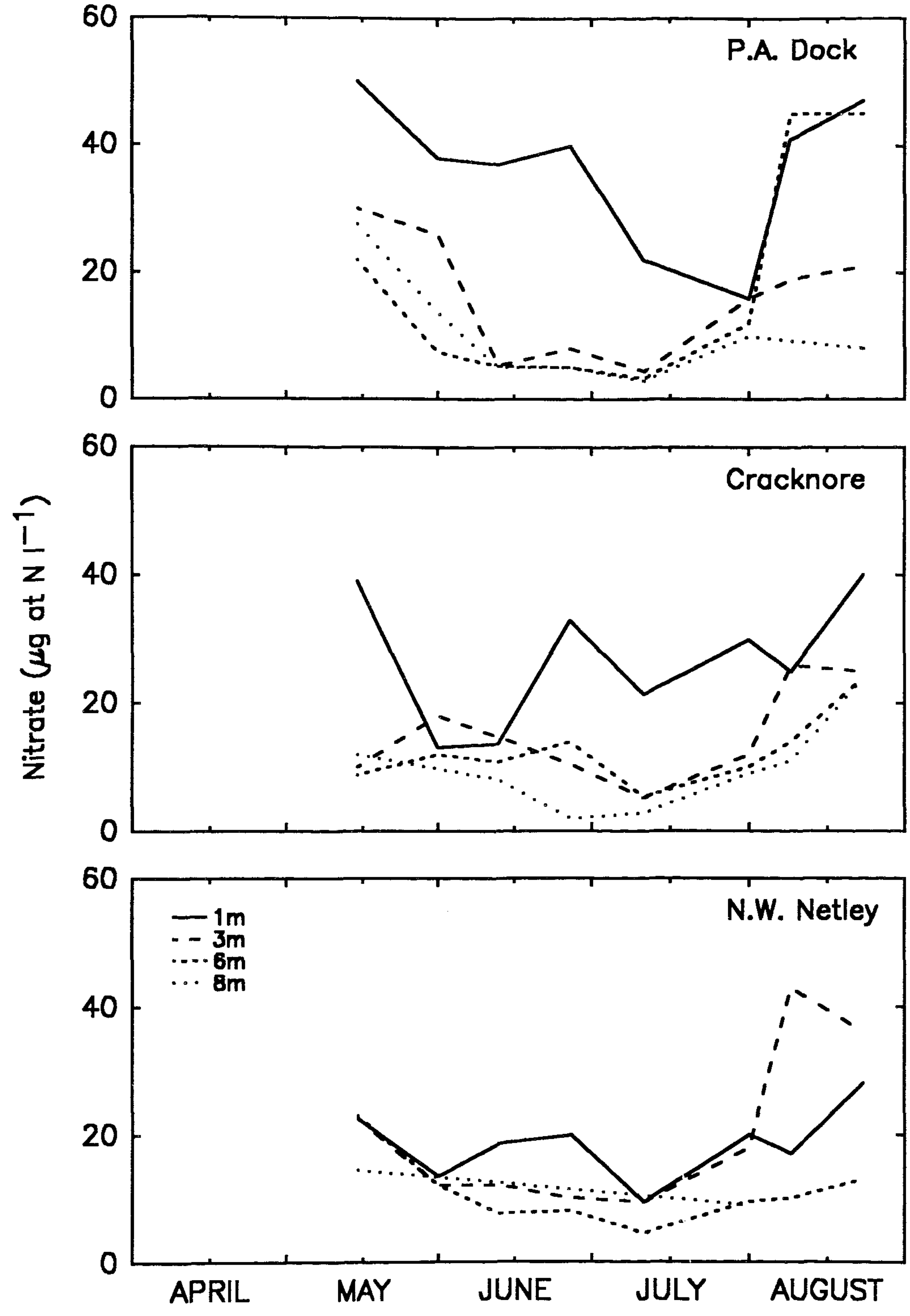


Figure 2.12.

Variation in concentration of nitrate-nitrogen at 4 discrete depths over spring/summer 1985.

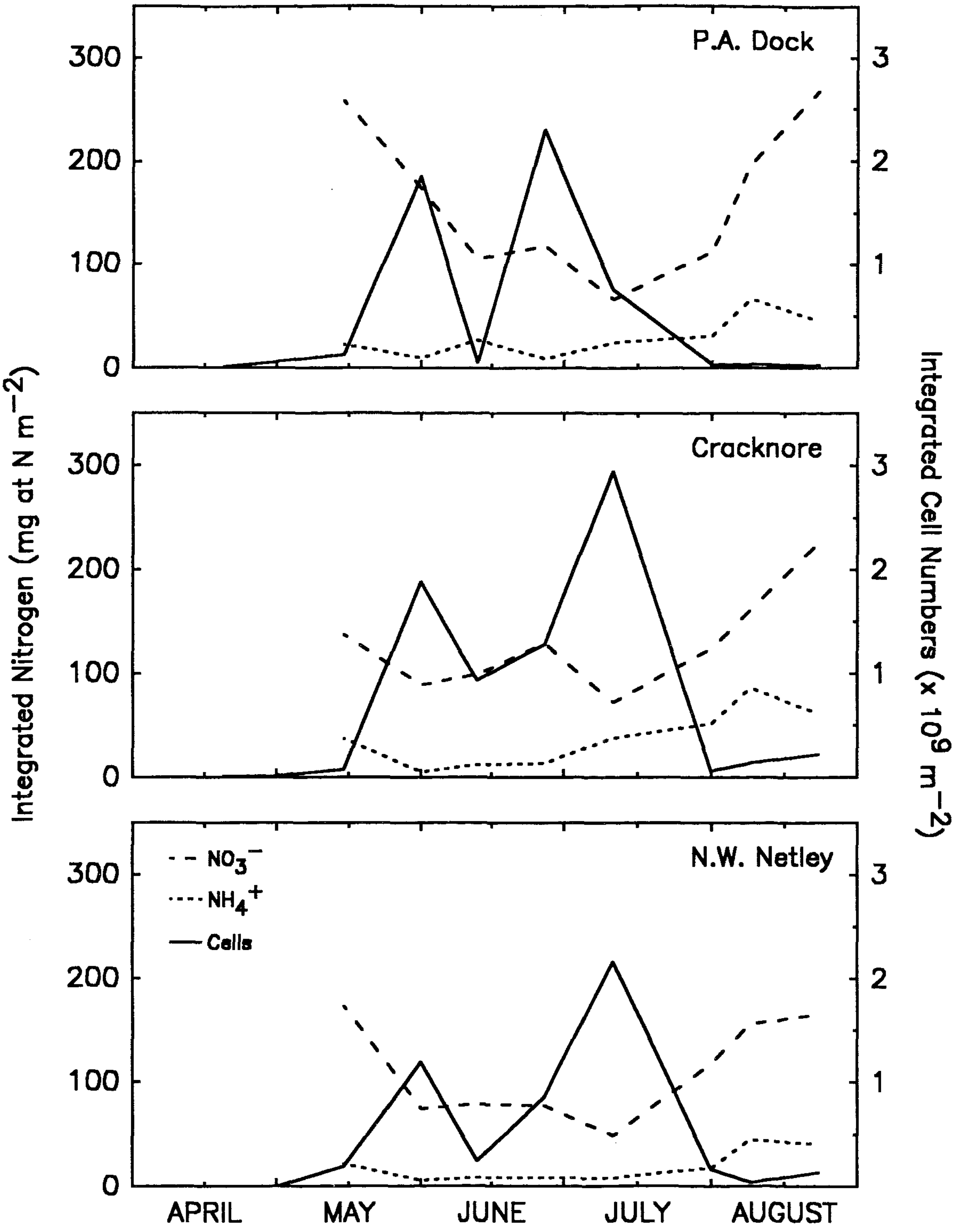


Figure 2.13.

Relationship between total integrated numbers of *Mesodinium rubrum* and total integrated dissolved inorganic nitrogen (nitrate + ammonia) over spring/summer 1985).

July. These data are more clearly summarised in Figure 2.13 which shows changes in total integrated nitrate, ammonia (μg at N m^{-2}) and cell numbers (m^{-2}), at each station. The decline in nitrate levels at P.A. Dock appears to occur later than at the other two stations, and this may reflect a greater input and thus utilisation of ammonia at this station, which is downstream of the Itchen River sewage works. However, given the much greater concentrations of nitrate in the estuary, it appears that this would still provide a major fraction of the inorganic nitrogen assimilated by the bloom, even with ammonia as the preferential source of nitrogen. Levels of both ammonia and nitrate increased sharply as the bloom declined.

The peak in ammonia in mid-June at the surface at P.A. Dock (Figure 2.11), coincided with the period of rough weather which resulted in the decline in cell numbers mentioned earlier. However, this peak in ammonia appeared not to be caused by regeneration within the water column, or, resuspension of bottom sediments, since it was only present in the surface sample. It is possible that a surface input from the Itchen river sewage works could have caused such a peak; this contention is supported by a lack of comparable signal at the other two stations.

2.3.2. 1986 Study

Water Column Structure

The variation in temperature and salinity with depth throughout June 1986 is shown in Figure 2.14. It is clear that surface temperature increases sharply through June creating a strong temperature stratification; this was particularly marked over the period of the onset of the bloom. Salinity also showed increased stratification during this period.

Temperature and salinity profiles are transformed into density profiles in Figure 2.15, alongside profiles of cell numbers. This shows marked density stratification throughout June, but particularly on 17th, when the bloom was first detected, both visually and in cell densities.

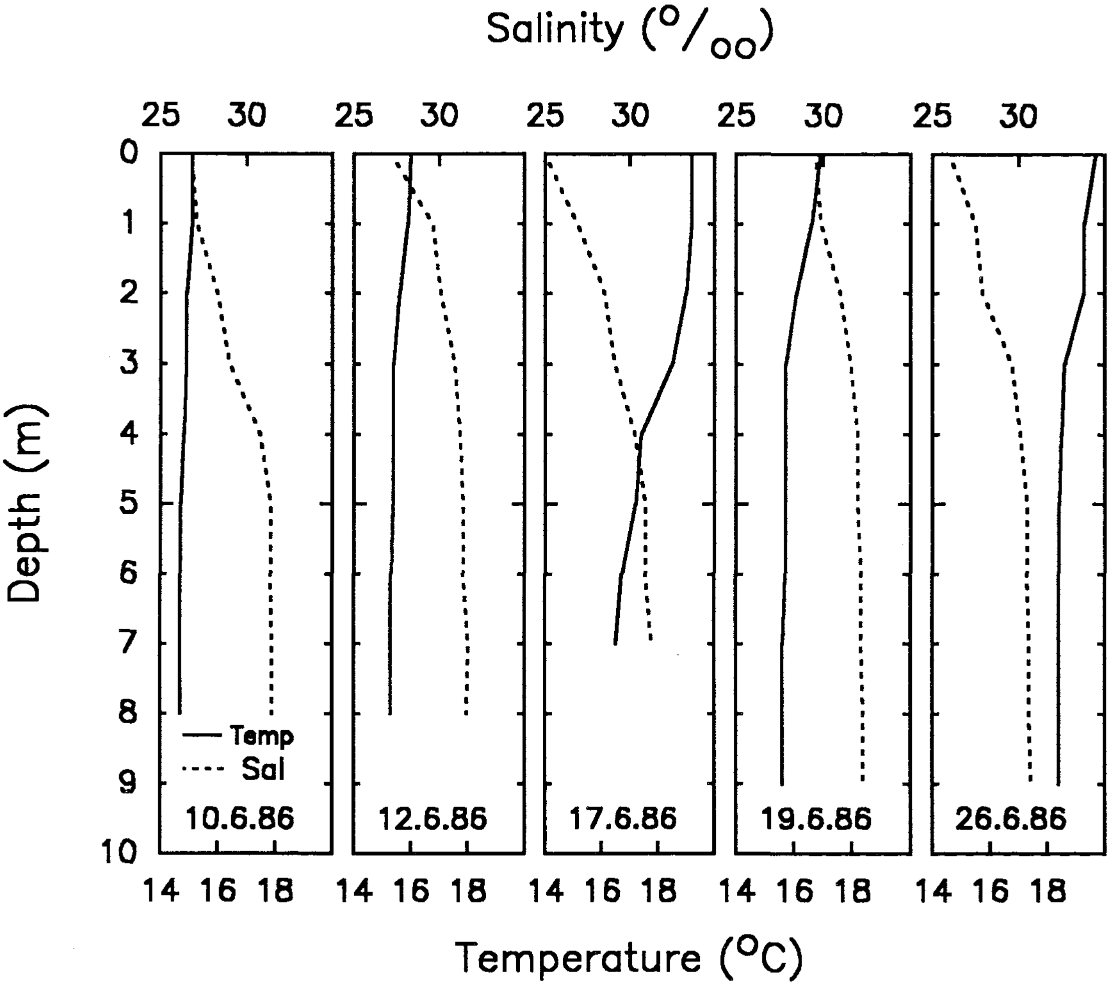


Figure 2.14.

Sequence of temperature and salinity profiles through June 1986 at P.A. Dock.

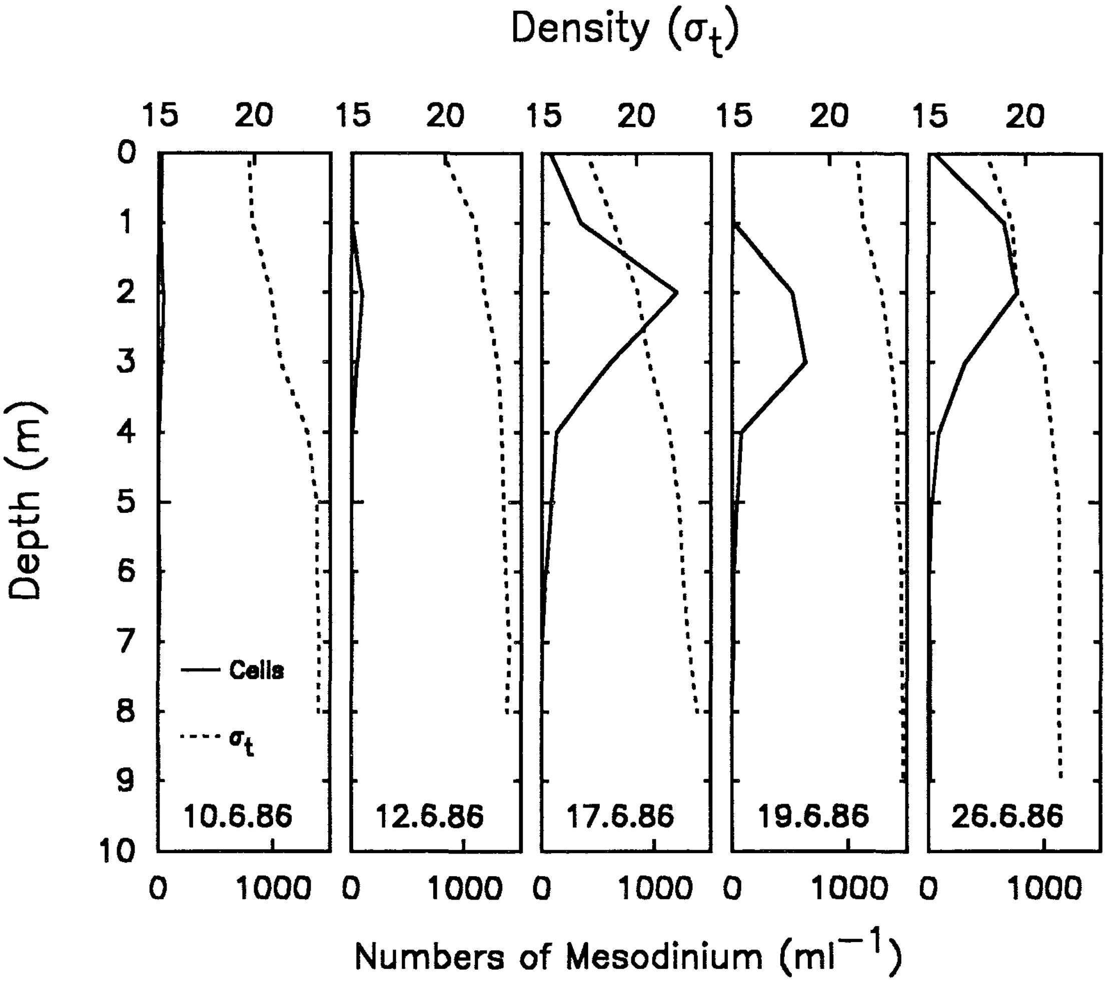


Figure 2.15.

Sequence of profiles of water column density and abundance of *Mesodinium rubrum*.

Cell Numbers

Figure 2.15 shows cell numbers superimposed on the density profiles. Numbers gradually increased through early June before an explosive increase in growth of the population between the 12th and 17th June. Typical pre-bloom numbers of *M. rubrum* were 1-10 ml^{-1}, whereas numbers during the bloom peaked at over 1000 ml^{-1}, as in 1985.

The profiles for June 1986 clearly demonstrate the degree of aggregation shown by *M. rubrum* at a preferred depth range of between 0-3 m; this was not so clearly shown in 1985, as water samples were taken only at 4 depths during that survey. During June 1986, sampling was conducted with a much greater degree of resolution (1 m intervals) than in 1985, and suggests that a lower degree of resolution could significantly underestimate, or sometimes even overestimate, the total depth integrated population. The extent of such under- or overestimation will depend critically upon the degree of co-incidence between sampling depth and the degree and depth of population aggregation. Vertical distribution of *M. rubrum* in differing environments is more fully explored in Chapters 3 and 4.

As the relationship between cell numbers of *M. rubrum* and chlorophyll was fairly well established in 1985, chlorophyll was not measured in 1986.

Oxygen

Figure 2.16 shows depth profiles of abundance of *M. rubrum* against oxygen saturation. Oxygen levels are high prior to the bloom, with an excess of 100% saturation throughout the water column. It is possible that these high saturation values are the result of a poorly calibrated electrode, but are more likely caused by a transient bloom of another phytoplankton species such as *Phaeocystis* sp. or possibly a diatom. The main difference between the depth profiles of oxygen prior to the bloom, on 10th and 12th June, and those during the bloom, is that oxygen levels become much more stratified, as a result of photosynthetic oxygen production by the subsurface population maximum of *M. rubrum*. The profiles on 17th and 26th June show this feature, whereas the profile on 19th June is an example of a curious

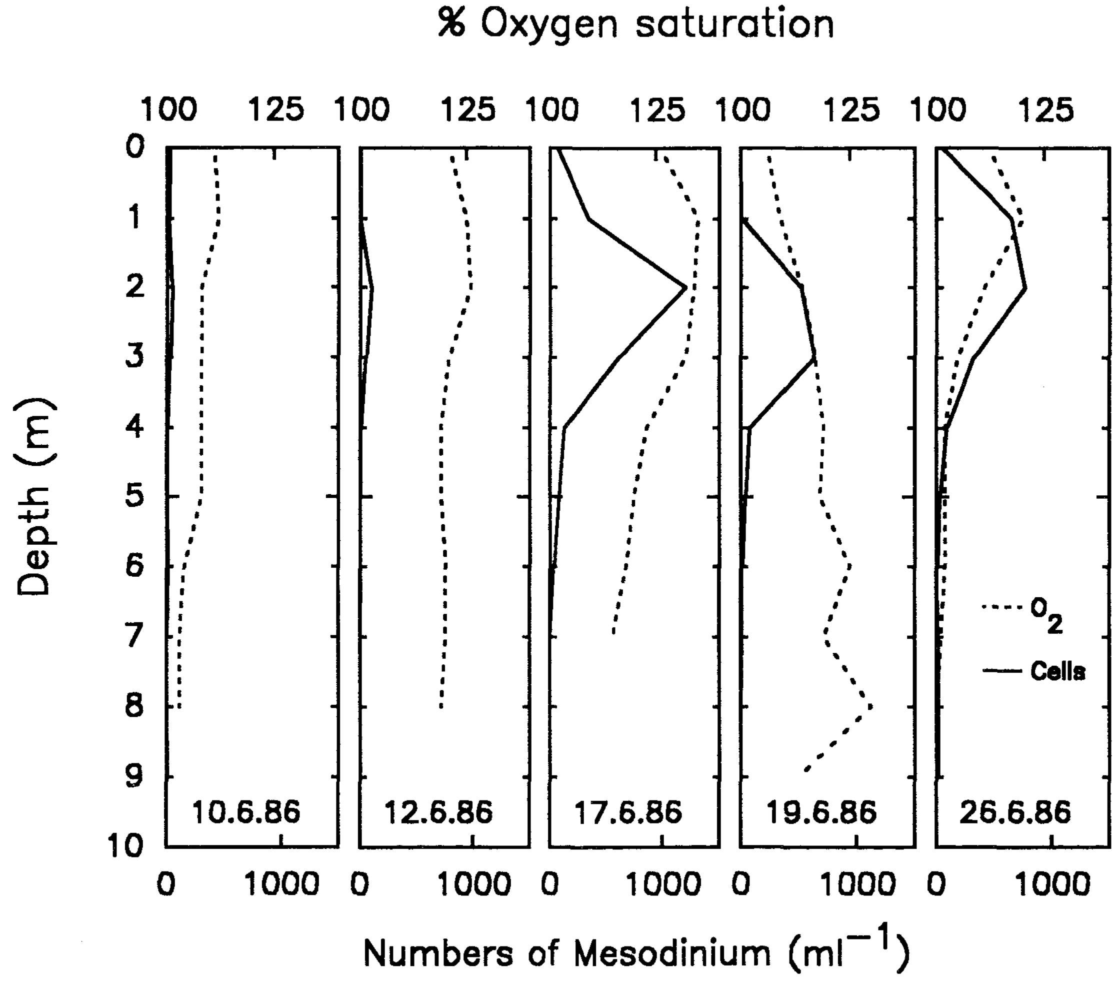


Figure 2.16.

Sequence of profiles of oxygen saturation and abundance of *Mesodinium rubrum* through June 1986.

anomaly, but one which has occurred on several occasions. Oxygen peaks here are found at depth and do not coincide with the abundance peak of *M. rubrum* as is usually the case. Possible explanations for this are discussed in later chapters in relation to vertical migration of the ciliate.

Oxygen levels at depth on 26th June are much reduced at around 100% and may indicate the early stage of the oxygen 'sag' in the estuary, as described in other studies (Rees & Williams 1982, Soulsby *et al.* 1984).

Inorganic Nitrogen

Figure 2.17 presents profiles of cell numbers in relation to those of nitrate and ammonia-nitrogen through June 1986. The profiles suggest, as do those during 1985, that ammonia appears to be the preferential source of nitrogen during the development of the bloom. However, the profiles from 1986 have a much greater degree of vertical and temporal resolution, and demonstrate the almost complete removal of ammonia below 2 m by the developing bloom on 17th June. Some ammonia remains at the surface, and this probably represents a persistent surface freshwater input. Once ammonia has been removed from the water column, the levels of nitrate fall more rapidly. Levels of both nutrients increase again by the 26th June; this is more difficult to explain, but occurred when the weather was deteriorating, and so may represent regeneration, or input from the sediments. Moreover, by this stage, the bloom did not seem to be continuing to grow but was simply maintaining itself in the estuary, and thus nutrient levels would not be expected to decline further.

If the integrated totals of cell numbers, ammonia and nitrate-nitrogen are considered over the onset of the bloom, as in Figure 2.18, it appears that although ammonia may be the preferential source of nitrogen, nitrate is the major source of inorganic nitrogen, as already suggested for the 1985 study. This is simply due to the much higher levels of nitrate in the estuary. Figure 2.18 also supports the data from 1985 in confirming that the bloom does not appear to be nitrogen limited. In contrast to the limited study of 1985, this represented a relatively frequent sampling program,

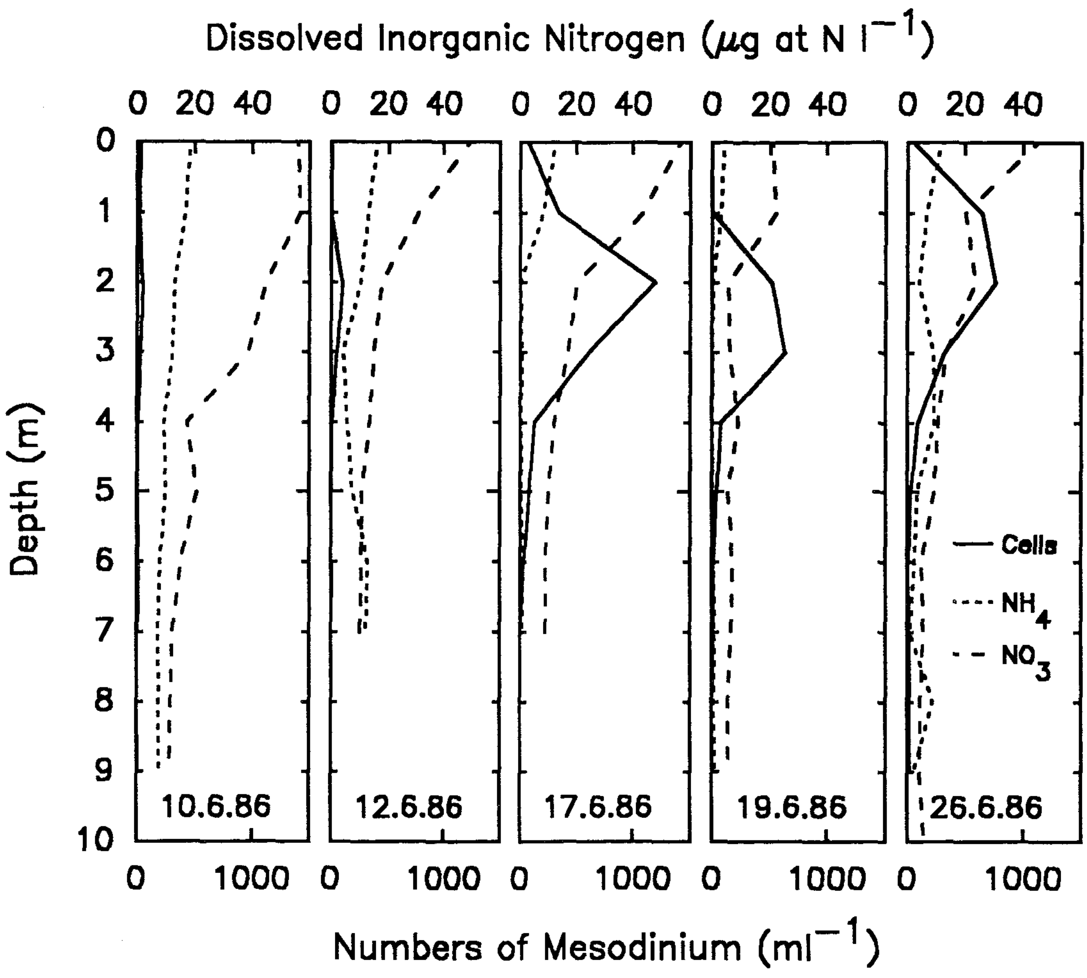


Figure 2.17.

Sequence of profiles of dissolved inorganic nitrogen (ammonia and nitrate) and abundance of *Mesodinium rubrum* through June 1986.

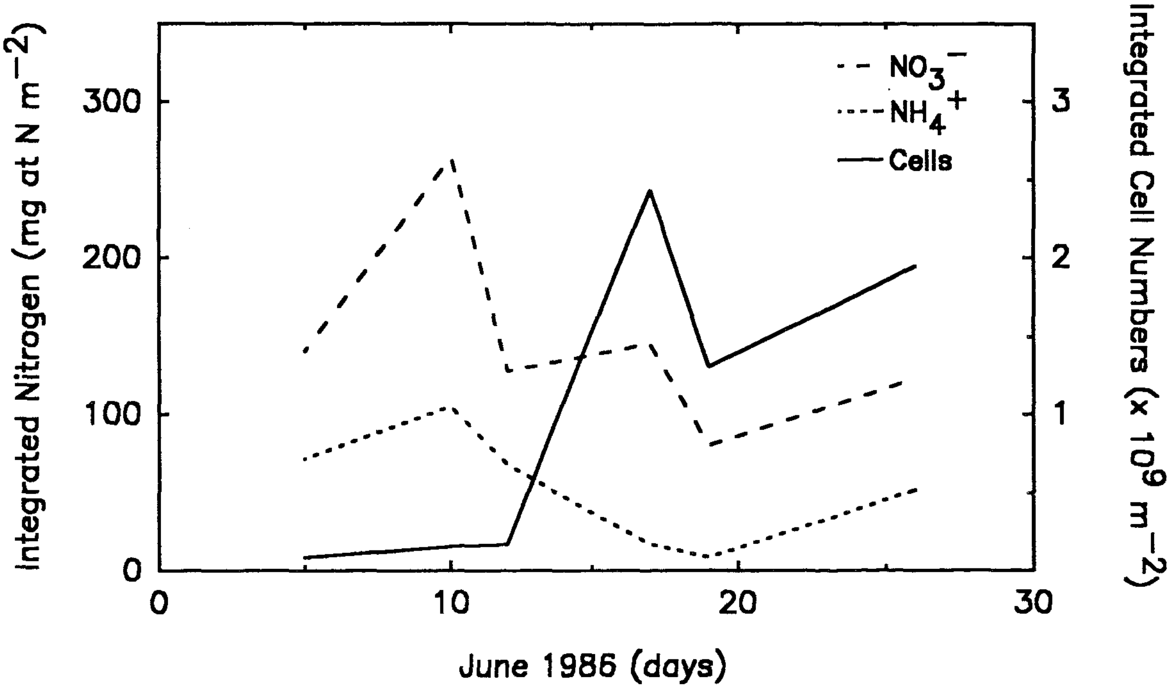


Figure 2.18.

Variation in integrated totals of inorganic nitrogen (nitrate + ammonia) and numbers of *Mesodinium rubrum* through June 1986.

and confirmed that a considerable quantity of nitrate remained in the water column at the peak of the bloom.

2.4. DISCUSSION

Red-Water Appearance

The cell numbers and levels of chlorophyll encountered in Southampton Water are typical of those described (see Taylor *et al.* 1971, Lindholm 1985 for reviews) in other reports of red-water caused by *M. rubrum* to date (Figures 2.2, 2.3, 2.4). Maximum cell numbers tend to be between 1 - 2 x 10^6 l^{-1}, and these represent chlorophyll levels of some 100 - 200 μg l^{-1} (Figure 2.4 & 2.5). A strong and significant relationship ($r = 0.8286$, $p < 0.001$) exists between cell numbers of *M. rubrum* and chlorophyll (Figure 2.4); if the lower scatter, below 1 μg l^{-1} and 10 cells ml^{-1}, is removed from Figure 2.4a (this representing pre-bloom levels characterized by diatoms in spring), then the resulting correlation between chlorophyll and numbers of *M. rubrum*, shown in Figure 2.19 (for values between May and October), is even stronger ($r = 0.8679$, $p < 0.001$). Using this correlation, and assuming spherical cells with an average cell diameter of 40 μm (typical of cells in the bloom), a chlorophyll content of 2.7 fg μm^{-3} cell volume can be estimated. This compares very closely with a figure of around 2.6 fg μm^{-3} determined for isolated and washed *M. rubrum* cells (Stoecker *et al.* 1991). These figures strongly support the contention that chlorophyll in Southampton Water, during red-water events, is overwhelmingly dominated, in terms of biomass, by *M. rubrum*. The chlorophyll also appeared to be in a healthy and active state throughout the bloom, as shown by the plot of percent chlorophyll *a* in Figure 2.4b; decrease in percent chlorophyll is indicative of an increase in phaeopigments, which are degradation products of chlorophyll. Figure 2.4b shows a significant relationship between numbers of *M. rubrum* and percent chlorophyll ($r = 0.8014$, $p < 0.001$). Some increase in percent chlorophyll appeared to occur as the bloom developed (Figure 2.5b), but there was no sudden increase in the level of phaeopigments as the bloom declined. This is a feature which might be expected if the bloom had 'crashed' and decomposed

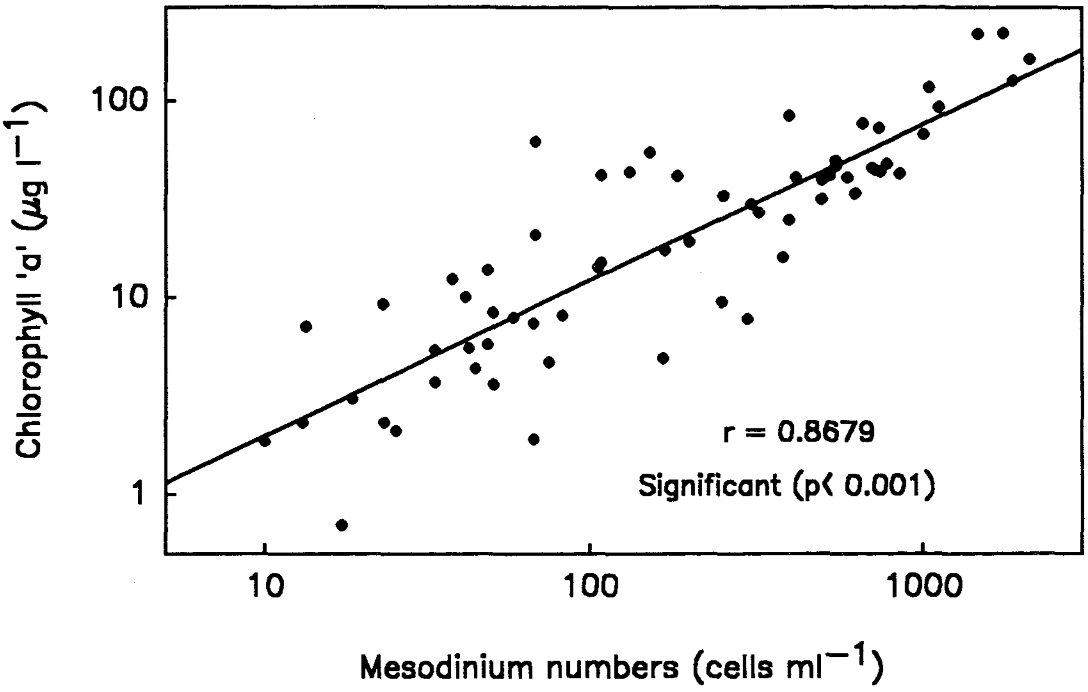


Figure 2.19.

Log:log regression of chlorophyll *a* concentration on abundance of *Mesodinium rubrum*; lower scatter of Figure 2.4 removed by considering only samples between May and October.

suddenly, or had been removed by a significant increase in zooplankton grazing activity.

Although chlorophyll is probably a reasonable index of biomass of *M. rubrum*, assuming a constant carbon:chlorophyll ratio, the use of cell numbers, in retrospect, may be questionable to some extent. Unfortunately, the significance of variation in cell volume (Lindholm 1985; Montagnes & Lynn 1989) was not fully appreciated from the literature, or from personal observation, until later in the study, and thus cell size was not routinely determined on preserved samples during 1985 and 1986. The question of variation in cell size in *M. rubrum* is more fully addressed in a later chapter. However, the sheer magnitude of increase in cell numbers as the red-water appeared probably reduced the influence of cell size on biomass change to a level of lesser significance. The influence of cell size variation on biomass is probably a much more critical factor for non-bloom populations.

In May 1985, sampling frequency was not sufficient to allow examination of growth rate of the population, but for 1986 a crude estimate of the doubling rate of the population was determined from the change in integrated cell numbers (m^{-2}) over the 5 day period between 12th and 17th June. This assumes a closed system in this dock, which clearly is not wholly accurate, but the exercise is a useful one:

generation time g (days) = $(T_1-T_0)\ln 2/(\ln N_1-\ln N_0)$

growth rate (divisions day^{-1}) = g^{-1} (Parsons *et al.* 1984*b*)

- where cell numbers N_0, N_1 are at times T_0, T_1 respectively.

Taking T_0 as 12th and T_1 as 17th June respectively, this gives a minimum estimated net growth rate of 0.8 divisions day^{-1} during the development of the bloom. This seems a reasonable estimate, and probably represents a rate of at least 1 division day^{-1}, allowing for some grazing, mortality and flushing losses. A regression of ln cell number against time through June gives a significant correlation ($r = 0.8886$, $p < 0.05$).

The timing of the red-water appearance was typical of these recurrent summer blooms in the estuary (Rees & Williams 1982, Soulsby *et al.* 1984), though perhaps slightly earlier. However, the observations and data from 1985 and 1986 do not clearly identify either a 'trigger' factor responsible for the appearance of the red-water, or, an explanation for why it should always be delayed until summer months. The question can thus be posed why the population of *M. rubrum* cannot develop in spring? By virtue of its motility it should be able to outcompete the diatoms for light by means of aggregation at the surface.

Subsurface light levels actually fell through April and May prior to the occurrence of the bloom (Figure 2.9). Although light intensity at the surface did appear to rise sharply between mid- and late May, the PAR to which individual cells were subjected, even close to the surface, was decreasing during this period probably due to the self shading effect of increasing cell numbers (Figure 2.9). Moreover, because of this, most of the water column received a level of PAR less than the I_k value of 275 μE m^{-2} s^{-1} determined for isolated and washed *M. rubrum* cells by Stoecker *et al.* (1991); Rees & Williams (1982) arrived at a similar value of 255 μE m^{-2} s^{-1} for samples incubated from Southampton Water, although this study did not specify the degree of dominance by *M. rubrum*. It appears then that virtually all of the *M. rubrum* population (other than those above 0.5m) were light limited after mid-April; that is, the reduction in observed subsurface PAR would result in a decreasing growth rate over this period. A greater degree of near surface aggregation may have offset this to some extent, but clearly the increase in surface PAR at the end of May seems unlikely to have been a sufficient driving force behind the rapid appearance of the bloom. If light were such a major controlling factor, then the question can again be posed why the bloom did not develop in April when subsurface PAR was quite sufficient for almost maximum growth? Indeed, when conditions are favourable, the 1986 study showed that the bloom develops in just a few days. This question will be examined in detail in later chapters.

Levels of dissolved inorganic nitrogen and phosphorus remained relatively high throughout the winter and spring (Rees & Williams 1982, Antai 1989), and are not even severely depleted by the diatom bloom in March/April; such nutrients are thus unlikely to represent a factor preventing the appearance of the bloom. Unlike the diatoms, ciliates and cryptomonads do not have a major structural requirement for silicon, therefore dissolved inorganic silicate is not a significant factor. Other inorganic or organic nutrients, vitamins, or trace elements not determined here, could conceivably be important; however, it is difficult to envisage any such substance becoming limiting in spring, with such a high freshwater input, when sufficient concentrations were present to allow massive red-tides to develop in summer. The only likely candidates might be substances such as vitamins excreted or released as breakdown products by preceding diatom blooms, as has been described in other studies of phytoplankton successions (e.g. Swift 1980).

The 1986 profiles demonstrate, as during 1985, that ammonia appears to be the preferential source of nitrogen during the development of the bloom. As the cell numbers increase sharply, ammonia is almost completely removed from the water column, excepting at the surface, caused by an apparently persistent freshwater input. However, if the integrated totals of cell numbers, ammonia and nitrate are considered over the onset of the bloom, it appears again that nitrate seems to be the predominant source of nitrogen overall. Some crude estimates of relative contributions can be made over the onset of the bloom in June 1986 (Figure 2.18); assuming spherical cells of *M. rubrum* of 40 μm diameter, a carbon:volume ratio of 0.19 pg C μm^{-3} for ciliates and *M. rubrum* (Putt & Stoecker 1989; Stoecker *et al.* 1991), and a carbon:nitrogen (Redfield) ratio of 7. Between 10th and 17th June, cell nitrogen increased by 0.15 g at N m^{-2}, while nitrogen decreased by 0.21 g at N m^{-2}, a reasonable comparison. This consisted of 0.09 g at N m^{-2} ammonia, and 0.12 g at N m^{-2} nitrate. As the bloom progresses, and ammonia becomes limiting, even more nitrate is assimilated, resulting in further depletion in the water column (Figures 2.17 & 2.18). The fairly close agreement between total nitrogen removed and assimilated during the development of the bloom supports the contention that these red-water events result from *in situ* growth of the population rather than physical concentration.

However, it is possible that the small difference between assimilated and depleted nitrogen may represent the DON known to be excreted by *M. rubrum* (Wilkerson & Grunseich 1990). Also, other phytoplankton, epiphytes and seaweeds might have contributed to removal of inorganic nitrogen.

The observations that the bloom coincided with periods of increased stratification of temperature, salinity and thus density, are particularly interesting. It was also noted, subjectively, that red-water in both years, as in most years since, occurred during neap tides when tidal mixing was minimal. Moreover, the blooms tended to decline as stratification weakened through the summer (Figures 2.6, 2.7, 2.8), and were temporarily dispersed in mid-June during a period of spring tides and wind-induced instability. In this context it is interesting to note that the increase in sunshine at the end of May 1985, which did not appear to have a significant effect in increasing subsurface PAR, because of self-shading, probably played a role in stabilising the water column. In a similar manner, freshwater input promotes stability of the water column through density gradients; however, the observation that red-tides often occur after heavy rainfall is often interpreted to suggest that dissolved nutrients or other trace substances are the critical factors, when this also promotes water column stability. The blooms of *M. rubrum* in Southampton Water have, at least subjectively, been observed to occur after heavy rainfall in some years, but no data is available to support this.

One observation that can be substantiated to some extent is that the blooms are often observed to develop when tidal mixing is low, i.e. during neap tides. For example, the 1985 bloom developed on the neap portion of the cycle in late May (Figure 2.20a), and the dispersal of the bloom in mid-June also coincided with spring tides, in addition to poor weather. Moreover, the red-water also occurs at a time of year, mid-summer, when the tidal range of both springs and neaps are at their lowest; i.e. when the smallest difference between springs and neaps occurs (Figure 2.20b). Thus at this point, tidal mixing is probably at the annual minimum.

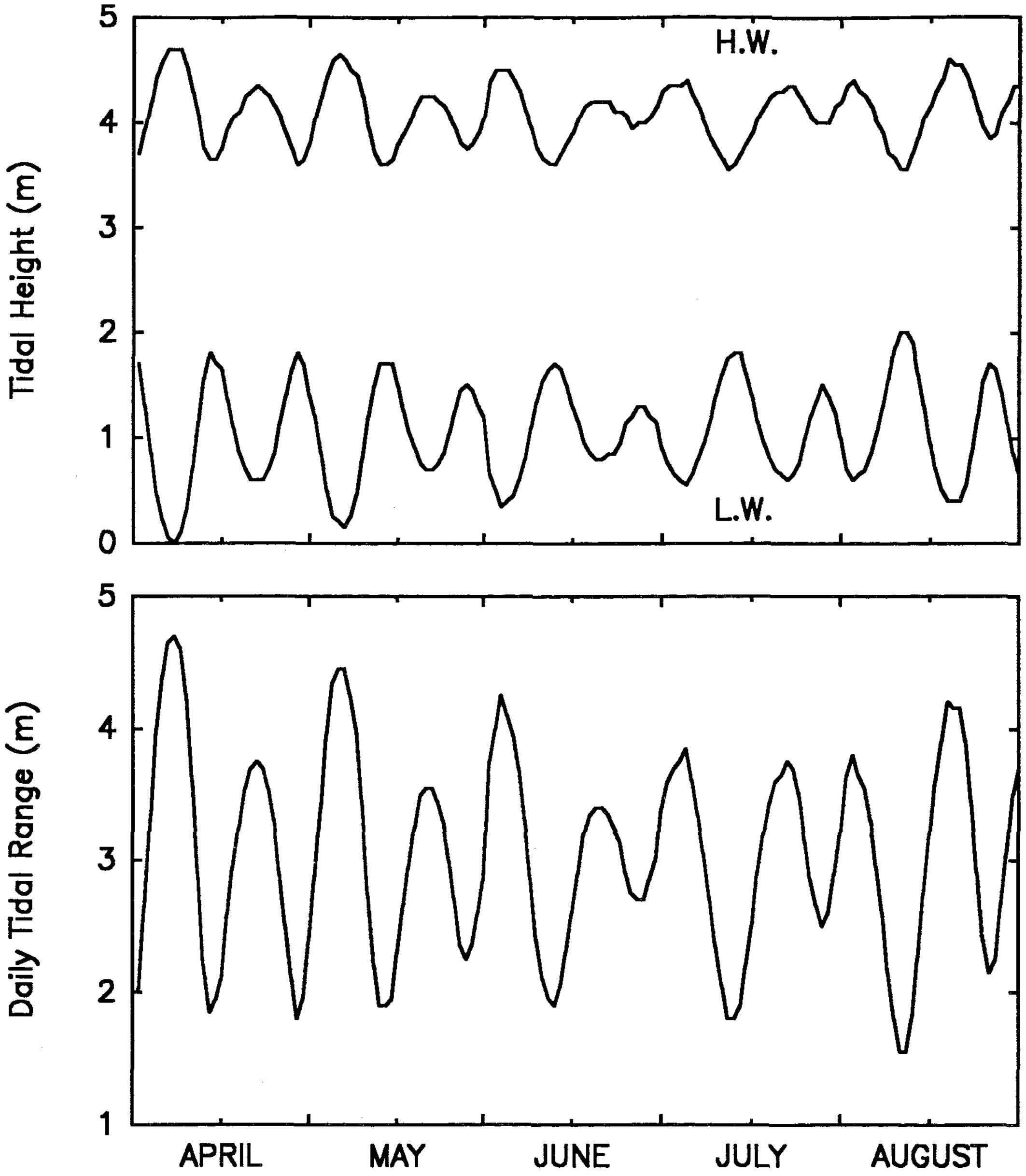


Figure 2.20.

Daily heights of high and low water, and the daily tidal range for Southampton Water over spring/summer 1985.

The problem with using static stability, that is density gradients, as an index of water column stability is that it does not account for dynamic processes. It gives an index of the degree to which the water column has been mixed, but not any indication of whether mixing is taking place. To give an indication of dynamic stability of the water column, mean current velocities, or velocity profiles must be available. Intuitive or empirical estimates of static stability are often derived from density gradients during plankton studies, but velocity profiles, allowing direct assessment of dynamic stability, are rarely measured (Heaney & Butterwick 1985). When current velocities are recorded, various indices are available, such as the stratification parameter proposed by Simpson & Hunter (1974), which quantifies tidal mixing by the formula h/u^3; where h is tidal height and u is mean current speed. Perhaps a better parameter for estuaries, where density gradients may be significant, is the Richardson Number (as described in chapter 3). This is simply an index of turbulence given by the stabilising effect of the buoyancy generated by the density gradient, against the destabilising effect of current shear. The lack of quantification of such an important parameter as turbulence in plankton studies may be one reason why its effects are so poorly understood, but represented by a wealth of anecdotal evidence in phytoplankton bloom studies.

Current velocities were not available here but a semi-quantitative index can be generated for illustrative purposes, and for a preliminary examination into the effect of dynamic stability on the development of red-water. If the difference in density between surface and bottom of the water column is taken as a simple index of static stability, and the degree of tidal mixing assumed to depend to some extent on tidal height (at high water for example), a simple semi-quantitative index can be given by:

$$\text{Dynamic stability} = \text{density difference/tidal height}$$

Tidal height is actually presented as the mean height over the 5 days preceding measurement of cell numbers (predicted tidal heights taken from tide tables).

The results of this are presented in Figure 2.21a; the index of dynamic stability is plotted against cell abundance for P.A. Dock for 1985. For 1986, a close relationship is shown in Figure 2.21b between total cell numbers and temperature stratification over the development of the bloom. Although this is a very crude semi-quantitative index, it appears that water column stability is an important factor during the development of the bloom. Also there seems to be a time lag between breakdown in stability and the decline in the bloom. These observations suggest that in some way turbulence might interfere with the growth of *M. rubrum*. Thus development of red-water seems directly proportional to the increase in stability, but a subsequent decrease in stability does not have an immediate effect on the population.

In the context of recent studies on the effects of turbulence on red-tide dinoflagellates these observations are particularly interesting. Turbulence has been shown to interfere with growth (White 1976), cell division (Pollinger & Zemel 1981) and motility (Thomas & Gibson 1990*a,b*) in some dinoflagellates. Moreover, throughout the red-tide literature are scattered anecdotal accounts of the role of calm conditions and water column stability as a prerequisite for red-tide appearance. Potential reasons for the beneficial effects of reduced turbulence on *M. rubrum* will be discussed in detail in later chapters.

Persistence of Red-Water

Once the red-water became established established, it remained for a considerable period - about two months. How it persisted at such densities is an interesting question. At first sight, one might expect that the bloom suffered from nitrogen limitation, as is common with many phytoplankton blooms. Although ammonia was almost completely removed from the water column, a small surface input remained, and even at the peak of the bloom a considerable quantity of nitrate was still present throughout the water column. Moreover, *M. rubrum* can migrate vertically to within close proximity of the sediments (Soulsby *et al.* 1984), which may be rich in inorganic and organic nitrogen, and as *M. rubrum* has been shown to utilise DON (Smith & Barber 1979, Wilkerson & Grunseich 1990), there appears little

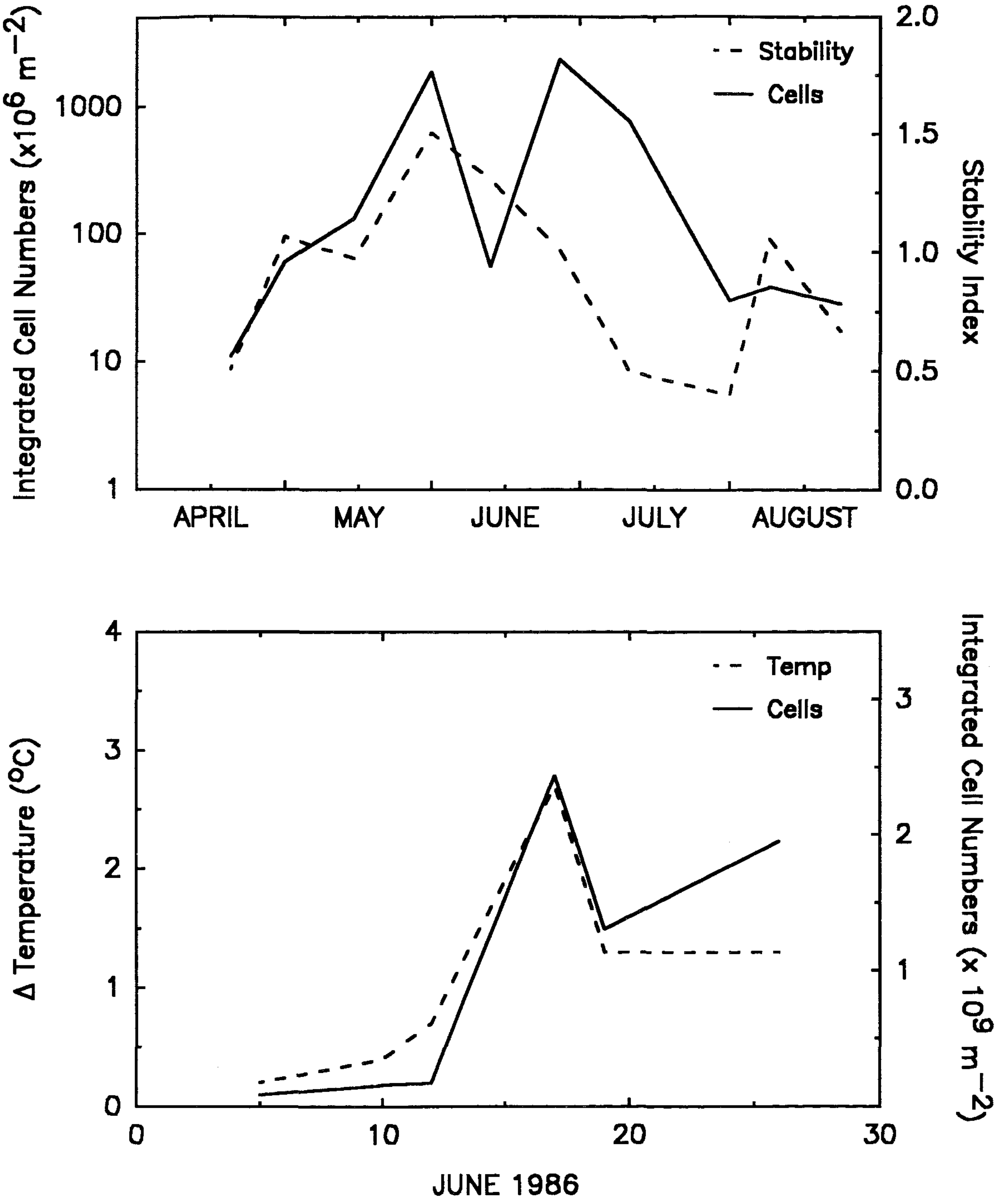


Figure 2.21.

a) total cell numbers of *Mesodinium rubrum* and a semi-quantitative index of stability (see text) over spring/summer 1985 at P.A. Dock.

b) total cell numbers of *Mesodinium rubrum* and temperature difference (between surface and bottom) at P.A. Dock during June 1986.

evidence to invoke nitrogen limitation as a limiting factor. Phosphate concentrations were not determined in this study, but other evidence suggests that phosphate does not become limiting; phosphate declines to about 0.3 - 0.5 μg at l^{-1} during the bloom (Rees & Williams 1982, Antai 1989), values still within the range for phosphate rich upwelled water (Bieleski 1973). Moreover, it would appear that should the bloom be nutrient limited, the decline would occur much sooner. The bloom appears to 'tick over' for some time, without further growth, after reaching maximum densities; this suggests that some form of 'steady state' is reached, at least temporarily.

In this context, the light profiles are particularly interesting; the light levels recorded as red-water appeared suggested that below the surface metre, there was insufficient light for significant growth of the cell densities present. This is due to the rapid attenuation of light with depth caused by the self-shading effects of such high numbers of cells. The system may have reached its capacity in terms of efficient conversion of solar to organic energy. The total quantity of chlorophyll 'a', per square metre of sea surface, reached a maximum of about 300 mg m^{-2} during red-water events; given the inherent sampling errors, this approximates to the level of 333 mg m^{-2} given by Lorenzen (1972) as the theoretical maximum achievable. At the population maximum, typically between 1-3 m depth, light levels during red-water events of about 30 μE m^{-2} sec^{-1}, were only approximately 2% of those at the surface, and about 10% of the I_k values given by Stoecker *et al.* (1991). Assuming a nominal respiration rate of at least 1% cell carbon hr^{-1}, as determined for other ciliates (see Chapter 9), and an I_k value of 275 μE m^{-2} sec^{-1} (Stoecker *et al.* 1991), then *M. rubrum* would require a light level of 20 μE m^{-2} sec^{-1} in order for photosynthesis just to cover respiratory demands. Clearly the light levels encountered once the bloom is fully developed, were only just sufficient to acheive this; cells could only theoretically realise a maximum net growth of less than 1% cell C h^{-1} during shaded red-water conditions, compared to a saturation photosynthetic rate of well over 10% cell C h^{-1}. This gives a doubling time of the order of about 10 days rather than 1 day or less during the bloom onset. These arguments thus support the contention that once developed, the bloom may just be 'ticking over'.

Another interesting point relates to the morphological variations described later in Chapter 5; larger and more irregular cells are observed to occur during red-water events when compared to 'normal' conditions. It has recently been shown that larger algal cells can achieve greater maximal population biomass under self-shaded conditions than small cells (Agustí 1991). Thus the morphological variation could be an adaptation to low light levels.

Impact upon the Estuary

The red-water events have been shown by this study to have a major impact upon the distribution of light, oxygen and nutrients in the estuary. The rapid attenuation of light with depth, and the uptake of dissolved inorganic and presumably organic nutrients and trace elements, must have a major competitive impact upon other photosynthetic plankton. However, although the chlorophyll regressions discussed earlier suggest *M. rubrum* to be overwhelmingly dominant, other species of phytoplankton do persist, most notably the small dinoflagellate *Scrippsiella trochoidea* (Kifle pers. comm.).

The bloom also has a major impact on the abundance of bacterioplankton in the estuary (Antai 1989) which presumably thrive on the high levels of DOC known from other studies to be excreted by *M. rubrum* (Smith & Barber 1979). In fact the numbers of bacterioplankton reached 1.9×10^{10} l^{-1} during a red-water event in 1987; these levels are as high as have been recorded in the marine environment (Antai 1989). The impact of these bacteria upon other organisms in the estuary are not known, but Crawford *et al.* (1992) have suggested that they are flushed out of the estuary and provoke a major immunological response in the Solent oyster population. This is supported by observations from Rias along the West coast of Spain which suggest shellfish and human pathogens of the *Vibrio* genus are associated with red-tides of *M. rubrum* (Romalde *et al.* 1990*a,b*). Strains of *V. alginolyticus*, *V. anguillarum* and *V. tubiashii* were particularly abundant during red-water patches, and their numbers were elevated by two orders of magnitude above those outside the red-water. *V. alginolyticus* is a well recognised opportunistic pathogen for humans (Molitoris *et al.* 1985), and *V. anguillarum* pathogenic to certain fish species

(Romalde *et al.* 1990*b*). *V. alginolyticus* is also thought to be the major producer of tetrodotoxin (TTX), one of the most potent natural neurotoxins known to man, principally in symbiosis with venomous marine organisms (e.g. Narita *et al.* 1987, Noguchi *et al.* 1987, Thuesen & Kogure 1989). Since Romalde *et al.* (1990*b*) showed that *V. alginolyticus* only displayed cytotoxicity against homoiothermic cell lines and not against those of poikilotherms, this could explain why no acute toxic effects have been reported to date on finfish or shellfish. Rausch de Traubenberg & Lassus (1991) have recently suggested a bacterial origin for PSP toxins, such as saxitoxin, since toxicity has been shown to vary widely depending upon environmental conditions, and since some phytoplankton species previously accepted to be non-toxic have recently 'become' toxic, as for example during the major bloom of *Chrysochromulina polylepis* in Scandinavian coastal waters. Because of the above considerations it may be prudent to remain cautious over the non-toxic status of *M. rubrum* observed to date.

There appears to be an inverse relationship between numbers of *M. rubrum* and macrozooplankton during the bloom (P. Wisemann pers. comm.; see Figure 2.22a). Whether this represents some form of exclusion or avoidance is not known. It may simply be that the herbivorous zooplankton themselves are heavily grazed by *Pleurobrachia* sp. and *Aurelia* sp. coming into the estuary at that time of year (J. Williams pers. comm.). However, the observation of an additional inverse relationship with depth during the bloom, but not at other times (Figure 2.22b,c), is more suggestive of an avoidance effect.

In terms of oxygen distributions, the potential effects could be widespread. Not only does *M. rubrum* produce oxygen supersaturation, through photosynthesis, but oxygen is gradually depleted lower in the water column through the respiration of the ciliate as the bloom progresses; high levels of bacterioplankton must also contribute significantly to this. This was not recorded to any major extent in 1985 or 1986, but other studies have demonstrated severe depletion in the estuary (Rees & Williams 1982, Soulsby *et al.* 1984), and the sequence of oxygen profiles in Figure 2.23 shows gradual depletion from a brief investigation in summer 1987 (from Hayes

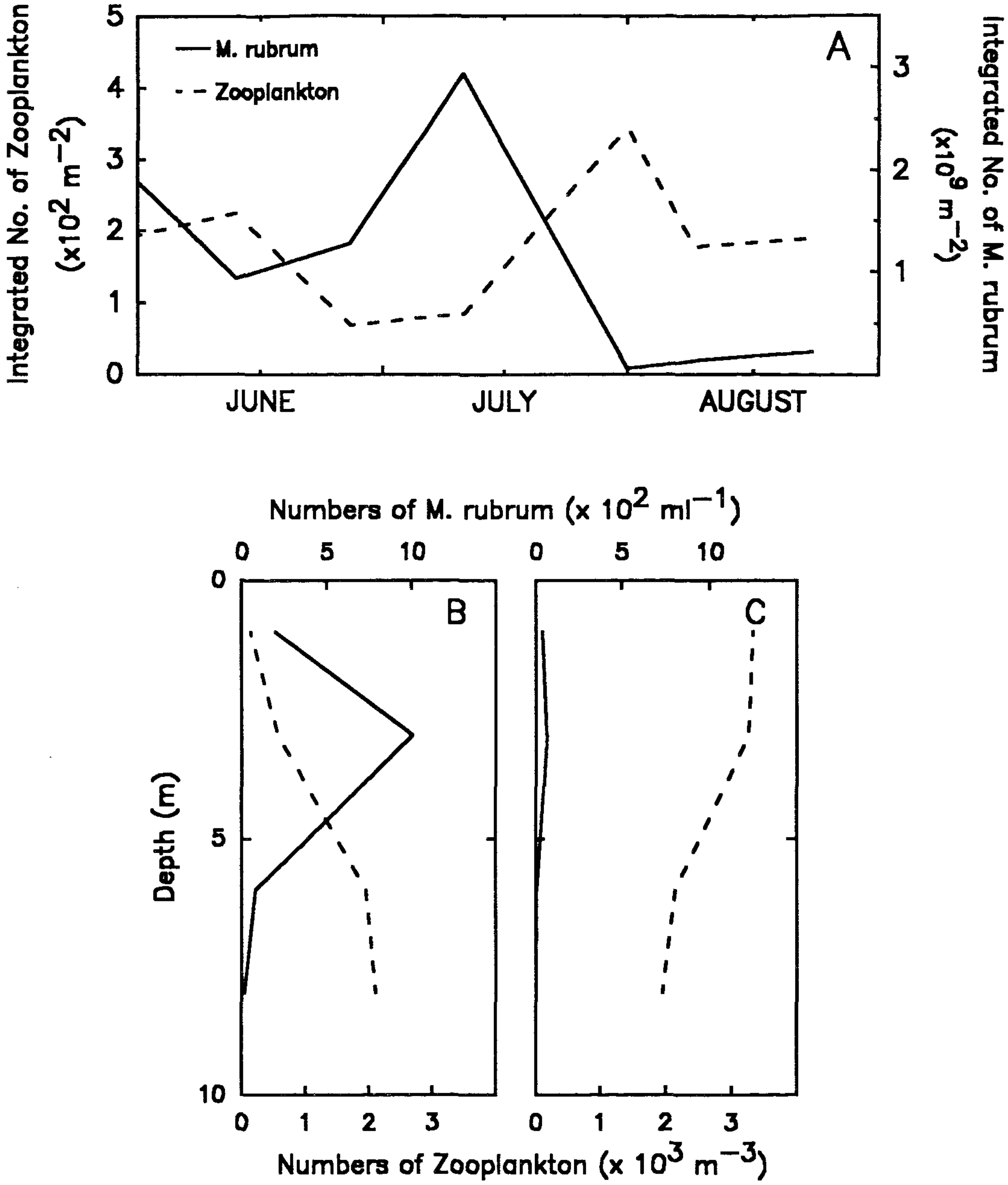


Figure 2.22.

Relationship between abundance of *Mesodinium rubrum* and total zooplankton at Cracknore buoy over spring/summer 1985.

A) Total numbers integrated through the water column

B) Depth profile during red-water

C) Depth profile after decline of red-water

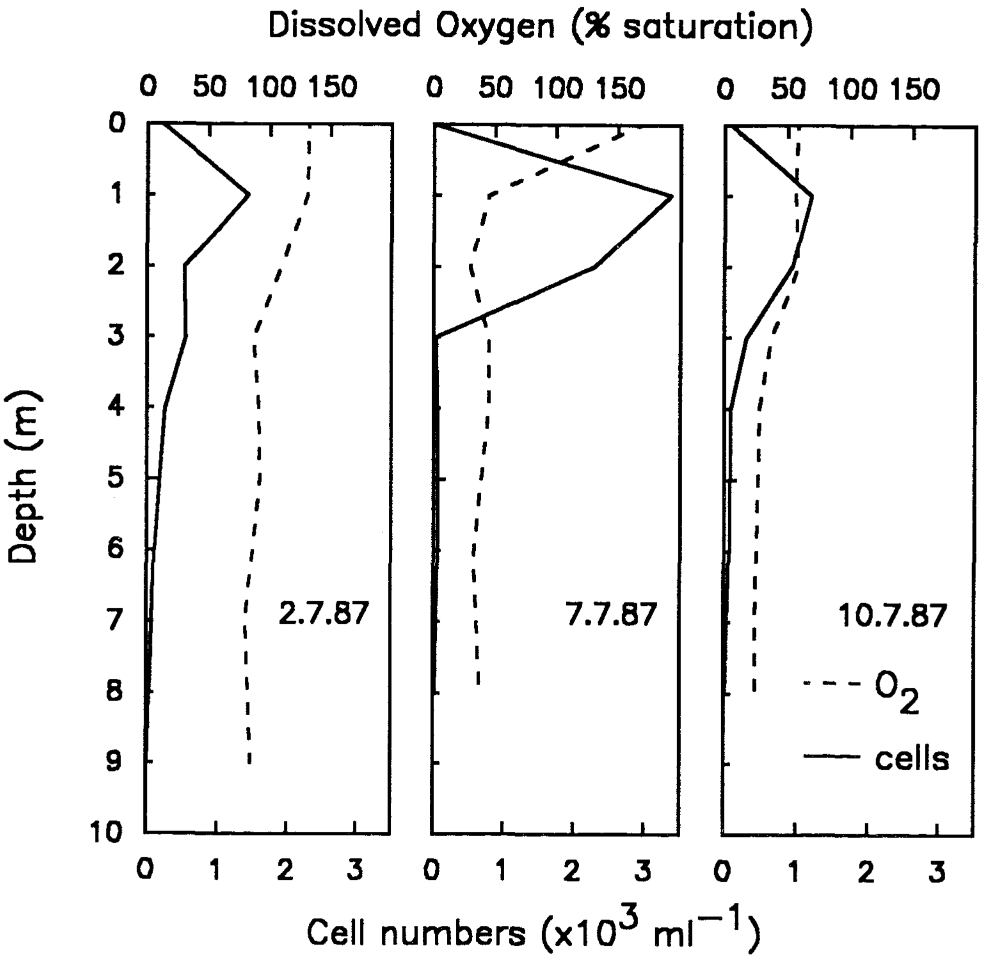


Figure 2.23.

Sequence of profiles of abundance of *Mesodinium rubrum* and oxygen saturation at Cracknore buoy, July 1987 (Data from Hayes 1987).

1987). After initial conditions characterised by supersaturated surface water, this declines to a state with supersaturated surface water and hypoxic bottom water, then culminates in an oxygen 'sag' throughout the whole water column. Soulsby *et al.* (1984) modelled these events and suggested that oxygen depletion resulted from respiration of the ciliate (and presumably bacteria) in the water column, and was not due to mass sedimentation and subsequent microbial decomposition processes in the sediment. Although oxygen levels have been recorded as low as 20% saturation, during some blooms, no adverse effects on other organisms have been demonstrated to date. However, Horstmann (1980) attributed mass mortalities of macrorganisms with hypoxia and anoxia caused by blooms of *M. rubrum* off the coast of South Africa. Oxygen supersaturation is another potential hazard to other organisms, for example gas-bubble disease in fish, however, Hayes *et al.* (1989) found no significant difference between the abundance of postlarval gobies in saturated, undersaturated, or superaturated conditions during a red-tide in the estuary.

Dense blooms of *M. rubrum* clearly have a major impact on the estuary and the organisms within it. However, the details of these effects are not obvious at this stage, and further study is clearly desirable. It seems likely that the red-water events have effects of potential chronic physiological stress rather than the acute toxic or anoxic effects typified by many other algal blooms, although the possibility of acute effects cannot be ruled out for future blooms.

Decline of Red-Water

The decline of the red-water seems to be rather more gradual than previously implied (Williams 1980, Rees & Williams 1982). There is little evidence of a major decline over a short period as has been observed for some other algal blooms around the world; levels of phaeopigments do not increase dramatically as might be expected if the bloom were to have suddenly 'crashed'. This tends to rule out the demise of the bloom by nutrient limitation, parasitic infestation or intense zooplankton grazing. Nutrient limitation has already been shown (see persistance of red-water) to be an unlikely factor, as has zooplankton grazing. Moreover, a recent study has shown that grazing of *M. rubrum* (at non-bloom densities) by copepods, only proceeds at about

20% of the rate for other ciliates due to the strong avoidance reaction to the feeding appendages (Jonsson & Tiselius 1990).

The disappearance of red-water can be tentatively linked again to water column stability; the bloom seemed to decline gradually over the summer as water column stability subsided, though with a considerable time lag. This lag would be expected if turbulence were to be shown to be a factor inhibitory to growth. Once red-water was established, a subsequent increase in mixing would not cause immediate mortality of cells, but would inhibit further growth and lead to a decline in the population after a time lag. It is interesting also to note that despite high numerical abundance later in the bloom, oxygen production was significantly lower than earlier, suggesting that net oxygen production (i.e. growth) was lower, possibly resulting from a higher respiration rate.

The implications of these observations will be further examined in the latter chapters of this thesis with regard to possible limiting factors for this species.

CHAPTER THREE:
VERTICAL DISTRIBUTION - DYNAMIC ESTUARY

"We perished, each alone:
but I beneath a rougher sea,
and whelmed in deeper gulfs than he"

William Cowper (1731-1800)

3.1. INTRODUCTION

Tidal flushing is accepted to be a fundamental factor controlling the development of plankton populations in estuaries (Ketchum 1954). Several bloom forming dinoflagellate species reduce flushing losses indirectly, through surface avoidance, by means of combinations of motile responses to various abiotic parameters, such as light, temperature, salinity and nutrients (e.g. Eppley *et al.* 1968, Chang & Carpenter 1985, Anderson & Stolzenbach 1985).

Although the study described in Chapter 2 was the first to investigate the recurrent red-tides occurring in the estuary in any detail, others have briefly described particular aspects of these events before (e.g. Williams 1980, Rees & Williams 1982, Soulsby *et al.* 1984). Unlike the spring bloom typical of coastal waters, including the Solent, the bloom caused by *M. rubrum* occurs in mid-summer in Southampton Water. Williams (1980), and Rees and Williams (1982) suggested that initiation of this bloom was limited until the growth rate exceeded the rate of flushing losses. That is, the generation time should become less than the flushing time of the estuary. This was based upon the studies of de Souza Lima & Williams (1978) and Bryan (1979) neither of which appeared aware of the causative organism of these blooms, or indeed of its peculiarities. Moreover, many anecdotal accounts have suggested that the bloom concentrates at the head of the estuary, and that flushing losses, based upon the observed distribution of red-water within the estuary, appear to be minimal (Antai 1989, Leakey 1989, Kifle pers. comm., Crawford pers. obs.); thus there seems to be a paradox. Since avoidance of flushing from the estuary by *M. rubrum* would negate the argument that bloom appearance is limited by flushing losses, it was considered critical to address this phenomenon. In particular, the vertical distribution of migration of *M. rubrum* over a tidal cycle was the most fundamental factor to examine.

Despite interest in its swimming speed, factors controlling vertical migration patterns of *M. rubrum* are not understood in any detail. *M. rubrum* attains phenomenal swimming speeds of over 8 mm s^{-1} (ca 200 body lengths s^{-1}; Lindholm 1985; Jonsson & Tiselius 1990), an order of magnitude greater than the majority of dinoflagellates (Smith & Barber 1979) and several times quicker than most ciliates (Sleigh & Blake 1977, Dale 1987*a*). Such swimming speeds enable *M. rubrum* to perform diel vertical migrations of some 40 m in the Peru upwelling zone (Smith & Barber 1979). *M. rubrum* is usually described as forming discrete surface or sub-surface (1-2 m) maxima during the day, apparently in response to light (Bary & Stuckey 1950, Smith & Barber 1979, Dale 1987*a*), and swimming down or dispersing at night (Smith & Barber 1979, Soulsby *et al.* 1984, Dale 1987*a*), although Passow (1991) has indicated that other factors may complicate this pattern. Moreover, such a behaviour pattern would clearly place the bulk of the population in a zone of intense near-surface currents during daytime ebb tides, thus maximizing the potential flushing rate to a level considerably greater than that of the estuary as a whole. Here, the evidence is examined for a mechanism by which *M. rubrum* could maintain itself in the estuary, faced with such potential flushing losses.

More generally, the role of vertical migration in the ecology of marine ciliates, and of red-tides dynamics, are matters of pertinent contemporary interest. The role of ciliates in marine ecosystems has attracted much interest as an important component of the microplankton (e.g. Beers *et al.* 1980, Smetacek 1981, Sherr *et al.* 1986*b*), but the adaptive significance of their considerably greater motile capacity has received only limited attention to date (e.g. Dale 1987*a*, Jonsson 1989). Moreover, recent observations on the abundance of mixotrophic and phototrophic ciliates (Stoecker *et al.* 1987, 1989, Crawford 1989) suggest that significant quantities of planktonic chlorophyll may be packaged in forms with much greater mobility than previously anticipated.

3.2. SAMPLING AND SITE DESCRIPTION

Sampling was conducted from 'Labrax' anchored at Eling buoy (see Figure 2.1), close to the head of the estuary[1], in July 1985 and July 1986. This prevented the need to move the boat which would have been necessary lower down the estuary because of shipping movements. Moreover, potential interference from shipping movements on the vertical distribution of *M. rubrum* was also minimised.

In such a dynamic estuary, it is impossible to track a body of water over the tidal cycle using a drifting boat for example, although this was attempted for a short period in an earlier study. The strong surface currents carry the boat swiftly downstream on the ebb tide, giving no advantage over the anchored boat. It had to be accepted that repeated sampling of a population within a fixed 'body of water' is impossible under these circumstances; the varying extent of differential flow with depth over the tidal cycle will constantly be 'smearing' horizontally the vertically migrating population.

3.3. RESULTS

Tidal advection past the fixed anchor station revealed significant horizontal fluctuations in the abundance of *M. rubrum*. To account for this, vertical distribution of *M. rubrum* (Figures 3.1 & 3.2) was represented as percentages of the total numbers integrated from the surface to 10 m; cell numbers (m^{-3}) were first expressed,

---

[1] details of sampling and methodology are given in the appendices.

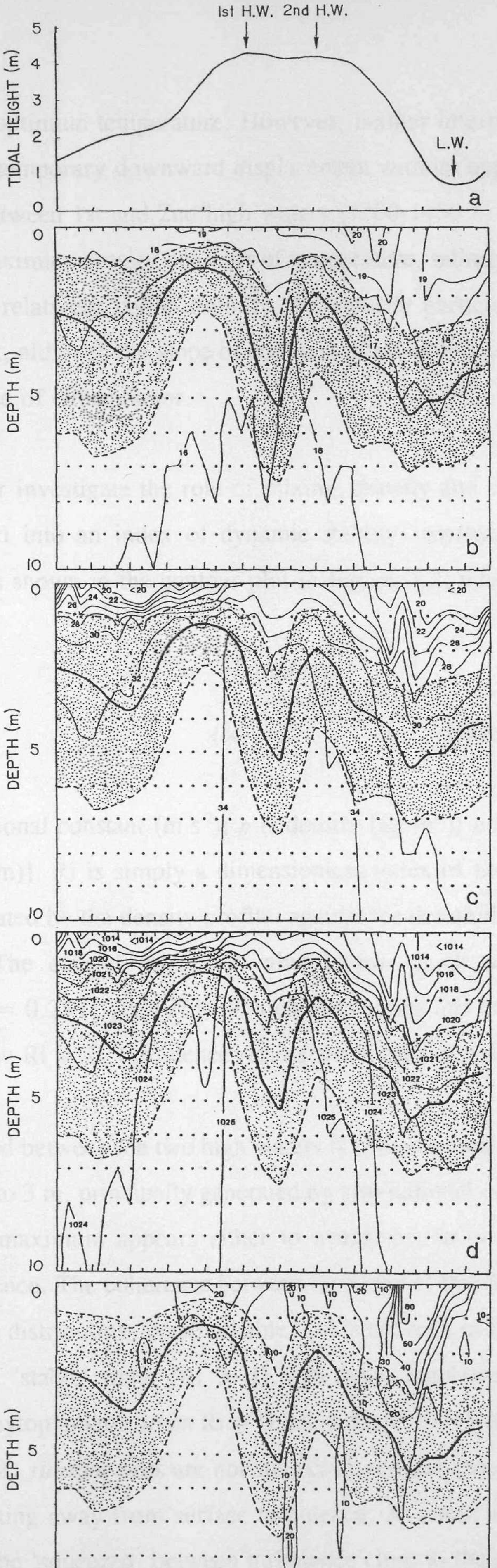
at any given metre depth interval, as a percentage of the total integrated population (cells m^{-2}). The depth, from the surface, of the 50% 'centre of gravity' of the population (thick solid line) was then derived, together with the 20% and 80% levels (stippled area between upper and lower dashed lines) to show degree of aggregation. This representation of population distribution is likely to considerably underestimate the actual degree of aggregation, since only 4 depths were sampled; this species aggregates to such an extent that a sampling interval of 0.5 - 1 m is preferable (Crawford 1989, Lindholm & Mörk 1990), though not always logistically feasible.

Figure 3.1 (a-e) shows the relationship, over a tidal cycle, between vertical distribution of *M. rubrum* (as described above) and the physical properties of the water column. The use of contours allows visual separation of migrational and advective phenomena i.e. whether population contours follow those of water mass properties. It is evident that during the ebb tide, between 1500-1800 h, the population deepens considerably, and thus is not exposed to the near surface flow, where flushing is greatest. Some coherence is apparent between the population maximum and the physical parameters during the ebb tide. The relationship with the 17°C isotherm appears strongest and could represent either a migration, with temperature as a cue, or, an artifact resulting from advective changes, with the population residing

---

Figure 3.1. (next page)

Relationship between vertical distribution of *M. rubrum* and contours of physical parameters of the water column over a tidal cycle during a red-water event at Eling buoy in July 1985. Fine line contours represent: a) tidal height (m), b) temperature (°C), c) salinity ($^{o}/_{oo}$), d) density (g l^{-1}), e) current speed (cm s^{-1}). Data points represent frequency of physical measurements. The thick solid line represents the depth, from the surface, of the 50% 'centre of gravity' of the integrated population of *M. rubrum*; the degree of aggegation is indicated by the stippled area between upper and lower dashed lines, representing the 20% and 80% levels respectively (see text for further explanation).


1st H.W.
2nd H.W.
L.W.
TIDAL HEIGHT (m)
a
b
c
d
e
DEPTH (m)
DEPTH (m)
DEPTH (m)
DEPTH (m)
TIME (hours)
8:00
10:00
12:00
14:00
16:00
18:00
16
18
19
20
<20
22
24
26
28
30
32
34
<1014
1014
1016
1018
1020
1021
1022
1023
1024
1025
1026
10
20
30
40
50
60
80

at a preferential optimum temperature. However, neither interpretation is supported by the sharp but temporary downward displacement without apparent cue, occurring approximately between 1st and 2nd high waters (1200-1400 h). During this period, the population maximum crosses contours of temperature, salinity and density, current speed remaining relatively stable. Salinity, and density particularly, show evidence of a mixing event, although the slope of contours of population displacement is much sharper than those of either factor.

To further investigate the role of mixing, density and current speed profiles were transformed into an index of dynamic stabilty, represented by 'Richardson Number' (Ri), as shown in the contour plot in Figure 3.2, where:

$$Ri = \frac{-g/\rho(\delta\rho/\delta z)}{(\delta u/\delta z)^2} \qquad \text{(Dyer 1973)}$$

[g is the gravitational constant (m s^{-2}); ρ is density (kg m^{-3}); u is current velocity (m s^{-1}); z is depth (m)]. Ri is simply a dimensionless index of the stabilizing effect of buoyancy, generated by the density profile, against the destabilizing effect of vertical current shear. The critical threshold value below which shear forces generate turbulence is Ri = 0.25 (Dyer 1973), though in estuaries this is accepted to be closer to Ri = 1. Below Ri = 0, turbulence results from static instability.

The period between the two high waters is characterised by surface turbulence extending down to 3 m, principally generated by gravitational instability (Figure 3.2). The population maximum appears either to avoid, or, to be mixed down by this increased turbulence. The coherence between the slope of the Ri contours, with those of the population distribution, is remarkable. Since the bulk of the population remains within a zone of 'stable' water (Ri > 1), and depth regulation is possible even by weakly motile phytoplankton when Ri = 0.5-1.0 (Ganf 1974), this would suggest that the majority of *M. rubrum* cells are not subject to vertical mixing, but rather may be actively aggregating away from surface turbulence. At about 1200 h, the population even appears to be 'squeezed' between turbulence close to the surface, and a zone of

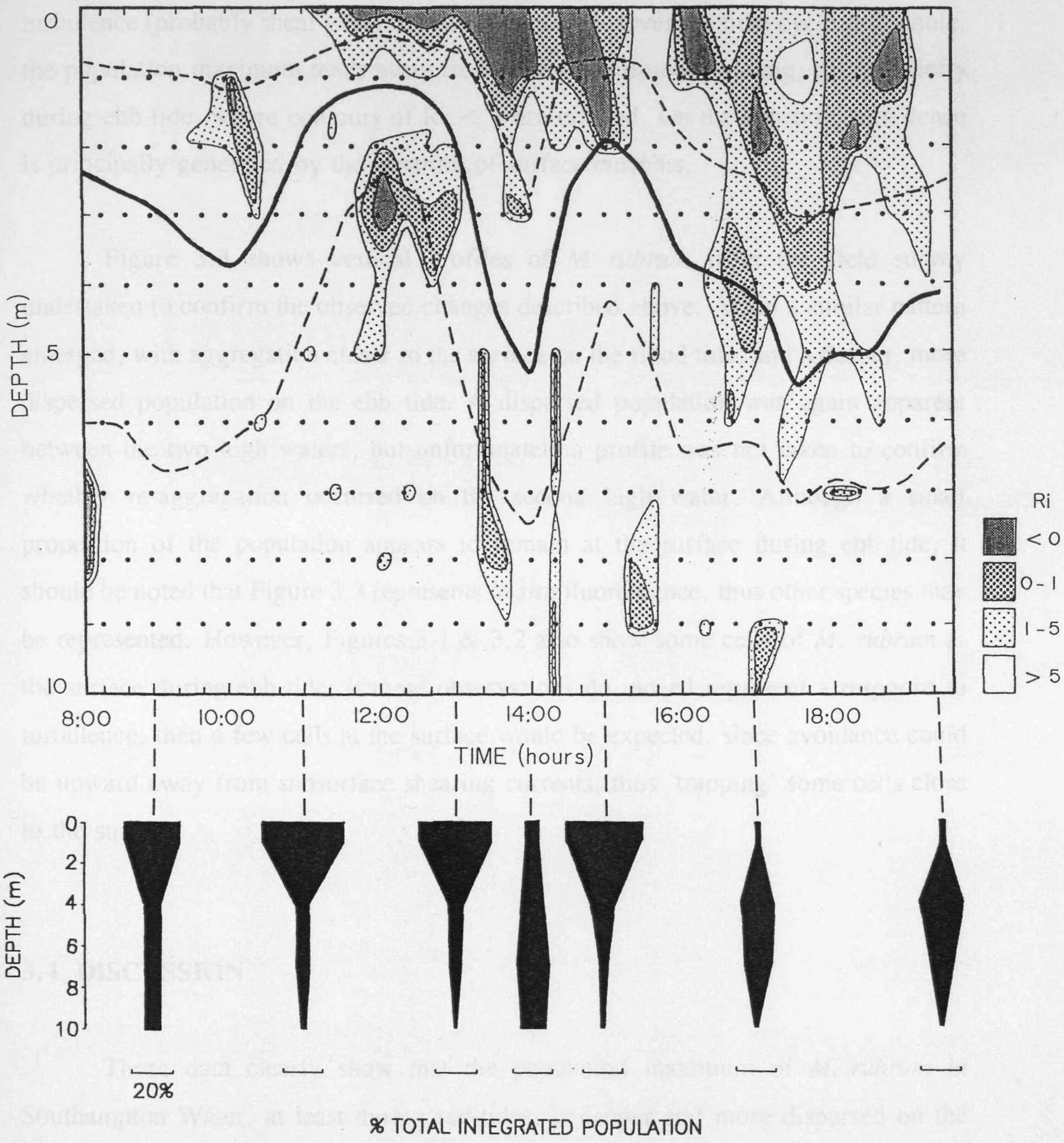


Figure 3.2.

Relationship between vertical distribution of *M. rubrum* (as indicated in Figure 3.1) and dynamic stability of the water column, represented by Richardson Number (see text for definition). The black shaded 'silhouettes' below emphasize more graphically the vertical distribution of *M. rubrum* at certain points during the tidal cycle; vertical distribution is represented as percentage of total integrated population.

turbulence (probably shear generated) at about 2-3 m. Over the tidal cycle as a whole, the population maximum tends away from zones of turbulent mixing, except briefly during ebb tide, where contours of $Ri < 1$ are crossed. On the ebb-tide, turbulence is principally generated by the shearing of surface currents.

Figure 3.3 shows vertical profiles of *M. rubrum* from the field survey undertaken to confirm the observed changes described above. Again a similar pattern emerged, with aggregation closer to the surface on the flood tide, and a deeper, more dispersed population on the ebb tide. A dispersed population was again apparent between the two high waters, but unfortunately a profile was not taken to confirm whether re-aggregation occurred on the second high water. Although a small proportion of the population appears to remain at the surface during ebb-tide, it should be noted that Figure 3.3 represents *in situ* fluorescence, thus other species may be represented. However, Figures 3.1 & 3.2 also show some cells of *M. rubrum* at the surface during ebb-tide; if these observations do indeed represent a response to turbulence, then a few cells at the surface would be expected, since avoidance could be upward away from subsurface shearing currents, thus 'trapping' some cells close to the surface.

3.4. DISCUSSION

These data clearly show that the population maximum of *M. rubrum* in Southampton Water, at least during red-tides, is deeper and more dispersed on the ebb than on the flood-tide or during slack water. In terms of the dynamics of the estuary, and perhaps in retrospect, such an observation was to be almost an inevitability; a behaviour pattern governed simply by phototactic aggregation close to the surface, as previously suggested (see Lindholm 1985 for review), would result in the advection by near-surface currents of the bulk of the population at least 5 km downstream on each daytime ebb tide. In contrast, the red-water typically maintains itself most intensely at the head of the estuary for several weeks without evidence of extensive flushing losses; the mouth of the estuary is characterised by very low

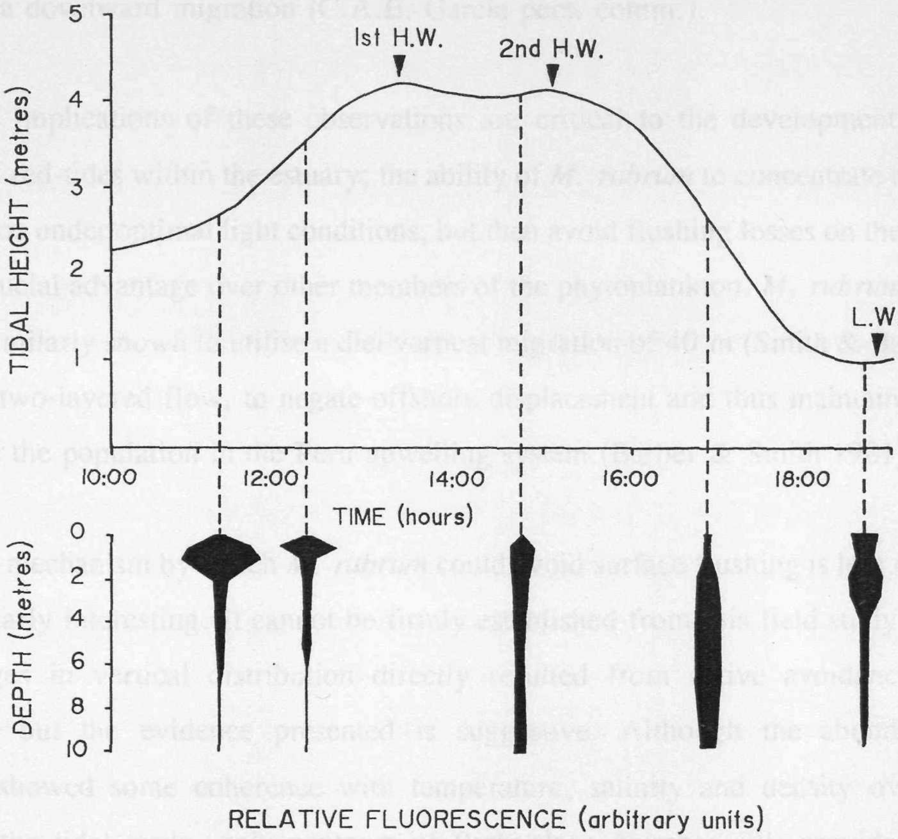


Figure 3.3

Vertical profiles of chlorophyll fluorescence (relative units) over a portion of the tidal cycle taken at Eling buoy during a red-tide caused by *M. rubrum* in July 1986. Fluorescence is adopted as an indicator of abundance of *M. rubrum*, which dominated the phytoplankton.

numbers of *M. rubrum*. The observations are further supported by data obtained during a survey of the estuary using the airborne thematic mapper to determine the spatial distribution of the bloom (Purdie & Garcia 1988); surface chlorophyll throughout the upper estuary was shown to be significantly reduced on the ebb tide, suggesting a downward migration (C.A.E. Garcia pers. comm.).

The implications of these observations are critical to the development and retention of red-tides within the estuary; the ability of *M. rubrum* to concentrate close to the surface under optimal light conditions, but then avoid flushing losses on the ebb tide, is a crucial advantage over other members of the phytoplankton. *M. rubrum* has also been similarly shown to utilise a diel vertical migration of 40 m (Smith & Barber 1979) in a two-layered flow, to negate offshore displacement and thus maintain and concentrate the population in the Peru upwelling system (Barber & Smith 1981).

The mechanism by which *M. rubrum* could avoid surface flushing is less clear but particularly interesting. It cannot be firmly established from this field study that such changes in vertical distribution directly resulted from active avoidance of turbulence, but the evidence presented is suggestive. Although the abundance maximum showed some coherence with temperature, salinity and density over a portion of the tidal cycle, only contours of Richardson Number (Ri) provided an explanation for the vertical displacements throughout the tidal cycle. Surface incident light intensity was not determined; however, the survey was undertaken on a cloudless sunny day with insufficient variation in light intensity to result in vertical migration of several metres at midday. The return of the population toward the surface in decreasing light, in the evening after the ebb tide (Figures 3.1 - 3.3), also suggests that light was not a major cue during daylight hours. However, this does not necessarily argue for light-independence for the vertical migration of *M. rubrum*; on the contrary, it appears to be strongly phototactic (see Lindholm 1985 for review) and has been reported to exhibit a diurnal migration pattern in Southampton Water (Soulsby *et al.* 1984) with near surface accumulation during the day, and downward dispersal during darkness. Such a pattern would, to some extent, reduce flushing losses in itself when ebb tide occurs during dark hours. This study provides considerably greater temporal resolution than acheived previously however, and it may be that an active response to turbulence could be superimposed upon the diurnal pattern i.e. when positive phototaxis draws the population into unstable near-surface waters. In fact, in a recent field study of vertical migration of *Gonyaulax catenata* and *M. rubrum*, Passow (1991) suggested that light dependant migration by *M. rubrum* appeared to be complicated by another unmeasured environmental factor. These observations could be explained by the present findings; turbulence is not necessarily a diurnal or tidal phenomenon and thus could interrupt profiles in an unpredictable fashion.

The limitations of the present study are acknowledged in terms of the lack of repeated surveys when the tidal and diurnal cycles are in a different phase; for example, when ebb-tide occurs during darkness or early morning. Thus, the afternoon descent of *M. rubrum* described here could simply represent a phenomenon typical of many migrating dinoflagellates. Nevertheless, the sharp downward displacement between 1200-1400 h (Figures 3.1 & 3.2), apparently with only turbulence as a cue, and the return of the population toward the surface after ebb-tide (Figures 3.1 - 3.3), support the contentions presented here. As has been described for some dinoflagellates, the observed distributions could represent the resultant pattern of a combination of interacting factors, and controlled studies would clearly be desirable. However, the relationship with turbulence is of sufficient novel interest to merit attention.

Experimental verification of turbulence avoidance has not been feasible at this stage; *M. rubrum* has not yet been cultured, is extremely fragile, and does not respond well to laboratory containment (Lindholm 1985). However, the interpretation of aggregation away from turbulence is supported by other published evidence. By means of rapid swimming, in response to small scale water movements, *M. rubrum* avoids isolation by micropipette (Lindholm 1985, Taylor *et al.* 1971, Crawford unpubl.), and substantially reduces capture efficiency by the feeding appendages of the copepod *Acartia tonsa* (Jonsson & Tiselius 1990). Since the motile behaviour of *M. rubrum* consists of alternation between rapid 'jumps' and periods of motionlessness (Lindholm 1985, Crawford 1989, Jonsson & Tiselius 1990), an increase in the frequency of 'jumps' in response to turbulence would result in a net aggregation into more stable zones of the water column (i.e. a directional response is not required). The apparent upward 'squeezing' of the population by turbulence from below at 1200 h supports this contention. Such increased 'escape' responses under turbulent conditions has recently been documented for the marine copepod *Centropages hamatus* (Costello *et al.* 1990). The mechanism by which so small an organism as *M. rubrum* (15-70 μm cell diameter) could sense fluid deformations is more difficult to resolve. Detection of microscale shear currents must be suspected, though this would be on a scale (< 100 μm) poorly understood by hydrodynamicists. The smallest turbulent length scale shown to stimulate dinoflagellate bioluminescence is of the order of 33 μm (Rohr *et al.* 1990), thus such small-scale shear could feasibly act as a cue. Sensing of acceleration forces using statocyst-like bodies, as described in the ciliate *Loxodes* sp. (Fenchel & Finlay 1986), is also conceivable, though no such structures have been described in ultrastructural studies on *M. rubrum* to date. Jonsson (1989) presented a hypothesis for the surface accumulation of oligotrichs and tintinnids based upon net upward swimming or passive geotaxis, which depended on the asymmetry of cell morphology. This seems unlikely to be a major factor explaining migration in *M. rubrum* which is both a more symmetrical cell (equatorial band of cilia and cirri), and, capable of much more extended diurnal migrations than other species (see Lindholm 1985).

The role of turbulence in red-tide ecology has clearly been underestimated, and is only now becoming fully apparent. Turbulence has been shown to interfere with growth (White 1976), cell division (Pollinger & Zemel 1981) and motility (Thomas & Gibson 1990*a,b*) in some dinoflagellates. Further research is imperative, since turbulence is not a routinely quantified parameter in plankton studies. Other studies have already documented the retention of dinoflagellates in estuaries through surface avoidance, however, the mechanisms appear to have been different, although turbulence was not measured. Anderson & Stolzenbach (1985) suggested that *Alexandrium (Gonyaulax) tamarensis* and *Heterocapsa triquetra* reduced flushing losses by means of avoidance of high surface light intensities. Using a dye injection technique in a small estuarine embayment, Garcon *et al.* (1986) demonstrated that *A. tamarensis*, whose growth rate approximated the rate of tidal flushing, could only bloom by such avoidance of outflowing surface waters.

More generally, the implications of these observations may be far-reaching; this study is quite atypical in providing an opportunity to simultaneously examine the vertical distribution of a motile microplankter, together with the physical properties defining water column stability on a scale relevant to the organism. Intuitive or empirical estimates of static stability are often derived from density gradients during plankton studies, but velocity profiles, allowing direct assessment of dynamic stability, are rarely measured (Heaney & Butterwick 1985). Mechanoreceptor induced escape reactions are a widespread phenomenon among the macro- and microplankton; however, the potential combined role of turbulence with such responses as a factor influencing microscale patchiness surely merits further investigation.

In terms of the present thesis, the implications are particularly interesting in the context of the conclusions of Chapter 2; this suggested that the decline in turbulent mixing may be a critical factor in the development of the red-tide in Southampton Water. The vertical migration study described here suggests that turbulence itself may elicit a greater frequency of motile jumps in *M. rubrum*. The combination of these ideas with regard to limitation of red-water appearance will be further developed later in the thesis in Chapters 7 - 10.

CHAPTER FOUR:
VERTICAL DISTRIBUTION - STABLE LAKE

> *"Though hast not gain'd a real height,*
> *nor art thou nearer to the light,*
> *because the scale is infinite"*
>
> Tennyson (1809-1892)

4.1. INTRODUCTION

The vertical migration of *M. rubrum* is poorly understood, and the implications of Chapter 3 that turbulence could be an important overriding factor complicates the matter even more. In order to further comprehend the factors governing vertical migration, it was desirable firstly to examine the distribution and potential migration patterns in a more stable environment, where the influence of turbulence was minimal.

Dale (1987*a*) and Lindholm & Mörk (1990) have provided preliminary information on the distribution of *M. rubrum* in stable enclosed environments, but dynamic variations in distribution on smaller temporal and spatial scales have not yet been described. The data presented by Lindholm & Mörk (1990) suggested that vertical migration in a brackish lake did not appear to be occurring regularly on a diel basis. However, photosynthetically healthy cells were found at depth, and thus it seems likely that these must be visiting the upper illuminated layers on a fairly regular basis, if not actually with a diel periodicity.

As *M. rubrum* is such a fast swimmer, vertical migration is not limited by depth, at least down to 40 m (Smith & Barber 1979, Sorokin & Kogelschatz 1979, Barber & Smith 1981), and temperature, salinity and oxygen gradients seem to pose no barrier (Taylor *et al.* 1971, Lindholm 1985). In fact, *M. rubrum* is able to traverse a temperature gradient of 20°C (20 to 2°C) in less than 1 hour (Lindholm & Mörk 1990).

Because of the abundance and potential swimming speeds of planktonic ciliates, their vertical migration is now generally of some interest. However, little has been published on the subject to date other than preliminary distribution patterns with a review of speeds (Dale 1987*a*), and also an experimental analysis of potential mechanisms involved (Jonsson 1989).

Much more is known on vertical migration of dinoflagellates; under controlled conditions this has been shown to be regulated by a number of complex interacting factors (e.g. Eppley *et al.* 1968, Heaney & Eppley 1981, Cullen 1985). Migration itself seems to be controlled primarily by phototaxis, but migration speed and distance are mediated by temperature, nutrient concentration and daily irradiance (Heaney & Eppley 1981, Kamykowski & McCollum 1986). Under limiting nutrient conditions, cells seem to spend less time in the euphotic zone, beginning upward migration later, and downward migration earlier than under nutrient replete conditions (Eppley *et al.* 1968, Heaney & Eppley 1981). It is suggested that such migration patterns represent a compromise between light and nutrient requirements, and are a behavioural adaptation to optimize growth when surface nutrients are depleted. Photosynthate produced in the light close to the surface is stored as carbohydrate during the day to be utilised to support nocturnal uptake of nitrate at depth (Cullen 1985).

As *M. rubrum* is a phototrophic ciliate, it is of interest to examine its vertical distribution and migration under conditions of vertical stability, when surface nutrients are limiting, in order to contrast this with the established pattern for dinoflagellates. In this study, distribution of the ciliate was examined in a brackish lake for which Lindholm & Mörk (1990) have already provided preliminary information. These conditions contrast with those for Southampton Water described in Chapter 3, as at least during summer months, the role of turbulence can be assumed to be minimal as a strong thermocline develops, and wind speeds are normally low. The semi-enclosed stable lake provided almost 'mesocosm'-like conditions, since the role of tidal, and thus advective disturbance of the population, was minimal. In addition, the fact that *M. rubrum* cannot yet be cultured means that such studies under 'semi-controlled' field conditions currently provide the only source of reliable information on vertical migration mechanisms. More generally, *M. rubrum* is a common and cosmopolitan species around the world, occurring in stable semi-enclosed coastal water bodies, as well as in more well-mixed coastal waters (Lindholm 1985). As such, vertical migration mechanisms of this species could at times be an important component in the functioning of such ecosystems, particularly in the form of a 'nutrient pump' from deep to surface waters.

4.2. SAMPLING AND SITE DESCRIPTION

This study was based at the Husö Biological Station on the Åland Islands, which are situated in the Bothnian Sea off southwestern Finland (Figure 4.1). Sampling was conducted about 25 km from the field station, in Inre Verkviken, a 20 m deep fjord-like brackish inlet, connected to the Bothnian Sea via a 200 m long, 1 m deep canal. Salinity tends to be between 5 and 6‰, usually with a very weak halocline, but a strong thermocline in summer.

All sampling was conducted from a rowing boat, situated approximately in the centre and deepest part of the lake[1].

4.3. RESULTS

Figure 4.2 shows a depth profile of abundance of *M. rubrum*, temperature and salinity taken on 27 May 1991 at about 15:00 h. This is a typical profile of physical

---

[1] full details of sampling and analyses are given in the appendices.

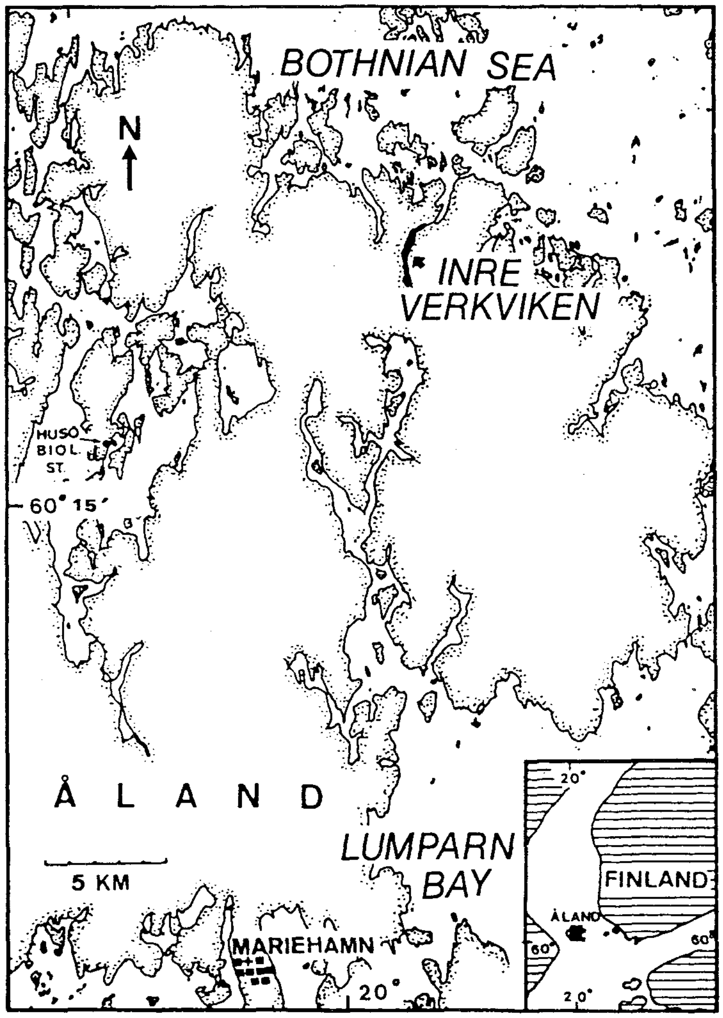


Figure 4.1.
Location of the field station and brackish lake in which sampling was conducted.

parameters for summer; the halocline is very weak, and stability is maintained by a strong thermocline. The population appears to be distributed into three distinct maxima, though with the upper one poorly defined. However, significant numbers of cells are present at all depths. Standard deviation of cell counts at each depth is also presented and represents the S.D. of 5 replicate transects across the base of the counting chamber. The sampling error was also examined by taking 4 replicate bottle samples; this gave counts (cells ml^{-1}) with S.D.'s of 96.5 (± 14.3), 99.9 (±31.9), 102.5 (±12.2), and 99.0 (±2.8). This gives a mean count of 99.0 cells ml^{-1} with a S.D. of 2.8, indicating that the error for multiple bottle sampling was quite low, and well below the order of variation in abundance observed with depth.

In Figure 4.3, the same profile as that in Figure 4.2 is shown (without error bars) in relation to profiles of chlorophyll, oxygen, light and nutrients. Cell numbers are relatively high for a non-bloom situation (up to ca. 100 ml^{-1}), though not quite high enough (ca. 200 ml^{-1}) to discolour the water (McAlice 1968). Chlorophyll clearly shows the same trend as cell numbers, suggesting that *M. rubrum* is probably the predominant source of chlorophyll. With cell numbers of 100 ml^{-1}, and assuming a cell diameter of 30 μm and chlorophyll content of 2.6 fg μm^{-3} (Stoecker *et al.* 1991), this should theoretically give a chlorophyll value of 3-4 $\mu g\ l^{-1}$ solely from *M. rubrum*; the actual value of 5-6 $\mu g\ l^{-1}$ suggests that over 50% is provided by the population of *M. rubrum*. However, the chl*a*:cell ratio falls with depth, implying either that other photosynthetic species are more important at the surface, or that the level of cellular chlorophyll of *M. rubrum* falls with depth. The fact that the cellular chlorophyll of deep cells (2-3 fg μm^{-3}) is about the same as determined in Chapter 2 and by Stoecker *et al.* (1991), suggests that the former explanation is the more probable. In fact, a small *Gymnodinium*-like dinoflagellate was present in high numbers in samples taken close to the surface.

From the light profiles taken, the irradiance of 20 $\mu E\ m^{-2}\ s^{-1}$ was plotted as a horizontal line on the profiles shown in Figure 4.3, as this is the level estimated in Chapter 2 to represent the light required for photosynthesis to just exceed respiratory

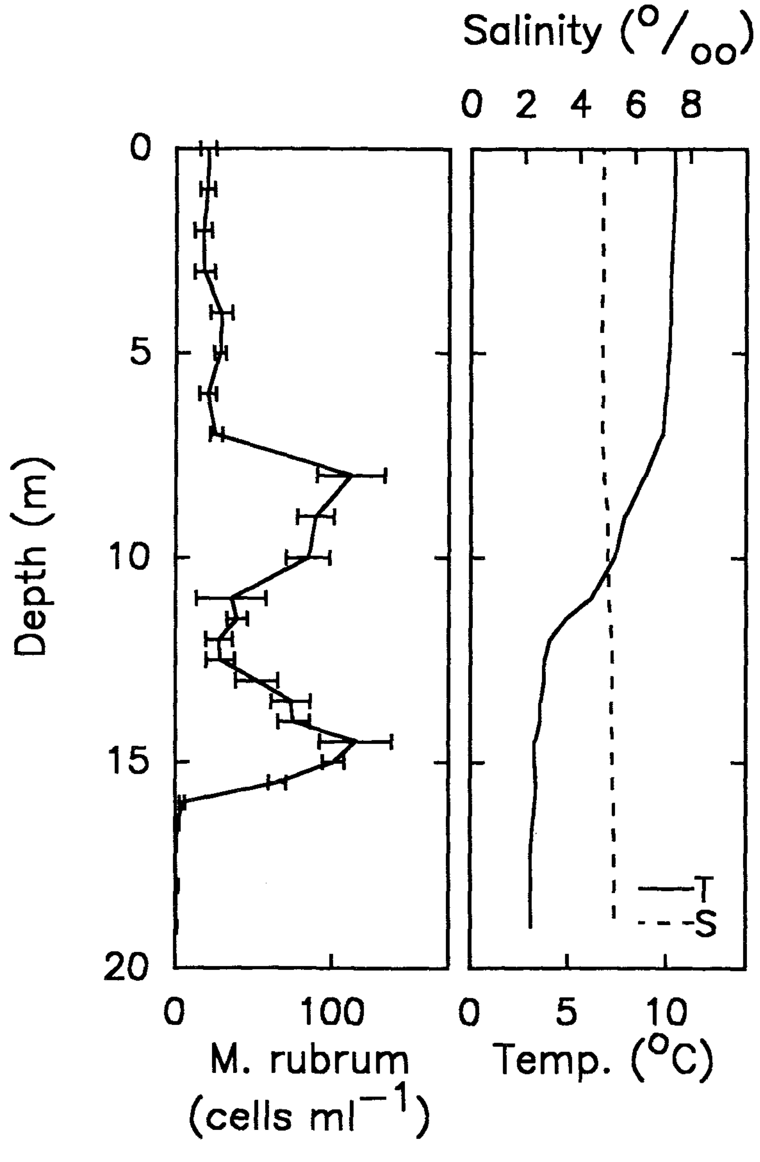


Figure 4.2.

Depth profiles of abundance of *Mesodinium rubrum* (with S.D. of counts), temperature and salinity, in Inre Verkviken on 27 May 1991 at 1500 h.

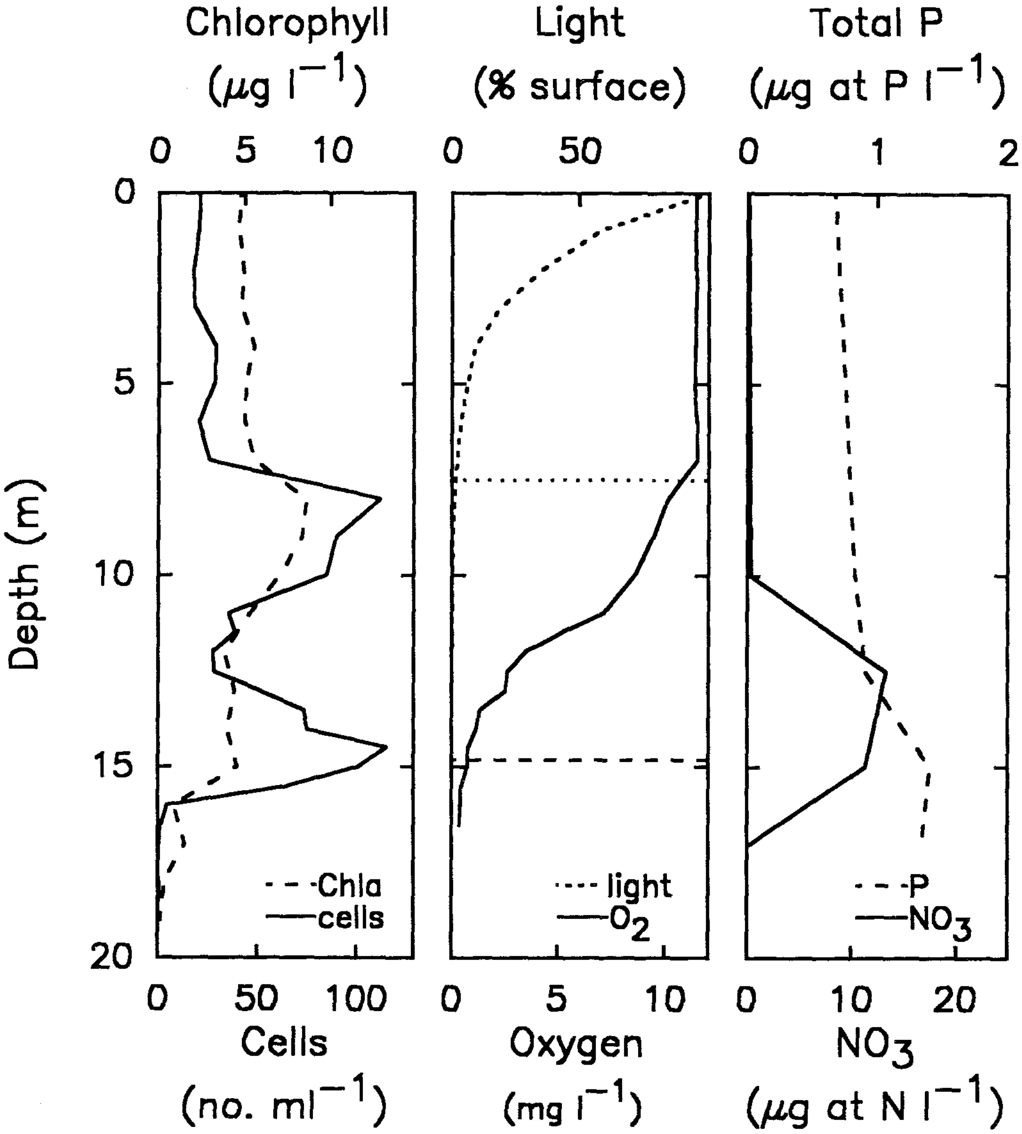


Figure 4.3.

Depth profiles of abundance of *Mesodinium rubrum*, chlorophyll, irradiance, oxygen concentration, total phosphorus and nitrate; taken at Inre Verkviken on 27 May 1991. The horizontal dotted line indicates the depth where irradiance is 20 μE m^{-2} s^{-1}; horizontal dashed line indicates depth where O_2 concentration is 0.5 mg l^{-1}.

demands. Here, this irradiance level occurs at about 7.5 m; the second cell abundance maximum occurs just below this level, whereas the upper one at about 4 m, and the cells occurring above, were subject to light intensities of between 70 and 700 μE m^{-2} s^{-1}, irradiances which are quite sufficient for growth. Oxygen saturation was fairly high in surface waters (120-130%), and remained at this level down to a depth of 7 m, below this it declined rapidly with depth; at about 15 m the water was almost totally anoxic. A dashed horizontal line indicates the level of 0.5 mg O_2 l^{-1} (about 5% saturation), this was used as an index of the depth of the anoxic boundary as O_2 never quite reached zero saturation. It is possible that the sampling technique adopted introduced small amounts of oxygen into the bottles. It is likely that water with an O_2 concentration of less than this probably was in fact anoxic, as it had a very strong odour of H_2S. The deeper abundance maximum of *M. rubrum* occurred just on this anoxic boundary, although some cells were also observed in the anoxic water itself, as previously noted by Lindholm & Mörk (1990).

Surface nutrient levels shown in Figure 4.3 are low, as has been shown already by Lindholm & Mörk (1990) for this lake in summer. Nitrate is between about 0.1 and 0.5 μg at l^{-1} in the surface 10 m, but increases sharply below this to over 10 μg at l^{-1} in deeper less oxygenated water. It is interesting that the middle peak in abundance of *M. rubrum* lies just between the estimated compensation irradiance for growth of 20 μE m^{-2} s^{-1}, and this nitracline. Nitrate levels decline sharply again below the anoxic boundary, probably due to anaerobic microbial denitrification processes in the reducing environment. Unfortunately, only total phosphorus (particulate + dissolved) was determined, rather than dissolved inorganic phosphate, but this also suggested low levels in surface water and an increase with depth. The problem with this approach is that with the relatively high cell numbers present, variations in cellular phosphorus could mask variations in dissolved inorganic phosphorus.

Figure 4.4 shows a sequence of profiles taken overnight on 28/29 May. The observed vertical distribution took the form of a single peak at about 10 m depth at 18:00; by 03:00 this appeared to have split into 3 peaks, though with the upper one

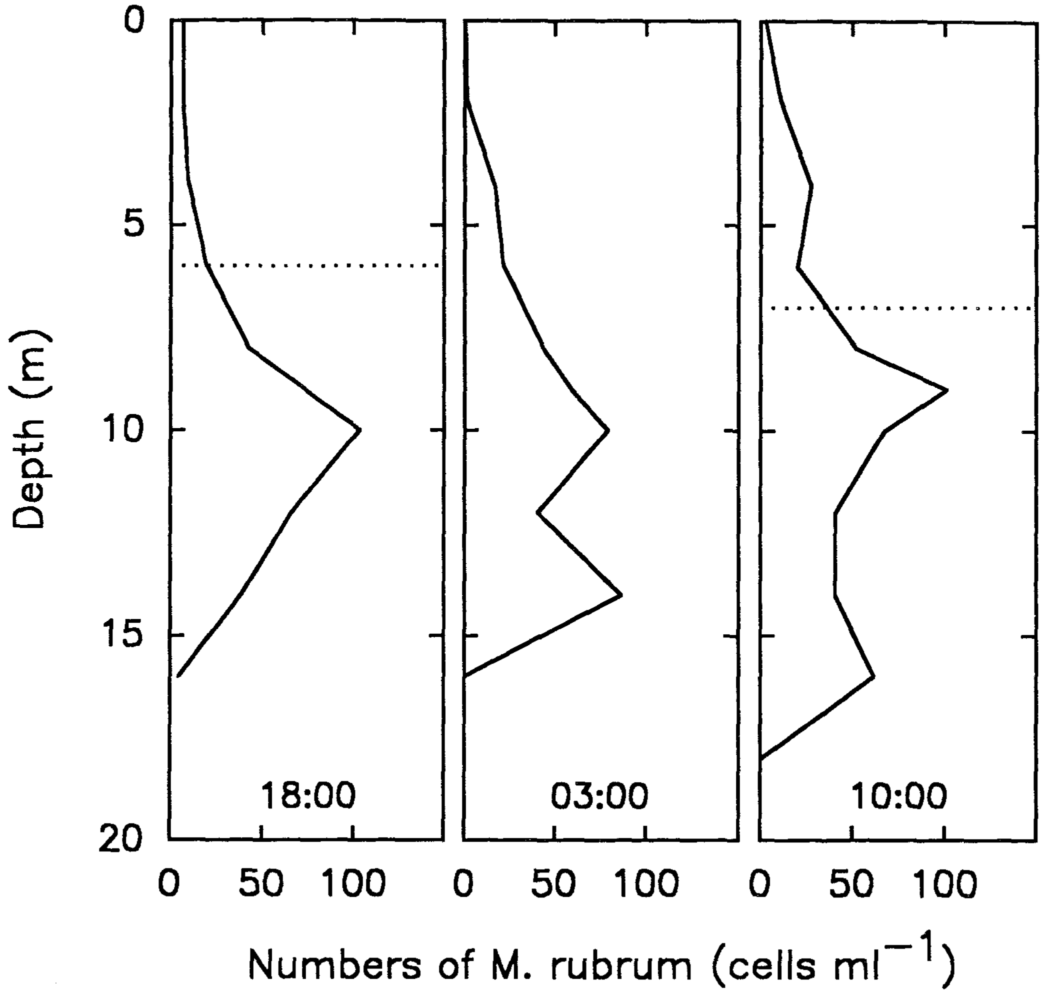


Figure 4.4.

Sequence of profiles of abundance of *Mesodinium rubrum* taken at Inre Verkviken on 28/29 May 1991. Horizontal dotted lines indicate irradiance of 20 μE m^{-2} s^{-1}.

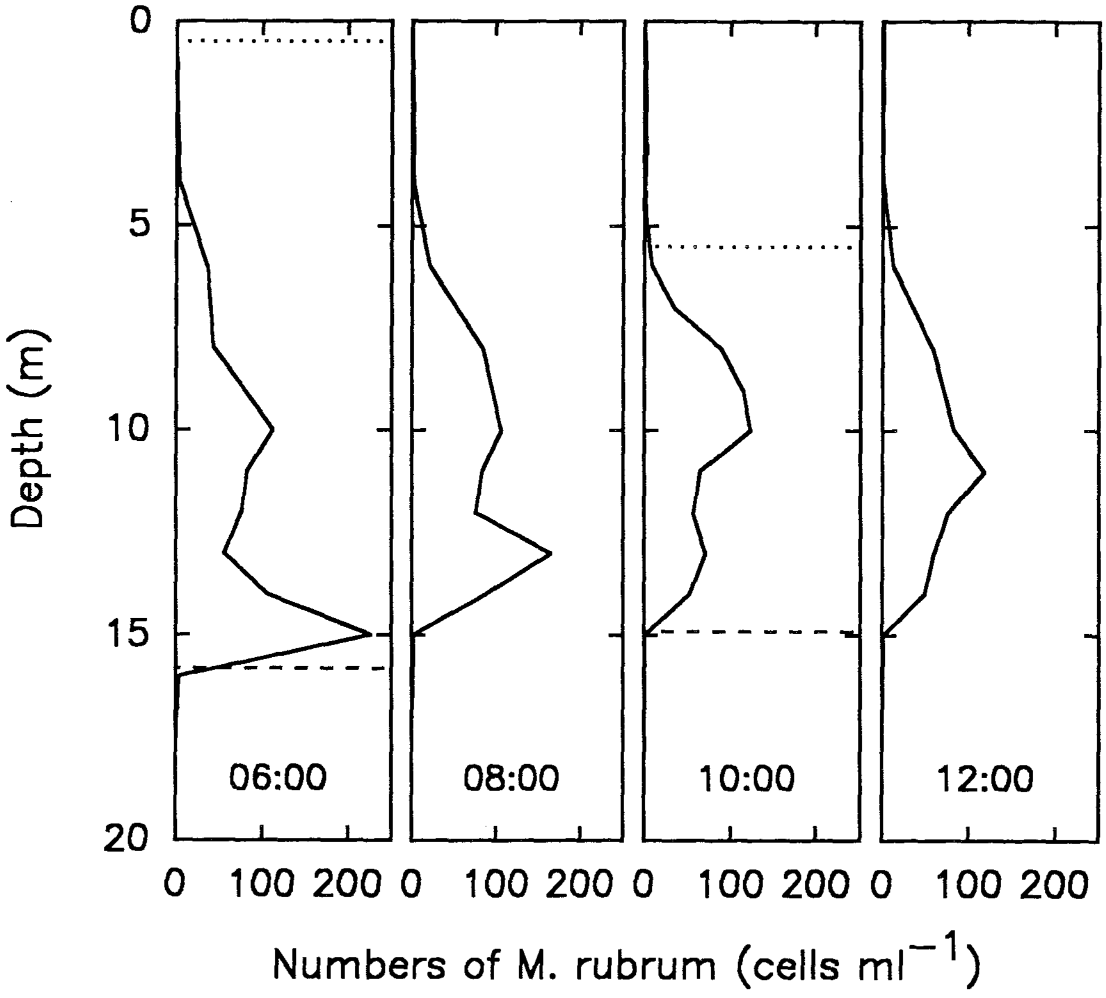


Figure 4.5.

Sequence of profiles of abundance of *Mesodinium rubrum* taken at Inre Verkviken on 4 June 1991. Horizontal dotted lines indicate irradiance of 20 μE m^{-2} s^{-1}; horizontal dashed line indicates O_2 concentration of 0.5 mg l^{-1}.

poorly defined, and appearing rather as a 'bulge'. Then by 10:00, the 3 peaks were still present but with the upper one more pronounced than before. The peaks had also become more separated, and the lower one again occured on the anoxic boundary. It should be noted however, that despite the presence of these distinct maxima, significant numbers of cells (>20 ml^{-1}) occurred from the surface right down to the anoxic boundary. In the absence of turbulence (wind speeds were low), this suggests that behavioural mechanisms must be regulating this distribution.

Figure 4.5 shows a further sequence of profiles of cell abundance, taken a few days later on the morning of 4 June 1991; other variables were recorded for some of these profiles e.g. light, oxygen, but since these were conducted at intervals of 2 hours, it was not logistically feasible to record each parameter on every profile, particularly with about 20 Ruttner samples to be taken for each profile. At 06:00, Figure 4.5 suggests the presence of 3 abundance maxima, as shown already for 27 May, again the upper 'bulge' seems rather poorly defined. The upper peak and the lower peak then appeared to migrate downward and upward respectively, gradually merging with the central peak at about 10 m depth by 08:00 - 10:00. In the final profile at 12:00, the three seemed to have almost completely merged at 11 m. The anoxic boundary has also shallowed slightly with the lower abundance maximum, possibly suggesting consumption of oxygen by the high numbers of cells present. Light was obviously low at 06:00, only just exceeding 20 μE m^{-2} s^{-1} at the surface. By 10:00, this compensation level had reached about 5-6 m, but almost all of the population was below this depth, implying that there was insufficient light for growth at this time.

4.4. DISCUSSION

Although from the data presented there is evidence of changes in vertical distribution with time, there seems no clear diel pattern, and this supports the preliminary observations of Lindholm & Mörk (1990) for *M. rubrum* in this lake. Depth profiles of cell numbers seemed to vary considerably, particularly between situations characterized by between 1 and 3 abundance maxima. However, the patterns were contradictory; in Figure 4.4 a single peak appeared to divide into three peaks overnight, with the lower peak migrating downwards and the upper peak migrating upwards in the morning. In contrast, Figure 4.5 suggests that during approximately the same period (06:00 - 10:00) a few days later, the opposite was occurring; the upper peak was migrating downward and the lower one upward, finally merging with the central peak at 10-11 m depth by 12:00. The patterns thus seem complicated, as has been shown for dinoflagellates (e.g. Heaney & Eppley 1981, Cullen 1985), and, as for dinoflagellates, vertical distribution and migration does not appear to be a process governed simply by phototaxis alone as implied by early studies (e.g. Bary & Stuckey 1953, Smith & Barber 1979).

These observations are therefore to a certain extent consistent with the notion that the population may be compromised between nutrient limitation at the surface, and light limitation below 10 m depth in the nitracline. This is supported by the work of Lindholm & Mörk (1990) who took samples from nutrient rich deep water, incubated them at the surface, and found them to be highly productive. It should be pointed out that considerations based upon 20 μE m^{-2} s^{-1} representing a light level where photosynthesis just exceeds respiration costs may be misleading; firstly, true respiration costs are not known, and secondly, *M. rubrum* may be much more photosynthetically efficient at depth than this suggests. The limited studies on P vs I curves have not provided any information on the spectral quality of the source light provided in relation to that encountered in the field. The photosynthetic efficiency in light at the blue end of the spectrum (i.e. deep in the water column) may be considerably greater than that in simulated surface light.

As *M. rubrum* is such a phenomenal swimmer, it may not necessarily be compromised by having to perform migrations with a diel periodicity in order to optimise growth in this stratified environment. For example with a swimming speed of 5 mm s^{-1} or greater (Lindholm 1985), theoretically it could perform a migration of 18 m h^{-1}; this is sufficient to exploit the whole of the oxygenated water column within 1 hour. Thus, the profiles could represent the dynamic summation of a population in which cells are undergoing migration on a continuous individual basis. That is, each cell migrating according to balancing its own individual needs of carbon and nitrogen; in fact, with a high swimming speed and high efficiency of photosynthesis, a diurnal pattern may not be necessary. Assuming a respiration rate of about 1% cell C h^{-1}, and a light saturated photosynthetic rate (P_{max}) of about 12% cell C h^{-1}, cells would only require a spell of about 2 h each day in the upper 5 m (where $I_k > 275$ μE m^{-2} s^{-1}) in order to satisfy respiratory demands. The actual respiratory rates estimated in Chapter 10 suggest that 1% cell C h^{-1} may even be an overestimate, so that the time required at the surface might in fact be less than this. Any additional time spent in surface waters would be contributing to net growth. The fact that cells found at depth in almost zero light are photosynthetically normal and rich in chlorophyll, suggests that these must be visiting the surface fairly regularly. Lindholm (1985) has pointed out that cells kept in darkness for several days show apparent reduction in chlorophyll and numbers of chloroplasts (also Crawford pers. obs.). Obviously, given these considerations, cells could either migrate once or several times per day, or perhaps more likely, only undergo migration once every few days, but remain at the surface for longer periods.

In this sense, the pattern of migration for *M. rubrum* could be quite at variance to that established for dinoflagellates; it may indeed be necessary for the latter to migrate on a diel basis in order to optimise growth in stratified environments. With migration speeds only of the order of 1 m h^{-1} for the majority of species (e.g. Sournia 1982), and an apparently lower photosynthetic efficiency than *M. rubrum*, dinoflagellates could not feasibly undertake migrations on shorter time scales and thus a diurnal cycle is probably the optimal strategy.

The alternative explanation for the observed multiple population maxima of *M. rubrum* is that cells are in fact migrating in phase to some extent; thus, the multiple peaks could represent separate 'cohorts' of either light or nutrient limited cells, with the central peak at about 10 m representing a point where crossing over occurs. This central maximum appears to be present in all profiles, and at approximately the same depth in each; this could represent a 'compromise' or 'ticking over' depth. Here, although both light and nutrients would be limiting to some extent, both would be supplied in small amounts. In support of this contention, this point usually occurs exactly between the assumed limiting light level of 20 μE m^{-2} s^{-1} and the depth of the nitracline (see Figure 4.3). In the context of such a theory of distinct migrating cohorts of cells, it would have been interesting to investigate variation in size distribution with depth, but unfortunately time was not available for such a laborious investigation.

Clearly there is insufficient data from this study to clarify the patterns and mechanisms involved in vertical migration for this species. If some form of size-dependant migration is occurring, this complicates the matter and suggests that patterns will only be elucidated by a program of very high resolution temporal and spatial sampling of both population and physico-chemical parameters. Temporal and spatial sampling scales of resolution less than 1 hour and 1 m, respectively, will be insufficient to resolve these patterns, as will survey durations of less than 24 hours. Since size distribution analysis would also be required for each depth sampled, this suggests a daunting task for future surveys.

CHAPTER FIVE:
SOME OBSERVATIONS ON MORPHOLOGY

"We see only what we know"

Goethe (1749-1832)

5.1. INTRODUCTION

Descriptions of the morphology of living cells of *M. rubrum* are rather limited, principally for the same reasons as the lack of understanding of bloom dynamics; as such events are highly unpredictable, and all attempts to culture *M. rubrum* have failed, many aspects of its basic biology and physiology remain poorly understood. Moreover, its extreme fragility and motility make observations on living material a particular challenge. This, together with poor cell preservation in many routine fixatives has resulted in the dynamics of it's life-cycle being virtually unknown (Lindholm 1985). In fact, despite populations achieving immense numerical densities during blooms, cell division has yet to be observed. This could be partly attributable to red-water in some instances being partially a behavioural and/or physical concentration process. However, in most examples of red-water formation, intense growth of the population does seem to occur, and doubling times extrapolated from rates of photosynthesis can be as low as 2-4 hours (e.g. W.O. Smith pers. comm., Stoecker *et al.* 1991); thus the absence of division stages is curious. Another enigma yet to be clarified concerning this species is the highly variable size distribution (discussed in more detail in Chapter 6) noted by several authors and considered by Lindholm (1985) to suggest that *M. rubrum* in fact represents a species complex.

Lindholm (1985) has described considerable morphological variation, mainly in terms of cell size, and has tentatively attributed this variation to a range of potential geographical genotypes within a 'species complex'. However, the factors governing morphological variation have not been resolved, and no evidence has been presented to support the contentions that the observed variation is genotypic rather than phenotypic.

The more or less predictable recurrent blooms in Southampton Water presented a good opportunity to observe the morphology of living cells. The high population densities in these blooms overcame the associated problem of observing a rapidly moving single cell; there was always a sufficient number of cells to observe. Although a detailed programme of microscopical study was not undertaken, it was hoped that the opportunistic observations made may make some contribution to the limited information presently available.

5.2. SAMPLING

Sampling was undertaken as a component of the regular two weekly programme, although some additional visits to the Dockside were also made during red-tide events to collect material for morphological studies[1].

5.3. RESULTS AND DISCUSSION

Cyst-like bodies

Frequently, but predominantly later in the bloom, numerous individuals possessed spherical, colourless, but opaque bodies, apparently attached or adherent to the outer cell membrane of *M. rubrum*. Cells observed prior to the bloom, or at other times of the year, were never observed to possess such structures.

---

[1] procedures for light and electron microscopy are given in the appendices

A typical *M. rubrum* cell, bearing one of the spherical structures, is shown in Figure 5.1a. Structures ranged in diameter from 5 - 10 μm, with some cells possessing two or more. The point of attachment was always on the 'oral' hemisphere (rudimentary cytostome - posterior relative to swimming direction; see Lindholm 1985) of the ciliate, usually close to the base of the cirri. The structures remained rigidly attached to the cell, even during rapid swimming, and the behaviour of such cells appeared normal. Appearance and attachment seemed unaffected by preservation in Lugol's iodine (Figure 5.1b), although the ciliate's cirri became relaxed and the ciliary belt disorganised. Athough Figures 5.1a & b may suggest some shrinkage upon preservation, both of the ciliate and the attached structure, it should be noted that the size of either in living material is highly variable.

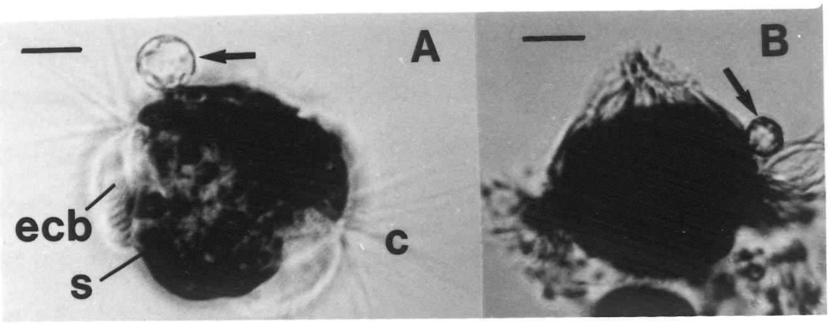
In glutaraldehyde, cell fixation was generally poor, although the spherical structures fixed rather better and could readily be discerned in section (Figure 5.2a). Invariably, they were packed with an amorphous, lipid-like material, surrounded by a thin but poorly preserved layer of granular cytoplasm with a remnants of enclosing plasma membrane. Internal membranous inclusions were apparent in some sections (Figure 5.2b), such as nucleus, Golgi body and mitochondria, but no unique diagnostic features could be distinguished. Serial sections were not available, therefore a detailed examination of the relative extent of lipid-like material and an unambiguous documentation of the ultrastructure was not feasible.

Figure 5.1. (next page)

Live and preserved cells of *Mesodinium rubrum* showing curious spherical bodies (marked by arrows) associated with it's 'oral' hemisphere. Scale bars = 10 μm.

(A) Live ciliate cell packed with chloroplasts of the endosymbiont (s), and surrounded by characteristic equatorial ciliary belt (ecb) and cirri (c) radiating out in three planes. See Taylor *et al.* (1971) and Lindholm (1985) for further morphological details.

(B) Cell preserved in Lugol's iodine (foreign material adhering below); cell stains black due to the presence of starch.


A
ecb
s
c
B

These observations suggest the bodies to be of biogenic origin, perhaps some form of cyst or spore, however, this cannot be confirmed on the basis of the present description. One can simply speculate that they either represent a parasitic infestation, perhaps resulting in bloom decline, or, that the cryptomonad endosymbiont of *M. rubrum* is encysting due to unfavourable conditions associated with bloom decline. Reports of parasitic infestations of ciliates are rare, although an ectoparasitic flagellate has been described on a soil ciliate (Foissner & Foissner 1984). More commonly encountered as infecting both marine (Johnson 1966), and freshwater (Reynolds 1973) microplankton are the chytrid fungi; moreover, some chytrids can possess a large lipid body (Kazama 1972). However, most ultrastructural studies on *M. rubrum* have also indicated the presence of large lipid droplets associated with the endosymbiont (Taylor *et al.* 1969, 1971, White *et al.* 1977, Oakley & Taylor 1978, Grain *et al.* 1982).

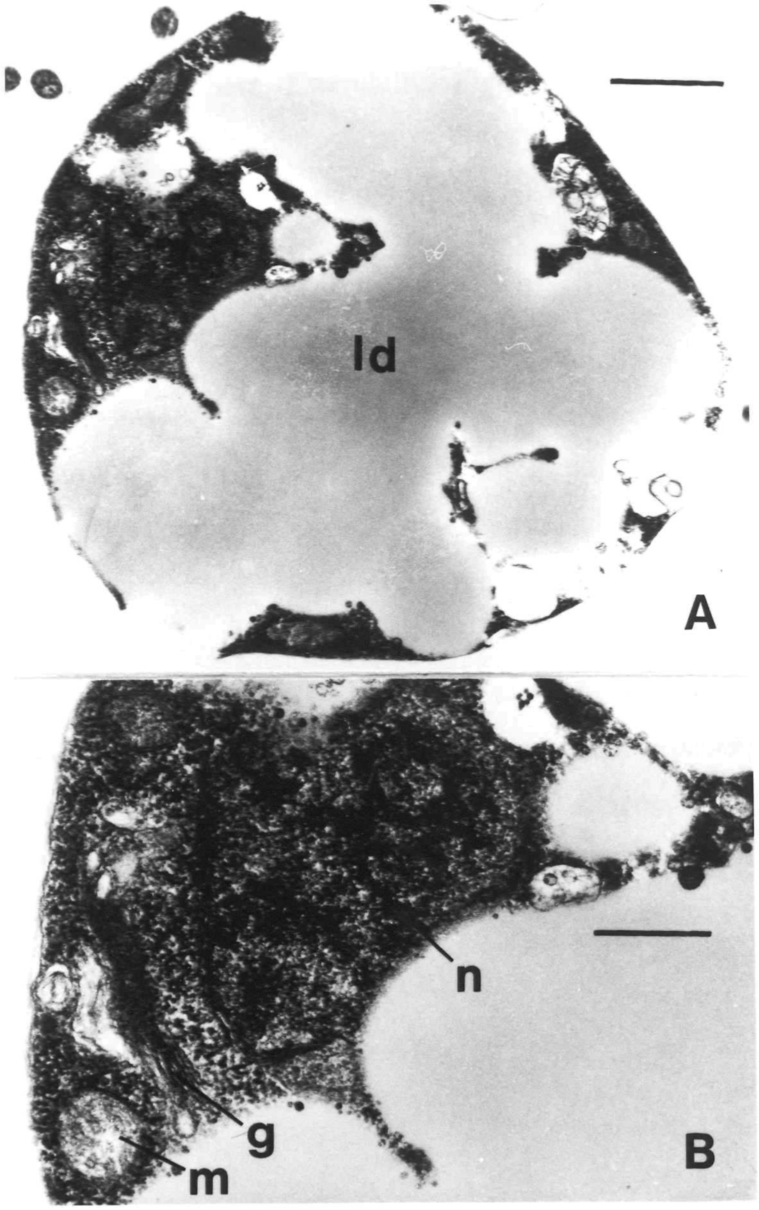
The nature of these bodies certainly requires further investigation beyond the preliminary observations described here. The dynamics of blooms of *M. rubrum* are poorly understood (Lindholm 1985), thus whatever their source, these structures seem likely to be of significance. If derived from the cryptomonad endosymbiont, then they may have far-reaching implications in an evolutionary context regarding debate on the nature of this interesting cytobiosis (see Taylor 1983).

Figure 5.2. (next page)

Transmission electron micrographs of a section of the structure shown in Figure 5.1; fixation is rather poor and the periphery is damaged.

(A) Complete body; note large lipid accumulation (ld). Scale bar = 1 μm.

(B) Close up of 'pocket' of cytoplasm shown in Figure 5.2A above. Though fixation is poor, nucleus (n), Golgi body (g) and mitochondria (m) are present. Scale bar = 0.5 μm.


ld
A
n
g
m
B

Cell morphology

Light micrographs of typical live cells of *M. rubrum* are shown in Figure 5.3a & b, in both 'oral' (rudimentary cytostome) and lateral views. Morphological aspects of this species are fully discussed by Taylor *et al.* (1971) and Lindholm (1985) and the details will not be repeated here. Essentially, Figure 5.3 shows the obvious features of a typical cell packed with chloroplasts of the cryptomonad endosymbiont. Cirri radiate out in three planes from around the equatorial circumference, and arise from a position slightly anterior (with respect to 'oral' hemisphere) to that of the equatorial ciliary belt.

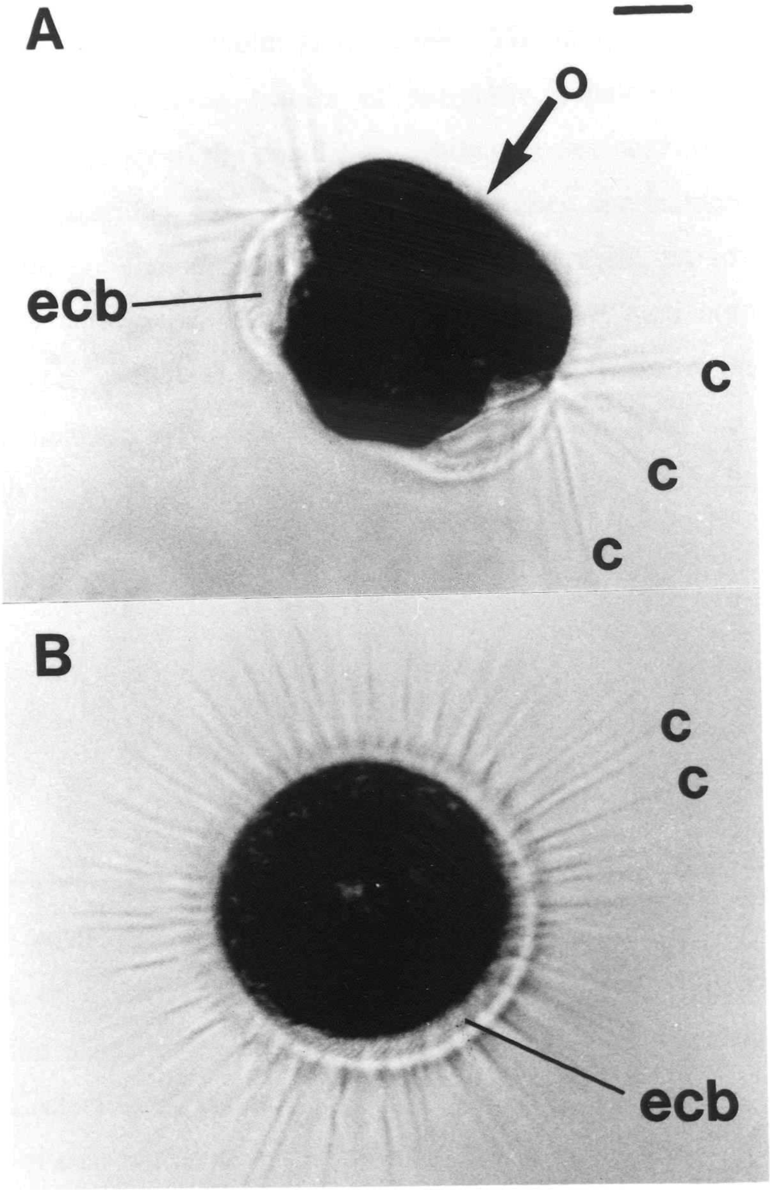
However, immediately preceding and during red-water events, notable morphological variations could be observed in some, but not all cells. Figure 5.4a-f shows six individuals with progressive stages of development of such morphological variation, in both lateral and 'oral' views. This variation manifests itself in its simplest form through irregularity of the 'oral' hemisphere (Figure 5.4a & b). Some cells develop a series of finger-like projections around the circumference of the cell (Figure 5.4c & d), and in extreme cases these seem to form discrete packages of the chloroplasts of the endosymbiont although somehow still attached to the ciliate (Figure 5.4e & f). These observations were confirmed by fluorescence microscopy, the packages still showed the orange autofluoresence characteristic of the cryptomonad endosymbiont.

Figure 5.3. (next page)

Photomicrographs of living cells of *Mesodinium rubrum*; scale bar = 10 μm in both plates.

(A) Lateral view, showing oral (rudimentary cytostome) region (O), equatorial ciliary belt (ecb), cirri (c).

(B) Oral view; note rudimentary cytostome or oral depression shows as central clear zone among densely packed chloroplasts.

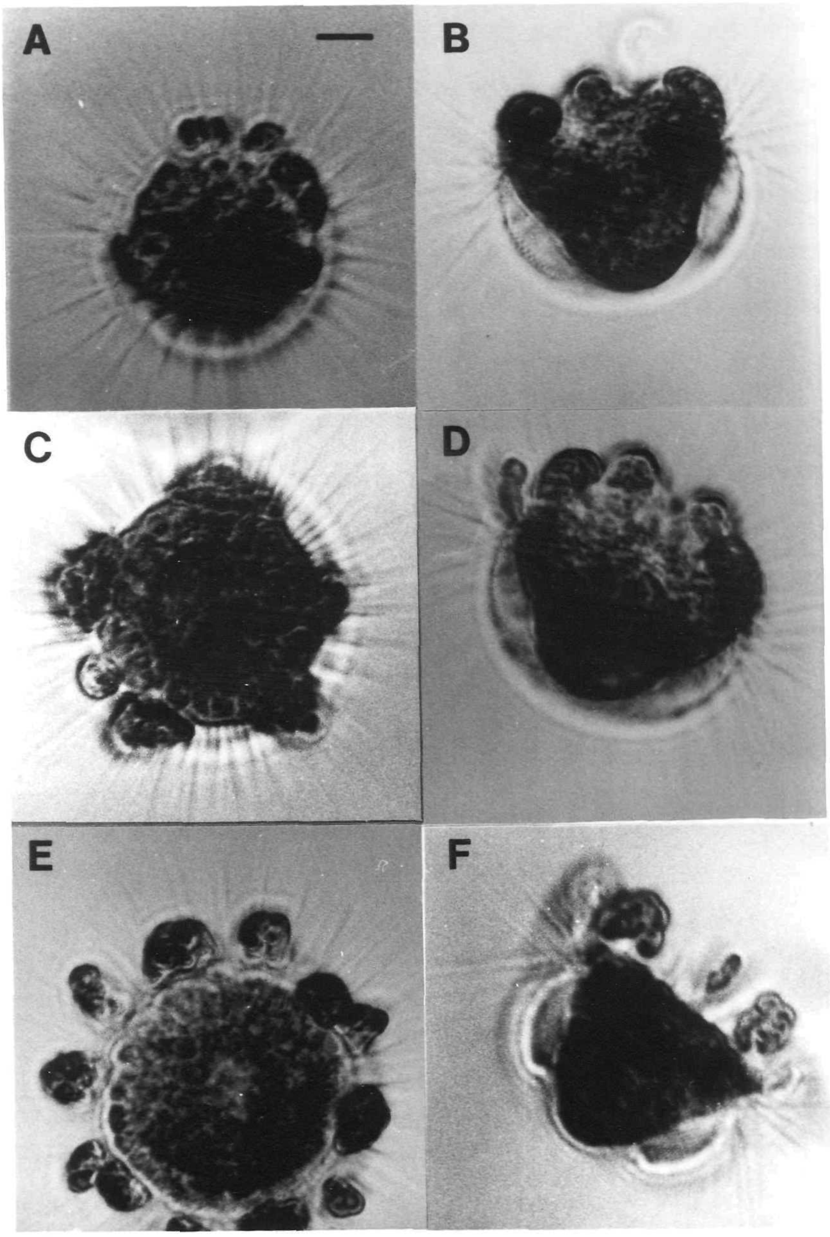

A
o
ecb
c
c
c
B
c
c
ecb

It should be emphasised that these cells were not exploding or damaged specimens, but were quite normal in terms of their behaviour and swimming activity. They were noted however to be rather more fragile than usual, perhaps resulting from a higher degree of the cytoplasmic vacuolation already noted for this species (Taylor *et al.* 1971, Hibberd 1977, Lindholm *et al.* 1988). The morphological variation described above was a common feature of red-water populations, and could sometimes affect the majority of the population, while on other occasions few or no cells were affected. Sometimes, upon return to the laboratory, the features would be widespread among the population, but after some time or overnight, the irregularities would be apparently withdrawn and cells would 'round-up' to their normal form. Although it could be speculated that these variations represent a response to disturbance during transport to the laboratory, it should be emphasised that they were also clearly observed in samples preserved in Lugol's iodine immediately after sampling.

Figure 5.4. (next page)

Photomicrographs of a series of individual living cells of *Mesodinium rubrum* showing progressive stages in development of irregularity in 'oral' hemisphere. Scale bar = 10 μm and refers to all plates.

(A) Periphery of oral region shows slight irregularity; oral view.
(B) Same degree of irregularity as A, but in lateral view.
(C) Distinct sometimes finger-like protrusions project from perphery; oral view.
(D) As in C; lateral view.
(E) Protrusions have become apparently distinct 'packages', though remaining attached during swimming activity. Isolated packages still contain cryptophyte chloroplasts; oral view.
(F) As in E; lateral view.


A
B
C
D
E
F

The size variation of individual cells during red-water was very pronounced; cell diameter usually varied between about 15 - 70 μm, with all sizes in between being represented at various times. Unfortunately, size frequency analysis was not undertaken. Some individuals were as small as 10 μm, though not photographed. These were narrow pencil-like individuals often with fewer than 5 chloroplasts. Also, what appeared to be fragments of larger cells (again checked with fluoresence microscope for intact cryptophyte chloroplasts) could be observed occasionally free-swimming in red-water samples. Sometimes these were observed swimming out of the debris of larger lysed cells. These fragments lacked the cirri characteristic of 'normal' cells, or else the cirri had separated into component cilia, and also showed a rather disorganised arrangement of the cilia themselves (Figure 5.5a). Swimming activity of fragments was irregular and lacked the jumping ability of normal cells. However, the viability of such fragments could not be checked for a long period of time since, just like the larger cells, they did not survive long under the microscope. Cells with a variety of shapes and sizes seemed to occur in bloom samples. Figure 5.5b shows a small cell in which the ciliary belt has formed around the long axis of a slender cell, while in Figure 5.5c a similar sized and shaped cell has the ciliary belt forming around the short axis. Figure 5.5d shows a slightly larger, but rather more 'normally' shaped cell.

Figure 5.5. (next page)

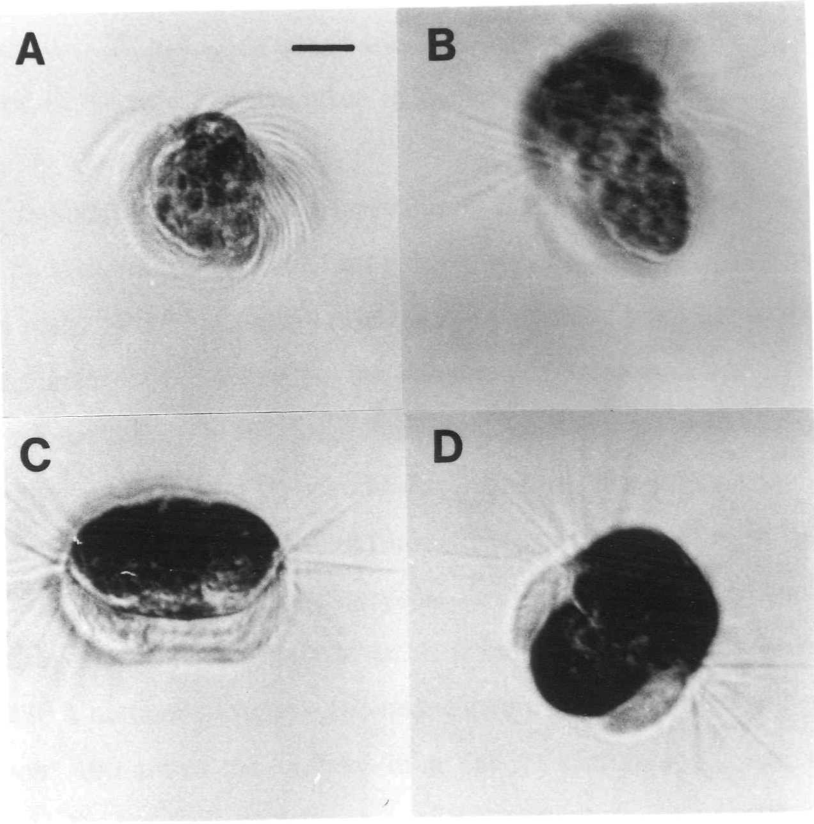
Morphological variation in small living *Mesodinium rubrum*. Scale bar = 10 μm and refers to all plates.

(A) Small free swimming 'fragment' of cell, lacking cirri, but possessing portion of ciliary belt.

(B) Elongated cell with cirri formed around the long axis; lateral view.

(C) Cell similar in size and shape to that in B, but with cirri formed around short axis; lateral view.

(D) Cell of more 'normal' shape, similar in length but wider in diameter than that in B; lateral view.


A
B
C
D

In agreement with Taylor *et al.* (1971), cell size was fairly predictable during winter/spring, tending to be in the region of 15 - 20 μm diameter, but highly variable just preceding and during red-water events. During such events cell diameter varied between about 10 - 70 μm. It was notable that cells larger than 15 - 20 μm were never observed in the few samples taken in winter.

One implication of these observations could be that *M. rubrum* was multiplying, at least to some extent, by a form of budding or fragmentation; this could explain many of the apparently disparate observations recorded in the literature to date. In particular, it could explain the absence of observed cell division, at least in the 'conventional' sense, in that large individuals could be splitting into many small fragments which might then be overlooked. In this way, the extremely high production values recorded to date could in fact represent growth of small cells into larger ones, rather than a series of binary fissions. One multiple division of a large cell, followed by subsequent growth of small fragments back into large cells would be equivalent to a number of binary fissions. Since the volume of a cell of diameter 70 μm is about 100 times the volume of a cell of diameter 15 μm, then fewer divisions would be required to achieve a given population biomass than if binary fission were the sole mode of reproduction.

Whether such a form of budding or multiple fission could be feasible is not clear; the difficulty of maintaining bloom samples of *M. rubrum* in the laboratory has precluded any studies of whether small cells or fragments can grow into larger cells. However, some evidence from the literature suggests that this may be the case. Fonds & Eisma (1967) found evidence of what they referred to as 'young *Mesodinium*' within large individuals from a fixed bloom sample from the North Sea. Montagnes & Lynn (1989) showed that the mean cell volume of *M. rubrum* varied by an order of magnitude throughout the year, suggesting that such variation could result from growth of individuals. Budding or multiple fission among ciliates is not common, except among the suctorians; however, given the peculiar structure of the cell covering of *M. rubrum*, such a type of reproduction is feasible. This species is highly unusual among ciliates in that the pellicle is very reduced and lacks alveoli (Taylor *et al.* 1971, Lindholm 1985); the cell is simply surrounded by a plasma membrane. This presumably makes the cell very 'plastic', and thus amenable to potential cell volume changes; perhaps the only limit imposed would be whether the kineties, or rows of kinetosomes (ciliary basal bodies), are able to replicate sufficiently around the circumference of the cell to keep pace with volume changes. This was questioned by Montagnes & Lynn (1989) who suggested that this could be a major developmental problem if the mean cell volume variation they observed were caused by cell growth. Lindholm (1985) has pointed out that many populations have a relatively stable number of cirri (36), and thus kinetosomes, but also noted that in some populations a range of cirral numbers occurred up to a maximum of 112 in large individuals. A range of numbers of kineties, or rows of kinetosomes, have been noted in the literature; Powers (1932) recorded 52-60, Bary & Stuckey (1950) observed a wider range of 43-106, and Fenchel (1968*a*) noted 30-40 for small individuals, with 8-10 kinetosomes in each. Thus a wide range does occur under certain circumstances, and suggests that numbers of ecb or cirral kineties could conceivably increase with cell volume.

Another precondition for multiple fission to occur is that nuclear DNA would have to be divided among several potential 'daughter' cells; studies on *M. rubrum* are not even close to a progression at the cellular level sufficient to answer such questions. However, the early cytological studies of Powers (1932) and Bary & Stuckey (1950), employing a range of staining techniques, did describe the presence of 'extra-nuclear vesicles' which stained lightly with the Feulgen reaction. Bary & Stuckey (1950) suggested that these were degenerative stages of the macronucleus during pycnosis; they also described other cells as possibly representing stages in karyorrhexis, in which chromatin becomes distributed throughout the cytoplasm. Although both conditions were implicated as common pathological stages during necrosis, an alternative interpretation is the allocation of macronuclear chromatin to a number of potential daughter cells prior to multiple fission. Fenchel (1968*a*) also remarked on the presence of extranuclear DNA in individuals from red-water in Danish waters. In an ultrastructural study of *M. rubrum*, Taylor *et al.* (1971) only observed a maximum of four macronuclei, however they did not employ staining techniques likely to highlight the presence of extra-nuclear DNA.

Even if some form of multiple fission occurs, this need not necessarily preclude binary fission as the normal means of reproduction; elongated cells are sometimes observed, and these certainly show the characteristics of what dividing cells might be expected to resemble (Lindholm 1985, Crawford pers. obs.), being in appearance similar to the pre- binary fission stages of *Mesodinium acarus* described by Tamar (1987). The smaller cells observed under 'normal' conditions in many environments (Taylor *et al.* 1971, Lindholm 1985), and in winter in Southampton Water (Crawford pers. obs.), are unlikely to divide by multiple fission since they barely exceed 15 μm in diameter and presumably must divide by binary fission. Maybe multiple division only occurs immediately prior to or during bloom conditions; perhaps production by the endosymbiont under certain circumstances becomes too rapid for the normal cell division process to keep pace? Generation times of 2-4 h as suggested (W.O. Smith pers. comm.) by production data (Stoecker *et al.* 1991, Smith & Barber 1979) are certainly much lower than those of in the range 5-40 h which can be predicted assuming theoretical considerations of intrinsic growth and cell volume (Fenchel 1968*b*) for ciliates generally. However, Banse (1982) has reviewed the major groups of microplankton and shown that ciliates do have much higher potential intrinsic growth rates than diatoms or dinoflagellates.

An alternative explanation for the observed irregular cells of *M. rubrum* is that they could be increasing surface area for more efficient photosynthesis (or nutrient uptake) in an analagous fashion to radiolarians which expose their symbionts by day and retract them at night (Anderson 1983). This hypothesis has particular appeal in view of the observation in Chapter 2 that the blooms appear to be light limited; and perhaps also in view of the recent contention by Agustí (1991) that by reducing self-shading, larger phytoplankton cells can support greater maximal biomass than smaller ones. However, against this hypothesis are observations that irregular cells also occur prior to the bloom in Southampton Water (Crawford pers. obs.) when cell densities are relatively low, and when light and nutrients are non-limiting. These irregular cells have also been observed in Spring at Millport, Scotland (Crawford pers. obs.) again under non-limiting conditions and at relatively low cell densities.

Clearly the issue is yet to be clarified, and much of this discussion is tentative speculation. *M. rubrum* is not a species which can simply be plucked from its environment and observed in the comfort of the research laboratory, and developing methods for its maintenance in culture only appear to be a long-term prospect. Thus perhaps an understanding of its life cycle can best progress by indulging in more speculation than is usually scientifically acceptable. In this way it is hoped that the observations presented here may throw some new light on the problem and offer some alternative directions than those pursued to date.

CHAPTER SIX:
A QUESTION OF SIZE

"All animals are equal, but some animals
are more equal than others"

George Orwell (Eric Blair, 1903-1950; Animal Farm)

6.1. INTRODUCTION

The question of size distribution of *M. rubrum* was mentioned in the last chapter with reference to the observed morphological variation. Many studies on *M. rubrum* have noted the unusually variable size distribution of this species (e.g. Leegard 1920, Michanek 1965, Taylor *et al.* 1971, Lindholm 1985, Revelante & Gilmartin 1987, Dale 1988*a*, Montagnes & Lynn 1989, Lindholm & Mörk 1990). In an early study, Leegard (1920) referred to larger and smaller cells as 'forma major' and 'forma minor' respectively, and this interpretation has been perpetuated by Michanek (1965) and then Lindholm (1985), who tentatively proposed that *M. rubrum* represents a 'species complex' of about four genotypes, separated on the basis of cell size. All of these studies observed populations of either 'small' cells, 'large' cells, or an assemblage of the two; the latter situation apparently being most commonplace. The size ranges usually quoted for populations of both small and large cells are highly variable, and intermediate forms are often present.

Despite the above observations, a comprehensive study of size distribution is notably lacking; size frequency distributions have not been published to date for this species. Moreover, the concept that both forms could represent different life stages of the same genotype does not appear to have been seriously approached. However, Montagnes & Lynn (1989) did suggest that the variation in mean cell volume of an order of magnitude over the annual cycle could have resulted from growth of the endosymbiont controlling cell volume of the ciliate. In view of their findings, together with the observations of the last chapter and the noted absence of 'large' cells in winter in some populations (Taylor *et al.* 1971, Crawford pers. obs.), this seems a particularly pertinent question to address.

Here, some examples of size distributions of populations of *M. rubrum* are presented together with preliminary studies on changes in such distributions on a daily basis.

6.2. SAMPLING AND SITE DESCRIPTIONS

Samples for size distribution were taken from the following locations; Cape Cod, USA (Eel Pond, Woods Hole) July 1990; Gulf of Maine, USA (Frenchmens Harbour, Maine) July 1990; Åland Islands, Finland (Inre Verkviken) June 1991; Firth of Clyde, Scotland (Keppel Pier, Millport) March 1991.

All of these samples represented populations of *M. rubrum* at non-bloom densities, except that from Frenchmens Harbour which was taken from a declining red-tide. Most samples were simply taken by bucket from the surface[1]; however, that from Inre Verkviken was taken by Ruttner sampler at 12 m depth (Day 0) and incubated in a polyethylene bag on the surface of Husöviken (Day 1).

6.3. RESULTS

A fairly typical size distribution of *M. rubrum* is shown in Figure 6.1 for two successive days, for samples taken at Millport. The population is dominated by small forms of mean diameter about 12-15 μm, with a second peak at about 25 μm. On the

---

[1] further details on methodology are given in appendix 2.

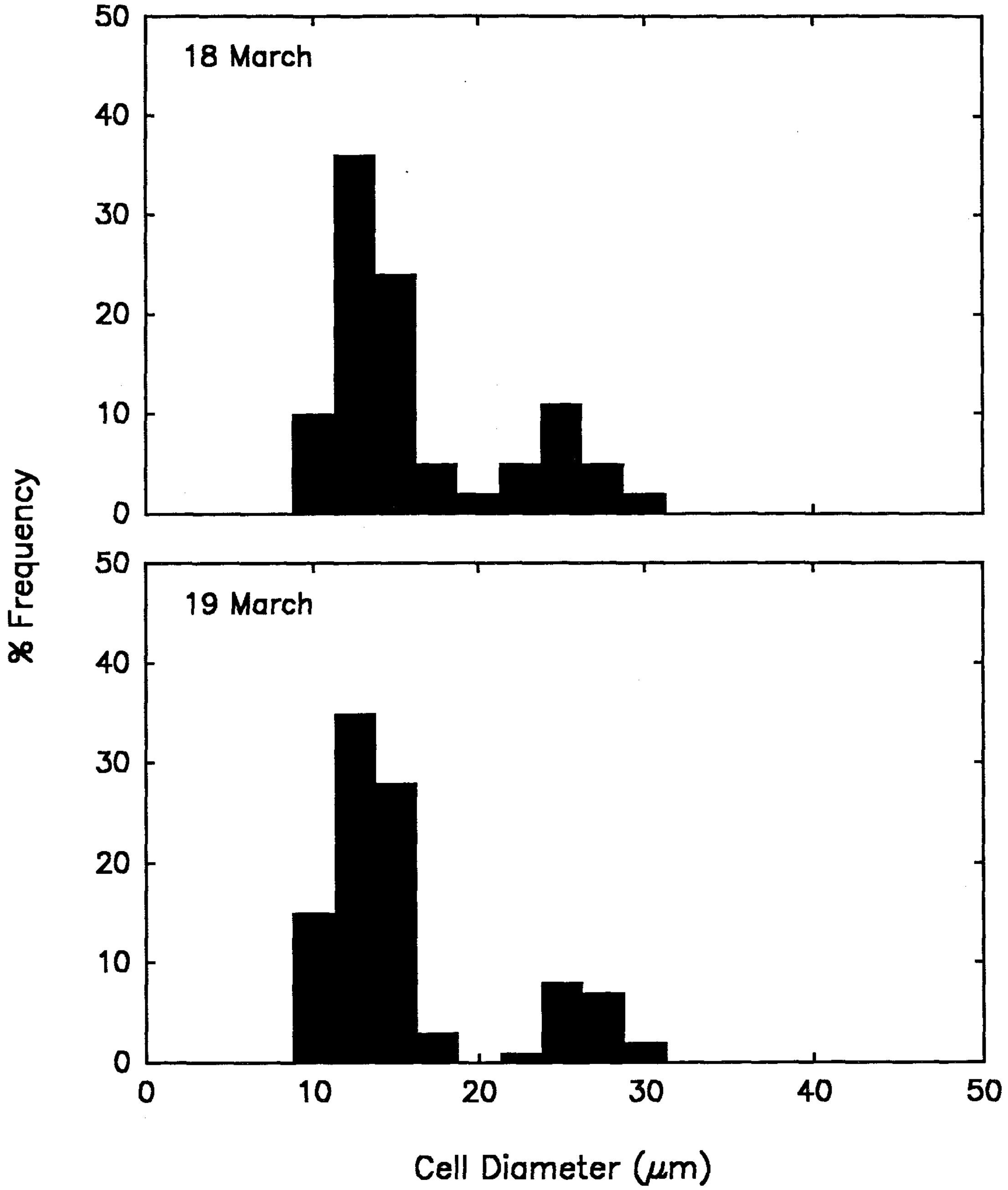


Figure 6.1.

Size frequency distribution of a population of *Mesodinium rubrum* from the Firth of Clyde on two successive days.

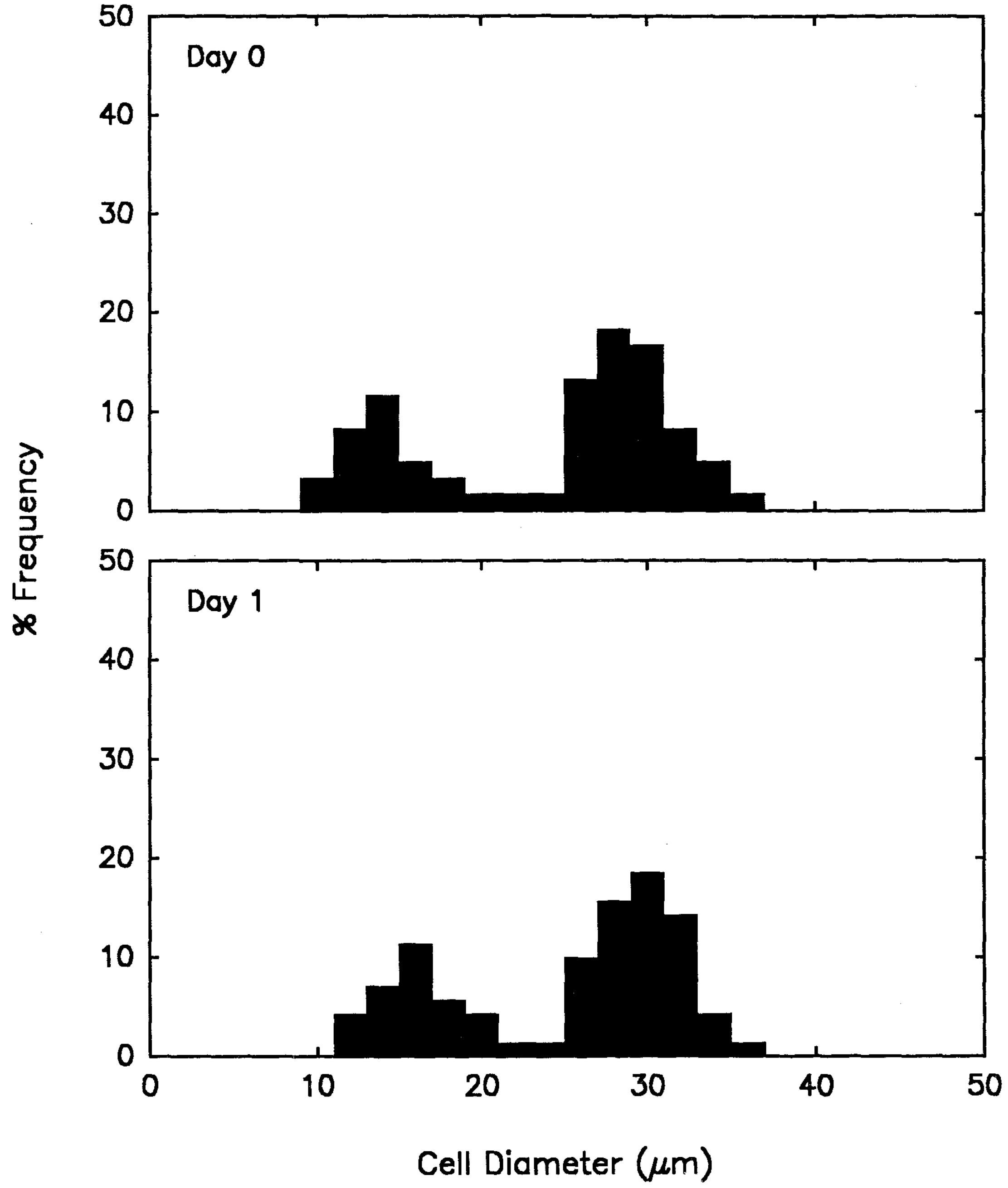


Figure 6.2.

Size frequency distribution of a population of *Mesodinium rubrum* taken from 12 m depth at Inre Verkviken, Finland (Day 0) and the same population the following day (Day 1) after being incubated at the surface in a polyethylene bag.

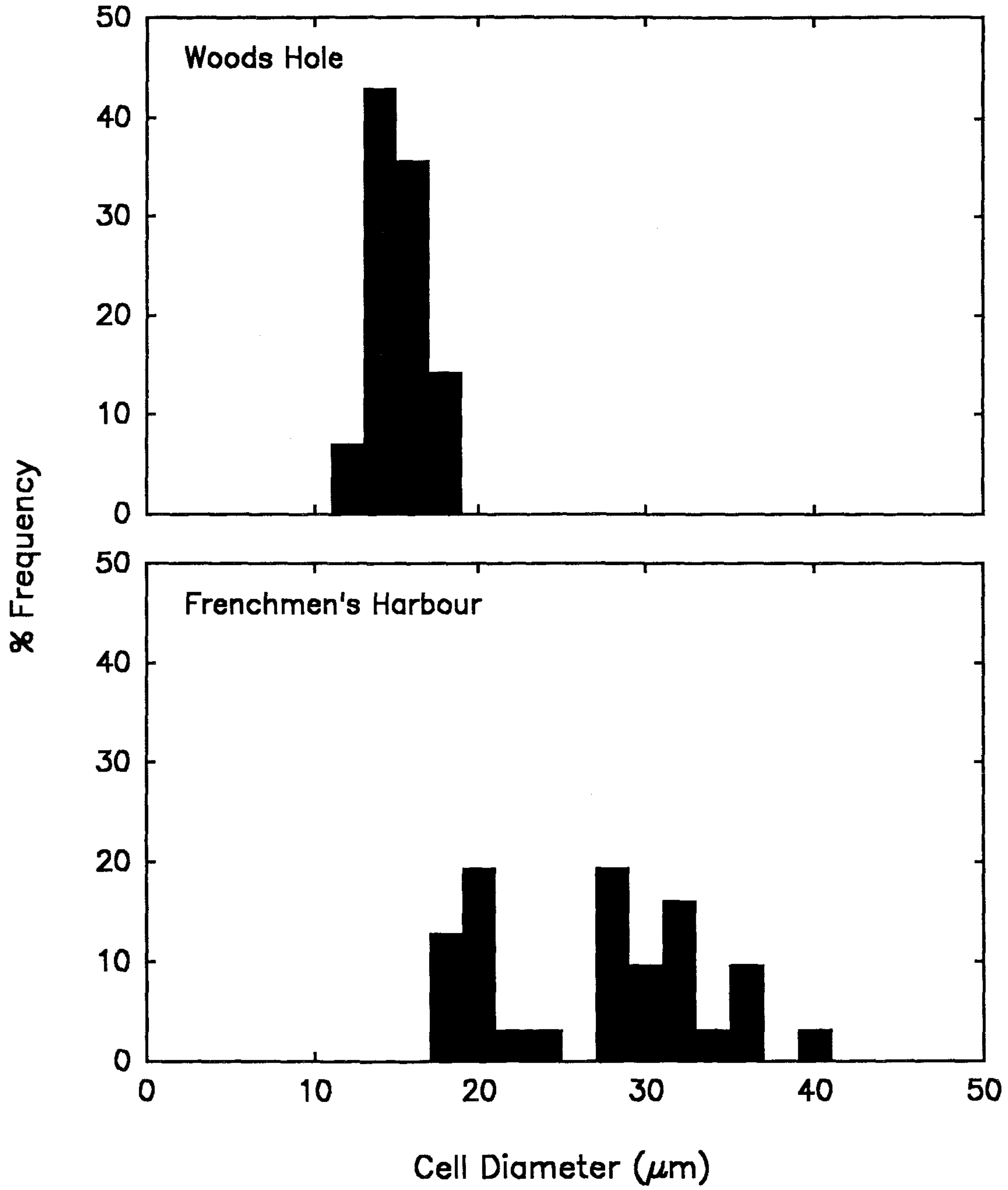


Figure 6.3.

Size frequency distributions of two populations of *Mesodinium rubrum*; the first from Eel Pond, Woods Hole, and the second from a declining red-tide in Frenchmen's Harbour, Gulf of Maine.

18 March there are intermediate forms of diameter about 20 μm, but these were absent on the 19 March.

In contrast, the population from Inre Verkviken, shown in Figure 6.2, has a similar size distribution though with small cells being slightly larger than at Millport; however, this population has a much higher proportion of large cells, and these are considerably larger (about 30 μm diameter) than those from Millport. Although the large and small groups are fairly distinctly separated, there are intermediate forms present in each of the size categories. The distribution on day 1, which is a day 0 sample incubated at the surface, suggests an increase in mean size of both small and large cells.

The population from Woods Hole shown in Figure 6.3 is composed entirely of the small group which shows a very narrow distribution. The sample from the declining red-tide from the Gulf of Maine has a very irregular size distribution of the whole population. The small cells (20 μm diameter) are considerably larger than those in any of the other populations described above, and the larger cells have a very wide size distribution broken up into a number of peaks. It is interesting to note that each of these peaks represents approximately a doubling of cell volume over the preceding peak, possibly suggesting a sequence of divisions.

Figure 6.4 shows a sequence of size distributions taken daily at Millport for 10 days; this includes the two distributions already shown. The sizes of both small and large cells can be observed to fluctuate over the 10 day period. It is interesting to note that as the distribution of small cells shifts to the right, suggesting cell growth, the relative abundance of the larger cells also increases, as does their maximum size and size range. This is particularly evident on the 14 March when the whole population seems to show a 'smeared' distribution across a wide size range, and intermediate sized cells are more prevalent. It is also interesting that this occurred just 2 days after the middle of the cycle of neap tides (lowest H.W.).

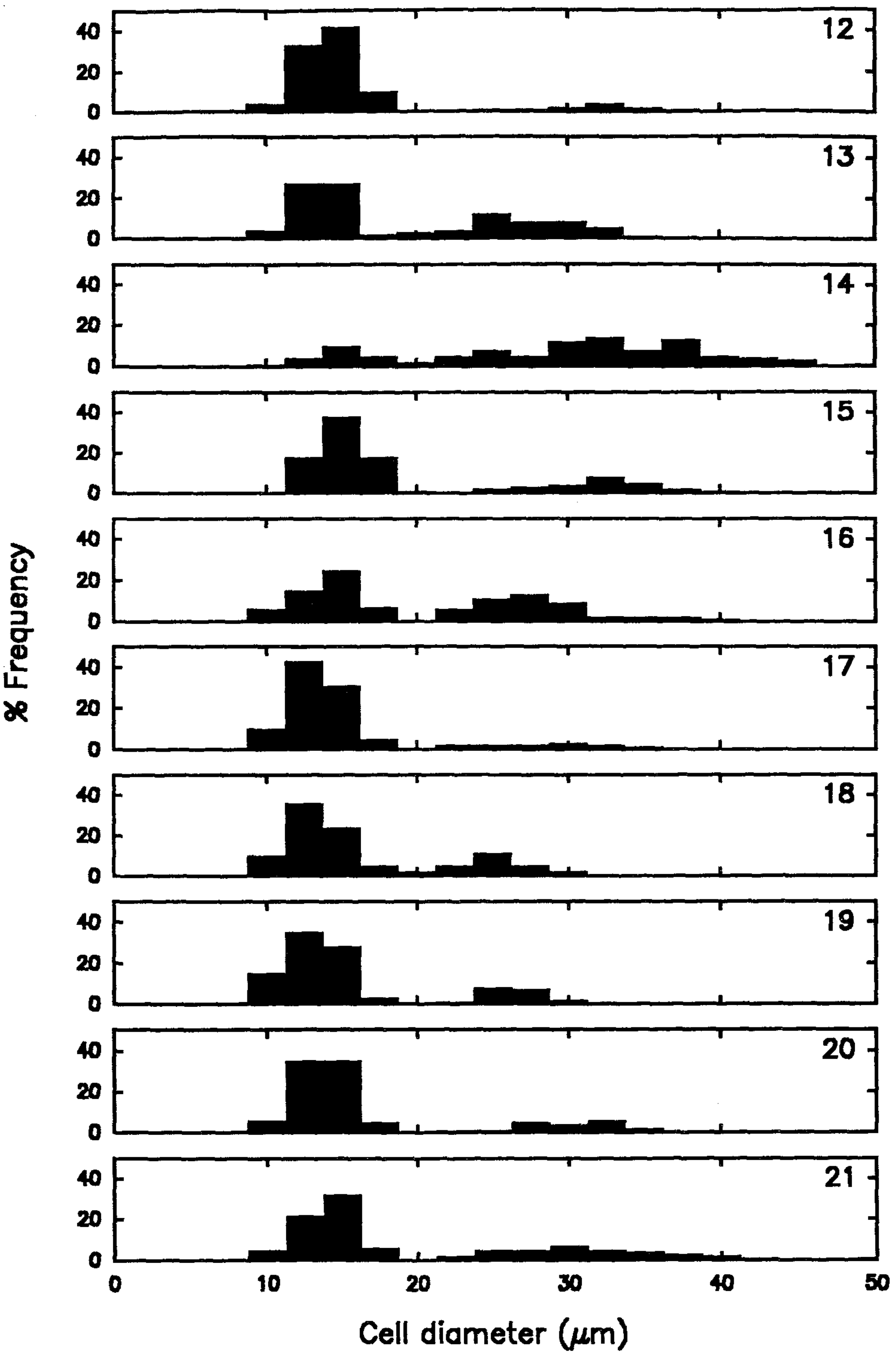


Figure 6.4.

Sequence of size distributions of a population of *Mesodinium rubrum* from the Firth of Clyde taken daily for 10 days in March 1991.

6.4. DISCUSSION

The data clearly support the previous observations of populations comprising small and large cells (Leegard 1920, Michanek 1965, Lindholm 1985, Revelante & Gilmartin 1987). The various environments studied gave varying proportions of large and small forms, and in one instance only the small form. The point of interest is that this is the first study to present size frequency data for *M. rubrum* rather than subjective descriptions of cell size. What this shows is that there is considerable overlap in size between the two forms, and that the size of each form varies greatly, particularly the large cells.

What is clear therefore from the data presented here is that there seems little evidence to support the separation of this species into a number of separate genotypes based solely on size, as suggested by Lindholm (1985), at least until the question of size distributions has been clarified. Cell volume variation both within and between populations, and over time, is just too great for this to be adopted as a useful criterion. For instance, the larger cells observed at Millport over 10 days (Figure 6.4) can be seen to vary from about 20 μm to about 45 μm in diameter, this represents a volume increase of some 11 fold, and equals almost exactly the annual variation in mean cell volume described by Montagnes & Lynn (1989). The smaller cells varied less, from about 10 μm to 18 μm in diameter, a volume increase of about 6 fold. The problem with the approach of Montagnes & Lynn (1989) was that only mean cell volume was presented; clearly, if the population size distribution was bimodal as at Millport, then the use of a single 'mean' will give very limited information and will be numerically biased in favour of the more abundant size group.

The important question is in essence the same as that posed by Chapter 5; whether small forms can in fact grow into larger cells; that is, is the variation observed phenotypic or genotypic? Clearly there is insufficient data available here to answer this, but that which is presented is useful in an illustrative sense. It suggests that at certain times the distribution of small cells seems to 'spill over' into that of larger cells. The distribution in the declining red-tide is also interesting and suggests that significant growth of small cells seems to have occurred, up to a diameter of about 20 μm; most populations at non-bloom densities seem to have a resident group of cells of about 15 μm diameter. Large cells are also somewhat larger, though with an irregular distribution. It would be difficult to explain such a distribution of cell size by means of a 'genotypic' hypothesis, and certainly not without a multitude of co-existing subspecies! These observations also lend support to the subjective impressions (Crawford pers. obs.) of a wide variation of cell size observed during red-tides in Southampton Water; however, during such events cell size attains a maximum of about 70 μm, in accordance with that noted by Taylor *et al.* (1971) for red-tide populations in British Columbia.

The variations certainly seem far too great for such forms to be separated on size alone, and there are few alternative distinguishing features. Even the fact that the population is divided into distinct peaks is not a sufficient criterion for a genotype hypothesis; cell size could be governed by a variety of non-linear physiological constraints, with growth perhaps occurring in intermittent bursts under favourable environmental conditions. In this way, the peaks of larger cells could represent 'cohorts' of cells which have 'broken through' some physiological barrier normally imposing an upper size limit of about 20 μm to the small cells.

Much of this discussion is speculation and it may be that the observed size groups are eventually shown to be genetically distinct, however, it is interesting to note that other species of microplankton maintained in clonal culture have also been shown to have bimodal population distributions. Genetically identical small and large cells have been observed in cultures of the dinoflagellate *Gymnodinium* cf. *nagasakiense* (*Gyrodinium aureolum*); large cells generate small cells by means of budding, and small cells can grow back into large cells, alternatively both forms have been shown to divide asexually independently (Partensky *et al.* 1988, Partensky & Vaulot 1989, Videau & Partensky 1990). Moreover, the mixotrophic ciliate *Strombidium capitatum* has also been shown to have a wide and bimodal size distribution (see Chapter 9).

Clearly much more data is required to resolve this issue for *M. rubrum*, as the life cycle could prove to be very complex. High resolution sequences of size distribution variation with time would be desirable, preferably from within some form of enclosed mesocosm, where advective population exchange would be minimized. In this context it is unfortunate, as mentioned in Chapter 2, that size frequency analysis was not undertaken on the Southampton Water red-tide population; in retrospect, this might have been particularly revealing since considerable dynamic variation in cell size seems to occur as the bloom develops. A diel sequence of hourly size frequency distributions, both leading up to and during the bloom, may have clarified some of the questions of both this chapter and Chapter 5.

CHAPTER SEVEN:
WHAT LIMITS *MESODINIUM RUBRUM*?

"The greatest and noblest pleasure which men can have in this world is to discover new truths; and the next is to shake off old prejudices"

Frederick the Great (1712-1786)

What is quite apparent both from the previous chapters and from the literature, is that there seems little to limit the growth or survival either of individual *M. rubrum* cells, or of its populations.

Table 7.1 reviews available data on the photosynthetic physiology of *M. rubrum*; it has been shown to be a remarkably efficient primary producer during blooms, typically with a very high chlorophyll specific maximum rate of photosynthesis (P_m^B or assimilation number) of up to about 20 mg C (mg chl*a*)$^{-1}$ h^{-1} (Packard *et al.* 1978, Smith & Barber 1979, Platt *et al.* 1980, Cabeçadas *et al.* 1983, Wilkerson & Grunseich 1990); however, lower values have also been recorded, perhaps during scenescent blooms. These figures agree well with those of Stoecker *et al.* (1991) which is the only study to date which has examined photosynthetic physiology of non-bloom cells; rates were determined on isolated individual cells, giving values for P_m^B of up to 8.6 mg C (mg chl*a*)$^{-1}$ h^{-1}. Although this is only about 50% of some of the red-water values there is always considerable 'noise' in bulk physiological estimates for *M. rubrum* because of subsampling artifacts associated with fragility and behaviour. This non-bloom value is nevertheless in the upper range of estimates observed both for exponential-phase phytoplankton cultures (Glover 1980) and natural phytoplankton assemblages (Malone 1980, Platt *et al.* 1980). Table 7.1 also shows that *M. rubrum* shows high photosynthetic efficiency at low light

Table 7.1. Summary of published estimates of photosynthetic parameters for *Mesodinium rubrum*

Author	P_{max} [mg C (mg chl*a*)$^{-1}$ h^{-1}]	α [mg C (mg chl*a*)$^{-1}$ h^{-1} (μE m^{-2} s^{-1})$^{-1}$]	I_k [μE m^{-2} s^{-1}]	$\beta(.10^3)$ [mg C (mg chl*a*)$^{-1}$ h^{-1} (μE m^{-2} s^{-1})$^{-1}$]
Packard *et al.* (1978)	5.3	-	-	-
Smith & Barber (1979)	6.0 - 16.8	-	-	-
Platt *et al.* (1980)	9.4	0.040[a]	235	0.12
Cabeçadas *et al.* (1983)	0.7 - 19.5	-	-	-
Wilkerson & Grunseich (1990)	18.1[b]	-	-	-
Stoecker *et al.* (1991)	1.8[c] - 8.6	0.004 - 0.030	275 - 450	-

---

[a] converted assuming approximate conversion of 1 Wm^{-2} = 5 μE m^{-2} s^{-1} (McCree 1981)

[b] changes in pH used to estimate *in situ* CO_2 fixation; mean chl*a* concentration of 88.8 μg l^{-1} assumed

[c] lower values represent cells concentrated by centrifugation prior to experiment

intensity, given by the initial slope of the P vs I curve (α), and shows minimal photoinhibition at high light intensity, given by the photoinhibition parameter (β) (Smith & Barber 1979; Platt *et al.* 1980; Harrison *et al.* 1981; Stoecker *et al.* 1991). The relatively high I_k (irradiance where photosynthesis is saturated) values of 235-450 μE m^{-2} s^{-1} is a reflection more of the high P_m^B values than a low value for α; the slope α is in fact high when compared to other groups of phytoplankton (Platt *et al.* 1980). The lower rates for P_m^B determined by Stoecker *et al.* (1991) represented cells concentrated by centrifugation prior to experiments; it is significant that this resulted both in significantly more scatter in P vs I curves, and rates considerably lower than community rates.

The above considerations, in agreement with the results of Chapter 2, suggest that light is not likely to be limiting the appearance of blooms, and in fact Rees & Williams (1982) came to the same conclusion for the Southampton Water population, that light is non-limiting to bloom development in spring. Even if light were to be limiting at depth, by virtue of its motility, *M. rubrum* can aggregate at a preferential light intensity close to the surface (Lindholm 1985). Thus because of its capability for extended vertical migrations of up to 40 m (Smith & Barber 1979, Sorokin & Kogelschatz 1979), *M. rubrum* need neither be light nor nutrient limited (see Chapter 4), except when nutrients are depleted over greater depths.

According to Chapter 2, the bloom appears to be characterised by low numbers of zooplankton, with some evidence for exclusion of zooplankton with depth. Moreover, by means of the 'escape' reaction *M. rubrum* appears to significantly reduce grazing losses compared to those for other ciliates (Jonsson & Tiselius 1990). The highly variable size distribution (see Chapter 6) may also minimise the impact of any size-dependent grazing activity by micro- or macrozooplankton; the above considerations thus suggest grazing not to be a major limitation to population growth.

Why the bloom in Southampton Water should then be delayed until summer is puzzling. The hypothesis that the bloom is limited until generation time is less than flushing time (Williams 1980, Rees & Williams 1982) has been shown in Chapter 3 to be unlikely, due to avoidance of surface flow on the ebb tide. Other studies have also shown that through migration in differential flows *M. rubrum* is able to minimize advective losses from upwelling systems (e.g. Barber & Smith 1981). The hypothesis by Williams (1980) and Rees & Williams (1982), even if it were to be valid, gives no indication of what factor does in fact stimulate growth of the population in order to overcome flushing losses; that is, an explanation of what is the trigger factor is not given.

Inorganic nutrients have been shown in this and in other studies to be reasonably high throughout the spring and unlikely to be limiting; the spring diatom outbursts are of insufficient magnitude to significantly deplete their levels in Southampton Water. Moreover, with the vast array of advantages described above, *M. rubrum* should be best able to outcompete the diatoms for light and thus nutrients. Other inorganic or organic nutrients or trace elements are possible limiting factors, but it is difficult to envisage any such substance becoming limiting in spring when freshwater input is high, but in sufficient conentrations to allow massive red-tides to develop in summer. Moreover, since red-water has been shown to appear through rapid growth of the population over only about 5 days, sudden appearance of a growth limiting factor seems unlikely. However, some role for the excretion of growth promoting substances by preceding phytoplankton blooms cannot be discounted at this stage.

The observations here, in 1985 and 1986, that the bloom coincided with periods of increased stratification of temperature and salinity, are particularly interesting (Chapter 2). However, there is no evidence of any direct effects of temperature and salinity being limiting factors; *M. rubrum* is known to be remarkably tolerant to variation in both factors (Taylor *et al.* 1971, Lindholm 1985), and red-water has even been recorded under the ice in Antarctica at a temperature of -1.6°C and salinity of ca. 7‰ (Satoh & Watanabe 1991). It was also subjectively noted that red-water in both years, as in most years since, occurred during neap tides when tidal mixing was minimal. Moreover, the blooms tended to decline as stratification weakened through the summer, and were temporarily dispersed in mid-June during a period of wind-induced instability. These observations are supported by a wealth of other anecdotal evidence summarised in Lindholm (1985); these suggest that blooms of *M. rubrum* around the world occur during 'exceptionally calm and sunny weather'. Such comments have previously been interpreted as suggesting that light is the critical factor, whereas the role of sunshine in promoting vertical stability, through increasing thermal gradients, has been ignored.

It is clear that in some way the stability of the water column may be important for the development and persistence of blooms of this species. This is intriguing since *M. rubrum* swims at a speed almost an order of magnitude quicker than even the fastest dinoflagellates, and thus has often been quoted as the species most capable of *counteracting* the effects of turbulence, and maintaining itself in optimum light (e.g. Margalef *et al.* 1979). But does it? Though not too clear from Figures 2.3 (on a log scale), *M. rubrum* does in fact usually maintain a maximum close to the surface throughout most of the spring. Thus it appears that the relaxation of turbulence as a factor promoting improved light climate for motile species, as has been implicated for flagellates, appears not to be significant here; *M. rubrum* was already optimising its light environment during April and early May without evidence of a developing bloom.

At this point a hypothesis will be presented that does not conform to existing paradigms in microbial plankton ecology; that is, could the growth of individual cells of *M. rubrum* be limited by energy expended on increased swimming when the water column is unstable, but promoted when less swimming is required under conditions of vertical stability? Intuitively, this hypothesis has appeal, since *M. rubrum* appears to swim more, by means of an increased frequency of rapid 'jumps', in turbulent zones of the water column, compared to stable zones (Chapter 3). Moreover, many observations suggest that *M. rubrum* swims rapidly in response to water disturbance or sub-optimal light conditions, but remains stationary in calm water (Lindholm 1985) or in optimum light (Smith & Barber 1979). A decline in water column stability would thus elicit an increase in motility in response to small scale fluid deformations, and moreover require *M. rubrum* to spend more time swimming in order to maintain itself at a preferential light intensity. Since *M. rubrum* either swims at almost maximum speed, or remains stationary, then such a dichotomy in energy expenditure between swimming and non-swimming situations would be most pronounced.

The critical unknown in this hypothesis is whether the swimming costs of locomotion for *M. rubrum* are indeed substantial enough to account for differences in growth, or even significant at all. However, since *M. rubrum* appears to be the fastest of all microplankton, then this particular species is one most likely to be subject to such costs, and a thorough examination of this whole matter is imperative.

Clearly what is now required is a series of tests of this hypothesis, either using single species techniques, if possible, and in the field on bloom populations where variation in bulk physiological parameters approximate to those for *M. rubrum* itself. The remainder of the thesis will address the validity of this hypothesis as a potential limiting factor both to the growth of individual cells, and to the dynamics of red-water appearance.

CHAPTER EIGHT:
COST OF MOTILITY IN PLANKTONIC PROTISTS - SOME THEORETICAL ESTIMATES

"Theory is the general, experiments are the soldiers"

Leonardo da Vinci (1452-1519)

8.1. INTRODUCTION

Since it has proved impossible to provide empirical estimates of the energy required for locomotion by *M. rubrum* to date, it seems useful instead to consider the problem on theoretical grounds for motile protists generally. This should at least provide some framework for future empirical examinations of the cost for *M. rubrum* when suitable techniques become available.

More generally, the physiological ecology of autotrophic and heterotrophic members of the pico, nano- and microplankton is now of particular interest because of the sheer magnitude of their biomass and metabolic activity, and thus potential role in global CO_2/O_2 budgets. Respiration rates of these smaller members of the plankton are now attracting widespread attention after receiving limited study in the past. Discrepencies between oxygen and ^{14}C methodologies, and the debated assumption that plankton community respiration represents a constant proportion of photosynthesis (Iriarte *et al.* 1991) require that plankton ecologists provide more detailed information on factors influencing metabolic losses both for individual species and communities. Recent studies have underlined the important, though previously underestimated, role of planktonic ciliates as heterotrophic (Sorokin 1981), mixotrophic (Stoecker *et al.* 1987, 1989) and phototrophic (Crawford 1989) members of the microplankton, and as food for metazoa (Sherr *et al.* 1986*a*), and thus imply the necessity of a thorough understanding of metabolic processes for this group.

Since the ciliates and dinoflagellates are probably the quickest of the motile microplankton, their respiration rates are intuitively of some interest. However, there appear to be no empirical studies on the subject, and theoretical ones available have tended to suggest a very low energetic cost for motility (Fenchel & Finlay 1983, Purcell 1977, Raven & Beardall 1981, Raven & Richardson 1984). Fenchel & Finlay (1983), in a comprehensive review of respiration rates of heterotrophic protists, suggested that "motility accounts for an insignificant fraction of the total metabolic rate". Similarly, Raven & Beardall (1981), using a slightly more empirical approach, concluded that "flagellar motility is not a major contributor to the energy requirements of dinoflagellates".

The debate on metabolic costs of motility for protists is reminiscent of that for larger members of the plankton; theoretical estimates (Vlymen 1970) of the cost of normal locomotion for planktonic copepods suggested this to be very low (<1% metabolism), but drag, and thus energy consumption, was shown to increase some 400 times during the rapid 'escape' reaction (Strickler 1974). For rotifers, despite low theoretical (<1% total metabolism) energy costs for locomotion, empirical estimates were 62% of total metabolism (Epp & Lewis 1984).

Here the question of energy costs of swimming is addressed theoretically in some detail for protists generally, and some of the limitations of the approaches mentioned above are critically examined. Further, implications for the photosynthetic ciliates, and in particular *M. rubrum*, are addressed.

8.2. THE MODEL

Fenchel & Finlay (1983; and Raven & Richardson 1984, see p. 263) adopted a theoretical approach to the problem by calculating the power (P) required to pull a sphere of diameter D, through water with viscosity η and velocity v, using Stokes' law:

$$P = 3\pi D v^2 \eta \qquad ..... (1)$$

This can be given in SI units of Joules sec^{-1} (or Watts) if D is given in metres, v in metres sec^{-1}, and η is given in N s m^{-2}; however, if η is assumed to be 0.01 poise (Fenchel & Finlay 1983), this is divided by 10 to give units of Newton s m^{-2}. For direct comparison with Fenchel & Finlay (1983), the units are converted to cgs units of ergs sec^{-1}, assuming 1 erg = 10^{-7} Joules.

An overall efficiency (hydrodynamical efficiency x efficiency of transforming chemical work into mechanical work) of the ciliary propulsion mechanism of 1% was assumed (Fenchel & Finlay 1983). The power required for motility was then expressed as a percentage of total metabolic rate.

Here, exactly the same model has been used as that given above by Fenchel & Finlay (1983). Total metabolic rate was determined assuming spherical cells, and using an empirically derived volume dependant relationship (Fenchel & Finlay 1983) for growing cultures of heterotrophic protists:

$$\log_{10}R\ (\mathrm{nl\ O_2\ cell^{-1}\ h^{-1}}) = 0.75[\mathrm{Log_{10}vol}(\mu m^3)] - 4.09 \qquad ..... (2)$$

The major drfference to the approach of Fenchel & Finlay (1983) is that the relative cost of motility is scaled, using the above theoretical equations, over typical ranges of both cell diameter (1 - 100 μm) and swimming speed (100 - 5000 μm sec^{-1}) encountered for planktonic protists.

8.3. RESULTS

Figure 8.1a shows a log:log plot of metabolic rate and cost of swimming against cell size. Here, the cost of swimming at any given speed is clearly observed to increase, relative to total metabolic rate, with decreasing cell size. This results from metabolic rate increasing as a power function of length with an exponent of between 2 and 3, whereas the energetic cost of swimming is proportional to length and to velocity squared. For a speed of 1000 μm sec^{-1}, the cost appears to approach total metabolic rate when cell diameter decreases to between 1 - 10 μm. The cost of swimming at two other speeds, 100 and 5000 μm sec^{-1} is also given, and all vary with the same slope.

When expenditure is expressed as a percentage of total metabolism, as in Figure 8.1b, it is clear that the cost of swimming at 1000 μm sec^{-1} increases significantly above 1% as cell diameter decreases below 100 μm. Thus, the choice of the ciliate *Didinium* as an example by Fenchel & Finlay (1983) was rather unfortunate; in their study, the cost of swimming of this species (diameter 120 μm) was quoted as about 0.7%, as can be verified by Figure 8.1b, but this is unrepresentative of the cost of swimming at such a speed for the much smaller ciliates which predominate in aquatic environments. In fact, the cost of swimming at 1000 μm sec^{-1} predicted by this model for ciliates in the typically encountered size range of 20 - 30 μm would be about 5 - 10% total metabolism. At 100 μm sec^{-1}, the relative cost only just exceeds 1% total at a size of between 1 - 10 μm diameter. For 5000 μm sec^{-1}, the cost is always substantial; about 60% at 50 μm and 460% at 10 μm cell diameter.

The other example quoted by Fenchel & Finlay (1983), at the lower end of the size spectrum, is the flagellate *Ochromonas* of diameter 8 μm and swimming speed 60 μm sec^{-1}. As can be observed in Figure 8.1b, the energy expended on motility by this species is unlikely to be significant, and in fact was given as 0.1% (Fenchel & Finlay 1983). Like *Ochromonas*, many flagellates swim at a speed of the order of 10 body lengths sec^{-1}; theoretical considerations have thus been expressed

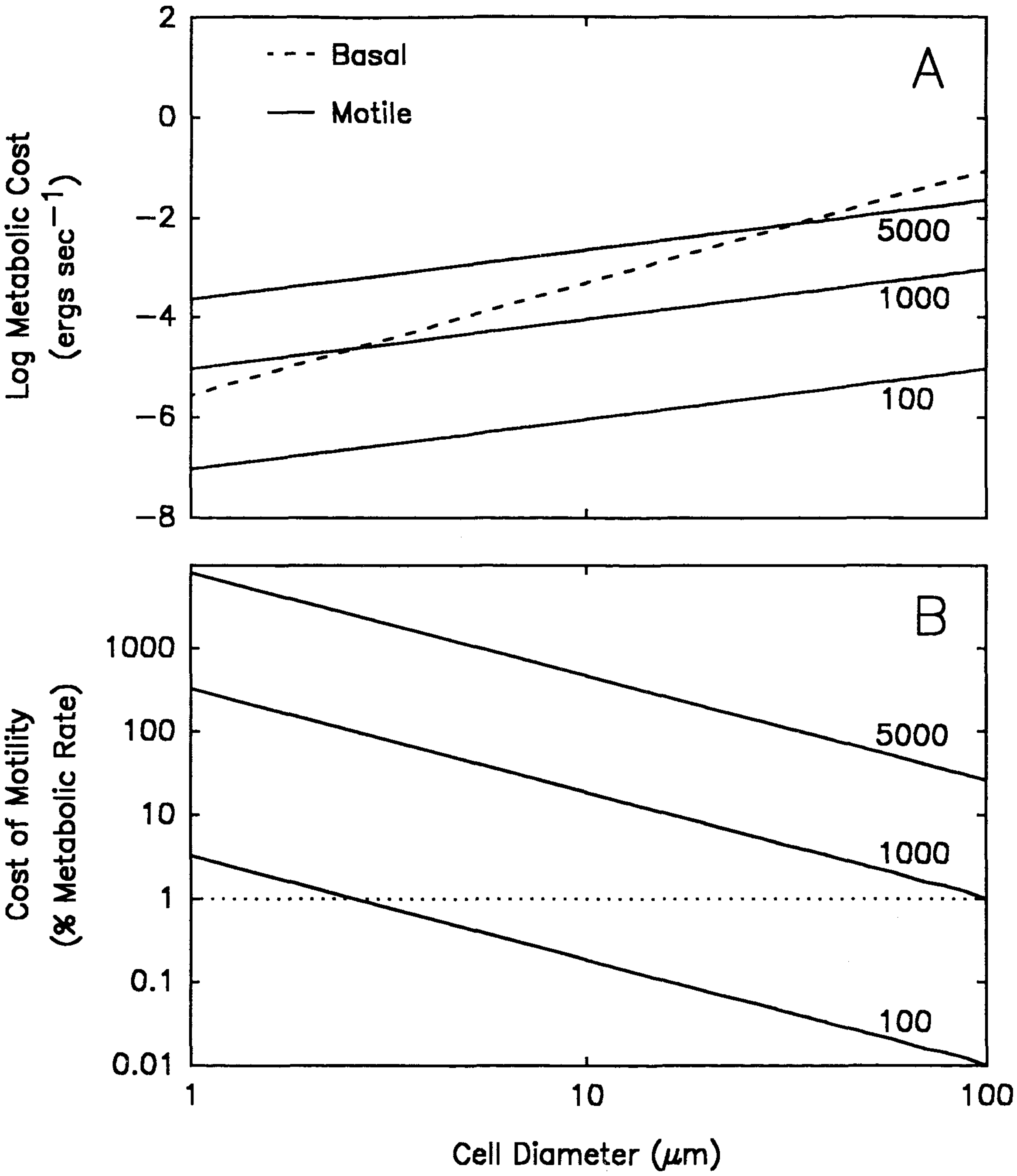


Figure 8.1.

(A) Log:log plot of 'basal' metabolic rate against cell diameter (dashed line). Solid lines represent theoretical costs of motility for a range of three swimming speeds (μm sec^{-1}), scaled over the size range.

(B) Theoretical costs of swimming at speeds given in Figure 8.1a, as percent total metabolic rate. Dotted line represents 1% total metabolic rate.

also on this basis in Figures 8.2a & b. In contrast to using a fixed speed against cell size, relative cost of swimming at 10 and 100 body lengths sec^{-1} increases with increasing cell size. The cost of swimming at 10 body lengths sec^{-1} does not exceed 1% total metabolism over the size range given. However, the cost at 100 body lengths sec^{-1} is always significantly above 1%, and above a cell diameter of 10 μm becomes quite substantial. For example, protists in the range 30 - 50 μm swimming at 100 body lengths sec^{-1} would be predicted to expend the equivalent of between 40-60% total metabolic rate on swimming.

8.4. DISCUSSION

It is not clear from the literature whether or not swimming speed varies as a function of cell diameter; speed has been suggested to be independent of cell size for flagellates (100-200 μm sec^{-1}) (Wu 1977), and for ciliates (1000 μm sec^{-1}) (Sleigh & Blake 1977). However, these studies were based on a limited number of examples, and there are certainly many forms which do not conform to these patterns, for example many flagellates swim at about 10 body lengths sec^{-1}. Thus, for clarification, alternative approaches of considering speed both as a fixed entity against size, and, as a function of body lengths sec^{-1} have been applied.

The model presented here, insofar as being identical to that of Fenchel & Finlay (1983), confirms their predictions in the context of their two 'fixed point' estimates. However, the extension of this model to cover the spectra of cell size and swimming speed typically encountered for planktonic protists suggests that certain forms may be expending substantial quantities of energy upon locomotion. Table 8.1 presents typical ranges of cell size and swimming speed for various broad groupings of motile protists, and summarises predictions of the model for each group, both for growing cells and for starving or light limited cells (discussed later).

The group highlighted for highest energetic costs are the planktonic ciliates of cell diameter 10 - 50 μm, and swimming speed greater than 1000 μm sec^{-1}. Sleigh &

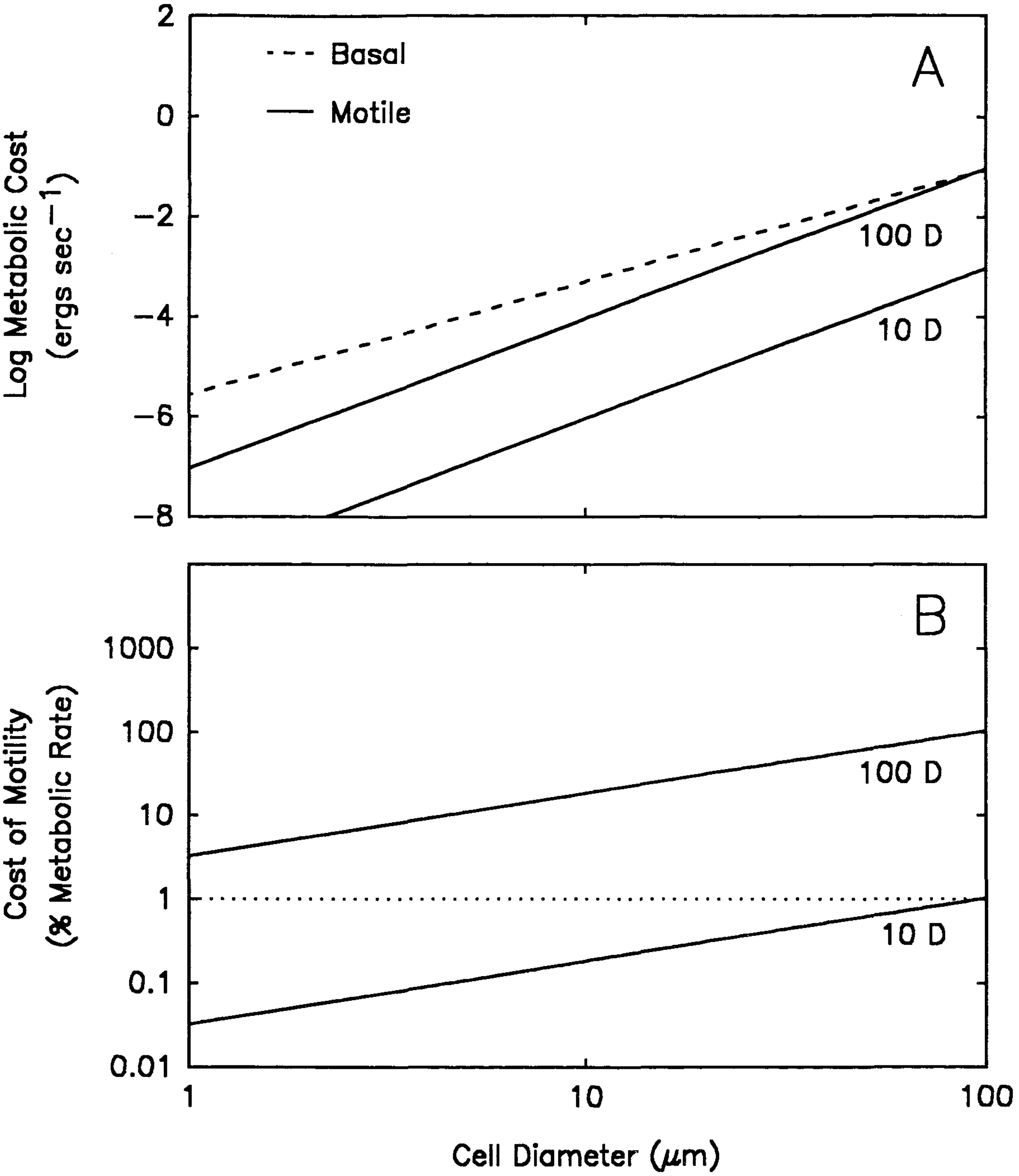


Figure 8.2.

(A) Log:log plot of 'basal' metabolic rate against cell diameter (dashed line). Solid lines represent theoretical costs of motility at 10 and 100 body lengths (D) sec^{-1}, scaled over the size range.

(B) Theoretical costs of swimming at speeds given in Figure 8.2a, as percent total metabolic rate. Dotted line represents 1% total metabolic rate.

Table 8.1. Typically encountered ranges of cell sizes and swimming speeds for major groups of planktonic protists; predicted cost of swimming, relative to total metabolism, given for 'growing' and 'starving'cells.

Protistan Group [with refs]	Cell Diameter Range (μm)[a]	Swimming Speed Range (μm sec^{-1})[a]	Predicted Cost (as % Basal R)	
			Growing[b]	Starved[c]
Nanoflagellates	1-20	1-100	0.00001-3	
(Bauerfeind *et al.* 1986, Throndsen 1973)				
e.g. *Ochromonas*	8	60	0.01	1.8[d]
Dinoflagellates	10-100	10-1000	0.0001-19	
(Levandowsky & Kaneta 1987, Sournia 1982)				
e.g. *Dinophysis*	40	500	0.6	1.3[e]
Ciliates (simple cilia)	10-100	500-1500	0.3-42	
(Roberts 1981, Sleigh & Blake 1977)				
e.g. *Uronema*	25	1200	8	51[f]
Ciliates (compound cilia)	10-100	1000-4000	1-297	
(Dale 1987*a*)				
e.g. *Strombidium*	30	3000	42	254[f]

---

[a] general guide to typically encountered ranges for each group; however, it is acknowledged that many examples fall outside these ranges

[b] 'basal' rate derived from empirical formula of Fenchel & Finlay (1983). Range represents max. and min. for sizes and speeds given

[c] or light limited autotrophs. Predicted costs computed assuming no change in swimming speed, but using estimates of reduced cell volumes and respiration rates for 'starving' cells; assumptions outlined below

[d] assuming estimate of reduction in cell volume by 80% and respiration rate by 97% (Fenchel & Finlay 1983)

[e] data not available for dinoflagellates; conservative assumption of reduction in volume by 50% and respiration by 50%

[f] no relevant data available; assuming estimates of reduction in volume by 80% and respiration by 90% for *Paramecium* (Fenchel & Finlay 1983)

Blake (1977) have suggested that propulsion by simple cilia acheives a fairly constant speed of 1000 μm sec^{-1}, although there are many ciliates which swim at much greater speeds (Dale 1987*a*). For example, the oligotrichs are generally rapid swimmers, and are now of realised to be of considerable ecological significance both in marine and freshwater environments. Rapid swimming is acheived by means of a circumoral ring of cilia, cirri or membranelles. Many oligotrichs have been shown to swim at speeds of 2000 - 3000 μm sec^{-1} (Dale 1987*a*), and even up to 4000 μm sec^{-1} (Fauré-Fremiet 1948). These figures suggest the energetic cost of motility to be the equivalent of some 10 - 75% total metabolic rate, while swimming, for cells of 30 - 40 μm diameter. *Mesodinium rubrum* an even more impressive swimmer, though perhaps an exception, swims at about 5000 μm sec^{-1} (Lindholm 1981), but has also been recorded at 8000 - 8500 μm sec^{-1}, or about 200 body lengths sec^{-1} (Jonsson & Tiselius 1990, Lindholm 1985). This gives costs of the equivalent of 80 - 120% (at 5000 μm sec^{-1}), and 200 - 340% metabolic rate (at 8500 μm sec^{-1}) for cells of 30 - 40 μm diameter! Speeds of species such as the holotrich *Askenasia* and the oligotrich *Halteria* appear not to have been determined, but can be observed to swim almost as quickly as *M. rubrum*, and certainly at speeds of several mm sec^{-1} (pers. obs.). It is interesting that genera such as *Mesodinium*, *Askenasia*, *Halteria*, *Strombidium*, *Laboea*, and *Tontonia*, all capable of very rapid swimming in short bursts (Klekowski 1981, Tamar 1979), do not swim continuously at such speeds. This, at least circumstantially, supports the contention that very rapid swimming could become energetically costly. It is also noteworthy that many of these fast swimmers harbour algal endosymbionts or retain plastids. Some species of the oligotrich genera *Strombidium*, *Laboea*, and *Tontonia* are mixotrophic (Jonsson 1987, Laval-Peuto & Rassoulzadegan 1988, Stoecker *et al.* 1988), and *M. rubrum*, the fastest of all, is an obligate functional phototroph (Lindholm 1985).

For dinoflagellates, the model predicts energetic costs of swimming to be substantially lower than for ciliates (see Table 8.1), principally due to the lower speeds acheivable through flagellar propulsion. Most dinoflagellates swim at about 10 body lengths sec^{-1} or less (Bauerfeind *et al.* 1986, Sournia 1982), suggesting a maximum energy cost of about 1% for a cell of 100 μm diameter, and much less for a cell of 10 - 20 μm diameter. At 100 body lengths sec^{-1} a cell of 100 μm would expend the equivalent of just over 100% total metabolism (Figure 8.2b) although this is an unrealistically high swimming speed for dinoflagellates. However, the swimming speed of the red-tide dinoflagellate *Protoperidinium* cf. *quinquecorne* has been given as 1500 μm sec^{-1} (Horstmann 1980), and the rock pool dinoflagellate *Peridinium gregarium* recorded at up to 1800 μm sec^{-1} (Lombard & Capon 1971). These are remarkable speeds for dinoflagellates giving estimated energetic costs for motility (sizes not given; assuming size of 30 μm diameter) in the order of about 10% total metabolic rate.

The nanoflagellates tend to acheive greater relative swimming speeds than the dinoflagellates, generally in the range 0.7 - 42 body lengths sec^{-1} (Bauerfeind *et al.* 1986), although 100 μm sec^{-1} for *Micromonas pusilla*, a cell of only 1 - 3 μm diameter has been recorded (Throndsen 1973). This represented a speed of up to 75 body lengths sec^{-1}, which gives an estimated cost of some 1 - 3 % total metabolism. Since most flagellates do not seem to swim much faster than 100 μm sec^{-1}, Figure 8.1b and Table 8.1 suggest relative costs will rarely exceed 1 - 10% basal metabolism. It is likely that for such small cells the cost of swimming even at 50 - 100 body lengths sec^{-1} is masked by the very high specific metabolic rates associated with small size.

Slightly more empirical approaches have been used to estimate cost of locomotion. Fenchel & Finlay (1983) supported their contentions with considerations of the mechanical power output and chemical power input of flagella/cilia. For the two examples quoted, these estimates were again less than 1% total metabolic rate. However, a standard flagellum was used without giving the length. Raven & Beardall (1981) considered the maximum ATP consumption based on the number of dynein/ATPase molecules per unit flagellar length for *Gonyaulax polyedra*, giving a relative cost of about 1.5%. These approaches again are limited by being point estimates; relative cost is not considered over the whole size range involved. Assuming flagellar length varies linearly to some extent (e.g. 3 - 15 x body length; Wu 1977) with cell size, and chemical power input is dependent upon flagellar length

(Wu 1977), then relative metabolic cost of swimming will increase with decreasing cell size, since total metabolic rate is volume dependent. The argument applies in an analogous manner to the ciliates; Sleigh & Blake (1977) suggest that "one might regard the product of ciliary length and ciliary number as a measure of the total energetic machinery of the organism". Many fast swimming forms have reduced or absent somatic ciliation, and possess an equatorial or oral girdle of cilia, cirri or membranelles around the cell. Since individual cilia or basal bodies vary relatively little in diameter or length (e.g. 5 - 15 μm length; Sleigh & Blake 1977), the number of cilia will be approximately dependent upon the circumference of the cell (i.e. linear dependence on diameter), thus as cell volume decreases, total cilium length:cell volume ratio, and thus potential energy expenditure on swimming will increase. Again, because of this, the choice of *Didinium* (Fenchel & Finlay 1983) was unrepresentative of smaller fast swimming ciliates in terms of its ciliary length:cell volume ratio.

The above discussions imply that the majority of planktonic protists will expend the equivalent of a low ($<1\%$) or moderate (1 - 10%) proportion of their total metabolic rate on motility (see Table 8.1). Some forms, such as the small fast swimming ciliates could be expending substantial amounts of energy (equivalent to 10 - 100%+ total metabolic rate) while swimming. Clearly, for these latter forms, rapid swimming, and thus energy expended, is not continuous, but this surely begs the question of what factors control the frequency and velocity of fast locomotory 'bursts'. For example, Chapter 3 suggests that the frequency of motile 'jumps' of *M. rubrum* may increase in response to turbulence. The implications of such potential metabolic costs, for the very fast forms, are clear and considerable, both in terms of the ecophysiology of individual species, and, the methodology and assumptions concerning determination of bulk physiological parameters of plankton assemblages.

For forms even with more modest motile capabilities, the implications may be considerable; Table 8.1 presents relative costs expressed for growing cells and for starving, or light limited cells in the case of these being phototrophs. Starving protists may reduce their cell volumes and specific respiration rates (Fenchel & Finlay 1983), both of which would increase the relative cost of swimming at a given speed. Similarly, Raven & Richardson (1984) considering the analagous situation of the effect of light limitation or continuous darkness on phototrophic protists, concluded that "the cost of having or using flagella ..... may not be negligible in relation to (for example) survival of an obligately photolithotrophic dinophyte in prolonged darkness". Fenchel & Finlay (1983) suggested that small starving protists may reduce their metabolic rates to 2% of that of growing cells; since the cost of swimming at a given speed cannot be similarly reduced, it is clear from Table 8.1 that relative costs of motility may increase substantially under such conditions, particularly for small fast moving oligotrichs ciliates such as *Strombidium*. In starvation experiments (see Chapter 9), the ciliates *Strombidium capitatum* and *Favella ehrenbergii* were observed to swim more slowly after 24 hours in the dark without food (pers. obs.), as did *Paramecium* sp. (M. Sleigh, pers. comm.), thus supporting the above contention. However, others have observed faster swimming with starvation, thus comparisons of anecdotal evidence is clearly of limited value; this may in fact represent a size specific phenomenon however. For small flagellates, Table 8.1 suggests again that high specific respiration rates associated with small size tend to mask costs of motility, even for starving cells.

With metabolic rate being strongly temperature dependant, low temperatures and accordingly elevated viscosities will further increase the cost of swimming (or reduce acheivable speed) relative to total metabolism. Thus it appears that for many even moderately motile planktonic protists, swimming could potentially represent a significant or major drain on resources during periods of the year when food and/or light is scarce, cell size is reduced and temperature is low.

What is now required are more empirical tests of these theoretical estimates, firstly on a selection of highly motile forms. In terms of presenting a testable hypothesis for such studies, Table 8.1 would suggest that respiration rates of oligotrich ciliates as well as *M. rubrum*, for example, should be significantly greater during bursts of fast swimming activity. This could be tested by determining rates of respiration in the presence/absence of inhibitors of motility. An alternative approach would be to determine those factors controlling the frequency of such rapid 'jumps' and then to utilise these as variables during determination of respiration rates.

The limitations of the present approach are acknowledged in terms of the numerous assumptions made; hydrodynamic and chemical conversion efficiencies are critical to the model, and little or no evidence is available to determine whether or not the value of 1% is conservative, and to what extent this efficiency varies. The model also assumes that the regression derived for heterotrophic protists (Fenchel & Finlay 1983) holds for all protists. However, the assumptions made are exactly those made in previous studies which have tended to extrapolate limited data to imply a low energetic expenditure for all planktonic protists. The purpose of this theoretical study is not to provide absolute estimates of energetic costs of locomotion, but to identify the limitations of previous theoretical approaches, particularly those of not scaling cell size and swimming speed, and to highlight those fast swimming protists which may be expending considerably more energy on motility than previously anticipated.

Looking at *M. rubrum* in particular, it is clear from these theoretical considerations that this species, more than any other, is likely to be expending a considerable portion of its energy budget on locomotion. Even despite the limitations of the model as described above, it is unlikely that such energy costs for *M. rubrum* could be trivial or insignificant. For example, even assuming an overall efficiency of ciliary propulsion of 10%, rather than 1%, the metabolic costs would still be of an order equal to the total (stationary) metabolic rate. The implications of such potential energy expenditure will be examined in detail in Chapter 10.

CHAPTER NINE:
EXPERIMENTAL DETERMINATION OF RESPIRATION RATES IN PLANKTONIC CILIATES

"An experiment is a device to make Nature speak intelligibly"

George Wald (1967 Nobel Lecture)

9.1. INTRODUCTION

The implications of Chapter 8 were that *M. rubrum* and a number of other ciliates could be expending a significant proportion of their energy budget on motility. Given the size variation shown in Chapter 6, it is clear that to comprehend the problem further, a technique for determining respiration rates is required which gives a precise estimation of rate per unit carbon on individual cells. Without this, cell size variation becomes an unacceptable bias. Fortunately, such a technique has just become available, but due to the technical difficulties in maintaining *M. rubrum* in the laboratory, and in achieving sufficient growth to evenly label the cells, the method is yet to be employed on *M. rubrum*. However, the technique has been utilised here on other planktonic ciliates readily available in culture; it is anticipated that this will be adopted in the future to address the question of respiration of *M. rubrum*.

For the microplankton generally, much attention is now becoming focussed upon metabolic activity of smaller members of the marine plankton. The contribution of these pico- nano- and microplankton to global fluxes of CO_2 and O_2 is becoming increasingly recognised, and thus it is imperative to provide estimates of metabolic activity for dominant forms.

For the phytoplankton, most estimates have been derived from bulk oxygen changes in dark bottles in natural communities or in cultures. One problem with such incubations has been the assumption that the contribution of microheterotrophs to these rates is negligible (see Peterson 1980). However, many recent studies have underlined the quantitative occurrence of ciliates (e.g. Sorokin 1981) and nanoflagellates (e.g. Fenchel 1982), and some suggest that at least at low chlorophyll levels, microheterotrophs may make an important contribution to community rates of respiration (Williams 1981, Iriarte *et al.* 1991). Of the larger microheterotrophs, the oligotrich ciliates appear now to be one of the more conspicuous groups, their role previously having been seriously underestimated (Smetacek 1981). To complicate matters, many oligotrichs have also recently been shown to function as mixotrophs, that is, they are partially photosynthetic by means of retained functional plastids (Stoecker *et al.* 1987; Laval-Peuto & Rassoulzadegan 1988). The situation in natural planktonic assemblages has thus become much more complex than previously anticipated, and clearly a plausible approach is now to determine rates of respiration on selected individual species.

Few data are presently available for respiration rates of individual species of marine microplankon, although some useful information can be taken from the freshwater literature. Much of such data has been provided either by studies of bulk physiology of cultured organisms, or by using diver respirometery on single cells or small groups of cells (for review see Fenchel & Finlay 1983). Although such information can be usefully employed in estimating generalized potential rates for marine forms, it clearly is no substitute, since variability in physiology is likely between marine and freshwater species. Moreover, there are several limitations to the use of data from bulk cultures and diver respirometry; firstly, they may not provide precise enough estimates of carbon content of individuals. Instead, cell carbon is estimated from linear dimensions and approximations to simple geometric shapes (e.g. using Strathmann 1967), and determinations of cell densities; this approach suffers from inherent variability of carbon:volume ratios, and from the imprecise and inaccurate method of computing cell volume. Secondly, significant starvation induced reduction in specific respiration rates have been reported in the literature (for reviews see Fenchel & Finlay 1983; Caron *et al.* 1990); this could conceivably result more from cell volume variation than previously anticipated if this is imprecisely determined or if carbon:volume ratios vary during the incubations.

In a comprehensive review of respiration rates of protists, Fenchel & Finlay (1983) emphasised the necessity for respiration studies on cells with exactly measured biomass. In this context, the recent approaches outlined for determination of cell carbon (Putt & Stoecker 1989) and respiration rates (Stoecker & Michaels 1991) appear promising. The beauty of these techniques is that they provide the required precision in estimation of cell carbon, as advocated by Fenchel & Finlay (1983), and thus can determine exact carbon specific respiration rates on individual cells.

Here this technique is adopted to determine the rate of respiration in starved cells of the mixotrophic oligotrich *Strombidium capitatum* (Leegard) Kahl and the heterotrophic tintinnid *Favella ehrenbergii* Claparède & Lachmann. The influence of variation in cell size upon respiration rate is also addressed, and the bearing of this upon rates of mortality of such starved cells is investigated.

9.2. OUTLINE OF TECHNIQUE

This relies on evenly labelling protists with ^{14}C, by means of growing them on labelled prey, and then either picking out individuals for direct carbon determination by scintillation counting, or monitoring loss of $^{14}CO_2$ into a KOH trap. Full details are given in Appendix 2.

9.3. RESULTS

For *S. capitatum*, the progressive deviation of the mean organic carbon (cell + excreted dpm in inner vial) from the total carbon (inner + outer vial dpm) over

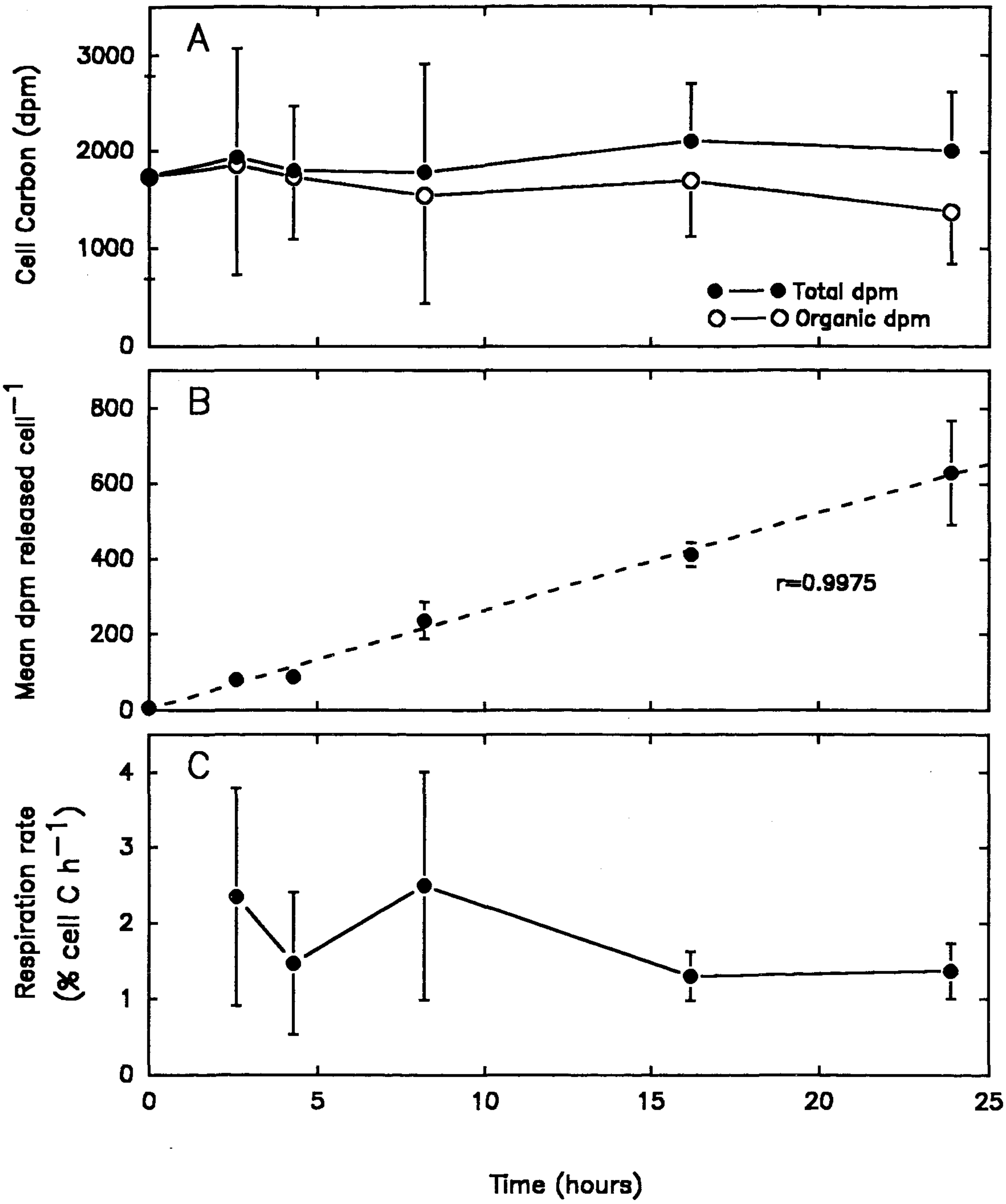


Figure 9.1.

Respiration rates of starved, individual *Strombidium capitatum* cells over a period of 24 hours:

(A) depletion of individual organic carbon with respect to total carbon

(B) increase in mean CO_2 respired per hour

(C) respiration rate expressed as percent cell carbon respired per hour. Points represent means of 7-12 cells ± S.D.

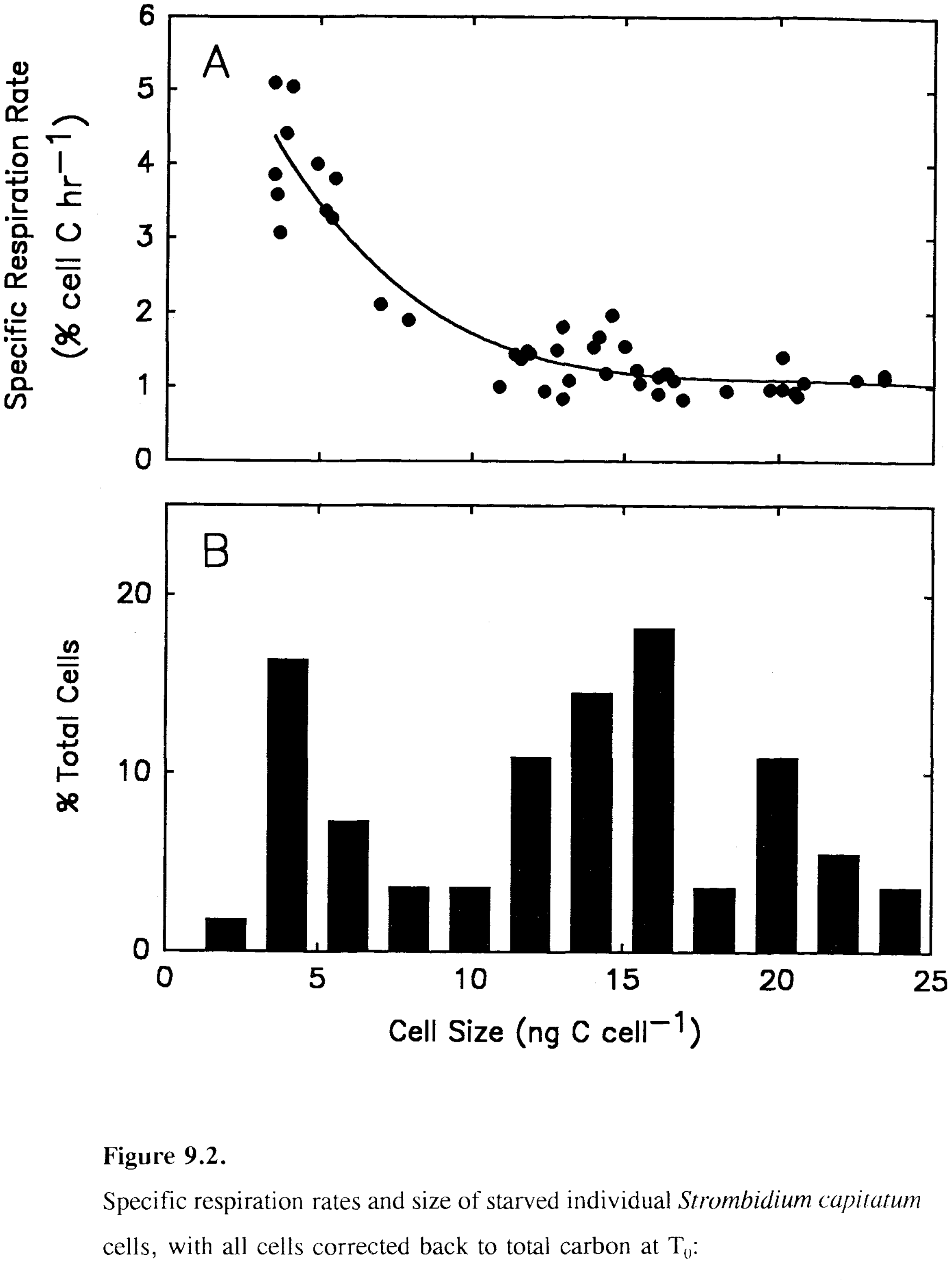


Figure 9.2.

Specific respiration rates and size of starved individual *Strombidium capitatum* cells, with all cells corrected back to total carbon at T_0:

A) specific respiration rates of cells expressed as a function of cell size

B) frequency histogram of size distribution; this will underestimate frequency of 'small' cells since these suffered higher rates of mortality.

the 24 h period is shown in Figure 9.1a. This deviation represents the inorganic $^{14}CO_2$ respired and collected in the KOH fraction. This is shown more clearly in Figure 9.1b; mean respiration rate (dpm cell^{-1}) was linear over the 24 h period and showed little evidence of a reduction of respiration with starvation. When expressed in Figure 9.1c as percent cell carbon respired per hour, evidence of such a reduction was slightly more apparent; however, during the initial 8 hours of the incubation, there was considerable variation in the means of total and organic carbon dpm, and in percent cell carbon respired. It appeared from the raw data that much of this variation resulted from a dependence of specific respiration upon cell size, so this was examined in more detail.

It is clear even from Figure 9.2a that cell size was highly variable and that such variation had a strong influence on specific respiration rate. Size frequency distribution in Figure 9.2b showed a bimodal peak; a distinct group of small cells occurred (< 7 ng C cell^{-1}) with high specific respiration rates (3 - 5 % cell C h^{-1}), whereas larger cells (7 - 25 ng C cell^{-1}) respired at 1 - 2 % cell C h^{-1}. Although the distribution did tend to form two fairly distinct groups, some intermediate sized cells were present with correspondingly intermediate rates of respiration. From these observations it followed that a variation in the proportion of smaller to larger cells at each time interval during the incubation could have caused the degree of variation observed in Figure 9.1c. For the purpose of further analysis and discussion, cells are defined as 'small'(< 7 ng C cell^{-1}), or large (> 7 ng C cell^{-1}) according to the natural division in the size frequency distribution (Figure 9.2b). For this discrimination, carbon for all cells was first corrected back to initial cell carbon at T_0.

Looking at rates of mortality, Figure 9.3a shows that this increases sharply in the first 8 hours but then levels off to a plateau. Upon closer examination it was clear that the bulk of the mortality is confined to the small cells of < 7 ng C cell^{-1}; these represent 40% of all cells at T_0 but none survived for more than 8 hours. Large cells appeared to have lower rates of mortality.

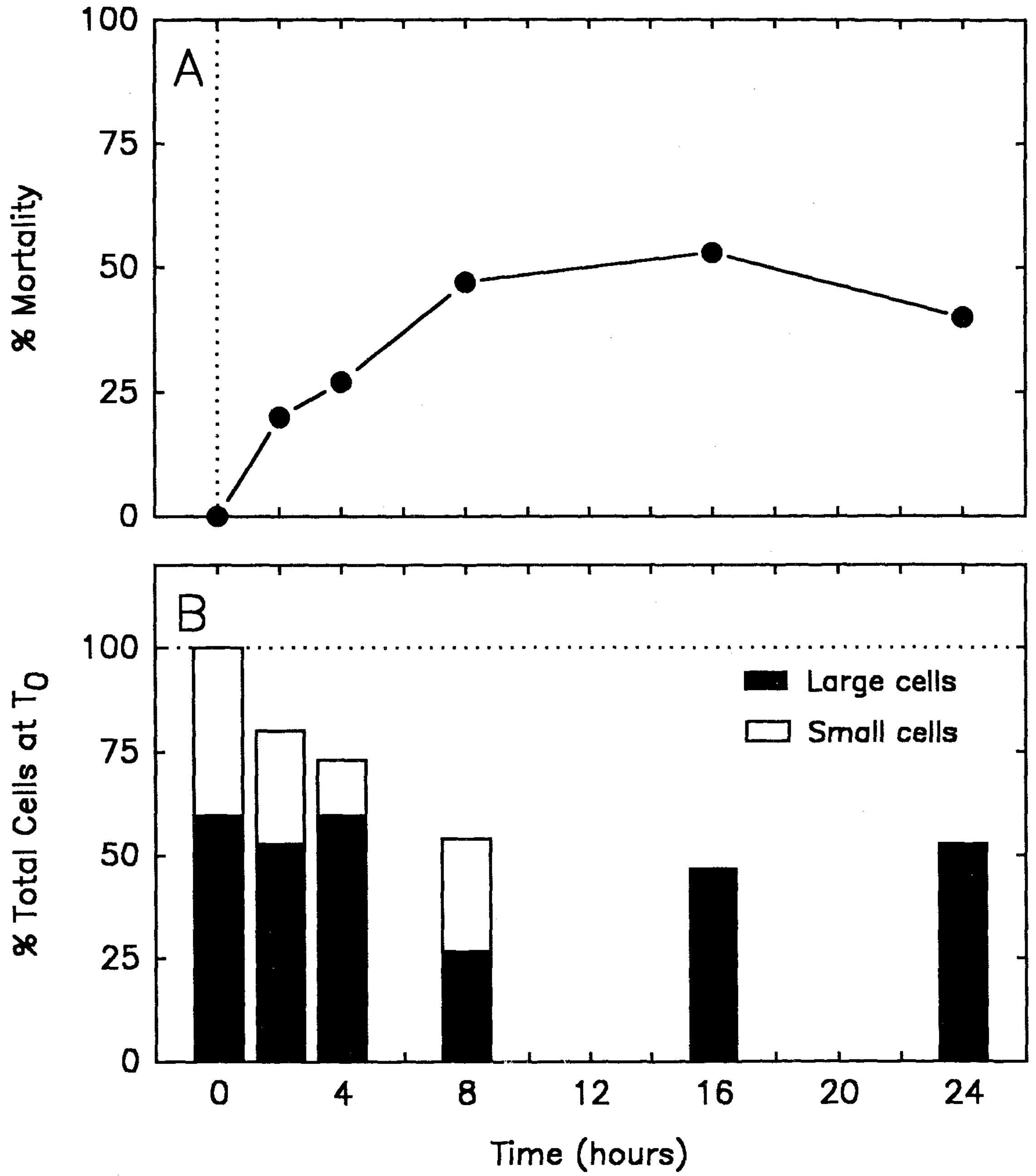


Figure 9.3.

Rates of mortality of starved *Strombidium capitatum* cells:

A) percent mortality expressed as a function of incubation time

B) percent survival expressed as proportions of 'small' and 'large' cells surviving over period of incubation.

Figure 9.4 shows changes in size distribution over time; there were insufficient cells for size frequency analysis at each time interval, therefore cells from 0 and 2 h incubations were pooled, as were those from 4 and 8 h, and 16 and 24 h. This gave three successive size frequency histograms. The size of cells decreased through the incubations, with an apparent loss of the smaller cells below 7 ng C cell^{-1} by 16 - 24 h, as also shown in Figure 9.3b. From the raw data it can be determined that the two 'small' cells remaining by 16 - 24 h represented cells which had commenced as 'large' cells and respired away a significant portion of their cell carbon thus becoming small cells. The changes in size distribution of cell carbon shown in Figure 9.4 closely reflect those predicted by the respiration rates shown in Figure 9.2a; for example at a rate of 1 % cell C h^{-1}, the larger cells of about 22 ng C cell^{-1} would be expected to decrease in carbon by over 20% to less than 18 ng C cell^{-1} over 24 h, and this is confirmed by Figure 9.4c. Small cells of less than 7 ng C cell^{-1}, respiring at around 4% cell C h^{-1}, would be predicted not to survive for 24 h since they would theoretically respire virtually all of their cell carbon in this time! This is in fact what is suggested by Figure 9.4; small cells appear to decrease in size after 4 - 8 h, but then disappear by 16 - 24 h. Since the influence of size variation and mortality appeared to be so critical for this species, specific respiration rates for 'large' cells were then separated from those of smaller cells. This is shown in Figure 9.5 which is a re-plot of Figure 9.2c showing that the more 'refined' specific respiration rates for larger cells clearly did not vary significantly over the 24 h period, and remained around 1 - 1.5 % cell C h^{-1}. The rates for 'small' cells were more variable, but also did not show evidence of a significant reduction over the 8 hours of their survival. This refinement clearly reduced the errors associated with the means of specific respiration rates in Figure 9.5 and thus emphasises the precision of the technique when 'normal' cell size variation is encountered.

In contrast to *S. capitatum*, Figure 9.6b shows *F. ehrenbergii* to have a non-linear release of $^{14}CO_2$ over 36 h. Release tends to plateau with time, and Figure 9.6c shows that this manifests itself as a gradual decrease in specific respiration rate; at 36 h, specific rate declines to about 50% of its 6 h value. However, at around

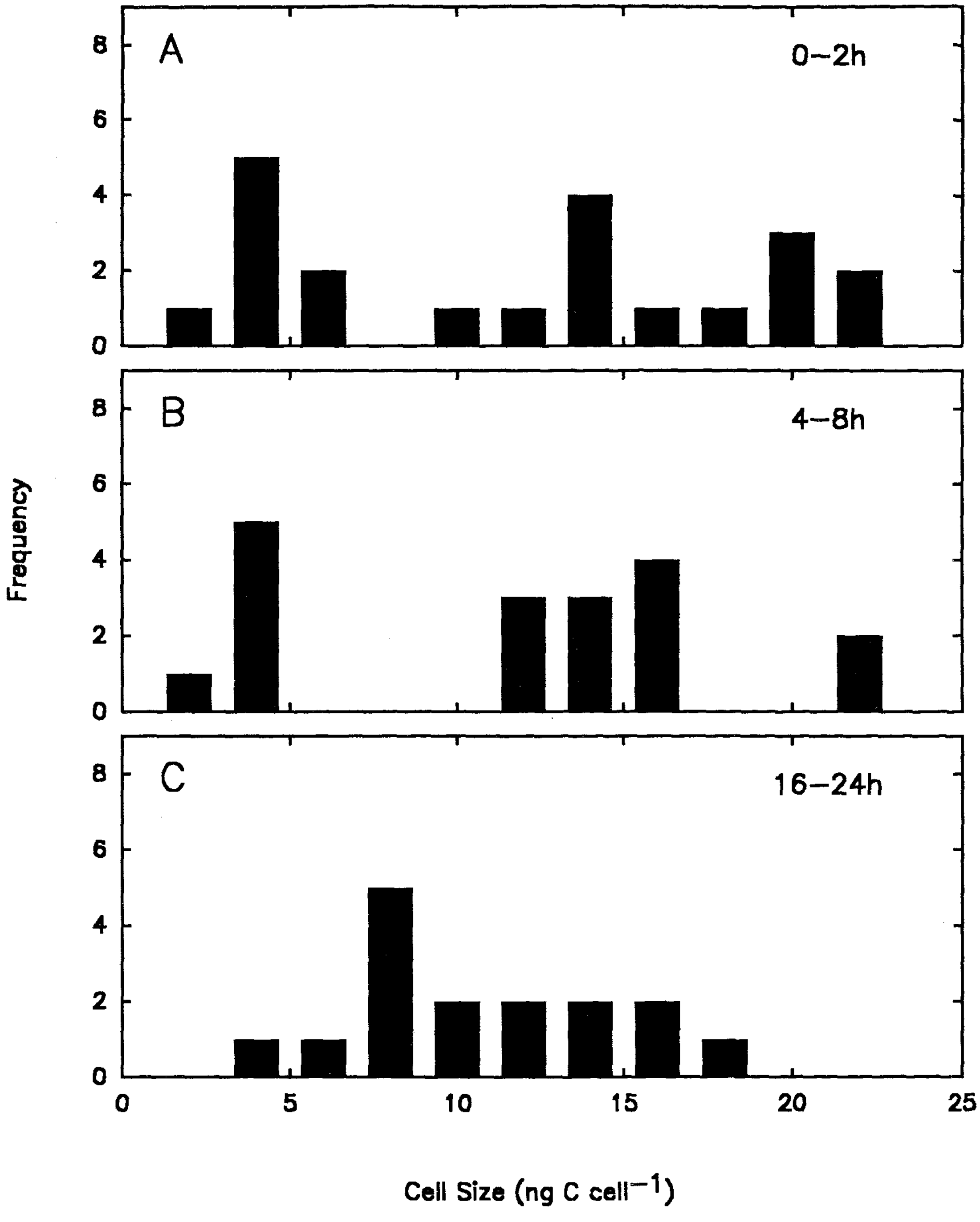


Figure 9.4.

Size frequency analysis of starved *Strombidium capitatum* cells surviving over the period of incubation, data pooled as follows:

(A) T_0 and 2 h incubations

(B) 4 and 8 h incubations

(C) 16 and 24 h incubations.

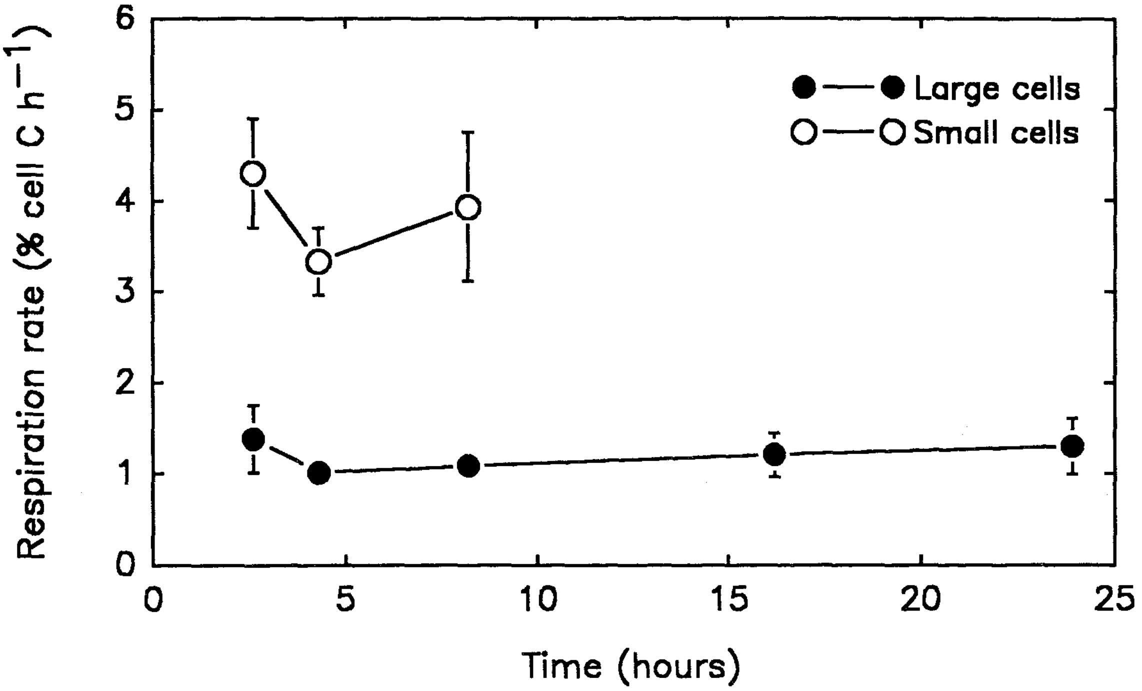


Figure 9.5.

Specific respiration rates of starved *Strombidium capitatum* cells; re-plot of Figure 1C with rates of 'small' (< 7 ng C cell^{-1}) and 'large' cells (> 7 ng C cell^{-1}) treated separately (± S.D.).

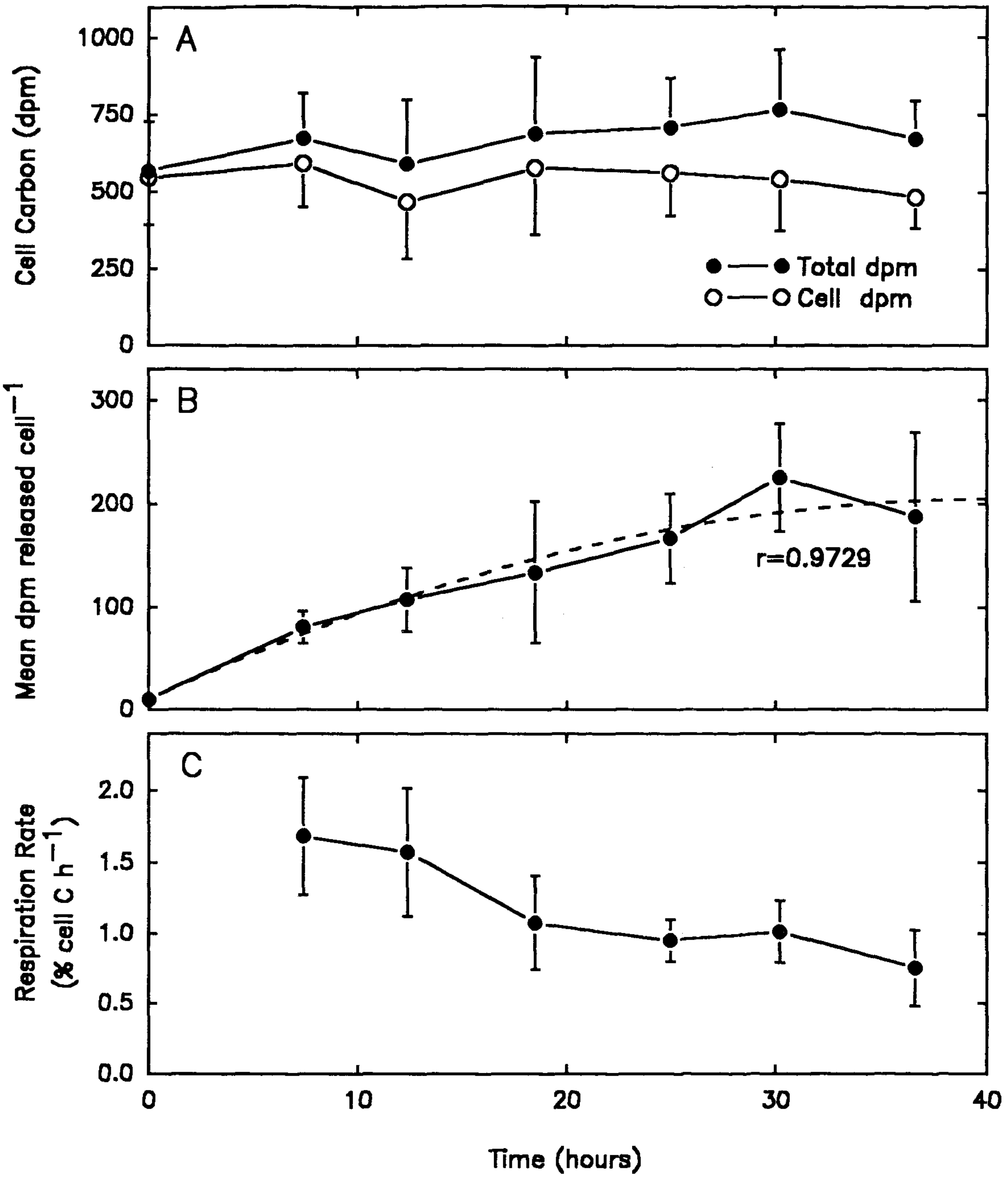


Figure 9.6.

Respiration rates of starved, individual *Favella ehrenbergii* cells over a period of 36 hours:

(A) depletion of individual organic carbon with respect to total carbon

(B) increase in mean CO_2 respired per hour

(C) respiration rate expressed as percent cell carbon respired per hour. Points represent means of 8-15 cells ± S.D.

1-1.5% cell C h^{-1}, the rate is of a similar order to that of the larger *S. capitatum* cells. Figure 9.7 shows that in contrast to *S. capitatum*, *F. ehrenbergii* has a rather more unimodal size distribution, but with evidence of some bimodality, perhaps indicating divided cells. Since the size range is narrower than for *S. capitatum*, very small cells and corresponding high specific rates of respiration are absent; however, there is still evidence of size dependent specific rate of respiration, the smallest cells having rates of about 3% cell C h^{-1} (Figure 9.7a).

9.4. DISCUSSION

The advantage of the technique described here over others is that it provides precise estimates of cell carbon and respiratory carbon loss at the end of the incubation. By back calculation to T_0, that is, summing organic (inner vial) and respired inorganic carbon (outer vial), initial cell carbon and thus carbon specific respiration rates can be computed (e.g. Figure 9.1) without the inherent problems associated with estimation of cell volume and use of carbon:volume ratios. The effect of intraspecific cell size variation on respiration rates can also be more fully explored than has been possible in previous studies.

The specific respiration rates determined here for *Strombidium capitatum* agree well with general rates determined for similar sized ciliates. Caron *et al.* (1990) quote rates of 0.4 - 20.0 x 10^{-6} nl O_2 μm^{-3} h^{-1} for cells in the range 1.0 -10.0 x 10^4 μm^3; this study gives rates of 0.8 - 5.1 % cell C h^{-1}, which converts to rates of about 2.5 - 20.1 x 10^{-6} nl O_2 μm^{-3} for cells of between 2.4 - 14.9 x 10^4 μm^3; assuming 0.19 pg C μm^3 and 20% cell volume shrinkage in Lugol's (Putt & Stoecker 1989), an RQ of 1.0 and Q_{10} of 2.0 (Fenchel & Finlay 1983). These rates of 3 - 5 % cell C h^{-1} for cells of 4 - 6 ng C also compare favourably with those of Stoecker & Michaels (1991) who determined rates for *S. capitatum* averaging 4.2 % cell C h^{-1} on cells of 6.5 ng C. Stoecker & Michaels (1991) did not describe larger cells such as those we describe here of up to 23.5 ng C, and respiring at 1 -1.5 cell C h^{-1}, but such size variation may depend upon the physiological stage of the culture.

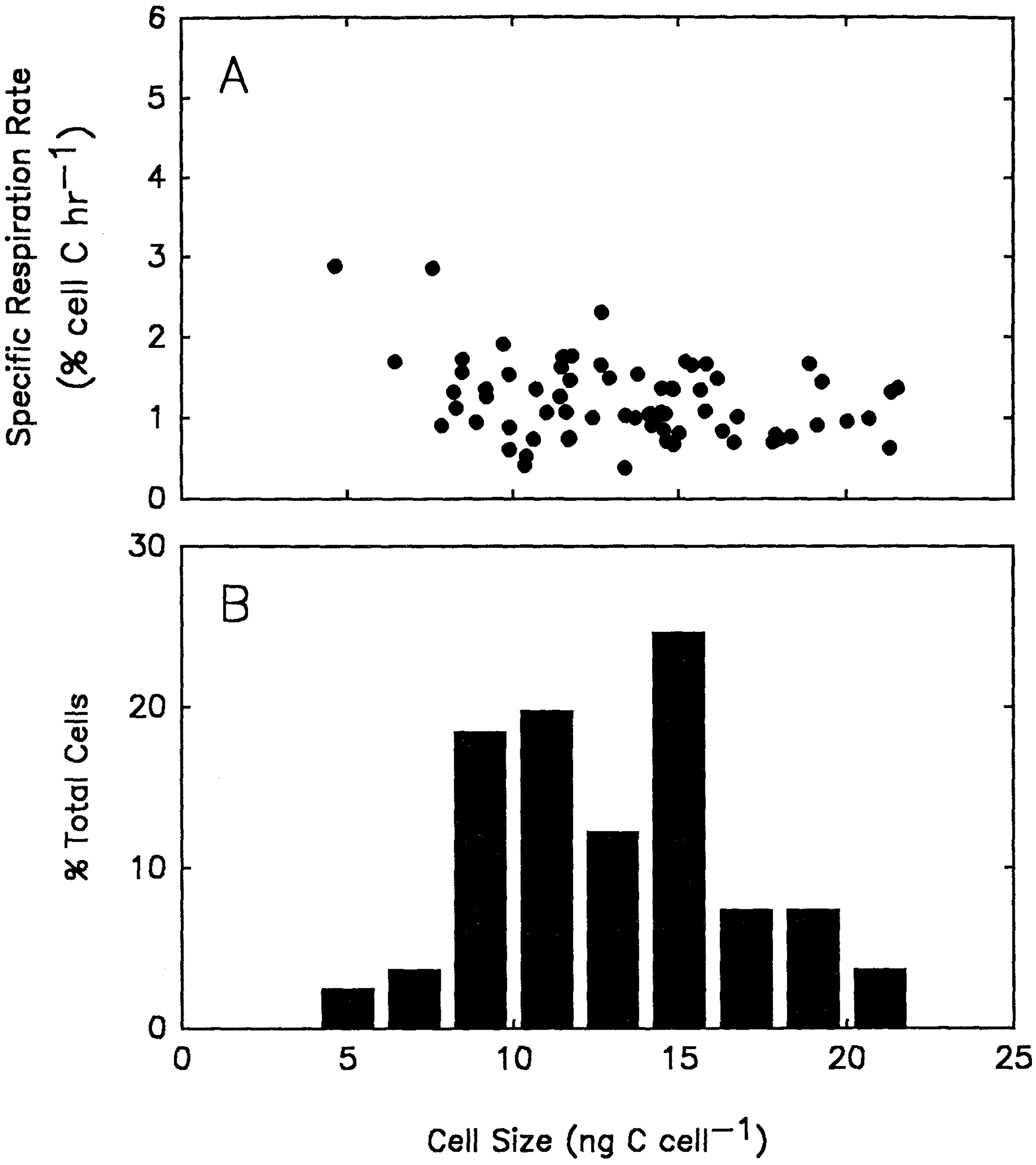


Figure 9.7.

Specific respiration rates and size of starved individual *Favella ehrenbergii* cells, with all cells corrected back to total carbon at T_0:

A) specific respiration rates of cells expressed as a function of cell size

B) frequency histogram of size distribution.

The study of Scott (1985) found the gross growth efficiency of a *Strombidium* sp. to be only 6.5 %, such a low figure could possibly implicate a high rate of respiration as a causative factor; the present study however gives a rate of respiration (discussed above) for *S. capitatum* at least, well within the normal range for similar sized ciliates. Chapter 8 hypothesized on theoretical grounds that small, fast swimming oligotrichs such as *Strombidium* spp. could expend a significant proportion of their metabolic rates on motility, particularly starving cells. Although the present empirical data do not in fact suggest a rate higher than that for other ciliates, these techniques do not allow costs of motility to be discriminated from overall metabolism, and furthermore, the behaviour of cells in dark incubations relative to that in the light is presently impossible to determine, thus the relative role of motility remains an unknown factor.

It is possible that the large variation in cell size (carbon content) observed here could be caused by even labelling of cells not being fully acheived, however, the study by Putt & Stoecker (1989) demonstrated that these techniques were in fact sufficient to evenly label several species of *Strombidium*, including *S. capitatum*, in three days of growth. Moreover, a review of data available from other studies on *S. capitatum*, presented in Table 9.1, suggests that cell size can be a highly variable feature of this species, although none of the studies quoted presented size frequency data. The cell dimensions of *S. capitatum* quoted by Montagnes *et al.* (1988*a*) do in fact suggest a cell volume variation of approximately 1.9 - 14.6 x 10^4 μm^3; this is of a similar magnitude (about 7 fold between largest and smallest cells) to that observed in this study. A close examination of the data presented by Putt & Stoecker (1989) and Stoecker *et al.* (1988/89) also reveals variations in cell volume spanning similar ranges. In view of these considerations, and the high specific respiration rates which we determined for small cells, as predicted by allometric size scaling, this suggests that the size variation shown in Figure 9.2 is real. Whether growth occurs between the various sizes (i.e. same genotype) is not known, but the absence of small cells in some cultures and of large cells in others, and the presence of both, with intermediates, in this study suggests that that such growth may occur. Variable size distribution could be a function of the physiological status of the culture or

Table 9.1.

Comparison of values determined in this study with review of other published ranges of individual cell volumes and carbon contents for *Strombidium capitatum*.

Cell Volume ($\times 10^4$ μm^3)[a]	Cell Carbon (ng C $cell^{-1}$)[b]	Reference
1.9 - 14.6[c]	3.6 - 27.7	Montagnes *et al.* (1988*a*)
2.5 - 9.5[d] 3.9 (± 2.2 SEM)[e] 4.7 (± 1.6 SEM)[f]	4.8 - 18.1	Putt & Stoecker (1989)
9.2 (± 2.7 SD)[g] 4.5 - 10.0[h] 2.6 - 6.4[h]	3.6 - 14.0	Stoecker *et al.* (1988/89)
-	6.5	Stoecker & Michaels (1991)
-	2.7 - 23.5	This study

---

[a] assuming spherical cells (Stoecker *et al.* 1988/89)

[b] C:vol ratio assumed of 0.19 pg μm^{-3} for Lugol's fixed cells, or 0.14 pg μm^{-3} for formalin fixed cells (Putt & Stoecker 1989)

[c] assuming Bouin's:Lugol's cell volume shrinkage ratio of about 75% (Leakey 1989)

[d] read approximately from their Fig. 2

[e] determined on live cells

[f] determined microscopically on Lugol's fixed cells

[g] already corrected for shrinkage, live:Lugol's volume ratio of about 80% adopted (Putt & Stoecker 1989)

[h] determined microscopically on formalin fixed cells

population, as for the dinoflagellate *Gyrodinium aureolum* (Partensky & Vaulot 1989). In fact Stoecker *et al.* (1988/89) provided evidence to suggest that cell volume of *S. capitatum* was largely dependent upon algal food concentration and ambient light level.

The data presented here is rather novel in that it suggests a size dependent variation both in specific respiration rate and in mortality rate of starved cells. A causal link between the two may also be evident; the specific respiration rates of small cells (3 - 5% cell C hr^{-1}) appear to be too high for these cells to survive a 24 hour incubation, and Figure 9.5 presents little evidence for these cells to be able to significantly reduce their respiration rates in reponse to starvation. Caron *et al.* (1990) suggested that protists may suffer respiratory loss of up to 80% cell biomass before death occurs, and the small cells here will have lost at least 80% of their original carbon. Some mortality may have resulted from damage during transfers to the vials, but Figure 9.3 suggests that this is not an immediate post-transfer effect, but rather is a gradually accumulating one. Moreover, it is rather unlikely that cell damage would be a size specific phenomenon. In an interspecific comparison of 7 protist species, Jackson & Berger (1984) presented evidence for a weak inverse relationship between 50% survivorship time and weight specific respiration rate, but not with cell volume. This study supports the above contention by removing the interspecific 'noise' and considering only intraspecific variation within a single species showing a large variation in cell volume. The concept of an inverse relationship between specific respiration rate and survivorship has always been intuitively an appealing one (Caron *et al.* 1990), but this study appears to be the first to present convincing intraspecific evidence.

When the variation in specific respiration rate caused by 'small' cells is removed, as in Figure 9.5, larger cells clearly show no reduction in specific respiration rate in response to starvation. This is interesting, since such ability to reduce respiration has been described for several species and may well be inversely related to cell volume (Fenchel & Finlay 1983; Caron *et al.* 1990); *S. capitatum* is of a size which could be expected to show such an adaptation. Perhaps a longer incubation may have resulted in some reduction, since starved *S. capitatum* can survive up to 40 h (Stoecker & Silver 1990). It is also possible that specific respiration was high during the first 2 h, before the first measurement was taken, and then declined. However, the linearity of carbon release (dpm cell^{-1}) in Figure 9.1a suggests this to be unlikely.

In contrast to *S. capitatum* the heterotrophic ciliate *Favella ehrenbergii* provides a useful comparison (Figure 9.6). This shows that there is a starvation induced reduction in specific respiration rate with time of incubation, suggesting that the absence of such a reduction for *S. capitatum* is not an artifact of the experiment but appears to be a real effect. *F. ehrenbergii* does not show such a marked cell size variation as *S. capitatum* (Figure 9.7) and thus the specific respiration rates do not need to be 'adjusted' for two distinct size groups of cells.

Since *S. capitatum* is a ciliate which retains functional plastids from its prey and thus is mixotrophic (Montagnes *et al.* 1988*a*; Stoecker *et al.* 1988/89), one explanation could be that these plastids are gradually digested during 'apparent' starvation and thus prevent the metabolism of the ciliate from transferring into a 'starvation' state. This contention is supported by the observations of Stoecker & Silver (1990) who found that sequestered prasinophyte and prymnesiophyte plastids disappeared rapidly in starved *S. capitatum* cells, and that cryptophyte plastids were also considerably depleted in numbers, though the latter did still persist in small irregularly shaped cells which had been starved for up to 40 hours. Gradual rounding, and reduction in size, fluorescence and abundance (no. cell^{-1}) of plastids, suggested that digestion may have been occurring. Cannibalism as a means of overcoming starvation was impossible since individuals were isolated prior to incubation. The possibility of feeding on bacteria cannot be discounted if these had grown in the fresh media; however, it is not known if *S. capitatum* is bactivorous.

It is unclear what the ecological implications may be of such size dependence on respiration and mortality under conditions of starvation; the size variation or extent of starved cells in natural populations of marine protists are rarely described.

However, it is likely that such physiological constraints will play a role in shaping the size distribution of a given species, and that these constraints may vary seasonally. It appears that the growth of plastidic oligotrichs will depend on the complex interaction of a number of non-linear factors, several of which appear to be more variable than for corresponding heterotrophic forms. Such variables include; photosynthesis and food intake (Stoecker *et al.* 1988/89), C:N ratio (Putt & Stoecker 1989), cell size and rate of respiration (this study) and motility (see Chapter 8). Thus the continued study of plastidic oligotrichs is certainly worthwhile, and may provide some unexpected results.

In retrospect, it may have been fruitful to continue incubations for a longer period to examine the continued effects of starvation on respiration and mortality, since *S. capitatum* may survive for 40 h or more under starved conditions (Stoecker & Silver 1990). However, the information presented is of novel interest, and the technique itself could certainly be utilised for studying metabolic rates in a range of other protists species, and, perhaps just as importantly, their various life cycle stages.

The technique described here has clear potential for providing precise determinations of the respiration rate of *Mesodinium rubrum*. *M. rubrum* shows an even more pronounced cell size variation than *S. capitatum* (Taylor *et al.* 1971, Lindholm 1985) and thus such isolation of single cells is imperative for meaningful data. Moreover, since blooms of *M. rubrum* are associated with extraordinarily high levels of bacterioplankton (Antai 1989, Crawford *et al.* 1992), such isolation is also desirable to minimise amplification of metabolic rates determined on bulk samples. Whether the technique is capable of resolving metabolic costs of motility remain to be seen; by stimulating swimming using turbulence for example, and by chemical inhibition of motility (e.g. see Lindholm 1982), there is certainly some potential for further investigations here.

CHAPTER TEN:
IS *MESODINIUM RUBRUM* RESPIRATION LIMITED?

"The great tragedy of science - the slaying of a beautiful hypothesis by an ugly fact"

T.H. Huxley (1825-1895)

The latter two chapters have implied both that *M. rubrum* expends a significant and substantial amount of energy while swimming, and that novel techniques may now be available, with modifications, to measure such costs. One remaining problem however, is that of preliminary labelling of cells of *M. rubrum* with ^{14}C. The organism must grow and divide in the presence of $H^{14}CO_3$ in the medium, in order to evenly label individual cells; since *M. rubrum* cannot yet be cultured, this is anticipated to be a major problem. Moreover, the fortunate co-incidence of suitable location, experimental facilities, and sufficient abundance of organisms is rarely realised, again particularly with one which cannot be cultured.

Therefore, in the absence of such an opportunity to date to actually determine respiration rates of *M. rubrum*, a useful alternative approach is to review available literature and data in order to examine, in a preliminary fashion, the feasibility of the hypothesis that motility is energetically costly for this species.

The notion that swimming of *M. rubrum* could be costly is certainly not a novel one; Smith & Barber (1979) speculated that red-tides of *M. rubrum* in the Peru Upwelling may provide relatively little energy to higher trophic levels if a high respiration rate were to recycle much of the fixed carbon back into the water column. However, after observing a diurnal migration of some 40 m by *M. rubrum*, Smith & Barber (1979) determined its respiration rate by measuring dark decrease in ^{14}C

Table 10.1.

Comments from the literature regarding the respiration rate of *Mesodinium rubrum*.

Authors	**Comments**
Setchell *et al.* (1978)	"The corresponding P/R ratios (13 & 2.2) fall within the range of P/R ratios for natural assemblages of marine phytoplankton (Steemann Nielsen & Hansen 1959)".
Margalef (1979)	"Probably it allocates more energy to swimming than to reproduction. The high relative concentration of respiratory enzymes (ETS) in these organisms (Packard *et al.* 1978) sustains this view".
Smith & Barber (1979)	"The *M. rubrum* population respired 4.6% of the previously fixed carbon hr^{-1}. This rate seems rather high; however, other studies demonstrate similar respiration rates (Eppley & Sharp 1976; Smith 1977)".
Taylor (1982)	"In upwelling zones *M. rubrum* is able to utilise its strong swimming ability (at high energetic cost) to exploit freshly upwelled water". "Because of strong swimming it might be expected that the respiration rate of *M. rubrum* might be high, but a figure of 4.6% is not excessive".

Table 10.1. (Continued)

Authors	**Comments**
Lindholm (1985)	"As well as producing oxygen, *M. rubrum* has a high respiration rate (Smith & Barber 1979; Setchell *et al.* 1979). According to Smith & Barber (1979) *M. rubrum* respires 4.6% of the photosynthetically fixed carbon hr^{-1}, a rate similar to that of 'conventional' phytoplankton".
Lëppanen & Bruun (1986)	"For *M. rubrum*, the ratio between respiration and production seems to be too low (cf. Smith & Barber 1979)".

labelled particulate material during a red-tide. This amounted to only 4.6% of P_{max}, a figure of about the same order as other less or non-motile phytoplankton.

This study by Smith & Barber (1979) epitomises the paradox; intuitively, an organism swimming so fast *should* be expected to expend considerable energy on locomotion, but empirical evidence suggests otherwise. There also appears evidence of considerable confusion in the literature; Table 10.1 summarises conflicting statements as to whether the published estimates for respiration of *M. rubrum* to date represent 'high' or 'low' rates. Some authors even present contradictory statements within the same publication.

Clearly, this is a problem resulting either from the lack of *normalised* respiration rates in the literature, or else data being normalised to different parameters. The high biomass levels associated with blooms of *M. rubrum* obviously result in extremely elevated absolute rates of respiration, and this has confused the issue. Surprisingly few, if any, studies have investigated the respiration rate of *M. rubrum* in any systematic and objective manner. Packard *et al.* (1978) did present respiration rates as carbon specific rates of turnover for the population as a whole, and Smith & Barber (1979) normalised respiration to rate of photosynthesis, but for any objective assessment, rates must be normalised on a cell specific basis for comparison with some estimate of swimming costs, such as those given in Chapter 8. With cell size being such a particularly variable feature under different conditions for this species (Lindholm 1985), this is particularly critical.

Table 10.2 presents cell specific rates of respiration for a range of microplankton groups and compares these to rates predicted by the Fenchel & Finlay (1983) regression for a spherical protist of about the same size as a *M. rubrum* cell (40 μm diameter). The energy cost for swimming at 5 mm s^{-1} is also presented for comparison, as computed from Chapter 8.

A close examination of the literature reveals a number of estimates of rate of respiration for *M. rubrum* which, with certain assumptions, can be normalised to cell

Table 10.2. Ranges of cell specific respiration rates for various groups of microplankton.

Reference	Group/species	Respiration (R)	Standard R ($M\ O_2\ cell^{-1}\ s^{-1} x 10^{-15}$)
Sorokin (1981)	Ciliates	2 - 10 $\mu l\ O_2\ h^{-1}$ (mg wet wt)$^{-1}$	0.8 - 4.2[a]
Fenchel & Finlay (1983)	Heterotrophic Protozoa	log R (nl O_2 cell^{-1} h^{-1}) = 0.75[log vol(μm^{-3})] - 4.09	0.1 - 19.8[b] 2.5[a]
Taylor (1987)	Dinoflagellates	---	2 - 20
---	Spherical protist (40 μm diameter)	energetic cost of swimming at 5 mm s^{-1}	2.1

---

[a] assuming 40 μm diameter cell

[b] assuming cell size range of 10 - 100 μm diameter

specific rates. These are presented in Table 10.3, normalised to the same cell specific units as in Table 10.2; it is clear that the rates determined to date tend to be rather low when compared to rates either for dinoflagellates or ciliates, as shown in Table 10.2. The mean cell specific rate for *M. rubrum* from Table 10.3 is 1.0 M O_2 cell^{-1} s^{-1} (x10^{-15}) ($\pm$ 0.56 SD), and on a carbon specific basis 0.7 % cell C h^{-1} ($\pm$ 0.38 SD). This is only 40% of the rate of 2.5 M O_2 cell^{-1} s^{-1} (x10^{-15}) predicted by the Fenchel & Finlay (1983) regression in Table 10.2. Moreover, such rates will be overestimates since these were determined principally by incubation of samples from blooms which are associated with extremely high levels of bacterioplankton (Antai 1990, Crawford *et al.* 1992). Although these bacteria account for only about 5% (up to 400 μg C l^{-1}) of the biomass of *M. rubrum*, the high specific rates of respiration associated with such small sized organisms would be predicted (extrapolating regression of Fenchel & Finlay 1983 to bacteria) to equal or exceed that of the ciliate, and thus account for approximately 50% of bloom respiration (ignoring organisms other than bacteria and *M. rubrum*). A conservative estimate would suggest that rates for *M. rubrum* cells alone probably represent only about 0.5 M O_2 cell^{-1} s^{-1} (x10^{-15}), or 0.35% cell C h^{-1}, some 20% of that predicted by the Fenchel & Finlay (1983) regression for protists.

This estimate suggests that the cell specific respiration rate only represents about 25% of the predicted cell specific metabolic cost of swimming at 5 mm s^{-1}, and thus it seems unlikely that the respiration rates determined to date are actually measuring swimming costs, unless the assumptions of Chapter 8 are out by several orders of magnitude. The relatively low rates could partially be accounted for by the fact that *M. rubrum* does not swim continuously, but alternates between fast swimming and motionlessness. However, the study by Smith & Barber (1979) did record a vertical migration of some 40 m in 2.5 hours which must have required a *continuous* swimming speed of over 4 mm s^{-1}, suggesting that such swimming can indeed be maintained over long periods. What Tables 10.2 & 10.3 and the above discussion suggest, by implication, is that the studies to date have measured a '*basal*' metabolic rate for *M. rubrum*, a concept rejected by Fenchel & Finlay (1983) for protists. Since *M. rubrum* appears to be strongly phototactic (Bary & Stuckey 1950,

Table 10.3. Cell specific respiration of *Mesodinium rubrum*; normalised from estimates quoted in literature.

Reference	Respiration (R)	Standard R[a] ($M\ O_2\ cell^{-1}\ s^{-1}x10^{-15}$)	Standard R[a] % cell C h^{-1}
De Souza Lima & Williams (1978)	400 - 600 $\mu l\ O_2\ l^{-1}\ day^{-1}$	1.0 - 1.5[b]	0.7 - 1.0
Packard *et al.*	326 $\mu l\ O_2\ l^{-1}\ h^{-1}$	(1.2[c])	(0.8)
(1978)	725 $\mu l\ O_2\ l^{-1}\ h^{-1}$	(9.0[d])	(6.1)
Setchell *et al.*	222 $\mu l\ O_2\ l^{-1}\ h^{-1}$	0.8[e]	0.5
(1979)	493 $\mu l\ O_2\ l^{-1}\ h^{-1}$	(6.1[c])	(4.1)
Smith & Barber (1979)	77.7 mg C $m^{-3}\ h^{-1}$	1.8[f]	1.2

---

[a] calculations assume C:vol ratio of 0.19 pg μm^{-3} (Putt & Stoecker 1989), an RQ of 1.0 and a cell diameter of 40 μm

[b] chl*a* of 20 - 30 $\mu g\ l^{-1}$ assumed to represent *M. rubrum*

[c] assuming 3,500 cells ml^{-1} derived from POC

[d] 1000 cells ml^{-1} derived from chl*a*

[e] recalculated from Packard *et al.* (1978) using R/ETS ratio derived for *M. rubrum*

[f] assuming 1000 cells ml^{-1}

Table 10.3. (continued)

Reference	Respiration (R)	Standard R (M O_2 cell^{-1} s^{-1}x10^{-15})	Standard R % cell C h^{-1}
Sellner (1981)	23.7[g] mg C m^{-3} h^{-1}	0.3	0.2
	58.1[h] mg C m^{-3} h^{-1}	0.6	0.4
Hendriksson *et al.* (1982)	800 mg C m^{-3} day^{-1}	0.3[i]	0.2
Rees & Williams (1982)	86 μg O_2 l^{-1} h^{-1}	1.5[j]	1.0
Whitfield (1985)	---	1.5 - (5.2[k])	1.0 - (3.5)
		Mean = 1.0[l] (± 0.56 SD)	Mean = 0.7[l] (± 0.38 SD)

---

[g] from primary productivity, assuming mean of 9.8% R/P ratio for Peru waters (Sellner 1981), and 1600 cells ml^{-1} derived from chl*a*

[h] as note g, but 2300 cells ml^{-1} derived from chl*a*

[i] assuming 2,300 cells ml^{-1} derived from chl*a*

[j] 500 cells ml^{-1} given

[k] scenescent bloom

[l] data in brackets ignored: Setchell *et al.* (1979) recalculated data of Packard *et al.* (1978) who considered second estimate to represent stressed population. Second estimate of Whitfield (1985) represented senescent bloom

Smith & Barber 1979) and also seems to swim in response to water movements (Taylor *et al.* 1971, Lindholm 1985, see also Chapter 3), it could be hypothesised that confinement in a dark bottle for respiration determination isolates the cells from the very stimuli which normally elicit motility, namely light and turbulence. This is supported by observations by Lindholm (1985) who noted "in totally quiet water and in darkness .... *M. rubrum* seems to remain almost motionless". Venrick *et al.* (1977) have discussed the artefacts of containing microplankton in incubation bottles, but this sort of consideration has not been mentioned.

It may be if the above hypothesis is correct, that *M. rubrum* is nevertheless a rather 'special' case, in that it is probably the quickest of all motile microplankton, but can also remain motionless; thus respiration costs may in fact cycle between two extremes. The true total respiration rate while swimming could therefore perhaps be approximated by summing the basal rate with the swimming cost in Tables 10.2 & 10.3. These arguments may account for the rather low estimates for basal rates; with some exceptions, few other fast moving forms have the ability to remain totally motionless. If swimming costs are significant for these species too, then unlike *M. rubrum* such costs will probably be included to a greater extent in the 'basal' rates.

This hypothesis, although not proven, has profound implications for the ecophysiology of *M. rubrum*, and its feasibility can be further examined by considering some field evidence.

Figure 10.1 presents changes in cell numbers and oxygen over a tidal cycle in the Southampton Water estuary during a red tide event; this data was recorded during the same survey as that presented in Chapter 3. What is interesting here is that if the changes in cell numbers represent a vertical migration pattern, as suggested by Chapter 3, rather than an advective phenomenon, then the oxygen changes are particularly curious. Superficially, a correlation between cell numbers and oxygen would be expected, due to photosynthetic production, but what is more difficult to explain is the decreases in oxygen associated with decreases in cell numbers. Considering only the 1 m plot in Figure 10.1, for simplification, then some crude

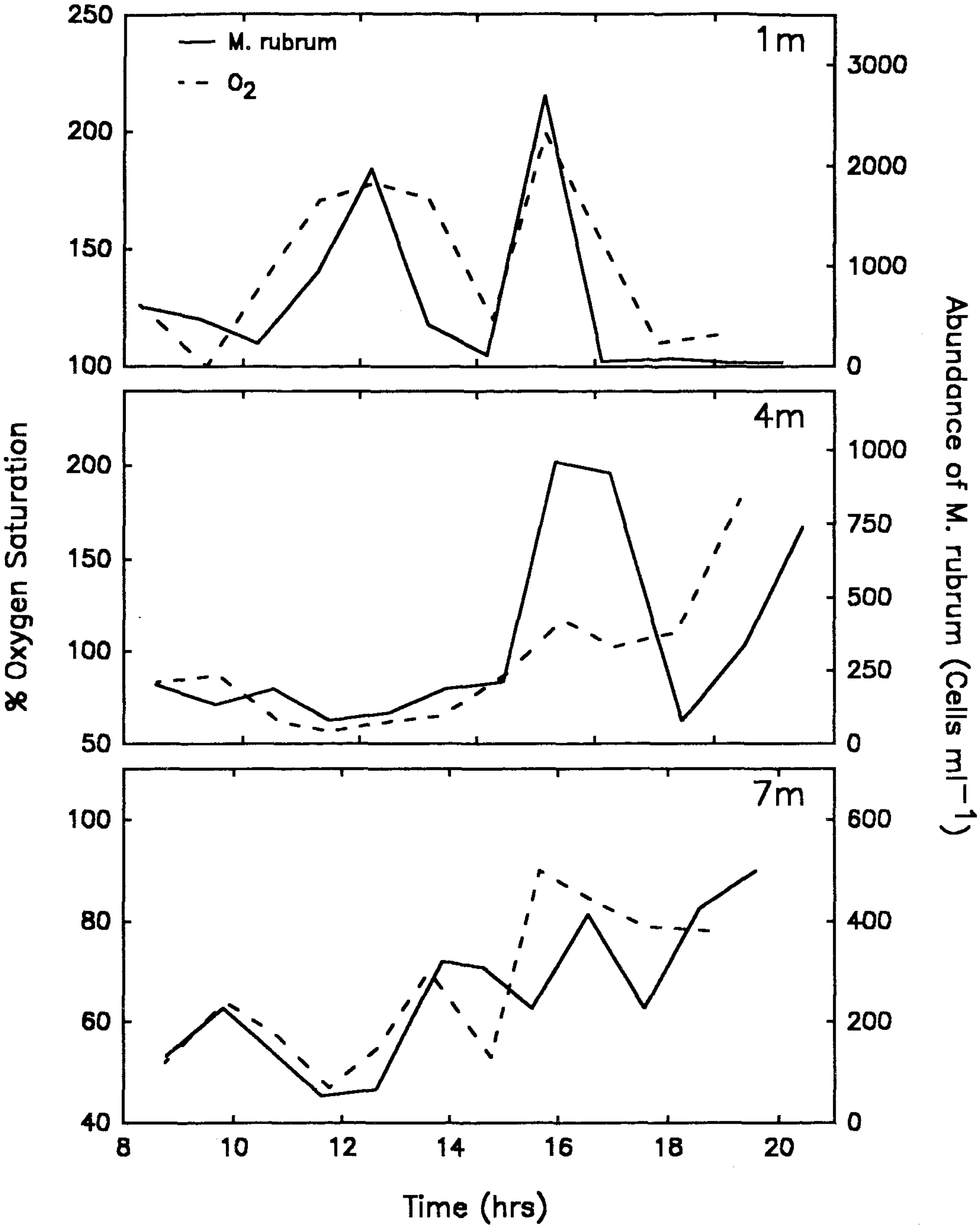


Figure 10.1.

Variation in abundance of *Mesodinium rubrum* and oxygen saturation over a tidal cycle at depths, taken at Eling buoy, Southampton Water, in July 1985.

calculations can be made. Between 09:00 and 12:00 oxygen increases by about 70% saturation, this represents a change of about 2.0 mg O_2 l^{-1} h^{-1}; with a mean cell concentration of about 1000 cells ml^{-1} over this period, this translates to a photosynthetic rate of 750 mg C m^{-3} h^{-1} or about 12% cell C h^{-1}. This compares very favourably with the rates of 11 & 14% for isolated cells of *M. rubrum*, at saturating irradiances, given by Stoecker *et al.* (1991), and thus supports the contention that the oxygen increase is of photosynthetic rather than advective origin. If it is accepted that such an increase is caused by photosynthesis of migrating cells arriving at the surface, then the sharp decrease in oxygen between 12:00 and 14:00 must also be addressed. This occurred between 1st and 2nd high waters, with minimal horizontal advection occurring at the time, but coinciding with a sharp downward displacement of the population (see Chapter 3). This could be explained by the hypothesis of motionless cells at the surface with net oxygen evolution, but migrating cells having a net consumption of oxygen. However, from Figure 10.1 this would require an oxygen consumption, for motility alone, of about double the rate of photosynthesis; this represents a rate of respiration of about 24% cell C h^{-1} to explain the observed changes. Following the calculation through, this would require a cost of some 69 times greater than the rates concluded for *M. rubrum* from Table 10.3, and corrected for bacteria. This would seem a huge requirement, but from the model given in Chapter 8, a cell of 40 μm diameter would in fact be predicted to expend 64 times more energy swimming at 8 mm s^{-1} (speed for *M. rubrum* given by Lindholm 1981) than at 1 mm s^{-1}; the cost at 1 mm s^{-1} would be predicted to be only 5% of the 'basal' rate and these can be approximated as equal in the context of these rather 'crude' caluculations. Thus, the changes at 1 m depth could be fairly closely explained by the hypothesis; variations at the other two depths are rather more difficult to interpret because of, for example, self shading and overlapping migrations, and this is reflected in the complicated distributions. However, there is still a similar tendency, that is for oxygen to decrease when cell numbers fall, as for example at about 12:00 when most of the population concentrates at the surface.

Although the observed oxygen changes in the estuary could therefore be explained by the hypothesis above, it is accepted that many dynamic processes are occurring simultaneously over a tidal cycle, such as advection, turbulence, self-shading etc..., and that these will also be contributing to the observed patterns.

Looking at a non-bloom situation, obviously oxygen cannot be used as a 'tracer' of metabolic activity since *M. rubrum* will not be dominant. Instead changes in cell size distribution (e.g. from Chapter 6) could perhaps give some indication of whether cells are growing; however, it is conceded that a number of other factors will influence size distribution. Figure 10.2 shows variation in mean cell size of both 'small' and 'large' cells of *M. rubrum* over the spring/neap cycle at Keppell Pier, Millport in March 1991. This is essentially a reassessment of the information given in Figure 6.4 in Chapter 6; the bimodally distributed population was divided into two size groups, large and small cells, and the mean cell size of each was plotted against time over a spring/neap cycle. This Figure suggests that the mean size of both large and small cells varied cyclically over the spring/neap cycle. When tidal height (of high water) is highest, that is during the spring portion of the cycle, cell size of both groups is smallest, and during neaps it is largest. Although the standard deviation of these estimates of mean size may appear too large for these differences to be significant, much of this variation resulted from the available resolution of size determination on the microscope employed (at x400). Clearly there are other factors contributing to variation in mean cell size, such as cell division, but these results do tend to suggest that growth may be less efficient on springs than on neaps; this is consistent with the rather subjective impressions of bloom formation (Chapter 2) and increase in cell size (Chapters 5 & 6) during neap tides. Moreover, recent observations of non-bloom dynamics of *M. rubrum* in Southampton Water (Kifle unpubl.) suggest that population peaks occur on neaps but not on springs. These observations seem to contradict the contentions of Margalef *et al.* (1979) and others, that *M. rubrum*, being such a fast swimmer, will have a competitive advantage in more turbulent conditions. The observations do however support the contention that the growth of *M. rubrum* may be limited by turbulence, and may in fact be negative, that is respiration greater than gross photosynthesis. The field evidence from Chapter 2 is also supportive; Figures 2.10 shows that the initial peak in cell numbers in late May is accompanied by a massive production of oxygen, whilst the second peak, despite

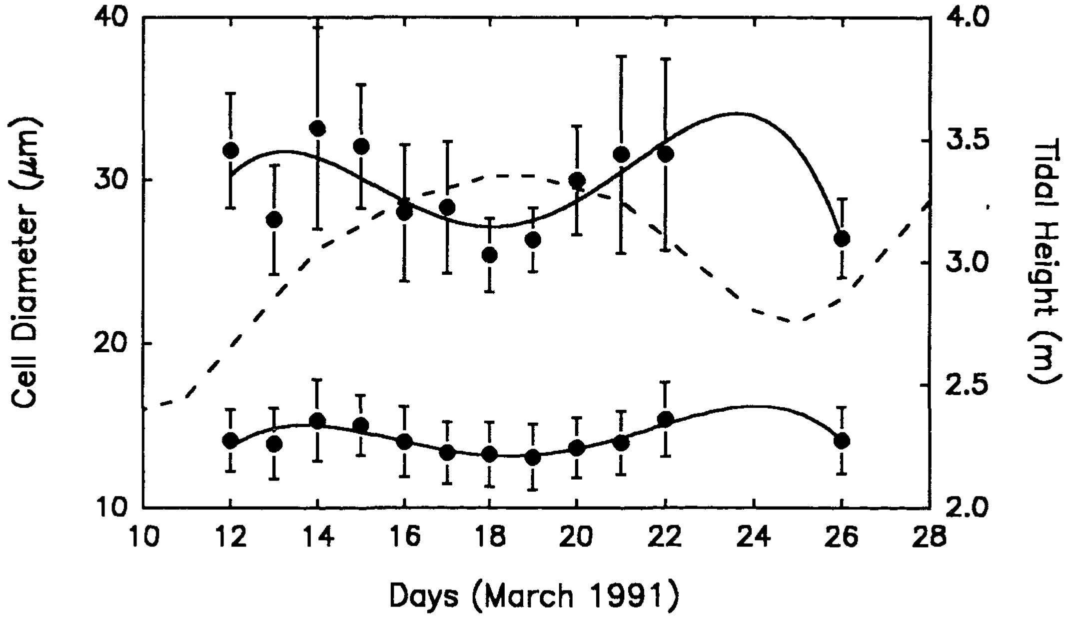


Figure 10.2.

Variation in mean cell diameter of both large and small forms of *Mesodinium rubrum* (with error bars of S.D.) over a spring/neap cycle from the Firth of Clyde. Data taken from histograms of Figure 6.4. Dashed line represents height of high water, as indication of the spring/neap cycle. The two means for each sampling date represent a total of about 100 cells.

higher numbers, is associated with much lower levels of oxygen. Since the first peak occurred during more intense stratification, it could be speculated that such high net oxygen production resulted from reduced respiratory expenditure on swimming, that is a higher net photosynthetic rate than during the second peak. It may be that a greater abundance of microheterotrophs were present during the second peak, thus contributing to oxygen demand. However, the numbers off bacteria, at least, seem to be proportional to the magnitude of the bloom of *M. rubrum* (Antai 1989), and so might be expected to consume roughly a constant proportion of the bloom respiration.

Examining the literature for support of the hypothesis, we can find several examples. As stated in Chapter 7, the swimming capabilities of *M. rubrum* should place it in a position to outcompete other species in moderately turbulent conditions, but the literature suggests the opposite; most reports of red-water caused by *M. rubrum* suggest that it occurred during exceptionally calm and/or stratified conditions, or upon stratification after mixing (see Lindholm 1985 for review). The reports of red-water formation in upwelling areas record blooms of *M. rubrum* around the stratified periphery of the upwelling plume as upwelling relaxes; diatoms tend to characterise the turbulent core of the upwelling in its early stages. The observations of Vaas (1968) are also worth mentioning; between 1964-1967 in the Dutch Veerse Meer, the role of phosphate, temperature and salinity were discounted as factors regulating the occurrence of blooms of *M. rubrum*. Instead there was a strong negative correlation between such events and the turbulence produced by westerly winds.

Support can also be found from the literature for non-bloom conditions; Revelante & Gilmartin (1983) recorded *M. rubrum* as the numerically dominant species of ciliate during stratified conditions, but made particular note of its near absence under well mixed conditions. Grice *et al.* (1980) also drew attention to the marked abundance of *M. rubrum* in an unmixed 'CEPEX' enclosure, compared to its near absence in a mixed enclosure. Similar observations were made by Dale (1988*a*) in Norwegian waters, who noted a marked increase in the abundance of *M. rubrum* in a control enclosure relative to surrounding water. In related studies, Dale (1987*b*

& 1988*b*) also mentioned the upward displacement of the population of *M. rubrum* within a control enclosure relative to that observed outside. These observations could be consistent with those of Chapter 3; relaxation of surface mixing (in control enclosure) allows upward displacement of the population.

Some useful supporting evidence can also be extracted from ultrastructural studies, and this appears to be unambiguous; several authors have commented on the very marked concentration of mitochondria in close association with the kinetosomes of the equatorial ciliary belt of *M. rubrum* (Taylor *et al.* 1971, Hibberd 1977, Oakley & Taylor 1978, Grain *et al.* 1982, Lindholm *et al.* 1988). This strongly suggests that swimming is the predominant energy consuming process of the cell.

The above discussions certainly support the idea that *M. rubrum* is in fact sensitive to turbulence, and that if it cannot avoid and 'seek refuge' lower in the water column, then it can only at most 'break even' energetically under such conditions. Turbulence could have two effects; firstly an increase in 'jumps' could result from sensitivity to fluid deformations, as suggested in Chapter 3, and secondly a greatly increased level of swimming activity might be required to maintain the ciliate in optimal light, if indeed it is phototaxic, as maintained at least anecdotally by several authors.

In more stable conditions, such as those described for the brackish lake in Finland in Chapter 4, *M. rubrum* is less likely to be limited by metabolic costs of swimming unless light and nutrients are vertically separated by great depths. Here *M. rubrum* is probably limited by pulses of perhaps wind induced nutrients into the euphotic zone. In fact mass development of *M. rubrum* in other Finnish lakes has been observed to occur immediately after turnover (Lindholm 1978).

The appeal of this hypothesis is that it appears to integrate a number of confusing observations from previous studies and this thesis, and suggests that cell physiology and thus growth may be regulated by interaction of a number of non-linear factors; thus complicated feedback mechanisms could be involved at the cellular level.

For instance, the model presented in Chapter 8 predicts lower carbon specific metabolic cost of motility with increasing cell diameter, this could explain why *M. rubrum* has such an unusually large size variation of 15-70 μm (Lindholm 1985), particularly during blooms. Cells physiologically constrained to a small size under normal conditions could experience 'runaway growth' under low turbulence conditions, when respiration costs are minimised and perhaps decline further with increasing cell size. Such rapid growth of the endosymbiont could result in normal cell division processes becoming unable to cope, thus perhaps explaining the morphological peculiarities observed in Chapter 5.

On a larger scale, dynamic considerations could also be important, and feedback mechanisms might be involved at the water column scale. For instance, the close association between development of the bloom and increasing temperature gradient (Figure 2.21b) may not just be a one way process; Dugdale *et al.* (1987) have noted that red-water patches caused by *M. rubrum* in the Peru upwelling actually trap heat in surface waters. Over 99% of solar radiation is trapped in the surface 1 m by dense surface aggregations of this organism; this results in temperature increases of 1-2°C within red-water patches, compared to outside of them. The net result of this is that an increasing abundance of *M. rubrum* at the surface will surely stabilise the water column by increasing the superficial thermal gradient. Moreover, the excretion of significant quantities of DOM (e.g. Smith & Barber 1979, Sellner 1981, Hendrikson 1982) could also influence the viscosity and thus stability of surface waters. If the growth of *M. rubrum* is in fact enhanced by declining turbulence, as hypothesised in this study, then this could represent a positive feedback mechanism; the development of the red-tide being promoted by its own presence. This could explain the exponential increase in cell numbers, despite light limitation caused by self-shading. In contrast, it is also interesting that Owen *et al.* (1992) noted that due to the presence of a subsurface maximum of *M. rubrum*, mixing above this layer actually increased because heat trapped by the layer at 1-2 m depth created a thermal instability. Although not recognised by Owen *et al.* (1992), who attributed it to temperature gradient avoidance, the absence of *M. rubrum* from the surface layer could have resulted from avoidance of mixing (analagous to the situation in Chapter

3) but actually created by the presence of the organisms themselves; that is a negative feedback occurred. Since *M. rubrum* blooms have been recognised by remote sensing techniques (Lin *et al.* 1984, Robinson & Holligan 1987, Purdie *et al.* 1988) it is possible that these could be used in conjunction with thermal imagery to examine these larger scale processes.

Thus a number of phenomena could be co-occurring on a variety of scales, with consequences difficult to predict, and depending upon the individual circumstances. The behaviour of *M. rubrum* appears not simply controlled by phototaxis, and in retrospect it appears that no studies have ever unambiguously proved that vertical migration is mediated by phototaxis. The alternative role of solar energy in influencing dynamics of thermal gradients, and thus stability, has not been approached in the context of vertical migration.

Clearly, understanding of the processes involved in the growth of this organism and in bloom formation will only proceed if such considerations are firstly recognised, and secondly incorporated into the design of future field sampling programs. Although the present thesis has not generated definitive solutions to the many unknowns concerning this curious species, it has certainly provided a number of interesting new questions, and posed them in a more dynamic context than have previous studies.

APPENDIX ONE:
FIELD SAMPLING

Southampton Water

At each station, profiles of temperature, salinity, oxygen and light intensity were recorded, initially at depths of 1, 3, 6 & 8 metres (up to 30/5/85), then at metre intervals from the surface down to 10m, or the bottom if shallower. Because of this lack of continuity, data is presented in Chapter 2 for 1985 only for the four depths of the original sampling.

Temperature, salinity, oxygen and light probes were mounted on a wooden board to give simultaneous profiles. Temperature and salinity were recorded using an in-house design T/S probe. Oxygen levels were measured using a YELLOW SPRINGS INSTRUMENTS (YSI) oxygen meter (model 57) and probe, attached to a submersible magnetic stirrer. The meter was calibrated in saturated air immediately prior to use on each sampling trip. Oxygen readout (mg l^{-1}) in the field was adjusted for ambient salinity using the salinity offset on the meter. Photosynthetically active radiation (PAR) was recorded over a defined waveband from 400-700nm (μE m^{-2} s^{-1}), using a MACAM (Model Q101) light meter with underwater quantum probes; however, these readings were only recorded up to the beginning of June, after which sensor malfunction prevented further field measurements.

Water samples were taken with a VAN-DORN type water bottle at 1, 3, 6 & 8 m. at each station in 1985, and at 1m intervals from the surface to bottom (approximately 9 m) in June 1986. 1 litre samples were dispensed from the sampler into polyethylene bottles, and stored in the dark, in a cool box until returned to the laboratory. Once in the laboratory, subsamples were immediately taken for cell enumeration, and for determination of chlorophyll, nitrate and ammonia concentrations.

In July 1985, sampling was undertaken over a complete tidal cycle (Chapter 3). Water samples were taken with a VAN-DORN bottle at four depths (1, 4, 7, 10 m), staggered at fifteen minute intervals, each depth sampled once every hour. Subsamples of 125 ml were dispensed directly into amber glass bottles containing preservative (see appendix 2) immediately following sampling. Every twenty minutes at 1 metre intervals, vertical profiles of temperature and salinity (conductivity) were taken using a NBA CTU-1 profiling CTD, and current velocity with a NBA current meter.

In July 1986, *M. rubrum* distribution was monitored over about 8 h during a red-water event, using *in situ* chlorophyll fluorescence as an index of abundance. Approximately every 2 h at 1 m intervals, water samples were pumped through a 'Turner Designs' flow-through fluorometer and changes in relative fluorescence recorded allowing for the residence time of the tubing.

Inre Verkviken

Only one station was sampled in this lake, firstly a single vertical profile was taken, followed by two separate sequences of profiles for vertical migration studies. Water samples were taken with a Ruttner type sampler down to 18-20 m, at 0.5 or 1.0 m intervals, in order to minimise underestimation of population peaks. Subsamples were removed for cell counts, oxygen concentration, chlorophyll, total nitrogen and phosphorous, and for some profiles nitrate.

Profiles of physical parameters were also taken at intervals of 1 m; temperature and salinity were recorded with a YSI probe, light with a LI-COR 188B radiometer.

APPENDIX TWO:
LABORATORY METHODOLOGY

Cell Counts

Samples from the 1985 study were returned to the laboratory in cool polyethylene bottles prior to subsampling and preservation. However, for the tidal survey in July 1985, and for subsequent studies, samples for enumeration of *M. rubrum* were dispensed directly from the water sampler into 125 ml amber glass bottles containing preservative. Since *M. rubrum* is a particularly fragile organism (Lindholm 1985), this minimized the possibilty of damage or loss of cells during transport back to the laboratory. *M. rubrum* was enumerated from 100 ml. subsamples (125 ml for 1986 study), preserved with 1% v/v Lugols iodine solution (Throndsen 1978; see also Crawford 1989). When cell abundance was insufficient for direct enumeration, 100 ml subsamples were concentrated to 10 ml for 2-3 days in measuring cylinders; the upper 90 ml was drawn off under suction, using a 'U' shaped glass pasteur pipette to avoid disturbance of sedimented material. Cells were enumerated from well-mixed 1 ml. subsamples in a SEDGEWICK-RAFTER counting chamber under an OLYMPUS BH2 microscope.

For studies undertaken at Inre Verkeviken, Cape Cod, Maine and Millport (Chapter 6), samples of 100 ml (or 500 ml at Millport) were taken either with buckets or RUTTNER type samplers and subsamples preserved as above. Subsamples were enumerated using 10 or 25 ml HYDROBIOS settling chambers, after a 10 fold concentration for the Millport samples, and examined under OLYMPUS, NIKON or ZEISS inverted microscopes.

Cell Size Determination

Cell sizes were determined at x400, using calibrated ocular micrometers on the inverted microscopes described above. Size-frequency distributions were based upon at least 30 individuals, and for the samples from Millport, about 100 individuals. All size-distributions shown represent sizes of Lugol's fixed cells, and are uncorrected for cell shrinkage. Putt & Stoecker (1989) and Choi & Stoecker (1989) have suggested that oligotrich ciliate cell volume may shrink by about 20-30% upon fixation in Lugol's, although this only represents about 10% shrinkage in linear dimensions. Since only relative changes between 'large' and 'small' cells were examined, and carbon:volume ratios were not necessary, shrinkage corrections were not applied. Cells were usually measured at least 1-2 days after preservation; Ohman & Snyder (1991) also showed significant cell shrinkage of *Strombidium* sp. in acid Lugol's, but showed that cells actually swelled during the first 24 h after preservation before shrinkage and stabilization occurred. They thus recommended determination of cell dimensions at least 24 h after preservation.

Chlorophyll

For chlorophyll determination, water samples were filtered under low vacuum (< 10 cm Hg), to prevent rupture of cells, onto 22 mm. diameter GF/C filters. The volume filtered depended to a greater extent on the abundance of *M. rubrum*; i.e. that volume required to cause visible colouration of the filter without resulting in clogging. Filters were folded (seston inside), wrapped in aluminium foil, frozen for up to two months, then processed according to the fluorometric method, with acidification for phaeopigments, as described by Parsons *et al.* (1984*a*).

Filters were homogenised in about 5 ml 90% Analar acetone, the homegenates transferred to clean polypropylene centrifuge tubes, covered in parafilm and left in the dark for 30 - 60 minutes to complete the extraction. The tubes were then centrifuged at 3,000 - 4,000 rpm for 10 minutes, and the supernatant decanted into small volumetric flasks and made up to volume with 90% acetone. The fluorescence of this extract was then measured on an AMINCO fluorometer, zeroed against 90% acetone. Phaeopigment concentration was determined after measuring chlorophyll fluorescence, by adding two drops of 10% v/v HCl to the cuvette and mixing, to degrade chlorophyll to phaeopigment, and remeasuring fluorescence. The fluorometer was calibrated with a known concentration of SIGMA chlorophyll standard, determined on a UNICAM SP 500 spectrophotometer according to Parsons *et al.* (1984*a*).

Oxygen

The water samples taken at Inre Verkviken (Finland) were analysed for oxygen concentration by the Winkler method according to Parsons *et al.* (1984*a*).

Total Phosphorus

Unfiltered water samples taken at Inre Verkviken (Finland) were analysed for total phosphorus (particulate + dissolved) according to the method of Menzel & Corwin (1965).

Inorganic Nitrogen

Inorganic nutrients were determined using the filtrate from the chlorophyll determination; 100 ml. subsamples were either stored below 4°C for ammonia analysis within 24 hours, or frozen and analyzed within 2-3 months for nitrate.

Samples for determination of ammonia in 1986 were filtered in the field and dispensed directly into 125 ml. bottles containing a phenol/ethanol solution for preservation, as recommended by Parsons *et al.* (1984*a*). This allowed a period of up to two weeks for subsequent analysis of samples. Duplicate 25 ml. subsamples were analyzed for ammonia according to the phenol/hypochlorite method described by Parsons *et al.* (1984*a*). The seawater subsamples were treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of a sodium nitroprusside catalyst. The extinction of the resulting blue indophenol colour formed with ammonia was determined against an ammonium standard at 640 nm using a UNICAM SP 500 spectrophotometer. The detection limit was approximately 0.1 μg at N l^{-1}.

Nitrate was determined using a TECHNICON autoanalyzer using a colorimetric technique after reduction to nitrite. A FROST Instrument A40 autosampler passed the sample through a reduction column using a "copperised" cadmium wire in transmission tubing. Nitrite reacts with Sulphanilamide resulting in a diazonium ion being formed, which is coupled with 1-nepthylethylenediamine hydrochloride, forming an intense pink azo dye (Stainton 1974). Colour was measured using a CHEMLAB 4 channel colorimeter. The detection limit was approximately 0.4 μg at N l^{-1}.

For samples taken at Inre Verkviken (Finland), dissolved nitrate was determined on filtered aliquots according to Parsons *et al.* (1984*a*).

Morphological Features

Cells bearing these bodies were photographed live, or after preservation in Lugol's iodine, using the phase contrast optics of an OLYMPUS BH2 photomicroscope at x400 magnification. A ZEISS fluorescence microscope was used for examination of autofluorescence of living cells.

Transmission Electron Microscopy

For a more detailed examination, a sample of red-water was fixed for 4 hours in 3% glutaraldehyde in 0.1M sodium cacodylate buffer (adjusted isosmotic to the sea-water with sodium chloride), rinsed in buffer, and postfixed in 2% osmium tetroxide in Palade's buffer. The fixed material was then stained in 1.5% uranyl acetate, washed and dehydrated through an ethanol series to histosol, before finally being embedded in Spurr's resin. Silver sections for transmission electron microscopy were cut on a REICHART ultracut microtome, stained with lead citrate, and viewed on a PHILLIPS 300 electron microscope.

Radiotracer Experiments

The technique principally consists of growing ciliates in ^{14}C labelled algal cultures until evenly labelled, then picking out individual ciliates for determination of loss of label (as $^{14}CO_2$), in the dark, over varying time intervals. *Strombidium capitatum* (strain 'GRG') was grown on a mixed algal culture of the cryptophyte *Pyrenomonas salina* Santore (strain '3C'), the prymnesiophyte *Isochrysis galbana* Parke (strain 'ISO'), and a small *Gymnodinium* - like dinoflagellate (strain 'GYMNO') in 75% seawater. *Favella ehrenbergii* was grown on the dinoflagellate *Heterocapsa pygmaea* (strain 'HT984') in 100% seawater. Vineyard Sound seawater was used for all cultures; this was filtered (1.0 μm), autoclaved in teflon and supplemented with 0.1 ml l^{-1} f/2 iron-EDTA trace metal solution (Guillard 1975). Cultures were maintained at a temperature of 15°C and a light intensity of approximately 100 μE m^{-2} s^{1} on a 14:10 hr light/dark cycle. ^{14}C bicarbonate ($NaH^{14}CO_3$) was added initially to algal cultures to achieve an estimated activity of about 1 μCi ml^{-1}.

Ciliates were allowed to grow until they had become evenly labelled according to the criteria utilised by Putt & Stoecker (1989); that is that specific activity of cell carbon becomes equal to that of inorganic carbon in the medium. Then individuals were picked out with a drawn-out pasteur pipette under a dissecting microscope, then washed through three changes of sterile, unlabelled medium. Each individual cell was finally transferred in a 50 μl aliquot, using an automatic pipette, to a small glass shell vial containing 2 ml of sterile, unlabelled medium. For the blanks, 50 μl of the final wash water was transferred, without a cell, into the 2 ml medium in the vial. Shell vials were placed upright within 20 ml glass scintillation vials, which contained 1 ml 10% KOH as a trap for carbon dioxide; this method is adapted from that of Manahan (1983). These outer scintillation vials were capped with a serum stopper, wrapped in aluminium foil to exclude light, and then incubated at 15 °C for time intervals of between 2 and 24 hours. The time of the start of the incubation was recorded exactly for each vial. 15 replicates and 5 blanks were prepared for each time interval. At T_0, and at the end of each incubation period, vials were checked for a live ciliate under a dissecting microscope; only vials with a live ciliate were further processed, but the proportion of vials with and without living ciliates was recorded at each time interval for rates of mortality. 0.5 ml of 0.1 N HCl was then injected through the serum stopper, using a hypodermic syringe and needle, into the shell vial. This killed the ciliate and lowered the pH, thus driving off any CO_2 from the seawater medium and trapping it in the KOH. The time at which the acid was injected into each vial was recorded exactly giving times of incubation to the nearest minute. After keeping sealed for 24 hours, to allow transfer of all CO_2 into the KOH trap, the inner shell vial was removed and the outside rinsed into the KOH with 1 ml of distilled water. 10 ml of scintillation fluor (UNIVERSOL, ICN) was then added to the KOH vial. The organic carbon fraction in the shell vial was then transferred into a second scintillation vial and the inside of the shell vial rinsed with 1 ml distilled water into this second vial. This was then dried at 60°C to remove residual inorganic ^{14}C, and 1 ml distilled water followed by 10 ml scintillation fluor added to the vial. Inner and outer blank vials were processed in a similar manner. Specific activity was determined for the medium (dpm) in which the ciliates were cultured; 5 replicate 0.1 ml samples of culture media were each placed in 0.2 ml of phenethylamine in a scintillation vial, followed by 10 ml scintillation fluor.

Radioactivity was determined for all vials on a BECKMAN LS5000 TD scintillation counter, using automatic quench correction and correction for chemoluminescence.

APPENDIX THREE:
DATA MANIPULATION

Density

Seawater density was calculated from a transformation of temperature and salinity data. The density (ρ) is expressed as the variable σ_t which specifies the excess mass over the same volume of distilled water at 4°C and normal atmospheric pressure:

$$\sigma_t = 1000(1-\rho) \text{ kg m}^{-3}$$

Ignoring the effect of pressure (and hence depth) for shallow water, temperature and salinity were transformed to σ_t using the equations given by Tett (1987) as follows:

TEMP = Temperature (°C)

SAL = Salinity ($^{o}/_{oo}$)

T_1, S_1, E_1, B_1, B_2 are variables defined by the following equations:

$$T_1 = \text{TEMP} + [4.4*10^{-6}*\text{TEMP}*(100 - \text{TEMP})]$$

$$S_1 = \text{SAL}*\{\text{SAL}*[(6.7678614*10^{-6}*\text{SAL})-4.8249614*10^{-4}]+0.81487658\} -0.093445863$$

$$E_1 = T_1*\{T_1*[T_1*((-1.438031*10^{-7}*T_1) - 1.982484*10^{-3}) - 0.5439391] + 4.53164843\}$$

$$B_1 = T_1*\{T_1*[(-1.0843*10^{-6}*T_1) + 9.8185*10^{-5}] - 4.7867*10^{-3}\} + 1.0$$

$$B_2 = T_1*\{T_1*[(1.677*10^{-8}*T_1) - 8.614*10^{-7}] + 1.803*10^{-5}\}$$

$$\sigma_t = [E_1/(T_1 + 67.26)] + \{S_1*[(B_2*S_1) + B_1]\}$$

Chlorophyll

Concentrations of chlorophyll *a* and phaeopigments in the original water sample were then computed using the following equations:

$$\text{Chlorophyll } a\ (\mu g\ l^{-1}) = F_D \times n(R_B - R_A) \times v/V$$

$$\text{Phaeopigments } (\mu g\ l^{-1}) = F_D \times n(yR_A - R_B) \times v/V$$

where:

F_D = calibration factor for fluorometer Ca/R

Ca = concentration of chlorophyll standard

R = fluorescence of chlorophyll standard

R_B = fluorescence of sample

R_A = fluorescence of sample after adding acid

v = volume of acetone extract (ml)

V = volume of sample filtered (l)

$n = (R_B/R_A)/(R_B/R_A-1)$ for standard

$y = R_B/R_A$ for standard

Oxygen

Percentage oxygen saturation was determined from oxygen concentration, in mg l^{-1}, by converting first to ml O_2 l^{-1} by multiplying by 22.4/32. Solubility of O_2 at the given salinity and temperature was determined according to Weiss (1970):

$$\ln C = [A_1 + A_2(100/T) + A_3.\ln(T/100) + A_4(T/100)] + S[B_1 + B_2(T/100) + B_3(T/100)2]$$

where:

C is solubility at STP (ml O_2 l^{-1}) from water saturated air at a total pressure of one atmosphere.

T is absolute temperature

S is salinity $(^{\circ}/_{oo})$

A's and B's are solubilty constants; A_1 = -173.4292, A_2 = 249.6339, A_3 = 143.3488, A_4 = 21.8492, B_1 = 0.033096, B_2 = 0.014259, B_3 = -0.0017

Oxygen concentration determined in the water column (ml l^{-1}) was then expressed as a percentage of the solubility for the temperature and salinity determined at that depth.

Light

Light extinction coefficients were determined at each station from field measurements of PAR (μE m^{-2} s^{-1}), by exponential regression analysis using the formula:

$$I_d = I_0.e^{-kd} \qquad \text{(Parsons \textit{et al.} 1984\textit{b})}$$

where I_d and I_0 are light intensities at depth (d) and the surface respectively, and k is the extinction coefficient (m^{-1}).

Radiotracer Experiments

The ^{14}C content $^{*}S$ (dpm $cell^{-1}$) at T_0 was calculated from:

$$^{*}S = (K-B1)+(L-B2)$$

where K is dpm in KOH fraction (outer vial), B1 is the mean blank dpm for the KOH fraction, L is the dpm in the organic carbon fraction (inner vial), and B2 is the mean blank dpm for the organic carbon fraction. K-B1 gives the loss of $^{14}CO_2$ (dpm/cell) at each time interval, and L-B2 gives the carbon content (dpm) of the cell at time T.

Carbon specific rates of respiration R (% cell C h^{-1}) were then given by:

$$R = \frac{100(K-B1)}{[(K-B1)+(L-B2)]T}$$

where T is the time in hours between commencing the incubation and injecting the acid. T was recorded exactly (to the nearest minute) for each individual vial.

Carbon content S (ng C cell^{-1}) was then calculated from:

$$S = \frac{{}^{*}S\ (DIC)\ (1.05)}{{}^{*}M}$$

where DIC is the dissolved inorganic carbon content of the medium (18.1 μg C ml^{-1} for 75% seawater, and 22.5 μg C ml^{-1} for 100% seawater, determined on a Dohrmann PR-1 carbon analyzer), *M is the ^{14}C content of the medium in which the ciliates were uniformly labelled (dpm ml^{-1}) and 1.05 is an isotopic discrimination factor (Welschmeyer & Lorenzen 1984).

APPENDIX FOUR:
GLOSSARY

P_{max}	- light saturated maximum rate of photosynthesis. - units: mg C^{-1} m^{-3} h^{-1}
P_m^B	- P_{max} normalised per unit chlorophyll. - units: mg C (mg chl*a*)$^{-1}$ h^{-1}
α	- intial slope of the photosynthesis versus irradiance curve. - units: mg C (mg chl*a*)$^{-1}$ h^{-1} (μE m^{-2} s^{-1})$^{-1}$
β	- degree of photoinhibition of photosynthesis versus light curve. - units: mg C(mg chl*a*)$^{-1}$ h^{-1} (μE m^{-2} s^{-1})$^{-1}$
I_k	- irradiance where photosynthesis is saturated. - units: μE m^{-2} s^{-1}
PAR	- photosynthetically active radiation over wavelengths 400-700nm. - units μE m^{-2} s^{-1}
k	- extinction coefficient of water column given by $I_d = I_0.e^{-kd}$, where I_d and I_0 are light intensities at depth (d) and the surface respectively. - units: m^{-1}
% chl*a*	- concentration of chlorophyll *a* expressed as a percentage of concentration of chlorophyll *a* + phaeopigments.

LITERATURE CITED:

"Read not to contradict and confute,
nor to believe and take for granted,
nor to find talk and discourse,
but to weigh and consider"

Francis Bacon (1561-1626)

Agamaliev, F.G. (1967). Faune des ciliés mésopsammiques de la côte ouest de la Mer Caspienne. *Cahiers de Biologie Marine* **8**: 359-402.

Agustí, S. (1991). Light environment within dense algal populations: cell size influences on self-shading. *Journal of Plankton Research* **13**: 863-871.

Anang, E.R., E.K. Obeng-Asamoa & D.M. John (1976). Observations on the red-water ciliate *Mesodinium rubrum* Lohmann in Ghanian coastal water. *Bulletin de l'Institut Fondamental d'Afrique Noire Série A* **38**: 235-240.

Andersen, P. & H.M. Sørensen (1986). Population dynamics and trophic coupling in pelagic microorganisms in eutrophic coastal waters. *Marine Ecology - Progress Series* **33**: 99-109.

Anderson, D.M. & K.D. Stolzenbach (1985). Selective retention of two dinoflagellates in a well-mixed coastal embayment: the importance of diel vertical migration and surface avoidance. *Marine Ecology - Progress Series* **25**: 39-50.

Anderson, O.R. (1983). *Radiolaria*. Springer-Verlag, New York, 355pp.

Antai, E.E. (1989). *Seasonal trophodynamics of bacterioplankton and heterotrophic microflagellates in Southampton Water.* PhD Thesis, University of Southampton, 200pp.

Apstein, C. (1908). *Supplement to Parts XVIII-XXI, Pflanzendes. Nordischen Planktons.* Nordisches Plankton XXII.

Avaria, S. (1970). Observacion de un fenomeno de marea roja en la Bahia de Valparaiso. *Revista de Biologia Marina Valparaiso* **14**: 1-5.

Avaria, S. (1979). Red tides off the coast of Chile. In: Taylor, D.L. & H.H. Seliger (eds) *Toxic Dinoflagellate Blooms.* Elsevier, Amsterdam, pp.161-164.

Bakker, C. (1966). Een protozo in symbiose met algen in het Veerse Meer. *De Levende Natuur* **69**: 180-187.

Bakker, C. (1967*a*). Massale ontwikkeling van ciliaten met symbiontische algen in het Veerse Meer. *De Levende Natuur* **70**: 166-173.

Bakker, C. (1967*b*). Veranderingen in milieu en plankton van het Oosterscheldegebied. *Vakblad voor Biologien* **47**: 181-192.

Banse, K. (1982). Cell volumes, maximal growth rates of unicellular algae and ciliates, and the role of ciliates in the marine pelagial. *Limnology and Oceanography* **27**: 1059-1071.

Barber, R.T. & W.O. Smith (1981). The role of circulation, sinking and vertical migration in physical sorting of phytoplankton in the upwelling centre at 15N. In: Richards, F.A. (ed.) *Coastal Upwelling: Coastal and Estuarine Sciences 1.* Geophysical Monography Bd, Washington D.C., pp. 366-371.

Barber, R.T., A.W. White & H.W. Siegelman (1969). Evidence for a cryptomonad symbiont in the ciliate *Cyclotrichium meunieri*. *Journal of Phycology* **5**: 86-88.

Baars, M.A. & H.G. Franz (1984). Grazing pressure of copepods on the phytoplankton stock of the central North Sea. *Netherlands Journal of Sea Research* **18**: 120-142.

Bary, B.M. (1953). Sea-water discolouration by living organisms. *New Zealand Journal of Science* **34**: 393-407.

Bary, B.M. & R.G. Stuckey (1950). An occurrence in Wellington Harbour of *Cyclotrichium meunieri* Powers, a ciliate causing red water, with some additions to its morphology. *Transactions of the Royal Society of New Zealand* **78**: 86-92.

Bauerfeind, E., M. Ellbrächter, R. Steiner & J. Throndsen (1986). Application of Laser Doppler Spectroscopy (LDS) in determining swimming velocities of motile phytoplankton. *Marine Biology* **93**: 323-327.

Beers, J.R. & G.L. Stewart (1967). Microzooplankton in the euphotic zone at five locations across the California current. *Journal of the Fisheries Research Board of Canada* **24**: 2053-2068.

Beers, J.R. & G.L. Stewart (1969*a*). The vertical distribution of microzooplankton and some ecological observations. *Journal du Conseil permanent international pour l'Exploration de la Mer* **33**: 30-44.

Beers, J.R. & G.L. Stewart (1969*b*). Microzooplankton and its abundance relative to the larger zooplankton and other seston components. *Marine Biology* **4**: 182-189.

Beers, J.R. & G.L. Stewart (1970). Numerical abundance and estimated biomass of microzooplankton. In: Strickland, J.D.H. (ed.) The ecology of the plankton off La Jolla, California, in the period April through September, 1967. *Bulletin of the Scripps Institution of Oceanography* **17**: 67-87.

Beers, J.R. & G.L. Stewart (1971). Microzooplankters in the plankton communities of the upper waters of the eastern tropical Pacific. *Deep Sea Research* **18**: 861-883.

Beers, J.R., F.M.H. Reid & G.L. Stewart (1975). Microplankton of the North Pacific central gyre. Population stucture and abundance, June 1973. *Internationale Revue der gesamten Hydrobiologie* **60**: 607-638.

Beers, J.R., F.M.H. Reid & Stewart, G.L. (1980). Microplankton population structure in southern Californian nearshore waters in late spring. *Marine Biology* **60**: 209-226.

Beers, J.R., M.R. Stevenson, R.W. Eppley & E.R. Brooks (1971). Plankton populations and upwelling off the coast of Peru, June 1969. *Fishery Bulletin* **69**: 859-876.

Bieleski, R.L. (1973). Phosphate pools, phosphate transport and phosphate availability. *Annual Reviews in Plant Physiology* **24**: 225-252.

Blackbourn, D.J., F.J.R. Taylor & J. Blackbourn (1973). Foreign organelle retention by ciliates. *Journal of Protozoology* **20**: 286-288.

Blasco, D., M. Estrada & B. Jones (1980). Phytoplankton distribution and composition in the northwest African upwelling near Cabo Corbeiro. *Deep Sea Research* **27**: 799-821.

Blasco, D., M. Estrada & B. Jones (1981). Short time variability of phytoplankton populations in upwelling regions - the example of N.W. Africa. In: Richards, F.A. (ed.) *Coastal Upwelling: Coastal and Estuarine Sciences 1*. Geophysical Monography Board, Washington D.C., pp. 339-347.

Braarud, T. & A. Bursa (1939). The phytoplankton of the Oslo Fjord 1933-1934. *Hvalrådets Skrifter* **19**: 1-63.

Braarud, T. & B. Hope (1952). The annual phytoplankton cycle of a landlocked fjord near Bergen (Nordåsvatn). *Fiskeridirektoratets Skrifter Serie Havundersøkelser* **9**: 1-26.

Braarud, T., K. R. Gaarder & J. Grøntved (1953). The phytoplankton of the North Sea and adjacent waters in May 1948. *Rapports et Procès-verbaux des Réunions du Conseil permanent international de l'Exploration de la Mer* **133**: 1-87.

Braarud, T., K.R. Gaarder & O. Nordli (1958). Seasonal changes in the phytoplankton at various points off the Norwegian west coast. *Fiskeridirektoratets Skrifter Serie Havundersøkelser* **12**: 1-77.

Bryan, J.R. (1979). *The production and decomposition of organic material in an estuary - Southampton Water.* PhD Thesis, University of Southampton.

Buchanan, R.J. (1966). *A study of species composition and ecology of the protoplankton of a British Columbia inlet.* Ph.D. Thesis, University of British Columbia, Vancouver, 268pp.

Burkill, P.H. (1982). Ciliates and other microplankton components of a nearshore food-web: standing stocks and production processes. *Annales de l'Institut Océanographique* **Fascicule Supplémentaire 58**: 335-350.

Bursa, A. (1961*a*). Phytoplankton of the *Calanus* expeditions in Hudson Bay, 1953 and 1954. *Journal of the Fisheries Research Board of Canada* **18**: 51-83.

Bursa, A. (1961*b*). The annual oceanographic cycle at Igloolik in the Canadian arctic. 2. The phytoplankton. *Journal of the Fisheries Research Board of Canada* **18**: 563-615.

Bursa, A. (1963). Phytoplankton in the coastal waters of the Arctic Ocean at Point Barrow, Alaska. *Arctic* **16**: 239-262.

Cabeçadas, G., M.E. Cunha, M.T. Moita, J. Pissarra & M.A.de M. Sampayo, M.A.de (1983). Red tide in Cascais Bay, Portugal. *Boletim do Instituto Nacional de Investigação das Pescas, Lisboa* **10**: 81-123.

Campodonico, I., L. Guzman & G. Lembeye (1975). Una discoloracion causada por el ciliado *Mesodinium rubrum* (Lohmann) en Ensenada Wilson, Magallanes. *Anales del Instituto de la Patagonia, Punta Arenas (Chile)* **6**: 225-239.

Caron, D.A., J.C. Goldman & T. Fenchel (1990). Protozoan respiration and metabolism. In: Capriulo, G.M. (ed) *Ecology of Marine Protozoa*, Oxford University Press, New York, pp. 307-322.

Chang, J. & E.J. Carpenter (1985). Blooms of the dinoflagellate *Gyrodinium aureolum* in a Long Island estuary: Box model analysis of bloom maintenance. *Marine Biology* **89**: 83-93.

Chester, A.J. (1978). Microzooplankton relative to a subsurface chlorophyll maximum layer. *Marine Science Communications* **4**: 275-292.

Choi, J.W. & D.K. Stoecker (1989). Effects of fixation on cell volume of marine planktonic protozoa. *Applied and Environmental Microbiology* **55**: 1761-1765.

Clemens, W.A. (1935). Red water bloom in British Columbia waters. *Nature (London)* **152**: 473.

Cloern, J.E., B.E. Cole, R.L.J. Wong & A.E. Alpine (1985). Temporal dynamics of estuarine phytoplankton: A case study of San Francisco Bay. *Hydrobiologia* **129**: 153-176.

Costello, J.H., J.R. Strickler, C. Marrasé, G. Trager, R. Zeller & A.J. Freise (1990). Grazing in a turbulent environment: Behavioural response of a calanoid copepod, *Centropages hamatus*. *Proceedings of the National Academy of Science of the U.S.A.* **87**: 1648-1652.

Crawford, D.W. (1989). *Mesodinium rubrum* - the phytoplankter that wasn't. *Marine Ecology - Progress Series* **58**: 161-174.

Crawford, D.W.; L.E. Hawkins; S. Hutchinson; E.E. Antai; D.A. Purdie & A.P.M. Lockwood (1992). Red-tides of *Mesodinium rubrum*: evidence for remotely imposed stress on the oyster *Ostrea edulis*. In: *Toxic Phytoplankton; Proceedings of the Fifth International Conference*, Newport, Rhode Island, USA. Elsevier. **in press**.

Cullen, J.J. (1985). Diel vertical migration by dinoflagellates: roles of carbohydrate metabolism and behavioural flexibility. In: Rankin, M.A. (ed) Migration: mechanisms and adaptive significance. *Contributions in Marine Science* **27 (Supplement)**: 135-152.

Cullen, J.J., F.M.H. Reid & E. Stewart (1982). Phytoplankton in the surface and chlorophyll maximum off southern California in August, 1978. *Journal of Plankton Research* **4**: 665-695.

Dale, T. (1987*a*). Diel vertical distribution of planktonic ciliates in Lindåspollene, western Norway. *Marine Microbial Food Webs* **2**: 15-28.

Dale, T. (1987*b*). Oil pollution and plankton dynamics. II. Abundance pattern of ciliates inside and outside enclosures and the responses of ciliates to oil during the 1980 spring bloom in Lindåspollene, Norway. *Sarsia* **72**: 197-202.

Dale, T. (1988*a*). Oil pollution and plankton dynamics. V. Controlled ecosystem pollution experiments in Lindåspollene, Norway, June 1980: Effects of oil, oil/nutrients, and oil/dispersant on microplankton. *Sarsia* **73**: 169-178.

Dale, T. (1988*b*). Oil pollution and plankton dynamics. VI. Controlled ecosystem pollution experiments in Lindåspollene, Norway, June 1981: Effects on planktonic ciliates following nutrient addition to natural and oil-polluted enclosed water columns. *Sarsia* **73**: 179-191.

Dale, T. & E. Dahl (1987). Mass occurrence of planktonic oligotrichous ciliates in a bay in southern Norway. *Journal of Plankton Research* **9**: 871-879.

De Sousa Lima, H. & P.J.LeB. Williams (1978). Oxygen consumption by the planktonic population of an estuary - Southampton Water. *Estuarine and Coastal Marine Science* **6**: 515-523.

Dobell, C. (1932). *Anthony van Leeuwenhoek and his "little animals"*. Staples Press, London, 435pp.

Dugdale, R.C., F.P. Wilkerson, R.T. Barber, D. Blasco & T.T. Packard (1987). Changes in nutrients, pH, light penetration and heat budget by migrating photosynthetic organisms. *Oceanologica Acta Special Issue* **No. 6**: 103-107.

Dyer, K. (1973). *Estuaries: A Physical Introduction*. John Wiley & Sons, London, 140 pp.

Elmgren, R. (1984). Trophic dynamics in the enclosed, brackish Baltic Sea. *Rapports et Procès-verbaux des Réunions du Conseil permanent international de l'Exploration de la Mer* **183**: 152-169.

Epp, R.W. & W.M. Lewis (1984). Cost and speed of locomotion for rotifers. *Oecologia (Berlin)* **61**: 289-292.

Eppley, R.W., O. Holm-Hansen & J.D.H. Strickland (1968). Some observations on the vertical migration of dinoflagellates. *Journal of Phycology* **4**: 333-340.

Eppley, R.W. & J.H. Sharp (1976). Photosynthetic measurements in the central North Pacific: the dark loss of carbon in 24-h incubations. *Limnology and Oceanography* **20**: 981-987.

Eriksson, S., C. Sellei & K. Wallström (1977). The structure of the plankton community of the Öregrundsgrepen (southwest Bothnian Sea). *Helgoländer wissenschaftliche Meeresuntersuchungen* **30**: 582-597.

Fauré-Fremiet, E. (1948). Le rhythme de marée du *Strombidium oculatum* Gruber. *Bulletin biologique de France et de Belgique* **82**: 3-23.

Fenchel, T. (1968*a*). On 'red-water' in the Isefjord (inner Danish waters) caused by the ciliate *Mesodinium rubrum*. *Ophelia* **5**: 245-253.

Fenchel, T. (1968*b*). Reproductive potential of ciliates. *Ophelia* **5**: 123-136.

Fenchel, T. (1982). Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. *Marine Ecology - Progress Series* **9**: 35-42.

Fenchel, T. & B.J. Finlay (1983). Respiration rates in heterotrophic, free-living protozoa. *Microbial Ecology* **9**: 99-122.

Fenchel, T. & B.J. Finlay (1986). The structure and function of Muller vesicles in loxodid ciliates. *Journal of Protozoology* **33**: 69-79.

Foissner, W. & I. Foissner (1984). First record of an ectoparasitic flagellate on ciliates: an ultrastructural investigation of the morphology and the mode of attachment of *Spiromonas gonderi* nov. spec. (Zoomastigophora, Spiromonadidae) invading the pellicle of ciliates of the genus *Colpoda* (Ciliophora, Colpodidae). *Protistologica* **20**: 635-648.

Fonds, M. & D. Eisma (1967). Upwelling water as a possible cause of red plankton bloom along the Dutch coast. *Netherlands Journal of Sea Research* **3**: 458-463.

Føyn, B.R. (1929). Investigation of the plankton at Lofoten. *Skrifter utgitt av det Norske Videnskaps-Akademi i Oslo* **1(10)**.

Ganf, G.G. (1974). Diurnal mixing and the vertical distribution of phytoplankton in a shallow equatorial lake (Lake George, Uganda). *Journal of Ecology* **62**: 611-629.

Garcon, V.C., Stolzenbach, K.D., Anderson, D.M. (1986). Tidal flushing of an estuarine embayment subject to recurrent dinoflagellate blooms. *Estuaries* **9**: 179-187.

Gaarder, K.R. (1932). Phytoplankton studies from the Tromsø district 1930-31. *Tromsø Museums Årshefter* **55**: 1-159.

Gieskes, W.W.C. & G.W. Kraay (1983). Dominance of Cryptophyceae during the phytoplankton Spring bloom in the central North Sea detected by HPLC analysis of pigments. *Marine Biology* **75**: 179-185.

Glover, H. E. (1980). Assimilation numbers in cultures of marine phytoplankton. *Journal of Plankton Research* **2**:69-79.

Gran, H.H. (1929*a*). Quantitative plankton investigations carried out during the expedition with the "Michael Sars", July-Sept. 1924. *Rapports et Procès-verbaux des Réunions du Conseil permanent international de l'Exploration de la Mer* **56**(4): 1-50.

Gran, H.H. (1929*b*). Investigation of the production of plankton outside the Romdalsfjord 1926-27. *Rapports et Procès-verbaux des Réunions du Conseil permanent international de l'Exploration de la Mer* **56**(5): 1-112.

Grain, J., P. de Puytorac & C.A. Grolière, C.A. (1982). Quelques précisions sur l'ultrastructure et la position systématique du cilié *Mesodinium rubrum*. et sur la constitution des symbiontes chloroplastiques. *Protistologica* **18**: 7-21.

Grice, G.D., R.P. Harris, M.R. Reeve, J.F. Heinbokel & C.O. Davis (1980). Large-scale enclosed water-column ecosystems. An overview of foodweb I, the final CEPEX experiment. *Journal of the Marine Biological Association of the United Kingdom* **60**: 401-414.

Grøntved, J. (1952). Investigations on the phytoplankton in the southern North Sea in May 1947. *Meddelelser fra Kommissionen for Danmarks Fiskeri og Havundersøgelser Serie Plankton* **5**: 1-49.

Grøntved, J. & E.S. Nielsen (1957). Investigations on the phytoplankton in sheltered Danish marine localities. *Meddelelser fra Kommissionen for Danmarks Fiskeri og Havundersøgelser Serie Plankton* **5**(6): 1-52.

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L. & M.H. Chanley (eds) *Culture of marine invertebrate animals*. Plenum Publishing Co., New York, pp. 29-60.

Guzman, L. & I. Campodonico (1978). Mareas rojas en Chile. *Interciencia* **3**: 144-151.

Halme, E. (1958). Planktologische Untersuchungen in der Pojo-Bucht und angrenzenden Gewässern. IV. Zooplankton. *Annales Zoologici Societatis Zoologicae-Botanicae Fennicae, Vanamo* **19**(3): 1-62.

Hamburger, C. & W. von Buddenbrock (1911). Nordische Ciliata mit Ausschluss der Tintinnoidea. *Nordisches Plankton* **13**: 1-152.

Harrison, W.G., T. Platt, R. Calienes & N. Ochoa (1981). Photosynthetic parameters and primary production of phytoplankton populations off the northern coast of Peru. In: Richards, F.A. (ed.) *Coastal Upwelling: Coastal and Estuarine Sciences 1*. Geophysical Monography Board, Washington D.C., pp. 303-311.

Hart, T.J. (1934). Red "water-bloom" in South African seas. *Nature (London)* **134**: 459-460.

Hart, T.J. (1943). Darwin and "water-bloom". *Nature (London)* **152**: 661-662.

Hart, T.J. & R.I. Currie (1960). The Benguela current. *Discovery Reports* **31**: 123-298.

Hayes, G.C. (1987). *The distribution of icthyoplankton and ctenophores in Southampton Water: the effect of a* Mesodinium rubrum *bloom*. Undergraduate dissertation, University of Southampton.

Hayes, G.C, D.A. Purdie & J.A. Williams (1989). The distribution of ichthyoplankton in Southampton Water in response to low oxygen levels produced by a *Mesodinium rubrum* bloom. *Journal of Fish Biology* **34**: 811-813.

Heaney, S.I. & R.W. Eppley (1981). Light, temperature and nitrogen as interacting factors affecting diel vertical migration of dinoflagellates in culture. *Journal of Plankton Research* **3**: 331-345.

Heaney, S.I. & C. Butterwick (1985). Comparative mechanisms of algal movement in relation to phytoplankton production. In: Rankin, M.A. (ed) Migration: mechanisms and adaptive significance. *Contributions in Marine Science* **27 (Supplement)**: 114-134.

Hendrikson, P., K.G. Sellner, B. Rojas de Mendiola, N. Ochoa & R. Zimmermann (1982). The composition of particulate organic matter and biomass in the Peruvian upwelling region during ICANE 1977 (Nov.14 - Dec.12). *Journal of Plankton Research* **4**: 163-186.

Hernández-Becerril, D.V. (1987). Un caso de marea roja en el Golfo de California. *Revista Latinoamericana de Microbiologia* **29**: 171-174.

Hibberd, D.J. (1977). Ultrastructure of the cryptomonad endosymbiont of the red-water ciliate *Mesodinium rubrum*. *Journal Marine Biological Association of the United Kingdom* **57**: 45-61.

Holm-Hansen, O., F.J.R. Taylor & R.J. Barsdate (1970). A ciliate red-tide at Barrow, Alaska. *Marine Biology* **7**: 37-46.

Horstman, D.A. (1981). Reported red-water outbreaks and their effects on fauna on the west and south coasts of South Africa, 1959-1980. *Fisheries Bulletin, Sea Fisheries Institute, Republic of South Africa* **15**: 71-88.

Horstmann, U. (1980). Observations on the peculiar diurnal migration of a red tide Dinophyceae in tropical shallow waters. *Journal of Phycology* **16**: 481-485.

Iriarte, A., G. Daneri, V.M.T. Garcia, D.A. Purdie & D.W. Crawford (1991). Plankton community respiration and its relationship to chlorophyll *a* concentration in marine coastal waters. *Oceanologica Acta* **14**: 379-387.

Jackson, K.M. & J. Berger (1984). Survival of ciliate protozoa under starvation conditions and at low bacterial levels. *Microbial Ecology* **10**: 47-59.

Jankowski, A.W. (1976). Revision of a system of cyrtophorines. In: *Materials of the II All-Union Conference of Protozoologists, Part I, General Protozoology*, A.P. Markevich, Yu.I. Poljansky *et al.* (eds), Naukova Dumka, Kiev, pp. 167-168.

Jimenez, R. & P. Intriago (1987). Observations on blooms of *Mesodinium rubrum* in the upwelling area off Ecuador. *Oceanologica Acta* **Special Issue No. 6**: 145-154.

Johnsson, P.R. (1987). Photosynthetic assimilation of inorganic carbon in marine oligotrich ciliates (Ciliophora, Oligotrichina). *Marine Microbial Food Webs* **2**: 55-68.

Jonsson, P.R. (1989). Vertical distribution of planktonic ciliates - an experimental analysis of swimming behaviour. *Marine Ecology - Progress Series* **52**: 39-53

Jonsson, P.R. & P. Tiselius (1990). Feeding behaviour, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. *Marine Ecology - Progress Series* **60**: 35-44.

Kahl, A. (1930-1935). Urtiere oder Protozoa. I. Wimpertiere oder Ciliata (Infusoria), eine Bearbeitung der freilebenden und ectocommensalen Infusorine der Erde, unter Ausschluss der marinen Tintinnidae. In: Dahl, F. (ed.) *Die Tierwelt Deutschlands*, vol. 18 (1930), vol. 21 (1931), vol. 25 (1932), vol. 30 (1935). Gustav Fischer, Jena, pp. 1-886.

Kamykowski, D. & S.A. McCollum (1986). The temperature-acclimated swimming speed of selected marine dinoflagellates. *Journal of Plankton Research* **8**: 275-287.

Kazama, F. (1972). Development and morphology of a chytrid isolated from *Bryopsis plumosa*. *Canadian Journal of Botany* **50**: 499-505.

Ketchum, B.H. (1954). Relation between circulation and planktonic populations in estuaries. *Ecology* **35**: 191-200.

Kivi, K. (1986). Annual succession of pelagic protozoans and rotifers in the Tvarminne Storfjarden, SW coast of Finland. *Ophelia* **Supplement 4**: 101-110.

Klekowski, R.Z. (1981). Size dependence of metabolism in protozoans. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **21**: 1498-1502.

Lackey, J.B. (1963). The microbiology of a Long Island bay in the summer of 1961. *Internationale Revue der gesamten Hydrobiologie* **48**: 577-601.

Lackey, J.B. (1967). The microbiota of estuaries and their roles. In: Lauff, G. (ed.) *Estuaries*. AAAS. Washington, D.C., pp. 291-302.

Lackey, J.B. & K.A. Clendenning (1963). A possible fish-killing yellow tide in Californian waters. *Quarterly Journal of the Florida Academy of Science* **26**: 263-268.

Lackey, J.B. & K.A. Clendenning (1965). Ecology of the microbiota of San Diego Bay, California. *Transactions of the San Diego Society for Natural History* **14**: 9-40.

Lackey, J.B. & E.W. Lackey (1963). Microscopic algae and Protozoa in the waters near Plymouth in August 1962. *Journal of the Marine Biological Association of the United Kingdom* **43**: 797-805.

Laval-Peuto, M. & F. Rassoulzadegan (1988). Autofluorescence of marine planktonic Oligotrichina and other ciliates. *Hydrobiologia* **159**: 99-110.

Laval-Peuto, M., P. Salvano, P. Gayol & C. Greuet (1986). Mixotrophy in marine planktonic ciliates: ultrastructural study of *Tontonia appendiculariformis* (Ciliophora, Oligotrichida). *Marine Microbial Food Webs* **1**: 81-104.

Leakey, R.J.G. (1989). *The ecology of planktonic ciliates in Southampton Water.* PhD Thesis, University of Southampton, 130pp.

Leegard, C. (1920). Microplankton from the Finnish waters during the month of May 1912. *Acta Societatis scientiarum Fennicae* **48**: 1-45.

Leppänen, J.M. & J.E. Bruun (1986). The role of pelagic ciliates including the autotrophic *Mesodinium rubrum* during the spring bloom of 1982 in the open northern Baltic Sea proper. *Ophelia* **Supplement 4**: 147-149.

Levandosky, M. & P.J. Kaneta (1987) Behaviour in dinoflagellates. In: Taylor, F.J.R. (ed) *The Biology of Dinoflagellates*, Botanical Monographs, Vol. 21, Blackwell Scientific Publications, pp. 360-397.

Lin, S., G.A. Borstad & J.F.R. Gower (1984). Remote sensing in the red spectral region. In: Nihoul, J.C.J. (ed.) *Remote Sensing of Shelf Sea Hydrodynamics.* Elsevier, Amsterdam, pp. 317-336.

Lindholm, T. (1978). Autumnal mass development of the 'red-water' ciliate *Mesodinium rubrum* in the Åland archipelago. *Memoranda Societatis pro Fauna et Flora Fennica* **54**: 1-5.

Lindholm, T. (1981). On the ecology of *Mesodinium rubrum* (Lohmann) (Ciliata) in a stagnant brackish basin on Åland, SW Finland. *Kieler Meeresforschungen, Sonderheft* **5**: 117-123.

Lindholm, T. (1982). EDTA and oxalic acid - two useful agents for narcotizing fragile and rapid microzooplankton. *Hydrobiologia* **86**: 297-298.

Lindholm, T. (1985). *Mesodinium rubrum* - a unique photosynthetic ciliate. *Advances in Aquatic Microbiology* **3**: 1-48.

Lindholm, T. & A.C. Mörk (1989). Symbiotic algae and plastids in planktonic ciliates. *Memoranda Societatis pro Fauna et Flora Fennica* **65**: 17-22.

Lindholm, T. & A.C. Mörk (1990). Depth maxima of *Mesodinium rubrum* (Lohmann) Hamburger & Buddenbrock - examples from a stratified Baltic Sea inlet. *Sarsia* **75**: 53-64.

Lindholm, T.; P. Lindroos & A.C. Mörk (1988). Ultrastructure of the photosynthetic ciliate *Mesodinium rubrum*. *BioSystems* **21**: 141-149.

Lohmann, H. (1908). Untersuchungen zur Festellung des vollstandigen Gehaltes des Meeres an Plankton. *Wissenschaftliche Meeresuntersuchungen* **10**: 129-370.

Lombard, E.H. & B. Capon (1971). Observations on the tidepool ecology and behaviour of *Peridinium gregarium*. *Journal of Phycology* **7**: 188-194.

Lorenzen, C.J. (1972). Extinction of light in the ocean by phytoplankton. *Journal du Conseil permanent international pour l'Exploration de la Mer* **34**: 262-267.

Mackenzie, A.L. & P.A. Gillespie (1986). Plankton ecology and productivity, nutrient chemistry, and hydrography of Tasman Bay, New Zealand, 1982-1984. *New Zealand Journal of Marine and Freshwater Research* **20**: 365-395.

Mackenzie, A.L., H.F. Kaspar & P.A. Gillespie (1986). Some observations on phytoplankton species composition, biomass, and productivity in Kenepuru Sound, New Zealand, 1982-83. *New Zealand Journal Marine and Freshwater Research* **20**: 397-405.

Malone, T. (1980). Algal size. In: Morris, I. (ed) *The physiological ecology of phytoplankton*. University of California Press, Berkley, pp. 433-463.

Mamaeva, N.V. (1985). Infusorians as a component of the planktonic community in the Baltic Sea. *Doklady Biological Sciences* **281**: 200-201.

Manahan, D.T. (1983). The uptake and metabolism of dissolved amino acids by bivalve larvae. *Biological Bulletin of the Marine Biological Laboratory, Woods Hole* **164**: 236-250.

Margalef, R. (1956). Estructura y dinámica de la "purga de mar" en el Rio de Vigo. *Investigacion Pesquera* **5**: 113-134.

Margalef, R., M. Estrada & D. Blasco (1979). Functional morphology of organisms involved in red tides, as adapted to decaying turbulence. In: Taylor, D.L. & H.H. Seliger (eds.) *Toxic Dinoflagellate Blooms*. Proceedings of the 2nd International Conference, Elsevier North Holland, Inc., Amsterdam, pp. 89-94.

McAlice, B.J. (1968). An occurrence of ciliate red-water in the Gulf of Maine. *Journal of the Fisheries Research Board of Canada* **25**: 1749-1751.

McCree, K.J. (1981). Photosynthetically active radiation. In: Lange, O.L., P. Nobel, B. Osmond & H. Ziegler (eds) *Encyclopedia of plant physiology*, Volume 12A (new series), Springer-Verlag, Berlin.

McManus, G.B. & J.A. Fuhrmann (1986). Photosynthetic pigments in the ciliate *Laboea strobila* from Long Island Sound, USA. *Journal of Plankton Research* **8**: 317-327.

Menzel, D.W. & N. Corwin (1965). The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. *Limnology and Oceanography* **10**: 280-282.

Meunier, A. (1910). *Microplankton des Mers de Barents et de Kara.* Duc d'Orléans Campagne Arctique de 1907, Charles Bulens, 355pp. + 37pl.

Michanek, G. (1965). Zooplankton of the Baltic - observations on some smaller species. In: Levring, T. (ed) Proceedings of the Fifth Marine Biological Symposium, *Botanica Gothoburgensia* **3**: 157-160.

Molitoris, E., S.W. Joseph, M.I. Krichovsky, W. Sindhuhardja & R.R. Colwell (1985). Characterization and distribution of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* isolated in Indonesia. *Applied and Environmental Microbiology* **50**: 1388-1394.

Montagnes. D.J.S. & D.H. Lynn (1987). A quantitative protargol stain (QPS) for ciliates: method description and test of its quantitative nature. *Marine Microbial Food Webs* **2**: 83-93.

Montagnes, D.J.S. & D.H. Lynn (1989). The annual cycle of *Mesodinium rubrum* in the waters surrounding the Isles of Shoals, Gulf of Maine. *Journal of Plankton Research* **11**: 193-201.

Montagnes, D.J.S., D.H. Lynn, D.K. Stoecker & E.B. Small (1988*a*). Planktonic ciliates with chloroplasts: taxonomic descriptions of one new species and redescriptions of four species in the family Strombidiidae (Ciliophora, Oligotrichida). *Journal of Protozoology* **35**: 189-197.

Montagnes, D.J.S., D.H. Lynn, J.C. Roff & W.D. Taylor (1988*b*). The annual cycle of heterotrophic planktonic ciliates in the waters surrounding the Isles of Shoals, Gulf of Maine: an assessment of their trophic role. *Marine Biology* **99**: 21-30.

Narita, H., S. Matsubara, N. Miwa, S. Akahane, M. Murakami, T. Goto, M. Nara, T. Noguchi, T. Saito, Y. Shida & K. Hashimoto (1987). *Vibrio alginolyticus*, a TTX-producing bacterium isolated from the starfish *Astropecten polyacanthus*. *Nippon Suisan Gakkaishi* **53**: 617-621.

Noguchi, T., D.F. Hwang, O. Arakawa, H. Sugita, Y. Deguchi, Y. Shida & K. Hashimoto (1987). *Vibrio alginolyticus*, a tetrodotoxin producing bacterium, in the intestines of the fish *Fugu vermicularis vermicularis*. *Marine Biology* **94**: 625-630.

Oakley, B.R. & F.J.R. Taylor (1978). Evidence for a new type of endosymbiotic organisation in a population of the ciliate *Mesodinium rubrum* from British Columbia. *BioSystems* **10**: 361-369.

Ohman, M.D. & R.A. Snyder (1991). Growth kinetics of the omniverous oligotrich ciliate *Strombidium* sp.. *Limnology and Oceanography* **36**: 922-935.

Owen, R.W., S.F. Gianesella-Galvão & M.B.B. Kutner (1992) Discrete, subsurface layers of the autotrophic ciliate *Mesodinium rubrum* off Brazil. *Journal of Plankton Research* **14**: 97-105.

Packard, T.T., D. Blasco & R.T. Barber (1978). *Mesodinium rubrum* in the Baja California upwelling system. In: Boje, R. & M. Tomczak (eds.) *Upwelling Ecosystems*. Springer-Verlag, Berlin, pp. 73-89.

Parsons, T.R. & D.J. Blackbourn (1968). Pigments of the ciliate *Mesodinium rubrum* (Lohmann). *Netherlands Journal of Sea Research* **4**: 27-31.

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

Parsons, T.R., M. Takahashi & B. Hargrave (1984*b*). *Biological Oceanographic Processes, 3rd Edition*. Pergamon Press, 330 pp.

Partensky, F. & D. Vaulot (1989). Cell size differentiation in the bloom-forming dinoflagellate *Gymnodinium* cf. *nagasakiense*. *Journal of Phycology* **25**: 741-750.

Partensky, F, D. Vaulot, A. Couté & A. Sournia (1988). Morphological and nuclear analysis of the bloom-forming dinoflagellates *Gyrodinium* cf. *aureolum* and *Gymnodinium nagasakiense*. *Journal of Phycology* **24**: 408-415.

Passow, U. (1991). Vertical migration of *Gonyaulax catenata* and *Mesodinium rubrum*. *Marine Biology* **110**: 455-463.

Paulsen, O. (1909). Plankton investigations in the waters round Iceland and in the North Atlantic in 1904. *Meddelelser fra Kommissionen for Danmarks Fiskeri- og Havundersøgelser Serie Plankton* **1**(8): 1-57.

Paulsen, O. (1934). Red "water-bloom" in Iceland Seas. *Nature (London)* **134**: 974.

Peterson, B.J. (1980). Aquatic primary productivity and the ^{14}C-CO_2 method: a history of the productivity problem. *Annual Review of Ecology and Systematics* 11: 359-385.

Platt, T., C.L. Gallegos & W.G. Harrison (1980). Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *Journal of Marine Research* **38**: 687-701.

Pollingher, U. & E. Zemel (1981). In-situ and experimental evidence of the influence of turbulence on cell division processes of *Peridinium cinctum* forma *westii* (Lemm.) Lefevre. *British Phycological Journal* **16**: 281-287.

Powers, P.B.A. (1932). *Cyclotrichium meunieri* sp. nov. (Protozoa, Ciliata); cause of red-water in the Gulf of Maine. *Biological Bulletin* **63**: 74-80.

Purcell, E.M. (1977). Life at low Reynolds number. *American Journal of Physics* **45**: 3-11.

Purdie, D.A., C.A.E. Garcia & D.W. Crawford (1988). The distribution of *Mesodinium* blooms in Southampton Water: Remote sensing studies using the airborne thematic mapper. *Proceedings of the NERC 1986 airborne campaign workshop*, Institute of Hydrology, Wallingford, U.K., pp. 143-147.

Purdie, D.A. & C.A.E. Garcia (1988). The use of airborne thematic mapper data in studying the effect of tidal state on the distribution of phytoplankton blooms in Southampton Water. *Proceedings of the NERC 1987 airborne campaign workshop*, University of Southampton, U.K., pp. 195-203.

Putt, M. (1990*a*). Metabolism of photosynthate in the chloroplast-retaining ciliate *Laboea strobila*. *Marine Ecology - Progress Series.* **60**: 271-282.

Putt, (1990*b*). Abundance, chlorophyll content and photosynthetic rates of ciliates in the Nordic Seas during summer. *Deep Sea Research* **37**: 1713-1731.

Putt, M. & D.K. Stoecker (1989). An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnology and Oceanography* **34**: 1097-1103.

Raven, J.A. & J. Beardall (1981). Respiration and photorespiration. *Canadian Bulletin of Fisheries and Aquatic Science* **210**: 55-82.

Raven, J.A. & K. Richardson (1984). Dinoflagellate flagella: a cost-benefit analysis. *New Phytologist* **98**: 259-276.

Rausch de Traubenberg, C. & P. Lassus (1991). Dinoflagellate toxicity: are marine bacteria involved? Evidence from the literature. *Marine Microbial Food Webs* **5**: 205-226.

Rees, T.A.V. & P.J.LeB. Williams (1982). *The role of phytoplankton in the Test Estuary*. Report to the Southern Water Authority. University of Southampton.

Reid, F.M.H., E. Stewart, R.W. Eppley & D. Goodmann (1978). Spatial distributions of phytoplankton species in chlorophyll maximum layers off southern California. *Limnology and Oceanography* **23**: 219-226.

Revelante, N. & M. Gilmartin (1983). The microzooplankton distribution in the northern Adriatic Sea with emphasis on the importance of ciliated protozoans. *Oceanologica Acta* **6**: 407-417.

Revelante, N. & M. Gilmartin (1987). Seasonal cycle of the ciliated protozoan and micrometazoan biomass in a Gulf of Maine estuary. *Estuarine and Coastal Shelf Science* **25**: 581-598.

Reynolds, C.S. (1973). The seasonal periodicity of planktonic diatoms in a shallow eutrophic lake. *Freshwater Biology* **3**: 89-110.

Roberts, A.M. (1981) Hydrodynamics of protozoan swimming. In: Levandowsky, M. & S.H. Hutner (eds) *Biochemistry and physiology of protozoa*, Academic Press, pp. 5-66.

Robinson, I.S. & P.M. Holligan (1987). Developments in ocean colour research in the U.K.. Ocean Colour. In: *Proceedings of an ESA Workshop*, Villefranche-sur-Mer, November 1986, ESA Publications.

Rodriguez, L. (1978). "Marea roja" en la Bahia de San Jorge, Antofagasta, Chile. *Noticario Mensual Museo Nacional de Historia Natural* **266**: 6-9.

Rohr, J., J. Losee & J. Hoyt (1990). Stimulation of bioluminescence by turbulent pipe flow. *Deep Sea Research* **37**: 1639-1646.

Romalde, J.L., A.E. Toranzo & J.L. Barja (1990*a*). Changes in bacterial populations during red tides caused by *Mesodinium rubrum* and *Gymnodinium catenatum* in North West Coast of Spain. *Journal of Applied Bacteriology* **68**: 123-132.

Romalde, J.L., J.L. Barja & A.E. Toranzo (1990*b*). Vibrios associated with red tides caused by *Mesodinium rubrum*. *Applied and Environmental Microbiology* **56**: 3615-3619.

Ryther, J.H. (1967). Occurrence of red-water off Peru. *Nature (London)* **214**: 1318-1319.

Sampayo, M.A. de M. & G. Cabeçadas (1981). Occurrence of red-water off Algarve. *Boletim Instituto Nacional de Investigação das Pescas, Lisboa* **5**: 63-87.

Sanders, R.W. (1987). Tintinnids and other microzooplankton - seasonal distributions and relationships to resources and hydrography in a Maine estuary. *Journal of Plankton Research* **9**: 65-77.

Satoh, H. & K. Watanabe (1991). A red-water bloom caused by the autotrophic ciliate, *Mesodinium rubrum*, in the austral summer in the fast ice near Syowa Station, Antarctica, with note on their photosynthetic rate. *Journal of the Tokyo University of Fisheries* **78**: 11-17.

Scott, J.M. (1985). The feeding rates and efficiencies of a marine ciliate, *Strombidium* sp., grown under chemostat steady state conditions. *Journal of Experimental Marine Biology and Ecology* **90**: 81-95.

Sellner, K.G. (1981). Primary productivity and the flux of dissolved organic matter in several marine environments. *Marine Biology* **65**: 101-112.

Setchell, F.W., D.W. Boisseau & T.T. Packard (1979). Respiration and ETS activity in the ciliate *Mesodinium rubrum*. In: Packard, T.T., D. Blasco & V. Jones (eds.) *Experiments with nitrate reductase and ETS in plankton from the Peru upwelling system*. CUEA Technical Report No. **54**, pp. 79-83.

Sherr, E.B., B.F. Sherr & G.A. Paffenhöfer (1986*a*). Phagotrophic protozoa as food for metazoans: A "missing" trophic link in marine pelagic food webs? *Marine Microbial Food Webs* **1**: 61-80.

Sherr, E.B., B.F. Sherr, R.D. Fallon & S.Y. Newell (1986*b*). Small aloricate ciliates as a major component of the marine heterotrophic nanoplankton. *Limnology and Oceanography* **31**: 177-183.

Sieburth, J.McN. & K.W. Estep (1985). Precise and meaningful terminology in marine microbial ecology. *Marine Microbial Food Webs* **1**: 1-16.

Sieburth, J.McN., V. Smetacek & F. Lenz (1978). Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnology and Oceanography* **23**: 1256-1263.

Simpson, J.H. & J.R. Hunter (1974). Fronts in the Irish Sea. *Nature (London)* **250**: 404-406.

Sleigh, M.A. & J.R. Blake (1977). Methods of ciliary propulsion and their size limitations. In: Pedley, T.J. (ed.) *Scale effects in animal locomotion*. Academic Press, pp. 243-256.

Slobodkin, L.B. (1953). A possible initial condition for red-tides on the coast of Florida. *Journal of Marine Research* **12**:148-155.

Small, E.B. & D.H. Lynn (1985). Phylum Ciliophora. In: Lee, J.J., S.H. Hutner & E.C. Bovee (eds.) *An illustrated guide to the Protozoa*. Society of Protozoologists, Lawrence, Kansas, pp. 393-440.

Smetacek, V. (1981). The annual cycle of protozooplankton in the Kiel Bight. *Marine Biology* **63**: 1-11.

Smith, W.O. (1977). The respiration of photosynthetic carbon in eutrophic areas of the ocean. *Journal of Marine Research* **35**: 557-565.

Smith, W.O. & R.T. Barber (1979). A carbon budget for the autotrophic ciliate *Mesodinium rubrum*. *Journal of Phycology* **15**: 27-33.

Sorokin, Yu.I. (1979). The 'red-tide' in the region of the Peruvian upwelling. *Doklady Biological Sciences* **249**: 253-256. English translation, *Doklady Akademii Nauk SSSR Series Biology* (1980): 1263-1266.

Sorokin, Yu.I. (1981). Microheterotrophic organisms in marine ecosystems. In: Longhurst, A.R. (ed.) *Analysis of Marine Ecosystems*. Academic Press, London, pp. 293-342.

Sorokin, Yu.I. & J. Kogelschatz, J. (1979). Analysis of heterotrophic microplankton in an upwelling area. *Hydrobiologia* **66**: 195-208.

Soulsby, P.G., M. Mollowney, G. Marsh & D. Lowthion (1984). The role of phytoplankton in the dissolved oxygen budget of a stratified estuary. *Water Science and Technology* **17**: 745-756.

Sournia, A. (1982). Form and function in marine phytoplankton. *Biological Reviews* **57**: 347-394.

Stainton, M.P. (1974). Efficient reduction column for use in the automated determination of nitrate in water. *Analytical Chemistry* **46**: 1616.

Steemann Nielsen, E. & V.K. Hansen (1959). Light adaptation in marine phytoplankton populations and its interrelation with temperature. *Physiologia Plantarum* **12**: 353-370.

Stein, F.R. (1862). Neue oder nicht genügend bekannte Infusorienformen. *Verhandlungen der Gesellschaft für Naturforschung Artze Karlsbad* **1862**: 161-162.

Stoecker, D.K. (1991). Mixotrophy in marine planktonic ciliates: physiological and ecological aspects of plastid-retention by oligotrichs. In: Reid, P.C., C.M. Turley & P.H. Burkill (eds) *Protozoa and their role in marine processes*, NATO ASI Ecological Series, Springer-Verlag, New York, pp.161-179.

Stoecker, D.K. & A.E. Michaels (1991). Respiration, photosynthesis and carbon metabolism in planktonic ciliates. *Marine Biology* **108**: 441-447.

Stoecker, D.K. & M.W. Silver (1990). Replacement and aging of chloroplasts in *Strombidium capitatum* (Ciliophora: Oligotrichida). *Marine Biology* **107**: 491-502.

Stoecker, D.K., A.E. Michaels & L.H. Davis (1987). Large proportion of marine planktonic ciliates found to contain functional chloroplasts. *Nature (London)* **326**: 790-792.

Stoecker, D.K., A. Taniguchi & A.E. Michaels (1989). Abundance of autotrophic, mixotrophic, and heterotrophic planktonic ciliates in shelf and slope waters. *Marine Ecology - Progress Series* **50**: 241-254.

Stoecker, D.K., M. Putt, L.H. Davis & A.E. Michaels (1991). Photosynthesis in *Mesodinium rubrum*: species-specific measurements and comparison to community rates. *Marine Ecology - Progress Series* **73**: 245-252.

Stoecker, D.K., M.W. Silver, A.E. Michaels & L.H. Davis (1988). Obligate mixotrophy in *Laboea strobila*, a ciliate which retains chloroplasts. *Marine Biology* **99**: 415-423.

Stoecker, D.K., M.W. Silver, A.E. Michaels & L.H. Davis (1988/1989). Enslavement of algal chloroplasts by four *Strombidium* spp. (Ciliophora, Oligotrichida). *Marine Microbial Food Webs* **3**: 79-100.

Strathmann, R.R. (1967). Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnology and Oceanography* **12**: 411-418.

Strickler, J.R. (1974). Swimming of planktonic *Cyclops* species (Copepoda, Crustacea): pattern, movements and their control. In: Wu, T.Y.-T. C.J. Brokaw & C. Brennen (eds) *Swimming and Flying in Nature*. Plenum Press, New York. pp. 599-613.

Swift, D.G. (1980). Vitamins and phytoplankton growth. In: Morris, I. (ed) *The Physiological Ecology of Phytoplankton*, Studies in Ecology, Vol. 7, Blackwell Scientific Publications, 625pp.

Takahashi, M. & K.D. Hoskins (1978). Winter condition of marine plankton populations in Saanich Inlet, B.C. Canada. II. Microzooplankton. *Journal of Experimental Marine Biology and Ecology* **32**: 27-37.

Tamar, H. (1979). The movements of jumping ciliates. *Archiv für Protistenkunde* **122**: 290-327.

Tamar, H. (1987). On division in a fresh-water *Mesodinium acarus*. *Acta Protozoologica* **26**: 213-218.

Taylor, F.J.R. (1982). Symbioses in marine microplankton. *Annales de l'Institut Océanographique* **Fascicule Supplémentaire 58**: 61-90.

Taylor, F.J.R. (1983). Some eco-evloutionary aspects of intracellular symbiosis. *International Review of Cytology* **Supplement 14**: 1-28.

Taylor, F.J.R. (1987). Ecology of dinoflagellates. In: Taylor, F.J.R. (ed) *The Biology of Dinoflagellates*. Botanical Monographs, Vol.21, Blackwell Scientific Publications, pp. 398-502.

Taylor, F.J.R., D.J. Blackbourn & J. Blackbourn (1969). Ultrastructure of the chloroplasts and associated structures within the marine ciliate *Mesodinium rubrum* (Lohmann). *Nature (London)* **224**: 819-821.

Taylor, F.J.R., D.J. Blackbourn & J. Blackbourn (1971). The red-water ciliate *Mesodinium rubrum* and its "incomplete symbionts": A review including new ultrastructural observations. *Journal of the Fisheries Research Board of Canada* **28**: 391-407.

Tett, P.B. (1987). Plankton. In: Baker, J.M. & W.J. Wolff (eds) *Biological Surveys of Estuaries and Coasts*. Estuarine and Coastal Sciences Association Handbook, Cambridge University Press, pp. 280-341.

Theusen, E.V. & K. Kogure (1989). Bacterial production of tetrodotoxin in four species of chaetognatha. *Biological Bulletin* **176**: 191-194.

Thomas, W.H. & C.H. Gibson (1990*a*). Quantified small-scale turbulence inhibits a red tide dinoflagellate, *Gonyaulax polyedra* Stein. *Deep Sea Research* **37**: 1583-1593.

Thomas, W.H. & C.H. Gibson (1990*b*). Effects of small-scale turbulence on microalgae. *Journal of applied Phycology* **2**: 71-77.

Throndsen, J. (1973). Motility in some marine nanoplankton flagellates. *Norwegian Journal of Zoology* **21**: 193-200.

Throndsen, J. (1978). Preservation and storage. In: Sournia, A. (ed.) *Phytoplankton manual. Monographs on oceanographic methodology 6.* Unesco Press, Paris, pp. 69-74.

Tumantseva, N.I. (1985). Red tide in the Black Sea. *Oceanology* **25**: 99-101.

Vaas, K.F. (1968). Annual Report, Division of Delta Research, Hydrobiological Institute of the Royal Netherlands Academy of Sciences for 1967. IV. Plankton. A. Veerse Meer. *Netherlands Journal of Sea Research* **4**: 274-276.

Venrick, E.L., J.R. Beers & J.R. Heinbokel (1977). Possible consequences of containing microplankton for physiological rate measurements. *Journal of Experimental Marine Biology and Ecology* **26**: 55-76.

Videau, C & F. Partensky (1990). Variability in the growth characteristics of *Gymnodinium* cf. *nagasakiense* (Dinophyceae) an its consequences for the determination of in situ growth rates. *Journal of Experimental Marine Biology and Ecology* **142**: 169-182.

Vlymen, W.J. (1970). Energy expenditure of swimming in copepods. *Limnology and Oceanography* **15**: 348-356.

Weiss, R.J. (1970). The solubility of nitrogen, oxygen and argon in water and seawater. *Deep Sea Research* **17**: 721-735.

Welschmeyer, N.A. & C.J. Lorenzen (1984). Carbon-14 labelling of phytoplankton carbon and chlorophyll *a* carbon: determination of specific growth rates. *Limnology and Oceanography* **29**: 135-145.

White, A.W. (1976). Growth inhibition caused by turbulence in the toxic marine dinoflagellate *Gonyaulax excavata. Journal of the Fisheries Research Board of Canada* **33**: 2598-2602.

White, A.W., R.G. Sheath & J.A. Hellebust (1977). A red tide caused by the marine ciliate *Mesodinium rubrum* in Passamaquoddy Bay, including pigment and ultrastructure studies of the endosymbiont. *Journal of the Fisheries Research Board of Canada* **34**: 413-416.

Whitfield, E.C. (1985). *A study of the oxygen changes associated with the respiration and photosynthetic activity of the ciliated protozoan* Mesodinium rubrum. M.Sc. Thesis, University of Southampton.

Wilkerson, F.P. & G. Grunseich (1990). Formation of blooms by the symbiotic ciliate *Mesodinium rubrum*: the significance of nitrogen uptake. *Journal of Plankton Research* **12**: 973-989.

Williams, P.J.LeB. (1980). Phytoplankton in Southampton Water. In: *The Solent Estuarine System.* NERC Publications, Series C, No. **22**: 73-75.

Williams, P.J.leB. (1981). Microbial contribution to overall marine plankton metabolism: direct measurements of respiration. *Oceanologica Acta* **4**: 359-364.

Wu, T.Y. (1977). Introduction to the scaling of aquatic animal locomotion. In: Pedley, T.J. (ed.) *Scale effects in animal locomotion.* Academic Press. pp. 203-232.

PUBLICATION LIST:

Thesis Publications

Crawford, D.W. (1989). *Mesodinium rubrum*: the phytoplankter that wasn't. *Marine Ecology - Progress Series* **58**: 161-174.

Crawford, D.W. & D.A. Purdie (1992). Evidence for avoidance of flushing from an estuary by a planktonic, phototrophic ciliate. *Marine Ecology - Progress Series* **79**: 259-265.

Crawford, D.W. (1992). An epibiont on the red-water ciliate *Mesodinium rubrum*. *Journal of the Marine Biological Association of the United Kingdom* **72**: 261-264.

Crawford, D.W. (1992). Metabolic cost of motility in planktonic protists: theoretical considerations on size scaling and swimming speed. *Microbial Ecology* **24**: 1-10.

Related Publications

Crawford, D.W.; D.A. Purdie & A.P.M. Lockwood (1992). Red-tides: recurrent red-water events in Southampton Water. *NERC News* **20**: 25-27.

Daneri, G.; D.W. Crawford & D.A. Purdie (1992). Algal blooms in coastal waters: a comparison between two adaptable phytoplankton species, *Phaeocystis pouchetii* and *Mesodinium rubrum*. *Science of the Total Environment* **(supplement 1992)**: 879-890.

Crawford, D.W.; L.E. Hawkins; S. Hutchinson; E.E. Antai; D.A. Purdie & A.P.M. Lockwood (1992). Red-tides of *Mesodinium rubrum*: evidence for remotely imposed stress on the oyster *Ostrea edulis*. In: *Proceedings of the Fifth International Conference on Toxic Marine Phytoplankton*, Newport, Rhode Island, 1991 (eds T.J. Smayda & Y. Shimuzu), Elsevier **in press**.

Vol. 58: 161–174, 1989
MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser.
Published December 15

REVIEW

Mesodinium rubrum: the phytoplankter that wasn't

David W. Crawford

Department of Oceanography, The University, Southampton SO9 5NH, United Kingdom

ABSTRACT: Recent reports suggest that the potential phototrophic role of plastidic ciliates in marine ecosystems may be considerable. A critical review of the literature demonstrates some confusion surrounding acceptance of the trophic position of even a well-established example of a photosynthetic ciliate, *Mesodinium rubrum*. Despite good evidence of obligate phototrophy from bloom studies, this species has, until recently, been omitted from the majority of routine phytoplankton counts, and has either been assigned to the microzooplankton or completely overlooked. Moreover, problems involved with sampling, enumeration and estimates of productivity for *M. rubrum* are also highlighted from the literature. These principally result from extremes of fragility, motility and vertical aggregation, which are commonly noted for this ciliate. Several recent studies, which have minimized some of these sampling problems and grouped the microplankton into more meaningful ecological categories, suggest that *M. rubrum* has an extremely widespread distribution and can be a very significant member of the phytoplankton. The combination of trophic and methodological difficulties appear to have compounded a serious underestimation of the contribution of *M. rubrum* to the primary productivity of coastal, estuarine and upwelling ecosystems, during both bloom, and perhaps more significantly, non-bloom conditions.

INTRODUCTION

Despite prolonged awareness of the presence of pigmented bodies in pelagic marine ciliates (e.g. Lohmann 1908, Leegard 1920, Kahl 1930–1935), it was not until the late 1960's that a potentially autotrophic role for chloroplast-bearing ciliates was first convincingly demonstrated through studies on blooms of *Mesodinium rubrum*[*] Lohmann (Bakker 1967a, Ryther 1967, Barber et al. 1969), an unidentified oligotrich (Burkholder et al. 1967), and a *Prorodon*-like species (Holm-Hansen et al. 1970).

Even though the photosynthetic capabilities of these forms, particularly *Mesodinium rubrum*, are now beyond doubt, most research has been carried out during blooms and surprisingly little is understood of their contribution as primary producers to the planktonic community under non-bloom conditions. However, several recent studies have demonstrated *M. rubrum* to be a significant, often dominant phototrophic member of the marine microplankton during certain periods of the year (e.g. Revelante & Gilmartin 1983, 1987, Leppänen & Bruun 1986, Stoecker et al. 1989).

An important ecological role has also been suspected for other chloroplast-bearing ciliates since the studies of Blackbourn et al. (1973) and later Mamaeva (1985). Laval-Peuto et al. (1986), Montagnes et al. (1988b), and Lindholm & Mörk (1989) have shown chloroplast retention in a number of ciliates at the morphological and ultrastructural levels, and, with a more physiological approach, Stoecker et al. (1988) demonstrated obligate mixotrophy in the ciliate *Laboea strobila* Lohmann. The ecological role of plastidic ciliates has been fully emphasized in recent studies by Jonsson (1987), Stoecker et al. (1987, 1989) and Laval-Peuto & Rassoulzadegan (1988).

Taylor et al (1971) and Lindholm (1985) have

---

[*] The name *Mesodinium rubrum* Lohmann is retained here rather than the more recently proposed *Myrionecta rubra* Jankowski (see Small & Lynn 1985), as many populations exhibit the bifurcate 'oral' tentacles which that description presumes to be lacking (Lindholm et al. 1988). The unique 14 microtubule structure of these oral tentacles has recently been described by Lindholm et al. (1988). *Cyclotrichium meunieri* Powers is considered conspecific with *M. rubrum* (Fenchel 1968, Taylor et al. 1971), and photosynthetic forms of *Mesodinium pulex* Claparède et Lachmann are assumed to represent *M. rubrum*. Taxonomic difficulties have been ignored for the purpose of this contribution. However, it is accepted that *M. rubrum* may eventually be shown to represent a species complex, rather than a single species (Lindholm 1985).

0171-8630/89/0058/0161/$ 03.00

reviewed the information relating to bloom occurrence, taxonomic problems and ultrastructure of *Mesodinium rubrum*, but did not consider in detail the relative importance of this species as a primary producer under 'normal' conditions. The present paper reviews literature concerning acceptance of the trophic position of *M. rubrum* under such conditions, with a view to stimulating examination of the role of phototrophic and mixotrophic ciliates generally, for which little ecological information is available.

In addition to these trophic considerations, a number of important methodological difficulties concerning *Mesodinium rubrum* are also addressed and reviewed, particularly those relating to behavioural, structural, and physiological peculiarities of this curious ciliate.

TROPHIC CONFUSION

Without its capacity to form red-water, *Mesodinium rubrum* could well have long remained unknown as a primary producer (Lindholm 1985).

The pigmented bodies noted by earlier authors (e.g. Lohmann 1908, Leegard 1920) were later identified as chromatophores with associated pyrenoid bodies (Powers 1932, Bary & Stuckey 1950). More recent studies also examined mass occurrences of *Mesodinum rubrum* (Bakker 1966, 1967a, Fonds & Eisma 1967, Ryther 1967, Fenchel 1968, McAlice 1968, Parsons & Blackbourn 1968, Barber et al. 1969, Taylor et al. 1969, 1971), all of which either suspected or verified its photosynthetic ability. Packard et al. (1978), Smith & Barber (1979), and Cabecadas et al. (1983) demonstrated it to be an exceptionally productive phototroph. During a dense oceanic bloom in the upwelling area off the coast of Peru, Smith & Barber (1979) measured values for chlorophyll *a* as high as 1000 $\mu g\ l^{-1}$, an assimilation number of 16.8 mg C (mg Chl *a*)$^{-1}$ h^{-1} and productivity of over 2000 mg C m^{-3} h^{-1}. Taylor (1982) considered this productivity rate to be the highest aquatic micro-organismal primary productivity on record. He also suggested the association between *M. rubrum* and its cryptomonad symbiont to be the most strongly integrated known, between host and photosynthetic cytobiont.

Despite this remarkable photosynthetic capacity, it is ironic that confusion has surrounded the phototrophic role of this ciliate under non-bloom conditions. The reasons for this are not clear, since no convincing evidence of heterotrophic feeding has been presented and intact chloroplasts appear to be present throughout the year (Taylor et al. 1971, Lindholm 1985, Crawford pers. obs. Southampton Water). It is possible that the highly variable size distribution (see Leegard 1920, Michanek 1965, Taylor et al. 1971, Lindholm 1985, Montagnes & Lynn 1989, Crawford pers. obs. Southampton Water) may, in part, have generated taxonomic and thus trophic uncertainty (e.g. see Revelante & Gilmartin 1987). Sorokin (1979, 1981) and Tumantseva (1985) suggested that *Mesodinium rubrum* combines phototrophy with phagotrophy upon bacterioplankton, but provide little clear supporting evidence. This view is not supported by observations that *M. rubrum* lacks a true cytostome (Bary & Stuckey 1950, Taylor et al. 1971, Hibberd 1977, Grain et al. 1982, Lindholm et al. 1988). Bakker (1966, 1967a, b) favoured the idea that *M. rubrum* is a temporary photosynthetic modification of the colourless *Mesodinium pulex* Claparède et Lachmann, with an accompanying degeneration of the cytostome. Tamar (1971) described such a dimorphism in the oral cone of a related species, *Mesodinium fimbriatum* Stokes, however, Taylor et al. (1971) have pointed out that the exceptional thinness of the pellicle of *M. rubrum*, with reduced alveoli, is a major modification unlikely to be accomplished on a short term basis. *M. rubrum* has only been shown to behave heterotrophically in the sense of active amino acid uptake (Smith & Barber 1979), a capability shared by a number of autotrophic forms (e.g. Hellebust 1970). However, it cannot survive in darkness in the laboratory for more than a few days (Taylor et al. 1969, 1971, Lindholm 1985).

Although earlier descriptive microplankton studies (e.g. Leegard 1920) did not specify the trophic mode of *Mesodinium rubrum* at non-bloom densities, some later ones (e.g. Michanek 1965) placed the ciliate within the microzooplankton category. However, despite the developing interest in the photosynthetic ability of *M. rubrum* in the late 1960's, many later studies continued to classify it as a microzooplankter (e.g. Takahashi et al. 1975, Beers et al. 1977a,b, Eriksson et al. 1977, Takahashi & Hoskins 1978). In some instances, this was due to cautious identification to genus level (e.g. Beers & Stewart 1971, Dale & Burkill 1982). Other studies, briefly noting the occurrence of *M. rubrum*, have not specified its trophic position (Parsons et al. 1977, Burkill 1982, Stoecker et al. 1984). The confusion can be emphasised by contrasting the study of Beers et al. (1971), which assigned relatively low numbers of *M. rubrum* to the phytoplankton, with later works by the same author (Beers et al. 1977a, b) placing it within the microzooplankton. A more recent study by Beers et al. (1980) correctly placed *M. rubrum* back with the phytoplankton.

Microzooplankton and more general microplankton studies from the late 1970's onwards have gradually acknowledged the autotrophic status of *Mesodinium rubrum* (e.g. Chester 1978, Lindholm 1981, Smetacek 1981, Revelante & Gilmartin 1983, 1987, Andersen & Sorensen 1986, Kivi 1986, Leppänen & Bruun 1986, McManus & Fuhrmann 1986, Sherr et al. 1986, Sanders 1987, Laval-Peuto & Rassoulzadegan 1988, Montagnes

& Lynn 1989, Stoecker et al. 1989). This may have partly resulted from a strong recommendation by Sieburth et al. (1978) that *M. rubrum*, being a functional phototroph, should be classified accordingly.

If the microzooplanktologists have appeared rather slow in releasing *Mesodinium rubrum* from their trophic grouping, then the phytoplanktologists have shown an even greater reluctance in its acceptance. Even now *M. rubrum* is often conspicuously absent from species lists of 'phytoplankton' surveys, even in areas where more specific studies have shown it to be relatively abundant throughout the year. This may in some instances have left *M. rubrum* in an ecological 'vacuum', acknowledged as phototrophic and thus ignored from some heterotrophic ciliate counts (e.g. Smetacek 1981, Kivi 1986), but then perhaps not recognised in corresponding phytoplankton surveys of the same area.

A good example of the complete omission of *Mesodinium rubrum* from routine counts is given by Gieskes & Kraay (1983), in a phytoplankton study of the central North Sea. This study demonstrated an absence of cryptophycean flagellates in cell counts, yet using HPLC pigment 'fingerprinting' they detected relatively high levels of alloxanthin, a pigment diagnostic of that algal class. Extremely low phytoplankton carbon : chlorophyll *a* ratios were also noted, suggesting possible omission of part of the phytoplankton community. Subsequent re-examination by the authors of the Lugol's iodine preserved samples revealed *M. rubrum* in relative abundance (25 to 50 cells ml^{-1}) and in sufficient numbers for its cryptomonad endosymbiont (Parsons & Blackbourn 1968, Barber et al. 1969) to contribute to much of the phytoplankton chlorophyll *a* and alloxanthin. The vast majority of phytoplankton studies do not have the benefit of HPLC as a chemotaxonomic tool. Consequently, significant numbers of *M. rubrum* may well have been ignored on many occasions.

In Southampton Water, UK, *Mesodinium rubrum* has apparently even been missed as the cause of annual red-water. These intense blooms (> 100 µg Chl *a* l^{-1}) have been known since the turn of the century (Royal Commission 1911), and were then assumed to be caused by a peridinean dinoflagellate, although *M. rubrum* was probably to blame (Williams 1980). *M. rubrum* was first noted as the causative species by Williams (1980) and Soulsby et al. (1984), but received no mention in earlier publications on phytoplankton (Savage 1965, 1967), zooplankton (Raymont & Carrie 1964, Barlow & Monteiro 1979), or planktonic oxygen consumption (De Sousa Lima & Williams 1978). This probably resulted both from confusion in its trophic position and inadequate sampling methods, but again emphasizes the potential underestimation of this species.

There are obviously a number of recent exceptions to the failure to include *Mesodinium rubrum* in phytoplankton species lists, for example, Reid et al. (1978) and Cullen et al. (1982) in the southern Californian bight, Blasco et al. (1980, 1981) in the North-West African upwelling, Holligan et al. (1984) in the Gulf of Maine, Cloern et al. (1985) in San Fransisco Bay, Mackenzie & Gillespie (1986) and Mackenzie et al. (1986) in New Zealand coastal waters.

Though acceptance of the autotrophic role of *Mesodinium rubrum* has improved considerably, problems persist with respect to the use of data from previous studies. In view of the relatively recent interest in ciliate trophic dynamics, considerable use can be made of sparse data, which may have been collected before the trophic modes of individual species were known in detail. For example, in a review of trophic dynamics in the Baltic Sea, Elmgren (1984) suggested that the role of ciliates as grazers of phytoplankton, described by Eriksson et al. (1977), was excessive since *M. rubrum* accounted for one third of the ciliate volume estimated in that study.

Failure to recognise the contribution of phototrophic and mixotrophic forms may also make re-analysis of data from previous studies problematical. Data from the earlier studies of Beers & Stewart (1969a, 1970, 1971) in the waters off southern California and in the eastern tropical Pacific has often been quoted in recent comparisons of heterotrophic ciliate abundances, despite the authors concession of the possibility of other modes of nutrition occurring in some species. In southern Californian coastal waters in March 1976, Reid et al. (1978) found *Mesodinium rubrum* to be an abundant phytoplankton species in the region generally, and the second most important species after *Exuviella* sp. (= *Prorocentrum*) with respect to the chlorophyll maximum. Cullen et al. (1982) also found *M. rubrum* to be abundant in this area in August 1978. However, Goodman et al. (1984) did not mention *M. rubrum* in this region during a study sampling at weekly intervals throughout the spring and summer. Closer examination reveals that this latter statistical study was actually based on a data set collected in 1967 by J. D. H. Strickland's Food Chain Research Group (Eppley et al. 1970, Reid et al. 1970). Although many other factors could be invoked, it would appear unlikely that *M. rubrum* was recorded in routine phytoplankton counts at that time. It is interesting to note that the corresponding microzooplankton contribution (Beers & Stewart 1970) to this study made no particular mention of *M. rubrum* either, though in retrospect the sampling methods (see subsequent sections) may not have been adequate for this species. Other reports do confirm the common occurrence of *M. rubrum* in this region (Lackey & Glendinning 1963, 1965, Lackey 1967, Beers et al. 1980). Future microplankton studies should adopt more ecologically appropriate categories, as recommended some time ago by Sieburth et al. (1978) and emphasised more recently by Sieburth & Estep (1985).

FIELD SAMPLING LIMITATIONS

Nets

The use of nets is not generally considered to be acceptable for the quantitative study of either phytoplankton (Tangen 1978) or microzooplankton (Beers 1978b). Ciliates are particularly susceptible (e.g. Margalef 1967, Beers & Stewart 1970), due either to physical damage or to cells passing through the mesh. Smetacek (1981) clearly demonstrated the detrimental effect of using nets, upon numerical estimates of non-loricate ciliates in the Kiel Bight. However, nets have been used extensively in semi-quantitative phytoplankton studies (Tangen 1978). The largest cells of *Mesodinium rubrum* occur during blooms and overlap the 'net phytoplankton' size category. This species has been noted by Lindholm (1985) to disintegrate totally in nets and as a consequence net sampling may have led to some erroneous reports of red-tide causal species (Lindholm 1985). The smaller cells of *M. rubrum*, usually encountered outside of bloom conditions, would either pass right through or disintegrate in even the finest nets used. The use of nets cannot therefore be considered acceptable even for semi-quantitative phytoplankton study, in view of the potential role of *M. rubrum* and possibly other ciliates as primary producers.

Pumps

Pump sampling is frequently employed as a means of investigating microplankton distributions. Samples for phytoplankton abundance, in particular, are often taken from the pump outflow for continuously monitored chemical and biological parameters. Beers (1978a) has given a detailed review of the use of pumps for sampling the phytoplankton.

Beers et al. (1967) described a pumping/ concentrating system based on a submersible centrifugal pump, for the quantitative sampling and concentration of microzooplankton and phytoplankton. This has been used in several subsequent studies (e.g. Beers & Stewart 1967, 1969a, b, 1970, 1971) which have contributed significantly to much of the current thinking on the role of ciliates in marine ecosystems. The use of these systems is defended by Beers et al. (1967) and Beers (1978a), who suggest that damage can be reduced to negligible proportions. However, Beers et al. (1967) did note significant damage to the more fragile forms such as the the dinoflagellate *Noctiluca* sp., while Beers et al. (1971) noted damage to aloricate ciliates in unconcentrated samples taken from a pump fitted to the ships hull. Sorokin (1981) has criticized the use of pumps generally, with even the most gentle concentrating procedures, for sampling ciliates, and further suggests that more than 95 % of both naked and loricate forms may be lost by such methods. In view of these criticisms, it appears unlikely that such a delicate species as *Mesodinium rubrum* (Lindholm 1985) could survive these sampling methods without a noticeable effect on cell numbers. Burkill (1982) did observe a significant reduction in the numbers of *M. rubrum* cells using a submersible centrifugal pumping system, whilst numbers of other microplankton species appeared unaffected. However, the centrifugal type pump is considered by Beers (1978a) to be potentially more damaging to plankters, because of the manner in which water is propelled.

Gentle pumping systems may be more promising, though quantitative information is lacking. For instance, Grice et al. (1980) noted a significant population of *Mesodinium rubrum* in a Controlled Ecosystem Pollution Experiment (CEPEX) enclosure in Saanich Inlet, British Columbia, Canada, when sampling with a peristaltic pump. However, such slower intake pumps are more susceptible to the problem of avoidance of the pump intake by the more motile forms (Beers 1978a). Though this problem is usually confined to the larger zooplankton, the well documented swimming capabilities and rheotactic behaviour of *M. rubrum* (Taylor et al. 1971, Lindholm 1985) render it a strong candidate for potential avoidance. Representative sampling of *M. rubrum* using any form of pump system must be considered questionable, at least until adequate quantitative comparisons have been undertaken between different methods. Furthermore, populations of *M. rubrum* enumerated from pumped samples should be considered minimal estimates.

Water bottles

Water bottles are the most widely adopted device for quantitatively investigating the distribution of phytoplankton and probably represent the most effective means of accurately sampling fragile species. A review of the use of water bottles in phytoplankton sampling is given by Venrick (1978).

Although water bottles may be considered potentially to have the least damaging effect on *Mesodinium rubrum*, these devices are not without drawbacks and care must be exercised during sampling. In water samplers such as the 'Van-Dorn', which may incorporate small 'windows' for the indication of water level, *M. rubrum* could quickly concentrate close to the light source and consequently be undersampled. Although this effect has not been described in a sampling study to date, phototaxis and motility are well documented (Lindholm 1985). This response to light has been effectively utilized by Lindholm (1981) to concentrate *M. rubrum* sampled in low numbers. Similarly, *M. rubrum* has been noted by Cabecadas et al. (1983) to accumulate at the top of a 'Van-Dorn' bottle whilst samples were drawn from the bottom, an effect considered by Lindholm (1985) to result from positive rheotaxis and high swimming speed. Thus, it is recommended that samplers be gently mixed if at all possible, immediately prior to subsampling, which should be done as soon as possible after sample collection.

Vertical distribution and migration

Though the sampling problems described are not insurmountable, and may be minimized, ignorance of the vertical distribution and migration of *Mesodinium rubrum* can lead to gross underestimates of its population density. Whilst this is a general caution for much of the motile plankton, *M. rubrum* is particularly susceptible as a result of its exceptionally high swimming speed (see Lindholm 1985). When hydrodynamical conditions permit, *M. rubrum* forms marked sub-surface and occasionally surface accumulations over extremely restricted depth intervals, both during blooms (Lindholm 1978, Packard et al. 1978, Smith & Barber 1979, Sorokin & Kogelschatz 1979, Cabecadas et al. 1983) and under normal conditions (Takahashi & Hoskins 1978, Lindholm 1981, McManus & Fuhrmann 1986, Dale 1987a). These characteristic vertical distributions have been shown to result from diurnal vertical migration apparently in response to light. It has been known for some time that *M. rubrum* exhibits a strong phototactic response (Bary & Stuckey, 1950). Smith & Barber (1979) further suggested that the diurnal migration resulted from positive phototaxis in increasing light, and negative phototaxis in decreasing light. In the Southampton Water estuary, Soulsby et al. (1984) have also shown an apparently phototactic diurnal migration of *M. rubrum*, while Crawford & Purdie (unpubl.) have evidence of vertical movements of the population in response to tidal motions, superimposed upon this diurnal pattern.

These migrations can lead to a vertical distribution in which the vast majority of the daytime population may be concentrated close to the surface. Although more pronounced during blooms, sub-surface maxima are a regular feature of the population in Southampton Water, except during strong winds (Crawford pers. obs.). Takahashi & Hoskins (1978) found an average of >65 % (mostly >90 %) of the *Mesodinium rubrum* population to be concentrated in the top 5 m throughout the winter in Saanich Inlet, British Columbia. In the Peru upwelling zone, *M. rubrum* has been shown to migrate vertically by as much as 30 to 40 m, commencing at dawn and arriving at the surface by late morning (Smith & Barber 1979, Sorokin 1979, Sorokin & Kogelschatz 1979, Barber & Smith 1981). This migration was monitored by Smith & Barber (1979) as a dense band of chlorophyll, about 60 $\mu g\ l^{-1}$ Chl *a*, while 10 m above and below this layer the chlorophyll concentration was 2 $\mu g\ l^{-1}$. Chlorophyll concentrations did reach 1000 $\mu g\ l^{-1}$ in a 10 to 20 cm thick layer (Smith & Barber 1979). Dale (1987a) demonstrated that the abundance maximum of *M. rubrum* in Lindåspollene, Norway, underwent a diurnal vertical migration from between 2 and 5 m depth during the day to 15 m at night. Vertical migration of *M. rubrum* has also been observed in Lindåspollene by Dale (1987b, 1988), and in New Zealand coastal waters by Mackenzie & Gillespie (1986) and Mackenzie et al. (1986). In the North Sea, Baars & Franz (1984) speculated that a diurnal fluctuation in surface chlorophyll could have been due to an internal rhythm of the symbiotic association in *M. rubrum*, which was abundant at the time. This fluctuation could equally have been caused by vertical migration of this species.

Given such distributions and migration, the depth intervals of bottle or pump sampling usually adopted in phytoplankton or microzooplankton studies (e.g. surface and 10 or 5 m intervals) could seriously underestimate the contribution of this species to the community. For instance Revelante & Gilmartin (1987) found *Mesodinium rubrum* to be an important species in the Damariscotta Estuary, Gulf of Maine, but sampling with a water bottle at the surface, 7, 15, and 22 m, could have significantly underestimated the population. Similarly, Montagnes & Lynn (1989) and Stoecker et al. (1989) sampled at ca 10 m intervals during examinations of the role in productivity of *M. rubrum* in other regions of the Gulf of Maine.

Smith & Barber (1979) suggested that the contribution to productivity of *Mesodinium rubrum*, even during blooms, could also be seriously underestimated, since at sea, samples for physiological rate incubations are often taken from the surface in the early morning before *M. rubrum* has completed its upward migration (see also Mackenzie et al. 1986). In situ fluorometry can overcome some of the problems of describing vertical distributions (e.g. Soulsby et al. 1984), but this can only be employed to give information on a single species during periods of total dominance, as encountered during blooms.

Some studies have taken integrated samples from pump intakes lowered over given depth ranges. Although this could potentially give a more accurate estimation of the total population in the water column, the procedure is nevertheless subject to the limitations of pumping samples already described. The potentially distorting effect of sampling upon determinations of the vertical distribution of *Mesodinium rubrum*, cannot be overstated. During design of field sampling programs, particular attention must be given to sample depth interval, time of day, and state of tide.

Horizontal distribution

The extremely inhomogenous horizontal distribution of *Mesodinium rubrum* is a further complication hindering sampling of this species. Blooms of *M. rubrum* have been noted to occur in irregular patches or clouds during calm conditions (Fenchel 1968, Packard et al. 1978), and aligned in streaks or 'windrows' when wind speed increases (Powers 1932, Packard et al. 1978). In a dynamic environment such as the Southampton Water estuary, streaks and patches of red-water caused by *M. rubrum* can be advected rapidly past the sampling station, on time and distance scales of seconds and metres respectively (Crawford pers. obs.). Clearly this presents considerable problems for representative sampling.

The potential of remotely sensed imagery for providing a more synoptic insight into the horizontal distribution and dynamics of blooms of *Mesodinium rubrum* appears impressive. In addition to providing some of the highest chlorophyll concentrations encountered in the marine environment, the cryptomonad endosymbiont of *M. rubrum* also possesses a red phycobiliprotein pigment, giving blooms their characteristic colour. Other than the cryptophyceae, this pigment is only found in the cyanophycean and rhodophycean classes of algae (White et al. 1977). Another pigment diagnostic of the cryptophyceae is alloxanthin, which has been detected by HPLC when *M. rubrum* is abundant in the North Sea (Gieskes & Kraay 1983).

Considerable success has been achieved in detecting blooms of *Mesodinium rubrum* in British Columbian coastal waters, using the Fluorescence Line Imager (Lin et al. 1984) and, with more limited success in Southampton Water, using the 'Daedalus' airborne thematic mapper (Purdie et al. 1988). The resolution obtained by Purdie et al. (1988) was limited by the sparsity of sea-truth measurements, which prohibited a statistically valid calibration of the spectral data set. However, more recent overflights using the same instrument (Purdie & Garcia 1988) have mapped, in detail, changes in the distribution of a bloom of *M. rubrum* over part of the tidal cycle in the Southampton Water estuary. A bloom of *M. rubrum* in this estuary in July 1984 was also detected by satellite, through processing of Landsat TM data (Robinson & Holligan 1987).

Although these preliminary remote sensing studies appear promising, difficulties associated with the vertical migration of motile phytoplankton, and particularly *Mesodinium rubrum*, must be considered. Diurnal migrations of 30 to 40 m, such as those undertaken by *M. rubrum* in the ocean upwelling off Peru (Smith & Barber 1979, Sorokin 1979, Sorokin & Kogelschatz 1979, Barber & Smith 1981), pose problems for the remote sensing of ocean colour, since this principally involves detection of integrated near surface colour. Because the blooms are only present close to the surface between about 11.00 and 15.00 h, any remotely sensed data collected outside of this period may completely ignore a substantial proportion of the depth integrated chlorophyll. Although vertical migration of this species in coastal waters and estuaries is more limited (e.g. Soulsby et al. 1984, Dale 1987a), and may be complicated by tidal effects, the penetrating capabilities of detectors is also reduced in these more turbid environments, and difficulties resulting from migration may also be envisaged. Meaningful interpretation of remotely sensed images from areas susceptible to blooms of *M. rubrum* are limited without detailed knowledge of the local dynamics of migrations of this species.

ENUMERATION

Live counting

The technique of live counting is advocated by some (e.g. Sorokin 1981) to be the only reliable means of enumerating pelagic ciliates. However, movement itself can be a problem in the live observation of highly motile ciliates. *Mesodinium rubrum* is a particular problem since it alternates between periods of total motionlessness, and such explosive bursts of swimming activity that the direction of these is impossible to follow out of the field of view (Lindholm 1985). Various narcotizing agents have been successfully employed to immobilize *M. rubrum*, such as hydrogen peroxide (Bary & Stuckey 1950), MS222 (Sandoz, Basel) (Lindholm 1978,1981), EDTA (ethylenediaminetetraacetate) and oxalic acid (Lindholm 1982) and droperiodol (Tumantseva 1985). Zaika & Averina (1968) apparently used, to their advantage, the rheotactic swimming behaviour exhibited by *M. rubrum* and other ciliates, by adjusting the rate of gravitational flow of water through a capillary tube until just sufficient to counter the movement of the ciliates, which could then be enumerated.

An additional problem is that *Mesodinium rubrum* is so delicate that it often explodes under the microscope, due to thermal effects and possibly physical damage (Taylor et al. 1971, Lindholm 1985). Dale & Burkill (1982) described an isothermal cell counting chamber in which ciliates can be enumerated in the live state and which overcomes the problem of thermal shock. With this technique they demonstrated an improvement of up to 20 % over counting of conventionally fixed pelagic ciliate species. However, numerical estimates of *Mesodinium* spp. were shown to be some 20 % lower than those from fixed samples. This they attributed either to the difficulty in observation of the very small forms of this genus when motionless, or to misidentification and overestimation of poorly fixed specimens of other genera in the fixed counts. Alternatively, it may simply be that *M. rubrum* and other *Mesodinium* spp., being so extremely delicate, are particularly sensitive to handling or containment of any description. Thus, live counting is arguably not the optimum method of enumeration for this species. Additionally, in any sampling program, the time constraints involved with live counting have to be 'weighed' against the convenience of fixing samples and the subsequent increase in resolution of sampling spatial and temporal distributions, which are of such critical importance in abundance estimates of *M. rubrum*.

Fixation and preservation

In common with the majority of naked ciliates (e.g. Sorokin 1981), the fixation and subsequent preservation of *Mesodinium rubrum* cells in a recognisable state is a major difficulty. This problem may be more serious for *M. rubrum* due to its structural weakness, probably resulting from the thin pellicle, reduced alveoli and high degree of internal vacuolation (Taylor et al. 1971, Hibberd 1977, Grain et al. 1982). *M. rubrum* has been noted not only to distort, as do other naked ciliates, but often to disintegrate or explode, both in commercial formalin (Taylor et al. 1971) and buffered formaldehyde (e.g. Ryther 1967, White et al. 1977, Sampayo & Cabecadas 1981). Although Fonds & Eisma (1967), Fenchel (1968), McAlice (1968) and Lindholm (1978) all found formaldehyde fixation adequate for counting cells during blooms, the relative fraction of cells that disintegrated is unknown. Moreover, positive identification of distorted cells under non-bloom conditions is much less certain. Thus the use of formaldehyde for routine microplankton counts must be considered questionable.

Several microplankton studies employing buffered formaldehyde as a fixative have noted numerous poorly fixed ciliate specimens, e.g. 'apparently not oligotrichs' (Beers & Stewart 1971) and 'predominantly holotrichs' (Beers et al. 1975). Beers & Stewart (1971) suggested that in their category of 'other ciliates' (other, that is, than sheathed or unsheathed oligotrichs), which included the holotrichs *Mesodinium* sp. and *Didinium* sp., almost 40 % of specimens were of questionable identification due to poor fixation. Jimenez & Intriago (1987) have commented on the lack of records of *Mesodinium rubrum* in phytoplankton counts in the eastern Pacific, caused by the use of 'formalin solution' as a routine fixative. Several studies recording *M. rubrum* as common have used buffered formaldehyde as a fixative and may be regarded as potential underestimates (e.g. Reid et al. 1978, Cullen et al. 1982).

The use of formaldehyde in fixation and enumeration of *Mesodinium rubrum* can be improved to some extent by combination with epifluorescence microscopy, with which the characteristic orange-red autofluorescence of the phycoerythrin pigment is apparent. This was utilised effectively by McManus & Fuhrmann (1986), Sherr et al. (1986), and Stoecker et al. (1989), all of whom recorded *M. rubrum* as abundant.

Lugol's iodine appears to be a rather more reliable routine fixative for *Mesodinium rubrum* (Jimenez & Intriago 1987, Crawford pers. obs.), and has been experimentally established to preserve non-loricate ciliates better than buffered formaldehyde, which caused 30 to 70 % loss of these forms (Revelante & Gilmartin 1983). Taylor et al. (1971) also noted cells to remain intact in Lugol's iodine, though with a serious disorganisation of the cirri and ciliary belt. Several recent studies employing Lugol's iodine as a fixative have recorded *M. rubrum* as an important component of the plankton (e.g. Gieskes & Kraay 1983, Revelante & Gilmartin 1983, 1987, Andersen & Sorensen 1986, Kivi 1986, Leppänen & Bruun 1986). Some caution should be excercised in the use of Lugol's iodine however, since Crawford (unpubl.) has preliminary evidence of a sensitivity in the fixation of *M. rubrum* cells to the concentration of Lugol's adopted. The weakest solutions (e.g. Kimor 1976) result in up to 100 % cell lysis. This is unfortunate, since guidelines in the literature for the concentration and quantity of Lugol's iodine required are rather vague, and can vary by at least an order of magnitude (e.g. contrast recommendations by Kimor 1976 with those of Throndsen 1978a). At least until adequate comparisons have been undertaken, the stronger acid version (e.g. Throndsen 1978a) is recommended (100 g KI, 50 g iodine, 100 ml glacial acetic acid, 1 l distilled water), in a ratio of 100:1 (sample : fixative).

A number of other fixatives have been utilized in cytological studies on *Mesodinium rubrum* such as Bouin's solution (Powers 1932, White et al. 1977), Schaudinn's fluid (Powers 1932, Bary & Stuckey 1950) and, more commonly, buffered glutaraldehyde (Taylor et al. 1969, 1971, Hibberd 1977, Oakley & Taylor 1978, Grain et al. 1982, Lindholm et al. 1988). Sampayo & Cabecadas (1981) and Cabecadas et al. (1983) found glutaraldehyde better than either formaldehyde or Lugol's iodine for routine fixation of *M. rubrum*. White et al. (1977) tried a range of fixatives on samples from a bloom of *M. rubrum*; the best results were obtained using osmium tetroxide. However, none of these studies gave any indication of the fraction of disintegrated cells. Moreover, the use of these fixatives for routine counts is normally precluded by their cost, their toxicity, and their more complex method of application (Kimor 1976).

For preservation of the characteristic pattern of cilia and cirri of living *Mesodinium rubrum* cells, the only fixative successfully employed to date has been Parducz's fixative (Parducz 1966), which was utilized extensively for light microscopy in the comprehensive study by Taylor et al. (1971).

The simplest and most reliable fixative to date for *Mesodinium rubrum* appears to be Lugol's iodine, though this may cause problems in its acid form for other groups such as coccolithophorids (Kimor 1976, Throndsen 1978a) and loricate oligotrichs (Dale & Dahl 1987). Recent developments with protargol staining after Bouin's fixation (Montagnes & Lynn 1987) appears promising for the study of *M. rubrum* (Montagnes & Lynn 1989) and ciliates generally (Montagnes et al. 1988a)

Future perspectives

Any form of enumeration that necessitates removal of microplankton samples from their environment, must introduce at least some bias and probably damages the more delicate forms. Perhaps the most valuable means of sampling fragile microplankton would be an in situ, non-destructive, perhaps photographic, type of detector. The only development in this direction has been that of Beers et al. (1970), who described the use of holography for providing a permanent record of the contents of settling chambers for the inverted microscope. However, this system was restricted in resolution to the larger phytoplankton (> 30 μm), and worked only in 1 plane. Beers (1978c) suggested that future developments in laser holography could theoretically provide the potential for 3-dimensional permanent records of the total size spectrum of phytoplankton, avoiding both concentration and fixation of samples. Whether these developments could be incorporated into an in situ device seems a question unlikely to be resolved in the foreseeable future, since no known recent progress has continued in this field

PHYSIOLOGICAL MEASUREMENTS

Sub-sampling

The behaviour and fragility of *Mesodinium rubrum* present experimental problems whenever this species is relatively abundant. Any subsampling for experimental purposes must take account of the extreme rapidity of aggregation, both in response to light and local currents (see Taylor et al. 1971, Lindholm 1985). This aggregation can be observed in bucket samples from dense blooms (Lindholm 1985, Crawford pers. obs.). Any sample to be divided which has stood for more than a few seconds, should be gently though thoroughly mixed, whilst avoiding severe agitation which may cause damage or lysis of cells. Ignorance of these procedures may lead to accumulation of cells in a sample being subsampled (Cabecadas et al. 1983), and poor replication of cell counts between subsamples (Crawford pers. obs.).

Productivity

The few studies to date which have examined the photosynthetic rate of *Mesodinium rubrum*, suggest this species to be exceptionally productive (Packard et al. 1978, Smith & Barber 1979, Cabecadas et al. 1983). Not only is the assimilation number (P_m^B) high and photoinhibition (β) apparently minimal (Smith & Barber 1979, Harrison et al. 1981, Platt et al. 1980), but the initial slope of the photosynthesis/irradiance (P/I) curve (α), a measure of photosynthetic efficiency at low light levels, appears comparable at least during blooms to that of the diatoms (Platt et al. 1980, Harrison et al. 1981).

This remarkable photosynthetic capacity, coupled with the elevated biomass levels encountered during blooms (see Lindholm 1985), is not without its drawbacks. For instance, Packard et al. (1978) and Smith & Barber (1979) have shown depressed photosynthesis in longer incubations of *Mesodinium rubrum* samples from blooms, compared to shorter ones, resulting either from lysis of cells or nutrient exhaustion. Smith & Barber (1979) estimated that incubations of these samples for more than 1 h, would result in nutrient exhaustion, since the level of inorganic nitrogen was 12.1 μg-at. N l^{-1} at the start of the incubation, whilst the calculated nitrogen demand of the samples was 15.5 μg-at. N l^{-1} h^{-1}. Dugdale et al. (1987) actually measured a nitrate uptake rate of 5 μg-at. N l^{-1} h^{-1} in samples from blooms of *M. rubrum*, thus supporting the contention that nutrients may be limiting during longer incubations.

Lysis of cells during containment, mentioned by Smith & Barber (1979), may be a general problem in physiological studies of microplankton (e.g. Venrick et al. 1977), though *Mesodinium rubrum* is probably particularly susceptible. Rupture of cells upon filtration is a further difficulty particularly for ^{14}C estimates of productivity. After gentle filtration, Smith & Barber (1979) were able to reject filters on the basis of red/pink pigment colouration to the edges, as an indication of mass lysis of cells. However, lysis on a smaller scale or rupture of cells during incubations at lower cell densities would be undetectable. Considerable distortion of physiological parameters could result when *M. rubrum* is abundant. In particular, this may cause underestimation of particulate production and consequential overestimation of dissolved excretion products in ^{14}C incubations. The red colouration to filters and elevation of extracellular excretion products has been noted by Sellner (1981) in blooms of *M. rubrum* off the coast of Peru. Throndsen (1978b) observed mass rupture of *M. rubrum* upon addition of slightly hypotonic rinsing water to fractionation screens. This resulted in overestimation of the ^{14}C productivity estimate for the < 5 µm fraction, caused by symbionts of *M. rubrum* (accounting for 54 % of productivity) passing through this screen.

A major limitation in the physiological studies on *Mesodinium rubrum* to date, has been the presence of other phytoplankton taxa and micro-organisms, even during major blooms. Single species techniques or pure cultures are now required to determine physiological properties of *M. rubrum* under controlled conditions. Stoecker et al. (1989) have provided the first such data through isolation of cells of *M. rubrum*. Using the ^{14}C technique, Stoecker et al. (1989) established a photosynthetic rate of 85 pg C $cell^{-1}$ h^{-1} for *M. rubrum*, at an irradiance of 100 µE m^{-2} s^{-1}. The prospects for establishment of pure cultures is remote however, since no known author has maintained cells of *M. rubrum* in the laboratory for an extended period (Taylor et al. 1971, Lindholm 1985).

Respiration

The respiration rate of *Mesodinium rubrum* is of particular interest in view of its motile capabilities, but has received little detailed attention. Although Margalef et al. (1979) speculated that *M. rubrum* probably allocates more energy to swimming than to reproduction, the studies to date (Packard et al. 1978, Setchel et al. 1978, Smith & Barber 1979) suggest the respiration rate to be rather low and within the range of conventional phytoplankton. This is perhaps not surprising in view of the claim by Fenchel & Finlay (1983) that motility in protozoa accounts for an insignificant fraction of the respiration rate. However, it should be noted that *M. rubrum* can swim at over 5 mm s^{-1} (Lindholm 1985), an order of magnitude faster than the majority of dinoflagellates (Smith & Barber 1979), and some 5 times quicker than most ciliates. Therefore, a more significant fraction could be anticipated for this species which, one could speculate, may not necessarily be swimming in incubation bottles. Should motility account for a significant proportion of the metabolic rate in *M. rubrum*, this may have important implications, not only for measurements of respiration rate, but also for determinations of photosynthetic rate. Smith & Barber (1979) noted cessation of swimming activity by *M. rubrum* at optimum light levels, thus distortions of P/I curves may result, since conventional techniques of photosynthesis determination assume a constant respiration rate. Clearly, the respiration rate of *M. rubrum* has aroused considerable speculation both in this paper and elsewhere, and deserves more detailed examination using single species techniques or pure cultures.

RECENT ESTIMATES OF ABUNDANCE

Although Taylor et al. (1971) commented upon the extremely widespread geographical distribution of *Mesodinium rubrum*, it has only recently become apparent how abundant this species may be. A preoccupation with bloom studies, in addition to the difficulties described in this paper, may have diverted attention from the potential role of *M. rubrum* as a regular component of the phytoplankton.

Mesodinium rubrum has often been noted to be the dominant member of the protozoan plankton during certain periods of the year (e.g. Michanek 1965, Eriksson et al. 1977, Takahashi & Hoskins 1978, Revelante & Gilmartin 1983, Mamaeva 1985, Andersen & Sorensen 1986, Leppänen & Bruun 1986). Ironically though, only a few recent studies minimizing some of the problems involved, have conceded its potential contribution to primary productivity (Reid et al. 1978, Grice et al. 1980, Revelante & Gilmartin 1983, 1987, Leppänen & Bruun 1986, Mackenzie & Gillespie 1986, Mackenzie et al. 1986, Stoecker et al. 1989).

Table 1 summarizes some recent studies which have attempted to quantify the contribution of *Mesodinium rubrum* to microplankton biomass or productivity.

In British Columbian waters near Vancouver, *Mesodinium rubrum* is often recorded during blooms (Taylor et al. 1969, 1971, Oakley & Taylor 1978) and was commonly encountered during many of the 'CEPEX' enclosure studies (Takahashi et al. 1975, Beers et al. 1977a,b, Parsons et al. 1977, Grice et al. 1980). The significance of *M. rubrum* in this area was further emphasised by Takahashi & Hoskins (1978), who, during winter months, demonstrated *M. rubrum* to represent 43 to 85 % of the microzooplankton carbon biomass, an order of magnitude greater abundance than

Table 1. *Mesodinium rubrum*. Some recent estimates of its contribution to microplankton biomass or productivity in marine ecosystems

Location	Period	Contribution of *M. rubrum*	Source
Saanich Inlet, British Columbia	Dec 1975–Feb 1976	43–85 % protozoan carbon biomass, an order of magnitude greater than other species. Averaged about 8 % phytoplankton biomass between 0 and 10 m	Takahashi & Hoskins (1978)
Saanich Inlet, British Columbia (CEPEX unmixed enclosure)	Summer 1978	Biomass peaked at 15 µg C l^{-1} throughout water column; 13 % average phytoplankton carbon; 205 % average heterotrophic ciliate carbon. Significantly less in mixed enclosure	Grice et al. (1980)
Adriatic Sea	Summer and Winter 1978–1982	Averaged 173 % total microzooplankton numerically, and 40 % biomass throughout water column, under stratified conditions. Not present under well-mixed conditions	Revelante & Gilmartin (1983)
Baltic Sea	Mar–Jun 1982	Ca 10 % total phytoplankton production, and 2 % biomass	Leppänen & Bruun (1986)
Damariscotta Estuary, Gulf of Maine	Mar 1981–Jun 1982	Within order of 100 % (from their Figs. 3 and 8) of total microzooplankton biomass between Dec and Apr. Sporadic occurrence during the rest of the year	Revelante & Gilmartin (1987)
Georges Bank Gulf of Maine	Summer 1987	*M. rubrum* & *Laboea strobila* (approx equal) contributed 1 to 7 % total phytoplankton carbon fixed, and 14 to 90 % microplankton carbon fixed. *M. rubrum* numerically 30 % of total ciliates at shallower stations, and 3 % at deeper stations off the bank	Stoecker et al. (1989)
Isles of Shoals, Gulf of Maine	May 1985–Aug 1986	Ca 0.3 % annual phytoplankton production	Montagnes & Lynn (1989)

any other species, and around 8 % phytoplankton biomass (between 0 and 10 m). In a 'CEPEX' enclosure study in the same area in summer, Grice et al. (1980) suggested that it constituted, at its peak abundance, about 13 % of the phytoplankton carbon in an unmixed enclosure.

Revelante & Gilmartin (1983) have shown that the numerical abundance of *Mesodinium rubrum* exceeded that of all the microzooplankton, under stratified conditions in the Adriatic Sea, and commented upon its potential importance as a primary producer.

Reports from the Baltic Sea have suggested *Mesodinium rubrum* to be a very significant species (e.g. Michanek 1965, Eriksson et al. 1977, Lindholm 1981, Smetacek 1981, Mamaeva 1985, Kivi 1986). During spring in the open northern Baltic, Leppänen & Bruun (1986) have estimated the contribution of *M. rubrum*, the dominant ciliate species, to be 10 % of the total primary productivity, and suggest this value may be even greater closer to the coast. However, this estimate was determined indirectly from production equations derived for other species and may, or may not, be appropriate for *M. rubrum*.

There are few records of *Mesodinium rubrum* from the North Sea other than the report of a bloom by Fonds & Eisma (1967). However, observations in some areas suggest it is sufficiently abundant, at least during May, to dominate surface chlorophyll (Gieskes & Kraay 1983, Baars & Franz 1984, Crawford pers. obs.).

In upwelling areas where *Mesodinium rubrum* forms massive blooms (Ryther 1967, Barber et al. 1969, Packard et al. 1978, Smith & Barber 1979, Sorokin 1979, Sorokin & Kogelschatz 1979), little is known of its occurrence at other times, other than brief mentions in a few studies (e.g. Beers et al. 1971, Blasco et al. 1980, 1981). However, Jimenez & Intriago (1987) have recently discussed the potential role of both bloom and pre-bloom levels of *M. rubrum* in the upwelling off Ecuador.

Several recent studies have examined the role of *Mesodinium rubrum* in different locations within the Gulf of Maine, USA. Revelante & Gilmartin (1987) found it to be a dominant ciliate in winter in the

Damariscotta Estuary, Gulf of Maine, and thus a potentially important phototroph. Montagnes & Lynn (1989) provided the first assessment of the annual contribution to productivity made by *M. rubrum*, in a study off the Isles of Shoals, Maine. The annual contribution was suggested to be only 0.3 % of phytoplankton production. However, as in the study by Leppänen & Bruun (1986), this was based on a rather indirect method of estimating production for heterotrophic ciliates, and may be inappropriate for *M. rubrum*. The study by Stoecker et al. (1989), during summer on the Georges Bank, Gulf of Maine, adopted the more objective method of comparing ^{14}C production estimates of isolated *M. rubrum* cells with those of intact water samples. They suggested that the photosynthetic ciliates *M. rubrum* and *Laboea strobila*, together contributed (approximately equal) 1 to 7 % total phytoplankton carbon fixed at stations on the bank.

Clearly, the quantity of useful non-bloom data presented to date is insufficient to determine the true role of *Mesodinium rubrum* in marine ecosystems, and precludes an assessment of whether its abundance has increased in recent years in response to coastal eutrophication. However, many reports do suggest it to be an important member of the phytoplankton, at least during certain periods of the year. In view of its potential contribution, considerably more research effort should be devoted to assessing its abundance and investigating its physiology, ecology, and taxonomy. Moreover, it must still be concluded, in agreement with Smith & Barber (1979), that the contribution of this enigmatic phototroph to the productivity of coastal, estuarine, and upwelling ecosystems has been seriously underestimated.

Acknowledgements. I would like to thank Dr D. A. Purdie, Professors A. P. M. Lockwood and M. A. Sleigh, for their encouragement and helpful critical reading of earlier drafts of the manuscript. I am indebted to Drs D. K. Stoecker, T. Lindholm, and 2 anonymous referees for the criticisms which shaped the final form of the manuscript. This paper was conceived as a result of N.E.R.C. grant no. GR3/5683

LITERATURE CITED

Andersen, P., Sørensen, H. M. (1986). Population dynamics and trophic coupling in pelagic microorganisms in eutrophic coastal waters. Mar. Ecol. Prog. Ser. 33: 99–109

Baars, M. A., Franz, H. G. (1984). Grazing pressure of copepods on the phytoplankton stock of the central North Sea. Neth. J. Sea Res. 18: 120–142

Bakker, C. (1966). Een protozo in symbiose met algen in het Veerse Meer. De Levende Natuur 69: 180–187

Bakker, C. (1967a). Massale ontwikkeling van ciliaten met symbiontische algen in het Veerse Meer. De Levende Natuur 70: 166–173

Bakker, C. (1967b). Veranderingen in milieu en plankton van het Oosterscheldegebied. Vakbl. Biol. 47: 181–192

Barber, R. T., Smith, W. O. (1981). The role of circulation, sinking and vertical migration in physical sorting of phytoplankton in the upwelling centre at ^{15}C. In: Richards, F. A. (ed.) Coastal Upwelling: Coastal and Estuarine Sciences 1. Geophysical Monography Bd., Washington D.C., p. 366–371

Barber, R. T., White, A. W., Siegelman, H. W. (1969). Evidence for a cryptomonad symbiont in the ciliate, *Cyclotrichium meunieri*. J. Phycol. 5: 86–88

Barlow, J. P., Monteiro, J. D. C. (1979). Selective grazing by zooplankton populations in Southampton Water, England U.K. Mar. Biol. 53: 335–344

Bary, B. M., Stuckey, R. G. (1950). An occurrence in Wellington Harbour of *Cyclotrichium meunieri* Powers, a ciliate causing red water, with some additions to its morphology. Trans. R. Soc. N.Z. 78: 86–92

Beers, J. R. (1978a). Pump sampling. In: Sournia, A. (ed.) Phytoplankton manual. Monographs on oceanographic methodology 6. Unesco Press, Paris, p. 41–49

Beers, J. R. (1978b). About microzooplankton. In: Sournia, A. (ed.) Phytoplankton manual. Monographs on oceanographic methodology 6. Unesco Press, Paris, p. 288–296

Beers, J. R. (1978c). Perspectives on holography. In: Sournia, A. (ed.) Phytoplankton manual. Monographs on oceanographic methodology 6. Unesco Press, Paris, p. 123

Beers, J. R., Knox, C., Strickland, J. D. H. (1970). A permanent record of plankton samples using holography. Limnol. Oceanogr. 15: 967–970

Beers, J. R., Reeve, M. R., Grice, G. D. (1977a). Controlled ecosystem pollution experiment: effect of mercury on enclosed water columns. IV. Zooplankton population dynamics and production. Mar. Sci. Communs 3: 355–394

Beers, J. R., Reid, F. M. H., Stewart, G. L. (1975). Microplankton of the North Pacific central gyre. Population stucture and abundance, June 1973. Int. Revue ges. Hydrobiol. 60: 607–638

Beers, J. R., Reid, F. M. H., Stewart, G. L. (1980). Microplankton population structure in southern Californian nearshore waters in late spring. Mar. Biol. 60: 209–226

Beers, J. R., Stewart, G. L. (1967). Microzooplankton in the euphotic zone at five locations across the California current. J. Fish. Res. Bd Can. 24: 2053–2068

Beers, J. R., Stewart, G. L. (1969a). The vertical distribution of microzooplankton and some ecological observations. J. Cons. perm. int. Explor. Mer 33: 30–44

Beers, J. R., Stewart, G. L. (1969b). Microzooplankton and its abundance relative to the larger zooplankton and other seston components. Mar. Biol. 4: 182–189

Beers, J. R., Stewart, G. L. (1970). Numerical abundance and estimated biomass of microzooplankton. In: Strickland, J. D. H. (ed.) The ecology of the plankton off La Jolla, California, in the period April through September, 1967. Bull. Scripps Instn Oceanogr. (New Ser.) 17: 67–87

Beers, J. R., Stewart, G. L. (1971). Microzooplankters in the plankton communities of the upper waters of the eastern tropical Pacific. Deep Sea Res. 18: 861–883

Beers, J. R., Stewart, G. L., Hoskins, K. D. (1977b). Dynamics of microzooplankton populations treated with copper: controlled ecosystem pollution experiment. Bull. mar. Sci. 27: 66–79

Beers, J. R., Stewart, G. L., Strickland, J. D. H. (1967). A pumping system for sampling small plankton. J. Fish. Res. Bd Can. 24: 1811–1818

Beers, J. R., Stevenson, M. R., Eppley, R. W., Brooks, E. R. (1971). Plankton populations and upwelling off the coast of Peru, June 1969. Fish. Bull. U.S. 69: 859–876

Blackbourn, D. J., Taylor, F. J. R., Blackbourn, J. (1973)

Foreign organelle retention by ciliates. J. Protozool. 20: 286–288

Blasco, D., Estrada, M., Jones, B. (1980). Phytoplankton distribution and composition in the northwest African upwelling near Cabo Corbeiro. Deep Sea Res. 27: 799–821

Blasco, D., Estrada, M., Jones, B. (1981). Short time variability of phytoplankton populations in upwelling regions – the example of N.W. Africa. In: Richards, F. A. (ed.) Coastal Upwelling. Coastal and Estuarine Sciences 1. Geophysical Monography Bd., Washington D.C., p. 339–347

Burkholder, P. R., Burkholder, L. M., Almodovar, L. R. (1967). Carbon assimilation of marine flagellate blooms in neritic waters of southern Puerto Rico. Bull. mar. Sci. 17: 1–15

Burkill, P. H. (1982). Ciliates and other microplankton components of a nearshore food-web: standing stocks and production processes. Annls Inst. océanogr., Paris, Fasc. Suppl. 58: 335–350

Cabecadas, G., Cunha, M. E., Moita, M. T. Pissarra, J., Sampayo, M. A. de M. (1983). Red tide in Cascais Bay, Portugal. Bol. Inst. Nac. Invest. Pescas, Lisboa 10: 81–123

Chester, A. J. (1978). Microzooplankton relative to a subsurface chlorophyll maximum layer. Mar. Sci. Communs 4: 275–292

Cloern, J. E., Cole, B. E., Wong, R. L. J., Alpine, A. E. (1985). Temporal dynamics of estuarine phytoplankton: a case study of San Francisco Bay. Hydrobiologia 129: 153–176

Cullen, J. J., Reid, F. M. H., Stewart, E. (1982). Phytoplankton in the surface and chlorophyll maximum off southern California in August, 1978. J. Plankton Res. 4: 665–695

Dale, T. (1987a). Diel vertical distribution of planktonic ciliates in Lindåspollene, western Norway. Mar. microb. Fd Webs 2: 15–28

Dale, T. (1987b). Oil pollution and plankton dynamics. II. Abundance pattern of ciliates inside and outside enclosures and the responses of ciliates to oil during the 1980 spring bloom in Lindåspollene, Norway. Sarsia 72: 197–202

Dale, T. (1988). Oil pollution and plankton dynamics. V. Controlled ecosystem pollution experiments in Lindåspollene, Norway, June 1980: effects of oil, oil/nutrients, and oil/dispersant on microplankton. Sarsia 73: 169–178

Dale, T., Burkill, P. H. (1982). Live counting - a quick and simple technique for enumerating pelagic ciliates. Annls Inst. océanogr., Paris, Fasc. Suppl. 58: 267–276

Dale, T., Dahl, E. (1987). Mass occurrence of planktonic oligotrichous ciliates in a bay in southern Norway. J. Plankton Res. 9: 871–879

De Sousa Lima, H., Williams, P. J. LeB. (1978). Oxygen consumption by the planktonic population of an estuary – Southampton Water. Est. cstl mar. Sci. 6: 515–523

Dugdale, R. C., Wilkerson, F. P., Barber, R. T., Blasco, D., Packard, T. T. (1987). Changes in nutrients, pH, light penetration and heat budget by migrating photosynthetic organisms. Oceanol. Acta Special Issue No. 6: 103–107

Elmgren, R. (1984). Trophic dynamics in the enclosed, brackish Baltic Sea. Rapp. P.-v. Réun. Cons. perm. int. Explor. Mer 183: 152–169

Eppley, R. W., Reid, F. M. H., Strickland, J. D. H. (1970). Estimates of phytoplankton crop size, growth rate and primary production. In: Strickland, J. D. H. (ed.) The ecology of the plankton off La Jolla, California, in the period April through September, 1967. Bull. Scripps Instn Oceanogr. (New Ser.) 17: 33–42

Eriksson, S., Sellei, C., Wallström, K. (1977). The structure of the plankton community of the Öregrundsgrepen (southwest Bothnian Sea). Helgoländer wiss. Meeresunters. 30: 582–597

Fenchel, T. (1968). On 'red-water' in the Isefjord (inner Danish waters) caused by the ciliate *Mesodinium rubrum*. Ophelia 5: 245–253

Fenchel, T., Finlay, B. J. (1983). Respiration rates in heterotrophic, free-living protozoa. Microb. Ecol. 9: 99–122

Fonds, M., Eisma, D. (1967). Upwelling water as a possible cause of red plankton bloom along the Dutch coast. Neth. J. Sea Res. 3: 458–463

Gieskes, W. W. C., Kraay, G. W. (1983). Dominance of Cryptophyceae during the phytoplankton Spring bloom in the central North Sea detected by HPLC analysis of pigments. Mar. Biol. 75: 179–185

Goodmann, D., Eppley, R. W., Reid, F. M. H. (1984). Summer phytoplankton assemblages and their environmental correlates in the southern California Bight. J. mar. Res. 42: 1019–1049

Grain, J., Puytorac, P. de, Grolière, C. A. (1982). Quelques précisions sur l'ultrastructure et la position systématique du cilié *Mesodinium rubrum*, et sur la constitution des symbiontes chloroplastiques. Protistologica 18: 7–21

Grice, G. D., Harris, R. P., Reeve, M. R., Heinbokel, J. F., Davis, C. O. (1980). Large-scale enclosed water-column ecosystems. An overview of foodweb I, the final CEPEX experiment. J. mar. biol. Ass. U.K. 60: 401–414

Harrison, W. G., Platt, T., Calienes, R., Ochoa, N. (1981). Photosynthetic parameters and primary production of phytoplankton populations off the northern coast of Peru. In: Richards, F. A. (ed.) Coastal Upwelling: Coastal and Estuarine Sciences 1. Geophysical Monography Bd., Washington D.C., p. 303–311

Hellebust, J. A. (1970). The uptake and utilization of organic substances by marine phytoplankters. In: Hood, D. W. (ed.) Organic Matter in Natural Waters. University of Alaska, p. 225–256

Hibberd, D. J. (1977). Ultrastructure of the cryptomonad endosymbiont of the red-water ciliate *Mesodinium rubrum*. J. mar. biol. Ass. U.K. 57: 45–61

Holligan, P. M., Balch, W. M., Yentch, C. M. (1984). The significance of subsurface chlorophyll, nitrate and ammonium maxima in relation to nitrogen for phytoplankton growth in the stratified waters of the Gulf of Maine. J. mar. Res. 42: 1051–1073

Holm-Hansen, O., Taylor, F. J. R., Barsdate, R. J. (1970). A ciliate red-tide at Barrow, Alaska. Mar. Biol. 7: 37–46

Jimenez, R., Intriago, P. (1987). Observations on blooms of *Mesodinium rubrum* in the upwelling area off Ecuador. Oceanol. Acta Special Issue No. 6: 145–154

Jonsson, P. R. (1987). Photosynthetic assimilation of inorganic carbon in marine oligotrich ciliates (Ciliophora, Oligotrichina). Mar. microb. Fd Webs 2: 55–68

Kahl, A. (1930–1935). Urtiere oder Protozoa. I. Wimpertiere oder Ciliata (Infusoria), eine Bearbeitung der freilebenden und ectocommensalen Infusorine der Erde, unter Ausschluss der marinen Tintinnidae. In: Dahl, F. (ed.) Die Tierwelt Deutschlands, vol. 18 (1930), vol. 21 (1931), vol. 25 (1932), vol. 30 (1935). Gustav Fischer, Jena, p. 1–886

Kimor, B. (1976). The fixation and preservation of marine protozoa: some problems and general considerations. In: Steedmann, H. F. (ed.) Zooplankton fixation and preservation. Monographs on oceanographic methodology 4. Unesco Press, Paris, p. 231–235

Kivi, K. (1986). Annual succession of pelagic protozoans and rotifers in the Tvärminne Storfjärden, SW coast of Finland. Ophelia, Suppl. 4: 101–110

Lackey, J. B. (1967). The microbiota of estuaries and their roles. In: Lauff, G. (ed.) Estuaries. AAAS, Washington, D.C., p. 291–302

Lackey, J. B., Glendinning, K. A. (1963). A possible fish-killing yellow tide in Californian waters. Q. J Fla Acad. Sci. 26: 263–268

Lackey, J. B., Glendinning, K. A. (1965). Ecology of the microbiota of San Diego Bay, California. Trans. S. Diego Soc. nat. Hist. 14: 9–40

Laval-Peuto, M., Rassoulzadegan, F. (1988). Autofluorescence of marine planktonic Oligotrichina and other ciliates. Hydrobiologia 159: 99–110

Laval-Peuto, M., Salvano, P., Gayol, P., Greuet, C. (1986). Mixotrophy in marine planktonic ciliates: ultrastructural study of *Tontonia appendiculariformis* (Ciliophora, Oligotrichida). Mar. microb. Fd Webs 1: 81–104

Leegard, C. (1920). Microplankton from the Finnish waters during the month of May 1912. Acta Soc. Sci. fenn. 48: 1–45

Leppänen, J. M., Bruun, J. E. (1986) The role of pelagic ciliates including the autotrophic *Mesodinium rubrum* during the spring bloom of 1982 in the open northern Baltic Sea proper. Ophelia, Suppl. 4: 147–149

Lin, S., Borstad, G. A., Gower, J. F. R. (1984). Remote sensing in the red spectral region. In: Nihoul, J. C. J. (ed.) Remote Sensing of Shelf Sea Hydrodynamics. Elsevier, Amsterdam, p. 317–336

Lindholm, T. (1978). Autumnal mass development of the 'red-water' ciliate *Mesodinium rubrum* in the Åland archipelago. Mem. Soc. Fauna Flora fenn. 54: 1–5

Lindholm, T. (1981). On the ecology of *Mesodinium rubrum* (Lohmann) (Ciliata) in a stagnant brackish basin on Åland, SW Finland. Kieler Meeresforsch., Sonderh. 5: 117–123

Lindholm, T. (1982). EDTA and oxalic acid – two useful agents for narcotizing fragile and rapid microzooplankton. Hydrobiologia 86: 297–298

Lindholm, T (1985). *Mesodinium rubrum* – a unique photosynthetic ciliate. Adv. aquat. Microbiol. 3: 1–48

Lindholm, T., Lindroos, P., Mörk, A. C. (1988). Ultrastructure of the photosynthetic ciliate *Mesodinium rubrum*. Biosystems (Amsterdam) 21: 141–149

Lindholm, T., Mörk, A. C. (1989). Symbiotic algae and plastids in planktonic ciliates. Mem. Soc. Fauna Flora fenn. 65: in press

Lohmann, H. (1908). Untersuchungen zur Feststellung des vollstandigen Gehaltes des Meeres an Plankton. Wiss. Meeresunters. 10: 129–370

Mackenzie, A. L., Gillespie, P. A. (1986). Plankton ecology and productivity, nutrient chemistry, and hydrography of Tasman Bay, New Zealand, 1982–1984. N.Z.J. mar. Freshwat. Res. 20: 365–395

Mackenzie, A. L., Kaspar, H. F., Gillespie, P. A. (1986). Some observations on phytoplankton species composition, biomass, and productivity in Kenepuru Sound, New Zealand, 1982–83. N.Z.J. Mar. Freshwat. Res. 20: 397–405

Mamaeva, N. V. (1985). Infusorians as a component of the planktonic community in the Baltic Sea. Dokl. Biol. Sci. 281: 200–201

Margalef, R. (1967). The food web in the pelagic environment. Helgoländer wiss. Meeresunters. 15: 548–559

Margalef, R., Estrada, M., Blasco, D. (1979). Functional morphology of organisms involved in red tides, as adapted to decaying turbulence. In: Taylor, D. L., Seliger, H. H. (eds.) Toxic Dinoflagellate Blooms. Proc. 2nd. Int. Conf. Elsevier North Holland, Inc., Amsterdam, p. 89–94

McAlice, B. J. (1968). An occurrence of ciliate red-water in the Gulf of Maine. J. Fish. Res. Bd Can. 25: 1749–1751

McManus, G. B., Fuhrmann, J. A. (1986). Photosynthetic pigments in the ciliate *Laboea strobila* from Long Island Sound, USA. J. Plankton Res. 8: 317–327

Michanek, G. (1965). Zooplankton of the Baltic – observations on some smaller species. Botanica Gothoburg 3: 157–160

Montagnes, D. J. S., Lynn, D. H. (1987). A quantitative protargol stain (QPS) for ciliates: method description and test of its quantitative nature. Mar. microb. Fd Webs 2: 83–93

Montagnes, D. J. S., Lynn, D. H. (1989). The annual cycle of *Mesodinium rubrum* in the waters surrounding the Isles of Shoals, Gulf of Maine. J. Plankton Res. 11: 193–201

Montagnes, D. J. S., Lynn, D. H., Roff, J. C., Taylor, W. D. (1988a). The annual cycle of hetertrophic planktonic ciliates in the waters surrounding the Isles of Shoals, Gulf of Maine: an assessment of their trophic role. Mar. Biol. 99: 21–30

Montagnes, D. J. S., Lynn, D. H., Stoecker, D. K., Small, E. B. (1988b). Planktonic ciliates with chloroplasts: taxonomic descriptions of one new species and redescriptions of four species in the family Strombidiidae (Ciliophora, Oligotrichida). J. Protozool. 35: 189–197

Oakley, B. R., Taylor, F. J. R. (1978). Evidence for a new type of endosymbiotic organisation in a population of the ciliate *Mesodinium rubrum* from British Columbia. Biosystems (Amsterdam) 10: 361–369

Packard, T. T., Blasco, D., Barber, R. T. (1978). *Mesodinium rubrum* in the Baja California upwelling system. In: Boje, R., Tomczak, M. (eds.) Upwelling Ecosystems. Springer-Verlag, Berlin, p. 73–89

Parducz, B. (1966). Ciliary movement and coordination in ciliates. Int. Rev. Cytol. 21: 91–128

Parsons, T. R., Blackbourn, D. J. (1968). Pigments of the ciliate *Mesodinium rubrum* (Lohmann). Neth. J. Sea Res. 4: 27–31

Parsons, T. R., Thomas, W. H., Siebert, D., Beers, J. R., Gillespie, P., Bawden, C. (1977). The effect of nutrient enrichment on the plankton community in enclosed water columns. Int. Rev. ges. Hydrobiol. 62: 565–572

Platt, T., Gallegos, C. L., Harrison, W. G. (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. mar. Res. 38: 687–701

Powers, P. B. A. (1932). *Cyclotrichium meunieri* sp. nov. (Protozoa, Ciliata): cause of red-water in the Gulf of Maine. Biol. Bull. mar. biol. Lab., Woods Hole 63: 74–80

Purdie, D. A., Garcia, C. A. E. (1988). The use of airborne thematic mapper data in studying the effect of tidal state on the distribution of phytoplankton blooms in Southampton Water. Proc. NERC 1987 airborne campaign workshop. University of Southampton, U.K., p. 195–203

Purdie, D. A., Garcia, C. A. E., Crawford, D. W. (1988). The distribution of *Mesodinium* blooms in Southampton Water: Remote sensing studies using the airborne thematic mapper. Proc. NERC 1986 airborne campaign workshop, Institute of Hydrology, Wallingford, U.K., p. 143–147

Raymont, J. E. G., Carrie, B. G. A. (1964). The production of zooplankton in Southampton Water. Int. Rev. ges. Hydrobiol. 49: 185–232

Reid, F. M. H., Fuglister, E., Jordan, J. B. (1970). Phytoplankton taxonomy and standing crop. In: Strickland, J. D. H. (ed.) The ecology of the plankton off La Jolla, California, in the period April through September, 1967. Bull. Scripps Instn Oceanogr. (New Ser.) 17: 51–66

Reid, F. M. H., Stewart, E., Eppley, R. W., Goodmann, D. (1978). Spatial distributions of phytoplankton species in chlorophyll maximum layers off southern California. Limnol. Oceanogr. 23: 219–226

Revelante, N., Gilmartin, M. (1983). The microzooplankton distribution in the northern Adriatic Sea with emphasis on the importance of ciliated protozoans. Oceanol. Acta 6: 407–417

Revelante, N., Gilmartin, M. (1987). Seasonal cycle of the ciliated protozoan and micrometazoan biomass in a Gulf of Maine estuary. Est. cstl Shelf Sci. 25: 581–598

Robinson, I. S., Holligan, P. M. (1987). Developments in ocean colour research in the U.K.. Ocean Colour. Proceedings of an ESA Workshop, Villefranche-sur-Mer, November 1986, ESA Publications

Royal Commission (1911). Nuisances due to excessive growth of green seaweeds in Belfast Lough. Seventh Report of the Royal Commission on Sewage Disposal, Wyman and Sons, London

Ryther, J. H. (1967). Occurrence of red-water off Peru. Nature, Lond. 214: 1318–1319

Sampayo, M. A. de M., Cabecadas, G. (1981). Occurrence of red-water off Algarve. Bol. Inst. Nac. Invest. Pescas, Lisboa 5: 63–87

Sanders, R. W. (1987). Tintinnids and other microzooplankton – seasonal distributions and relationships to resources and hydrography in a Maine estuary. J. Plankton Res. 9: 65–77

Savage, P. D. V. (1965). Preliminary observations on the phytoplankton of Southampton Water. Br. phycol. Bull. 2: 515–516

Savage, P. D. V. (1967). Some features of the phytoplankton and its production in Southampton Water. Challenger Rep. 19: 41

Sellner, K.G. (1981). Primary productivity and the flux of dissolved organic matter in several marine environments. Mar. Biol. 65: 101–112

Setchell, F. W., Boisseau, D. W., Packard, T. T. (1978). Respiration and ETS activity in the ciliate *Mesodinium rubrum*. In: Packard, T. T., Blasco, D., Jones, V. (eds.) Experiments with nitrate reductase and ETS in plankton from the Peru upwelling system. CUEA tech. Rep. No. 54, p. 79–83

Sherr, E. B., Sherr, R. D., Fallon, R. D., Newell, S. Y. (1986). Small aloricate ciliates as a major component of the marine heterotrophic nanoplankton. Limnol. Oceanogr. 31: 177–183

Sieburth, J. McN., Estep, K. W. (1985). Precise and meaningful terminology in marine microbial ecology. Mar. microb. Fd Webs 1: 1–16

Sieburth, J. McN., Smetacek, V., Lenz, F. (1978). Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnol. Oceanogr. 23: 1256–1263

Small, E. B., Lynn, D. H. (1985). Phylum Ciliophora. In: Lee, J. J., Hutner, S. H., Bovee, E. C. (eds.) An illustrated guide to the Protozoa. Society of Protozoologists, Lawrence, Kansas, p. 393–440

Smetacek, V. (1981). The annual cycle of protozooplankton in the Kiel Bight. Mar. Biol. 63: 1–11

Smith, W. O., Barber, R. T. (1979). A carbon budget for the autotrophic ciliate *Mesodinium rubrum*. J. Phycol. 15: 27–33

Sorokin, Yu. I. (1979). The 'red-tide' in the region of the Peruvian upwelling. Doklady Biol. Sci. 249: 253–256. English translation, Dokl. Akad. Nauk. SSSR, Ser. Biol. (1980): 1263–1266

Sorokin, Yu. I. (1981). Microheterotrophic organisms in marine ecosystems. In: Longhurst, A.R. (ed.) Analysis of Marine Ecosystems. Academic Press, London, p. 293–342

Sorokin, Yu. I., Kogelschatz, J. (1979). Analysis of heterotrophic microplankton in an upwelling area. Hydrobiologia 66: 195–208

Soulsby, P. G., Mollowney, M., Marsh, G., Lowthion, D. (1984). The role of phytoplankton in the dissolved oxygen budget of a stratified estuary. Wat. Sci. Tech. 17: 745–756

Stoecker, D. K., Davis, L. H., Anderson, D. M. (1984). Fine scale spatial correlations between planktonic ciliates and dinoflagellates. J. Plankton Res. 6: 829–842

Stoecker, D. K., Michaels, A. E., Davis, L. H. (1987). Large proportion of marine planktonic ciliates found to contain functional chloroplasts. Nature, Lond. 326: 790–792

Stoecker, D. K., Silver, M. W., Michaels, A. E., Davis, L. H. (1988). Obligate mixotrophy in *Laboea strobila*, a ciliate which retains chloroplasts. Mar. Biol. 99: 415–423

Stoecker, D. K., Taniguchi, A., Michaels, A. E. (1989). Abundance of autotrophic, mixotrophic, and heterotrophic planktonic ciliates in shelf and slope waters. Mar. Ecol. Prog. Ser. 50: 241–254

Takahashi, M., Hoskins, K. D. (1978). Winter condition of marine plankton populations in Saanich Inlet, B.C. Canada. II. Microzooplankton. J. exp. mar. Biol. Ecol. 32: 27–37

Takahashi, M., Thomas, W. H., Seibert, D. L. R., Beers, J. R., Koeller, P., Parsons, T. R. (1975). The replication of biological events in enclosed water columns. Arch. Hydrobiol. 76: 5–23

Tamar, H. (1971). *Mesodinium fimbriatum* Stokes, 1887, a ciliate with bifurcated and barbed cirri. Acta Protozool. 9: 209–222

Tangen, K. (1978). Nets. In: Sournia, A. (ed.) Phytoplankton manual. Monographs on oceanographic methodology 6, Unesco Press, Paris, p. 50–58

Taylor, F. J. R. (1982). Symbioses in marine microplankton. Annls Inst. océanogr., Paris, Fasc. Suppl. 58: 61–90

Taylor, F. J. R., Blackbourn, D. J., Blackbourn, J. (1969). Ultrastructure of the chloroplasts and associated structures within the marine ciliate *Mesodinium rubrum* (Lohmann). Nature, Lond. 224: 819–821

Taylor, F. J. R., Blackbourn, D. J., Blackbourn, J. (1971). The red-water ciliate *Mesodinium rubrum* and its 'incomplete symbionts': A review including new ultrastructural observations. J. Fish. Res. Bd Can. 28: 391–407

Throndsen, J. (1978a). Preservation and storage. In: Sournia, A. (ed.) Phytoplankton manual. Monographs on oceanographic methodology 6. Unesco Press, Paris, p. 69–74

Throndsen, J. (1978b). Productivity and abundance of ultra- and nanoplankton in Oslofjorden. Sarsia 63: 273–284

Tumantseva, N. I. (1985). Red tide in the Black Sea. Oceanology, Wash. 25: 99–101

Venrick, E. L. (1978). Water bottles. In: Sournia, A. (ed.) Phytoplankton manual. Monographs on oceanographic methodology 6. Unesco Press, Paris, p. 33–40

Venrick, E. L., Beers, J. R., Heinbokel, J. R. (1977). Possible consequences of containing microplankton for physiological rate measurements. J. exp. mar. Biol. Ecol. 26: 55–76

White, A. W., Sheath, R. G., Hellebust, J. A. (1977). A red tide caused by the marine ciliate *Mesodinium rubrum* in Passamaquoddy Bay, including pigment and ultrastructure studies of the endosymbiont. J. Fish. Res. Bd Can. 34: 413–416

Williams, P. J. LeB. (1980). Phytoplankton in Southampton Water. In: The Solent Estuarine System. NERC Publications, Series C, No. 22: 73–75

Zaika, V. Ye., Averina, T. Yu. (1968). Proportions of infusoria in the plankton of Sevastopol Bay, Black Sea. Oceanology, Wash. 8: 843–845

This review was presented by Dr D. K. Stoecker, Woods Hole, Massachusetts, USA

Manuscript first received: December 29, 1988
Revised version accepted: August 2, 1989

Vol. 79: 259-265, 1992
MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser.
Published January 23

Evidence for avoidance of flushing from an estuary by a planktonic, phototrophic ciliate

David W. Crawford*, Duncan A. Purdie

Department of Oceanography, The University, Highfield, Southampton, SO9 5NH, UK

ABSTRACT: The vertical distribution of the planktonic, phototrophic ciliate *Mesodinium rubrum* was monitored during a red-water event in daylight hours, over a complete tidal cycle Theoretical considerations based upon the hydrodynamics of the estuary, and upon the behaviour of the ciliate described to date, suggest that the population should not be able to resist flushing losses from the estuary. However, field observations reveal a pattern consisting of aggregation close to the surface during flood tide and high water, but dispersion away from the surface on the ebb tide thus minimizing flushing losses due to the strong superficial currents. Evidence is presented suggesting that the major cue for such migration may be the turbulence generated by shearing of surface currents, or gravitational (static) instability of surface water

INTRODUCTION

Tidal flushing is accepted to be a fundamental factor controlling the development of plankton populations in estuaries (Ketchum 1954). Several bloom-forming dinoflagellate species reduce flushing losses indirectly, through surface avoidance, by combinations of motile responses to various abiotic parameters, such as light, temperature, salinity and nutrients (e.g. Eppley et al. 1968, Anderson & Stolzenbach 1985, Chang & Carpenter 1985). Although the role of ciliates in marine ecosystems has also attracted much recent interest as an important component of the microplankton (e.g. Beers et al. 1980, Smetacek 1981), the adaptive significance of their considerably greater motile capacity has received only limited attention to date (e.g. Dale 1987, Jonsson 1989). Moreover, recent observations on the abundance of mixotrophic and phototrophic ciliates (Stoecker et al. 1987, 1989, Crawford 1989) suggest that significant quantities of planktonic chlorophyll may be packaged in forms with much greater mobility than previously anticipated.

The planktonic ciliate *Mesodinium rubrum* (Lohmann) Hamburger & Buddenbrock forms non-toxic red-water blooms around the world in coastal and upwelling ecosystems (Taylor et al. 1971, Lindholm 1985). *M. rubrum* is enabled to be an obligate, functional phototroph (Ryther 1967, Barber et al. 1969) by the presence of an algal (cryptomonad) endosymbiont (Hibberd 1977). It is thought to be the most strongly integrated association known between host and photosynthetic cytobiont (Taylor 1982), and has been responsible for some of the highest rates of planktonic primary productivity on record (Taylor 1982, Lindholm 1985). Thus, often erroneously grouped within the microzooplankton, *M. rubrum* is functionally a member of the phytoplankton (Sieburth et al. 1978, Lindholm 1985, Crawford 1989), and has been seriously neglected as such in routine surveys to date (Crawford 1989). *M. rubrum* attains phenomenal swimming speeds of over 8 mm s^{-1} (ca 200 body lengths s^{-1}; Lindholm 1985, Jonsson & Tiselius 1990), an order of magnitude greater than the majority of dinoflagellates (Smith & Barber 1979) and several times quicker than most ciliates (Sleigh & Blake 1977, Dale 1987). Such swimming speed enables *M. rubrum* to perform diurnal vertical migrations of some 40 m in the Peru upwelling zone (Smith & Barber 1979).

Mesodinium rubrum causes recurrent red-water events each summer and autumn in Southampton Water, England (Williams 1980, Soulsby et al. 1984), an estuary characterised by a peculiar tidal regime

---

* Present address: University Marine Biological Station, Millport, Isle of Cumbrae, Scotland, KA28 0EG, UK

0171-8630/92/0079/0259/$ 03.00

consisting of a double high water, some 2 h apart, followed by a short ebb tide with near-surface currents reaching 0.5 to 1.0 m s^{-1} (Dyer 1973). *M. rubrum* is usually described as forming discrete surface or subsurface (1 to 2 m) maxima during the day, apparently in response to light (Bary & Stuckey 1950, Smith & Barber 1979), and swimming down or dispersing at night (Smith & Barber 1979, Soulsby et al. 1984). However, this behaviour pattern would clearly place the bulk of the population in a zone of intense near-surface currents during daytime ebb tides. Here, we examine evidence for a mechanism by which *M. rubrum* could maintain itself in the estuary when faced with such potential flushing losses.

MATERIALS AND METHODS

Surveys were conducted during the summers of 1985 and 1986 from a small research launch, anchored at Eling buoy, at the head of the River Test/Southampton Water estuary (Fig. 1). In such a dynamic estuary, it is impossible to track a body of water over the tidal cycle, using a drifting boat for example; the strong surface currents carry the boat swiftly downstream on the ebb tide, giving no advantage over the anchored boat.

In July 1985, sampling was undertaken over a complete tidal cycle. Water samples were taken with a Van-Dorn bottle at 4 depths (1, 4, 7, and 10 m), staggered at 15 min intervals, each depth sampled once every hour. Subsamples of 125 ml were preserved with 1 ‰ Lugol's iodine (see Crawford 1989) immediately after sampling, and stored in amber glass bottles in the dark for subsequent cell counts. *Mesodinium rubrum* was enumerated (after concentration by sedimentation, when necessary) in a Sedgewick-Rafter counting chamber, using an Olympus BH2 microscope. Vertical profiles of physical parameters were taken at 1 m intervals every 20 min using a NBA CTU-1 profiling CTD and NBA current meter.

In July 1986, *Mesodinium rubrum* distribution was monitored over ca 8 h, using *in situ* chlorophyll fluorescence as an index of abundance; approximately every 2 h at 1 m intervals, water samples were pumped through a Turner Designs flow-through fluorometer. Only changes in relative fluorescence are shown; peaks represent chlorophyll levels in excess of 100 mg m^{-3} and were dominated by *M. rubrum* (100 mg m^{-3} chlorophyll *a* represents approximately 10^9 cells m^{-3}).

RESULTS

Tidal advection past the fixed anchor station revealed significant horizontal fluctuations in the abundance of *Mesodinium rubrum*. To account for this, vertical distribution of *M. rubrum* (Figs. 2 & 3) was represented as percentages of the total numbers integrated from the surface to 10 m; cell numbers (m^{-3}) were first expressed, at any given 1 m depth interval, as a percentage of the total integrated population (cells m^{-2}). The depth, from the surface, of the 50 % 'centre of gravity' of the population (thick solid line) was then derived, together with the 20 and 80 % levels (stippled area between upper and lower dashed lines) to show degree of aggregation. This representation of population distribution is likely to considerably underestimate the actual degree of aggregation, since only 4 depths were sampled; this species aggregates to such an extent that a sampling interval of 0.5 to 1 m is preferable (Crawford 1989, Lindholm & Mörk 1990), though not always logistically feasible.

Fig. 2 shows the relationship, over a tidal cycle, between vertical distribution of *Mesodinium rubrum*

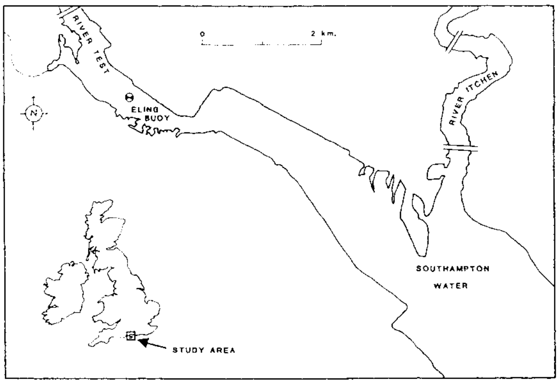


Fig. 1. Location of Eling buoy fixed anchor station at head of Test River/Southampton Water estuary

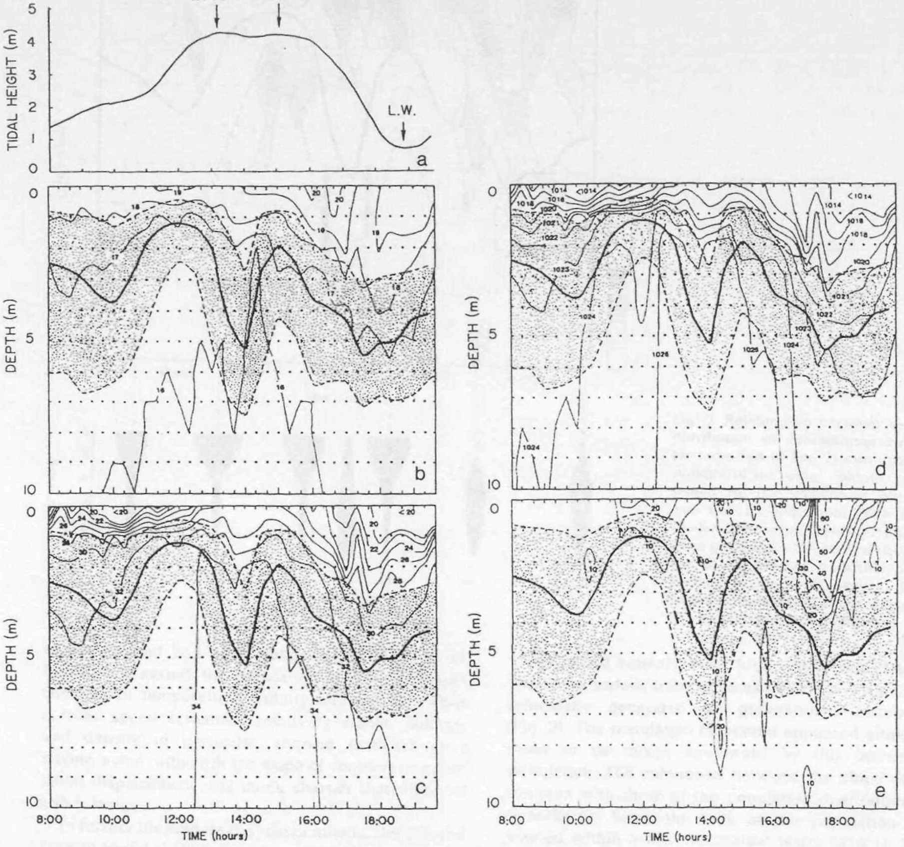


Fig. 2. Relationship between vertical distribution of *Mesodinium rubrum* and contours of physical parameters of the water column over a tidal cycle during a red-water event at Eling buoy in July 1985. Fine line contours represent: (a) tidal height, m; (b) temperature, °C; (c) salinity, ‰; (d) density, g l^{-1}; (e) current speed, cm s^{-1}. Data points represent frequency of physical measurements. The thick solid line represents the depth, from the surface, of the 50 % 'centre of gravity' of the integrated population of *M. rubrum*; the degree of aggegation is indicated by the stippled area between upper and lower dashed lines, representing the 20 % and 80 % levels respectively (see text for further explanation)

(as described above) and physical properties of the water column. Use of contours allows visual separation of migrational and advective phenomena, i.e. whether population contours followed those of water mass properties. It was evident that during the ebb tide, between 15:00 and 18:00 h, the population deepened considerably, and thus was not exposed to the near-surface flow, where flushing is greatest. Some coherence was apparent between the population maximum and the physical parameters during the ebb tide. The relationship with the 17 °C isotherm appeared strongest and could represent either a migration, with temperature as a cue, or an artifact resulting from advective changes, with the population residing at a preferential optimum temperature. However, neither interpretation is supported by the sharp but temporary downward displacement without apparent cue, which occurred approximately be-

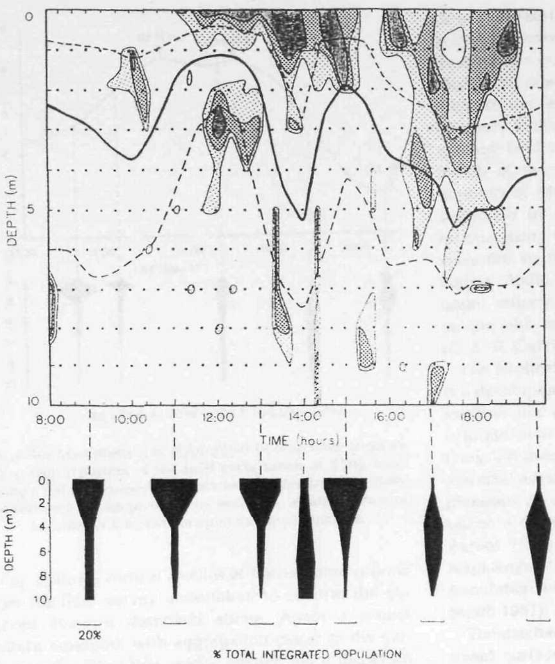


Fig. 3. Relationship between vertical distribution of *Mesodinium rubrum* (as indicated in Fig. 2) and dynamic stability of the water column, represented by the Richardson Number (see text for definition). The black shaded 'silhouettes' below emphasize more graphically the vertical distribution of *M. rubrum* at certain points during the tidal cycle; vertical distribution is represented as percentage of total integrated population

tween 1st and 2nd high waters (12:00 to 14:00 h). During this period, the population maximum crossed contours of temperature, salinity and density, while current speed remained relatively stable. Salinity, and density in particular, showed evidence of a mixing event, although the slope of contours of population displacement was much sharper than those of either factor.

To further investigate the role of mixing, density and current speed profiles were transformed into an index of dynamic stability, represented by the Richardson Number (*Ri*), as shown in the contour plot in Fig. 3:

$$Ri = \frac{-g/\rho(\delta\rho/\delta z)}{(\delta u/\delta z)^2} \quad \text{(Dyer 1973)}$$

where g = gravitational constant; ρ = density; u = current velocity; z = depth. *Ri* is a dimensionless index of the stabilizing effect of buoyancy, generated by the density profile, against the destabilizing effect of vertical current shear. The critical threshold value below which shear forces generate turbulence is $Ri = 0.25$ (Dyer 1973), though in estuaries this is accepted to be closer to $Ri = 1$. Below $Ri = 0$, turbulence results from static instability.

The period between the 2 high waters was characterised by surface turbulence extending down to 3 m, principally generated by gravitational instability (Fig. 3). The population maximum appeared either to avoid or be mixed downward by this increased turbulence. The coherence between the slope of *Ri* contours with those of the population distribution is remarkable. Since the bulk of the population remained within a zone of 'stable' water ($Ri > 1$), and depth regulation is possible even by weakly motile phytoplankton when $Ri = 0.5$ to 1.0 (Ganf 1974), this would suggest that the majority of *Mesodinium rubrum* cells were not subject to vertical mixing, but rather may have been actively aggregating away from surface turbulence. At about 12:00 h, the population even appeared to be 'squeezed' between turbulence close to the surface and a turbulence zone (probably shear-generated) at about 2 to 3 m. Over the tidal cycle as a whole, the population maximum tended away from turbulent mixing zones, except briefly during ebb tide, where contours of $Ri < 1$ were crossed. On the ebb tide, turbulence is principally generated by the shearing of surface currents.

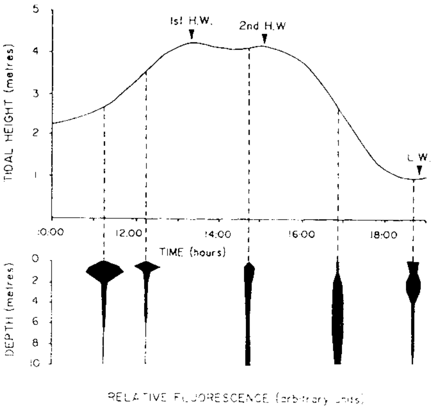


Fig. 4. Vertical profiles of chlorophyll fluorescence (relative units) over a portion of the tidal cycle taken at Eling buoy during a red-tide caused by *Mesodinium rubrum* in July 1986. Fluorescence is adopted as an indicator of abundance of *M. rubrum*, which dominated the phytoplankton

Fig. 4 shows vertical profiles of *Mesodinium rubrum* from the field survey undertaken to confirm the observed changes described above. Again a similar pattern emerged, with aggregation closer to the surface on the flood tide, and a deeper, more dispersed population on the ebb tide. A dispersed population was again apparent between the 2 high waters, but unfortunately a profile was not taken to confirm whether re-aggregation occurred on the 2nd high water. Although a small proportion of the population appeared to remain at the surface during ebb tide, it should be noted that Fig. 4 represents *in situ* fluorescence, thus other species may be represented. However, Figs. 2 & 3 also show some cells of *M. rubrum* at the surface during ebb tide: if our observations do indeed represent a response to turbulence, then a few cells at the surface would be expected, since avoidance could be upward away from subsurface shearing currents, thus 'trapping' some cells close to the surface.

DISCUSSION

Our data clearly show that the population maximum of *Mesodinium rubrum* in Southampton Water, at least during red tides, is deeper and more dispersed on the ebb than on the flood tide or during slack water. In terms of estuary dynamics, and perhaps in retrospect, such a finding was almost inevitable; a behaviour pattern governed simply by phototactic aggregation close to the surface, as previously suggested (see Lindholm 1985 for review), would result in the advection by near-surface currents of the bulk of the population at least 5 km downstream on each daytime ebb tide. In contrast, the red-water typically maintains itself most intensely at the head of the estuary for several weeks without evidence of extensive flushing losses; the mouth of the estuary is characterised by very low numbers of *M. rubrum*. The observations are further supported by data obtained during a survey of the estuary using the airborne thematic mapper to determine the spatial distribution of the bloom (Purdie & Garcia 1988); surface chlorophyll throughout the upper estuary was shown to be significantly reduced on the ebb tide, suggesting a downward migration (C. A. E. Garcia pers. comm.).

The implications of these observations are critical to the development and retention of red tides within the estuary; the ability of *Mesodinium rubrum* to concentrate close to the surface under optimal light conditions, but then avoid flushing losses on the ebb tide, is a crucial advantage over other members of the phytoplankton. *M. rubrum* has also been similarly shown to utilise a diurnal vertical migration of 40 m (Smith & Barber 1979) in a 2-layered flow to negate offshore displacement and thus maintain and concentrate the population in the Peru upwelling system (Barber & Smith 1981).

The mechanism by which *Mesodinium rubrum* could avoid surface flushing is less clear but particularly interesting. Although it cannot be firmly established from this field study that such changes in vertical distribution directly resulted from active avoidance of turbulence, the evidence presented is suggestive. Although the abundance maximum showed some coherence with temperature, salinity and density over a portion of the tidal cycle, only contours of Richardson Numbers (*Ri*) provided an explanation for the vertical displacements throughout tidal cycle. Surface incident light intensity was not determined; however, the survey was undertaken on a cloudless sunny day with insufficient variation in light intensity to result in vertical migration of several meters at midday. The return of the population toward the surface in decreasing light, in the evening after the ebb tide (Figs. 2 to 4), also suggests that light was not a major factor during daylight hours. However, this does not necessarily argue for light-independence for the vertical migration of *M. rubrum*; on the contrary, it appears to be strongly phototactic (see Lindholm 1985 for review) and has been reported to exhibit a diurnal migration pattern in Southampton Water (Soulsby et al. 1984) with near-surface accumulation during the day, and downward dispersal during darkness. Such a pattern would, to some extent, reduce flushing losses in itself when ebb tide occurs during dark hours. Our study provides considerably greater temporal resolution than acheived previously however, and we suggest that an active response to turbulence could be superimposed upon the diurnal pattern i.e. when positive phototaxis draws the population into unstable near-surface waters.

We acknowledge the limitations of our study due to the lack of repeated surveys when tidal and diurnal cycles are in a different phase, i.e. when ebb tide occurs during darkness or early morning. Thus, the afternoon descent of *Mesodinium rubrum* described here could simply represent a phenomenon typical of many migrating dinoflagellates. Nevertheless, the sharp downward displacement between 12:00 and 14:00 h (Figs. 2 & 3), apparently with only turbulence as a cue, and the return of the population toward the surface after ebb tide (Figs. 2 to 4) support our contentions. We cannot discount the possibility that the observed distributions may represent the resultant pattern of a combination of interacting factors; however, we feel that the relationship with turbulence is of sufficient novel interest to merit attention.

Experimental verification of turbulence avoidance has not been feasible at this stage; *Mesodinium rubrum* has not yet been cultured, is extremely fragile, and does not respond well to laboratory containment (Lindholm 1985). However, the interpretation of aggregation away from turbulence is supported by other published evidence. By means of rapid swimming, in response to small scale water movements, *M. rubrum* avoids isolation by micropipette (Taylor et al. 1971, Lindholm 1985, Crawford unpubl.) and substantially reduces capture efficiency by the feeding appendages of the copepod *Acartia tonsa* (Jonsson & Tiselius 1990). Since the motile behaviour of *M. rubrum* alternates between rapid 'jumps' and periods of motionlessness (Lindholm 1985, Crawford 1989, Jonsson & Tiselius 1990), an increase in the frequency of 'jumps' in response to turbulence would result in a *net* aggregation in more stable zones of the water column (i.e. a directional response is not required). The apparent upward 'squeezing' of the population by turbulence from below at 12:00 h supports this contention. Such increased 'escape' responses under turbulent conditions has recently been documented for the marine copepod *Centropages hamatus* (Costello et al. 1990). The mechanism by which so small an organism as *M. rubrum* (15 to 70 µm cell diameter) could sense fluid deformations is more difficult to resolve. Detection of microscale shear currents must be suspected, though this would be on a scale (< 100 µm) poorly understood by hydrodynamicists. The smallest turbulent length scale shown to stimulate dinoflagellate bioluminescence is on the order of 33 µm (Rohr et al. 1990), thus small scale shear could feasibly act as a cue. Sensing of acceleration forces using statocyst-like bodies, as described in the ciliate *Loxodes* sp. (Fenchel & Finlay 1986), is also conceivable, though no such structures have been described in ultrastructural studies on *M. rubrum* to date. Jonsson (1989) presented a hypothesis for the surface accumulation of oligotrichs and tintinnids based upon net upward swimming or passive geotaxis, which depended on the asymmetry of cell morphology. This seems unlikely to be a major factor explaining migration in *M. rubrum*, which is both a more symmetrical cell (equatorial band of cilia and cirri) and capable of much more extended diurnal migrations than other species (see Lindholm 1985).

The role of turbulence in red tide ecology has clearly been underestimated, and is only now becoming fully apparent. Turbulence has been shown to interfere with growth (White 1976), cell division (Pollinger & Zemel 1981) and motility (Thomas & Gibson 1990a, b) in some dinoflagellates. Further research is imperative, since turbulence is not a routinely quantified parameter in plankton studies. Other studies have already documented the retention of dinoflagellates in estuaries through surface avoidance; however, the mechanisms appear to have been different, although turbulence was not measured. Anderson & Stolzenbach (1985) suggested that *Alexandrium* (*Gonyaulax*) *tamarensis* and *Heterocapsa triquetra* reduced flushing losses by means of avoidance of high surface light intensities. Using a dye-injection technique in a small estuarine embayment, Garcon et al. (1986) demonstrated that *A. tamarensis*, whose growth rate approximated the rate of tidal flushing, could only bloom by such avoidance of outflowing surface waters.

More generally, the implications of our observations may be far-reaching. Our study is quite atypical in providing an opportunity to simultaneously examine the vertical distribution of a motile microplankter, together with the physical properties defining water column stability on a scale relevant to the organism. Intuitive or empirical estimates of static stability are often derived from density gradients during plankton studies, but velocity profiles, allowing direct assessment of dynamic stability, are rarely measured (Heaney & Butterwick 1985). Mechanoreceptor-induced escape reactions are a widespread phenomenon among the macro- and microplankton; however, the potential combined role of turbulence with such responses as a factor influencing microscale patchiness surely merits further investigation

Acknowledgements. This work was funded by N.E.R.C. grant no. GR3/5683. Drs N. Taylor and S. Boxall kindly provided the raw physical data. We thank Kate Saull and Rachel Scott for assistance with the figures. We are particularly grateful for the constructive comments of 2 anonymous reviewers

LITERATURE CITED

Anderson, D. M., Stolzenbach, K. D. (1985). Selective retention of two dinoflagellates in a well-mixed coastal embayment: the importance of diel vertical migration and surface avoidance. Mar. Ecol. Prog. Ser. 25: 39–50

Barber, R. T., Smith, W. O. (1981). The role of circulation, sinking and vertical migration in physical sorting of phytoplankton in the upwelling centre at 15N. In: Richards, F. A. (ed.) Coastal upwelling: coastal and estuarine sciences 1. Geophysical Monography Bd, Washington D.C., p. 366–371

Barber, R. T., White, A. W., Siegelman, H. W. (1969). Evidence for a cryptomonad symbiont in the ciliate *Cyclotrichium meunieri*. J. Phycol. 5: 86–88

Bary, B. M., Stuckey, R. G. (1950). An occurrence in Wellington Harbour of *Cyclotrichium meunieri* Powers, a ciliate causing red water, with some additions to its morphology. Trans. R. Soc. N. Z. 78: 86–92

Beers, J. R., Reid, F. M. H., Stewart, G. L. (1980). Microplankton population structure in southern California nearshore waters in late spring. Mar. Biol. 60: 209–226

Chang, J., Carpenter, E. J. (1985). Blooms of the dinoflagellate *Gyrodinium aureolum* in a Long Island estuary: box model analysis of bloom maintenance. Mar. Biol. 89: 83–93

Costello, J. H., Strickler, J. R., Marrasé, C., Trager, G., Zeller, R., Freise, A. J. (1990). Grazing in a turbulent environment: behavioural response of a calanoid copepod, *Centropages hamatus*. Proc. natn. Acad. Sci. U.S.A. 87: 1648–1652

Crawford, D. W. (1989). *Mesodinium rubrum*: the phytoplankter that wasn't. Mar. Ecol. Prog. Ser. 58: 161–174

Dale, T. (1987). Diel vertical distribution of planktonic ciliates in Lindaspollene, western Norway. Mar. microb. Fd Webs 2: 15–28

Dyer, K. (1973). Estuaries: a physical introduction. John Wiley & Sons, London

Eppley, R. W., Holm-Hansen, O., Strickland, J. D. H. (1968). Some observations on the vertical migration of dinoflagellates. J. Phycol. 4: 333–340

Fenchel, T., Finlay, B. J. (1986). The structure and function of Muller vesicles in loxodid ciliates. J. Protozool. 33: 69–79

Ganf, G. G. (1974). Diurnal mixing and the vertical distribution of phytoplankton in a shallow equatorial lake (Lake George, Uganda). J. Ecol. 62: 611–629

Garcon, V. C., Stolzenbach, K. D., Anderson, D. M. (1986). Tidal flushing of an estuarine embayment subject to recurrent dinoflagellate blooms. Estuaries 9: 179–187

Heaney, S. I., Butterwick, C. (1985). Comparative mechanisms of algal movement in relation to phytoplankton production. Contrib. mar. Sci. 27 (Suppl.): 114–134

Hibberd, D. J. (1977). Ultrastructure of the cryptomonad endosymbiont of the red-water ciliate *Mesodinium rubrum*. J. mar. biol. Ass. U.K. 57: 45–61

Jonsson, P. R. (1989). Vertical distribution of planktonic ciliates – an experimental analysis of swimming behaviour. Mar. Ecol. Prog. Ser. 52: 39–53

Jonsson, P. R., Tiselius, P. (1990). Feeding behaviour, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. Mar. Ecol. Prog. Ser. 60: 35–44

This article was presented by D. K. Stoecker, Cambridge, Maryland, USA

Ketchum, B. H. (1954). Relation between circulation and planktonic populations in estuaries. Ecology 35: 191–200

Lindholm, T. (1985). *Mesodinium rubrum* – a unique photosynthetic ciliate. Adv. aquat. Microbiol. 3: 1–48

Lindholm, T., Mörk, A. C. (1990). Depth maxima of *Mesodinium rubrum* (Lohmann) Hamburger & Buddenbrock – examples from a stratified Baltic Sea inlet. Sarsia 75: 53–64

Pollingher, U., Zemel, E. (1981). In-situ and experimental evidence of the influence of turbulence on cell division processes of *Peridinium cinctum forma westii* (Lemm.) Lefevre. Br. phycol. J. 16: 281–287

Purdie, D. A., Garcia, C. A. E. (1988). The use of airborne thematic mapper data in studying the effect of tidal state on the distribution of phytoplankton blooms in Southampton Water. In: Proc. NERC 1987 Airborne Campaign Workshop, Univ. Southampton, U.K., p. 195–203

Rohr, J., Losee, J., Hoyt, J. (1990). Stimulation of bioluminescence by turbulent pipe flow. Deep Sea Res. 37: 1639–1646

Ryther, J. H. (1967). Occurrence of red-water off Peru. Nature, Lond. 214: 1318–1319

Sieburth, J. McN., Smetacek, V., Lenz, F. (1987). Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnol. Oceanogr. 23: 1256–1263

Sleigh, M., Blake, J. R. (1977). Methods of ciliary propulsion and their size limitations. In: Pedley, T. J. (ed.) Scale effects in animal locomotion. Academic Press, London, p. 243–256

Smetacek, V. (1981). The annual cycle of protozooplankton in the Kiel Bight. Mar. Biol. 63: 1–11

Smith, W. O., Barber, R. T. (1979). A carbon budget for the autotrophic ciliate *Mesodinium rubrum*. J. Phycol. 15: 27–33

Soulsby, P. G., Mollowney, M., Marsh, G., Lowthion, D. (1984). The role of phytoplankton in the dissolved oxygen budget of a stratified estuary. Wat. Sci. Tech. 17: 745–756

Stoecker, D. K., Michaels, A. E., Davis, L. H. (1987). Large proportion of planktonic ciliates found to contain functional chloroplasts. Nature, Lond. 326: 790–792

Stoecker, D. K., Taniguchi, A., Michaels, A. E. (1989). Abundance of autotrophic, mixotrophic, and heterotrophic planktonic ciliates in shelf and slope waters. Mar. Ecol. Prog. Ser. 50: 241–254

Taylor, F. J. R. (1982). Symbioses in marine micropiankton. Annls Inst. oceanogr. Paris, Fasc. Suppl. 58: 61–90

Taylor, F. J. R., Blackbourn, D. J., Blackbourn, J. (1971). The red-water ciliate *Mesodinium rubrum* and its incomplete symbionts: a review including new ultrastructural observations. J. Fish. Res. Bd Can. 28: 391–407

Thomas, W. H., Gibson, C. H. (1990a). Quantified small-scale turbulence inhibits a red tide dinoflagellate, *Gonyaulax polyedra* Stein. Deep Sea Res. 37: 1583–1593

Thomas, W. H., Gibson, C. H. (1990b). Effects of small-scale turbulence on microalgae. J. appl. Phycol. 2: 71–77

White, A. W. (1976). Growth inhibition caused by turbulence in the toxic marine dinoflagellate *Gonyaulax excavata*. J. Fish. Res. Bd Can. 33: 2598–2602

Williams, P. J. leB. (1980). Phytoplankton in Southampton Water. In: The Solent estuarine system. NERC Publications, Series C, 22: 73–75

Manuscript first received: April 11, 1991
Revised version accepted: December 10, 1991

J. mar. biol. Ass. U.K. (1992), 72, 261-264
Printed in Great Britain

AN EPIBIONT ON THE RED-WATER CILIATE *MESODINIUM RUBRUM*

DAVID W. CRAWFORD

Department of Oceanography, The University, Southampton, SO1 5NH. Present address: University Marine Biological Station, Millport, Isle of Cumbrae, KA28 0EG

Spherical, opaque bodies (5-10 µm diameter) were frequently observed adhering to cells of the marine ciliate *Mesodinium rubrum*, particularly towards the end of red-water blooms in Southampton Water. Transmission electron microscope sections revealed these structures to be of biogenic origin, and packed with an amorphous, lipid-like material. No diagnostic features were observed to confirm their identity.

The phototrophic ciliate *Mesodinium rubrum* (Lohmann) forms recurrent red-tides each summer and autumn in the Southampton Water estuary, UK (Williams, 1980; Soulsby *et al.*, 1984). During a study of the impact of these blooms on the estuary, live cells were routinely observed microscopically. Frequently, but predominantly later in the bloom, numerous individuals possessed spherical, colourless, but opaque bodies, apparently attached or adherent to the outer cell membrane. Cells observed prior to the bloom, or at other times of the year, were never observed to possess such structures.

Cells bearing these bodies were photographed live, or after preservation in Lugol's iodine, using the phase contrast optics of an Olympus BH2 photomicroscope. For a more detailed examination, a sample of red-water was fixed for 4 hours in 3% glutaraldehyde in 0·1M sodium cacodylate buffer (adjusted isosmotic to the sea-water with sodium chloride), rinsed in buffer, and postfixed in 2% osmium tetroxide in Palade's buffer. The fixed material was then stained in 1·5% uranyl acetate, washed and dehydrated through an ethanol series to histosol, before finally being embedded in Spurr's resin. Silver sections for transmission electron microscopy were cut

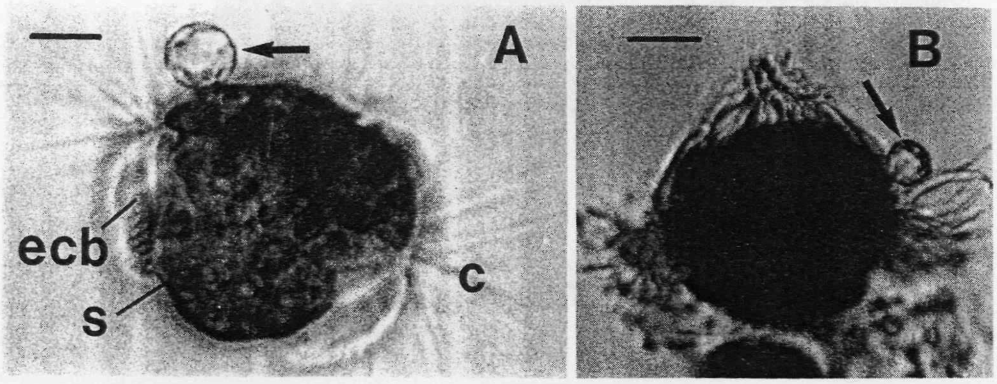


Figure 1. Live and preserved cells of *Mesodinium rubrum* showing curious spherical bodies (marked by arrows) associated with its 'oral' hemisphere. Scale bars = 10 µm. (A) Live ciliate cell packed with chloroplasts of the endosymbiont (s), and surrounded by characteristic equatorial ciliary belt (ecb) and cirri (c) radiating out in three planes. See Taylor *et al.* (1971) and Lindholm (1985) for further morphological details. (B) Cell preserved in Lugol's iodine (foreign material adhering below); cell stains black due to the presence of starch.

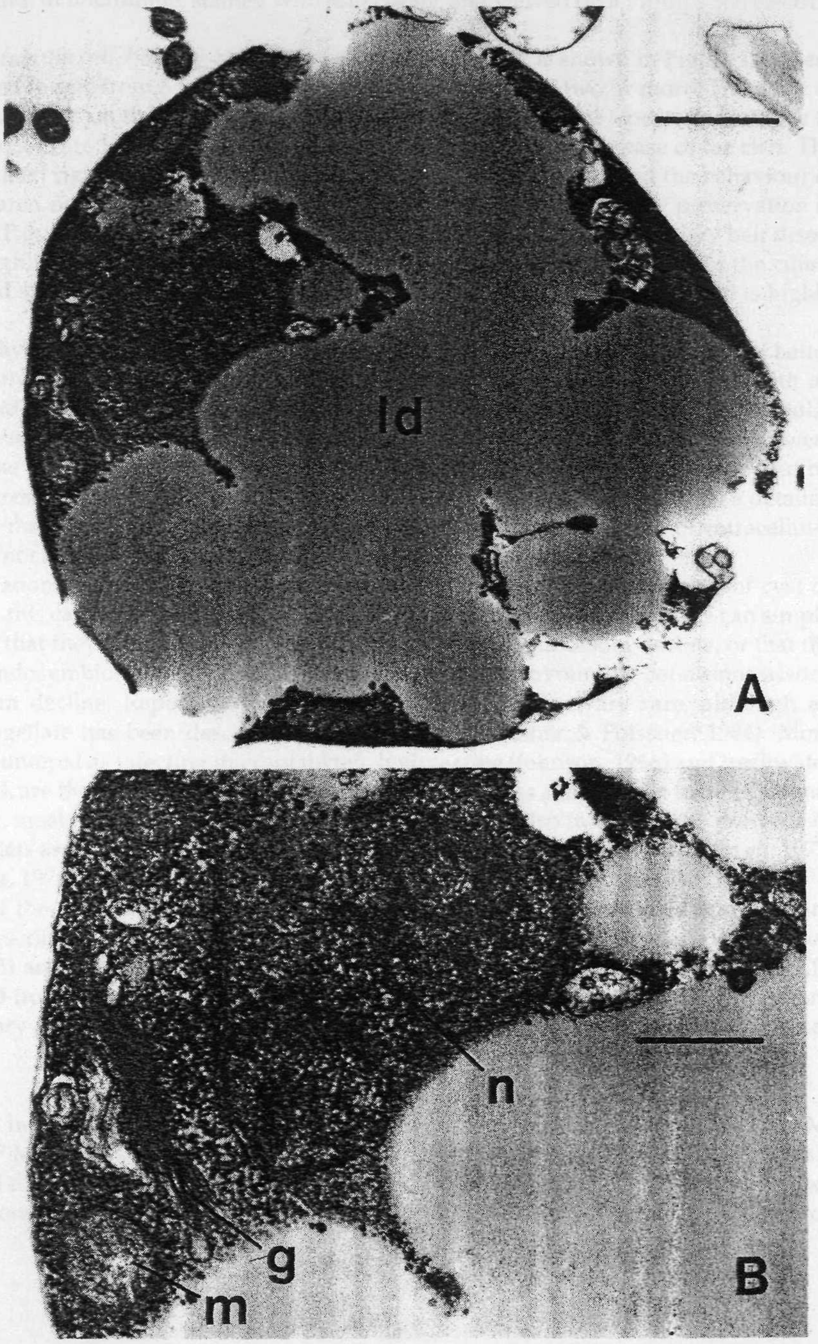


Figure 2. Transmission electron micrographs of a section of the structure shown in Figure 1. Fixation is rather poor and the periphery damaged. (A) Complete body; note large lipid accumulation (ld). Scale bar = 1 µm. (B) Close-up of 'pocket' of cytoplasm shown in (A). Though fixation is poor, nucleus (n), golgi body (g) and mitochondria (m) appear to be present. Scale bar = 0·5 µm.

on a Reichart ultracut microtome, stained with lead citrate, and viewed on a Phillips 300 electron microscope.

A typical *M. rubrum* cell, bearing one of the spherical structures, is shown in Figure 1A; these structures ranged in size from 5 to 10 μm, with some cells possessing two or more. The point of attachment was always on the 'oral' hemisphere (rudimentary cytostome - posterior relative to swimming direction; see Lindholm, 1985) of the ciliate, usually close to the base of the cirri. The structures remained rigidly fixed to the cell, even during rapid swimming, and the behaviour of such cells appeared normal. Appearance and attachment seemed unaffected by preservation in Lugol's iodine (Figure 1B), although the ciliate's cirri became relaxed and the ciliary belt disorganised. Athough Figure 1 may suggest some shrinkage upon preservation, both of the ciliate and the attached structure, it should be noted that the size of either in living material is highly variable.

In glutaraldehyde, cell fixation was generally poor; the spherical structures fixed rather better and could readily be discerned in section (Figure 2A). Invariably they were packed with an amorphous, lipid-like material, surrounded by a thin but poorly preserved layer of granular cytoplasm with a trace of enclosing membrane. Some internal membranous inclusions were apparent in some sections (Figure 2B), such as a nucleus, golgi body and mitochondria, but no diagnostic features could be distinguished. Serial sections were not available, therefore a detailed examination of the relative extent of lipid-like material and of the presence of intracellular organelles, was not feasible.

These observations suggest the bodies be of biogenic origin, perhaps some form of cyst or spore; however, this cannot be confirmed on the basis of the present description. One can simply speculate either that they represent a parasitic infestation, resulting in bloom decline, or that the cryptomonad endosymbiont of *M. rubrum* is encysting due to unfavourable conditions associated with bloom decline. Reports of parasitic infestations of ciliates are rare, although an ectoparasitic flagellate has been described on a soil ciliate (Foissner & Foissner, 1984). More commonly encountered as infecting microplankton, both marine (Johnson, 1966) and freshwater (Reynolds, 1973), are the chytrid fungi; moreover, these can possess a large lipid body (Kazama, 1972). However, most ultrastructural studies on *M. rubrum* have also indicated the presence of large lipid droplets associated with the endosymbiont (Taylor *et al.*, 1969, 1971; White *et al.*, 1977; Oakley & Taylor, 1978; Grain *et al.*, 1982).

The nature of these bodies certainly requires further investigation beyond the preliminary observations described here. The dynamics of blooms of *M. rubrum* are poorly understood (Lindholm, 1985) and thus whatever their source, these structures seem likely to be of significance. If derived from the cryptomonad endosymbiont then they may have further implications in an evolutionary context relevant to the debate on the nature of this interesting cytobiosis (see Taylor, 1983).

I thank Chris Inman for his invaluable assistance with the electron microscope, and Prof. M. Sleigh, Prof. A.P.M. Lockwood, Dr D.A. Purdie and Dr A. Rogerson for advice and comments. I am also grateful to Dr T. Lindholm and an anonymous referee for further comments on the text. These observations were made during tenure of a grant from the Natural Environment Research Council.

REFERENCES

Foissner, W. & Foissner, I., 1984. First record of an ectoparasitic flagellate on ciliates: an ultrastructural investigation of the morphology and the mode of attachment of *Spiromonas gonderi* nov. spec. (Zoomastigophora, Spiromonadidae) invading the pellicle of ciliates of the genus *Colpoda* (Ciliophora, Colpodidae). *Protistologica*, **20**, 635-648.

Grain, J., Puytorac, P. de & Grolière, C.A., 1982. Quelques précisions sur l'ultrastructure et la position systématique du cilié *Mesodinium rubrum*, et sur la constitution des symbiontes chloroplastiques. *Protistologica*, **18**, 7-21.

Johnson, T.W., 1966. Chytridiomycetes and Oömycetes in marine phytoplankton. *Nova Hedwigia*, **10**, 579-588.

Kazama, F., 1972. Development and morphology of a chytrid isolated from *Bryopsis plumosa*. *Canadian Journal of Botany*, **50**, 499-505.

Lindholm, T., 1985. *Mesodinium rubrum* - a unique photosynthetic ciliate. *Advances in Aquatic Microbiology*, **3**, 1-48.

Oakley, B.R. & Taylor, F.J.R., 1978. Evidence for a new type of endosymbiotic organisation in a population of the ciliate *Mesodinium rubrum* from British Columbia. *BioSystems*, **10**, 361-369.

Reynolds, C.S., 1973. The seasonal periodicity of planktonic diatoms in a shallow eutrophic lake. *Freshwater Biology*, **3**, 89-110.

Soulsby, P.G., Mollowney, M., Marsh, G. & Lowthion, D., 1984. The role of phytoplankton in the dissolved oxygen budget of a stratified estuary. *Water Science and Technology*, **17**, 745-756.

Taylor, F.J.R., 1983. Some eco-evolutionary aspects of intracellular symbiosis. *International Review of Cytology*, supplement 14, 1-28.

Taylor, F.J.R., Blackbourn, D.J. & Blackbourn, J., 1969. Ultrastructure of the chloroplasts and associated structures within the marine ciliate *Mesodinium rubrum* (Lohmann). *Nature, London*, **224**, 819-821.

Taylor, F.J.R., Blackbourn, D.J. & Blackbourn, J., 1971. The red-water ciliate *Mesodinium rubrum* and its 'incomplete symbionts': A review including new ultrastructural observations. *Journal of the Fisheries Research Board of Canada*, **28**, 391-407.

White, A.W., Sheath, R.G. & Hellebust, J.A., 1977. A red tide caused by the marine ciliate *Mesodinium rubrum* in Passamaquoddy Bay, including pigment and ultrastructure studies of the endosymbiont. *Journal of the Fisheries Research Board of Canada*, **34**, 413-416.

Williams, P.J.LeB., 1980. Phytoplankton in Southampton Water. In *The Solent Estuarine System*. NERC Publications, series C, no. 22, 73-75.

Submitted 19 April 1991. Accepted 10 July 1991

Microb Ecol (1992) 24:1–10

MICROBIAL ECOLOGY

Metabolic Cost of Motility in Planktonic Protists: Theoretical Considerations on Size Scaling and Swimming Speed

David W. Crawford

University Marine Biological Station, Millport, Isle of Cumbrae, Scotland KA28 0EG, United Kingdom

Received: December 2, 1991; Revised: February 17, 1992

Abstract. The metabolic cost of swimming for planktonic protists is calculated, on theoretical grounds, from a simple model based upon Stokes' law. Energetic expenditure is scaled over both typically encountered size ranges (1–100 μm) and swimming speeds (100–5,000 μm/sec). In agreement with previous estimates for typical flagellates, these estimates generally suggest a low ($<1\%$) cost for motility, related to total metabolic rate of growing cells. However, the cost of motility in small, fast-moving forms, such as some ciliates and flagellates, may be significant (1–10%) and even substantial (10–100%+) for certain species. In accordance with these predictions, many fast-moving ciliates restrict motility to bursts of activity or "jumps." In the absence of a reduction in swimming speed or in the frequency of jumps, it is predicted that this relative cost of motility will be significantly increased in starving heterotrophs or light-limited autotrophs, if such cells reduce cell volumes and specific rates of respiration.

Introduction

The physiological ecology of autotrophic and heterotrophic members of the pico-, nano- and microplankton is now of particular interest because of the sheer magnitude of their biomass and metabolic activity, and thus potential role in global CO_2/O_2 budgets. Respiration rates of these smaller members of the plankton are now attracting widespread attention after receiving limited study in the past. Discrepancies between oxygen and ^{14}C methodologies and the debated assumption that plankton community respiration represents a constant proportion of photosynthesis [9] require that plankton ecologists provide more detailed information on factors influencing metabolic losses for both individual species and communities. Recent studies have underlined the important, though previously underestimated, role of planktonic ciliates as heterotrophic [24], mixotrophic [26, 28], and phototrophic [2] members of the microplankton, and as food for metazoa [22]. They thus imply the necessity of a thorough understanding of metabolic processes for this group.

Because the ciliates and dinoflagellates are probably the quickest of the motile microplankton, their respiration rates are of interest. However, there appear to be no empirical studies on the subject, and available theoretical ones have tended to suggest a very low energetic cost for motility [7, 18–20]. Fenchel and Finlay [7], in a comprehensive review of respiration rates of heterotrophic protists, suggested that "motility accounts for an insignificant fraction of the total metabolic rate." Similarly, Raven and Beardall [19], using a slightly more empirical approach, concluded that "flagellar motility is not a major contributor to the energy requirements of dinoflagellates."

The debate on metabolic costs of motility for protists is reminiscent of that for larger members of the plankton; theoretical estimates [32] of the cost of normal locomotion for planktonic copepods suggest this to be very low ($<1\%$ metabolism), but drag, and thus energy consumption, increases 400 times during the rapid "escape" reaction [29]. For rotifers, despite low theoretical ($<1\%$ total metabolism) energy costs for locomotion, empirical estimates were 62% total metabolism [4].

This paper addresses the question theoretically in some detail for protists and examines some of the limitations of the approaches mentioned above.

The Model

Fenchel and Finlay [7] (and Raven and Richardson [20], see p. 263) adopted a theoretical approach to the problem by calculating the power (P) required to pull a sphere of diameter D through water with viscosity η and velocity v, using Stokes' law:

$$P = 3\pi D v^2 \eta \qquad (1)$$

This can be given in SI units of Joules $\sec^{-1}$ (or Watts) if D is given in meters, v in meters $\sec^{-1}$, and η is given in N s m^{-2}. However, if η is assumed to be 0.01 poise [7], this is divided by 10 to give units of N sec m^{-2}. For direct comparison with [7], the units are converted to cgs units of ergs $\sec^{-1}$, assuming 1 erg $= 10^{-7}$ Joules.

An overall efficiency (hydrodynamical efficiency × efficiency of transforming chemical work into mechanical work) of the ciliary propulsion mechanism of 1% was assumed. The power required for motility was then expressed as a percentage of total metabolic rate.

Here, exactly the same model has been used as that given above [7]. Total metabolic rate was determined assuming spherical cells and using an empirically derived volume-dependent relationship [7] for growing cultures of heterotrophic protists:

$$\log_{10} R\ (\text{nl } O_2 \text{ cell}^{-1} \text{ hour}^{-1}) = 0.75[\log_{10} \text{vol}(\mu m^3)] - 4.09 \qquad (2)$$

The major difference between this and the approach of Fenchel and Finlay [7] is that the relative cost of motility is scaled, using the above theoretical equations, over typical ranges of both cell diameter (1–100 μm) and swimming speed (100–5,000 μm $\sec^{-1}$) encountered for planktonic protists.

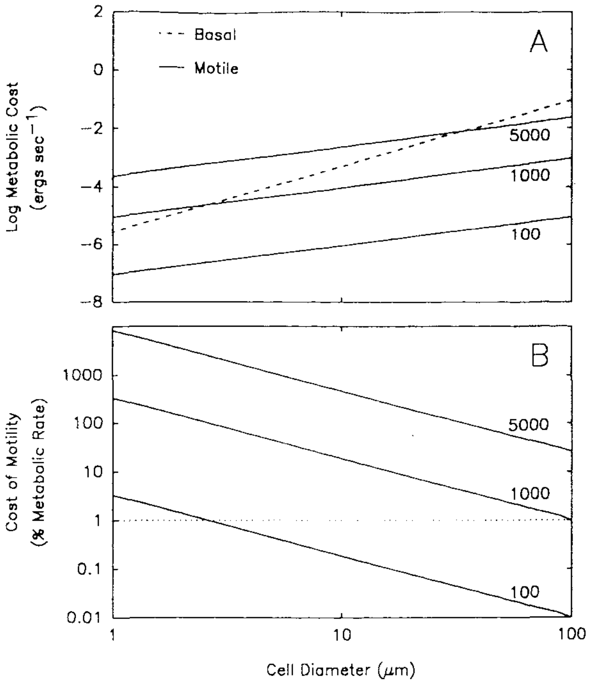


Fig. 1. A: Log : log plot of basal metabolic rate against cell diameter (dashed line). Solid lines represent theoretical costs of motility for a range of three swimming speeds (μm sec^{-1}), scaled over the size range. **B**: Theoretical costs of swimming at speeds given in A, as a proportion of total metabolic rate. Dotted line represents 1% total metabolic rate.

Results

Figure 1A shows a log : log plot of metabolic rate and cost of swimming against cell size. Here, the cost of swimming at any given speed is clearly observed to increase, relative to total metabolic rate, with decreasing cell size. This results from metabolic rate increasing as a power function of length with an exponent of between 2 and 3, whereas the energetic cost of swimming is proportional to length and to velocity squared. For a speed of 1,000 μm sec^{-1}, the cost appears to approach total metabolic rate when cell diameter decreases to between 1 and 10 μm. The costs of swimming at two other speeds, 100 and 5,000 μm sec^{-1}, are also given, and all vary with the same slope.

When expenditure is expressed as a percentage of total metabolism, as in Fig. 1B, it is clear that the cost of swimming at 1,000 μm sec^{-1} increases significantly above 1% as cell diameter decreases below 100 μm. Thus, the choice of the ciliate *Didinium* as an example by Fenchel and Finlay [7] was rather unfortunate; in their study, the cost of swimming of this species (diameter 120 μm) was quoted as about 0.7%, as can be verified by Fig. 1B. However, this is unrepresentative of the cost of swimming at such a speed for the much smaller ciliates, which predominate in aquatic environments. In fact, the cost of swimming at 1,000 μm sec^{-1} predicted by this model for ciliates in the typically encountered size range of 20–30 μm would be about 5–10% total metabolism. At 100 μm sec^{-1}, the relative cost only just exceeds 1% total at a size of between 1 and 10 μm diameter. For 5,000 μm sec^{-1}, the cost is always substantial; about 60% at 50 μm and 460% at 10 μm cell diameter.

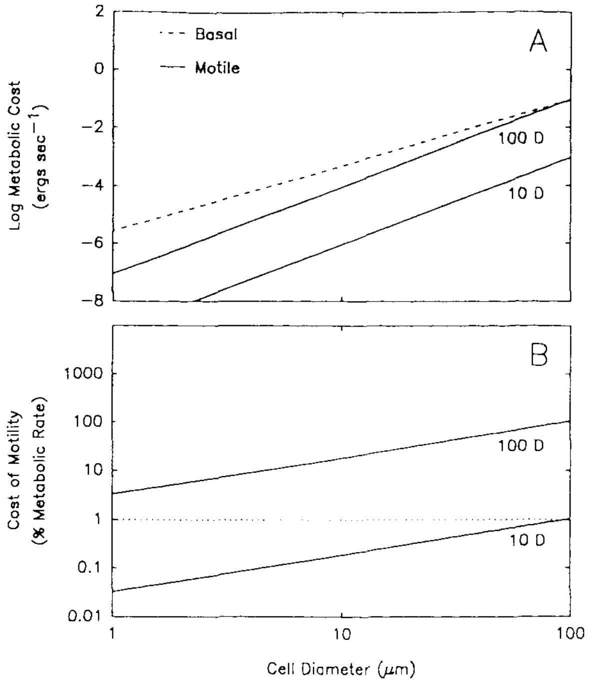


Fig. 2. **A**: Log : log plot of basal metabolic rate against cell diameter (dashed line). Solid lines represent theoretical costs of motility at 10 and 100 body lengths (D) sec^{-1}, scaled over the size range. **B**: Theoretical costs of swimming at speeds given in A, as a proportion of total metabolic rate. Dotted line represents 1% total metabolic rate.

The other example quoted by Fenchel and Finlay [7], at the lower end of the size spectrum, is the flagellate *Ochromonas* of diameter 8 μm and swimming speed 60 μm sec^{-1}. As can be observed in Fig. 1B, the energy expended on motility by this species is unlikely to be significant, and in fact was given as 0.1% [7]. Like *Ochromonas*, many flagellates swim at a speed of the order of 10 body lengths sec^{-1}; theoretical considerations have thus been expressed also on this basis in Fig. 2A and B. In contrast to using a fixed speed against cell size, relative cost of swimming at 10 and 100 body lengths sec^{-1} increases with increasing cell size. The cost of swimming at 10 body lengths sec^{-1} does not exceed 1% total metabolism over the size range given. However, the cost at 100 body lengths sec^{-1} is always significantly above 1%, and above a cell diameter of 10 μm becomes quite substantial. For example, protists in the range 30–50 μm swimming at 100 body lengths sec^{-1} would be predicted to expend the equivalent of between 40 and 60% total metabolic rate on swimming.

Discussion

It is not clear from the literature whether or not swimming speed varies as a function of cell diameter; speed has been suggested to be independent of cell size for flagellates (100–200 μm sec^{-1}) [33] and for ciliates (1,000 μm sec^{-1}) [23]. However, these studies were based on a limited number of examples, and

Table 1. Ranges of cell sizes (equivalent spherical diameter) and swimming speeds typically encountered for major groups of motile planktonic protists, and relative metabolic cost predicted by the model for an example of each group for growing and starving cells

Protistan group [with refs]	Cell diameter range (μm)[a]	Swimming speed range (μm sec^{-1})[a]	Predicted cost M (% basal R)	
			Growing[b]	Starved[c]
Nanoflagellates [1, 31]	1–20	1–100	0.00001–3.0	
e.g., *Ochromonas*	8	60	0.01	1.8[d]
Dinoflagellates [14, 25]	10–100	10–1,000	0.0001–19	
e.g., *Dinophysis*	40	500	0.6	1.3[e]
Ciliates (simple cilia) [21, 23]	10–100	500–1,500	0.3–42	
e.g., *Uronema*	25	1,200	8	51[f]
Ciliates (compound cilia) [5]	10–100	1,000–4,000	1–297	
e.g., *Strombidium*	30	3,000	42	254[f]

[a] General guide to typically encountered ranges for each group; however, it is acknowledged that many examples fall outside these ranges

[b] 'Basal' rate derived from empirical formula of Fenchel and Finlay [7]. Range represents max. and min. for sizes and speeds given

[c] Or light limited autotrophs. Predicted costs computed assuming no change in swimming speed, but using estimates of reduced cell volumes and respiration rates for 'starving' cells; assumptions given below

[d] Assuming estimate of reduction in cell volume by 80% and respiration rate by 97% [7]

[e] Data not available for dinoflagellates; conservative assumption of reduction in volume by 50% and respiration by 50%

[f] No relevant data available; assuming estimates of reduction in volume by 80% and respiration by 90% for *Paramecium* [7]

there are certainly many forms that do not conform to these patterns. For example, many flagellates swim at about 10 body lengths sec^{-1}. Thus, for clarification, alternative approaches of considering speed, both as a fixed entity against size and as a function of body lengths sec^{-1}, have been applied.

The model presented here, insofar as being identical to that of Fenchel and Finlay [7], confirms their predictions in the context of two "fixed point" estimates. However, the extension of this model to cover the spectra of cell size and swimming speed typically encountered for planktonic protists suggests that certain forms may be expending substantial quantities of energy upon locomotion. Table 1 presents typical ranges of cell size and swimming speed for various broad groupings of motile protists, and summarizes predictions of the model for each group, both for growing cells and for starving or light-limited cells (discussed below).

The group highlighted for highest energetic costs is the planktonic ciliates of cell diameter 10–50 μm and swimming speed greater than 1,000 μm sec^{-1}. Sleigh and Blake [23] have suggested that propulsion by simple cilia achieves a fairly constant speed of 1,000 μm sec^{-1}, although there are many ciliates that swim at much greater speeds [5]. For example, the oligotrichs are generally rapid swimmers, and are of considerable ecological significance both in marine and freshwater environments. Rapid swimming is achieved by means of a circumoral ring of cilia, cirri, or membranelles. Many oligotrichs have been shown to swim at speeds of 2,000–3,000 μm sec^{-1} [5], and even up to 4,000 μm sec^{-1} [6]. These figures suggest the energetic cost of motility to be the equivalent of some 10–75% total metabolic rate, while swimming, for cells of 30–40 μm diameter. An even more impressive swimmer, though perhaps an exception, is the holotrich ciliate *Mesodinium rubrum* that swims at about 5,000 μm sec^{-1} [15], but which has also been recorded at 8,000–8,500 μm sec^{-1}, or about 200 body lengths sec^{-1} [11, 16]. This gives costs of the equivalent of 80–120% (at 5,000 μm sec^{-1}) and 200–340% (at 8,500 μm sec^{-1}) metabolic rate for cells of 30–40 μm diameter. Speeds of species such as the holotrich *Askenasia* and the oligotrich *Halteria* appear not to have been determined, but they can be observed to swim almost as quickly as *Mesodinium*, and certainly at speeds of several mm sec^{-1} (personal observation). It is interesting that genera such as *Mesodinium, Askenasia, Halteria, Strombidium, Laboea,* and *Tontonia,* all capable of very rapid swimming in short bursts [12, 30], do not swim continuously at such speeds. This, at least circumstantially, supports the contention that very rapid swimming could become energetically costly. It is also noteworthy that many of these fast swimmers harbor algal endosymbionts or retain plastids. Some species of the oligotrich genera *Strombidium, Laboea,* and *Tontonia* are mixotrophic [10, 13, 27], and *Mesodinium rubrum,* the fastest of all, is an obligate functional phototroph [16].

For dinoflagellates, the model predicts energetic costs of swimming to be substantially lower than for ciliates (see Table 1), principally due to the lower speeds achievable through flagellar propulsion. Most dinoflagellates swim at about 10 body lengths sec^{-1} or less [1, 25], suggesting a maximum energy cost of about 1% for a cell of 100 μm diameter, and much less for a cell of 10–20 μm diameter. At 100 body lengths sec^{-1}, a cell of 100 μm would expend the equivalent of just over 100% total metabolism (Fig. 2B), although this is an unrealistically high swimming speed for dinoflagellates. However, the swimming speed of the red-tide dinoflagellate *Protoperidinium* cf. *quinquecorne* has been estimated to be 1,500 μm sec^{-1} [8], and the rock pool dinoflagellate *Peridinium gregarium* has been recorded at up to 1,800 μm sec^{-1} [17]. These are remarkable speeds for dinoflagellates, giving estimated energetic costs for motility (sizes not given; assuming size of 30 μm diameter) in the order of about 10% total metabolic rate.

The nanoflagellates tend to achieve greater relative swimming speeds than the dinoflagellates, generally in the range 0.7–42 body lengths sec^{-1} [1], although 100 μm sec^{-1} for *Micromonas pusilla,* a cell of only 1–3 μm diameter has been recorded [31]. This represented a speed of up to 75 body lengths sec^{-1}, which gives an estimated cost of some 1–3% total metabolism. As most flagellates do not seem to swim much faster than 100 μm sec^{-1}, Fig. 1B and Table 1 suggest relative costs will rarely exceed 1–10% basal metabolism. It is likely that for such small cells, the cost of swimming, even at 50–100 body lengths sec^{-1}, is masked by the very high specific metabolic rates associated with small size.

Slightly more empirical approaches have been used to estimate cost of locomotion. Fenchel and Finlay [7] supported their contentions with considerations of the mechanical power output and chemical power input of flagella/cilia. For the two examples quoted, these estimates were again less than 1% total metabolic rate. However, a standard flagellum was used without giving the length. Raven and Beardall [19] considered the maximum ATP consumption based on the number of dynein/ATPase molecules per unit flagellar length for *Gonyaulax polyedra,* giving a relative cost of about 1.5%. These approaches are limited by being point estimates; relative cost is not considered over the whole size range involved. Assuming flagellar length varies linearly to some extent (e.g., 3–15 × body length [33]) with cell size, and chemical power input is dependent upon flagellar length [33], then relative metabolic cost of swimming will increase with decreasing cell size, as total metabolic rate is volume dependent. The argument applies in an analogous manner to the ciliates; Sleigh and Blake [23] suggest that "one might regard the product of ciliary length and ciliary number as a measure of the total energetic machinery of the organism." Many fast swimming forms have reduced or absent somatic ciliation, and possess an equatorial or oral girdle of cilia, cirri, or membranelles around the cell. Because individual cilia or basal bodies vary relatively little in diameter or length (e.g., 5–15 μm length [23]), the number of cilia will be approximately dependent upon the circumference of the cell (i.e., linear dependence on diameter). Thus, as cell volume decreases, total cilium length : cell volume ratio, and thus potential energy expenditure on swimming, will increase. Thus, the choice of *Didinium* [7] was unrepresentative of smaller fast-swimming ciliates in terms of its ciliary length : cell volume ratio.

The above discussions imply that the majority of planktonic protists will expend the equivalent of a low (< 1%) or moderate (1–10%) proportion of their total metabolic rate on motility (see Table 1). Some forms, such as the small fast swimming ciliates could be expending substantial amounts of energy (equivalent to 10–100%+ total metabolic rate) while swimming. Clearly, for these latter forms, rapid swimming, and thus energy expended, is not continuous, but this surely begs the question of what factors control the frequency and velocity of fast locomotory "bursts." For example, Crawford and Purdie [3] have suggested that the frequency of motile "jumps" of *Mesodinium rubrum* may increase in response to turbulence. The implications of such potential metabolic costs for the very fast forms are clear and considerable, both in terms of the ecophysiology of individual species and for the methodology and assumptions concerning determination of bulk physiological parameters of plankton assemblages.

For forms with even more modest motile capabilities, the implications may be considerable; Table 1 presents relative costs expressed for growing cells and for starving or light-limited cells. Starving protists may reduce their cell volumes and specific respiration rates [7], both of which would increase the relative cost of swimming at a given speed. Similarly, Raven and Richardson [20], considering the analagous situation of the effect of light limitation or continuous darkness on phototrophic protists, concluded that "the cost of having or using flagella . . . may not be negligible in relation to (for example) survival of an obligately photolithotrophic dinophyte in prolonged darkness." Fenchel and Finlay [7] suggested that small starving protists may reduce their metabolic rates to 2% of that of growing cells. Because the cost of swimming at a given speed cannot be similarly reduced, it is clear from Table 1 that relative costs of motility may increase substantially under such conditions, particularly for small, fast-moving oligotrich ciliates such as *Strombidium*. In starvation experiments, the ciliates *Strombidium capitatum* and *Favella ehrenbergii* were observed to swim more slowly after 24 hours in the dark without food (personal observation), as did *Paramecium* sp. (M. Sleigh, personal communication), thus supporting the above contention. However, others have observed faster swimming with starvation. Thus, comparison of anecdotal evidence is clearly of limited value. For small flagellates, Table 1 suggests that high specific respiration rates associated with small size tend to mask costs of motility, even for starving cells.

With metabolic rate being strongly temperature dependent, low temperatures, and accordingly elevated viscosities, will further increase the cost of swimming (or reduce achievable speed) relative to total metabolism. Thus, it appears that for many even moderately motile planktonic protists, swimming could potentially represent a significant or major drain on resources during periods of the year when food and/or light is scarce, cell size is reduced, and temperature is low.

Now required are more empirical tests of these theoretical estimates, firstly on a selection of highly motile forms. In terms of presenting a testable hypothesis for such studies, Table 1 would suggest that respiration rates of oligotrich ciliates, for example, should be significantly greater during bursts of fast swimming activity. This could be tested by determining rates of respiration in the presence/absence of inhibitors of motility. An alternative approach would be to determine those factors controlling the frequency of such rapid jumps and then to utilize these as variables during determination of respiration rates.

The limitations of the present paper are acknowledged in terms of the numerous assumptions made. Hydrodynamic and chemical conversion efficiencies are critical to the model, and little evidence is available to determine whether or not the value of 1% is conservative and to what extent this efficiency varies. The model also assumes the regression that was derived for heterotrophic protists [7] applies to all protists. However, the assumptions made are exactly those made by previous studies which have tended to imply a low energetic expenditure for all planktonic protists. The purpose of this paper is not to provide absolute estimates of energetic costs of locomotion, but to identify the limitations of previous theoretical approaches, particularly those of not scaling cell size and swimming speed, and to highlight those fast swimming protists which may be expending considerably more energy on motility than previously anticipated.

Acknowledgments. I thank Prof. Michael Sleigh and Dr. Diane Stoecker for comments on an earlier draft of the manuscript, and two anonymous reviewers for critical improvements. University Marine Biological Station Millport provided financial support.

References

1. Bauerfeind E, Ellbrächter M, Steiner R, Throndsen J (1986) Application of Laser Doppler Spectroscopy (LDS) in determining swimming velocities of motile phytoplankton. Mar Biol 93:323–327
2. Crawford DW (1989) *Mesodinium rubrum*: The phytoplankter that wasn't. Mar Ecol Prog Ser 58:161–174

3. Crawford DW, Purdie DA (1992) Evidence for avoidance of flushing from an estuary by a planktonic phototrophic ciliate. Mar Ecol Prog Ser 79:259–265
4. Epp RW, Lewis WM (1984) Cost and speed of locomotion for rotifers. Oecologia (Berl) 61: 289–292
5. Dale T (1987) Diel vertical distribution of planktonic ciliates in Lindåspollene, Western Norway. Mar Microb Food Webs 2:15–28
6. Fauré-Fremiet E (1948) Le rhythme de marée du *Strombidium oculatum* Gruber. Bull Biol de France et de Belgique 82:3–23
7. Fenchel T, Finlay BJ (1983) Respiration rates in heterotrophic, free-living Protozoa. Microb Ecol 9:99–122
8. Horstmann U (1980) Observations on the peculiar diurnal migration of a red tide Dinophyceae in tropical shallow waters. J Phycol 16:481–485
9. Iriarte A, Daneri G, Garcia VMT, Purdie DA, Crawford DW (1991) Plankton community respiration and its relationship to chlorophyll *a* concentration in marine coastal waters. Oceanol Acta 14:379–387
10. Jonsson PR (1987) Photosynthetic assimilation of inorganic carbon in marine oligotrich ciliates (Ciliophora, Oligotrichina). Mar Microb Food Webs 2:55–68
11. Jonsson PR, Tiselius P (1990) Feeding behaviour, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. Mar Ecol Prog Ser 60:35–44
12. Klekowski RZ (1981) Size dependence of metabolism in protozoans. Verh Internat Verein Limnol 21:1498–1502
13. Laval-Peuto M, Rassoulzadegan F (1988) Autofluorescence of marine planktonic Oligotrichina and other ciliates. Hydrobiologia 159:99–110
14. Levandosky M, Kaneta PJ (1987) Behaviour in dinoflagellates. In: Taylor FJR (ed) The biology of dinoflagellates. Botanical Monographs, vol 21. Blackwell Scientific Publications, Oxford, pp 360–397
15. Lindholm T (1981) On the ecology of *Mesodinium rubrum* (Lohmann) (Ciliata) in a stagnant brackish basin on Åland, SW Finland. Kiel Meeresforsch 5:117–123
16. Lindholm T (1985) *Mesodinium rubrum*—A unique photosynthetic ciliate. Adv Aquat Microbiol 3:1–48
17. Lombard EH, Capon B (1971) observations on the tidepool ecology and behaviour of *Peridinium gregarium*. J Phycol 7:188–194
18. Purcell EM (1977) Life at low Reynolds number. Am J Phys 45:3–11
19. Raven JA, Beardall J (1981) Respiration and photorespiration. Can Bull Fish Aquat Sci 210: 55–82
20. Raven JA, Richardson K (1984) Dinoflagellate flagella: A cost–benefit analysis. New Phytol 98:259–276
21. Roberts AM (1981) Hydrodynamics of protozoan swimming. In: Levandowsky M, Hutner SH (eds) Biochemistry and physiology of protozoa. Academic Press, New York, pp 5–66
22. Sherr EB, Sherr BF, Paffenhöfer GA (1986) Phagotrophic protozoa as food for metazoans: A "missing" trophic link in marine pelagic food webs? Mar Microb Food Webs 1:61–80
23. Sleigh MA, Blake JR (1977) Methods of ciliary propulsion and their size limitations. In: Pedley TJ (ed) Scale effects in animal locomotion. Academic Press, New York, pp 243–256
24. Sorokin YI (1981) Microheterotrophic organisms in marine ecosystems. In: Longhurst AR (ed) Analysis of marine ecosystems. Academic Press, New York, pp 293–342
25. Sournia A (1982) Form and function in marine phytoplankton. Biol Rev 57:347–394
26. Stoecker DK, Michaels AE, Davis LH (1987) Large proportion of marine planktonic ciliates found to contain functional chloroplasts. Nature (Lond) 326:790–792
27. Stoecker DK, Silver MW, Michaels AE, Davis LH (1988) Obligate mixotrophy in *Laboea strobila,* a ciliate which retains chloroplasts. Mar Biol 99:415–423
28. Stoecker DK, Taniguchi A, Michaels AE (1989) Abundance of autotrophic, mixotrophic and heterotrophic planktonic ciliates in shelf and slope waters. Mar Ecol Prog Ser 50:241–254
29. Strickler JR (1974) Swimming of planktonic *Cyclops* species (Copepoda, Crustacea): Pattern, movements and their control. In: Wu TY, Brokaw CJ, Brennen C (eds) Swimming and flying in nature. Plenum Press, New York, pp 599–613

30. Tamar H (1979) The movements of jumping ciliates. Archiv für Protistenk 122:290–327
31. Throndsen J (1973) Motility in some marine nanoplankton flagellates. Norw J Zool 21:193–200
32. Vlymen WJ (1970) Energy expenditure of swimming in copepods. Limnol Oceanogr 15:348–356
33. Wu TY (1977) Introduction to the scaling of aquatic animal locomotion. In: Pedley TJ (ed) Scale effects in animal locomotion. Academic Press, New York, pp 203–232