UNIVERSITY OF SOUTHAMPTON

THE TAXONOMY AND SEASONAL DYNAMICS OF HETEROTROPHIC FLAGELLATES IN SOUTHAMPTON WATER, U.K.

by Susan Mary Tong

submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Department of Biology, December, 1994.

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

BIOLOGY

Doctor of Philosophy

THE TAXONOMY AND SEASONAL DYNAMICS OF HETEROTROPHIC FLAGELLATES IN SOUTHAMPTON WATER, U.K.

by Susan Mary Tong

The diversity of heterotrophic nanoflagellates and other protists was examined at various sites around Southampton Water from October 1991 to June 1994. A total of 122 species are described using light and electron microscopy, including 14 new species. The taxonomy, morphological variation and other aspects of poorly known taxa are discussed in detail.

The abundance of heterotrophic flagellates, autotrophic flagellates and bacteria at the N.W. Netley station was determined at approximately fortnightly intervals between March 1993 and June 1994, together with taxonomic analysis of the flagellate populations. The major taxa of heterotrophic flagellates were the chrysomonads, choanoflagellates, bicosoecids, bodonids and dinoflagellates. It was found that although the abundance of these taxa fluctuated because of seasonal changes in the overall abundance of heterotrophic flagellates, they accounted for similar proportions of the population throughout the year. The majority of heterotrophic flagellates were found to be small (mainly 2.5-5μm) and bacterivorous. Larger nanoflagellates (mostly 10-20μm) formed about 12% of the total population, but accounted for 75% of the biomass. It is concluded that heterotrophic flagellates have an equal grazing impact on the bacterial and nanoplankton populations in Southampton Water, and that the trophic role of larger non-bacterivorous flagellates has been greatly underestimated.

CONTENTS

Acknowledgements

I am greatly indebted to my supervisor, Prof. M.A. Sleigh, for his encouragement, patience and valuable advice and guidance. I am also grateful to Dr. P.H. Burkill for his part in arranging the grant which funded this study.

Dr. J. Eccleston and N.H. Larsen are thanked for the provision of bacterial isolates. Members of the Dept. of Oceanography, University of Southampton, are thanked for the collection of water samples, in particular, Andrew Hirst, Cathy Lucas and Don Hutchinson. I am grateful to Ray Cornick and Barry Lockyer for assistance with the production of graphs and photographs in this thesis.

Thanks are due to a large number of colleagues for advice and discussions about various aspects of this thesis, including Prof D.J. Patterson, Dr. H. Thomsen, Dr. B.S.C. Leadbeater, J. Ikävalko, Dr. J. Ekebom, Dr. D.A. Purdie, Dr. A. Rogerson, Dr. H. Butler, Dr. D. Leipe, Dr. C. O'Kelly and Dr. P.H. Hansen.

This volume is dedicated to Naja Vørs, in acknowledgement of her friendship, encouragement, her generous advice, and discussions about bugs and critters.

This study was funded by the Natural Environment Research Council (award GT4/91/ALS/30).

Chapter 1: Introduction

1.1 Marine microbial food webs and the role of heterotrophic flagellates as bacterivores.

In the last two decades our understanding of marine pelagic food webs and nutrient cycling in the sea has been revolutionised by the recognition and study of the role of microorganisms. Traditional models of marine food webs (e.g. Steele 1974) considered that most of the primary productivity was due to relatively large cells which were grazed by zooplankton, which in turn were preyed on by larger zooplankton, which were eaten by fish. Microorganisms were rarely noted because of sampling methods (using a net with a mesh of 60μm or more) and preservation techniques with fixatives such as alcohols and formalin which destroy many of the smaller more fragile cells (Porter *et al.* 1985, Graham 1991). Protozoa were more frequently described from the benthos however, presumably because core sampling and other collection techniques did not discriminate against smaller organisms (e.g. Mare 1942, Lackey and Lackey 1963).

Pomeroy (1974) was among the first to suggest that the classical model of planktonic food webs should be revised, due to recent observations that organisms of 60μm or less consistently accounted for 90% or more of the primary production in oceanic waters. In the late 1970s and early 1980s, the use of epifluorescence microscopy to enumerate natural populations of bacteria (e.g. Hobbie *et al.* 1977, Porter and Feig 1980), and the thymidine incorporation and frequency of dividing cells techniques to estimate their production (e.g. Fuhrman and Azam 1982, Sherr and Sherr 1983) showed that they were an important component of marine plankton. Exudates of dissolved organic matter (DOM) from phytoplankton were found to be a major source of food for bacteria (e.g. Larsson and Hagström 1979, Williams 1981).

It was found that the abundance of bacteria in surface waters was remarkably stable at about 10^6 ml^{-1} (e.g. Hobbie *et al.* 1977, Fuhrman and Azam 1980, Wright and Coffin 1984, Andersen and Fenchel 1985), despite their production often being a significant percentage of the primary production (Fuhrman and Azam 1982, Larsson and Hagström 1982). This led to the conclusion that there must be a sink for bacterial production, and grazing by small (<10µm) heterotrophic flagellates was found to be largely responsible.

The importance of small flagellates as bacterivores was first described in work on bacterial decomposition of detritus by Johannes (1965) and Fenchel (1977), where it was found that their presence enhanced the rate at which this process occurs. This was followed by observations that peaks in the abundance of bacteria and flagellates followed a cyclical pattern, with a few days lag between the maxima of the two populations, suggesting predator-prey oscillations (Fenchel 1982c, Andersen and Fenchel 1985). Further evidence was provided by the observation of bacteria in food vacuoles with electron microscopy (e.g. Haas and Webb 1979, Sieburth 1979) and epifluorescence microscopy (Sherr and Sherr 1983, 1984).

At the same time other potential grazers were shown to have little impact on marine bacterial production. Most metazoan zooplankton are incapable of efficiently extracting bacterial cells at *in situ* concentrations (e.g. King *et al.* 1980, Porter 1984, Sanders and Wickham 1993). Although cells of bacterial size may form a large part of the diet of small (<30µm) aloricate ciliates (Sherr and Sherr 1987, Rassoulzadegan *et al.* 1988), their localised distribution (only occurring in significant numbers in eutrophic near-shore sites) and slow metabolism in comparison to flagellates means that they have little impact on bacterial populations as a whole. Most ciliates have been shown to be more efficient at capturing larger particles (Capriulo and Carpenter 1980, Rassoulzadegan and Etienne 1981, Bernard and Rassoulzadegan 1990).

The use of epifluorescence microscopy to enumerate heterotrophic nanoflagellates (nano- refers to organisms between 2 and 20µm, similarly pico- to organisms of less than 2µm and micro- to organisms between 20 and 200µm after the scheme of Sieburth *et al.* 1978) revealed that previous techniques such as dilution cultures had led to a large underestimation of their abundance. Caron *et al.* (1989) found that the abundance of heterotrophic nanoflagellates calculated using dilution series averaged only 1% of that using epifluorescence microscopy. The latter method has shown that these flagellates are present in seawater at values averaging around 10^3 ml^{-1} (Burney *et al.* 1981, Sieburth and Davis 1982, Davis *et al.* 1985, Sherr *et al.* 1986). Clearance rates measured in the laboratory and *in situ* have shown that they can filter 10-100% of the water column for bacteria each day (e.g. Fenchel 1982b (100%), Sherr *et al.* 1986 (40-45%), McManus and Fuhrman 1988 (23% in summer, 100% in winter), Kuuppo-Leinikki 1990 (>75%)).

1.2 The wider trophic role of flagellates and the increasing complexity of the microbial food web.

In 1983 Azam *et al.* proposed the addition of a 'microbial loop' to the traditional picture of marine food webs. This involved the idea of DOM released from phytoplankton and as waste products from other organisms being passed on to the classical food web by the bacteria-flagellate-ciliate route. In addition to the evidence for flagellate bacterivory much support has been found for the flagellate-ciliate link (e.g. Davis and Sieburth 1984, Rassoulzadegan *et al.* 1988, Bernard and Rassoulzadegan 1990) and for consumption of ciliates by metazoa (e.g. Stoecker *et al.* 1987, Wiadnyana and Rassoulzadegan 1989). However, since the mid-1980s it has become clear that the microbial food web is more complex than this.

At the same time as the discovery of the importance of bacterial production in the microbial food web, evidence was published showing that a very large part of primary production in the oceans as a whole is due to cyanobacteria (Johnson and Sieburth 1979, Waterbury *et al.* 1979) and picoplanktonic eukaryotic algae (Johnson and Sieburth 1982, Li *et al.* 1983, Platt *et al.* 1983). The fate of this primary production received less interest than the fate of the bacterial secondary production, but recently studies have shown that small heterotrophic flagellates are also capable of consuming these organisms (Parslow *et al. 1986,* Hagström *et al.* 1988, Kuosa 1991, Rogerson and Laybourn-Parry 1992a). This means that in total a very large part of the entire oceanic production passes through heterotrophic nanoflagellates.

The trophic impact of heterotrophic flagellates is supplemented to some extent by photosynthetic flagellates. Although the phenomenon of mixotrophy (attaining nutrient and energy requirements from a mixture of autotrophy and heterotrophy) has been known amongst phytoflagellates for some time, its ecological impact has only recently been realised (Jones *et al.* 1993). Field studies have shown that in some situations these organisms may be responsible for a large part of the grazing on picoplanktonic populations (Sanders and Porter 1988, Sanders 1991 and refs within). Bacteria may also be lost from the water column through viral activity (Proctor and Fuhrman 1990, Wommack *et al.* 1992) or due to the activity of the predatory bacterium *Bdellovibrio* (Shilo 1984).

Additional links or pathways within the microbial food web include 'short-cuts' such as the uptake of DOM by heterotrophic flagellates (Sherr 1988), and the fact that some metazoa can predate on flagellates, bypassing the 'ciliate link' (Kopylov *et al.* 1981, Buck and Garrison 1988), although these are probably of little quantitative importance overall. There may also be grazing within particular size classes. Some heterotrophic flagellates can predate on other flagellates, (e.g. *Leucocryptos* spp. (Vørs 1992b), *Ciliophrys infusionum* (Larsen and Patterson 1990) and *Metopion fluens* (observations in this study)). Phytoflagellates, diatoms and other small plankton may also be consumed (e.g. Caron *et al.* 1990, Sherr *et al.* 1991). Some heterotrophic dinoflagellates, particularly those in the microplankton size category, can feed on organisms several times larger than themselves by using a pseudopodium which extends out of the flagellar pore and engulfs the prey.

Hence the microbial food web has emerged as a complex and dynamic series of interactions between protistan and prokaryotic microorganisms. But the role of heterotrophic nanoflagellates as the major grazers on heterotrophic and photosynthetic picoplankton is, as yet, undisputed.

1.3 The microbial food web in Southampton Water; previous studies.

Southampton Water is a shallow eutrophic estuary about 11km long, with a maximum width of 2.5km, and runs North West to South East in direction. It is supplied by three rivers: the Test and the Itchen discharge into the northern end and account for about 70% of the total inflow of freshwater (Webber 1980), whilst the Hamble enters about 4km from the mouth of the estuary. There are also inputs of treated domestic wastewater from four main sites around the estuary, and of industrial effluent, particularly on the Western side where the largest oil refinery in the U.K. is located (Antai 1989). Tidal currents are complex and characterised by a period of 2-3 hours of relatively little tidal movement; a 'stand' of high water or double high tide (Webber 1980). Surface samples of water were taken from various sites in the estuary (shown in fig. 1) between October 1991 and June 1994. One site, NW Netley, was chosen for a more intensive seasonal study, and was the only site sampled during the last 17 months of this study.

The abundance, biomass and trophic interactions of various components of the microbial food web in Southampton Water have been the subject of several previous studies, particularly at the sites marked by navigation buoys at Calshot (which is at the mouth of the estuary) and NW Netley. At NW Netley, bacteria, cyanobacteria, heterotrophic flagellates, ciliates and levels of chlorophyll a (as an indication of phytoplankton biomass) have all been found to exhibit a seasonal cycle of abundance and biomass, with minimum values in the winter, an initial increase in May associated with the a spring bloom of phytoplankton, and high values persisting through summer and early autumn (Antai 1989, Leakey 1989, Iriarte 1991, Kifle 1992 and Leakey *et al.* 1992). Maximum values often coincide with the *Mesodinium rubrum* bloom which occurs annually in the upper reaches of Southampton Water, often causing a red colouration of the water (Lindholm 1985, Kifle and Purdie 1994).

Antai (1989) measured bacterial numbers in 1987 and 1988 and found values of 0.27-19.4 x10^6 ml^{-1}. Leakey (1989) found slightly lower numbers (0.1-1.9 x10^6 ml^{-1}) from June 1986 to June 1987. He also enumerated cyanobacteria, which varied from 600 ml^{-1} in July to only 50-90 ml^{-1} in January. Iriarte (1991) reported a slightly higher maximum value of cyanobacteria (1.3 x 10^3 ml^{-1}) , but concluded that picoplankton formed a relatively small component of the total phytoplankton biomass and primary production in Southampton Water.

The seasonal abundance of heterotrophic flagellates was also measured by Antai (1989), and values were found to follow bacterial numbers fairly closely (which in turn correlated with temperature and chlorophyll a concentration). Flagellate density ranged from 0.7-9.8 x 10^3 ml^{-1}.

Leakey (1989, Leakey *et al.* 1993) found 55 morphotypes of ciliates during a years study of the NW Netley and Calshot sites in Southampton Water during 1986 and 1987, indicating their taxonomic diversity in this estuary. They dominated the microzooplankton, with rotifers and metazoan larvae amounting to less than 600 l^{-1}. Dinoflagellates were occasionally abundant (up to 1200 l^{-1}), but since samples were fixed in Lugols iodine it was not possible to determine whether these were heterotrophic or photosynthetic. Numbers of ciliates ranged from 1000 l^{-1} in January 1987 to 16000 l^{-1} in June 1986. Ciliate biovolume showed similar trends to abundance. Burkill (1982) found that the biomass of tintinnids was greater than that of naked ciliates, and that both groups had maximum values in May. However the sampling procedures used could have led to an underestimation of aloricate taxa (Leakey 1989).

Chlorophyll a values typically show a first peak in April or May, corresponding to blooms of diatoms, after low levels throughout the autumn and winter (Savage 1965, Burkill 1978, Kifle 1992). Iriarte (1991) and Kifle (1992) also report a bloom of the colonial haptophyte *Phaeocystis* in April 1988 and May 1990. Relatively high values of phytoplankton biomass are maintained until a second peak during July or August corresponding to a bloom of the photosynthetic ciliate *Mesodinium rubrum* which dominates chlorophyll levels and primary production at this time, particularly in the upper parts of the estuary (Kifle 1992, Kifle and Purdie 1994).

Iriarte (1991) and Kifle (1992) carried out size-fractionated studies of chlorophyll biomass. Iriarte found that on average 85.7% of biomass was in the >3μm fraction, accounting for some 80% of annual carbon fixation, and Kifle reports that 66-67% of primary production was carried out by the >10μm fraction in 1988, and these organisms were mostly larger than 20μm. However nanoplanktonic autotrophic flagellates (referred to as 'microflagellates') were often numerically dominant, and may account for a large proportion of primary production at times when the larger phytoplankton are not forming blooms (Kifle 1992).

Zooplankton have recently been studied in Southampton Water by Zinger (1989) and Lucas (1993). The herbivorous zooplankton seems to be dominated by calanoid copepods, and to a lesser extent, cirripede larvae, with peak abundances in early spring and autumn. The relative scarcity of mesozooplankton during the late spring and summer appears to be due to a succession of predatory coelenterates and ctenophores (Lucas 1993).

1.4 The taxonomic diversity of heterotrophic flagellates.

Recent ultrastructural studies have revealed the enormous taxonomic diversity of heterotrophic flagellates (Larsen and Patterson 1990, Patterson and Larsen 1991, Vørs 1992b and refs within) and have led to the complete demolition of traditional schemes of classification. The realisation that many taxa previously classified as 'algae' or 'fungi' are very closely related to certain heterotrophic flagellates has largely contributed to the integration of protistan taxonomy. The artificial separation of taxa into auto- and heterotrophic forms, and the resulting overlap of study by protozoologists and phycologists now means that some taxa have to be named under both the Botanical and Zoological codes (ambiregnal taxa; Patterson 1986). Because of the application of different taxonomic ranks to the same assemblages under the two codes, and the present instability of some groups which are proving to be polyphyletic, there is no acceptable standard for higher taxonomic titles. Therefore the use of colloquial names has been adopted in this report, and these are explained where necessary.

Since protists are unicellular, their adaptive radiation has occurred at the level of cell organelles, and is therefore particularly well documented by ultrastructural studies (Patterson 1989b). The fact that ultrastructural comparisons have led to the construction of robust taxa is supported by recent molecular data from the sequencing of ribosomal RNA (e.g. Sogin and Gundersen 1987, Schlegel 1991, Leipe and Hausmann 1993, Leipe *et al.* 1994). However, our knowledge of the diversity and relationships amongst heterotrophic flagellates remains incomplete. New species are still being discovered at a considerable rate, and the lack of electron microscopical studies on many named taxa has led to numerous synonyms and invalid groupings. There are a large number of species which cannot yet be assigned to any suprageneric assemblage (Protista *incertae sedis*), many of which are only known from light microscopical observations. Our limited knowledge of these organisms has recently been reviewed by Patterson and Zölffel (1991).

Knowledge of the taxonomy of heterotrophic flagellates is important for several reasons. Their pivotal role in marine food webs has already been discussed; the microbial food web provides a basis for the production of a large proportion of higher organisms, including commercially important species, and a very considerable proportion of primary and secondary production passes through heterotrophic flagellates. The fate of this production will affect global carbon cycling, and the amount of food available to higher organisms, and will depend on the proportions of the different flagellate taxa present. Some species may feed on other flagellates or nanophytoplankton, which reduces the resources available to larger organisms. Some taxa may be less favoured as a food source, particularly species with large siliceous loricas or spines (e.g. acanthoecid choanoflagellates, *Thaumatomastix* species). Also Eccleston-Parry and Leadbeater (1994) have shown that several common marine species have different growth rates and nutrient regeneration efficiencies under the same conditions. At present practically nothing is known about which taxa of heterotrophic flagellates are the most abundant in marine ecosystems, and a initial study of this was one of the major aims of this PhD.

Sequencing studies on ribosomal RNA (rRNA) have shown that the huge taxonomic diversity of flagellates is reflected in the variation of their rRNA. Since ribosomes are ubiquitous in living organisms (and also in mitochondria and chloroplasts) and the rRNA molecules are highly conserved, the genetic variation in these sequences from any organism can be compared. The variation in rRNA sequences of heterotrophic flagellates greatly exceeds that observed within all the multicellular organisms, and that within the prokaryotes (Schlegel 1991). This is of potential importance in view of the current interest in biodiversity, and maintenance of genetic diversity.

Finally, the diversity of ultrastructure amongst heterotrophic flagellates means that they are invaluable tools in cell biology, and in the study of early eukaryotic evolution. Some groups (the parabasalids and diplomonads) evolved before eukaryotic cells acquired mitochondria (Leipe and Hausmann 1993), and the location of heterotrophic species near the roots of hypothetical evolutionary sequences containing autotrophic flagellates suggests that chloroplast symbioses may have arisen on several occasions, and that they can be relatively easily lost or acquired.

1.5 Aims of this study.

(a) To document the diversity of flagellates in Southampton Water, with particular emphasis on new and poorly known species.

Detailed taxonomic studies on heterotrophic flagellates within a particular location are rare, and those which have been carried out have usually had a limited sampling period. Despite recent studies, new types of flagellates are still being discovered at a considerable rate (Patterson 1994). Many of the recently described species are poorly known. Therefore a detailed study of the diversity and taxonomy of heterotrophic flagellates in Southampton Water aimed to increase our knowledge of poorly known species, to describe new taxa, and to allow comparison with the species found in studies in other geographical locations.

Ninety-two species of heterotrophic flagellates are described in this study, together with a variety of other nanoplanktonic protists. Fourteen new species are presented (eleven flagellates and three other protists of uncertain taxonomic affinities).

(b) To determine which species of flagellates are numerically dominant, and whether this varies seasonally, and to combine studies of flagellate diversity and abundance to allow a greater understanding of their role in marine microbial food webs.

Heterotrophic flagellates play an important role in marine microbial food webs, but have been considered as a single functional group by most ecologists, despite recent studies showing their enormous diversity (e.g. Larsen and Patterson 1990, Patterson and Larsen 1991, Vørs 1992b). At present, practically nothing is known about which heterotrophic flagellate taxa are most important in terms of abundance and ecological impact. This would provide an increased understanding of the importance of different interactions and trophic pathways involving flagellates within the microbial food web, and allow research on topics such as energetics, food preferences, nutrient regeneration and molecular studies to be directed to species which are numerically important in marine ecosystems.

Efforts were made in this study to optimise the preservation of cells during the preparation of filters for epifluorescence microscopy to allow differentiation and enumeration of different taxa. The examination of light and electron microscope whole-mounts, and studies of live material and of enrichment cultures provided additional taxonomic information. The abundance of bacteria, nanoplanktonic diatoms and autotrophic nanoflagellates was also measured to study fluctuations in possible food resources for flagellates. Taxonomic differentiation of flagellates allowed an estimation of the relative grazing impact of heterotrophic nanoflagellates on phytoplankton, and on bacteria.

Chapter 2: Material and methods

Surface water samples were collected from a number of sites in Southampton Water between October 1991 and June 1994 (fig. 1). Initial samples were used to provide material for qualitative study, and then one site (NW Netley) was chosen for a detailed seasonal study of protistan abundance and dynamics. The methods specific to this seasonal study (samples NT6-NT31) are described in a separate section (Chapter 4), although the methods described below are also relevant for all of these samples. Details of sample locations and date of collection are shown in table 1.

Six different sites were sampled. Samples from Mayflower Park (MP) were collected at the edge of the shore; the site is close to ferry terminals and commercial docking areas. The Lee on Solent (LS) sample was also collected close to the shore, but the area is close to the mouth of the estuary and not industrialised. The Hythe (HY) samples were collected from the end of Hythe pier, and so were at some distance from the shore. Cracknore Buoy (CN), Greenland Buoy (GB) and NW Netley (NT) samples were collected in open water sites in the estuary, at sites marked by navigation buoys.

Two enrichment cultures were established from each sample by adding four wheat or four rice grains to about 100ml of untreated water. The wheat or rice caused growth of bacteria in the sample, which in turn stimulated the growth of bacterivorous flagellates, on which the study was concentrated, although other protists were sometimes encountered. The cultures were kept in the dark, at 10°C.

Light microscopy was carried out on living cells using an Olympus BH2 microscope. Observations were documented through annotated drawings, photography and video recordings.

A number of species in the enrichment cultures were isolated into mono-culture (table 2) and maintained in autoclaved, 0.8μm filtered natural seawater usually with a single species of marine bacterium for food, although for one species (*Developayella elegans*) it was found that growth was poor without allowing the continued growth of a

Figure 1: Map of sampling sites

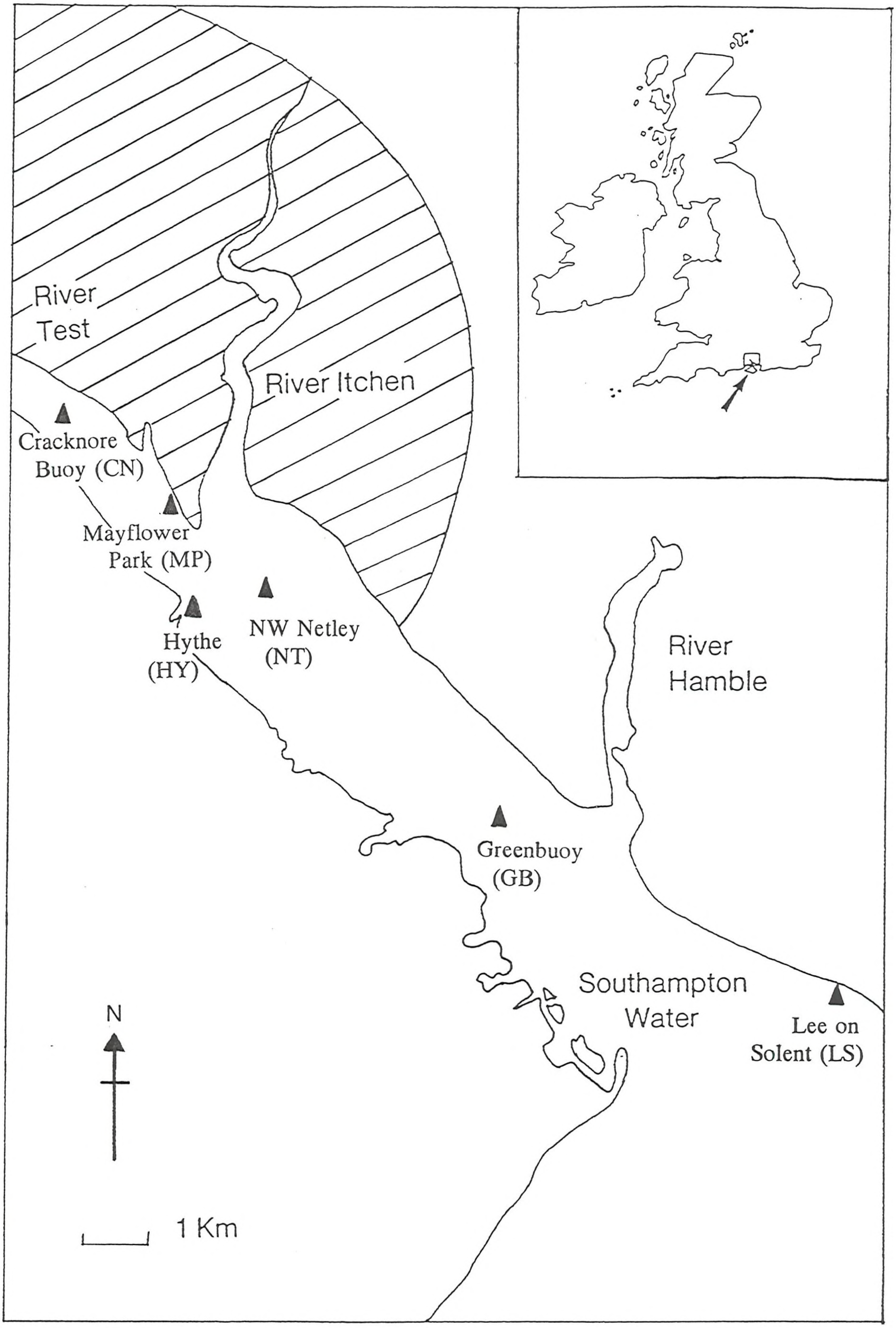


Table 1: Samples collected in Southampton Water, 1991-1994

SAMPLE	DATE COLLECTED
MP1	18.10.91
LS1	06.01.92
MP2	07.01.92
CN1	19.02.92
GB1	20.03.92
NT1	27.04.92
NT2	26.05.92
CN2	07.08.92
HY1	08.09.92
MP3	13.10.92
MP4	29.10.92
HY2	15.01.93
NT3	26.01.93
NT4	17.02.93
NT5	25.02.93
NT6	12.03.93
NT7	26.03.93
NT8	23.04.93
NT9	06.05.93
NT10	21.05.93
NT11	04.06.93
NT12	22.06.93
NT13	21.07.93
NT14	09.08.93
NT15	19.08.93
NT16	29.09.93
NT17	15.10.93
NT18	01.11.93
NT19	15.11.93
NT20	01.12.93
NT21	16.12.93
NT22	11.01.94
NT23	28.01.94
NT24	11.02.94
NT25	08.03.94
NT26	22.03.94
NT27	07.04.94
NT28	26.04.94
NT29	09.05.94
NT30 (a)	24.05.94
NT30 (b)	24.05.94
NT31 (0m)	08.06.94
NT31 (2m)	08.06.94
NT31 (7m)	08.06.94

Key to locations: MP = Mayflower Park, LS = Lee on Solent, CN = Cracknore Buoy, GB = Greenbuoy, NT = NW Netley, HY = Hythe cyanobacterium isolated at the same time as the protist. Protists were isolated by serial dilution, or, when they were less abundant, by picking out individual cells. Serial dilutions were made in test-tubes, by adding 1ml of the enrichment culture, or the previous dilution, to 10ml of autoclaved natural seawater. To encourage the growth of the protist, a grain of wheat or rice was added to stimulate growth of bacteria isolated from the enrichment culture, or else 1ml of a dense suspension of bacteria was added to the tube. Cells were picked out from enrichment cultures using a glass pipette drawn out to a diameter of 30-50μm. They were located and identified under the x40 lens of a light microscope, and then picked out whilst viewed under the x10 lens; a binocular microscope could not be used because of the insufficient magnification. Once the cell had been picked out, the tip of the pipette was snapped off into the culture medium.

Sub-culturing was carried out every month to six weeks, whereby 1ml of the original culture was added to 10ml of autoclaved 0.8μm filtered seawater and 1ml of bacterial suspension, with a grain of wheat. Two isolates of bacteria were used: 'B1', an unnamed marine isolate was supplied by Dr. J. Eccleston-Parry, University of Lancaster, U.K. and maintained on Oxoid Nutrient Agar, and *Vibrio natriegens* was supplied by N.H. Larsen, Københavns Universitet, Denmark, and maintained on Difco Marine Agar 2216.

From NT1 (27.04.92) onwards the protistan species in freshly collected material were studied in addition to those grown up in enrichment cultures. After being passed through a 40μm net, 192 ml of the sample was centrifuged, at the temperature measured on collection, in two stages (ten minutes each) at 2200rpm (502g) to 2ml. Part of the material was examined immediately under the light microscope, and the rest was used to make electron microscope whole-mounts (CN2 onwards) and light microscopical whole-mounts (MP4 onwards).

Electron microscopical whole-mounts were prepared as described by Moestrup and Thomsen (1980). Small drops of the concentrate were placed on pioloform-coated copper mesh grids and fixed in osmium vapour for approximately 20 seconds. They were then dried rapidly at around 40°C (to prevent copper in the grid bars reacting with constituents of seawater, which causes the sample to become obscured by crystal

Table 2: Protistan species isolated from Southampton Water and maintained in mono-culture

SPECIES	Name of original sample and date isolated	Notes
Bicosoeca maris	NT4, 08.03.92	
Bodo designis	CN2, 24.08.92	
Bodo saliens	MP1, 16.01.92	Studied ultrastructurally
Caecitellus parvulus	MP1, 31.03.92	
Cafeteria roenbergensis	MP1, 14.01.92	
Ciliophrys infusionum	MP1, 03.04.92	Used in growth kinetics experiments by Eccleston-Parry and Leadbeater (1994)
Developayella elegans	CN2, 28.08.92	Studied ultrastructurally; type culture for this species
Massisteria marina	NT20, 20.12.93	
Ministeria 'vibrans'	II: MP1, 20.03.92 IV: GB1, 24.04.92	Several isolates made, only two maintained. Strain II is the type culture for this species
Paraphysomonas butcheri	HY2, 12.03.93	V_4 region of 18S rRNA gene sequenced by J. Rice
Paraphysomonas foraminifera	NT24, 21.04.94	Complete 18S rRNA gene sequenced by J. Rice
Paraphysomonas imperforata	HY1, Oct. 1992	V_4 region of 18S rRNA gene sequenced by J. Rice
Paraphysomonas vestita	MP1, 10.01.92	Complete 18S rRNA gene sequenced by J.Rice
Pteridomonas danica	CN2, 28.08.92	
'Pendulomonas adriperis'	NT24, 21.04.94	Studied ultrastructurally, type culture of this species

formation). Grids were then placed on a wire support in distilled water for about 5 minutes, before being rinsed several times in clean distilled water, and then blotted dry with filter paper which was applied to the edge of the grid. Grids were examined (without metal shadowing) in a JEOL JEM 1200 electron microscope. In addition to the examination of freshly collected material, electron microscope whole-mounts were also routinely prepared from enrichment cultures in order to identify species which could not be distinguished by light microscopy, and to provide more detailed information about the morphology of species with loricas or scales.

Light microscope whole-mounts were prepared in a similar manner. Drops (30µl) of the material were placed on clean coverslips, fixed in osmium vapour and then dried at room temperature. The coverslips were then placed in distilled water for 5 minutes, then dipped several times in clean distilled water. After drying at room temperature, the coverslips were inverted and mounted on microscope slides using drops of nail varnish at the corners. This method was used to identify acanthoecid choanoflagellates, since their loricas are often too thin to be resolved sufficiently for identification in water samples.

Four species isolated into mono-culture were examined ultrastructurally. Dense cultures of the species were concentrated by gentle centrifugation to a volume of about 1ml, and then fixed as described below.

1) *Ministeria* 'vibrans' was fixed for 30 minutes in 5% glutaraldehyde, 0.8% osmium tetroxide, 0.35M sucrose, and 0.05% MOPS (3-[N-Morpholino]propanesulphonic acid) buffer at pH7.4.

2) *Bodo saliens* Larsen and Patterson, 1990 was fixed for one hour in 2.5% glutaraldehyde, 0.6M sucrose and 0.05% MOPS buffer, followed by a rinse in a sucrose/buffer mixture, and post-fixation for one hour in 1% osmium tetroxide, 0.6M sucrose and MOPS.

3) *Developayella elegans* Tong, in press, was fixed for 45 minutes in 2.5%

glutaraldehyde, 0.4% osmium tetroxide, 0.4M sucrose and 0.05% MOPS buffer at pH7.2.

4) 'Pendulomonas adriperis' (an undescribed stramenopile) was fixed in the same manner as *Developayella elegans*.

After fixation cells were washed in a series of sucrose/buffer mixtures, with a decreasing concentration of sucrose, and finally in a dilute buffer solution. The cells were then embedded in agar, dehydrated through a graded series of alcohols, with staining in 2% uranyl acetate for one hour at the 50% dehydration stage, and embedded in araldite resin via propylene oxide. With the latter three species the procedure was carried out in an ice bath until the 99% dehydration stage, and thereafter at room temperature. With *Ministeria*, the whole procedure was carried out at room temperature.

Ultrathin sections were cut with a glass knife (*Ministeria*, *Bodo*) or with a diamond knife (*Developayella*, 'Pendulomonas') and stained with uranyl acetate (2% solution in 50% methanol, 20 mins) and lead citrate (20 mins) at about 35°C.

Chapter 3: The taxonomy of heterotrophic flagellates and other protists in Southampton Water

This section describes the protists encountered in enrichment cultures of water collected from various sites around Southampton Water. A few forms encountered only in freshly collected material, or seen on whole-mounts of fresh material are also described. This taxonomic part of the study of nanoplankton in Southampton Water was concentrated on heterotrophic nanoflagellates, excluding dinoflagellates, and this is reflected in the species discussed below.

Approximately 150 morphotypes of heterotrophic nanoflagellates have been distinguished during the course of this study. Ninety-two species are described below. Others are not referred to since insufficient information has been collected to identify them, or in many cases, to provide a diagnosis of the unique/defining characteristics necessary for the description of a new taxon. Eleven new species of flagellates are described. Two of these have only been given colloquial names (under undescribed species) since more information is required to describe them formally, but they have been included because they occurred quite commonly in cultures, and are likely to be found in any subsequent study of flagellates in Southampton Water. Four other species have been mentioned in the literature previously (three *Thaumatomastix* species and *Telonema* 'antarctica'), but have not yet been officially named.

The flagellates described below are grouped into what are currently conceived to be monophyletic taxa, except for those of uncertain taxonomic affinities, which are described under 'Incertae sedis taxa'. Most of the taxa are referred to by colloquial titles because of the current instability of the nomenclature of higher ranking taxa in protistology.

A variety of other protists were encountered: amoebae, ciliates, autotrophic flagellates, diatoms and protists of uncertain taxonomic affinities (Protista *incertae sedis*). A few of these are mentioned in this section, including three new species belonging to genera of uncertain taxonomic affinities: *Luffisphaera* 'hamatus', *Luffisphaera* sp. and *Ministeria* 'vibrans'.

The occurrence of flagellates and other protists in different samples is shown in Appendix 1. The information in this table combines observations from all the types of methodology used: observations on freshly collected centrifuged material, on enrichment cultures, on light and electron microscope whole-mounts and on epifluorescence microscopy filters. (See chapter 4 for epifluorescence methodology.) In earlier samples some of these methods were not used (see Chapter 2), and therefore the diversity of protists recorded is lower.

3.1 AMOEBAE

The word 'amoebae' (like 'flagellates'), is not intended as a taxonomic term, since it refers to a large diversity of unrelated protists. For the purpose of this study it refers to protists whose dominant locomotive form is aflagellate and which move by the means of pseudopodia. Amoebae occurred in most enrichment cultures and were principally bacterivorous. The most common forms were vahlkampfiids and members of *Vanella/Platyamoeba* (these two genera can only be confidently distinguished by using electron microscopy). The taxonomy of marine amoebae, particularly small naked forms, which were common in this study, has been largely neglected (Rogerson and Laybourn-Parry 1992b, Rogerson 1993). Therefore it was not possible to identify most of the forms encountered; at least some of these were undescribed species (A. Rogerson, personal communication).

Hartmannella sp.

Figs: 2a, 2b

Size of motile form: c1.5 x 9µm

Hartmannella species are limax amoebae with non-eruptive locomotion and an anterior hyaline cap and they may possess uroidal filaments. They can be distinguished from species of the related genus *Saccamoeba* which has a bulbous uroid, and a reduced hyaline cap (Page 1983). This species was fairly common in enrichment cultures.

Thecamoeba orbis Schaeffer, 1926

Fig. 2c

Size of motile form: c 15 x 18μm

Thecamoeba species can be distinguished by conspicuous folds or wrinkles in the surface. The species in the present study was in accord with Page (1983) and was found in two enrichment cultures.

Neoparamoeba sp.

Fig. 2d

Size of motile form: c3 x 8μm

This genus can be distinguished from *Mayorella*, which also has a 'duck's foot' shape, with subpseudopodia produced from the anterior hyaline zone, by the parasome (small DNA containing body adjacent to the nucleus). It may have prominent longitudinal ridges.

Paulinella ovalis (Wulff) Johnson, Hargraves and Sieburth, 1988

Fig. 2e

Size of lorica: c 3x 4.5μm

This species was found in two samples. A detailed description is given by Johnson *et al.* (1988). Their ultrastructural investigation revealed that this species, previously

Figure 2: Amoebae, ciliates and undescribed flagellates

a-b: *Hartmanella* sp., LM of motile cells x2000.

c: *Thecamoeba orbis*, LM x1500.

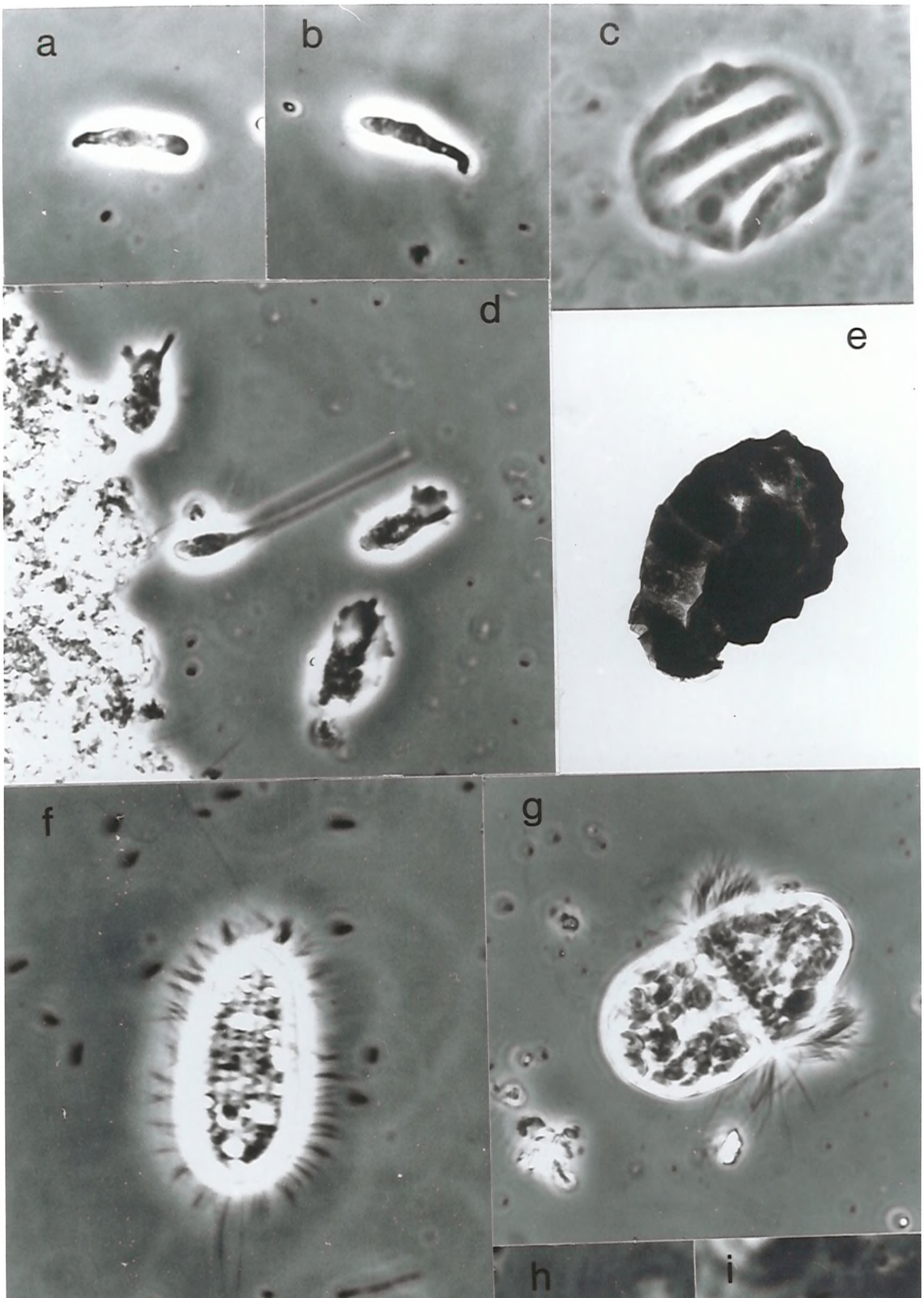
d: *Neoparamoeba* sp. LM of gliding and floating forms (the latter represented by the cell with a long pseudopod) x2000.

e: *Paulinella ovalis*, EM of test x10,000.

f: *Uronema* sp., LM x1500.

g: *Mesodinium rubrum*, LM x1500.

h-i: 'Cyrano', an undescribed flagellate of unknown taxonomic affinities (section 3.16) LM x2000.


a
b
c
d
e
f
g
h
i

classified in the chrysophytes (as *Calycomonas ovalis*) is a testate amoeba.

3.2 CILIATES

Several species of ciliates were observed during the study, particularly in freshly collected centrifuged material, but were generally not identified. A *Euplotes* species was seen on two occasions in enrichment cultures, and a *Vorticella* species on one occasion. A *Uronema* species (c15 x 30µm) was frequently seen in enrichment cultures, and *Mesodinium rubrum* was often seen in freshly collected material; these two species are shown in figs 2f and 2g. The *Mesodinium* cells seen were usually about 15-20 x 30-40µm; it is likely that larger cells occurred, but these would not have passed through the screening mesh used after samples had been collected.

3.3 APUSOMONADS

A group containing *Amastigomonas* and *Apusomonas* species which have been shown to be closely related through ultrastructural studies (Karpov and Mylnikov 1989).

Amastigomonas debruynei De Saedeleer, 1931

Figs: 3a-d, 3h, 4a

Size: 2-3.5 x 3.5-7.5µm

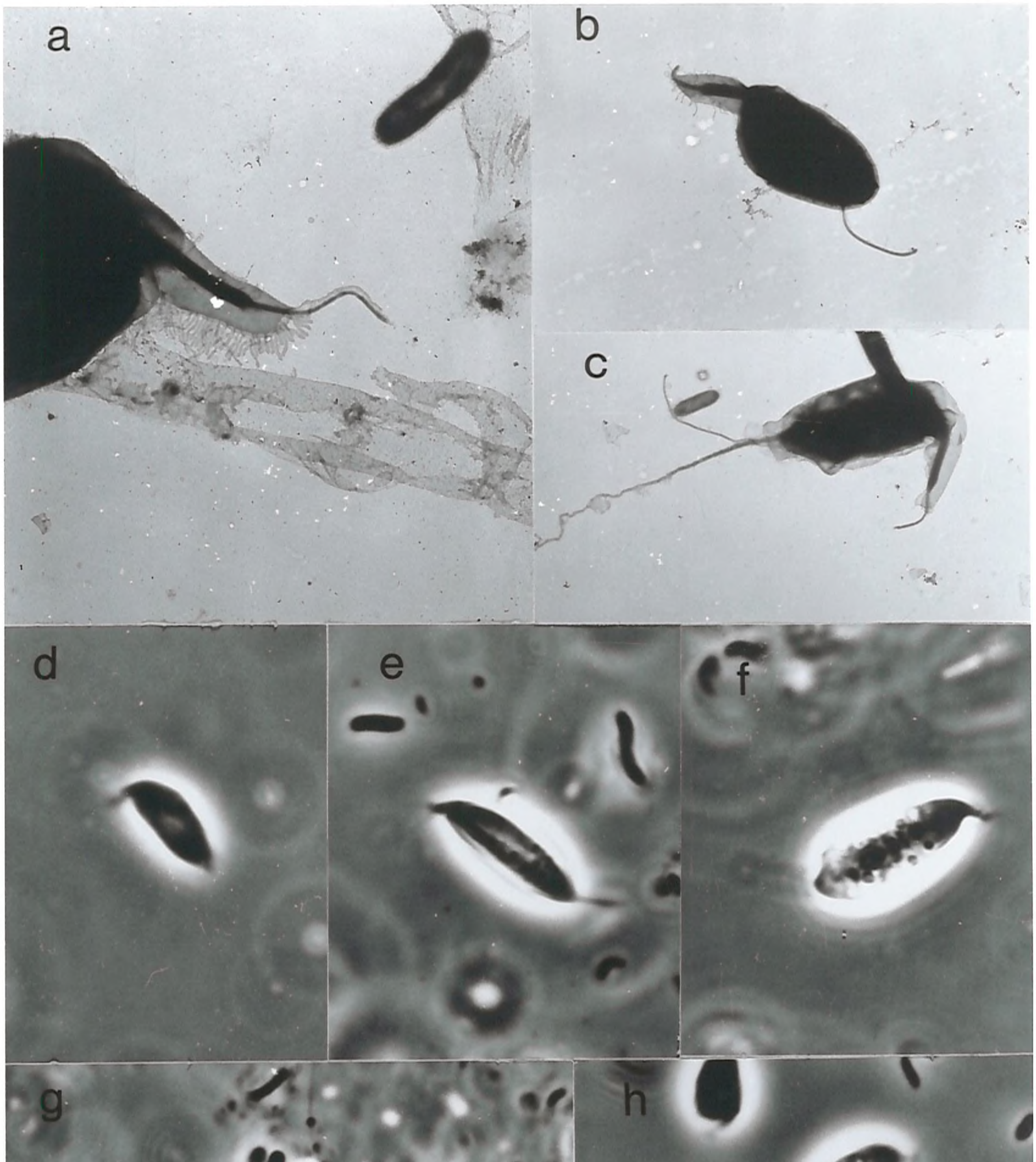
Observations: *A. debruynei* was encountered in nearly every culture, although rarely in great numbers. The cells are oval, flexible, and may produce pseudopodia from the ventral surface, which are used to engulf prey. Pseudopodia may also trail behind the cell (figs 3c, 3h). Two flagella insert sub-apically. The anterior one is enclosed within a sleeve of cytoplasm, forming the snout or proboscis. This flagellum may extend a short distance from the end of the snout, and since this varies within individual cells it would appear that the flagellum is somewhat retractable. The snout waves slowly up and down as the cell glides forward. The posterior flagellum lies across the cell, and may trail

Figure 3: Apusomonads and cercomonads

a-d, h: *Amastigomonas debruynei.* a-b: EM of cells with unusually wide snouts, notice the threads on the lower part of the snout, a: x10,000, b: x7500. c: EM of 'normal' cell, notice the pseudopod trailing behind the cell, x5000. d,h: LM x2000.

e-f: *Amastigomonas mutabilis*, LM x2000.

g,i: *Massisteria marina*, LM showing sessile cells with radiating pseudopodia bearing extrusomes x2000.


a
b
c
d
e
f
g
h
i

behind the cell for a short distance. Both flagella are difficult to see with light microscopy.

The characters of this species are somewhat variable, but a continuum of variation suggests that cells should not be divided into more than one species. On some occasions the snout was wider and clearer than usual, and the path of the flagellum inside the cytoplasmic sleeve was apparent. When examined in electron microscopical whole-mounts it was found that these cells bore small threads on the lower surface of the snout (figs 3a,b). The nature and function of these threads is unknown.

Remarks: This species is superficially similar to the bodonid *Rhynchomonas nasuta*, but may be distinguished by the fact that *Rhynchomonas* has a thicker, longer and acronematic flagellum, a proboscis which dilates at the distal end, and does not form pseudopodia. The author regards *A. debruynei* as being con-specific with *A. trahens* (Larsen and Patterson 1990) Molina and Nerad, 1991 since the only distinguishing feature is the habitat from which these species were first reported (freshwater or marine).

Amastigomonas mutabilis (Griessmann) Molina & Nerad, 1991

Figs: 3e-f, 4b

Size: 3-4.5 x 8-14.5μm

Observations: Found sporadically in cultures, in small numbers. Cells are more elongate than in *A. debruynei*, and the two flagella are more easily seen. The posterior flagellum is attached along the body next to a row of refractile granules (fig. 4b), and extends behind the cell for a short distance. The snout is longer than in *A. debruynei*, and the anterior flagellum can be clearly seen protruding from it (usually for a distance of about 2.5-3μm). Cells are flexible and become distorted when changing direction.

Remarks: The row of refractile granules beside the posterior flagellum is one of the characteristic features of *A. mutabilis* (Larsen and Patterson 1990), but a few cells were

Figure 4: Diagrams of apusomonads and cercomonads

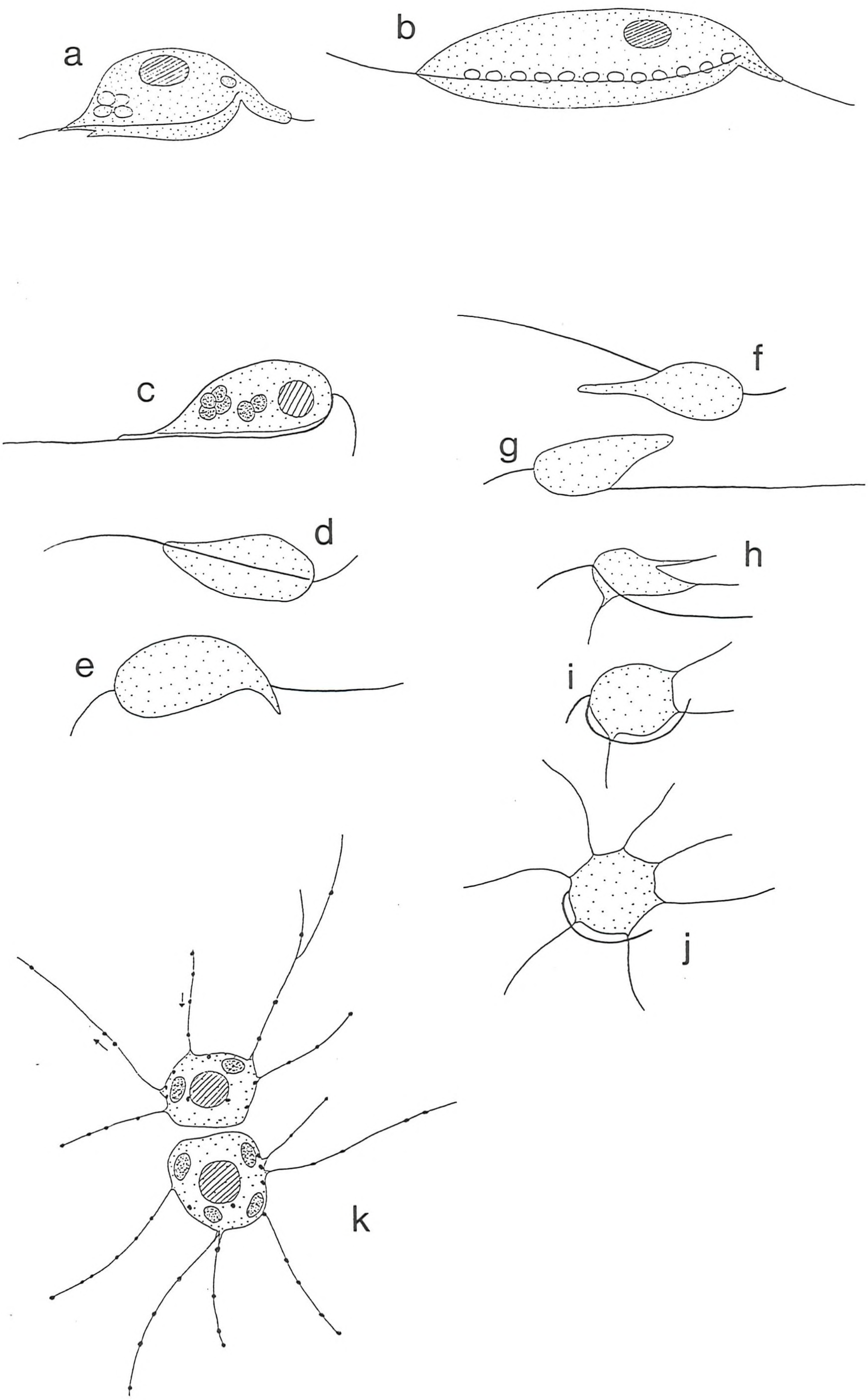
a: *Amastigomonas debruynei.*

b: *Amastigomonas mutabilis.*

c-e: *Cercomonas* sp., showing the cell from a lateral view (c), ventral view (d) and dorsal view (e).

f-k: *Massisteria marina,* showing 'swarmer' cells from the dorsal (f) and lateral views (g), stages in transformation between 'swarmer' cells and sessile cells (g-j) and sessile cells (k).

All diagrams x5000.


a
b
c
d
e
f
g
h
i
j
k

encountered where the granules were not visible, although normal in all other respects.

3.4 CENTROHELIDS/CENTROHELIDA Kühn, 1926

These are heliozoa characterised by the possession of hexagonally arranged bundles of microtubules in the pseudopodia, a centroplast (region where the microtubules terminate at the centre of the cell), ribbon like cristae in the mitochondria, and various other ultrastructural features (Dürrschmidt and Patterson 1987). A detailed description of centrohelid species is given in Siemensma (1991).

Meringosphaera mediterranea Lohmann, 1902

Fig. 5a

Observations: Seen in electron microscope whole-mounts from two samples. The cell surface is covered by long undulating spine scales, and flattened plate scales (both visible in fig. 5a).

Pterocystis sp.

Figs: 5c-d

Observations: Found in electron microscopical whole-mounts of the NT23 sample. Cells bear two types of scales: flattened plate scales, and similar scales with short extentions on them, creating a spade-like shape.

Raphidocystis tubifera Penard, 1904

Fig: 5f

Observations: This species bears three types of scales (Rees *et al.* 1980). Two of these (flat oval scales and 'funnel' shaped scales) were seen in electron microscope whole-

Figure 5: Centrohelids, coccolithophorids and prasinophytes

a: *Meringosphaera mediterranea*, EM of plate and spine scales x5000.

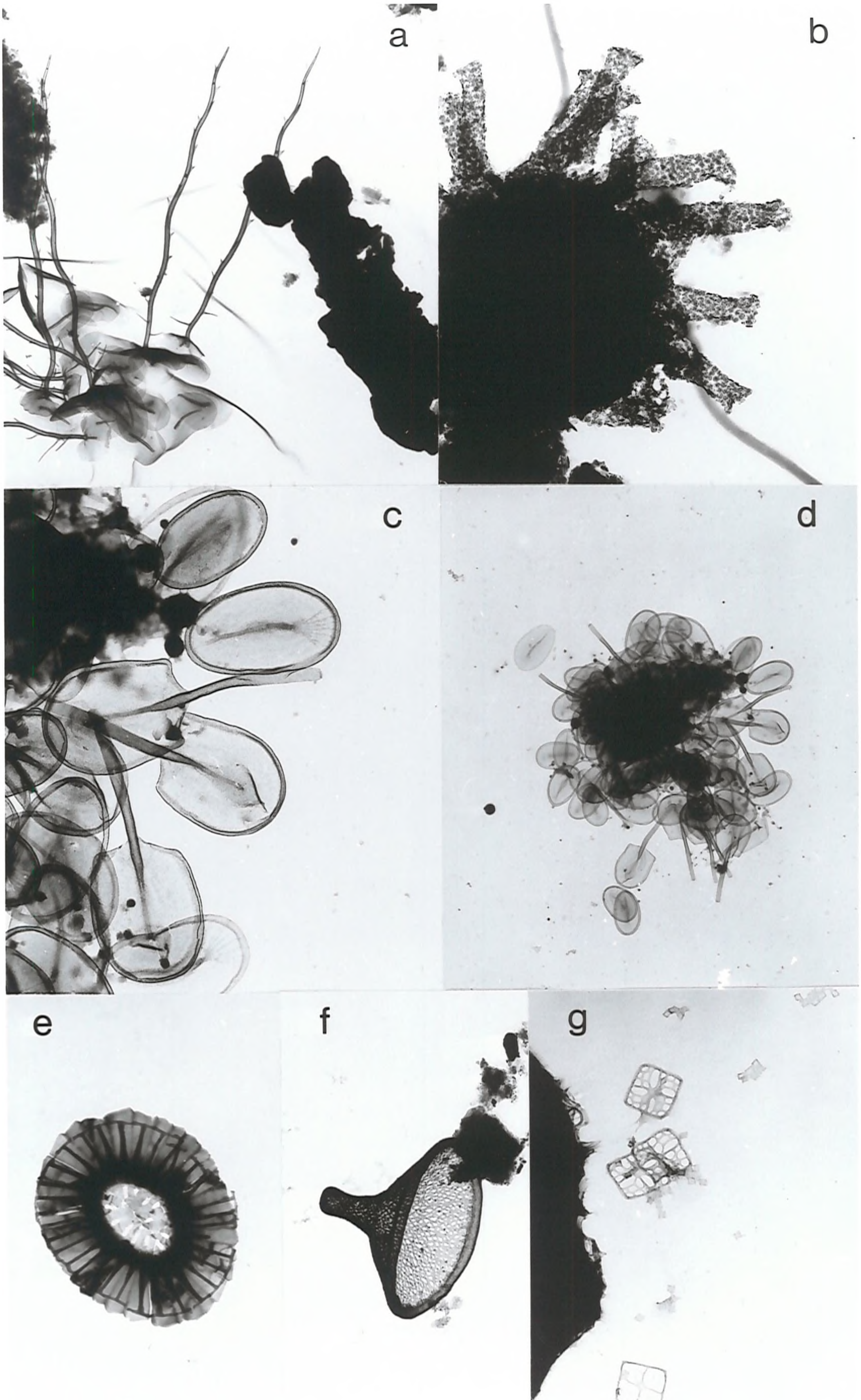
b: *Papposphaera* (*Turrisphaera*) sp., EM of scales composed of hexagonal crystallites, the two flagella are also visible x10,000.

c-d: *Pterocystis* sp., EM of cell surrounded by plate scales, and spade-like scales (d) x3000, and detail of scales (c) x 10,000.

e: *Emiliania huxleyi*, EM of a single scale x10,000.

f: *Raphidocystis tubifera*, EM of a single funnel shaped cell x10,000.

g: *Pyramimonas cirolanae*, EM of middle body-layer scales and smaller inner-layer scales x30,000.


a
b
c
d
e
f
g

mounts of the NT22 sample. One of the 'funnel' shaped scales is shown in fig. 5f.

3.5 CERCOMONADS

This group contains three genera: *Cercomonas*, *Heteromita* and *Massisteria* (Patterson and Zölffel 1991). The taxonomy, and to some extent the identity, of the first two genera is confused, with many species described, and many probable synonyms. Members of the genus *Cercomonas* were seen quite regularly, but could not be identified. The most common species is described below.

Cercomonas sp.

Figs: 4c-e

Size: 2-2.5 x 3-7.5µm, anterior flagellum 4.5-5µm, posterior flagellum 11-14.5µm

Observations: Seen in enrichment cultures of several samples. Cells are flattened ventrally and broadest at the anterior, elongating into a posterior projection of variable length. A narrow strip of transparent cytoplasm lies over, and is attached to the posterior flagellum on the ventral surface; this strip projects along the flagellum behind the more dense cytoplasm (fig. 4c), and is characteristic of this species. Two flagella insert apically. The anterior flagellum waves stiffly from side to side as cells glide. The posterior flagellum is attached to the ventral side of the cell, and is conspicuously acronematic. Pseudopodia may be produced from any part of the body (particularly on the dorsal posterior side), except for the strip of transparent cytoplasm on the ventral side, and ingestion of large bacteria (c1 x 3µm) was observed on the dorsal side of the body. This is contrary to the observations of Mignot and Brugerolle (1975) who found that prey was phagocytosed on the ventral side in a different species of *Cercomonas*. Swimming was not observed.

Massisteria marina Larsen & Patterson, 1990

Figs: 3g, 3i, 4f-k

Size: sessile cells: 2-5.5µm; swarmer cells: 1.5-2.5 x 3-7µm.

Observations: *M. marina* was one of the most commonly encountered flagellates in enrichment culture. It may be easily overlooked because of its close association with detritus; cells generally become visible several minutes after a slide has been prepared, when the cell has moved (by unknown means) above the detritus and on to the cover slip. Feeding cells are roughly spherical, and flattened, with fine pseudopodia radiating out around the body, bearing small granules (extrusomes) (figs 3g, 3i, 4k). These extrusomes may sometimes be seen inside the cell body, and can move quite rapidly up and down pseudopodia. Different granules on a single pseudopodium may move in opposite directions at the same time. Motile bacteria can become stuck to the pseudopodia, and sessile bacteria are engulfed by the growth of the distal end of a pseudopodium to surround them. A tiny vacuole containing the prey item is then translocated along the pseudopodium, in association with several extrusomes, and fuses with the cell body. Two short inactive flagella are sometimes observed curved over the cell body.

Cells may absorb their pseudopodia and transform into motile cells or 'swarmers' (figs 4f,g) which may glide, or swim, rotating about the longitudinal axis with the posterior flagellum trailing behind. Swarmer cells show much resemblance to members of *Cercomonas*. The anterior flagellum is short, and beats stiffly from side to side, causing the elongated posterior of the body, which is raised above the substrate, to wag from side to side. The posterior flagellum is 2-3½ times the body length and is acronematic. It attaches to the cell for a short length at the proximal end. Stages in the transformation from motile to sessile cells are shown in figs 4f-j; gliding becomes irregular, and then ceases, the posterior flagellum becomes shorter, and pseudopodia are gradually produced around the cell as it assumes a rounded form.

Remarks: Details of food capture, and movement of the extrusomes have not previously been reported. Patterson and Zölffel (1991) place *Massisteria* within the cercomonads on the basis of ultrastructural features shared with *Cercomonas* and *Heteromita*.

3.6 CHOANOFLAGELLATES

Choanoflagellates are commonly found in marine waters throughout the world, and often form a large part of the nanoplankton (e.g. Booth *et al.* 1982, Marchant 1985, Buck and Garrison, 1988). Three families are recognised on the basis of the covering surrounding the cell. Members of the Acanthoecidae (with siliceous loricae) are particularly conspicuous in marine environments, whilst the Codosigidae ('naked' cells, although a thin investment is sometimes observed with electron microscopy) and Salpingoecidae (thecate cells) are more prevalent in fresh water (Thomsen 1992, Vørs 1992b).

CODOSIGIDAE Kent, 1880

Kentrosiga 'echina' (Tong, in prep.)

Figs: 6a-c, 6e, 15a

Size: cell 2.5-7.5 x 4.5-9.5µm, flagellum 23.5-31.5µm, pseudopodial collar 6.5-7.5µm, posterior bristles (rather rigid fine cytoplasmic extensions) up to 14µm

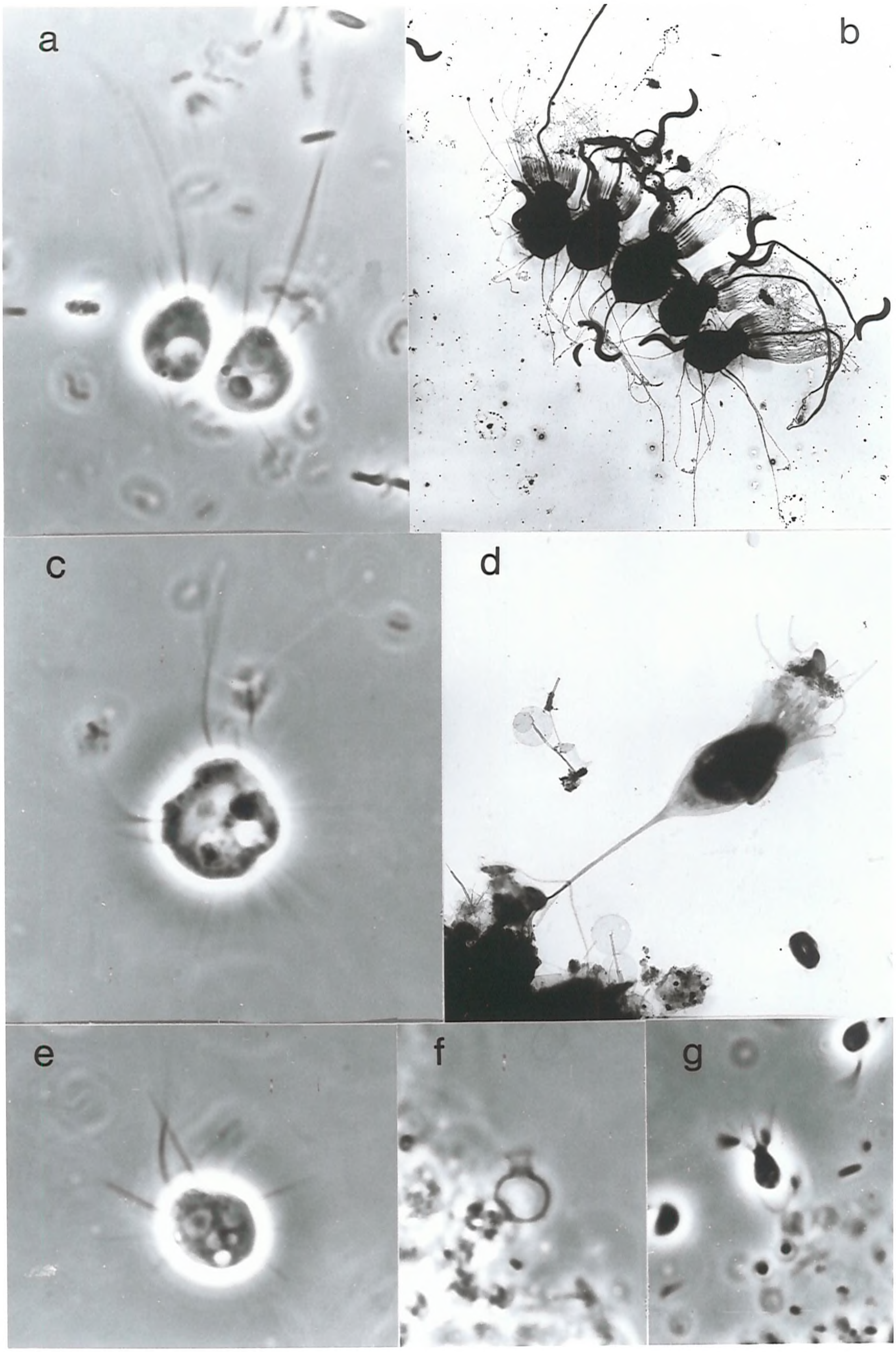
Observations: Seen sporadically in enrichment cultures. Cells were usually in pairs, but occasionally seen in groups of four or five, or singly. The groups of cells were often motile, but usually settled after they had been observed for some time under the microscope. Although there is quite a variation in cell size, small individuals (2.5 x 4.5µm) were seen on only one occasion, and larger individuals (c7 x 9µm) were also rare. The largest cell seen (9.5µm) was in the process of division (fig. 6c). Despite the confusion surrounding the identity of many 'naked' choanoflagellates, this species has several conspicuous features: the bristles which are found all over the cell, but particularly over the posterior half, the long flagellum, the conspicuous nucleus (e.g. figs

Figure 6: Codosigidae and Salpingoecidae

a-c, e: *Kentrosiga* 'echina'. a,c,e: LM of cells showing 'bristles' on cell surface, prominent nuclei (c,e) and long flagella (a), all x2000. b: EM of colony of five cells, notice the 'bristles' on the cell surface, and the thin cell covering, x2000.

d,g: *Salpingoeca marina*. d: EM x5000, g: LM x2000.

f: *Salpingoeca amphoridium*, LM of empty theca x2000.


a
b
c
d
e
f
g

6a,e), and the habit of appearing in small groups. The bristles were occasionally seen to bend sharply, and then straighten, and seemed to allow sessile cells to reposition themselves. Each cell is enclosed in a thin investment, which is visible in electron microscope whole-mounts (fig. 6b) looking rather like a shadow around the cell.

Remarks: The present species appears to be identical to *Desmarella moniliformis* sensu Throndsen (1974, 1983). However, there is no mention of any 'bristles' on the cell in the original description of *D. moniliformis* (Kent 1880), nor do they appear in more recent diagnoses of the genus (e.g. Thomsen and Buck 1991). Schiller (1953) created the genus *Kentrosiga* to accommodate choanoflagellates forming small colonies and bearing bristles from the posterior of the cell. The species in this study is most similar to *K. setifera*, but differs in having more bristles (described species only have 2-5 per cell), a relatively longer flagellum, and a much smaller cell size (*K. setifera* is about 20μm). Therefore it is proposed that this taxon is described as a new species of *Kentrosiga*.

SALPINGOECIDAE Kent, 1880

Salpingoeca amphoridium James-Clark, 1867

Figs: 6f, 8d

Size: cell 3.5 x 5.5-7.5μm, width of 'neck' of cell 1.5μm, length of neck 2.5μm

Observations: Seen sporadically in culture. As with many *Salpingoeca* species, the theca is often difficult to see around living cells. Fig. 6f shows an empty theca, which illustrates the flask-like shape that characterises this species. This mirrors the shape of living cells (fig. 8d).

Remarks: Vørs (1992b) discusses how illustrations by several authors have led to confusion about the identity of this species, and refers readers to the original description by James-Clark (1867) and that of Kent (1880).

Salpingoeca infusionum Kent, 1880

Figs: 7a-g, 8a-c

Size: cell: 2.5-5 x 4-6.5μm, lorica chamber 4.5-5 x 9.5-12.5μm, lorica stalk 11.5-27μm, flagellum 12-43.5μm, pseudopodial collar 9-13μm, 'cyst' 3.5-4.5 x 4.5-6.5μm, 'cyst flagella' 2.5-5μm, swarmer cells 2-3 x 4.5-5μm

Observations: The theca is relatively thick, and therefore more readily distinguished with light microscopy than in some other members of this genus. It has an ovate chamber which tapers posteriorly. In some cells there is a small 'bulb' just above the attachment to the pedicle (fig. 8a). The pedicle is generally 1½-2 times the length of the theca, and is attached to the substratum by a number of fibres (fig. 7d). The theca surface appears amorphous in electron microscope whole-mounts. The cell is ovoid and extends to occupy the anterior of the theca during feeding, remaining attached to the base of the chamber by one or more fine cytoplasmic threads (figs 7e, 8a). The length of the flagellum varies but is often very long (5-7 times the length of the cell).

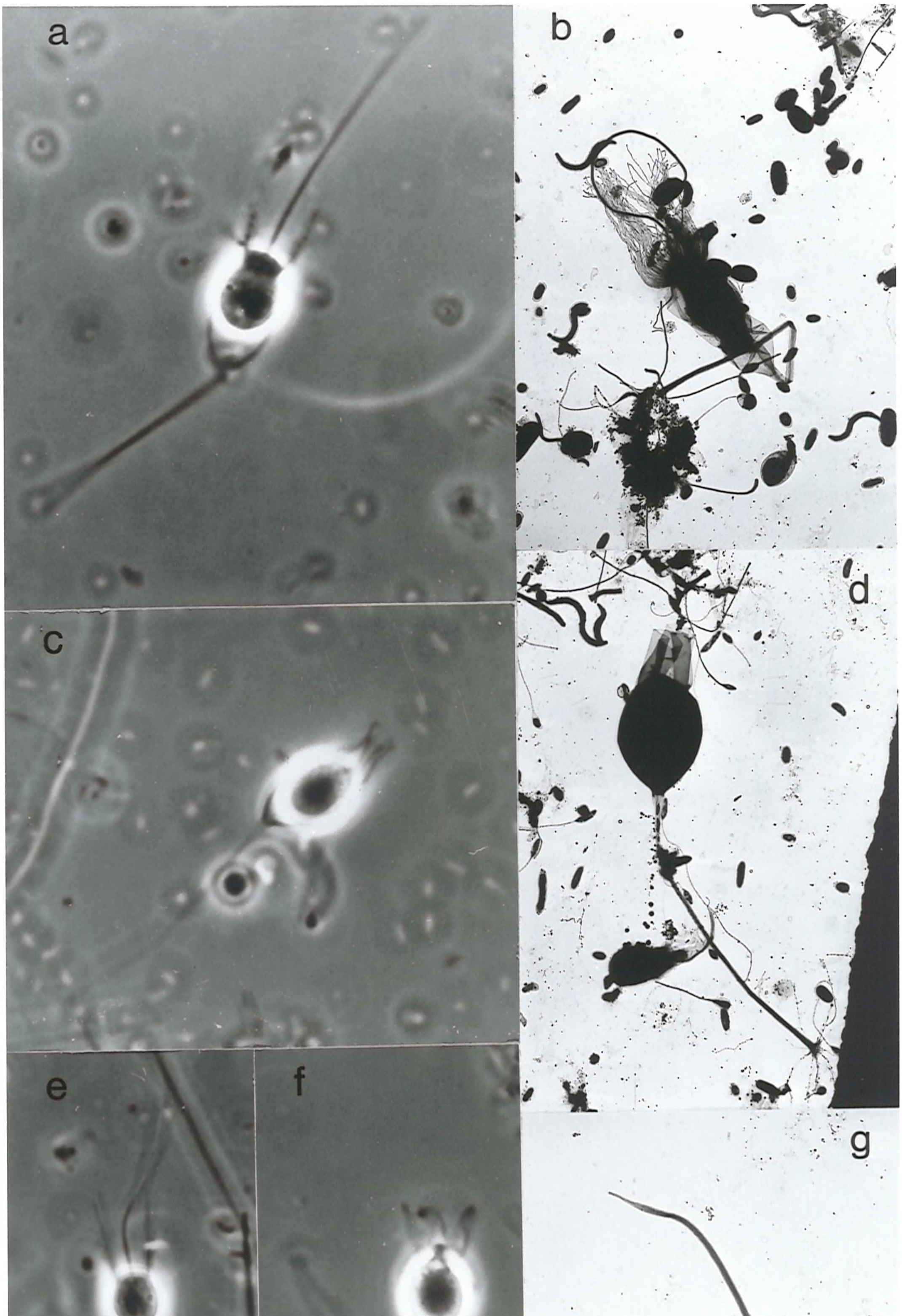
This species was observed to form structures resembling cysts within the theca (figs 7c,d). The 'cysts' were oval, with a small papilla at the anterior end and usually bore two short inactive structures which appeared to be flagella (fig. 7c). Stages intermediate between the encysted and normal forms were occasionally seen, with two longer active flagella; the pseudopodial collar was absent or rudimentary (fig. 8c). The formation of cysts in choanoflagellates is a subject of some dispute, and therefore confident interpretation of the phenomena seen in this species must await electron microscopy of fine sections. 'Swarmer' cells were also seen. They were somewhat smaller than normal cells, had a shorter flagellum (about 10μm), and appeared to lack pseudopodia, although this may an artifact of fixation (fig. 7g).

Although several forms have been observed in this species, it is not possible to confidently reconstruct a life-history, and transitions have not been observed between all stages. However, the fact that cysts possess two flagella, and that swarmer cells are

Figure 7: ***Salpingoeca infusionum***

a,b,e: LM (a,e) and EM (b) of normal cells, notice the relatively long flagellum and theca stalk. c,f: LM of encysted cells, notice the two short flagella inside the anterior part of the theca (c) and the papilla at the top of the cyst (f). d: EM of encysted cell, notice the thin fibres at the distal end of the theca stalk. g: EM of swarmer cell, notice the absence of a pseudopodial collar.

All figures x2000, except g, x3000.


a
b
c
d
e
f
g

usually smaller than loricate cells suggests that the cyst gives rise to two motile daughter cells.

Remarks: The description of *S. longipes* (Kent 1880) only differs from that of *S. infusionum* in that the stalk of the lorica is longer (4-5 times lorica length as opposed to 1-2). The specimens seen in this study had stalks equal to 1-4 times the lorica length, with a continuous series of intermediate lengths, and therefore these species are likely to represent the same organism.

Cells in this study differed from previous descriptions of the species in the possession of a 'bulb' at the base of the theca (this occurs only in some cells and is easily overlooked), the exceptional length of the flagellum, and in the lorica dimensions. Kent (1880) noted that the lorica length was generally 1½ times the width. In the individuals seen in this study, the length was 2-2.5 times the width. However, these differences are not considered to be significant.

S. infusionum can be distinguished from *S. marina* by possessing a relatively more substantial theca and by minor differences in the shape of the cell and the lorica, which are both wider at the anterior in *S. infusionum* (compare fig. 8a and fig. 8e). In this study the flagellum of *S. infusionum* was much longer than that of *S. marina* (4-5 times cell length as opposed to 1-2), but other authors have reported shorter flagella in *S. infusionum*. *S. marina* has a slight 'neck' at the top of the theca, but the theca of *S. infusionum* also assumes this shape when cells are fixed, or when the lorica contains a 'cyst' and it seems simply to be determined by the relative position and shape of the protoplast, so this does not appear to be a useful feature to distinguish the two species. Although differences between the two species are relatively minor, it was always immediately possible to distinguish between the two forms with light microscopy, and so it does not seem necessary to merge them into a single species as proposed by Griessmann (1913) and Boucaud-Camou (1967). The organism described as *S. infusionum* in Patterson *et al.* (1993) is regarded by this author to be *S. marina* (note the short flagellum, relatively insubstantial theca, and the shape of the cell in fig. 8H, p85).

The formation of cysts in this genus has not been reported since the work of Griessmann (1913), who also found them in *S. infusionum*. One drawing (Fig. 20.II) is very similar to the cysts observed in this study, although using only light microscopy, he was not able to resolve the two 'flagella', and instead represents them as an extension of the cytoplasm. Also the anterior part of the theca retains its usual shape, whereas in this study it was observed to enclose around the cyst to give a 'waisted' shape. Swarmer cells were observed by Kent (1880). He also notes the presence of cysts, but his description implies that they are athecate.

Salpingoeca marina James-Clark, 1867

Figs: 6d, 6g, 8e

Size: cell 2-3 x 3-6.5µm, flagellum 7-10µm, pseudopodial collar 5.5-9µm

Observations: Appeared sporadically in culture. The lorica of this species is quite fine and often difficult to see with light microscopy. It is vase-shaped, and fits closely to the cell at the anterior end. The lorica stalk is relatively short (around 9µm).

Remarks: See under *S. infusionum*.

Salpingoeca tuba Kent, 1880

Fig. 8f

Size: cell 3.5-4 x 8-13.5µm, flagellum 30-35µm, pseudopodial collar 8.5-14µm (usually similar to cell length)

Observations: Appeared sporadically in culture. Cells were in accordance to those described by Kent (1880). This species can be regarded as synonymous with *S. cylindrica* and *S. petiolata* (Boucaud-Camou 1967, Vørs 1993b). The posterior of the lorica was often embedded in detritus. Cells occupied varying proportions of the lorica

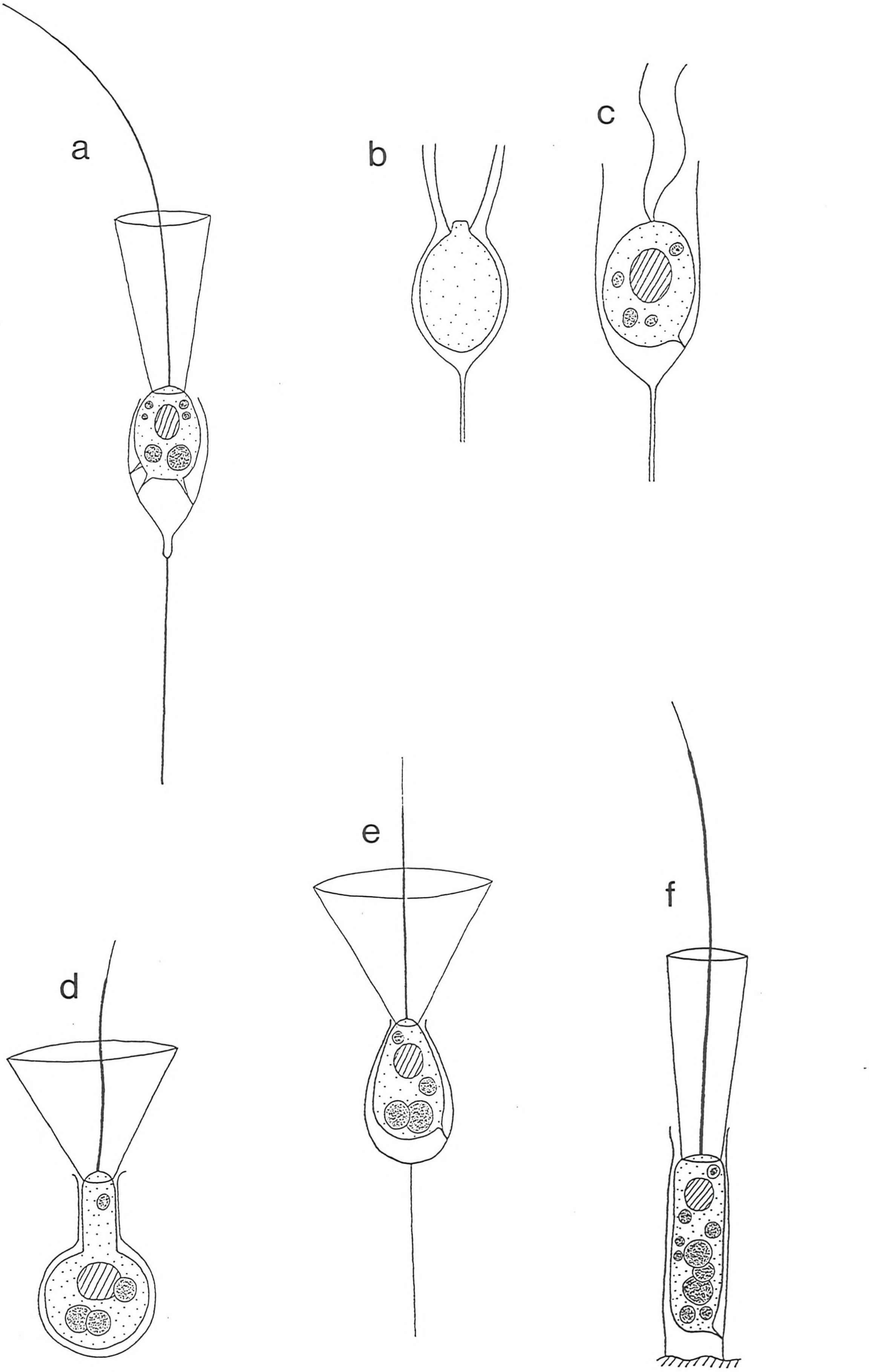
Figure 8: Diagrams of *Salpingoeca* species

a-c: *Salpingoeca infusionum*, showing normal cell (a), x2500, encysted cell (b), with two short inactive flagella, x5000 and 'intermediate' stage (c), with two short active flagella, x5000.

d: *Salpingoeca amphoridium*, notice how the cell shape fits closely to the theca, x5000.

e: *Salpingoeca marina*, x5000.

f: *Salpingoeca tuba*, x2500.


a
b
c
d
e
f

length, but fitted closely to the lorica width. Cells were sometimes anchored to the side of the lorica by a slender thread, others were anchored to the posterior or else were freely suspended, and could sometimes be seen to rise to the top of the lorica from the posterior. The anterior end of the cell was usually less constricted than in previous diagrams of this species, but this is not considered to be significant.

ACANTHOECIDAE Norris, 1965

Acanthoecid choanoflagellates are distinguished by the morphology of their loricas including the form and dimensions of the lorica, the shape, number and types of joints of the costal strips and whether or not there are anterior or posterior projections. Division may be nudiform (the daughter cell leaves the parent lorica before new costal strips are manufactured) or, as in the majority of species, tectiform, where costal strips are accumulated in a bundle in the parent lorica before cell division occurs. See Thomsen and Buck (1991) and references therein for descriptions of all the genera mentioned below, and definitions and discussion of the various taxonomic criteria used to define species in this group.

All cell sizes refer to living specimens, and therefore tend to be greater than many previously reported values where the dried protoplast has been used for measurements.

Acanthocorbis apoda (Leadbeater) Hara & Takahashi, 1984

Figs: 9a-c

Size: (of cell) 4.0-5.5 x 4.5-6.5μm.

Observations: This species was seen in nearly every sample, and was the acanthoecid most frequently found in enrichment culture. Most of the individuals found in Southampton Water appear to have 15 anterior spines, although individuals with 14, 16 and 17 have also been seen. The lorica has a typical 'waisted' shape (figs 9a-c) as in the type drawing (Leadbeater 1972a). Many other individuals reported from brackish waters are U-shaped, and may represent a different species (H.A. Thomsen, personal communication). *A. apoda* undergoes tectiform division and it appears that the production of new costal strips occupies much of the cell cycle, since they were seen in the lorica of nearly every individual (from fresh samples and enrichment cultures). Two stages in division are represented by figs 9c and 9b. After a bundle of costae is accumulated (fig. 9c), the cell divides (fig. 9b) and is pushed out of the parent lorica. The daughter cell then constructs a new lorica.

Remarks: *A. apoda* is closely related to *A. asymmetrica* but can be distinguished by the lack of a pedicel, and minor differences in the arrangement and length of the costae (Thomsen 1979).

Acanthocorbis campanula (Espeland) Thomsen (in Thomsen *et al.*, 1991)

Figs: 9d-e

Size: (of cell) 2.5-3.0 x 3.5-4.5µm

Observations: This species was observed quite regularly in whole mounts made from freshly collected material, but only once in enrichment culture. The loricas were similar to those found in California by Thomsen *et al.* (1991), possessing more longitudinal costae (10-14) than specimens from the type location in Norway (Espeland and Throndsen 1986), and lacking a posterior spine.

Acanthocorbis haurakiana Thomsen (in Thomsen *et al.*, 1991)

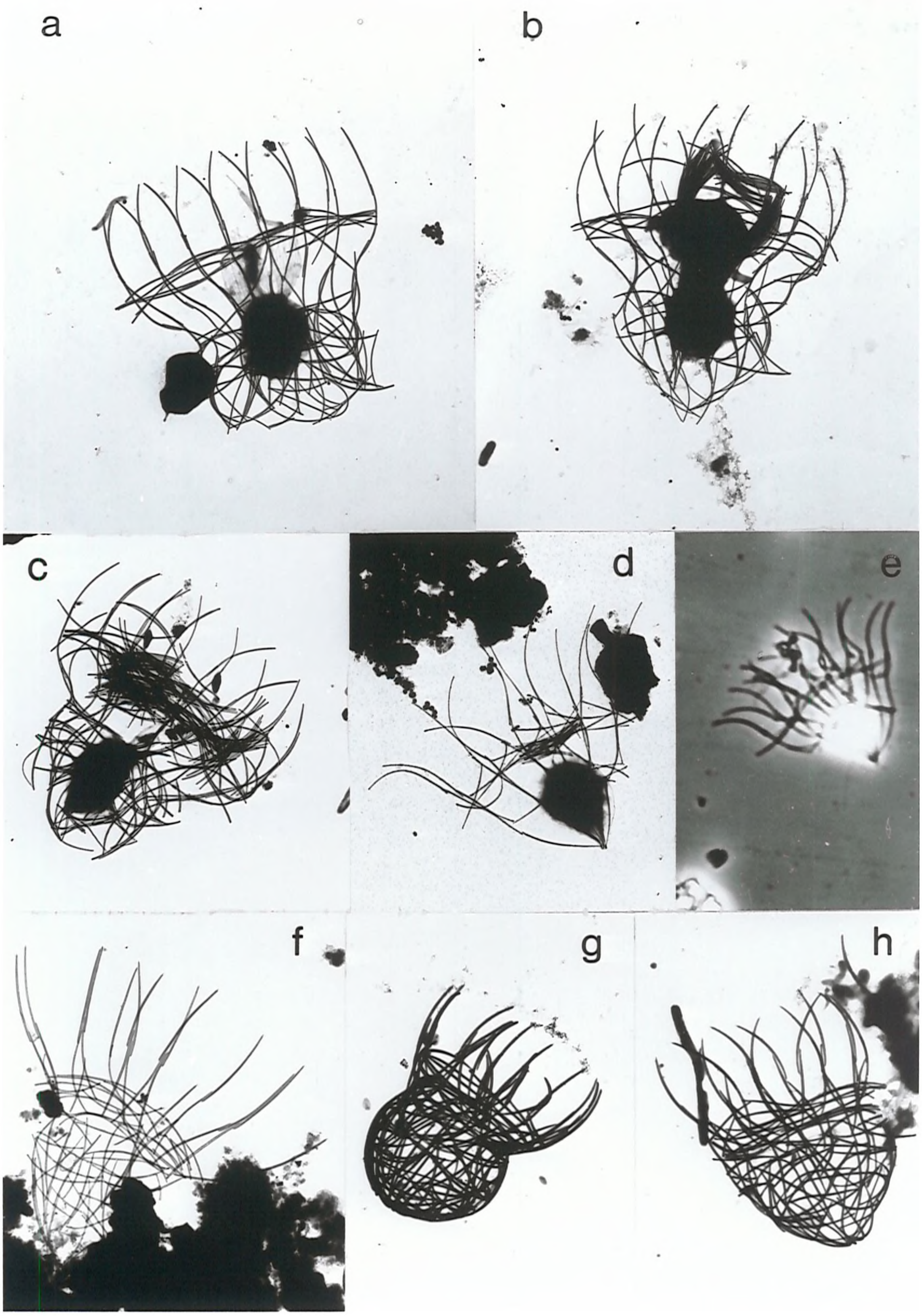
This species was observed on only one occasion, in light microscope whole-mounts made from freshly collected material (sample NT13). It is similar to *A. campanula*, but can be distinguished by tapering anterior longitudinal costae, and a cup-shaped costal strip cluster around the protoplast (Thomsen *et al.* 1991).

Figure 9: ***Acanthocorbis*** **species**

a-c: *Acanthocorbis apoda*, EM of cells showing typical lorica form (a), cell with a accumulated bundle of costae (c) and dividing cell (b), all x3000.

d-e: *Acanthocorbis campanula*, EM and LM of cells, x3000 and x2000 respectively.

f-h: *Acanthocorbis unguiculata*, EM of empty loricas, notice the thickness of the costae in g,h compared to those in f, all x3000.


a
b
c
d
e
f
g
h

Acanthocorbis unguiculata (Thomsen) Hara and Takahashi, 1984

Figs: 9f-h

Size: (of cell) 4.5-7.0 x 5.0-10.5µm

Observations: Found frequently in preparations of freshly collected material, and sometimes appeared in enrichment cultures. The lorica may be cup-shaped (fig. 9h) or have a 'waisted' appearance (fig. 9g). The costae of individuals from enrichment culture were appreciably thicker than those from freshly collected material (compare fig. 9f (freshly collected) with figs 9g,h (from culture)).

Remarks: Cells were only rarely observed with accumulations of costal strips within the lorica. This is in contrast to *A. apoda* (see above).

Acanthoeca brevipoda Ellis, 1930

Fig. 10a

A few individuals were seen in electron microscope whole-mounts made from an enrichment culture of the NT2 sample. The lorica is cup-shaped and formed of densely packed spirally arranged costae, as is characteristic of the genus (Thomsen and Buck 1991).

Acanthoeca spectabilis Ellis, 1930

Figs: 10b-c

Size: (of loricate cells) 1.5-4 x 4.5-9µm, flagellum 3.5-6µm, (of naked juvenile cells) 2.5-3 x 5.5-8µm, flagellum about 15µm

Observations: This species was found quite commonly in culture. Leadbeater (1979) noted that the length of the lorica stalk could vary considerably, but found that only short-stalked forms survived in mixed culture. However individuals in enrichment cultures in this study generally had long stalks (as in fig. 10c). Short stalked forms were sometimes encountered in freshly collected material (fig. 10b). Cells are elongated, and widest at the top. They attach to one side of the base of the widened part of the lorica by a thin extension of cytoplasm (fig. 10c).

Remarks: Unlike the majority of acanthoecid choanoflagellates, *A. spectabilis* undergoes division by nudiform replication. The flagellum appears to degenerate somewhat after the cell has settled and produced a lorica (see under 'size' above).

Bicosta minor (Reynolds) Leadbeater, 1978

Fig. 10i

Size: (measurements from one cell) 2.5 x 6.5μm, flagellum 14.5μm

Observations: Found in whole-mounts prepared from freshly collected material throughout the year. A single cell was once observed in an enrichment culture (NT25).

Remarks: There is considerable variation in the size and other characteristics of the loricas of all *Bicosta* species (Manton *et al.* 1980, Thomsen and Larsen 1992). It is difficult to distinguish between *B. minor* and *B. spinifera* on occasions (see below).

Bicosta spinifera (Throndsen) Leadbeater, 1978

Figs: 10d-h

Observations: Observed occasionally in whole-mounts.

Remarks: *B. spinifera* is distinguished from *B. minor* by the fact that the longitudinal costae cross over in the middle of the lorica chamber (as in fig. 10d). Also, the anterior

Figure 10: ***Acanthoeca*** **and** ***Bicosta*** **species**

a: *Acanthoeca brevipoda*, EM x5000.

b,c: *Acanthoeca spectabilis*, EM of cell from freshly collected material (b), x3000, and cell from an enrichment culture (c), x2000.

d-h: *Bicosta spinifera*. d: EM of an empty lorica with 'typical' morphology, x1200. e: EM of lorica with the crossing over point of the costae behind the cell, x2000. f: EM of lorica with a barely perceptible crossing over pint of the costae, x2000. g: LM of cell with a barely perceptible crossing over point of the costae, x1500. h: LM of cell with a lorica where the costae do not cross over, x1500.

i: *Bicosta minor*, LM, notice how the longitudinal costae do not cross over, the more narrow angle between the anterior spines (compared to atypical *B. spinifera* cells, figs e-h), and also the curved posterior spine, x2000.


a
b
c
d
e
f
g
h
i

and posterior projections (spines) are usually relatively longer in *B. spinifera.* In some *Bicosta* cells, which typically have a wide angle between the two anterior spines, the longitudinal costae cross over very close to the point at which they attach to the pedicel (posterior spine), or may not actually cross over at all (fig. 10h). The continuum of variation between the loricas of these forms and those of typical *B. spinifera* cells (figs 10h, 10g, 10f, 10e) means that they are currently assigned to this species (H. Thomsen, personal communication).

Calliacantha longicaudata (Leadbeater) Leadbeater, 1978

A single cell was seen in a light microscope whole-mount prepared from the NT24 sample. It differs from other *Calliacantha* species in lacking anterior spines, and in having a pedicel composed of numerous costal strips (although *C. simplex* may have several costal strips in the pedicel too, see below).

Calliacantha multispina Manton & Oates, 1979a

Figs: 11a-b

Observations: Frequently found in whole-mounts of freshly collected material, although it was less abundant than *C. natans* or *C. simplex*.

Remarks: *C. multispina* cells are often difficult to distinguish from *C. simplex* cells in light microscope whole-mounts, particularly as the number of spines can vary (Manton and Oates 1979a, and see under *C. simplex*).

Calliacantha natans (Grøntved) Leadbeater, 1978

Fig. 12a

Size: (of cell) 5 x 6.5-7μm.

Observations: Frequently observed in whole-mounts. Living cells were occasionally seen in freshly collected, centrifuged material.

Remarks: *C. natans* is sometimes difficult to distinguish from *C. multispina* and *C. simplex*. The most important characteristic of *C. natans* is that the longitudinal costae are not continuous with the anterior spines as in the other two species. Other features are discussed by Thomsen (1982).

Calliacantha simplex Manton & Oates, 1979b

Figs: 11c-d

Observations: Frequently seen in whole-mounts. Some species showed small differences from the type material (Manton and Oates 1979b), possessing five spines (see below), or a pedicel made of four costal strips rather than a single strip. The latter type of variation was also seen by Thomsen *et al.* (1991) in Californian material.

Remarks: The major features listed by Manton and Oates (1979a,b) which distinguish *C. simplex* from *C. multispina* are as follows:
1) *C. simplex* always has four spines. *C. multispina* may occasionally have four but more usually has five.

2) In *C. simplex* the longitudinal costae pass to the back of the lorica chamber without numerical reduction. In *C. multispina* the longitudinal costae are reduced in number in the hind end of the chamber (figs 11a,b)

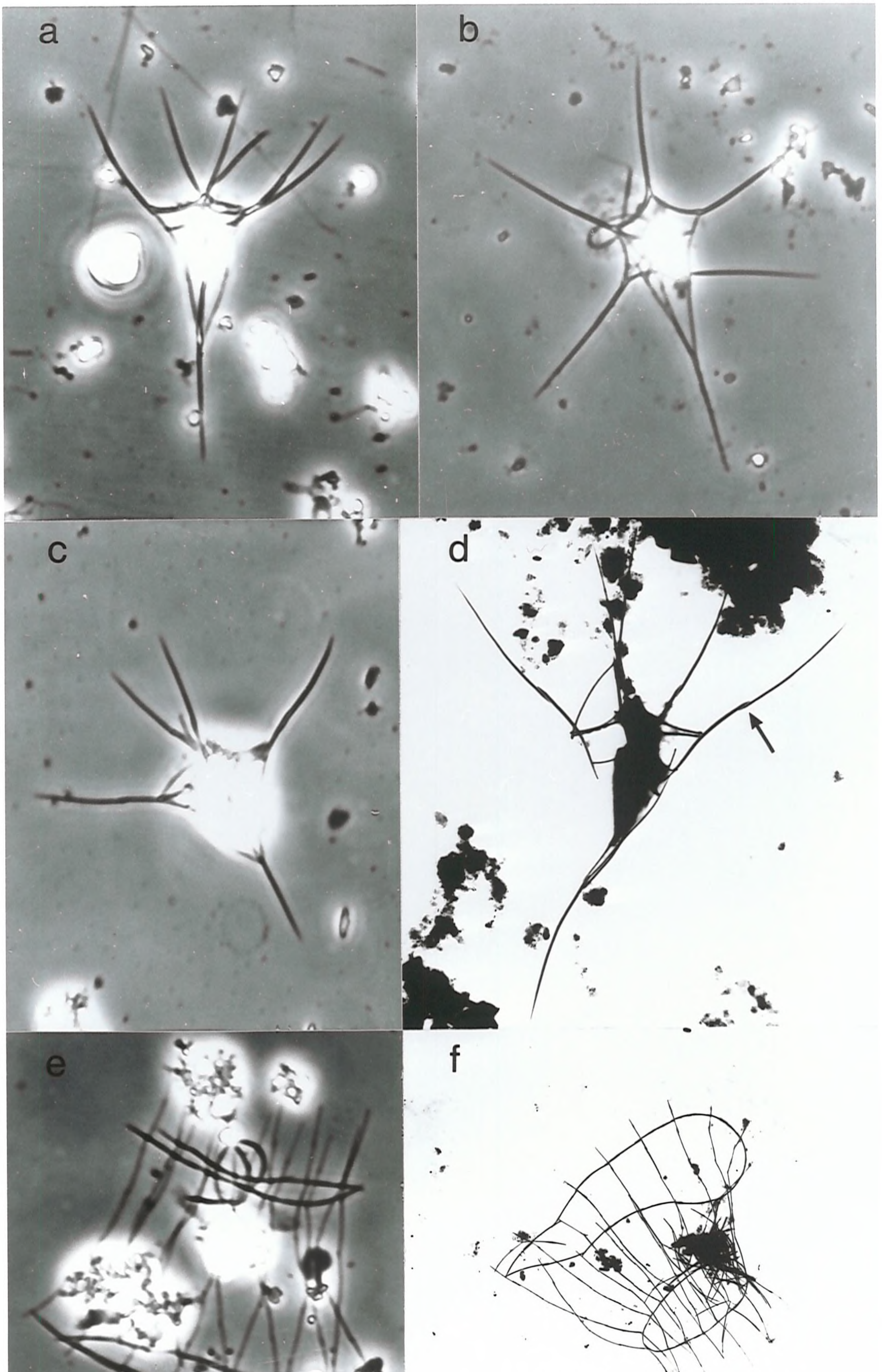
3) In *C. simplex* the anterior spines are composed of more than a single costa (usually one and a half costae). In *C. multispina* the spines are formed from a single costal strip, which attaches directly near its base to an anterior transverse costa. 'Detection of a joint in the structure of an anterior spine in a position anterior to that of any transverse costa is virtually diagnostic of this species as at present construed' (Manton and Oates, 1979b, referring to *C. simplex*).

Figure 11: *Calliacantha* species and *Crinolina isefiordensis*

a,b: *Calliacantha multispina*, LM, notice the five anterior spines, and the reduction in the number of longitudinal costae posteriorly, x2000.

c,d: *Calliacantha simplex*. c: LM of a typical cell with four anterior spines, x2000. d: EM of lorica with five anterior spines, arrow shows joint between two costal strips forming a spine, as is diagnostic for the species, x3000.

e,f: *Crinolina isefiordensis*, e: LM showing cell with curled flagellum, x2000. f: EM of lorica with a bundle of accumulated costae, x2000.


a
b
c
d
e
f

Some of the *Calliacantha* cells encountered looked very like *C. simplex*, but had five spines. These were usually seen in light microscope whole-mounts, and so it was not possible to determine the exact position and pathway of the costae. However fig. 11d shows a cell with five spines which are composed of more than a single costal strip (see arrow at joint between two costal strips). It seems logical to interpret this as a *C. simplex* cell with five spines since the number of spines can also vary in *C. multispina*, and the replication/deletion of particular costal strips is common amongst acanthoecids (Leadbeater, 1991). Unfortunately parts of the posterior of the lorica are missing, meaning that it is not possible to ascertain if the number of longitudinal costae reduces in the hind end of the lorica chamber.

Cosmoeca norvegica Thomsen (in Thomsen & Boonruang, 1984)

Figs: 12b, 12f-g

Observations: Seen frequently in whole-mount preparations. Details of the lorica morphology typical for this species can be seen in fig. 12g including the end to end joints and the relative thickness of the anterior transverse costal strips compared to other costal strips.

Cosmoeca ventricosa Thomsen (in Thomsen and Boonruang, 1984)

Fig. 12d

Observations: Seen in whole-mount preparations on a few occasions. Although the lorica in fig. 12d is partly obscured it is possible to see two costal strips between the upper and the middle transverse costae (see arrow at joint) compared to the single costal strip separating these two transverse costae in *C. norvegica* (fig. 12f), and the relatively larger size of the lorica is apparent.

Crinolina isefiordensis Thomsen, 1976

Figs: 11c-f

Size: (of cell) 4-7 x 5-9.5µm

Observations: Found in nearly every water sample. It occasionally occurred in enrichment cultures. Small (moribund) flagellate cells were sometimes observed within the lorica. Ingestion was not observed, but the ingestion of nanoplankton has been recorded for *Diaphanoeca grandis* (Marchant, 1985) and these two genera are closely related (Thomsen and Buck, 1991).

In living cells the proximal end of the pseudopodial collar is level with the anterior transverse costa and the flagellum is curled as in fig. 11e. As the cell dies the lorica shape rapidly becomes distorted. This phenomenon has also been observed in *Diaphanoeca grandis* and *Diaphanoeca undulata.*

Remarks: Despite its conspicuous nature, this species has only previously been reported from four other locations: Denmark (Thomsen 1976), New Zealand (Moestrup 1979), West Greenland (Thomsen 1982) and Norway (Espeland and Thomsen, 1986).

Crucispina cruciformis (Leadbeater) Espeland (in Espeland & Throndsen, 1986)

Figs: 12c, 12e

Size: total length of lorica 13.5-19µm

Observations: Seen frequently in whole-mounts, usually in small numbers, but it is probable that some cells were overlooked due to the inconspicuous nature of the lorica (particularly in light microscope whole-mounts and when a lot of detritus was present in the sample). The maximum length of the lorica was slightly greater than the

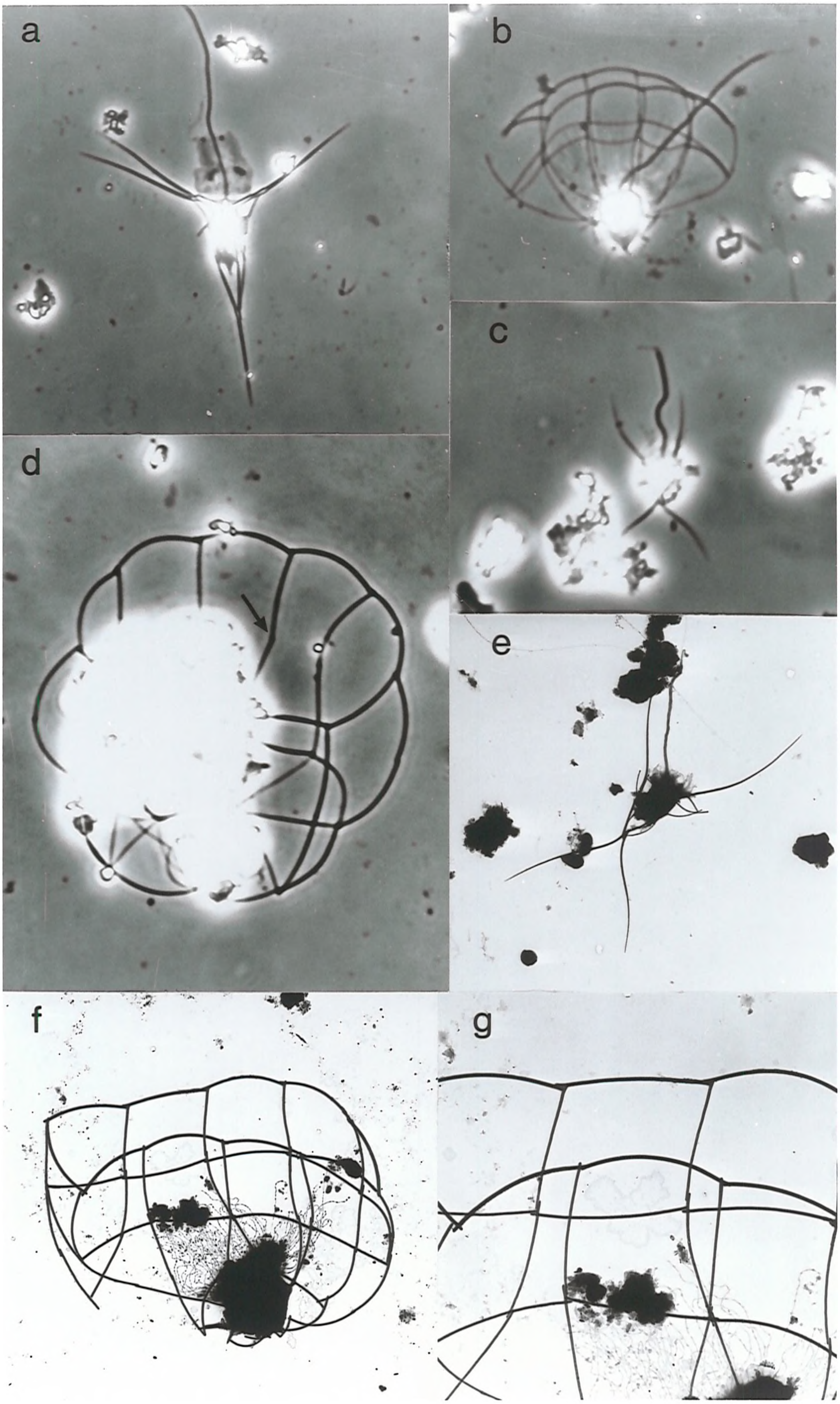
Figure 12: ***Calliacantha, Cosmoeca*** **and** ***Crucispina*** **species**

a: *Calliacantha natans*, LM, notice the three anterior spines, x1500.

b,f,g: *Cosmoeca norvegica*. b: LM x2000. f: EM x3000. g: Enlargement of anterior part of lorica, showing detail of joints between costae. Notice the thickness of the anterior transverse costae relative to the other costae, x6000.

d: *Cosmoeca ventricosa*, LM of partially obscured lorica, notice the joint between the two longitudinal costae between the upper and middle transverse costae (arrow), x2000.

c,e: *Crucispina cruciformis*, LM (c), x2000 and EM (e), x3000.


a
b
c
d
e
f
g

previously reported value of 17µm (Leadbeater 1974, Moestrup 1979).

Diaphanoeca grandis Ellis, 1930

Figs: 13a-b

Size: cell 4-6 x 6-8µm, pseudopodia 3.5-6µm, lorica 14.5-17.5 (at widest point) x 23.5-30.5µm (including anterior spines)

Observations: Seen sporadically in whole-mounts, but observed more frequently in enrichment cultures. In culture most cells had the posterior end of the lorica embedded in detritus suggesting that the feeding mechanism proposed by Andersen (1989), where water enters at the rear of the lorica, does not always apply. It is possible that *D. grandis* feeds on dissolved organic matter (as reported by Marchant and Perrin 1990) in eutrophic conditions such as those in culture, and this, together with large numbers of motile bacteria, may eliminate the need for creating feeding currents. The anterior transverse costa appears to be made of doubled costal strips (fig. 13a), but this arises from the fact that the strips overlap considerably with one another (Thomsen 1982). The number of transverse costae at the posterior of the lorica is reported as being variable (Thomsen 1982). They are usually difficult to differentiate once the lorica has collapsed (as in whole mounts), but in all living cells seen in this study there were three transverse costae at the posterior end of the lorica (making a total of four).

Diaphanoeca pedicellata Leadbeater, 1972b

Seen in whole-mounts from three samples. This species has a similar lorica morphology to *D. undulata* (below), but differs in having two transverse costae in the upper part of the lorica (instead of one) and there are minor differences in the lorica shape of the two species (Thomsen 1982).

Diaphanoeca undulata Thomsen, 1982

Figs: 13d-e

Size: (of cell) 4.5-5 x 5-8μm

Observations: Frequently found in whole-mounts prepared from freshly collected material. The flagellum appears to be curled in living cells, as in *Crinolina isefiordensis*. The proximal end of the pseudopodial collar is level with the anterior transverse costa. In living cells the anterior spines curve inwards slightly, and the main part of the lorica is cylindrical, but this shape soon becomes distorted when the cell dies (see *Crinolina isefiordensis*). In specimens seen in electron microscope whole-mounts, the first two costal strips of the pedicel overlapped to the extent that they almost appeared to be a double strip (figs 13d,e). This may also occur in *D. pedicellata* (e.g. fig. 36 in Thomsen 1982).

Remarks: This species was found far more frequently than the closely related *Diaphanoeca pedicellata*, despite the fact that it has not often been reported in the literature.

Diplotheca costata Valkanov, 1970

Figs: 13c, 13f-g

Size: cell 3-3.5 x 4.5-6μm, lorica 8 (at widest point) x 13-14μm

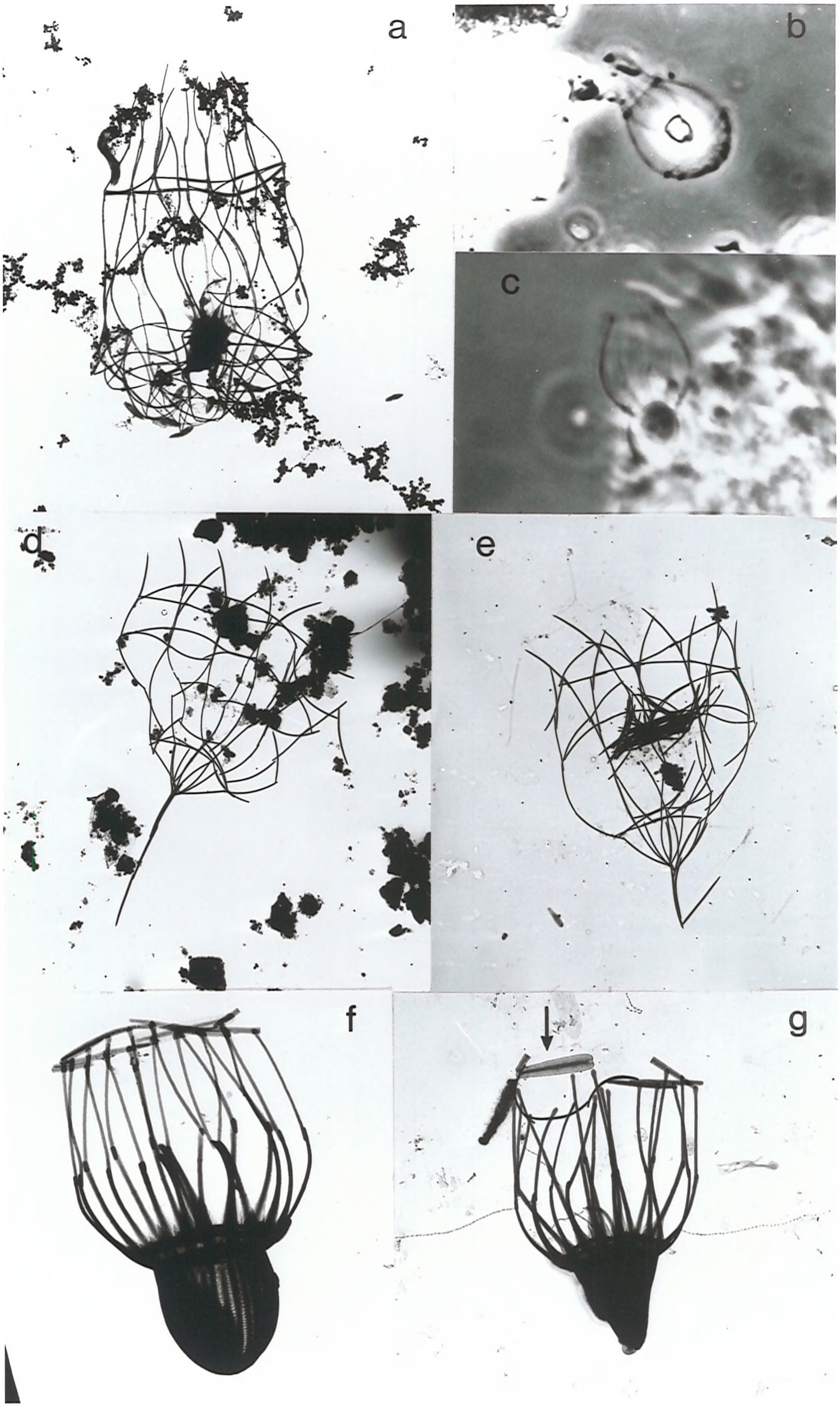
Observations: Found occasionally in enrichment cultures, but not observed in preparations of freshly collected material. In living cells the posterior part of the lorica (that composed of broad ribbed costal strips) was cup-shaped (as in fig. 13f). However, most dried specimens had a pointed end to the lorica, which is caused by distortion of the costal strips (Jackson and Leadbeater 1991). Five different types of costal strips are present in the lorica (Jackson and Leadbeater 1991). Those forming a cup at the bottom

Figure 13: ***Diaphanoeca*** **and** ***Diplotheca*** **species**

a,b: *Diaphanoeca grandis*. (a): EM, notice the overlapping strips forming the anterior transverse costa, x2000. (b): LM of a living cell, x1000

d,e: *Diaphanoeca undulata*, EM of loricas, notice the overlapping costal strips at the proximal end of the pedicel, x2000.

c,f,g: *Diplotheca costata*, LM of living cell (c) 2000, and EM of empty loricas (f) x5000 and (g) x3000.


a
b
c
d
e
f
g

are broad (being slightly wider at the top), flattened and bear a row of perforations on either side of a thicker middle strip (see arrow pointing to detached costal strip in fig. 13g). At the top of the cup a number of transverse costae are organised into two bands (fig. 13f). They overlap considerably, and are rather thick, but otherwise conventional. The longitudinal costae are composed of two strips; the lower ones are wide, and have very broad spatulate ends around the region of the transverse bands, and the upper ones are thinner, with a slightly spatulate end connecting to the anterior transverse costa. Finally, the strips which form the anterior costa are quite broad and somewhat flattened.

Nannoeca minuta (Leadbeater) Thomsen, 1988

Seen on three occasions in light microscope whole-mounts. It is possible that other specimens were overlooked due to the small size of the lorica. The lorica is conical, with two transverse costae and 6-10 longitudinal costae (Thomsen 1988).

Parvicorbicula circularis Thomsen, 1976 emend. Thomsen *et al.* 1990.

Observed on three occasions in light microscope whole-mounts. The lorica of this species is similar to that of *P. quadricostata* (see below).

Parvicorbicula quadricostata Throndsen, 1970

Fig. 16f

Observations: Seen in whole-mounts prepared from the NT31 sample. This species is closely related to *P. circularis*, but the posterior transverse costa of *P. circularis* is made of six to nine costal strips, and is circular, whereas that of *P. quadricostata* contains only four strips (unless one is duplicated), and forms a distinctive quadrangle (Thomsen *et al.* 1990).

Parvicorbicula socialis (Meunier) Deflandre, 1960

Fig. 14f

Observations: Seen in whole-mounts from three samples. The material encountered in this study differs slightly from that previously described. Only single specimens were observed although *P. socialis* is usually colonial. Colonies could have been disrupted by passing the sample through a net (40µm pore) or by centrifugation, but since very small numbers of cells were encountered this was probably unlikely. Fig. 14f shows a cell with at least two consecutive costal strips at the posterior end of the lorica (it is partly obscured). Figures presented by many other authors (e.g. Throndsen 1970, Thomsen 1973, Thomsen *et al.* 1990) show loricas without pedicels, and Manton *et al.* (1976) note that, if present, the stipe may consist of a single or of two or more parallel strips, but never of two consecutive strips.

Parvicorbicula superpositus Booth, 1990

Figs: 17a-c

Size: (measurements from one cell) 3.5 x 6µm, flagellum 15µm

Observations: Found in whole-mounts made from most samples, sometimes in large numbers, particularly in late winter and spring. This species was recently described by Booth (1990), although it had been previously noted (as a form related to *P socialis*) by Moestrup (1979) and Espeland and Throndsen (1986). The specimens found in this study had several differences from the type material. Booth (1990) reports that every other longitudinal costa had a fourth strip (forming anterior spines), which means that there should be five spines. This is also the case in the fig. 23 in Thomsen *et al.* (1991). However, although the Southampton material had ten longitudinal costae, more than half of these bore spines (figs 17a,b). In fact the number of spines varied between 7 and 9. The pedicel usually consisted of two overlapping costal strips, but individuals with one or three strips were also seen. The posterior transverse costa was generally composed of seven strips, but in many cases extra costae, which were presumably being accumulated for replication, were present in this region, making the transverse costa appear thickened.

Parvicorbicula 'manubriata' (Tong, in prep.)

Figs: 14a-e, 15b

Size: cell 3.5-5.5 x 4.5-7μm, pseudopodial collar 8μm, flagellum 13-19μm

Observations: This undescribed species of *Parvicorbicula* had a very marked seasonal occurrence (see remarks). It has also been seen in Danish North Sea saltmarshes, where it forms colonies, in W. and N.E. Greenland, and in the Arctic by H. Thomsen, and in Canada by I. Manton (H. Thomsen, personal communication). It is also probably the same species as that shown in fig. 18.3 in Thomsen and Buck (1991), although there are slight differences to the specimens seen in this study (e.g. no duplicated transverse costal strip).

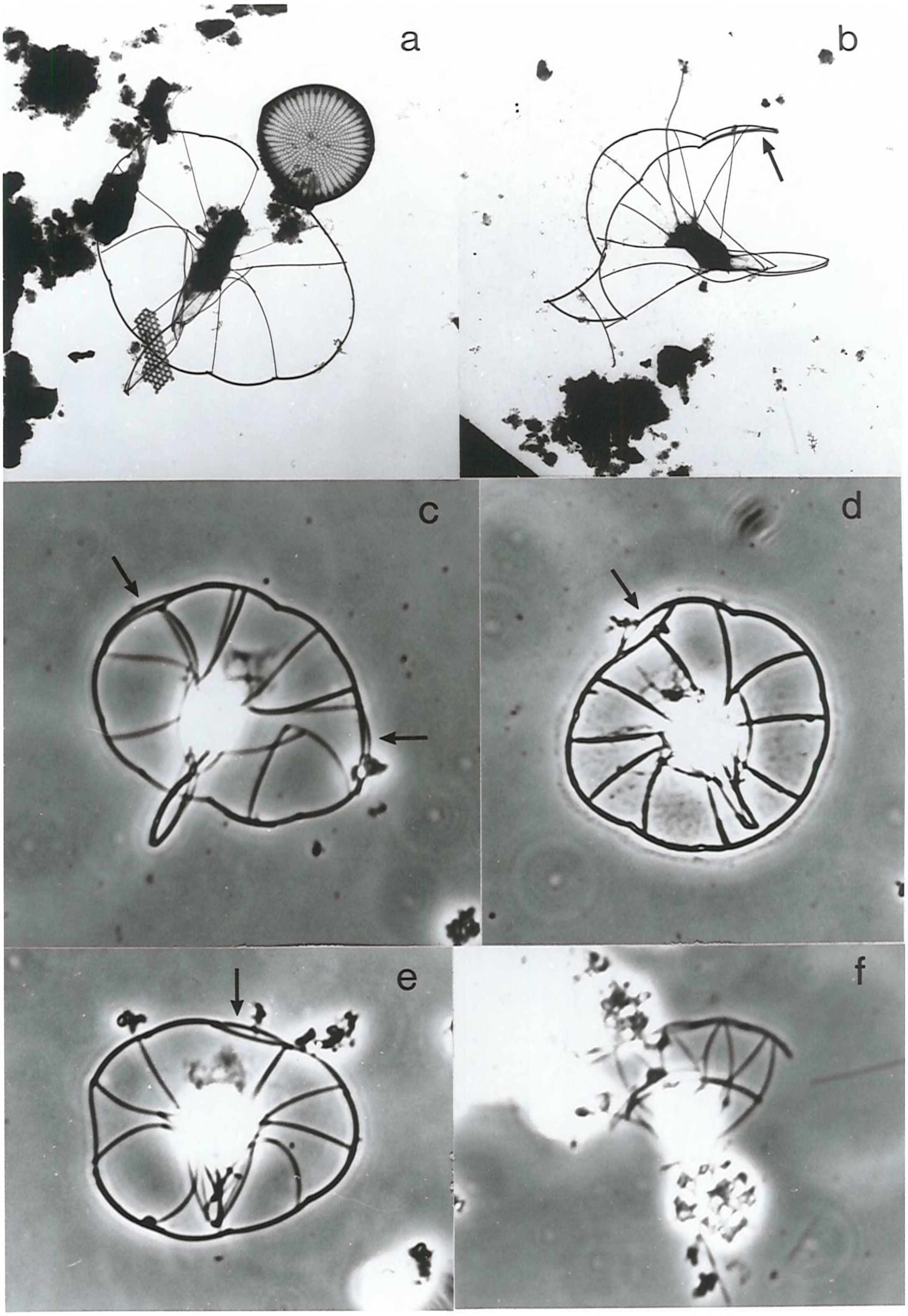
The anterior transverse costa is formed of 10 or 11 costal strips (13 in two specimens), one of which is duplicated (figs 14b-e, arrows) giving a total of 11 or 12. These strips are slightly curved, forming a perfect circle in living specimens, but giving the lorica a slightly convoluted appearance when flattened in whole-mount preparations. There are 8 to 10 longitudinal costae, but no regular pattern of attachment. The longitudinal costa attaching to the duplicated costal strip usually does so with an end to end joint, but this is not always so. Otherwise, the number and position of the different joints varies; in fig. 14e they are all end to end joints (one is slightly intermediate), in fig. 14d they are all T-joints, or intermediate, in fig. 14a there are 5 T-joints and 4 end to end joints. Fig. 14c shows a somewhat aberrant lorica with 13 transverse costal strips, and two duplications (giving a total of 15) and a total of 12 longitudinal costae (with three pairs or duplications).

The arrangement of costae in the lower part of the lorica is less apparent; many details

Figure 14: *Parvicorbicula* 'manubriata' and *P. socialis*

a-e: *Parvicorbicula* 'manubriata', notice the duplicated transverse costae (arrows) and the 'handle' at the bottom of the lorica (particularly figs b,c). a,b: EM and c-e: LM, all x2000.

f: *Parvicorbicula socialis*, LM of partially obscured cell, x2000.


a
b
c
d
e
f

are obscured by the protoplast. However, fig. 15b shows a reconstruction of the probable form of the lorica, using a combination of observations on living cells, and information from electron micrographs. The number of costae in the posterior part of the lorica below the protoplast is variable (3-7), although the most common figure is four. This conspicuous grouping like a 'handle' at the bottom of the lorica led I. Manton to call the species *Parvicorbicula* 'manubriata', as above (H. Thomsen, personal communication).

In living cells the protoplast occupies the middle part of the lorica, with the pseudopodial collar extending a short way above the transverse costa. Electron micrographs show evidence of a membrane which lines the middle part of the lorica around the cell, and then draws out into a thinner projection which anchors to the bottom of the lorica (figs 14a,b). The cell contains numerous food vacuoles, and a nucleus which is conspicuously off-centre (fig. 15b).

This species falls into the group of *Parvicorbicula* species where the number of longitudinal costae is similar to the number of costal strips in the anterior transverse costa (see Thomsen and Buck 1991). However, it differs from all other *Parvicorbicula* species in lacking a second transverse costa, and in having no regular way of joining the anterior transverse costae and the longitudinal costae.

Remarks: This species was one of two acanthoecids which had a markedly seasonal occurrence (see also *Parvicorbicula* 'aculeatus'). In 1993 cells were found in samples from 17th February to 23rd April (with individual cells in whole-mounts of samples taken on 4th June, 15th November and 16th December). In 1994 cells were found in samples from 11th February to 26th April. The species was present in very high numbers during these periods, particularly in 1993.

There are several possible explanations as to how the species recurred in samples after an absence of almost ten months: cells may have been washed in from elsewhere by tidal action, or could have formed cysts as resting stages, or else persisted in very low numbers. Since single cells were observed on three occasions through the 'absence period', the third option seems more probable, although other explanations cannot be

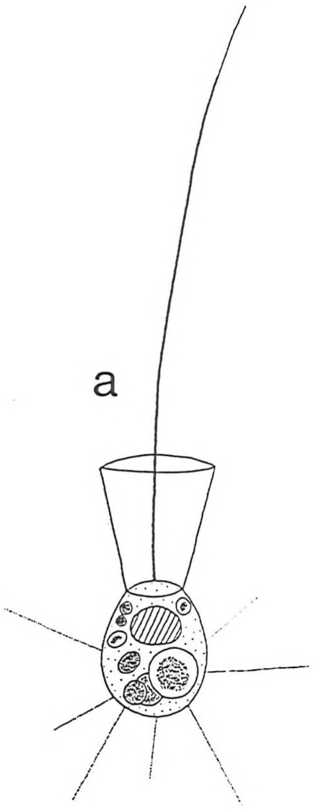
Figure 15: Diagrams of new choanoflagellate species, and of *Oxyrrhis marina*.

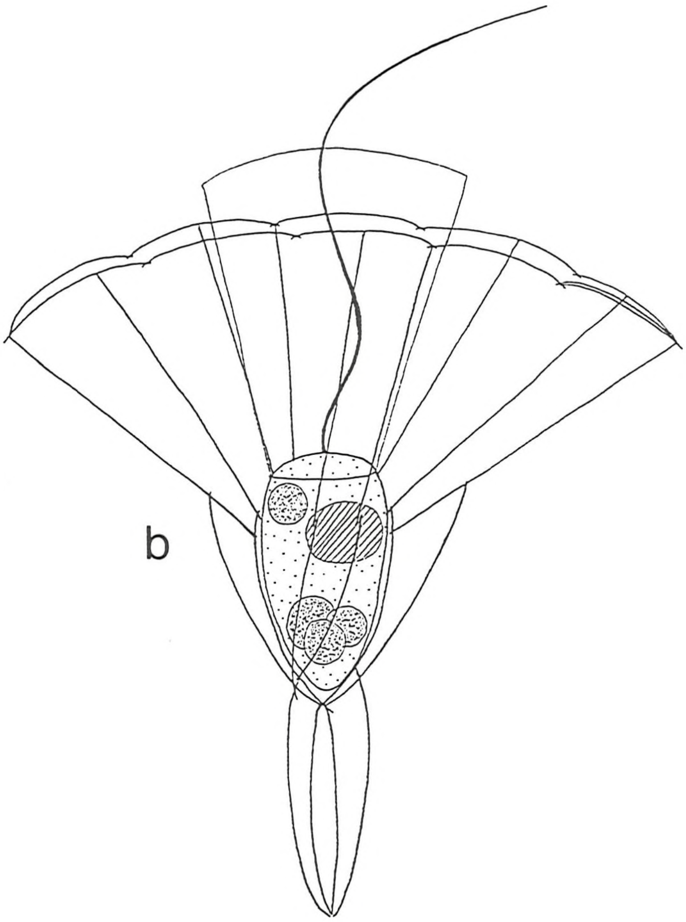
a: *Kentrosiga* ’echina’, x2500.

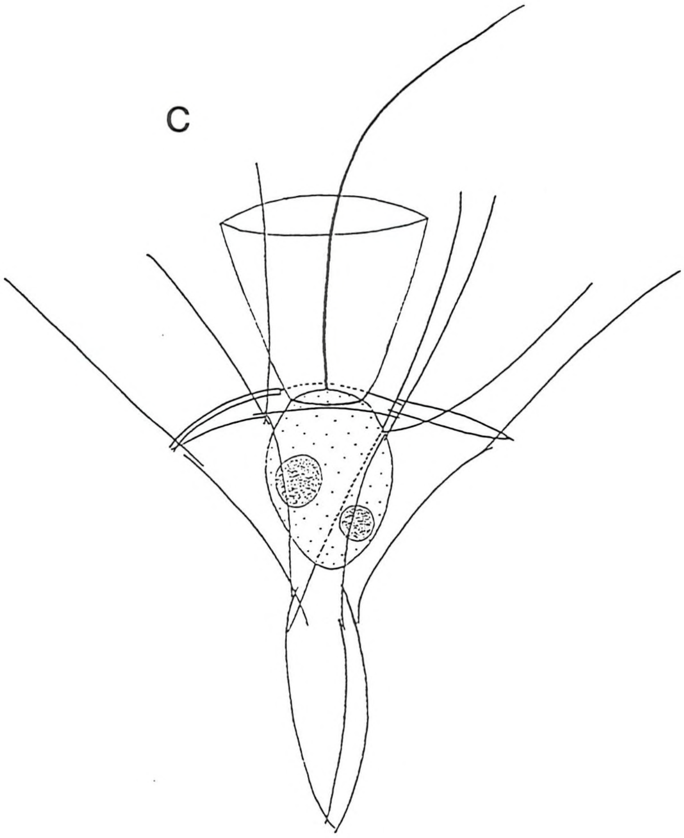
b: *Parvicorbicula* ’manubriata’, x5000.

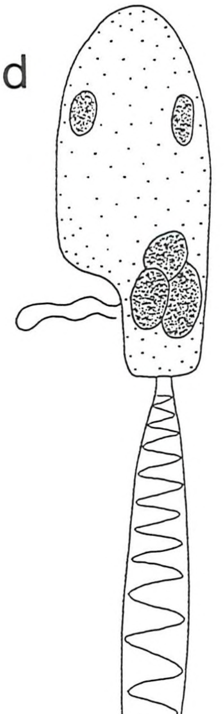
c: *Parvicorbicula* ’aculeatus’, x5000.

d: *Oxyrrhis marina*, x1250.


a


b


c


d

ruled out.

Parvicorbicula 'aculeatus' (Tong, in prep.)

Figs: 15c, 16a-e

Size: cell 3.5-4.5 x 4.5-5.5µm, pseudopodial collar 4.5-7.5µm, flagellum c16µm

Observations: This species had a very marked seasonal occurrence similar to that of *Parvicorbicula* 'manubriata' (above). In 1993 it was found in samples collected from 12th March to 21st May (with a single cell seen in the NT14 sample on 9th August), and in 1994 it occurred from 8th March to 24th May.

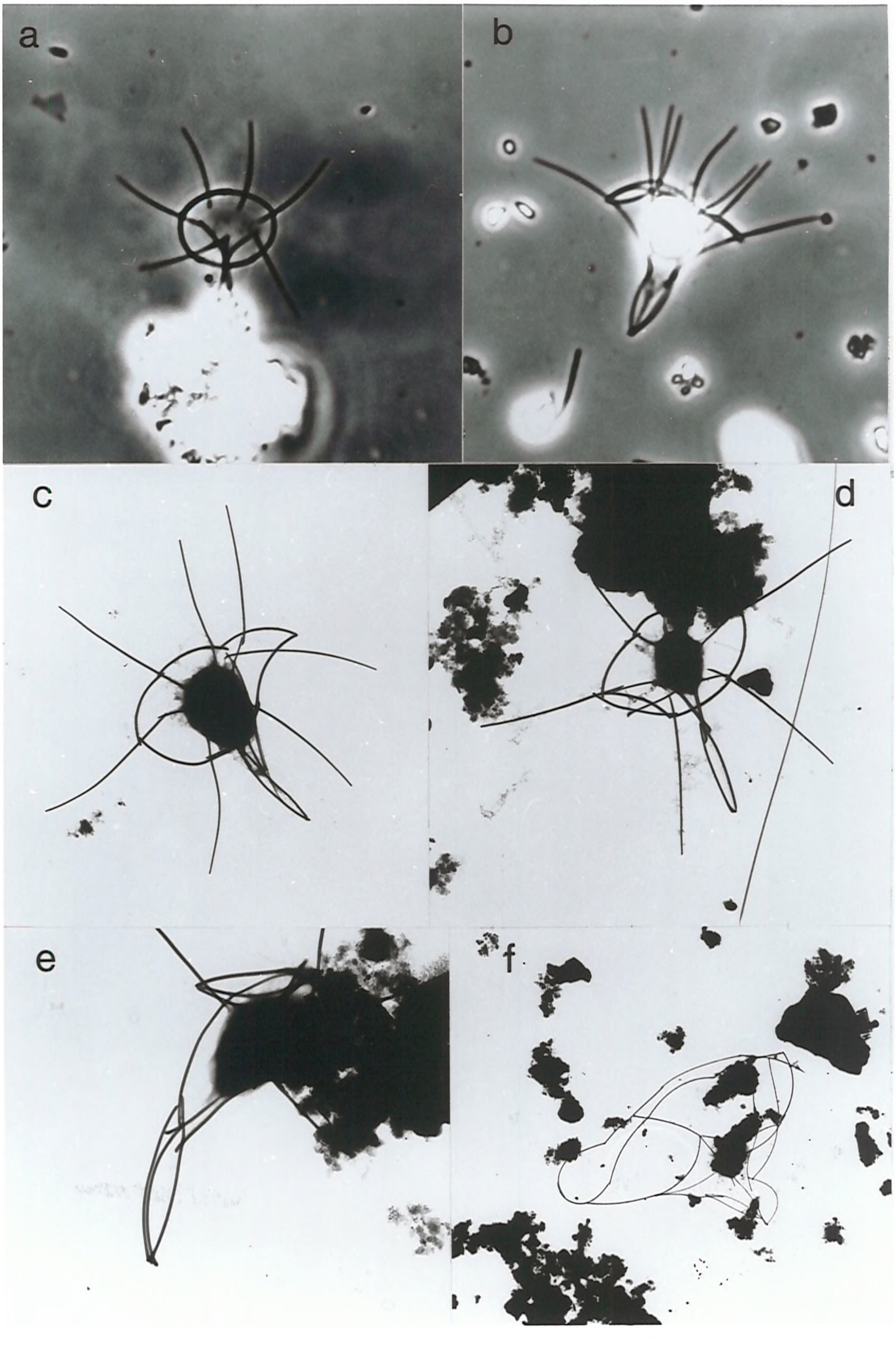
The single transverse costa consists of 5-7 costal strips (most commonly 6), and one of these strips is usually duplicated (making a total of 6-8 strips). 6-8 (usually 7) longitudinal costae project above the main chamber of the lorica as spines. Since the number of transverse costal strips is usually less than the number of spines, the joints in this region vary, being mostly 'end to end', but sometimes with a few 'T' joints. It should be noted that these joints are unconventional in themselves, since about 1/7th of the spine strip (usually) projects below the transverse costa. Below the level of the spine strips the number of longitudinal costae is frequently reduced (to 3-6), and then further reduced to 2-3 costal strips below the protoplast, although duplications, or the continuation of some of the second longitudinal costal strips in this region sometimes increases the number to 4 or 5. All costal strips are narrow rods, but vary in length and are frequently curved. Cell division is tectiform.

Remarks: *P.* 'aculeatus' is similar to many members of the genus *Parvicorbicula*, particularly to *P.* 'manubriata', but differs from all these species by lacking a transverse costa at the top of the lorica (so that the longitudinal costae project as spines), and from all species except *P.* 'manubriata', in having only one transverse costa and in possessing variable types of joints between the longitudinal and transverse costae.

Figure 16: *Parvicorbicula* 'aculeatus' and *P. quadricostata*

a-e: *Parvicorbicula* 'aculeatus'. a,b: LM x2000. c,d: EM x3000. e: EM of detail of arrangement of costae at the posterior of the lorica, x5000.

f: *Parvicorbicula quadricostata*, EM x1500.


a
b
c
d
e
f

Similarities to *P.* 'manubriata' include: a single transverse costa, a duplicated transverse costal strip, lack of consistency in the joints of the longitudinal costae with the transverse costa, posterior reduction in the number of longitudinal costae and the tendency for some of the second longitudinal costal strips to curve across the lorica in the region containing the protoplast. Similarity between the two species would seem to encompass their ecology as well as lorica structure, since both species were present for only a short period in 1993 and in 1994 (mid-February to the end of April for *P.* 'manubriata', and early March to the end of May for *P.* 'aculeatus'), and absent or very rare for the rest of the study period.

Pleurasiga minima Throndsen, 1970

Figs: 17e-f

Size: (measurements from one cell) 3 x 5µm

Observations: Seen in whole-mounts of most samples. The lorica is composed of two transverse and seven longitudinal costae (Thomsen *et al.* 1990). This species differs from *P. reynoldsii* (below) in the smaller size of the lorica, so that the flagellum projects some way beyond the anterior transverse costa (fig. 17f).

Pleurasiga reynoldsii Throndsen, 1970

Observations: Appeared sporadically in whole-mounts, usually in low numbers.

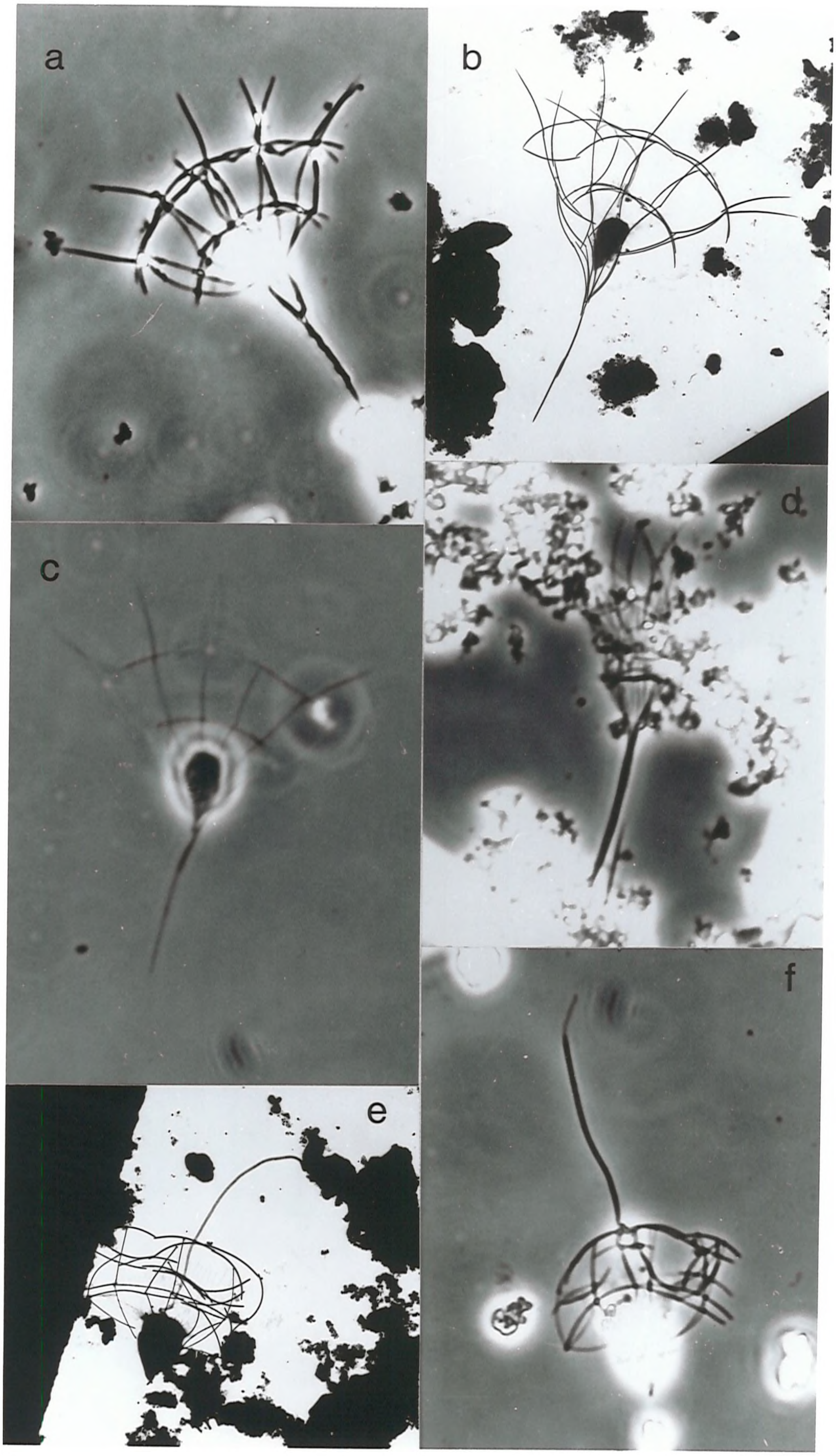
Remarks: *P. reynoldsii* can be distinguished from *P. minima* by its greater size and a more bulbous lorica, but there are also differences in the detail of the morphology of the posterior end of the lorica (Manton *et al.* 1976). These two species show many similarities to *Parvicorbicula*, and should probably be transferred to this genus (Thomsen and Boonruang 1984).

Figure 17: *Parvicorbicula superpositus, Polyoeca dichotoma* **and *Pleurasiga minima***

a-c: *Parvicorbicula superpositus*, a,b: EM (b) and LM (a), notice that the number of anterior spines is greater than five (7 in fig. a and 9 in fig. b), x2000. c: LM of living cell x2000.

d: *Polyoeca dichotoma*, LM of partially obscured cell, notice the thick pedicel of the lorica, x2000.

e,f: *Pleurasiga minima*, EM (e) and LM (f), notice the projection of the flagellum beyond the top of the lorica, x2000.


a
b
c
d
e
f

Polyfibula sphyrelata (Thomsen) Manton (in Manton & Bremer, 1981)

Figs: 18a-e

Size: cell 3 x 4.5-5µm, flagellum 8.5-9.5µm, lorica 6.5-9 x 13-14µm.

Observations: Present in nearly every sample. In living cells the protoplast is drawn out into a fine point at the posterior, with which it attaches to the lorica. The top of the pseudopodial collar is level with the second transverse costa, and the flagellum extends to the height of the anterior transverse costa. The facets, or bosses which define this genus are clearly visible in the middle of the costal strips forming the anterior transverse costa in fig. 18d. Manton and Bremer (1981) say that exceptionally eight, rather than seven, longitudinal costae may be present in *P. sphyrelata*. However, approximately half the specimens seen in this study possessed eight longitudinal costae (e.g figs 18a, 18e). In one specimen (fig. 18c) there appeared to be a single costal strip projecting from the posterior end of the lorica. Since the cell possessed seven longitudinal costae, it could be interpreted as *P. stipitata*. However, I would prefer to interpret it as a variant of *P. sphyrelata* for reasons outlined below.

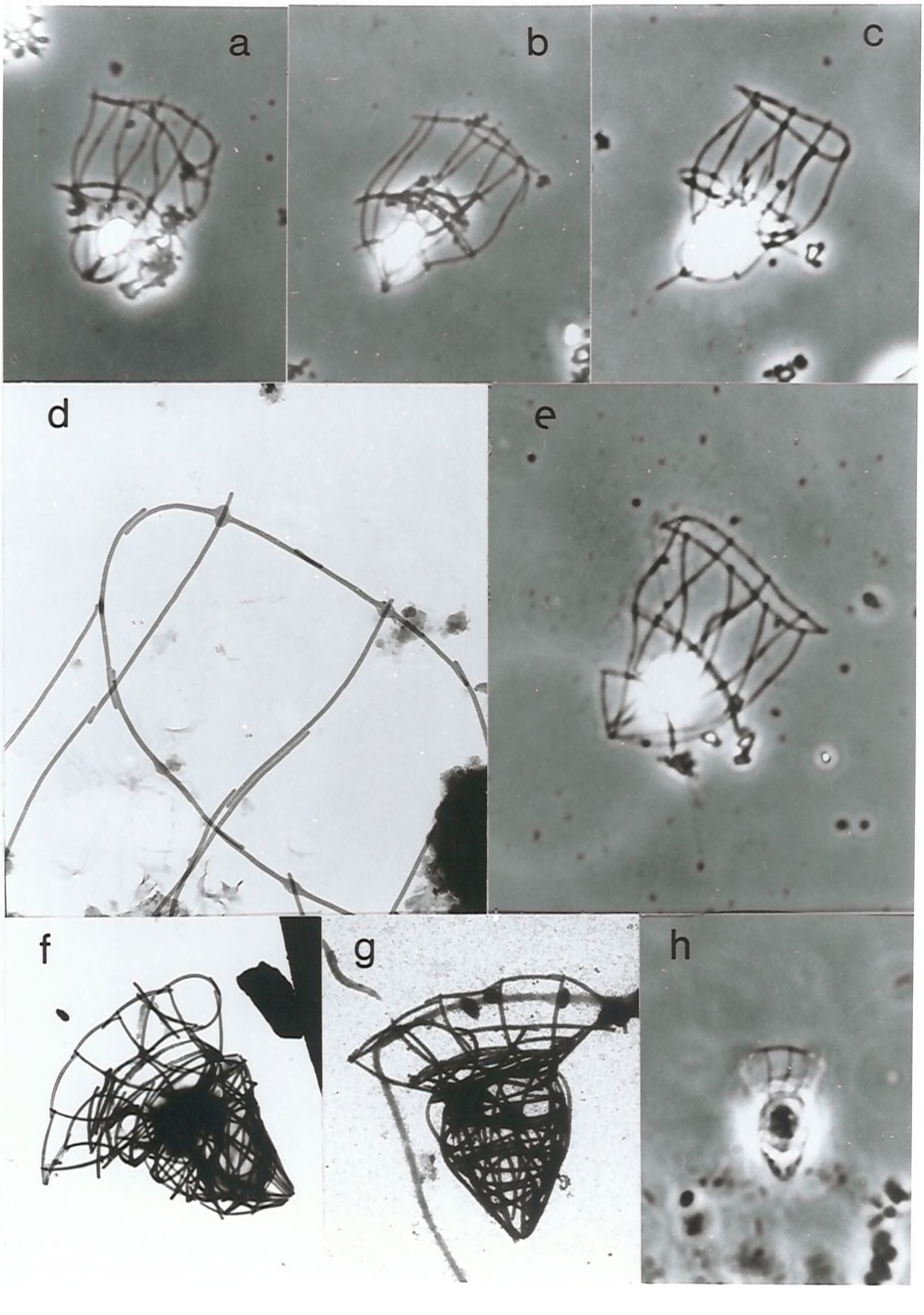
Remarks: Manton and Bremer (1981) distinguish five species of *Polyfibula*. Of these perhaps only *P. elatensis* has a distinct identity, distinguished from all the other species by significant anterior projections of the longitudinal costae. The other four species are distinguished by the number of longitudinal costae, and whether or not the cell possesses a pedicel.

The presence or absence of a pedicel, particularly if consisting of only a single costal strip, is not considered by this author as sufficient grounds, on its own, for separating two taxa at the species level since this character may vary within other species (e.g. *Parvicorbicula socialis* above), and pedicels may be easily lost during the preparation of cells for examination, or artificially produced if the posterior part of the lorica is damaged. Manton and Bremer (1981) suggest that the four species in question may need to be reduced to two, each with a sub-species depending on whether or not a pedicel is

Figure 18: *Polyfibula sphyrelata* and *Saepicula pulchra*

a-e: *Polyfibula sphyrelata*. a-c,e: LM of cells with seven (b,c) or eight (a,e) longitudinal costae. Notice the 'pedicel' on the specimen in fig. c, all x2000. d: EM of the anterior part of the lorica showing the 'bosses' at junctions between costae, x10,000.

f-h: *Saepicula pulchra*, f,g: EM of empty loricas, notice the 'T' joints between the longitudinal and top transverse costae, x5000. h: LM of living cell, x2000.


a
b
c
d
e
f
g
h

present.

Variation in the number of longitudinal costae is already accepted in *P. sphyrelata*, which may have seven or eight, and it seems unlikely that this number is sufficiently stable within other members of the genus to be used as a single defining character for species. Thomsen *et al.* (1991) and Smith and Hobson (1994) found specimens with eight longitudinal costae (which would designate them as *P. sphyrelata*), but which appeared to have a costal strip at the posterior end, which does not occur in *P. sphyrelata* as presently defined. Since Manton and Bremer organised the *Pleurasiga sphyrelata* complex into five species in 1981, there have only been two reports of any of the four species in question other than *P. sphyrelata* (*P. stipitata* in Booth 1990, and the specimen mentioned above in Smith and Hobson, which they interpreted as *P. stipitata*).

For the reasons outlined above, it seems difficult to justify the separation of the genus *Polyfibula* into five species. It is therefore recommended that *P. caudata*, *P. hexacostata* and *P. stipitata* become reabsorbed into *P. sphyrelata* as variants or subspecies (formal recommendations in paper in preparation).

Polyoeca dichotoma Kent, 1881

Fig. 17d

Observations: A single cell was observed in a light microscope whole-mount made from the NT19 sample. The lorica is composed of numerous longitudinal costae, which are continuous with an elongated pedicel (fig. 17d). The transverse costae are organised into three bands in the specimen seen in this study (there may be two or more bands, Thomsen and Buck, 1991). The loricas of this species often form linear or dendroid colonies.

Saepicula pulchra Leadbeater, 1980

Figs: 18f-h

Size: Cell 2.5-3.5 x 3-5.5µm, lorica 6-7 (at widest point) x 9-11µm

Observations: Seen in enrichment cultures on several occasions. Living cells occupy much of the posterior chamber of the lorica. Bundles of costae, presumably accumulated in preparation for tectiform division, were occasionally seen just above the lorica, resting on the pseudopodial collar which extends to the level of the anterior transverse costa. This species has only been reported once (Thomsen 1992) since the description of the type material from South Brittany, France. Leadbeater (1980) found that the longitudinal costae could either attach at the joints of the strips forming the anterior transverse costa, or else attach to the middle of these strips; Thomsen (1992) found the latter arrangement to be more common. Specimens found in this study all had longitudinal costae attaching to the joints of the anterior transverse costae (figs 18f,h). Although the numerous costae in the posterior chamber of the lorica can have a rather disorganised appearance in fixed cells, observations on living cells suggest that they are arranged horizontally and longitudinally.

Savillea micropora (Norris) Leadbeater, 1975

Figs: 19a-b

Size: cell 1.5-3µm (spherical), lorica 3.5-4.5 x 4.5-6.5µm

Observations: Seen sporadically in enrichment cultures, but never observed in freshly collected material. It is possible that cells in light microscope whole-mounts were overlooked because of their small size, and the dense construction of the lorica. Living cells are rounded in shape and are located at the base of the lorica, with the pseudopodia extending nearly to the top of the lorica (fig. 19b). As noted by Leadbeater (1975), cells frequently lack flagella. Flagella were observed on less than one third of living cells (numerous individuals were examined), and when present, were short and inactive.

Savillea parva (Ellis) Loeblich III, 1967

Figs: 19c, 19f

Size: cell 2.5-4µm (spherical), lorica 10-10.5µm in length

Observations: Found in enrichment cultures of two samples. In living cells the protoplast is situated at the bottom of the lorica, the pseudopodial collar extends to about two-thirds of the way up the lorica, and the flagellum extends to the top of the lorica. Unlike *S. micropora*, a flagellum was always present, and active.

Stephanoeca cupula (Leadbeater) Thomsen, 1988

Figs: 19d-e

Observations: Occurred in two samples. This species can be distinguished by the characteristic joints of the longitudinal costae with the anterior transverse costa (fig. 19d, arrow), with the slight projection of the longitudinal costae above the transverse costae, and one end of each transverse costa curving down to attach to a longitudinal costa a short distance below the position of the rest of the transverse ring. Costal strips are arranged in a fairly strict horizontal and vertical manner, compared to the disorganised appearance of the anterior chamber of the lorica in some other *Stephanoeca* species, such as *S. diplocostata*, once specimens have been fixed. Costae tend to overlap for at least one third of their length.

Stephanoeca diplocostata Ellis, 1930

Figs: 19g-i, and 19j-k (*S. diplocostata* var.)

Size: cell 2-4 x 4.5-6µm, lorica 8-9 (at widest point) x 14.5-15.5µm

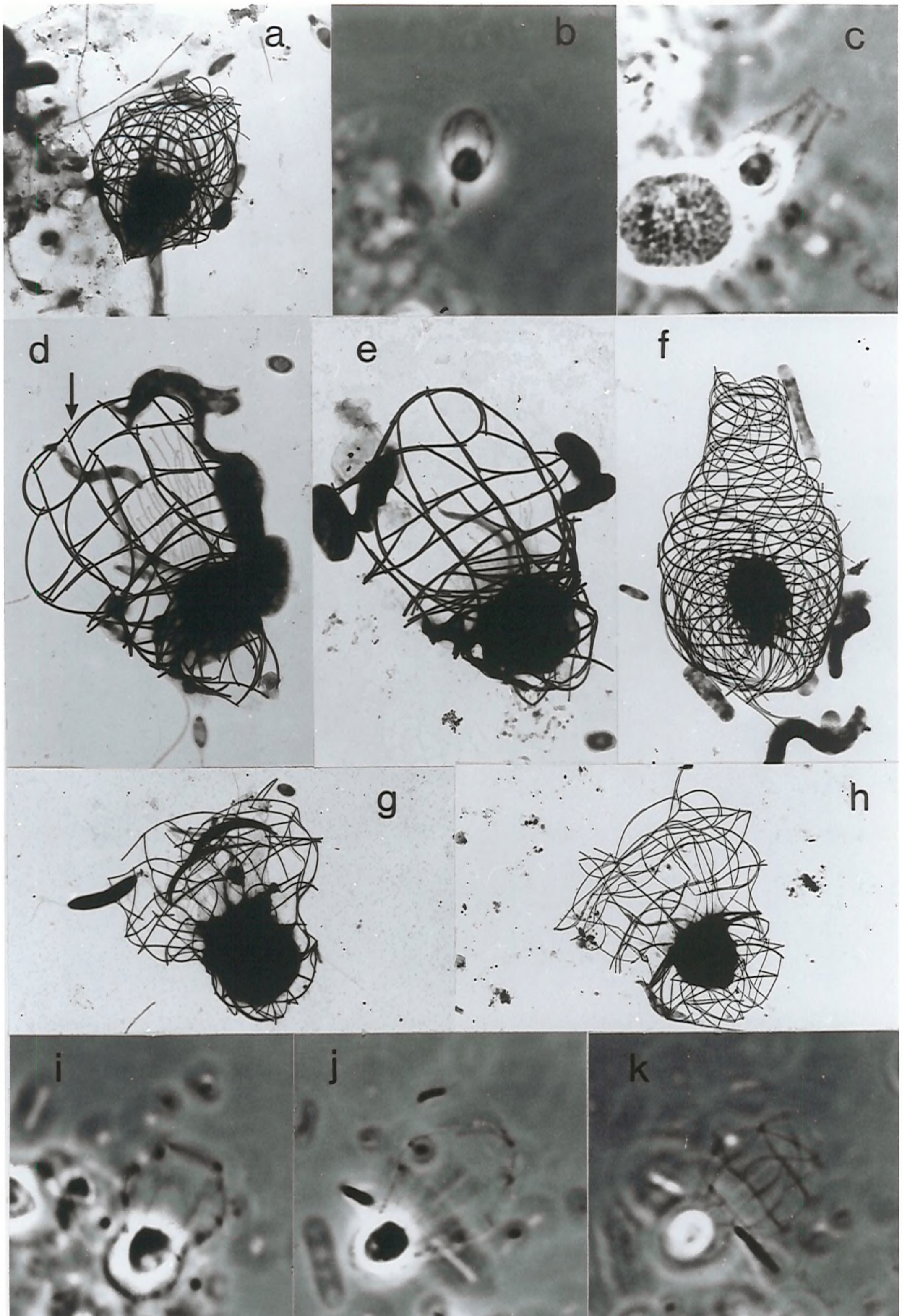
Figure 19: *Savillea* and *Stephanoeca* species

a,b: *Savillea micropora*. a: EM showing spirally arranged costae, x5000. b: LM of living cell, notice the absence of a flagellum, x2000.

c,f: *Savillea parva*. c: LM of partially obscured living cell, notice the presence of a flagellum, x2000. f: EM showing spiral arrangement of costae, x4000.

d,e: *Stephanoeca cupula*, EM, notice the rather regular arrangement of costae compared to *S. diplocostata* (below), and the characteristic junctions between the longitudinal and top transverse costae (fig. d, arrow), x5000.

g-k: *Stephanoeca diplocostata*. g,h: EM of typical cells, notice the thickened transverse costae just above the cell (h), x3000. i: LM of living (typical) cell, x2000. j,k: LM of living cells, showing a form related to *S. diplocostata*, notice the relative length of the lorica (j), and the obliquely arranged costae visible in a different plane of focus on the same cell (k), x2000.


a
b
c
d
e
f
g
h
i
j
k

Observations: Seen frequently in enrichment cultures, but never observed in preparations of freshly collected material. The lorica of this species has a characteristic 'waist' between the anterior and posterior chambers, which is usually delineated by a dense transverse costa (fig. 19h); the density is due to these costal strips being thicker than those used in the rest of the lorica, as well as there being a double ring of strips, as in the other transverse costae (Leadbeater 1975).

A form clearly related to *S. diplocostata* was found in an enrichment culture of the HY2 sample. It had four transverse costae in the anterior chamber of the lorica (fig. 19k) with the protoplast (2.5 x 3.5µm) occupying the lower chamber, and pseudopodia extending to the level of the first transverse costa above it. A number of oblique costae were visible between the upper two transverse costae (fig. 19k). The lorica was somewhat longer than those of typical *S. diplocostata* cells (17µm), and the width of the orifice at the top was smaller (4µm as opposed to 5-6µm). The arrangement of costae in the lower part of the lorica was obscured by the protoplast but there does appear to be some similarity to *Stephanoeca complexa* (Norris) Throndsen, 1974 (the lorica is about 16µm long in fig. 21 in Norris, 1965).

Stephanoeca elegans (Norris) Throndsen, 1974 (var. 'constricta', Tong, in prep.)

Figs: 20d, 20f-g

Observations: The specimens presented here are clearly related to *Stephanoeca elegans*, but differ from the accepted form of the species as interpreted after electron microscopical examination by Leadbeater (1972a) and Thomsen (1973). The lorica of the present taxon differs in shape from *S. elegans*, due to the lower transverse costa being reduced in size, so that the posterior chamber of the lorica is constricted. There is also an additional transverse costa at the level of the joint between the first and second costal strips of the longitudinal costae. As in *Stephanoeca elegans* there are 16-18 longitudinal costae, each consisting of two costal strips above the posterior transverse costa. Many costal strips have spatulate ends, although those at the anterior ends of the upper longitudinal strips are the most prominent (fig. 20d, arrow). Posteriorly the number of longitudinal costae becomes reduced from 18 to 7-13; in *Stephanoeca elegans*, there are 15-18 longitudinal costae in the posterior chamber (Leadbeater 1972a, Thomsen *et al.* 1991). The anterior transverse costa consists of six, or occasionally five curved costal strips forming a circular orifice at the top of the lorica (see fig. 20f). There are generally three longitudinal costae attached to each transverse strip. The middle (additional) transverse costa is formed from 9 costal strips, and it is therefore wider than the anterior transverse costa. Strips forming the middle transverse costa are somewhat loosely attached and can easily be displaced. Unfortunately the protoplast is in a position which obscures much of the posterior transverse costa. However, there appear to be around six costal strips, with a greater degree of overlap than in the anterior transverse costa, and hence a smaller diameter. It is worth noting that the original description of *S. elegans* (as *Pleurasiga elegans*) by Norris (1965) shows a lorica with a shape somewhat similar to that of the specimens presented here; although the middle transverse costa is not represented it is quite possible that if this were present it would not be visible using light microscopy. However, his diagram does not suggest that the longitudinal costae reduce in number posteriorly, and the protoplast seems to occupy a rather more posterior position, as in the micrographs of Leadbeater (1972a) and Thomsen *et al.* (1991).

The present taxon differs from *S. elegans* in three main aspects: the possession of an extra transverse costa, the constriction at the posterior transverse costa and the posterior reduction in the number of longitudinal costae. However, the two forms are obviously closely related, and therefore it is recommended that the variant in this study is retained within *S. elegans* as variety 'constricta' (referring to the constricted posterior chamber of the lorica).

Stephanoeca norrisii Thomsen, 1973

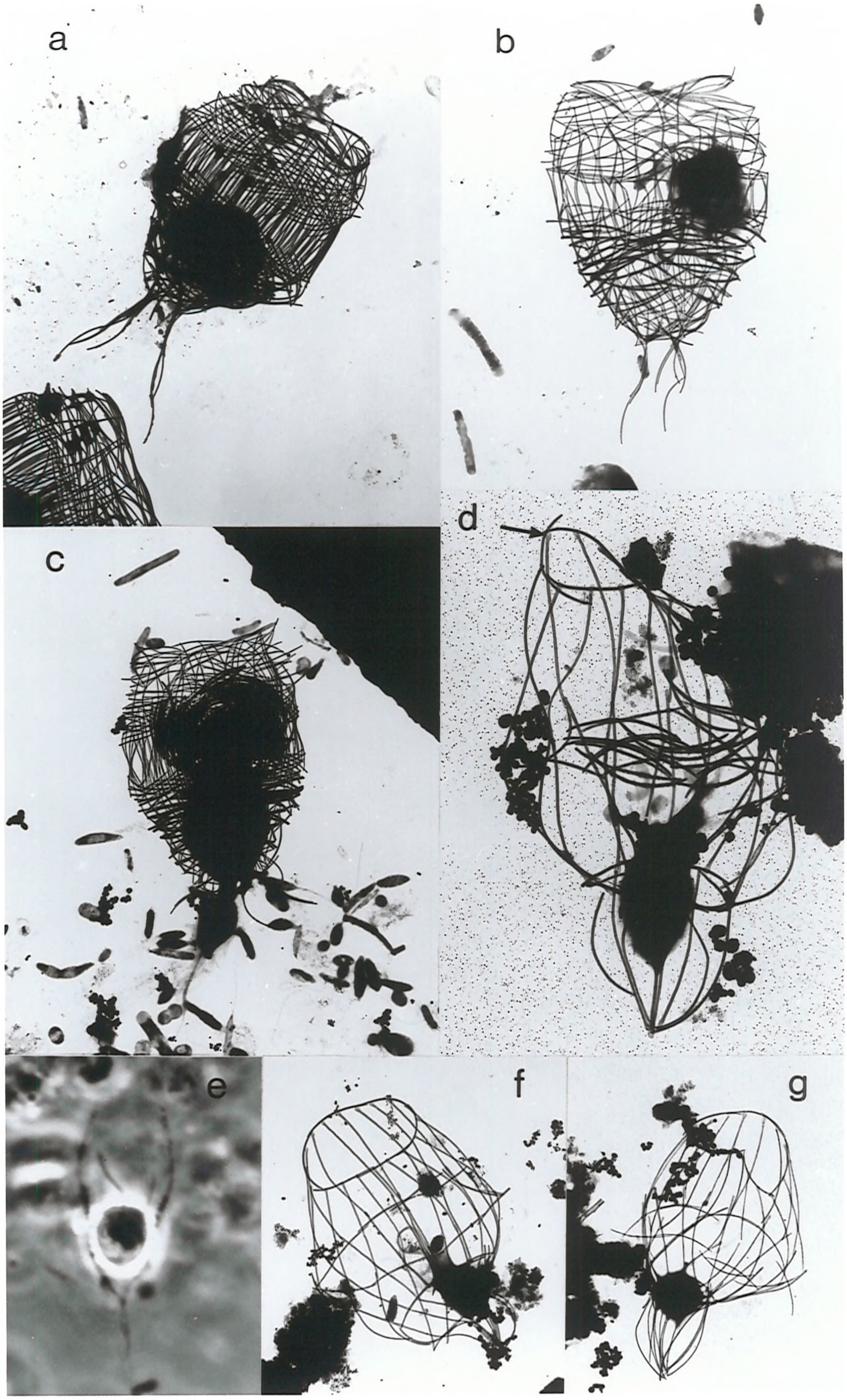
Figs: 20a-c, 20e

Size: cell 4-5 x 4.5-6μm, lorica 8-9 (at widest point) x 13.5-17.5μm, stalk/'tails' of lorica up to 8.5μm

Figure 20: *Stephanoeca norrisii* and *S. elegans* (var.)

a-c, e: *Stephanoeca norrisii.* a-c: EM showing the densely packed longitudinal costae characteristic of the species, variation in the overall number of costae (compare a,b) and accumulation of costae before division (c), x3000. e: LM of living cell, notice the single pedicel, x2000.

d,f,g: *Stephanoeca elegans* var. 'constricta', EM, notice the spatulate end of the costae (arrow, d), the extra transverse costa above the cell, and the reduction of the number of longitudinal costae in the posterior chamber of the lorica (causing it to be 'constricted'), x5000 (d) and x3000 (f,g).


a
b
c
d
e
f
g

Observations: This species occurred in three samples, and was seen in enrichment cultures as well as in whole-mounts of freshly collected material. It has only been reported once (Fenchel 1982c) since the original description by Thomsen (1973). The lorica contains a very large number of coastal strips, so that considerable variation might be expected, both in the number and position of costae within different individuals, and also as a result of changes in the lorica after fixation. There are obviously considerably more costae in the lorica of the specimen in fig. 20a than that in fig. 20b, although it is practically impossible to count them. In all cases, however, the species can be identified by the region of closely packed longitudinal costae in the middle of the lorica, although the number of these strips may be considerably less than the figure of 80 given by Thomsen (1973). A variable number of costae project from the posterior of the lorica, and may be arranged into a single stalk as in fig. 20e, or into two 'tails' as in figs 20a,b. The vast number of costae in the lorica must mean that the cell is not adapted to a planktonic existence, and the posterior costae could well serve to attach the cell to detritus or other surfaces. Loricas frequently contained large bundles of costae as in fig. 20c, in preparation for division.

3.7 COCCOLITHOPHORIDS/COCCOLITHOPHORALES Lemmermann, 1908

Haptophytes were found in every sample (observed in freshly collected material or epifluorescence studies). The coccolithophorids were particularly conspicuous because their scales were preserved in electron microscope whole-mounts.

Emiliania huxleyi (Lohmann) Hay and Mohler, 1967

Fig. 5e

Observations: Complete cells of this species were never seen, but their scales were found in whole-mounts of nearly every sample.

Papposphaera (*Turrisphaera*) sp.

Fig. 5b

Observations: Found in electron microscopical whole-mounts of two samples. This species has hexagonal crystallites arranged to form tubular structures, as in members of the genus *Turrisphaera*, but does not resemble any of the three species named so far (H. Thomsen, personal communication). Organisms described as *Turrisphaera* species have recently been found to represent a stage in the life cycle of *Papposphaera* species, so that these two genera are now considered synonymous, with *Papposphaera* taking priority (Thomsen, Østergaard and Hansen, 1991).

Phaeocystis spp.

The scales of this genus are less conspicuous in whole-mounts, and so may have been overlooked on some occasions. However, *Phaeocystis* was common during early summer, and formed a large bloom in May 1994.

3.8 DINOFLAGELLATES

Heterotrophic dinoflagellates were only observed in enrichment culture on one occasion, but were commonly seen in light microscope whole-mounts of samples collected during the summer. Epifluorescence studies reveal that dinoflagellates may sometimes form quite a proportion of the larger autotrophic flagellates, and to a lesser extent, the heterotrophic population.

Oxyrrhis marina Dujardin, 1841

Fig. 15d

Size: cell 13.5-18 x 24-38.5μm, posterior flagellum 34.5-50μm

Observations: Seen occasionally in freshly collected material, and in large numbers in an enrichment culture of the NT8 sample. Cells are generally bullet-shaped, with an asymmetrical indentation on the ventral side (fig. 15d), but the shape can vary quite considerably when prey are ingested, since the theca is of an unusually simple and flexible structure for a dinoflagellate (Dodge and Crawford 1970). Two flagella insert within the indentation; the posterior flagellum is about 1½ times the cell length, and the transverse flagellum is unattached at the distal end and executes irregular looping movements. *Oxyrrhis* preyed on other (smaller) heterotrophic flagellates and frequently contained a large number of refractile particles.

3.9 EUGLENOZOA Cavalier-Smith 1981

The euglenozoa contains the kinetoplastid flagellates (bodonids and trypanosomes) and the eugenids which have been shown to be closely related by ultrastructural and molecular studies (e.g. Kivic and Walne 1984, Sogin and Gundersen 1987, Triemer and Farmer 1991). Larsen and Patterson (1990) include the genus *Diplonema* (=*Isonema*), and the genus *Cryptaulax* may be closely related to *Diplonema*, and so also included here (D.J. Patterson, personal communication; *Cryptaulax* is described under *Incertae sedis* taxa).

PLICOSTOME EUGLENOZOA

An informal grouping suggested by Larsen and Patterson (1990) to include euglenozoa with a plicate mouth, and organisms descended from them.

Diplonema ambulator Larsen & Patterson, 1990

Figs: 21f, 22a

Size: (measurements from two cells) 6 x 14µm, 6.5 x 12.5µm

Observations: Single cells of this species were found in two cultures (of the NT2 and

Figure 21: Euglenozoa, *Cryptaulax marina* and *Telonema* species

a: *Petalomonas pusilla*, LM, notice how the flagellum beats only at the distal end, x2000.

b: *Bodo designis*, LM, notice how the flagella curl around the cell, x2000.

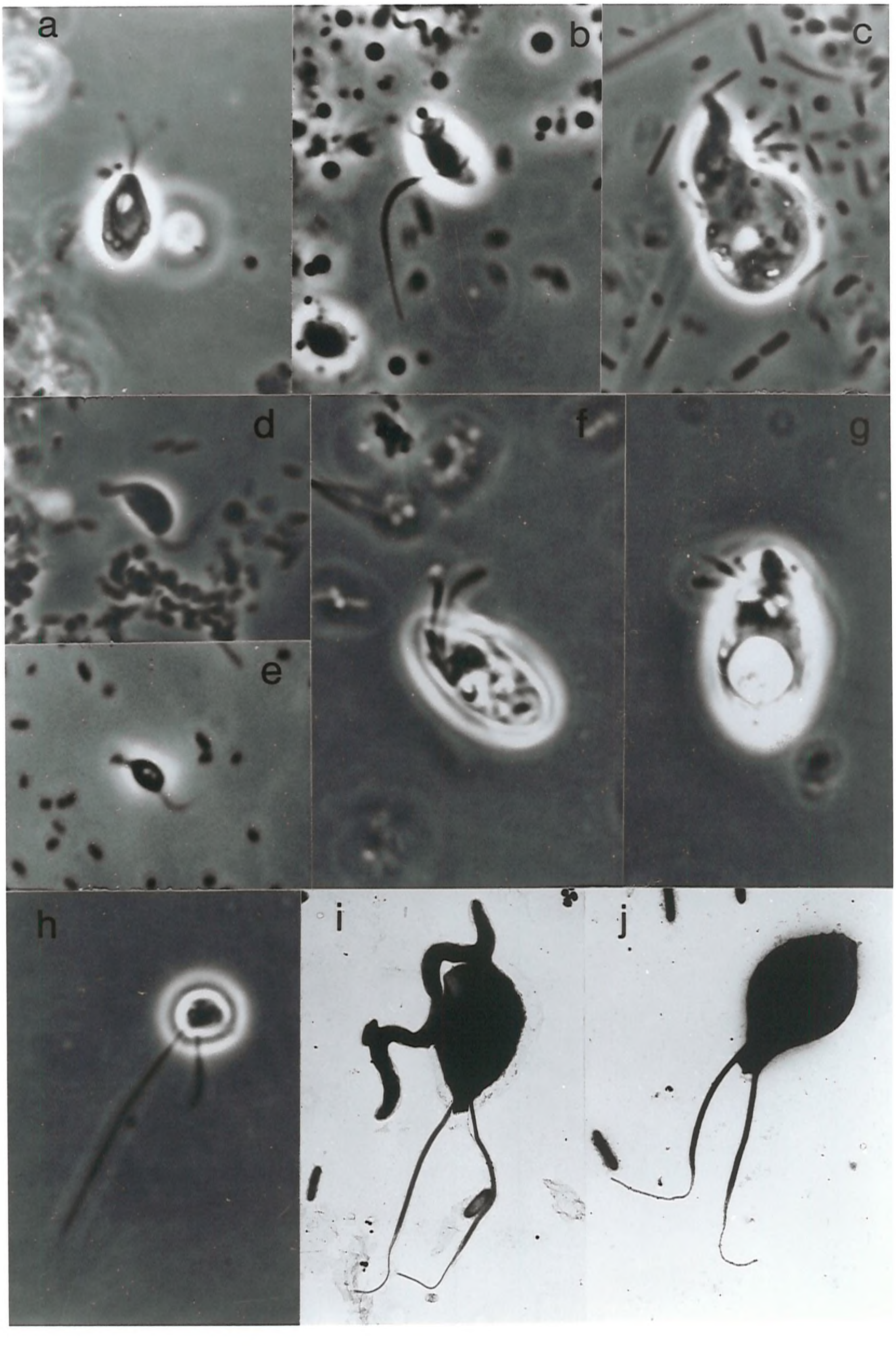
c,g: *Cryptaulax marina*, LM, showing a cell squirming in detritus (c) and flagellar insertion in a stationary cell (g), x2000.

f: *Diplonema ambulator*, LM of cell showing flagellar insertion, notice the similarity to *Cryptaulax marina* in fig. g, x2000.

d,e: *Rhynchomonas nasuta*, LM of cells showing characteristic 'snout', x2000 (d) and x1500 (e).

h: *Telonema* 'antarctica', LM of cell, notice the two flagella of unequal length, x2000.

i,j: *Telonema subtile*, EM, notice the two equal, acronematic flagella and characteristic cell shape, x4000 (i) and x5000 (j).


a
b
c
d
e
f
g
h
i
j

NT16 samples). Cells are cylindrical with two short thickened flagella (about 8μm) inserting into a prominent flagellar pocket (fig. 22a). The body is metabolic, and contains conspicuous food vacuoles containing bacteria and algal cells.

Remarks: This organism can be easily confused with *Cryptaulax marina*, particularly when the length of the flagella is not apparent (see under *incertae sedis* taxa); the flagellar insertion is remarkably similar (compare figs 21f and 21g) and these two species may be closely related.

Petalomonas minuta Hollande, 1942

Fig. 22c

Size: (of two cells) 2.5 x 4 and 3.5 x 6.5μm

Observations: Seen in two cultures; on both occasions *P. pusilla* was also present. Cells are oval to pyriform with a prominent flagellar pocket, and a longitudinal furrow on the ventral side. The distal half of the thickened flagellum beats stiffly from side to side as cells glide.

Petalomonas pusilla Skuja, 1948

Figs: 21a, 22d

Size: 2.5-4.5 x 6-8μm, flagellum protrudes 5.5-7μm from cell

Observations: Seen sporadically in culture and occasionally in freshly collected, centrifuged material. This species is similar to *P. minuta*, but distinguished by the lack of a ventral furrow. As with *P. minuta*, only the distal part of the flagellum is active during gliding (fig. 21a).

Figure 22: Diagrams of euglenids and of *Cryptaulax marina*

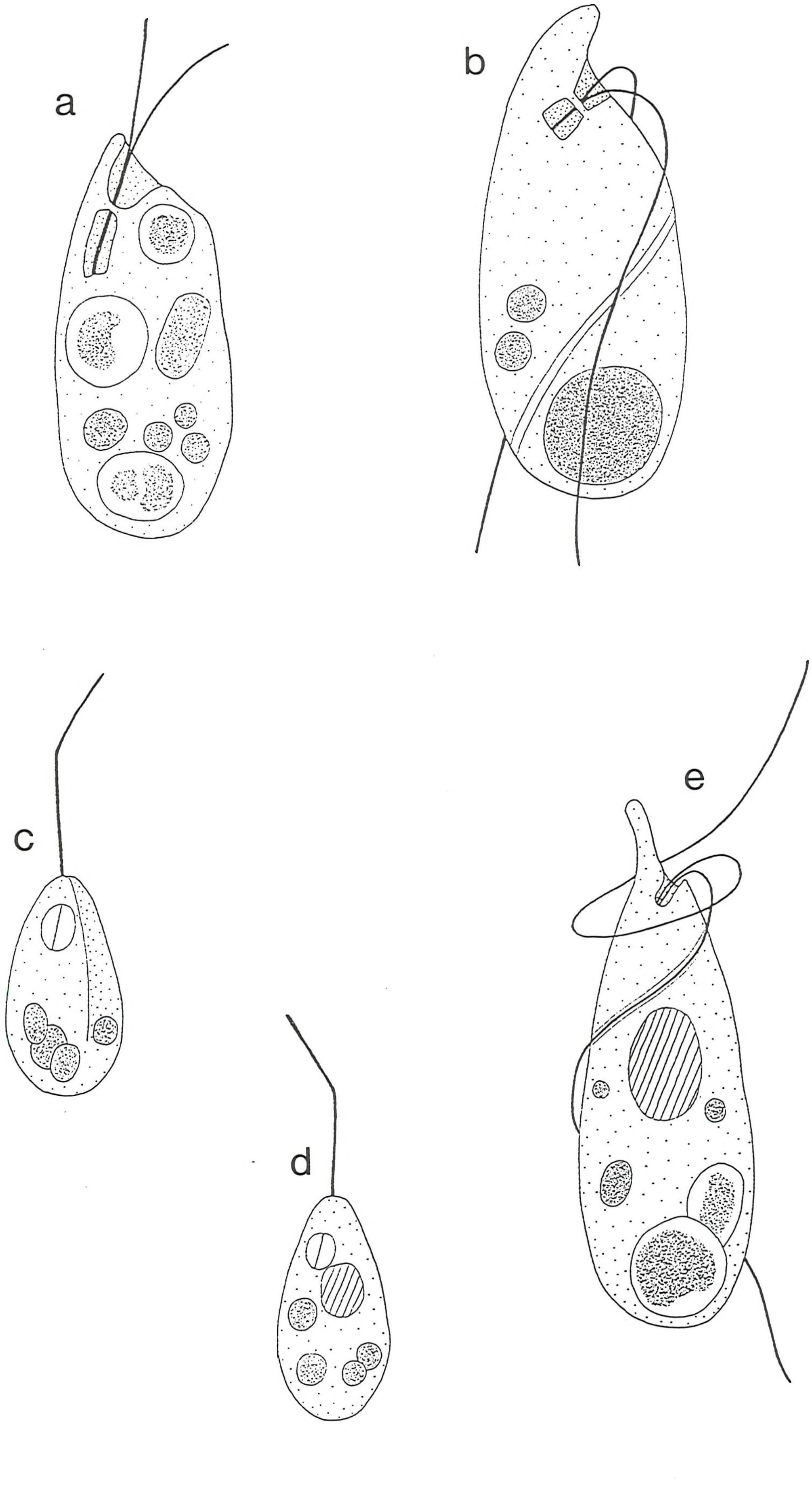
a: *Diplonema ambulator*, notice the prominent flagellar insertion.

b,e: *Cryptaulax marina*, showing the prominent flagellar insertion (b), flexible 'snout' (e) and spiralling grooves around the cells.

c: *Petalomonas minuta*, notice the longitudinal groove.

d: *Petalomonas pusilla*, notice the lack of a longitudinal groove.

All diagrams x5000.


a
b
c
d
e

KINETOPLASTIDS.

This group contains parasitic flagellates (mainly trypanosomes) and the free-living bodonids, which contain a mass of DNA within the mitochondrion (a kinetoplast). Some 200 species have been described within the genus *Bodo* (Zhukov 1991), but many of these are of uncertain identity, and several of the more distinctive species have recently been removed from the genus following ultrastructural studies (e.g. Patterson and Zölffel 1991, Patterson *et al.* 1993). The diversity of bodonids in marine environments appears to be quite low (Larsen and Patterson 1990, Vørs 1992b).

Bodo curvifilus Griessmann, 1913

Fig. 23a

Size: 2.5-8 x 5-13μm (mostly c3.5 x 7.5μm)

Observations: Found frequently in culture. Cells are oval or reniform, and slightly flattened. The posterior of the cell may be elongated into a spine (as also noted by Griessmann 1913 and Burzell 1973), particularly when cells are stressed. Two flagella insert sub-apically in a rather more dorsal position than most *Bodo* species (fig. 23a). The posterior flagellum is about twice as long as the cell, and markedly acronematic. The anterior flagellum is about one-third of the body length, and moves with a characteristic paddling motion (rather like a beckoning finger), as cells glide with the posterior flagellum in contact with the substratum. During gliding, the cell is usually also in contact with the surface, in contrast to *B. designis* and *B. saliens*, although the posterior end waggles slightly. Cells frequently undergo squirming movements when amongst detritus. The prominent cytostome is visible at the cell anterior (fig. 23a) and used to pick bacteria off surfaces. Swimming, as with all *Bodo* species seen, occurs with rotation about the longitudinal axis, with the anterior flagellum undergoing a corkscrewing motion, and the posterior flagellum trailing behind.

Bodo designis Skuja, 1948

Figs: 21b, 23d

Size: 3.5-7 x 6-12μm (typically 4 x 8μm)

Observations: Seen in nearly every culture and was the most common *Bodo* species in this study. Cells are elliptical, usually twice as long as wide, and sometimes have a rather flattened posterior. Two flagella insert sub-apically behind a rostrum, which may be particularly distinct and beak-shaped in some cells. Cells in monoculture tend to retain their characteristic shape, and have never been seen to form 'pseudopodia' as in *B. saliens*. The posterior flagellum is about three times as long as the cell, with a tapered distal portion (fig. 23d).

Swimming is as described for *B. curvifilus*. *B. designis*, like *B. saliens*, does not have a true gliding motion. It skims along surfaces, with the distal part of the posterior flagellum in contact with the substratum, and the anterior flagellum undergoing 'lassoing' movements. Cells periodically stop, coil the anterior flagellum around the rostrum, and apply this to the substrate and ingest food particles. *B. designis* usually feeds on bacteria, but on one occasion was seen ingesting a *Bodo curvifilus* cell. Once captured, the *B. curvifilus* cell rapidly became moribund and rounded, and then part of the cell was taken into the cytostome area by the *B. designis* cell, and the prey cell rapidly became vacuolated. Finally the vacuolated sphere was released; the cytostome of *B. designis* is not sufficiently wide to allow the ingestion of a particle of this size.

Remarks: *Bodo designis* is very similar to *B. saliens*. See below for distinctions.

Bodo saliens Larsen & Patterson 1990

Figs: 23e-f, 24a-d

Size: 2-4 x 3.5-12.6μm. Small cells are often found when the organism is growing

Figure 23: Diagrams of kinetoplastids

a: *Bodo curvifilus.*

b: *Rhynchomonas nasuta.*

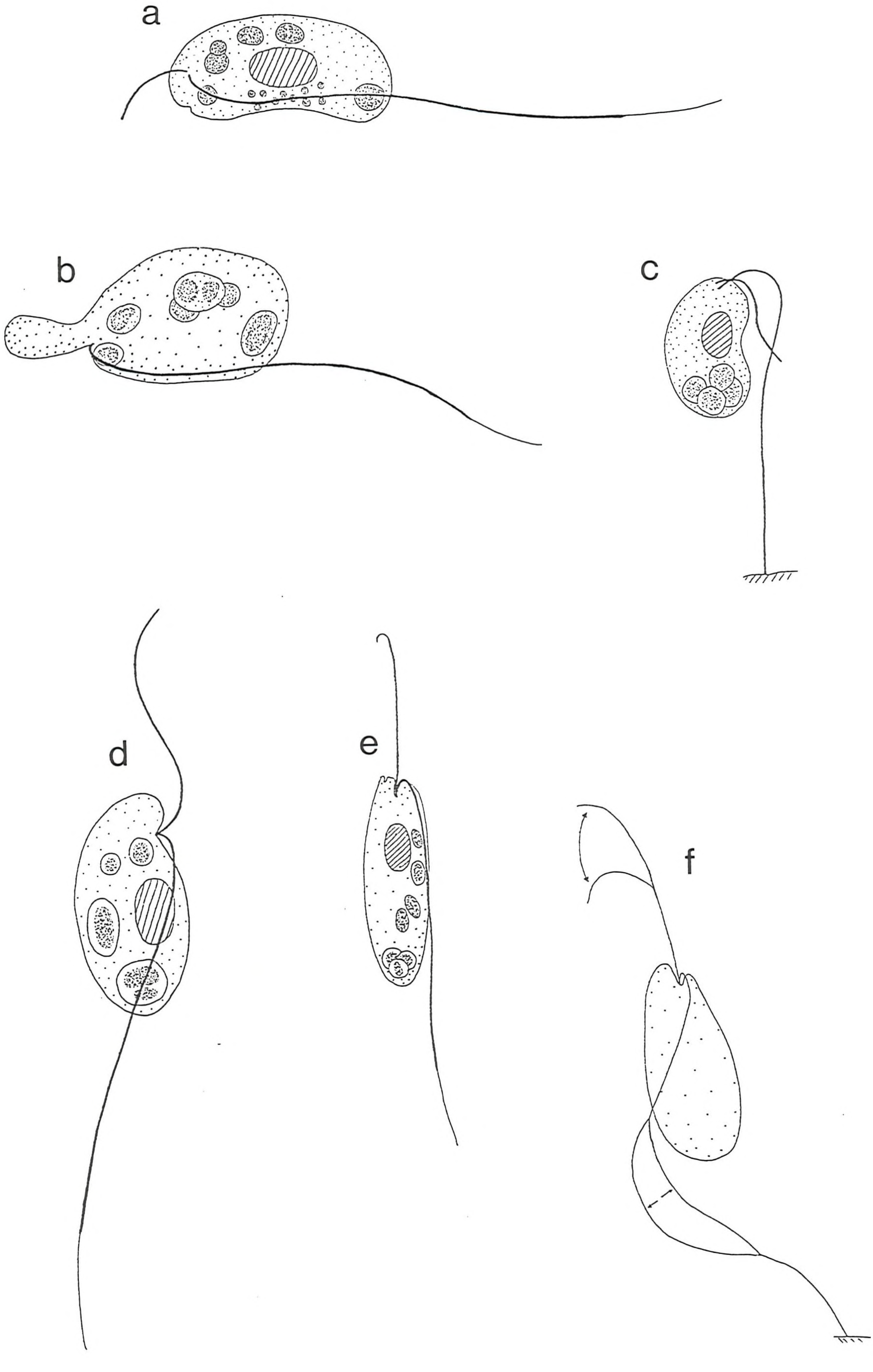
c: *Bodo saltans*, the posterior flagellum is attached to the substratum at its distal tip.

d: *Bodo designis.*

e: *Bodo saliens*, notice the hook at the distal end of the anterior flagellum, and the relative slimness of the cell compared to *B. designis.*

f: *Bodo saliens*, showing typical behaviour for cells in monoculture, notice that the posterior flagellum is not acronematic as in cells in mixed culture (e).

All diagrams x5000.


a
b
c
d
e
f

rapidly in monoculture (with bacteria).

Observations: This organism was frequently seen in enrichment cultures, often in the presence of *Bodo designis* with which it can be confused (see below). Cells are usually relatively elongate (length typically 2½-3 times the width) with two flagella inserting in a subapical depression (fig. 23e). The anterior flagellum is about body length and is held out straight except for a rigid hook at the end, which curves towards the dorsal side of the body (the ventral side being where the flagella insert). This behaviour is particularly distinct when cells skim along near surfaces with rapid darting movements, and the posterior flagellum in contact with the substratum. When feeding the anterior flagellum curves around the top of the cell, in a similar manner to *B. designis*. The two species are practically impossible to distinguish when swimming.

Cells maintained in monoculture exhibit a greater variety of cell shapes, and parts may become quite irregular. They are frequently seen attached to the substratum by the posterior flagellum, or a 'pseudopodial' extension of the body, with the anterior flagellum beating in a curve towards the cell (fig. 23f). Gliding (or 'skidding') and swimming occur only for a few seconds once cells are transferred to a slide; a phenomenon which occurs regardless of incubation temperature (between 4 and 25°C). When in enrichment cultures cells can be observed swimming or gliding for several minutes under the microscope. The flagella of cells in monoculture are only rarely acronematic.

Larsen and Patterson (1990) suggest that the organism studied ultrastructurally as *Bodo curvifilus* by Burzell (1975) was in fact *B. saliens*, but the light microscopical description in this paper seems to be in good accord with that of Griessmann (1913), and my own observations on *B. curvifilus*. *B. saliens* was fixed for electron microscopy as described in the materials section. The fixation obtained was not particularly good, so that not all the details of cell structure could be determined, but the information obtained revealed that *B. saliens* has a very similar ultrastructure to other *Bodo* species. Figs 24a-d show some of these features: the prominent kinetoplast (fig. 24a), an elongate mitochondrion with discoid cristae (figs 24a,b), a slightly ovoid nucleus (fig. 24c), as in *B. designis* (Eyden 1977), and pellicular microtubules (fig. 24d, and in grazing section, fig. 24c).

Remarks: This organism was renamed by Larsen and Patterson (1990) because a variety of similar organisms had been described under different (and often invalid) names. Sectioning has revealed that it appears to be closely related to other species of *Bodo*, supporting its placement in this genus. The organism is similar to *Bodo designis*, but can be distinguished by the following characteristics:

1) The action of the anterior flagellum when 'skidding' (skimming along near a surface). In *B. saliens* it is held out rigidly, with a hook at the distal end, and in *B. designis* it coils in a lassoing movement.

2) Cells are more elongate in *B. saliens*.

Monocultures of these two species reveal other minor, but consistent differences, although both species tend to show slightly different characteristics to cells which are in mixed cultures. *B. saliens* may also be confused with *Bodo curvifilus*, particularly when squirming in detritus, but the anterior flagellum of *B. curvifilus* is shorter, curved towards the ventral side of the body and its paddling action bears no resemblance to the rigid hook of the anterior flagellum in *B. saliens*.

Bodo saltans Ehrenberg, 1832

Fig. 23c

Size: 2.5-3μm x 3.5-7μm

Observations: *B. saltans* appeared sporadically in culture, and was associated with larger pieces of detritus. Although widely reported from freshwater and soil (e.g.Sandon 1927, Brooker 1971 and Hänel 1979) it appears to be less characteristic of marine waters than the other three *Bodo* species above (see also Vørs 1992b, Patterson *et al.* 1993).

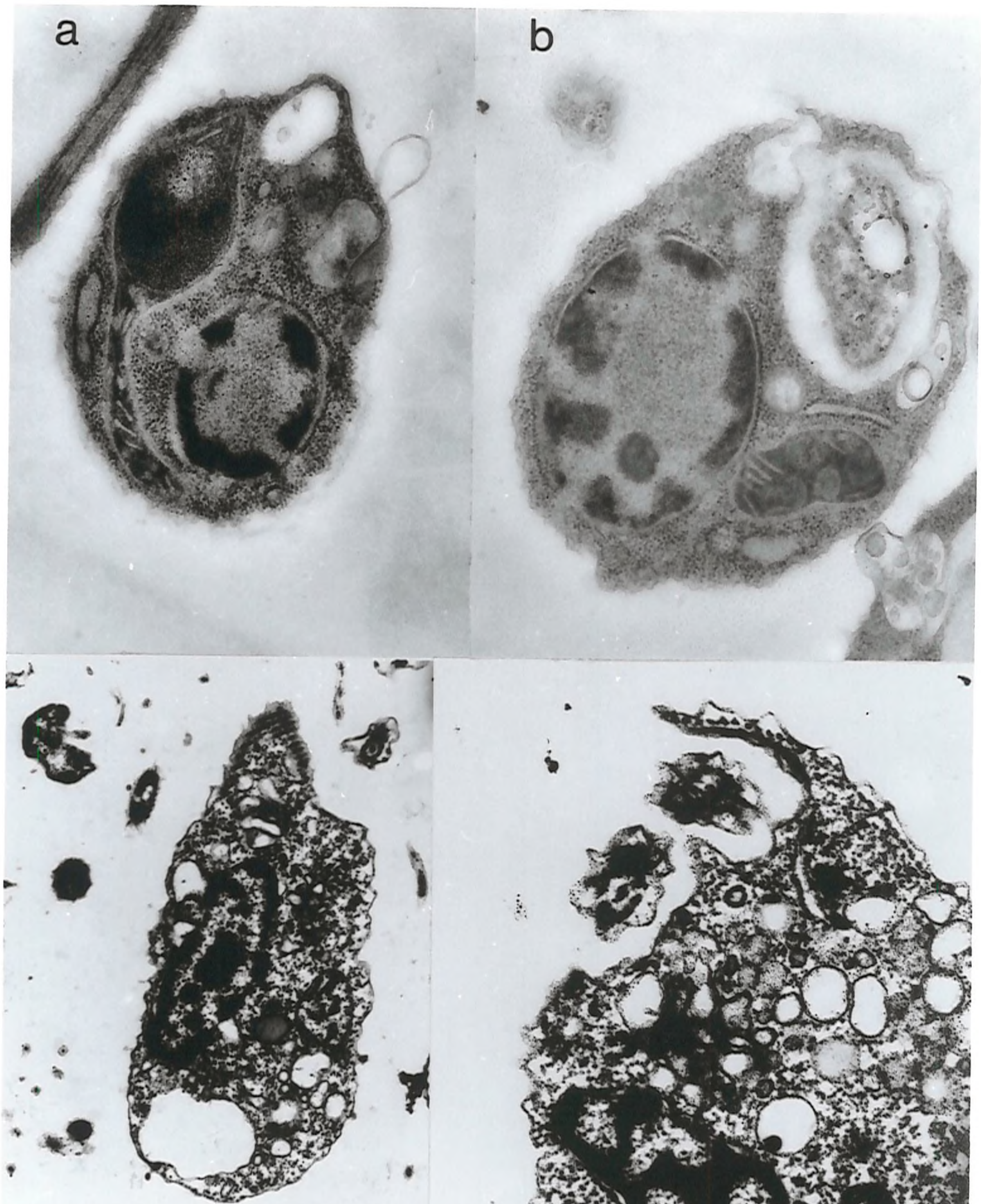
Figure 24: Ultrastructure of *Bodo saliens*

a: Section through anterior part of cell, showing prominent kinetoplast, x30,000.

b: Transverse section through cell, showing discoidal cristae in the mitochondrion, x30,000.

c: Longitudinal section of cell showing the ovoid nucleus, and pellicular microtubules cut in grazing section at the anterior, 15,000.

d: Transverse section through the anterior part of the cell, showing pellicular microtubules, x30,000.


a
b
c
d

Cells are oval to reniform, with a short anterior flagellum (about cell length) and a longer posterior flagellum (about 2.5 times cell length). Both flagella insert into a shallow pocket near the anterior end of the cell. The anterior flagellum sometimes lies across the body in a shallow groove and may be difficult to see. This species can be easily identified by its jerking ('jumping') movements, when attached by the tip of the posterior flagellum to a substratum. Its rotating swimming is similar to that of other *Bodo* species, although it tends to be slightly irregular, and the cell jerks from side to side. This can be attributed to the shortness of the anterior flagellum compared to that of species such as *B. designis*.

Rhynchomonas nasuta (Stokes) Klebs,1892

Figs: 21d-e, 23b

Size: cell 2-3 x 3-6μm (excluding snout), snout 2.2-2.8μm long, flagellum 10-15μm

Observations: Frequently seen in culture, but usually in small numbers. Cells are ovoid and rather flattened, with two flagella inserting at the base of a mobile snout or proboscis. The anterior flagellum lies along the proboscis but is rarely visible with light microscopy, and hence not shown in fig. 23b. In one cell it was seen to protrude beyond the snout by about 2μm. The posterior flagellum is about three times as long as the body, and acronematic. Cells glide, with the proboscis sweeping up and down; occasionally the bulbous tip of the proboscis is applied to the substratum against potential food items (bacteria) which may, or may not be ingested suggesting some process of selection. Swale (1973) also noted discrimination between bacteria in this species. Cells are pliable and capable of squeezing between detrital particles, but squirming was rarely observed.

Remarks: A number of species have been assigned to this genus, but currently *R. nasuta* is the only one which has an accepted identity. Some have been referred to other genera; see Larsen and Patterson (1990) for discussion of this.

3.10 HEMIMASTIGOPHORIDS/HEMIMASTIGOPHORA Foissner, Blatterer and Foissner, 1988

An assemblage containing flagellates grouped by the possession of cilia-like flagella arranged in slightly spiralled kineties, a cortex of plicate plates with rotational symmetry and other ultrastructural features (Foissner and Foissner 1993). They are possibly related to euglenids (Foissner *et al.* 1988, Foissner and Foissner 1993).

Stereonema sp.

Figs: 25a-b

Size: 3-5.5 x 25-45µm (including posterior spine)

Observations: Seen in enrichment cultures of three samples. Cells are highly elongated, with the posterior end drawn out into a long tapering spine. The spine is fairly rigid, but may curve. Two rows of flagella attach to the anterior half of the main part of the cell along grooves which curve around the cell (fig. 25a). The flagella beat assynchronously and tend to spiral around the cell so that the number and length cannot be determined, although those at the anterior of the cell (which occasionally beat away from the cell surface) are about 5µm long. A small contractile vacuole is present just above the spine portion of the cell. It was never seen to contract, but its identity can be inferred from the description of related species in Foissner and Foissner (1993). Contractile vacuoles are rarely seen in marine flagellates, even if they are visible in the same species in other habitats. Cells burrow into detritus with the spiralling of the flagella producing a corkscrewing movement. Swimming may also occur, with rotation about the longitudinal axis. Gliding was not observed.

The species preys on other flagellates (heterotrophic and autotrophic). Feeding on *Pseudobodo tremulans* was observed in detail: the anterior of the *Stereonema* cell came into contact with the *Pseudobodo* cell, and widened, with the flagella creating a propulsive force towards the prey so that the cell became shorter. The anterior of the cell then opened and partially sucked in the prey cell, and a bolus of food passed into the cell before the remains of the prey were released from the interior of the *Stereonema* cell, although they remained attached to the apex. The *Pseudobodo* cell was then taken into the *Stereonema* cell once again, and another portion of its cell contents ingested. This occurred several times with the same prey cell until only a tiny part of its contents remained. This feeding behaviour is very similar to that described in *Hemimastix amphikineta* (Foissner *et al.* 1988), which is a member of the same phylum (Foissner and Foissner 1993).

Remarks: The present species differs from all the described members of the family Spironemidae, to which it clearly belongs, and is the first record of a hemimastigid in a marine location. Foissner and Foissner (1993) revise the current knowledge about this group and divide species into two genera depending on whether the body is acontractile (*Stereonema*) or shows euglenoid movement (*Spironema*). Although the species in this study can shorten its body length when feeding, this is due to the propulsive pressure of the flagella acting against the prey item; the organism is not actually contractile, and certainly does not undergo euglenoid movements. It differs from the only described member of the genus *Stereonema* (*S. geiseri*) in several respects: *S. geiseri* cannot swim, the tail is proportionally longer in the present species (usually accounting for half to two-thirds of the cell length), the shape of *S. geiseri* is shorter and relatively wider, and finally, it is found in fresh water (although this latter difference is not necessarily significant).

3.11 *INCERTAE SEDIS* TAXA

The taxa described below cannot currently be assigned to any suprageneric assemblage.

Allantion tachyploon Sandon, 1924

Fig. 25e

Size: 3.5 x 7-8.5µm

Figure 25: Diagrams of *Stereonema* sp. and various *incertae sedis* flagellates

a,b: *Stereonema* sp., showing two rows of flagella attached to spiral grooves (a); the flagella are only drawn in on one of the grooves, for clarity, x2500.

c: *Kiitoksia ystava*, showing two gliding cells (the right hand one has an acronematic flagellum), x5000.

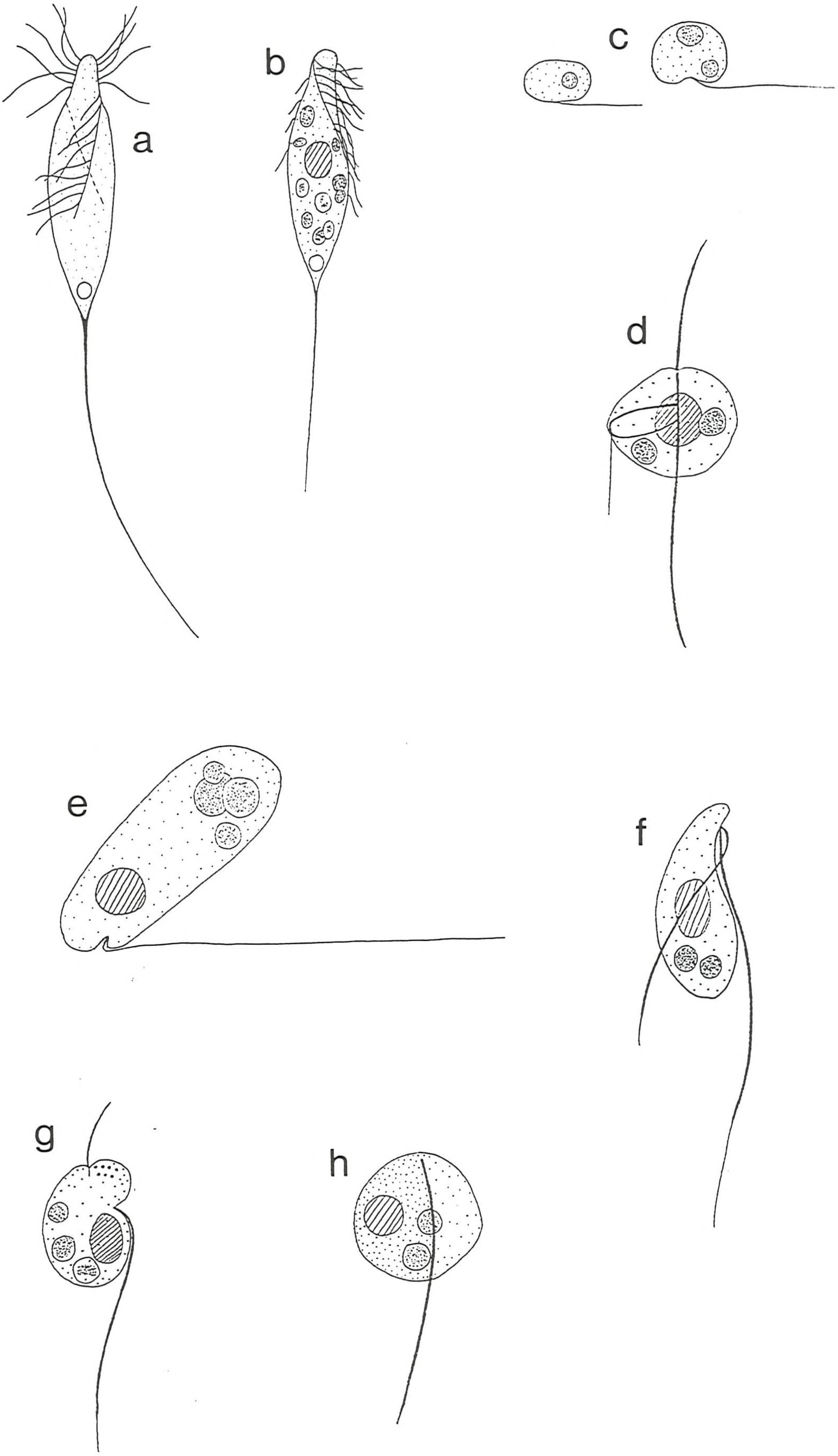
d: *Caecitellus parvulus*, notice the 'mouth' on the right-hand side of the cell (LHS in fig.), and the fine thread trailing from this, x5000.

e: *Allantion tachyploon*, showing gliding cell, notice how the cell is raised above the substratum to which the flagellum is attached, x5000.

f: *Colpodella* sp., x5000.

g: *Ancyromonas sigmoides*, notice the dark granules at the anterior of the snout, and fine anterior flagellum, x5000.

h: *Discocelis saleuta*, notice the thinner left-hand side of the cell (on RHS in fig.), x5000.


a
b
c
d
e
f
g
h

Observations: Found in two samples. Cells are cylindrical with rounded ends. A single flagellum inserts sub-apically in a small depression. Cells glide swiftly with the whole of the flagellum in contact with the substrate, but with most of the posterior part of the cell raised above the surface. Gliding was sometimes interspersed by periods of a waggling movement, as was noted by Sandon (1924). Cells were observed to swim short distances, with rotation about the longitudinal axis, and the flagellum trailing behind. Swimming has not previously been reported in this species.

Remarks: This species is widely distributed in soils (Sandon 1924, 1927) and has also been found in marine locations in Finland, Denmark and Greenland (Vørs 1992b).

Ancyromonas sigmoides Kent, 1880

Figs: 25g, 26d-e, 26h

Size: 2.1-3.5 x 2.9-4.5μm

Observations: This organism was frequently found in enrichment cultures, sometimes in large numbers. One flagellum (the posterior) emerges from behind an apical prominence, or snout, and is two to three times the length of the cell and acronematic (fig. 25g). The anterior flagellum is thinner, emerges at the apex of the cell, and beats stiffly from side to side. It may be easily overlooked, but is actually absent in some cells. The cells are reniform and dorso-ventrally flattened, and glide with the posterior flagellum in contact with the substratum. Two rows of tiny dark granules can be seen on the snout (fig. 25g and fig. 26e, arrow), which may be the extrusomes referred to by Mylnikov (1990) (see below).

Remarks: The organism referred to as *Bodo cephaloporus* in Larsen and Patterson (1990), was later assigned to this taxon (Patterson and Zölffel, 1991). It may have been studied ultrastructurally by Mylnikov (1990) under the name *Heteromita*. *Ancyromonas* may be confused with *Metopion*, but can be distinguished by the anterior flagellum, when present, the acronematic posterior flagellum and the row of 'extrusomes' on the snout.

Caecitellus parvulus (Griessmann) Patterson, Nygaard, Steinberg & Turley, 1993

Figs: 25d, 26f

Size: 2.0-4.7 x 2.0-4.3μm, anterior flagellum: 2.7-4.5μm, posterior flagellum: 6.8-9.5μm.

Observations: Cells glide with the posterior flagellum in contact with the substratum and the anterior flagellum beating stiffly from side to side. The anterior flagellum is about body length and the posterior flagellum is about twice as long. The body is slightly irregular in shape due to a mouth on the left ventral side of the body. The dark band running around the mouth (figs 25d, 26f) appears to be attached to the posterior flagellum, and although it has not previously been observed by light microscopy, its presence is supported by the observations of Patterson *et al.* (1993) who found an arc of microtubules supporting the mouth when they examined the organism by electron microscopy. The mouth can extend and is squeezed over bacterial cells adhering to the substratum. Bacteria may then be ingested or 'rejected', suggesting some form of selection. The organism appears to prefer rod shaped bacteria of about 1.5 x 0.5μm. A very fine thread is seen trailing from the far left hand edge of the mouth (fig. 25d). This has not previously been reported, but is easily overlooked, although it appears to be present in all cells. The thread varies in length (from 1 to 7μm), but is generally 1-1.5μm. The function of this thread is unknown, but could conceivably be involved in food selection.

Remarks: Electron microscopy by Patterson *et al.* (1993) indicated that the organism was incorrectly placed in the genus *Bodo* by Griessmann (1913).

Colpodella sp.

Figs: 25f, 26a-c

Size: 2-4.5 x 4-6μm

Observations: Seen in large numbers in a single enrichment culture of the MP4 sample. Cells are rather elongated, acuminate anteriorly and rounded at the posterior end. The ventral side of the cell is rather convex (fig. 25f). Two flagella insert sub-apically. The anterior flagellum is about 1½-2 times the cell length and the posterior flagellum is slightly longer (about 2½ times cell length) and acronematic (fig. 26c). Cells were commonly seen to attach to *Paraphysomonas* cells, but did not appear to feed since when they detached after a short time the *Paraphysomonas* cells were still living and unchanged in appearance. Feeding was observed on a larger cell of unknown identity (it was already dead, and gradually changed shape and decreased in size as feeding proceeded). Individuals (up to three at one time) attached at their apex to the prey item, with their flagella coiled around the cell (figs 26a,b). Swimming is rapid, with the anterior flagellum coiling around the cell anterior, the posterior flagellum trailing, and rotation about the longitudinal axis; it is rather similar to the swimming in *Bodo* species. Encystment was not observed.

Remarks: See Patterson and Zölffel (1991) for a discussion of various synonyms of members of this genus. The species seen in the present study shows some resemblance to *Colpodella perforans* (as described as *Spiromonas perforans* in Brugerolle and Mignot, 1979) but differs in the feeding behaviour observed, with cells detaching after a relatively short period of feeding although this may have been due to a different (less appropriate?) host. Also, the flagella in the present species are longer, and Brugerolle and Mignot remark that the swimming of their species is very different from that of *Bodo* cells. *Colpodella* may be related to the apicomplexans (Patterson 1989a).

Cryptaulax marina Throndsen, 1969

Figs: 21c, 21g, 22b, 22e,

Size: 5.5-10.5 x 15-24μm.

Observations: Found in nearly every sample. When swimming the cell is a long ovoid shape with a flexible snout (fig. 22c), but it can deform considerably when amongst detritus or in contact with a surface (fig. 21c). As the cell squirms and twists the course of the groove may vary from spiral (with a variable number of rotations, but usually 1-1¼) to longitudinal. Two thickened flagella of roughly equal length insert into a flagellar pocket which is conspicuous when the cells are stationary (figs 21g, 22b). The anterior flagellum loops around the cell during the characteristic rotating and zig-zagging swimming, which is quite rapid. The organism periodically stops and presses its snout against a surface, with the anterior flagellum wound around it in a manner reminiscent of *Bodo designis*.

All cells from freshly collected material contained algal remains, although cells also grew in enrichment cultures, and here predated on heterotrophic flagellates, including other *C. marina* cells. This species appears to be a significant predator on nanophytoplankton in Southampton Water. A range of algal prey was ingested, including the euglenid *Eutreptiella marina,* chrysophytes, cryptophytes, haptophytes, dinoflagellates and centric diatoms. Single prey items sometimes occupied a large proportion of the cell (e.g. a centric diatom of 9.2µm diameter was seen in a cell of 10.6 x 23.8µm). The food contents of cells usually reflected the algal species dominant in the water sample at that time, and the population seemed able to adapt to potential food sources in terms of size, with the largest cells seen associated with the bloom of *Eutreptiella marina* seen in NT11.

Remarks: This species is very similar to *C. thiophila* Skuja 1956, except that extrusomes have not been observed in the flagellar pocket in *C. marina*. Extrusomes have not been observed in any other *Cryptaulax* species, but their presence or absence needs to be established before conclusions about conspecificity can be drawn (Vørs 1992b). *Cryptaulax* shares similarities with at least three other described genera, and further study, particularly electron microscopy is urgently needed. The insertion of the flagella into a pocket in *Diplonema ambulator* is very similar to that of *C. marina* (see figs 21f,g). There is also similarity to *Entomosigma* Schiller 1925, which was originally and erroneously described with a chloroplast (Patterson and Zölffel 1991). In freshly

Figure 26: Various *incertae sedis* flagellates

a-c: *Colpodella* sp. a,b: LM of three cells attached to a prey cell, x2000. c: EM of cell, notice the acronematic flagella, x3000.

d,e,h: *Ancyromonas sigmoides*. d,e: LM of cells showing the fine anterior flagellum (d) and granules at the anterior of the snout (arrow, fig. e), x2000. h: EM, x5000.

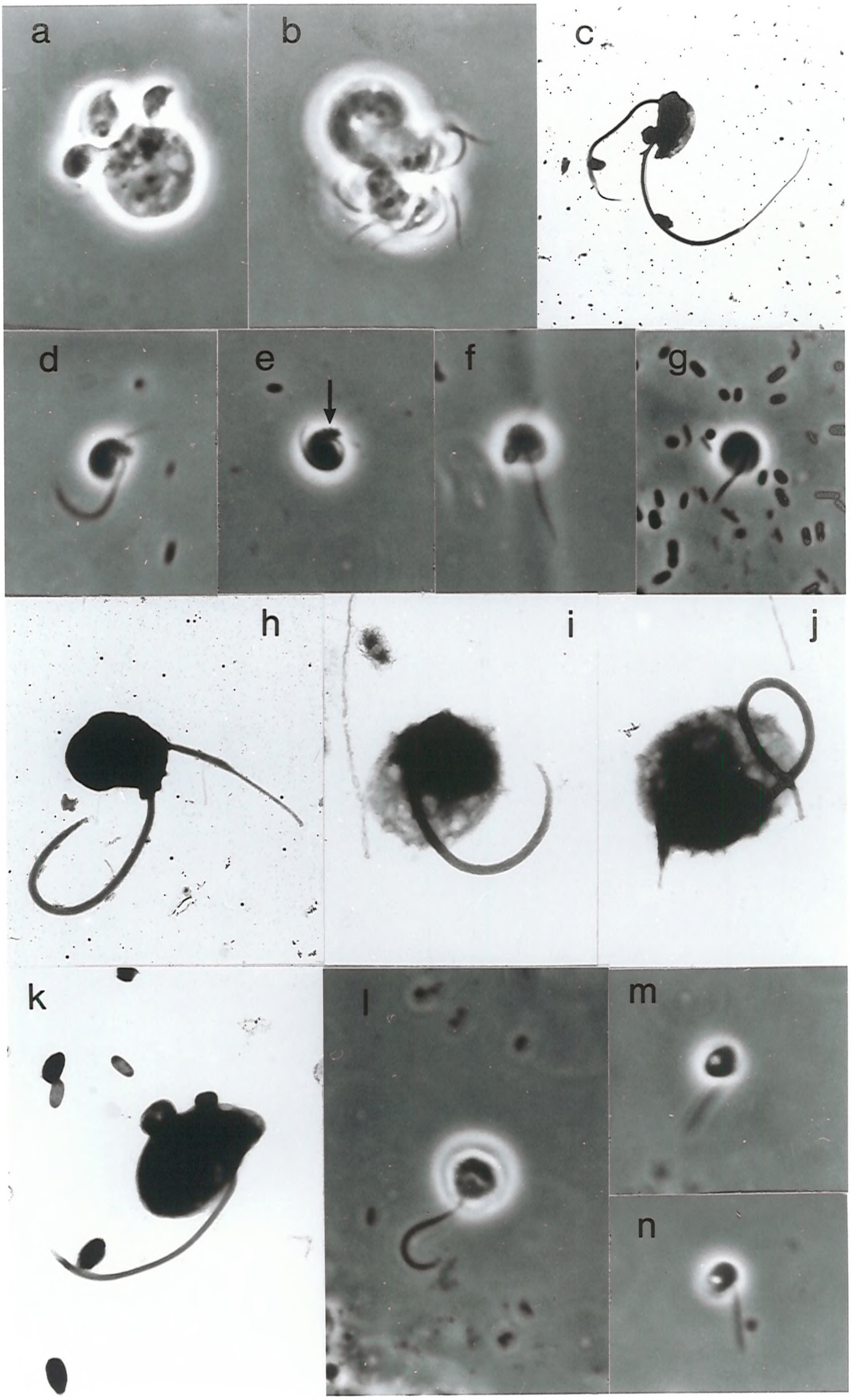
f: *Caecitellus parvulus*, LM, notice the band of microtubules forming the mouth on the right-hand side of the cell (LHS of fig.), x2000.

g,i,j: *Discocelis saleuta*. g: LM x2000. i,j: EM of cells, notice the second short flagellum in fig. j, x10,000.

k: *Metopion fluens*, EM x5000.

l: *Metromonas simplex*, LM of cell attached to the substratum by the longer flagellum, and waving from side to side, x2000.

m,n: *Kiitoksia ystava*, x2000.


a
b
c
d
e
f
g
h
i
j
k
l
m
n

collected samples, *C. marina* has been frequently observed with algal food contents in a large vacuole at the posterior, resulting in an appearance very similar to that of *E. peridinoides* as presented by Sournia (1986). Griessmann (1913) described a species, *Hemistasia klebsii*, which only differs from *C. marina* in the fact that most cells have two grooves. Griessmann's detailed description of the swimming and squirming movements of the organism are identical to those observed in the present species.

The similarity of *C. marina* to *Diplonema ambulator*, and the ultrastructural study on *Rhynchobodo taeniata* (=*Cryptaulax taeniata* Skuja 1956) in Vørs (1992b) which shows that this species is a kinetoplastid suggests that *C. marina* may belong to the euglenozoa.

Discocelis saleuta Vørs, 1988

Figs: 25h, 26g, 26i-j

Size: 2.5-4.5µm; posterior flagellum 7-10µm.

Observations: Cells are rounded and dorso-ventrally flattened. The left side of the cell may be slightly pointed and is frequently thinner than the right, and may be almost transparent when viewed with phase contrast optics. The nucleus is relatively small, rounded and often visible in living cells on the right side of the cell (fig. 25h). One flagellum, about 2.5 times the cell diameter in length inserts at the anterior of the cell, and trails behind it, attaching the cell to the substratum. A second flagellum was observed on one electron microscopical whole mount of a cell (fig. 26j), but never in living cells or in other whole mounts. This is contrary to the observations of Vørs (1988) and Larsen and Patterson (1990) who found that two flagella were typically present, although Larsen and Patterson remark that the anterior flagellum may be absent from smaller cells. This erratic occurrence means that the anterior flagellum is unlikely to be responsible for cell motion as suggested by Vørs (1988).

Kiitoksia ystava Vørs, 1992b

Figs: 25c, 26m-n

Size: cell 1.5-3.5μm, flagellum 5-8μm (12μm in one individual, see below)

Observations: Found quite regularly in enrichment cultures, particularly in those which developed large detrital flocs. Cells are spherical, ovoid or reniform. A single flagellum inserts into a small depression. The flagella on some cells had a very fine hair-like portion distally; this was not noted on all cells, but could be easily overlooked. On one cell (3.5μm, the largest seen) the thinner portion was 6μm long (total length of flagellum 12μm). Cells glide, slightly jerkily, with the flagellum trailing behind and adhering to the substratum for the whole of its length, and the cell raised slightly above the surface. Since this species is so small, very little is known about its cytological details, but at least one refractile granule was present in every cell.

Leucocryptos marina (Braarud) Butcher, 1967

Fig. 28a

Size: 5-10 x 9-20.5μm

Observations: Seen frequently in freshly collected centrifuged material, and in epifluorescence preparations. Cells are droplet-shaped with two thick flagella, about the same length as the body, inserting subapically. One or two rows of extrusomes were sometimes visible on the ventral side. When swimming, the anterior flagellum spirals round vigorously at the front of the cell causing the cell to rotate, and the posterior flagellum trails passively.

Remarks: Vørs (1992c) found that maximum numbers of *L. marina* coincided with maximum numbers of nanophytoplankton, and that the species formed a substantial proportion of the nanoplankton biomass during 1988 and 1989 in the southern Kattegat.

In this study there was no real correlation of the numbers of *Leucocryptos* with phytoplankton. It would appear that *Cryptaulax marina* is a far more important predator on nanophytoplankton in Southampton Water (see observations on this species above).

Leucocryptos remigera Vørs, 1992b

Fig. 28b

Size: 4.5-8 x 7-16μm

Observations: Seen frequently in freshly collected centrifuged material, and in epifluorescence preparations. Cells are ovoid-oblong with two thickened flagella inserted subapically. The anterior flagellum is about twice cell length, and the posterior flagellum two to three times cell length; both coil around the cell during swimming, although movement is more vigorous in the anterior flagellum. Most cells contained green-brown food particles, and two rows of extrusomes were usually visible on the ventral side. See Vørs (1992b) for a detailed description.

Remarks: *L. remigera* may be easily distinguished from *L. marina* by its shape and the possession of longer flagella.

Luffisphaera Belcher & Swale, 1975

This genus contains spherical or elliptical cells of around 1.5-3.5μm. They bear a single layer of two types of hollow meshwork scales ('basal' and 'spine' scales), lack flagella, and have tubulocristate mitochondria (Belcher and Swale 1975). This genus may be easily overlooked because of its small size, and has so far only been reported four times since its initial description (Thomsen 1982, Vørs 1992a,b, Vørs 1993b). The two species found in the present study do not conform to any of the nine taxa previously described.

Figure 27: Electron microscope whole-mounts of *Luffisphaera* 'hamatus'

a,c: Cells showing the basal scales (a), with scattered spine scales, x7500 (a) and x10,000 (c).

b,d: Detail of spine scales, notice the hexagonal perforation in the proximal part, with one large circular perforation, and the tiny hook at the end of the spine, x20,000.

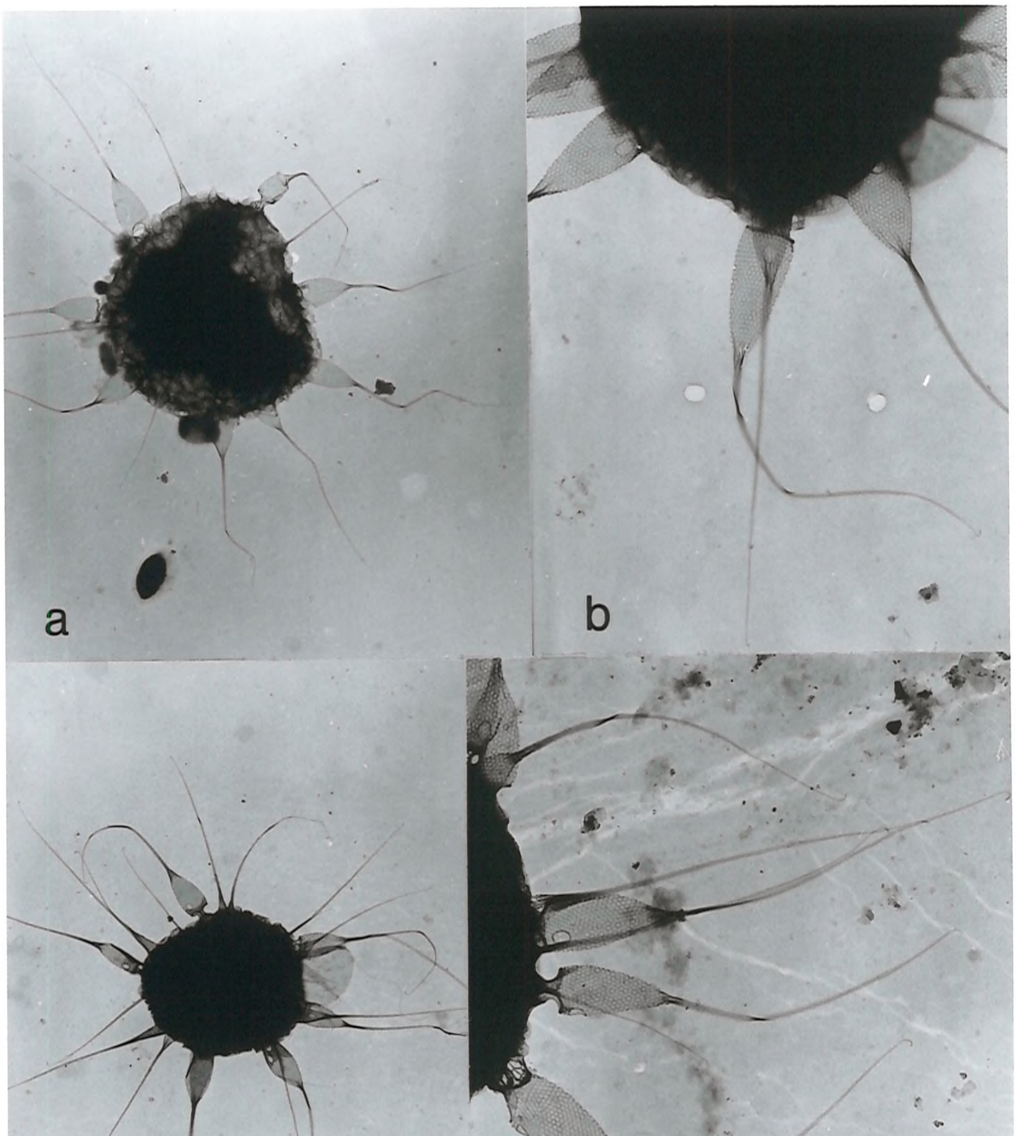

a
b
c
d

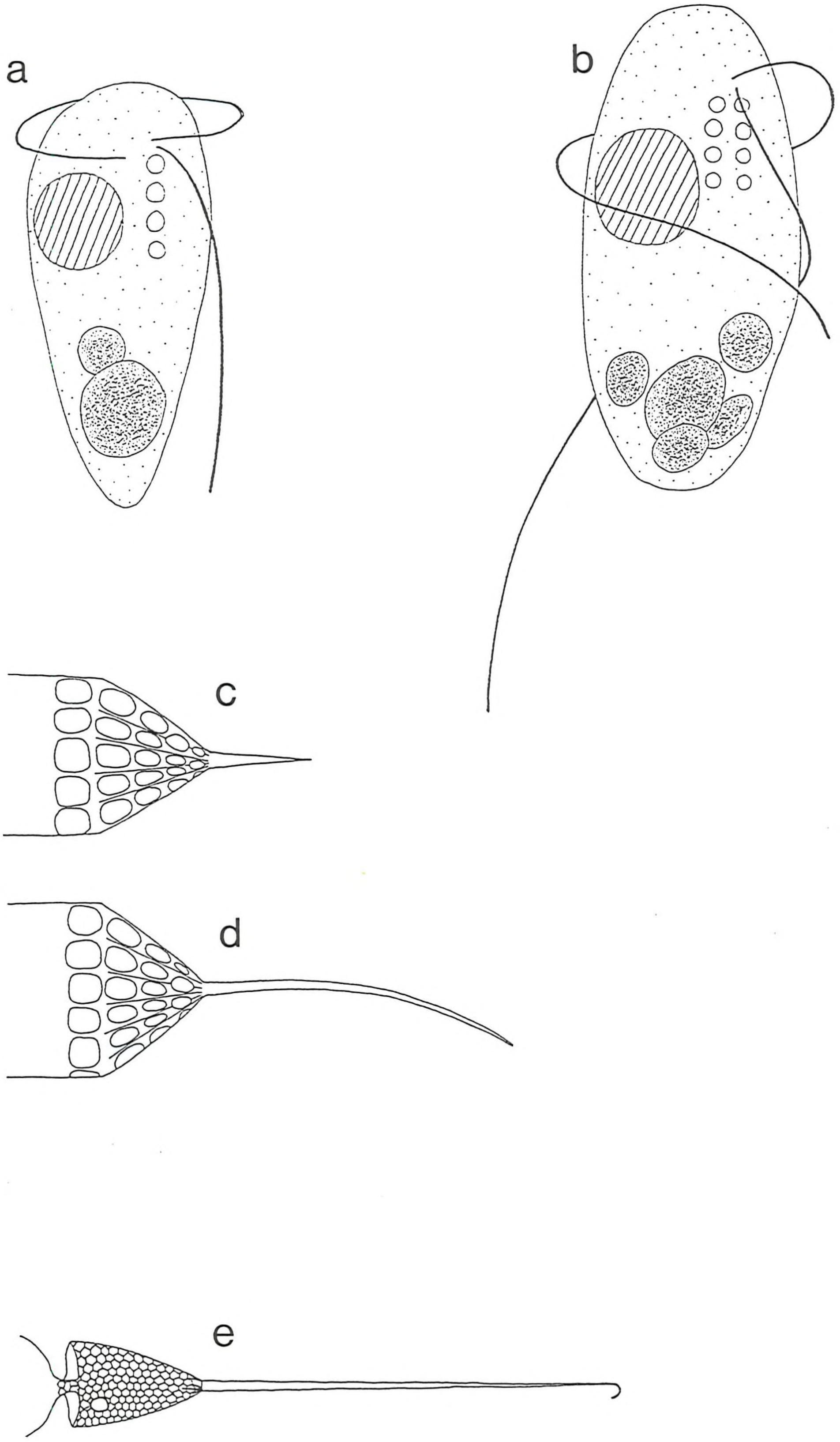
Figure 28: Diagrams of *Leucocryptos* species, and scales of *Luffisphaera* species

a: *Leucocryptos marina*, notice the pointed posterior end of the cell, and relatively short flagella, x5000.

b: *Leucocryptos remigera*, notice the rounded posterior end of the cell, and relatively long flagella, x5000.

c,d: Spine scales of a *Luffisphaera* species, with a short spine (c) and a long spine (d), x100,000.

e: Spine scale from *Luffisphaera* 'hamatus', x25,000.


a
b
c
d
e

Luffisphaera 'hamatus' (Tong, in prep.)

Figs: 27a-d, 28e

Size: (fixed cells on electron microscope whole-mounts) 2.3-4.2µm

Observations: Found in electron microscope whole-mounts of an enrichment culture of the NT2 sample. Cells are roughly spherical and densely covered in basal scales, with scattered spine scales (figs 27a,c). Spine scales consist of a bulbous base, supporting a dome-shaped part on two broad struts. The dome has a regular lattice pattern with hexagonal perforations, and a single larger circular perforation (figs 27c,d, 28e). The basal part appears to have irregularly shaped perforations. Distally the dome carries a long smooth slightly flattened spine which gradually tapers, and terminates in a small but distinctive hook, after which the species is named. Spine scales measure about 3-4µm in length, with the dimensions of the dome-shaped part being approximately 0.4 x 0.8µm. Basal scales are oval and have a single row of square perforations around the outside, with larger, irregular perforations inside.

Remarks. This species most closely resembles *L. myosorus* Belcher and Swale 1975, and *L. bulbochaete* Vørs 1993b. However it may be distinguished from *L. myosorus* by the struts carrying the dome-shaped part, the large circular perforation in the dome part, the longer spine, the hook at the end of the spine and the shape of the perforations. It may be distinguished from *L. bulbochaete* by all but the last of these features. Vørs (1992b) reported a species which she identified as *L. myosorus*, but which clearly conforms to the description of the new species.

Luffisphaera sp.

Figs: 28c-d, 29a-d

Size: (fixed cells on electron microscope whole-mounts) 1.5-2 x 6.5-7µm, 'pseudopodia' up to 13µm

Observations: Found in electron microscope whole-mounts of the MP4 sample. Cells are spindle-shaped with a long thread of cytoplasm projecting from either pole. The whole of the cell, except for the threads is covered in scales. The scaly part of the cell may deform (fig. 29b). The structure of the basal scales is not really apparent from the available micrographs, but they appear to be oval, with numerous hexagonal perforations (fig. 29c). Spine scales are relatively short; the basal part supports a cone-shaped lattice, which terminates in a short spine or a thinner, more elongate and frequently curved thread (figs 28c,d). The scales with the thread-like tips particularly occur at the two poles. Spine scales are about 350-470nm in length (with short spines) or 590-760nm (those with thread-like tips).

Remarks: Although the cells of all other species of *Luffisphaera* are spherical or oval, and have never been seen with fine extensions of the cytoplasm as in this species, the base scales are very similar to those found in *Luffisphaera* species and the morphology of the spine scales shows some resemblance to *L. bulbochaete* Vørs 1993b, although they are shorter than those of any named species of *Luffisphaera.* It seems judicious to await further information on this taxon particularly in connection with the polar cytoplasmic extensions, before a formal description.

A single cell very similar to this taxon has been seen in a sample from Belize (N. Vørs, personal communication).

Metopion fluens Larsen & Patterson, 1990

Figs: 26k, 30b

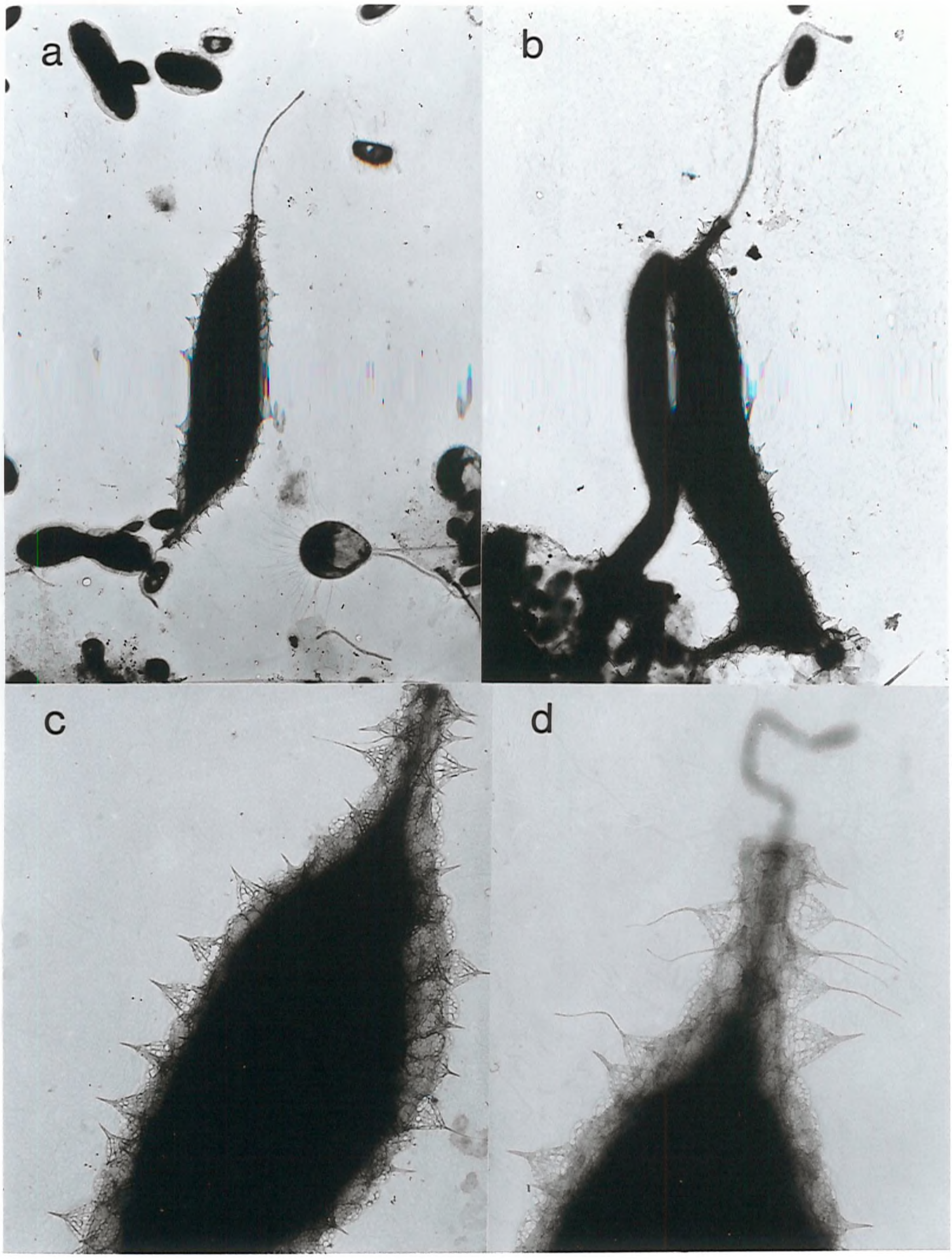
Size: 3-5 x 4.5-8μm; flagellum 8.5-11.5μm

Observations: This species appeared in enrichment cultures on several occasions, generally in fairly high numbers. Cells are oval, rather flattened dorso-ventrally and have a small protrusion or snout near the cell anterior. A single flagellum emerges from a ventral groove and is directed posteriorly. The ventral part of the cell adjacent to the

Figure 29: Electron microscope whole-mounts of *Luffisphaera* sp.

a,b: Whole cells, notice the polar 'pseudopods', and deformation of the cell shape in fig. b, x5000 (a) and x7500 (b).

c,d: Detail of the scales, notice that there are two types of spine scales, x20,000 (c) and x30,000 (d).


a
b
c
d

flagellum is often thinner than the rest of the cell (fig. 30b). Cells glide with a nodding motion. *Metopion* appears to ingest food through the tip of the snout and a chain of refractile particles is frequently seen in the most anterior part of the cell, along the snout. This species is able to prey on flagellates as well as bacteria, and on one occasion was observed feeding on small *Bicosoeca* cells. Living cells are often refractile, and hence difficult to photograph.

Remarks: *Metopion* may be confused with *Ancyromonas sigmoides* (see description of this species).

Metromonas simplex (Griessmann) Larsen & Patterson, 1990

Figs: 26l, 30c

Size: cell 4-9.5μm, long flagellum 11-16μm, short flagellum 0.5-3μm (usually c1μm)

Observations: Seen sporadically in cultures, and once in freshly collected centrifuged material. Cells are rounded or leaf-shaped and are flattened or sometimes slightly concave. Two flagella insert at the thinnest point of the cell. A second flagellum was always present, although it was sometimes extremely short (see above). Fine, oblique striations were sometimes visible on the cell surface.

The behaviour of *Metromonas* is distinctive. Cells may glide, with the long flagellum in contact with the substratum, and the anterior of the cell raised slightly above the surface and waving from side to side, or may attach by the distal end of the long flagellum (fig. 26l) and wave from side to side like a pendulum. This organism is probably predatory. It was isolated into culture with *Amastigomonas debruynei* and various bacteria, and the two protistan species underwent coupled oscillations in abundance, with *Metromonas* lagging about eight days behind *Amastigomonas*.

Remarks: The cells seen in this study were very similar to those described by Griessmann (1913; as *Phyllomitus simplex*), except that he only occasionally found a second short flagellum, and interpreted this as a pre-division stage. Cells in this study which were preparing to divide retained the short flagellum whilst a second long flagellum appeared. Cells seen by Larsen and Patterson (1990) and Vørs (1992b) usually only had one flagellum, and had a thinner pear-shaped profile.

Larsen and Patterson (1990) differentiate *Metromonas grandis* from *M. simplex* by its greater size (9-14μm), the fact that it always has a second short flagellum, and a different cell shape. Cells in this study overlap the size categories given for both *Metromonas* species, and always had a second flagellum, but were shaped identically to those described by Griessmann suggesting that there is variation within M. simplex, and that the only remaining distinction for *M. grandis* is the fact that the flagella insert on the broadest side of the cell.

Ministeria 'vibrans' (Tong, in prep.)

Figs: 30d-j, 31f

Size: cell 1.2-4μm (usually 2.5μm), arms 3.5-8μm, stalk 2-16.5μm

Observations: Appeared sporadically in enrichment cultures, more commonly in autumn and winter. Cells are spherical with between 16 and 30 (20 seems usual) fine radiating arms spaced at regular intervals. The arms vary in length, but are all of the same length on an individual cell. Cells were frequently seen attached to surfaces (diatoms, filamentous cyanobacteria or detrital flocs) by a stalk. The stalk was thicker than the arms at the proximal end, but was frequently extended by a fine cytoplasmic thread which seemed identical to the arms except that it could greatly exceed them in length (fig. 30d). When attached, cells were sometimes seen to vibrate; the purpose and mechanism of this activity is unknown. Cells in monoculture only rarely have stalks. When cells are in contact with a surface (e.g. on a microscope slide) they can shift position slightly, with the aid of the arms, which bend slightly during the process.

Ministeria is bacterivorous and seems to preferentially consume motile rods. Bacteria

Figure 30: Diagrams of various *incertae sedis* protists

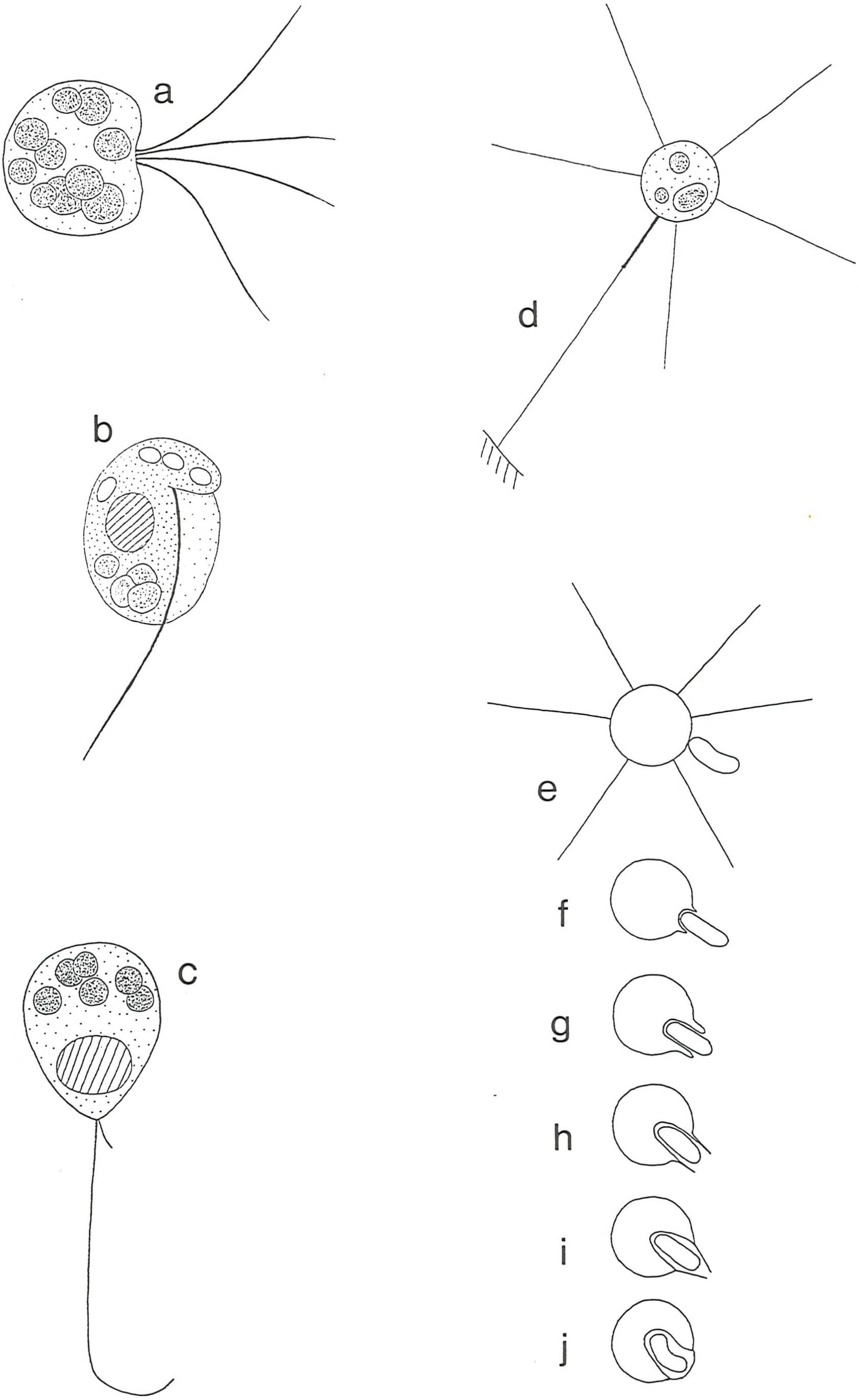
a: *Quadricilia rotundata.*

b: *Metopion fluens*, notice the thinner part of the cell delineated by the flagellum.

c: *Metromonas simplex*, notice the second, short flagellum.

d-j: *Ministeria* 'vibrans'. d: Cell attached to the substratum by a stalk. e-j: the mechanism of food ingestion; a bacterium becomes attached to the cell surface (e), the cell surface indents and two pseudopodia grow out around the bacterium (f,g), the pseudopodia become longer and narrower (h), and then bend to close behind the bacterium (i), they then join together and retract, pushing the bacterium into the cell (j).

All diagram x5000.


a
d
b
e
f
c
g
h
i
j

stick to the cell surface, the cell indents, and pseudopodia grow out on either side of the prey. The pseudopodia then bend, and meet together behind the prey, and as they are withdrawn, the bacterium is pushed into the cell (figs 30e-j). Preliminary ultrastructural studies have revealed that the cell contains a nucleus and a mitochondrion with flattened cristae, thus confirming the observations of Patterson *et al.* (1993) on *M. marisola.* As yet the structure of the arms, and the stalk have not been determined.

Remarks: This species differs from the type of the genus, *Ministeria marisola* Patterson *et al.* (1993) in the possession of a stalk, and the associated vibrating movement, and in having a greater number of arms.

Quadricilia rotundata (Skuja) Vørs, 1992b

Fig. 30a

Size: 9.5-13µm

Observations: Frequently seen in freshly collected centrifuged material (where cells were moribund and few observations could be made) or in epifluorescence preparations; more commonly in spring and summer. Cells were rounded or slightly reniform, with four flagella of equal length which tapered slightly to form pointed tips distally. Cells became more irregular and often produced pseudopodia when in contact with a surface. Food contained within the cell was frequently pigmented, but feeding was never observed. Vørs (1992b) found that this species often coincided with diatom blooms, but no evidence for this was found in Southampton Water.

Rigidomastix 'devoratum' (Tong, in prep., from the Latin 'to swallow')

Figs: 31a-e, 31g, 32a-c

Size: cell 2.5-5 x 3-5.5µm, flagellum up to 20µm

Observations: Seen frequently in enrichment cultures, sometimes in large numbers. Cells are rounded to pyriform, with a rather flattened anterior, which has a small lip on one side (figs 31a, 32a). The lip marks the edge of an opening into the cell (fig. 31g), which is probably where food is ingested, although this has not been observed. A single flagellum is inserted apically in the middle of the anterior side. Its total length usually amounts to 3½-4 times the cell length. However, cells may be seen with very short flagella, or without flagella, because the flagellum can be taken inside the cell through the anterior opening described above. This particularly occurs when cells are stressed, for example in anoxic culture, or it may be induced by turning the light up to full power on a microscope. The flagellum stops beating for a few seconds and then is smoothly and rapidly withdrawn into the cell; the cytoplasm can be seen spiralling inside the cell as this occurs (fig. 32b). A short thread projects from the flagellum near the point of insertion (figs 31c, 32c); this is particularly visible when cells are gliding (see below) but unfortunately has not been seen in electron microscope whole-mounts. The nucleus lies slightly to the anterior of the cell, and is median or slightly lateral. Food vacuoles at the posterior of the cell frequently had a brownish tint, when viewed under phase-contrast microscopy and those towards the anterior of the cell frequently contained bacteria.

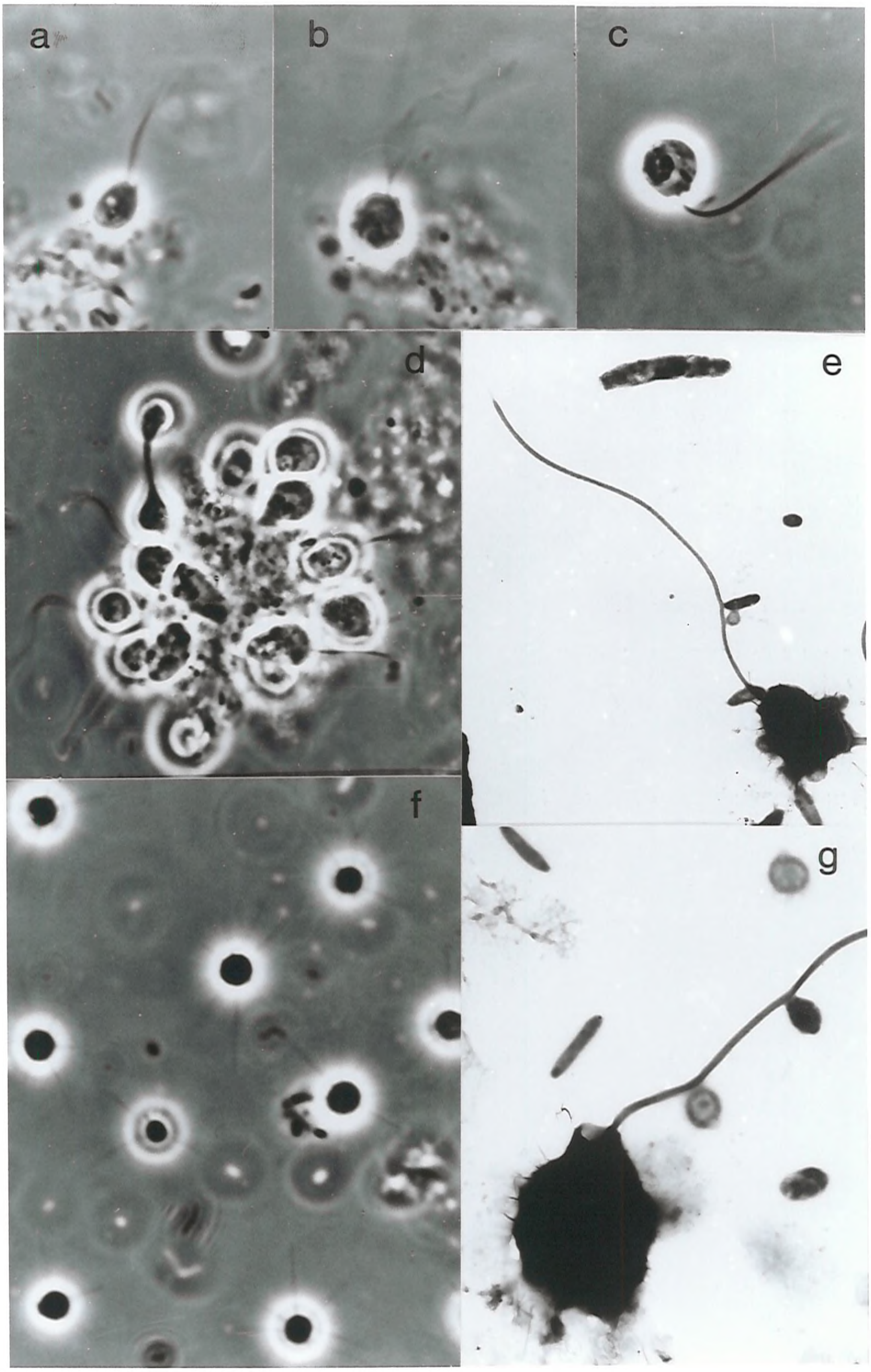
Cells are usually embedded in detrital flocs in small groups; if the flagella have been withdrawn they become very difficult to identify. They may also glide, smoothly, with the flagellum in contact with the substratum and trailing behind the cell, which is raised slightly above the surface by a curving of the proximal part of the flagellum (fig. 32c). Intervals of gliding are usually interspersed with periods of irregular 'nodding' of the cell, and beating of the flagellum. When swimming, the flagellum is held in front of the cell, and curved, and the cell undergoes conspicuous rotation about the longitudinal axis. Although the flagellum is in front of the cell, swimming motion is not reminiscent of stramenopiles, and electron microscopy reveals that the flagellum is smooth. After this fairly rapid swimming, cells may circle slowly near a surface, with the flagellum behind the cell, before settling on the substratum.

Remarks: Although this organism has not been examined ultrastructurally, the

Figure 31: ***Rigidomastix*** **'devoratum' and** ***Ministeria*** **'vibrans'**

a-e,g: *Rigidomastix* 'devoratum'. a-c: LM showing lip at one side of the cell anterior (a), flagellar beat (b) and the 'thread' attached to the proximal part of the flagellum (c). Figs a and b show cells embedded in detritus and fig. c shows a gliding cell, all x2000. d: LM of group of cells embedded in detritus, notice that some of the cells have shorter (partially retracted) flagella, x1500. e,g: EM of cells showing the length of the flagellum (e) and the anterior opening into the cell through which the flagellum can be retracted (g), x5000 (e) and x10,000 (g).

f: *Ministeria* 'vibrans', LM of a group of cells, notice the fine radiating filaments on the cells, x2000.


a
b
c
d
e
f
g

peculiarities of its appearance under the light microscope (in particular the lip at the cell anterior and the thread on the side of the single flagellum) and of many aspects of its behaviour (swallowing of the flagellum, gliding, and swimming with the flagellum in front of the cell) mean that it is easily distinguished from any flagellate described so far.

This species shows some similarity to *Rhizomonas setigera* as interpreted by Patterson *et al.* (1993): the association with detritus, a single flagellum, and the general appearance of cells shown in fig. 10 in this paper. However, this organism does not have a lip at the cell anterior and has never been observed to glide or swim (D.J. Patterson, personal communication), and the species in this study does not produce pseudopodia.

There is also similarity to the coprozoite *Rigidomastix coprocola* (Aléxéieff 1929) in terms of size, shape and the general appearance presented in fig. XI in this paper (particularly the cell in XI.4 which appears to have a 'lip' near the flagellum). Aléxéieff did not see the flagellum moving (hence the genus name); in *Rigidomastix* 'devoratum' cells are frequently seen with stationary flagella, particularly when they are stressed (before the flagellum is taken into the cell). Aléxéieff also mentions that he found colonies of aflagellate cells embedded in mucus, which otherwise seemed to resemble *R. coprocola*. This habit is also found in the present species, when flagella have been taken inside the cell. Therefore it seems likely that these two species are closely related. A new species has been created since Aléxéieff did not report gliding, swimming, a hair on the flagellum, or the swallowing of the flagellum.

The *Rigidomastix* species added by Bovee and Telford (1962) is clearly an amoeboflagellate, and bears little similarity to the description of the type of the genus, which does not show any amoeboid tendencies (despite the fact that Bovee and Telford call it an amoeboflagellate), and should thus be placed in another genus.

Telonema 'antarctica' (Thomsen, in prep)

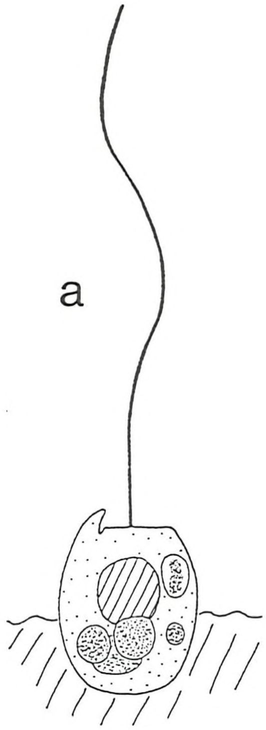
Figs: 21h, 32d

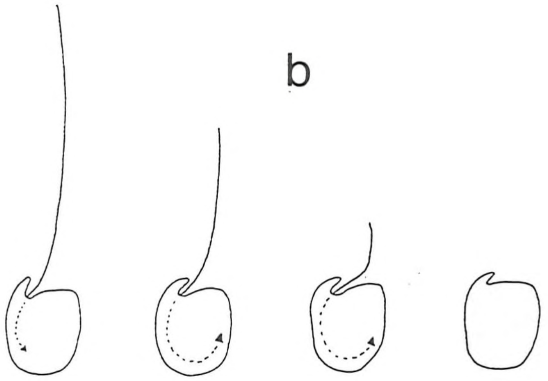
Figure 32: Diagrams of *Rigidomastix* 'devoratum' and *Telonema* species

a-c: *Rigidomastix* 'devoratum'. a: Cell embedded in detritus, notice the lip at one side of the anterior of the cell, x5000. b: Cell undergoing retraction of the flagellum through an opening at the cell anterior, notice how the cytoplasm spirals inside the cell as this occurs, x2500. c: Gliding cell, notice the thread attached to the proximal region of the anterior flagellum, x5000.

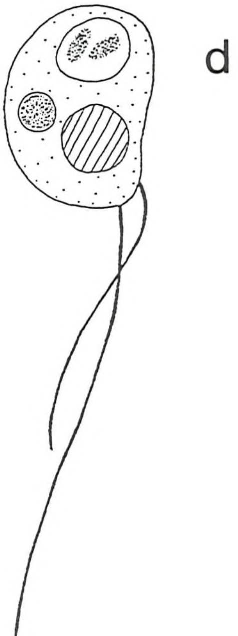
d: *Telonema* 'antarctica', x5000.

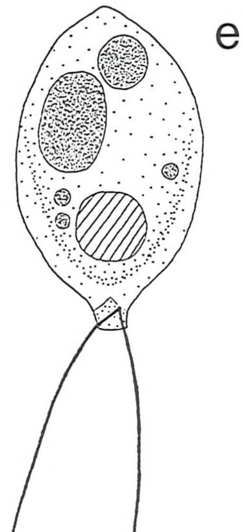
e: *Telonema subtile*, x5000.


a


b


c


d


e

Size: 3.5-8μm, short flagellum 4.5-8μm, long flagellum 12.5-17.5μm

Observations: Frequently seen in freshly collected material (including in epifluorescence preparations), but not usually as abundant as *T. subtile*. Cells are droplet shaped, reniform, or almost spherical. Two flagella insert at the base of a small projection, although this is rarely as pronounced as the 'snout' of T. subtile. One flagellum is usually considerably shorter than the other. Swimming is almost identical to that of *T. subtile*.

Remarks: This is the 'droplet-shaped flagellate' reported by Buma *et al.* (1989). It was also seen by Vørs (1992a) in the Gulf of Finland, although the cells in her study were larger (10-20μm). Ultrastructural studies have revealed that this species is closely related to *Telonema subtile* (H. Thomsen, personal communication). *Telonema* 'antarctica' can feed on bacteria, algal cells or other flagellates.

Telonema subtile Griessmann, 1913

Figs: 21i-j, 32e

Size: cell 4-12 x 6-20μm (mostly about 5 x 8μm)

Observations: This species was one of the flagellates most commonly observed in freshly collected centrifuged material, and was also conspicuous in epifluorescence preparations. It also appeared quite frequently in enrichment cultures. Cells are oval, droplet-shaped or leaf-shaped, and slightly flattened, or slightly convex. Two flagella of roughly equal length emerge from either side of a small projection or 'snout' (although both are inserted on one side; see fig. 32e) and are tapered distally (figs 21i,j). The flagella are generally about body length in cells in enrichment culture, but usually 2-3½ times the body length in fresh material. Cells spiral conspicuously as they swim, with both flagella behind the cell, twisting round each other. This species appears to be predatory, although ingestion was not observed. In culture, *T. subtile* was usually first observed about two weeks after the fresh sample was inoculated. After this the abundance of other flagellates in the culture decreased, and the diversity of species was considerably reduced; usually only acanthoecid choanoflagellates remained in any abundance, implying that their loricas afford some protection against predation.

3.13 PRASINOPHYTES

Prasinophytes were frequently seen in freshly collected material, and in epifluorescence studies, but rarely noted on electron microscope whole-mounts. More than one species was seen in freshly collected material, and that described below was probably present in samples in addition to the one in which it was identified.

Pyramimonas cirolanae Pennick, 1982

Fig. 5g

Observations: Found in the NT9 sample. This species possesses three types of body scales and three types of flagellar appendages. It was identified from the 'middle layer body scales' (as described by Pennick, 1982); the smaller 'inner layer body scales' are also shown (fig. 5g).

3.14 STRAMENOPILES

An informal grouping created by Patterson (1989a) for all organisms bearing tubular tripartite hairs on the cell surface. The majority of organisms in this assemblage carry such hairs on the anterior flagellum and therefore belong to the heterokonts *sensu* Moestrup and Andersen (1991), but the term stramenopiles is used in preference here as it includes the opalinids which show certain similarities to *Developayella elegans* (see below).

BICOSOECIDS/BICOSOECALES Grassé, 1926

Recent ultrastructural studies (Fenchel 1982a, Fenchel and Patterson 1988) have shown that two genera of naked flagellates, *Cafeteria* and *Pseudobodo*, are related to the loricate genus *Bicosoeca*.

Members of the genus *Bicosoeca* are distinguished by the morphology of the lorica, but descriptions of many species contain inadequate information, and little is known about the extent of intraspecific variation. This genus is in urgent need of taxonomic revision. Species have been frequently distinguished by differences in the length of the lorica pedicel (which has often been observed to vary within species e.g *B. lacustris* in James-Clark 1867 and Skuja 1948), and by small differences in the shape of the lorica. Some species are distinguished by whether the anterior margin of the lorica curves in or out, or is straight. This is unlikely to be a stable character; in fixed specimens the lorica margin is usually straight, even when curved in living specimens, suggesting that this part of the lorica is flexible. Picken (1941) and Skuja (1948) note that the anterior margin of the lorica of *B. lacustris* closes inwards when the cell retracts to the posterior of the lorica. As yet the diversity of *Bicosoeca* species reported from marine habitats is relatively small.

Bicosoeca epiphytica Hilliard 1971/*Bicosoeca pulchra* Hilliard 1971

Figs: 33c, 33f, 34b

Size: cell 2.5-4.5 x 3.5-6.5µm, flagellum 8-13.5µm, lorica 3.5-5 x 8-10µm

Observations: Found in an enrichment culture of the MP4 sample. The lorica of this species was crenated (fig. 33c) and so differed from all the other *Bicosoeca* species seen. Unfortunately cells were not found on electron microscopical whole-mounts and so it was not possible to confirm whether the crenations corresponded to horizontal striations of the lorica. The lorica had approximately 8-10 crenations and was fairly rounded at the posterior. It tapered slightly at the anterior, before a flared aperture. The lorica had a short pedicel which was ⅓-1 times the lorica length, and was terminated with a distal 'button' as is characteristic in this genus. The cell and lorica sizes of this species were smaller than those reported in either *B. epiphytica* or *B. pulchra*, but this is not considered significant.

Remarks: Although Hilliard (1971) states that *B. pulchra* can be separated from *B. epiphytica* by its smaller size, shorter stipe, and ecological characteristics, it is unlikely that this position is defensible. Stipe length can vary within species according to substrate (e.g. Skuja 1948), and lorica size is also variable. In other flagellates, such as choanoflagellates, it has been suggested that lorica size may actually be controlled by environmental conditions, so the difference in habitat of the two *Bicosoeca* species could possibly have actually caused the differences in their loricas, rather than being a distinguishing feature. There is also some similarity to *B. parva* Hilliard 1971, which differs in having smaller dimensions, a more narrow lorica and in the lorica striations being less apparent. These three species could probably be regarded as synonymous.

Bicosoeca gracilipes James-Clark, 1867

Figs: 33a-b, 34a

Size: cell 2-5 x 2.5-8.5μm, flagellum 25-36μm, length of lorica pedicel 10-21.5μm

Observations: Seen in enrichment cultures of three samples. The characteristic features of this species were the smooth lorica (striations were just visible on the better fixed specimens with electron microscopy, but never with light microscopy), the fact that the lorica aperture did not close inwards when the cell retracted, and the exceptional length of the flagellum. The size of the cell varied considerably. On some occasions it filled the lorica (fig. 33b), and on others it was only half the size (fig. 33a; cell on left hand side). Chains of small food vacuoles were often seen down one side of the cell beneath the cytostome (fig. 34a).

Remarks: The present species seems to resemble *B. gracilipes* more closely than any other described species of *Bicosoeca*, except that it has a rather long anterior flagellum, and the lorica is relatively wider. There is also some similarity with *B. exilis* (Penard 1921), which does have a long anterior flagellum, but the lorica aperture of the present species curves outward slightly when the cell is retracted, rather than curving inwards as described by Penard, although the lorica is closely appressed to the cell when it is in its extended state. As indicated above, it is not clear at present how much lorica shape varies in individual species, and therefore how much weight can be placed on this as a taxonomic criterion. Similarly it is not known how much variation there is in the length of the flagellum in *Bicosoeca* species.

Bicosoeca lacustris James-Clark, 1867

Fig. 34c

Size: cell 2.5-4 x 4-7µm, flagellum 13.5-27µm

Observations: Found in an enrichment culture of the NT10 sample. This species could be distinguished from the others seen in this study by the relative thickness of the lorica (thus being clearly visible with light microscopy), its elongate shape, and the fact that the anterior margin of the lorica always curved inwards, and shut when the cell retracted. The flagellum tended to be shorter than in *B. gracilis* (above), and the lorica pedicel was consistently shorter, rarely exceeding half the length of the lorica. The anterior flagellum tended to be held out at an oblique angle, and the posterior flagellum either narrowed at the distal tip, or was connected to the lorica by a fine filament (fig. 34c); interpretation of this phenomenon was not possible as the organism was not examined with electron microscopy.

Remarks: This species is practically identical to *B. kepneri* Reynolds, 1926. The two species differ in the substructure of the lorica, and the thinner lorica of *B. kepneri* means that it is difficult to see in living cells (Mignot 1974). Although confident identification of the two species therefore requires sectioning of the material for electron microscopy the lorica was easily resolved with light microscopy (was relatively thick) and the material seen was in good accord with the descriptions of James-Clark (1867), Skuja (1948) and Mignot (1974).

Figure 33: Bicosoecids

a,b: *Bicosoeca gracilipes*, LM showing cells attached to detritus by the lorica pedicel. The cell in fig. b, and the right-hand cell in fig. a are fully retracted into the lorica, whilst the left-hand cell in fig.a is partially extended, notice how the anterior flagellum coils when cells are retracted.

c,f: *Bicosoeca epiphytica*, LM of retracted cell (c) and extended cell (f), notice the crenated surface of the lorica.

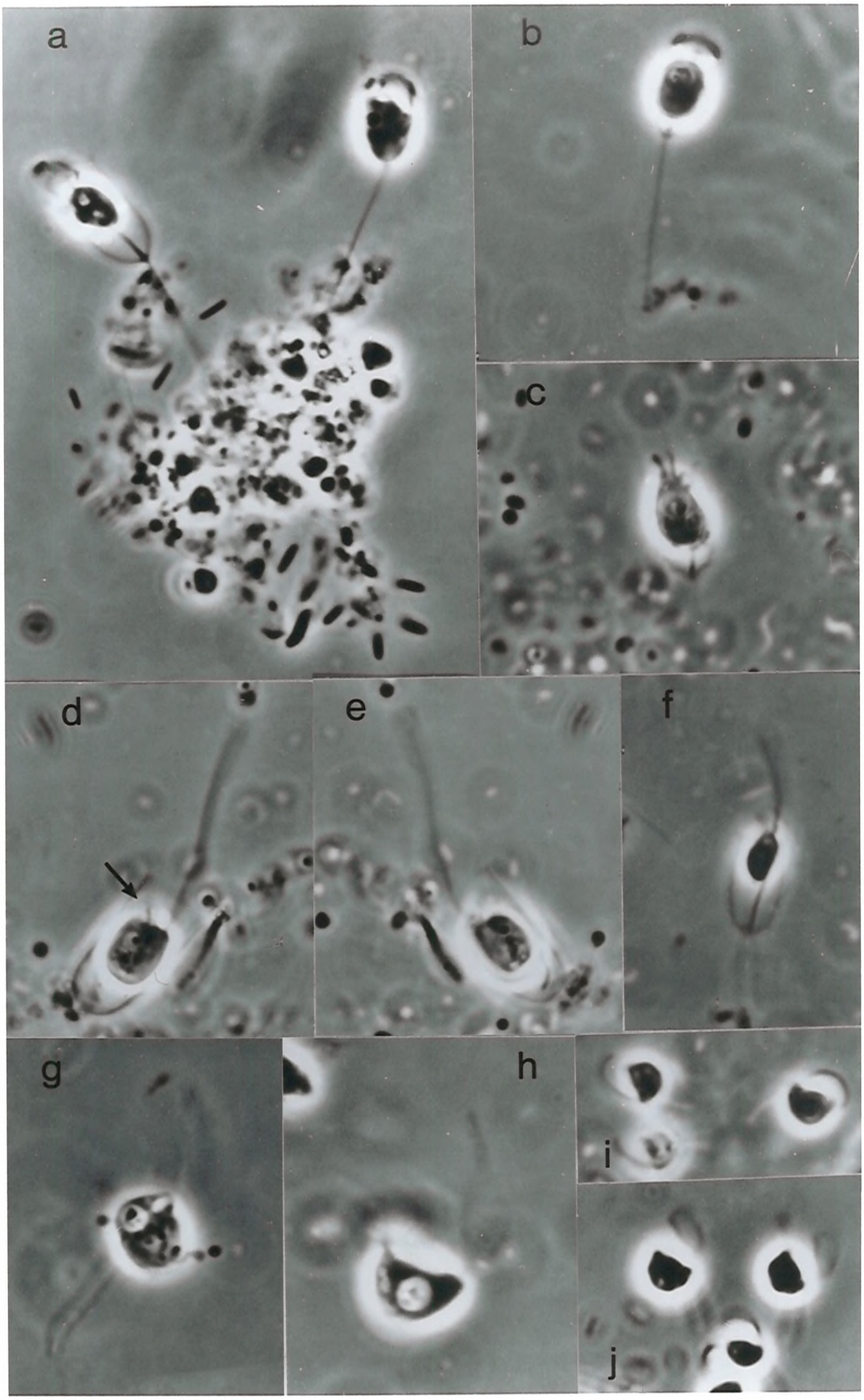
d,e: *Bicosoeca maris*, LM of cells showing a bacterium being ingested (d, arrow) and the attachment of the cell to the lorica by the posterior flagellum (e).

g: *Pseudobodo tremulans*, LM of cell showing the prominent collar around the cytostome.

h: *Cafeteria marsupialis*, showing the prominent groove or 'pouch'.

i,j: *Cafeteria roenbergensis*, showing cells attached to the substratum by the posterior flagellum.

All figures x2000.


a
b
c
d
e
f
g
h
i
j

Bicosoeca maris Picken, 1941

Figs: 33d-e, 34d

Size: cell 3-5 x 3.5-6.5µm, anterior flagellum 6.5-13µm, lorica 4.5-6 x 8-14.5µm

Observations: Appeared in enrichment cultures of many of the samples taken between October and February, but was not seen at other times. The posterior flagellum lies along the cell in a shallow groove, and attaches to the lorica (fig. 33e) a short way behind it. Unlike the other species of *Bicosoeca* seen, the anterior flagellum does not exhibit any 'lassoing' behaviour; it was occasionally seen loosely coiled within the lorica (as noted by Moestrup and Thomsen (1976), but the posterior flagellum remained extended (it appears to 'contract' in other species when the anterior flagellum coils, drawing the protoplast back to the posterior of the lorica).

Remarks: *Bicosoeca pocillum* Kent, 1880 is very similar to *B. maris* but has a short pedicel on the lorica and is shown with two anterior flagella, as in all the species of *Bicosoeca* which he describes. It is possible that this second anterior flagellum was a misinterpretation of the cytostomal area; the structure in fig. 33d (arrow) is a bacterium being taken into the cytostomal area, but looks rather like a short flagellum. Griessmann (1913) also mentions that a short stalk is sometimes present on the lorica of *B. pocillum*, but shows cells with only one flagellum. Moestrup and Thomsen (1976) consider *B. pocillum* sensu Griessmann to be synonymous with *B. maris* but give no clear reason for giving the latter species priority.

Cafeteria marsupialis Larsen & Patterson, 1990

Figs: 33h, 34g

Size: 4.5-7.5 x 7.5-10.5µm

Observations: Found in an enrichment culture of the CN1 sample. This sample was stored at 4°C for two months before being inoculated with rice grains. *C. marsupialis* is distinguished by the prominent groove or 'pouch', in which the posterior flagellum lies. It is also much larger than other *Cafeteria* species, although *C. roenbergensis* may occasionally attain similar dimensions (Larsen and Patterson 1990, Vørs 1993b). Cells in this study were in good accord with the type description, except that the posterior flagellum did not attach to the substrate; attachment was achieved by fine filaments on the left side of the cell (in a similar manner to *Developayella elegans*). Food particles entered the cell at the opposite side of the pouch to the flagella, and were very rapidly conducted to the dorsal side of the cell, suggesting the presence of a cytostome along the posterior margin of the cell. *C. marsupialis* fed on large rod-shaped bacteria and small detrital particles in suspension, and ingested material more rapidly than any other flagellate seen in this study (at rates exceeding 4 particles a minute). As a consequence, much of the cell was occupied with food vacuoles, which tended to fuse together to create large vacuoles (up to 3µm) with several bacteria in them.

This is the first report of this species since the type description of material from various tropical locations (Larsen and Patterson 1990).

Cafeteria roenbergensis Fenchel & Patterson, 1988

Figs: 33i-j, 34f

Size: 1.5-6.5µm (usually 3-3.5µm)

Observations: Occurred in enrichment cultures of nearly every sample, and generally accounted for about 6-8% of the heterotrophic flagellate population in freshly collected samples. Cells are D-shaped, with two equal flagella. One (the posterior) attaches to the substratum, and the other curves over the cell and creates a current directing food particles to the posterior ventral margin.

Figure 34: Diagrams of bicosoecids

a: *Bicosoeca gracilipes*, x2500.

b: *Bicosoeca epiphytica*, x5000.

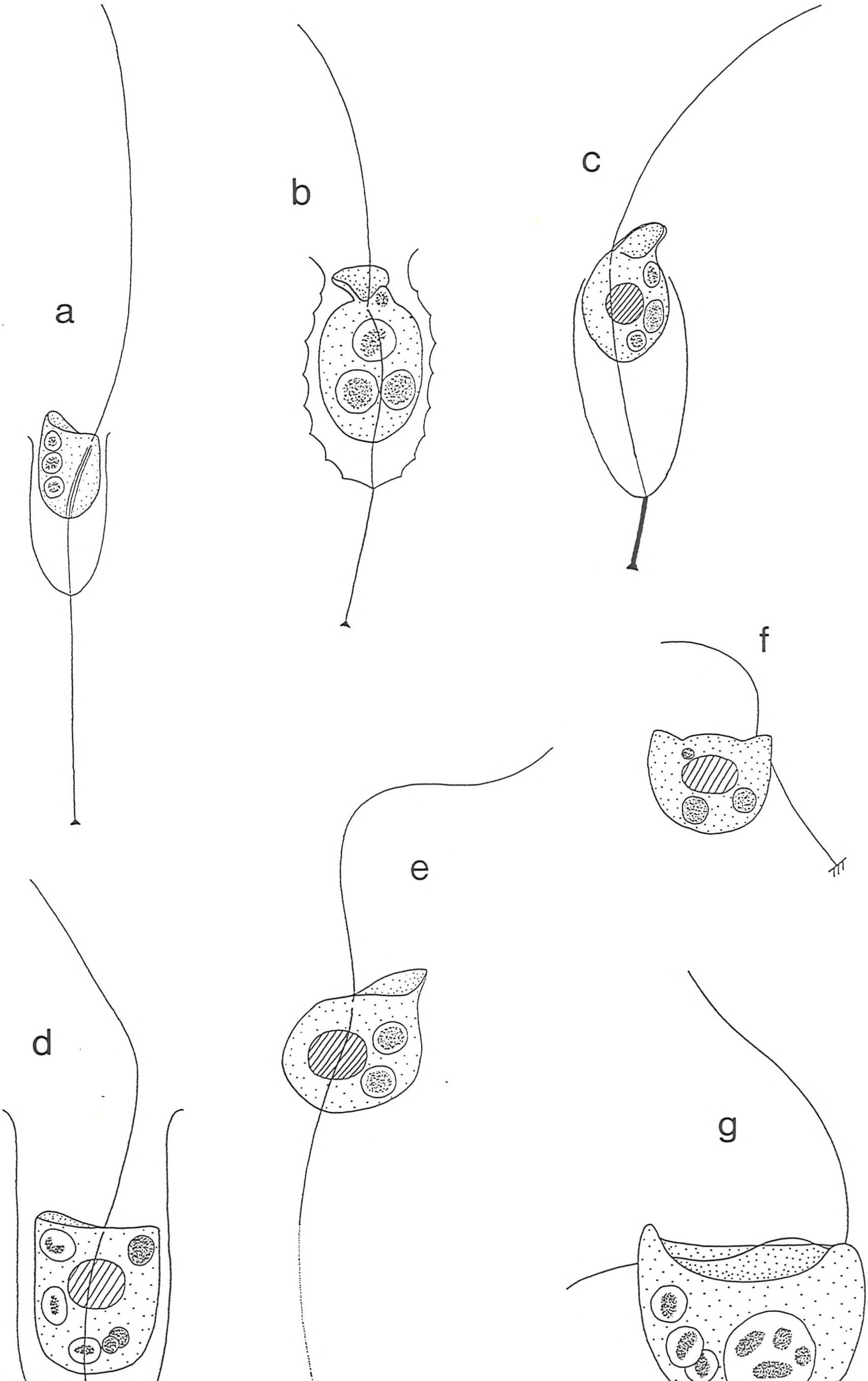
c: *Bicosoeca lacustris*, notice how the posterior flagellum narrows at the distal end, x5000.

d: *Bicosoeca maris*, x5000.

e: *Pseudobodo tremulans*, notice how the cell is attached to the substratum by a fine mucoid extension of the posterior flagellum, x5000.

f: *Cafeteria roenbergensis*, x5000.

g: *Cafeteria marsupialis*, x5000.


a
b
c
d
e
f
g

Pseudobodo tremulans Griessmann, 1913

Figs: 33g, 34e

Size: 2-6.5µm

Observations: Appeared frequently in enrichment cultures. This species can be distinguished from other naked bicosoecids (members of the genus *Cafeteria*) by the presence of a prominent collar around the cytostome (fig. 33g). The periodic jerking movement of the cell back towards the substratum (as described by Griessmann, 1913) is also characteristic. Cells attach to the substratum by the posterior flagellum, or sometimes by a fine mucus thread attached to the flagellum (fig. 34e). Occasionally the flagella of two cells are joined to a single filament. When swimming, the anterior flagellum is held out very rigidly in a straight line to the front (differing from many other stramenopiles where the anterior flagellum is curved, but similar to *Ciliophrys infusionum*).

Remarks: Cells in one culture were distinctly smaller than previously reported for this species (2-2.5µm). Ruinen (1938) described two new very small species of *Pseudobodo*. The diagrams of one (*Pseudobodo minima*) resemble the appearance of *Bodo saltans*, but very few details are given. The other, *Pseudobodo minuta* (from the diagrams) is far more like *P. tremulans*, but again few details are given. Larsen and Patterson (1990) interpret this organism as a *Cafeteria* species (*C. minuta*) since they found a similar (but larger) organism which lacked a prominent collar around the cytostome. The small *Pseudobodo* cells in the present study were identical in all other respects to larger specimens of *P. tremulans*.

CHRYSOPHYCEAE *sensu stricto* e.g. Hibberd, 1986 (CHRYSOPHYTES AND CHRYSOMONADS).

A variety of taxa have been placed within the chrysophytes at different times. Subsequently, some have been removed altogether, and others, although clearly related, show major differences in ultrastructure to the 'core chrysophytes'. In this study, the term refers to forms with the basic '*Ochromonas* type' of organisation (Hibberd 1976, 1986).

Ollicola vangoorii (Conrad) Vørs, 1992b

Fig. 35a

Size: (of cell) 4-5.5 x 5-6.5µm

Observations: Found frequently in samples, particularly during the summer and autumn. Cells were occasionally seen (living) in enrichment cultures, in contrast to the observations of Vørs (1992b). This species is bacterivorous, and also contains a chloroplast (Vørs 1992b) but is unlikely to be able to photosynthesise because of the lorica surrounding the cell. Many of the specimens seen had heavily mineralised loricas, so that the protoplast could not be seen inside, but swimming cells were seen to project from the top of the lorica, and bore one short and one long flagellum. The thickness, size and shape of the lorica may vary (Espeland and Throndsen 1986, Vørs 1992b).

Remarks: Since the type of the genus *Calycomonas* Lohmann (designated by Lund (1960)) has been transferred to the rhizopodian genus *Paulinella*, *Calycomonas* has been reduced into synonymy and renamed *Ollicola* (Vørs 1992b).

Paraphysomonas De Saedeleer, 1929

Paraphysomonas is one of the most commonly reported genera of heterotrophic flagellates in both marine and freshwater locations, although the diversity of species found in fresh water is usually greater. Species are distinguished by the morphology of scales, which lie over the whole of the body surface. The detailed structure of the scales cannot be resolved by light microscopy, and so identification requires electron microscopy, except perhaps for species bearing larger scales, such as *P. vestita*. Scales of different isolates vary in size, and other details, and these variations may be partly due to environmental conditions (Moestrup 1979, Vørs 1993b). Monospecific cultures were established for four of the species encountered in this study. Each species exhibited minor differences in form and/or behaviour, but these were not so distinct that they could be used to confidently identify species without electron microscopy.

Paraphysomonas antarctica Takahashi, 1987

Fig. 35c

Size: (of scales) diameter 1.1-1.3μm, length of thicker basal part of spine 1.5-3.1μm, length of thin spine tip 0.3-0.5μm

Observations: Found several times in whole-mounts of enrichment cultures of samples collected in winter and early spring. The length of thicker basal part of the scale spine is slightly longer than that recorded for the type material (Takahashi 1987). Vørs (1993a,b) lists other variations in the spine.

Remarks: *P. antarctica* has scales very similar to those of *P. imperforata*. Vørs (1993b) found forms of *P. antarctica* and *P. imperforata* which invalidate two of the three criteria listed by Takahashi (1987) to distinguish the two types of spine, so that the only way in which they can unambiguously be separated at present is by the ratio of the spine tip to the total length of the spine (the tip is relatively shorter in *P. antarctica*). However, the material in this study also satisfied Takahashi's other two criteria (an abrupt change in spine thickness and the dimensions of the basal part of scale and the spine, which are twice as large as those in (most) *P. imperforata* cells).

Paraphysomonas butcheri Pennick & Clarke, 1972

Fig. 35b

Size: cell 3.5-7.5μm (usually c4.5μm), short flagellum 2-4μm, long flagellum 7.5-14μm

Figure 35: *Ollicola vangoorii* and *Paraphysomonas* species

a: *Ollicola vangoorii*, EM of lorica; the long flagellum is protruding from the anterior of the lorica, x10,000.

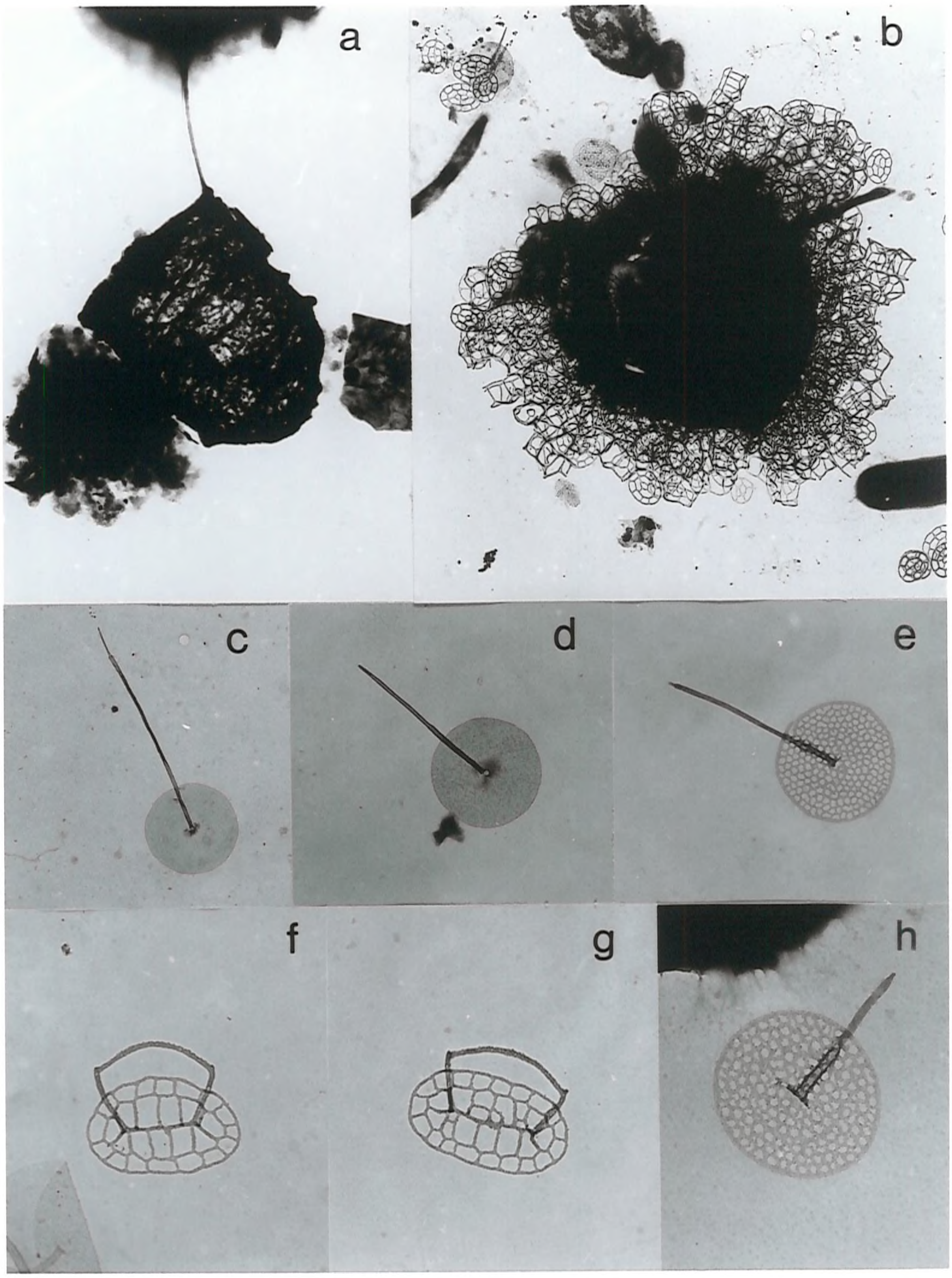
b: *Paraphysomonas butcheri*, EM of cell showing crown and plate scales, x10,000.

c: *Paraphysomonas antarctica*, EM of a single scale, notice the abrupt change of thickness at the distal end of the spine, x10,000.

d: *Paraphysomonas imperforata*, EM of single scale, notice the change in thickness half way along the spine, x25,000.

e,h: *Paraphysomonas foraminifera*, EM of single scales, x25,000 (e) and x 40,000 (h).

f,g: *Paraphysomonas siderophora*, EM of single scales, x25,000.


a
b
c
d
e
f
g
h

Observations: Seen quite regularly in whole-mounts of freshly collected samples and of enrichment cultures. Cells in crude culture were generally slightly larger than those (in exponential growth phase) in monospecific culture; average diameter: crude culture 5.1µm, monoculture 4.0µm (ten cells measured in each case). *P. butcheri* bears two types of scales, crown scales, and flattened plate scales (fig. 35b); see Pennick and Clarke (1972) and Preisig and Hibberd (1982b) for a detailed description. There may be considerable variation in the number and arrangement of perforations in the plate scales. One specimen in the process of division was seen on a whole-mount preparation. The daughter cell was slightly smaller than the parent, and lacked any scales, although the two cells were already partially separated.

Observations from monospecific culture: Cells are usually spherical, but may be slightly wider than long when sessile. Large food vacuoles (like those in *P. foraminifera* and *P. vestita*) were rare. Swimming is rarely observed, but when it occurs, is in a similar manner to *P. foraminifera*. Feeding is as described for *P. vestita*, type 1 (below).

Paraphysomonas foraminifera Lucas, 1967

Figs: 35e, 35h

Size: cell 3-6.5µm, short flagellum 3-4µm, long flagellum 9-16.5µm, scale base 0.6-0.7µm, scale spine 0.45-1.3µm

Observations: Occurred in two samples, and was found in large numbers in an enrichment culture from one of these samples, allowing a monospecific culture to be established by serial dilution. The scale bases were smaller than those described in the type material (Lucas 1967), and some of the scale spines were shorter than the previously reported minimum value of 0.65µm (Vørs, 1993a). In some cases the spines did not appear to taper, except for the point at the distal end. The scales are just visible with light microscopy under optimal conditions, although it is not possible to distinguish them from those of other *Paraphysomonas* species without electron microscopy.

Observations from monospecific culture: cells tend to have a perfectly spherical shape, although they may be slightly oval or triangular when swimming. They frequently attach to surfaces by a stalk from the posterior of the cell. When swimming, the axis of most of the flagellum is in the same direction as the cell movement (compare *P. imperforata*). Cells may contain large food vacuoles with relatively small prey items inside. Feeding as described for *P. vestita*, type 1 (below).

Paraphysomonas imperforata Lucas, 1967

Fig. 35d

Size: cell 2.5-5µm, short flagellum 2.5-4.5µm, long flagellum 11-15.5µm, scale base 0.55-0.65µm, scale spine 0.6-1.65µm

Observations: Occurred in nearly every sample. The bases of the scales can just be resolved with light microscopy. The scales are very similar to those of *P. vestita* but lack a rim around the outside of the basal disc and the thickness of the spine changes more abruptly (rather than tapering gradually), although it does not change as abruptly as in *P. antarctica*; compare figs 35c and 35d. Also the scales of *P. imperforata* were considerably smaller than any of the *P. vestita* scales found at this location. As with all *Paraphysomonas* species, the scales exhibit morphological variation and Thomsen (1975) and Preisig and Hibberd (1982a) list a far greater range of scale dimensions in *P. imperforata.* Preisig and Hibberd also remark that marine forms are generally smaller than those found in fresh water (as in this study). However Choi and Peters (1992) isolated two cold waters ecotypes of this species which were considerably larger than those in this study (c7 and c8µm). As in *P. butcheri*, cells seen on electron microscopical whole-mounts in the process of division lacked scales on the daughter cell.

Observations from monospecific culture: cells tend not to be spherical, especially when swimming, but oval or asymmetrical. When swimming, the proximal part of the flagellum is usually at an angle to the main axis of swimming (as well as being curved in the distal part), which means that swimming motion is slightly irregular compared to other *Paraphysomonas* species. Cells frequently attach to surfaces by a fine thread from the posterior. At the distal end the stalk splits into a number of thinner fibres (this can sometimes be resolved even with light microscopy). This is in contrast to the observations of Hibberd (1979) who found this sort of arrangement in *P. foraminifera*, but found the stalk in *P. imperforata* terminated in a circular foot.

Paraphysomonas siderophora Thomsen, 1975

Figs: 35f-g

Size: (of scales) 0.55-0.6 x 0.85-0.95μm

Observations: Found in whole-mounts of enrichment cultures of two samples. The scales were slightly smaller than in the type material from Denmark (Thomsen 1975). One scale (fig. 35g) was slightly aberrant in that there were extra, smaller perforations inside the outer ring of perforations, instead of two parallel rows as in fig. 35f.

Paraphysomonas vestita (Stokes) De Saedeleer, 1929

Figs: 36a-f, 38d

Size: cell 4.5-17μm, scale base 1.0-1.4μm, scale spine 2.5-6μm.

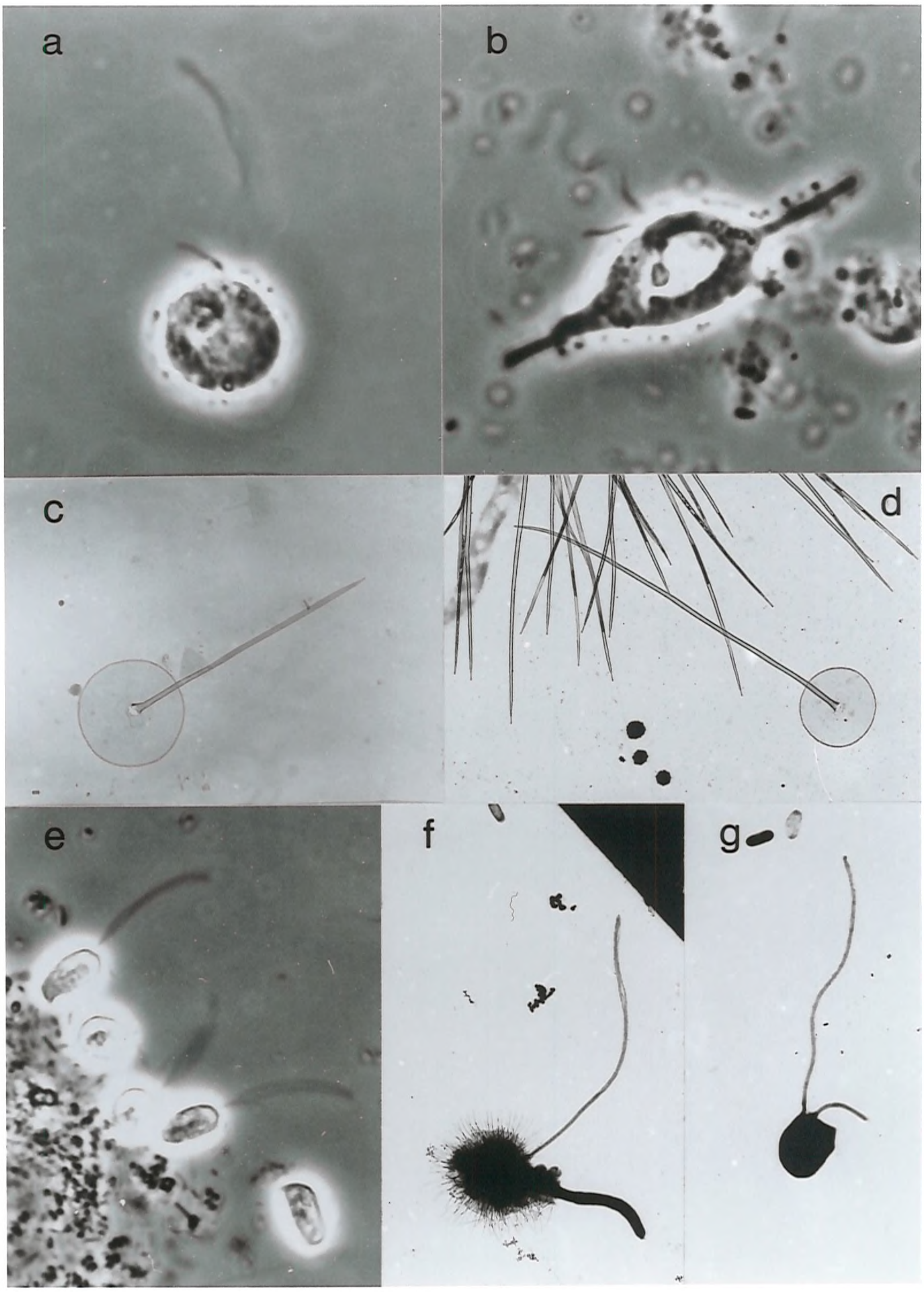
Observations: Found frequently in enrichment cultures. Cells are usually spherical, but may be elongate, and occasionally become extremely polymorphic when embedded in detritus, where attachment may occur through large branched extensions of the cell, rather than a slender posterior stalk as is usual. Two types of feeding were exhibited:

Type 1. The long flagellum creates a current drawing potential food particles towards the cell. These are 'held' briefly between the long and short flagellum, and then rejected by a sharp flicking movement of the flagella, or ingested. The short flagellum usually loops over the particle whilst it is being taken into the cell. Food is ingested near the

Figure 36: *Paraphysomonas vestita* and *Spumella* sp.

a-f: *Paraphysomonas vestita.* a: LM of cell, notice the dark granules around the cell periphery; they are the basal parts of the scales, x2000. b: LM of cell which has just ingested a diatom, x2000. c,d: EM of two single scales, notice the thin rim around the circular basal disc, and the gradual tapering of the spine, x15,000 (c) and x 10,000 (d). e: LM of group of cells attached to detritus, x1000. f: EM of cell in the process of ingesting a bacterium (N.B. osmium fixation has caused the *Paraphysomonas* cell to shrink, so that the bacterium seems disproportionately large), x3000.

g: *Spumella* sp., notice the lack of any scales, and the flattened anterior surface of the cell x5000.


a
b
c
d
e
f
g

flagellar bases.

Type 2. Food items are ingested through any part of the cell. It occurs when the cell is closely associated with detritus (so normal flagellar currents can not be created) and/or with the ingestion of large prey items.

P. vestita will ingest a wide variety of particles; bacteria are taken most frequently (fig. 36f), but the organism has also been observed to ingest diatoms (fig. 36b), photosynthetic flagellates of varying sizes, and occasionally, other heterotrophic flagellates. Under cultural conditions, the population can be seen to become adapted to a particular prey item. For example, cells may start feeding on bacteria, and have an average cell diameter of around 7μm, then switch to feeding on other flagellates, once large detrital flocs have developed, and have an average diameter of about 12μm (a five-fold increase in cell volume). Goldman and Dennett (1990) found that *Paraphysomonas imperforata* fed preferentially on the two smallest of three algal species offered in mixed culture, but switched to the largest prey once the abundance of the smaller cell had been substantially reduced, suggesting similar adaptability in feeding strategies in this species.

P.vestita is unusual amongst members of the genus in that its surface scales are usually visible with light microscopy (fig. 36a). Electron microscope whole-mounts reveal scales with a circular disc with a slightly raised rim, and a long spine (figs 36c,d). The relatively large diameter of the basal plate of the scale explains why they are often visible under the light microscope; the basal plates of *P. imperforata* scales found during this study were only about half the diameter of those of *P. vestita*.

Remarks: The use of the flagella for prey 'selection' has been noted in another (but mixotrophic) chrysophyte, *Epipyxis pulchra* (Wetherbee and Andersen 1992). The genus *Paraphysomonas* and the closely related genera *Spumella* (*Monas*) and *Ochromonas* have been known to be capable of omnivory for some time, with reports of ingestion of diatoms and photosynthetic flagellates (e.g.Ansell *et al.* 1963, Goldman *et al.* 1985, Caron *et al.* 1990), and even cannibalism (Fenchel 1982a). The observations above suggest that there are two separate feeding mechanisms for the ingestion of small and large prey items.

'Collared' Paraphysomonas spp.

Figs: 37a-e

Size: cell 2-6µm

Observations: *Paraphysomonas* cells were observed in several cultures with a crown of small 'scales' around the long flagellum. Electron microscopy revealed that the rest of the cell surface is covered by scales identical to *P. imperforata* (compare fig. 35d (*P. imperforata*) with fig. 37c), or, in one case, similar to those of *P. vestita* (fig. 37a). However the *P. vestita*-like scales differ slightly from the scales of *P. vestita* seen in this study in that they are smaller (base diameter only c0.5µm), and the tips of the spines are blunter, as in *P. vestita* ssp. *truncata* Preisig and Hibberd, 1982.

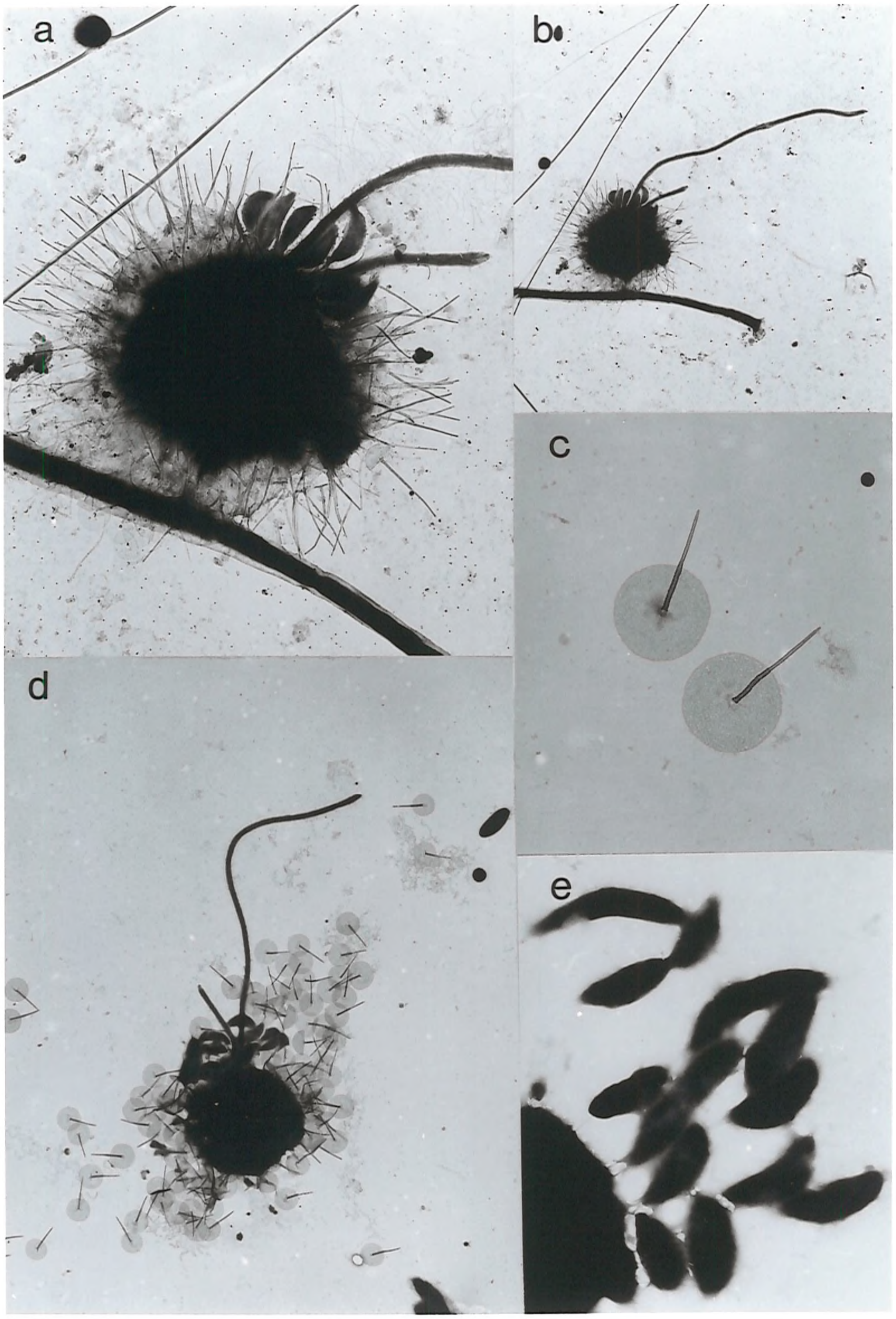
The 'crown' is composed of about 7 (in 'vestita' type) or up to 14 (in 'imperforata' type) wedge shaped structures. (figs 37a,b and 37d,e). If these structures around the flagellum are in fact bacteria, and not scales, division stages would be expected, so that they are passed on to new 'hosts'. This is also a possible interpretation of fig. 37e where some of the wedges are about twice as long as usual, and two are joined together (dividing?). Feeding of these flagellate cells is as in type 1 under *P. vestita.*

Remarks: It may well be significant that the 'collared' cells were found in enrichment cultures containing normal *P. imperforata* or *P. vestita* cells respectively. Hypothetically, bacteria present in the enrichment culture could then 'infect' normal cells, or else they could perhaps be released from inside the cell; intracellular bacteria have been reported in two other species of *Paraphysomonas* (Preisig and Hibberd 1983). If the structures around the long flagellum are bacteria they could benefit from nutrients released by the flagellate. The possible effects on the flagellate host are obscure. Cells with collars were usually smaller (by 1-1.5µm) than other *Paraphysomonas* cells in the same cultures. The percentage of cells with collars usually increased with time, so that at first just a few

Figure 37: 'Collared' *Paraphysomonas* species

a,b: EM of a *Paraphysomonas vestita*-like cell, notice the collar of wedge-shaped segments around the long flagellum, x10,000 (a) and x3000 (b).

c-e: EM of a *Paraphysomonas imperforata*-like cell, showing a complete cell (d), detail of scales (c) identical to those of *P. imperforata* (see fig. 35d), and detail of the wedge shapes (bacteria?) (e), x25,000 (c,e) and x5000 (d).


a
b
c
d
e

were noted in a larger population of normal cells, and after some days they were often the dominant form. This could occur through some competitive advantage of collared cells, through spreading 'infection' if the wedges were bacteria present in the culture, or through increasing environmental pressure of some sort to release the wedges if they were intracellular bacteria.

Spumella sp. Cienkowski, 1870 (syn. *Monas* Müller 1773)

Fig. 36g

Size: (of cells in culture only) 1.5-2.5 x 3-4.5μm, short flagellum 2-2.5μm, long flagellum 7.5-11μm

Observations: Small chrysomonad cells, similar to *Paraphysomonas* were observed in nearly every sample, (but were not actually recorded before NT23), particularly in freshly collected centrifuged material, and formed a large proportion of the 'chrysomonad' category in epifluorescence counts. Those appearing in enrichment cultures had a distinctive form, with a consistently elongated shape, flattened anterior end and a very short and stiff second flagellum, and probably all belonged to the same species. However this species could not be identified because the taxonomy of the genus is in a state of confusion at present (Preisig *et al.* 1991). When examined by electron microscopy there were no scales present on the cell surface (fig. 36g) and cells were in a good state of preservation so that it is unlikely that scales were lost. Cells often attached to surfaces by stalks from the posterior of the cell. Feeding was as 'type 1' described for *Paraphysomonas vestita*.

Remarks: See Preisig *et al.* (1991) for a discussion about the taxonomy of this genus. Although the name *Monas* has priority over *Spumella*, they argue that the identity of the former genus is unclear, and recommend that the latter name should be adopted.

PEDINELLIDS/PEDINELLALES Zimmermann *et al.*, 1984

Actinomonas mirabilis Kent, 1880/*Pteridomonas danica* Patterson & Fenchel, 1985

Figs: 38a-b and 39e (*A. mirabilis*), 38c, 39d and 39f (*P. danica*)

Size: cell 3-8.5µm

Observations: One or both of these species were observed in cultures of nearly every sample. Although separate descriptions are given of these species, they could not always be distinguished, and hence the information on their occurrence in samples is combined (appendix 1).

A. mirabilis: cells are rather cuboid in shape (fig. 39e), but slightly broader anteriorly. The flagellum is thickened, although this is usually only obvious in motionless (moribund) cells. There is a double ring of pseudopodia around the flagellum, and a single ring about half way down the cell. Pseudopodia in both regions bear small granules. When the stalk of *A. mirabilis* contracts, as happens periodically in both of the above species when attached to a surface, the pseudopodia (anterior and median) also contract, and can then be observed slowly unfurling (fig. 38b).

P. danica: cells are rounded, or slightly oval, but slightly broader anteriorly. The posterior is not as flattened as in *A. mirabilis*. There is a single ring of pseudopodia around the flagellum. Pseudopodia may be present further down the cell as in *A. mirabilis*, but lack granules, and are not so stiff (they are easily bent by water currents) suggesting that they lack the microtubular supports reported by Patterson and Fenchel (1985) in the anterior pseudopodia. On one occasion four cells were seen fused together (as also noted by Patterson and Fenchel 1985).

Although it is usually possible to distinguish between the two species with careful observation, swimming cells appear very similar (both retract the pseudopodia, and other details are obscured). Also cell shape may be distorted by the presence of food vacuoles,

Figure 38: Diagrams of *Actinomonas, Pteridomonas* and *Paraphysomonas vestita*

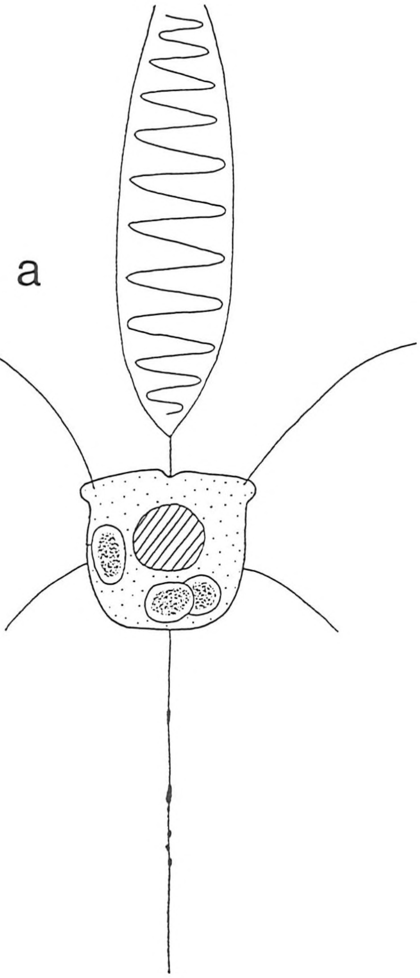
a,b: *Actinomonas mirabilis*, indicating the position of the pseudopodia (a), and showing the unfurling of coiled pseudopodia (b), notice the cuboid shape of the cell.

c: *Pteridomonas danica*, notice the pear-shaped form of the cell.

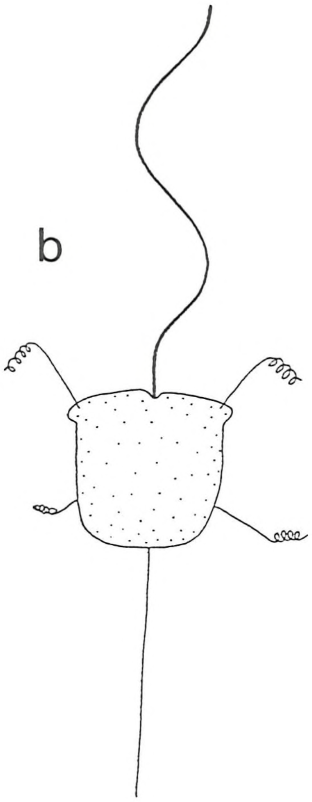
d: *Paraphysomonas vestita*, notice the circular basal discs of the scales which are visible on the cell surface.

All diagrams x5000.

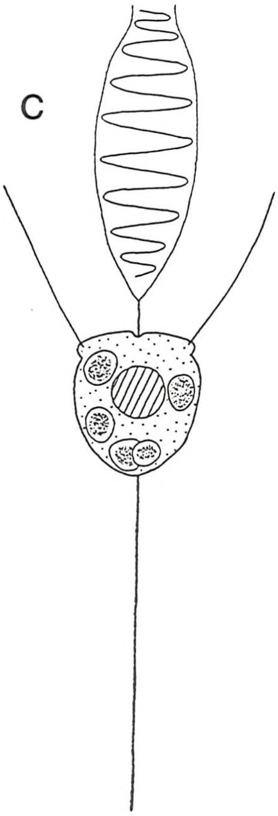
a



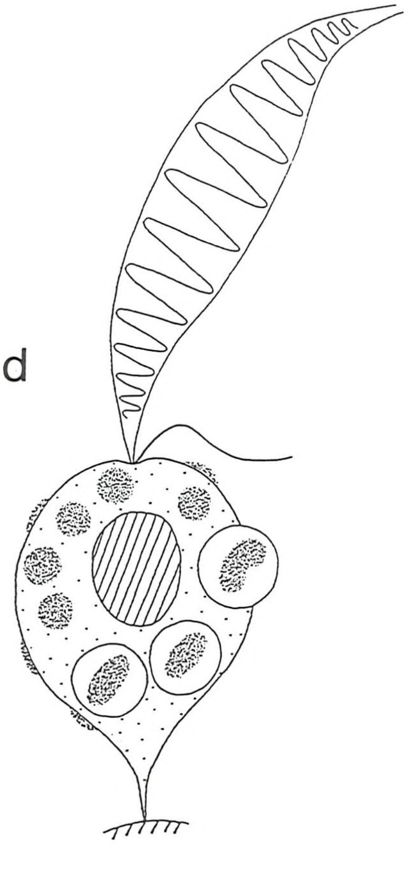
b



c



d



and it is difficult at times to discern whether there is a single or double ring of pseudopodia anteriorly, or whether there are granules on the median pseudopodia. On occasions some of the pseudopodia are retracted even when cells are not swimming and small granules can be seen on the cell surface marking their position (particularly with the anterior pseudopodia). Both species may have two or more threads trailing posteriorly, although one is more common.

Both species feed on bacteria or small protists. Ingestion was only observed around the middle portion of the cell. When food was caught on the anterior pseudopodia they were curved back to bring the object into contact with the middle region of the cell, where they were ingested.

Remarks: These two genera can be distinguished at the ultrastructural level by the presence of a well developed paraxial rod in *Actinomonas* (hence the thicker flagellum), and the presence of flagellar transitional rings in *Pteridomonas* (Larsen and Patterson 1990).

Although contraction of the pseudopodia has been reported widely in helioflagellates (e.g. Skuja 1948, Patterson and Fenchel 1985), coiling of pseudopodia has not been reported since the description of *Pteridomonas pulex* (Penard 1890). Penard makes no mention of pseudopodia on any part of the cell other than around the flagellum, but states that the use of a 'small student's microscope' may have led to them being overlooked (Penard 1921). He also described the pseudopodia arising from a depression around the flagellum; a phenomenon which has not been supported by any modern-day observations on pedinellid helioflagellates.

Since all other observations on this organism are in accord with previous descriptions of A. mirabilis (=*A. pusilla* in Larsen 1985), it is thought appropriate that cells with the ability to coil pseudopodia are retained within this species, at least until the investigation of cultural material allows more thorough examination of any variation in this genus.

Apedinella radians (Lohmann) Campbell, 1973

Fig. 42d

Observations: Seen in light and electron microscopical whole-mounts of several samples, particularly during summer, when substantial numbers of cells were found on some occasions. This species has a single flagellum, like all pedinellids, and bears two types of scales: flattened circular or oval scales, and long spine scales, which are both visible in fig. 42d. See Throndsen (1969) for a general description (as *Pseudopedinella spinifera*).

Ciliophrys infusionum Cienkowski, 1876

Figs: 39a-c, 41a-b

Size: 4.5-10.5µm

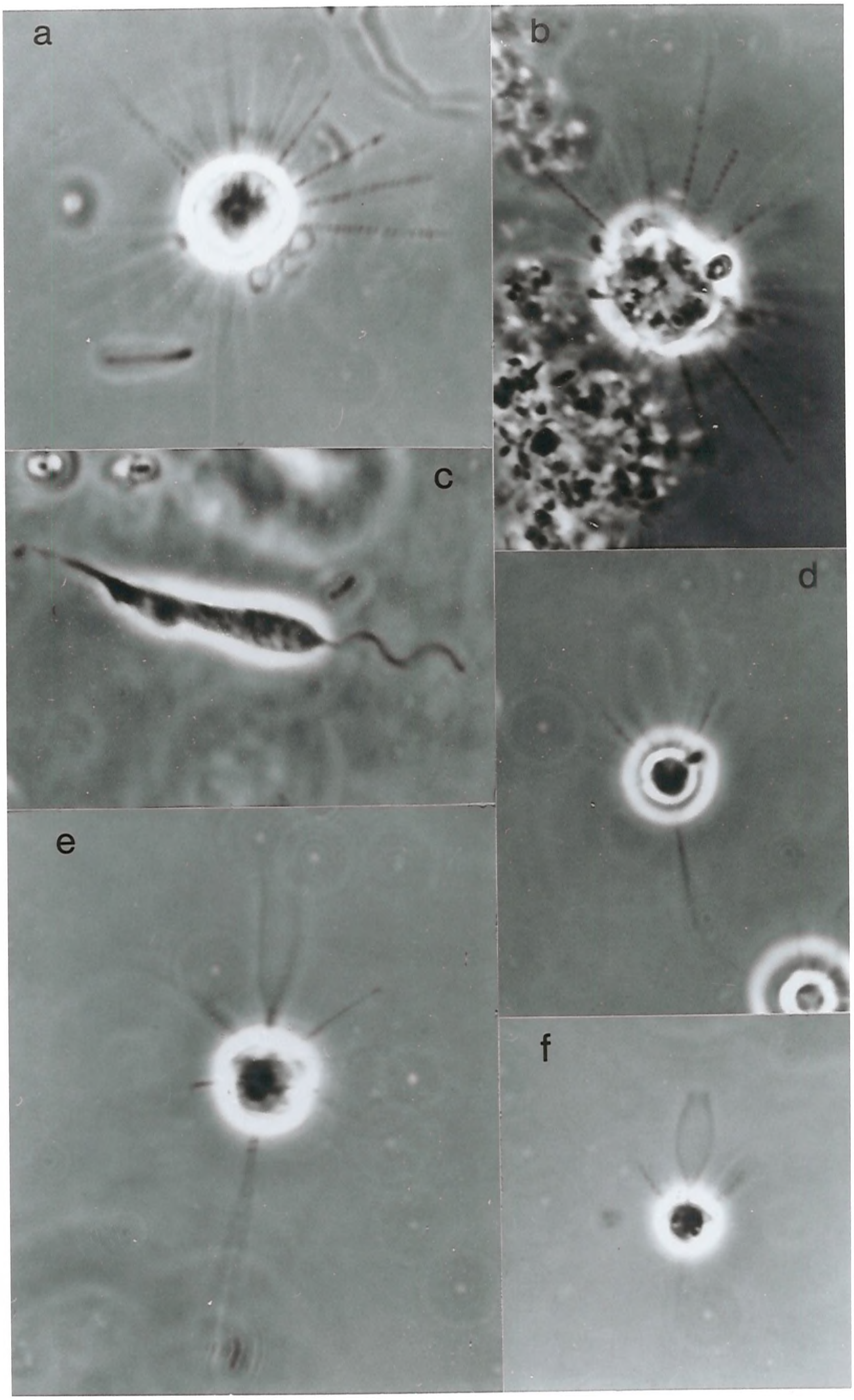
Observations: Seen sporadically in cultures, particularly those prepared from samples collected during autumn and winter. When in the sessile state *Ciliophrys* resembles a small heliozoan cell, being rounded with numerous stiff radiating arms or axopodia which bear small granules (figs 39a,b, 41a) and are used to catch prey. However, careful focusing reveals the flagellum, which is inactive or moves very slowly and is curled into a figure of eight. The pathway of the axonemes (microtubular structures which support the arms) is sometimes apparent inside the cell; they terminate on a central spherical nucleus (fig. 41a). The peripheral cytoplasm of the cell is often highly vacuolated. Cells may rapidly transform into the motile state (in a matter of seconds); the arms are withdrawn and the flagellum extends in a straight line in front of the cell. Swimming cells may have highly irregular shapes, particularly soon after transformation, but eventually assume a more regular elongate shape. When swimming cells return to the sessile state, flagellar beating becomes laboured and irregular, the arms are produced once more, and the flagellum waves feebly for a short time before curling up into its figure of eight shape. *C. infusionum* may fed on bacteria, small suspended detrital

Figure 39: Pedinellids

a-c: *Ciliophrys infusionum*, LM of sessile (feeding) cell (a,b) and motile cell (c). Notice the coiled flagellum in sessile cells (a), granules on the pseudopodia (a,b), and the irregular shape of the motile cell (c), all x2000.

d,f: *Pteridomonas danica*, LM showing the general form of the cell (f) and the ring of pseudopodia around the flagellum (d), x2000.

e: *Actinomonas mirabilis*, notice the second ring of pseudopodia situated half way down the cell, x2000.


a
b
c
d
e
f

particles, amoebae (these are usually 3-5µm), or other flagellates. The cells seen in this study were somewhat smaller than the values usually reported.

Remarks: This species is regarded as con-specific with *C. marina* Caullery, 1909 (see Larsen and Patterson 1990 for a discussion of this). Davidson (1982) gives a detailed description of the light microscopical and ultrastructural characteristics and behaviour of *Ciliophrys* (as *C. marina*).

STRAMENOPILES, *incertae sedis*

Bordnamonas tropicana Larsen & Patterson, 1990

Fig. 41c

Size: 3-6 x 6.5-14µm

Observations: Seen occasionally in enrichment cultures. One flagellum is held in an arc in front of the body, the other trails behind. There is a small protuberance at the anterior end of the cell, which is periodically applied to the substrate, and used for ingestion of food items. From a side-on view *Bordnamonas* looks rather like *Bodo curvifilus*, but differs markedly in its behaviour, particularly the swimming motion. Swimming is rapid, and in smooth curves, with the anterior flagellum held in a curve to the front of the cell in a manner reminiscent of many stramenopiles, hence its tentative inclusion in this group by Larsen and Patterson (1990). (Swimming motion is very similar to that of *Developayella elegans*, but the latter species can be easily distinguished by the prominent groove at the anterior of the cell.)

On one occasion cells were seen gliding with both flagella trailing behind the cell. Cells then resumed normal swimming activity, so it does not appear that this behaviour occurred in stressed individuals. This observation, together with the method of feeding with a manipulative mouth casts some doubt on whether this organism is in fact a stramenopile.

Remarks: Despite its name, this species has a widespread distribution (Larsen and Patterson 1990, Vørs 1992b). In the present study it was found mainly in samples collected during autumn and winter, and was closely associated with detrital particles in cultures.

Developayella elegans Tong, in press.

Figs: 40a-g, 43e

Size: 2-6 x 3.5-8.5μm

Observations: This previously undescribed organism has been found frequently in samples taken during this study, and has been isolated into monospecific culture, feeding on *Vibrio natriegens* and cyanobacteria isolated with the flagellate. Its ultrastructure and phylogenetic relationships are discussed in detail in Tong (1995). Two flagella, measuring 1½-2½ times the length of the cell, insert into a conspicuous depression which occupies much of the anterior half to two-thirds of the right-hand part of the ventral side (figs 40a, 43e). The posterior flagellum is attached along the bottom of the depression, whilst the anterior flagellum leaves the cell freely and is directed forward and curved when swimming, or directed posteriorly and held in a broad curve to the right hand side when the cell is feeding. The nucleus is visible close to the point of flagellar insertion, and the posterior of the cell contains food vacuoles of varying sizes in which bacteria are often visible. Small dark granules are sometimes visible around the periphery of the cell.

The main ultrastructural features of the organism are shown in figs 40d-g. The flagella insert at an angle of about 60° to each other, with the proximal end of the basal body of the smooth (posterior) flagellum associated with the side of the anterior one (fig. 40e). The position of the single Golgi body, anterior to the nucleus and close to the proximal end of the basal body of the anterior flagellum, and of a mitochondrion overlying the nucleus beside the posterior basal body is characteristic (figs 40d,e). The flagellar transition zones have a transverse partition at the level at which the flagella enter the

Figure 40: ***Developayella elegans***

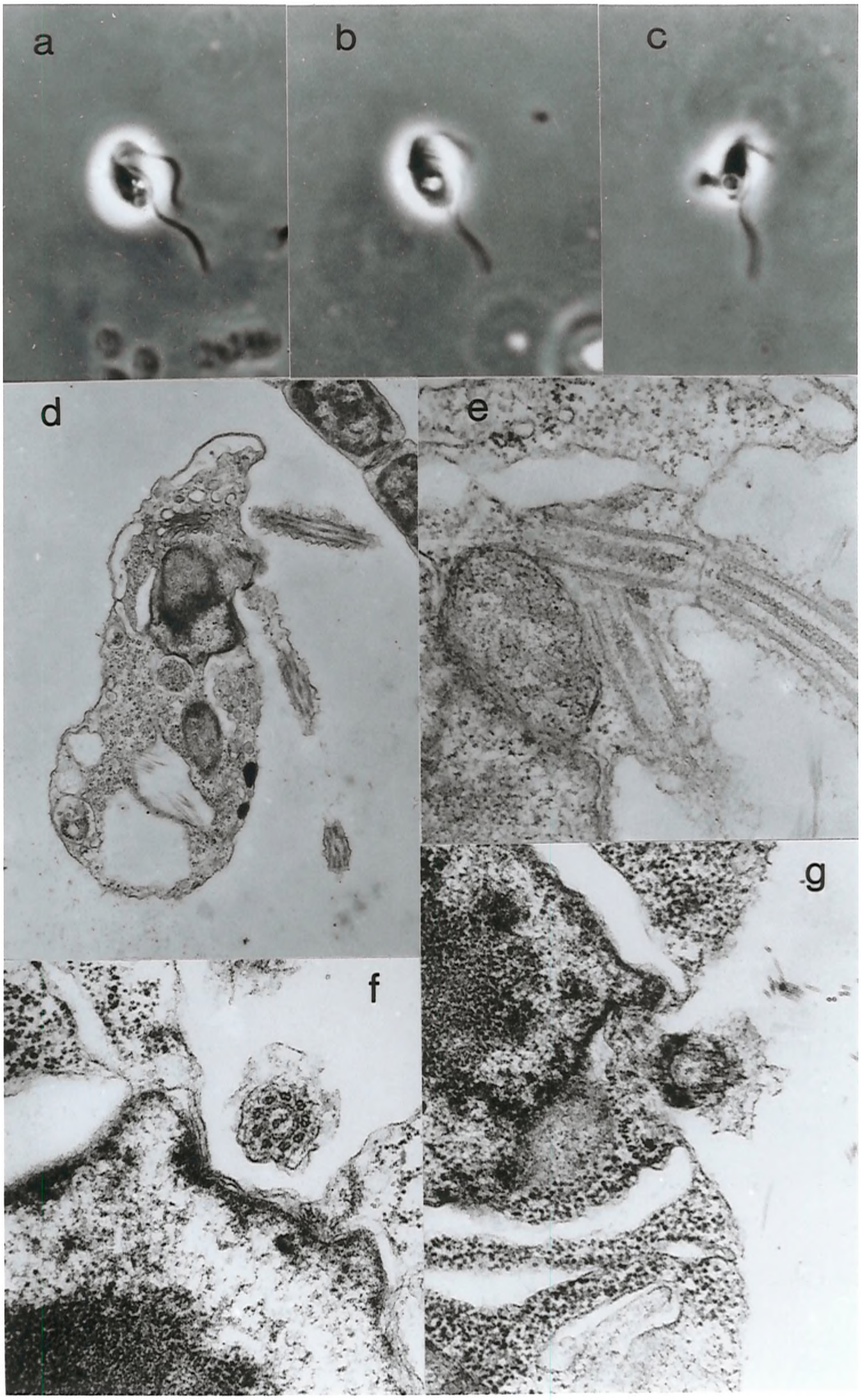
a-c: LM of cells showing insertion of the flagella into a prominent depression (a), attachment to the substratum by a thread at the cell posterior (b) and attachment to the substratum by a larger extension of cytoplasm (c), all x2000.

d: EM of longitudinal section through a cell, showing a vacuole containing mastigonemes, and the characteristic arrangement of a mitochondrion and Golgi body near the point of flagellar insertion, x15,000.

e: EM section showing detail of flagellar insertion, notice the double transitional helix, present as a series of dots either side of the central pair of microtubules in the flagellum, x50,000.

f: EM transverse section through the posterior flagellum and part of the nucleus, notice the transitional helix, visible as a ring around the central pair of microtubules in the flagellum, x50,000.

g: EM, transverse section of the complex flagellar root which runs down the left-hand margin of the ventral depression. Notice the large vacuoles around the nucleus, and the cell periphery in this and other figures, x50,000.


a
b
c
d
e
f
g

cell, and a double transitional helix just above this (figs 40e,f) The anterior flagellum bears tubular tripartite hairs (figs 40e,g), which can also often be seen in a vacuole below the nucleus (fig. 40d). Only one microtubular root has been observed associated with the bases of the flagella. In cross-section (fig. 40g) it is seen as a semi-circle of 10 microtubules (with additional structures inside this at the end nearest the basal bodies), and it runs parallel to the cell surface along the left hand margin of the ventral depression for about 1.8μm.

Remarks: The swimming of *D. elegans* is reminiscent of certain cryptophytes such as *Goniomonas*, and of *Bordnamonas tropicana*, which is believed to be a stramenopile (Larsen and Patterson 1990). However, when the organism is attached to a surface it is easily distinguished from any other type of heterotrophic flagellate described so far, due to the insertion of the flagella into the sub-apical groove and their characteristic beating. Various features of the cell ultrastructure (tripartite tubular hairs, transverse partition, transitional helix, angle and manner of flagellar insertion, flagellar root) show that *D. elegans* is related to the stramenopiles, but ultrastructurally it does not seem to be closely related to any particular taxon within this group. Preliminary studies of its ribosomal RNA sequence suggest that it may be related to oomycetes (D. Leipe, personal communication).

Labyrinthula sp.

Figs: 42a-c, 43a-d

Size: (of cells) 2-4.5 x 7-15.5μm

Observations: Seen sporadically in cultures, associated with detrital flocs. As in all *Labyrinthula* species, cells are fusiform and glide in a network of ectoplasmic channels (figs 42a,b). The nucleus is circular, with a prominent nucleolus, and lies in the centre of the cell (figs 42a,b, 43a). The cytoplasm may be filled with small spherical granules (figs 42a,b). Single cells were observed to join their ectoplasmic networks to form small colonies (figs 43b and 43c, 42b), and after several days these colonies were extensive,

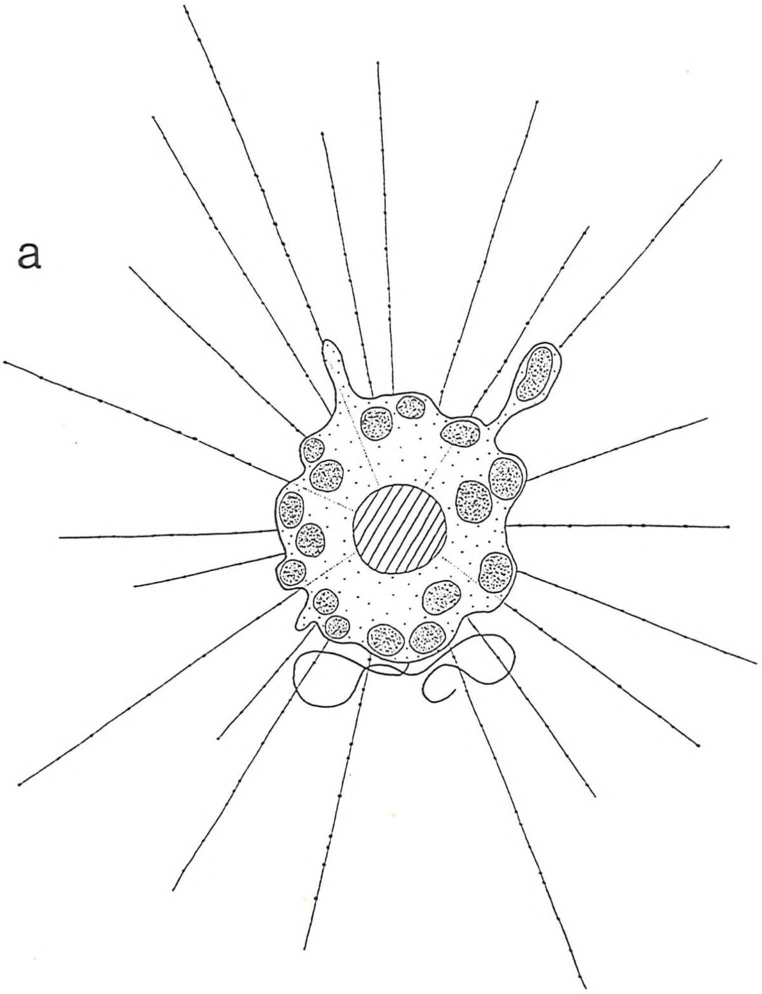
Figure 41: Diagrams of *Ciliophrys, Bordnamonas* and 'Pendulomonas'

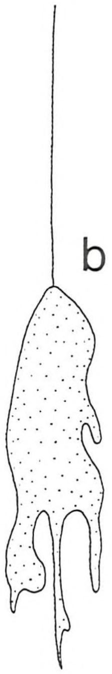
a,b: *Ciliophrys infusionum*, showing the sessile form with a coiled flagellum (a) and the motile form, with an irregular cell shape (b).

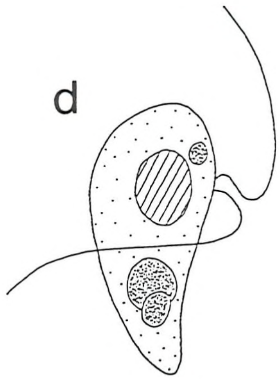
c: *Bordnamonas tropicana.*

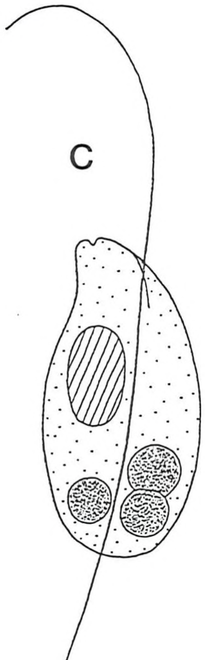
d,e: 'Pendulomonas adriperis', showing cell hanging from the substratum by its posterior flagellum (d) and a vibrating cell (e).

All diagrams x5000.


a


b


d


c

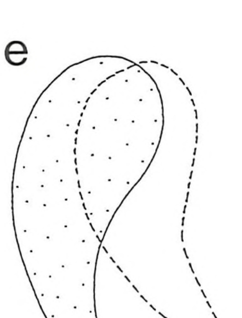
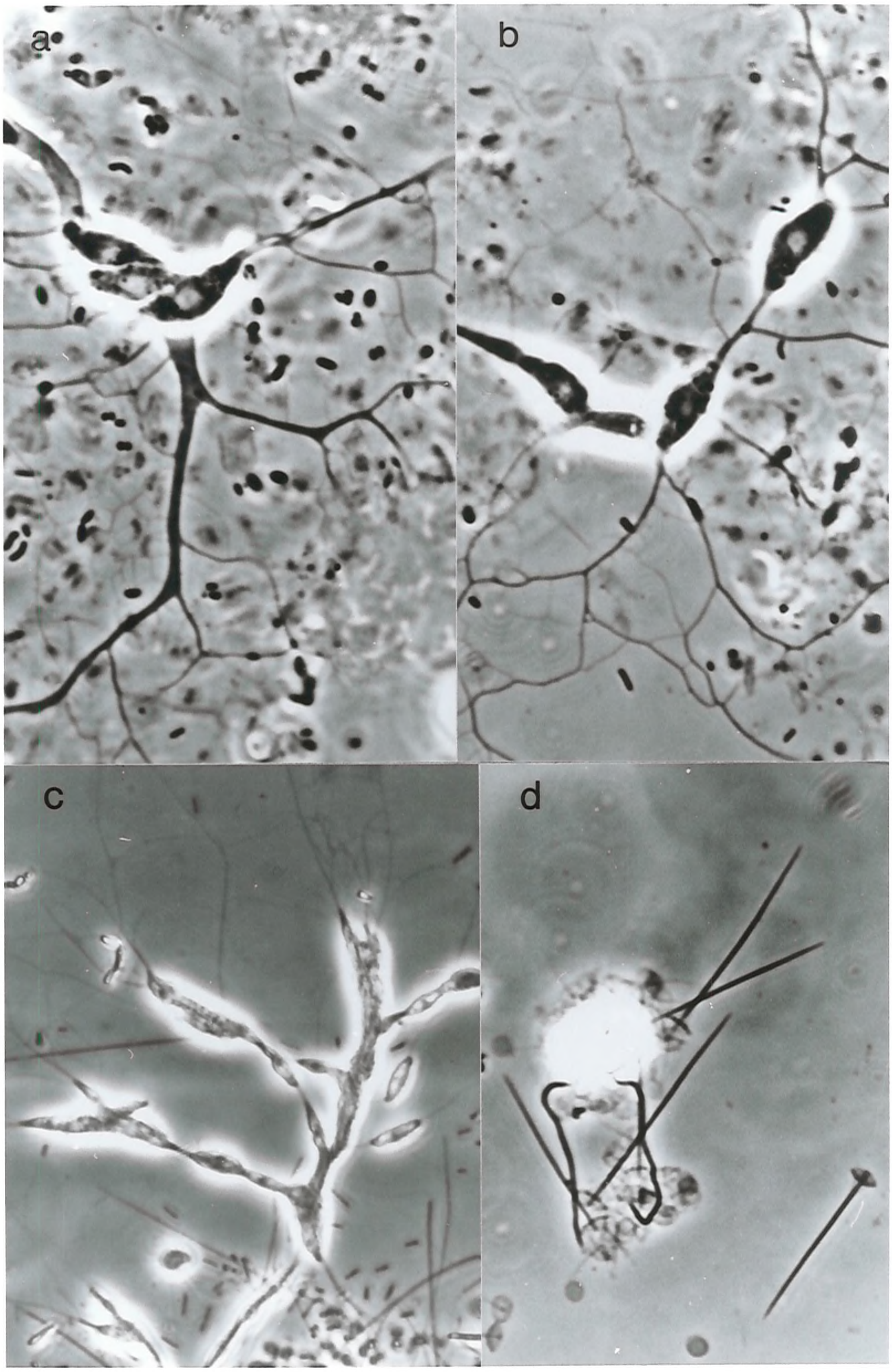

e

Figure 42: *Labyrinthula* sp. and *Apedinella radians*

a-c: *Labyrinthula* sp., LM showing fusiform cells gliding in a network of channels (a,b), notice the prominent central nuclei, and LM showing cells beginning to aggregate together (c), all x2000.

d: *Apedinella radians*, LM whole-mount of cell showing scattered plate and spine scales, x2000.


a
b
c
d

suggesting the cells inside the network had undergone mitotic divisions as described by Porter (1989). After some days cells began to aggregate (figs 42c, 43d), but stages in the life cycle subsequent to this (the formation of cysts and spores) were not observed.

Remarks: Species of *Labyrinthula* are distinguished by their cell size, cell colour and developmental stages (Porter 1989). The first two characters may overlap, or vary under different conditions, and the third requires a time consuming study of cultured material. The cells in this study were colourless, but did not conform exactly to the cell dimensions of any of the species with colourless cells described in Porter (1989).

Labyrinthula has zoospores bearing tubular tripartite hairs, and has therefore been associated with the heterokont/stramenopile grouping (e.g. Patterson 1989a). Its position in this group is supported by rRNA sequence data, which suggests that *Labyrinthuloides minuta* diverged early in stramenopile evolution, before the acquisition of a transitional helix in the flagella (Leipe *et al.* 1994). *Labyrinthuloides* probably belongs to the thraustochytrids rather than the labyrinthulids, but these two groups are closely related (Chamberlain and Moss 1988, Moss 1991).

'Pendulomonas adriperis' (Tong, in prep.)

Figs: 41d-e, 44a-d

Size: 3.5-5 x 5-8.5μm, flagella about 1.5 times cell length

Observations: Seen in an enrichment culture of the NT24 sample, and isolated into pure culture. Cells are generally an elongated ovoid to droplet shape with a pointed posterior end, but they can become distorted by food particles. Two flagella of roughly equal length insert about one third of the way down the cell in a shallow groove. The nucleus is visible close to the point of flagellar insertion. When swimming, cells tend to become more elongate. The swimming action is smooth and rapid, with rotation about the longitudinal axis. The anterior flagellum is held in a curve in front of the cell, and draws the cell towards it in a manner reminiscent of other stramenopiles such as

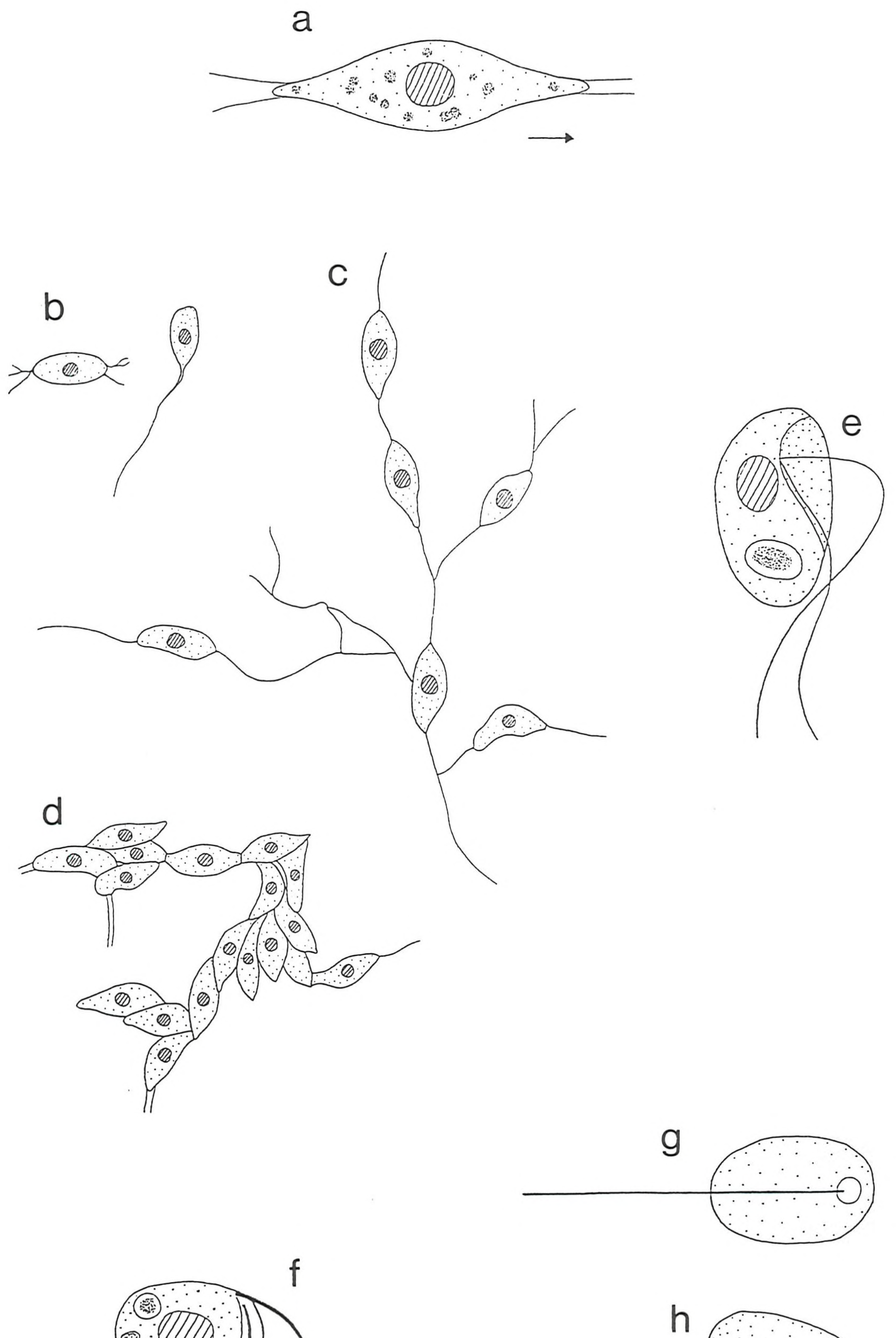
Figure 43: Diagrams of *Labyrinthula*, *Developayella* and undescribed flagellates

a-d: *Labyrinthula* sp. a: detail of single cell, x5000. b-d: Stages in the life cycle: single cells start to form the ectoplasmic network (a), cells link up and/or divide to form a large network (c), and then start to aggregate (d), all x1250.

e: *Developayella elegans*, notice the large depression into which the flagella insert, x5000.

f: 'Cyrano', an undescribed flagellate, notice the insertion of the flagella into a thin, clear region at the anterior of the cell, x5000.

g,h: 'Glissander', an undescribed flagellate, showing the gliding cells from the ventral view (g) and the lateral view (h), notice how the posterior of the cell is raised above the substratum, x5000.


a
b
c
d
e
f
g
h

Paraphysomonas or *Developayella*. The posterior flagellum is trailed passively behind, except when cells are in close proximity to a surface, when it may provide the propulsive force, rather than the anterior flagellum.

When stationary, cells are attached to the substratum by the distal two-thirds of the posterior flagellum, with the cell suspended below this. The anterior flagellum creates a feeding current which draws water down to the cytostome in the posterior part of the cell. This part of the cell can be seen to be elongated beyond its normal position, and rapidly retracts once food is captured. However, the action of the anterior flagellum causes the cell to vibrate rapidly (being more pronounced at the posterior of the cell (fig. 41e)), so that the details of food capture are obscured. This species feeds on bacteria or other flagellates; cannibalism was occasionally observed in monoculture, even when bacteria were in high concentrations. 'Pendulomonas adriperis' is most easily identified by its vibrating movement when attached to a surface by the posterior flagellum, which causes the outline of the cell to become blurred (fig. 41e).

Preliminary ultrastructural studies reveal that this organism is a stramenopile. There is a single mitochondrion with tubular cristae, which surrounds most of the nucleus (fig. 44b). The single Golgi body lies anterior to the nucleus. The flagella have a transverse plate at the level at which they enter the cell, and a single stranded transitional helix (fig. 44a). Mastigonemes are borne on the anterior flagellum, which inserts into a shallow depression on the nucleus, whilst the posterior flagellum is smooth. Mastigonemes are often also visible in vacuoles in the anterior half of the cell. There appear to be several flagellar roots. One (root 'a') appears to curve around the anterior of the cell (fig. 44c) and consist of only a few microtubules. Another large root ('b') consists of a number of microtubules forming a semi-circle. It arises between the two basal bodies and passes to the posterior of the cell. Other roots are shorter, and consist of only one or two microtubules.

Remarks: 'Pendulomonas' shows similarity to several other stramenopile species. The insertion of the anterior flagellum into a small depression on the nucleus is similar to the condition found in the chrysomonad genus *Paraphysomonas*, as is the flagellar

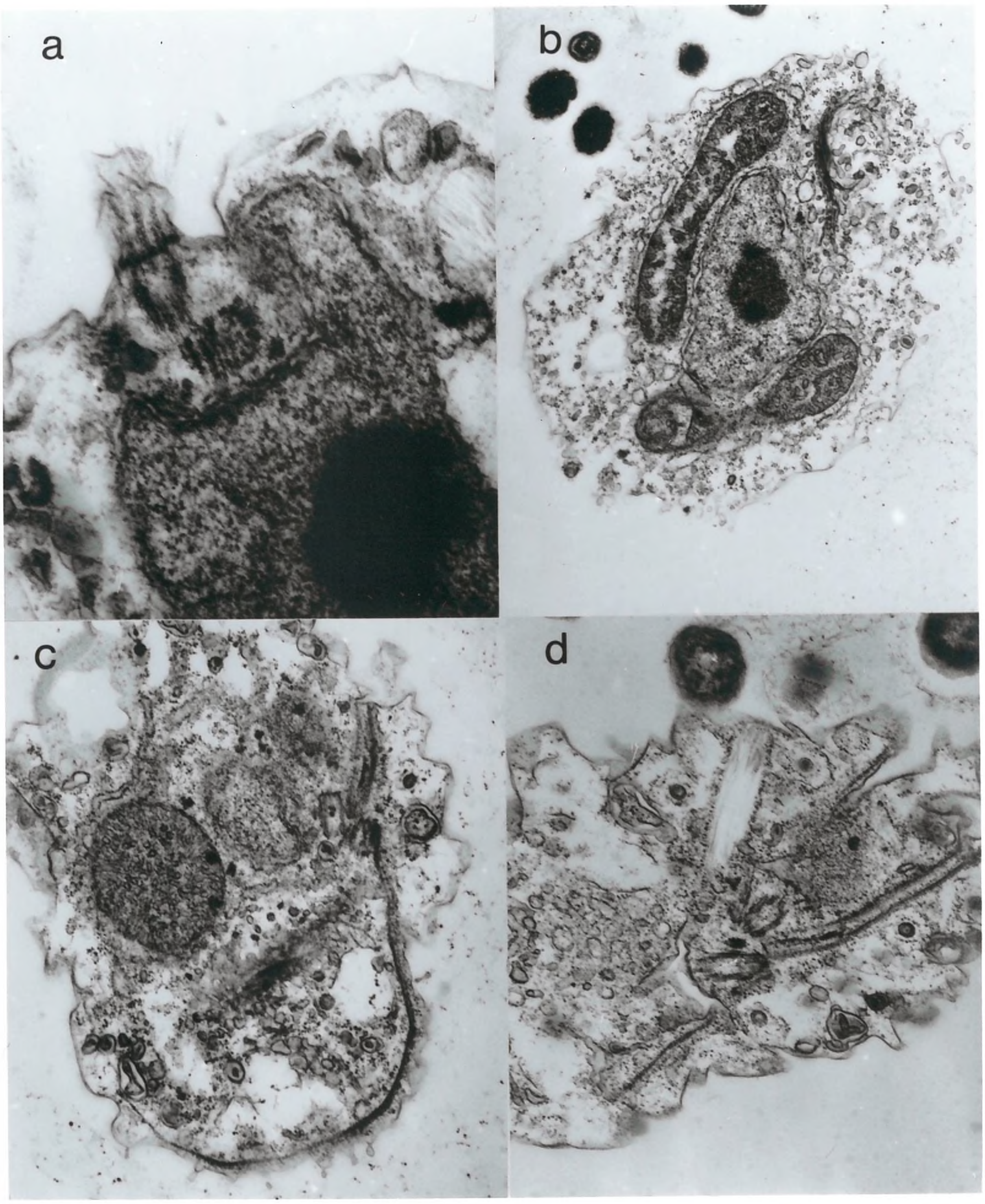
Figure 44: The ultrastructure of 'Pendulomonas adriperis'

a: Section through cell showing the insertion of the basal body of the anterior flagellum into a depression in the nucleus, and the rings of the transitional helix in this flagellum, x50,000.

b: Section showing the arrangement of the single mitochondrion forming a cup around the nucleus (the posterior part of this cup is not visible in this section), x15,000.

c: Section showing the path of one of the microtubular flagellar roots around the anterior of the cell (curving around the bottom of the cell in this fig.), the two basal bodies are also visible, x20,000.

d: Oblique section through the basal bodies showing a flagellar root running towards the posterior of the cell (on RHS of fig.) and part of the root which runs around the cell anterior (LHS of basal bodies in fig.), x25,000.


a
b
c
d

transition region. The semi-circle of microtubules arising near the basal body of the anterior flagellum (root 'b') is similar to the flagellar root described in *Developayella* (Tong 1995). Root 'a' appears to be similar to one of the roots described in the bicosoecid genus *Cafeteria* (Fenchel and Patterson 1988), but a transitional helix has only once (tentatively) been reported in this group (Moestrup and Thomsen 1976). The ultrastructure of *Cafeteria* is about to be revised (C. O'Kelly, personal communication), and it seems prudent to await this before completing the taxonomic analysis and description of this species. Analysis of its ribosomal RNA is also being carried out.

3.14 THAUMATOMONADS

The seven genera currently believed to belong to this group are discussed in Larsen and Patterson (1990) and Patterson and Zölffel (1991) (the Thaumatomastigaceae). The distinctions between some genera are insubstantial, and further work, particularly electron microscopy, may demonstrate synonymy.

Protaspis Skuja, 1939

Species of *Protaspis* are oval or circular and slightly flattened, with a longitudinal furrow on the ventral side (which may be indistinct in some species, particularly when the cell is filled with food vacuoles). The anterior flagellum has a characteristic stiff beating from side to side as cells glide with the posterior flagellum trailing behind. Feeding occurs through pseudopodia which emerge from the furrow. Cells lack scales, but may appear slightly granular at the surface when viewed with light microscopy. Cells often possess a large number of small refractile particles.

Protaspis glans Skuja, 1939

Figs: 45a-b, 46a

Size: 9-11.5 x 11.5-17.5μm

Observations: This was the only *Protaspis* species to be found regularly in cultures. Cells are oval, and slightly flattened, particularly on the ventral side. Two flagella insert subapically; the anterior one is about body length, or occasionally only half this, and the posterior flagellum is 1 ½ to 2 times the body length. This species has a very conspicuous median groove (figs 45a,b). The surface of the cell is covered in fine granules (which are smaller than those in *P. verrucosa* Larsen and Patterson, 1990). The nucleus of cells seen in this study was at the anterior of the cell and in a median position as indicated by Skuja (1939), but in contrast to the observations of Larsen and Patterson (1990), although they indicate that this feature may be variable in members of this genus. Food is ingested by pseudopodia which are produced from the posterior part of the groove, and includes other flagellates. Material in food vacuoles is frequently coloured (usually brown or green) when viewed with phase-contrast optics.

Protaspis metarhiza Skuja, 1939

Figs: 46f-g

Size: (two cells seen) 11 x 27.5 and 13 x 28.5µm

Observations: Seen in a single culture. The cell, when gliding, is elongated and slightly pointed posteriorly and the longitudinal groove is indistinct. The anterior flagellum is about one-third of the cell length, and the posterior, about cell length. The nucleus is anterior and median. There is a greater tendency for the cell to produce pseudopodia than in the other species seen in this study, and cells appear to be more flexible, frequently undergoing squirming movements. Skuja (1939) also noted that this species underwent rather distinct contractions.

Protaspis obliqua Larsen and Patterson, 1990

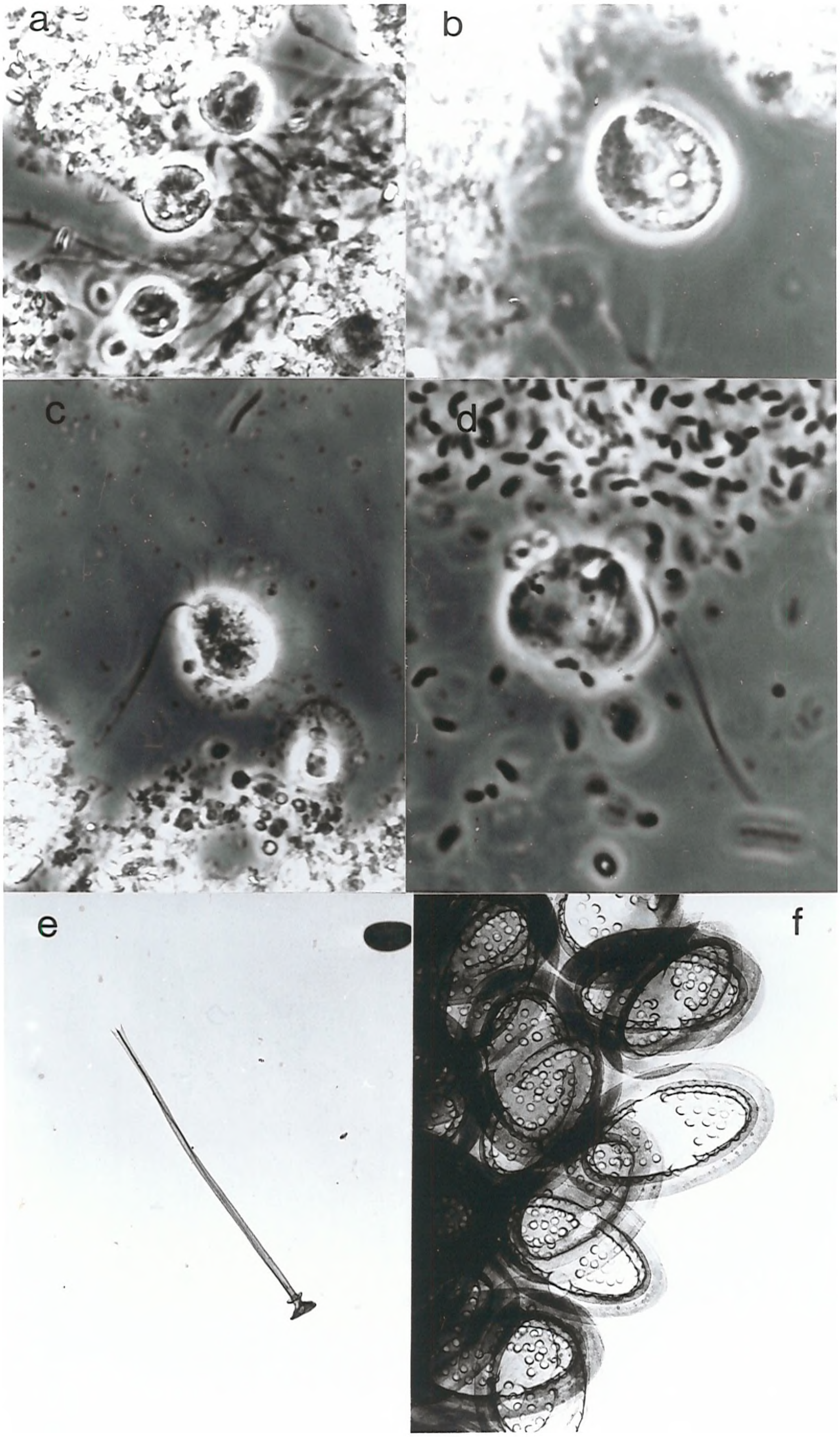
Fig. 46b

Size: 8.5-12.5µm

Figure 45: *Protaspis glans* and *Thaumatomastix salina*

a,b: *Protaspis glans*, LM of cell showing prominent longitudinal furrows, and nuclei (flagella are not visible) x1000 (a) and x2000 (b).

c-f: *Thaumatomastix salina*. c,d: LM of cell showing spines (c) and a longitudinal furrow arising near the point of flagellar insertion (d), x2000. e: EM of a single spine scale, x10,000. f: EM of plate scales, x25,000.


a
b
c
d
e
f

Observations: Seen in a single enrichment culture. Cells are rounded or slightly oval, and flattened on the ventral surface. The anterior flagellum is about cell length, and the posterior flagellum about one and a half times as long. Flagellar insertion is conspicuously assymetrical, as described by Larsen and Patterson (1990). The nucleus is rather large, and anterior.

Protaspis simplex Vørs, 1992b

Fig. 46c

Size: 4-6 x 7-10μm

Observations: Appeared sporadically in cultures. Cells are oval or occasionally rounded, with an inconspicuous furrow. The anterior flagellum is about half the cell length and beats more to the left side of the cell, as described by Vørs (1992b) and the posterior flagellum is two to three times cell length. The nucleus is anterior and median. When gliding, the posterior part of the cell is raised slightly above the surface.

Thaumatomastix Lauterborn, 1896

Species of *Thaumatomastix* are oval with two subapically inserted flagella. One is relatively short (sometimes barely visible) and bears small scales, whilst the longer one is devoid of surface structures, although sometimes surrounded by a layer of mucus (Thomsen *et al.* 1993). The cell is covered by siliceous scales.

Thaumatomastix salina (Birch-Andersen) Beech & Moestrup, 1986

Figs: 45c-f, 46d

Size: 7-15 x 9-17.5μm

Observations: Frequently found in electron microscope whole-mounts of freshly collected

Figure 46: Diagrams of thaumatomonads

a: *Protaspis glans*, notice the prominent longitudinal furrow.

b: *Protaspis obliqua*, notice the assymmetical flagellar insertion.

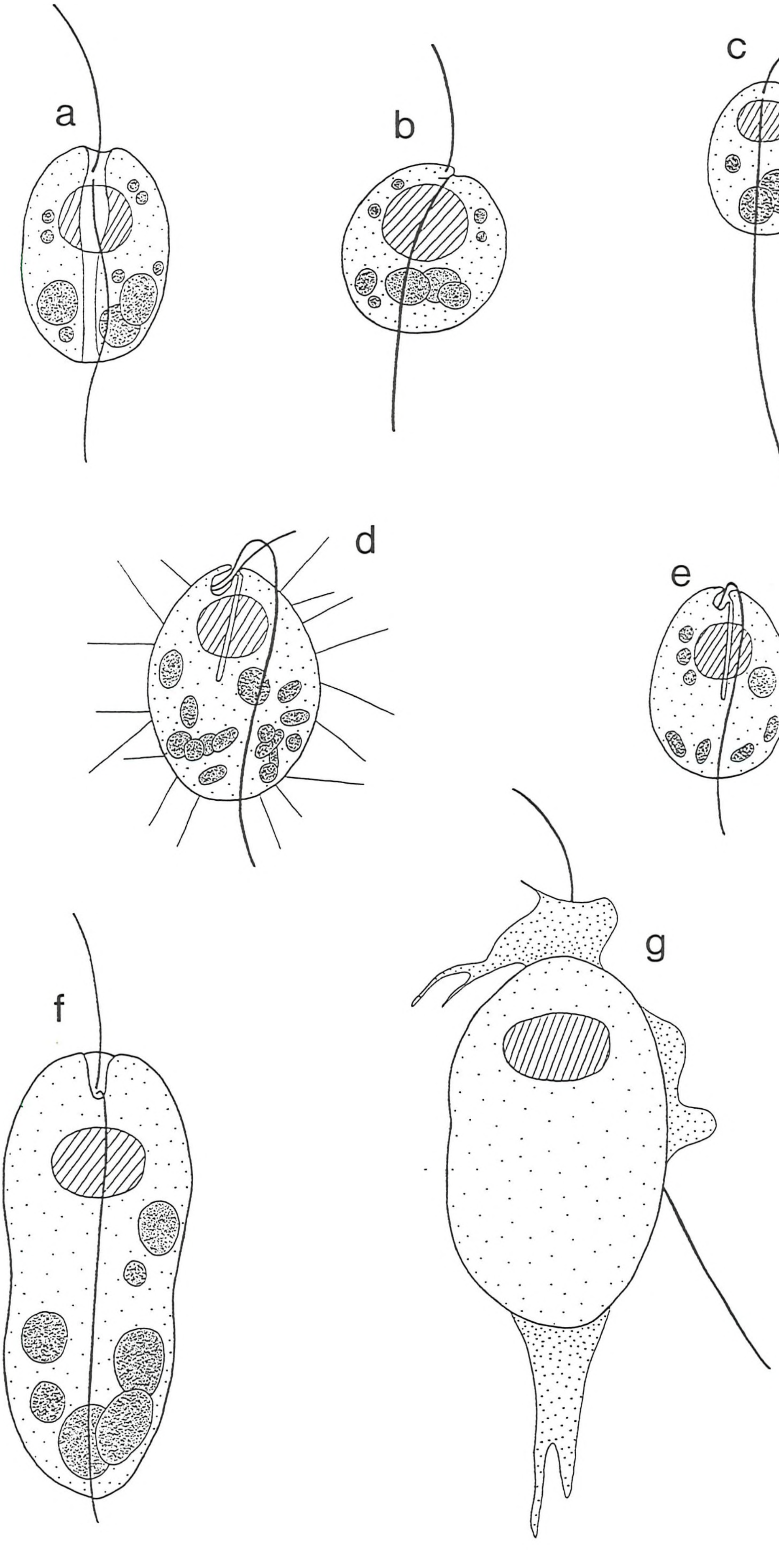
c: *Protaspis simplex.*

d: *Thaumatomastix salina, notice the spines projecting from the cell surface.*

e: *Thaumatomastix* 'thomseni'.

f,g: *Protaspis metarhiza*, showing a gliding cell (f) and a cell producing pseudopodia (g).

All diagrams x2500.


a
b
c
d
e
f
g

material, and sporadically, in enrichment cultures. The light microscopical appearance was exactly as described by Beech and Moestrup (1986), with the furrow arising near the flagellar bases visible in most cells (fig. 45d) and a pale orange area (when viewed with phase-contrast microscopy) visible in the centre of cells which were not filled with refractile particles. *T. salina* has two types of body scales: spine scales (fig. 45e) and plate scales (fig. 45f). A detailed description of the scales is given in Beech and Moestrup (1986) and Thomsen *et al.* (1993).

Thaumatomastix 'thomseni' (Tong, in prep.)

Figs: 46e, 47a-b, 47d

Size: 7-10 x 10-12.5µm

Observations: Found in three samples. Cells are oval and slightly flattened with a prominent median anterior nucleus. A narrow furrow arises near the point of flagellar insertion (as described by Beech and Moestrup (1986) for *T. salina*). Only a single (long) flagellum has been observed, but the presence of a short flagellum is implicated by the presence of scales typical of those found on the short flagellum of other *Thaumatomastix* species. Cells glide with the long flagellum trailing behind, or slightly to one side. The long flagellum is surrounded by a mucilage sheath (fig. 47a, arrows), similar to that reported by Beech and Moestrup (1986) in *T. salina*.

The body scales are oval (500-600 x 800-960nm) and formed from two partially fused plates with a slightly thickened central longitudinal stripe, and a roughly oval thickening where the two plates join (figs 47a,b). Fine striations, formed from tiny dots, were visible either side of the longitudinal line in some scales (e.g. in fig. 47b). There are no spine scales; the spine in fig. 47d is from *T. salina*. Flagellar scales are oval (165-195 x 250-310nm), with a ring of tiny perforations just inside the rim (fig. 47d, arrow).

Remarks: This is the 'unknown flagellate' (Ukendt flagellat) in Vørs (1992a), fig 6.48. (The scales shown by Vørs are slightly smaller than the ones found in this study.) It can be placed in the genus *Thaumatomastix* through the possession of flagellar scales, body scales which bear some resemblance to those of other species where they are composed of two partially fused plates (e.g. *T. salina* and the species shown in fig. 47 in Thomsen *et al.* (1993)), and a light microscopical appearance typical of the genus (particularly the flagellar insertion and possession of a furrow arising from the side of the flagellar pocket, as described in *T. salina*).

Thaumatomastix sp. 1

Figs: 47f, 47g

This species appeared in an enrichment culture of the NT22 sample, along with *Thaumatomastix* sp. 2 below. It is the same species (related to *T. spinosa*) reported in Thomsen *et al.* (1993), from Thailand (see figs 8 and 13 in this paper). The body scales are complex (figs 47f,g); see Thomsen *et al.* (1993) for a detailed description. Unfortunately, as in the material examined by Thomsen *et al.* (1993), flagellar scales were not observed, and so no formal description of this taxon is possible.

Thaumatomastix sp. 2

Figs: 47c, 47e

This is the same species as in figs 16 and 17 in Thomsen *et al.* (1993), and also in figs 6.47B and 6.47C (as *T. sagittifera*) in Vørs (1992a). It appears to be related to *T. dybsoeana*. The body scales have a triangular base, with tiny serrations on the inner side of a peripheral rim, and a smaller triangular plate raised above the base on a series of struts, and rotated through 60° relative to the base, which has a border of small oval perforations just inside the edge. The flagellar scales are circular to oval, with tiny perforations, a rim around the outside and a slightly thickened middle region (which is wider and less distinct than the knob-like projections in *T. dybsoeana*).

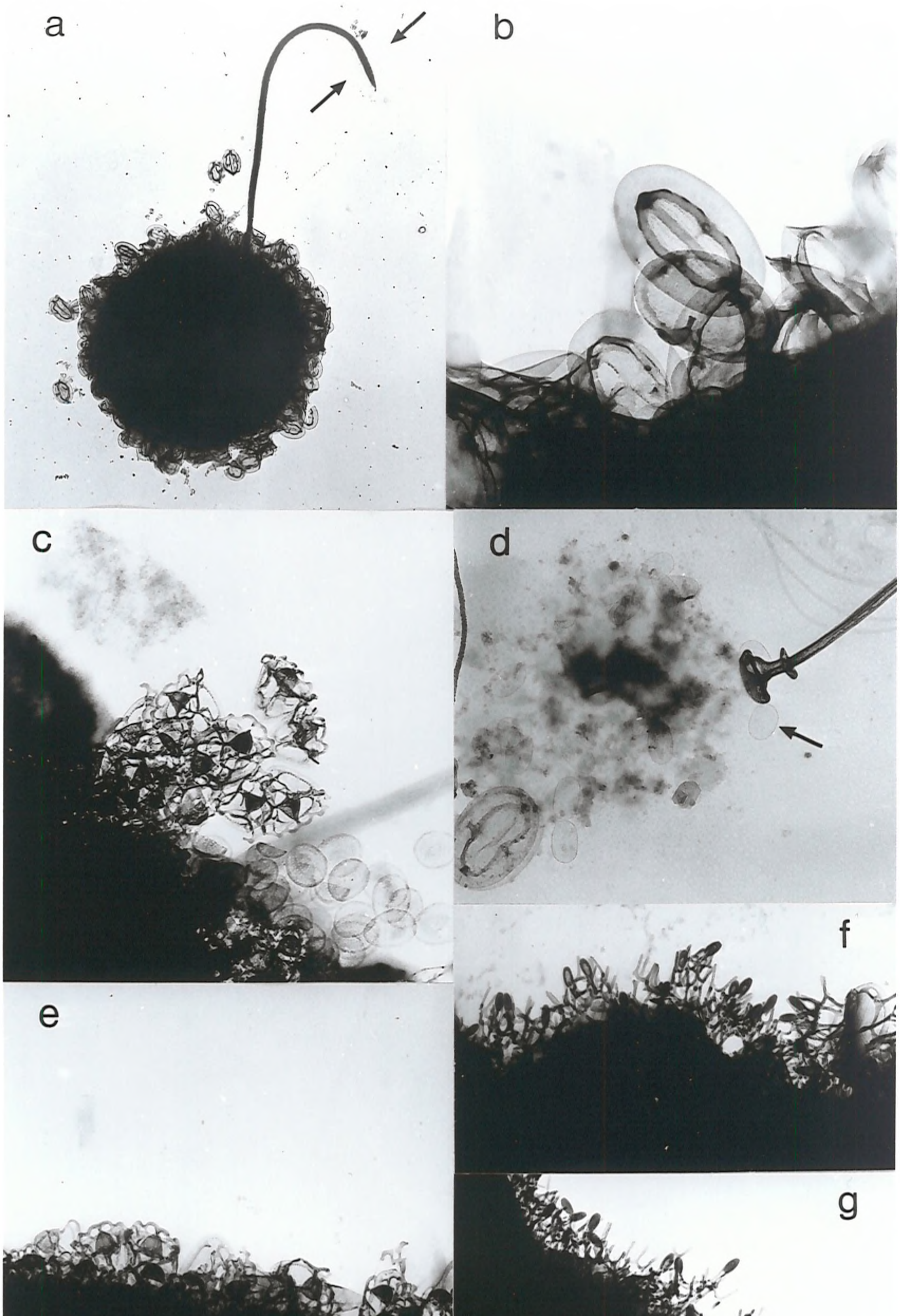
Remarks: The description of *T. dybsoeana* is based on a single cell (Thomsen *et al.*

Figure 47: *Thaumatomastix* species

a,b,d: *Thaumatomastix* 'thomseni'. a: EM of whole cell, x5000. b: EM of detail of scales, x 40,000. d: EM of flagellar scales (e.g. scale arrowed), the spine on the RHS of the fig. belongs to *T. salina*, x25,000.

c,e: *Thaumatomastix* sp. 2, EM of scales on cell surface, notice the oval flagellar scales in fig c., x25,000.

f,g: *Thaumatomastix* sp. 1, EM of scales on cell surface, x25,000.


a
b
c
d
e
f
g

1993), and therefore the extent of variation within this species is not known. Although the scales of 'sp. 2' do show differences to those of *T. dybsoeana*, it seems judicious to await more information on the two taxa, before deciding whether they justify placement in separate species.

3.15 UNDESCRIBED SPECIES

A large number of flagellate species were seen in addition to those described above. Many of these were seen in very small numbers and/or for a short time, so that insufficient information has been accumulated to describe them formally, or identify them with any previous described taxa. However, two of these species were particularly distinctive, and found on a number of occasions, so that it seems appropriate to mention them here. The names of these previously undescribed taxa are merely colloquial.

'Cyrano'

Figs: 2h-i, 43f

Size: 2.5-4 x 3-4.5μm, anterior flagellum about body length, Posterior flagellum 2-2.5 times body length

Observations: Seen frequently in enrichment cultures. Cells are oval or rounded, and rather flattened. The anterior flagellum is about body length, and thickened. Although it is not enclosed within a sleeve, its thickness, and its motion gives it an appearance reminiscent of the anterior flagellum in *Amastigomonas*. The posterior flagellum is thickened, and about twice the body length. It inserts slightly below the anterior flagellum, in a transparent band of cytoplasm which lies along the cell anterior (fig. 43f). The nucleus is quite prominent and usually located around the middle of the cell, although it may be slightly dorsal or ventral. The posterior part of the cell often contains small refractile granules. Cells glide slowly, with the anterior flagellum held in a curve in front of the cell and beating or flickering towards it. Bacteria are ingested in the transparent region of cytoplasm, just beneath the anterior flagellum.

Remarks: This tiny flagellate resembles *Amastigomonas* species in the appearance of the anterior flagellum, and gliding motion but differs in the possession of a thickened, highly visible posterior flagellum and the lack of a sleeve enclosing the anterior flagellum. There is also some similarity to *Rhynchomonas nasuta*, but there is no bulbous snout, and the posterior flagellum is not acronematic.

'Glissander'

Figs: 43g-h

Size: 3.5-4.5 x 5-7µm

Remarks: Seen in enrichment cultures of several samples. Cells are oval, and slightly flattened, and glide smoothly and rapidly with the single flagellum adhering to the substratum and trailing behind the cell and the posterior of the cell raised slightly above the flagellum (fig. 43h). The flagellum appears to be thickened, and inserts into a very prominent circular depression on the ventral side of the cell. The cell occasionally deforms to produce bumps on the surface or small pseudopodia. Other details are unknown.

3.16 <u>The diversity of heterotrophic flagellates and other protists in Southampton Water</u>

Although this study concentrated on the taxonomy of heterotrophic flagellates, a number of other protists were seen, and some of these are described above.

Amoebae occurred in nearly every enrichment culture. They were typically bacterivorous, and small (<20µm). The occurrence and ecological role of these small amoebae has until recently been overlooked, and their taxonomy is in urgent need of attention (Rogerson and Laybourn-Parry 1992b, Rogerson 1993). At least five morphotypes occurred commonly which were smaller than 10µm in their locomotive form and only two species have been described in this size class so far (Rogerson 1993). Slightly larger species (10-25µm) were also common, and were mostly vahlkampfiids, or *Vanella/Platyamoeba* species.

Only three species of ciliates occurred in the enrichment cultures: *Uronema* sp., *Euplotes* sp. and *Vorticella* sp. The first two species fed on bacteria in the culture but *Vorticella* was not observed feeding. Several other species of ciliates were seen in live 40µm-screened, centrifuged material, particularly *Mesodinium rubrum*, a small *Tintinnopsis* species (c25 x 70µm), *Lohmanniella* sp. and *Strombidium* sp.

Two genera which do not fall into any convenient niche in current schemes of classification were encountered (*Ministeria* and *Luffisphaera*). Three new species in these genera are described under '*incertae sedis*' taxa. A large number of autotrophic species (flagellates and diatoms) were seen during the study. A few of these are mentioned above, but little effort was made to identify most of them. Further information on autotrophic nanoplankton (the abundance of diatoms and of autotrophic flagellates, which are separated into various taxonomic groups) is contained in chapter 5.

Ninety-two species of heterotrophic flagellates are described in this study. Four of these species have been mentioned in the literature previously, but have not yet been officially named (*Telonema* 'antarctica', *Thaumatomastix* 'thomseni', and *Thaumatomastix* 'sp. 1' and 'sp. 2'). Eleven of the flagellates discussed above have not previously been described. Two of these have only been given colloquial names (under undescribed species) since more information is required to describe them formally, but they have been included because they occurred quite commonly in cultures, and are likely to be found in any subsequent study of flagellates in Southampton Water. The other new species have been named provisionally (awaiting formal diagnoses in a publication as required by the ICZN), with the exception of *Developayella elegans* (Tong, 1995).

A variety of other heterotrophic flagellates were seen during the study, but were seen in small numbers, or for only a short time so that it was not possible to collect sufficient information (including the extent of variation within the species) to allow identification, or to diagnose a new taxon. In all it is estimated that over 150 species of flagellates were seen during the study. New species, or species not previously seen in this study were encountered in enrichment cultures from every sample, even after the flagellates in Southampton Water had been studied for over two and a half years.

Studies of flagellate diversity in marine sites are rare, and those that exist were generally conducted over a relatively short time-period, meaning that it is difficult to compare the list of species found in this study with any other. Even within this study, the species list is not complete; other species were seen but not identified, the sample size on each occasion was low, the methodology used to study the flagellates was selective in various ways, and the fact that new species were being encountered after two and a half years study of the plankton in Southampton Water suggests that subsequent study would lead to the addition of more species.

The acanthoecid choanoflagellates have been relatively well studied. The taxa found in this study compare closely to those found in the Kattegat around Denmark as reported in Thomsen (1992). Thirty-five of the thirty-eight species found in this study have also been found in Denmark (two of the others are new species).

Heterotrophic flagellates were studied over several relatively short time periods in the Tvärminne Archipelago, Finland by Vørs (1992b). In this investigation sediment samples were studied in addition to plankton samples. However, the diversity of flagellates found shows some similarity to those found in Southampton Water. Within the choanoflagellates, one species of naked choanoflagellate was seen in each study, but these were two different species, two species of *Salpingoeca* were found in both studies, and two additional species in each study and 11 of the 17 acanthoecid species in the Finnish study were also seen in this study. Within the euglenids, 7 of the 9 species seen in Finland were also seen in this study, with one additional species. The chrysomonad and pedinellid species within the two studies are very similar, but only one of the three bicosoecid taxa reported by Vørs (1992b) was found in this study, along with six additional species. The '*incertae sedis*' and apusomonad taxa are very similar in the two studies, but only half the thaumatomonads in the Finnish study were found in this one, along with five others. In all, 49 of the 74 flagellate species reported in Vørs (1992b) have been found in this study.

A similar study of flagellates (excluding dinoflagellates and acanthoecids) was made by Vørs (1992a) around the coast of Denmark. The species reported are similar to those in the Finnish study (above) and to those found in this study. Three-quarters of the heterotrophic flagellates found in the Danish study have also been seen in Southampton Water.

Larsen and Patterson (1990) described the heterotrophic flagellates found in samples of sediment from five tropical sites and several temperate sites. A large diversity of euglenid and dinoflagellate taxa were found; these species are characteristic of sediments and were not seen in this study. Excluding these species, just under half the species in Larsen and Patterson (1990) were also seen in this study.

It appears that most heterotrophic flagellates are cosmopolitan in distribution, with the exception of the acanthoecid choanoflagellates (Vørs 1992b, 1993a,b). Thomsen (1982) and Thomsen *et al.* (1991) reported nine cosmopolitan acanthoecids, (which have all been seen in this study), eight species confined to warmer waters (none of these have been seen in this study), and five species restricted to colder waters (of which two have been seen in this study; *Calliacantha longicaudata* when the water temperature was 6.3°C, and *Parvicorbicula quadricostata* when the water temperature was 15.2°C).

Vørs (1993b) compared the flagellate species found in studies in Belize and Tenerife to those found in other studies in the Arctic (Vørs 1993a) and the Baltic (Vørs 1992a,b) and found surprising similarity in the species diversity of the three regions, particularly amongst the small bacterivorous flagellates. The differences between the species found in these studies, that of Larsen and Patterson (1990) and this study of flagellates in Southampton Water might be further reduced with more intensive sampling.

3.17 Ecological implications of heterotrophic flagellate diversity.

The taxonomic diversity of heterotrophic flagellates in Southampton Water implies that there is a similar wealth of ecological niches. Flagellates may coexist because of differences in feeding strategies or preferences for certain types of food, or may be favoured above other species with similar nutritional habits because of different responses to abiotic factors, or because of factors making them less susceptible to predation.

The majority of marine nanoflagellate species feed in association with detrital particles or other surfaces. Only a few larger (mostly >10μm) non-bacterivorous species, and some acanthoecid choanoflagellates can be considered as truly planktonic organisms. It is advantageous for small species to be associated with particles because they are often foci of microbial activity and have higher concentrations of bacteria than the surrounding water (e.g. Silver *et al.* 1978, Caron *et al.* 1982, Rogerson and Laybourn-Parry 1992a). In addition, the efficiency of flagellar movements used to create feeding currents is increased when flagellates which feed on suspended bacteria are attached to a surface (Fenchel 1982a).

Several non-bacterivorous species of nanoflagellates were encountered during this study. They were characteristically large (>10μm), and planktonic. Although they may feed on cells associated with particles, they are highly motile, and also feed on other protists (mainly autotrophs) which are not associated with surfaces. Flagellates within this category include *Cryptaulax marina*, which appeared regularly in enrichment cultures, but rarely in high numbers, *Telonema* species, which appeared in culture in large numbers on occasions, and *Leucocryptos* species and *Quadricilia rotundata*, which did not appear in culture. Since these planktonic 'predatory' species are generally larger than bacterivorous flagellates, they may account for a substantial proportion of the flagellate biomass, despite the fact that they are less abundant than the bacterivores. The occurrence and ecological role of these flagellates is discussed further in sections 6.11 and 6.13.

Planktonic acanthoecids are particularly conspicuous in oligotrophic marine sites, where particles of detritus, and other organisms which could serve as sites for attachment are rare (e.g. Booth 1990, Thomsen *et al.* 1991). They show several adaptations to a planktonic existence (B.S.C. Leadbeater personal communication). Other choanoflagellates (including some acanthoecids) need to be attached to a surface before the naked cell can construct a covering (in non-loricate species), or before the costal strips forming the lorica can be assembled (loricate species undergoing nudiform replication). However species which undergo tectiform replication do not require a surface for the construction of the lorica, although it is likely that attachment sometimes also occurs in these species. Another disadvantage of a planktonic existence is the reduced efficiency of feeding on suspended food items (Fenchel 1982a) but this may be partially overcome by the drag produced by the lorica, so the flagellar force acts to create feeding currents without also causing locomotion of the cell. The structure of the loricas of some acanthoecids (particularly species with long spines such as *Bicosta* and *Calliacantha*) may also serve to reduce the sinking velocity of the cell.

Flagellates which are associated with particles during feeding may be sub-divided into four main groups in terms of different feeding strategies (after Fenchel 1991). (1) Species which pick food (generally bacteria) off surfaces using a cytostome e.g. bodonids and *Caecitellus*, or using pseudopodia e.g. *Amastigomonas*, *Cercomonas* and the thaumatomonads. (2) Interception feeders; the flagellar currents cause water to flow past an area where food is intercepted e.g. *Paraphysomonas*, *Cafeteria* and *Developayella*. (3) Diffusion feeders; species which are sessile whilst feeding, and catch food on the cell surface, which is usually equipped with radiating arms and extrusomes e.g. *Ciliophrys*, *Massisteria*. (4) Filter feeders; flagellar currents cause water to be directed through a sieving mechanism, where food is intercepted e.g. some choanoflagellates. True filter feeding is rare. *Actinomonas* and *Pteridomonas* are sometimes considered to be filter feeders (e.g. Fenchel 1986, 1991) but the fact that the size of the gap between adjacent pseudopodia is bigger than the dimensions of their prey means that particles are not really filtered; the 'collar' increases the efficiency of interception feeding. Choanoflagellates are considered to be filter-feeders because it is intuitive that the collar of pseudopodia around the flagellum acts as a sieving device. However, it appears that feeding is not so easily explained in some of the loricate taxa. For example, *Savillea micropora* frequently lacks a flagellum, or else the flagellum is very short and immobile, and therefore could not create a feeding current (Leadbeater 1975, observations in this study). Other species such as *Savillea parva*, *Acanthoeca* *brevipoda* and *Diaphanoeca grandis* either have very densely packed costal strips, or have membraneous structures inside the lorica which would seem to impair the passage of food particles to the pseudopodial collar.

Another factor which segregates the ecological niches of flagellates is the choice of food. The sort of food which is ingested may be determined physically (e.g. the diameter of a cytostome, or the gap between adjacent elements of a filtration device) or by (largely uninvestigated) means of selection. There is a large body of evidence that different flagellates can discriminate between bacteria and fluorescent particles of similar dimensions (Nygaard *et al.* 1988), or between live and dead prey (Landry *et al.* 1991), and that they may feed preferentially on a particular size and/or shape of prey when offered a mixed source of food (Andersson *et al.* 1986, Goldman and Dennett 1990, Simek and Chrzanowski 1992). Observations on several of the flagellates in this study also suggest that at least some species have the ability to select certain types of prey. *Caecitellus parvulus*, which feeds by gliding across a surface and picking off bacteria with a mouth on the left ventral side of the cell, sometimes presses the mouth around a bacterial cell for a few seconds, and then rejects it, whilst on other occasions the food is taken into the cell. A similar phenomenon has been seen in *Rhynchomonas nasuta* in this study, and by Swale (1973) whereby only a few of the bacteria 'tested' by the tip of the proboscis are ingested. *Paraphysomonas* species all seem to be capable of 'testing' bacteria by rotating them between the short and long flagellum just above the cell surface. Bacteria are then 'rejected' with a flick of the flagella, or else held on the cell surface by the short flagellum and ingested. This behaviour has also been seen in *Epipyxis pulchra*, a mixotrophic chrysophyte (Wetherbee and Andersen 1992).

Heterotrophic nanoflagellates are currently considered to be the major grazers of picoplankton (see sections 1.1, 1.2). However many species are able to prey on other flagellates. Some feed mainly on smaller cells but are able to ingest larger cells when they are encountered (e.g. chrysomonads, *Metopion*), but others can be regarded as 'predatory' species and may have a significant impact on the population of other flagellates present. In some cultures the growth of *Metromonas simplex*, *Telonema* 'antarctica' or, most commonly, *Telonema subtile* coincided with a decrease in the abundance and diversity of other flagellates in the culture. When *Telonema* species appeared in large numbers in a culture, acanthoecid choanoflagellates were usually the only other species remaining after 5-7 days, suggesting that they were able to avoid predation. The acanthoecid species which occurred in enrichment cultures were all species with dense loricas, and so it appears that this protected them from the predatory species (which were not large enough to ingest the lorica as well).

When different species are competing for a similar food source, variations in their growth kinetics can influence their abundance and survival. Eccleston-Parry and Leadbeater (1994) showed that six nanoflagellate species had different growth characteristics when subjected to the same experimental conditions, using a single species of marine bacterium as food. For example, *Jakoba libera* (Protista *incertae sedis*) had a higher specific growth rate when bacteria were below a concentration of 4.2 x 10^6 ml^{-1} than *Codosiga gracilis* (choanoflagellate), but *Codosiga* had a higher growth rate than *Jakoba* when concentrations of bacteria were greater than this. There were also different thresholds at which growth could occur; for example the theoretical value for *Paraphysomonas vestita* was 2 x 10^4 bacteria per ml, 9.9 x 10^4 for *Bodo designis* and 1.3 x 10^7 for *Ciliophrys infusionum*. If the value for *Ciliophrys* was similar for other bacterial species, this would suggest that growth could only occur in eutrophic environments, and even then probably only at certain times of year. It is possible that the six species used in the above study would respond differently to different species of bacteria.

Variations in the growth kinetics of flagellates will lead to a greater diversity of species being present over a spatial and temporal scale. For example, one species may be favoured in the environment of a small piece of decaying matter, with particular bacterial species associated at a particular concentration, whilst another may be favoured in the environment of another piece of detritus associated with different species and/or abundances of bacteria. Other species may be favoured at a particular time of year, for example, when bacterial concentrations are lower over the winter and early spring, or they may have a more competitive growth rate at a particular temperature.

3.18 Taxonomy and diversity of heterotrophic flagellates; concluding remarks.

Despite the recent interest in the diversity and taxonomy of marine heterotrophic flagellates (e.g. Fenchel and Patterson 1988, Larsen and Patterson 1990, Vørs 1992a,b,c, Patterson *et al.* 1993, Vørs 1993a,b) there still appear to be a large number of undescribed species. In addition, the ultrastructural identity, and taxonomic relationships of many named species are poorly known, and the extent of intra-specific variation amongst many of the recently described species is not yet clear (Patterson and Zölffel 1991, Vørs 1992b). This study has shown that a diverse population of heterotrophic nanoflagellates is present in Southampton Water. Some of these have not previously been described, and many others are known only from one or two previous reports. Even observations on relatively well known taxa have yielded new information about the extent of morphological variation within species, and about their behaviour and ecology. The fact that new species were encountered this study even after two and a half years of sampling indicates the value of a long-term study of a single site.

Further studies on the taxonomy of heterotrophic marine flagellates are likely to reveal new types of ultrastructural organisation as well as new members of existing assemblages. There is a need for many species to be studied ultrastructurally, particularly the Protista *incertae sedis*, so that their phylogenetic relationships can be discovered, or in the case of species already assigned to a larger taxon, so that their position can be confirmed. The information gained from ultrastructural studies is now increasingly being supported by molecular studies, particularly the sequencing of ribosomal RNA (e.g. Schlegel 1991, Leipe *et al.* 1994). The combination of data from ultrastructural and molecular studies should lead to a stable system of classification for protists (Patterson 1994, Tong 1995).

Despite their taxonomic diversity, heterotrophic nanoflagellates are still considered as a single functional unit (bacterivores) in most models of marine microbial food webs (Patterson 1993, Eccleston-Parry and Leadbeater 1994). This ignores their role as grazers on other protists, including primary producers. Larger (>10μm) nanoflagellates feed mainly on other nanoplankton, and, although less abundant than the smaller bacterivores, may have a significant biomass. This additional ecological role of heterotrophic nanoflagellates is discussed further in sections 6.11 and 6.13.

Chapter 4: Material and methods used in the seasonal study of plankton abundance and taxonomy at NW Netley.

The NW Netley site was chosen for a detailed seasonal study of plankton dynamics, because of concurrent and previous studies of various components of the food web at this site, and the fact that despite its proximity to the outflow of the rivers Itchen and Test salinity rarely falls below 28‰. Samples were taken, when possible at fortnightly intervals coinciding with the Spring tide, and close to the first stand of high water. However, this was not always possible, and on occasions samples were collected during other parts of the tidal cycle, rather than having a long gap between successive samples. Regular sampling was carried out between 17th Feb. 1993 and 8th June 1994, although some of the techniques described below were not carried out on the first few samples.

A decision was taken at the beginning of the study to limit samples to one site, and one depth, because of the time taken to carry out the various procedures on each sample. However, two samples were collected from the surface on 24.05.94 (NT30) to give some indication of the variation in plankton numbers over a small spatial scale. The samples were collected in an identical manner, in two different containers, and have been labelled NT30 (a) and NT30 (b). On 08.06.94 (NT31) samples were taken from the surface (as usual), 2m and 7m depth to investigate variation in plankton numbers and taxonomy with depth. The sample at 0m was collected as usual, by filling a container hung over the side of the boat, and the samples at 2 and 7m were collected in a 5l Niskin water bottle.

Temperature and salinity were measured (at the surface) for most samples using a temperature/salinity probe. Temperature, salinity and the part of the tidal cycle when the sample was collected are shown in Appendix 2.

Chlorophyll a concentrations were measured at 5m and 10m depths at the same time as many of the samples in this study were taken. Water samples were collected in 5l Niskin water bottles and kept in a dark cool box until they were returned to the laboratory where 50ml of each sample was filtered through a 1.2μm pore-size glass microfibre filter. Filters were frozen for a maximum of 3 months before fluorometric analysis of acetone extractions of pigments using the methodology of Kifle 1992. (A. Hirst, personal communication).

The following procedures were carried out on each of the NW Netley samples:

1) Centrifugation.
The procedure was as described in Chapter 2. Part of this material was used to prepare whole-mounts (see below).

2) Electron microscope whole-mounts.
Prepared as described in chapter 2. These preparations were used to identify heterotrophic flagellates with scales or loricas (most naked flagellates are difficult to identify when fixed with osmium), and also some autotrophic flagellates, particularly those with conspicuous scales.

3) Light microscope whole-mounts.
These were prepared as described in chapter 2. The light microscope whole-mounts were used to examine changes in the diversity of acanthoecid choanoflagellates. Five drops were examined for each sample (giving a total of 150μl of concentrated material; equivalent to 14.5ml of the original sample) and the numbers of each choanoflagellate species noted.

4) Epifluorescence microscopy.
Freshly collected material was passed through a 40μm mesh, and approximately 50mls were fixed with ice-cold electron microscopical-grade glutaraldehyde to a final concentration of 2.5%. The glutaraldehyde was stored frozen until a few hours before use, to maintain its purity and stability as recommended by Gillett and Gull (1972) and colleagues carrying out similar studies.

After 5-30 minutes 5 or 10ml sub-samples were filtered by gravity, or using minimal suction pressure, onto black Poretics polycarbonate membrane filters with a pore size of 1µm. A 1µm pore size was chosen because a smaller pore size required a greater suction pressure for the sample to pass through, and the aim of the preparation was to maintain maximum numbers of fragile cells, and to preserve them in an optimum state so that some identification was possible. Larger pore sizes would have meant that many of the flagellates were lost. A backing filter of 8µm pore size was used to promote the even distribution of material on the filter.

For the enumeration of bacteria, fixed material was diluted 20 times with 0.2µm filtered autoclaved seawater, and 2 or 5 ml of this were concentrated onto a filter of 0.2µm pore size, using a gentle suction.

At first filters were stained with DAPI and primulin. DAPI stains DNA, causing the nucleus of eukaryotic cells to fluoresce bright blue when excited by ultraviolet light, and causing similar fluorescence of complete bacterial cells whilst primulin stains polysaccharides, so that other details of eukaryotic cells may be discerned (e.g. Martinussen and Thingstad 1991). However it was found that DAPI caused detritus to fluoresce a weak yellow colour (as was also noted by Porter and Feig, 1980), which was similar to that of cells stained with primulin. Because samples were collected in a eutrophic estuarine environment they contained a lot of detritus, and this meant that cells were often difficult to see with this staining procedure. Therefore only DAPI was used, at a fairly high concentration (50µl/ml). This allowed cells to be quickly located by bright fluorescence of the nucleus and also stained the rest of the cell sufficiently brightly to observe its outline, details of the flagella, and to allow the image to be captured on a video screen for measurement. Staining was carried out with a minimal light intensity to prevent the fading of the fluorochromes.

Filters were stained for three minutes, and then rinsed several times with distilled water. During this procedure care was taken not to allow the filter to dry completely, as this can cause cells to explode (e.g. Goldman and Dennett 1985). After the final rinse the filter was rapidly transferred to a microscope slide and embedded between two drops of paraffin oil. A coverslip was placed on top of the second drop of oil, and then slides were stored in the dark at 4°C until they were used (within 12-18 hours). Filters were not examined immediately as it was found that the visibility of cells on the filter improved one or two hours after preparation, and also that small creases in the filter gradually disappeared, which would have otherwise led to inaccuracies in calculating cell abundances. Some decrease in the autofluorescence of autotrophic cells was observed after storage for 18 hours, but it was still possible to distinguish between autotrophic and heterotrophic cells.

Filters were examined under oil immersion using a x100 lens. Ten fields of view were examined to enumerate bacteria (equivalent to 1.08 x 10^{-4}ml), and 100 fields (or 200 fields when 5ml was filtered instead of 10ml) to enumerate other categories of plankton (equivalent to 0.108ml of the original sample). Three categories of nanoplankton (<20µm) were enumerated; heterotrophic and autotrophic flagellates, and diatoms. Flagellates were divided into taxonomic groups using characters such as the shape of the cell, the number, location, size and relative length of flagella, the position of the nucleus and the number, position and colour of autofluorescence of chloroplasts in autotrophic flagellates (see tables 3a and 3b).

After a few weeks it became apparent that the heterotrophic flagellate population underwent rapid changes in cell size as well as in total numbers, and therefore the diameters of 50 cells were measured from each sample. Measurements were made by freezing images on a video screen, since the fluorescence faded rapidly and low light levels meant that it was not possible to measure cells directly down the microscope lens.

Table 3a: Features used to distinguish various taxa of autotrophic flagellates with epifluorescence microscopy

PLANKTON GROUP	Diagnostic features
cryptophytes	cell shape, flagellation and single chloroplast with slightly orange fluorescence
chrysophytes	spherical cell, flagellation, one or two chloroplasts with red fluorescence
dinoflagellates	condensed chromosomes in nucleus, cell shape, chloroplast with bright red fluorescence
euglenids	cell shape, numerous chloroplasts, thickened flagella
haptophytes	'Butterfly' appearance, with two chloroplasts with bright red fluorescence
prasinophytes	four flagella, cell shape

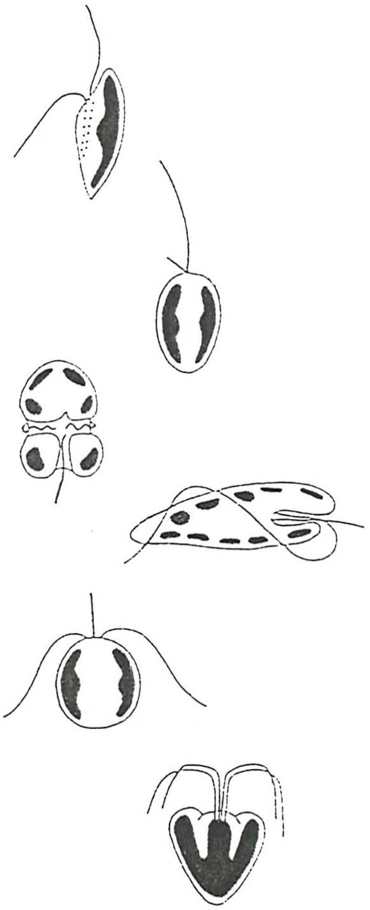


Table 3b: Features used to distinguish various taxa of heterotrophic flagellates in epifluorescence studies

PLANKTON GROUP	Diagnostic features	Comments
chrysomonads	spherical shape, central nucleus, relative length of flagella	short flagellum not always visible, pedinellids also included in this group.
dinoflagellates	condensed chromosomes in nucleus	cell shape also useful as preserved well after fixation
acanthoecid choanoflagellates	pseudopodial collar, presence of lorica	
other choanoflagellates	pseudopodial collar, absence of lorica	the thecas of *Salpingoeca* spp. were often difficult to see, and so not distinguished from codosigids.
bodonids	kinetoplast, thickness and tapering of flagella, elongated shape	
bicosoecids	combination of the three genera described below	
Bicosoeca spp.	relative length of flagella, lorica	
Cafeteria roenbergensis	cell shape, two equal short flagella	
Pseudobodo tremulans	relative length of flagella, irregular cell shape	membranelle sometimes visible
Cryptaulax marina	size and shape of cell, thickness and length of flagella	
Telonema spp.	characteristic cell shapes, relative length of flagella	
Leucocryptos spp	cell shape, thickness and differing lengths of flagella	*L. marina* has a droplet shape, *L.remigera* is bullet shaped and has longer flagella
Massisteria marina	circular cell with central nucleus, pseudopodia with granular thickenings	
Amastigomonas spp.	cell shape, thin flagella	
Quadricilia rotundata	cell shape, 4 flagella tapering at tips	
Caecitellus parvulus	cell shape and size, two equal flagella	the flagella tend to lie in a different manner to those of *Cafeteria* allowing the two to be distinguished; cell shape also preserves differently

Chapter 5: Results from the seasonal study of plankton abundance at NW Netley

5.1 Physical variables

Measurements of temperature and salinity were made for most of the Netley samples, and these data are summarised in appendix 2 and fig. 48. Surface temperature followed a typical annual cycle, with lower values in the winter (minimum of 6.0°C on 1.12.93), and maximum values in the summer (up to 18.6°C on 19.08.93). Temperatures measured at 5m and 10m depth at the same time as many of the samples in this study were very similar, with a maximum of 0.4°C difference between 0 and 10m (A. Hirst, personal communication), reflecting the lack of stratification at this site.

Salinity at the surface ranged between 22.4 and 33.4‰, but was only below 28‰ on two occasions. The salinity tended to be slightly higher during the summer, reflecting the lower rainfall, and smaller input of freshwater into the estuary.

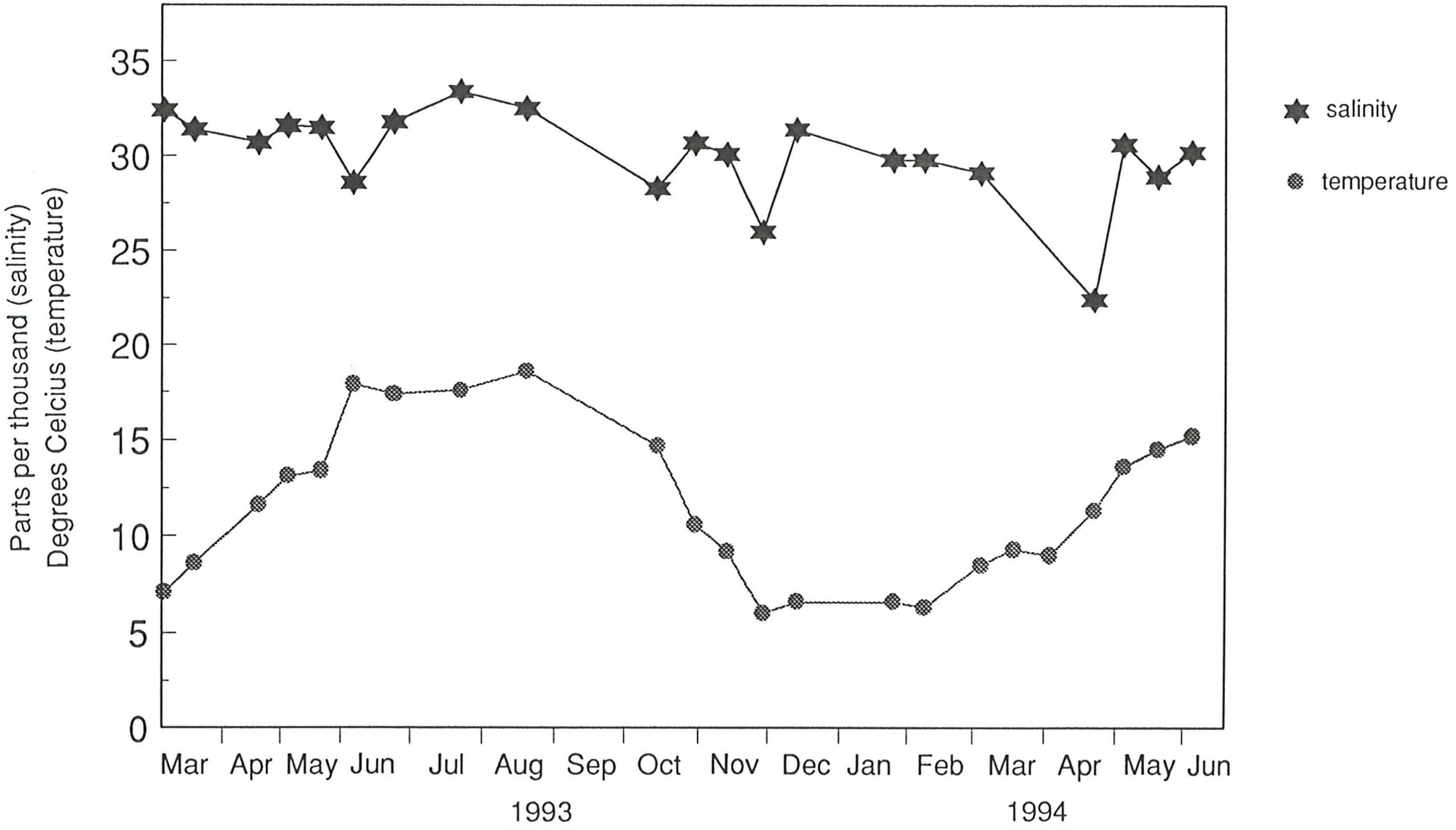
5.2 Chlorophyll a concentration

Chlorophyll a data are available for many of the earlier samples because of concurrent studies on NW Netley by A. Hirst of the Dept. of Oceanography, University of Southampton. Appendix 3 shows the concentration of chlorophyll a at 5 and 10m depths, measured when the corresponding samples were taken at the surface for this study. Although data are not available for surface chlorophyll concentrations, it does give some idea of the seasonal variation in phytoplankton biomass in the water column over the study period. Values ranged from 0.51-7.59 mg m^{-3} at 5m, and 0.56-12.29 mg m^{-3} at 10m between February 1993 and February 1994, with maximum values in July and August.

5.3 Results from centrifuged material

It is necessary to concentrate marine samples to get a sufficient density of heterotrophic

Figure 48: Variation in temperature and salinity of NW Netley samples
March 1993-June 1994



flagellates to study them, but this process (whether by centrifugation or filtration) kills many of the more fragile forms. Therefore the diversity of protists seen in centrifuged samples was poor, and consisted mainly of autotrophic flagellates (which are generally more robust than heterotrophic forms) and diatoms. Characteristic heterotrophic flagellates were dinoflagellates, the acanthoecid choanoflagellates, which were particularly numerous in spring, *Paraphysomonas* and *Spumella* species (in rather low numbers), *Telonema* species, *Cryptaulax marina* and *Leucocryptos* species. However, on occasions when there was relatively little detritus in the water column, other more fragile forms were also seen including *Bodo*, *Petalomonas*, *Cafeteria*, *Actinomonas*, *Pteridomonas* and *Caecitellus*. *Ollicola vangoorii* and *Quadricilia rotundata* were common in summer collections; this appeared to reflect their actual numbers in the water column, rather than damaging effects of centrifugation at other times of the year. Ciliates, particularly tintinnids and small (<30µm) scuticociliates were also common in summer collections, as was *Mesodinium rubrum*. Ciliates appeared to be less affected by centrifugation than the smaller flagellates, since they appeared in similar numbers to flagellates in centrifuged material from some samples, despite the fact that they would be expected to be a thousand times less abundant (e.g. data from Leakey *et al.* 1992).

5.4 Results from electron microscope whole-mounts

This method was intended to allow more detailed study of the loricas of the acanthoecids seen in the light microscope whole-mounts and to allow the identification of forms which can not be distinguished by light microscopy, such as different species of *Paraphysomonas*. Some data from the electron microscopical whole-mounts are presented in the taxonomy section in the form of micrographs of various species.

Heterotrophic flagellates which were identified from the use of electron microscopy alone included all the *Paraphysomonas* species, the *Spumella* species (light microscopy revealed that the species lacked chlorophyll and electron microscopy revealed the absence of scales on the cell surface, thereby distinguishing it from *Paraphysomonas*), and the *Thaumatomastix* species.

A large number of other protists were seen; comparatively little effort was made to identify these as the main focus of study was on heterotrophic flagellates. However, species listed in the taxonomy section identified by electron microscopy were the centrohelids *Meringosphaera mediterranea*, *Pterocystis* sp. and *Raphidocystis tubifera*, the coccolithophorids *Emiliana huxleyi* and *Papposphaera* sp. (only isolated scales were seen for the former species, although these were common), the *Luffisphaera* species (Protista *incertae sedis*), the prasinophyte *Pyramimonas cirolanae* and the pedinellid *Apedinella radians*.

Information from electron microscope whole-mounts of freshly collected material and enrichment cultures contributed to the list of species present in each sample, as shown in appendix 1.

5.5 Results from light microscope whole-mounts

Light microscope whole-mounts were used to study the seasonal variation in the taxa of acanthoecid choanoflagellates present at NW Netley. These data are summarised in table 4. Other protists, such as *Paraphysomonas vestita* and *Thaumatomastix salina* could also be identified on occasions, and this information contributed to the species lists given in appendix 1.

A total of 32 acanthoecid species were seen in light microscope whole-mounts, out of a total of 38 species seen by the combined methods used in this study. They can be placed into four broad groups on the basis of their seasonal occurrence during the study period (March 1993-June 1994).

1) Species present throughout the year.

Five species were present throughout the year (found in most samples), with no obvious seasonal pattern: *Acanthocorbis apoda*, *Calliacantha natans*, *Crinolina isefiordensis*, *Crucispina cruciformis* and *Polyfibula sphyrelata*. Others were present throughout the year but most numerous in spring: *Calliacantha multispina* (seemed to be rare in winter), *Calliacantha simplex* and *Parvicorbicula superpositus,* or most numerous in summer: *Bicosta minor* and *Cosmoeca norvegica.*

2) Species which were rarely seen.

Thirteen species were seen in only 1-3 samples during the study period: *Acanthocorbis haurakiana*, *Acanthoeca spectabilis*, *Calliacantha longicaudata*, *Cosmoeca ventricosa*, *Diaphanoeca pedicellata*, *Nannoeca minuta*, *Parvicorbicula circularis*, *Parvicorbicula quadricostata*, *Parvicorbicula socialis*, *Polyoeca dichotoma*, *Saepicula pulchra*, *Stephanoeca cupula* and *Stephanoeca elegans.*

3) Species which were seen sporadically.

Acanthocorbis campanula was seen sporadically, but appeared to be more common in spring and summer. *Bicosta spinifera* was seen in only five samples, but in fairly large numbers in two summer samples (NT13, NT30). *Pleurasiga reynoldsii* was seen in low numbers on several occasions. *Diaphanoeca grandis* appeared in low numbers between June 1993 and January 1994, and *Acanthocorbis unguiculata*, between August and November 1993, with a single cell seen in April 1994 (NT28).

4) Species with a seasonal occurrence.

Diaphanoeca undulata occurred mainly in the summer, although one or two specimens occurred at other times. *Pleurasiga minima* occurred mainly in the winter and spring and was rare during summer and autumn.

Two species, which have not previously been described, had a very strict seasonal appearance. *Parvicorbicula* 'manubriata' appeared in samples NT4-NT8 (17th February-23rd April 1993) and NT24-NT28 (11th February-26th April 1994), with only single cells being seen in three samples in between these dates. Although light microscope whole-mounts of the NT4 and NT5 samples were made, the methodology meant that these results were not strictly comparable with those of later samples and have therefore

Table 4: Acanthoecid choanoflagellate species seen in light microscope whole-mounts (samples NT6-NT14)

SPECIES	NT 6	NT 7	NT 8	NT 9	NT 10	NT 11	NT 12	NT 13	NT 14
Acanthocorbis apoda	3	2	4	2		1			1
A. campanula	1		1				1		
A. haurakiana								3	
A. unguiculata									
Acanthoeca spectabilis									
Bicosta minor	4	3	2		1	23			
B. spinifera					1			20	1
Calliacantha longicaudata									
C. multispina	10	9	8			3	3	1	
C. natans		2				13	18		
C. simplex	20	9	10	9		5	7	16	5
Cosmoeca norvegica			3			14	1	1	
C. ventricosa						4			
Crinolina isefiordensis	1			1	3	16	7	1	4
Crucispina cruciformis	1		2	2	1	13	5		
Diaphanoeca grandis						2		1	
D. pedicellata									
D. undulata			2			6	16	3	1
Nannoeca minuta									
Parvicorbicula circularis									
P. quadricostata									
P. socialis									
P. superpositus	8	3	2	1		16	2		
P. 'manubriata'	102	65	2			1			
P. 'aculeatus'	6	35	8	5	1				1
Pleurasiga minima	3	1	3	1	1				
P. reynoldsii						2		1	1
Polyfibula sphyrelata									
Polyoeca dichotoma									
Saepicula pulchra									
Stephanoeca cupula									
S. elegans									1

Table 4 (cont.): Acanthoecid choanoflagellate species seen in light microscope whole-mounts (samples NT15-NT23)

SPECIES	NT 15	NT 16	NT 17	NT 18	NT 19	NT 20	NT 21	NT 22	NT 23
Acanthocorbis apoda		1	9		2		1	2	1
A. campanula			1						
A. haurakiana									
A. unguiculata	4		1	2	4				
Acanthoeca spectabilis									
Bicosta minor		1		10	4	2		6	2
B. spinifera	4								
Calliacantha longicaudata									
C. multispina				1			1	1	
C. natans		3	4			1	5	3	
C. simplex	4	8	2		7	3	3		1
Cosmoeca norvegica	1	1	2	7					2
C. ventricosa					3				
Crinolina isefiordensis	3	2	12	2	1	1	1	2	
Crucispina cruciformis			1	2	1		1	4	6
Diaphanoeca grandis		4	4		1			4	
D. pedicellata	1								
D. undulata									
Nannoeca minuta								1	
Parvicorbicula circularis								7	
P. quadricostata									
P. socialis								1	
P. superpositus	1		1	9	1		5	7	3
P. 'manubriata'					1		1		
P. 'aculeatus'									
Pleurasiga minima	1			2	1	2	1	20	12
P. reynoldsii						2	1		2
Polyfibula sphyrelata				13	2	3	1	8	2
Polyoeca dichotoma						1			
Saepicula pulchra						1			
Stephanoeca cupula								1	
S. elegans	5		5						

Table 4 (cont.): Acanthoecid choanoflagellate species seen in light microscope whole-mounts (samples NT24-NT31)

SPECIES	NT 24	NT 25	NT 26	NT 27	NT 28	NT 29	NT 30	NT 31
Acanthocorbis apoda	1		4	21	17	21	2	2
A. campanula			1	6	4	16	4	
A. haurakiana								
A. unguiculata					1			
Acanthoeca spectabilis		1		1				
Bicosta minor	3	1	3	1	1	1	62	1
B. spinifera							13	
Calliacantha longicaudata	1							
C. multispina			3	6		7	3	
C. natans		3	10	8	3	4		7
C. simplex	1	4	17	27	10	1	2	
Cosmoeca norvegica	1	1	2	2	1		12	2
C. ventricosa								
Crinolina isefiordensis	3	6	16	8		9	5	11
Crucispina cruciformis				1	1	3	1	
Diaphanoeca grandis								
D. pedicellata						1		7
D. undulata								
Nannoeca minuta				1				1
Parvicorbicula circularis				2				1
P. quadricostata								3
P. socialis			2					
P. superpositus	10	14	27	26	6	3	22	7
P. 'manubriata'	2	12	35	6	2			
P. 'aculeatus'		4	58	7	15	8	39	
Pleurasiga minima	4	1	4	3			1	
P. reynoldsii				4			1	4
Polyfibula sphyrelata	1	9	2	3	3	24	6	20
Polyoeca dichotoma								
Saepicula pulchra								
Stephanoeca cupula								
S. elegans								

not been included in table 4. *Parvicorbicula* 'aculeatus' was present in samples NT6-NT10 (12th March-21st April 1993) and NT25-NT30 (8th March-24th May 1994), with a single cell in NT14 (9th August 1993).

Although the occurrence of acanthoecid species in the whole-mounts is thought to broadly reflect the actual situation in a particular sample, certain species are likely to be under-represented in the data (see discussion). The appearance of several species in enrichment cultures, which were not seen in whole-mounts from the same sample reflects this. For a full record of the occurrence of acanthoecids in various samples using combined methodologies see appendix 1.

5.6 RESULTS FROM EPIFLUORESCENCE MICROSCOPY COUNTS

The abundance of bacteria, hetero- and autotrophic nanoflagellates and nanoplanktonic diatoms was estimated in each NW Netley sample by enumerating DAPI-stained cells with epifluorescence microscopy. The abundance of different flagellate taxa was also calculated, allowing some insight into which taxa are most numerous in the estuary, and whether this varies seasonally.

5.6a Abundance of nanoplankton and bacteria

Total numbers of bacteria and nanoplanktonic autotrophic flagellates, diatoms and heterotrophic flagellates were measured for samples NT8-NT31 (from NT11 for bacteria). These data are summarised in table 5 and fig. 49 and are discussed further below.

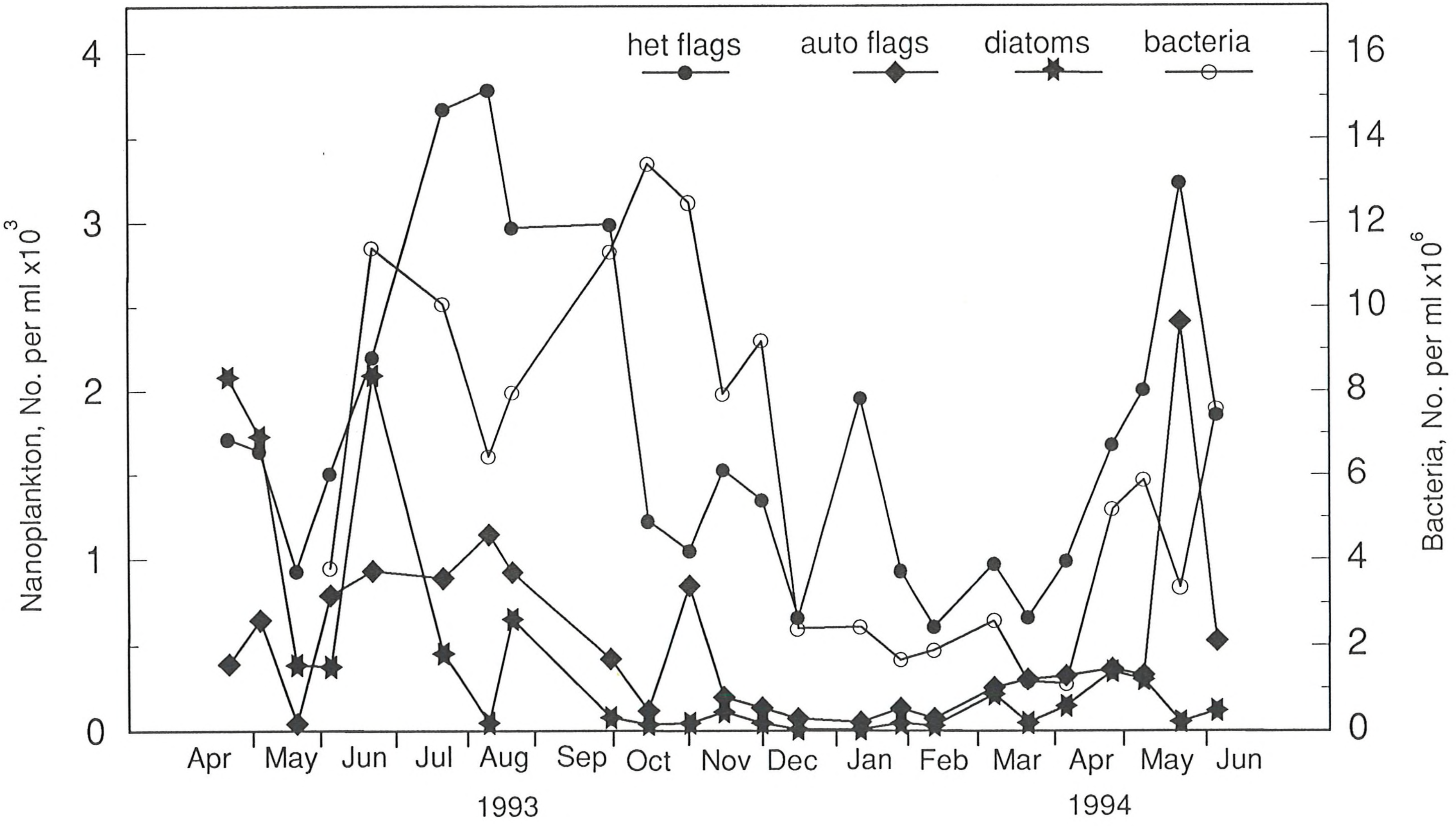
Bacteria (1.11-13.4 x10^6 per ml)

The abundance of bacteria at NW Netley was measured over one year from June 1993-June 1994. Numbers ranged from 1.11 x10^6 ml^{-1} (April 1994) to 13.4 x10^6 ml^{-1} (October 1993). Abundance was quite high over the summer, but then somewhat unexpectedly

Table 5: Abundance of bacteria and nanoplanktonic protists (No./ml) at NW Netley 1993-1994

SAMPLE	Bacteria (x10^6)	Heterotrophic flagellates	Autotrophic flagellates	Diatoms
NT8 23.04.93		1714	381	2086
NT9 06.05.93		1642	649	1729
NT10 21.05.93		934	45	389
NT11 04.06.93	3.82	1557	795	378
NT12 22.06.93	11.42	2202	935	2094
NT13 21.07.93	10.08	3669	893	455
NT14 09.08.93	6.44	3788	1146	47
NT15 19.08.93	7.96	2975	927	655
NT16 29.09.93	11.33	2994	424	79
NT17 15.10.93	13.40	1227	119	40
NT18 01.11.93	12.49	1052	847	46
NT19 15.11.93	7.94	1529	197	112
NT20 01.12.93	9.19	1350	134	44
NT21 16.12.93	2.40	662	71	(9)
NT22 11.01.94	2.44	1957	51	(9)
NT23 28.01.94	1.67	936	132	43
NT24 11.02.93	1.89	612	73	32
NT25 08.03.94	2.58	975	252	216
NT26 22.03.94	1.16	665	301	45
NT27 07.04.94	1.11	992	321	147
NT28 26.04.94	5.19	1683	365	348
NT29 09.05.94	5.89	2008	328	302
NT30 (a) 24.05.94	3.37	3240	2414	54
NT30 (b) 24.05.94	3.06	3107	2650	63
NT31 (0m) 08.06.94	7.59	1863	530	120
NT31 (2m) 08.06.94	7.44	1195	359	137
NT31 (7m) 08.06.94	7.85	1265	487	231

Figure 49: Abundance of nanoplankton and bacteria at NW Netley April 1993-June 1994



increased during autumn and early winter of 1993. Values plummeted during December, and remained low for the rest of the winter and early spring, until a sudden increase occurred in late April. High values then persisted until the end of the sampling period in June 1994.

Autotrophic flagellates (45-2414 cells per ml)

The abundance of nanoplanktonic autotrophic flagellates was measured from April 1993 to June 1994. They showed a seasonal cycle of abundance, with low values during the winter and highest values during late spring and summer. However there were sudden increases in abundance on several occasions (between two consecutive samples). These reflected short-term blooms of particular species.

The abundance of different taxa of autotrophic flagellates was recorded in the NT14 sample, and from NT18 onwards, as it was found that relatively few forms were present, and that these were easy to distinguish with epifluorescence microscopy. These results are presented in table 6. Flagellates were divided into cryptophytes, dinoflagellates, euglenids (these were often 30-40µm long, and therefore could not really be considered as nanoplankton), haptophytes (prymnesiophytes), prasinophytes and chrysophytes (including pedinellids). Other taxa were occasionally seen, but in low numbers. The diversity of species within each category appeared to be low. The cryptophytes were dominated by three species, which had cell lengths of about 5, 10 and 15µm. Two species of euglenids were seen, as also noted by Kifle (1992): *Eutreptiella marina* and an unidentified *Euglena* species (c7 x 40µm) Dinoflagellates were nearly all *Gymnodinium*-like species of about 15µm, and most prasinophytes belonged to three species, tentatively identified as *Pyramimonas* spp. Haptophytes and chrysophytes were slightly more diverse, with *Phaeocystis* (haptophyte) and *Apedinella radians* (pedinellid chrysophyte) being particularly conspicuous during the spring and summer respectively.

Particularly high numbers of flagellates were seen in the NT14 and NT30 samples. In NT14 this was mainly due to two species of cryptophytes (of c5µm and c15µm) and dinoflagellates. The bloom of flagellates in NT30 was due to the haptophyte *Phaeocystis*,

Table 6: Types of autotrophic flagellates detected in epifluorescence counts (numbers per ml.)

SAMPLE	chr	dino	eug	hapt	pras	cry	auto flags (total)
NT14 09.08.93	40	324	32	0	55	402	1146
NT18 01.11.93	31	593	(8)	(15)	31	139	847
NT19 15.11.93	120	0	0	(17)	0	60	197
NT20 01.12.93	24	47	0	0	0	63	134
NT21 16.12.93	27	0	0	0	0	35	71
NT22 11.01.94	(9)	(9)	0	0	0	(18)	51
NT23 28.01.94	106	0	0	0	0	(18)	132
NT24 11.01.94	(8)	33	0	0	0	(16)	73
NT25 08.03.94	27	(18)	(18)	(9)	0	117	252
NT26 22.02.94	0	27	37	37	0	100	301
NT27 07.04.94	27	(9)	0	37	0	211	321
NT28 26.04.94	80	(18)	0	(18)	(9)	205	365
NT29 09.05.94	124	(9)	0	71	(9)	98	328
NT30 (a) 24.05.94	118	81	(9)	1682	(18)	326	2414
NT30 (b) 24.05.94	90	72	(9)	2324	0	154	2650
NT31 (0m) 08.06.94	137	34	0	188	26	60	530
NT31 (2m) 08.06.94	68	51	0	196	(9)	26	359
NT31 (7m) 08.06.94	90	34	0	256	0	68	487

KEY: 'chr' = chrysophytes, 'dino' = dinoflagellates, 'eug' = euglenids, 'hapt' = haptophytes, 'pras' = prasinophytes, 'cry' = cryptophytes. Numbers in brackets refer to abundances calculated from the presence of only one or two cells.

although quite high numbers of cryptophytes were also present. A bloom of flagellates was also noted in the NT18 sample in November 1993 which was due to a large number of dinoflagellates, which were entirely absent in the next sample two weeks later.

Diatoms (9-2094 cells per ml)

The abundance of nanoplanktonic diatoms was measured from April 1993 to June 1994. They showed a similar pattern to autotrophic flagellates in that whilst values were generally high in the summer and low in the winter, the population was capable of undergoing rapid short-term increases in abundance. A bloom of small pennate diatoms in NT8 (April 1993) was preceded by extremely low numbers in March (data not shown), and a second bloom in NT12 (June) was also preceded by relatively low numbers a fortnight before. The second bloom appeared to be caused by the same species as in April. Abundance then decreased during July and early August before a third bloom in mid-August, and then low values persisted from September to February 1994. Numbers of nanoplanktonic diatoms showed small increases during March, April and May of 1994, but in contrast to the previous year, there were no distinct blooms. In spring and summer of 1993, blooms of nanoplanktonic diatoms generally coincided with low numbers of autotrophic nanoflagellates, and vice versa.

Nanoplanktonic diatoms were dominated by short pennate forms, although centric, and chain-forming diatoms were present in significant numbers on some occasions during summer. Small diatoms were also conspicuous in electron microscope whole-mounts. Most of the nanoplankonic species (seen with epifluorescence and electron microscopy) were of less than 12μm in length, and centric diatoms seen in whole-mounts were frequently smaller than 5μm in diameter.

Heterotrophic flagellates (612-3788 cells per ml)

The abundance of heterotrophic flagellates at NW Netley was measured from April 1993 to June 1994. Numbers followed a fairly conventional seasonal cycle with low values throughout the winter, an increase in mid-spring associated with rising numbers of bacteria, and highest values during the summer.

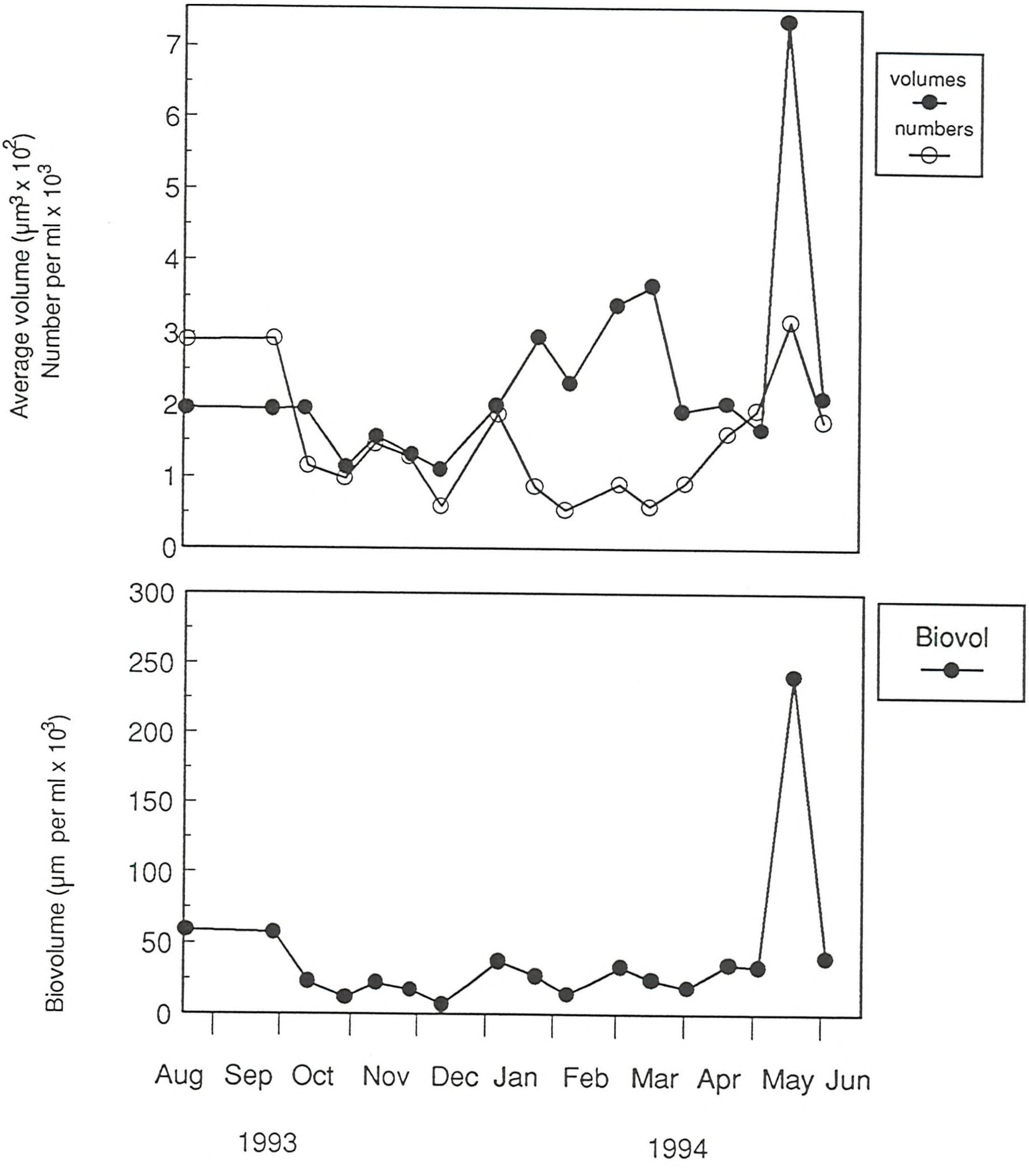
The diameters of 50 cells were measured on epifluorescence filters from each sample from August 1993 to June 1994. Cell diameters ranged from 1.4 to 12.6μm (in glutaraldehyde-fixed cells). The average volume of 50 cells was combined with the abundance of flagellates in the sample to give an estimation of biovolume (μm^3/ml), shown in appendix 4 and fig. 50. The average volume of cells underwent some change; there appeared to be no clear seasonal pattern, but rather the average measurements were affected by the abundance of various groups of larger nanoplanktonic flagellates (>10μm). In the NT30 sample for example, the value for the average volume of cells was considerably greater than at any other time because of the presence of a large number of *Cryptaulax marina* cells. Since this coincided with a high abundance of flagellates in general, the estimated value for heterotrophic flagellate biovolume per ml was over four times greater than in any other sample. Fig. 50 demonstrates the relative contribution of changes in cell volume and cell abundance to the overall biovolume.

Over the period measured (August 1993-June 1994), estimated biovolume ranged from 7000 to 240500 μm^3/ml, with an average of 41000μm^3/ml for the 17 samples. Using this figure as a rough guide, and the conversion factor of Børsheim and Bratbak (1987) of 0.22pg C μm^{-3}, the standing carbon-biomass of nanoplanktonic heterotrophic flagellates at NW Netley is estimated to be in the region of 10,000pg C ml^{-1} (or 10μg C l^{-1}) Biomass estimates from the individual samples range from 1.5 to 52.9μg C l^{-1} (NT21 and NT30 respectively).

It should be noted that fixation in glutaraldehyde causes heterotrophic flagellate cells to shrink by about ⅓-½ of the volume of living cells (Børsheim and Bratbak 1987, Choi and Stoecker 1989). The shrinkage is probably mainly due to the egestion of food particles (Sieracki *et al.* 1987, observations in this study). The volume of fixed cells may actually be a more realistic parameter than the volume of living cells for the calculation of biomass, since the volume of food vacuoles is not included (Kuuppo 1994).

Heterotrophic flagellates were subdivided into several taxa which are discussed below.

Figure 50: Abundance, average cell volume and biovolume of heterotrophic flagellates at NW Netley August 1993-June 1994



All percentage values refer to the percentage of the total heterotrophic nanoflagellate population which the group accounted for. It should be borne in mind that abundances of less than 30 cells per ml were frequently calculated from the observation of only one or two cells on a filter and therefore have little statistical validity.

5.6b The abundance of the major groups of heterotrophic flagellates

Six taxa (chrysomonads, dinoflagellates, acanthoecid choanoflagellates, non-loricate choanoflagellates, bodonids and bicosoecids) were found to account for a large percentage of the total heterotrophic flagellate population. The seasonal abundance of these taxa is given in appendix 5 and shown in fig. 51. The six groups collectively ranged in abundance from 403 to 2742 cells per ml and accounted for 56 to 82 percent of the flagellate population (average 71.3%) between March 1993 and June 1994. Although numbers fluctuated in each taxon, the proportion of the total flagellate population which each taxon accounted for remained more constant. The percentage abundance of each taxon is shown in appendix 6 and fig. 52. Further details of the groups are given below.

1) Chrysomonads (280-2007 cells per ml, 31.1-61.9%; 44.6% on average).

This group contains chrysomonads in the typical sense (e.g. *Paraphysomonas*), but also the pedinellids. Although pedinellids (like chrysomonads) are stramenopiles, they form a distinct group in terms of ultrastructure. They are included in this category because it was often not possible to distinguish between the two groups. The second short flagellum was sometimes difficult to see on chrysomonads, particularly when there was a lot of detritus in the sample, and the general shape of the cell, and location of the nucleus is similar in members of both groups.

Two distinct size categories were noted in this group: those of less than 2μm, and those greater than 2μm (usually 3μm or more). The abundance of chrysomonads falling into these two size categories is given in appendix 7, and shown in fig. 53. The <2μm category was composed mainly of members of the genus *Spumella*, and the ≥2μm

Figure 51: Abundance of the major taxonomic groups of heterotrophic flagellates at NW Netley, May93-June94

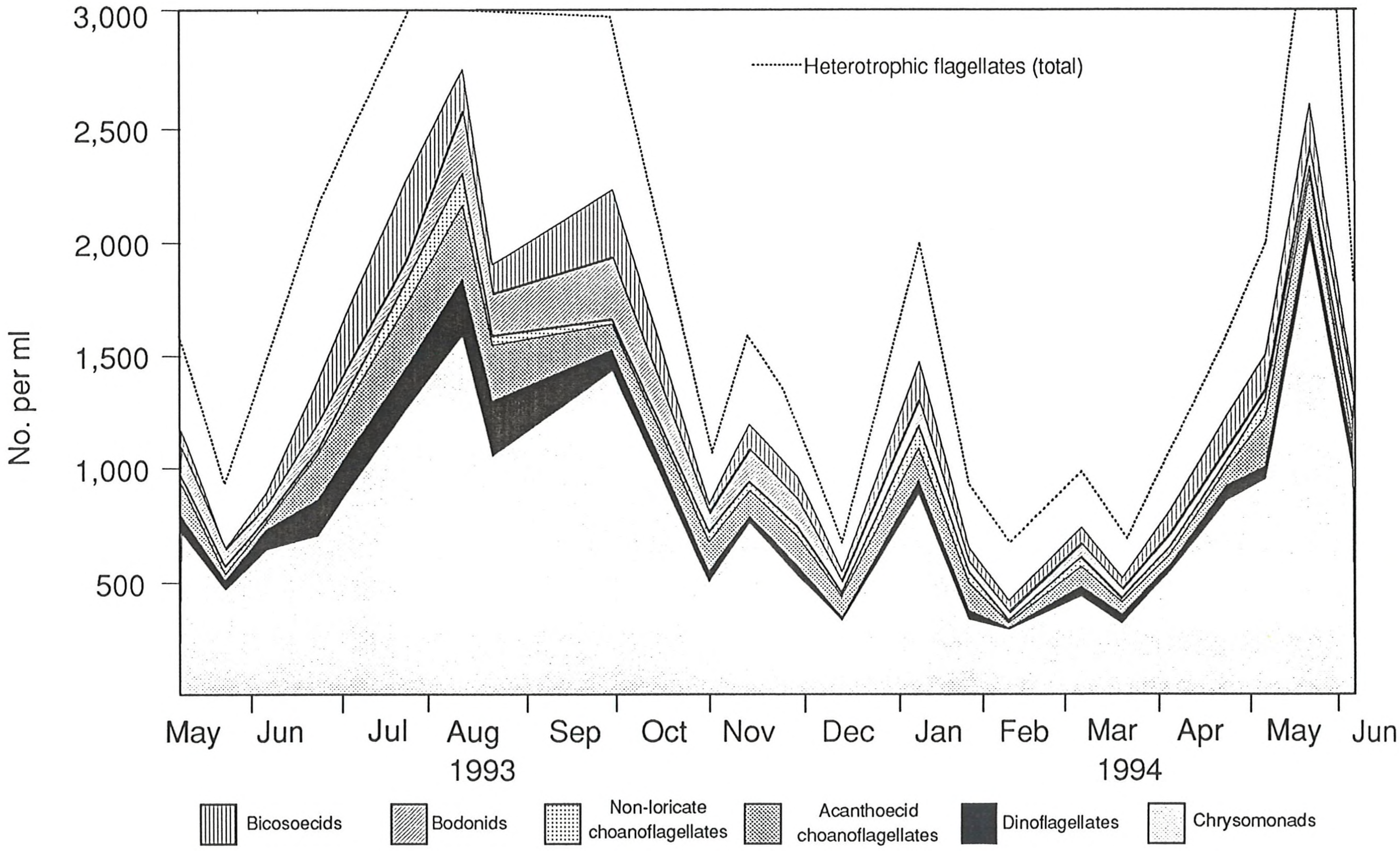


Figure 52: Abundance of the major taxonomic groups as a percentage of all heterotrophic flagellates at NW Netley, May93-June94

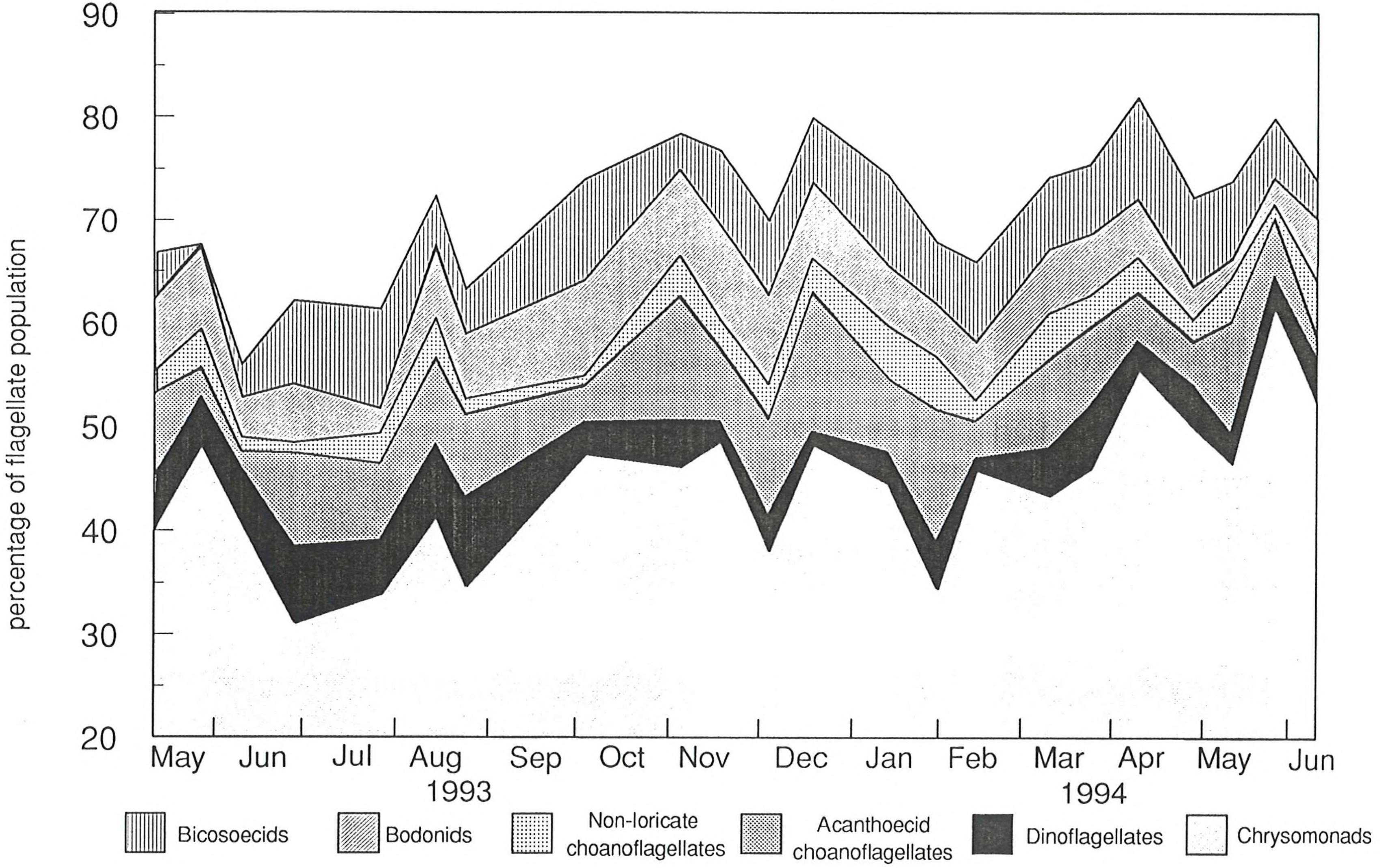
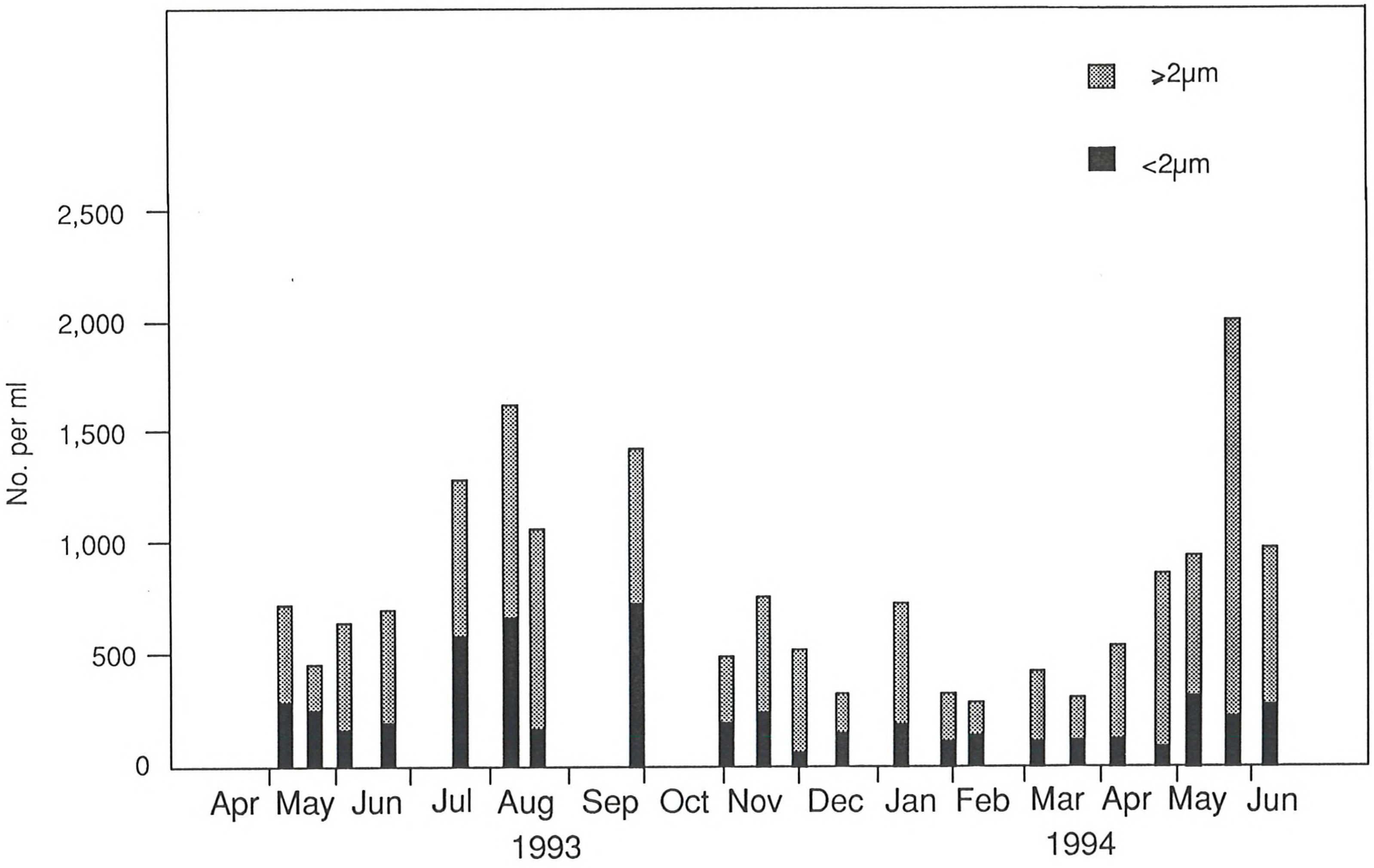


Figure 53: Abundance of two size classes of chrysophytes at NW Netley
May 1993-June 1994



category mainly of *Paraphysomonas* species. These two genera accounted for a very large proportion of the total heterotrophic nanoflagellate population. Within the genus *Paraphysomonas*, *P. imperforata* was by far the most common and numerous species. It was recorded on electron microscope whole-mounts of nearly every sample, and on one occasion (NT30) reached concentrations of around 1500 cells per ml. Pedinellids (*Actinomonas*, *Ciliophrys* and *Pteridomonas*) and chrysomonads other than the two genera mentioned above formed a minor part of the group. Chrysomonads were by far the most abundant heterotrophic flagellate group, accounting for about 45% of the total population on average.

2) Dinoflagellates. (6-253 cells per ml, 1.0-8.3%; 4.0% on average)

Heterotrophic dinoflagellates smaller than 20µm occurred in all samples. They were mostly *Gymnodinium* or *Gyrodinium* species, although *Oxyrrhis marina* was occasionally seen, and fell within this size category. Dinoflagellates which were greater than 20µm were occasionally encountered (since the sample was passed through a mesh of 40µm), but were not enumerated. There was a tendency for dinoflagellates to be less abundant during the winter, both in terms of actual numbers (see fig. 51) and percentage abundance (fig. 52).

3) Acanthoecid choanoflagellates. (23-325 cells per ml, 1.9-13.7%; 7.4% on average)

Acanthoecids occurred at all times of the year, with the greatest abundances occurring in spring and summer, mainly because of blooms of two previously undescribed species of *Parvicorbicula*, which occurred only at this time of year. Further details of the occurrence of different species are discussed in the results from light microscope whole-mounts section above.

4) Non-loricate choanoflagellates. (12-144 cells per ml, 0.8-5.3%; 3.0% on average)

Members of the Salpingoecidae and Codosigidae were difficult to distinguish on occasions, as the theca of *Salpingoeca* species is often thin, and was rarely visible with epifluorescence microscopy. Therefore, these two groups were combined into one category. It is also possible that the category contained those members of the Acanthoecidae which undergo nudiform replication in their non-loricate 'swarmer' stage. However these types of acanthoecids were rarely encountered in light microscope whole-mounts or other studies, so it is unlikely that this was significant. Concentrations of 30-40 cells per ml seemed typical, with higher values in late spring and early summer.

5) Bodonids. (39-274 cells per ml, 2.0-9.2%; 6.0% on average)

Numbers of bodonids tended to be lower during the winter (around 40-60 per ml) than during the summer, but the abundance did not follow a strictly seasonal cycle. It was noted that relatively high values occurred in the autumn and winter samples which contained a large amount of detritus.

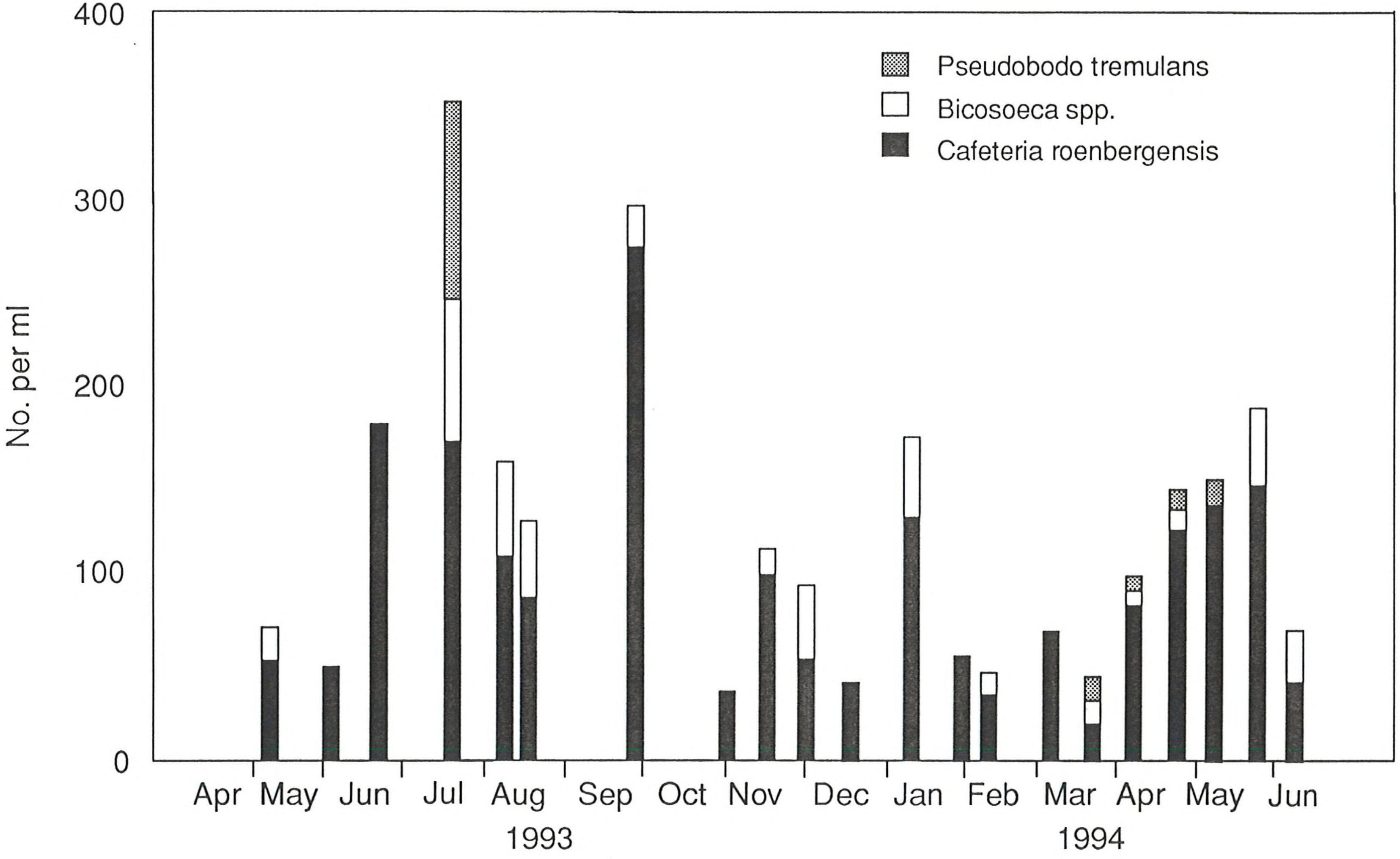
6) Bicosoecids. (42-353 cells per ml, 3.2-9.9%; 6.7% on average)

Bicosoecids showed a relatively typical seasonal cycle of abundance with lower values over the winter and higher values during the summer, however numbers in a few summer samples (NT9, NT11, NT31) were rather low. *Cafeteria roenbergensis* was the most abundant type of bicosoecid, occurring at densities of up to 179 cell per ml. *Pseudobodo tremulans* was periodically common, but undetectable in many samples. *Bicosoeca* species were found in most samples, generally in low numbers. The sub-division of the bicosoecids into these three genera is shown in appendix 8 and fig. 54. Bicosoecids were not distinguished from chrysomonads in NT9.

5.6c <u>Abundance of 'non-bacterivorous' flagellates</u> (2.1-12.2%, 7.7% on average)

These are flagellates which feed to a large extent on other nanoplankton. They are usually larger than bacterivorous flagellates (10µm+), although the *Telonema* species may be considerably smaller than this. Results are presented in appendix 9 and fig. 55.

Figure 54: Abundance of bicosoecid taxa at NW Netley May 1993-June1994



1) *Cryptaulax marina* (0-167 cells per ml, 0-5.2%)

C. marina was seen in most samples in low numbers, but attained high numbers on two occasions, coinciding with a bloom of *Eutreptiella marina* in NT11, and a bloom of haptophytes (*Phaeocystis*) in NT30. All individuals in freshly collected material had algal remains in food vacuoles, although the species often occurred in enrichment cultures where it fed on heterotrophic flagellates. The algal remains could often be distinguished, and included centric diatoms, cryptophytes, haptophytes, euglenids, chrysophytes and dinoflagellates. *C. marina* appeared to be able to exploit the dominant nanoalgal group in each sample. In NT11 when it was feeding on *Eutreptiella*, cells were almost twice the usual dimensions.

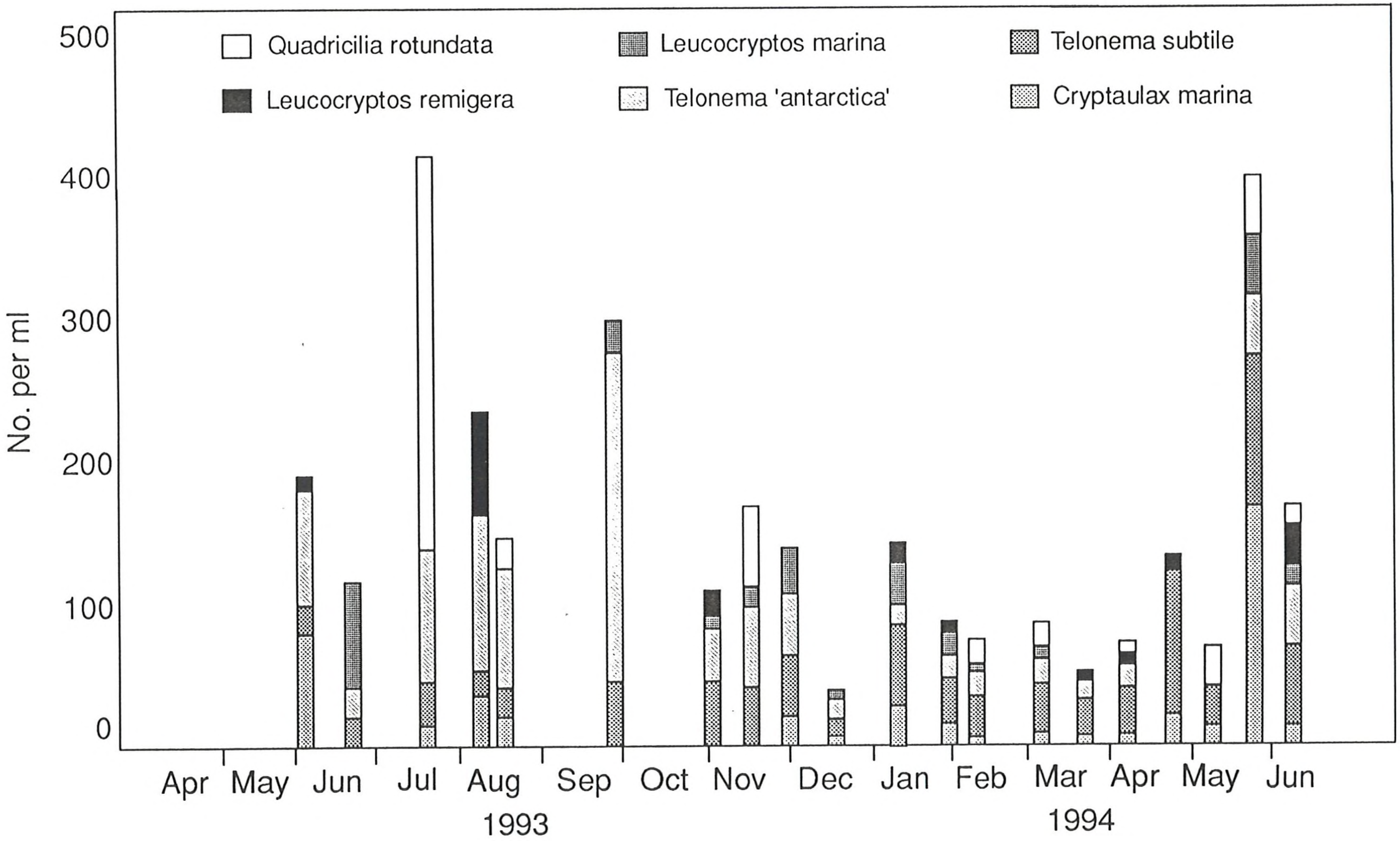
2) *Telonema* 'antarctica' (0-229 cells per ml, 0-7.6%)

Although this species has not been observed feeding it is presumed to be predatory because of its affinities with *T. subtile*, and because of observations of a reduction in flagellate numbers and diversity when it appeared in enrichment cultures, as seen for *T. subtile*. *Telonema* 'antarctica' was seen in nearly every sample, often in high numbers during the summer of 1993 (80, 92, 108 and 229 cells per ml in samples NT11, NT13, NT14 and NT16 respectively).

3) *Telonema subtile* (14-105 cells per ml, 0.5-5.9%)

This species was seen in every sample, and is one of the easiest heterotrophic species to recognise with epifluorescence microscopy because it retains its characteristic shape after fixation. It appears to feed mainly on heterotrophic cells, although algal remains were occasionally observed in food vacuoles. *T. subtile* was present in high numbers in two samples during the summer of 1994 (100 and 105 cells per ml in samples NT28 and NT30 respectively).

Figure 55: Abundance of 'non-bacterivorous' flagellates at NW Netley
June 1993-June1994



4) *Leucocryptos marina* (0-74 cells per ml, 0-3.4%)

This species is easily identified with epifluorescence microscopy because of its characteristic droplet shape with two equal, thickened flagella inserted near the broadest end. It appeared in most samples, but was sometimes below the level of detection in epifluorescence counts (i.e. less than one cell per 100 fields of view). The greatest abundance (74 per ml) occurred in June 1993, although it was below the level of detection in the preceding and following samples.

5) *Leucocryptos remigera* (0-72 cells per ml, 0-1.9%)

This species could be distinguished from *L. marina* by its cylindrical shape and longer flagella. It occurred sporadically, in high numbers on one occasion (72 cells per ml, NT14). *L. remigera* and *L. marina* appeared to feed mainly on autotrophic nanoplankton.

6) *Quadricilia rotundata* (0-276 cells per ml; 0-7.5%)

Quadricilia occurred sporadically, usually in low numbers, although cells reached an abundance of 276 per ml in NT13, and 56 per ml in NT19. Cells seen in living centrifuged material usually had algal food contents.

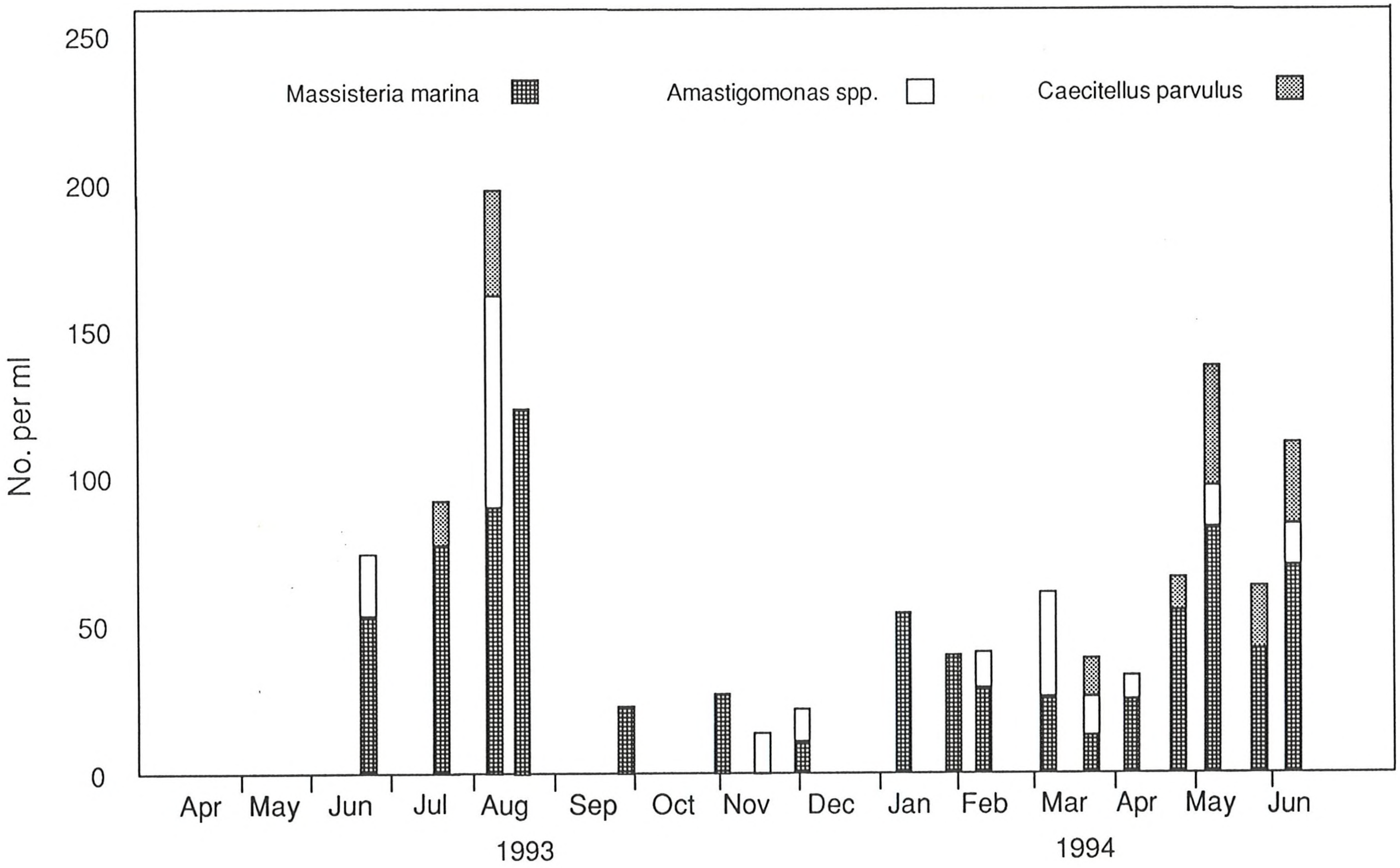
5.6d Abundance of other commonly occurring species

Three bacterivorous species, which did not fall into any of the major flagellate taxa (in 5.6b) were frequently identified in epifluorescence counts. The relative abundance of these three species is presented in appendix 10 and fig. 56.

1) *Massisteria marina* (0-124 cells per ml, 0-4.7%)

This cercomonad was distinguished in epifluorescence counts by its sessile form (circular cell with central nucleus and radiating pseudopodia). The granules borne on the pseudopodia were often visible. Figures for the abundance of this species are

Figure 56: Abundance of three taxa of heterotrophic flagellates at NW. Netley June1993-June1994



underestimates, because it also occurs in a motile gliding or swimming form. The motile form could not be distinguished from *Cercomonas* species, however large numbers of '*Cercomonas*' occurred on only one occasion (NT29), and it is thought that this was the same species which later developed in enrichment cultures of this sample (*Cercomonas* sp., section 3.6). Abundances of greater than 50 cells per ml were common during the summer.

2) *Amastigomonas* spp. (0-72 cells per ml; 0-3.6%)

Amastigomonas species (apusomonads) were frequently detected in epifluorescence counts (usually *A. debruynei*). However the values presented in appendix 10 may be underestimates since its amoeboid changeable form means that some cells may not have been identified. *A. debruynei* occurred frequently in enrichment cultures, although usually in low numbers. Abundances calculated from epifluorescence counts are also low.

3) *Caecitellus parvulus* (0-41 cells per ml)

Caecitellus (Protista *incertae sedis*) was detected in several summer samples, in low numbers. However, it occurred in enrichment cultures from most samples, and it is likely that cells were often not identified.

5.6e Abundance of unidentified flagellates and minor groups

The percentage of the total flagellate population in this group drops from about 30% in samples NT9-NT13, to about 15% (or less) in the remaining samples (table 7). This reflects the increased ability to identify different taxonomic groups in epifluorescence counts resulting from a combination of experience and greater competence in preparing the sample.

In some cases individual species could be identified, but their occurrence was sporadic and/or abundance low, so they were not recorded as separate categories but grouped with

Table 7: Information on less abundant groups and forms which could not be identified with epifluorescence microscopy (No. per ml.)

SAMPLE	het flags (total)	small groups and unknowns (?)	'?' as % of total	Other groups distinguished (not included in other tables)
NT9 12.03.93	1649	441	26.7	
NT10 21.05.93	934	274	29.3	
NT11 04.06.93	1557	472	30.3	
NT12 22.06.93	2202	643	29.2	
NT13 21.07.93	3669	906	24.7	
NT14 09.08.93	3788	595	15.7	
NT15 19.08.93	2975	372	12.5	456 of single unknown taxon
NT16 29.09.93	2994	434	14.5	
NT17 15.10.93	1227	-	-	
NT18 01.11.93	1052	91	8.7	
NT19 15.11.93	1529	168	10.9	
NT20 01.12.93	1350	246	18.2	
NT21 16.12.93	662	98	14.8	
NT22 11.01.94	1957	300	15.3	157 *Rigidomastix*
NT23 28.01.94	936	169	18.1	
NT24 11.02.94	612	94	15.3	
NT25 08.03.94	975	104	10.7	
NT26 22.03.94	665	65	9.8	
NT27 07.04.93	992	90	9.1	
NT28 26.04.94	1683	266	15.8	
NT29 09.05.94	2008	248	12.4	69 *Cercomonas* sp.
NT30 (a) 24.05.94	3240	188	5.8	
NT31 (0m) 08.06.94	1863	126	6.8	84 *Rigidomastix*
NT31 (2m) 08.06.94	1195	108	9.0	36 *Rigidomastix*
NT31 (7m) 08.06.94	1265	67	5.2	38 *Rigidomastix*

'unknown' flagellates. Occasions where large numbers of distinct/identifiable species occurred are recorded in table 7.

5.6f Variation between two samples of water collected at the same time

On 24.05.94 (NT30) two samples of water ('a' and 'b') were taken from the same location, and the abundance of bacteria, hetero- and autotrophic flagellates and diatoms was measured in both, as were the proportions of the various taxa of autotrophic flagellates. The abundance of plankton groups in the two samples is shown in fig. 57 (data also in table 5) and the abundance of different autotrophic flagellate taxa is shown in table 6.

Variation between estimated abundance of the four plankton groups in the two samples was relatively small; the number of bacteria counted in sample a was about 10% more than that in sample b, the number of flagellates was estimated to be about 4% more in sample a than sample b, and the number of autotrophic flagellates and diatoms were estimated to be 10% and 17% more in sample b than sample a respectively.

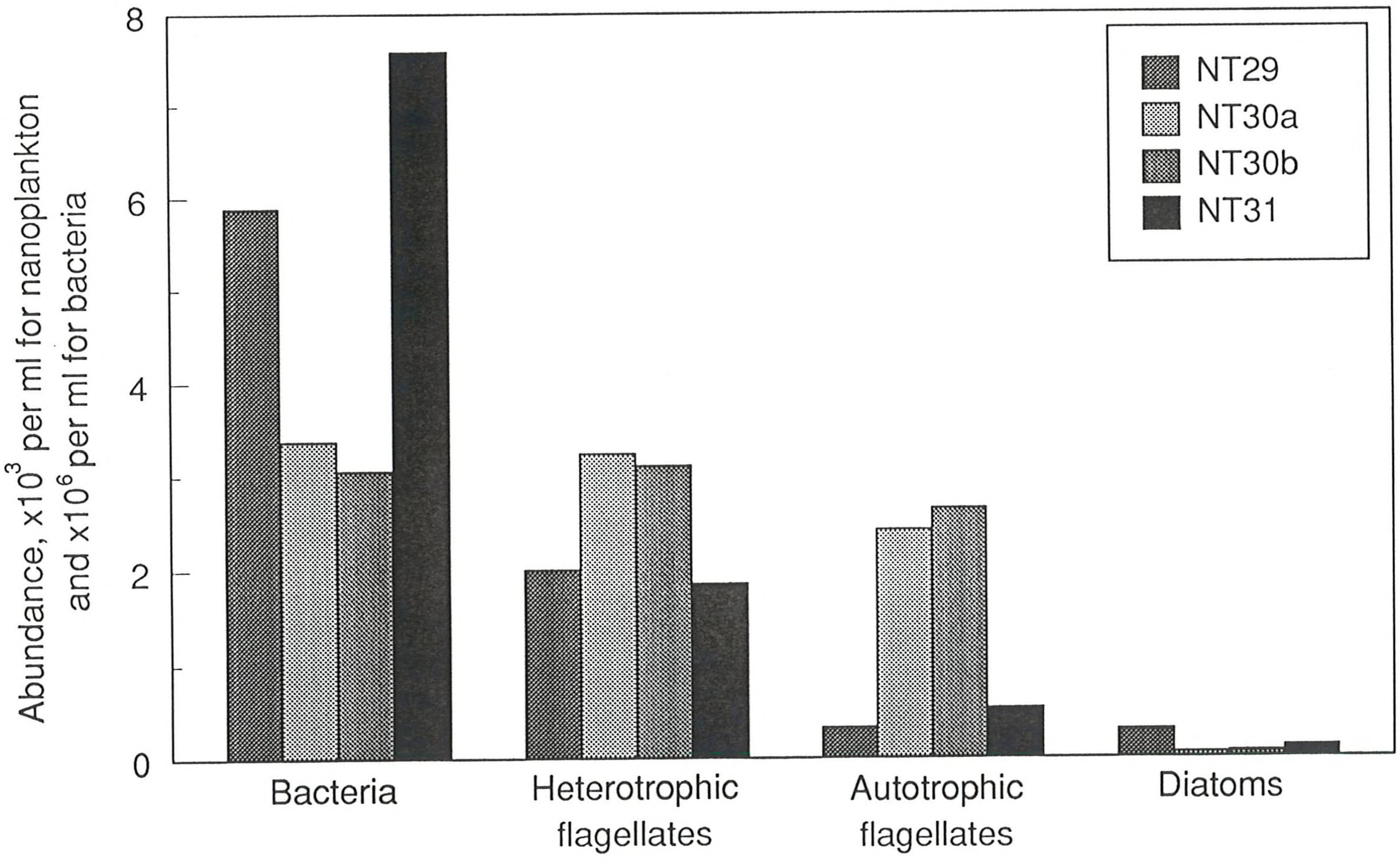
Since counts were not replicated for each of the two NT30 samples, it is not possible to suggest how much of the variation is due to sample preparation and to statistical error, and how much is due to actual variation between the two samples. However, the difference in abundance of each of the four plankton groups is substantially less than differences between the NT30 samples and the preceding and subsequent samples, NT29 and NT31 (0m), as shown in fig. 57.

Although total numbers of autotrophic flagellates were similar in both samples, the abundance of different taxa showed more variation, with greater numbers of cryptophytes and chrysophytes in sample a, and greater numbers of haptophytes in sample b (table 6).

5.6g Variation in samples collected at different depths

Water samples were collected from three depths: 0m, 2m and 7m, on 08.06.94 (NT31).

Figure: 57: Variation in plankton within the NT30 sample, and comparison with the NT29 and NT31 samples



The abundance of bacteria, hetero- and autotrophic flagellates and diatoms at these depths is shown in fig. 58. The numbers of bacteria showed little variation between the three depths, with a difference of only 5.5% between the lowest value (at 2m) and greatest value (at 7m). Heterotrophic flagellates were most numerous at the surface, where the abundance was 56% greater than 2m (the lowest value). Autotrophic flagellates were also most numerous at the surface, where the abundance was 48% greater than at 2m. Diatoms were most abundant at 7m, and least abundant at the surface with about half the number found at 7m.

The estimated biovolume of heterotrophic flagellates per ml was greatest for the sample collected at 2m (fig. 59c), despite having the lowest abundance of flagellates. This reflects a greater number of cells with large diameters, which may have been due to a greater number of *Cryptaulax marina* and *Leucocryptos marina* cells at this depth (fig. 59b), which are two of the largest species recorded in the sample.

Variation in the numbers of heterotrophic flagellates in the six major taxa is shown in fig. 59a. The number of chrysomonads was far greater at 0m than at the other two depths, and this largely accounted for the greater number of heterotrophic flagellates at this depth. Numbers of dinoflagellates and the two choanoflagellate categories were similar at each depth. Bodonids were more abundant at the surface than the other two depths, and bicosoecids were more abundant at 7m, due the presence of a larger number of *Pseudobodo* cells.

The abundance of 'non-bacterivorous' flagellates species is shown in fig. 59b. Since all six species were present in low numbers, variation between the different depths is difficult to assess. However, the values for *Cryptaulax marina*, *Leucocryptos marina* and *Telonema subtile* were similar at 0 and 7m, with the first two species being more abundant at 2m, and *T. subtile* being less abundant at this depth.

The abundance of other flagellate taxa at the three depths is shown in appendix 10 and table 7. The number of *Massisteria marina* and *Rigidomastix* 'devoratum' cells was slightly greater at 0m.

Figure 58: Abundance of bacteria and nanoplankton in the NT31 sample

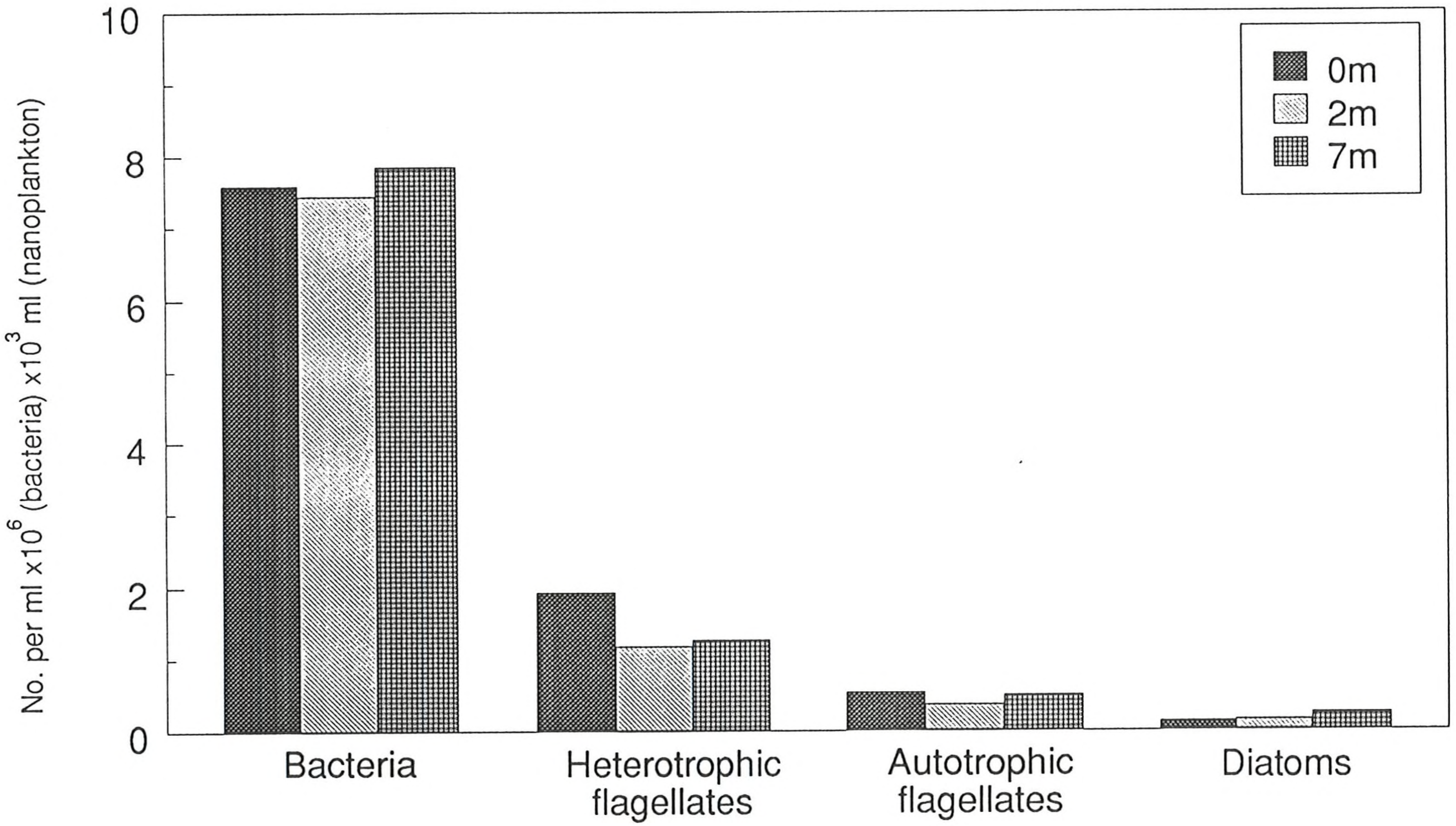


Figure 59a: Abundance of the major taxa of heterotrophic flagellates in NT31

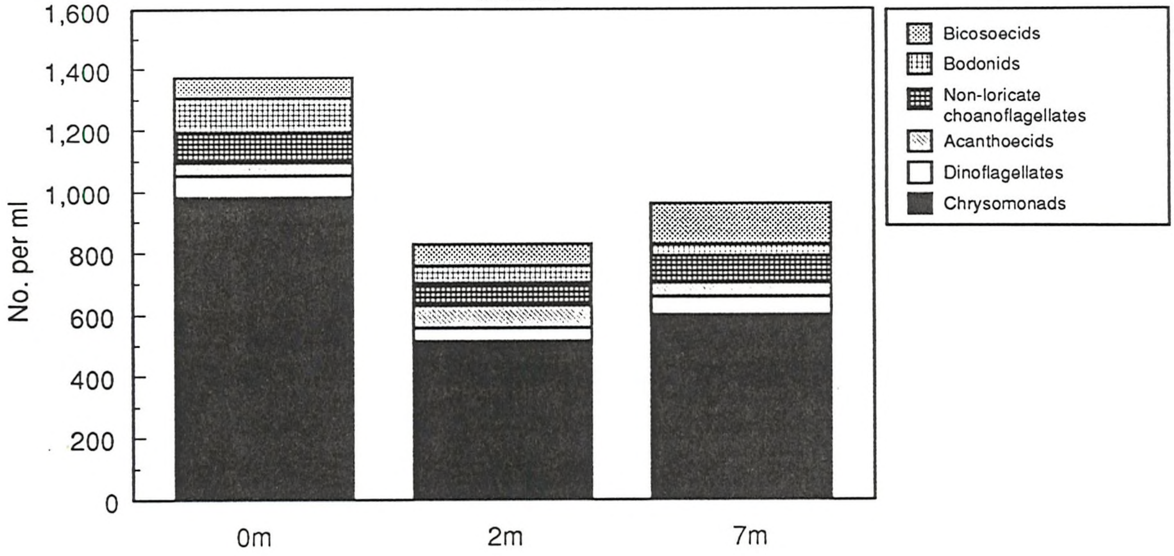


Figure 59b: Abundance of 'non-bacterivorous' flagellates in NT31

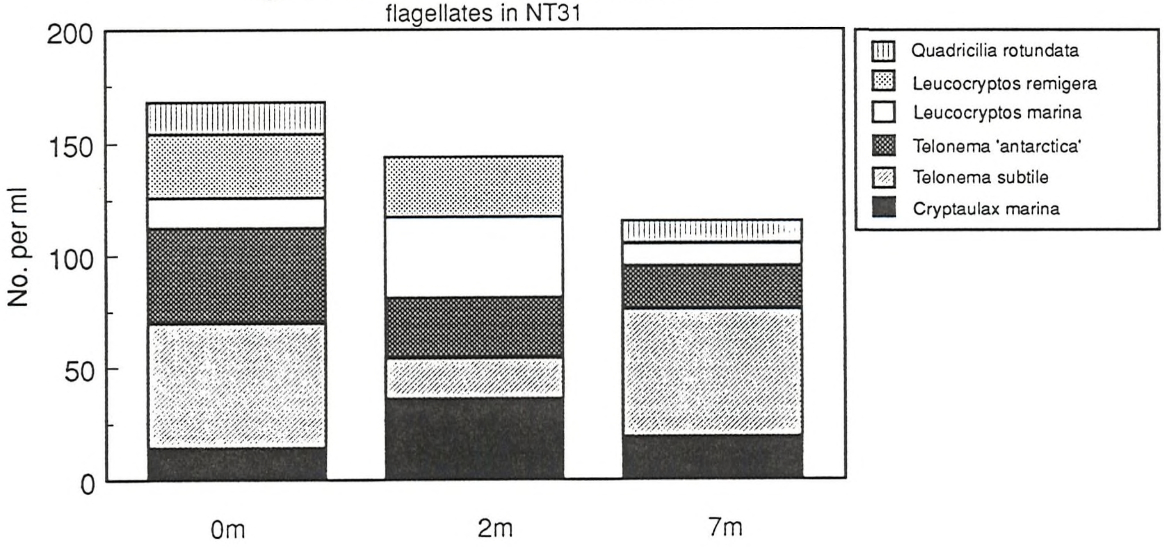
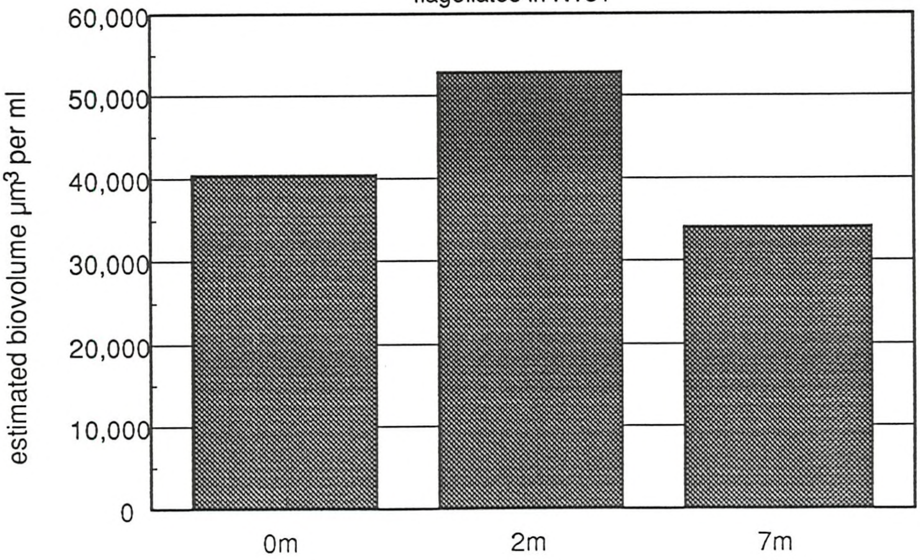


Figure 59c: Estimated biovolume of heterotrophic flagellates in NT31



Variation in the different taxa of autotrophic flagellates is shown in table 6. Euglenids and prasinophytes were absent or in low numbers at each depth. Cryptophytes were most abundant at 0m and 7m, chrysophytes most abundant at 0m and haptophytes most abundant at 7m. Dinoflagellates were slightly more abundant at 2m than the other depths.

There are no clear patterns in the variation of the abundance of plankton groups, or their constituent taxa and increasing depth in the NT31 sample. Rather, there seems to be more similarity between the samples at 0m and 7m, than between the 2m sample and either of the other two depths. The abundances of bacteria, heterotrophic and autotrophic flagellates were all lowest at 2m, although there is not a great deal of difference from values at the other depths. However, the estimated biovolume of flagellates was greatest at 2m, probably due to the presence of a greater number of the largest nanoflagellate species in the sample.

Chapter 6: Discussion of the study of plankton abundance and taxonomy at NW Netley

A study of bacteria and nanoplankton at NW Netley was achieved through measurements of plankton abundance by epifluorescence microscopy, and taxonomic studies on this material supplemented by observations on living, centrifuged material, and on fixed material on whole-mount preparations. The methodology used often had to be adapted from existing procedures because of the effects of large amounts of detritus in the estuary. The application of other techniques (light microscope whole-mounts, centrifugation of living material) was also affected by this. In addition to a discussion of the results obtained by each technique, information from all the types of methodology used is pooled to try and give an overall understanding of factors affecting the abundance of heterotrophic nanoflagellates, and their impact on potential prey groups (bacteria, autotrophic nanoflagellates and nanoplanktonic diatoms).

6.1 Physical variables and chlorophyll a concentration

Chlorophyll a concentrations were measured at NW Netley at depths of 5 and 10m (Feb 1993-Feb 1994) and varied from 0.5-12.3 mg m^{-3} (or $\mu g\ l^{-1}$), with measurements at 10m generally being higher. It is likely that values at the surface would differ from these, but they give some idea of the size of the standing stock of primary producers at the site. The data were obtained from samples collected on spring tides, coinciding with sampling in this study. However, it is possible that much higher levels of chlorophyll occurred, since intensive sampling within tidal cycles has shown that levels of chlorophyll in the estuary are at a maximum at neap tides, and then decrease before each spring tide (D.A. Purdie, personal communication). This seems to indicate that there is a build up of phytoplankton when tidal flushing from the estuary is reduced. It is probable that the rest of the microbial community is similarly affected because of direct or indirect interactions with the phytoplankton and/or because populations are subject to similar flushing effects of the tides. Therefore, the fact that most samples in this study were collected during spring tides may have led to lower estimates of plankton abundance than would have been obtained if sampling had been carried out during neap tides. Chlorophyll a concentration (using an average of the 5m and 10m data) correlates quite closely to the total abundance of heterotrophic nanoflagellates (fig. 60a). This may partly be as a result of certain taxa of flagellates feeding on phytoplankton (see section 6.11). It is probably also because of indirect effects on flagellates, including the exudation of DOM by phytoplankton, leading to the growth of bacteria, on which many flagellates feed. And the fact that the production of all plankton groups in the estuary show similar seasonal trends in their magnitude, because of the effects of temperature on metabolism (and other factors) would also cause these two variables to be correlated.

Numerous factors besides the tidal cycle cause conditions within an estuary to vary, and will have an effect on the numbers and taxa of plankton groups. The extent and the mechanisms of the effects of such variables on heterotrophic flagellates remain largely uninvestigated. Records of temperature and salinity have been made for the samples in this study whenever possible, but little is known about the range of tolerance of these two factors within different taxa of flagellates, although the ranges of tolerance for some of the species seen in this study are listed in Vørs (1992b). The evidence available would suggest that many species are very tolerant of such variations. The same species are often found in both sea water and fresh water (e.g. *Ciliophrys infusionum*, *Paraphysomonas vestita*, *Petalomonas pusilla* and *Rhynchomonas nasuta*). Similarly, many species have been found in different locations with widely differing water temperatures, and in temperate locations at different times of the year. Laboratory observations also support concepts of tolerance to these factors. Most species in enrichment cultures or mono-culture can be maintained at any temperature between 4 and 25°C (other temperatures were not investigated), and of eight species inoculated accidentally into artificial seawater which was five times too concentrated (i.e. 175‰), five species survived. One exception in the tolerance shown to salinity and temperature may be the acanthoecid choanoflagellates (those with a siliceous lorica). This group is only found in marine and brackish waters, and there is some indication that some species are restricted to either cold or warm waters (see section 3.17). The total abundance of heterotrophic flagellates is quite closely correlated to temperature (fig. 60b), probably reflecting the effect of temperature on metabolism and growth, and also similar effects on the metabolism of other plankton groups serving as prey for the flagellates.

Figure 60a: Regression of chlorophyll a concentration versus heterotrophic flagellate abundance

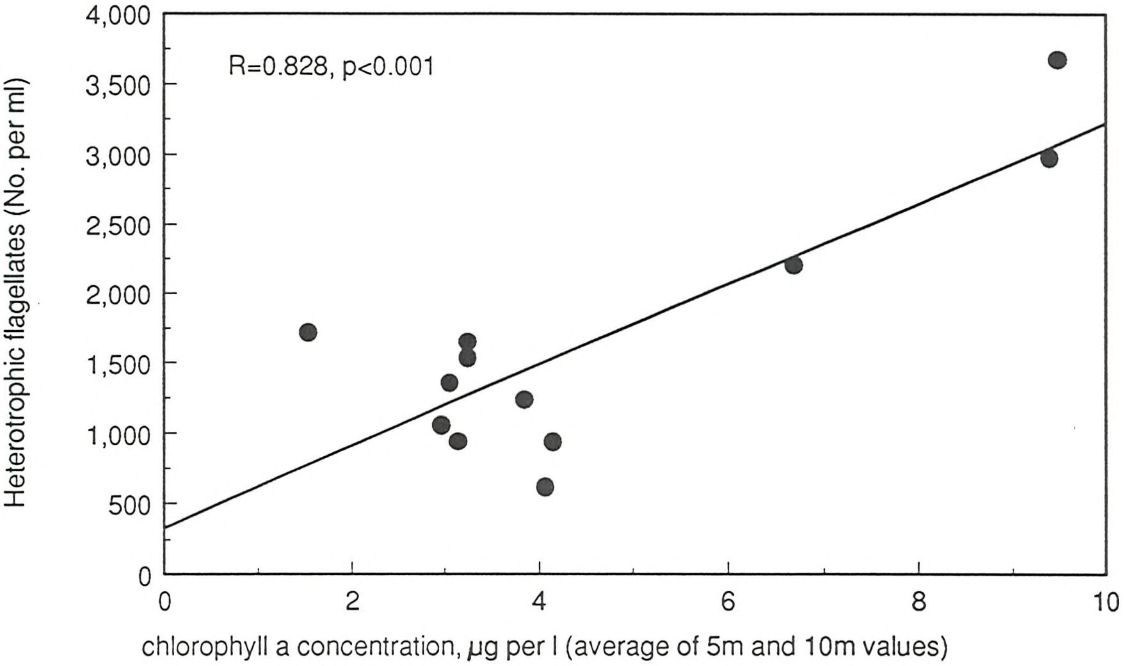
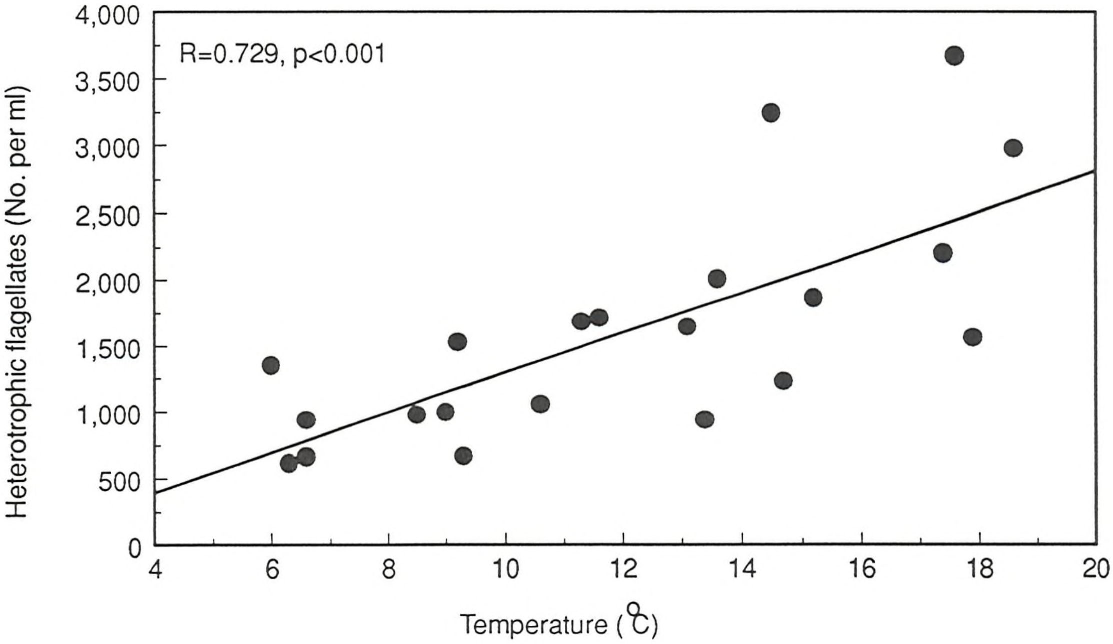


Figure 60b: Regression of temperature (°C) versus heterotrophic flagellate abundance



In shallow estuarine locations, weather conditions may also affect the types of species found in the water column. If the wind is sufficiently strong for the sediment to be disturbed, the flagellates which are typically benthic species become temporarily suspended. In fact there seem to be very few taxa which are exclusively benthic; many species feed in contact with a surface, but can utilise flocs of detritus or other organisms within the water column for this purpose. However the large (>10μm) heterotrophic euglenids, certain dinoflagellates, and a few *incertae sedis* taxa such as *Discocelis* appear to be truly benthic (Larsen and Patterson 1990, Vørs 1992b). One of the Netley samples, NT9, was collected in particularly windy weather, and several of the species found in this sample are characteristic of sediment habitats, including *Allantion tachyploon*, *Metromonas simplex* and the *Petalomonas* species. The Mayflower Park samples, which were collected at the waters edge, also contained a few species characteristic of sediments.

6.2 Centrifuged material

It was necessary to concentrate material to provide a suitable density of cells for light and electron microscope whole-mounts, but the centrifuged material was found to be of little use to examine the diversity of living heterotrophic flagellates. The diversity of autotrophic flagellates and diatoms was found to be similar to that seen in epifluorescence studies, although taxonomic data were not actually recorded. However, amongst the heterotrophs only forms with loricas (acanthoecid choanoflagellates) or more 'rigid' cell surfaces reinforced by sub-pellicular microtubules (e.g. dinoflagellates, *Leucocryptos* and *Telonema* species) were commonly seen. These were also the sorts of species most frequently reported by Vørs (1992b) in centrifuged material from the coast of Finland.

The diversity of heterotrophic flagellates in centrifuged material in this study seemed to be linked to the amount of detritus in the sample, i.e. diversity was highest in samples with the smallest amount of detritus. Generally, the amount of detritus in samples was high during autumn and spring, very high during winter, and relatively low during summer. Correspondingly, flagellate diversity in centrifuged material was lowest during the winter, although the diversity of flagellates seen by other methods remained high. Vørs (1992b) also reported a lower diversity of flagellates in centrifuged material from winter samples, in comparison to samples collected during the summer. The area which was sampled in the Finnish study can contain substantial amounts of detritus in the water column (J. Ekebom and J. Ikävalko, personal communication), but it is not known whether this was a factor affecting flagellates in individual samples.

6.3 Light microscope whole-mounts and the occurrence of acanthoecid choanoflagellates

The use of light microscope whole-mounts allows identification of acanthoecid choanoflagellates, without resorting to electron microscopy, and means that a relatively large proportion of the sample can be examined. However, it cannot yield quantitative results since when samples are rinsed after fixation, the majority of the material is washed away, leaving only a small percentage. In addition, many of the cells in this study were obscured by detrital flocs and could not be enumerated. A small number of cells had loricas which were damaged and could not be identified; this became more significant in the autumn and winter, as increased amounts of detritus and sediment appeared to damage the loricas (and all types of flagellates) during centrifugation. It is possible that rinsing disproportionately removes different taxa due to the morphology or weight of their loricas conferring different adhesive properties. Also, forms with small loricas or very dense loricas may be overlooked (the latter because they blend into the background of detritus).

The taxonomic information derived from the whole-mounts was broadly supported from observations on living centrifuged material and electron microscope whole-mounts, although relatively few cells were seen in these studies. The species which appeared only rarely in enrichment cultures such as *Bicosta minor*, *Crinolina isefiordensis* and *Parvicorbicula* 'manubriata' did so when particularly high numbers of that taxon appeared on the whole-mounts. The species which grew well in enrichment cultures were those with dense loricas composed of numerous costal strips: *Acanthocorbis apoda*, *A. unguiculata*, *Acanthoeca spectabilis*, *Diaphanoeca grandis*, *Diplotheca costata*, *Saepicula pulchra*, *Savillea micropora*, *S. parva*, *Stephanoeca diplocostata*, *St. elegans*, and *St.*

norrisii. These species were recorded less frequently, or even not at all, in light microscope whole-mounts. This was perhaps partly due to the fact that species with dense loricas are difficult to distinguish from detritus when scanning a preparation, but also seems to reflect that these species were generally only present in small numbers in the sample, and attained large numbers only when presented with the more eutrophic conditions of the enrichment culture. These species also seem to be associated with estuarine or near-coastal sites; only species with more open loricas are recorded from truly oceanic sites (see, for example, the species listed in Leadbeater 1972a, Thomsen 1973, 1979, Thomsen *et al.* 1991 (coastal sites) and compare with those reported from Buck and Garrison 1988, Booth 1990, Thomsen *et al.* 1990 (open water sites)).

In conclusion, the use of light microscope whole-mounts is a useful way of examining acanthoecid diversity at a particular site, but would probably reflect the abundance of particular species more accurately in off-shore and open water sites, where there is less detritus to obscure and damage loricas. It should be used in conjunction with other methods such as electron microscope whole-mounts, where the smallest species are less likely to be overlooked, and observations on enrichment cultures which reveal the presence of species with dense loricas, which become distorted when cells are killed, and are then difficult to resolve with light microscopy.

6.4 Epifluorescence microscopy counts in a eutrophic marine environment

The methodology used in the preparation of epifluorescence filters had to be adapted from standard techniques because of problems associated with working in a eutrophic estuarine environment, with a large amount of detritus suspended in the water column in comparison to locations where similar studies have been carried out. A double-staining technique using DAPI and primulin (e.g. Martinussen and Thingstad 1991) was not possible because detritus was stained yellow by the DAPI and obscured protistan cells which were stained yellow by primulin. This led to some decrease in the amount of detail which could be distinguished in flagellate cells. Although the same features could be seen in both primulin and DAPI stained cells, the cytoplasm and flagella were only weakly stained by DAPI, and the fluorescence faded rapidly so that interpretation was sometimes hindered. A 1μm filter pore size was used as a compromise between retaining the maximum number of protistan cells, and allowing the sample to be concentrated on the filter with the minimal amount of pressure, to reduce damage to the cells. Concentration of samples through a 0.2μm filter (e.g. Andersen and Sørensen 1986, Kuuppo-Leinikki and Kuosa 1989) was only possible by applying a moderate suction, and so these filters were only used in the enumeration of bacteria, which are more robust. It is possible that some protistan cells were lost through the 1μm filter. Living heterotrophic flagellates may pass through pore sizes of 1μm, or even 0.4μm (Cynar *et al.* 1985, Rassoulzadegan and Sheldon 1986). Rassoulzadegan and Sheldon (1986) report that flagellates seem to be able to 'squeeze through' pores which are supposedly half the size of their smallest diameter. However, once cells are fixed they may not be so flexible, although they are still very fragile. In the current study the smallest protistan cells seen in enrichment cultures, and in epifluorescence studies were 1.3μm. Cells smaller than 1.5μm were rare, but there were an appreciable number of cells smaller than 2μm, which fell almost exclusively into the chrysomonad category in epifluorescence studies.

6.5 Bacterial abundance

The abundance of bacteria found at NW Netley over the course of one year varied between 1.11 and 13.4 $\times 10^6$ ml^{-1}. These figures are typical for estuarine and near-shore locations e.g. Delaware Estuary, USA: 1-8 $\times 10^6$ ml^{-1} (Coffin and Sharp 1987), Limfjord, Denmark: 0.5-15.2 x 10^6 ml^{-1} (Andersen and Sørensen 1986), Chesapeake Plume, USA: 1.1-9.1 x 10^6 ml^{-1} (McManus and Fuhrman 1988). They are also within the range reported by Antai (1989) for NW Netley during January 1987 to October 1988 (0.27-19.4 x 10^6 ml^{-1}), but the values reported by Leakey (1989) from June 1986-June 1987 at NW Netley were much lower (0.08-1.9 $\times 10^6$ ml^{-1}). Antai found a typically seasonal pattern of abundance, with low values over the winter, small peaks associated with each of the spring blooms, and a very large peak of abundance during the summer maximum of chlorophyll a, associated with the annual *Mesodinium rubrum* bloom. The pattern was similar in this study (although values did not fall quite so low during the winter) except for the fact that the greatest abundance of bacteria was found during the autumn (11.3, 13.4 and 12.5 x 10^6 ml^{-1} on 29th September, 15th October and 1st November 1993, compared to the summer maximum of 11.4 x 10^6 ml^{-1}). These high numbers of bacteria coincided with and followed a period of intense rainfall that caused flooding along much of the South coast. The freshwater input during this time from the rivers feeding the estuary must have been very high, and probably carried a large amount of suspended sediment from land surrounding the rivers. It is therefore possible that this unseasonably large abundance of bacteria was due, at least in part, to allochthonous input.

6.6 Abundance and taxonomy of autotrophic nanoflagellates and diatoms

The abundance of autotrophic flagellates at NW Netley was measured from April 1993 to June 1994, and varied from 45 to 2414 cells per ml. Diatoms were sampled over the same time-period as autotrophic flagellates and had a similar range of abundance (9-2094 cells per ml). Overall abundances followed a conventional seasonal pattern, with low values during the winter, and several peaks in abundance during spring and summer. Peaks in abundance were associated with blooms of various species, and usually lasted only a short time (i.e. the bloom had disappeared by the next sample). For example during November 1993 there were approximately 600 dinoflagellates per ml in the NT18 sample, but none recorded in the NT19 sample two weeks later (table 6). The bloom of *Phaeocystis* in May 1994 fell from around 2000 cells per ml in the NT30 sample, to around 200 cells per ml in the NT31 sample. A bloom of *Eutreptiella marina* was evident in NT11 (4.6.93; not enumerated), but very few cells were seen two weeks later.

This pattern of short-term blooms associated with particular species was also found at NW Netley during 1988 by Kifle (1992), although his study was more concerned with larger (>20µm) species. It appears that the duration of blooms in Southampton Water is affected by the spring-neap tidal cycle, which affects the residence time of water in the estuary (Kifle and Purdie 1994).

Kifle (1992) also found that diatoms and flagellates had a "reciprocal relationship" in terms of cell numbers, a phenomenon which is also evident in this study, both over a short time scale (i.e. in individual samples) and in the fact that the major nanoautotrophic blooms were mediated by diatoms in 1993, and by flagellates (*Phaeocystis* sp.) in 1994.

Although much of Kifle's taxonomic work was on microplanktonic species, it is possible to make some comparisons with the occurrence of some of the taxa found in this study. Kifle noted a bloom of the euglenid *Eutreptiella marina* in late May 1988 (with abundances of up to 1560 cells per ml at NW Netley). A similar bloom was observed during early June 1993 (NT11), although not enumerated, and appeared to be grazed heavily by the heterotrophic flagellate (probably also a euglenid; section 3.12) *Cryptaulax marina*. However, *Eutreptiella* was not observed during the course of the study (to 8th June) in 1994; a bloom may have occurred slightly later than this. Kifle (1992) reported a bloom of *Phaeocystis* during April of 1988. A bloom of this haptophyte was also seen in May 1994, but only low abundances were observed during 1993. Iriarte (1991) also noted a spring bloom of *Phaeocystis* in May 1990. *Cryptomonas* species were found to be numerous in Kifle's study, reaching an abundance of 1000 per ml in February and March, and dominating the phytoplankton during October of 1988. Cryptophytes were also frequently the most numerous autotrophic flagellates during 1993 and 1994 (table 6). The centric diatom *Skeletonema costatum* formed relatively small blooms during March and April of 1988 (Kifle 1992). Individual cells of *S. costatum* were seen in epifluorescence microscopy preparations, and on electron microscope whole-mounts during early spring of 1993, but since the cells of this species form chains it is likely that many were unable to get through the 40μm mesh with which samples were filtered.

Size-fractionated measurements of chlorophyll a in Southampton Water (Savage 1967, Leakey 1989, Kifle 1992) have suggested that a substantial proportion of primary productivity is carried out by organisms of 20μm or less. Leakey *et al.* (1992) estimated that approximately 61% of phytoplankton biomass was accounted for by the <10μm size fraction on an annual basis. Kifle (1992) found relatively lower amounts of chlorophyll in this size category, and estimated that around 33-34% of primary production was due to organisms of less than 10μm, and that most of the rest of the production was carried out by organisms greater than 20μm in size. The amount of primary production carried out by organisms of less than 3µm (roughly equivalent to the picoplankton) seems to be around 13-18% (Iriarte 1991, Kifle 1992) which is somewhat surprising in view of the low numbers of cyanobacteria and other picophytoplankton reported (Leakey 1989, Iriarte 1991). Cyanobacteria were not enumerated in this study as Iriarte (1991) found that they were typically present at concentrations of only 1000 per ml. This is 1000 times less than the minimum concentrations of bacteria found in this study, and therefore cyanobacteria were not considered to be likely to be a significant food source for flagellates.

This study reveals that the nanoplanktonic primary production can be attributed to a relatively small diversity of species. Diatoms were dominated by three pennate morphotypes, and two or three centric species. Cryptophytes were the dominant group of autotrophic flagellates on a yearly basis, as also found by Kifle (1992), and a very large percentage of the specimens seen belonged to just three species. Chrysophytes (including pedinellids) were the second most important group on an annual basis, and although not studied in detail, appeared to be the most diverse autotrophic taxon of nanoplankton. Dinoflagellates were periodically common, and were dominated by only three or four morphotypes. The only haptophyte which appeared in large numbers (for a short period) was *Phaeocystis* sp. Euglenids were only present in low numbers, except for a short bloom of *Eutreptiella* in 1993, and only two species were seen. Prasinophytes were only found in low numbers, and only three morphotypes were noted. Other taxa of autotrophic flagellates were rarely seen. It should be noted that this study was concentrated on the taxonomy of heterotrophic nanoplankton, and therefore it is possible that the diversity of autotrophic flagellates was somewhat underestimated. It is also likely that other species of autotrophs (particularly diatoms and euglenids) are associated with the benthos.

6.7 Abundance of heterotrophic nanoflagellates

The abundance of heterotrophic flagellates at NW Netley ranged from 0.6-3.7 x 10^3 cells ml^{-1}. These figures are within the range reported for estuarine and near-shore locations in other studies, e.g. Delaware estuary, USA: 0.4-9 x 10^3 ml^{-1} (Coffin and Sharp 1987), Limfjord, Denmark: 0.5-15 x 10^3 ml^{-1} (Andersen and Sørensen 1986), Chesapeake Plume, USA: 0.8-4.4 x 10^3 ml^{-1} (McManus and Fuhrman 1988), although the maximum value is somewhat lower than might be expected for a eutrophic estuary. Antai (1989) found a slightly wider range of flagellate numbers at NW Netley between January 1987 and October 1988 (0.1-8.7 x 10^3 ml^{-1}).

Flagellate numbers followed a general seasonal cycle of low values during the winter and high values during summer, except perhaps for an unseasonably high abundance in NT22 (11.01.94). In this respect they followed the annual cycle of abundance of bacteria, except for the fact that there was no corresponding increase in abundance in response to the high numbers of bacteria in autumn 1993. Plankton dynamics in estuaries, as already indicated in section 6.1, will be affected by the tidal cycle which affects the length of time for which water is resident in the estuary, and also by the input of nutrients, water and organisms from freshwater inflows. Flagellate numbers during the autumn of 1993 may not have been able to increase at a sufficiently high rate to respond to the greater potential food source because of a combination of rapid flushing of cells out of the estuary (and rapid inflow of fresh water), and a lowered rate of metabolism due to reduced water temperature, and perhaps the effects of lower salinity. Another possibility is that for some reason, resident flagellate populations were unable to graze the species of bacteria imported from freshwater and terrestrial environments.

The total abundance of heterotrophic nanoflagellates showed poor correlation to the abundance of bacteria ($R=0.369$, $p<0.1$). Antai (1989) found that there was some correlation between the abundance of the two groups ($R= 0.597$, with a 99% significance level), but that, as might be expected, the abundance of flagellates was more closely correlated to measurements of bacterial production. Other reasons for poor correlation may include the fact that if flagellates are preying on bacteria the two groups would be expected to undergo coupled oscillations in abundance (Fenchel 1982c, Andersen and Fenchel 1985), which would mean that peaks and troughs in the abundance of the two groups would not coincide on a temporal scale. Also, plankton dynamics and abundance will be affected by the import and export of material in an estuary, as mentioned above.

Samples in this study were collected on spring tides, when the flushing of water is at a maximum. Flagellates may also have been feeding on organisms other than bacteria; the likely magnitude of this in Southampton Water is discussed below.

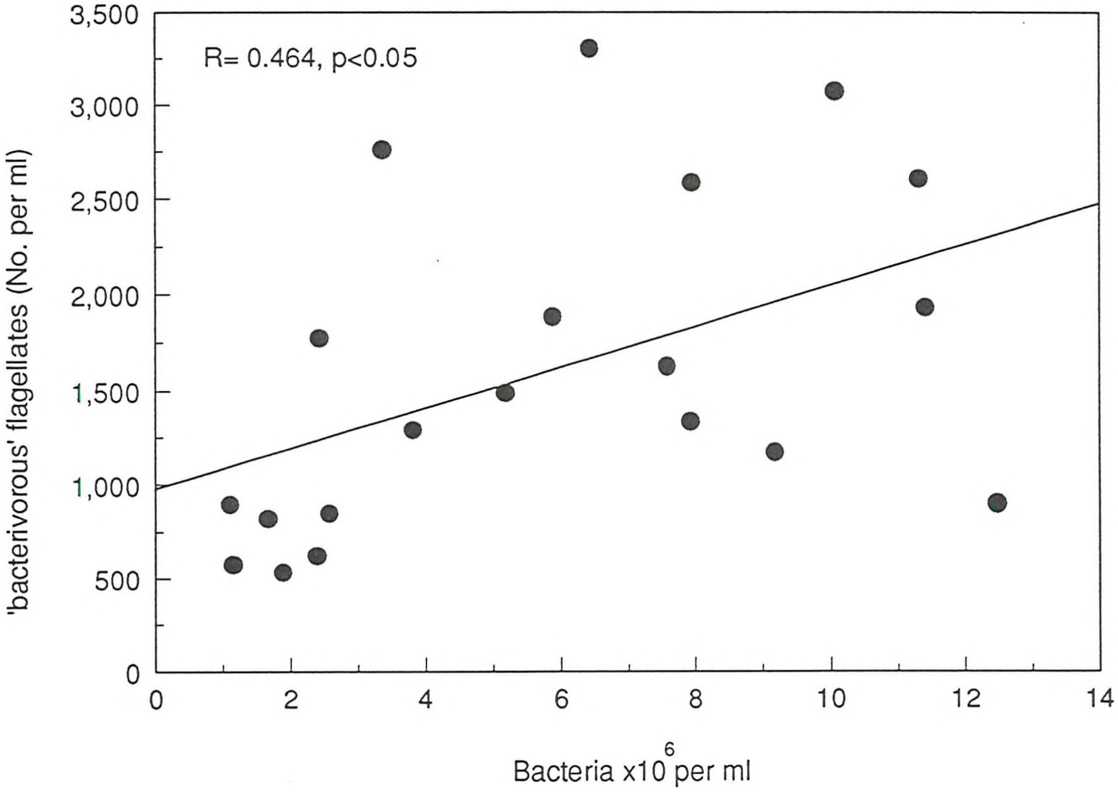
Small heterotrophic flagellates have been shown to graze autotrophic cells in the same size range as bacteria (e.g. Parslow *et al.* 1986, Hagström *et al.* 1988, Kuosa 1991). Picoplanktonic cells may account for a large part of the primary production in open oceanic locations. However, their abundance and contribution to productivity seems to decrease markedly in coastal and estuarine areas (Iriarte 1991). Picoplanktonic eukaryotic autotrophs were very rarely seen in this study, and cyanobacteria are only present in low numbers in Southampton Water (Leakey 1989, Iriarte 1991). Therefore it is unlikely that autotrophic picoplankton constituted a significant food source for flagellates during this study, or contributed to their seasonal dynamics (section 6.6).

Some of the species of heterotrophic flagellates seen in this study are able to graze on nanoplanktonic cells, and so variation in the abundance of organisms larger than bacteria could also affect flagellate numbers. However, analysis of the abundance of the major flagellate taxa in Southampton Water (section 5.b,c) suggests that 'non-bacterivorous' flagellates account for a relatively small proportion (around 12%) of the total population. Some other taxa which feed mainly on bacteria are capable of taking larger prey items; this has particularly been reported in the chrysomonads (e.g. Goldman *et al.* 1985, Suttle *et al.* 1986). However, observations on chrysomonads in water samples collected during this study suggest that this is a rare phenomenon in Southampton Water, even during phytoplankton blooms. The removal of flagellate taxa which are presumed to feed on nanoplanktonic cells yields slightly better (but still poor) correlation between the abundance of bacteria and the abundance of heterotrophic (bacterivorous) flagellates (fig. 61).

The abundance of heterotrophic flagellates would also have been affected by predation. The zooplankton in Southampton Water is dominated by calanoid copepods and cirripede larvae (Zinger 1989, Lucas 1993) which are unlikely to have any substantial impact on nanoflagellate populations (Sanders and Wickham 1993, and references within). However

Figure 61.

Regression of the abundance of bacteria against the abundance of 'bacterivorous' flagellates.



Leakey (1989) has shown that ciliates at NW Netley are dominated by forms with a volume of 10^3-10^5 μm^3 (i.e. of 10-45μm in diameter). Although ciliates of less than 20μm seem to graze almost exclusively on picoplankton (e.g. Sherr and Sherr 1987, Rassoulzadegan *et al.* 1988), small nanoplankton forms a substantial part of the diet of larger ciliates (Rassoulzadegan and Etienne 1981, Rassoulzadegan *et al.* 1988, Bernard and Rassoulzadegan 1990). Therefore at least some of the ciliates present at NW Netley could potentially graze on heterotrophic nanoflagellates, which are dominated by forms of less than 4μm (observations in this study).

6.8 Biovolume and biomass of heterotrophic flagellates at NW Netley

During the first few weeks of the study of plankton dynamics at NW Netley, it was found that the cell size of the heterotrophic flagellate population underwent rapid changes as well as the abundance, and so the diameters of fifty (random) cells were measured in subsequent samples. The volume of 50 cells was combined with measurements of cell abundance, to give an estimated population biovolume. The average volume was greatly influenced by the abundance of certain larger species of flagellates, particularly dinoflagellates, *Cryptaulax marina*, and *Leucocryptos* species, which similarly affected estimates of the population biovolume. Kuuppo (1994) also found that the abundance of large (>7μm) flagellates was responsible for changes in the mean cell volume of nanoflagellates off the coast of Tvärminne, Finland.

The biovolume of flagellates was estimated from measurements on cells fixed in glutaraldehyde, which, although it causes shrinkage of cells, may actually reflect the volume of the flagellate itself, rather than including the volume of prey and vacuole space (see section 5.6a). Flagellate biovolume remained rather stable between mid-October and early May. From January to early May changes in the abundance of flagellates tended to be compensated by changes, in the inverse direction, in the average cell size, causing overall biovolume to remain stable (fig. 50). Although measurements did not cover a full seasonal cycle, there seemed to be a tendency for flagellate biovolume to be greater during the summer.

Biomass estimates (section 5.6a) based on the biovolume of flagellates range from 1.5-52.9μg C l^{-1} in different samples (average 10μg). This compares to ranges of biomass over an annual cycle at NW Netley of 1.3-393.2μg C l^{-1} for bacteria (Antai 1989), and 1.4-219μg C l^{-1} for ciliates (Leakey *et al.* 1992). Although the upper range of biomass for flagellates is lower than for these two groups, the highest biomass of flagellates might be expected during the summer peak of chlorophyll a, during the annual *Mesodinium rubrum* bloom, and biovolume was not measured at this time.

6.9 Spatial variation in plankton abundance

It was not possible to replicate counts of plankton groups within particular samples, or to assess samples from different depths, because of the time involved in making detailed taxonomic studies of each sample (both through epifluorescence studies and the other methods employed). However, two samples were taken on one occasion (NT30) to try to examine the magnitude of variation between two concurrent samples on a small spatial scale, and samples were taken from two additional depths on another occasion (NT31) to examine whether surface samples could be considered to be at all representative of the state of the rest of the water column.

The difference between counts of plankton in the two samples in NT30 was around 10% for bacteria and autotrophic flagellates, 17% for diatoms, and 4% for heterotrophic flagellates. The larger variation between the two counts of diatoms is not surprising, since they were in low numbers, meaning that the observation of only one extra cell on one filter could account for the difference. Otherwise the variation between the two samples was very low, although the fact that counts were not replicated within the two samples means that statistical error may have affected this. Kuuppo-Leinikki (1993) found that variation due to sample preparation seemed to influence the estimated abundance of microbial plankton groups more than where the sample was collected from on a scale of 100-200m. The differences in abundance of plankton on duplicate filters in Kuuppo-Leinikki's study were of a similar magnitude to the differences between the two NT30 samples in this study; 13, 11, and 13% for bacteria, autotrophic and heterotrophic flagellates respectively. Kuuppo-Leinikki (1993) concluded that although one sampling point could not precisely represent her study area, it gave a reliable estimation, with only 3 to 5 fold deviation of microbial densities on a scale of <1km. Comparison of data from the preceding and subsequent samples to NT30 (fig. 57) show that variation between samples on a temporal scale of around 14 days was far greater than variation between two duplicate samples at a particular time. Andersen and Sørensen (1986) also found that spatial heterogeneity in the abundance of microorganisms was low (usually of a similar size to sampling variation) compared to temporal changes. Therefore, although it would have obviously been preferable to replicate all the measurements of abundance made in this study, the evidence available suggests that this would not have revealed a great amount of variation.

Estimates of the abundance of bacteria, diatoms, autotrophic and heterotrophic flagellates at 0m, 2m and 7m at NW Netley on 8th June 1994 revealed relatively little variation (fig. 58, section 5.6g). Numbers of diatoms were low so that it was difficult to assess whether variation in counts was due to actual differences or statistical error and sample preparation. Heterotrophic flagellates showed more variation with depth than the other plankton groups. They were most abundant at the surface, mainly because of a greater number of chrysomonad cells at this depth (section 5.6g). This was despite the fact that the abundance of the presumed prey of the chrysomonads (bacteria) showed no great variation in abundance at different depths. Heterotrophic flagellate biovolume was greatest at 2m, because of a greater number of cells with large diameters, possibly due to a greater abundance of *Cryptaulax marina* and *Leucocryptos marina* cells at this depth.

No clear trends in the variation of microbial plankton with depth were apparent from this limited study. It is likely that benthic taxa could influence 'plankton' abundance at 7m, since the depth of water at NW Netley does not exceed 15m (A. Hirst, personal communication), and 'benthic' species were sometimes seen even in samples collected at the surface. Antai (1989) found that differences in bacterial numbers sampled from 1, 4 and 6m at NW Netley were small, apart from a few occasions during the summer. Numbers of heterotrophic flagellates in Antai's study also showed relatively little variation at different depths during winter samples, but varied more during the summer. The greater variation during the summer may have reflected a smaller degree of water mixing. Leakey (1989) measured the abundance of ciliates at different depths at NW Netley in early April 1986. He found that ciliates increased in numbers below the surface at 1m, then showed some decline below this, although the abundance at 10m was greater than that at 5m. In conclusion, further study of the variation of microbial plankton abundance with depth in Southampton Water would be advantageous, particularly during summer when some stratification may occur. The present study, together with that of Antai (1989) suggests that variation in the abundance of heterotrophic flagellates at different depths may be significant during the summer.

6.10 Abundance of the major taxa of heterotrophic flagellates

There is very little quantitative taxonomic data on heterotrophic flagellates. This reflects both the lack of taxonomic expertise amongst ecologists, and problems in methodology. Epifluorescence microscopy is currently regarded as one of the best ways of enumerating flagellates, but the fixation necessary to preserve cells through this procedure renders many species unidentifiable. Examinations of living material are not practical since natural densities of flagellates mean that very few cells would be encountered at magnifications sufficient to allow identification, and stationary cells, when sparsely distributed, tend to be overlooked. If living material is concentrated by centrifugation or filtration many species are destroyed.

To date, the only taxa to be enumerated (if any) have generally been the choanoflagellates (particularly the acanthoecids) and the dinoflagellates. This is because the loricas of acanthoecids and the rather rigid cell-shapes of dinoflagellates are preserved when material is fixed or concentrated. With the use of fluorescent stains in epifluorescence microscopy, the pseudopodial collar of choanoflagellates, and the condensed chromosomes of dinoflagellates can serve to confirm their identities. The only detailed studies of the abundance of other taxa, previous to this work, have been in the Limfjord, Denmark (Fenchel 1982c, Andersen and Sørensen 1986), and these cover only part of the seasonal cycle. Therefore only a limited comparison of data is possible with taxa other than the choanoflagellates and dinoflagellates.

Acanthoecid choanoflagellates occurred at concentrations ranging from 23-325 cells per ml, with maximum densities occurring during the summer and autumn. They accounted for 1.9-13.7% of the total flagellate population, but values of 7-8% were typical. The non-loricate choanoflagellates accounted for about 3% of the total heterotrophic flagellate population, although actual and relative abundance fluctuated somewhat through the year, with no obvious seasonal pattern. Non-loricate choanoflagellates only exceeded the acanthoecid choanoflagellates in abundance on two occasions: in May 1993 (NT10) and June 1994 (NT31).

Marine salpingoecid and codosigid choanoflagellates have received very little attention in comparison to the acanthoecids. Although they may have been neglected because their cell coverings render them less conspicuous than acanthoecids, the sparsity of records in papers on the taxonomy of choanoflagellates in marine locations does suggest that they play a relatively minor role in comparison to fresh water environments where acanthoecids do not occur (Thomsen and Buck 1991, Vørs 1992b).

Choanoflagellates, particularly acanthoecids, have been frequently reported to form a large proportion of oceanic nanoplankton. For example, Buck and Garrison (1983) found they were the third most abundant group in the ice-edge region of the Weddell Sea, Booth *et al.* (1982) found that they were the fifth most important group in the Gulf of Alaska and Hara *et al.* (1986) found that choanoflagellates and amoebae were the dominant heterotrophic protists in the Indian sector of the Southern Ocean. Although choanoflagellates, particularly the acanthoecids, are undoubtably an abundant group of heterotrophic flagellates in many regions, with concentrations of up to 2.5 $\times 10^4$ ml^{-1} reported (Garrison and Buck 1989), views of their importance may have been biased by the sorts of methodology employed to enumerate plankton in such studies. For example, the use of settling chambers (e.g. Buck and Garrison 1983) is likely to lead to relatively high counts of acanthoecids, since their loricas make them conspicuous, and probably increase their sedimentation rates. Buck and Garrison (1988) found that counts of acanthoecids in settling chambers were 83% those of counts on filters, but it is likely that this percentage would be smaller for other flagellate taxa. Hara *et al.* (1986) froze and thawed plankton samples before they were fixed in mercuric chloride. This process is likely to have destroyed a large proportion of heterotrophic flagellates, perhaps leading to invalid assumptions about the importance of the taxa which remained.

There seems to be something of a trend in the literature that acanthoecid choanoflagellates are particularly prevalent (as a percentage of the total flagellate population) during winter (e.g. Andersen and Sørensen 1986, McManus and Fuhrman 1990, Smith and Hobson 1994), and that (where enumerated) other choanoflagellates are more important during the summer (Fenchel 1982c, Andersen and Sørensen 1986). One exception to this is the study of Kuuppo (1994), where choanoflagellates were found to account for 23% of the flagellate population during June-July (compared to an average yearly percentage of 6.8%), and this was mainly due to a large number of *Bicosta/Calliacantha* cells. In the present study non-loricate choanoflagellates remained at low levels throughout the year, whilst acanthoecids accounted for a greater proportion of the total flagellate population, but there were no clear seasonal patterns of variation.

There has been a surge of interest in marine heterotrophic dinoflagellates in the last few years, particularly in their complex and varied feeding mechanisms, and their potential role in the microbial food web (e.g. Bjørnsen and Kuparinen 1991, Hansen 1991, Lessard 1991, and references within). The abundance of dinoflagellates falling into the microplankton size category (20-200µm) appears to be similar to that of ciliates (in the region of one cell per ml). However recent studies have revealed that the abundance of dinoflagellates in the nanoplankton size category is far greater. Their importance may have been previously overlooked because they were difficult to distinguish from photosynthetic forms once fixed, or by conventional light microscopy, and because they were too small to be included in microplankton counts, and ignored by those studying smaller flagellates which were considered to be bacterivorous (Bjørnsen and Kuparinen 1991).

Verity *et al.* (1993) found dinoflagellates ranging in abundance from 2-414 cells per ml during the North Atlantic spring blooms of 1989 and 1990. Nanoplanktonic cells accounted for 99% of the abundance and 70-88% of total dinoflagellate carbon biomass. It was estimated that their grazing impact could account for 25% of the primary production. Abundances at other open water sites include 130 cells per ml in the Weddell Sea during the austral summer (Bjørnsen and Kuparinen 1991) and 13-217 and 6-123 cells per ml in the Gulf of Alaska and the Atlantic Ocean respectively during July 1986 (Shapiro *et al.* 1989).

To date, the only detailed seasonal study of nanoplanktonic dinoflagellates in an inshore location is by Hansen (1991) in the southern Kattegat. He found that large (>20µm) forms dominated the biomass for most of the year except during the summer when <20µm naked forms were found at the surface at abundances of up to 200 per ml (P.J. Hansen, personal communication). Rogerson and Laybourn-Parry (1992a) also report abundances of dinoflagellates of up to 200 per ml in the Clyde estuary, Scotland. In the present study, dinoflagellates smaller than 20µm were found at abundances of 6-253 per ml, although they only fell below 40 per ml on two occasions. Peak abundances were found during the summer (late June to August 1993).

Chrysomonads were by far the most abundant group of heterotrophic nanoflagellates in this study. Although pedinellids were included in this category, they formed a small percentage of the group, so that just two genera (*Paraphysomonas* and *Spumella*) typically accounted for around 40% of the total flagellate population. Electron microscope whole-mounts of the samples revealed that *Spumella* species were about half as abundant as *Paraphysomonas* species, and generally smaller (cells usually 1.5-3µm when observed in epifluorescence preparations, compared to *Paraphysomonas* cells which were generally 2.5-4µm). For some reason, *Spumella* species rarely proliferated in enrichment cultures. In contrast *Paraphysomonas* species were always the first cells to be seen (after cultures had been incubated for 2-4 days), and always occurred in high numbers in the early stages of species-succession in the culture. Observations on freshly collected material suggest that these flagellates were almost exclusively bacterivorous despite numerous reports of their ability to ingest larger cells (e.g. Caron *et al.* 1986, Suttle *et al.* 1986), and observations of predation on other flagellates and autotrophs in enrichment cultures.

Bodonids were present in abundances of 39-274 cells per ml, and usually accounted for about 6% of the total flagellate population. Abundances tended to be slightly lower during the winter, except when there was a particularly large amount of detritus in the sample. Bodonids are adapted to be closely associated with particles since they feed by picking bacteria off surfaces, and are poor swimmers (Caron 1987, observations in this study). Rogerson and Laybourn-Parry (1992a) found that forms with a trailing flagellum accounted for 20-40% of the heterotrophic flagellate population in the Clyde estuary, and that bodonids were the dominant species within this assemblage. Their observations were based on centrifuged material, which may have disproportionately destroyed some of the taxa. However, observations in this study suggest that bodonids are one of the groups which tend to be affected (destroyed) by centrifugation, and therefore it is unlikely that their importance as a group was overestimated during the study in the Clyde. In contrast Fenchel (1982c) found that bodonids were rare in the Limfjord, Denmark.

Bicosoecids occurred at similar abundances to the bodonids, with numbers often fluctuating in a similar manner in successive samples (appendix 5). *Cafeteria roenbergensis* usually dominated the group numerically, and often attained high numbers in enrichment cultures, although there appeared to be some sort of lag in its growth, so that large numbers of cells were not seen until about 10 days after inoculation, whereas *Bodo* species (particularly *B. designis*) often reached high densities in culture after only 6 or 7 days. Fenchel (1982c) and Andersen and Sørensen (1986) found that bicosoecids were fairly common during the summer in the Limfjord, Denmark, but that they were dominated by *Pseudobodo tremulans*, as opposed to *Cafeteria roenbergensis* in this study.

Cryptomonads have frequently been cited as a major taxon of marine heterotrophic flagellates (e.g. Buck and Garrison 1983, Sherr *et al.* 1984, McManus and Fuhrman 1990). However, no cryptomonads were seen in this study, in epifluorescence studies, or in enrichment cultures. Although cryptomonads undoubtably do occur in marine environments, it is possible that their importance has been overestimated because of the occurrence of forms which are morphologically similar to autotrophic cryptophytes and which have only recently been removed from this assemblage on the basis of ultrastructural studies. Such species include *Cryptaulax*, which may be related to the euglenids (D.J. Patterson, personal communication), and *Leucocryptos*, now placed in Protista *incertae sedis* (Vørs 1992b,c).

Taxa of heterotrophic flagellates have been quantified in the Limfjord, Denmark by Fenchel (1982c) and Andersen and Sørensen (1986), during August-September 1981 and March-November 1983 respectively. The Limfjord is a long and shallow sound. The two studies were carried out at sites with maximum depths of 7.5 and 5m. During the second study period (March-November 1983), the range of temperature (3-22°C) was slightly greater than found in Southampton Water, and the salinity slightly lower (c25‰), and between April and November the chlorophyll a concentration varied between 1 and 12μg chl l^{-1}. Therefore, the abiotic factors, and trophy of the Danish site appear to be similar to the conditions found at NW Netley, although there is less comparison between the tidal regimes at the two sites.

During winter, early spring and autumn Andersen and Sørensen found the flagellate population to be dominated by loricate choanoflagellates (acanthoecids). During late spring and summer of 1983, and during the summer of 1981 the dominant flagellates were naked choanoflagellates (*Monosiga* species), chrysomonads (particularly *Paraphysomonas*) and the naked bicosoecid *Pseudobodo*. Bodonids and helioflagellates (pedinellids) were rare (Fenchel 1982c).

In Southampton Water, there appears to be very little seasonal difference in the relative importance of different flagellate taxa. The actual number of each taxon varies with the total number of heterotrophic flagellates, which does show a seasonal pattern, but the percentage of the total flagellate abundance which each taxon contributes remains far more stable (fig. 52, appendix 6). Therefore the contributions of the most abundant taxa to the total heterotrophic nanoflagellate population can be summarised over the study period: chrysomonads 40-50%, dinoflagellates c4%, loricate choanoflagellates c7.5% (varies more than the other taxa, but with no clear seasonal pattern), non-loricate choanoflagellates 3-4%, bodonids 5-7% and bicosoecids 6-9%.

The lack of similarity between the heterotrophic flagellate communties at Limfjord and

NW Netley; two temperate inshore sites with relatively similar physical parameters, and levels of chlorophyll a; means that at present it is not possible to speculate about the relative importance of different flagellate taxa even in fairly eutrophic near-shore locations. In Southampton Water it appears that the relative contribution of the six most abundant groups of heterotrophic flagellates remains rather stable throughout the year, although further studies would be required to confirm this.

6.11 Abundance of 'non-bacterivorous' flagellates

The main emphasis of this study, particularly the taxonomic part, was on small bacterivorous flagellates. However in studies of freshly collected material it became evident that a substantial portion of the biomass of heterotrophic nanoflagellates was due to forms (usually greater than 10μm) which typically preyed on larger cells (although they have also occasionally been observed to prey on bacteria). In addition to the dinoflagellates (discussed above) the species which fell into this category were the *Leucocryptos* and *Telonema* species, *Cryptaulax marina* and *Quadricilia rotundata*. Other 'predatory' species were observed in enrichment cultures, but these forms are typically benthic, and there was no indication that they were numerically important in the samples collected in this study.

As a group (including the dinoflagellates) these large 'non-bacterivorous' flagellates ranged in abundance from 70 to 598 per ml. Typically, they accounted for 12% of total heterotrophic nanoflagellate abundance (range 6.2-17.3%). If an average diameter of 10μm is assumed for the group, and an average of 3.5μm for the more numerous bacterivorous species (the majority were 3-3.5μm), the 'non-bacterivorous' flagellates would have a biomass roughly three times that of the bacterivorous species in a typical sample.

Observations on freshly collected material (section 5.6c) indicate that 'non-bacterivorous' flagellates feed mainly on phytoplankton in Southampton Water (although they may predate on heterotrophic cells in enrichment cultures). Similar observations have been made by Smetacek (1981), Kuosa and Marcussen (1988) and Vørs (1992b,c). The abundance of 'non-bacterivorous' flagellates at NW Netley showed some correlation with the total abundance of autotrophic nanoplankton (R=0.503, $p<0.02$). However, when the autotrophic nanoplankton is divided into diatoms and flagellates, there is virtually no correlation between 'non-bacterivorous' flagellates and diatoms (R=0.192, $p>0.1$), but quite good correlation with autotrophic nanoflagellates (R=0.716, $p<0.01$). This implies that much of the grazing of these larger heterotrophic flagellates is on autotrophic nanoflagellates. Vørs (1992c) also found that peaks in the abundance of one of the large flagellates seen in this study, *Leucocryptos marina*, coincided with peaks in autotrophic flagellates, but that there was no correlation with the abundance of diatoms. Further observations on living, freshly collected cells could provide further information about any food preferences amongst the larger heterotrophic nanoflagellates, since they are fairly robust and survive concentration procedures (such as centrifugation) far better than many smaller taxa (section 6.2). It may be that the size of prey is a factor; although very small diatoms (3-5µm) were observed in this study, they were comparatively rare, whilst the majority of autotrophic flagellates were less than 6µm. Kuosa and Marcussen (1988) found that large (8-14µm) flagellates off the coast of Finland fed mainly on the smaller nanophytoplankton (3-4µm). Some 'non-bacterivorous' flagellates ingest food through a cytostome (e.g. *Leucocryptos* species (Vørs 1992c) and *Telonema* species (Vørs 1992b)) and the diameter of this may limit the size of prey which can be taken. *Cryptaulax marina*, however, seems to be an exception to this. The ultrastructure of this flagellate has not been investigated, and so the architecture of organelles associated with the uptake of food is not known. However, in this study individuals were observed with relatively large, complete phytoplankton cells in food vacuoles, including a centric diatom with a diameter of 9µm, and *Eutreptiella marina* cells, which had a minimum width of about 7µm, and a length of about 30µm.

6.12 Abundance of unidentified flagellates and minor taxa and the use of epifluorescence microscopy for quantitative taxonomy

The taxa described in sections 6.10 and 6.11 accounted for a very large proportion of the heterotrophic flagellates in most samples. However, there were also a number of minor groups which could be distinguished with epifluorescence microscopy, and a residue of flagellates which could not be identified. The number of flagellates which could not be identified decreased through the study, particularly during the first few samples, reflecting an increased ability to distinguish taxa, and improved preparation techniques, so that better preservation of cell form was achieved. Subsequent to this, unidentified forms usually accounted for 10-15% of the total number of heterotrophic flagellates examined.

Three genera (*Massisteria, Amastigomonas* and *Caecitellus*) were identified in many of the samples. *Massisteria* was frequently present in abundances exceeding 30 cells per ml, although it never exceeded 5% of the total flagellate population. The other two genera were less abundant, but their occurrence may have been underestimated since they are not particularly easy to identify once fixed in glutaraldehyde. The fact that they appeared frequently in enrichment cultures in samples where they were not recorded on epifluorescence filters would tend to support this. Other taxa could sometimes be identified. These were included in the 'unknown' category unless more than 3 or 4 cells were seen in a particular sample. *Rigidomastix* occurred in fairly large numbers in the NT22 and NT31 samples (table 7), and a *Cercomonas* sp., in NT29.

Many taxa of heterotrophic flagellates which developed in enrichment cultures were not identified in epifluorescence studies. Studies on the abundance of taxa have been extremely limited because of the belief that epifluorescence studies can only yield very limited taxonomic information (e.g. Kuuppo 1994). It is not possible to identify many flagellates down to species level with epifluorescence microscopy, but in this study it was possible to assign 85% of the population to a taxonomic grouping. The taxa ranged in size from individual species to large collections of fairly distantly related forms (e.g. 'chrysomonads'), but more detailed analysis of the species within the larger taxa is possible through other concurrent studies on material such as light and electron microscope whole-mounts. In conclusion, epifluorescence studies can provide a wealth of information about the relative importance of different groups of heterotrophic flagellates in a particular location. This information should allow a more detailed picture of the importance of different interactions in microbial food webs to emerge, and can also be used to direct physiological, genetic and other studies to quantitatively important species.

6.13 Size structure of the heterotrophic flagellate population and the trophic role of heterotrophic nanoflagellates

Heterotrophic nanoflagellate populations (in marine and fresh waters) are numerically dominated by small (5µm) cells which have been shown to be major predators on picoplankton (section 1.2). However, more recent studies have shown that flagellates of 5-20µm, although less abundant, may have an equivalent or greater biovolume to the smaller forms. Sherr and Sherr (1991) calculated that although <5µm flagellates accounted for 86% of total abundance in a variety of marine habitats, 75% of the flagellate biovolume was due to forms of greater than 5µm. Similarly, in the present study, nanoflagellates larger than 5µm (mostly larger than 10µm) accounted for about 12% of the population, but are estimated to represent around 75% of nanoflagellate biomass (section 6.11). These cells are capable of ingesting picoplankton but seem to feed predominantly on >2µm (nanoplanktonic) protists (e.g. Smetacek 1981, Sherr and Sherr 1991, Sherr *et al.* 1991, observations in this study). Sherr and Sherr calculated that if >5µm flagellates have a metabolic rate of about ⅓ that of <5µm flagellates (based on the relationship of cell volume and respiration rate of phagotrophic protists proposed by Fenchel and Finlay, 1983), half the grazing impact of heterotrophic flagellates should be on picoplankton, and half on >2µm autotrophs and other nanoplankton. These calculations could also apply to Southampton Water, and should be considered in models of carbon flow within the estuary.

Taxonomic analysis of the >5µm nanoflagellates in this study suggests that, in addition to their food source, they are further segregated from <5µm bacterivorous flagellates by being 'planktonic', rather than being associated with particles (section 3.18).

It appears therefore, that while more detailed taxonomic analysis of flagellate communities would be advantageous, ecologists lacking taxonomic expertise could improve current models of microbial food webs by dividing heterotrophic flagellates into forms of less than 5µm (bacterivores feeding in association with particles) and greater than 5μm (which feed on free-living nanoplankton, particularly phytoplankton). The studies of Sherr and Sherr (1991) and the present study suggest that approximately 15% of nanoflagellates fall into the latter category, accounting for about 75% of the biomass, and that they have a grazing potential which is equivalent to that of bacterivorous flagellates.

Appendix 1: Protists found in samples taken from Southampton Water 1991-1994 (dates of samples in table 1, p13)

SPECIES	MP 1	LS 1	MP 2	CN 1	GB 1	NT 1	NT 2	CN 2	HY 1	MP 3	MP 4	HY 2	NT 3	NT 4	NT 5	NT 6	NT 7	NT 8	NT 9	NT 10
APUSOMONADS																				
Amastigomonas debruynei	√	√	√			√	√	√	√		√	√	√	√	√	√	√	√	√	√
A. mutabilis	√	√	√					√			√	√	√		√			√		
CERCOMONADS																				
Cercomonas sp.		√	√																	
Massisteria marina	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
CHOANOFLAGELLATES; CODOSIGIDAE																				
Kentrosiga 'echina'							√			√		√			√					
CHOANOFLAGELLATES; SALPINGOECIDAE																				
Salpingoeca amphoridium	√										√									
S. infusionum							√					√		√	√					
S. marina																				√
S. tuba	√						√				√									
CHOANOFLAGELLATES; ACANTHOECIDAE																				
Acanthocorbis apoda											√	√		√		√	√	√	√	√
A. campanula											√			√		√		√	√	
A. haurakiana																				
A. unguiculata												√				√				
Acanthoeca brevipoda							√													
A. spectabilis		√												√				√		
Bicosta minor														√	√	√	√	√		√
B. spinifera																				√
Calliacantha longicaudata																				
C. multispina																√	√			
C. natans																	√			
C. simplex														√	√	√	√	√	√	
Cosmoeca norvegica											√			√		√	√	√		
C. ventricosa																				
Crinolina isefiordensis											√	√		√	√	√			√	√
Crucispina cruciformis														√	√	√		√	√	√
Diaphanoeca grandis																		√	√	√
D. pedicellata																				
D. undulata						√												√		
Diplotheca costata												√								
Nannoeca minuta																				
Parvicorbicula circularis																				
P. quadricostata																				
P. socialis																				
P. superpositus						√								√	√	√	√	√	√	
P. 'manubriata'														√	√	√	√	√	√	
P. 'aculeatus'																√	√	√	√	√
Pleurasiga minima														√	√	√	√	√	√	√
P. reynoldsii																				
Polyfibula sphyrelata														√		√	√	√	√	√
Polyoeca dichotoma																				
Saepicula pulchra												√						√		
Savillea micropora											√	√		√					√	
S. parva												√							√	
Stephanoeca cupula							√										√			
S. diplocostata	√										√							√		
S. elegans																				
S. norrisii												√								
CILIATES																				
Mesodinium rubrum												√								
Uronema sp.	√	√	√			√	√	√		√	√		√				√			√
COCCOLITHOPHORIDS																				
Emiliania huxleyi														√		√	√	√		
Papposphaera sp.																				
DINOFLAGELLATES																				
Oxyrrhis marina									√									√		
EUGLENOZOA																				
Diplonema ambulator							√													
Petalomonas minuta											√									
P. pusilla											√		√						√	
Bodo curvifilus	√	√		√				√			√	√		√		√	√			
B. designis	√	√	√	√		√		√	√		√	√	√	√	√		√	√	√	√
B. saliens	√	√						√	√		√	√	√		√			√		√

Appendix 1 (cont.): Protists found in samples taken from Southampton Water 1991-1994 (dates of samples in table 1, p13)

SPECIES	NT 11	NT 12	NT 13	NT 14	NT 15	NT 16	NT 17	NT 18	NT 19	NT 20	NT 21	NT 22	NT 23	NT 24	NT 25	NT 26	NT 27	NT 28	NT 29	NT 30	NT 31
APUSOMONADS																					
Amastigomonas debruynei	√	√		√		√	√	√	√	√	√	√	√	√	√	√	√	√	√		√
A. mutabilis							√		√	√	√	√	√	√		√		√			√
CERCOMONADS																					
Cercomonas sp.								√										√	√		
Massisteria marina	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
CHOANOFLAGELLATES; CODOSIGIDAE																					
Kentrosiga 'echina'				√						√								√			
CHOANOFLAGELLATES; SALPINGOECIDAE																					
Salpingoeca amphoridium					√		√								√					√	
S. infusionum					√		√	√		√	√										
S. marina	√											√					√		√		√
S. tuba	√								√	√										√	
CHOANOFLAGELLATES; ACANTHOECIDAE																					
Acanthocorbis apoda	√	√	√	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√
A. campanula	√	√			√	√	√									√	√	√	√	√	
A. haurakiana			√																		
A. unguiculata					√		√	√	√						√	√		√	√	√	√
Acanthoeca brevipoda							√														
A. spectabilis	√							√				√			√		√			√	
Bicosta minor	√					√		√	√	√	√	√	√	√	√	√	√	√	√	√	√
B. spinifera			√	√	√								√							√	
Calliacantha longicaudata														√							
C. multispina	√	√	√					√			√					√	√	√	√	√	
C. natans	√	√	√			√	√			√	√	√	√	√	√	√	√	√	√	√	√
C. simplex	√	√	√	√	√	√	√		√	√	√		√	√	√	√	√	√	√	√	
Cosmoeca norvegica	√	√	√		√	√	√	√					√	√	√	√	√	√		√	√
C. ventricosa	√								√							√					
Crinolina isefiordensis	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Crucispina cruciformis	√	√					√	√	√		√	√	√				√	√	√	√	
Diaphanoeca grandis	√	√	√		√	√	√		√		√	√	√	√	√	√	√	√	√		√
D. pedicellata					√														√		√
D. undulata	√	√	√	√	√	√		√			√	√								√	√
Diplotheca costata					√			√					√								
Nannoeca minuta												√					√				√
Parvicorbicula circularis												√					√				√
P. quadricostata																					√
P. socialis												√				√		√			
P. superpositus	√	√			√		√	√	√		√	√	√	√	√	√	√	√	√	√	√
P. 'manubriata'	√								√		√		√	√	√	√	√	√	√		
P. 'aculeatus'				√											√	√	√	√	√	√	
Pleurasiga minima					√			√		√	√	√	√	√	√	√	√	√		√	
P. reynoldsii	√	√	√	√						√	√		√				√			√	√
Polyfibula sphyrelata	√	√	√	√		√		√	√	√	√	√	√	√	√	√	√	√	√	√	√
Polyoeca dichotoma									√												
Saepicula pulchra							√		√				√						√		
Savillea micropora	√						√			√		√									
S. parva																					
Stephanoeca cupula												√									
S. diplocostata		√	√	√	√			√			√	√	√		√					√	
S. elegans				√	√	√	√													√	√
S. norrisii									√											√	
CILIATES																					
Mesodinium rubrum		√		√						√				√	√	√		√	√		√
Uronema sp.					√			√					√	√	√				√		
COCCOLITHOPHORIDS																					
Emiliania huxleyi	√	√	√		√		√	√	√		√	√	√	√	√	√	√	√		√	√
Papposphaera sp.																					
DINOFLAGELLATES																					
Oxyrrhis marina				√		√						√				√		√			
EUGLENOZOA																					
Diplonema ambulator						√															
Petalomonas minuta												√			√						
P. pusilla				√								√			√						
Bodo curvifilus			√			√	√		√	√		√		√	√	√	√	√	√	√	√
B. designis	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
B. saliens			√	√			√	√	√	√	√	√	√	√	√	√		√	√		√

Appendix 1 (cont.): Protists found in samples taken from Southampton Water 1991-1994 (dates of samples in table 1, p13)

SPECIES	MP 1	LS 1	MP 2	CN 1	GB 1	NT 1	NT 2	CN 2	HY 1	MP 3	MP 4	HY 2	NT 3	NT 4	NT 5	NT 6	NT 7	NT 8	NT 9	NT 10
EUGLENOZOA (cont.)																				
B. saltans														✓		✓				
Rhynchomonas nasuta	✓		✓				✓				✓	✓		✓				✓	✓	✓
HEMIMASTIGOPHORA																				
Stereonema sp.												✓						✓		
INCERTAE SEDIS																				
Allantion tachyploon																			✓	
Ancyromonas sigmoides	✓	✓	✓		✓		✓	✓		✓	✓	✓	✓		✓	✓		✓	✓	
Caecitellus parvulus	✓		✓			✓	✓	✓	✓		✓	✓	✓	✓	✓			✓		
Colpodella sp.											✓									
Cryptaulax marina	✓	✓	✓				✓		✓	✓	✓	✓	✓	✓	✓	✓			✓	
Discocelis saleuta	✓											✓								
Kiitoksia ystava	✓						✓					✓						✓	✓	
Leucocryptos marina																		✓		✓
L. remigera														✓		✓			✓	
Luffisphaera 'hamatus'							✓													
Luffisphaera sp.																				✓
Metopion fluens	✓				✓		✓							✓						
Metromonas simplex	✓	✓	✓								✓	✓						✓		
Ministeria 'vibrans'	✓	✓					✓				✓		✓	✓						
Quadricilia rotundata																		✓	✓	
Rigidomastix 'devoratum'			✓							✓				✓	✓	✓		✓	✓	✓
Telonema 'antarctica'							✓	✓	✓	✓	✓	✓					✓			
T. subtile			✓					✓				✓	✓	✓	✓	✓	✓	✓	✓	✓
STRAMENOPILES; BICOSOECIDS																				
Bicosoeca epiphytica											✓									
B. gracilipes	✓				✓	✓	✓			✓		✓	✓	✓				✓		
B. lacustris																				✓
B. maris	✓									✓	✓	✓		✓						
Cafeteria marsupialis				✓										✓						
C. roenbergensis	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓
Pseudobodo tremulans	✓		✓				✓	✓		✓		✓					✓			
STRAMENOPILES; CHRYSOMONADS																				
Ollicola vangoorii									✓	✓	✓							✓	✓	
Paraphysomonas antarctica														✓						✓
P. butcheri											✓	✓	✓		✓			✓	✓	
P. foraminifera																				
P. imperforata									✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. siderophora														✓						
P. vestita	✓						✓		✓	✓	✓			✓						
Paraphysomonas spp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
'Collared' *Paraphysomonas*			✓			✓	✓	✓												
Spumella sp.																				
STRAMENOPILES; PEDINELLIDS																				
Actinomonas/Pteridomonas	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	
Apedinella radians																	✓			
Ciliophrys infusionum	✓	✓						✓			✓		✓							
STRAMENOPILES; INCERTAE SEDIS																				
Bordnamonas tropicana												✓								
Developayella elegans		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓				
Labyrinthula sp.						✓		✓			✓	✓								
'*Pendulomonas adriperis*'																				
THAUMATOMONADS																				
Protaspis glans											✓	✓							✓	
P. metarhiza												✓								
P. obliqua																				
P. simplex													✓							
Thaumatomastix salina											✓	✓		✓		✓	✓	✓		
Thaumatomastix 'thomseni'																				
Thaumatomastix sp.1																				
Thaumatomastix sp.2																				
UNDESCRIBED SPECIES																				
'Cyrano'					✓	✓	✓					✓	✓					✓	✓	
'Glissander'												✓								

Appendix 1 (cont.): Protists found in samples taken from Southampton Water 1991-1994 (dates of samples in table 1, p13)

SPECIES	NT 11	NT 12	NT 13	NT 14	NT 15	NT 16	NT 17	NT 18	NT 19	NT 20	NT 21	NT 22	NT 23	NT 24	NT 25	NT 26	NT 27	NT 28	NT 29	NT 30	NT 31
EUGLENOZOA (cont.)																					
B. saltans	√						√			√		√							√		
Rhynchomonas nasuta	√					√		√		√		√	√		√	√		√		√	√
HEMIMASTIGOPHORA																					
Stereonema sp.											√										
INCERTAE SEDIS																					
Allantion tachyploon																			√	√	
Ancyromonas sigmoides	√					√	√	√	√	√	√	√	√	√	√	√			√	√	√
Caecitellus parvulus			√	√	√	√	√	√		√			√	√	√	√	√	√	√	√	√
Colpodella sp.																					
Cryptaulax marina	√	√	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√
Discocelis saleuta								√			√										
Kiitoksia ystava			√				√					√	√	√		√			√	√	
Leucocryptos marina	√	√		√	√	√	√	√	√	√	√	√	√	√	√					√	√
L. remigera	√		√	√			√	√				√	√	√			√	√			√
Luffisphaera 'hamatus'																					
Luffisphaera sp.																					
Metopion fluens					√	√						√	√	√					√	√	
Metromonas simplex	√			√																	
Ministeria 'vibrans'						√						√									
Quadricilia rotundata			√	√	√		√		√					√	√	√	√		√	√	√
Rigidomastix 'devoratum'	√	√	√	√		√	√	√	√	√	√	√	√	√				√	√	√	√
Telonema 'antarctica'	√	√	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√		√	√
T. subtile	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
STRAMENOPILES; BICOSOECIDS																					
Bicosoeca epiphytica																					
B. gracilipes		√	√			√	√					√		√							√
B. lacustris																					
B. maris									√	√											
Cafeteria marsupialis																					
C. roenbergensis	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Pseudobodo tremulans		√	√	√	√	√				√		√				√	√	√	√	√	√
STRAMENOPILES; CHRYSOMONADS																					
Ollicola vangoorii	√	√	√	√	√	√	√		√												√
Paraphysomonas antarctica												√			√	√					
P. butcheri					√		√	√						√					√		√
P. foraminifera													√	√							
P. imperforata	√		√	√			√	√		√	√	√			√	√	√	√	√	√	√
P. siderophora																	√				
P. vestita			√					√	√	√		√	√		√			√	√	√	√
Paraphysomonas spp.	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√
'Collared' *Paraphysomonas*							√	√				√				√			√		
Spumella sp.													√	√	√	√	√	√	√	√	√
STRAMENOPILES; PEDINELLIDS																					
Actinomonas/Pteridomonas	√		√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Apedinella radians		√	√	√	√							√									√
Ciliophrys infusionum								√						√	√						
STRAMENOPILES; INCERTAE SEDIS																					
Bordnamonas tropicana								√				√	√		√				√		√
Developayella elegans			√	√	√		√	√	√		√			√	√			√	√	√	
Labyrinthula sp.										√	√			√							
'*Pendulomonas adriperis*'												√		√					√		
THAUMATOMONADS																					
Protaspis glans		√		√			√	√		√	√		√	√	√	√			√	√	
P. metarhiza																					
P. obliqua								√													
P. simplex				√															√		
Thaumatomastix salina	√							√	√			√	√		√			√	√	√	√
Thaumatomastix 'thomseni'																			√		√
Thaumatomastix sp.1												√									
Thaumatomastix sp.2												√									
UNDESCRIBED SPECIES																					
'Cyrano'								√	√	√	√		√		√				√		√
'Glissander'				√						√	√										

Appendix 2: Samples collected at NW Netley, Feb. 1993-June 1994

SAMPLE	Date collected	Temp (°C)	Salin (‰)	Spring high tide? (part of tidal cycle)
NT5	25.02.93	7.4	31.8	yes
NT6	12.03.93	7.1	32.4	yes
NT7	26.03.93	8.6	31.4	yes
NT8	23.04.93	11.6	30.7	yes
NT9	06.05.93	13.1	31.6	yes
NT10	21.05.93	13.4	31.5	yes
NT11	04.06.93	17.9	28.6	no: not Spring, tide going out
NT12	22.06.93	17.4	31.8	yes
NT13	21.07.93	17.6	33.4	yes
NT14	09.08.93	-	-	no
NT15	19.08.93	18.6	32.5	yes
NT16	29.09.93	c16.0	-	no: not Spring, just after HT
NT17	15.10.93	14.7	28.3	yes
NT18	01.11.93	10.6	30.7	yes
NT19	15.11.93	9.2	30.1	yes
NT20	01.12.93	6.0	26.0	yes
NT21	16.12.93	6.6	31.4	no: just after Spring, at HT
NT22	11.01.94	-	-	no: not Spring, half way between HT and LW
NT23	28.01.94	6.6	29.8	yes
NT24	11.02.94	6.3	29.8	yes
NT25	08.03.94	8.5	29.1	no: Neap, low water
NT26	22.03.94	9.3	-	no: Neap, low water
NT27	07.04.94	9.0	-	no: not Spring, high water
NT28	26.04.94	11.3	22.4	no: day before Spring, just below HW
NT29	09.05.94	13.6	30.6	yes
NT30 (a)	24.05.94	14.5	28.9	no: Spring, tide going out
NT30 (b)	24.05.94	14.5	28.9	as NT30a
NT31 (0m)	08.06.94	15.2	30.2	yes
NT31 (2m)	08.06.94	15.2	30.9	yes
NT31 (7m)	08.06.94	14.9	32.3	yes

Appendix 3: Chlorophyll a concentrations at NW Netley Feb. 1993-Feb. 1994 (courtesy of A. Hirst, Dept of Oceanography, University of Southampton)

DATE	Corresponding sample in this study	Chlorophyll a at 5m (mg.m^{-3})	Chlorophyll a at 10m (mg.m^{-3})
25.02.93	NT5	0.51	0.56
12.03.93	NT6	0.98	0.98
26.03.93	NT7	1.18	1.60
23.04.93	NT8	1.63	1.45
06.05.93	NT9	3.25	3.25
21.05.93	NT10	1.44	6.87
22.06.93	NT12	6.51	6.87
21.07.93	NT13	6.69	12.29
19.08.93	NT15	7.59	11.20
15.10.93	NT19	3.62	4.09
01.11.93	NT18	2.82	3.11
15.11.93	NT19	2.86	3.65
01.12.93	NT20	3.29	2.82
28.01.94	NT23	2.02	4.27
11.02.94	NT24	4.23	3.91

Appendix 4: Estimated biovolume of heterotrophic flagellates ($\mu m^3/ml$), calculated from abundance and average volume of cells in epifluorescence counts

SAMPLE	Average diameter (μm) *	Average volume (μm^3)	Number per ml	Estimated biovolume ($\mu m^3/ml$)
NT15 19.08.93	3.3	19.8	2975	58,905
NT16 29.09.93	2.9	19.0	2994	57,023
NT17 15.10.93	3.3	18.6	1227	22,825
NT18 01.11.93	2.7	11.2	1052	11,759
NT19 15.11.93	2.8	14.7	1529	22,454
NT20 01.12.93	3.0	12.8	1350	17,280
NT21 16.12.93	2.7	10.6	662	7,007
NT22 11.01.94	3.5	19.0	1957	37,220
NT23 28.01.94	3.5	29.1	936	27,236
NT24 11.02.94	3.4	23.3	612	14,230
NT25 08.03.94	3.7	34.1	975	33,240
NT26 22.03.94	3.6	37.1	665	24,673
NT27 07.04.94	3.3	19.0	992	18,876
NT28 26.02.94	3.3	20.8	1683	35,030
NT29 09.05.93	3.1	16.7	2008	33,622
NT30 (a) 24.05.94	4.3	74.2	3240	240,466
NT31 (0m) 08.06.94	3.4	21.7	1863	40,375
NT31 (2m) 08.06.94	3.5	44.2	1195	52,821
NT31 (7m) 08.06.94	3.5	26.9	1265	34,087

*diameters measured from cells fixed in glutaraldehyde

Appendix 5: Abundance of the major groups of heterotrophic flagellates (No. per ml) at NW Netley, March 1993-June 1994

SAMPLE	chryso	dino	acanth choanos	other choanos	bod	bico
NT9 12.03.93	705	70	141	(36)	124	71
NT10 21.05.93	540	42	28	35	77	-
NT11 04.06.93	633	80	30	(20)	60	50
NT12 22.06.93	685	158	200	(22)	126	179
NT13 21.07.93	1243	184	276	107	92	353
NT14 09.08.93	1569	253	325	144	271	180
NT15 19.08.93	1033	248	248	(42)	186	127
NT16 29.09.93	1417	91	114	(23)	274	297
NT17 15.10.93	-	-	-	-	-	-
NT18 01.11.93	485	46	128	46	82	37
NT19 15.11.93	743	(28)	112	42	140	112
NT20 01.12.93	514	43	129	43	118	97
NT21 16.12.93	320	(7)	91	21	49	42
NT22 11.01.94	871	57	143	100	114	172
NT23 28.01.94	323	40	121	48	48	56
NT24 11.02.94	280	(6)	23	(12)	35	47
NT25 08.03.94	423	43	86	43	60	69
NT26 22.03.94	306	39	52	20	39	46
NT27 07.04.94	533	25	49	33	57	98
NT28 26.04.94	842	66	76	33	55	144
NT29 09.05.94	935	55	220	83	41	150
NT30 (a) 24.05.94	2007	84	188	42	84	188
NT31 (0m) 08.06.94	981	70	42	98	112	70
NT31 (2m) 08.06.94	512	45	72	72	54	72
NT31 (7m) 08.06.94	599	57	86	86	38	133

KEY: 'chryso' = chrysomonads and pedinellids, 'dino' = dinoflagellates, 'acanth choanos' = acanthoecids, 'other choanos' = salpingoecids and codosigids, 'bod' = bodonids, 'bico' = bicosoecids. Numbers in brackets where abundance calculated from observations of only one or two cells.

Appendix 6: Abundance of the major groups of heterotrophic flagellates as a percentage of the total population

SAMPLE	chrysos	dinos	acanth choanos	other choanos	bods	bicos
NT9 12.03.93	41.1	4.1	8.2	2.1	7.2	4.1
NT10 21.05.93	48.2	4.5	3.0	3.7	8.2	-
NT11 04.06.93	40.6	5.1	1.9	1.3	3.9	3.2
NT12 22.06.93	31.1	7.2	9.1	1.0	5.7	8.1
NT13 21.07.93	33.9	5.0	7.5	2.9	2.5	9.6
NT14 09.08.93	41.4	6.7	8.6	3.8	7.1	4.8
NT15 19.08.93	34.7	8.3	8.3	1.4	6.3	4.3
NT16 29.09.93	47.3	3.0	3.8	0.8	9.2	9.9
NT17 15.10.93	-	-	-	-	-	-
NT18 01.11.93	46.1	4.4	12.2	4.4	7.8	3.5
NT19 15.11.93	48.6	1.8	7.3	2.7	9.1	7.3
NT20 01.12.93	38.1	3.2	9.6	3.2	8.7	7.2
NT21 16.12.93	48.3	1.1	13.7	3.2	7.4	6.3
NT22 11.01.94	44.5	2.9	7.3	5.1	5.8	8.8
NT23 28.01.94	34.5	4.3	12.9	5.1	5.1	6.0
NT24 11.02.94	45.8	1.0	3.8	2.0	5.7	7.7
NT25 08.03.94	43.4	4.4	8.8	4.4	6.2	7.1
NT26 22.03.94	46.0	5.9	7.8	3.0	5.9	6.9
NT27 07.04.93	55.7	2.5	4.9	3.3	5.7	9.9
NT28 26.04.94	50.0	3.9	4.5	2.0	3.3	8.6
NT29 09.05.94	46.6	2.7	11.0	4.1	2.0	7.5
NT30 (a) 24.05.94	61.9	2.6	5.8	1.3	2.6	5.8
NT31 (0m) 08.06.94	52.7	3.8	2.3	5.3	6.0	3.8
NT31 (2m) 08.06.94	42.8	3.8	6.0	6.0	4.5	6.0
NT31 (7m) 08.06.94	47.4	4.5	3.6	6.8	3.0	10.5

KEY: as for appendix 5.

Appendix 7: Proportions of chrysomonads* in two size classes (abundances given as No./ml)

SAMPLE	chrysos (total)	chrysos <2μm	chrysos ≥2μm	% chrysos <2μm
NT9 12.03.93	705	282	423	40.0
NT10 21.05.93	450	246	204	54.7
NT11 04.06.93	633	161	472	25.4
NT12 22.06.93	685	190	495	38.4
NT13 21.07.93	1243	568	675	45.7
NT14 09.08.93	1569	649	920	41.4
NT15 19.08.93	1033	165	868	16.0
NT16 29.09.93	1417	731	686	51.6
NT17 15.10.93	-	-	-	-
NT18 01.11.93	485	192	293	39.6
NT19 15.11.93	743	238	505	32.0
NT20 01.12.93	514	64	450	12.5
NT21 16.12.93	320	146	174	45.6
NT22 11.01.94	871	186	528	21.4
NT23 28.01.94	323	113	210	35.0
NT24 11.02.94	280	134	146	47.9
NT25 08.03.94	423	112	311	26.5
NT26 22.03.94	306	117	189	38.2
NT27 07.04.94	533	123	410	23.1
NT28 26.04.94	842	89	753	10.6
NT29 09.05.94	935	316	619	33.8
NT30 (a) 24.05.94	2007	230	1777 #	11.5
NT31 (0m) 08.06.94	981	280	700	28.5
NT31 (2m) 08.06.94	512	180	332	35.2
NT31 (7m) 08.06.94	599	181	418	30.2

* 'chrysomonads' category also includes pedinellids, however the majority of this group belong to the genera *Paraphysomonas* and *Spumella*.

electron microscopical whole-mounts show that most of these were *Paraphysomonas imperforata*

Appendix 8: Abundance (No. per ml) of different taxa of bicosoecids at NW Netley, March 1993-June 1994

SAMPLE	Bicosoecids (total)	Bicosoeca spp.	Pseudobodo tremulans	Cafeteria roenbergensis
NT9 12.03.93	71	(18)	0	53
NT10 21.05.93	-	-	-	-
NT11 04.06.93	50	0	0	50
NT12 22.06.93	179	0	0	179
NT13 21.07.93	353	77	107	169
NT14 09.08.93	180	72	0	108
NT15 19.08.93	127	(41)	0	86
NT16 29.09.93	297	(23)	0	274
NT17 15.10.93	-	-	-	-
NT18 01.11.93	37	0	0	37
NT19 15.11.93	112	(14)	0	98
NT20 01.12.93	97	43	0	54
NT21 16.12.93	42	0	0	42
NT22 11.01.94	172	43	0	129
NT23 28.01.94	56	0	0	56
NT24 11.02.94	47	(12)	0	35
NT25 08.03.94	69	0	0	69
NT26 22.03.94	46	(13)	(13)	20
NT27 07.04.94	98	(8)	(8)	82
NT28 26.04.94	144	(11)	(11)	122
NT29 09.05.94	150	0	(14)	136
NT30 (a) 24.05.94	188	(42)	0	146
NT31 (0m) 08.06.94	70	(28)	0	42
NT31 (2m) 08.06.94	72	(9)	(18)	45
NT31 (7m) 08.06.94	133	(19)	57	57

Numbers in brackets refer to values calculated from observation of only one or two cells.

Appendix 9: Abundance of 'non-bacterivorous' flagellates (No./ml) at NW Netley, June 1993-June 1994.

SAMPLE	Cryptaulax marina	Telonema 'antarctica'	Telonema subtile	Leucocryptos marina	Leucocryptos remigera	Quadricilia rotundata
NT11 04.06.93	80	80	20	0	10	0
NT12 22.06.93	0	21	21	74	0	0
NT13 21.07.93	15	92	31	0	0	276
NT14 09.08.93	36	108	18	0	72	0
NT15 19.08.93	21	83	21	0	0	21
NT16 29.09.93	0	229	46	23	0	0
NT17 15.10.93	-	-	-	-	-	-
NT18 01.11.93	0	37	46	9	18	0
NT19 15.11.93	0	56	42	14	0	56
NT20 01.12.93	21	43	43	32	0	0
NT21 16.12.93	7	14	14	7	0	0
NT22 11.01.94	29	14	57	29	14	0
NT23 28.01.94	16	16	32	16	8	0
NT24 11.01.94	6	17	29	6	0	17
NT25 08.03.94	9	17	35	9	0	17
NT26 22.03.94	7	13	26	0	7	0
NT27 07.04.94	8	16	33	0	8	8
NT28 26.04.94	22	0	100	0	11	0
NT29 09.05.94	14	0	28	0	0	28
NT30 (a) 24.05.94	167	42	105	42	0	42
NT31 (0m) 08.06.94	14	42	56	14	28	14
NT31 (2m) 08.06.94	36	27	18	36	27	0
NT31 (7m) 08.06.94	19	19	57	10	0	10

Appendix 10: Seasonal abundance of three taxa (No. per ml) forming a minor, but significant proportion of the total heterotrophic flagellate population at NW Netley, June 1993-June 1994

SAMPLE	Massisteria marina	Amastigomonas species	Caecitellus parvulus
NT12 22.06.93	53	(21)	0
NT13 21.07.93	77	0	(15)
NT14 09.08.93	90	72	(36)
NT15 19.08.93	124	0	0
NT16 29.09.93	(23)	0	0
NT17 15.10.93	-	-	-
NT18 01.11.93	27	0	0
NT19 15.11.93	0	(14)	0
NT20 01.12.93	(11)	(11)	0
NT21 16.12.93	0	0	0
NT22 11.01.94	54	0	0
NT23 28.01.94	40	0	0
NT24 11.02.94	29	(12)	0
NT25 08.03.94	26	35	0
NT26 22.03.94	(13)	(13)	(13)
NT27 07.04.94	25	(8)	0
NT28 26.04.94	55	0	(11)
NT29 09.05.94	83	(14)	41
NT30 (a) 24.05.94	(42)	0	(21)
NT31 (0m) 08.06.94	70	(14)	(28)
NT31 (2m) 08.06.94	45	0	(18)
NT31 (7m) 08.06.94	46	29	(10)

Numbers in brackets refer to abundances calculated from the observation of only one or two cells.

REFERENCES.

Aléxéieff, A. (1929). Matériaux pour servir a l'étude des protistes coprozoites. Archives de Zoologie Experimentale et Générale, 68: 609-698.

Andersen, P. (1989). Functional biology of the choanoflagellate *Diaphanoeca grandis* Ellis. Mar. Microb. Food Webs, 3: 35-50.

Andersen, P. and Fenchel, T. (1985). Bacterivory by microheterotrophic flagellates in seawater samples. Limnol. Oceanogr., 30: 198-202.

Andersen, P. and Sørensen, H.M. (1986). Population dynamics and trophic coupling in pelagic microorganisms in eutrophic coastal waters. Mar. Ecol. Prog. Ser., 33: 99-109.

Andersson, A., Larsson, U. and Hagström, Å. (1986). Size-selective grazing by a microflagellate on pelagic bacteria. Mar. Ecol. Prog. Ser., 33: 51-57.

Ansell, A.D., Raymont, J.E.G., Lander, K.F., Crowley, E. and Shackley, P. (1963). Studies on the mass culture of *Phaeodactylum* II. The growth of *Phaeodactylum* and other species in outdoor tanks. Limnol. Oceanogr., 8: 184-206.

Antai, A.E. (1989). Seasonal trophodynamics of bacterioplankton and heterotrophic microflagellates in Southampton Water. PhD. thesis, University of Southampton, U.K.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. and Thingstad, F. (1983). The ecological role of water column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257-263.

Beech, P.L. and Moestrup, Ø. (1986). Light and electron microscopical observations on the heterotrophic protist *Thaumatomastix salina* comb. nov. (syn. *Chrysosphaerella salina*) and its allies. Nord. J. Bot., 6: 865-877.

Belcher, J.H. and Swale, E.M.F. (1975). *Luffisphaera* gen. nov., an enigmatic scaly microorganism. Proc. R. Soc. Lond. B, 188: 495-499.

Bernard, C. and Rassoulzadegan, F. (1990). Bacteria or microflagellates as a major food source for marine ciliates: possible implications for the microzooplankton. Mar. Ecol. Prog. Ser., 64: 147-155.

Bjørnsen, P.K. and Kuparinen, J. (1991). Growth and herbivory by heterotrophic dinoflagellates in the Southern Ocean, studied by microcosm experiments. Mar. Biol., 109: 397-405.

Booth, B.C. (1990). Choanoflagellates from the subarctic North Pacific Ocean, with descriptions of two new species. Can. J. Zool., 68: 2393-2402.

Booth, B.C., Lewin, J. and Norris, R.E. (1982). Nanoplankton species predominant in the subarctic Pacific in May and June 1978. Deep-Sea Res., 29: 185-200.

Børsheim, K.Y. and Bratbak, G. (1987). Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar. Ecol. Prog. Ser., 36: 171-175.

Boucaud-Camou, E. (1967). Les choanoflagellés des cotes de la manche: I. Systematique. Bull. Soc. Linn. Normandie 1966, 10: 191-209.

Bovee, E.C. and Telford, S.R. (1962) Protozoan inquilines from Florida reptiles. III. *Rigidomastix scinorum* n. sp.; *Cercobodo stilosomorum* n. sp.; and *Cryptobia geccorum* n. sp. Quart. J. Florida Acad. Sci., 25: 180-191.

Brooker, B.E. (1971). Fine structure of *Bodo saltans* and *Bodo caudatus* (Zoomastigophora: Protozoa) and their affinities with the Trypanosomatidae. Bulletin of the British Museum of Natural History, 22: 89-102.

Brugerolle, G. and Mignot, J.P. (1979). Observations sur le cycle l'ultrastructure et la position systématique de *Spiromonas perforans* (*Bodo perforans* Hollande 1938), flagellé parasite de *Chilomonas paramecium*: ses relations avec les dinoflagellés et sporozoaires. Protistologica, 15: 183-196.

Buck, K.R. and Garrison, D.L. (1983). Protists from the ice-edge of the Weddell Sea. Deep-Sea Res., 30: 1261-1277.

Buck, K.R. and Garrison D.L. (1988). Distribution and abundance of choanoflagellates (Acanthoecidae) across the ice-edge zone in the Weddell Sea, Antarctica. Mar. Biol., 98: 263-269.

Buma, A., Estrada, M., Larsen, J., Riebesell, U., Schloss, I. and Thomsen, H.A. (1989). Unicellular organisms studied alive using photographic and video techniques. Reports on Polar Research. The Expedition Antarktis VII/3 (Epos leg 2) of RV 'Polarstern' in 1988/89, pp102-110.

Burkill, P.H. (1978). Quantitative aspects of the ecology of marine planktonic ciliated protozoa with special reference to *Uronema marinum* Dujardin. PhD thesis, University of Southampton, U.K.

Burkill, P.H. (1982). Ciliates and other microplankton components of a nearshore food-web: standing stocks and production processes. Ann. Inst. Océanogr., 58: 335-350.

Burney, C.M., Davis, P.G., Johnson, K.M. and Sieburth, J.McN. (1981). Dependence of dissolved carbon concentration upon small scale nanoplankton and bacterioplankton distributions in the Western Sargasso Sea. Mar. Biol., 65: 289-296.

Burzell, L.A. (1973). Observations on the proboscis-cytopharynx complex of *Rhynchomonas metabolita* Pshenin 1964 (Zoomastigophorea: Bodonidae). J. Protozool., 20: 385-393.

Burzell, L.A. (1975). Fine structure of *Bodo curvifilus* Griessmann (Kinetoplastida: Bodonidae). J. Protozool., 22: 35-39.

Capriulo, G.M. and Carpenter, E.J. (1980). Grazing by 35-202μm microzooplankton in Long Island Sound. Mar. Biol., 56: 319-326.

Caron, D.A. (1987). Grazing of attached bacteria by heterotrophic microflagellates. Microb. Ecol., 13: 203-218.

Caron, D.A., Davis, P.G., Madin, L.P. and Sieburth, J. McN. (1982). Heterotrophic bacteria and bacterivorus protozoa in oceanic macroaggregates. Science, 218: 795-797.

Caron, D.A., Davis, P.G. and Sieburth J. McN. (1989). Factors responsible for the differences in cultural estimates and direct microscopical counts of populations of bacterivorous nanoflagellates. Microb. Ecol., 18: 89-104.

Caron, D.A., Goldman, J.C., and Dennett, M.R. (1986). Effect of temperature on growth, respiration and nutrient regeneration by an omnivorous microflagellate. Appl. Environ. Microbiol., 52: 1340-1347.

Caron, D.A., Goldman, J.C. and Dennett, M.R. (1990). Carbon utilisation by the omnivorous flagellate *Paraphysomonas imperforata*. Limnol. Oceanogr., 35: 192-201.

Chamberlain, A.H.L. and Moss, S.T. (1988). The thraustochytrids: a protist group with mixed affinities. Biosystems, 21: 341-349.

Choi, J.W. and Peters, F. (1992). Effects of temperature on two psychrophilic ecotypes of a nanoflagellate, *Paraphysomonas imperforata*. Appl. Environ. Microbiol., 58: 593-599.

Choi, J.W. and Stoecker, D.K. (1989). Effects of fixation on cell volume of marine planktonic protozoa. Appl. Environ. Microbiol., 55: 1761-1765.

Coffin, R.B. and Sharp, J.H. (1987). Microbial trophodynamics in the Delaware estuary. Mar. Ecol. Prog. Ser. 41: 254-266.

Cynar, F.J., Estep, K.W. and Sieburth, J.McN. (1985). The detection and characterisation of bacteria-sized protists in 'protist-free' filtrates and their potential impact on experimental marine ecology. Microb. Ecol., 11: 281-288.

Davidson, L.A. (1982). Ultrastructure, behaviour and algal flagellate affinities of the helioflagellate *Ciliophrys marina* and the classification of the helioflagellates (Protista, Actinopoda, Heliozoea). J. Protozool., 29: 19-29.

Davis, P.G., Caron, D.A., Johnson, P.W. and Sieburth, J.McN. (1985). Phototrophic and apochlorotic components of picoplankton and nanoplankton in the North Atlantic: geographical, vertical and seasonal and diel distributions. Mar. Ecol. Prog. Ser., 21: 15-26.

Davis, P.G. and Sieburth, J.McN. (1984). Estuarine and oceanic microflagellate predation of actively growing bacteria: estimation by frequency of dividing-divided bacteria. Mar. Ecol. Prog. Ser., 19: 237-246.

Dodge, J.D. and Crawford, R.M. (1970). A survey of thecal fine structure in the Dinophyceae. Bot. J. Linn. Soc., 63: 53-67.

Dürrschmidt, M. and Patterson, D.J. (1987). On the organisation of the heliozoa *Raphidiophrys ambigua* Penard and *R. pallida* Schulze. Annls Sci. Nat. Zool. Paris, 13. Ser, 1986-1987, 8: 135-155.

Eccleston-Parry, J. and Leadbeater, B.S.C. (1994). A comparison of the growth kinetics of six marine heterotrophic nanoflagellates fed with one bacterial species. Mar. Ecol. Prog. Ser., 105: 167-177.

Espeland, G. and Throndsen, J. (1986). Flagellates from Kilsfjorden, Southern Norway, with description of two new species of Choanoflagellida. Sarsia, 71: 209-226.

Eyden, B.P. (1977). Morphology and ultrastructure of *Bodo designis* Skuja 1948. Protistologica, 13: 169-179.

Fenchel, T. (1977). The significance of bacterivorous protozoa in the microbial community of detrital particles. In: Cairns, J. (ed.): Aquatic microbial communities. Garland, pp529-544.

Fenchel, T. (1982a). Ecology of heterotrophic microflagellates I. Some important forms and their functional morphology. Mar. Ecol. Prog. Ser., 8: 211-223.

Fenchel, T. (1982b). Ecology of heterotrophic flagellates II: Bioenergetics and growth. Mar. Ecol. Prog. Ser., 8: 225-231.

Fenchel, T. (1982c). Ecology of heterotrophic flagellates IV: Quantitative occurrence and importance as bacterial consumers. Mar. Ecol. Prog. Ser., 9: 35-42.

Fenchel, T. (1986). The ecology of heterotrophic microflagellates. Adv. Microb. Ecol., 9: 57-97.

Fenchel, T. (1991). Flagellate design and function. In: Patterson, D.J. and Larsen, J. (eds.). The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp7-19.

Fenchel, T. and Finlay, B.J. (1983). Respiration rates in heterotrophic, free-living protozoa. Microb. Ecol., 9: 99-122.

Fenchel, T. and Patterson, D.J. (1988). *Cafeteria roenbergensis* nov. gen., nov. sp., a heterotrophic microflagellate from marine plankton. Mar. Microb. Food Webs, 3: 9-19.

Foissner, W., Blatterer, H. and Foissner, I. (1988). The Hemimastigophora (*Hemimastix amphikineta* nov. gen., nov. spec.), a new protistan phylum from Gondwanian soils. Europ. J. Protistol., 23: 361-383.

Foissner, I. and Foissner, W. (1993). Revision of the family Spironemidae Doflein (Protista, Hemimastigophora), with description of two new species, *Spironema terricola* N. Sp. and *Stereonema geiseri* N. G., N. Sp. J. Euk. Microbiol., 40: 422-438.

Fuhrman, J.A. and Azam, F. (1980). Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol., 39: 1085-1095.

Fuhrman, J.A. and Azam, F. (1982). Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol., 66: 109-120.

Garrison, D.L. and Buck, K.R. (1989). The biota of the Antarctic pack ice in the Weddell Sea and Antarctic Peninsula regions. Polar Biol., 10: 211-219.

Gillett, R. and Gull, K. (1972). Glutaraldehyde - its purity and stability. Histochemie, 30: 162-167.

Goldman, J.C., Caron, D.A., Andersen, O.K. and Dennett, M.R. (1985). Nutrient cycling in a microflagellate food chain: I. Nitrogen dynamics. Mar. Ecol. Prog. Ser., 24: 231-242.

Goldman, J.C. and Dennett, M.R. (1985). Susceptibility of some marine phytoplankton species to cell breakage during filtration and post-filtration rinsing. J. Exp. Mar. Biol. Ecol., 86: 47-58.

Goldman, J.C. and Dennett, M.R. (1990). Dynamics of prey selection by an omnivorous flagellate. Mar. Ecol. Prog. Ser., 59: 183-194.

Graham, J.M. (1991). Symposium introductory remarks: A brief history of aquatic microbial ecology. J. Protozool., 38: 66-69.

Griessmann, K. (1913). Über marine Flagellaten. Arch. Protistenk., 32: 1-78.

Haas, L.W. and Webb, K.L. (1979). Nutritional mode of several non-pigmented microflagellates from York River Estuary, Virginia. J. Exp. Mar. Biol. Ecol., 39: 125-134.

Hagström, Å., Azam, F., Andersson, A., Wikner, J. and Rassoulzadegan, F. (1988). Microbial loop in an oligotrophic pelagic marine ecosystem: possible roles of cyanobacteria and nanoflagellates in the organic fluxes. Mar. Ecol. Prog. Ser., 49: 171-178.

Hänel, K. (1979): Systematics and ecology of colourless flagellates in sewage. Arch. Protistenk., 121: 73-137.

Hansen, P.J. (1991). Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagic food web. Mar. Ecol. Prog. Ser., 73: 253-261.

Hara, S., Tanoue, E., Zenimoto, M., Komaki, Y. and Takahashi, E. (1986). Morphology and distribution of heterotrophic protists along 75°E in the Southern Ocean. Mem. Natl. Inst. Polar Res., Spec. Issue, 40: 69-80.

Hibberd, D.J. (1976). The structure and taxonomy of the Chrysophyceae and Prymnesiophyceae (Haptophyceae): a survey with some new observations on the ultrastructure of the Chrysophyceae. Bot. J. Linn. Soc., 72: 55-80.

Hibberd, D.J. (1979). Notes on the genus *Paraphysomonas* (Chrysophyceae) with special reference to *P. bandaiensis* Takahashi. Arch. Protistenk., 121: 146-154.

Hibberd, D.J. (1986). Ultrastructure of the Chrysophyceae - phylogenetic implications and taxonomy. In: Kristiansen, J. and Andersen, R.A. (eds.): Chrysophytes: Aspects and problems. Cambridge University Press, pp23-36.

Hilliard, D.K. (1971). Notes on the occurrence and taxonomy of some planktonic chrysophytes in an Alaskan lake, with comments on the genus *Bicoeca*. Arch. Protistenk., 113: 98-122.

Hobbie, J.E., Daley, R.J. and Jasper, S. (1977). Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol., 33: 1225-1228.

Iriarte, A. (1991). Picoplankton distribution and primary production in coastal and estuarine waters. Ph.D. thesis, University of Southampton, U.K.

Jackson, S.M. and Leadbeater, B.S.C. (1991). Costal strip accumulation in the marine choanoflagellate *Diplotheca costata* Valkanov. J. Protozool., 38: 97-104.

James-Clark, H. (1867). On the Spongiae Ciliatae as Infusoria Flagellata. Mem. Boston Soc. Nat. Hist., 1: 305-342.

Johannes, R.E. (1965). Influence of marine protozoa on nutrient regeneration. Limnol. Oceanogr., 10: 434-442.

Johnson, P.W., Hargreeves, P.E. and Sieburth, J. McN. (1988). Ultrastructure and ecology of *Calycomonas ovalis* Wulff, 1919, (Chrysophyceae) and its redescription as a testate rhizopod *Paulinella ovalis* N. Comb. (Filiosea: Euglyphina). J. Protozool., 35: 618-626.

Johnson, P.W. and Sieburth, J.McN. (1979). Chroococcoid cyanobacteria in the sea: a ubiquitous phototrophic biomass. Limnol. Oceanogr., 24: 928-935.

Johnson, P.W. and Sieburth, J.McN. (1982). *In situ* morphology and occurrence of eukaryotic phototrophs of bacterial size in the picoplankton of estuarine and oceanic waters. J. Phycol., 18: 318-327.

Jones, H.L.J., Leadbeater, B.S.C. and Green, J.C. (1993). Mixotrophy in a marine species of *Chrysochromulina* (Prymnesiophyceae): ingestion and digestion of a small green flagellate. J. Mar. Biol. Ass. U.K., 73: 283-296.

Karpov, S.A. and Mylinikov, A.P. (1989). Biology and ultrastructure of colourless flagellates Apusomonadida ord. n. Zoologischkei Zhurnal, 58: 5-17. [in Russian.]

Kent, W.S. (1880). A manual of the infusoria. Bogue, London (three vols).

Kifle, D. (1992). Seasonal and spatial variations in species composition, abundance, biomass and primary production of phytoplankton in Southampton Water, U.K. Ph.D. thesis, University of Southampton.

Kifle, D. and Purdie, D.A. (1994). The seasonal abundance of the phototrophic ciliate *Mesodinium rubrum* in Southampton Water, England. J. Plank. Res., 15: 823-833.

King, K.R., Hollobaugh, J.T. and Azam, F. (1980). Predator-prey interactions between the larvacean *Oikopleura dioica* and bacterioplankton in enclosed water columns. Mar. Biol., 56: 49-57.

Kivic, P.A. and Walne, P.L. (1984). An evaluation of a possible phylogenetic relationship between the Euglenophyta and the Kinetoplastida. Origins of Life, 13: 269-288.

Kopylov, A.I., Pasternak, A.F. and Moiseyev, Ye. V. (1981). Consumption of zooflagellates by planktonic organisms. Oceanogr., 21: 269-271.

Kuosa, H. (1991). Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing. Mar. Ecol. Prog. Ser., 73: 269-276.

Kuosa, H. and Marcussen, B. (1988). Grazing of bacteria and phytoplankton by heterotrophic nanoflagellates in a Baltic Sea sample. Hydrobiol., 161: 211-216.

Kuuppo, P. (1994). Annual variation in the abundance and size of heterotrophic nanoflagellates on the SW coast of Finland, the Baltic Sea. J. Plank. Res., in press.

Kuuppo-Leinikki, P. (1990). Protozoan grazing on picoplanktonic bacteria and its impact on bacterial production. Mar. Ecol. Prog. Ser., 63: 227-238.

Kuuppo-Leinikki, P. (1993). Horizontal distribution of photo- and heterotrophic microorganisms on the coastal area of the northern Baltic Sea - a case study. J. Plank. Res., 15: 27-35.

Kuuppo-Leinikki, P. and Kuosa, H. (1989). Preservation of picoplanktonic cyanobacteria and heterotrophic nanoflagellates for epifluorescence microscopy. Arch. Hydrobiol., 144: 631-636.

Lackey, J.B. and Lackey, E.W. (1963). Microscopic algae and protozoa in the waters near Plymouth in August 1962. J. Mar. Biol. Ass. U.K., 43: 797-805.

Landry, M.R., Lehner-Fournier, J.M., Sundstrom, J.A., Fagerness, V.L., and Selph, K.E. (1991). Discrimination between living and heat-killed prey by a marine zooflagellate, *Paraphysomonas vestita* (Stokes). J. Mar. Biol. Ecol., 146, 139-151.

Larsen, J. (1985). Ultrastructure and taxonomy of *Actinomonas pusilla*, a heterotrophic member of the Pedinellales (Chrysophyceae). Br. Phycol J., 20: 341-355.

Larsen, J. and Patterson, D.J. (1990): Some flagellates (Protista) from tropical marine sediments. J. Nat. Hist., 24, 801-937.

Larsson, U. and Hagström, Å. (1979). Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. Mar. Biol., 52: 199-206.

Larsson, U. and Hagstrom, Å. (1982). Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic marine environment. Mar. Biol., 67: 57-70.

Leadbeater, B.S.C. (1972a). Fine-structural observations on some marine choanoflagellates from the coast of Norway. J. Mar. Biol. Ass. U.K., 52: 67-79.

Leadbeater, B.S.C. (1972b). Ultrastructural observations on some marine choanoflagellates from the coast of Denmark. Br. Phycol. J., 7: 195-211.

Leadbeater, B.S.C. (1974). Ultrastructural observations on nanoplankton collected from the coast of Yugoslavia and the Bay of Algiers. J. Mar. Biol. Ass. U.K., 54: 179-196.

Leadbeater, B.S.C. (1975). A microscopical study of the marine choanoflagellate *Savillea micropora* (Norris) comb. nov., and preliminary observations on lorica development in *S. micropora* and *Stephanoeca diplocostata* Ellis. Protoplasma, 83: 111-129.

Leadbeater, B.S.C. (1979). Developmental and ultrastructural observations on two staked marine choanoflagellates, *Acanthoecopsis spiculifera* Norris and *Acanthoeca spectabilis* Ellis. Proc. R. Soc. Lond. B. 204: 57-66.

Leadbeater, B.S.C. (1980). Four new species of loricate choanoflagellates from South Brittany, France. Cahiers de Biologie Marine, 21: 345-353.

Leadbeater, B.S.C. (1991). Choanoflagellate organisation with special reference to loricate taxa. In: Patterson, D.J. and Larsen, J. (eds.): The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp241-258.

Leakey, R.J.G. (1989). The ecology of planktonic ciliates in Southampton Water. PhD. thesis, University of Southampton, U.K.

Leakey, R.J.G., Burkill, P.H. and Sleigh, M.A. (1992). Planktonic ciliates in Southampton Water: abundance, biomass, production, and role in pelagic carbon flow. Mar. Biol., 114: 67-83.

Leakey, R.J.G., Burkill, P.H. and Sleigh, M.A. (1993). Planktonic ciliates in Southampton Water: quantitative taxonomic studies. J. Mar. Biol. Ass. U.K., 73: 579-594.

Leipe, D. and Hausmann, K. (1993). Neue Erkenntnisse zur Stammesgeschichte der Eukaryoten. Biologie in unserer Zeit, 23: 178-183.

Leipe, D.D., Wainwright, P.O., Gunderson, J.H., Porter, D., Patterson, D.J., Valois, F., Himmerich, S. and Sogin, M.L. (1994). The stramenopiles from a molecular perspective: 16s-like rRNA sequences from *Labyrinthuloides minuta* and *Cafeteria roenbergensis*. Phycologica, 33: 369-377.

Lessard, E.J. (1991). The trophic role of heterotrophic dinoflagellates in diverse marine environments. Mar. Microb. Food Webs, 5: 49-58.

Li, W.K.W., Subba-Rao, D.V., Harrison, W.G., Smith, J.C., Cullen, J.J., Irwin, B. and Platt, T. (1983). Autotrophic picoplankton in the tropical ocean. Science, 219: 292-295.

Lindholm, T. (1985). *Mesodinium rubrum* - a unique photosynthetic ciliate. Adv. Aquat. Microbiol., 3: 1-48.

Lucas, C. (1993). The biology of gelatinous predators and their impact on the mesozooplankton community of Southampton Water. Ph.D. thesis, University of Southampton, U.K.

Lucas, I.A.N., (1967). Two new marine species of *Paraphysomonas*. J. Mar. Biol. Ass. U.K., 47: 329-334.

Lund, J.W.G. (1960). Concerning *Calycomonas* Lohmann and *Codonomonas* Van Goor. Nova Hedw., 1: 423-430.

Manton, I. and Bremer, G. (1981). Observations on lorica structure and aspects of replication in the *Pleurasiga sphyrelata* Thomsen complex (= *Polyfibula* spp., gen. nov.) (Choanoflagellata). Zool. Scripta, 10: 273-291.

Manton, I. and Oates, K. (1979a). Further observations on choanoflagellates in the genus *Calliacantha* Leadbeater, with special reference to *C. multispina* sp. nov. from South Africa and Britain. J. Mar. Biol. Ass. U.K., 59: 207-213.

Manton, I. and Oates, K. (1979b). Further observations on *Calliacantha* Leadbeater (Choanoflagellata), with special reference to *C. simplex* sp. nov. from many parts of the world. Proc. R. Soc. Lond. B. 204: 287-300.

Manton, I., Sutherland, J. and Leadbeater, B.S.C. (1976). Further observations on the fine structure of marine collared flagellates (Choanoflagellata) from arctic Canada and west Greenland: species of *Parvicorbicula* and *Pleurasiga*. Can. J. Bot., 54: 1932-1955.

Manton, I., Sutherland, J. and Oates, K. (1980). A reinvestigation of collared flagellates in the genus *Bicosta* Leadbeater with special reference to correlations with climate. Phil. Trans. R. Soc. Lond. B. 290: 431-447.

Marchant, H.J. (1985). Choanoflagellates in the Antarctic marine food chain. In: Siegfried, W.R., Condy, P.R. and Laws, R.M. (eds.): Antarctic nutrient cycles and food webs. Springer-Verlag, Berlin Heidelberg, pp271-276.

Marchant, H.J. and Perrin, R.A. (1990). Seasonal variation in abundance and species composition of choanoflagellates (Acanthoecidae) at Antarctic coastal sites. Polar Biol. 10: 499-505.

Mare, M.F. (1942). A study of a marine benthic community with special reference to microorganisms. J. Mar. Biol. Ass. U.K., 25: 517-554.

Martinussen, I. and Thingstad, T.F. (1991). A simple double staining technique for simultaneous quantification of auto- and heterotrophic nano- and picoplankton. Mar. Microb. Food Webs, 5: 5-11.

McManus, G.B. and Fuhrman, J.A. (1988). Clearance of bacteria-sized particles by natural populations of nanoplankton in the Chesapeake Bay outflow plume. Mar. Ecol. Prog. Ser., 42: 199-206.

McManus, G.B. and Fuhrman, J.A. (1990). Mesoscale and seasonal variability of heterotrophic nanoflagellate abundance in an estuarine outflow plume. Mar. Ecol. Prog. Ser., 61: 207-213.

Mignot, J.P. (1974). Étude ultrastructurale des *Bicoeca*, protistes flagellés. Protistologica, 10: 543-565.

Mignot, J.P. and Brugerolle, G. (1975). Étude ultrastructurale de *Cercomonas* Dujardin (=*Cercobodo* Krassilstchick) protiste flagellé. Protistologica, 11: 547-554.

Moestrup, Ø. (1979). Identification by electron microscopy of marine nanoplankton from New Zealand, including the description of four new species. New Zealand Journal of Botany, 17: 61-95.

Moestrup, Ø. and Andersen, R.A. (1991). Organisation of heterotrophic heterokonts. In: Patterson, D.J. and Larsen, J. (eds.): The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp333-360.

Moestrup, Ø. and Thomsen, H.A. (1976). Fine structural studies on the flagellate genus *Bicoeca*. Protistologica, 12: 101-120.

Moestrup, Ø. and Thomsen, H.A. (1980). Preparation of shadow cast whole mounts. In: Gantt, E. (ed.): Handbook of phycological methods. Cambridge Univ. Press, Cambridge, pp385-390.

Moss, S.T. (1991). Thraustrochytrids and other zoosporic marine fungi. In: Patterson, D.J. and Larsen, J. (eds.): The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp415-425.

Mylnikov, A.P. (1990): Characteristic features of the ultrastructure of the colourless flagellate *Heteromita* sp. Tsitologiya, 32, 567-571. [In Russian.]

Norris, R.E. (1965). Neustonic marine Craspedomonadales (choanoflagellates) from Washington and California. J. Protozool., 12: 589-602.

Nygaard, K., Børsheim, K.Y. and Thingstad, T.F. (1988). Grazing rates on bacteria by marine heterotrophic microflagellates compared to uptake rates of bacterial-sized monodispersed fluorescent latex beads. Mar. Ecol. Prog. Ser., 44: 159-165.

Page, F.C. (1983). Marine Gymnamoebae. Institute of Freshwater Ecology, Cambridge.

Parslow, J.S., Doucette, G.J., Taylor, F.J.R. and Harrison, P.J. (1986). Feeding by the zooflagellate *Pseudobodo* sp. on the picoplanktonic prasinomonad *Micromonas pusilla*. Mar. Ecol. Prog. Ser., 29: 237-246.

Patterson, D.J. (1986). Some problems of ambiregnal taxonomy and a possible solution. Symposia Biologica Hungarica, 33: 87-93.

Patterson, D.J., (1989a). Stramenopiles: chromophytes from a protistan perspective. In: Green, J.C., Leadbeater, B.S.C. and Diver, W.L. (eds.): The chromophyte algae: problems and perspectives. Clarendon Press, Oxford, pp357-379.

Patterson, D.J. (1989b). The evolution of protozoa. Mem. Inst. Oswaldo Cruz., 83: 580-600.

Patterson, D.J. (1993). The current status of free-living heterotrophic flagellates. J. Euk. Microbiol., 40: 606-609.

Patterson, D.J. (1994). Protozoa: evolution and systematics. In: Hausmann, K. and Hülsmann, N. (eds.): Progress in Protistology, Proceedings of the IX International Congress of Protozoology, Berlin 1993. Gustav Fischer Verlag, Stuttgart, pp1-14.

Patterson, D.J. and Fenchel, T. (1985). Insights into the evolution of heliozoa (Protozoa, Sarcodina) as provided by ultrastructural studies on a new species of flagellate from the genus *Pteridomonas*. Biol. J. Linn. Soc., 34: 381-403.

Patterson, D.J. and Larsen, J. (1991). The biology of free-living heterotrophic flagellates. Systematics Association special volume No. 45, Clarendon Press, Oxford.

Patterson, D.J., Nygaard, K., Steinberg, G. and Turley, C.M. (1993): Heterotrophic flagellates and other protists associated with oceanic detritus throughout the water column in the mid North Atlantic. J. Mar. Biol. Ass. U.K., 73: 67-95.

Patterson, D.J. and Zölffel, M. (1991): Heterotrophic flagellates of uncertain taxonomic position. In: Patterson, D.J. and Larsen, J. (eds.): The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp427-475.

Penard, E. (1890). Über einige neue oder wenig bekannte Protozoën. Jahrbuch Nassauischer Verein für Naturforschung, 43: 73-91.

Penard, E. (1921). Studies on some flagellata. Proc. Acad. Natural Sciences Philadelphia, 73: 105-168.

Pennick, N.C. (1982). Studies on the external morphology of *Pyramimonas* 6. *Pyramimonas cirolanae* sp. nov. Arch. Protistenk., 125: 87-94.

Pennick, N.C. and Clarke, K.J. (1972). *Paraphysomonas butcheri* sp. nov., a marine colourless scale-bearing member of the Chrysophyceae. Br. Phycol. J., 7: 45-48.

Picken, L.E.R. (1941). On the Bicoecidae: a family of colourless flagellates. Phil. Trans. R. Soc. Ser. B, 230: 451-473.

Platt, T., Subba-Rao, D.Y. and Irwin, B. (1983). Photosynthesis of picoplankton in the oligotrophic ocean. Nature, 301: 702-704.

Pomeroy, L.R. (1974). The ocean's food web; a changing paradigm. Bioscience, 24: 499-504.

Porter, D. (1989). Phylum Labyrinthulomycota. In: Margulis, L., Corliss, J.O., Melkonian, M. and Chapman, D.J. (eds.): Handbook of Protoctista. Jones and Bartlett, Boston, pp388-398.

Porter, K.G. (1984). Natural bacteria as food resources for zooplankton. In: Klug, M.J. and Reddy, C.A. (eds.): Current perspectives in microbial ecology. Amer. Soc. Microbiol, Washington D.C., pp340-345.

Porter, K.G. and Feig, Y.S. (1980). The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25: 943-948.

Porter, K.G., Sherr, E.B., Sherr, B.F., Pace, M. and Sanders, R.W. (1985). Protozoa in planktonic food webs. J. Protozool., 32: 409-415.

Preisig, H. R. and Hibberd, D.J. (1982a). Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera 1. Nord. J. Bot., 2: 397-420.

Preisig, H. R. and Hibberd, D.J. (1982b). Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera 2. Nord. J. Bot., 2: 601-638.

Preisig, H. R. and Hibberd, D.J. (1983). Ultrastructure and taxonomy of *Paraphysomonas* (Chrysophyceae) and related genera 3. Nord. J. Bot., 3: 695-723.

Preisig, H.R., Vørs, N. and Hällfors, G. (1991). Diversity of heterotrophic heterokonts. In: Patterson, D.J. and Larsen, J. (eds.): The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp361-399.

Proctor, L.M. and Fuhrman, J.A. (1990). Viral mortality of marine bacteria and cyanobacteria. Nature, 343: 60-62.

Rassoulzadegan, F. and Etienne, M. (1981). Grazing rates of the tintinnid *Stenosomella ventricosa* (Clap. & Lachm.) Jorg. on the spectrum of the naturally occurring particulate matter from a Mediterranean neritic area. Limnol. Oceanogr., 26: 258-270.

Rassoulzadegan, F., Laval-Peuto, M. and Sheldon, R.W. (1988). Partitioning of the food ration of marine ciliates between pico- and nanoplankton. Hydrobiologica, 159: 75-88.

Rassoulzadegan, F. and Sheldon, R.W. (1986). Predator-prey interactions of nanozooplankton and bacteria in an oligotrophic marine environment. Limnol. Oceanogr., 31: 1010-1021.

Rees, A.J.J., Donaldson, D.A. and Leedale, G.F. (1980). Morphology of the scales of the freshwater heliozoan *Raphidocystis tubifera* (Heliozoa, Centrohelidia) and organisation of the intact scale layer. Protistologica, 16: 565-570.

Rogerson, A. (1993). *Parvamoeba rugata* n.g., n. sp., (Gymnamoebia, Thecamoebidae): an exceptionally small marine naked amoeba. Europ. J. Protistol., 29: 446-452.

Rogerson, A. and Laybourn-Parry, J. (1992a). Aggregate dwelling protozooplankton communities in estuaries. Arch. Hydrobiol., 125: 411-422.

Rogerson, A. and Laybourn-Parry, J. (1992b). The abundance of marine naked amoebae in the water column of the Clyde estuary. Estuarine, Coastal and Shelf Science, 34: 187-196.

Ruinen, J. (1938). Notizen über Salzflagellaten II. Über die Verbreitung der Salzflagellaten. Arch. Protistenk., 90: 210-258.

Sanders, R.W. (1991). Mixotrophic protists in marine and freshwater ecosystems. J. Protozool., 38: 76-81.

Sanders, R.W. and Porter, K.G. (1988). Phagotrophic phytoflagellates. In: Marshall, K.C. (ed.): Microbial ecology, vol. 10. Plenum Press, New York, pp167-192.

Sanders, R.W. and Wickham, S.A. (1993). Planktonic protozoa and metazoa: predation, food quality and population control. Mar. Microb. Food Webs, 7: 197-223.

Sandon, H. (1924). On some protozoa from the soils and mosses of Spitzbergen. J. Linn. Soc. (Zool.)., 35: 449-475.

Sandon, H. (1927). The composition and distribution of the protozoan fauna of the soil. Oliver and Boyd, Edinburgh.

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. Rep. Challenger Soc., 3: 19-41.

Schiller, J. (1953). Über neuen Craspedomonaden (Choanoflagellaten). Arch. Hydrobiol., 48: 248-259.

Schlegel, M. (1991). Protist evolution and phylogeny as discerned from small subunit ribosomal RNA sequence comparisons. Europ. J. Protistol., 27: 207-219.

Shapiro, L.P., Haugen, E.M. and Carpenter, E.J. (1989). Occurrence and abundance of green-fluorescing dinoflagellates in surface waters of the Northwest Atlantic and Northeast Pacific Oceans. J. Phycol., 25: 189-191.

Sherr, B.F. and Sherr, E.B. (1984). Role of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems. In: Klug, M.G. and Reddy, C.A. (eds.): Current perspectives in microbial ecology. Amer. Soc. Microbiol., Washington, pp412-423.

Sherr, B.F. and Sherr, E.J. (1991). Proportional distribution of total numbers, biovolume and bacterivory among size classes of 2-20μm non-pigmented marine flagellates. Mar. Microb. Food Webs, 5: 227-237.

Sherr, B.F., Sherr, E.B., Andrew, T.A., Fallon, R.D. and Newell, S.Y. (1986). Trophic interaction between heterotrophic protozoa and bacterioplankton in estuarine water analyzed with selective metabolic inhibitors. Mar. Ecol. Prog. Ser., 32: 169-180.

Sherr, B.F., Sherr, E.B. and Newell, S.Y. (1984). Abundance and productivity of heterotrophic nanoplankton in Georgia Waters. J. Plank. Res., 6: 195-202.

Sherr, E.B. (1988). Direct use of high molecular weight polysaccharides by heterotrophic flagellates. Nature 335, 348-351.

Sherr, E.B. and Sherr, B.F. (1983). Double-staining epifluorescence technique to assess frequency of dividing cells and bacterivory in natural populations of heterotrophic microprotozoa. Appl. Environ. Microbiol., 46: 1388-1393.

Sherr, E.B. and Sherr, B.F. (1987). High rates of consumption of bacteria by pelagic ciliates. Nature, 325: 710-711.

Sherr, E.B., Sherr, B.F. and McDaniels, J. (1991). Clearance rates of <6μm fluorescently labelled algae (FLA) by estuarine protozoa: potential grazing impact of flagellates and ciliates. Mar. Ecol. Prog. Ser., 69: 81-92.

Shilo, M. (1984). *Bdellovibrio* as a predator. In: Klug, M.J. and Reddy, C.A. (eds.): Current perspectives in microbial ecology. American Soc. Microbiol., Washington, pp334-339.

Sieburth, J. McN. (1979). Sea microbes. Oxford Univ. Press, N. York.

Sieburth, J.McN. and Davis, P.G. (1982). The role of heterotrophic nanoplankton in the grazing of planktonic bacteria in the Sargasso and Caribbean Seas. Ann. Inst. Océanogr. Paris, 58: 285-296.

Sieburth, J.McN., Smetacek, V. and Lenz, J. (1978). Pelagic ecosystem structure: heterotrophic compartments and their relationship to plankton size fractions. Limnol. Oceanogr., 23: 1256-1263.

Siemensma, F.J. (1991). Heliozoea. In: Page, F.C., and Siemensma, F.J. (eds.): Nackte Rhizopoda und Heliozoa. Protozoenfauna Band 2. Gustav Fischer Verlag.

Sieracki, M.E., Haas, L.W., Caron, D.A. and Lessard, E.J. (1987). Effect of fixation on particle retention by microflagellates: underestimation of grazing rates. Mar. Ecol. Prog. Ser., 38: 251-258.

Silver, M.W., Shanks, A.L. and Trent, J.D. (1978). Marine snow: microplankton habitat and source of small-scale patchiness in pelagic populations. Science 201: 371-373.

Simek, K. and Chrzanowski, T.H. (1992). Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. Appl. Environ. Microbiol., 58: 3715-3720.

Skuja, H. (1939). Beitrag zur Algenflora Lettlands II. Acta Horti botanici Universitatis latviensis, 11/12: 41-169.

Skuja, H. (1948). Taxonomie des Phytoplanktons einiger Seen in Upplands, Schweden. Symbolae botanicae Uppsaliensis, 9: 1-399.

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

Smith, P.J. and Hobson, L.A. (1994). Temporal variations in the taxonomic composition of flagellated nanoplankton in a temperate fjord. J. Phycol., 30: 369-375.

Sogin, M. and Gunderson, J.H. (1987). Structural diversity of eukaryotic small subunit ribosomal RNAs. Ann. N.Y. Acad. Sci., 503: 125-139.

Sournia, A. (1986). Atlas du phytoplancton marin. vol. I: Cyanophycées, Dictyophycées, Dinophycées et Raphidophycées. CNRS, Paris.

Steele, J.H. (1974). The structure of marine ecosystems. Harvard Univ. Press, Cambridge, Mass.

Stoecker, D., Michaels, A.E. and Davis, L.H. (1987). Grazing by the jellyfish *Aurelia aurita* on microzooplankton. J. Plank. Res., 9: 901-915.

Suttle, C.H., Chan, A.M., Taylor, W.D. and Harrison, P.J. (1986). Grazing of planktonic diatoms by microflagellates. J. Plank. Res., 393-398.

Swale, E.M.F. (1973). A study of the colourless flagellate *Rhynchomonas nasuta* (Stokes) Klebs. Biol. J. Linn. Soc., 5: 255-264.

Takahashi, E. (1987). Loricate and scale bearing protists from Lützow-Holm Bay, Antarctica II. Four marine species of *Paraphysomonas* (Chrysophyceae) including two new species from the fast-ice covered coastal areas. Jap. J. Phycol. (Sôrui), 35: 155-166.

Thomsen, H.A. (1973). Studies on marine choanoflagellates I. Silicified choanoflagellates of the Isefjord (Denmark). Ophelia, 12: 1-26.

Thomsen, H.A. (1975). An ultrastructural survey of the chrysophycean genus *Paraphysomonas* under natural conditions. Br. J. Phycol., 10: 113-127.

Thomsen, H.A. (1976). Studies on marine choanoflagellates II. Fine structural observations on some silicified choanoflagellates from the Isefjord (Denmark), including the description of two new species. Norw. J. Bot., 23: 33-51.

Thomsen, H.A. (1979). Electron microscopical observations on brackish-water nanoplankton from the Tvärminne area, S.W. coast of Finland. Acta Bot. Fennica, 10: 11-37.

Thomsen, H.A. (1982). Planktonic choanoflagellates from Disko Bugt, West Greenland, with a survey of the marine nanoplankton of the area. Meddr. Grønland, Biosci., 8: 3-35.

Thomsen, H.A. (1988). An electron microscopical study of marine loricate choanoflagellates: *Nannoeca minuta* (Leadbeater) gen. et comb. n. and *Stephanoeca cupula* (Leadbeater) comb. n. Zoologica Scripta, 17: 315-323.

Thomsen, H.A. (1992). Loricabærende Choanoflagellater (Kraveflagellater). In: Thomsen, H.A. (ed.): Plankton i de indre danske farvande. Miljøministeriet, Miljøstyrelesen, pp157-194.

Thomsen, H.A. and Boonruang, P. (1984). A light and electron microscopical investigation of loricate choanoflagellates (Choanoflagellida, Acanthoecidae) from the Andaman sea, S.W. Thailand and Denmark: species of *Cosmoeca* gen. n. Zoologica Scripta 13: 165-181.

Thomsen, H.A. and Buck, K.R. (1991). Choanoflagellate diversity with particular emphasis on the Acanthoecidae. In: Patterson, D.J. and Larsen, J. (eds.): The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp259-284.

Thomsen, H.A., Buck, K.R., Coale, S.L., Garrison, D.L. and Gowing, M.M. (1990). Loricate choanoflagellates (Acanthoecidae, Choanoflagellida) from the Weddell Sea, Antarctica. Zoologica Scripta, 19: 367-387.

Thomsen, H.A., Buck, K.R. and Chavez, F.P. (1991). Choanoflagellates of the central Californian waters: taxonomy, morphology and species assemblages. Ophelia, 33: 131-164.

Thomsen, H.A., Hällfors, G., Hällfors, S. and Ikävalko, J. (1993). New observations on the heterotrophic protist genus *Thaumatomastix* (Thaumatomastigaceae, Protista *incertae sedis*), with particular emphasis on material from the Baltic Sea. Ann. Bot. Fennici, 30: 87-108.

Thomsen, H.A. and Larsen, J. (1992). Loricate choanoflagellates of the Southern Ocean with new observations on cell division in *Bicosta spinifera* (Throndsen, 1970) from Antarctica and *Saroeca attenuata* Thomsen, 1979, from the Baltic Sea. Polar Biol., 12: 53-63

Thomsen, H.A., Østergaard, J.B. and Hansen, L.E. (1991). Heteromorphic life histories in arctic coccolithophorids (Prymnesiophyceae). J. Phycol., 27: 634-642.

Throndsen, J. (1969). Flagellates of Norwegian coastal waters. Nytt. Mag. Bot. 16: 161-216.

Throndsen, J. (1970). Marine planktonic acanthoecaceans (Craspedophyceae) from arctic waters. Nytt Mag. Bot., 17: 103-111.

Throndsen, J. (1974). Planktonic choanoflagellates from North Atlantic waters. Sarsia, 56: 95-122.

Throndsen, J. (1983). Ultra- and nanoplankton from coastal waters of southern Honshu and Kyushu, Japan (including some results from the western part of the Kuroshio off Honshu): working party on taxonomy in the Akashiwo Mondai Kenyukai fishing ground preservation division (Research Dept., Fisheries Agency, Japan) 4: 1-68.

Tong, S.M. (1995). *Developayella elegans* gen. nov., spec. nov., a new type of heterotrophic flagellate from marine plankton. Europ. J. Protistol., 31.1: *(in press).*

Triemer, R.E. and Farmer, M.A. (1991). An ultrastructural comparison of the mitotic apparatus, feeding apparatus and cytoskeleton in euglenoids and kinetoplastids. Protoplasma, 164: 91-104.

Valkanov, A. (1970). Beitrag zur Kenntnis des Protozoen des Schwarzen Meeres. Zool. Anz., 184: 241-290.

Verity, P.G., Stoecker, D.K, Sieracki, M.E., and Nelson, J.R. (1993) Grazing, growth and mortality of microzooplankton during the 1989 North Atlantic spring bloom at 47°N 18°W. Deep Sea Res. I, 40: 1793-1814.

Vørs, N. (1988). *Discocelis saleuta* gen. nov. et sp. nov. (Protista incertae sedis). Europ. J. Protistol. 23, 297-308.

Vørs, N. (1992a). Heterotrofe protister (ekskl. dinoflagellater, loricabærende choanoflagellater og ciliater). In: Thomsen, H.A. (ed.): Plankton i de indre danske farvande. Miljøministeriet, Miljøstyrelesen, pp195-250.

Vørs, N. (1992b). Heterotrophic amoebae, flagellates and heliozoa from the Tvärminne area, Gulf of Finland in 1988-1990. Ophelia, 36: 1-109.

Vørs, N. (1992c). Ultrastructure and autecology of the marine heterotrophic flagellate *Leucocryptos marina* (Braaud) Butcher 1967 (Katablepharidaceae/Kathablepharidae), with a discussion of the genera *Leucocryptos* and *Katablepharis/Kathablepharis*. Europ. J. Protistol., 28: 369-389.

Vørs, N. (1993a): Heterotrophic amoebae, flagellates and heliozoa from Arctic marine waters (North-West Territories, Canada and W. Greenland). Polar Biol., 13, 113-126.

Vørs, N. (1993b). Marine heterotrophic amoebae, flagellates and heliozoa from Belize (Central America) and Tenerife (Canary Islands), with descriptions of new species, *Luffisphaera bulbochaete* N. Sp., *L. longihastis* N. Sp., *L. turriformis* N. Sp. and *Paulinella intermedia* N. Sp. J. Euk. Microbiol., 40: 272-287.

Waterbury, J.B., Watson, S.W., Guillard, R.L. and Brand, L.E. (1979). Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. Nature, 277: 293-294.

Webber, N.B. (1980). Hydrography and water circulation in the Solent. In: The Solent estuarine system: an assessment of present knowledge. N.E.R.C. publication series C, 22: 23-35.

Wetherbee, R. and Andersen, R.A. (1992). Flagella of a chrysophycean alga play an active role in prey capture and selection; direct observations on *Epipyxis pulchra* using image enhanced video microscopy. Protoplasma, 166: 1-7.

Wiadnyana, N.N. and Rassoulzadegan, F. (1989). Selective feeding of *Acartia clausi* and *Centropages typicus* on microzooplankton. Mar. Ecol. Prog. Ser., 53: 37-45.

Williams, P.J. (1981). Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. Kiel. Meeresforsch. Sonderh., 5: 1-28.

Wommack, K.E., Hill, R.T., Kessel, M., Russel-Cohen, E. and Colwell, R.R. (1992). Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol., 58: 2965-2970.

Wright, R.T. and Coffin, R.B. (1984). Measuring microzooplankton grazing on planktonic marine bacteria by its impact on bacterial production. Microb. Ecol., 10: 137-149.

Zhukov, B.F. (1991). The diversity of bodonids. In: Patterson, D.J. and Larsen, J. (eds.): The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp177-184.

Zinger, I. (1989). Zooplankton community structure in Southampton Water and its potential response to estuary chronic oil pollution. Ph. D. thesis, University of Southampton, U.K.