

Joe

# Protistan Community Structure

DAVID A. CARON

30

Protistan assemblages of aquatic ecosystems have become the focus of a concerted research effort in aquatic ecology. One stimulus for this work has been the recognition that phototrophic protists (the microalgae) constitute a major fraction of the primary productivity within aquatic ecosystems. Another incentive has been the growing realization that protozoa (heterotrophic protists) play a pivotal role in the flow of energy and elements in these communities (59). Studies of the abundance, biomass, and feeding activity of protozoa have been conducted in a wide range of ecosystems in recent years. In addition, many laboratory studies have examined the general biology and physiology of various protozoa grown under carefully controlled conditions. The synthesis of this information into useful models of how protozoan assemblages are structured and how they function in nature has begun.

Protozoa typically are defined as heterotrophic, eukaryotic, single-celled organisms. It is important to recognize that the term "protozoa" now has more historical significance than phylogenetic or ecological meaning. Protozoa are distinguished from other heterotrophic eukaryotes by their ability to exist as unicells and from the microscopic algae by their inability to photosynthesize. These distinctions are rather ambiguous in many cases. For example, many "protozoan" taxa occur among the "algal" taxa. Numerous species of heterotrophic chrysomonads and dinoflagellates exist. These species are closely related to chloroplast-bearing species on the basis of ultrastructural features and DNA sequence data (3, 44, 65). They are separated from the algae solely on the basis of the absence of a chloroplast. In addition to the existence of apochlorotic "algal" species, chloroplast-bearing genera that are capable of phagotrophy (i.e., heterotrophic nutrition) in addition to photosynthesis exist within the chrysophyte, dinoflagellate, prymnesiophyte, cryptophyte, and euglenophyte algae (62). This "mixotrophic" behavior obscures the distinction between traditional definitions of algae and protozoa.

There are also difficulties in the classification of ciliated protozoa as phototrophs or heterotrophs. Some ciliates ingest and digest algal prey but are able to retain the chloroplasts of these prey in a functional state, thereby providing the ciliates with a photosynthetic capability (62, 73). Photosynthesis in these "green ciliates" contributes significantly to the overall nutrition of the protozoan and also forms a

notable fraction of the primary productivity of some planktonic communities (24).

The close phylogenetic affinities of some flagellated protozoa with algal taxa, as well as the mixed nutrition of many protists, indicate the artificiality of the historical distinction between the algal and protozoan taxa (44). For these reasons, consideration of the "purely protozoan" species in an assemblage is not appropriate from a phylogenetic or an ecological perspective. This discussion will, therefore, include references to chloroplast-bearing protists (i.e., algae).

## ASSESSING PROTISTAN COMMUNITY STRUCTURE

Defining "community structure" in protistan communities is a difficult task. Some of this difficulty is related to problems associated with identifying species in mixed, natural assemblages, while some of it is related to the fact that the appropriate analysis of protistan community structure depends to some extent on the goals of the investigator.

There are at least three major considerations for assessing protistan species diversity in natural samples: the large number of species present in most aquatic environments, the disparate methodologies that are necessary for sampling these species, and the variety of morphological criteria that are used for identifying them. Free-living protists range in size from approximately 2- $\mu\text{m}$  flagellated protists up to some species of radiolaria that can form cylindrical gelatinous colonies measuring 0.01 by 1 m. This 6-order-of-magnitude size range makes it necessary to apply a variety of sampling techniques in order to adequately sample all of the protistan species in an environment. The unique physical and chemical characteristics of different aquatic environments (e.g., planktonic and benthic environments) also contribute to the varied protocols that are necessary to sample protistan assemblages. In addition, identification keys for the major protistan taxa are based on morphological criteria that differ among the various protistan groups. Taxonomic expertise among protozoologists is often limited to one of the major groupings of protozoa (e.g., flagellates, amoebae, and ciliates) or some portion of one of these major categories. Recognizing the difficulties posed by species number, sampling, and taxonomy is central to understanding the present state of our knowledge concerning the structure of natural assemblages of aquatic protists.

Methods for sampling protistan communities typically are tailored for specific groups of protists and often for specific environments. Very distinct sampling methods typically are used for planktonic and benthic environments. Some of these sampling protocols are necessary because of the wide range of protistan abundances in different ecosystems. Abundances of protists in oxygenated surface sediments typically are several orders of magnitude greater than abundances in an equivalent volume of water above the sediment environment.

Even within an environment, sampling protocols must be adapted for particular groups of protists. For example, enumerating species of benthic protozoa among the sedimentary particles in which they exist has been a long-standing problem in assessing protistan species diversity in these environments. Various methods for extracting and concentrating protozoa from sediments have relied on the mobility of the community in response to changing salinity, extraction by centrifugation, or enrichment culture (1, 32, 61, 72). Such approaches have resulted in reasonable estimates of the protozoan diversity of some sediment environments, but the success of these methods is usually group specific. The extraction of protozoa by the sea ice method may work well for highly mobile species such as benthic ciliates, but this method may be less useful for more slowly moving forms such as small amoebae. For the latter forms, enrichment cultivation appears to be the most appropriate method.

Adjustments to sampling protocols also exist for sampling different protistan groups in plankton communities. Sample volumes of 200 to 500 ml are usually sufficient for flagellated protists (typical abundances are hundreds to thousands per milliliter), and volumes of 0.5 to 2 liters are usually sufficient for ciliated protists (typical abundances are tens to thousands per liter), but sarcodine protozoa (amoebae, actinopods, and foraminifera) usually must be concentrated by using plankton nets or filters. These latter techniques, however, are damaging to delicate species of planktonic ciliates (38). Several common methods for sampling protistan assemblages are reviewed by Sieburth (68).

Preservation, fixation, and other manipulations are prerequisites for the identification of most protistan species once appropriate samples have been collected. Notable exceptions to this generalization are the "naked" amoebae (primarily the Gymnamoebae), in which some of the characteristics that are essential for proper identification are present only in living specimens. For the remaining protist groups, correct preservation is dependent on the protistan group under consideration. For flagellated (and often ciliated) protists, aldehyde fixatives (formaldehyde and glutaraldehyde) are commonly used, often followed by osmium tetroxide when electron microscopy is planned (43, 67). A variety of fixatives have been developed for ciliated protozoa, most of which are usually employed in combination with postfixation staining methods that are used to visualize cytological features of the cells (44, 75).

The preservation of some protistan taxa requires special consideration. A preservative that does not promote dissolution of skeletal structures must be used for those species which possess such structures (e.g., actinopods and foraminifera). Careful adjustment of the pH of the preservative is necessary to prevent dissolution of foraminiferan tests (9), while addition of strontium is necessary to prevent dissolution of acantharian skeletons (50). When these requirements conflict, subsamples must be preserved separately for the different groups. For example, samples for planktonic foraminifera (which require alkaline pH) typically would be

preserved differently than samples for planktonic ciliates, which are often preserved in acid Lugol's solution (75).

The identification of protistan species in mixed natural assemblages depends on criteria that are often as different as the methodologies used to sample and preserve these assemblages. Ciliates typically possess morphological features that provide sufficient taxonomic criteria for identifying species by light microscopy. Cell shape, size, location, and characteristics of the oral area, presence of a lorica, and particularly the arrangement of the somatic ciliature are useful features for species identification (44). Ciliates are often easier to identify than many of the flagellated and amoeboid protists because of the presence of these features, and extensive species lists exist for various environments (see reference 57 for a review).

Flagellated protists typically possess fewer morphological features that can serve as useful taxonomic criteria when they are observed by light microscopy. Cell size and shape, chloroplast arrangement, and flagellation are important criteria for identification by light microscopy. Some diagnostic features (e.g., flagellar mastigonemes and body scales), however, are visible only by electron microscopy. Electron microscopy is often necessary for distinguishing the numerous genera and species of small heterotrophic flagellates (<10  $\mu\text{m}$ ). The need to establish these features by using electron microscopy makes it difficult to process large numbers of samples. Moreover, many of the latter taxa have not been adequately described. There is considerable uncertainty about the validity of numerous genera (56) and, thus, the true species diversity of small heterotrophic flagellates in many environments.

The amoeboid protists are a polyphyletic collection of species, and the methods of identification applied to these species are heterogeneous. The naked amoebae are identified on the basis of features of the living organisms: cell size and shape during locomotion, arrangement and type of pseudopodia, morphology of the floating form, etc. The requirement for live material for species identification has made the determination of species diversity of natural assemblages of amoebae a difficult topic, but the taxonomy, distribution, and general ecology of these species are slowly emerging (5, 20, 53). Identification of the many types of testate amoeboid protists (testacea, foraminifera, radiolaria, heliozoa, and others) is based on the skeletal structures that are present in these species and on features of cellular organization such as the pseudopodia that characterize this diverse assemblage. The presence of a rigid skeletal structure in many of these species makes it possible to use plankton nets or screens for collecting and concentrating these specimens from the plankton or sediment.

Difficulties associated with sampling and identifying the entire spectrum of protists (as described above) in natural communities hampers the documentation of true protistan species diversity of any natural ecosystem. Very few, if any, studies have attempted to characterize all of the protistan species present in an aquatic ecosystem or have documented even all of the heterotrophic protistan species. Exceptions to this generalization might be found in environments in which protozoan diversity is greatly reduced as a result of severe environmental factors such as anaerobic conditions (36), but it is safe to say that the vast majority of studies of natural communities have underestimated total protistan species diversity.

Analyses of species diversity for particular taxa of protists (i.e., the ciliates, flagellates, or amoebae), however, have been more accurately determined. The most complete infor-

mation exists for plankton communities, for which extensive lists of ciliated protozoa, chloroplast-bearing flagellates, and skeleton-bearing sarcodines (foraminifera and actinopods) have been obtained (8, 29, 48, 54, 55, 57).

### Protozoan Abundance and Biomass

Identification of the protistan species present in an aquatic environment provides useful but limited information on their potential contribution to the structure and function of the total biological community because of the tremendous size range and varied trophic activities of protistan species. A much greater understanding of their importance can be obtained by combining species lists with estimates of abundance and biomass. Most modern methods for collection and identification of protists have been designed with this goal in mind. Loss of protistan cells during collection, enrichment, preservation, and sample processing continues to be a major concern in studies attempting to measure protistan species diversity and abundance, but generally accepted methods which minimize this problem for specific groups of protists and allow accurate estimates of protistan abundance to be obtained are now emerging.

The estimation of population abundances of amoebae presents a particularly difficult problem. Not only must these species be observed alive in order to be accurately identified, but their amorphous cell shape makes them difficult to enumerate in preserved samples. The few abundance estimates that are available for these species have been obtained by using a most probable number culture technique that relies on the growth of the amoebae in serial dilutions of the water or sediment samples (25, 61).

Protistan abundance measurements can be used to calculate total protistan biomass (typically expressed in units of carbon), using measurements of abundance, cell volume, and empirically derived carbon/volume conversion factors. Cell volume measurements obtained from microscopical studies are combined with abundance estimates to calculate the volume of particular protistan taxa, and carbon/volume conversion factors are then applied to calculate the carbon content. Carbon/volume conversion factors must take into account shrinkage due to fixation and the variable vacuolar space of protists. Shrinkage due to fixation can be both taxon and size specific. Recently published values for converting carbon to volume are 160, 240, and 360 fg of C  $\mu\text{m}^{-3}$  for flagellated protists of  $10^3$ ,  $10^2$ , and  $10^1 \mu\text{m}^3$ , respectively (80), and 190 fg of C  $\mu\text{m}^{-3}$  for ciliated protists (58) preserved appropriately. Carbon/volume conversion factors for larger sarcodines (acantharia, radiolaria, and foraminifera) are based on aspects of the cells that are resistant to net collection (51). Methods for estimating the cell volume of naked amoebae that directly relate the diameter of the nucleus to total cell volume have been proposed (60).

### Describing Protistan Community Structure

The term "community structure" implies that organized relationships exist between protists and other microorganisms within natural ecosystems. Indeed, the "niche" concept has been applied to protistan assemblages with the implication that the number of protistan species in an environment is indicative of the number of unique ecological roles for protists in these assemblages. Unfortunately, it is unrealistic to consider all protistan species in a community as separate entities at this time because of the great species diversity of these assemblages, the limited ecological information available on the realized niches of many protistan species, and the extreme difficulty in obtaining species identifications

and abundance/biomass information for all protistan species in an assemblage. For these reasons, various simplifying groupings of protists have been used as a way of reducing the complexity of protistan assemblages into manageable (and measurable) quantities.

Various manners of grouping species of protists have been used. The most popular types of these methods have attempted to group protists by trophic mode (phototrophic versus heterotrophic), size, and prey type (for heterotrophs), in keeping with the trophic-level concept of Lindeman (47). In recent years, however, there has been a recognition that these trophic categories must be somewhat more flexible than originally proposed because of the common behavior of mixed nutrition among protists (18, 79). Nevertheless, aggregation of species into "trophospecies" (79) is a useful and necessary procedure for compartmentalizing the assemblage in order to allow investigations of energy and elemental flow through aquatic communities in models of manageable size (22).

For heterotrophic protists, it is common to group species according to the type of prey that they consume. Bacterivorous flagellates and ciliates in plankton communities or bacterivorous flagellates and amoebae on suspended particles may be grouped together to represent a major sink for bacterial biomass in the plankton. Similarly, ciliate species may be grouped into bacterivorous, herbivorous, or predacious species (26). Such classifications based on "feeding guilds" ignore some of the details of protistan feeding behavior (such as omnivory), but they are useful for reducing the complexity of the assemblage. Feeding guilds are often treated as single species in biological or biogeochemical models of ecosystem function.

The organization of protists by size is a logical one for two reasons. Allometric dependence of growth and metabolism can be used to constrain the potential contribution of a particular size range of protists to biogeochemical cycles (7, 19, 34). In addition, predator-prey relationships are typically size dependent, with larger predators consuming smaller prey. This generalization is realistic. Many heterotrophic flagellates 2 to 20  $\mu\text{m}$  in size consume bacteria and cyanobacteria that are  $<2 \mu\text{m}$  in size, and many ciliate species 20 to 100  $\mu\text{m}$  in size consume algae and protozoa  $<20 \mu\text{m}$  in size.

There are some notable exceptions, however, to size-dependent grazing. Many species of heterotrophic dinoflagellates consume diatom prey that are considerably larger than themselves by employing a pseudopodial "feeding veil" (41). Similarly, some planktonic sarcodines (acantharia, radiolaria, and foraminifera), because they produce a sticky pseudopodial network that entangles and immobilizes prey items, are capable of consuming metazoan prey considerably larger than themselves (76).

Notwithstanding these exceptions, size-dependent grazing models are the most common means of organizing protistan populations into manageable units for inclusion into models of elemental flow in aquatic ecosystems (6, 28, 52). The aggregation of species into groups within models probably reduces the predictive capabilities of these models, but their outcomes thus far appear to be in reasonable agreement with field data. It remains to be seen how the reduction of species diversity in these models will affect predictions of the response of the community to internal and external perturbations, but the gradual disaggregation of these models into more ecologically relevant compartments should provide insight into this issue.

TABLE 1 Species list showing the range of protistan diversity of an oligotrophic oceanic environment<sup>a</sup>

Protistan taxon	Avg size (μm)	Chloroplasts present?	Phagotrophy?	Probable prey	Representative abundance (liter <sup>-1</sup> )
Flagellated and nonmotile protists					
Dinoflagellates					
<i>Protoperdinium</i> sp.	55	No	No	Dia	10 <sup>2</sup>
<i>Gymnodinium</i> sp.	50	Yes	Yes	C, Din, Dia	2 × 10 <sup>2</sup>
<i>Prorocentrum micans</i>	25 by 40	Yes	No		10 <sup>3</sup>
<i>Ornithocercus magnificus</i>	40	No <sup>b</sup>	Yes	Dia, Sf	10 <sup>2</sup>
Chrysophytes and chrysoomonads					
<i>Paraphysomonas imperforata</i>	7.0	No	No	B, Cc, Sf	10 <sup>5</sup>
<i>Ochromonas</i> sp.	6.0	Yes	Yes	B, Cc	10 <sup>5</sup>
Prymnesiophytes					
<i>Chrysochromulina ericina</i>	6.0	Yes	Yes	B, Cc	10 <sup>4</sup>
Chlorophytes					
<i>Nannochloris atomus</i>	4.0	Yes	No		10 <sup>5</sup>
Bacillariophytes (diatoms)					
<i>Minutocellus polymorphus</i>	3.0	Yes	No		10 <sup>3</sup>
<i>Coscinodiscus concinnus</i>	75 by 200	Yes	No		10 <sup>-2</sup>
<i>Ditylum brightwellii</i>	20 by 100	Yes	No		10 <sup>2</sup>
<i>Rhizosolenia clevei</i>	200 by 500	Yes	No		10 <sup>-3</sup>
<i>Ethmodiscus rex</i>	1,000	Yes	No		10 <sup>-4</sup>
Choanoflagellates					
<i>Diaphanoeca grandis</i>	2.5	No	Yes	B	10 <sup>3</sup>
Amoeboid protists					
Gymnamoebae (naked amoebae)					
<i>Platyamoeba weinsteini</i>	3 by 12	No	Yes	B	10 <sup>0</sup>
<i>Flabellula citata</i>	4 by 30	No	Yes	B	5 × 10 <sup>-1</sup>
Foraminifera <sup>c</sup>					
<i>Globigerina bulloides</i>	700 <sup>d</sup>	No	Yes	Omi, Mz	10 <sup>-3</sup>
<i>Globigerinoides sacculifer</i>	700 <sup>d</sup>	No <sup>e</sup>	Yes	Omi, Mz	10 <sup>-3</sup>
Acantharea <sup>c</sup>					
<i>Amphilonche elongata</i>	50 by 400	No <sup>e</sup>	Yes	Omi	10 <sup>-1</sup>
Spumellarian radiolaria <sup>c</sup>					
<i>Thalassicolla nucleata</i>	1,000 <sup>d</sup>	No <sup>e</sup>	Yes	Omi, Mz	10 <sup>-4</sup>
<i>Collozoum caudatum</i>	200 <sup>d,f</sup>	No <sup>e</sup>	Yes	Omi, Mz	10 <sup>-5</sup>
Ciliated protists					
Tintinnids					
<i>Tintinnopsis parva</i>	20 by 40	No	Yes	B, Cc, Sf	10 <sup>2</sup>
Oligotrichs					
<i>Strombidium sulcatum</i>	25 by 50	Yes <sup>g</sup>	Yes	B, Cc, Sf	10 <sup>2</sup>
<i>Loboea strobila</i>	50 by 150	Yes <sup>g</sup>	Yes	B, Cc, Sf	10 <sup>2</sup>
Hypotrichs					
<i>Euplotes woodruffi</i>	65 by 120	No	Yes	C	5 × 10 <sup>-1</sup>
Hymenostomatids					
<i>Uronema marinum</i>	10 by 20	No	Yes	B, Cc	10 <sup>3</sup>

<sup>a</sup> Only a partial list of representative species to exemplify the breadth of trophic modes in a real assemblage. Species are organized according to major taxa. Pertinent ecological information and realistic abundances are based on literature values. Dia, diatoms; C, ciliates; Din, dinoflagellates; Sf, small flagellated protists; B, bacteria; Cc, chroococcoid cyanobacteria; Mz, metazoan zooplankton; Omi, omnivorous on prokaryotic and eukaryotic unicells.

<sup>b</sup> Species harboring extracellular, symbiotic cyanobacteria that contribute to the photosynthetic nutrition of the host.

<sup>c</sup> Adult specimens only.

<sup>d</sup> Size does not take into account extensive pseudopodial network.

<sup>e</sup> Species harboring intracellular, symbiotic dinoflagellates that contribute to the photosynthetic nutrition of the host.

<sup>f</sup> Colonial species with colonies up to 1 cm in width and 1 m in length.

<sup>g</sup> Species that retain functional chloroplasts from ingested prey.

### An Example from the Plankton

A hypothetical example easily indicates the analytical approaches for examining protistan community structure and the limitations of these approaches. A species list of protists that are typical of an oceanic plankton community is given

in Table 1. This assemblage is not meant to be complete but rather indicative of the breadth of protistan sizes and nutritional modes in this type of ecosystem. Pertinent information on cell size, photosynthetic and phagotrophic ability, prey type(s), and typical abundances are also provided.

The species in this assemblage have been arranged according to major taxonomic categories (44).

As shown in Table 1, taxonomic groupings of the protists correspond poorly to the nutritional modes of the species. Reorganization of the same species into groups based on the nutritional modes of the species provides a very different view of this assemblage (Table 2). This reorganization indicates the classical dichotomy between phototrophs and heterotrophs, but it also indicates the more recent realization that many protistan species possess the ability for mixed nutrition. This ability results in some of the species occupying more than one trophic category.

The collection of species in this assemblage also demonstrates the enormous breadth of cell sizes that can be displayed by protistan assemblages (Fig. 1). The size range is not necessarily restricted for any particular trophic mode. In this assemblage, heterotrophs range from 2.5  $\mu\text{m}$  to >1 mm in size, phototrophs range from 3.0  $\mu\text{m}$  to >1 mm, and mixotrophs range from 6.0  $\mu\text{m}$  to >1 mm if one includes symbiont-bearing sarcodines in this last category. Commonly used plankton size class designations are also shown in this figure. These designations correspond to organisms 2 to 20  $\mu\text{m}$  (nanoplankton), 20 to 200  $\mu\text{m}$  (microplankton), 0.2 to 20 mm (mesoplankton), and 20 to 200 mm (macroplankton) in longest dimension (69). Protists occur in all of these size classes, as indicated in Fig. 1, although the majority of these species typically occur in the nano- and microplankton classes.

One generality that is clear from Table 1 is that small planktonic protists typically occur in greater abundances than large species. This relationship is shown clearly by differences of 1 to 2 orders of magnitude in abundances when the species in each plankton size class are summarized (Fig. 2A). The magnitudes of these differences are typical for both phototrophic and heterotrophic protists (and often for mixotrophs, as shown in Fig. 2). However, the abundances of phototrophic, mixotrophic, and heterotrophic protists within a size class are often similar (hatched and stippled columns within each size class).

The large disparity that is apparent when one compares the abundances of protists in different size classes (Fig. 2A) is greatly reduced when the total volumes of living protists are compared (Fig. 2B). Small cell size among nanoplankton is generally balanced by high abundances of these species, while low abundances of the larger protists are balanced by their larger volumes. These general relationships of protistan abundance and biovolume are consistent with data from natural assemblages of nanoplanktonic and microplanktonic protists (17).

The information summarized in Tables 1 and 2 and Fig. 1 and 2 can be used to construct a typical box model depicting the flow of materials from producers through consumers in this hypothetical protistan community (Fig. 3). The species have been grouped according to their nutritional modes and approximate predator-prey relationships. Arrows in the model indicate the presumed direction of energy and material flow (i.e., from producers to consumers and from small organisms to large consumers). Examination of this model exemplifies the advantages and disadvantages of this approach for describing nutritional modes and trophic relationships among protists in a mixed natural assemblage.

The goals for most of the work on modeling "microbial loop" processes have been the development of working models that accurately describe energy and elemental flow within these communities and the incorporation of microbial processes into classical models of aquatic community

TABLE 2 Planktonic protist species described in Table 1 but arranged according to trophic category<sup>a</sup>

#### Phototrophy

*Gymnodinium* sp.  
*Prorocentrum micans*  
*Ochromonas* sp.  
*Chrysochromulina ericina*  
*Nannochloris atomus*  
*Minutocellus polymorphus*  
*Coscinodiscus concinnus*  
*Ditylum brightwellii*  
*Rhizosolenia clevei*  
*Ethmodiscus tex*

#### Mixotrophy

Phagotrophic algal species  
*Gymnodinium* sp.  
*Ochromonas* sp.  
*Chrysochromulina ericina*  
Chloroplast-retaining species  
*Strombidium sulcatum*  
*Loboea strobila*  
Symbiont-bearing species  
*Ornithocercus magnificus*  
*Hastigerina pelagica*  
*Amphilonche elongata*  
*Thalassicolla nucleata*  
*Collozoum caudatum*

#### Heterotrophy

##### Bacterivory

*Paraphysomonas imperforata*  
*Ochromonas* sp.  
*Chrysochromulina ericina*  
*Diaphanoeca grandis*  
*Platyamoeba weinsteini*  
*Flabellula citata*  
*Amphilonche elongata*  
*Thalassicolla nucleata*  
*Collozoum caudatum*  
*Tintinnopsis parva*  
*Strombidium sulcatum*  
*Loboea strobila*  
*Uronema marinum*

##### Herbivory

*Protoperidinium* sp.  
*Gymnodinium* sp.  
*Ornithocercus magnificus*  
*Paraphysomonas imperforata*  
*Amphilonche elongata*  
*Thalassicolla nucleata*  
*Collozoum caudatum*  
*Tintinnopsis parva*  
*Strombidium sulcatum*  
*Loboea strobila*  
*Globigerina bulloides*

##### Carnivory

*Paraphysomonas imperforata*  
*Globigerina bulloides*  
*Amphilonche elongata*  
*Thalassicolla nucleata*  
*Collozoum caudatum*  
*Euploes woodruffi*

<sup>a</sup> Note that some species occur in more than one category.

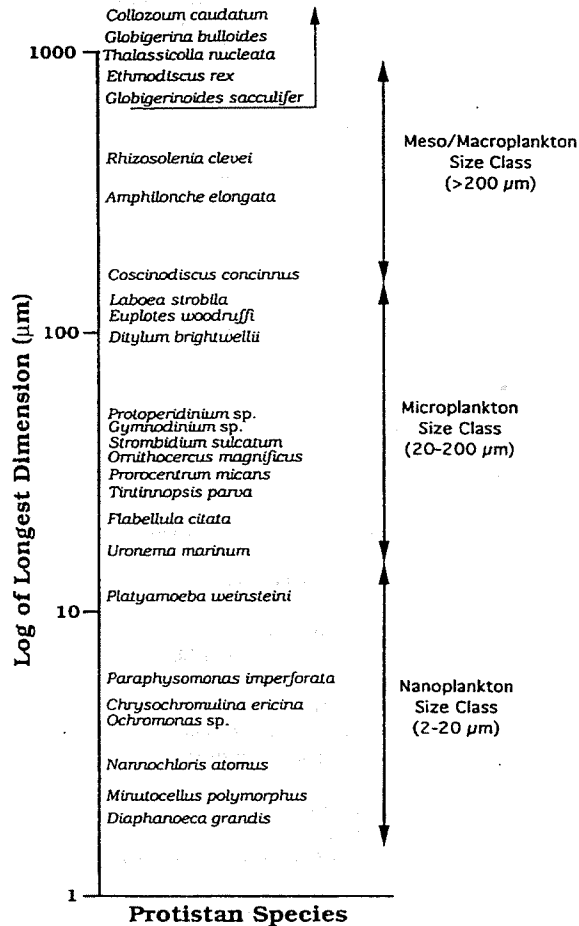


FIGURE 1 Approximate sizes (longest dimension) of the planktonic protistan species listed in Table 1. Commonly used size class designations are shown on the right. Note that the sizes of these protists span more than 3 orders of magnitude. The upward-pointing arrow under *Globigerinoides sacculifer* indicates that the group of five species enclosed by the arrow can be larger than 1,000  $\mu\text{m}$ .

structure and function. Models such as the one shown in Fig. 3 are appropriate for these purposes because they attempt to reduce a complex assemblage of microorganisms to a manageable number of trophic "compartments" and trophic interactions. These models, therefore, are strongly influenced by methodologies available for identifying protistan species (or trophospecies) and for investigating their trophic interactions.

The model in Fig. 3 might adequately describe energy or elemental flow in this hypothetical protistan assemblage if the biomass and flow parameters of the model could be determined. However, this type of depiction of community structure still has some inherent disadvantages. As referred to earlier, predator-prey relationships that are not size dependent are difficult to represent and measure. Energy is depicted as moving from smaller to larger size classes in this model, but this representation is incorrect for species such as *Protoperidinium sp.*, which can graze on diatoms larger than itself, and for the sarcodines *Globigerinoides sacculifer*, *Thalassicolla nucleata*, *Collozoum caudatum*, and *Globigerina bulloides*, which can consume metazoan prey. The double-

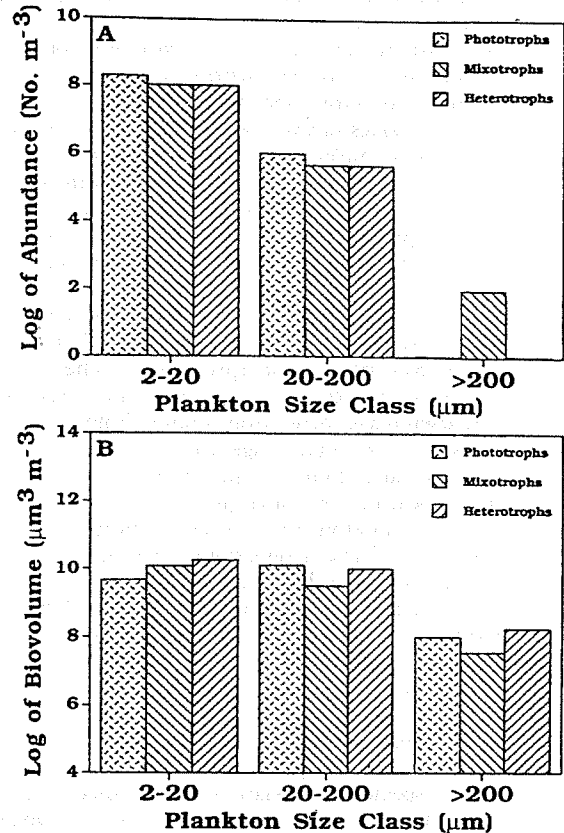


FIGURE 2 Abundance and biovolume relationships of the protistan assemblage listed in Table 1. The species have been grouped according to size class and according to trophic mode (phototrophic, mixotrophic, heterotrophic).

headed arrows connecting these latter compartments indicate the potential for the flow of energy in either direction. In practice, these measurements are difficult to make.

Similarly, selective grazing and omnivory are difficult to incorporate into this type of model. For example, *Euplotes woodruffi* is a predacious ciliate feeding primarily on other ciliates (in this assemblage, it might feed on *Uronema marinum*). On the other hand, *Tintinnopsis parva* may accept a variety of small protists and other microorganisms as prey. The distinction between these two rather different nutritional modes has been forfeited by placing them into the same trophic compartment. Clearly, if the goal of this modeling exercise was to understand the factors affecting the success or failure of either of these two species in plankton communities, then this model would be unsatisfactory. It is for reasons such as this last example that the appropriate conceptualization and representation of protistan community structure must take into account the goal of the investigator.

## TEMPORAL AND SPATIAL CHANGES IN COMMUNITY STRUCTURE

The most significant differences in the species composition and trophic relationships of protistan communities exist between different aquatic environments. However, there is also a rapidly increasing database on changes in community

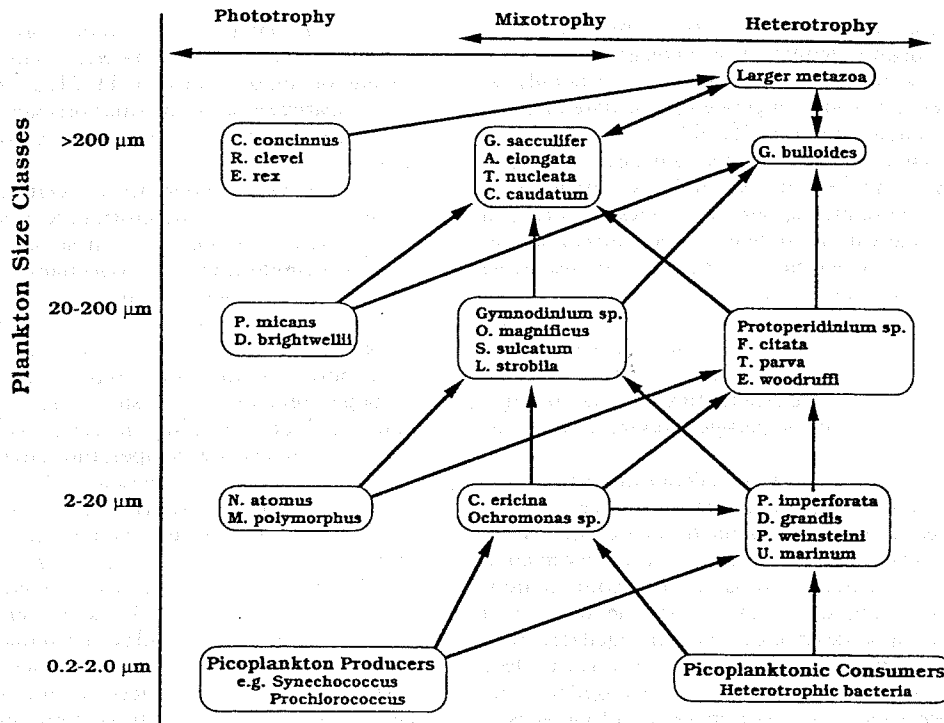


FIGURE 3 A box model approximating the major trophic interactions within the protistan assemblage listed in Table 1. The species have been grouped according to known or presumed size-dependent and trophy-dependent relationships. Arrows indicate the direction of energy flow in predator-prey interactions.

structure over seasonal and shorter time scales. These latter changes appear to be most significant in temperate and polar climates.

**Freshwater versus Marine Ecosystems**

Probably the most distinct difference between freshwater and marine protistan communities is the restriction of the larger sarcodines (acantharia, radiolaria, and foraminifera) to brackish and marine ecosystems. In tropical and subtropical oceanic plankton communities, adult sarcodines are often the most conspicuous macroscopic organisms in surface water, while swarmer cells and juvenile specimens of these species contribute to the entire size spectrum of protozooplankton (20). In marine sediments, benthic foraminifera constitute an important component of faunal assemblages (39, 45).

In contrast to large differences in the assemblages of larger sarcodine species in freshwater and salt water, there appears to be similarity with respect to types of ciliates and flagellates in these environments. Recent summaries from both environments have indicated that planktonic bacterial biomass and production are related to phytoplankton biomass and primary productivity (23). Likewise, the ecological roles of small protozoa in freshwater and marine plankton communities appear to be similar and related to bacterial production over very broad scales of examination (63). Most ciliates in the freshwater and marine plankton also appear to play grossly analogous ecological roles as consumers of small prokaryotes and eukaryotes (10, 57). Mixotrophic (phagotrophic) algae exist in both freshwater and marine water (4, 12, 13), as do species of chloroplast-retaining ciliates (35, 74). These generalizations do not mean that the

same species of flagellates or ciliates occur in both ecosystems, but rather that similar ecological niches have been filled by protistan species in these different environments.

**Benthic versus Pelagic Ecosystems**

Although there are species of protists that are commonly found in both benthic and pelagic environments, there are clearly numerous species within these assemblages that are uniquely suited for one environment or the other. Morphological adaptations of ciliates to life between sediment particles in the benthos have resulted in the evolution of cell forms that allow movement through this medium. Common adaptations include cylindrical or flattened shapes, flexible cell walls, and patterns of ciliature that allow "crawling" along surfaces and grazing on prey loosely associated with particulate material. Some species permanently attach to surfaces. In contrast, ciliates in pelagic environments (e.g., choreotrichs) tend to have more rounded shapes and patterns of ciliature that afford rapid swimming behavior and feeding on suspended particles.

There is great diversity among and within benthic environments as a consequence of sediment grain size, organic loading, oxygen gradients, etc. The number of microenvironments at one locale may be considerable. For this reason, the remoteness of many benthic environments, and the difficulties of sampling and concentrating protists from these environments, the ciliate (and other protist) fauna of many benthic ecosystems is still poorly characterized. There are extremely few observations of the protistan fauna of the deep-ocean benthos (39, 71, 78).

The amoebae are particularly well suited for existence in benthic environments. Locomotion and feeding of these

species take place on particles. Therefore, benthic environments tend to support significant assemblages of amoebae (64). Amoebae occurring in the plankton are generally assumed to be associated with suspended particulate material or with the air-water interface (25, 61).

Among the larger sarcodines, there are clear differences between pelagic and benthic assemblages. Foraminifera occur in both environments, but the species occurring in these two environments are different. The planktonic species are restricted to pelagic, oceanic ecosystems, while benthic species are common from salt marshes to abyssal depths. Most radiolaria (polycystines and phaeodaria) and acantharia are restricted to pelagic, oceanic ecosystems, but most heliozoa are coastal and/or benthic. There are relatively few exceptions to these generalities, making the larger sarcodine fauna of benthic and pelagic ecosystems quite distinct.

Beyond the obvious contribution of phototrophic protists to the flagellated protistan assemblages in surface waters of pelagic ecosystems, the heterotrophic flagellate assemblages of benthic and pelagic environments can also differ in composition. Many flagellated protozoa occur in both environments, but species that are capable of particle attachment or movement along surfaces (e.g., bodonid flagellates) tend to predominate in benthic environments. Forms that feed on suspended bacteria (e.g., chryomonad flagellates and choanoflagellates) tend to predominate in pelagic ecosystems.

Pelagic environments generally are considered to be more homogeneous than benthic ecosystems, but there are clearly sources of heterogeneity in the plankton. Epibiotic (and possibly enteric) protistan assemblages have not been adequately studied, but they contribute to protistan species diversity in the water column (77). Suspended particles also create unique microhabitats in pelagic ecosystems for some protozoan species that are more characteristic of the benthos. Macroscopic detrital aggregates in marine planktonic ecosystems (so-called marine snow) may create a false benthos for benthic species by creating microenvironments with elevated abundances of bacteria and other prey (16, 70). Similar oases for unique protozoan assemblages in the plankton may be established by using artificial foam substrates (14). It has been demonstrated that the colonization and species succession of protozoa on these natural and artificial substrates may follow a pattern similar to that found for the colonization of oceanic islands by higher organisms (14, 84).

### Depth and Seasonal Distributions

The seasonality of algal species composition and abundance in pelagic environments is well known. Distribution of the protistan algae with depth has also received considerable attention. In contrast, changes in total protozoan abundance or biomass with season or depth have been documented, but there is relatively little information on changes in species composition or community structure and function with depth. This paucity of information is not surprising given the difficulties mentioned previously with identification and high species diversity and the logistical problems associated with the collection of long-term data sets or multiple samples from discrete depths.

Most studies to date have been restricted to a particular group of heterotrophic protists because of either methodological approach or taxonomic expertise. Often these investigations have reported only changes in broad taxonomic or ecologically relevant categories (heterotrophic flagellates, mixotrophic flagellates, ciliates, etc.). For example, depth

and seasonal changes in abundance have been reported in a variety of marine and freshwater environments for flagellated and/or ciliated protozoa (11, 21, 27, 42). More detailed data on spatiotemporal distributions are available only for specific taxa for which identification is more straightforward (8, 40).

It is difficult to generalize concerning changes in the community structure of heterotrophic protistan assemblages as a function of season from these scattered reports. For temperate communities, seasonal changes in species composition and winter reductions in the intensity of grazing activity are likely, but the extent of these changes remains largely undetermined for most environments. Temperature is a strong controlling influence on processes within the microbial loop of temperate ecosystems (82), but diverse heterotrophic and phototrophic protistan assemblages abound even in extremely low temperature environments such as sea ice habitats near Antarctica (37).

The vertical distributions of heterotrophic protists typically demonstrate greater overall abundances in surface waters relative to abundances at depth. These distributions of abundance are clearly related to the production of organic material in surface waters. Fine-scale vertical distributions, however, can be complex. Elevated abundances of protozoa have been observed at the air-water interface (25), at oxic-anoxic boundaries within water columns (85), and at subsurface biological features such as deep chlorophyll maxima (30). Vertical distributions of protists in the sediments typically are related to physical and chemical gradients within the benthos (33). The exploitation of these chemical and physical features within the benthos and water column can increase the diversity of protistan assemblages of an environment by providing unique microhabitats for the growth of species able to exist there.

### NEW APPROACHES TO STUDYING PROTISTAN COMMUNITY STRUCTURE

The significant problems associated with determining species diversity, abundance/biomass, and trophic activity of protistan assemblages in aquatic ecosystems continue to hamper in-depth analyses of the structures and functions of these communities. As described above, classical methods of identification are time-consuming and often do not provide quantitative information on the occurrence of species. New conceptual and methodological approaches will be necessary to deal with these recalcitrant problems.

Conceptual approaches and methodologies from molecular biology and immunology offer hope for addressing some of these problems. For example, RNA-targeted oligonucleotide probes and PCR-based methods for determining the presence of specific microorganisms are becoming commonplace in medical and environmental bacteriology (2). At present, the application of these approaches to protistan ecology is largely confined to investigations designed to determine the presence or absence of species of interest to human health (49) or species that might have adverse environmental impact (66). Applications to species of purely ecological significance, however, are beginning (31). Development of immunological methods for determining the presence and abundance of specific microbiological taxa also has begun. This approach has been successfully applied to phototrophic protists (15).

The application of molecular and immunological approaches to protistan groups with problematic taxonomic features may be particularly helpful in the future. For exam-

ple, species of heterotrophic flagellates or amoebae which presently involve nonquantitative or impractical approaches such as electron microscopy or the observation of living specimens might be particularly suited for this approach (46).

Biochemical markers to indicate the presence, abundance, and activity of heterotrophic protists also may provide new methods for examining natural assemblages of protists. Detailed pigment analyses have provided useful insights into the contribution of microalgal taxa to total algal biomass (83). Lipid biomarkers have been applied to obtain information on the biomass and nutritional status of bacterial assemblages (81). Analogous methodologies for assessing community-level features of heterotrophic protistan assemblages would be useful for investigating natural, mixed assemblages of protists.

Support for the preparation of this chapter was provided by National Science Foundation grants OCE-9216270, 9314533, and 9310693.

## REFERENCES

- Alongi, D. M. 1986. Quantitative estimates of benthic protozoa in tropical marine systems using silica gel: a comparison of methods. *Estuarine Coastal Shelf Sci.* 23: 443-450.
- Amann, R. I., W. Ludwig, and K.-H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59:143-169.
- Andersen, R. A., G. W. Saunders, M. P. Paskind, and J. P. Sexton. 1993. Ultrastructure and 18S rRNA gene sequence for *Pelagomonas calceolata* gen. et sp. nov. and the description of a new algal class, the Pelagophyceae classis nov. *J. Phycol.* 29:701-715.
- Arenovski, A. L., E. L. Lim, and D. A. Caron. 1995. Mixotrophic nanoplankton in oligotrophic surface waters of the Sargasso Sea may employ phagotrophy to obtain major nutrients. *J. Plankton Res.* 17:801-820.
- Arndt, H. 1993. A critical review of the importance of rhizopods (naked and testate amoebae) and actinopods (heliozoa) in lake plankton. *Mar. Microb. Food Webs* 7: 3-29.
- Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* 10: 257-263.
- Banse, K. 1982. Cell volumes, maximal growth rates of unicellular algae and ciliates, and the role of ciliates in the marine pelagial. *Limnol. Oceanogr.* 27:1059-1071.
- Bé, A. W. H. 1977. An ecological, zoogeographic and taxonomic review of recent planktonic foraminifera, p. 1-100. In A. T. S. Ramsay (ed.), *Oceanic Micropaleontology*. Academic Press, London.
- Bé, A. W. H., and O. R. Anderson. 1976. Preservation of planktonic foraminifera and other calcareous plankton, p. 250-258. In H. F. Steedman (ed.), *Zooplankton Fixation and Preservation*. UNESCO Press, Paris.
- Beaver, J. R., and T. L. Crisman. 1989. The role of ciliated protozoa in pelagic freshwater ecosystems. *Microb. Ecol.* 17:111-136.
- Bernard, C., and F. Rassoulzadegan. 1994. Seasonal variations of mixotrophic ciliates in the northwest Mediterranean Sea. *Mar. Ecol. Prog. Ser.* 108:295-301.
- Berninger, U.-G., D. A. Caron, and R. W. Sanders. 1992. Mixotrophic algae in three ice-covered lakes of the Pocono Mountains, USA. *Freshwater Biol.* 28:263-272.
- Bird, D. F., and J. Kalff. 1986. Bacterial grazing by planktonic lake algae. *Science* 231:493-495.
- Cairns, J., Jr., D. L. Kuhn and J. L. Plafkin. 1979. Protozoan colonization of artificial substrates, p. 34-57. In R. L. Wetzel (ed.), *Methods and Measurements of Periphyton Communities: a Review*. Special Technical Publication 690. American Society for Testing and Materials, Philadelphia.
- Campbell, L., L. P. Shapiro, and E. Haugen. 1994. Immunohistochemical characterization of eukaryotic ultraplankton from the Atlantic and Pacific oceans. *J. Plankton Res.* 16: 35-51.
- Caron, D. A. 1991. Heterotrophic flagellates associated with sedimenting detritus, p. 77-92. In D. J. Patterson and J. Larsen (ed.), *The Biology of Free-Living Heterotrophic Flagellates*, special vol. 45. Clarendon Press, Oxford.
- Caron, D. A., H. G. Dam, P. Kremer, E. J. Lessard, L. P. Madin, T. C. Malone, J. M. Napp, E. R. Peele, M. R. Roman, and M. J. Youngbluth. 1995. The contribution of microorganisms to particulate carbon and nitrogen in surface waters of the Sargasso Sea near Bermuda. *Deep-Sea Res.* 42:943-972.
- Caron, D. A., and B. J. Finlay. 1994. Protozoan links in food webs, p. 125-130. In K. Hausmann and N. Hülsmann (ed.), *Progress in Protozoology. Proceedings of the IX International Congress of Protozoology, Berlin 1993*. Gustav Fischer Verlag, Stuttgart.
- Caron, D. A., and J. C. Goldman. 1990. Protozoan nutrient regeneration, p. 283-306. In G. M. Capriulo (ed.), *Ecology of Marine Protozoa*. Oxford University Press, New York.
- Caron, D. A., and N. R. Swanberg. 1990. The ecology of planktonic sarcodines. *Rev. Aquat. Sci.* 3:147-180.
- Carrick, H. J., and G. L. Fahnenstiel. 1990. Planktonic protozoa in Lakes Huron and Michigan: seasonal abundance and composition of ciliates and dinoflagellates. *J. Great Lakes Res.* 16:319-329.
- Christian, R. R. 1994. Aggregation and disaggregation of microbial food webs. *Microb. Ecol.* 28:327-329.
- Cole, J. J., S. Findlay, and M. L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.* 43:1-10.
- Crawford, D. W. 1989. *Mesodinium rubrum*: the phytoplankton that wasn't. *Mar. Ecol. Prog. Ser.* 58:161-174.
- Davis, P. G., D. A. Caron, and J. M. Sieburth. 1978. Oceanic amoebae from the North Atlantic culture, distribution, and taxonomy. *Trans. Am. Microsc. Soc.* 96: 73-88.
- Dolan, J. R. 1991. Guilds of ciliate microzooplankton in the Chesapeake Bay. *Estuarine Coastal Shelf Sci.* 33: 137-152.
- Dolan, J. R., and D. W. Coats. 1990. Seasonal abundances of planktonic ciliates and microflagellates in mesohaline Chesapeake Bay waters. *Estuarine Coastal Shelf Sci.* 31: 157-175.
- Ducklow, H. W. 1994. Modeling the microbial food web. *Microb. Ecol.* 28:303-319.
- Dworetzky, B. A., and J. J. Morley. 1987. Vertical distribution of radiolaria in the Eastern Equatorial Atlantic: analysis of a multiple series of closely-spaced plankton tows. *Mar. Micropaleontol.* 12:1-19.
- Fairbanks, R. G., and P. H. Wiebe. 1980. Foraminifera and chlorophyll maximum: vertical distribution, seasonal succession, and paleoceanographic significance. *Science* 209:1524-1526.
- Fell, J. W., A. Statzell-Tallman, M. J. Lutz, and C. P. Kurtzman. 1992. Partial rRNA sequences in marine yeasts: a model for identification of marine eukaryotes. *Mol. Mar. Biol. Biotechnol.* 1:175-186.
- Fenchel, T. 1967. The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with

- metazoans in various types of sediments. *Ophelia* 4: 121-137.
33. Fenchel, T. 1969. The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. *Ophelia* 6:1-182.
  34. Fenchel, T., and B. J. Finlay. 1983. Respiration rates in heterotrophic, free-living protozoa. *Microb. Ecol.* 9: 99-122.
  35. Finlay, B. J., U.-G. Berninger, L. J. Stewart, R. M. Hindle, and W. Davison. 1987. Some factors controlling the distribution of two pond-dwelling ciliates with algal symbionts (*Frontonia vernalis* and *Euplotes daidaleos*). *J. Protozool.* 34:349-356.
  36. Finlay, B. J., K. E. Clarke, E. Vicente, and M. R. Miracle. 1991. Anaerobic ciliates from a sulfide-rich solution lake in Spain. *Eur. J. Protistol.* 27:148-159.
  37. Garrison, D. L., and M. M. Gowing. 1993. Microzooplankton, p. 123-166. In E. I. Friedmann (ed.), *Antarctic Microbiology*. Wiley-Liss, New York.
  38. Gifford, D. J. 1985. Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotrichida). *Mar. Ecol. Prog. Ser.* 23:257-267.
  39. Gooday, A. J., L. A. Levin, P. Linke, and T. Heeger. 1992. The role of benthic foraminifera in deep-sea food webs and carbon cycling, p. 63-91. In G. T. Rowe (ed.), *Deep-Sea Food Chains—and the Global Carbon Cycle*. Kluwer Academic, Dordrecht, The Netherlands.
  40. Gowing, M. M. 1993. Seasonal radiolarian flux at the VERTEX North Pacific time-series site. *Deep-Sea Res.* 40: 517-545.
  41. Jacobson, D. M., and D. M. Anderson. 1986. Thecate heterotrophic dinoflagellates: feeding behavior and mechanisms. *J. Phycol.* 22:249-258.
  42. Jürgens, K., and G. Stolpe. 1995. Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacteria in a shallow, eutrophic lake. *Freshwater Biol.* 33: 27-38.
  43. Leadbeater, B. S. C. 1993. Preparation of pelagic protists for electron microscopy, p. 241-251. In P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (ed.), *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton, Fla.
  44. Lee, J. J., S. H. Hutner, and E. C. Bovee. 1985. *An Illustrated Guide to the Protozoa*. Society of Protozoologists, Lawrence, Kans.
  45. Lee, J. J., K. Sang, B. ter Kuile, E. Strauss, P. J. Lee., and W. W. Faber, Jr. 1991. Nutritional and related experiments on laboratory maintenance of three species of symbiont-bearing, large foraminifera. *Mar. Biol.* 109:417-425.
  46. Lim, E. E., L. A. Amaral, D. A. Caron, and E. F. DeLong. 1993. Application of rRNA-based probes for observing marine nanoplanktonic protists. *Appl. Environ. Microbiol.* 59: 1647-1655.
  47. Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. *Ecology* 23:399-418.
  48. Maeda, M. 1986. An illustrated guide to the species of the families Halteriidae and Strobilidiidae (Oligotrichida, Ciliophora), free swimming protozoa common in the marine environment. *Bull. Ocean Res. Inst. Univ. Tokyo* 21: 1-67.
  49. McLaughlin, G. L., S. Montenegrojames, M. H. Vodkin, D. Howe, M. Toro, E. Leon, R. Armijos, I. Kakoma, B. M. Greenwood, M. Hassanking, J. Marich, J. Ruth, and M. A. James. 1992. Molecular approaches to malaria and babesiosis diagnosis. *Mem. Inst. Oswaldo Cruz* 87:57-68.
  50. Michaels, A. F. 1991. Acantharian abundance and symbiont productivity at the VERTEX seasonal station. *J. Plankton Res.* 13:399-418.
  51. Michaels, A. F., D. A. Caron, N. R. Swanberg, F. A. Howse, and C. M. Michaels. 1995. Planktonic sarcodines (Acantharia, Radiolaria, Foraminifera) in surface waters near Bermuda: abundance, biomass and vertical flux. *J. Plankton Res.* 17:131-163.
  52. Moloney, C. L., and J. G. Field. 1991. The size-based dynamics of plankton food webs. 1. A simulation model of carbon and nitrogen flux. *J. Plankton Res.* 13:1003-1038.
  53. Page, F. C. 1983. *Marine Gymnamoebae*. Institute of Terrestrial Ecology, Cambridge.
  54. Parke, M., and P. S. Dixon. 1976. Checklist of British marine algae—third revision. *J. Mar. Biol. Assoc. U.K.* 56:527-594.
  55. Patterson, D. J., and J. Larsen. 1991. *The Biology of Free-Living Heterotrophic Flagellates*. Clarendon Press, Oxford.
  56. Patterson, D. J., and M. Zöllfel. 1991. Heterotrophic flagellates of uncertain taxonomic position, p. 427-475. In D. J. Patterson and J. Larsen (ed.), *The Biology of Free-Living Heterotrophic Flagellates*. Clarendon Press, Oxford.
  57. Pierce, R. W., and J. T. Turner. 1992. Ecology of planktonic ciliates in marine food webs. *Rev. Aquat. Sci.* 6: 139-181.
  58. Putt, M., and D. K. Stoecker. 1989. An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnol. Oceanogr.* 34:1097-1103.
  59. Reid, P. C., C. M. Turley, and P. H. Burkill. 1991. *Protozoa and Their Role in Marine Processes*. Springer-Verlag, Berlin.
  60. Rogerson, A., H. G. Butler, and J. C. Thomason. 1994. Estimation of amoeba cell volume from nuclear diameter and its application to studies in protozoan ecology. *Hydrobiologia* 284:229-234.
  61. Rogerson, A., and J. Laybourn-Parry. 1992. The abundance of marine naked amoebae in the water column of the Clyde estuary. *Estuarine Coastal Shelf Sci.* 34:187-196.
  62. Sanders, R. W. 1991. Mixotrophic protists in marine and freshwater ecosystems. *J. Protozool.* 38:76-81.
  63. Sanders, R. W., D. A. Caron, and U.-G. Berninger. 1992. Relationships between bacteria and heterotrophic nanoplankton in marine and fresh water: an inter-ecosystem comparison. *Mar. Ecol. Prog. Ser.* 86:1-14.
  64. Sawyer, T. K. 1980. Marine amebae from clean and stressed bottom sediments of the Atlantic Ocean and Gulf of Mexico. *J. Protozool.* 27:13-32.
  65. Schlegel, M. 1991. Protist evolution and phylogeny as discerned from small subunit ribosomal RNA sequence comparisons. *Eur. J. Protistol.* 27:207-219.
  66. Scholin, C. A., and D. M. Anderson. 1994. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU rRNA genes. *J. Phycol.* 30:744-754.
  67. Sherr, E. B., and B. F. Sherr. 1993. Preservation and storage of samples for enumeration of heterotrophic protists, p. 207-212. In P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (ed.), *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton, Fla.
  68. Sieburth, J. M. 1979. *Sea Microbes*. Oxford University Press, New York.
  69. Sieburth, J. M., V. Smetacek, and J. Lenz. 1978. Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol. Oceanogr.* 23:1256-1263.
  70. Silver, M. W., M. M. Gowing, D. C. Brownlee, and J. O. Corliss. 1984. Ciliated protozoa associated with oceanic sinking detritus. *Nature (London)* 309:246-248.
  71. Small, E. B., and M. E. Gross. 1985. Preliminary observa-

- tions of protistan organisms, especially ciliates, from the 21°N hydrothermal vent site. *Biol. Soc. Wash. Bull.* 6: 401-410.
72. Starink, M., M.-J. Bär-Gilissen, R. P. M. Bak, and T. E. Cappenberg. 1994. Quantitative centrifugation to extract benthic protozoa from freshwater sediments. *Appl. Environ. Microbiol.* 60:167-173.
73. Stoecker, D., A. E. Michaels, and L. H. Davis. 1987. Large proportion of marine planktonic ciliates found to contain functional chloroplasts. *Nature (London)* 326: 790-792.
74. Stoecker, D., A. Taniguchi, and A. E. Michaels. 1989. Abundance of autotrophic, mixotrophic and heterotrophic planktonic ciliates in shelf and slope waters. *Mar. Ecol. Prog. Ser.* 50:241-254.
75. Stoecker, D. K., D. J. Gifford, and M. Putt. 1994. Preservation of marine planktonic ciliates: losses and cell shrinkage during fixation. *Mar. Ecol. Prog. Ser.* 110:293-299.
76. Swanberg, N. R., and D. A. Caron. 1991. Patterns of sarcodine feeding in epipelagic oceanic plankton. *J. Plankton Res.* 13:287-312.
77. Taylor, F. J. R. 1982. Symbioses in marine microplankton. *Ann. Inst. Oceanogr. (Paris)* 58(Suppl.):61-90.
78. Turley, C. M., and K. Lochte. 1990. Microbial response to the input of fresh detritus to the deep-sea bed. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 89:3-23.
79. Turner, J. T., and J. C. Roff. 1993. Trophic levels and trophospecies in marine plankton: lessons from the microbial food web. *Mar. Microb. Food Webs* 7:225-248.
80. Verity, P. G., C. Y. Robertson, C. R. Tronzo, M. G. Andrews, J. R. Nelson, and M. E. Sieracki. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.* 37:1434-1446.
81. White, D. C. 1994. Is there anything else you need to understand about the microbiota that cannot be derived from analysis of nucleic acids? *Microb. Ecol.* 28:163-166.
82. Wiebe, W. J., W. M. Sheldon, Jr., and L. R. Pomeroy. 1992. Bacterial growth in the cold: evidence for an enhanced substrate requirement. *Appl. Environ. Microbiol.* 58:359-364.
83. Wright, S. W., S. W. Jeffrey, F. C. Mantoura, C. A. Llewellyn, T. Bjørnland, D. Repeta, and N. Welschmeyer. 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar. Ecol. Prog. Ser.* 77:183-196.
84. Yongue, W. H., Jr., and J. Cairns, Jr. 1978. The role of flagellates in pioneer protozoan colonization of artificial substrates. *Pol. Arch. Hydrobiol.* 25:787-801.
85. Zubkov, M. V., A. F. Sazhin, and M. V. Flint. 1992. The microplankton organisms at the oxic-anoxic interface in the pelagial of the Black Sea. *FEMS Microbiol. Ecol.* 101: 245-250.