

MANUAL OF ENVIRONMENTAL MICROBIOLOGY
Section IV: AQUATIC ENVIRONMENTS
Chapter 6: PROTISTAN COMMUNITY STRUCTURE

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Protistan assemblages of aquatic ecosystems are the focus of extensive research in aquatic ecology. One stimulus for this work has been the long-standing recognition that phototrophic protists (the unicellular algae) constitute a major fraction of the primary productivity within aquatic ecosystems. Another incentive has been the realization that protozoa (heterotrophic protists) play a pivotal role in the flow of energy and elements in these communities (109, 118). Studies of the abundance, biomass and trophic activities of protists have now been conducted in a wide range of aquatic ecosystems. In addition, many laboratory experiments have examined the general biology and physiology of various protistan species grown under carefully controlled conditions. The synthesis of this information into models of how protistan assemblages are structured and how they function in nature has advanced considerably during the last few decades.

Protozoa traditionally have been distinguished from unicellular eukaryotic algae as heterotrophic organisms that exist by absorbing dissolved organic substances (osmotrophy) or more commonly by engulfing prey (phagocytosis). It is important to recognize that the term Protozoa now has more historical significance than phylogenetic or ecological meaning. Modern phylogenetic schemes have merged many 'protozoan' taxa with 'algal' taxa and vice versa. For example, numerous heterotrophic species of chrysophytes and dinoflagellates exist within these traditionally-algal taxa. Heterotrophic species within each group are closely related to chloroplast-bearing species based on ultrastructural features and DNA sequence data but obviously have quite different modes of nutrition than their photosynthetic counterparts (4, 80, 99, 115). In addition to the existence of 'apochlorotic algae', chloroplast-bearing genera exist within the chrysophyte, dinoflagellate, prymnesiophyte, cryptophyte and euglenophyte algae that are capable of phagotrophy in addition to photosynthesis (112, 129, 130). This mixotrophic behavior obscures the distinction between traditional definitions of algae and protozoa and has lead ecologists to conceptualize protistan trophic activity as a continuum of nutritional modes (76).

There are also difficulties in the classification of some ciliated protozoa as phototrophs or heterotrophs. Some ciliate species ingest and digest algal prey but are able to retain the chloroplasts of their prey in a functional state, thereby providing those ciliates with limited photosynthetic ability (112, 127). Photosynthesis in green ciliates contributes significantly to the overall nutrition of these protozoa, and may form a notable fraction of the primary productivity of some planktonic communities (33).

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 (80). Therefore, use of the term 'protist' in reference to both chloroplast-bearing forms (i.e. algae) and heterotrophic forms (protozoa) has gained popularity in recent years.

ASSESSING PROTISTAN COMMUNITY STRUCTURE

Protistan species diversity

Complete taxonomic

characterization of the protistan assemblage

has been achieved for few, if any, aquatic communities. There are at least three major, interrelated reasons for this situation: (1) the large number of protistan species present in most aquatic environments, (2) the tremendous range in size and abundance of these species, and (3) the disparate methodologies that are required for sampling and identifying them. Overcoming the difficulties posed by these issues is central to improving the present state of our knowledge concerning the structure of natural assemblages of aquatic protists.

Literally thousands of species of protists have been described and many, perhaps all of these species, have sympatric distributions (52). In addition, free-living protists range in size from approximately 0.8 μm prasinophytes up to some species of radiolaria that can form cylindrical gelatinous colonies ≈ 1 centimeter in diameter and >1 meter in length. Similarly, relative abundances of protistan species within a habitat may vary by several orders of magnitude. These immense ranges of size and abundance make it necessary to apply a number 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 vs. benthic environments) also contribute to the varied protocols that are necessary to sample protistan assemblages (120).

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 those 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, 48, 111, 126, 144). Such approaches have resulted in reasonable estimations of the protozoan diversity of some sediment environments, but the success of these methods is usually group-specific (22). The extraction of protozoa by the sea-ice method, or centrifugation and staining, may work well for large or highly mobile species such as benthic ciliates but this method may be less useful for slower moving forms such as small amoebae. For the latter forms, enrichment cultivation appears to be the most appropriate method (97).

Adjustments to sampling protocols are also necessary for adequately sampling different protistan groups within plankton communities. Sample volumes of 200-500 ml are usually sufficient in oceanic waters for flagellated protists (typical abundances are hundreds to thousands per milliliter), and volumes of 0.5-2 liters are usually sufficient for ciliated protists (typical abundances are tens to thousands per liter), but actinopods and foraminifera usually must be concentrated using plankton nets or filters. These latter techniques, however, are damaging to delicate species of planktonic ciliates (62), and some actinopods that lack mineralized skeletal structures may be significantly undersampled. At present, visualization and enumeration *in-situ* may be the only tractable means of obtaining accurate counts of these latter forms (36). Common methods for sampling planktonic protists have been recently reviewed (63).

Preservation, fixation and other manipulations are necessary 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 identification are present only in living specimens (97). For the remaining protistan groups, appropriate preservation is dependent on the taxon under consideration (63). For flagellated (and often ciliated) protists, aldehyde fixatives are commonly employed (formaldehyde, glutaraldehyde) often followed by osmium tetroxide when electron microscopy is planned (79, 119). A variety of fixatives have been developed for ciliated protozoa, most of which are usually employed in combination with post-fixation staining methods that are used to visualize cytological features of the cells (82, 94, 131, 144).

The preservation of some protistan taxa requires special consideration. A preservative that does not promote the dissolution of skeletal structures must be employed for species which possess such structures because they are important diagnostic parameters (e.g. some actinopods and foraminifera). Careful adjustment of the pH of the preservative is necessary to prevent dissolution of foraminiferan tests (11), while addition of strontium is necessary to prevent dissolution of acantharian skeletons (91). When these requirements conflict, subsamples must be preserved separately for the different groups. For example, planktonic foraminifera (which require alkaline pH) typically would be preserved differently than planktonic ciliates which are often preserved in acid Lugol's solution (131).

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. Therefore, taxonomic expertise among protistologists is often limited to one of the numerous protistan groups (e.g. diatoms, dinoflagellates, amoebae, ciliates, foraminifera) or some portion of one of these major categories.

Ciliates typically possess morphological features that provide sufficient taxonomic criteria for identifying species using 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 (80). 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 (55, 104).

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, however, are only visible using electron microscopy (e.g. flagellar hairs, body scales). Electron microscopy is often necessary for distinguishing the numerous genera and species of small heterotrophic flagellates (<10 μm). The need to establish these features 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 (99, 102), 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 based on 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 new methodological approaches are slowly yielding information on the taxonomy, distribution and general ecology of these species (6, 19, 97, 111, 123, 124). Identification of the many types of testate amoeboid protists (testacea, foraminifera, radiolaria, and others) is largely based on the skeletal structures that are present in many of these species, and to features of their cellular organization. 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. Nevertheless, there is mounting evidence from the application of molecular biological approaches that traditional methods may be missing much of the diversity of these groups (70, 73, 103).

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. Exceptions to this generality might be found in environments where protozoan diversity is greatly reduced due to severe environmental factors such as anaerobic conditions (54), extreme pH (2), and in some enteric environments (3). However, it is safe to generalize that the vast majority of studies of natural communities have underestimated total protistan species diversity.

Analyses of species diversity for particular taxa of protists, however, have been more complete (i.e. the ciliates, flagellates or amoebae). The most complete information exists for plankton communities where extensive lists of ciliated protozoa, chloroplast-bearing flagellates and skeleton-bearing sarcodines (foraminifera and actinopods) have been obtained (10, 43, 88, 98, 100, 105).

Protistan 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 broader 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. Generally accepted methods are now emerging which minimize problems associated with loss of cells during collection, enrichment, preservation and sample processing for specific groups of protists, thus allowing accurate estimates of protistan abundance.

As with species identification, estimating population abundances of amoebae presents the most formidable problem. The amorphous shapes of these microorganisms in preserved samples make them difficult to recognize and enumerate in natural samples, and their association with detritus and other particles also can obscure them from view. The few abundance estimates that are available for

these species have been obtained using a Most Probable Number culture technique that relies on the growth of the amoebae in serial dilutions of the water or sediment samples (34, 111).

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 shrinkage due to fixation into account and the variable vacuolar space of protists. Shrinkage due to fixation can be both taxon- and size-specific. Typical values for converting carbon to volume range between 160 and 360 fg C μm^{-3} for flagellated protists (90, 139), and 190 fg C μm^{-3} for ciliated protists (108). Carbon:volume conversion factors for larger sarcodines (acantharia, radiolaria and foraminifera) are based on aspects of the cells that are resistant to net collection (92). A method has been proposed for estimating the cell volume of naked amoebae that directly relates the diameter of the nucleus to total cell volume (110). Commonly-used conversion factors have been summarized and reviewed recently (63).

Describing protistan community structure: classical approaches

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, but this is a problem common to many ecological studies. 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 has resulted in the use of various simplifying groupings as a way of reducing the complexity of protistan assemblages into manageable (and measurable) quantities.

Various means of simplifying protistan community structure have been used. The most popular approaches have grouped protists by trophic mode (e.g., phototrophic vs. heterotrophic), size, and prey type (for heterotrophs) in keeping with the trophic-level concept of Lindeman (84). Trophic categories must be somewhat more flexible than simply 'phototrophy vs. heterotrophy' because of the common behavior of mixed nutrition among protists (24). Nevertheless, aggregation of species into "trophospecies" (136) is still a useful and necessary procedure for partitioning the assemblage in order to allow investigations of energy and elemental flow through aquatic communities in models of manageable size (30).

For heterotrophic protists, it is a common procedure 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 (39). Such "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 (9, 27, 51, 72). In addition, predator-prey relationships are typically size-dependent, with larger predators consuming smaller prey. For example, many heterotrophic flagellates 2-20 μm in size consume bacteria and cyanobacteria that are $<2 \mu\text{m}$ in size, and many ciliate species 20-100 μm in size consume algae and protozoa $<20 \mu\text{m}$ in size.

While this generalization is realistic, it is not absolute. Many species of heterotrophic dinoflagellates consume diatom prey that are considerably larger than themselves by employing a pseudopodial "feeding veil" (75). Similarly, some planktonic sarcodines (acantharia, radiolaria and foraminifera) are capable of consuming metazoan prey considerably larger than themselves due to the production of a sticky pseudopodial network that entangles and immobilizes prey items (132).

Notwithstanding these exceptions, size-dependent grazing models are the most common manner of organizing protistan populations into manageable units for inclusion into models of elemental flow in aquatic ecosystems (8, 42, 74, 93). 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.

An example from the plankton

A hypothetical example indicates the analytical approaches for examining protistan community structure and the limitations of these approaches. A species list of protists that is representative of an oceanic plankton community is given in Table 1. This assemblage is not meant to be realistic or complete, but rather indicative of the breadth of protistan sizes and nutritional modes that exist in this type of ecosystem. Pertinent information on cell size, photosynthetic and/or phagotrophic ability, prey type(s) and typical abundances are also provided (abundances do not take bloom conditions into account, which are common for phytoplankton). The species in this assemblage have been arranged according to major taxonomic (i.e. phylogenetic) affinities (80).

As shown in Table 1, taxonomic groupings of the protists correspond poorly to the nutritional modes and ecological roles of the species. Reorganization of the same species into groups based on the nutritional modes of the species provides a very different classification 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. Note that the latter ability results in some of the species occurring in more than one 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 $\approx 1.8 \mu\text{m}$ to $>1 \text{ mm}$ in size, phototrophs range from $\approx 0.8 \mu\text{m}$ to $>1 \text{ mm}$, and mixotrophs from $\approx 6.0 \mu\text{m}$ to $>1 \text{ mm}$ if one includes symbiont-bearing sarcodines in this last category. Commonly employed plankton size class designations are also shown in this figure. These designations correspond to organisms $0.2\text{-}2.0 \mu\text{m}$ (pico-), $2\text{-}20 \mu\text{m}$ (nano-), $20\text{-}200 \mu\text{m}$ (micro-), $0.2\text{-}20 \text{ mm}$ (meso-), $20\text{-}200 \text{ mm}$ (macro-) in longest dimension (121). Protists occur in all of these size classes as indicated in Figure 1, although the majority of these species occur in the nano- and micro- size 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 1-2 orders of magnitude differences in abundances when individuals <20 , $20\text{-}200$, and $>200 \mu\text{m}$ are tallied according to plankton size class (Fig. 2A). Phototrophic, heterotrophic and mixotrophic protists also exhibit large differences in their abundance between size classes, although the abundances of these groups within a size class are often similar.

The large disparity that is apparent when comparing the abundances of protists in different size classes (Fig. 2A) is greatly reduced when the total volume of living protists is compared (Fig. 2B). Small cell size among nanoplankton is generally balanced by high abundances of these species, while larger cellular volumes of large protists compensates for their low abundances. These general relationships of protistan abundance and biovolume are consistent with data from natural assemblages of nanoplanktonic and microplanktonic protists (18, 25, 37, 60).

The information summarized in Tables 1, 2 and Figures 1, 2 can be used to construct a typical box model depicting the flow of materials from producers through consumers for this hypothetical protistan community (Fig. 3). The species have been grouped according to their nutritional modes, sizes 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). These types of models are now common in the literature. They indicate the fundamental role of bacteria, cyanobacteria, phototrophic and heterotrophic protists as producers and consumers of organic matter in aquatic ecosystems. These depictions also recognize the important role of bacteria and their consumers as a means of converting detrital material back into living biomass thus making it available for reentry into food webs, a fundamental role of 'the microbial loop' (8).

Major goals for 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 structure and function. Models such as the one

shown in Figure 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 Figure 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 flaws. 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-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 that feeds 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 were 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 in constructing such models.

Box models that approximate trophic relations within planktonic communities can often be tested. For example, if we hypothesize that ciliates in the microplankton size class are the dominant consumers in the plankton, we would expect that their removal (e.g., by size fractionation) would result in an increase in the abundance of species in the nanoplankton size class. This relationship constitutes the basis of a 'trophic cascade' which assumes that changes in the activity of organisms at one trophic level will result in alternating positive and negative effects on the assemblages in lower trophic levels. For example, the removal of microplankton and larger zooplankton in the community depicted in Figure 3 would be expected to result in increases in nanoplanktonic species (2.0-20 μm) which would in turn result in decreases in the abundance of picoplanktonic species (0.2-2.0 μm). The strength with which a trophic cascade propagates through a food web has been shown to depend on the complexity of trophic relationships within the assemblage (71, 96, 106).

Describing protistan community structure: molecular approaches

We have learned a great deal about the taxonomic composition and trophic structure of aquatic protistan communities through the application of traditional

approaches of morphological analysis and culture. Nevertheless, the tremendous diversity of protistan assemblages, and the varied methods required for identifying these species, their abundances, biomass and trophic activity continue to hamper in-depth understanding of the structure and function of these communities. As a result, new conceptual and methodological approaches are coming into use that help deal with these recalcitrant problems.

Increasingly valuable are culture-independent, molecular methods that allow characterization of community structure without the need for culture and/or extensive morphological analyses. Several approaches to accomplish this task have been pursued. Two common ones are various methods of DNA fragment analyses, which provide a quick “snapshot” of the relative diversity of the community as a whole, and the cloning and sequencing of specific genes in order to obtain detailed information on the species (phylotypes) present in an assemblage (Fig. 4). Both approaches employ the extraction of nucleic acids directly from samples, avoiding biases inherent in traditional taxonomic characterizations due to fixation, preparation or culture. Nonetheless, most extant molecular approaches possess potential biases due to efficiency of nucleic acid extraction and (if used) PCR amplification.

Fragment analysis approaches rely on distinguishing microbial taxa from unique characteristics (e.g. migration rate through a gel) of their genes or gene fragments when amplified from a natural assemblage. Several approaches have been employed for eukaryote assemblages including the common methods of denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphisms (T-RFLP) (31, 38, 61, 138). By far, these approaches have focused primarily on ribosomal RNA genes, and in particular on small subunit ribosomal RNA genes. The intergenic spacer regions have also been employed for fragment analyses, but these regions may actually resolve differences at the strain level, rather than the species level. The number of DNA fragments in such analyses is indicative of the number of Operational Taxonomic Units (OTUs) present in the sample. Ideally, OTUs can be equated to species although this assumes that each species produces a single, unique fragment. An example of a T-RFLP pattern is shown in Figure 5, where several cultured protists have been combined into a single sample prior to extraction and analysis. Fragment sizes for the species were determined using monocultures of the protists, and thus the identity of each fragment can be assigned (as shown in the Figure).

The utility of DNA fragment analysis is the relative speed with which these analyses can be accomplished. For this reason, these approaches are useful in ecological studies that generate large numbers of samples, and for comparative studies where the goal is to obtain a rapid comparison between environmental samples or experimental treatments. However, these methods typically assess only the numerically dominant taxa within an assemblage (rare species may produce fragments that cannot be detected), and they usually cannot provide taxonomic information without additional work (such as sequencing fragments produced in the analyses).

The cloning and sequencing of genes from environmental samples has recently yielded a large amount of information on the structure of microbial communities, especially prokaryotic assemblages (57). This approach also constitutes a powerful

method for analyzing microbial eukaryote assemblages as well. Cloning and sequencing is more time- and cost-intensive than fragment analysis but provides improved phylogenetic/taxonomic resolution. Comparison of sequences with publicly available databases provides molecular-based taxonomic identities that describe protistan diversity within a sample (Fig. 6).

Cloning/sequencing of microbial species has largely involved work on ribosomal RNA genes (usually the small subunit, but also the large subunit), in contrast to work on higher organisms that has focused on the cytochrome oxidase I gene such as in the Barcode of Life project. Some crossover in approaches is warranted but few studies have been performed to date using CO1 genes to assess microorganismal diversity. Studies of natural assemblages has yielded exciting new information on the diversity and structure of protistan assemblages. These insights have included the detection of previously unknown diversity and many novel phylotypes in oceanic plankton (31, 69, 95), freshwater plankton (47), and shallow and deep-sea benthic communities (44, 70, 87). This approach has also revealed the unexpected existence of 'marine' taxa in freshwater ecosystems (73), and possibly undescribed, kingdom-level diversity in anoxic ecosystems (35).

A logical extension of gene sequencing has been the use of sequence information in methods targeting specific taxa of microorganisms. These techniques do not examine protistan community structure per se, but rather attempt to quantify a particular taxon (or taxa) in a natural assemblage. Methods such as fluorescent *in-situ* hybridization (FISH) and quantitative real-time PCR (qPCR) allow estimates of species abundances that are highly sensitive, accurate, and often much more rapid than traditional microscopy (17, 32, 83, 107, 116). The development of DNA arrays for assaying the presence of species in natural samples is, in some ways, an extension of these approaches. Arrays greatly expand the number of taxa that can be assayed at one time. Thus far, the application of 'phylochips' has been restricted largely to prokaryote assemblages (45, 85, 137), but the development of protistan arrays is virtually assured as sequence information amasses for eukaryotes.

In addition to gene-based approaches, immunological methods for determining the presence and abundance of specific taxa have contributed to our knowledge of protistan species. This approach has been successfully applied to phototrophic protists (21, 26, 117). Biochemical markers to indicate the presence, abundance and activity of protists also may provide new methods for examining natural assemblages. Detailed pigment analyses have provided useful insights into the contribution of specific taxa to total algal biomass (146) and lipid biomarkers may also be useful for obtaining information on the biomass and nutritional status of microbial assemblages (143).

The success of using molecular (genetic/immunological) signatures for assessing the community structure of natural protistan assemblages will ultimately depend on linking these signatures to classical (morphological) species descriptions, and to the physiological abilities of protistan phylotypes. Ultimately, molecular approaches, in combination with classical methods, will provide new tools for studying the emergent physiological, ecological and biogeochemical processes that are created and/or affected by protistan community structure (Fig. 4).

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

Freshwater vs. 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. Adult sarcodines are often the most conspicuous macroscopic organisms in surface waters of tropical and subtropical oceanic plankton communities, while swarmer cells and juvenile specimens of these species contribute to the entire size spectrum of protozooplankton (28). In marine sediments, benthic foraminifera can constitute an important component of faunal assemblages (65, 70, 81). In addition, recent molecular analyses have indicated that many previously-undetected foraminiferal taxa may exist in some freshwater ecosystems (73).

In contrast to large differences in the assemblages of sarcodine species in fresh and salt water, there appears to be a fair degree of similarity with respect to types of ciliates and flagellates in these environments. 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 (113). Most ciliates in the freshwater and marine plankton also appear to play grossly analogous ecological roles as consumers of small prokaryotes and eukaryotes (12, 104). Mixotrophic (phagotrophic) algae exist in both fresh and marine waters (5, 14, 16), as do species of chloroplast-retaining ciliates (40, 53, 127, 128). These generalizations do not necessarily 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 vs. 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 (50). Morphological adaptations of ciliates to life between sediment particles in the benthos has 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 facilitate movement on surfaces and grazing on prey loosely associated with particulate material. Numerous species permanently attach to surfaces. In contrast, ciliates in pelagic environments tend to have more rounded shapes, and patterns of ciliature that afford rapid swimming behavior and feeding on suspended particles.

There is great protistan biodiversity among and within benthic environments as a

consequence of differences in various environmental gradients such as sediment grain size, organic loading and oxygen gradients. In addition, the number of microenvironments at one locale may be considerable. Spatial heterogeneity, the remoteness of many benthic environments, and difficulties associated with sampling, concentrating and observing benthic protists continues to limit our knowledge of the protistan fauna of many benthic ecosystems. There are extremely few observations of the protistan fauna of the deep ocean benthos (7, 44, 64, 65, 86, 87, 125, 135).

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 (114). Amoebae occurring in the plankton are generally assumed to be associated with suspended particulate material or with the air/water interface (19, 34, 111).

Among the larger sarcodines, there are clear dichotomies 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. There are relatively few exceptions to these generalities, making the larger sarcodine fauna of benthic and pelagic ecosystems quite distinct.

The contribution of phototrophic protists to flagellate assemblages in surface waters of pelagic ecosystems clearly differentiate them from flagellate assemblages of benthic environments in which phototrophs may be reduced or absent. However, the heterotrophic flagellate assemblages of these two habitats 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, while forms that feed on suspended bacteria (e.g. chryomonad flagellates, choanoflagellates) tend to predominate in pelagic ecosystems (23, 78, 101, 134, 140-142).

Pelagic environments generally are considered more homogeneous ecosystems 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 (133). 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 abundances (23, 122). Similar "oases" for unique protozoan assemblages in the plankton may be established using artificial foam substrates (20). It has been demonstrated that the colonization and species succession of protozoa on these natural and artificial substrates may follow a pattern similar to the colonization of oceanic islands by higher organisms (147).

Depth and Seasonal Distributions

The seasonality of algal species composition and abundance in pelagic environments is well known. Distributions 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/function with depth. This paucity of information is not surprising given the logistical problems associated with the collection of long-term data sets or multiple samples from considerable depth.

Most studies to date have been restricted to a particular group of heterotrophic protists either because of 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 (13, 29, 41, 77). More detailed data on the spatiotemporal distributions are available only for taxa for which identification is more straightforward (10, 66).

It is difficult to generalize concerning changes in the community structure of protozoan 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 (145), but diverse heterotrophic and phototrophic protistan assemblages abound even in extremely low temperature environments such as marine habitats of Antarctica (58, 59, 61). In the latter environment, rather unique assemblages of phototrophic and heterotrophic protists have adapted to existence in association with sea ice.

The vertical distributions of protozoa 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 (34), at oxic/anoxic boundaries within water columns (148), and at subsurface biological features such as deep chlorophyll maxima (46). Vertical distributions of protists in the sediments typically are related to physical and chemical gradients within the benthos (15, 49). The exploitation of these chemical/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.

FUTURE DIRECTIONS AND CHALLENGES

We have learned a great deal about the taxonomic composition and trophic structure of aquatic protistan communities since the discovery of these microbes by Antonie van Leeuwenhoek more than three centuries ago. Much of this work was descriptive in nature prior to 1970, but significant progress has been made within the last three decades towards an understanding of the trophic activities and ecological roles of numerous protistan species.

Despite these advances, large gaps in our knowledge still exist with respect to the overall diversity and distribution of aquatic protistan assemblages. For example, an accurate account of the overall diversity of protists has yet to be established. Modern molecular biological approaches have revealed unexpected, and as yet largely uncharacterized, protistan diversity from a wide variety of ecosystems (35, 73). Establishing the (morphological) identity of these taxa, their physiological abilities, and the overall diversity of microbial eukaryotes in nature constitute active areas of research at this time. Most importantly, *linking* molecular and/or morphological signatures to physiological/ecological function will help establish the biogeochemical roles played by protists. In this way, it will be possible to relate changes in protistan community structure to changes in nutrient and elemental cycles in nature. One recent study has indicated that dominance structure of planktonic protistan assemblages can be highly responsive to containment and changes in environmental conditions (31). These results imply the existence of a large 'reservoir' of rare species in natural plankton assemblages and the potential for rapid shifts in dominance (and possibly ecosystem function) as a consequence of changes in environmental conditions.

Minute protists (< 10 μm) are likely the least understood of all eukaryotes with respect to diversity in natural aquatic ecosystems, and will continue to receive attention in the near future (47, 69, 87, 89, 95). These species have been greatly underestimated using traditional approaches of identification (microscopy) largely because they possess few distinctive morphological characteristics. The use of molecular approaches, coupled with novel culturing approaches, is rapidly expanding our knowledge of the diversity and ecological roles of these taxa (67, 68).

Finally, significant uncertainty also remains regarding the distribution of protists on our planet. Finlay and Fenchel (52, 56) have speculated that most (if not all) protistan species have global distributions because their small size facilitates dispersal. The debate over whether protists have cosmopolitan or endemic distributions is, to some degree, a matter of the species concept applied to protists, and the amount of intraspecific variability (morphological, DNA sequence, or physiological) one is willing to accept. Nevertheless, the present emphasis in microbial ecology on studies of prokaryotic and protistan diversity in a wide range of ecosystems around the planet will provide us with a much better understanding of protistan biodiversity and the distribution of these species.

ACKNOWLEDGEMENTS

Support for the preparation of this manuscript was provided in part by National Science Foundation grants MCB-0084231, OPP-0125437, The Center for Embedded Networked Sensing (CENS) under the NSF Cooperative Agreement CCR-0120778, National Oceanic and Atmospheric Administration grant NA160P2790, and Environmental Protection Agency grant RD-83170501. We are grateful to Patrick Vigil and Peter Countway for providing the T-RFLP pattern used in Figure 5.

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FIGURE LEGENDS

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

FIGURE 2. Abundance (A) and biovolume (B) 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).

FIGURE 3. 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 trophic-dependent relationships. Arrows indicate the directions of energy flow in predator-prey interactions.

FIGURE 4. Conceptual framework for using culture independent and dependent methods to study protistan community structure. Arrows indicate order in which this goal can be accomplished (see detail in text).

FIGURE 5. T-RFLP pattern for an artificial protistan community. The 18S rRNA gene of six known species was PCR amplified and digested using a single restriction enzyme. The unique T-RFLP fragment size for each of the species could be recognized in the mixed sample (as indicated).

FIGURE 6. Relative contribution (% of total number of clones) of various protistan taxa to a theoretical clone library ($n = 27$) created for the protistan assemblage presented in Table 1.

Figure 1

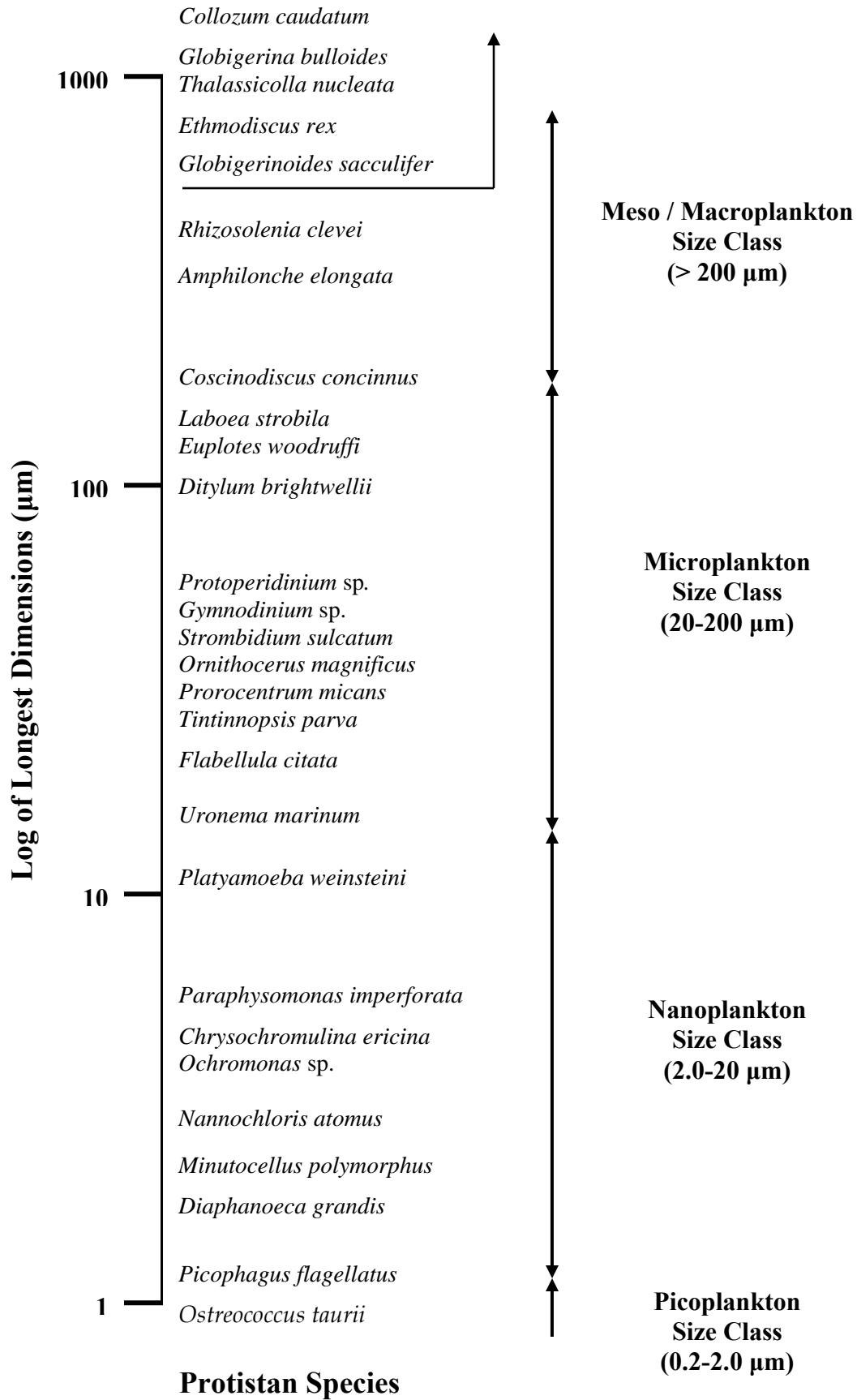


Figure 2

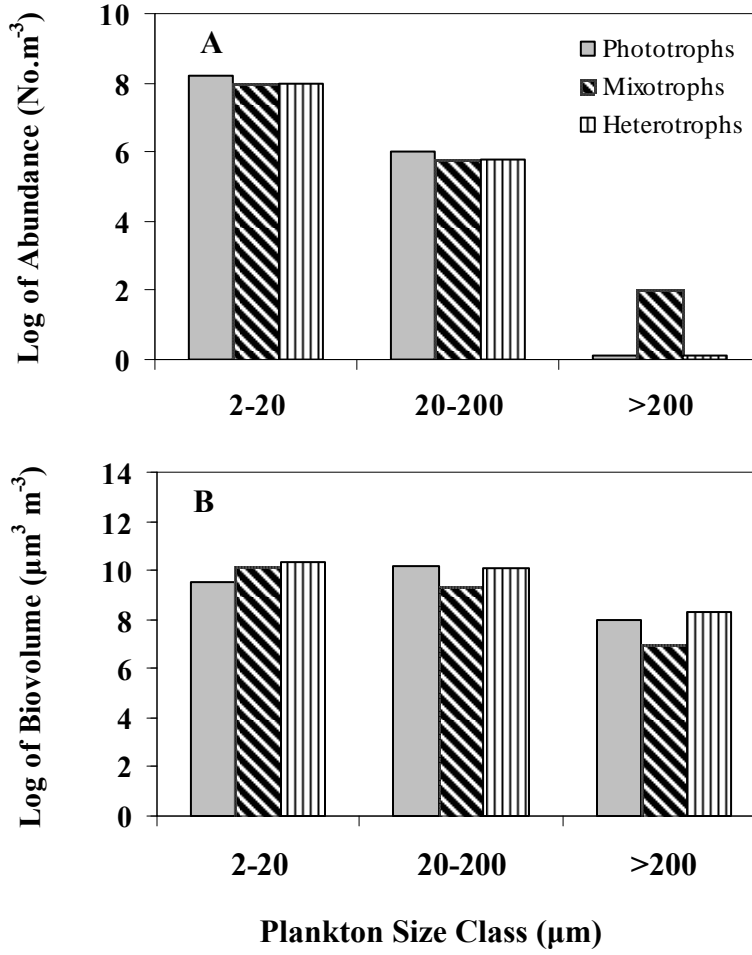


Figure 3

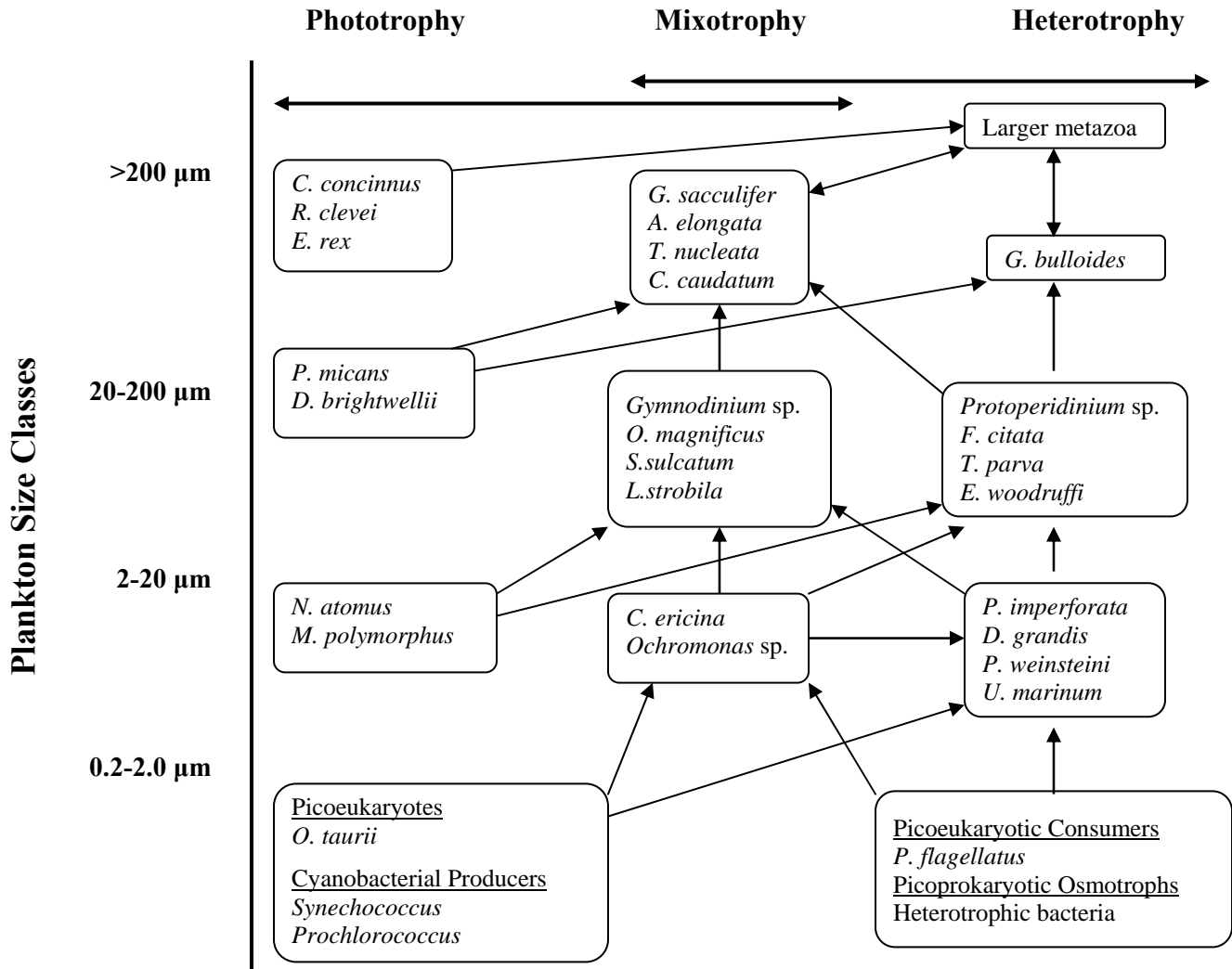


Figure 4

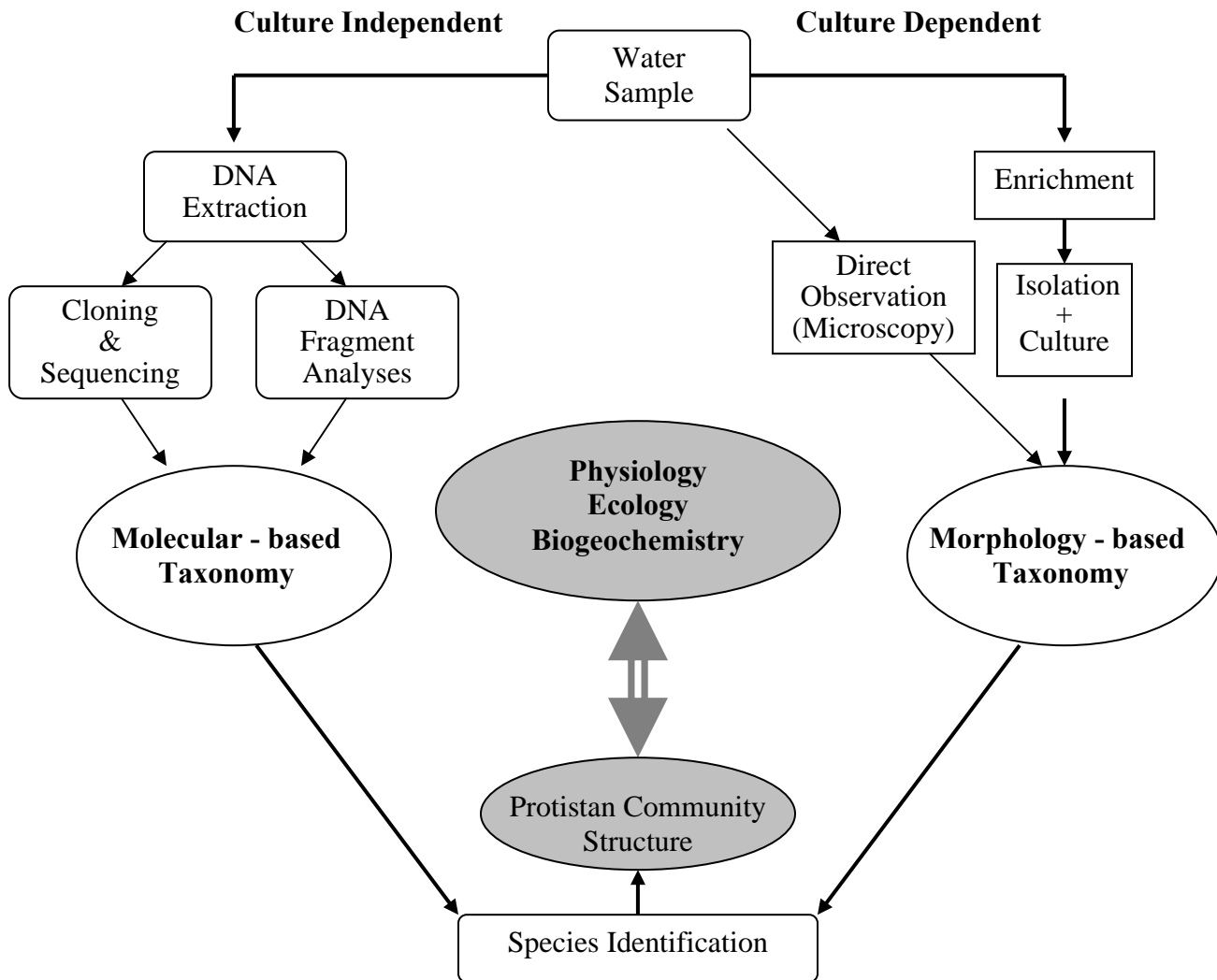


Figure 5

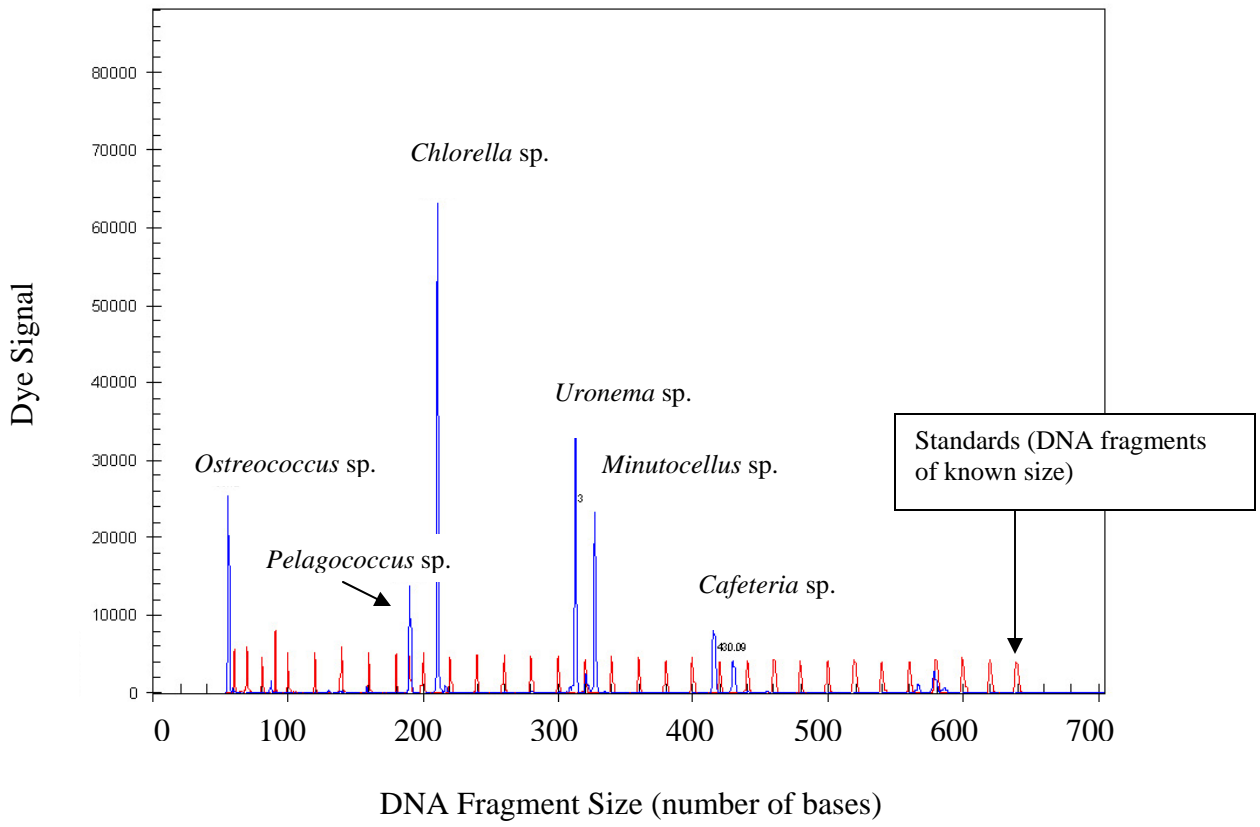


Figure 6

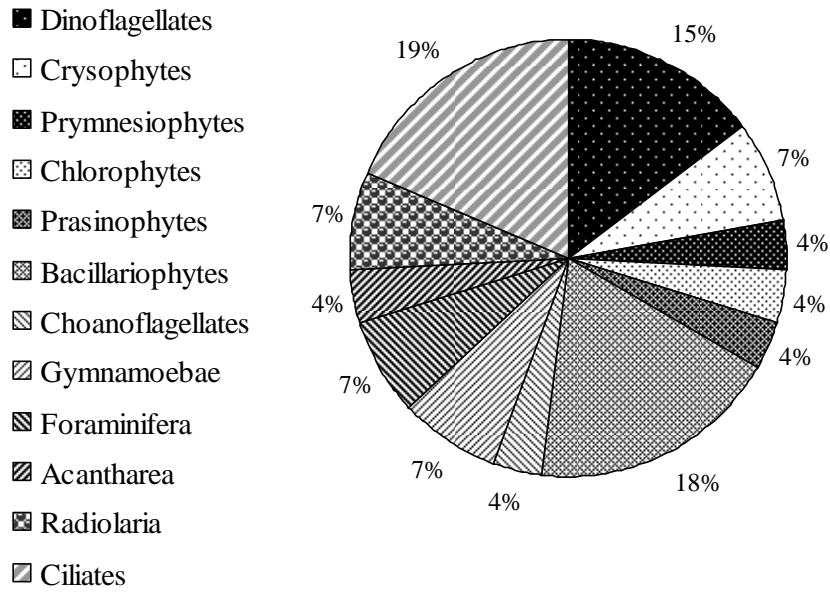


Table 1. Species list showing the range of protistan diversity of an oligotrophic oceanic environment. This list is only a partial list of representative species to exemplify the breadth of trophic modes in a real assemblage. Species are organized according to major taxa. Pertinent ecological information and realistic abundances are provided 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.

<u>Protistan Taxon</u>	<u>Average Size (µm)</u>	<u>Chloroplasts Present?</u>	<u>Phagotrophy?</u>	<u>Probable Prey</u>	<u>Representative Abundances (liter⁻¹)</u>
<u>Flagellated/Non-motile Protists</u>					
Dinoflagellates					
<i>Protoeridinium</i> sp.	55	No	No	Dia	1x10 ²
<i>Gymnodinium</i> sp.	50	Yes	Yes	C, Din, Dia	2x10 ²
<i>Prorocentrum micans</i>	25x40	Yes	No	-	1x10 ³
<i>Ornithocercus magnificus</i>	40	No*	Yes	Dia, Sf	1x10 ²
Chrysophytes/Chrysomonads					
<i>Paraphysomonas imperforata</i>	7.0	No	No	B, Cc, Sf	10 ⁵
<i>Picophagus flagellatus</i>	1.8	No	Yes	B	10 ⁵
<i>Ochromonas</i> sp.	6.0	Yes	Yes	B, Cc	10 ⁵
Prymnesiophytes					
<i>Chrysochromulina ericina</i>	6.0	Yes	Yes	B, Cc	10 ⁴
Table 1 (continued).					
<u>Protistan Taxon</u>	<u>Average Size (µm)</u>	<u>Chloroplasts Present?</u>	<u>Phagotrophy?</u>	<u>Probable Prey</u>	<u>Representative Abundances (liter⁻¹)</u>
Chlorophytes					
<i>Nannochloris atomus</i>	4.0	Yes	No	-	10 ⁵
<i>Ostreococcus taurii</i>	0.8	Yes	No	-	10 ⁵
Bacillariophytes (Diatoms)					
<i>Minutocellus polymorphus</i>	3.0	Yes	No	-	10 ⁵
<i>Coscinodiscus concinnus</i>	75x200	Yes	No	-	10 ⁻²
<i>Ditylum brightwellii</i>	20x100	Yes	No	-	10 ²
<i>Rhizosolenia clevei</i>	200x500	Yes	No	-	10 ⁻³
<i>Ethmodiscus rex</i>	1,000	Yes	No	-	10 ⁻⁴
Choanoflagellates					

<i>Diaphanoeca grandis</i>	2.5	No	Yes	B	10 ⁵
Amoeboid protists					
Gymnamoebae (naked amoebae)					
<i>Platyamoeba weinsteini</i>	3x12	No	Yes	B	1x10 ⁰
<i>Flabellula citata</i>	4x30	No	Yes	B	5x10 ⁻¹
Foraminifera					
<i>Globigerina bulloides</i> §	700†	No	Yes	Omi, Mz	10 ⁻³

Table 1 (continued).

<u>Protistan Taxon</u>	<u>Average Size (µm)</u>	<u>Chloroplasts Present?</u>	<u>Phagotrophy?</u>	<u>Probable Prey</u>	<u>Representative Abundances (liter⁻¹)</u>
<i>Globigerinoides sacculifer</i> §	700†	No**	Yes	Omi, Mz	10 ⁻³
Acantharea					
<i>Amphilonche elongata</i> §	50x400	No**	Yes	Omi	1x10 ⁻¹
Spumellarian Radiolaria					
<i>Thalassicolla nucleata</i> §	1,000†	No**	Yes	Omi, Mz	10 ⁻⁴
<i>Collozoum caudatum</i> §	200†¥	No**	Yes	Omi, Mz	10 ⁻⁵
Ciliated protists					
Tintinnids					
<i>Tintinnopsis parva</i>	20x40	No	Yes	B, Cc, Sf	10 ²
Oligotrichs					
<i>Strombidium sulcatum</i>	25x50	Yes***	Yes	B, Cc, Sf	10 ²
<i>Loboea strobila</i>	50x150	Yes***	Yes	B, Cc, Sf	10 ²
Hypotrichs					
<i>Euplotes woodruffi</i>	65x120	No	Yes	C	5x10 ⁻¹
Hymenostomatids					
<i>Uronema marinum</i>	10x20	No	Yes	B, Cc	10 ³

*Species harboring extracellular, symbiotic cyanobacteria that contribute to the photosynthetic nutrition to the host. **Species harboring intracellular, symbiotic dinoflagellates that contribute to the photosynthetic nutrition to the host. ***Species that retain functional chloroplasts from ingested prey. †Size does not take into account extensive pseudopodial network. ¥Colonial species with colonies up to 1 cm in width and 1 m in length. §Considers adult specimens only.

Table 2. Planktonic protist species list described in Table 1 but arranged according to trophic category. Note that some species occur in more than one category.

Phototrophy

Gymnodinium sp.
Prorocentrum micans
Ochromonas sp.
Chrysochromulina ericina
Nannochloris atomus
Ostreococcus tauri
Minutocellus polymorphus
Coscinodiscus concinnus
Ditylum brightwellii
Rhizosolenia clevei
Ethmodiscus rex

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

Carnivory

Paraphysomonas imperforata
Globigerina bulloides
Amphilonche elongata
Thalassicolla nucleata
Collozoum caudatum
Euplotes woodruffi

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
Picophagus flagellatus

Herbivory

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