Marine Protists: A Hitchhiker's Guide to their Role in the Marine Microbiome

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Charles Bachy, Elisabeth Hehenberger, Yu-Chen Ling,
David M. Needham, Jan Strauss, Susanne Wilken,
and Alexandra Z. Worden

Abstract

Diversity within marine microbiomes spans the three domains of life: microbial eukaryotes (i.e., protists), bacteria, and archaea. Although protists were the first microbes observed by microscopy, it took the advent of molecular techniques to begin to resolve their complex and reticulate evolutionary history. Symbioses between microbial entities have been key in this journey, and such interactions continue to shape the ecology of marine microbiomes. Nowadays, photosynthetic marine protists are appreciated for their activities as primary producers, rivalling land plant contributions in the global carbon cycle. Predatory protists are known for consuming prokaryotes and other protists, with some combining metabolisms into a mixotrophic lifestyle. Still, much must be learned about specific interactions and lifestyles, especially for uncultured groups recognized just by environmental sequences. With respect to the fate of protists in food webs, there are many paths to consider. Despite being in early stages of identifying

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C. Bachy · E. Hehenberger · Y.-C. Ling · D. M. Needham · J. Strauss
Ocean EcoSystems Biology Unit, RD3, GEOMAR Helmholtz Centre for Ocean Research Kiel,
Kiel, Germany
e-mail: ehehenberger@geomar.de; yling@geomar.de; dneedham@geomar.de; jstrauss@geomar.de

S. Wilken
Faculty of Science, Institute for Biodiversity and Ecosystem Dynamics, Amsterdam, Netherlands
e-mail: s.wilken@uva.nl

A. Z. Worden (✉)
Ocean EcoSystems Biology Unit, RD3, GEOMAR Helmholtz Centre for Ocean Research Kiel,
Kiel, Germany

Kiel University, Kiel, Germany

Max Planck Institute for Evolutionary Biology, Plön, Germany
e-mail: azworden@geomar.de

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interactions, whether mutualistic or death-inducing infections by parasites and viruses, knowledge is advancing rapidly via methods for interrogation in nature without culturing. Here, we review marine protists, their evolutionary histories, diversity, ecological roles, and lifestyles in all layers of the ocean, with reference to how views have shifted over time through extensive investigation.

Keywords

Carbon cycle · Eukaryotic evolution · Marine food webs · Phytoplankton · Protistan evolution · Protistan interactions

> *Many were increasingly of the opinion that they'd all made a big mistake in coming down from the trees in the first place. And some said that even the trees had been a bad move, and that no one should ever have left the oceans.*
>
> From *A hitchhiker's guide to the galaxy—by Douglas Adams, 1979.*

4.1 Introduction: The Poetry and Beauty of Protists Through Time

Microbial communities comprise three domains of life: the Archaea and the Bacteria—domains containing cells that lack a nucleus—and single-celled Eukarya—organisms that do possess a nucleus—which are also known as protists. Protists are found throughout the ocean and in terrestrial environments, where they fulfill a vast array of ecological roles due to the wide variety of physiological capacities they collectively possess. The rise of eukaryotes is linked to the great oxygenation event (GOE), wherein cyanobacterial oxygenic photosynthesis drove a massive change in environmental conditions (Luo et al. 2016). The GOE spurred the advent of new metabolisms that functioned in an oxygenated environment, through merging of different cell types, resulting in eukaryotic cells which used oxygen for respiration (Planavsky et al. 2021). Symbioses played and still play major roles in diversification, including most known unicellular eukaryotic marine lineages. Protists not only have a nucleus, but other organelles as well, such as a mitochondrion where respiration occurs and a chloroplast (plastid) if photosynthetic. They can also have other "arrangements" involving more ephemeral capture and use of plastids. Interactions of protists with bacteria, archaea, viruses as well as with multicellular taxa in the ocean influence biogeochemical cycling, mortality, and even the metabolic potential of the protists themselves. Finally, protists can adopt a variety of cellular "organizations" ranging from free-living cells to colonial forms, with some being able to transform into truly multicellular forms and back to single cells. This breadth of interactions, behaviors, and relationships, connecting into deep time, drive the remarkable level of diversity that we see today. Known ecological roles of protists range from primary producers to predators, as well as from parasites and decomposers to organisms that blend multiple trophic modes. Although different "supergroups" can be parsed across eukaryotes based on their phylogenetic relationships, these divisions do not necessarily link to distinct trophic strategies, generating an even greater complexity with which microbial ecologists and microbiome scientists must grapple. In this chapter, key facets behind protistan diversity are introduced with a sprinkling of historical concepts as they have emerged and shifted through the centuries.

Through the 19th and 20th centuries scientists moved beyond skepticism about Antonie van Leeuwenhoek's (1632–1723) initial reports of having observed microbes or "animalcules"—as he called them—to deep appreciation of unicellular diversity. The studies by this Dutch microbiologist shifted from being infamous to famous as other scientists gained the possibility to observe microscopic cells, something upon which van Leeuwenhoek had a marked lead. Scholars and scientists then focused on how such microorganisms might influence the health of humans or on what these organisms could tell us about how cells function. Notable studies from individuals like Ernst Haeckel, who is credited with introducing the term "Protista", had a profound impact on both the scientific community and the public. Indeed, these scientific discussions and illustrations brought to light the infinite variety of microscopic organisms in which artists found objects of wonder, providing inspiration for Art Nouveau, a style of art and architecture that spread in Europe in the late nineteenth century. There are still traces today of how it took to the streets (Fig. 4.1), as well as how it permeated the salons of Europe as captured in the works of literary greats like Marcel Proust (Box 4.1). A little over 100 years from the publications of Haeckel and Proust, knowledge of protistan diversity and how protists arose and evolved is still advancing.

Box 4.1

"Ma tante n'habitait plus effectivement que deux chambres contiguës, restant l'après-midi dans l'une pendant qu'on aérait l'autre. C'étaient de ces chambres de province qui,—de même qu'en certains pays des parties entières de l'air ou de la mer sont illuminées ou parfumées par des myriades de protozoaires que nous ne voyons pas,—nous enchantent des mille odeurs qu'y dégagent les vertus, la sagesse, les habitudes, toute une vie secrète, invisible, surabondante et morale que l'atmosphère y tient en suspens; . . ."

"My aunt's life was now practically confined to two adjoining rooms, in one of which she would rest in the afternoon while the other was being aired. They were rooms of that country order which (just as in certain climes whole tracts of air or ocean are illuminated or scented by myriads of protozoa which we cannot see) fascinate our sense of smell with the countless odors springing from their own special virtues, wisdom, habits, a whole secret system of life, invisible, superabundant and profoundly moral, which their atmosphere holds in solution;. . .".

From *Swann's Way—Combray—by Marcel Proust, first published in 1913.*

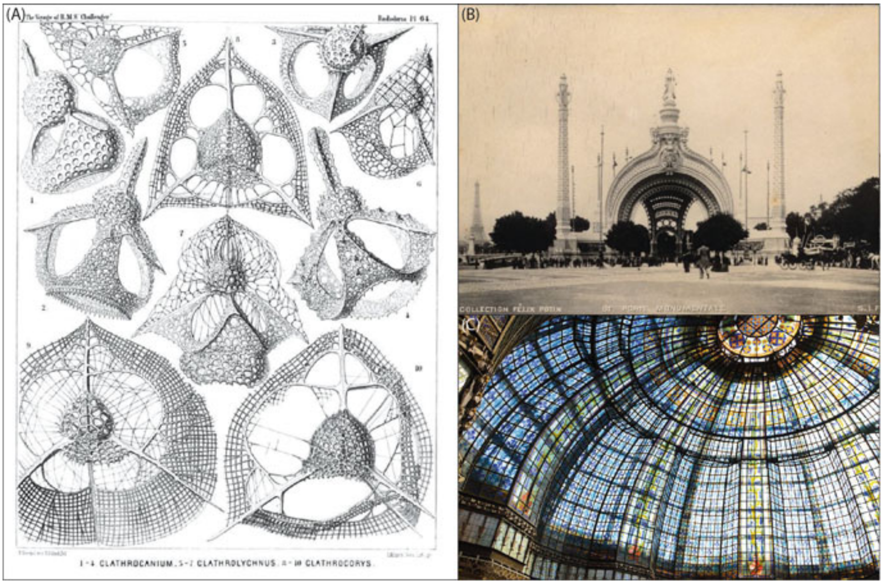

Fig. 4.1 Art Nouveau inspired by protists—The first images of protists, even though "just" drawings, had a massive influence on the Art Nouveau (also called Modern style). (**a**) The plate depicts Radiolaria specimens as provided by Ernst Haeckel to the report on the scientific expedition made aboard the sailing ship *H.M.S.* Challenger (Haeckel 1887), which circumnavigated the globe between 1872 and 1876, traversing 70,000 nautical miles. This expedition is widely considered to have laid the foundations of oceanography as a discipline. (**b**) The radiolarian named *Clathrocanium reginae* inspired the architect René Binet to design the entrance for the 1900 Paris Exposition (photographer unknown), which was dismantled after the exhibition. There are still traces of the Art Nouveau architectural style in Europe, for example (**c**) the ceiling at the Brasserie Printemps in Paris, that appears to be inspired by the skeleton of the Radiolarian *Sethophormis eupilium* (*Litharachnium eupilium*) described by Haeckel

Marine protists are typically categorized by size class, trophic mode, or phylogenetic lineage, or using trait-based approaches which might integrate information from more than one of these categories. To what extent do marine microbiome scientists need to think about the evolutionary origins and relationships between protists, or between protists and other biological entities? Understanding of evolutionary relationships between eukaryotic groups has been advancing rapidly (Brown et al. 2018; Burki et al. 2020; Strassert et al. 2019). After more than a century of observations with the naked eye and successive generations of microscopes (Fig. 4.2), this improvement is largely due to application of the latest technologies in genomics, single cell/population genomics and transcriptomics to protists, as well as improvements in evolutionary models and methods for inferring phylogenetic relationships (Bhattacharya et al. 2014; Burki 2017; Cooney et al. 2020; Cuvelier et al. 2010; Gawryluk et al. 2019; Keeling et al. 2014; Krabberød et al. 2017).

Nevertheless, it might still seem attractive to keep studies of the dynamics in marine environments and future trajectories to a purely mechanistic science, in this

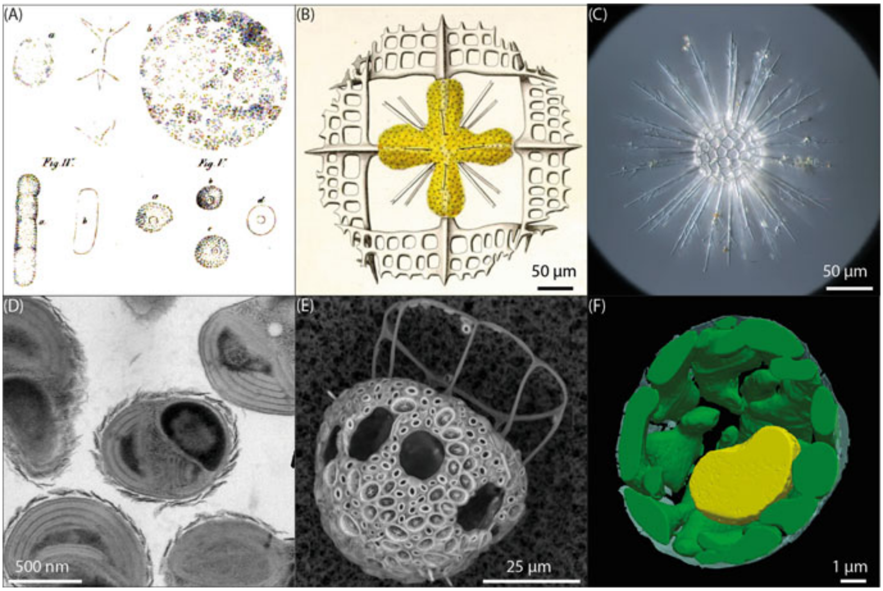

Fig. 4.2 The beauty of protists—Antonie van Leeuwenhoek interpreted his first descriptions of motile microbial eukaryotes observed through his microscope as being little animals or "animalcules" (Van Leeuwenhoek 1677). Thereafter, a panoply of methods made it possible to show others the beauty and meticulous details of marine protists. (**a**) One of the first formal descriptions depicting planktonic specimens of collodarians (from Meyen (1834)). (**b**) Haeckel's drawing of *Lithoptera mulleri* and the symbiotic algae it contains (Haeckel and Ernst Heinrich Philipp 1862). (**c**) A *Cladococcus* skeleton from deep waters of Villefranche-sur-Mer imaged using an optical microscope (courtesy of John R. Dolan). (**d**) A Transmission Electron Microscopy image of the picophytoplanktonic prasinophyte alga *Bathycoccus calidus* (Worden Lab in collaboration with Danielle M. Jorgens). (**e**) Scanning Electron Microscopy image of a ciliate, the heterotrophic tintinnid *Dictyocysta*, covered with coccoliths from its haptophyte prey (image, Charles Bachy). (**f**) A three-dimensional representation of a haptophyte in the *Phaeocystis* genus and its subcellular structures (green, plastids and yellow, nucleus) found in endosymbiosis with a radiolarian similar to that in panel B (from Decelle et al. 2019, Copyright: J. Decelle & C. Uwizeye)

case by characterizing the cell biology and activities of microbial eukaryotes that exist today. However, information on the evolutionary origins of microbial eukaryotes provides important insights into how roles and capabilities change and how they might continue to change with accelerated perturbations to the ocean environment. Just as an example, evolutionary studies have revealed that the apicomplexan parasites that causes Malaria (*Plasmodium*) are descended from free-living algae (Dorrell et al. 2014; Gardner et al. 2002; McFadden et al. 1996). *Plasmodium* species still maintain a chloroplast vestige (termed apicoplast), although it has lost the genes related to photosynthetic function. These evolutionary changes to the primary nutrition of *Plasmodium* are fascinating and profoundly important in terms of the change in its ecological role even if the timeframe and triggers remain unclear. In fact, loss of photosynthesis has been documented in multiple marine protistan lineages, many having close relatives that are still photosynthetic, as well as other relatives that never were photosynthetic (Hadariová et al. 2018; Worden et al. 2015).

Recently, efforts have been made to summarize how genomic and transcriptomic datatypes could improve exploration of the physiology of marine protists (Caron et al. 2017) and be applied to study the ecological consequences of their activities and interactions with other biological entities (Worden et al. 2015). Indeed, single cell methods are being used to query possible trophic modes and cell attributes (Labarre et al. 2021; Seeleuthner et al. 2018; Sieracki et al. 2019; Wideman et al. 2019, 2020) and to identify host–virus pairs alongside virally-encoded molecular pathways that shape host biology (Castillo et al. 2019; Needham et al. 2019a, b; Yoon et al. 2011). Based on science to date we know that the ocean harbors a wealth of complex interactions that determine the composition and functioning of the marine microbiome, and most of which await elucidation. The importance of understanding ocean ecosystems was outlined by Dawn Wright in her examination of the challenges that lie ahead in ocean science (Box 4.2). These challenges extend to ocean microbial interactions and the need to study them in a contextualized manner reaching far beyond discovery science, because ocean productivity and biogeochemical cycling are driven by the microbiome and the evolution of the organisms within!

Box 4.2

“Today we map the ocean not only to increase fundamental scientific understanding of the ocean system but also to protect life and property, promote economic vitality, and inform ecosystem-based management and policy.”

From *Swells, Soundings, and Sustainability, but... “Here Be Monsters”—by Dawn Wright, published in Oceanography (Wright* 2017*).*

4.2 Evolutionary Relationships among Protists

The term “Protista” has been widely embraced since its introduction by Haeckel, who felt a term was needed to describe and group together the living beings that were neither animals nor plants (the latter then also including fungi). However, alongside this regrouping and other early attempts to classify organisms is the notion that protists represent “lower” and/or more ancient life forms, a view that ignores their immense diversity and complexity.

4.2.1 A Historical Perspective on Protistan Diversity

For many years the only described species of eukaryotes belonged to well-studied lineages of macroscopic animals, plants, and fungi, which represent only a minority of eukaryotic diversity. Study of the microbial world including the plethora of unicellular eukaryotes was hampered by the tiny size of its members and the availability of technology with appropriate resolution powers. After Leeuwenhoek's discoveries, protists were typically classified into distinct lineages based on their morphology and nutritional mode. In many studies, their naming and placement depended on the researcher's primary discipline, with zoologists and botanists using different taxonomic contexts to group photosynthetic taxa (named algae or phytoplankton) apart from heterotrophic taxa (named protozoa, Box 4.3).

Box 4.3

"Es ist schon von verschiedenen Seiten darauf aufmerksam gemacht worden, dass es sowohl für die Zoologie als für die Botanik ein großer Gewinn sein würde, wenn man die vielen zweifelhaften Lebewesen, die weder echte Tiere noch echte Pflanzen sind, in einem besonderen Mittelreiche oder Urwesenreiche vereinigen würde; . . .".

"It has been pointed out by various parties, that both the fields of zoology and botany would greatly benefit, if the many dubious life forms, that are neither true animals nor true plants, were united in a special middle kingdom or kingdom of primeval life forms. . .".

From *Generelle Morphologie der Organismen: allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von C. Darwin reformierte Descendenz-Theorie. 1. Band (Berlin: Druck und Verlag von Georg Reimer)—by Ernst H. P. Haeckel, 1866.*

Major strides were made with comparative morphology studies and ultimately the application of transmission electron microscopy to photosynthetic protists, which was pioneered by Irene Manton in the 1950s. Manton was soon joined by Dorothy Pitelka, who studied heterotrophic protists, and collectively their efforts gave access to the subcellular scale, allowing morphological descriptions to be greatly improved. However, the enormous morphological diversity and often inconsistent descriptors thwarted attempts to elucidate the relationships between those groupings leading to protists being brought together as one kingdom of "lower" organisms at the base of animals, plants, and fungi (Whittaker 1969) (Fig. 4.3). Ultimately, protist lineages were regrouped and refined, although with disagreement, based on ultrastructural characteristics (Corliss 1984).

4.2.2 Developments in the Understanding of Evolution of Protists

The scientific view of protists being "lower" organisms was challenged by results from molecular phylogenetic analyses, the first of which used the gene coding for the small subunit of the ribosome, i.e., 18S rRNA, to resolve relationships, as reviewed in Taylor (2003). Using 18S rRNA genes, several research teams simultaneously

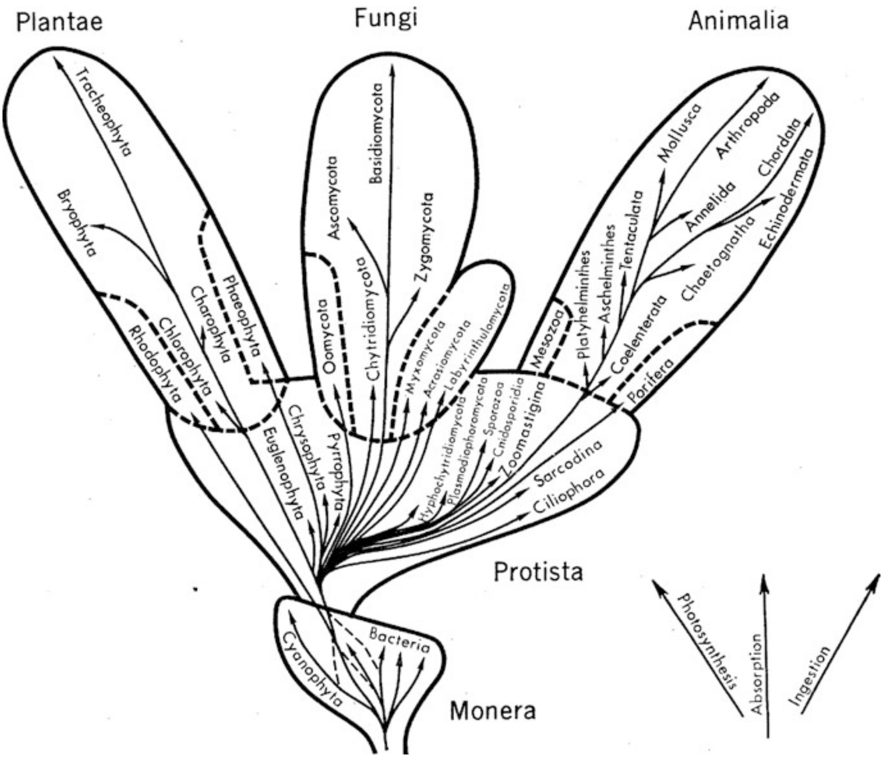

Fig. 4.3 The classical five-kingdom system of the twentieth century—Now outdated and replaced, this view of the relationships between different life forms, as depicted by Robert Whittaker (Whittaker 1969), was a standard feature of biology textbooks in the last decades of the twentieth century. The advent of molecular phylogenetics and sequencing led to multiple revisions, and only with phylogenetics and studies of uncultivated taxa has a more stable framework been resolved, although there is still much to be learnt—especially as we try to understand the relationships between eukaryotes and how they arose from an evolutionary perspective

reported that there was considerable marine eukaryotic diversity even after pre-filtering water samples through 5, 3, or even 2 μm pore size filters to capture just the cells smaller than these pore sizes (Díez et al. 2001; López-García et al. 2001; Moon-van der Staay et al. 2001). While marine microbiologists had long been aware of small eukaryotic cells, particularly phytoplankton (Knight-Jones and Walne 1951; Murphy and Haugen 1985; Takahashi and Bienfang 1983), the extent of their diversity was shocking to the scientific world, as was the identification of sequences coming from small presumably heterotrophic protists. Collectively, environmental molecular 18S rRNA sequencing of marine and other habitats also revealed that the vast majority of eukaryotic phylogenetic diversity lies within the protists, see Sogin (2015).

It took the advent of "phylogenomics," phylogenetic reconstructions based on multiple homologous genes from each organism in the analysis (10s to 100s, or sometimes more), concatenated into datasets of ever-increasing size, to establish a clearer picture of the tree of eukaryotes (Baldauf 2003; Chan and Ragan 2013; Delsuc et al. 2005; Philippe et al. 2011). This picture was very different from prior conceptions wherein there was a concept of "crown eukaryotes" (bringing together many multicellular and macroscopic groups) versus "simple eukaryotes," which were protistan. These views were dismantled with implementation of improved methods for estimating evolutionary distances. Increasingly protists were shown to be interspersed with various macroscopic lineages throughout the eukaryotic tree, or in basal branches, or large groups with no known macroscopic lineages, e.g., Baldauf (2003). Indeed, phylogenomic analyses distributed eukaryotic diversity into a small number of enormous groups that were informally termed "supergroups." Thus, in contrast to the tumultuous history of eukaryotic tree structure through the centuries, its overall structure now changes more modestly, due to the establishment of the supergroup concept (Adl et al. 2005; Keeling et al. 2005; Simpson and Roger 2004). Nevertheless, the resolution of relationships continues to improve due to analytical advancements and genome and transcriptome sequencing of new groups of protists (Burki et al. 2020; Janouškovec et al. 2017; Lax et al. 2018).

4.2.3 Major Groups of Eukaryotes as of "Currently"

A well-resolved framework amenable to the phylogenetic study of eukaryotic relationships now exists, see Burki et al. (2020) and Strassert et al. (2019). This is central to the study of the numerous aspects of eukaryote evolution such as the invention of multicellularity (Brunet and King 2017; Ros-Rocher et al. 2021) and sex (Goodenough and Heitman 2014), but it is also an essential tool to interpret the diversity of environmental sequence data in ecological communities (Worden et al. 2015). A factor that hinders understanding of eukaryotic evolution is the propensity of protists to engage in endosymbioses events (Worden et al. 2015), just as eukaryogenesis itself is hypothesized to have occurred through endosymbiosis, or potentially other mechanisms of fusion, between different prokaryotic cell types (Imachi et al. 2020; López-García et al. 2017; Spang et al. 2015; Zaremba-Niedzwiedzka et al. 2017). Eukaryogenesis and discussions about the Last Eukaryotic Common Ancestor (LECA) will not be covered in this chapter; several excellent publications are available on these topics (López-García et al. 2017; O'Malley et al. 2019).

4.2.4 The Contribution of Plastid Acquisition and Evolution to the Generation of Eukaryotic Diversity

A key event in the natural history of eukaryotes was the uptake and ultimate integration of a cyanobacterium by a eukaryotic cell about a billion years ago. The endosymbiosis between a cyanobacterium and its eukaryotic host gave rise to the so-called primary plastid (or chloroplast) that is found in land plants, green and red

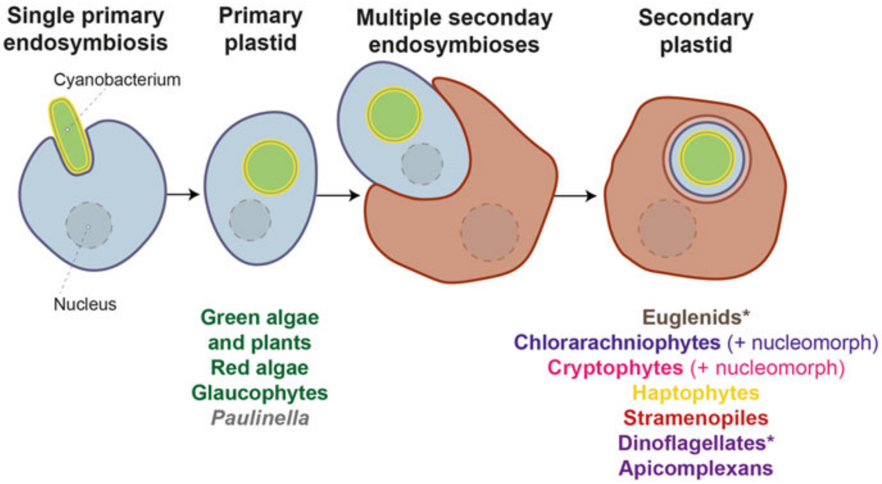

Fig. 4.4 Evolutionary history of algal endosymbiosis and putative plastid losses—Mitochondria and plastids both are hypothesized to have arisen from the endosymbiotic uptake of different bacteria (an alphaproteobacterium and a cyanobacterium, respectively). These and subsequent events are still difficult to resolve, for example integration of the alphaproteobacterium that gave rise to the mitochondrion has recently been proposed to have occurred by a mechanism other than endosymbiosis. While plastid origins are still considered as being through endosymbiosis, tracing the evolution of plastids has been complicated by additional endosymbiosis events, and loss of photosynthesis in some lineages. The original or “primary” plastid that descended directly from the cyanobacterial endosymbiont is found in the archaeplastids (glaucophytes, red algae, green algae, and plants). But green and red algae have themselves been taken up by other eukaryotic lineages, resulting in “secondary” plastids characterized by the additional membranes and more complex protein-targeting systems present in euglenids, chlorarachniophytes, cryptophytes, haptophytes, stramenopiles, dinoflagellates, and apicomplexans. Some members of these groups are predatory mixotrophs, and others are purely heterotrophic (predatory, saprotrophic, or even parasitic) because photosynthesis or plastids have been lost, or had never been acquired. Although green algae are common in marine environments (e.g., picoprasinophytes such as *Bathycoccus*, *Ostreococcus*, and *Micromonas*), lineages resulting from secondary endosymbiotic partnerships include other important marine primary producers (e.g., diatoms, pelagophytes, haptophytes, and dinoflagellates) and represent incredible metabolic versatility. Secondary plastids are usually surrounded by four membranes but the asterisk (*) denotes those with three membranes, specifically the euglenids and dinoflagellates (Keeling 2013). It is interesting to speculate that the redundancy and reshuffling of characteristics resulting from mergers of distinct eukaryotic lineages favors new combinations of traits with strong ecological potential

algae, and glaucophytes (Fig. 4.4). Thus, this event was the source of photosynthesis as we know it in plants and algae, see, e.g., Sagan (1967); Sibbald and Archibald (2020) and the starting point for the evolution of diverse algal groups spread all over the eukaryotic tree of life, most of which thrived in the ocean. Much of that diversity arose because primary plastids have subsequently been “moved into” various other lineages via a eukaryote–eukaryote endosymbiosis, i.e., when an alga with a primary plastid was engulfed by another non-photosynthetic eukaryote, which then retained that alga (after quite some reductions in gene content) in what is termed a "secondary" plastid (Sibbald and Archibald 2020). Some lineages took this strategy a step further and engulfed secondary plastid-bearing algae in a tertiary endosymbiosis. The existence of even higher-order endosymbioses and plastids has been proposed in attempts to explain the distribution of plastids as seen in the current tree of eukaryotes (Archibald 2006; Ševčíková et al. 2015; Stiller et al. 2014).

Did multiple and higher-order endosymbiotic events occur? The answer to this question lies in the incongruence between the phylogenies of plastids themselves and the phylogenies of the algae hosting said plastids. While plastid phylogenies indicate that all plastids are seemingly closely related, the nuclear genomes of the algal hosts are not. Plastid-hosting lineages are found across the eukaryotic tree nested within lineages lacking plastids and heterotrophic lineages, resulting in a seemingly random distribution across the eukaryotic tree (Fig. 4.5). It is thought that there must have been at least two additional endosymbiotic events after the primary endosymbiosis, if not more, because both red and green algae have been taken up by other eukaryotes (Keeling 2013; Lane and Archibald 2008). Although the spread of the green primary plastid via secondary endosymbiosis is generally well understood, there are still some mysterious features, likely because we are still discovering photosynthetic lineages (Choi et al. 2017; Kim et al. 2011a), and because other taxa that are keys to the puzzle may now be extinct.

The evolutionary history of red algal plastids is highly contentious and has been discussed in dedicated publications, e.g., Sibbald and Archibald (2020). While the exact evolutionary path that plastids of red algal origin forged through different parts of the eukaryotic tree may not be known, detection of their presence (albeit not their absence) is usually more straightforward. Hence, the discovery of plastids in unexpected areas of the eukaryotic tree has provided essential pieces that bring the field closer to solving the puzzle of plastid evolution. "Red" plastids originating from secondary or even higher-order symbioses are found in cryptophytes (supergroup Cryptista), haptophytes (supergroup Haptista), and several lineages of alveolates and stramenopiles (Fig. 4.5). Application of multiple molecular biology methods allowed the discovery of novel branches of uncultured marine protists bearing plastids of probable "red" origin, such as the Rappemonads (Kawachi et al. 2021; Kim et al. 2011a) and other deep-branching plastid lineages (DPL1 and 2 (Choi et al. 2017)). However, these environmental clades await formal morphological descriptions. Most of the described lineages with red plastids are free-living photosynthetic algae, but not all plastid-bearing protists have retained the ability to perform photosynthesis and some are not even free-living. For example, it was discovered that a group of obligate intracellular parasites, the apicomplexans (e.g., *Plasmodium* and *Toxoplasma*), which are part of the major group Alveolates, have a plastid (McFadden et al. 1996). Subsequently, a free-living marine coral-sediment associated genus of photosynthetic protists, *Chromera*, was discovered, and found to be a close relative of apicomplexans and also helped to establish the red algal origin of the *Plasmodium* plastid (Janouskovec et al. 2010; Moore et al. 2008). Comparisons between *Plasmodium* and the chromerids (Janouškovec et al. 2015) have since led to the proposal that disruptions to the unique chloroplast transcript

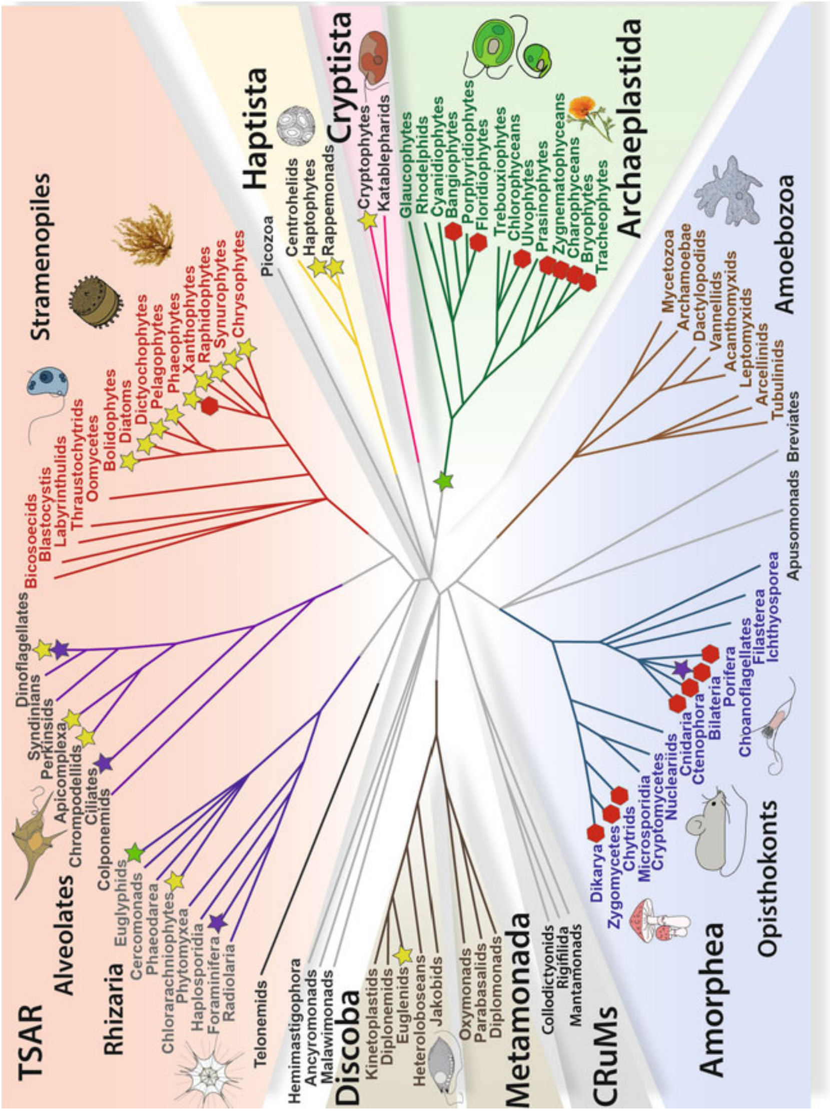
TSAR
Stramenopiles
Bicosoecids
Blastocystis
Labyrinthulids
Thraustochytrids
Oomycetes
Bolidophytes
Diatoms
Dictyochophytes
Pelagophytes
Phaeophytes
Xanthophytes
Raphidophytes
Synurophytes
Chrysophytes
Alveolates
Dinoflagellates
Syndinians
Perkinsids
Apicomplexa
Chrompodellids
Ciliates
Colponemids
Rhizaria
Euglyphids
Cercomonads
Phaeodarea
Chlorarachniophytes
Phytomyxea
Haplosporidia
Foraminifera
Radiolaria
Telonemids
Haptista
Picozoa
Centrohelids
Haptophytes
Rappemonads
Cryptista
Cryptophytes
Katablepharids
Archaeplastida
Glaucophytes
Rhodelphids
Cyanidiophytes
Bangiophytes
Porphyridiophytes
Floridiophytes
Trebouxiophytes
Chlorophyceans
Ulvophytes
Prasinophytes
Zygnematophyceans
Charophyceans
Bryophytes
Tracheophytes
Amoebozoa
Mycetozoa
Archamoebae
Dactylopodids
Vannellids
Acanthomyxids
Leptomyxids
Arcellinids
Tubulinids
Breviates
Apusomonads
Amorphea
Opisthokonts
Ichthyosporea
Filasterea
Choanoflagellates
Porifera
Bilateria
Ctenophora
Cnidaria
Nucleariids
Cryptomycetes
Microsporidia
Chytrids
Zygomycetes
Dikarya
CRuMs
Mantamonads
Rigifilida
Collodictyonids
Metamonada
Diplomonads
Parabasalids
Oxymonads
Discoba
Jakobids
Heteroloboseans
Euglenids
Diplonemids
Kinetoplastids
Malawimonads
Ancyromonads
Hemimastigophora

Fig. 4.5 The eukaryotic tree of life—Eukaryote diversity has been subdivided into major lineages, often referred to as "supergroups." The supergroup concept emerged in large part due to support gained for relationships through the advent of phylogenomic methods. The Archaeplastida, containing land plants and green algae, red algae and glaucophytes, is distinguished by their primary plastids (green stars). A second, independent origin of a primary plastid has been reported within the Rhizaria, in the amoeba *Paulinella* (Nowack et al. 2008). The primary plastids within the Archaeplastida were spread across the tree of eukaryotes via secondary endosymbiotic events, i.e., the uptake of (red or green) primary algae by other eukaryotic lineages (see Fig. 4.4), and can be found in ecologically important marine lineages such as diatoms and dinoflagellates. Plastid remnants are also present in some unexpected groups such as the obligatory parasitic apicomplexans. Secondary plastids have spread further by tertiary endosymbiotic events and potentially even higher-order events (secondary and higher plastids are indicated by yellow stars). Purple stars mark lineages performing kleptoplasty, a specific form of mixotrophy. Such lineages sequester plastids from their prey and maintain them for variable amounts of time. Lineages containing at least one taxon that is multicellular are indicated by red hexagons. Although multicellularity has arisen more than once, and thus appears in a variety of lineages, the vast majority of eukaryotic life is unicellular. Figure adapted from Worden et al. (2015) with updates from Burki et al. (2020); Derelle et al. (2016); Keeling and Burki (2019); Parfrey and Lahr (2013); Sibbald and Archibald (2020) processing machinery seen in chromerids resulted in the loss of photosynthesis in the ancestors of parasitic apicomplexans (Dorrell et al. 2014).

The major eukaryotic groups that are recognized today can be briefly characterized as follows. Note that these groupings are constantly being revised as new information and methods become available.

TSAR represents a combination of SAR, which stands for the clade uniting Stramenopiles, Alveolates, and Rhizaria, with the Telonemids. SAR emerged early after the implementation of phylogenomic methods and has been estimated to encompass half of all eukaryote diversity. The Telonemids, which contain only two described species and are considered to be a widespread (but not abundant) lineage of heterotrophic flagellates, were proposed to be a sister lineage of SAR, creating the even larger group TSAR (Strassert et al. 2019).

Stramenopiles comprise well-known microbial algae (e.g., diatoms and chrysophytes) but also macroscopic multicellular seaweeds (e.g., kelps) as well as an enormous diversity of free-living heterotrophic and mixotrophic protists. They also include several important pathogens of animals and plants, such as the infamous oomycete *Phytophthora*, causative agent of the potato blight that led to the Great Famine in the mid-nineteenth century Ireland. Stramenopiles also comprise many sequences from enigmatic groups called MASTs, which stands for MArine STramenopiles. MASTs were initially identified in environmental clone libraries and the organisms themselves remain largely uncultured (Massana et al. 2002). Currently there are 18 recognized MAST clades and only a few have been further elucidated in terms of functional roles, although most are considered to be heterotrophic and some have been demonstrated to consume bacterial cells using culture independent methods (Labarre et al. 2021; Lin et al. 2012; Massana et al. 2014). A distinctive feature of stramenopiles are two flagella (although they are not always present!) of unequal length (hence the alternative name "heterokont") and hair-like structures termed mastigonemes on the longer flagellum (hence stramenopiles, or "straw-like pili") (Cavalier-Smith 2018).

Alveolates include three hugely diverse and well-studied protist groups (ciliates, dinoflagellates, and apicomplexans) plus several smaller groups of parasites and flagellates. Ciliates are a major group of microbial predators and grazers in all known environments. Dinoflagellates are also extremely abundant in nature with a variety of lifestyles and include probably the most abundant marine eukaryotes, the parasitic MALVs (for Marine Alveolates). All apicomplexans described thus far are obligately associated with animals, most commonly as intracellular parasites (e.g., *Plasmodium*).

Rhizarians comprise a wide diversity of predominantly amoeboid protists with thin pseudopodia used more for feeding than for locomotion. This group also includes parasites of crop plants and invertebrates (e.g., *Plasmodiophora*) and even algae (chlorarachniophytes). In marine ecosystems the most prominent members of rhizarians are planktonic organisms such as the foraminifera and radiolarians that have been extensively described from the fossil record and are observed in open ocean and intertropical waters (Burki et al. 2016; Cavalier-Smith et al. 2015).

Haptista contains two main lineages: the haptophytes, which includes the still enigmatic uncultured rappemonads (Kim et al. 2011a), and the centrohelids (Burki et al. 2016; Cavalier-Smith et al. 2015). Haptophytes are mostly marine species that perform photosynthesis and can bloom to high density. Much attention has been given to the calcifying coccolithophorids (e.g., *Emiliania huxleyi*) because of their unique role in the biogeochemical cycles (connected to their calcium carbonate coccoliths) and their resulting sensitivity to climate change (Read et al. 2013; Taylor et al. 2017). Marine non-calcifying haptophytes are also important and diverse pico- and nano-phytoplankton (Cuvelier et al. 2010; Jardillier et al. 2010; Liu et al. 2009), and some appear to consume other cells (Hartmann et al. 2013). The other main Haptista lineage, the centrohelids, contains free-living heterotrophic protists characterized by distinctive radiating pseudopodia most often found in freshwater environments (Cavalier-Smith and von der Heyden 2007).

Cryptista contains the cryptomonads, algae best known for their red algal-derived plastids that retain a relict endosymbiont nucleus (the so-called nucleomorph) and which are central to the study of plastid origin and spread across eukaryotes. The group also contains the enigmatic heterotrophic katablepharids and the lone genus *Palpitomonas*. Cryptophytes are also noted for their importance in Antarctic waters (Mendes et al. 2018).

Archaeplastids are defined by the presence of the so-called primary plastids directly derived from the primary endosymbiosis with a cyanobacterium. They include green algae (from which land plants evolved), red algae and glaucophytes. A new group, *Rhodelphis*, was discovered that branches as sister group to red algae in phylogenomic analyses (Gawryluk et al. 2019). *Rhodelphis* cells are heterotrophic flagellates, but sequence data suggest that they have a non-photosynthetic primary plastid.

Amoebozoans are the second primarily amoeboid group besides the Rhizaria and include groups with pseudopodia for feeding and locomotion (Cavalier-Smith et al. 2016). The group also includes slime molds and flagellates as well as some important pathogens (e.g., *Entamoeba* causing amoebic dysentery). Several marine genera isolated from sediments have also been described (Kudryavtsev et al. 2018).

Opisthokonts include several protistan lineages, including a variety of heterotrophic flagellates, amoeboid protists, and parasites, and fungi as well as all multicellular animals. The term opisthokont refers to the presence of a single posterior flagellum. Opisthokonts, amoebozoans, and a few other small lineages are grouped as Amorphea. In marine and freshwater ecosystems, choanoflagellates have long been recognized as predators of bacteria and structural similarity with sponges was noted before molecular analysis confirmed that they are related to sponges, corals, and the rest of the animals (Brunet and King 2017; Leadbeater 2015).

CRuMS is an amalgamation of several former "orphan" taxa (see below): the Collodictyonids, Rigifilida, and the marine genus *Mantamonas* (Glücksman et al. 2011). Thus far these groups comprise solely free-living protists that have morphologically little in common, but it was recently found that they branch together in molecular phylogenies (Brown et al. 2018).

Discobids and Metamonads are two groups previously classified as the Excavate supergroup based on their distinctive morphology. Molecular phylogenies have mostly failed to support this grouping and therefore they are currently treated as two separate but possibly related groups. Discobids include photosynthetic euglenids (e.g., *Euglena*), parasites (e.g., *Trypanosoma*), and many free-living heterotrophic flagellates, such as the diplonemids, a great diversity of which are present in marine environments (Flegontova et al. 2016). Metamonads contain anaerobic protists including several pathogens (e.g., *Giardia* and *Trichomonas*) and symbionts in animal guts (e.g., *Trichonympha*).

Hemimastigophora or "hemimastigotes" are free-living soil protozoa with two rows of flagella. They were first noted in the nineteenth century but not cultured and genetic data were therefore lacking. Phylogenomic analyses based on transcriptomes sequenced from hand-picked cells of two genera indicated that hemimastigotes are one of the deepest branches within the eukaryotes. Because these analyses failed to place them as sister to any one of the "established" supergroups (or any "orphan"), it has been proposed that they should be considered as a new supergroup (Lax et al. 2018).

Orphan Taxa refers to several seemingly species-poor taxa for which phylogenomic analyses have thus far failed to provide convincing evolutionary relationships with other lineages. These so-called orphan taxa are all free-living heterotrophic protists and include e.g., the tiny and widespread marine group of Picozoa (Moreira and López-García 2014; Not et al. 2007b; Seenivasan 2013; Seenivasan et al. 2013).

4.3 Traits Distinguishing Protists from Other Marine Microbiome Members: Size and Cell Structure

The adequate representation of diversity in studies with the aim to address ecological questions has been a long-lasting challenge. Questions such as how elemental cycles function, how ecosystem stability is achieved, or what are the biogeography distributions of (micro)organisms all require some kind of simplification of the immense diversity of taxa, and the delineation of a tractable set of ecological roles fulfilled by diverse protists is no exception. Trait-based approaches have gained ground in ecology and oceanography (Kiørboe et al. 2018; Martini et al. 2020). These offer a representation of functional diversity that is independent from characterization of a large number of individual species and their assignment to pre-defined groups. Insights into community ecology thereby can be gained by understanding the traits that best characterize an ecological niche, how those traits affect fitness, and how they relate to one another in potential trade-offs. The resultant insights into community ecology include the potential for predicting response scenarios under future environmental conditions that could result in novel combinations of traits that are the most favorable under those conditions.

Detailed overviews of traits that are considered relevant based on current knowledge have been assembled for phytoplankton (Litchman and Klausmeier 2008) and zooplankton (Litchman et al. 2013) as well as for potential trade-offs found in mixotrophic organisms capable of both photosynthesis and phagocytosis (see below) (Andersen et al. 2015). Relevant traits are often classified into morphological, physiological, behavioral, and life-history traits, many of which are not yet understood for the uncultured majority of protistan diversity. Moreover, they are difficult to predict from genomic information alone (Keeling and Campo 2017). Thus, a major research pursuit is to identify functional traits in protists. An important feature of this type of research is to study mechanisms at the relevant scale. For example, results from the analysis of flow fields generated by beating flagella of swimming protists suggested a trade-off between maximizing resource acquisition (via swimming) and minimizing predation risk from flow-sensing predators (Nielsen and Kiørboe 2021). Below, we highlight just a few of the traits that perhaps have a larger influence on the biology and function of marine protists relative to their influence on the biology of bacteria and archaea.

4.3.1 Cell Size of Marine Protists

Unlike the size of marine archaea and most marine bacteria, the cell diameter of protists ranges from less than one to several hundred micrometers (Caron et al. 2017; Finkel et al. 2009). Thus, it has become standard practice to group microbial eukaryotes according to a series of size fractions. These fractions are based on cell diameter with the prefix pico- indicating 0.2–2.0 μm cell diameter, nano- indicating 2.0–20 μm, and micro- indicating 20–200 μm (Sieburth et al. 1978). Of course, the grouping "picoplankton" includes eukaryotes as well as bacteria and archaea and therefore further precision is needed. Thus, picoeukaryotes is the term used for the smallest eukaryotic cells and by current practice their size range is often defined as either 0.2–2.0 μm or 0.2–3.0 μm depending on the filter pore size used. Historically, other terms have also been employed for example, "ultraplankton" (variously, 0.2–3 to 10 μm, (Murphy and Haugen 1985; Pitta and Karakassis 2005; Reynolds 1973; Takahashi and Bienfang 1983), but usually <5 μm) and these terms have their own value because all of these fractions are to some extent arbitrary.

While it is somewhat arbitrary to lump organisms solely based on size, it can be helpful for considering the various life strategies and the competition processes that go on among different microbial populations, something eloquently addressed in 1992 by Sallie Chisholm (see Box 4.4). Moreover, due to their tiny size picoplanktonic organisms have low Reynolds numbers (Re) meaning that their movement is dominated by viscous forces rather than by inertial forces (Aris 1990). Because of their low Re, picoplankton does not sink through the water column as individual cells. They sink when aggregated into larger material (e.g., through predation, fecal pellet, and marine snow) or when other mechanisms occur, such as downwelling or other mesoscale oceanographic features (Omand et al. 2015). The viscosity of seawater also has evolutionary consequences for the energetics and mechanisms of directional movement by motile microbes such as the structure and placement of the flagellar apparatus (Barry et al. 2015; Brumley et al.

2015; Febvre-Chevalier and Febvre 1994). A corresponding cell size also puts different species of photosynthetic picoeukaryotes, such as the prasinophyte *Ostreococcus*, and photosynthetic bacteria, such as the cyanobacterium *Synechococcus*, under somewhat similar constraints in terms of the ratio of cell surface area to volume. This ratio impacts the efficiency of nutrient acquisition (since nutrient transporters are located on the surface of cells in cytoplasmic membranes) as well as cellular packaging of photosynthetic pigments (Raven 1998). These factors are critical to the success of these organisms in oligotrophic environments and overall, such size-based considerations are important for integrating organism dynamics into food webs and global biogeochemical cycles.

Box 4.4

"In reviewing this subject, it became clear to me that plankton ecologists fall out into two groups: Those who delight in finding the patterns in nature that can be explained by size, and those who delight in finding exceptions to the established size-dependent rules. I came to appreciate the degree to which the satisfaction of both groups is equally justified. The mechanisms underlying the size-dependent patterns have undoubtedly steered the general course of phytoplankton evolution, but the organisms that do not abide by the rules reveal the wonderful diversity of ways in which cells have managed to disobey the "laws" scripted for them. The simplicity of the general relationships serves as a stable backdrop against which the exceptions can shine. By understanding the forces that have driven the design of these exceptions, we can begin to understand the ecology that has shaped past and present planktonic ecosystems."

From *Phytoplankton Size—by Sallie W. Chisholm, published in 1992.*

4.3.2 Cellular Structure and Mosaic Genomes

Historically, the cell structure of Eukarya has been viewed as being different from Bacteria and Archaea (Doolittle 1998a). The cytological classification system formalized by Stanier and van Niel (Stanier and Van Niel 1962) laid out the criteria for distinguishing bacteria (including archaea) from protists. In this view, eukaryotic cells have complex structural features such as the membrane-enveloped true nucleus, a complex endomembrane system, and a cytoskeleton, while Bacteria and Archaea do not possess such features. These differences are not quite as clear-cut anymore, because some features that were considered to be characteristic for eukaryotes have also been discovered in Bacteria and Archaea, blurring the boundaries between the three domains of life (Grant et al. 2018; Oikonomou et al. 2016; Vellai and Vida 1999). Still, there are typical features of eukaryotes, such as the mitochondrion, although deviations have been identified (Karnkowska et al. 2016), as well as the capacity for endocytosis and exocytosis (Vellai and Vida 1999).

While partial compartmentalization has also been observed in Bacterial and Archaeal lineages, it is different from the membrane-delimited compartments that characterize eukaryotes (Diekmann and Pereira-Leal 2013). Eukaryotes originate from what is thought to have been a highly compartmentalized cell, LECA, a hypothetical lineage already containing a complex endomembrane system, thought to have given rise to all modern eukaryotes (Field and Dacks 2009). Protists and other eukaryotes have more or less retained this compartmentalized cell plan with multiple organelles, whereas Bacteria and Archaea tend to have only one compartment. Furthermore, endosymbionts or their remnants are ubiquitous in eukaryotes, such as mitochondria and plastids. In contrast, endosymbiosis appears to be rare in Bacteria and Archaea. Thus, the compartmentalized cell plan creates fundamental differences from the bulk of the known Bacteria and Archaea (Diekmann and Pereira-Leal 2013).

Cellular complexity has consequences for the protistan cell. Compounds acquired exogenously must be trafficked through multiple membranes, affecting both nutrient acquisition and energy allocation. Cell complexity also determines how ecological interactions can manifest, as well as possibilities for evolutionary adaptation. For example, the transient contribution of Auxiliary Metabolic Genes (AMGs) brought to their hosts by viruses is much discussed (Breitbart et al. 2018; Zimmerman et al. 2020). The fact that photosynthetic protists have a membrane around the plastid means that proteins must have a specific transit peptide to cross that membrane, and hence it would be difficult for a virally-encoded photosynthesis-related gene to function in the host cell, because it would not localize to the correct compartment (the plastid). In contrast, once a virus enters a cyanobacterium there is no such additional boundary for the AMGs it brings, and indeed photosynthesis-related genes are commonly seen in cyanophages and are highly active in augmenting host photosynthesis during infection (see below).

An expansion of genetic information, relative to prokaryotes, and its residence in the nucleus is a feature of eukaryotes, and indeed central to the evolution of eukaryotic cells. In 1986, Lynn Margulis and her son Dorion Sagan conveyed the intricate entwining of life forms and the importance of the nucleus to the lay audience (Box 4.5). Although controversial in the theory of eukaryotic evolution (Lynch and Marinov 2017), the expansion of genetic information in eukaryotic cells alongside increased cytoplasmic complexity and compartmentalization is proposed to have been facilitated by the increased energy supply that occurred through symbiotic integration of endocytosed bacteria (Lane 2011; Lane and Martin 2010; Vellai and Vida 1999). This proposed relief from energy constraints was purportedly paralleled by a more K-selected lifestyle of the Eukarya, compared to Bacteria and Archaea (Carlile 1982), and physiological optimization resulting in lower death rates (Kerszberg 2000).

It should be noted that following endosymbiosis there is usually a dramatic reduction of the gene content of the genome of the endosymbiont. In fact, the remnant genomes of endosymbionts can contain <5% of the genes found in their free-living relatives. Gene transfer from organelle "ancestors" to the "host" nucleus, a process called endosymbiotic gene transfer (EGT), also leads to genetic variation in eukaryotes (Gould et al. 2008; Kleine et al. 2009; Timmis et al. 2004). Overall, EGT from bacteria to eukaryotes has caused episodic transfer of bacterial genes to eukaryotic genomes (Ku et al. 2015) and also takes place between eukaryotes (Archibald 2015; Gould et al. 2008).

Endosymbiosis aside, it has been unclear to what extent protists and other eukaryotes transfer genetic material laterally between them. In the evolution of Bacteria and Archaea lateral or horizontal gene transfer (LGT or HGT) is known to play a major role (Soucy et al. 2015). Viruses can also act as vectors for gene transfer and likely facilitate LGT between eukaryotes (Gilbert and Cordaux 2017). Moreover, protists may acquire foreign DNA by ingesting and digesting prey (Doolittle 1998b). Collectively, EGT and potentially these other mechanisms of LGT are important modes of gene acquisition in eukaryotes and underpin the apparent mosaicism seen in the genomes of protists that reflects acquisition and retention of genetic material from different biological entities.

Box 4.5

"Life on earth is such a good story you cannot afford to miss the beginning. . .Beneath our superficial differences we are all of us walking communities of bacteria. The world shimmers, a pointillist landscape made of tiny living beings. Giant redwoods and whales, mosquitoes and mushrooms are intricate symbiotic networks, modular manifestations of the nucleated cell."

From *Four Billion Years of Evolution From Our Microbial Ancestors—by Lynn Margulis & Dorion Sagan, published in 1986.*

4.4 Metabolic Exchanges Between Microbiome Members

When considering microbiomes, the aim often is to understand the present function of that microbiome and consequences for the local habitat (e.g., most research on the human gut microbiome). However, it is important to recall that interactions between microorganisms in the early ocean are what eventually led to eukaryogenesis—the rise of protists—and similarly the plethora of symbioses and organismal functions seen in modern time have been shaped by cell-to-cell interactions in ancestral microbiomes. Interactions between microorganisms may simply involve exchange of metabolites or signaling compounds in the water column, but also occur through the range of physical interactions known as symbioses. The nuances of the term symbiosis have shifted over the years, and today the meaning tends toward being quite broad as outlined below.

4.4.1 Symbioses: Manifestation Is a Status Not an Identity

Symbioses can be mutually beneficial (mutualism), beneficial for one partner without incurring a cost for the other (commensalism), or beneficial for only one partner at a cost to the other (parasitism/pathogenicity). These distinctions are generally not clear-cut, in part because the costs and benefits can shift with changing environmental conditions. Examples are photo-endosymbiotic associations that are usually considered mutualistic since a heterotrophic host gains access to photosynthetically-fixed carbon from its (photosynthetic) endosymbiont, which receives nutrients in return. However, these associations may also represent an exploitation of the endosymbionts by their host (Decelle 2013) or under certain conditions can even be costly for the host, such as under low light conditions where photosynthesis does not operate well, but the endosymbiont still receives nutrients from the host (Lowe et al. 2016).

Parasites—One marine example of parasites is chytrid fungi that parasitize common marine diatoms (Garvetto et al. 2019; Hanic et al. 2009; Scholz et al. 2017). Chytrids are found throughout the ocean especially during and following diatom blooms and may play a role in the collapse of these phytoplankton blooms. Additionally, the chytrids and their host-specificity appear to influence diatom bloom dynamics and diversity (Chambouvet et al. 2019; Gsell et al. 2013).

Pathogens—Disease or death causing microorganisms are the subject of many biomedical studies but receive less attention in studies of the marine environment. One example is bacteria belonging to the newly discovered candidate phylum Dependentiae (also known as TM6) which can cause rapid infection and death of a heterotrophic flagellate host, the stramenopile *Spumella elongata* (Deeg et al. 2019), a stramenopile lineage with marine relatives. While this particular example is a freshwater pathogen-host system, similar interactions may also occur in the ocean. In regard to protists rather than bacteria that may be considered pathogens of protists, examples include the Oomyceta genera *Lagenisma* and *Olpidiopsis*, which infect the diatoms *Coscinodiscus* and *Rhizosolenia*, respectively (Buaya et al. 2017; Scholz et al. 2014).

Mutualists/Commensualists—An example of mutualism in the marine plankton is between diatoms and nitrogen-fixing bacteria (diazotrophs) also known as DDAs (diatom-diazotroph associations). This type of symbiosis is well documented between several diatom genera, especially *Rhizosolenia* and *Chaetoceros*, and the cyanobacteria *Richelia intracellularis* and *Calothrix rhizosoleniae* (Foster et al. 2011; Foster and Zehr 2006; Jahson et al. 1995). DDAs are found throughout the tropical and subtropical ocean (Monteiro et al. 2010). In addition, there are examples where the real impact of a symbiosis for each partner is still unclear with respect to the possible spectrum of commensalism to mutualism, principally because of the lack of knowledge of the cell biology underpinning the relationship.

4.4.2 Phycosphere and Metabolic Exchanges

The first attempts to maintain diverse photosynthetic protists in culture already revealed dependence on particular supplements. For example, for many years organic supplements in media for eukaryotic phytoplankton included B-vitamins (Droop 1957). Later it was realized that many of these phytoplankton did not actually need the vitamin, but did need one of the precursor moieties, for example hydroxylmeythl-pyrimidine (HMP) in the case of many haptophyte algae, or for some prasinophytes the thiazole moiety (Gutowska et al. 2017; McRose et al. 2014; Paerl et al. 2018). In nature these compounds would have been supplied by other microbes, while the phytoplankton themselves produce other compounds utilized by microbiome members. At this stage a wide range of metabolic interactions are known (Johnson et al. 2020), including the exchange of growth factors, essential nutrients, and carbon sources, which can either become available to other microbes as “public goods” upon release by the producer, or be exchanged in more targeted “trading relationships.” In case of the photosynthetic protists unable to grow on a purely mineral medium, requirements can sometimes be met by the supply of filtered natural seawater indicating that the required compounds are present in sufficient concentrations and thus could be regarded as “public good.” However, if the metabolite is depleted in the environment, the concentration gradient around the producer becomes steep and metabolite exchange requires a close cell proximity (van Tatenhove-Pel et al. 2021). Proximity can be attained by motile cells in brief encounters, but can also be semi-permanent in the case of stable co-associations such as symbioses.

Beyond the role of symbioses in protistan ecology, and the top-down controls on protists exerted by viruses and predation (see below), there are other mechanisms of interaction that occur in the aquatic environment. For example, many photosynthetic protists are large enough to have a viscous boundary layer termed the “phycosphere,” in which bacteria can encounter higher concentrations of phytoplankton exudates and utilize them for growth (Raina et al. 2019; Seymour et al. 2017). Furthermore, a currency exchange can occur whereby compounds produced by particular bacteria can be used by the phytoplankton cells, while the bacteria themselves acquire other compounds from the phytoplanker, e.g., Amin et al. (2012, 2015).

4.4.3 The Holobiont Concept

The holobiont concept offers a contextual shift in biology that can help to describe and to understand biological interactions in marine ecosystems. This concept applies to organisms ranging from protists (Dittami et al. 2021) to animals, including humans (Pride et al. 2010; Simon et al. 2019; van de Guchte et al. 2018). The term “holobiont” was conceived in the 1940s (Meyer-Abich 1943) and later independently by Margulis (Margulis 1990) who coined the term in the context of symbiosis-driven evolutionary innovations. Today it usually refers to a close association between different individuals that form an anatomical, physiological, immunological, or evolutionary unit (Simon et al. 2019). The discovery of the extent of marine molecular microbial diversity and microbiome inter-connectivity suggests that the holobiont concept may apply more frequently in marine ecosystems than currently recognized (Lima-Mendez et al. 2015). Studies on corals (Apprill 2017; Thompson et al. 2014) and sponges (Pita et al. 2018) alongside fascinating foundational studies on squid (McFall-Ngai 2014; Nyholm and McFall-Ngai 2004; Tischler et al. 2019) have demonstrated that microbes that associate with this suite of multicellular marine animals are an integral part of the living system: the holobiont.

4.5 Shifting from a Functional Dichotomy to Recognizing the True Complexity of Marine Protists

Although the famous drawings by Haeckel made the wonders of protistan diversity accessible to a wide audience, a contemporary of Haeckel, Viktor Hensen was potentially the first to raise awareness of the functional importance of protists in the ocean. Hensen coined the term "plankton" for organisms drifting in the water and unable to swim against currents. He described plankton as "without doubt of great importance for the entire metabolism of the sea" (Smetacek 1999) and he hoped to link fisheries yields to the plankton productivity that supported them. At the time his idea was ridiculed, but is now seen as the foundation of quantitative ecology and biological oceanography. Hensen's work led to phytoplankton (and especially diatoms) being considered the "pasturage of the sea"—making them the first protists to be recognized as having global importance, as they support secondary production by metazoan zooplankton, such as copepods, which in turn are fed upon by fish. The importance of photosynthetic protists has now been proven and it is estimated that ~45% of global photosynthetic CO_2 fixation is performed by planktonic marine eukaryotes and cyanobacteria (Field et al. 1998).

It took much longer for the heterotrophic protists constituting the smallest size fractions of zooplankton to gain a comparable recognition if they even have by now. The realization that the larger size classes of plankton collected in nets, such as metazoan zooplankton, contributed only a minor fraction of overall respiration in seawater, steered attention toward microbes as metabolic hubs in the ocean (Pomeroy 1974). Additionally, it was recognized that phytoplankton excrete part of their photosynthetically-fixed carbon as dissolved organic matter (DOM) and that both DOM and particulate organic matter (POM) from these taxa could also become available when they are lysed by viruses, or during "sloppy feeding" by zooplankton. In turn, this free DOM and POM provid an energy source for heterotrophic microorganisms. Iterations between and through a web of carbon exchanges were recognized as involving consumption of bacterial and archaeal cells by heterotrophic nanoflagellates and consumption of these and other cells by larger protists, such as ciliates. Collectively, recognition of this complexity resulted in conceptualization of the microbial web (Pomeroy 1974; Sherr and Sherr 1988) and the microbial loop (Azam et al. 1983), both of which called into question the traditional view that aquatic microbial food webs were primarily a three-step food chain from primary producers to zooplankton and fish (Box 4.6). Further development of the microbial loop concept captured alternative flows of carbon to both lower and higher trophic levels that were less efficient than direct transfer from primary producers. Today, diverse protists are recognized as efficient bacterivores often controlling bacterial standing stocks and influencing community composition (Pernthaler 2005). Many of these protists can also feed efficiently on other eukaryotes collectively dominating herbivory in the ocean (Sherr and Sherr 2007). It has been estimated that about two-thirds of global planktonic primary production is removed through predation by protistan microzooplankton (Steinberg and Landry 2017). Thinking back to Hensen, if photosynthetic microbes are the "pasturage of the sea" then predatory protists would by analogy represent the "cows of the sea."

An important paradigm shift in plankton ecology addressed misconceptions inherent to the assignment as either "phytoplankter" or "zooplankter" (Flynn et al. 2012) which rooted back to the plant–animal dichotomy introduced by Carl von Linné in his "system of nature" first published in 1735 (von Linné 1735). The theory of endosymbiosis suggests that life forms could exist that maintain or combine both types of nutritional strategies (photosynthesis and phagocytosis). However, the maintenance of a mixed nutritional strategy was not considered because it was thought that as soon as the plastid was acquired, photosynthetic nutrition alone would be sufficient. This view is illustrated in the symbiont theory presented by Konstantin Mereschkowsky who concluded that plants evolved from animals through invasion by cyanobacteria, and that once photosynthetic, even a lion would become a peaceful creature, thriving by photosynthesis, with no interest in prey (Mereschkowsky 1905). Alas, Mereschkowsky and others at the time had no knowledge that many photosynthetic protists are also efficient predators!

As early as the 1950s, Ernst Georg Pringsheim used the term "mixotrophy" to describe a variety of lifestyles among flagellates that used preformed organic substances next to photosynthesis (Pringsheim 1958). Pringsheim's interest in these different nutritional requirements was rooted in an ambition to grow diverse photosynthetic flagellates in culture, which he found often required supplementation with organic compounds. Although photosynthetic protists with the capacity to ingest microbial prey had already been discovered (Biecheler 1936), their importance as bacterivores in aquatic microbial food webs was only reported in the late 1980s. Sparked by a seminal paper reporting high rates of bacterivory by photosynthetic protists (Bird and Kalff 1986), many reports of feeding by mixotrophic flagellates on bacteria or other groups of phytoplankton followed for the marine environment (Stoecker et al. 2017). The term "mixoplankton" was then introduced in an attempt to better represent the diverse planktonic protists capable of both photosynthesis and phagocytosis, clearly delineating between predatory mixotrophy and mixotrophy as a term referring to use of dissolved organic compounds by phytoplankton (Flynn et al. 2019). The combination of both predation and photosynthesis in the same cell has many consequences for the functional role of these protists. For instance, mixotrophs that acquire nutrients bound in their prey and use them to support a mainly photosynthetic lifestyle invalidate the assumption that primary productivity relies largely on the direct availability of dissolved inorganic nutrients. Moreover, the direct link between consumption of prey and photosynthetic carbon fixation in the same cell results in less release and recycling of nutrients and more efficient trophic transfer, potentially supporting higher biomass of top consumers (Mitra et al. 2014; Ward and Follows 2016). Both the access of mixotrophs to alternative resources (Rothhaupt 1996a) and their interaction with their prey by predation and competition (Wilken et al. 2014) can result in stronger suppression of prey abundances and allow them to outcompete specialists in resource-poor environments (Rothhaupt 1996b).

Box 4.6

"Unseen Strands in the Food Web: The new paradigm of the ocean's food web that is developing, as a result of recent studies of protistan activities and alternative pathways of organic matter, may contain many unseen strands. We are not certain how the long-recognized food web of diatoms and copepods fits into the expanded web which is gradually appearing. Quantitatively, large diatoms seem to be minor contributors to production, and net plankton seems to be a minor component of respiration; but if this is not the major link of photosynthesis to nekton, what is? Are the communities of upwellings really more efficient producers of nekton, and is the food web really different in them? These questions are important not only to the basic ecologist but to the fisheries scientist."

From *The Ocean's Food Web, A Changing Paradigm—by Larry Pomeroy,* 1974.

Predatory mixotrophs differ in their relative reliance on photosynthesis versus (phago)-heterotrophy for their nutrition and their inherent potential to perform photosynthesis (Mitra et al. 2016; Stoecker 1998). Constitutive predatory mixotrophs engage in phagocytosis and have an inherent photosynthetic potential through possession of their own stably integrated vertically inherited plastids, while non-constitutive mixotrophs derive their photosynthetic potential from their prey via either kleptoplasty or photo-endosymbiosis (Mitra et al. 2016). These different forms of mixotrophy imply different functionalities, for instance, in the degree to which their capacity to photosynthesize depends on prey availability.

With every paradigm shift, past or recent, the interaction network among protists as well as how they interact with Bacteria, Archaea, protists, and even multicellular eukaryotes has become more complex. Elemental flow no longer follows neatly distinguishable trophic levels but can be merged or arranged in loops and of course through the various manifestations of symbiotic interactions (Fig. 4.6). There is still much to learn about the functioning of microbial networks and particularly about the quantities and routes of carbon flow (Worden et al. 2015). One consideration is the balance between photosynthetic fixation of CO_2 into biomass of living cells and its release and remineralization through the action of heterotrophic bacteria. Small

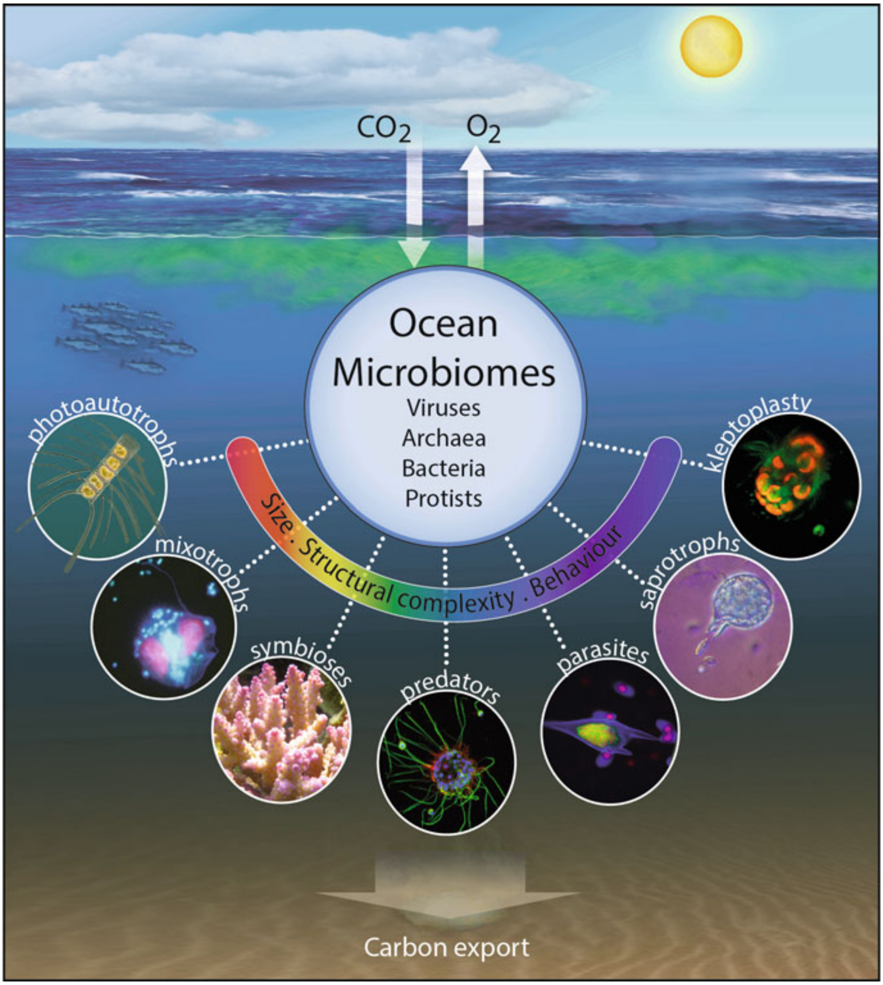

Fig. 4.6 The functional roles of protists in the ocean. Protists influence biogeochemical cycles in multiple ways, many of which involve direct interactions with other biological entities. This schematic depicts some of the major protistan roles discussed herein, with images of example organisms in white circles, as they link to the broader marine microbiome and the network of direct and indirect interactions it entails. Note that some of these categories overlap—for example, here we show a coral/dinoflagellate symbiosis, under symbiosis, but other categories can be considered symbioses, such as parasitism (see within chapter text). The image illustrating kleptoplasty is a confocal fluorescence micrograph of *Mesodinium rubrum* with cryptophyte organelles (courtesy of Matthew Johnson). Other images, as well as the overall figure, are adapted from Worden et al. (2015)

changes in this balance determine how much biomass will be moved into deep waters and potentially buried in sediments over long time scales, or released back into the atmosphere from the ocean surface waters. Although predatory protists are increasingly receiving attention, we still have much more knowledge, more cultured taxa, and even more genomic information from photosynthetic protists (Bhattacharya et al. 2014; Burki 2017; Cooney et al. 2020; Gawryluk et al. 2019; Keeling et al. 2014; Krabberød et al. 2017).

4.5.1 Pursuing Lines of Protistan Heterotrophy in the Sea

The role of heterotrophic protists as major bacterivores and herbivores in the ocean is established, but heterotrophic protists can have non-predatory modes for gaining nutrition. This includes protistan parasites that infect multicellular eukaryotes or other protists, and saprotrophs that utilize detrital organic matter often via the excretion of exoenzymes. The latter break down macromolecules into small enough units for uptake via osmotic transport, specialized transporters, or via endocytosis. The detailed characterization of these nutritional strategies traditionally relies on controlled laboratory studies of cultured protists. However, even the first molecular surveys of marine protistan communities revealed a large diversity of novel groups of small (presumably) heterotrophic protists that passed through filters of only a few micrometer pore size (Díez et al. 2001; López-García et al. 2001; Moon-van der Staay et al. 2001). Two groups that were and continue to be commonly retrieved at high sequence abundances were affiliated with the alveolates and stramenopiles, respectively, and were named accordingly MALV (Marine ALVeolates) and MAST (MArine STramenopiles). MALV include known species of Syndiniales (Guillou et al. 2008) that are obligate parasites, and using the guilt-by-association principle all MALV are commonly assumed to share this lifestyle although the possibility of more varied trophic modes has been suggested based on their paraphyly (Strassert et al. 2018). The MAST are also paraphyletic (Not et al. 2007a) and contain several independent lineages that appear to represent basal heterotrophic stramenopiles (Massana et al. 2014), some of which have been shown to be bacterivores (Lin et al. 2012; Massana et al. 2006).

While predation on other microbes and parasitism are probably the most common forms of nutrition in heterotrophic protists, novel nutritional modes are still being found. For instance, the analysis of the genome sequence of individual cells directly isolated from their natural environment suggested that some MAST lineages are photoheterotrophs. This idea arose because genes encoding microbial rhodopsins were observed, and these were hypothesized to use sunlight for photoheterotrophy, although their true cell biological role has yet to be shown (Labarre et al. 2021). Another study used single cell sequencing approaches on a few cells of the uncultured Picozoa and concluded that they may "feed" on phages (Brown et al. 2020). This evolutionarily distinct lineage was briefly in culture during which studies indicated that it lives on particulate organic matter (POM) (Seenivasan et al. 2013).

Predatory protists are perhaps the best characterized heterotrophic protists in pelagic marine environments. Their role in the carbon cycle is through the ingestion of prey via phagocytosis. Phagotrophic nutrition requires the internalization of prey through the invagination of the cell membrane and formation of a food vacuole followed by the modification of vacuolar conditions for the digestion of prey, absorption of nutrients from the vacuole into the cytoplasm, and finally egestion of any remaining material (Flannagan et al. 2012). The process of phagocytosis has been well characterized in metazoans because of its important role in immune responses. The variations occurring across the diverse protists that rely on phagocytotic processes for their nutrition are less well resolved despite the first observations of the process in the mid-twentieth century that came from the ciliate *Paramecium* (Mast 1947).

There are many different strategies and morphological adaptations within protistan lineages for finding, capturing, and ingesting their prey. Among the nano- and pico-sized flagellates that represent the majority of bacterivores in the ocean (Jürgens and Massana 2008), three main strategies are known for capturing prey in the dilute marine environment: filter feeding, direct interception feeding, and diffusion feeding. The first two rely on creating a feeding current toward the cell using undulating flagella. In the filter-feeding choanoflagellates, close relatives of metazoans, the feeding current passes through a collar of finely spaced microvilli from where the retained prey is transported to the cell surface and phagocytosed (Pettitt et al. 2002). The fine spacing of the microvilli allows capture of small food particles and also causes a strong flow resistance. How choanoflagellates create a flow strong enough to accomplish their high filtration rate remains unresolved and the presence of a flagellar vane has been postulated as a potential explanation (Nielsen et al. 2017). Direct interception feeding is common among cultured flagellates within the stramenopiles (Boenigk and Arndt 2002) and is speculated to be the feeding mode of many of the uncultured MAST groups (Labarre et al. 2021). Interception feeding requires direct contact with the prey at the protist's cell surface upon which ingestion is initiated. Since each food item is handled individually this allows selectivity based on physical or chemical properties of the prey item and preferential feeding based on size, prey species, or prey quality has been reported in many species of interception feeders (Gonzáez et al. 1993; Monger and Landry 1991). Finally, diffusion feeding predators remain motionless, waiting for prey to collide with pseudopodia extended from their cell body. This feeding strategy underlies the sun-like appearance of heliozoans and is also found among planktonic foraminifera, many of which are mixotrophs that host photosynthetic endosymbionts, and are able to capture prey ranging in size from bacteria to copepods.

Two alveolate groups have perhaps the widest array of different feeding strategies, the ciliates and dinoflagellates (Hansen and Calado 1999; Leander 2020). Both groups contain bacterivores that can dominate consumers of both heterotrophic and photosynthetic microorganisms in some marine habitats. Additionally, they contain specialized predators that are able to hunt and consume prey larger than themselves via phagocytosis. This usually requires immobilization of the prey, for which extrusive organelles are used, and appears to have evolved independently several times. The ciliate *Didinium* feeds on other ciliates at which it first shoots "toxicysts" to inject a toxin into its prey (Wessenberg and Antipa 1970). In a number of specialized dinoflagellates, extremely complex cell organelles termed nematocysts are used similarly. Nematocysts bear overall similarity to the harpoon-like cells of cnidarians with which they share their name. Nematocysts in dinoflagellates and cnidarians do not share their evolutionary origins, rather they are examples of convergent evolution over an enormous phylogenetic distance. Even colonial or multicellular prey organisms too large to be phagocytosed can be consumed by protists, for example chain-forming diatoms are fed upon by the dinoflagellate *Protoperidinium*, which extrudes a pseudopod-like structure, termed the pallium, to accomplish this (Gaines and Taylor 1984). The pallium stretches along the surface of the prey to enclose it, followed by the digestion and uptake of the cellular content, leaving only empty diatom frustules behind. Finally, large prey can also be consumed via injection of a tube-like structure to suck out prey cell contents as done by some dinoflagellates, using what is termed the peduncle.

Next to ciliates and dinoflagellates there are many less explored groups of small heterotrophic flagellates that feed upon other eukaryotes. These perform the impressive feat of "swallowing" cells that are almost their own size. In contrast to the heterotrophic flagellates identified by environmental surveys (e.g., MASTs), several predatory eukaryotrophic flagellates (i.e., feeding on eukaryotic prey) have been discovered and isolated using elaborate culturing efforts (Tikhonenkov et al. 2021). These efforts include identifying the eukaryotic prey for the eukaryotrophic predator of interest, providing this prey as well as its own source of nutrition (which in some cases can be bacteria). Predatory eukaryotrophic flagellates are rapid feeders and reproduce quickly in culture, hence they may play ecologically important roles in controlling other small flagellates (Tikhonenkov 2020). The position of eukaryotrophic flagellates in the "upper" trophic levels of the microbial food web may help to explain their relative scarcity in molecular surveys. In addition, such species often occupy regions of the eukaryotic tree that are not well resolved or are found at the "base" of large groups, which makes identification and placement of 18S rRNA gene amplicon sequences from them difficult, so that they are sometimes simply passed over in molecular diversity studies. However, from an evolutionary standpoint these small flagellates are frequently positioned as sisters to major eukaryotic groups, aiding understanding of the evolution of those groups (Tikhonenkov et al. 2020b). The complex life cycles and eukaryotrophic nutrition of novel unicellular animal-relatives can further help to understand possible features of the ancestor of animals and how the multicellularity of animals evolved (Hehenberger et al. 2017; Tikhonenkov et al. 2020a). An exciting example in the context of the origin of photosynthetic eukaryotes was the discovery of the aquatic eukaryotroph flagellate genus *Rhodelphis* which appears to be closely related to the typically photoautotrophic red algae (supergroup Archaeplastida). The discovery of a non-photosynthetic primary plastid in *Rhodelphis* combined with its predatory lifestyle suggests that the ancestor of red algae and *Rhodelphis* may have been a predatory mixotroph, raising questions about the long-standing idea that phagotrophy was lost early in the evolution of the Archaeplastida, before the divergence of the three major lineages (Fig. 4.4) (Gawryluk et al. 2019).

The large range of feeding mechanisms found among protists allows them to target different prey groups and size classes. Through this diversity in feeding strategies, predatory protists can collectively act as the main consumers of both bacteria and phytoplankton in the ocean (Calbet and Landry 2004), as well as feeding on other protists and archaea. However, because the size ranges of the prey organisms in these groups can overlap, hence many protists are in fact omnivores rather than exclusively bacterivores or herbivores. Conversely, not a single predatory protist would be able to feed on all of the diverse primary producers because they span several orders of magnitude in size. While bacterivorous protists are often treated as one functional group, the fact that they use different feeding strategies has implications for feeding preferences and strong prey size selectivity has been observed in many heterotrophic flagellates. This in turn can shape bacterial community size structure in which cells of intermediate size (~1 μm) are preferentially consumed by flagellate grazing, while both smaller and larger colony-forming cells persist (del Giorgio et al. 1996; Jürgens and Matz 2002). Prey motility is also thought to influence selectivity or at least feeding success, as motile prey presumably has both higher encounter rates with other cells, such as predators, and has the potential to escape ingestion (Harvey et al. 2013; Matz et al. 2002). Other prey characteristics influencing feeding selectivity by protists are not well understood. These include prey cell surface properties or compounds detected prior to ingestion, likely through the receptors that initiate phagocytosis by the predator (Roberts et al. 2011). Selective feeding also has technical implications for the methods to detect and quantify grazing rates. For example, some protists do not ingest the fluorescently labeled and heat-killed bacteria often used as tracers to detect and quantify consumption rates in field experiments, even if they do feed on the same bacterial strain when it is offered as live prey (Bock et al. 2021).

Parasitic protists—Parasitic protists are found across the eukaryotic tree of life and they can appear to infect a similar diversity of unicellular and multicellular hosts. Not surprisingly parasitic protists were first described in metazoan hosts. The first systematic description of parasitic life forms was by Francesco Redi who noted "animals living in animals" in 1648, and pointed to gregarines, which are now classified as apicomplexans (supergroup Alveolata). The Apicomplexa are a large phylogenetic group with many marine members and appear to consist predominantly of parasites, some of which reside in the intestines or coelom of invertebrates (Leander 2008).

The Alveolata contain other parasites, such as the perkinsids, a sister group to dinoflagellates, which includes parasites of other protists as well as bivalves. The basal dinoflagellate group syndiniales, also referred to as Marine ALVeolates (MALVs), harbors examples of obligate parasites. However, because most MALVs have not been cultured, the idea that they are parasitic comes from field studies (Chambouvet et al. 2008) and by analogy to their few described members (John et al. 2019). Syndiniales are particularly abundant in 18S rRNA PCR-based studies, including amplicon studies from marine ecosystems when sequencing the nano- and picoplankton size fraction (Guillou et al. 2008; Massana et al. 2004; Moon-van der Staay et al. 2001). Although syndiniales contain parasites of plankton ranging from protists to copepods, the host ranges of specific lineages generally remain unknown. Both syndinian and perkinsid parasites can infect dinoflagellates that cause harmful algal blooms and these parasites have also been implicated in rapid dinoflagellate species succession during blooms and even in being responsible for bloom termination (Chambouvet et al. 2008; Jephcott et al. 2016).

Other protists infect phytoplankton hosts and potentially play a role in controlling bloom formation or initiating bloom termination. Some members of the oomycetes, a stramenopile group once thought to be fungi, parasitize marine diatoms (Hanic et al. 2009). The chytrid fungi are important parasites of freshwater phytoplankton (Ibelings et al. 2004) and some chytrids infect marine diatoms (Gutiérrez et al. 2016) as do the novel chytrid-like-clade-1 (NCLC1) (Chambouvet et al. 2019). An awareness raised by these discoveries was that likely $<10\%$ of fungi species on the planet have been described (Blackwell 2011; Hawksworth and Lücking 2017; Jones 2011). Moreover, the ecological impact of those fungi infecting diatoms remains underexplored, although infections have been reported in upwelling regions where diatom blooms occur, suggesting a potentially important alteration of classical expectations of carbon cycling and food web dynamics in these regions. Finally, in the Arctic chytrid abundances reportedly correlate with sea-ice associated diatoms and predominantly occur in areas with ice melt, leading to questions about how parasite infection networks might change with future ice retreat (Kilias et al. 2020).

Osmotrophic and saprotrophic protists—Nearly all protists show some form of osmotrophy, which describes nutrition through direct uptake of dissolved organic substrates from the environment (Richards and Talbot 2018). In contrast to what the term might imply, this is not a passive process but rather involves specialized transport systems to facilitate active uptake of substrates. Lysotrophy (also called chemoheterotrophy) is a common form of osmotrophy that involves secretion of enzymes into the extracellular environment to break down larger substrates such as polymers (e.g., cellulose, lignin, lipids, and proteins) into their building blocks (e.g., sugars, fatty acids, amino acids) so that they can be taken up (Richards et al. 2006). When this mode of nutrition is used to utilize detrital organic matter, it is also referred to as saprotrophy. Extracellular digestion via lysotrophy can also be used to feed on living organisms in which case it would be considered parasitism.

The oomycetes, hyphochytriomycetes, and labyrinthulomycetes exhibit variations in their manner of osmotrophy (Amend et al. 2019; Cavalier-Smith 2018; Raghukumar 2002). Once thought to be fungi these lineages belong to the stramenopiles, unlike fungi which belong to the opisthokonts. Labyrinthulomycetes, which include both labyrinthulids and thraustochytrids, have been observed residing alongside fungi on marine snow (Bochdansky et al. 2017) and in marine sediments (Rodríguez-Martínez et al. 2020), raising the possibility that they utilize different enzymes than fungi, and thus different forms of organic matter which would result in effective niche partitioning. Environmental sequences of basal oomycetes are frequently detected in marine environments but generally cannot yet be associated with known species (Thines 2018). Additionally, ichthyosporeans, which are relatives of metazoans, have evolved osmotrophic nutrition independently of fungi and are found in the digestive tracts of some marine invertebrates (de Mendoza et al. 2015). While saprotrophic protists are present in marine habitats ranging from the surface layers to the sediments, there is still much to learn about their specific enzymes, activities, and substrate preferences.

Although it is known that different forms of osmotrophic nutrition are represented among protistan marine fungi, the ecological impacts of marine saprotrophic fungi are less well understood than of those that are parasitic (Grossart et al. 2019; Richards et al. 2012). Fungi isolated from seaweed have been demonstrated to degrade plant and algal biomass (Patyshakuliyeva et al. 2019); marine fungi also utilize phytoplankton derived polysaccharides (Chrismas and Cunliffe 2020; Cunliffe et al. 2017) and can be associated with phytoplankton blooms (Priest et al. 2021). Some marine fungi reside on aggregates of organic and detrital material referred to as marine snow (Bochdansky et al. 2017). Moreover, Arctic fungi have been reported to carry genes for degrading refractory compounds such as lignin and naphthalene alongside genes for nitrate assimilation. Active fungi have been detected in marine sediments, based on presence of ribosomal RNA (Rodríguez-Martínez et al. 2020), and can grow on zooplankton fecal pellets, a common carbon source in sediments (Hassett et al. 2019), suggesting they contribute to the degradation of organic matter in these deep-sea habitats. However, active diatoms (based on ribosomal RNA) have also been reported in sediments below 1000 m of overlying waters. Hence, it is important to tease apart sequence data coming from the resident community versus that coming from recently deposited surface water cells (Rodríguez-Martínez et al. 2020). Apart from their ecological roles, there is considerable interest in marine fungi for possible medical and industrial use of their enzymes. Marine fungi generate compounds that have been reported to have antibiotic and anticancer properties (Deshmukh et al. 2017). Additionally, their extracts can break down the cell wall of skin bacterial pathogens (Agrawal et al. 2020) and can degrade crude oil (Maamar et al. 2020).

Multiple lineages branching near the base of the fungal portion of the tree have been discovered in the last decade, including taxa grouped into the endoparasitic Opisthosporidia (Karpov et al. 2014), which contains the NCLC1 mentioned above that infect marine diatoms (Chambouvet et al. 2019). The Opisthosporidia as a whole relate to fungi in a manner similar to how choanoflagellates relate to animals (Brunet and King 2017). Another Opisthosporidia lineage detected in marine waters is the Cryptomycota, which includes the parasitic genus *Rozella* (Livermore and Mattes 2013; Richards et al. 2015). These and other discoveries have led to considerable restructuring of the fungal portion of the eukaryotic tree and the number of recognized fungal phyla has tripled over the last 20 years (James et al. 2020).

4.5.2 Non-constitutive Mixotrophy (Via Photosynthetic Endosymbionts and Kleptoplasty)

The British naturalist and poet Henry Baker was lucky that a friend sent him a specimen that seems to be the first recognized dinoflagellate (Box 4.7). The animalcule described in his friend's letter is now known as *Noctiluca*, a globally distributed marine dinoflagellate. It attracted the attention of Baker and others because of its bright bioluminescent blooms, earning it the common name "sea sparkle." Unusually big for a dinoflagellate (up to 2 mm), these "bladder"-like cells can host large populations of free-swimming endosymbionts that are green algae. This form of *Noctiluca* is called "green" *Noctiluca* in contrast to the "red" form that does not harbor endosymbionts (both types can form massive blooms). The photosynthetic endosymbionts contribute to growth, yet the green *Noctiluca* is still a voracious predator of other microbial eukaryotes including some species of their own dinoflagellate sisters (do Rosário Gomes et al. 2018). The green form of *Noctiluca* thus combines phototrophic and heterotrophic strategies to grow, representing a mixotrophic lifestyle. Because *Noctiluca* does not inherently possess the ability to fix carbon but needs to acquire that ability by engulfing and hosting photosynthetic symbionts, it has been termed a "non-constitutive" mixotroph. This strategy has resulted in blooms of the green mixotrophic *Noctiluca* over enormous expanses and appears to profit from changing oceanic conditions resulting from warming and anthropogenic inputs (do Rosário Gomes et al. 2014).

Box 4.7

"In the Glass of Sea Water I send with this are some of the Animalcules which cause the Sparkling Light in Sea Water; they may be seen by holding the Phial up against the Light, resembling very small Bladders or Air Bubbles..."

From *a letter from Mr. Joseph Sparshall to Henry Baker in "Employment for the microscope" (Henry Baker, 1753, Dodsley, London).*

A conceptual question arises in trying to place dinoflagellates in terms of functional classification. About half of the known dinoflagellates are photosynthetic and the other half lead a heterotrophic life. Those that are photosynthetic have plastids of a hodgepodge of various algal origins and "levels" of endosymbioses (Waller and Kořený 2017). As a whole, dinoflagellates display probably the most peculiar and complex plastid evolution among all plastid-bearing groups. Several lineages have either completely lost their ancestral plastid of red algal origin or have just lost the ability to perform photosynthesis but still harbor a more or less stably integrated plastid from a different alga. They are seemingly not too selective about which algal group they host as endosymbionts. Plastids from almost every other photosynthetic lineage have been identified in independent dinoflagellate lineages. This impressive flexibility of dinoflagellates with respect to losing or gaining a plastid from a variety of sources has likely played a key role in their niche expansion across aquatic environments. It also means that dinoflagellate lineages are distributed throughout the different functional modes we describe herein.

The relationship between non-constitutive mixotrophs and their endosymbionts can be complex, involving adaptations on several levels from both partners. A particularly captivating example is the interaction observed between the foraminiferan genera *Orbulina* and *Globigerinoides* and their dinoflagellate endosymbionts. Some foraminifera and several groups of radiolarians (both supergroup Rhizaria) can form easily-discernible associations with photosynthetic protists that were observed during the nineteenth century Challenger- Expedition (Tizard et al. 1885). Both foraminifera and radiolarians build intricate mineral skeletons composed of long spines and possess highly dynamic cytoplasmic strands that they can extend outside of their shells. In symbiotic foraminifera, the dinoflagellate endosymbionts dwell within vacuoles that are in turn attached to their network of cytoplasmic strands. The interaction between these two partners is characterized by a compelling diurnal pattern: at dawn the dinoflagellate symbionts move along the spines of the host to reside outside its shell during the day, at dusk they return into the inner cytoplasm inside the shell (Roger Anderson and Be 1976). Foraminifera and radiolarians are increasingly recognized as important players in open ocean communities due to their predatory activity. The mixotrophic (symbiotic) lineages also contribute to primary productivity and their large cells house up to several thousand photosynthesizing symbionts (Decelle et al. 2015).

One of the more peculiar ways to gain access to photosynthate occurs in non-constitutive mixotrophs that perform kleptoplasty or the “stealing of plastids.” Specifically, kleptoplasty involves the sequestration and retention of the plastid of an algal prey (plus sometimes other useful bits of the prey cell) while the rest is digested. Unlike the non-constitutive mixotrophy based on endosymbiosis, the road for the “stolen” alga, providing the photosynthetic ability, ends with uptake by the “host” cell. This behavior is found in a range of protists (and even animals). However, it is often challenging to distinguish lineages with kleptoplasts from those containing partially digested algae in their food vacuoles, unless observations are made for an extended period in culture.

The best described protistan examples of kleptoplasty are found in ciliates and dinoflagellates. *Mesodinium rubrum* is a globally distributed marine ciliate and represents one of the most common and abundant protists engaging in kleptoplasty. It can dominate ciliate biomass in the plankton and, during blooms, can dominate primary production, see Stoecker et al. (2017). This ciliate has evolved an elaborate scheme to make the best use of its stolen plastids, sequestering not just the photosynthetic organelles, but also the mitochondria and nuclei from its cryptophyte prey. Microscopy observations have revealed that the nuclei and plastids (together with the mitochondria) are packaged into two separate complexes surrounded by membranes upon ingestion. The ciliates and the stolen plastids divide as long as the prey nuclei are present. This indicates that the stolen nuclei still function in maintaining the plastids from the prey. Because the stolen prey nuclei begin to disappear before plastid numbers begin to decline, *Mesodinium* must recurrently steal cryptophyte nuclei (Johnson et al. 2007).

Unfortunately, the intricate strategy of *Mesodinium* to gain the benefits of photosynthesis has attracted a follower, the dinoflagellate *Dinophysis*, which appears to exploit the work done by the ciliate. *Dinophysis* has kleptoplasts of cryptophyte origin, but culturing efforts using a variety of prey, including cryptophytes, have not succeeded. Only when offered *Mesodinium* as a prey item did the dinoflagellate begins to grow and it was then shown that *Dinophysis* uses its peduncle to extract the cell contents of the ciliate, including the organelles it has stolen previously from its cryptophyte prey (Park et al. 2006), of which it only retains the plastids. It also needs to constantly reacquire these plastids, for which it has evolved the capacity to detect its prey via chemoreception. It appears to approach *Mesodinium* at low speed, which nevertheless can evade capture through escape jumps, to which *Dinophysis* responds by releasing mucus and/or using capture filaments to slow down and eventually immobilize the ciliate. The release of toxins is suspected to play a role as well (Jiang et al. 2018; Mafra et al. 2016).

Unlike *Dinophysis*, an abundant Antarctic dinoflagellate, the Ross Sea Dinoflagellate (RSD), can maintain its kleptoplasts for at least 30 months when starving (Sellers et al. 2014). The observed retention time of the kleptoplasts, which are stolen from the haptophyte *Phaeocystis antarctica*, is longer than in any other kleptoplastic systems currently known and suggests a tight integration of the kleptoplasts within the "host," putatively on the way to becoming fully integrated and stable plastids. Transcriptomic analyses of this singular relationship have revealed that RSD seems to maintain and employ kleptoplasts for photosynthetic functions as well as harbor and use the original secondary plastid found in "standard" photosynthetic dinoflagellates for plastidial metabolic pathways (Hehenberger et al. 2019). Kleptoplasty is not unique to protists since it has also been observed in sacoglossan sea slugs, which retain the plastid of their algal food (Händeler et al. 2009), and in two species of marine flatworms (Van Steenkiste et al. 2019).

4.5.3 Constitutive Mixotrophy

Because constitutive mixotrophs are defined as possessing an inherent capability to photosynthesize, they align with the description as being microalgae that feed on other microbes. This type of mixotrophy is found in the stramenopile groups chrysophytes and dictyochophytes, as well as dinoflagellates, cryptophytes, and haptophytes (Choi et al. 2020). Because the absence of a phagocytotic potential is difficult to prove (Wilken et al. 2019), the number of microalgae that were traditionally considered as purely photosynthetic but later found to also ingest prey has increased. An example are reports of feeding by coccolithophores (Avrahami and Frada 2020), although other mixotrophic members of the haptophytes had been known for many years (Frias-Lopez et al. 2009; Hansen and Hjorth 2002).

Much of the knowledge on the physiology of specific groups of constitutive mixotrophs comes from controlled laboratory experiments with cultured representatives. These data have been used to construct conceptual models of mixotrophy based on the relative importance of photosynthesis and phagotrophy and the environmental trigger that induces feeding (Jones 2000; Stoecker 1998). While a balanced contribution of both photosynthesis and phagotrophy to the overall nutrition seems to be rare, most constitutive mixotrophs are currently lumped into the category of being primarily photosynthetic. The haptophyte genus *Chrysochromulina*, the chrysophyte *Dinobryon*, many dinoflagellates, and probably most cryptophytes are considered capable of purely, or at least dominantly, photosynthetic growth. Current experimental work indicates that these taxa ingest prey when light for photosynthesis is inadequate, or when dissolved inorganic nutrients and other growth factors are limiting (Hansen 2011; Hansen and Hjorth 2002). However, some constitutive mixotrophs show a stronger reliance on heterotrophy, as is the case for several chrysophytes. Unfortunately, evolutionary relatedness does not align well with differences in physiological strategies of mixotrophs, and closely related strains can show divergent ecophysiologies differing in both resource requirements and responses to environmental conditions (Moeller et al. 2019; Wilken et al. 2020). This makes inferences of functional roles in nature difficult. Further, most species available in culture represent coastal rather than oceanic taxa, and it is unclear how the differences in nutrient availability in the latter might influence mixotrophic adaptations.

Constitutive mixotrophs, also known as “phagotrophic phytoflagellates”, have been studied in natural communities through amendments with surrogate prey that have been fluorescently or radioactively labeled. This allows quantification of ingestion rates by heterotrophic versus pigmented flagellates, and the resulting studies have confirmed the important contribution of predatory mixotrophs to overall bacterivory in many marine habitats, especially the open ocean. However, these approaches often do not allow the taxonomic groups responsible for this predation to be distinguished, especially if morphological differences cannot be observed by fluorescence microscopy. Constitutive mixotrophs are often the main bacterivores in oligotrophic ecosystems as shown in the Atlantic subtropical gyres (Hartmann et al. 2012) and the Mediterranean Sea (Unrein et al. 2007), where fluorescence in-situ hybridization (FISH) approaches have suggested the quantitative importance of haptophytes to consumption, alongside dinoflagellates and chrysophytes (Hartmann et al. 2013; Unrein et al. 2014). Another important group in the open ocean are the dictyochophytes (see below). While the relative importance of constitutive mixotrophs in oligotrophic waters seems intuitive due to the benefit of feeding as a route of nutrient acquisition, there are also many examples of constitutive mixotrophs at high abundance in more eutrophic and coastal waters. In fact, many harmful algal bloom (HAB) species especially those belonging to dinoflagellates, haptophytes, and raphidophytes are mixotrophs (Flynn et al. 2018). Although many HAB species are intensively monitored, reports of their feeding behavior mainly come from experiments with cultured isolates and the role of mixotrophy in bloom formation is not well understood (Burkholder et al. 2008). Below, two important marine groups are discussed that are particularly complicated to categorize functionally, as described for dinoflagellates above.

Dictyochophytes are planktonic stramenopiles about which there is still much to be learned. The dictyochophytes display a variety of lifestyles ranging from planktonic photoautotrophs to mixotrophs (as shown for *Florenciella*) and bacterivores. With fossil records dating back to the Cretaceous period 145 to 66 million years ago (Preisig 1994), large scale molecular surveys have now revealed that dictyochophytes are abundant and diverse in the ocean, with most clades lacking any cultured representatives (Carradec et al. 2018; Choi et al. 2020; de Vargas et al. 2015). Because their pigments overlap with those of diatoms, pigment-based analyses thus far have incorporated dictyochophyte contributions as being from diatoms. Studies in the North Pacific subtropical gyre have shown that mixotrophic dictyochophytes graze on picocyanobacteria (Frias-Lopez et al. 2009). Furthermore, ecophysiological characterization of a dictyochophyte isolated from the same region, *Florenciella*, demonstrated increased prey ingestion rates under nutrient limitation (Li et al. 2021). Mixotrophic nutrition might thus explain the success of diverse dictyochophytes in the oligotrophic surface layer of strongly stratified subtropical oceans, as detected in a survey based on amplicon sequencing of the plastid 16S rRNA gene and single cell sorting of field samples using a flow cytometer (Choi et al. 2020).

Dictyochophytes can have a siliceous skeleton during one phase of their life cycle, leading to the entire lineage often being referred to as silicoflagellates, and spines on these skeletons are thought to reduce sinking rates (Han et al. 2019; Preisig 1994). Yet, so far, it is estimated that the silica skeletons of dictyochophytes make up a minor fraction (ca. 1–2%) of the siliceous component of marine sediments, indicating that they may be less abundant than diatoms, less prone to sinking, or are more actively degraded and utilized in the water column by other organisms. Most cultured representatives appear to propel themselves forward using their flagellum and some dictyochophytes (e.g., *Pseudochattonella*) can produce potent ichthyotoxins that detrimentally impact economically important fish species such as Atlantic salmon (Eckford-Soper and Daugbjerg 2016). A nuclear genome sequence for dictyochophytes is still lacking making it difficult to elucidate more of their cell biology, but complete plastid genomes of four cultured species and one uncultured dictyochophyte have been sequenced and analyzed (Choi et al. 2020; Han et al. 2019).

Chrysophytes are also difficult to assign to any one functional category. This diverse group of stramenopiles has more than 1000 described species including some marine representatives (Kristiansen and Škaloud 2017). Molecular surveys of plankton diversity have revealed the presence of novel clades of marine chrysophytes in particular in picoplanktonic cells with no cultured representatives (Choi et al. 2020; del Campo and Massana 2011; Seeleuthner et al. 2018). Chrysophytes contain both purely photosynthetic, mixotrophic and heterotrophic species, and among the photosynthetic chrysophytes that have been cultured, it seems that some become heterotrophic in the absence of light (Wilken et al. 2020). They typically live as solitary cells that are free-swimming but there are also filamentous and colonial forms that can grow as branched or unbranched chains. The cell surface of some chrysophytes is covered by silica scales and chrysophytes also produce siliceous resting cysts that accumulate in sediments. These cysts are abundant in deposits from the Paleocene (66–56 million years ago) while the oldest are from the Cretaceous (~145–66 million years ago).

4.5.4 Diversity and Importance of Photosynthetic Protists

Throughout their evolutionary history photosynthetic protists have been an important part of life and modifications of terrestrial ecosystems that facilitated the rise of animals. Through their photosynthetic activity following the rise of cyanobacteria, gaseous oxygen released has gradually changed the Earth's atmosphere and redox status to create the world as we know it (Lyons et al. 2014). As outlined above, today, marine phytoplankton contribute ~ half of annual global carbon fixation into organic carbon compounds, providing the basis for the marine food web and maintaining the oxygenated atmosphere and current CO_2 drawdown (Field et al. 1998). Although once thought to use only inorganic compounds, it is now widely accepted that most phytoplankton also use organic compounds. Based on the above sections, we see that the trophic modes of some photosynthetic lineages are complex because they can consume other cells (predatory mixotrophs). Photosynthetic taxa can also live in symbiosis with animals. A prominent example is *Symbiodinium*, a genus of dinoflagellates which is found in association with corals worldwide and with other marine animals, such as sea anemones (Baker 2003; Dixon et al. 2013; Liu et al. 2018; Pontasch et al. 2014), and with the calcifying ciliate *Tiarina* in open ocean waters (Mordret et al. 2016).

Photosynthetic protists are diverse in their size range, spanning three orders of magnitude from picoplankton to mesoplankton, and have representatives in almost all branches of the eukaryotic tree (Fig. 4.5). Historically, the diversity of photosynthetic protists was determined using microscope-based methods and morphological features. Applying such methods about 5000 species have been described (Sournia et al. 1991). Yet, this number largely underestimates the true biodiversity of photosynthetic protists because molecular surveys have revealed undescribed diversity including lineages for which we currently have only environmental sequence data and no cultured representatives, e.g., Massana and Pedrós-Alió (2008). It should be noted that molecular markers like 18S rRNA gene sequences can still underestimate diversity, with amplicon sequencing of some variable regions doing so even more (Monier et al. 2016), and also more generally for organisms with large population sizes and fast turnover rates, such as the prasinophyte algae (Leray and Knowlton 2016; Piganeau et al. 2011). Methods for studying photosynthetic protist communities have employed microscope-based morphological analysis, measurements of photosynthetic pigment signatures, flow cytometric cell counting and cell sorting, and molecular surveys, including species- or group-specific quantitative PCR and FISH, all of which added to our understanding of general ecological patterns (Karlusich et al. 2020).

Green algae were among the earliest eukaryotic algae in the ocean and are the product of the primary endosymbiosis event (Fig. 4.4). Prasinophytes are broadly distributed in the modern ocean and have been observed in the geological record

(Brocke et al. 2006), although their record is considered weaker than for some other algal groups with more robust cell structures. These unicellular green algae are also proposed to bear resemblance to the ancestral alga that gave rise to land plants (Lewis and McCourt 2004; Worden et al. 2015). The model green alga *Chlamydomonas reinhardtii* belongs to the Chlorophyceae or chlorophytes, alongside several other groups. The phylogenetic relationships and taxonomic levels of these classes and orders are under constant revision in part because the prasinophytes are clearly paraphyletic. It will likely take comprehensive phylogenomic analyses with even sampling of the different lineages to resolve this branch of the Archaeplastida tree.

Despite their ecological and evolutionary importance phylogenetic relationships within the prasinophytes are poorly resolved and information on physiological, morphological, and cellular characteristics are lacking for most so-called species (Duanmu et al. 2014; Marin and Melkonian 2010). The exception is the Mamiellophyceae class, for which a considerable body of literature exists for three genera that belong to the picoplankton size class, and therefore often termed "picoprasinophytes". Isolates of *Bathycocccus*, *Micromonas*, and *Ostreococcus* also have particularly small genomes (13–22 Mb) for eukaryotic cells and *Bathycocccus* and *Ostreococcus* especially seem to have engaged in an intriguing evolutionary process to reduce genome size, while *Micromonas* seems to have simply not expanded protein families as extensively as larger and multicellular archaeplastids (Moreau et al. 2012; Worden et al. 2009). The diminutive cell size of the Mamiellophyceae taxa renders a low ratio of cell surface area to volume, which provides a competitive uptake advantage relative to other eukaryotic phytoplankton in open ocean areas where nutrients can be scarce. Use of qPCR was instrumental in demonstrating that there are different *Ostreococcus* clades that rarely co-occur in nature, although initially they had been proposed to co-reside by partitioning the water column vertically, based on growth versus irradiance experiments on isolates in the laboratory. It now appears that one of the clades is better adapted to nutrient-rich "mesotrophic" conditions while the other is found in more nutrient poor "oligotrophic" environments (Demir-Hilton et al. 2011). Moreover, both clades are found in surface and deeper waters in their respective environments. A similar trend has been observed for two *Bathycoccus* clades using qPCR, with the difference that co-occurrence of both types is more common and they genetically less diverged than the *Ostreococcus* clades (Limardo et al. 2017; Simmons et al. 2016). Apart from the Mamiellophyceae, there are multiple other clades of prasinophytes, some with few cultured representatives (Tragin et al. 2016). Cell sizes within these clades appear to range from 2 to 20 μm. Prasinophytes as a whole abound in a wide range of marine habitats, serving not only as important primary producers but also as food for the predatory protists and in turn contribute to the food web of marine fauna (Bock et al. 2021; Tragin and Vaulot 2018; Worden et al. 2004). In addition, several Mamiellophyceae have recently been observed at high relative abundances in the North Atlantic spring bloom, which traditionally had been considered as diatom dominated (Bolaños et al. 2020).

While comprehensive quantitative maps of prasinophyte distributions are still lacking, changes have already been detected in connection to climate change. For example, in the Canadian Arctic, *Micromonas* has been increasing while larger algae such as diatoms are declining (Li et al. 2009; Worden et al. 2015). In addition, infection of prasinophytes by viruses (see below) is among the earliest known examples of viruses with marine algal hosts (Mayer and Taylor 1979). Finally, there is direct evidence for sexual reproduction in both *Nephroselmis olivacea* and *C. reinhardtii* (Goodenough et al. 2007; Suda et al. 1989), however, otherwise any evidence has largely been indirect. For example, the Mamiellophyceae genomes contain sex-related and meiotic genes (Worden et al. 2009) and comparative genome analyses indicate that sexual reproduction occurs in nature (Grimsley et al. 2010) but with a high prevalence of asexual division. For example, in *Ostreococcus* a minimum of 1 meiosis has been estimated for every 100,000 mitoses (Blanc-mathieu et al. 2017).

Green algae are sometimes found in close association with other protists. Some prasinophytes are observed in photosymbiosis with ciliates (Stoecker et al. 1988), but so far most symbiotic green algae are related to the “core chlorophytes” lineages, which are more common to freshwater environments. For example, the symbiont of the “green” *Noctiluca* is related to the class Pedinophyceae (Sweeney 1976; Wang et al. 2016) and members of the same class have been observed in association with radiolarians (Cachon and Caram 1979), although the latter has not been confirmed with molecular methods. Finally, although symbiotic green algae are found in a number of benthic foraminifers (Hallock 1999), in the iconic relationship with the flatworm *Symsagittifera roscoffensis* (Parke and Manton 1967), and in terrestrial lichens that cover a significant surface of land, they appear to participate in symbioses relatively infrequently in the marine water column.

Diatoms are one of the best studied photosynthetic protistan groups and belong to the stramenopiles. Diatoms are widespread in the plankton and benthos of marine and freshwater habitats occurring as solitary cells or chains of cells that are linked by hollow silica tubes (setae), mucilage, or chitin filaments. Fossil records and molecular phylogenetic analyses have been used to establish that the centric diatoms are the most ancient among diatoms, appearing ~150 million years ago (Cermeño 2016). Diatoms endured the Cretaceous-Tertiary mass extinction event 66 million years ago and thereafter their diversity increased (Benoiston et al. 2017). Their extraordinarily modern-day diversity comprises an estimated 100,000 species (Malviya et al. 2016) and, due to the many chain-forming species and species with large size (>100 μm), some were well represented in the early sampling campaigns of oceanographers like Viktor Hensen. Thus, the ecological relevance of diatoms was recognized early on and today their importance for fisheries and marine food chains is well established.

Diatoms have a diplontic life cycle that is often characterized by long periods (up to years) during which diploid cells divide mitotically alternated with brief periods (days) of sexual reproduction. They have an intricate silica cell wall, which is called a frustule and consists of two halves (called thecae) that overlap like a Petri dish (Hildebrand and Lerch 2015; Karlusich et al. 2020). It is now known that diatoms take up dissolved silicic acid and concentrate it in the cytoplasm within silica deposition vesicles (SDVs) near the plasma membrane (Heintze et al. 2020). Frustules may have a defensive role (Pančić et al. 2019) and are thought to impose a limitation that prevents diatoms from phagocytosing other cells.

Planktonic diatoms are important bloom-formers in nutrient-rich regions, such as coastal regions. They are well adapted to growth in mixed turbulent water where cells are shortly exposed to light and pulsed availability of nutrients because they can use their large central vacuole for nutrient storage. Coastal planktonic diatoms contribute importantly to total long-term organic carbon sequestration because considerable parts of coastal blooms sink rapidly (Armbrust 2009). They can also have "destructive" food web roles for example some species produce domoic acid, which is a neurotoxin that accumulates in higher trophic levels (Brunson et al. 2018). Finally, some genera including *Fragilariopsis* and *Pseudo-nitzschia* contain both benthic and planktonic species. The existence of versatility between benthic and planktonic lifestyles suggests that traits acquired while living in the benthos can also be beneficial during a planktonic lifestyle. Diatoms that have both benthic and planktonic lifestyles have been termed tychoplankton (Cahoon 2016).

Pelagophytes described to date are all marine. Two genera that have been successfully isolated are *Pelagomonas* and *Pelagococcus* which occur in the open ocean as well as transition zones beyond the truly coastal environment (Choi et al. 2020; Dupont et al. 2015; Worden et al. 2012). Their abundances suggest considerable contributions, and in the open ocean these are particularly important in the deep chlorophyll maximum (DCM) (Choi et al. 2020). The size of pelagophytes can range from about 3 to 5 μm, such as *Pelagomonas* and *Aureococcus*, to macroscopic sheets and flowing colonies up to 5 cm long (Schaffelke et al. 2004). Distinct morphological features within the pelagophytes are not known as there are multiple environmental clades that lack cultured representatives. An extracellular perforated theca has been proposed as a common feature of pelagophytes (Wetherbee et al. 2020). From an evolutionary perspective, the genome sequences of two pelagophytes (Grigoriev et al. 2021) provided new insights into how they differentiate from diatoms and to understanding of brown tide species, since one of the sequenced pelagophytes was *Aureococcus anophagefferens* a HAB that causes economic damage (Gobler et al. 2011). In the context of symbiosis, the dinoflagellate *Amphisolenia bidentata* hosts cyanobacteria and an undescribed pelagophyte species closely related to *Pelagomonas calceolata*, in an uncommon triumvirate association (Daugbjerg et al. 2013).

Haptophytes are also referred to as prymnesiophytes and have garnered much attention due to the coccolithophores, which form intricate calcified scales (coccoliths) that cover the cell. Coccoliths are built within Golgi vesicles prior to exocytosis on the cell surface and are major component of global biogenic calcium carbonate production (Billard and Inouye 2004). The unique light diffraction of coccoliths allows recognition of the presence of coccolithophores in satellite data. For example, annual blooms of the coccolithophore *E. huxleyi* have been observed in temperate North Atlantic waters near the UK. These blooms are thought to be supported by a high affinity for inorganic nutrients and mechanisms to maintain growth under high light that have been reported in *E. huxleyi* (Paasche 2001; Read et al. 2013). However, there are many other haptophyte groups, including many uncultured clades, that do not have coccoliths and these comprise a major fraction of global primary producer communities in several biogeochemical ocean provinces (Cuvelier et al. 2010). A characteristic feature of haptophytes as a whole is the haptonema (from Greek *hapis* touch and *nema* thread), which is similar to a flagellum but shows a different ultrastructural arrangement of microtubules and is used for swimming, surface attachment, or, in some lineages, for capturing prey (Kawachi et al. 1991).

The origin and evolutionary affiliation of haptophytes are contentious. Based on their plastids surrounded by four membranes, containing the chlorophylls a and c, various carotenoids, and the presence of the carbohydrate storage product beta-1,3-linked glucan, they were initially grouped with the stramenopiles (Cavalier-Smith 1981). Newer analyses place the haptophytes with a newly discovered deep-branching lineage, the rappemonads (Kim et al. 2011b), and the centrohelids, in a group termed the haptista (Burki et al. 2016) and new hypotheses have been developed for origins of their plastids (Dorrell et al. 2017). The earliest records of coccolith fossils correspond to the origin of calcifying haptophytes at ca. 220 mya. Coccolithophores have been used to calibrate molecular clock analyses and bio-stratigraphic dating since they are abundant microfossils in sediments. Haptophytes are estimated to have diverged around the onset of the Cryogenian “snowball Earth” (1031–637 mya) and extant haptophyte lineages diverged about 543 mya, early in the Cambrian period (Liu et al. 2010). This period was characterized by rapid and widespread diversification of life, however extant coccolithophores likely diversified from just a few lineages that survived the major extinction event at the Cretaceous-Tertiary boundary. In contrast, non-calcifying haptophytes were less affected by this extinction event (Medlin et al. 2008), possibly due to their ability to switch from phototrophy to mixotrophy.

Most haptophytes are non-calcifying, and many of these are predatory mixotrophs unlike the coccolithophores which generally lack this capacity (Anderson et al. 2018; Frias-Lopez et al. 2009; Kamennaya et al. 2018). In general, the mechanism of phagocytosis in mixotrophic haptophytes is not well known because cultures are lacking for the important marine lineages (Cuvelier et al. 2010; Frias-Lopez et al. 2009). Field studies indicate that uncultured pico- and nano-planktonic haptophytes are exceptionally diverse and they contribute considerably to primary production in the open ocean (Liu et al. 2009). Among cultured non-calcifying haptophytes that are environmentally important is the colony-forming genus *Phaeocystis*, which is found from the poles to the tropics. It forms dense blooms that are considered detrimental to growth and reproduction of zooplankton and shellfish (Schoemann et al. 2005). *Phaeocystis* has also been reported to live in close association with radiolarian hosts, in which the alga has “super-developed” plastids (see Fig. 4.2f) as compared to its free-living counterpart. Finally, in terms of the life cycle, most haptophytes are characterized by haploid and diploid stages that may occupy distinct ecological niches (Nöel et al. 2004). Both life cycle stages can grow independently by asexual division and can have distinct scale morphologies during each stage.

4.6 Distribution and Vertical Dimension of Protistan Diversity and Ecology: From the Sea Surface to Sediments

Protists are involved in major biogeochemical reactions and acclimate to environmental changes. As discussed throughout the above sections, many protists contribute to photosynthesis, generating organic matter in the photic zone (the sunlit portion of the water column) that fuels the marine food webs throughout the water column (Azam 1998; Ducklow et al. 2001; Gooday et al. 2020; Worden et al. 2015). Those with mineral structures often sink rapidly, bringing labile organic carbon to the deep ocean and sometimes accumulating in large deposits that are observed in the geological record. Thus, by sinking, other physical transport mechanisms, or by trophic interactions with other microbes and viruses that cause aggregation, or with multicellular zooplankton, protists contribute importantly to the biological carbon pump. The biological carbon pump refers to carbon dioxide that is removed from the atmosphere, fixed into organic material, and exported to the deep ocean where it is buried for millennia. Based on a back of the envelope calculation done in 2012, it has been estimated that if phytoplankton would stop its activities, the concentration of carbon dioxide in the atmosphere would rise by another 200 ppm and further accelerate global warming (Falkowski 2012). Photic zone processes, whether it be the fueling of higher trophic levels or contributions to the sequestration and burial of carbon in the deep sea, are intimately connected with ocean physics. Ocean physics from the small to large scale influences cell movement and metabolism, aggregation, dispersal, and many more aspects of ecology and biogeochemistry.

Vertical gradients in the ocean reflect the “dominance” of various microbial metabolisms, which shift dramatically from the surface to the dark ocean and into the ocean floor. Collectively, the resident microbes including protists influence the exchange and cycling of elements that occur in these connected but distinct environments. The photic zone is in fact a small portion of the ocean compared to these other deeper zones, with the dark ocean occupying 94.7% of the total ocean volume (Whitman et al. 1998). Microbial cell concentrations in the dark ocean are much lower than in the surface water, as are those of protists, albeit based on relatively little data. However, just by sheer volume the dark ocean contains about 1.8 times as many bacteria and archaea as does the photic zone (Orcutt et al. 2011; Sogin et al. 2006; Whitman et al. 1998).

4.6.1 Protists in the Photic Zone

In the open ocean, the photic zone ranges from the surface seawater to ~200 m deep (Fig. 4.7). A vertical gradient is often seen, with the DCM receiving less light than the surface water, but greater nutrient availability, in stratified water columns. As the name implies, the DCM contains the maximum amount of chlorophyll and typically displays the highest absolute abundances of protists (Rocke et al. 2015). In ecosystems that have pronounced seasonal stratification changes, protists tend to be distributed throughout the photic zone when “winter” mixing occurs and peak

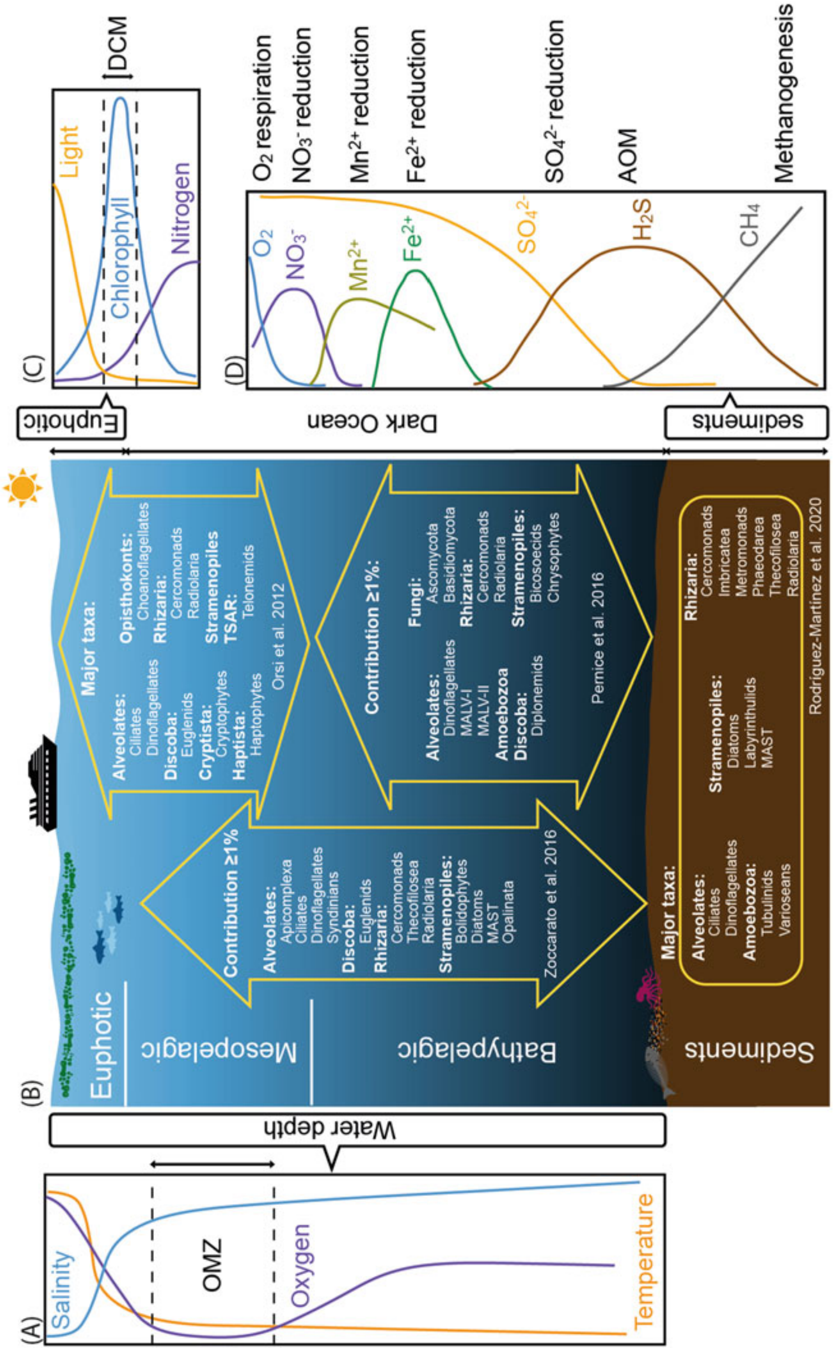
(A)
Salinity
Oxygen
Temperature
OMZ
Water depth
(B)
Euphotic
Mesopelagic
Bathypelagic
Sediments
Major taxa:
Alveolates: Ciliates Dinoflagellates
Discoba: Euglenids
Cryptista: Cryptophytes
Haptista: Haptophytes
Opisthokonts: Choanoflagellates
Rhizaria: Cercomonads Radiolaria
Stramenopiles
TSAR: Telonemids
Orsi et al. 2012
Contribution ≥1%
Alveolates: Apicomplexa Ciliates Dinoflagellates Syndinians
Discoba: Euglenids
Rhizaria: Cercomonads Thecofilosea Radiolaria
Stramenopiles: Bolidophytes Diatoms MAST Opalinata
Zoccarato et al. 2016
Contribution ≥1%:
Alveolates: Dinoflagellates MALV-I MALV-II
Amoebozoa
Discoba: Diplonemids
Fungi: Ascomycota Basidiomycota
Rhizaria: Cercomonads Radiolaria
Stramenopiles: Bicosoecids Chrysophytes
Pernice et al. 2016
Major taxa:
Alveolates: Ciliates Dinoflagellates
Amoebozoa: Tubulinids Varioseans
Stramenopiles: Diatoms Labyrinthulids MAST
Rhizaria: Cercomonads Imbricatea Metromonads Phaeodarea Thecofilosea Radiolaria
Rodríguez-Martínez et al. 2020
Euphotic
Dark Ocean
Sediments
(C)
Light
Chlorophyll
Nitrogen
DCM
(D)
O_2
NO_3^-
Mn^{2+}
Fe^{2+}
SO_4^{2-}
H_2S
CH_4
O_2 respiration
NO_3^- reduction
Mn^{2+} reduction
Fe^{2+} reduction
SO_4^{2-} reduction
AOM
Methanogenesis

Fig. 4.7 Current understanding of protistan distributions from surface marine waters to the seafloor. (**a**) Archetypical profile of temperature, oxygen, and salinity in oceanic environments. (**b**) The diversity described for protists reported thus far in each indicated depth horizon and in sediments. Boxes reflect the results of individual studies that employed amplicon sequencing (not visual observation). In that with results from (Orsi et al. 2012), protistan taxa shared between the photic and mesopelagic zones are shown. The boxes representing results of Zoccarato et al. (2016) and Pernice et al. (2016) include protistan taxa that contributed $\geq 1\%$ of the relative amplicon abundance in 18S rRNA gene sequencing surveys. The most abundant taxa within each major group (in bold) in marine sediments are displayed in the box based on results from Rodríguez-Martínez et al. (2020). (**c**) More resolved profiles of light, chlorophyll, and nitrogen of a representative photic zone section. The maximum concentration of chlorophyll is typically located within the photic zone, however note that biomass can be subducted to below the photic zone (Johnson and Omand 2021; Omand et al. 2015)! (**d**) Representative redox transitions in marine sediments on the scale of cm to km below the seafloor (Inagaki et al. 2016; Jørgensen and Kasten 2006). *OMZ* oxygen minimum zone, *DCM* deep chlorophyll maximum, *AOM* anaerobic oxidation of methane. Concentrations and distances not to scale during the spring blooms triggered by the infusion of nutrients that comes along with winter mixing. In coastal areas and transition zones (moving from the coast to offshore) protistan abundances are often higher than in the open ocean due to overall higher nutrient availability.

The central focus of research on phytoplankton has long been photosynthesis and its metabolic product, O_2. Since the great oxygenation event, many complex life forms have evolved that are dependent on availability of oxygen (Sánchez-Baracaldo and Cardona 2020), although permanently anoxic environments also exist in which O_2 is toxic for the inhabitants. Primary producers are a source of food for zooplankton in the photic zone, including for heterotrophic protists that belong to the zooplankton and reportedly consume between 8 and 100% of the phytoplankton standing stock biomass (microscope counts) (Sherr and Sherr 2002). Vertical migrators also exist that come from darker waters below to graze in the photic zone. Limited data exists where specific or even bulk heterotrophic marine protists have been enumerated. This is because many are not amenable to fixation and time-consuming counting by microscopy is still the best method for enumerating heterotrophic taxa. To identify the taxon beyond being a heterotrophic predator, target sequences and FISH probes targeting those sequences are required.

Photosynthetic taxa do not extend below the photic zone (except as they exit the system as sinking blooms, aggregations, detritus, or through mesoscale processes). Laterally though, there is great variation with coastal and continental shelf regions often dominated by diatoms, dinoflagellates, and calcifying haptophytes (coccolithophores) that are able to form large blooms, while the open ocean is often dominated by cyanobacteria alongside picoplanktonic eukaryotic phytoplankton such as small prasinophytes, chrysochromulina-like haptophytes, as well as small stramenopiles like pelagophytes and chrysophytes. This emphasizes the important role of cell size in determining the global ecological patterns of photosynthetic protists (Peter and Sommer 2013), as discussed above. Protists have other adaptations to the low nutrient concentrations that frequently occur in the surface of stratified photic zones, such as high-affinity transporters, capacity to use organic nutrients and ingestion of particles (e.g., Arenovski et al. 1995; Finkel et al. 2009; Palenik and Morel 1990; Wilken et al. 2019; Zubkov and Tarran 2008). Each lineage and their individual members employ a plethora of diverse ecological strategies.

4.6.2 Protists in the Dark Ocean: Oxygen Minimum Zones and Sediments

Based on microscope and flow cytometry cell counts the absolute cell abundance of protists in the dark ocean appears to decline proportionally with that of bacteria and archaea. This indicates that predation by protists is taking place (Pernice et al. 2015) although little is known about protistan functional roles in the dark ocean. Without the availability of sunlight, microbial metabolisms in the dark ocean and sediments are based on redox reactions and rely on organic matter sinking from the surface (Orcutt et al. 2011).

The regions with the lowest oxygen saturation are called oxygen minimum zones (OMZs, Fig. 4.7). While there is no general agreement concerning threshold concentrations of oxygen, the major OMZs are defined by $O_2 < 20$ M and can reach concentrations as low as 1 M O_2 in the core (Paulmier and Ruiz-Pino 2009). With respect to protists, abundances in OMZs can fluctuate with seasonal shifts, as observed for example in the Canadian Pacific, relative abundances of ciliates and euglenozoans (i.e., diplonemids and symbiontids) increased in anoxic zones of the water column during summer (Orsi et al. 2012). In OMZs heterotrophic metabolism relies on other electron acceptors than oxygen such as nitrate and nitrite, while chemotrophic metabolisms can utilize different types of nitrogen (and other) compounds such as nitrite and ammonia as energy sources (Lam and Kuypers 2011). This metabolic diversity so far appears largely to be confined to bacterial and archaeal communities, although dissimilatory nitrate reduction has been reported in a fungus isolated from an OMZ (Kamp et al. 2015). Regardless, protists appear to control the abundance of nitrate-reducing and ammonia-oxidizing bacteria by preying on them (Orsi et al. 2012), and protists can consume up to 28% of the bacterial biomass based on data from microscope cell counts (Medina et al. 2017).

Going a little bit deeper, if most studies regarding marine protist diversity have focused on planktonic environments, the subseafloor sediments are estimated to contain 2.9×10^{29} cells (Kallmeyer et al. 2012). This assessment is about five orders of magnitude higher than the numbers estimated for the entire ocean waters, i.e., 1.37×10^{24} cells (Whitman et al. 1998). However, it should be noted that few studies have quantified protists in any of these environments, rather most of what we know is about their diversity through early environmental clone libraries and through amplicon sequencing. At this stage, a comprehensive systematic characterization of ocean habitats using amplicon sequence variants (ASVs) (Amir et al. 2017; Callahan et al. 2016; Eren et al. 2015) and metagenomics would be most beneficial for improving ecological resolution of different taxon distributions (Needham et al. 2017), even if broad-scale differences can be similar to earlier approaches of grouping sequences (Glassman and Martiny 2018).

To date, most deep-sea studies have found little overlap in community composition of protists in planktonic versus subseafloor environments; and that there are few relevant reference sequences from cultured organisms for categorizing these taxa (Forster et al. 2016). Among the classified protists, Rhizaria appear to be the dominant eukaryotes in surface sediments at depths ranging from 79 to 2939 m (Wu and Huang 2019). In subseafloor ecosystems, microorganisms have long been thought to compete for limited energy sources (Hoehler et al. 1998; Bradley et al. 2019, 2020), and differing dominant "metabolic guilds" are thought to have shaped the different redox zones over depth gradients (Jørgensen and Kasten 2006). These redox zones start with oxygen respiration at the subseafloor surface followed by nitrate-, iron-, and sulfate-reduction, anaerobic oxidation of methane, and methane generation (methanogenesis, Fig. 4.7), reflecting the typical profile of marine sediments. While some of this metabolic diversity again appears to be confined to bacteria and archaea, ciliates have symbiotic relationships with bacteria and archaea that are capable of aerobic methane oxidation, sulfate reduction, and methanogenesis in sulfidic marine sediments (Edgcomb et al. 2011). Additionally, in anoxic sediments, a new type of symbiosis between south-seeking magnetotactic protists and magnetite-containing *Deltaproteobacteria* has been observed (Monteil et al. 2019). Although the benefit for protists is not fully understood, it is thought that the motility of this symbiotic consortium along the geomagnetic field allows the protist to move toward locations that are optimal to them and allows the sulfate reducers to grow using the protist's metabolic products (Monteil et al. 2019). Finally, in marine sediments protists defy the dogma of redox zonation and often aggregate bacteria or archaea that would generally be partitioned over the three different redox zones.

4.6.3 Diversity of Marine Protists in the Vertical Dimension

We know by now that protist abundance and community composition are controlled by different environmental factors that are still poorly understood, especially in the dark ocean and marine sediments (Fig. 4.7). Based on the relative amplicon abundances, ciliates, dinoflagellates, and stramenopiles are considered important in deep and sediment ecosystems (Orsi et al. 2012; Pernice et al. 2016; Rodríguez-Martínez et al. 2020). In sediments underlying different water column depths the radiolarian Acantharea show higher relative amplicon abundances in cores collected from greater depths (881 and 957 m) than lesser depths (200 and 650 m) (Rodríguez-Martínez et al. 2020). They are also evenly detected in both the photic and mesopelagic zone which may be due to life cycle stages where flagellated swarmers are released from sinking cysts in deep waters (Decelle et al. 2013). Within stramenopiles, amplicon relative abundances indicate that in the dark ocean MAST, chrysophytes, bicosoecids, and diatoms are present, although it is difficult to discern which members reflect recent export versus in-situ growth (Pernice et al. 2016). Marine sediments also contain these stramenopile taxa (Rodríguez-Martínez et al. 2020). Cercozoans (members of the Rhizaria) display much higher relative amplicon sequences in the ocean water column (Orsi et al. 2012; Zoccarato et al. 2016) than in sediments (Rodríguez-Martínez et al. 2020). A molecular survey directly comparing diversity of benthic and pelagic environments suggests there is a higher diversity of protists in the former than the latter (Forster et al. 2016).

Differences in numerical abundances for inferring importance can be misleading. For example, in NE Atlantic deep-sea sediments at 2170 m depth, foraminifera and bacteria both account for 50% of algae degradation, but the biomass of the former is negligible compared to that of the bacteria (Moodley et al. 2002). Finally, rare populations are thought to maintain the diversity in different environments (Lennon and Jones 2011) and can quickly respond to environmental changes such as redox fluctuations (DeAngelis et al. 2010), tidal cycling (Ling et al. 2018), and even the deep-sea oil well blowout (Kleindienst et al. 2016). Rare protists have been reported to shift in relative amplicon abundances in response to fluctuations in OMZs (Orsi et al. 2012), chaotic flows (Villa Martín et al. 2020), and seasonal variations in surface seawater (Genitsaris et al. 2015).

Further investigation of protistan diversity, cell biology, and ecology, alongside their metabolic interactions with other microorganisms will advance the knowledge required to evaluate the efficiency and magnitude of global biogeochemical cycles. In just one study that addressed multiple marine zones including the mesopelagic and bathypelagic, it was determined that protists graze significantly upon bacterial and archaeal prey across the DCM (80–130 m), the upper mesopelagic zone (220 m), the deep Antarctic Intermediate Water (750 m), and the bathypelagic North Atlantic Deep Water (2500 m) (Rocke et al. 2015). Compared to the 8–100% removal rates reported in the photic zone (Sherr and Sherr 2002), these removal values ranged from 3.8–31.1% of the bacterial and archaeal standing stock biomass in the deep ocean (Rocke et al. 2015), or about 20% of the in-situ abundance of bacteria and archaea in the deep ocean oxycline (Edgcomb 2016).

4.7 Forces of Mortality

Biomass in the oceans is ~1.2% of that in terrestrial systems, yet productivity is roughly equal (Bar-On et al. 2018). This surprising fact is primarily due to ocean biomass being mainly microbial and having a fast turnover. High turnover rates in marine ecosystems come from strong top-down biomass "removal" imposed by grazing and viral infections, leading to turnover times of the microbial components on the order of days. Understanding the ecological and evolutionary implications of how these removal processes act on protists is important for understanding their influences on the marine microbiome and biogeochemical cycles.

4.7.1 Timeline of Virus Discovery

While viruses escaped the observations of Haeckel and Leeuwenhoek, they found their way into art and economic movements in the seventeenth century Europe. Indeed, when Leeuwenhoek was 5 years old the popularity of tulips in Holland had reached a zenith as extremely prized possessions ("tulip mania"). The most coveted tulips were those whose deep color was broken with white and the source of these stripes was chlorosis caused by a plant virus, one of the first documented viruses. As knowledge of viruses has increased it has become clear that their influence lies far beyond simply infecting and killing their hosts. Indeed, most viruses harbor genes that can augment host metabolism and influence the ecosystem in surprising ways.

The first formal description of a virus ("*Contagium vivum fluidum*" from latin: "contagious living fluid") was of the Tobacco Mosaic Virus (TMV) in 1892. TMV was defined as tiny (originally defined as being able to pass through a porcelain Chamberland filter of ~0.2 μm) obligate pathogen dependent on intracellular multiplication in hosts. After TMV, viruses of bacteria (bacteriophages) were discovered in ~1910 and subsequently revolutionized molecular biology. As early as 1958 there were observations that suggested viruses may infect the aquatic photosynthetic green alga *Chlorella* (Brown and Malcolm Brown 1972; Zavarzina and Protsenko 1958).

However, it was not until 1972 that the first virus of a protist was enriched and shown to be the causative agent of mortality in the amoebozoan *Entamoeba histolytica* (Diamond et al. 1972; Diamond and Mattern 1976; Wang and Wang 1991). The first virus isolated on a photosynthetic protist came from the multicellular green alga *Chara* (Gibbs et al. 1975) and shortly after the first virus of a marine phytoplankter was discovered, which infected the prasinophyte *Micromonas pusilla* (Mayer and Taylor 1979).

Despite these discoveries, the realization of the importance of viruses in ocean ecosystems did not become clear until two publications showed extraordinarily high abundances of free "living" particles of $>10^7$–10^8 per mL (Bergh et al. 1989) and estimated that up to 70% of marine microorganisms were infected at any given time (Proctor and Fuhrman 1990). Further experimental evidence of their importance came from experiments where the addition of concentrated viruses to natural seawater samples resulted in a considerable decrease in primary productivity by phytoplankton (Suttle et al. 1990). Over the subsequent decades, in-situ experiments, culturing studies, and natural observations have shown that viruses are an important source of mortality in the ocean and can impact community composition as well as facilitating genetic exchanges. Thus, these entities, which are only active once they have infected a host, contribute importantly to the ecology and evolution of the marine microbiome as well as to the cycling of nutrients and energy in the ocean (Breitbart et al. 2018; Zimmerman et al. 2020).

4.7.2 Current Perspectives on Viruses of Marine Protists

The current diversity of viruses of marine protists is substantial with most of the cultured viruses being those that infect photoautotrophs (Hyman and Abedon 2012). The diversity of known viruses of protists includes viruses made up of ssDNA, dsDNA, ssRNA, and dsRNA and ranges in size from a few genes (thousands of bases) to the thousands of genes (1–2 million bp), with the latter being akin to the size of small bacterial genomes. The characteristics of each of these virus types vary in terms of infectivity and burst sizes but rigorous comparative studies are still lacking.

Redirection of the carbon flow by viral infection of protists has been linked to phytoplankton bloom demise in the ocean (Brussaard 2004; Suttle et al. 1990; Weynberg 2018). For example, viruses associated with *E. huxleyi* blooms studied in the field and in mesocosm experiments appear to end these blooms (Brussaard et al. 1996; Schroeder et al. 2003; Vardi et al. 2012). Other phytoplankton species for which viruses are implicated in bloom decline include the pelagophyte *Aureococcus anophagefferens* (Gastrich et al. 2004; Gobler et al. 2007) and the haptophyte *Phaeocystis* (Brussaard et al. 2005; Ruardij et al. 2005). Still, there is much to learn about the degree to which viruses are the causative agent of phytoplankton bloom declines, especially since viruses commonly co-exist with their hosts without causing such dramatic events. Much less is known about viruses that infect heterotrophic protists although complex defenses against viral infection, e.g., virophages

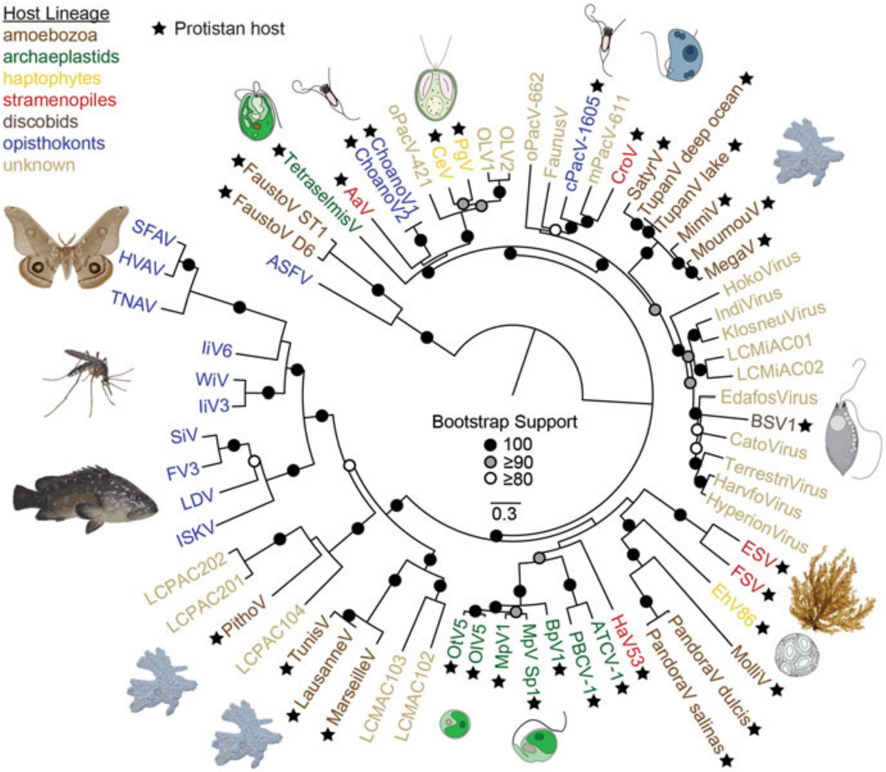

Fig. 4.8 Maximum Likelihood phylogenomic tree of Nucleocytoplasmic Large DNA viruses (NCLDV) based on 10 putatively vertically inherited proteins. To build this type of tree, individual protein sequences need to be curated carefully to retain only those that are of high quality (for example, not truncated) and are not paralogs. This can be an issue because the growing number of NCLDV genomes are derived from metagenomics assemblies (MAGs). After curation, sequences are aligned, trimmed to remove ambiguously aligned or non-homologous positions, and an evolutionary model is selected for each individual gene, or on the entire group after concatenation (as done here). Subsequently, a phylogenetic tree is constructed (in this case with IQ-tree, under the model LG + C20 + F + G-PMSF) with calculations of bootstrap support (here, non-parametric bootstraps with 500 replicates). Here, statistical support is indicated when it exceeds 80% bootstrap support. The scale bar indicates the number of substitutions per site. NCLDV infect a variety of eukaryotic hosts, mainly protists, as indicated here for those recovered from hosts using cultivation and cultivation-independent studies, modified from Needham et al. (2019a). The color of each virus label indicates the host lineage with which the virus has been found to associate, as well as those with unknown hosts. Black stars indicate protistan hosts. The drawings of critters are not to scale. It is likely that the skew towards higher relative contributions of photosynthetic protists as hosts of NCLDV results from there being more photosynthetic protists in culture relative to heterotrophic and/or mixotrophic protists

have been observed (Mougari et al. 2019; Yau et al. 2011). Overall, viruses of protists that belong to different trophic modes or roles should be considered in conceptualizations and models of ecosystem dynamics (Taylor et al. 2014, 2018).

4.7.3 Diversity of Viruses Infecting Marine Protists

Probably the best studied group of viruses of marine protists are the dsDNA viruses from the nucleocytoplasmic large DNA virus (NCLDV) family. These include a wide diversity of viruses that infect different taxa within the haptophytes, amoebozoans, stramenopiles, and archaeplastids (Fig. 4.8). While bacteriophages and other viruses are generally small, the Mimiviridae is a family of NCLDV that is remarkable because of their large physical- and genome size, some of which are similar to small bacteria in these characteristics. In fact, their original discovery in association with the discovery of Amoebozoa was delayed for almost 10 years because they were erroneously considered to be bacteria (La Scola et al. 2003; Raoult et al. 2004). Mimiviruses (for "mimicking" bacteria) now have been isolated from a variety of environments and those from the ocean have been found in association with or co-cultured with a similar host range to all other NCLDVs (Mihara et al. 2018). Based on community surveys this viral group has been proposed to be as diverse or more diverse than bacteria and archaea together (Mihara et al. 2018). Notably, genes once thought to be hallmarks of cellular life are encoded by viruses: genes involved in transcription and translation are common across the Mimiviridae family including tRNAs, amino-acyl tRNA synthetases, transcription factors (Abrahão et al. 2018; Schulz et al. 2017), in addition to the replication machinery encoded by most viruses (such as DNA polymerases).

NCLDVs with sequenced genomes show an incredible array of genome potential with the ability to supplement functions their hosts already encode or bring new functions to the infected host. These can include genes to help with nutrient acquisition such as nitrogen- (in the form of ammonium) (Monier et al. 2017; Needham et al. 2019a) and phosphate transporters (Bachy et al. 2018) as well as supplementation of carbohydrate and fermentation metabolism (Schvarcz and Steward 2018). Diverse Mimiviruses also encode chitinases that may either be involved in degradation of host chitinaceous structures or the prey of heterotrophic protists that are infected (Needham et al. 2019b; Van Etten et al. 2017).

One striking difference between viruses of marine protists versus cyanobacteria is that the former do not carry genes involved in photosynthesis, genes that are common in cyanophages (Lindell et al. 2005; Sharon et al. 2009). This may be due to difficulties in viral acquisition of a photosystem protein that possesses the "correct" transit peptide, a peptide required to bring proteins across the chloroplast membrane of photosynthetic protists in order to function in photosynthesis (Bhattacharyya and Chakraborty 2018; Patron and Waller 2007). However, viruses of protists do encode other proteins that use light, not to be mistaken for photosystems involved in oxygenic photosynthesis. Viral rhodopsins were first reported in a virus of *Phaeocystis globosa* and in metagenome contigs putatively from eukaryotic viruses (Yutin and Koonin 2012). They have now been reported in uncultured viruses from choanoflagellates that were single cell sorted from the wild (Needham et al. 2019a, b). The presence of these microbial rhodopsins is independent of whether or not the host encodes such proteins. Hence, they can bring a new metabolic function to the host (Needham et al. 2019b). Furthermore, some

Mimiviruses encode the proteins required for biosynthesis of the photoreceptor pigments that rhodopsins need to function, i.e., retinal (Needham et al. 2019a, b). This is notable because likely host organisms do not appear to synthesize beta-carotene which is cleaved to retinal, and thus the viruses must encode multiple genes to do so which would then also be expressed by the infected host.

The dsRNA and ssRNA viruses are other types which infect aquatic protists including fungi, stramenopiles, and ciliates (Short 2012). In contrast to the NCLDV, these viruses are small, typically encoding only a few genes. The hallmark gene of RNA viruses is the RNA-dependent RNA polymerase (Gustavsen et al. 2014; Kaneko et al. 2021). While much of virus research in the ocean has focused on dsDNA viruses, RNA viromes are of great interest and there are some indications that these viruses may be much more abundant than previously thought (Kaneko et al. 2021; Steward et al. 2013; Urayama et al. 2018; Wolf et al. 2020; Zeigler Allen et al. 2017).

Finally, viruses influence host evolution by integrating into genomes, a phenomenon that has been described for the human genome as well (Griffiths 2001), and in plant genomes (Maumus et al. 2014). Likewise, the ability of viruses to integrate into protist genomes has been observed in cultured host–virus pairs (Delaroque and Boland 2008; Filée 2014; Gallot-Lavallée and Blanc 2017; Meints et al. 2008; Sharma et al. 2014). The consequences of this integration are unclear, but the viral material appears to reflect remnants of ancient infections and is not expressed at the same frequency as other genes. This suggests that these ancient remnant genes may have become nonfunctional and may not influence the host greatly, or are only expressed under certain conditions. Nevertheless, these observations complicate studies aiming at the description of extant viral diversity through metagenomic sequencing and must be considered when investigating protistan genomes.

4.7.4 Death of a Protist Via Predation

Forces of mortality are plentiful in the oceans. Protists can die from being eaten either by metazoans or by other protists, from being parasitized, through viral lysis, or through aggregation and sinking. The quantitative impact of mortality on protist populations has mainly been investigated for primary producers, which often appear to be eaten as fast as they reproduce, keeping populations stable despite rapid growth rates. A synthesis study suggested about two-thirds of the global phytoplankton productivity is removed through consumption by protistan zooplankton, while a large part of the remainder is eaten by larger metazoan zooplankton (Schmoker et al. 2013; Steinberg and Landry 2017). In this scenario, grazing already accounts for the loss of the majority of primary production. However, studies that also take viral lysis into account often find its importance quantitatively similar to grazing (Brussaard 2004). The relative importance of the forces causing protistan mortality also likely varies, for example, viruses may terminate phytoplankton blooms or have impacts that vary in different marine habitats or ocean regions (Mojica et al. 2016).

Despite the quantitative importance of grazing mortality for biogeochemical fluxes in the ocean, there is surprisingly little information about the organisms that consume protists. While there is some information on consumers of photosynthetic protists, this information is lacking for the predators that consume heterotrophic protists. In general, the dilution assays used to quantify community level grazing impact on phytoplankton are rarely combined with techniques that identify the actively grazing organisms. However, when combined with a detailed characterization of the microzooplankton community composition (Neuer and Cowles 1994) predators can be identified, leading to data suggesting that dinoflagellates are important herbivores in some environments (Sherr and Sherr 2007). Nevertheless, despite estimates of grazing mortality over large geographical ranges, the specific interactions responsible for these often remain elusive. It should be noted that if mixotrophic grazers control their grazing rates in response to nutrient or light availability (Anderson et al. 2017; Li et al. 2021), then, these environmental conditions will introduce considerations distinct from those for heterotrophic grazers (Edwards 2019). In cases where predator protistan–prey interactions have been tracked, the focus has been on predation on the smallest primary producers. For example, dinoflagellates, ciliates, telonemids, and the stramenopile MAST clades 1 and 3 have been shown to consume the picoeukaryote *Micromonas* in the Pacific Ocean (Orsi et al. 2018).

Knowledge of specific predator–prey interactions is also important in cases where overall community grazing mortality is low. Predators that selectively feed on rare community members or avoid dominant species can influence community composition and bloom dynamics. For example, despite high microzooplankton abundances and grazing on larger phytoplankton species, it has been suggested that low grazing pressure on the coccolithophore *E. huxleyi* is an important factor for bloom formation by this organism (Olson and Strom 2002). Results from laboratory experiments were taken to suggest that the haploid life-cycle stage of *E. huxleyi* is an inducible anti-predator response that might underpin low grazing pressure on this organism (Kolb and Strom 2013). The mechanistic basis for this hypothesis remains unknown as do the field implications. Both constitutive and inducible defenses against predators are common among photosynthetic protists (Pančić and Kiørboe 2018; Van Donk et al. 2011). For instance, the solid silica frustule of diatoms or the long spines found in some species may deter predators. Induced colony formation to increase particle size, or production of toxins may also both contribute to deterrence or negative effect on grazers.

As mentioned above almost nothing is known about predation on heterotrophic protists and their specific loss rates are rarely quantified. In contrast to the widespread assumption that protistan predators prefer prey of about a tenth of their own size, the diversity of feeding strategies found among different protists includes many predator-prey size relationships with large deviations from this assumption. Many heterotrophic protists are fed upon by other similarly sized protists as detected using double FISH staining for visualization of both predators and the protistan prey in their food vacuoles (Piwosz et al. 2021). Such trophic interactions among equally sized protists imply that increasing trophic levels in microbial food webs are not necessarily paralleled by increases in size. Hence, carbon may flow through the microbial food web via several trophic levels before reaching larger metazoan zooplankton which are fed upon by fish (Piwosz et al. 2021). Because organic carbon is considered lost at each trophic transfer (although utilized by other microbiome members in one way or another), a higher number of trophic levels will decrease food web efficiency. In contrast, a larger proportion of mixotrophic protists in the food web has been predicted to have the opposite effect—exhibiting higher efficiency (Stoecker et al. 2017; Ward and Follows 2016). Quantitation of the "exact" routes of carbon flow is needed to infer the food web efficiency that supports life at the highest trophic levels in the ocean, including fisheries of commercial interest.

4.8 Looking Forward

Studies of marine microbiomes are governed by prevailing conceptual and methodological challenges. Each of the topics below focuses on protists and could fill additional chapters, but will be discussed here in brief and without attention to prioritization order.

4.8.1 Classics: The Delineation of Protistan Species

Although the species is a basic unit and currency in ecological and evolutionary research in any environment, there is no consensus on how a microbial species is best defined. Traditionally, protists have been defined by their morphological features, but we now know that morphological features can change under varying environmental conditions (Pizay et al. 2009), and comparisons of morphological and molecular data as well as mating experiments have provided evidence for cryptic diversity (Amato et al. 2019; Sarno et al. 2005). Not least, small cells such as picoeukaryotic cells often lack distinctive features. The classical biological concept by Ernst Mayr (Mayr 1996) that defines a species as a member of an interbreeding population that is reproductively isolated from other such groups and capable of producing fertile descendants cannot be readily applied to most protists, due to a lack of knowledge on their sexual reproduction (Silva 2008). Thus, alternative species concepts have been proposed (De Queiroz 2007; Samadi and Barberousse 2006) but operationally objective criteria to define a microbial species are still lacking. Advances in resolving cryptic diversity have utilized metabarcoding/amplicon datasets for reconstructing phylogenetic networks (De Luca et al. 2021) and progress in this field will be crucial for understanding the population biology of protists that underlies their adaptive responses to contemporary and future environmental conditions.

4.8.2 Classics: Everything Is Everywhere, but, the Environment Selects Versus Endemism

Another challenging and highly debated concept is that of "*everything is everywhere*, but, *the environment selects*," which roots back to the nineteenth century (O'Malley 2007). It postulates that the abundance of microbial species is so large that their dispersal is never restricted by geographical barriers (Finlay 2002). This concept still dominates the ecological and evolutionary understanding of microbial distribution based on culture studies (de Wit and Bouvier 2006). However, environmental molecular surveys have provided evidence that barriers likely do exist for dispersal and while some species might be globally distributed, others are not (Casteleyn et al. 2010) or may exhibit niche partitioning so that they can co-reside (Foulon et al. 2008). For example, phytoplankton like the diatom *Fragilariopsis cylindrus* (Lundholm and Hasle 2010; Mock et al. 2017) and the prasinophyte *Micromonas polaris* (Simmons et al. 2015) are found in both Arctic and Antarctic environments, while the cryptophyte *Geminigera cryophilia* has only been observed in Antarctic environments (Taylor and Lee 1971), a finding which has held up in amplicon sequencing studies.

4.8.3 Classics: Diversity and Stability of Plankton Communities

The dazzling diversity of protists found in the ocean has long fascinated ecologists and led G. Evelyn Hutchinson to formulate the paradox of the plankton. He asked "why do so many planktonic species co-exist in a supposedly homogeneous habitat?" (Hutchinson 1961). Based on the competitive exclusion principle, phytoplankton species competing in a well-mixed environment for only few inorganic resources should outcompete each other resulting in a winner. Of course, we now know that most phytoplankton species utilize dissolved organic resources, not just inorganic compounds, and that many of them are capable of feeding on other microbes. Additional explanatory factors for the high diversity of photosynthetic protists include: (1) the temporal and spatial variability of surface ocean waters, which are now recognized as not being homogeneously mixed (Azam 1998); (2) the dynamics of species interactions that can result in oscillations and chaos, and thereby never settle toward an equilibrium (Record et al. 2013); (3) toxins produced by phytoplankton resulting in maintenance of diversity (Roy and Chattopadhyay 2007). Collectively, these findings might thus have (partly) resolved the paradox. Nevertheless, the mechanisms generating and maintaining the diversity of marine protists, and the relationship between diversity and ecosystem stability (another long-standing debate in ecology) require greater attention. With respect to marine microbial science and phytoplankton diversity, genomics and metagenomics have made clear that functional redundancy, which lumps organisms by what is perceived as their "main" biogeochemical function, is an inappropriate term that likely over predicts system resiliency and stability. Functional redundancy ignores evolutionary trajectories of organisms, the diversity of proteins they contain (many of which have uncharacterized functions that are likely important to how they make a living in the ocean), and different overall gene content in the genomes of different phytoplankton species. These factors underpin the biology of each species and determine how individual microbial species acclimate and adapt to changing ocean conditions as well as how they thrive or not in the novel community assemblies that arise from change. Hence, predicting the degree of diversity an ecosystem can sustain, or maybe more importantly the degree of diversity an ecosystem can afford to lose without ecosystem functions collapsing, is an urgent frontier for microbiome science given that we have entered the sixth period of mass extinction (Cavicchioli et al. 2019; Ceballos et al. 2015).

4.8.4 The Uncultured Majority: Quantifying Activities and Trophic Transfer

Beyond known microbiome interactions involving eukaryotes, there may be a plethora of interaction types that have yet to be discovered. In the course of studies on choanoflagellates aimed at improving understanding of the origins of animal multicellularity, it has become clear that bacteria can influence the behavior of these single-celled heterotrophic protists in culture. In particular, specific lineages of bacteria produce a compound that stimulates sexual reproduction in choanoflagellates (Woznica et al. 2017) as well as the transition to a truly multicellular state (Woznica et al. 2016). Although the degree and mechanisms by which such interactions occur in the dilute marine environment is still an open question, these types of discoveries highlight the fact that the many protists that remain uncultured to date may present many novel types of interactions. Unfortunately, this dearth of knowledge impedes identification of trophic linkages or the strength of interactions between different microbiome members, and how they might shift in future oceans.

What approaches can be used to tackle the uncultured majority? Advances in genomic and targeted metagenomic analyses of cultured, e.g., Bowler et al. (2010); Keeling et al. (2014); Mock et al. (2017); Moreau et al. (2012); Read et al. (2013); Worden et al. (2009) and wild algae (Cuvelier et al. 2010; Simmons et al. 2016; Teeling et al. 2012; Vannier et al. 2016; Worden et al. 2012), as well as marker-gene studies (Choi et al. 2017; de Vargas et al. 2015; Ibarbalz et al. 2019; Kim et al. 2011a; Not et al. 2007a; Pernice et al. 2016) have illustrated the tremendous diversity of uncultured taxa and have provided first insights into aspects of their biology and evolution. However, the natural distributions and activities of these algae are generally not known at the level of genetic differentiation that connects to their physiology and ecology. Importantly, much less is known from a genomic perspective about marine predatory protists, apart from e.g., the choanoflagellates (King et al. 2008; Richter et al. 2018) and there are few methodologies that quantify their activities in the field. Hence, the quantities of protistan carbon that moves into e.g., the microbial loop versus more directly into higher trophic levels are more or less unknown, although incredibly important for modeling efforts and for understanding how food networks might change as the ocean changes. Estimating the carbon flow along specific trophic interactions used to rely on tedious microscopy counts following FISH staining of groups of interest (Massana et al. 2009; Piwosz et al. 2021) but can now be supplemented by stable isotope techniques to track substrate uptake or prey ingestion into diverse consumers (Frias-Lopez et al. 2009; Orsi et al. 2018).

4.8.5 Bringing Cell Biology to Bear on the Protistan Role in the Marine Microbiome

As outlined throughout this chapter, protistan dynamics are essential parts of the carbon cycle and food networks, processes that determine the ultimate fate of the CO_2 fixed by algae through oxygenic photosynthesis (Behrenfeld et al. 2006; Field et al. 1998; Lomas et al. 2013; Steinberg et al. 2001; Worden et al. 2015). Despite the fundamental importance of their biological activities and the massive advances in the knowledge about their evolutionary relationships, mechanistic understanding of the factors that determine the growth, physiology, and fate of protists is often still lacking. Even actual host–virus pairs are typically unknown. Part of the challenge is the sheer diversity of marine protists and the fact that many remain uncultured. However, understanding the biology of protists is critically important for identifying how marine microbiomes will transition as the ocean changes. In addition to a lack of knowledge on specific field interactions with viruses and with predators, key parameters of population biology and sexual reproduction are mostly unknown. In particular, the ecological importance and evolutionary trade-offs of basic features such as the type of mixotrophy or even a motile versus non-motile lifestyle are unknown. Single-cell and targeted metagenomics have moved the field forward, as have transcriptomics and metaproteomics. However, only when these data types are engaged in ways that illuminate cellular responses can they be fully implemented in understanding physiology and ecology in a mechanistic manner. Without comprehension of organismal biology, many studies still rely on correlation analyses and yet most environmental parameters are not in steady state. Just imagine trying to address cancer biology without any knowledge of human physiology, cell boundaries, genome composition, genetics, or epidemiology. Marine science is well positioned to move beyond that place! A next step in this direction is to employ genetics to begin to recover functions for genes in marine protists. Genetic systems are now available for several environmentally relevant marine protists (Faktorová et al. 2020) that can be used to ascertain these functions and to perform studies examining mutant response relative to wild type to understand these functions in a cellular context.

4.8.6 Connecting Microbiome Members and Interactions to Ocean Physics and Chemistry

Understanding the interactions among marine protists as well as their interactions with bacteria, archaea, and viruses is challenging. Add to that their responses to the physical and chemical conditions of the environment and how that might modify activities and interactions and we start to tackle microbiome science. One important approach for addressing the complexity of these communities and their dynamics is through long-term time-series observations (Fuhrman et al. 2015; Giovannoni and Vergin 2012). Several marine time-series studies make measurements that are aimed at elucidating the diversity and dynamics of plankton alongside physical and chemical conditions. Unfortunately, protists are sometimes not included in time-series studies, while bacteria, archaea, and less commonly, viruses are more often considered. Nevertheless, time-series analyses have been used to examine protists at a variety of temporal scales. Here, we provide some examples for studies at different time scales, including diurnal (Hu et al. 2018; Madin et al. 2001; Needham et al. 2018), daily (Berdjeb et al. 2018; Fitzsimmons et al. 2015; Lie et al. 2013; Martin-Platero et al. 2018; Needham and Fuhrman 2016), monthly and inter-annual (Choi et al. 2020; Guadayol et al. 2009; Kim et al. 2013; Limardo et al. 2017; Massana et al. 2015; Pasulka et al. 2013; Steele et al. 2011; Wiltshire et al. 2008). Such time-series studies have often revealed strong seasonality in surface waters, which tend to be the focus. These studies also revealed strong correlations between different taxa or with physical and chemical conditions (Anderson and Harvey 2020; Genitsaris et al. 2015; Kim et al. 2013; Simon et al. 2015; Treusch et al. 2011), that can then be used to develop hypotheses on controls. Seasonal variations are also observed in bacterial, archaeal, and viral communities (Cram et al. 2014; Fuhrman et al. 2015; Giovannoni and Vergin 2012; Steinberg et al. 2001; Treusch et al. 2009). While the seasonality of these communities is often quite stable, they can be interrupted by periods of much higher variability, especially during bloom conditions (Lambert et al. 2019; Needham et al. 2018; Needham and Fuhrman 2016). Moreover, some locations such as the subtropical North Pacific Gyre (Ollison et al. 2021) reportedly have much weaker seasonality in bacterial, archaeal (Bryant et al. 2016), and viral communities (Luo et al. 2020). Hence, comprehensive, well-resolved, and long-term surveys from a variety of well-chosen global locations are needed to grasp the controls of short-term plankton dynamics and their influence on the ecosystem, as well as how these changes manifest under longer periods of natural variation (such as El Niño) and climate change (Fuhrman et al. 2015). Going forward, an understanding of ecosystem dynamics and of community composition will require integration of different scientific disciplines—with the diversity and activity of all three domains (Eukarya, Bacteria, and Archaea) and the viruses being addressed simultaneously (Needham et al. 2017). Small- and mesoscale physical phenomena as well as major currents, subduction, and upwelling must also be included because they all play a role in determining community composition (Bolaños et al. 2020) and influence carbon export (Guidi et al. 2016; Omand et al. 2015).

4.8.7 Climate Change and Conservation

Annual seasonal warming is a long-standing and important factor in determining phytoplankton community transitions and primary production (Lomas et al. 2013; Steinberg et al. 2001). What the marine microbiome is now confronted with is anthropogenically accelerated warming and acidification, which are predicted to change primary production over vast swaths of the ocean (Behrenfeld et al. 2006; Doney et al. 2009, 2012; Flombaum et al. 2013; Raven et al. 2005). Changes in primary production and species composition of resident communities have already been reported in the Arctic where the impact of warming is pronounced (Alexeev et al. 2012; Arrigo et al. 2008; Box et al. 2019; Graversen et al. 2008; Wassmann 2015). For example, picoeukaryotes like the tiny motile green alga *Micromonas* have increased in abundance in the Canadian Arctic while larger algae like diatoms have decreased (Li et al. 2009). These kinds of changes must have an impact on food webs but nothing is known about the consumers in the Arctic that might eat picoeukaryotes. Additionally, although conditions appear to be favorable now for *Micromonas* in the Canadian Arctic, a climate change laboratory study demonstrated that acidification via increased pCO_2 will result in a decreased motility of green algae, due to the loss of the flagellum (Wang et al. 2020). Hence, it is unclear how trajectories of Arctic phytoplankton will continue to change. The consequences of climate change for the myriad protists of the sea and the broader microbiome are grand challenges indeed.

How do we conserve communities that we still struggle to describe? Marine Protected Areas (MPAs) currently focus on fish and other economically important species (Edgar et al. 2014) or on hotspots of marine biodiversity such as coral reefs (McClanahan et al. 2006). It has been recommended that a substantial increase in ocean protection and strategic conservation planning that prioritizes highly protected MPAs would have multiple benefits on ecosystem services, protecting biodiversity, improving the yield of fisheries and securing marine carbon stocks (Sala et al. 2021). Modeling efforts could be used to start to tackle how protistan community composition, or invasion by one protistan group over another, might impact conservation efforts. Another approach is to recognize that there are likely more favorable versus less favorable biogeochemical states of marine habitats (Azam and Worden 2004). An example for a less favorable state brought about by human activity is coastal eutrophication, which can lead to mass occurrences of toxin producing photo- and mixotrophic protists in harmful algal blooms, which in turn impact other components of the marine food web (Glibert 2017). Identifying the “more” and the “less” desirable states requires assessment of the human populations living at the land–sea interface with respect to their needs and impacts, those of other living entities, and emergent feedback loops. As Cinda Scott explains, the best way to accomplish this recognition of the “whole living community” is to embrace the need for equity and for diverse voices to be heard (Box 4.8), something which should be prioritized in future marine microbiome research and more broadly across and between different shareholder groups and scientific disciplines.

Box 4.8

"At its very core, conservation has to be rooted in equity for ultimate success. Let's start reimagining how we can not only use Marine Protected Areas as a tool for conservation, but as a means to uplift, respect, and enhance others."

From *Cinda P. Scott, oral contribution, 2020.*

Note from the Authors— This chapter is not a comprehensive review but rather it reflects topics that the authors felt were interesting and important to consider with an emphasis on some of our favorites. Additionally, as authors, we struggled with inclusion of work by some scientists who have espoused despicable views with respect to human beings and origins or belief systems. We are aware of the concerning views and writings of these individuals, and in no way endorse them, rather we seek to present scientific ideas they formulated that facilitated the development of new concepts by the broader scientific community. In doing so, we recognize that rather than always thinking that we "stand on the shoulders of giants," it is important to remember that we are faulted human beings standing on the shoulders of other human beings and the faults of the individuals and systems in which they participated.

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