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Mixotrophy in the Marine Plankton

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Keywords

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Abstract

Mixotrophs are important components of the bacterioplankton, phytoplankton, microzooplankton, and (sometimes) zooplankton in coastal and oceanic waters. Bacterivory among the phytoplankton may be important for alleviating inorganic nutrient stress and may increase primary production in oligotrophic waters. Mixotrophic phytoflagellates and dinoflagellates are often dominant components of the plankton during seasonal stratification. Many of the microzooplankton grazers, including ciliates and Rhizaria, are mixotrophic owing to their retention of functional algal organelles or maintenance of algal endosymbionts. Phototrophy among the microzooplankton may increase gross growth efficiency and carbon transfer through the microzooplankton to higher trophic levels. Characteristic assemblages of mixotrophs are associated with warm, temperate, and cold seas and with stratification, fronts, and upwelling zones. Modeling has indicated that mixotrophy has a profound impact on marine planktonic ecosystems and may enhance primary production, biomass transfer to higher trophic levels, and the functioning of the biological carbon pump.

1. INTRODUCTION

DOC: dissolved organic carbon

GGE: gross growth efficiency

Mixotrophy refers to an organism's use of alternative forms of nutrition; it has traditionally been defined as alternative forms of carbon (C) acquisition but can also include acquisition of nitrogen (N), phosphorus (P), trace elements [e.g., iron (Fe)], trace nutrients (e.g., vitamins), and energy. Herein, we limit our consideration of mixotrophy to plankton living in the euphotic zone and organisms in which one of the trophic modes is phototrophy. In strict photoautotrophs, phototrophy involves C fixation, acquisition of chemical energy, and assimilation of inorganic nutrients. In mixotrophs, phototrophy can involve the use of light energy to produce chemical energy (ATP) and photoreductant (NADPH), either with or without C fixation, and may or may not involve uptake of dissolved N and P because these elements may be provided by ingestion of prey.

Mixotrophic plankton that combine phototrophic and heterotrophic nutritional modes are ubiquitous in the euphotic zones of estuaries, coastal seas, and the open ocean. They range in size from pico- to megaplankton and include prokaryotes, unicellular eukaryotic phytoplankton, protistan microzooplankton, and a few multicellular zooplankton. Some are obligate, whereas many others are facultative mixotrophs or are obligately mixotrophic during only part of their life cycle. There are many physiological types of mixotrophy among plankton (**Table 1**), and the benefits vary qualitatively as well as quantitatively (Stoecker 1998, Kirchman & Hanson 2013, Mitra et al. 2016).

Many phytoplankton and micro- and mesozooplankton can take up dissolved organic carbon (DOC) to some extent. However, herein we do not consider the uptake of DOC as a component of mixotrophy in protists, because it is ubiquitous and therefore not useful in discriminating among trophic strategies (Flynn et al. 2013). By contrast, uptake of DOC is the primary mode of nutrition in many bacteria and archaea; bacteria and archaea that additionally use light energy are mixotrophic (Eiler 2006).

Plankton biologists have long recognized that some organisms combine trophic modes, but the widespread occurrence of mixotrophy and its importance for energy budgets and elemental cycling in planktonic ecosystems was largely ignored until recently. Mixotrophy changes the basic trophic structure of euphotic marine ecosystems; most of the unicellular organisms at the base of the plankton food web can no longer be regarded as strictly producers or consumers (Flynn et al. 2013). Mixotrophic primary producers may use phagotrophy to supplement their inorganic nutrient acquisition and C budgets. Combining this behavior with phototrophy may boost consumers' gross growth efficiency (GGE, i.e., growth biomass divided by ingested biomass) (Stoecker et al. 2009, Schoener & McManus 2012). The overall effect is increased trophic transfer to higher levels in the food web compared with that expected in webs with only strict autotrophy and heterotrophy (Stoecker 1998, Mitra et al. 2016, Ward & Follows 2016). Mixotrophy may increase retention of N, P, and Fe in the upper water column; transfer organic matter more efficiently into larger size classes of plankton; and thereby improve the efficiency of the biological C pump that leads to C storage in the deep ocean (Flynn et al. 2013, Mitra et al. 2014b, Ward & Follows 2016).

One goal of this review is to present case studies of prominent types of mixotrophs that are important in coastal or oceanic ecosystems and describe their physiological and ecological properties so that their effects on ecosystem function can be better appreciated and modeled. A second goal is to provide an overview of the spatial and temporal distribution of mixotrophs, which should be useful to biological oceanographers and ecosystem modelers. A third goal is to encourage integrated physiological, ecological, and modeling studies of mixotrophy by outlining what is known and unknown about mixotrophy in the plankton. We also hope to dispel some common misconceptions about planktonic mixotrophs and their roles in marine ecosystems and to demonstrate why, where, and when mixotrophy is important.

Table 1 Planktonic mixotrophs that combine phototrophy and heterotrophy, along with terms used to describe them

General types	Includes	Case studies
I. Photoheterotrophic bacterioplankton	Bacteria with aerobic anoxygenic phototrophy Bacteria and archaea with proteorhodopsin-based phototrophy (Béja & Suzuki 2008, Kirchman & Hanson 2013)	*Roseobacter* spp. *Erythrobacter* spp. Gammaproteobacterial clade OM60 Widespread among bacteria and archaea
II. Phytoplankton that eat: photosynthetic protists with inherited plastids and the capacity to ingest prey (termed constitutive mixotrophs in Mitra et al. 2016)	Most mixotrophic nanoflagellates and dinoflagellates Mixotrophic protists from groups B, C, and D in Jones (1997) Phagotrophic algae and type II mixotrophic protists in Stoecker (1998)	*Ochromonas* spp. *Prymnesium parvum* Dinoflagellate examples: *Fragilidium subglobosum*, *Heterocapsa triquetra*, *Karlodinium veneficum*, *Neoceratium furca*, *Prorocentrum minimum*
III. Zooplankton that are photosynthetic: microzooplankton or metazoan zooplankton that acquire phototrophy through chloroplast retention[a] or maintenance of algal endosymbionts (Stoecker et al. 2009) [termed nonconstitutive mixotrophs (NCMs) in Mitra et al. 2016]	Mixotrophic protists from group A in Jones (1997) Photosynthetic protozoa and type III.B mixotrophic protists in Stoecker (1998)	
III.A. Generalists (termed GNCMs in Mitra et al. 2016)	Protists that retain chloroplasts and rarely other organelles from many algal taxa	Most plastid-retaining[a] oligotrich ciliates
III.B. Specialists (termed SNCMs in Mitra et al. 2016)	III.B.1. Protists that retain chloroplasts and sometimes other organelles from one algal species or very closely related algal species (termed pSNCMs in Mitra et al. 2016)	*Dinophysis* spp. *Mesodinium rubrum* (also called *Myrionecta rubra*)
	III.B.2. Protists or zooplankton with algal endosymbionts of only one algal species or very closely related algal species (termed eSNCMs in Mitra et al. 2016)	Metazooplankton with algal endosymbionts Most mixotrophic Rhizaria (Acantharea, Polycystinea, and Foraminifera) Green *Noctiluca scintillans*

[a]Chloroplast (or plastid) retention = sequestration = enslavement. Some plastid-retaining species also retain other organelles and prey cytoplasm.

2. CASE STUDIES OF MIXOTROPHY

2.1. Photoheterotrophic Bacteria and Archaea

AAP (or AAnP): aerobic anoxygenic phototrophy

PR: proteorhodopsin

PRP: proteorhodopsin-based phototrophy

Photoheterotrophy is a form of mixotrophy common in surface waters among bacteria, archaea, and some protists. It includes aerobic anoxygenic phototrophy (AAP or AAnP) and proteorhodopsin (PR)–based phototrophy (PRP) (Eiler 2006). In photoheterotrophs, the proton motive force generated by light can be used to drive active transport, motility, and ATP synthesis but is not used for C fixation; these microbes require organic C for growth.

AAP bacteria have bacteriochlorophyll and make up 1–30% of the bacteria in surface waters (Béja & Suzuki 2008, Kirchman & Hanson 2013). They belong to the alpha, beta, and gamma subclasses of the Proteobacteria, and common marine genera include *Roseobacter*, *Erythrobacter*, and the gammaproteobacterial clade OM60; these bacteria occur in estuaries, bays, and the open

ocean (Eiler 2006, Béja & Suzuki 2008). Not surprisingly, many AAP bacteria in culture grow faster in the light than in the dark (Hauruseu & Koblizek 2012, Kirchman & Hanson 2013). Based on theoretical calculations of costs and benefits, Kirchman & Hanson (2013) concluded that, although the cost of synthesizing the pigment complex is high, the net energy gain is sufficient to cover cell maintenance and prevent starvation. In general, AAP bacteria have relatively high growth rates and large cell sizes. They are probably highly susceptible to grazing and thus constitute an important component of microbial food webs. In culture, AAP bacteria are facultative mixotrophs because they can survive and grow in the dark when provided with DOC (Hauruseu & Koblizek 2012).

PR is present in 15% to >70% of all bacterioplankton in surface waters and has been reported in almost all bacterial and some archaeal taxa, although not all strains of a given species necessarily have PR; PR appears to be readily lost and gained through horizontal gene transfer (Kirchman & Hanson 2013). Some bacteria have a green PR with an absorption spectrum tuned to light in coastal waters, whereas others have a blue PR tuned to light in deep ocean waters (Béja & Suzuki 2008). PRP bacteria require DOC to grow but are thought to use the PRP to generate supplemental energy. In contrast to AAP bacteria, most cultured PRP bacteria do not grow faster in the light than in the dark, but light increases the survival of some cultured PRP strains during starvation (Gómez-Consarnau et al. 2010, Wang et al. 2012). Kirchman & Hanson (2013) calculated that, although the cost of synthesizing PR is low, the net energy gain is also low. PR bacteria are usually relatively small and slow growing compared with AAP bacteria, and many, such as members of the SAR11 clade, are adapted to oligotrophic conditions (Giovannoni et al. 2005). Under severe starvation conditions, PRP may be important for extending survival time, but it is difficult to evaluate whether the process makes a significant contribution to C cycling by bacterioplankton.

2.2. Mixotrophic Phytoflagellates

Mixotrophic phytoflagellates are motile protists that have well-integrated chloroplasts (evolutionarily and physiologically) but can also ingest particulate food. Algae capable of this type of mixotrophy are phylogenetically diverse, including many chrysophytes, haptophytes, cryptophytes, silicoflagellates (dictyophytes), and even some chlorophytes (Bell & Laybourn-Parry 2003, Sanders & Gast 2012, Gerea et al. 2016) (**Figure 1*a*,*d***). The occurrence of mixotrophy across these disparate algal lineages is presumably a testament to the effectiveness of this ecological strategy (Flynn & Mitra 2009). Mixotrophic algae possess a wide range of behaviors and abilities and may employ phagotrophy to meet a variety of cellular needs, including the acquisition of C (for energy or cellular growth), macronutrients (N and P), and various micronutrients (e.g., vitamins and trace elements). Most species engulf and digest bacteria, cyanobacteria, or tiny eukaryotes, but some—such as the haptophyte *Prymnesium parvum*—produce toxins that can immobilize and kill prey much larger than themselves (Tillmann 2003).

Demonstrating mixotrophy by algae in natural planktonic communities is problematic (see below), and what we know about the physiology of mixed nutrition of these species comes from work with cultures. We often conceptualize mixotrophic phytoflagellates as existing along a continuum from nearly pure phototrophy to nearly pure heterotrophy, but individual species typically exhibit particular strategies and/or constraints. For example, some species are obligately phototrophic; they consume prey but also require light in order to grow. Some aspects of their nutrition appear to be dependent on their own photosynthetic activity. Some of these obligate phototrophs can be grown completely phototrophically, without any particulate food, if nutrients and certain growth factors (e.g., some vitamins) are provided as dissolved substances in the culture medium. Others, such as some chrysophyte species within the genera *Ochromonas* and *Poterioochromonas*, are marginally dependent on photosynthesis and will grow readily in continuous darkness as long as

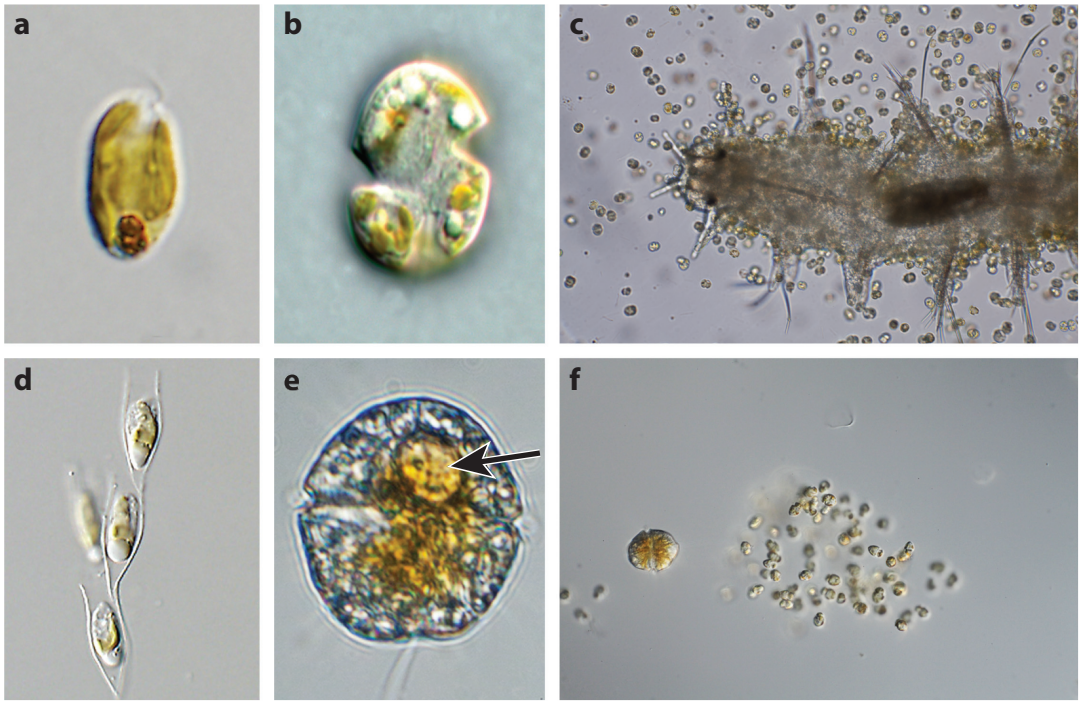

Figure 1

Examples of phagotrophic algae. (*a*) A bloom-forming and toxic haptophyte, *Prymnesium parvum* (10 μm in length), from Danish waters containing a reddish food vacuole in the posterior part of the cell. (*b*) A bloom-forming and toxic dinoflagellate, *Karlodinium armiger* (15 μm in length), from the Mediterranean. (*c*) Hundreds of *K. armiger* cells swarming around and attacking a polychaete. The polychaete will eventually be killed, and the dinoflagellates will consume the much larger prey. (*d*) A small colony (four cells) of the chrysophyte *Dinobryon* sp. (15 μm in length). (*e*) A dinoflagellate, *Alexandrium pseudogonyaulax* (30 μm in diameter), containing a food vacuole (*arrow*). (*f*) *A. pseudogonyaulax* using a mucus trap to catch *Heterocapsa rotundata* cells. The mucus trap is attached to the dinoflagellate via the longitudinal flagellum. Photos taken by Gert Hansen (panels *a* and *d*), Niels Daugbjerg (panel *b*), Terje Berge (panel *c*), and Hannah Blossom (panels *e* and *f*). Panel *e* reproduced from Blossom et al. (2012) with permission from Elsevier.

bacteria are present as prey (Caron et al. 1990, Rottberger et al. 2013). These latter mixotrophs appear to employ phototrophy largely as a survival strategy in the absence of abundant prey.

The distributions, abundances, and activities of phagotrophic phytoflagellates in plankton communities have been difficult to characterize. Taxonomic surveys of phytoplankton can establish the presence of some species that are known to be mixotrophic, but that does not necessarily mean that these species are acting heterotrophically at a particular time and place. Most studies have therefore attempted to estimate the abundances and heterotrophic activity of mixotrophic algae using the uptake of fluorescently labeled or radiolabeled prey (usually bacteria) to demonstrate phagotrophic activity in chloroplast-bearing protists. In the former approach, labeled prey are added to natural samples, incubated, and then examined by fluorescence microscopy to determine the number of small protists that display chlorophyll autofluorescence (indicating the presence of chloroplasts) and also contain ingested fluorescent prey. Given that the heterotrophic activity of algae in a sample can be confirmed only by the presence of ingested fluorescent prey, this method provides a minimum estimate of the number of mixotrophic algae.

Mixotrophic phytoflagellates have been known to constitute an important component of freshwater ecosystems for 30 years (Bird & Kalff 1986), and they have recently become a topic of intense

interest in biological oceanography because they have been shown to be an important component of some marine phytoplankton communities. Marine mixotrophic flagellates are globally distributed, occurring commonly in coastal and oceanic waters and latitudinally from the Arctic Ocean to Antarctic coastal waters (Arenovski et al. 1995, Safi & Hall 1999, Moorthi et al. 2009, Unrein et al. 2010, Sanders & Gast 2012). Studies have found that half of the small phytoplankton in the open ocean may be mixotrophic and account for a significant fraction of bacterial mortality (Zubkov & Tarran 2008; Hartmann et al. 2012, 2013; Unrein et al. 2014).

2.3. Mixotrophic Dinoflagellates

Mixotrophic nutrition among dinoflagellates is diverse. At least one phagotrophic dinoflagellate, *Oxyrrhis marina*, has PRP that is thought to have been gained through horizontal gene transfer from bacteria (Guo et al. 2014). *O. marina* grown in the light contains PR, which gives the cells a pink color. Without food, *O. marina* survives much longer in the light than in the dark, which suggests that phototrophy is important in supplying energy for metabolism (Guo et al. 2014). Some other nonplastidic dinoflagellates are pinkish, and it will be interesting to see whether they also use PRP to prolong survival when prey are scarce.

The mixotrophic dinoflagellates with plastids can be split into several groups based on their physiology and the origin of their plastids. The first group comprises species that have inherited chloroplasts (**Figure 1*b*,*c*,*e*,*f***). Dinoflagellates are often considered photoautotrophs, but many are facultative mixotrophs (reviewed in Jeong et al. 2010 and Hansen 2011). These species play an important role in marine waters, particularly during stratified conditions. They typically grow well on standard inorganic nutrient growth media (with nitrate as a N source) if light is supplied. All members of this group, with a few notable exceptions (e.g., *Fragilidium subglobosum*), depend on light for growth and survival and cannot grow in the dark even when supplied with prey (Hansen 2011). Prey intake is often influenced by light, generally increasing with increasing irradiance; thus, photosynthesis appears to stimulate feeding (Li et al. 1999). However, in many of the red tide dinoflagellates (e.g., *Prorocentrum minimum* and *Heterocapsa triquetra*), food uptake in inorganic growth media is low irrespective of irradiance. Thus, food uptake in these cases does not substitute C gained through photosynthesis. Additionally, prey selectivity and ingestion rates vary. For example, *Neoceratium furca* (formerly *Ceratium furca*) preferentially ingests small ciliates (Smalley et al. 2003, 2012), and ingestion rates for *P. minimum* are higher on *Teleaulax amphioxeia* than on other cryptophytes (Johnson 2015). Concentrations of inorganic nutrients (N or P) can also affect phagotrophy by this group of dinoflagellates. For example, species such as *F. subglobosum* and *Karlodinium veneficum* can depend on prey ingestion for the bulk of their C requirements even in the presence of ample inorganic nutrients (Skovgaard et al. 2000, Calbet et al. 2011). Other species do not feed at all, or at least very little, when dissolved inorganic nutrients are present in excess; the best-documented example of this is *N. furca* (Smalley et al. 2003, 2012), but many more dinoflagellates likely belong to this group. Thus, C gained from phagotrophy within the first group depends on species, irradiance, availability of suitable prey, and nutrient concentrations.

A second group comprises dinoflagellates that lack their own chloroplasts but harbor ecto- or endosymbionts. Dinoflagellates with ectosymbionts (e.g., *Ornithocercus* and *Histioneis*) can be found in warm N-poor oceanic surface waters (Tarangkoon et al. 2010). The ectosymbionts are all cyanobacteria but apparently are not involved in N_2 fixation, as the large majority of the *nifH* genes found in the dinoflagellates are typically of heterotrophic bacterial origin (Farnelid et al. 2010). The best example of a dinoflagellate with endosymbionts is the green *Noctiluca scintillans* (**Figure 2*c***), which forms huge blooms in Southeast Asian and Arabian seas (Harrison et al. 2011, Gomes et al. 2014). This species harbors thousands of free-swimming endosymbiotic green

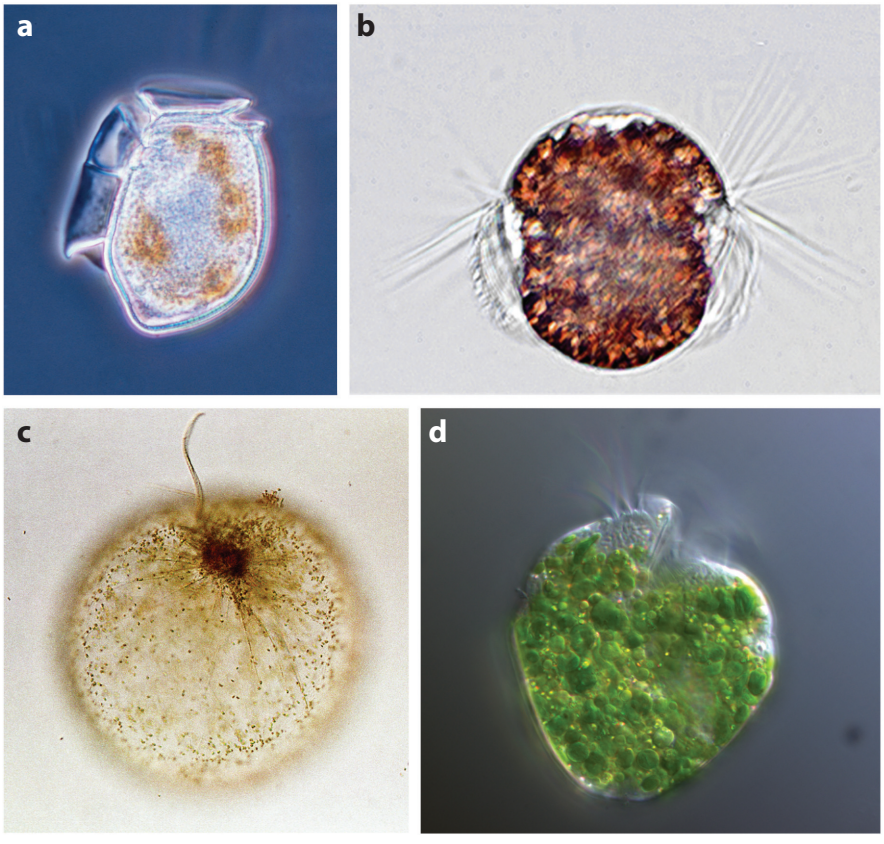

Figure 2

Examples of ciliates and dinoflagellates, which acquire chloroplasts and sometimes other cell organelles from their prey. (*a*) The toxic dinoflagellate *Dinophysis acuta* (60 μm in length). This dinoflagellate harbors chloroplasts of cryptophyte origin, which it sequesters from its prey. It cannot catch the cryptophytes by itself, and instead relies on ingesting ciliates (red *Mesodinium* spp.), which sequester their chloroplasts from a specific cryptophyte clade (*Geminigera/Plagioselmis/Teleaulax*). (*b*) The ciliate *Mesodinium major* (50 μm in diameter), one of the red *Mesodinium* species. (*c*) The green dinoflagellate *Noctiluca scintillans* (500–1,000 μm in diameter). In Southeast Asia, blooms of this dinoflagellate are common. It harbors thousands of intact green pedinophytes (*Protoeuglena noctilucae*), which swim freely around inside it. (*d*) The oligotrich ciliate *Strombidium rassoulzadegani* (60 μm in length). Oligotrich ciliates in marine waters often sequester chloroplasts and other cell organelles from algal prey. *S. rassoulzadegani* is common in marine tide pools and sequesters chloroplasts from a variety of green algal and cryptophyte prey. Photos taken by Per Juel Hansen (panels *a* and *c*), Lydia Garcia-Cuetos (panel *b*), and George McManus (panel *d*). Panel *b* reproduced from Garcia-Cuetos et al. (2012) with permission from Wiley; panels *c* and *d* reproduced from Hansen et al. (2004) and Schoener & McManus (2012), respectively, with permission from Inter-Research.

algae that were originally described as prasinophytes (*Pedinomonas noctilucae*) (Sweeney 1976), but recent molecular analyses have revealed that they are pedinophytes (*Protoeuglena noctilucae*) (Wang et al. 2016). *N. scintillans* depends mostly on photosynthesis for its C requirements (Hansen et al. 2004). Some strains lose their endosymbionts under prey-limiting conditions (Hansen et al. 2004), whereas others maintain their endosymbionts and can grow in inorganic media even in the absence of prey (Furuya et al. 2006).

A third group contains species that also lack their own chloroplasts but are able to sequester functional chloroplasts from their prey; all other cell organelles are lost (**Table 1**). In *Dinophysis* spp. (**Figure 2*a***), the sequestered chloroplasts are of cryptophyte origin; in *Phalacroma mitra* (formerly *Dinophysis mitra*), recent reports of chloroplast genes acquired from a variety of algal species indicate that the chloroplasts are primarily of haptophyte origin (Nishitani et al. 2012). *Dinophysis* spp., which are important diarrhetic shellfish toxin producers, acquire their cryptophyte chloroplasts by ingesting ciliates (red pigmented *Mesodinium* spp.) (Kim et al. 2012); they cannot sequester the cryptophyte chloroplasts directly from cryptophyte prey. *Dinophysis* spp. are marine obligate mixotrophs and require light for growth. Cells can divide three or four times when starved of prey and can remain photosynthetically active for several months (Park et al. 2008; Nielsen et al. 2012, 2013). At high prey concentrations, 70–90% of the gross C could be obtained through prey uptake. However, at lower prey concentrations, C derived from prey may be 0–55% of gross C uptake. This prolonged functioning of sequestered plastids in *Dinophysis* spp. appears to be supported by several dinoflagellate nucleus-encoded proteins that are involved in metabolite transport and protection of plastid photosystems from stress (Wisecaver & Hackett 2010).

A fourth group of dinoflagellates retains more or less intact prey cells (cryptophytes or haptophytes) and keeps them functional from a few hours to weeks (Jakobsen et al. 2000, Gast et al. 2007, Sellers et al. 2014). Some of these species can be grown in the dark, whereas others cannot. Nevertheless, growth rates and ingestion rates are often stimulated by light, and their growth response to light is similar to that of their prey (Jakobsen et al. 2000).

2.4. Mixotrophic *Mesodinium*

The ciliate genus *Mesodinium* contains a wide nutritional spectrum of species, ranging from almost entirely phototrophic species to completely heterotrophic species. The best-known species is *Mesodinium rubrum* (formerly *Myrionecta rubra*), a photosynthetic, red-pigmented ciliate (**Figure 2*b***) that has a worldwide distribution (Taylor et al. 1971, Lindholm 1985, Crawford 1989). *M. rubrum* can dominate ciliate biomass in the plankton and contribute to a substantial part of the chlorophyll biomass (reviewed in Stoecker et al. 2009). They are also well known as red tide formers in coastal temperate waters, and during blooms they can dominate chlorophyll biomass and primary production (Ryther 1967, Crawford et al. 1997).

Recent studies have shown that *Mesodinium rubrum* is actually a species complex. Two species, *M. rubrum* and *M. major* (**Figure 2*b***), are now well described (Garcia-Cuetos et al. 2012), and molecular studies indicate that many more species may exist within this complex (Herfort et al. 2011). The isolates studied so far all rely on red-pigmented cryptophytes within the *Geminigera/Plagioselmis/Teleaulax* clade as prey (Hansen et al. 2012). The ciliates sequester not only the chloroplasts from the cryptophyte prey, but also the cryptophyte nuclei, nucleomorphs, mitochondria, and some cytoplasm.

The red *Mesodinium* spp. are all obligate mixotrophs and rely on light and ingestion of cryptophyte prey for sustained growth (Gustafson et al. 2000, Hansen & Fenchel 2006, Park et al. 2007). Ingestion rates are generally low, and the ingestion of just one cryptophyte per day (equivalent to 1% of the C required for growth) can sustain one doubling per day, indicating that these ciliates can grow almost entirely phototrophically (Smith & Hansen 2007). The red *Mesodinium* spp. have some control of their acquired cryptophyte cell organelles and are able to divide the plastids three or four times when starved of cryptophyte prey. Studies have shown that the ingested cryptophyte nuclei are transcriptionally active inside the ciliate (Johnson et al. 2007) and that red *Mesodinium* spp. can photoacclimate (Johnson & Stoecker 2005, Moeller et al. 2011). Transcriptional changes in the stolen cryptophyte nucleus suggest that the ciliate host exerts

some control over the expression of cryptophyte genes and the delivery of cryptophyte proteins from the cryptophyte nucleus to the plastids (Lasek-Nesselquist et al. 2015). Red *Mesodinium* spp. assimilate dissolved inorganic nitrate and phosphate (Tong et al. 2015), but sustained ciliate growth is linked to the presence of cryptophyte nuclei (Johnson et al. 2006, Moeller et al. 2011). Nevertheless, red *Mesodinium* spp. can survive for up to four months without prey, depending on the temperature and light conditions (Johnson et al. 2006, 2007; Smith & Hansen 2007).

2.5. Chloroplast-Retaining Oligotrich Ciliates

Oligotrich ciliates usually dominate the planktonic ciliate assemblage and are important grazers on smaller cells in the plankton. On average, 30–40% of the oligotrichs in the euphotic zone are mixotrophic owing to sequestration of functional plastids from phytoplankton prey (Dolan & Perez 2000, Stoecker et al. 2009). Although not all types of plastids may be retained, sequestered plastids from more than one algal family can be observed within a species and within an individual oligotrich. Thus, they are generalists with respect to plastid retention (**Table 1**). The retained plastids do not divide in the ciliate, and plastid turnover is usually rapid in the light, with plastids continuously replaced through ingestion (Stoecker et al. 2009, Schoener & McManus 2012).

Chloroplast-retaining oligotrich ciliates include *Laboea strobila*, *Tontonia* spp., some *Strombidium* spp. (**Figure 2*d***), and *Cyrtostrombidium* spp. (reviewed in Stoecker et al. 2009). Volume-specific chlorophyll content and volume-specific photosynthetic capacity vary among species (Jonsson 1987, Stoecker et al. 1988/1989). Most (and perhaps all) chloroplast-retaining species seem to be obligate mixotrophs in culture (reviewed in Stoecker et al. 2009 and Schoener & McManus 2012). Enigmatically, at least one plastidic *Strombidium* species can grow in the dark when provided with a photosynthetic dinoflagellate as prey, although the same ciliate species cannot grow in the light with the dinoflagellate as the only prey; in the light, the ciliate requires phytoflagellates, from which it sequesters plastids (McManus et al. 2012). Another plastidic oligotrich, *L. strobila*, does not survive in culture without phytoflagellates as food and a source of plastids (Stoecker et al. 1988), but it does survive through the darkness of winter in polar waters, although it is not known to make cysts (Levinsen et al. 2000a). Some sequestered plastids are used more effectively than other types, affecting the GGE of the host (Schoener & McManus 2012). These observations suggest that the dependence of plastid-retaining species on mixotrophy is influenced in complex ways by both light and the type of prey (and source of plastids).

Chlorophyll-specific rates of photosynthesis in plastidic oligotrichs are usually similar to those in phytoplankton in the same environment (Putt 1990a). Light-saturated rates of photosynthesis are sufficient to increase survival and/or enhance growth in many species (Jonsson 1987, Stoecker et al. 2009). Photosynthate is used primarily to meet respiratory demands for C (Putt 1990b). Respiration experiments indicate that, in the light, cell C is spared from respiration (Stoecker & Michaels 1991). This may be due to very rapid turnover of photosynthate and/or production of ATP that is used by the ciliate. The chloroplast-retaining oligotrichs are thought to obtain most of their N and P from ingestion of prey, but quantitative data are lacking on their N and P budgets (reviewed in Stoecker et al. 2009).

Decrease in C loss caused by respiration probably delays starvation and prolongs survival of plastidic oligotrichs in postbloom periods, when phytoplankton biomass is low (Crawford & Stoecker 1996, Stoecker et al. 2009). Mixotrophy has the potential to increase the GGE of plastid-retaining oligotrichs over the ~30% estimated for heterotrophic ciliates (Putt 1990b, Stoecker et al. 2009). Depending on the algal food, GGE in the mixotroph *Strombidium rassoulzadegani* can be as high as 56% at low food concentrations (Schoener & McManus 2012). Measurements of body C spared from respiration indicate that GGE may sometimes be as high as 60% in some

mixotrophic oligotrichs (Stoecker & Michaels 1991) (**Figure 2*d***). Mixotrophic oligotrichs may have lower maximum growth rates than strictly heterotrophic ciliates of similar cell size (Pérez et al. 1997); however, at low or variable algal food concentrations, mixotrophs may have an advantage over strictly heterotrophic oligotrichs. Few data are available to compare the growth rates of mixotrophic and heterotrophic ciliates in situ, but at least in cold seas, mixotrophic oligotrichs can have growth rates that are the same or higher than those of heterotrophic oligotrichs (Franzè & Lavrentyev 2014).

2.6. Rhizarian Symbioses

Several lineages within the supergroup Rhizaria are composed of large, amoeba-like phagotrophic protists (Pawlowski & Burki 2009), including the Foraminifera and the Radiolaria (which are now rather broadly defined to include the Acantharea, Polycystinea, and Taxopodida). These species include some of the largest protistan single cells (>1 cm), and many form colonies that can be much larger (**Figure 3*f***). These Rhizaria construct large, complex, pseudopodial networks with which they entangle, engulf, and digest a wide variety of prey (Swanberg 1983, Swanberg & Caron 1991, Anderson 1993). In addition, most surface-dwelling species combine phototrophy and heterotrophy by harboring intracellular symbiotic algae (**Figure 3**). These associations, called holobionts, have been well known since Haeckel's time (Haeckel 1887).

Symbiont-bearing Rhizaria are relegated largely to oceanic waters and locations where oceanic waters impinge on islands and coastlines, but in the vast oligotrophic regions of the world ocean they are some of the most conspicuous organisms in the plankton. Nonetheless, the global abundances and biomass of symbiont-bearing Rhizaria have been grossly underestimated by traditional methods of plankton collection and preservation; these methods destroy these delicate forms or dissolve the skeletal structures, which are formed of celestite ($SrSO_4$), calcite ($CaCO_3$), or opal (SiO_2). A new appreciation for the importance of these species has been stimulated by a recent global gene sequencing survey that found high abundances of rhizarian sequences throughout the ocean (de Vargas et al. 2015) and by community networking analyses and in situ observations showing that they play an important role in the sinking of C from the upper layers of the ocean (Biard et al. 2016, Guidi et al. 2016).

Most individual rhizarian-symbiont associations appear to be rather specific, but algal endosymbionts vary among groups. For example, a recent study reported that haptophytes from the genus *Phaeocystis* are widespread symbionts in the Acantharea (Decelle et al. 2012) (**Figure 3*a***). In *Orbulina universa* and some other spinose Foraminifera (**Figure 3*d***), the symbionts are *Pelagodinium béii* (Siano et al. 2010), a photosynthetic dinoflagellate related to but distinct from *Symbiodinium* spp., which are symbionts in many corals. Many polycystines appear to harbor a different dinoflagellate symbiont, *Scrippsiella nutricula* (Gast & Caron 2001). Symbionts in larger planktonic Rhizaria appear to be horizontally transmitted (with acquisition of potential endosymbionts from the water column), and the molecular mechanisms controlling cell-cell recognition, establishment of these specific relationships, and maintenance are not known.

In species where it has been examined, symbiont morphology changes substantially in the symbiotic state (e.g., the loss of thecal plates in dinoflagellates), and symbionts continue to multiply in the cytoplasm throughout the life of the host when particulate food is present. This implies that the host's cytoplasm is a favorable microenvironment for symbiont activity and growth. Moreover, rhizarian holobionts are microenvironments of elevated primary production (Caron et al. 1995), in part because of the high density of algae within the cytoplasm of the host (**Figure 3**) and also possibly because of high rates of primary production per individual symbiont.

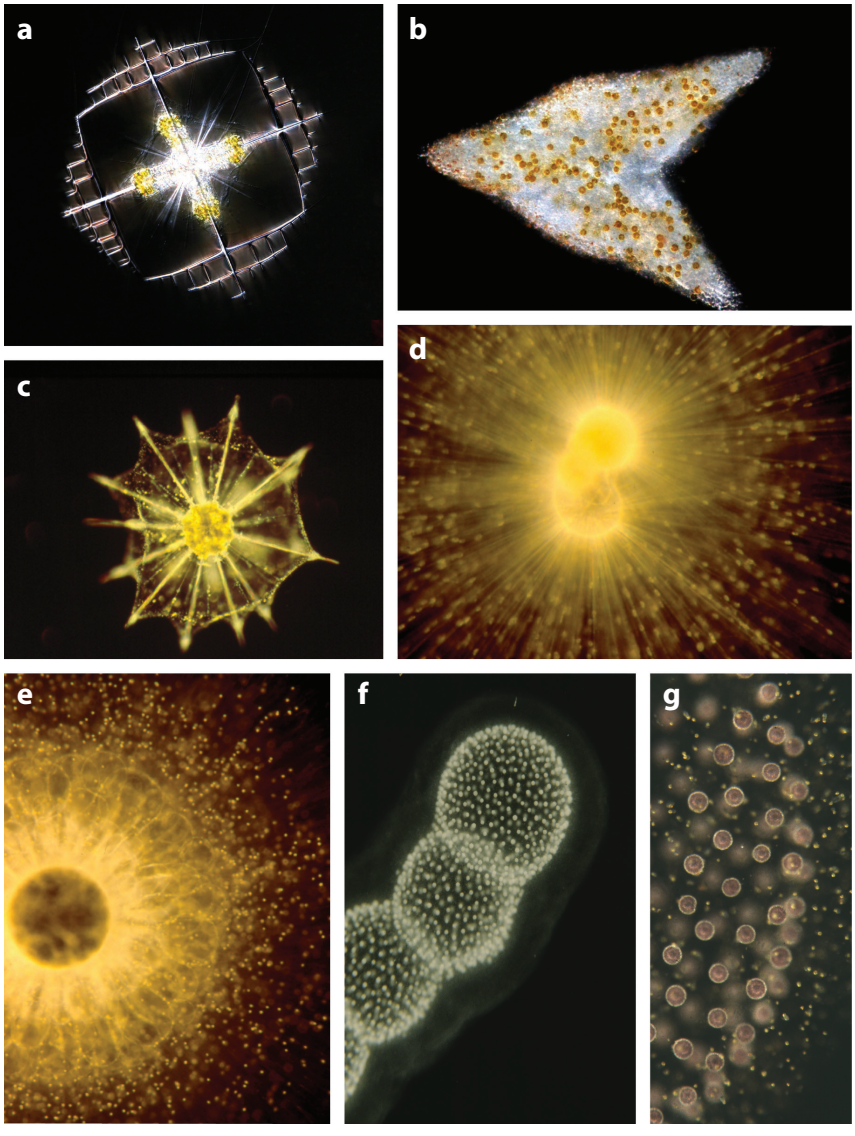

Figure 3

Examples of planktonic Rhizaria (Acantharea, Polycystinea, and Foraminifera) possessing endosymbiotic algae. (*a*) An ornate acantharian, *Lithoptera* sp. (~300 μm in diameter), showing the presence of numerous endosymbiotic algae at four locations within the endoplasm (*yellow-green*). (*b*) A solitary radiolarian, *Dictyocoryne elegans* (~300 μm in length), with golden endosymbionts. (*c*) An unidentified acantharian (~300 μm in longest dimension) with symbionts dispersed throughout the ectoplasm and concentrated around the endoplasm. (*d*) A multichambered life stage of the planktonic foraminifer *Orbulina universa* (200 μm in maximal length), with a few thousand dinoflagellate endosymbionts strewn within the host's cytoplasm along its calcite spines. (*e*) A solitary radiolarian, *Thalassicola nucleata*, and its symbiotic algae, visible as small golden cells in the pseudopodial networks extending from the radiolarian central capsule (dark central capsule, ~250 μm in diameter). (*f*,*g*) Two symbiont-bearing colonial radiolarians. The radiolarian central capsules (~100 μm in diameter) are visible as small white dots in panel *f*; panel *g* shows a higher magnification of a different colony (*Collosphaera* sp.) in which the endosymbiotic algae are visible as small golden dots within the radiolarian pseudopodial network. Photos by Johan Decelle (panels *a* and *b*) and David A. Caron (panels *c*–*g*).

HNLC regions: high-nutrient, low-chlorophyll regions (usually Fe-limited regions)

The nature of the relationship between rhizarian hosts and their symbionts—that is, whether these associations represent true mutualisms or merely farming of algae by the host as an energy source to be used later in ontogeny—is still a matter of debate. The available evidence appears to favor the latter in some species. Symbionts in at least some planktonic Foraminifera are digested at the time of host reproduction, which involves cellular division and reorganization into hundreds of thousands of minute, reproductive swarmer cells (Bé et al. 1983). The wholesale symbiont digestion that occurs at the onset of this life stage presumably supplies energy and C for this massive cell division.

3. THE REGIONAL AND SEASONAL IMPORTANCE OF MIXOTROPHS

3.1. Tropical and Subtropical Seas

Mixotrophy occurs in many bacterioplankton, phytoplankton, and microzooplankton and (to a lesser extent) in metazoan zooplankton inhabiting warm surface waters. Photoheterotrophic bacteria with PRs are major contributors to plankton biomass in warm oligotrophic oceans. Swan et al. (2013) have hypothesized that photoheterotrophy allows bacterioplankton to survive periods of starvation and permits genome streamlining and metabolic specialization. Small mixotrophic phytoflagellates are important contributors to photosynthesis and bacterivory in warm oligotrophic seas (Unrein et al. 2007, Stukel et al. 2011, Hartmann et al. 2012). Mixotrophic phytoflagellates and photosynthetic dinoflagellates can account for much of the grazing on picophytoplankton (Sanders et al. 2000, Unrein et al. 2007).

The tropical and subtropical microzooplankton include many species with acquired phototrophy. Plastidic oligotrich ciliates constitute 17–54% of all ciliates and contribute up to 20% of total chlorophyll in oligotrophic regions of the Mediterranean (Dolan & Marrase 1995, Pitta & Giannakouru 2000). However, in the oceanic subtropical gyres and high-nutrient, low-chlorophyll (HNLC) regions, plastidic ciliates are seldom abundant (reviewed in Stoecker et al. 2009). Phagotrophic dinoflagellates with algal ecto- and endosymbionts are typically found in warm oligotrophic waters, but at low abundances (Foster et al. 2006, Tarangkoon et al. 2010).

The large Rhizaria (Acantharea, Polycystinea, and Foraminifera) with acquired phototrophy are important grazers and predators as well as sites of enhanced primary production in oligotrophic warm oceans (Caron & Swanberg 1990, Anderson 1993, Caron et al. 1995). They also constitute important, often dominant components of the mixotrophic biomass in the micro- and mesoplankton size classes in these ecosystems. The calculated contribution of Rhizaria to total primary production is usually only a few percent, but because most primary production in oligotrophic waters is conducted by picoplankton, the contribution of Rhizaria to primary production in larger size classes can be significant (Caron et al. 1995). Although little is known about the trophic role of Rhizaria in food webs, they are clearly important components of the biological C pump (reviewed in Caron & Swanberg 1990 and Stoecker et al. 2009). In addition to their contribution to C flux, the skeleton-forming species are important contributors to the particulate flux of calcium carbonate and silicate and to biogeochemical cycling of strontium and barium (reviewed in Caron & Swanberg 1990 and Stoecker et al. 2009).

Mixotrophs also occur in nonoligotrophic warm waters. In coastal waters of Southeast Asia and India, green mixotrophic populations of the dinoflagellate *N. scintillans* form blooms (Harrison et al. 2011). In recent years, massive outbreaks of green *N. scintillans* have occurred offshore in the Arabian Sea; the blooms appear to be related to upwelling of hypoxic waters caused by changes in climate forcing. Green *Noctiluca* is a voracious predator of diatoms, and during the

winter monsoon, *Noctiluca* blooms have begun to replace diatom blooms, probably because of a combination of predation and changes in the environment that favor growth of the dinoflagellate over that of the diatoms; this change in plankton species composition is expected to disrupt food webs supporting regional fisheries (Gomes et al. 2014).

In warm waters, mixotrophy is not restricted to the protistan plankton. Planktonic planulae of many reef-building corals have dinoflagellate endosymbionts, which are important to the survival, dispersal, and settling success of the planulae (Gaither & Rowan 2010). Among the holoplanktonic invertebrates, several warm-water coastal medusae are mixotrophic and have dinoflagellate endosymbionts (Muscatine et al. 1986, Kremer et al. 1990). Planktonic mixotrophic acoel flatworms with algal endosymbionts are widespread in oligotrophic tropical and subtropical seas (Stoecker et al. 1989, Lima-Mendez et al. 2015).

3.2. Temperate and Subpolar Seas

Mixotrophs are a predictable part of plankton succession in seas with a strong seasonal cycle (Mitra et al. 2014b). During spring and autumn blooms, conditions are optimal for strictly phototrophic phytoplankton, such as diatoms. As inorganic nutrients become limiting after the spring bloom, conditions become favorable for mixotrophic phytoflagellates and dinoflagellates, which can obtain N and P from ingestion as well as from assimilation of dissolved inorganic nutrients (Havskum & Riemann 1996, Mitra et al. 2014b). During stratification, grazing by small phytoflagellates may account for 40% or more of bacterivory in surface waters (Zubkov & Tarran 2008). Some phytoflagellates and dinoflagellates ingest other protists, and by eating their competitors, they may reduce competition for inorganic nutrients and light (Thingstad et al. 1996). Most of the haptophyte, raphidophyte, and dinoflagellate species responsible for harmful or ecosystem-disruptive algal blooms are mixotrophs (Sunda et al. 2006, Burkholder et al. 2008, Jeong et al. 2010). Decreased irradiance and deep winter mixing following the autumnal bloom can result in light limitation of phytoplankton. During these conditions, mixotrophic phytoplankton may also have an advantage over strictly phototrophic phytoplankton in C acquisition (Hall et al. 1993, Czypionka et al. 2011).

As part of seasonal succession, mixotrophic microzooplankton can become as abundant as or more abundant than strictly heterotrophic microzooplankton (Mitra et al. 2014b). During the spring or autumn blooms, particulate food is plentiful, favoring strict phagotrophs, but when food becomes limiting, microzooplankton that can supplement their energy and C budget with acquired phototrophy increase in abundance (Mitra et al. 2014b). Under low-chlorophyll conditions, plastidic oligotrich ciliates often dominate ciliate biomass and can be important contributors to chlorophyll and photosynthesis in the microplankton size class (Stoecker et al. 2009, 2014).

The photosynthetic red *Mesodinium* spp. are present year round but are often particularly abundant under low-light conditions, such as in turbid coastal waters, in deep chlorophyll maximum layers, and during winter (Packard et al. 1978, Crawford 1989, Weber et al. 2014). *Mesodinium* spp. are often highly abundant where inorganic nutrients are plentiful, such as in upwelling/downwelling areas associated with fronts or in areas subject to tidal mixing (Herfort et al. 2012). Both mixotrophic oligotrichs and *Mesodinium* spp. are preferred prey of crustacean zooplankton and first-feeding fish larvae (Figueiredo et al. 2007, Fileman et al. 2007, Dutz & Peters 2008), which makes them an important part of food webs in temperate and boreal seas.

Temperate and boreal seas include two oligotrophic HNLC regions: the northeastern subarctic Pacific and the Southern Ocean. Some mixotrophic phytoflagellates can alleviate Fe limitation by ingesting bacteria (Maranger et al. 1998), but little information is available on the importance of

mixotrophic flagellates in cold-water HNLC regions. Large plastid-containing oligotrich ciliates often dominate the ciliate biomass in cold-water HNLC regions (Saito et al. 2005, Christaski et al. 2008, Assmy et al. 2014).

3.3. Polar Seas

The Arctic and Antarctic seas are characterized by extreme seasonality and low water temperatures. Bacterivorous mixotrophic protists are common in the water column and in sea ice microbial communities (Moorthi et al. 2009, Sanders & Gast 2012). In the oligotrophic central Arctic Ocean, *Micromonas* sp. (a picoplanktonic prasinophyte) can dominate bacterivory. The bacterivorous colonial chrysophyte alga *Dinobryon balticum* is often an important component of the plankton in deep, detritus-rich layers of the Arctic Ocean (Olli et al. 2002), and ingestion of bacteria presumably supplements C fixation. However, there is no evidence that the major bloom-forming phytoplankton in cold waters (diatoms and *Phaeocystis* spp.) are mixotrophic.

Interestingly, *Phaeocystis* spp. are an important source of algal endosymbionts and perhaps plastids for several polar species with acquired phototrophy. Polar Acantharea have *Phaeocystis* endosymbionts (Decelle et al. 2012). *Phaeocystis antarctica* is also the source of plastids for an Antarctic dinoflagellate that retains plastids (Gast et al. 2006). Ciliates with acquired phototrophy are typically abundant in subpolar and polar seas, particularly in the Arctic during summer stratification (Putt 1990a, Davidson et al. 2010, Stoecker et al. 2014). As in temperate waters, the plastidic oligotrichs and red *Mesodinium* spp. are preferred prey of zooplankton and fish larvae and are thus important in polar planktonic food webs (Levinsen et al. 2000b).

3.4. Upwelling, Fronts, and Mixing Events

Upwelling, fronts, and mixing events can produce blooms followed by a plankton succession that is similar in pattern to that which occurs seasonally. However, in contrast to spring blooms, blooms at fronts and in convergence zones are often dominated by motile mixotrophs, such as photosynthetic dinoflagellates and red *Mesodinium* spp. (Crawford et al. 1997). When inorganic nutrients and light are abundant, growth is probably supported primarily by phototrophy. When inorganic nutrients become limiting, the populations probably rely increasingly on phagotrophy for acquisition of N and P.

4. GENERALIZATIONS

Five main generalizations can be drawn from the studies discussed above. First, mixotrophs differ widely in their biology (**Table 1**) and therefore also differ widely in the roles they play in food webs (**Figure 4**) and in their impact on ecosystem processes. The photoheterotrophic bacteria acquire energy but not C from phototrophy. Phagotrophic phytoflagellates and many dinoflagellates are innately phototrophic, but under some conditions they may acquire C, N, P, trace elements, or other essential growth factors from prey. Mixotrophy is facultative in photoheterotrophic bacteria and in many (but not all) mixotrophic phytoflagellates and dinoflagellates. By contrast, mixotrophy is often obligate for sustained growth and reproduction in micro- and mesozooplankton with acquired phototrophy. Microzooplankton with acquired phototrophy obtain organic compounds and energy from their algal endosymbionts or plastids. The generalists usually need to ingest prey frequently, and their growth is highly dependent on ingested C and N. By contrast, some specialists are primarily phototrophic. In microzooplankton with acquired phototrophy, there may be additional benefits beyond C or energy acquisition, including capacity to take up inorganic N

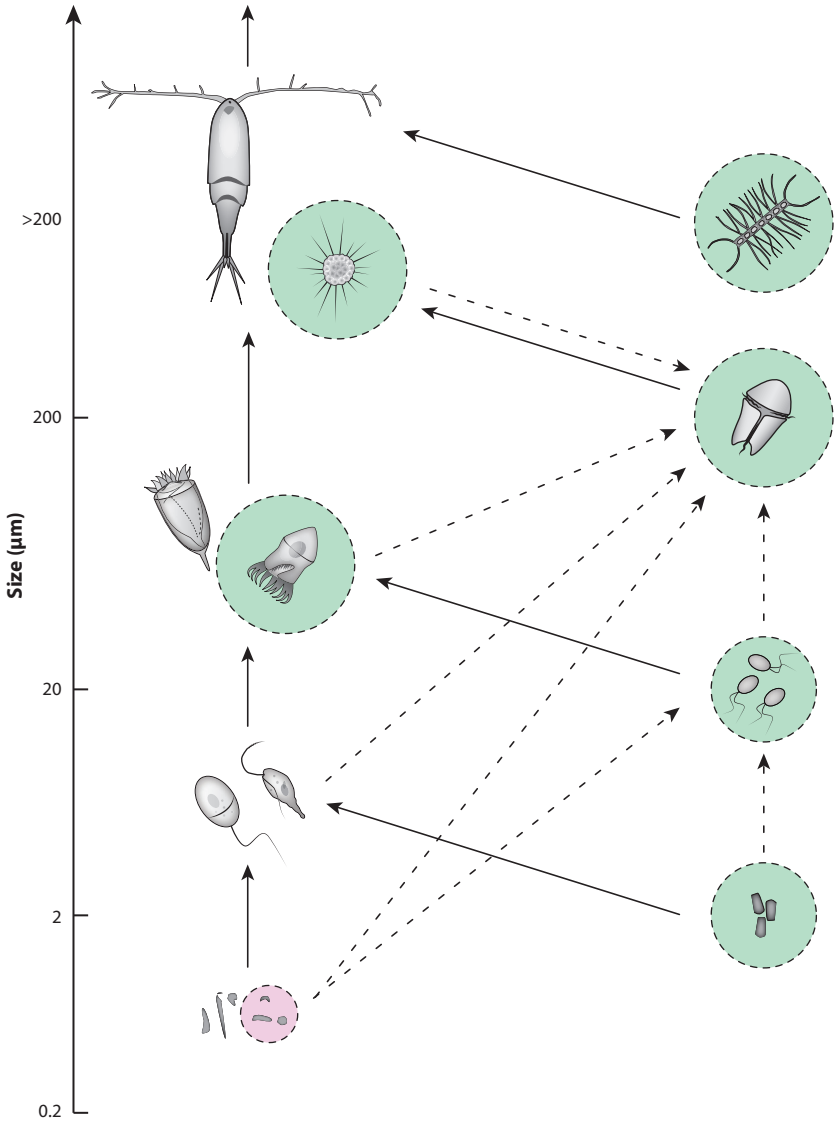

Figure 4

Food web diagram showing how mixotrophy alters our view of the structure of planktonic food webs. Photosynthesis (indicated by *green circles*) occurs in both the zooplankton (*left*) and the phytoplankton (*right*). Photoheterotrophy (indicated by the *pink circle*) occurs in many bacterioplankton (*left*). Solid arrows indicate ingestion of prey by zooplankton; dashed arrows indicate ingestion of prey by phytoplankton.

(Tong et al. 2015) and enhanced survival and growth in low-oxygen waters (Esteban et al. 2010, Gomes et al. 2014).

Second, mixotrophic pico- and nanophytoflagellates can dominate primary production and bacterivory in surface waters of oligotrophic oceans and during seasonal stratification in temperate and polar waters. Mixotrophy among phytoplankton can increase trophic transfer to higher trophic

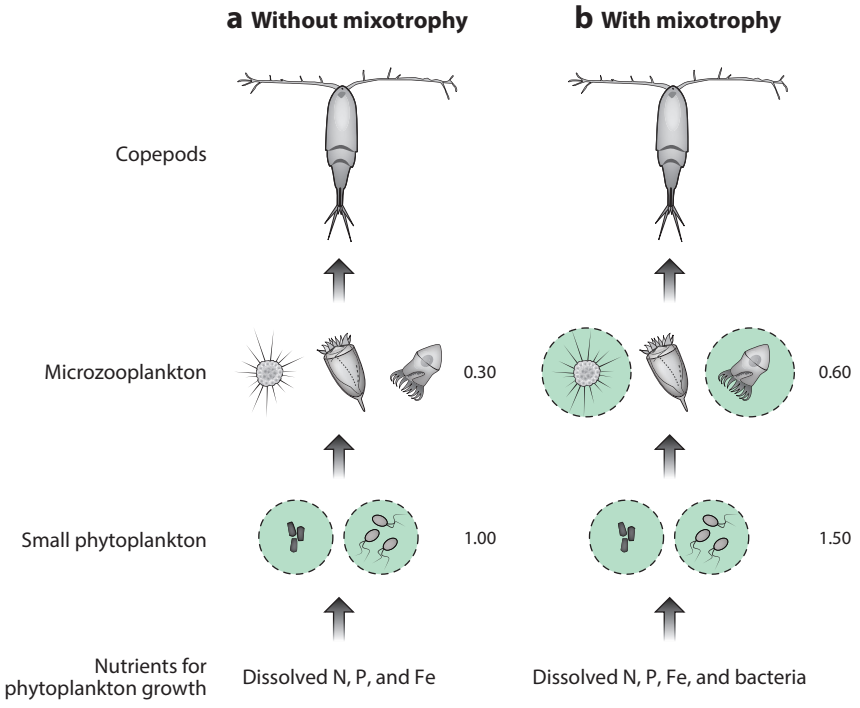

Figure 5

Conceptual model of a nutrient-limited open-ocean food chain (*a*) without mixotrophy and (*b*) with mixotrophy. The green circles indicate photosynthetic organisms, and the numbers to the right of each group show the calculated relative biomass of that group. This model assumes that mixotrophy among phytoplankton boosts primary production by 50% (high-N:P simulation) (Mitra et al. 2014b), that mixotrophs constitute 33% of the microzooplankton (based on data in Stoecker et al. 2009), and that the gross growth efficiencies of strictly heterotrophic microzooplankton and mixotrophic microzooplankton are 30% and 60%, respectively (Stoecker et al. 2009). In the food chain with mixotrophy, the potential microzooplankton biomass available as prey for copepods is twice that in the food web without mixotrophy; this is important because microzooplankton constitute more than 50% of the diet of copepods in oligotrophic waters (Saiz & Calbet 2011).

levels because of increases in primary production and repackaging of bacterioplankton C into larger eukaryotic cells that are ingested by most grazers (**Figure 5**).

Third, many of the bloom-forming dinoflagellates, raphidophytes, and haptophytes in estuarine and coastal water are mixotrophs. Although high levels of inorganic nutrients may be necessary for bloom initiation, mixotrophy contributes to the ability of these species to maintain dominance, especially once inorganic nutrients become limiting. Mixotrophy in some blooming phytoplankton may reduce prey availability to higher trophic levels, especially when the mixotrophs are harmful algal bloom species or consume phytoplankton or microzooplankton that are better foods for crustacean zooplankton or fish larvae than the mixotrophs.

Fourth, acquired phototrophy contributes disproportionately to primary production in larger size fractions and is thought to increase the GGE of microzooplankton; this should increase C transfer through the microzooplankton link to higher trophic levels (**Figure 5**). Without the C supplement of phototrophy, many large protistan predators and grazers probably could not survive in oligotrophic oceans.

Finally, it is difficult to quantify the effects of mixotrophy on marine planktonic ecosystems because of gaps in understanding and information. Most mixotrophs have not been cultured; there is little or no information on the physiology of most planktonic mixotrophs, and even less known about their in situ activities and contributions to ecosystem processes. This is particularly true for "phytoplankton that eat" because it is difficult to determine which cells are feeding, how much ingestion occurs, and the contributions of phototrophy and phagotrophy to their C, N, and P budgets. Although the presence of mixotrophy in microzooplankton is easier to detect, there are few measurements of both grazing/predation and photosynthesis by microzooplankton. The effects of acquired phototrophy on the average GGE of the microzooplankton link in planktonic food webs are unknown. This is an important gap because increases in GGE within the microbial food web (**Figure 5**) could dramatically increase the amount of C available to higher trophic levels (Landry & Calbet 2004), particularly in oligotrophic planktonic ecosystems in which microzooplankton are an important food source for crustacean zooplankton (Calbet & Saiz 2005, Saiz & Calbet 2011).

5. INCORPORATION OF MIXOTROPHY INTO BIOGEOCHEMICAL, FOOD WEB, AND HARMFUL ALGAL BLOOM MODELS

Most mechanistic plankton food web models are based on the classic nutrient-phytoplankton-zooplankton model. Within these models, the primary focus has been biogeochemistry, relying on the differentiation of the planktonic communities into phytoplankton or zooplankton functional types (Mitra et al. 2014a); this is analogous to the plant-animal dichotomy that is the norm in terrestrial ecosystems. Even with the current trend of expanding the classic nutrient-phytoplankton-zooplankton structure by subdividing the phytoplankton and zooplankton groups into more specific functional groups, mixotrophs as explicit entities are typically not modeled. The recognition that mixotrophic protists are major components of the phytoplankton and microzooplankton communities has provided a strong justification for describing mixotrophy in planktonic ecosystem models. Mixotrophy links the microbial food web to the larger plankton and ultimately to fisheries (Mitra & Flynn 2010, Våge et al. 2013, Mitra et al. 2014a). End-to-end modeling strategies to describe linkages from primary production to fisheries thus should include explicit descriptions of mixotrophy. Modeling of coastal and regional planktonic ecosystems, and specifically of harmful algal blooms, requires a consideration of mixotrophy because many of the motile bloom formers are mixotrophic protists (Burkholder et al. 2008). Detailed physiological modeling of the organisms involved in harmful algal blooms should aid understanding of the underlying processes of bloom formation. Irrespective of the application, the fundamental question is how best to integrate planktonic mixotrophic processes within biogeochemical, food web, and species-specific plankton models.

Various methods have been used to incorporate mixotrophy into models of planktonic ecosystem function. One approach has been to assign the phototrophic activity of these organisms to the phytoplankton functional group and the heterotrophic activity to the zooplankton component (e.g., Baretta-Bekker et al. 1998, Fulton et al. 2004). This method assumes that mixotrophy is an additive process in which the phototrophic and phagotrophic activities are independent of each other. Mixotrophy has also been modeled as an interactive "top up" mechanism in which the protist balances phototrophy and/or phagotrophy in order to acquire a limiting resource (e.g., Stickney et al. 2000, Kooijman et al. 2002, Flynn & Hansen 2013). This aspect has been considered in more detail by incorporating a trait trade-off strategy, based on the assumption of a metabolic cost associated with the maintenance of the two diverse strategies of phototrophy and phagotrophy for nutrient acquisition (e.g., Thingstad et al. 1996, Våge et al. 2013). This method has included the development of models in which cell size is a dominant trait that regulates the mode of resource

acquisition (Ward et al. 2011, Andersen et al. 2015). At the other extreme, Ward & Follows (2016) developed a global marine plankton model in which all plankton are potentially mixotrophic, with no trade-offs between phototrophy and phagotrophy. Mitra et al. (2016) proposed that phototrophy and phagotrophy in mixotrophs can provide synergistic and coupled support of multinutrient acquisition, which requires the deployment of a multinutrient (C, N, and P) currency in models and appropriate descriptions of prey stoichiometry.

Irrespective of the modeling approach, the incorporation of mixotrophy has a profound impact on simulations of planktonic ecosystems. In simulations with mixotrophy, the primary production (C fixation), bacterial production, transfer of biomass to larger size classes, and sinking C flux (the biological C pump) are usually enhanced in comparison with simulations without mixotrophy (e.g., Stickney et al. 2000; Mitra et al. 2014b, 2016; Ward & Follows 2016).

Empirical data are necessary to ensure that models describing the diverse mixotrophic strategies are fit for the purpose. Including explicit descriptions of mixotroph functional types within ecosystem simulators (e.g., Blackford et al. 2004) requires condensing the description of the complexity of mixotroph ecophysiology to a level where the computational load is acceptable. One approach is to utilize the available data to construct models that agree with the mixotroph descriptions given by Mitra et al. (2016). However, when good numeric data are absent (Mitra et al. 2014a), the construction of simple models that do not describe dysfunctional behavior is challenging; in this context, dysfunctionality refers to a model that through its construction describes features of system dynamics that run contrary to expectations from empirical evidence (Flynn 2010). One option is to use complex models (e.g., Flynn & Mitra 2009), the behavior of which has been evaluated and approved by scientists with a comprehensive appreciation of how these organisms behave in nature, to generate a "reality," i.e., a library of data against which simple models can be tested (Mitra & Flynn 2010). Such a complex-to-simple approach is in stark contrast to traditional modeling approaches, which tend to start with a simple construct and increase complexity only if the simple models fail. The test of failure for models in such instances is not robust (Franks 2009), and certainly it would not be so for descriptions of mixotrophy, where good numeric data are often lacking. Although exploiting phenomenological data is valuable, ultimately we need a better coupling of laboratory, field, and modeling efforts to enable us to form a more holistic view of plankton ecology.

SUMMARY POINTS

1. Mixotrophs are important components of the bacterioplankton, phytoplankton, and microzooplankton communities and thus are integral components of planktonic ecosystems.
2. Mixotrophy among the phytoplankton may be an important mechanism for alleviating inorganic nutrient limitation and increasing primary production in oligotrophic waters. In more eutrophic waters, mixotrophic bloom-forming phytoplankton may sometimes decrease food web transfer to higher trophic levels.
3. Acquired phototrophy is thought to increase the gross growth efficiency of microzooplankton; this should increase carbon transfer through the microzooplankton link to higher trophic levels.
4. Little information is available on the physiological ecology of mixotrophs, particularly those inhabiting the open ocean. With a few exceptions, progress has been hampered by a lack of sensitive methods to measure both ingestion and photosynthesis in mixotrophs in natural samples.

5. Mixotrophy may be favored in a changing ocean because of increased stratification or temperature, changes in nutrient availability, or hypoxia within the euphotic zone. Depending on the situation, the response of mixotrophs may either help ecosystems adapt or lead to ecosystem disruption.
6. It is important to explicitly include mixotrophic plankton functional types in ecosystem models because mixotrophy can profoundly influence biomass and size distributions, stoichiometry, cycling of carbon (particularly dissolved organic carbon), and trophic dynamics among bacteria, primary producers, and secondary producers.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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