Acknowledging and incorporating mixed nutrition into aquatic protistan ecology, finally

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We often instinctively think of life on our planet being composed of species capable of obtaining the organic matter and energy they need for growth from inorganic compounds (autotrophy) or species that utilize pre-formed organic matter produced by autotrophs to meet their energy and carbon needs (heterotrophy). This is particularly true for terrestrial, macroscopic life forms, where there exists an obvious dichotomy between most phototrophs (plants) and heterotrophs (animals, fungi). That perspective was also extrapolated to single-celled eukaryotic organisms (the protists) many years ago as one way of organizing the enormous diversity that exists among those species (Whittaker, 1969). That description was somewhat justified at the time by the fact that many protists exhibit either phototrophic nutrition (microalgae,) or heterotrophic nutrition (protozoa), and as a practical way to organize the enormous diversity of species within the kingdom Protista. However, recent phylogenies that have been proposed for the domain Eukarya (Burki, 2014) recognize that photosynthetic and heterotrophic ability are not always phylogenetically informative, and often not mutually exclusive behaviors. Many, perhaps even a dominant proportion of protists, it turns out, exhibit some combination of these nutritional modes (an ability generally referred to as mixotrophy).

Mixotrophic protists occur throughout the eukaryotic tree of life although, functionally, most of these species can be grouped into one of three general behavioral categories (Mitra *et al.*, 2016; Stoecker *et al.*, 2016). Firstly, phagotrophic phytoflagellates are species that possess chloroplasts but also engulf and digest small prey such as bacteria and cyanobacteria by phagocytosis. We have long been aware that species within several algal classes exhibit this behavior (Porter, 1988). Secondly, kleptoplastidic protists are heterotrophic species that feed on algae, partially digest them, but retain their chloroplasts in a functional state (and occasionally other organelles from their prey). These species (e.g. many dinoflagellates and ciliates) possess 'acquired phototro-phy' as a consequence of chloroplast retention. They can be generalists, consuming and acquiring chloroplasts from a variety of algal prey, or highly specialized in their preferred prey. Finally, numerous physically-intimate, often-mutualistic associations exist between heterotrophic protistan species and intact photosynthetic algae or cyanobacteria that are generally now included under the broad definition of mixotrophy. These associations constitute efficient and productive relationships in which feeding and nutrient remineralization by the heterotrophic host support photosynthesis and growth of the symbionts which in turn contribute to host nutrition. These associations (holobionts) have been described for well over a century for the larger Rhizaria (e.g. foraminifera and radiolaria) (Haeckel, 1887) but they also exist among other protistan phyla such as the ciliates, including the ciliate-symbiont model system of *Paramecium-Chlorella* (Brown and Nielson, 1974).

Given that mixotrophic nutrition and the organisms that conduct it have been known for quite some time, why is this subject only now receiving attention by a broad audience of aquatic microbiologists? One reason is that only recently have the three general categories of mixotrophy noted above been formally defined (Mitra *et al.*, 2016). Additionally, new information now appearing in the literature indicates that we may have grossly underestimated the collective abundances of these species in aquatic ecosystems, and therefore poorly characterized their impacts on food web structure and function in the plankton. Evidence over the last few years has indicated substantial abundances of phagotrophic phytoflagellates in the ocean (Unrein *et al.*, 2014). The ingestion of prey by these algae may provide them with a mechanism for obtaining vital nutrients for photosynthesis, relative to algae that do not possess phagotrophic ability. If so, mixotrophy bestows an ecological advantage in low-nutrient environments, and implicates those algae as a significant source of bacterial mortality in the plankton (overlapping with the ecological role attributed to small heterotrophic protists). Mixotrophic algae are also widespread in many freshwater ecosystems, although a clear generality across all freshwater ecosystems is not yet possible because of the vast number of

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those environments and the limited number of studies that have been conducted to date. For their part, klepto-plastidic ciliates and dinoflagellates are typically a significant, and sometimes dominant portion of the microzooplankton in planktonic ecosystem (Stoecker *et al.*, 1987; Stoecker, 1999). Photosynthesis by the acquired chloroplasts of these species increases the efficiency of trophic transfer of carbon and nutrients in the plankton (Stoecker, 1998). Finally, recent publications from a global ocean survey indicate a much greater contribution of large, symbiont-bearing Rhizaria to plankton communities in oceanic ecosystems (Biard *et al.*, 2016). These beautiful and delicate associations constitute microhabitats of high rates of photosynthesis, and the hosts are also important generalist consumers in the oceanic plankton (Caron and Swanberg, 1990).

Another reason for the recent upsurgence in scientific interest in mixotrophic protists is that global biogeochemical models of the ocean still do not have the predictive accuracy that we desire and need for the climatological challenges of the coming decades. The limitations of these models are certainly not solely a consequence of the fact that they lack mixotrophic behavior. However, some prescient work on this topic has shown that the incorporation of even a highly simplified form of mixotrophy into biogeochemical models changes modeled outcomes. It increases the transfer of carbon to higher trophic levels, and the sinking of particles (Mitra *et al.*, 2014; Ward and Follows, 2016). Such findings, if substantiated with further study and complexity, have important implications for a variety of ocean processes ranging from fisheries biology to removing carbon from surface waters of the ocean (the so-called 'biological carbon pump').

I suggest that the time is overdue to address the glaring omission of mixed nutrition among protists in our collective scientific psyche regarding plankton food web structure and, moreover, that we are uniquely poised to do so at this time. Recent decades of field and culture studies have opened our eyes to the tremendous diversity of these species, yielded some insights into their physiological abilities, and outlined the potential ecological consequences of mixotrophic nutrition. Cutting-edge approaches and techniques are now enabling new ways of documenting their global abundances, understanding the ecological advantages (and limitations) that mixed nutrition provides to these species, and incorporating this behavior into food web and biogeochemical models. Several 'omics approaches feature prominently in teasing apart the details of mixed nutrition among protists. These approaches are now being broadly applied to microbial eukaryotes (Caron *et al.*, 2016), and are yielding new means of addressing long-standing ecological questions involving microbial eukaryotes, including some mixotrophic species. How are photosynthetic and heterotrophic processes regulated within single-celled phagotrophic phytoflagellates? How does prey availability affect cellular metabolism in those species? How are prey chloroplasts stabilized and controlled in the host cytoplasm of kleptoplastidic heterotrophic protists? How are endosymbionts captured, recognized and maintained at the molecular level in symbiont-bearing protists? Genetic approaches to answer these questions are at the forefront. Such studies are identifying changes in gene expression that accompany shifts in the nutritional mode of mixotrophic algae (Liu *et al.*, 2015), transcriptional activity of chloroplasts in kleptoplastidic ciliates (Johnson *et al.*, 2007), and the molecular signaling that takes place between heterotrophic hosts and symbiotic algae in the establishment of mutualistic associations (Balzano *et al.*, 2015). At present, such applications make use primarily of transcriptomics because of the daunting size of eukaryotic genomes, but genome sequencing of microbial eukaryotes (including mixotrophic species) is rapidly becoming economically feasible. Functional genomics, enabled by sequenced genomes, will greatly expand the investigative tools available for understanding the physiology of mixotrophic protists.

The 'end game' of understanding and incorporating mixotrophy into the paradigm of the microbial food web will not be realized in the near future, of course, but it is at least entrenched in the minds of more researchers these days. Full acknowledgment of the importance of this behavior, and incorporation into biogeochemical models will entail an accurate determination of the contribution of mixotroph abundances and biomass, their impact on energy flow, organic carbon production and utilization, and nutrient cycling. Given its ubiquitous occurrence and important ecological consequences, the continued absence of mixed nutrition in our conceptualization of aquatic food webs seems unwarranted and unwise. Much work remains on properly defining and parameterizing these plankton categories, but I predict (and hope) that will change radically within the next decade.

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