From webs, loops, shunts and pumps to microbial mix-masters: Evolving paradigms of marine microbial ecology, mixoplankton and implications for a future ocean

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Condensed running head: Microbes and the mixoplankton paradigm

Abstract

Emerging knowledge of mixoplankton–ubiquitous microbes that employ phototrophy and phagotrophy synergistically in one cell–reshapes our knowledge of the flow of materials and energy, with wide-reaching impacts on marine productivity, biodiversity and sustainability, through provision of additional trophic linkages. Concepts of microbial interactions have evolved from the traditionally-conceived food chain, in which carbon-fixing phytoplankton are grazed upon by zooplankton, which in turn support fisheries and higher trophic levels, to microbial webs, loops and shunts as knowledge about abundance, activity and roles of microbial organisms– bacteria, archaea, viruses, phytoplankton, zooplankton, mixoplankton have increased. In a future world, phytoplankton that dominate in short food 'chains' and simple microbial 'webs' may be disproportionated stressed as temperatures rise, as thermal stratification reduces water column nutrient exchange, as oceanic waters become more acidic, and as land-based and atmospheric nutrient pollution shifts in amounts, forms and proportions. These warm, stoichiometrically imbalanced conditions strengthen the importance of mixoplankton, including those that form harmful algal blooms, and alter the likely pathways of organic carbon processing in the ocean. Understanding, measuring, and modeling the interconnectivity of microbes have direct consequences for understanding how nutrient cycling pathways, harmful algal blooms and fisheries may change in a globally-changing, anthropogenically-impacted world.

Introduction

In the plankton microbial world, there is competition for resources and winners achieve that status by avoiding death as much as from their inherent rate of growth. Persistent questions in plankton ecology and biological oceanography relate to how plankton interact, how these interactions modify biogeochemical cycling, affect species succession, and ultimately how they collectively determine the composition and productivity of higher trophic levels. In the past half-century, research into microbial planktonic warfare, or more precisely, microbial trophic interactions, has greatly advanced, with accelerating knowledge of newly-found organisms, pathways and interactions–and new consequences for aquatic ecosystem functioning.

There has been much debate about forces driving the biodiversity of plankton species with researchers focusing mainly on phytoplankton–the traditional primary producers of aquatic systems (including microbial prokaryotic cyanobacteria and eukaryotic autotrophs; Flynn 1988). The fact that many co-existing microbes occupy outwardly similar, but sometimes subtly different, niches led to various researchers in the 1940's - 1960's to question the validity of prevalent theories about competition and success of phytoplankton in aquatic systems. Hutchinson (1961) coined the phrase "paradox of the plankton", suggesting that the rules governing terrestrial life-forms were not suitable for describing phytoplankton success and diversity. That a number of species can co-exist in an apparently relatively unstructured aquatic environment, even though they are all competing for similar if not identical resources, is because of the lack of equilibrium in the system and wide diversity in physiology of different taxa. More recently our understanding of how the base of the food web has been questioned.

The marine food-chain was originally conceptualized based on our understanding of terrestrial dynamics where the plants are the producers and animals the consumers (Hairston et al. 1960). Thus, in aquatic systems we have traditionally thought of the phytoplankton as the producers, the zooplankton as the primary consumers, the fish as the secondary consumers etc. Based upon plant functional groupings, the marine microbial organisms have also been assigned functional groups, including phytoplankton, bacterioplankton, zooplankton. Over the last decade, there has been an increasing awareness about the existence of another important functional group–the mixoplankton, those protists that engage synergistically in phototrophy and phagotrophy within a single cell (Flynn et al. 2013, 2019; Mitra et al. 2016). Our emerging knowledge of mixoplankton has led to a re-imaging of the marine plankton food-web, and a reinterpretation of 'phytoplankton' and '(proto)zooplankton'.

With global climate changes, plankton diversity and succession are changing, making their understanding ever more important. Here, we review the evolution of conceptual models of plankton interactions from food chains to the mixoplankton paradigm, and we emphasize how this latest paradigm alters our understanding of how plankton interact and the importance of understanding these dynamics in predicting how the microbial community may change in a future ocean.

From food chains to loops and webs

The importance of plankton as feed for other marine organisms has long been known. In the traditional view, plant-like phytoplankton form the base of a linear food 'chain', and they are then consumed by the primary consumers (protozooplankton and mesozooplankton) which are then eaten by small fish, these in turn provide food for larger fish and higher trophic levels. In these linear food chain systems, bacteria–if considered at all–were only considered as minor players, for example, as the "decomposers". In this role, bacteria were viewed solely as decomposers what 'rained out' of the surface ocean, leading to a unidirectional flow of energy and materials. However, in the late 1970's, marine ecology saw the advent of the microbial 'loop' paradigm with bacteria identified as playing a more central role. This led to the food chain being re-imagined as a 'web', with the flow of energy and materials mediated at numerous steps by bacteria (Fig. 1b), with them serving as consumers as well as decomposers (a semantic difference perhaps, but still an important one).

The concept of the 'microbial loop' represented a major paradigm shift in marine ecology of the 20th century. This was not just a conceptual shift: it was based on acceptance of a greater abundance of bacteria in the ocean than previously recognized. Even though the abundance of bacteria in marine waters had been well documented using direct counts by Eastern (predominately Russian) investigators as early as the 1920's (e.g., Cholodny 1928), differences in bacteriological techniques (direct counts vs plate counts) between Eastern and Western scientists impeded acceptance of the importance of bacteria in the Western literature for many years (reviewed by Williams and Ducklow 2019). The microbial loop concept, introduced by Pomeroy (1974), and more formally described by Azam et al. (1983), highlighted the importance of grazing of bacteria by protozooplankton (2-20 μm size range) wherein these grazers formed an important linkage between the marine microbial production and higher trophic levels (Fig. 1a). The microbial web, which encompasses not only bacteria, but also viruses, archaea and heterotrophic protists, rather than unidirectionally decomposing materials, provides the pathway by which dissolved organic carbon (DOC; see Table 1 for list of abbreviations), largely derived from phytoplankton exudates, is recycled, and ultimately passed to higher trophic levels. In the microbial web, predator-prey interactions, via predation, parasitism, mutualism and other interactions, modulate not only the amount and rate of organic material available for bacterial growth, but also the numbers of bacteria, and ultimately the energy that is transferred to higher trophic levels. The heterotrophic protists, as consumers of bacteria, further contribute to the dissolved organic pool via excretion, but also to the particulate via production of fecal material. Thus, the acceptance of the role of bacteria thus evolved, from simply decomposers to serving as food for protozooplankton. This active flux only could be accounted for by an active and abundant bacterial community–a community that could be counted when methods advanced (Williams and Ducklow 2019). The advancement of methods also included the advent and proliferation of use of techniques such as dilution experiments which revealed that microzooplankton, rather than microzooplankton, were responsible for most of the consumption of primary production (Landry and Hassett 1982, reviewed by Schmoker et al. 2013).

From loops to shunts

Nearly two decades after the formalization of the microbial loop concept, the concept of the ‘viral shunt’ emerged. New data, again coupled with advances in techniques, led to enhanced understanding of the abundances of viruses and the processes by which viruses facilitate the movement of nutrients from organisms to pools of dissolved and particulate organic matter (Wilhelm and Suttle 1999; Jiao et al. 2010; Fig. 1b). From the initial discovery of the prevalence of marine viruses, shown to outnumber bacteria by an order of magnitude (e.g., Bergh et al. 1989; Suttle 2007), followed by identification of phages of specific taxa (e.g., Sullivan et al.

2003; Lindell et al. 2005), including phytoplankton-infecting viruses (e.g., Wilson et al. 2005; Derelle et al. 2008,), the role of viral interactions in marine microbial interactions came into prominence. The importance to the understanding of microbial trophic dynamics was that, depending on the specific compounds released by viral lysis, the cycling of organic compounds could be modified, as some these compounds could be readily used by bacteria, while other compounds were more calcitrant (Weitz and Wilhelm 2012). Viruses thus came to be recognized as playing an important role in the microbial loop, regulating organic matter cycling via cell lysis and its resultant release of organic material for further consumption by bacteria. It has also been suggested that viruses can be responsible for lysing harmful algal blooms (HABs) (e.g., Lawrence 2002; Baudoux et al. 2006) and thus the abundance of specific taxa could be modified through viral interactions (cf. blooms occurring due to a loophole in predatory activity; Irigoien et al. 2005). Viral metagenomics has advanced understanding of their geographic patterns (De Corte et al. 2016) and relationships with many environmental conditions (Mateus 2017).

From loops and shunts to pumps

The microbial loop concept led to a debate as to whether the loop represented a link, channeling fixed carbon (C) to higher trophic levels, or whether it represented a sink, a loss of fixed C from the system, the so-called ‘link-sink’ debate (cf., Sherr and Sherr 2000). The link-sink dichotomy has been further advanced with the differentiation between the biological carbon pump (BCP) and the microbial carbon pump (MCP). The former encapsulates the notion that bacteria mediate the transformation of DOC to more recalcitrant and particulate forms that are subsequently lost to the deep sea via marine snow or sinking fecal pellets (Honjo et al. 2014), a process considered important in under most oceanic–oligotrophic–conditions (e.g., Fenchel 2008). In contrast, the MCP is based on the microbial transformation of labile DOC to more recalcitrant forms which can remain as important sources of sequestered, but dissolved, C in the ocean (Jiao et al. 2010).

The balance between the BCP and MCP is a function of the production of DOC and its resulting quality by phytoplankton–and therefore the conditions on which the phytoplankton were growing and which type of phytoplankton were growing at any particular time or place. It has also been proposed that viruses can be significant drivers of the biological pump, via their facilitation of particle aggregation and transfer to the deep sea, leading to their dual 'shunt and pump' roles (Suttle 2007; Lomas and Moran 2011). The relationships between the BCP and MCP and of the viral shunt and pump in biogeochemical cycling is, nevertheless, yet to be determined, as diverse groups of eukaryotic viruses have been documented in the ocean, with different lineages functioning differently (Blanc-Mathieu et al. 2019), due to their host-strain specificity (Breitbart 2012; Thingstad et al. 2021).

Where large phytoplankton dominate, and where nutrients are sufficient, phytoplankton are most likely to be stoichiometrically balanced, and to be readily grazed by micro- or macro-zooplankton, leading to an enhanced BCP. When phytoplankton become limited by nutrients or light, their stoichiometric balance changes, they become less palatable for grazers, and they are more likely to release DOC (Fig. 2). Thus, under these condition, two factors contribute to an enhanced MCP. First, cells may release compounds rich in organic C as a stoichiometric rebalancing process (Glibert and Burkholder 2011), and second, as their food quality to grazers is reduced, the link with grazers is also weakened (Polimene et al. 2017).

Loops, shunts and pumps to mixotrophy

Plankton mixotrophy, as characterized by its early and broadest definition - a combination of autotrophy and heterotrophy, has been known for many decades (Raven et al. 2009 and references therein). As common with most concepts, the definition and description of mixotrophy in aquatic systems was mirrored on terrestrial examples. The most common use of this term, and reports thereof, refer to the combined capability of phototrophy plus osmotrophy, the uptake of sugars, dissolved free amino acids and other organic compounds. Yet, even with this definition, based on results from the occasional laboratory experiment, the early understanding was that bacteria were far superior to microalgae in competing for organic substrates (e.g., Wright and Hobbie 1965). Thus, it was generally concluded that osmotrophy was marginal in its importance for phytoplankton (reviewed by Syrett 1981), and so mixotrophy for non-bacteria microbes was marginalized as a concept. However, the ability of phytoplankton to be osmotrophs gained appreciation over time, again largely as a function of new approaches and methodologies (e.g., Flynn and Butler 1986; Antia et al. 1991; Glibert 1993; Berman and Bronk 2003; Burkholder et al. 2008). It now seems, from the perspective of osmotrophy, that all planktonic autotrophs can be recognized to be mixotrophs to some degree (Flynn et al. 2019; Mitra and Flynn 2021).

In the 1990's, the first conceptual models of mixotrophy were proposed by Jones (1997) and Stoecker (1998) for freshwater and marine systems, respectively. These conceptual models described the occurrence, under varying nutrient and/or light limiting conditions, of mixotrophy as a combination of phototrophy, osmotrophy and also of phagotrophy. Differentiating mixotrophy in this way becomes important when considering food-web interactions, as phago-heterotrophy results in death of other organisms in contrast to osmo-heterotrophy which is dependent on uptake of dissolved material. Just as mixotrophic activity based on the traditional definition involving phototrophy *plus* osmotrophy was initially considered minimal in importance (Syrett 1981), mixotrophic activity by protists engaging in phototrophy *plus* phagotrophy was initially considered nothing more than a curiosity with mixotrophic organisms considered to be inferior compared to the purely phototrophic and purely phagotrophic organisms. Thus, for one reason or another, mixotrophic plankton were rarely considered and when they were considered, they were invariably apportioned as part of the "phytoplankton" community. For example, based on the Stickney et al. (2000) models of mixotrophy, a fraction of dinoflagellates was assigned mixotrophic capabilities in the biogeochemical marine ecosystem model of Fulton et al. (2004).

Over the last decade the understanding of protistan mixotrophic activity has changed significantly. It is now recognized that most of the protist planktonic primary producers, excluding diatoms, are potential grazers, and over one-third of protozooplankton are capable of acquired phototrophy (Stoecker et al. 2009; Flynn et al. 2013). This led to a revision of the protist plankton functional classification (Mitra et al. 2016) and recently, the coining of the term "mixoplankton" (Flynn et al. 2019). Usage of the term mixoplankton is important, as it clearly flags organisms that are mixotrophic by virtue of killing other organisms; they are predators and their activity directly affects food-web structuring. Mixoplankton are thus major players within the microbial food web. They consume bacteria as well as phytoplankton or other microzooplankton, they release DOC as they digest their food, they also photosynthesize with their own or acquired chloroplasts, and they, in turn, become food for other micrograzers and other mixoplankton.

The mixoplankton paradigm

Mixoplankton are now recognized to comprise a marine community in their own right, similar to bacterioplankton, phytoplankton and proto/meso-zooplankton communities. Within the mixoplankton there is significant diversity with the community broadly being classified into (i) constitutive mixoplankton (CM) – those that have an inherent ability to photosynthesize (they have genetic ability to make their own chloroplasts) but that also acquire food through predation, and, (ii) non-constitutive mixoplankton (NCM) - protists that engage in predation and photosynthesis through acquired phototrophy (Mitra et al. 2016). Examples of CM include the iconic marine "phytoplankton" *Emiliania huxleyi*, *Tripos tripos* (previously known as *Ceratium furca*) and also various HAB taxa (e.g., *Karlodinium veneficum*, *Karenia brevis*, *Alexandrium minimum*; Leles et al. 2019). The NCM can be further divided into those that are generalist non-constitutive mixotrophs (GNCM) acquiring plastids from a range of prey (e.g., the fisheries-supporting *Laboea strobila*, *Strombidium rassoulzadegani*; Leles et al. 2017) and those that are specialist non-constitutive mixotrophs (SNCM). The specialists themselves are further categorized into two functional groups – the plastidic SNCM (pSNCM) that acquire plastids from specific species (e.g., *Mesodinium rubrum*, *Dinophysis acuta*; Leles et al. 2017), and, the endosymbiotic SNCMs (eSNCM), those that harbor photosynthetic endosymbionts (e.g., different rhizarians).

Conceptualizing and comprehending the importance of mixoplanktonic activity is much more than recognizing which protist plankton have this dual synergistic nutritional capability. The mixoplankton paradigm assumes mixoplankton dominate the base of microbial food-webs, and via their activity, short-circuit the flow of energy and materials to primary producers in ways that are fundamentally different than those envisioned in a microbial food-web dependent on bacteria for this recycling (Fig. 1c). At the simplest level, there are advantages to eating your competitor, and so-called phytoflagellates that consume other algae or bacteria may gain this advantage (Thingstad et al. 1996; Mitra et al. 2014). Productivity and/or growth may increase due to the dual channels by which the organism gains its C or nutrients (Fig. 3). Essential elements such as C, nitrogen (N) or phosphorus (P) are typically rich in microbial prey, and therefore upon feeding, the mixoplankton is provided not only an element that may have been otherwise in limiting proportion, but the consumer gains all elements pre-packaged in the food. Thus, CM mixoplankton gain C, N, P and micronutrients from grazing, while NCM mixoplankton may gain at least transitory inorganic C-fixing capacity after feeding on phototrophs (Leles et al. 2021). For example, the CM dinoflagellate *Margalefidinium* (reported as *Cochlodinium*) *polykrikoides* nearly doubles its growth rate when allowed to grow as a mixoplankton compared to when no prey was provided and therefore it was forced to survive on phototrophy only (Jeong et al. 2004). Similar observations have been reported for the CM dinoflagellates *Karlodinium veneficum* and *Karenia brevis* (Adolf et al. 2008; Glibert et al. 2009). As mixoplankton, species that were originally thought to have comparatively low growth rates because they were grown as phototrophs, may in reality have an advantage over competitors under nutrient limiting conditions, leading to compounded gains over time. This is one strategy by which HABs may form; indeed, most protist HAB species (i.e, excluding the cyanobacterial and diatom HABs) are now recognized to be mixoplankton (Mitra and Flynn 2021).

Mixoplankton may selectively feed on prey that have a nutritional composition complementary to themselves (e.g., Glibert and Burkholder 2011), and this, in turn, can alter the elemental stoichiometry of the mixoplankton and of its release products (e.g., Lundgren et al. 2016; Lin et al. 2017; Zhang et al. 2017). Availability of food with a differential stoichiometric composition may provide multiple advantages for a mixoplankton that itself may be limited in a particular elemental constituent. When a macrograzer consumes a mixoplankton, this upgraded trophic benefit is further transferred (Traboni et al. 2020). If the grazer is itself a mixoplankter, as in the case of the multi-link mixoplankton consortia of a bacterivorous CM crytophyte - pSNCM ciliate *Mesodinium* - pSNCM dinoflagellate *Dinophysis,* one may expect trophic upgrading to be accomplished at each step. On the other hand, when release products are altered, this can also create both positive and negative feedbacks that, in turn, alter the composition of the primary producers, potentially generating those that are unpalatable (e.g., Mitra and Flynn 2006). Ultimately, how the resource stoichiometric proportions compare with those of the mixoplankton could have propagating effects through the microbial loop, with implications for food-web structure and functioning (Mitra et al. 2014, Polimene et al. 2017). Such advantages afforded to mixoplankton may outweigh their supposedly comparatively poor growth rates in otherwise non-limiting nutrient conditions. In oceanic waters, mixoplanktonic activity may help to explain why oceanic ‘phytoplankton’ may have the ability to grow relatively rapidly even though inhabiting oligotrophic waters (Goldman et al. 1979); they may be grazing on bacteria to acquire nutrients (Zubkov and Tarran 2008; Hartmann et al. 2012).

Mixoplankton have other ecological advantages to phytoplankton. For those mixoplankton that form HABs, the relationship between phagotrophic nutrition and toxicity of many HAB taxa may also be synergistic. The toxin may harm or stun the prey, in turn making the prey easier to capture (Skovgaard and Hansen 2003; Tillmann 2003). Even for those species that are not directly toxic, mixoplanktonic activity and allelopathy may be synergistic: the mixoplankton gain while the competitors affected by allelopathic compounds do not (e.g., John et al. 2015). All of these interactions lay themselves open to interference from virus attack, and all of them will affect, in one way or the other, the functioning of the microbial loop (Flynn et al. 2021; Thingstad et al. 2021). Further, mixoplankton activity alters the stability of a plankton community, and models have suggested that open ocean plankton dynamics may have a more stable equilibrium and higher production rates due to enhanced nutrient feedbacks (e.g., Mitra et al. 2014).

Mixoplankton – global importance rising

That a large proportion of protist plankton are, in fact, mixoplankton suggests that they are able to thrive in conditions that are considered imbalanced from an inorganic-nutrient perspective (Burkholder et al. 2008; Jeong et al. 2010; Glibert and Burkholder 2011; Flynn et al. 2013). Thus, mixoplankton as a functional group are ubiquitous in the ocean and can be found from oligotrophic, where nutrients are limiting, to eutrophic environments, where nutrients may be available but perhaps stoichiometrically imbalanced (Burkholder et al. 2008; Glibert and Burkholder 2011; Leles et al. 2017, 2019; Faure et al. 2019). Mixoplanktonic activity displayed by CM may be continually expressed, or more intermittent, depending on conditions.

Accordingly, this nutritional route can also be important in the maintenance of blooms, allowing bloom taxa to be sustained for longer periods of time than might be the case if the dissolved inorganic nutrients were the only substrate available (e.g., Glibert et al. 2009; Fig. 2c).

Much is yet to be explored–empirically and in models–with respect to the significance of mixoplankton in ocean biogeochemistry, C cycling, and in trophic dynamics. Modelling studies have shown the importance of considering phototrophy and phagotrophy in mixoplankton synergistically (Flynn and Mitra 2009). Indeed, models which attribute a portion of phytoplankton activity or zooplankton activity to mixotrophy cannot capture the implications for biogeochemical cycling and trophic dynamics (Mitra and Flynn 2010). In the absence of mixoplankton, the C fixation and production of dissolved organics in the open oceans could be severely underestimated (Fig. 3) with incorrect predictions associated with the MCP (Mitra et al. 2014). Further, ecosystem models ignoring seasonality and biomass production of mixoplankton functional groups in coastal waters could provide contrary predictions which could ultimately be deleterious for ecosystem services (Leles et al. 2021).

The future ocean

Trends in numerous factors suggest that the ubiquitous mixoplankton (Leles et al. 2017, 2019; Faure et al. 2019) could become dominant in the globally changing (Flynn et al. 2014; Glibert 2020). In contrast to the 'pure' protist phytoplankton–the diatoms, mixoplankton tend to become proportionately more abundant as systems seasonally mature (become nutrient poor) rather than in immature (nutrient rich) ecosystems (e.g., Mitra et al. 2014). Moreover, in a future world, short food 'chains' and simple microbial 'webs' may be disproportionated stressed as temperatures rise, increasing thermal stratification and reducing water column nutrient exchange, as oceanic waters become more acidic, and as land-based and atmospheric nutrient pollution shifts in amounts, forms and proportions. These conditions strengthen the importance not only of mixoplankton, but also that of the MCP. Any enhancement of the MCP will further enhance mixoplankton dominance by enhanced bacterial activity and abundance, in turn increasing food availability for those microbial masters capable of eating them. If organisms evolve their growth rate potential to match the flows of resources in their environment (Flynn and Skibinski 2020), then mixoplankton would not necessarily have to have high growth rates to succeed in a changing world.

With a trajectory of altered nutrient stoichiometry due to eutrophication and/or de-eutrophication (e.g., Glibert and Burkholder 2011; Flynn et al. 2014; Glibert et al. 2014), concepts of stoichiometric imbalance and changes in the traits of growth and metabolism are at the fore in terms of understanding how systems will be shaped in the future. Meunier et al. (2017) predict that increasing N:P ratios should shift ecosystems towards systems with trait dominance of higher optimal N:P ratios, higher P affinity, decreasing N retention and increasing P storage. These are the traits of many mixotrophic harmful algae. Understanding, quantifying and predicting how plankton are interacting with changing climate and nutrients will continue to be challenging. Thus, not only may diatoms be disadvantaged in a future ocean, but those mixoplankton with harmful properties may emerge even more successful (Flynn et al. 2014; Glibert 2020).

Conclusions

The mixoplankton paradigm requires us to reconsider our conceptual understanding of the plankton system as the traditional phytoplankton-zooplankton dichotomy represents only a small, albeit important, component of the marine plankton community. While the microbial loop may be considered as at least relatively well understood, it still remains poorly described in models; bacteria are often not included, or, are included as a generic 'box' for remineralization. The same can be said of modeling the activity of viruses (Mateus 2017; Flynn et al. 2021), as they are either excluded in models, or where included, inadequately parameterized with respect to viral-host interactions. Of course, the requirement to include 'mixoplankton' as a functional group in models also demands inclusion of bacteria and viruses and the suite of interactions within the microbiome. The inclusion of mixoplankton alters the quality and fate of DOC and POC, it alters the production rate and abundance of bacteria, in turn impacting the growth and abundance of viruses, and growth of heterotrophic micrograzers, a role which mixoplankton themselves may play.

Inclusion of mixoplankton interactions in understanding microbial trophic dynamics is not simply a more complex version of our previous conceptual models, this new paradigm needs to be appropriately parameterized in models–but these models can only advance in tandem with the experimental data necessary to appropriately parameterize these models. A holistic effort integrating *in vivo*, *in vitro* and *in silico* work is needed to confront these challenges. Incorporating individual mixoplankton types and their specific prey, viruses and their hosts, and all the processes involved, adds tremendous complexity to models. While progress is advancing in characterizing metabolic versality via genomic data, incorporating such data in models, and these data to interpret who is doing what within organisms or communities remains problematic (e.g., Coles and Hood 2016). Nevertheless, ecosystem models including mixoplankton have advanced to the point where they can be applied in hypothesis testing, but their validation depends on data availability.

While recognizing complexity and parameterizing it appropriately may be the ultimate goal, it also must be recognized that experiments that explicitly explore the processes of photosynthesis, grazing and nutrient processes under varying environmental conditions–including conditions that are outside the traditionally considered 'normal' range of temperature, CO_2, nutrients, pH and other factors are needed. Experimentalists have to think beyond conditions of idealized 'balanced growth' (a lesson that perhaps should have been learned from Hutchinson 1961!) and exponential growth and challenge their microbial protists with the stresses, including multiple stressors, that are emerging in aquatic systems. Advancing the next generation ecological models will occur as these experiments proceed and as new approaches to incorporate new data streams (including 'omics data) in models become routine.

In this UN Ocean Decade, it is important to get the basics right – the base of the marine food-web that drives life in the oceans, impacting biogeochemical processes, higher trophic levels and associated ecosystem services. Understanding, measuring, and modeling the interconnectivity of microbes have direct consequences for understanding how nutrient cycling pathways, HABs and fisheries may change in a globally-changing, anthropogenically-impacted world. At the very least we will be in a position to better predict, even if we cannot control, the more frequent and intense blooms of mixotrophic, and indeed mixoplanktonic, HABs that are anticipated in the future with expanding anthropogenic footprints and climate extremes. Challenges remain, in identifying *who* contributes to the microbiome, the *extent* of their interactions, including the degree of mixoplanktonic activity, *how* these interactions contribute positively or negatively to ecosystem functioning and *how* these interactions change as the environment changes.

References

Adolf, J.E., T. Bachvaroff, and A.R. Place. 2008. Can cryptophytes trigger toxic *Karlodinium veneficum* blooms in eutrophic estuaries? Harmful Algae 81: 119–128, doi: 10.1016/j.hal.2008.08.003.

Antia, N.J., P.J. Harrison, and L. Oliveria. 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30: 1–89.

Azam, F., T. Fenchel, J.G. Field, J.S. Gray, L.A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257–263, doi.org:10.3354/meps010257.

Baudoux, A., A. Noordeloos, M. Veldhuis, and C. Brussaard. 2006. Virally induced mortality of *Phaeocystis globosa* during two spring blooms in temperate coastal waters. Aquat. Microb. Ecol. 44: 207–217.

Bergh, O., K.Y. Borsheim, G. Bratbak, and M. Heldal. 1989. High abundance of viruses found in aquatic environments. Nature 340: 467–468.

Berman, T. and D.A. Bronk. 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquat. Microb. Ecol. 31: 279–305.

Blanc-Mathieu, R. and others. 2019. Eukaryotic virus composition can predict the efficiency of carbon export in the global ocean. iScience 24: 102002. doi: 10.1016/j.isci.2020.102002.

Breitbart, M. 2012. Marine viruses: truth or dare. Mar. Sci. 4: 425–448, doi.org/10.1146/annurev-marine-120709-142805.

Burkholder, J.M., P.M. Glibert and H. Skelton. 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae 8: 77–93.

Cholodny, N. 1928. Contributions to the quantitative analysis of bacterioplankton. Trans. Sn. Biol. Diepre. 3: 157–171.

Coles, V.J. and R.R. Hood. 2016. Approaches and challenges for linking marine biogeochemical models with the "omics" revolution, pp. 45–66. In P.M. Glibert and T.M. Kana TM [eds.], Aquatic microbial ecology and biogeochemistry: A dual perspective. Geneva, Switzerland, Springer.

De Corte, D., E. Sintes, T. Yokokawa, I. Lekunberri and G.J. Herndl. 2016. Large-scale distribution of microbial and viral populations in the South Atlantic Ocean. Environ. Microbiol. Rep. 8: 305–315, doi: 10.1111/1758-2229.12381.

Derelle, E. and others. 2008. Life-cycle and genome of OtV5, a large DNA virus of the pelagic marine unicellular green alga *Ostreococcus tauri*. PLoS One 3: e2250.

Faure E, F. Not, A.-S. Benoiston, K. Labadie, L. Bittner and S.-D. Ayata. 2019. Mixotrophic protists display contrasted biogeographies in the global ocean. ISME J., doi: 10.1038/s41396-018-0340-5.

Fenchel, T. 2008. The microbial loop – 25 years later. J. Exp. Mar. Biol. Ecol. 366: 99–103, doi: 10.1016/j.jembe.2008.07.013.

Flynn K.J. 1988. The concept of "primary production" in aquatic ecology. Limnol. Oceanogr. 33: 1215–1216.

Flynn, K.J. and I. Butler. 1986. Nitrogen sources for the growth of marine microalgae; role of dissolved free amino acids. Mar. Ecol. Prog. Ser. 34: 281–304.

Flynn, K.J. and others. 2014. Ocean acidification with (de)eutrophication will alter future phytoplankton growth and succession. Proc. R Soc. B. 282: 20146204, doi:10.1098/rspb.2014.2604.

Flynn, K.J., S.A. Kimmance, D.R. Clark, A. Mitra, L. Polimene and W. Wilson. 2021. Modelling the effects of traits and abiotic factors on viral lysis in phytoplankton. Front. Mar. Sci. 8, doi.org/10.3389/fmars.2021.667184

Flynn, K.J. and A. Mitra. 2009. Building the "perfect beast": modelling mixotrophic plankton. J. Plankt. Res. 31: 965–992.

Flynn, K.J. and others. 2019. Mixotrophic protists and a new paradigm for marine ecology: where does plankton research go now? J. Plankt. Res. 41: 375–391, doi:10.1093/plankt/fbz026.

Flynn, K.J., and D.O.F. Skibinski. 2020. Exploring evolution of maximum growth rates in plankton. J. Plankt. Res. 42: 497–513, doi:10.1093/plankt/fbaa038.

Flynn, K.J., D.K. Stoecker, A. Mitra, J.A. Raven, P.M. Glibert, P.J. Hansen, E. Granéli and J.M. Burkholder. 2013. Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. J. Plankt. Res. 35: 3–11.

Fulton, E.A., J.S. Parslow, A.D.M. Smith and C.R. Johnson. 2004. Biogeochemical marine ecosystem models II: the effect of physiological detail on model performance. Ecol. Model. 173: 371–406.

Glibert, P.M. 1993. The interdependence of uptake and release of NH_4^+ and organic nitrogen. Mar. Microb. Food Webs 7: 53–67.

Glibert, P.M. 2020. Harmful algae at the complex nexus of eutrophication and climate change. Harmful Algae 91: 10153, doi:10.1016/j.hal.2019.03.001.

Glibert, P.M. and J.M. Burkholder. 2011. Eutrophication and HABs: Strategies for nutrient uptake and growth outside the Redfield comfort zone. Chinese J. Oceanol. Limnol. 29: 724–738.

Glibert, P.M., J.M. Burkholder, T.M. Kana, J.A. Alexander, C. Schiller, and H. Skelton. 2009. Grazing by *Karenia brevis* on *Synechococcus* enhances their growth rate and may help to sustain blooms. Aquat. Microb. Ecol. 55: 17–30.

Glibert, P.M., R. Manager, D.J. Sobota and L. Bouwman. 2014. The Haber-Bosch - Harmful algal bloom (HB-HAB) link. Environ. Res. Lett. 9: 105001(13 pp), doi.10.1088/1748-9326/9/10/105001.

Goldman, J.C., J.J. McCarthy and D.G Peavey. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279: 210–215.

Hairston, N.G., F.E. Smith, and L.B. Slobodkin. 1960. Community structure, population control, and competition. Amer. Nat. 94: 421–425.

Hartmann, M., C. Grub, G.A. Tarran, A.P. Martin, P.H. Burkill, D.J. Scanlan, and M.V. Zubkov. 2012. Mixotrophic basis of Atlantic oligotrophic ecosystem. Proc. Natl. Acad. Sci. USA 109: 5756–5760.

Hutchinson, G. E. 1961. The paradox of the plankton. Amer. Nat. 882, 137-145.

Irigoien, X., K.J. Flynn and R.P. Harris. 2005. Phytoplankton blooms: a ‘loophole’ in microzooplankton grazing impact? J. Plankt. Res. 27: 313–321.

Jeong, H.J., Y.D. Yoo, J.S. Kim, T.S. Kim, J.H. Kim, N.S. Kang and W. Yih. 2004. Mixotrophy in the phototrophic harmful alga *Cochlodinium polykrikoides* (Dinophyceae): prey species, the effects of prey concentration, and grazing impact. J. Eukaryot. Microbiol. 51: 563–569.

Jeong, H.J., Y.D. Yoo, J.S. Kim, K.A. Seong, N.S. Kang and T.H. Kim. 2010. Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. Ocean Sci. J. 45: 65–91.

Jiao, N. and others. 2012. Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat. Rev. Microbiol. 8: 593–599, doi.org/10.1038/nrmicro2386.

John, U., U. Tillmann, J. Hülskötter, T.J. Alpermann, S. Wohlrab and D.B. Van de Waal. 2015. Intraspecific facilitation by alleochemical mediated grazing protection with a toxigenic dinoflagellate population. Proc. Roy. Soc. B. 282: 20141268, doi:10.1018/rspb.2014.1268.2015.

Jones, H. 1997. A classification of mixotrophic protists based on their behaviour. Freshwat. Biol. 37: 35–43.

Lawrence, J.E., A.M. Chan and C.A. Suttle 2002. Viruses causing lysis of the toxic bloom-forming alga *Heterosigma akashiwo* (Raphidophyceae) are widespread in coastal sediments of British Columbia. Can. Limnol. Oceanogr. 47: 545–550.

Leles, S.G., J. Bruggeman, L. Polimene, J. Blackford, K.J. Flynn and A. Mitra. 2021. Differences in physiology explain succession of mixoplankton functional types and affect carbon fluxes in temperate seas. Progr. Oceanogr. 190: 102481.

Leles, S.G. and others. 2019. Sampling bias misrepresents the biogeographic significance of constitutive mixotrophs across global oceans. Glob. Ecol. Biogeogr. doi:10.1111/geb.12853.

Leles, S.G. and others. 2017. Oceanic protists with different forms of acquired phototrophy display contrasting biogeographies and abundance. Proc. Roy. Soc. B. 284: 20170664, doi: 10.1098/rspb.2017.0664.

Lin, C.-H., S. Accoroni and P.M. Glibert. 2017. Mixotrophy in the dinoflagellate *Karlodinium veneficum* under variable nitrogen:phosphorus stoichiometry: feeding response and effects on larvae of the eastern oyster (*Crassostrea virginica*). Aquat. Microb. Ecol. 79: 101–114, doi.10.3354/ameo01823.

Lindell, D., J.D. Jaffe, Z.I. Johnson, G.M. Church and S.W. Chisholm. 2005. Photosynthesis genes in marine viruses yield proteins during host infection. Nature 438: 86–89.

Lomas, M.W. and S.B. Moran 2011. Evidence for aggregation and export of cyanobacteria and nano-eukaryotes from the Sargasso Sea euphotic zone Biogeosci. 8: 203–216.

Lundgren, V. and others. 2016. Metabolic and physiological changes in *Prymnesium parvum* when grown under, and grazing on, prey of variable nitrogen:phosphorus stoichiometry. Harmful Algae 55: 1–12.

Mateus, M.D. 2017. Bridging the gap between knowing and modeling viruses in marine systems–An upcoming frontier. Front. Mar. Sci. doi.org/10.3389/fmars.2016.00284.

Meunier, C.L., M. Boersma, R. El-Sabaawi, H.M. Halvorson, E.M. Herstoff, D.B. Van de Waal, R.J. Vogt and E. Litchman. 2017. From elements to function: toward unifying ecological stoichiometry and trait-based ecology. Front. Env. Sci. 5: 18, doi:10.3389/fenvs.2017.00018.

Mitra, A. and K.J. Flynn. 2006. Promotion of harmful algal blooms by zooplankton predatory activity. Biol. Letts. 2: 194-197.

Mitra, A. and K.J. Flynn. 2010. Modelling mixotrophy in harmful algal blooms: More or less the sum of the parts? J. Mar. Syst. 83: 158–169.

Mitra, A. and K.J. Flynn. 2021. HABs and the Mixoplankton Paradigm. Harmful Algae News, doi.org/10.5281/zenodo.5109703.

Mitra, A. and others. 2014. The role of mixotrophic protists in the biological carbon pump. Biogeosci. 11: 995–1005, doi:10.5194/bg-11-995-2014.

Mitra, A. and others. 2016. Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition; incorporation of diverse mixotrophic strategies. Protist 167: 106–120, doi: 10.1016/j.protis.2016.01.003.

Pomeroy, L.R. 1974. The ocean's food web, a changing paradigm. BioSci. 24: 499–504, doi.org:10.2307/12996885.

Raven, J.A., J. Beardall, K.J. Flynn and S.C. Maberly. 2009. Phagotrophy in the origins of photosynthesis in eukaryotes and as a complementary mode of nutrition in phototrophs: relation to Darwin's insectivorous plants. J. Exp. Bot. 60: 3975–3987.

Sherr, E. and B. Sherr. 2000. Understanding roles of microbes in marine pelagic food webs: a brief history, pp. 27–44. In D.L. Kirchman [ed.], Microbial ecology of the ocean, 2nd ed. Wiley-Liss, New York.

Skovgaard, A. and P.J. Hansen. 2003. Food uptake in the harmful alga *Prymnesium parvum* mediated by excreted toxins. Limnol. Oceanogr. 48: 1161–1166.

Stickney, H.L., R.R. Hood and D.K. Stoecker. 2000. The impact of mixotrophy on planktonic marine ecosystems. Ecol. Mod. 125: 203–230.

Stoecker, D.K. 1998. Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. Eur. J. Prot. 34: 281–290.

Stoecker, D.K., M.D. Johnson, C. de Vargas and F. Not. 2009. Acquired phototrophy in aquatic protists. Aquat. Microb. Ecol. 37: 279–310.

Sullivan, M.B., J.B. Waterbury and S.W. Chisholm. 2003. Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. Nature 424: 1047–1051.

Suttle, C.A. 2007. Marine viruses--major players in the global ecosystem. Nat. Rev. Microbiol. 5: 801–812.

Syrett, P.J. 1981. Nitrogen metabolism of microalgae. Can. Bull. Fish. Aquat. Sci. 210: 182–210.

Thingstad, T.F., H. Havskum, K. Garde and B. Riemann. 1996. On the strategy of "eating your competitor": A mathematical analysis of algal mixotrophy. Ecol. 77: 2108–2118.

Thingstad, T.F., S. Våge, G. Bratbak, J. Egge, A. Larsen, J.C. Nejstgaard and R.A. Sandaa. 2021. Reproducing the virus-to-copepod link in Arctic mesocosms using host fitness optimization. Limnol. Oceanogr. 66: S303–S313.

Tillmann, U. 2003. Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum*. Aquat. Microb. Ecol. 32: 73–84.

Traboni, C., A. Calbet and E. Saiz. 2020. Effects of prey trophic mode on the gross-growth efficiency of marine copepods: the case of mixoplankton. Sci. Rep. 10: 1–14.

Weitz, J.S. and S.W. Wilhelm. 2012. Ocean viruses and their effects on microbial communities and biogeochemical cycles. F1000 Biol. Rep., doi:10.3410/B4-17.

Wilhelm, S.W. and C.A. Suttle. 1999. Viruses and nutrient cycles in the sea – viruses play critical roles in the structure and function of aquatic food webs. BioSci. 49: 781–788.

Williams, P.J. leB. and H. Ducklow. 2019. The microbial loop concept: A history, 1930-1974. J. Mar. Res. 77 (Suppl): 23–81.

Wilson, W.H. and others. 2005. Complete genome sequence and lytic phase transcription profile of a *Coccolithovirus*. Science 309: 1090–1092.

Wright, R.T. and J.E. Hobbie. 1965. The uptake of organic solutes in lake water. Limnol. Oceanogr. 10: 22–28

Zhang, S., H. Liu, P.M. Glibert, C. Guo and Y. Ke. 2017. Elemental stoichiometry and nutrient excretion of *Noctiluca scintillans* in response to prey of different quality. Sci. Rep. 7: 7622, doi:10.1038/s41598-017-05991-w.

Zubkov, M.V. and G.A. Tarran. 2008 High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. Nature 455: 224–227.

Authorship contributions

PMG: conceptualization (original idea; formulation and evolution of overarching research goals and aims), visualization, writing, AM: conceptualization (formulation and evolution of overarching research goals and aims.), visualization, writing.

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Table 1. Abbreviations used in text. Entries are ordered by their first use in text.

Abbreviation	**Definition**
DOC	Dissolved organic carbon
POC	Particulate organic carbon
HAB	Harmful algal blooms
BCP	Biological carbon pump– fixation of C into POC and its subsequent sinking from the euphotic zone
MCP	Microbial carbon pump– bacterially mediated transformation of labile DOC to recalcitrant DOC
CM	Constitutive mixotroph- those mixotrophs with an inherent ability to photosynthesize (they make their own chloroplasts)
NCM	Non-constitutive mixotroph–microzooplankton that can photosynthesize through acquired phototrophy
GNCM	Generalist non-constitutive mixotroph- NCM acquiring plastids from a range of prey
SNCM	Specialist non-constitutive mixotroph– NCM acquire plastids from specific species. There are two types, pSNCM and eSNCM
pSNCM	Specialist non-constitutive mixotroph– NCM acquire plastids from specific species.
eSNCM	Endosymbiotic specialist non-constitutive mixotroph–NCM that harbor photosynthetic endosymbionts
DIM	Dissolved inorganic matter
DOM	Dissolved organic matter

Figure Legend

Figure 1. (a). The traditionally-viewed food 'chain', now envisioned as a food 'web' with the introduction of the microbial loop (arrows with red outline). In the web, dissolved nutrients and organic matter are taken up by bacteria that are, in turn consumed by protozooplankton such as flagellates and ciliates, and they, in turn, also support higher trophic levels, and contribute to nutrient recycling. Note that the elements of the web are highlighted in color, while the traditionally-viewed food chain is depicted in gray-scale. (b). The microbial food-web and microbial loop with inclusion of the viral shunt. In addition to the pathways depicted in (a), the roles of viruses are represented. They infect bacteria, phytoplankton and protozooplankton (solid red arrows), in turn releasing particulate and dissolved organic matter during their growth and reproduction (dashed red arrows). (c). The microbial food-web, including the microbial loop and the viral shunt, under the mixoplankton paradigm (orange arrows). The phytoplankton now include diatoms and cyanobacteria. The mixoplankton community comprise constitutive mixoplankton (CM), that can graze a wide range of particles, from bacteria to other phytoplankton, and, non-constitutive mixoplankton (NCM) which in addition to grazing can also photosynthesize through acquired phototrophy. All these organisms form the microbiome and their interactions alter the flow of dissolved and particulate organic matter, as well as the nutritional quality of material transferred through the food-web. Organism icons are from the University of Maryland Center for Environmental Integration and Application Network, Algal Resources Collection (Wilmington, NC) and flicker, under a creative commons license.

Figure 2. Conceptual model linking phytoplankton stoichiometry, the biological and microbial carbon pumps (BCP and MCP), and propensity for mixoplankton. Modified from Polimene et al. 2017)

Figure 2. Three contrasting food web structures (upper panels) and the corresponding temporal patterns (lower panels) of the development of biomass (gC L^{-1}) in the simulated communities over 30 days. (a) The classic food-web paradigm in which phytoplankton (Phyto) and Protozooplankton (μZ) and Bacteria (B) are the dominant protist plankton functional types; (b) as for the panel (a) except that the μZ functional type is replaced by the generalist non-constitutive mixoplankton (GNCM; e.g., plastidic ciliates); and (c) as for panel (a) except that the μZ functional type is replaced by the constitutive mixoplankton (CM; e.g., dinoflagellates). The release and uptake of dissolved inorganic and organic matter (DIM and DOM, respectively) are indicated. Note the higher biomass of the GNCM and CM relative to phytoplankton and the sustained duration of the CM over the time period shown. Figures modified from Mitra et al. 2016.

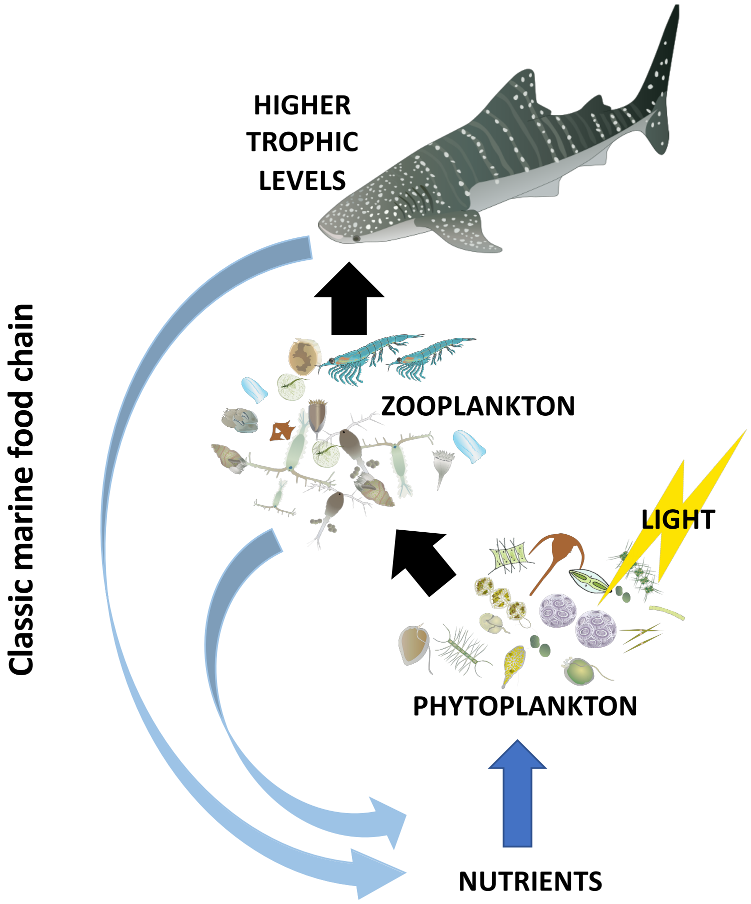


Figure 1. (a). The classic food chain in which dissolved inorganic and organic nutrients are taken up by phytoplankton, which in turn are grazed by zooplankton and they, in turn support higher trophic levels. Nutrient recycling occurs via the grazing processes of zooplankton and higher trophic levels. Organism icons are from the University of Maryland Center for Environmental Integration and Application Network, Algal Resources Collection (Wilmington, NC) and flicker, under a creative commons license.

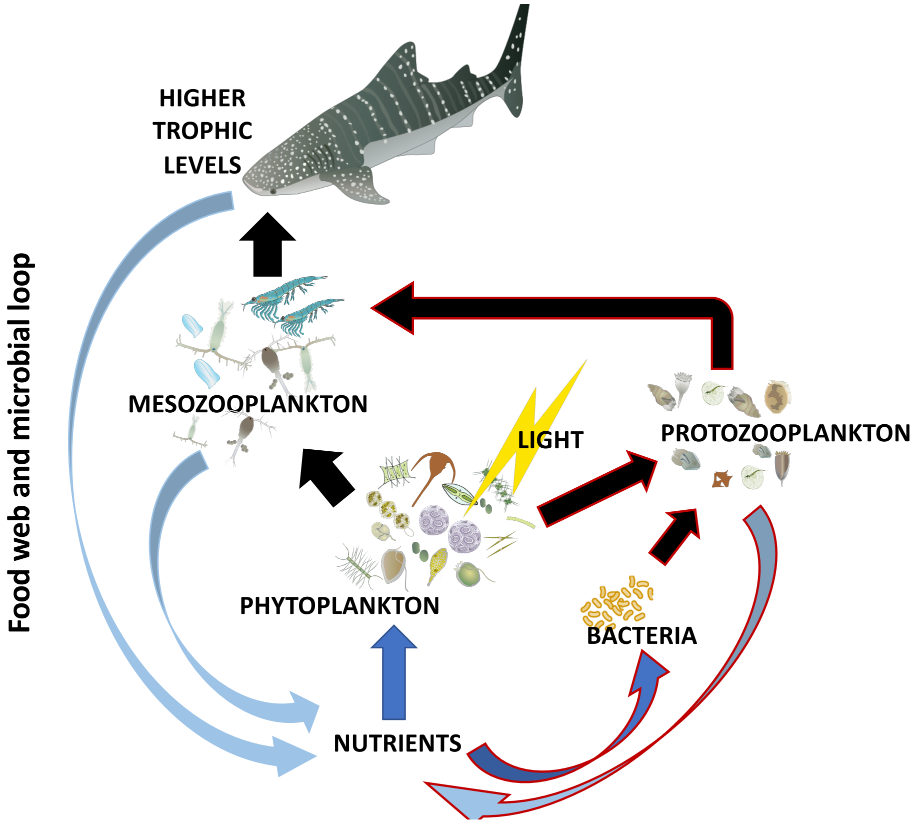
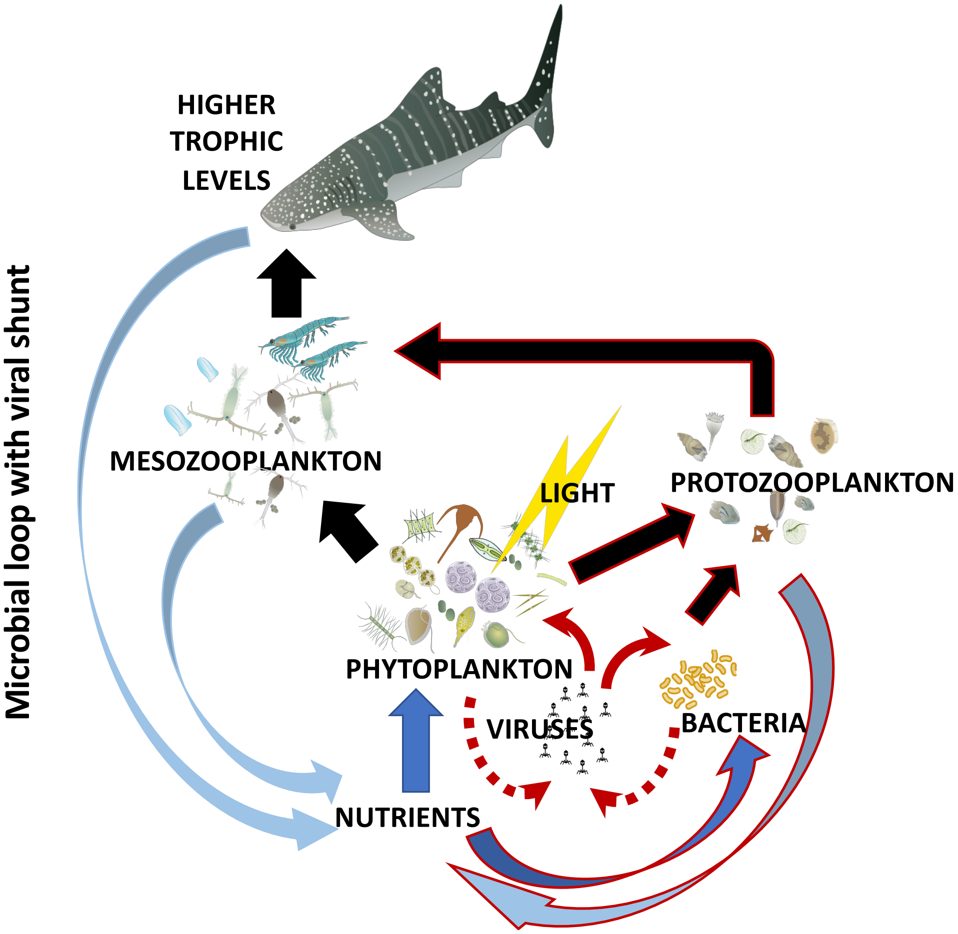


Figure 1. (b). The classic food chain, now envisioned as a food-web with the introduction of the microbial loop (arrows with red outline). In addition to the transfer of energy and materials depicted in (a), dissolved nutrients and organic matter are taken up by bacteria that are, in turn consumed by protozooplankton, such as flagellates and ciliates, and they, in turn, support higher trophic levels and contribute to nutrient recycling.



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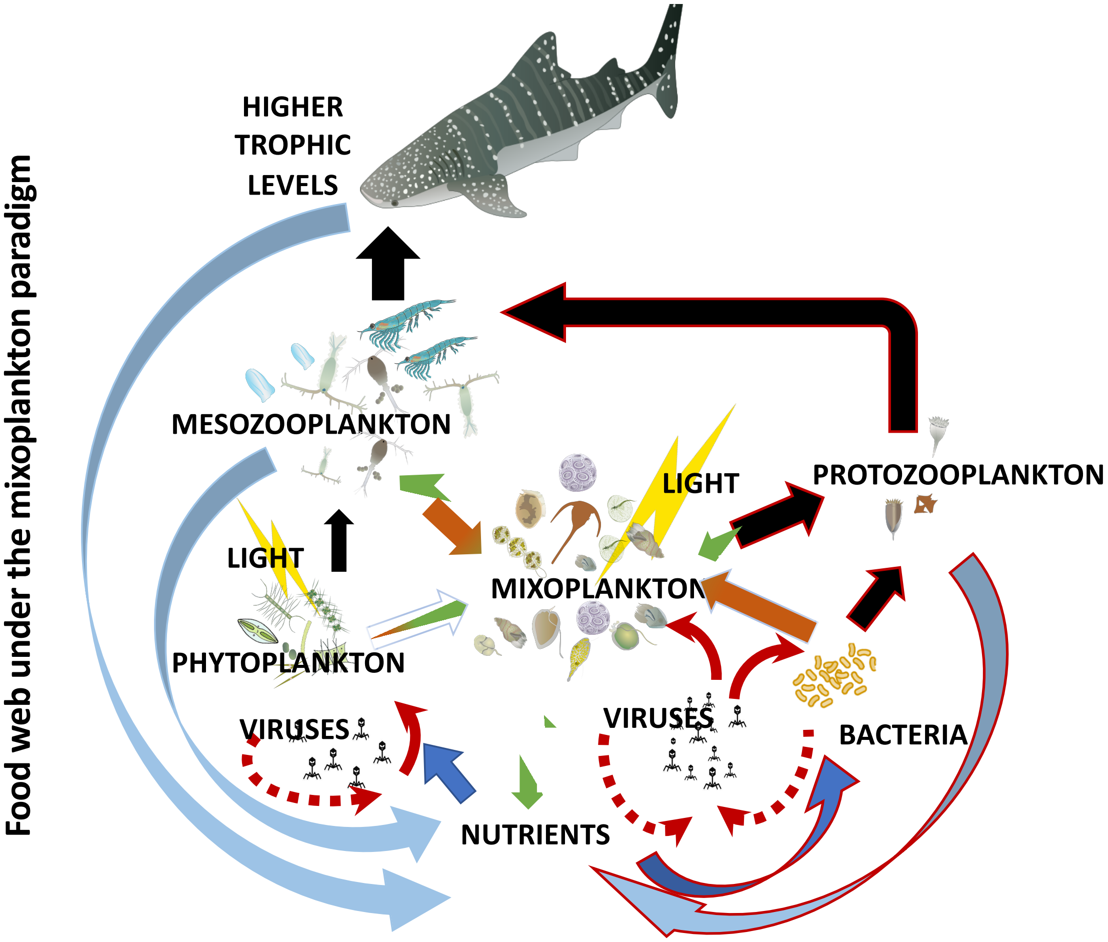
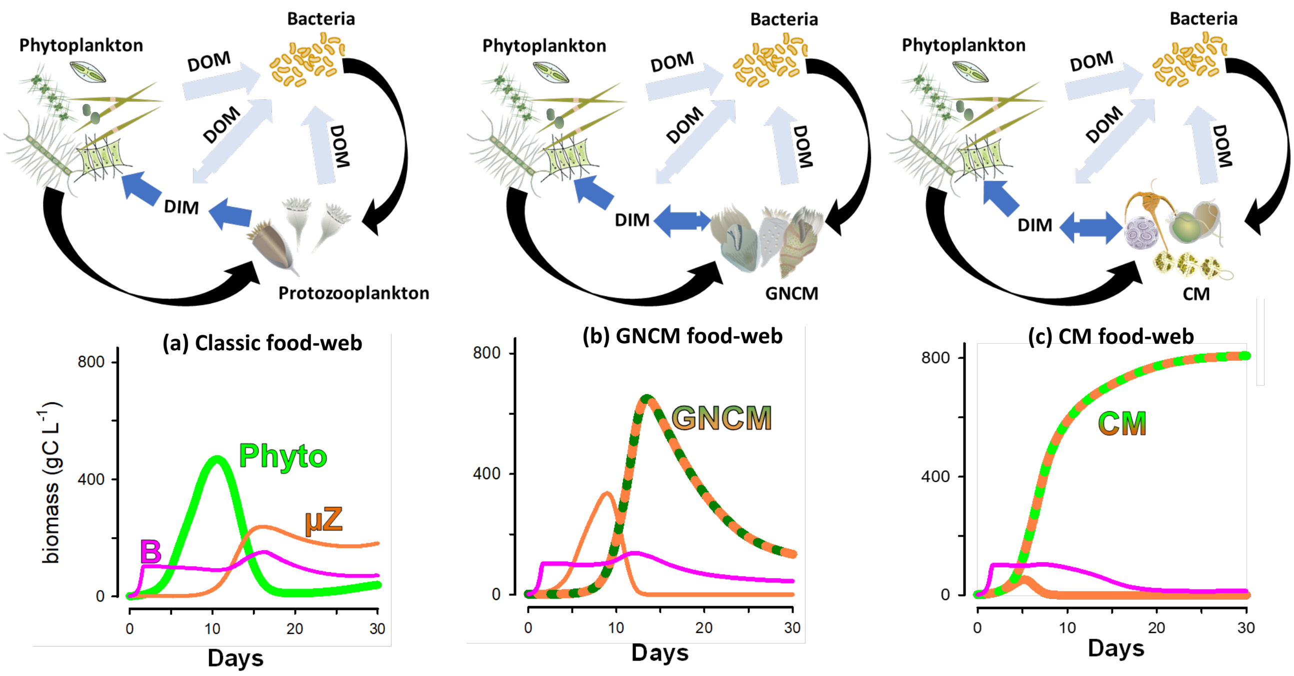


Figure 1. (d). The microbial biome, including the microbial loop and the viral shunt, under the mixoplankton paradigm (orange arrows). The phytoplankton now include diatoms and cyanobacteria. The mixoplankton community comprise constitutive mixoplankton (CM) that can graze a wide range of particles, from bacteria to other phytoplankton, and the non-constitutive mixoplankton (NCM) which in addition to grazing can also photosynthesize through acquired phototrophy. All these organisms form the microbiome and their interactions alter the flow of dissolved and particulate organic matter, as well as the nutritional quality of material transferred through the food-web.



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