

From webs, loops, shunts, and pumps to microbial multitasking: Evolving concepts of marine microbial ecology, the mixoplankton paradigm, and implications for a future ocean

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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. Conceptual models of microbial interactions have evolved from food-chains, where carbon-fixing phytoplankton are conceived as being grazed solely by zooplankton that, in turn, support fisheries and higher trophic levels, to microbial webs, loops, and shunts, as knowledge about abundance, activity, and roles of marine microbial organisms—as well as the complexity of their interactions—has increased. In a future world, plankton that depend on a single strategy for acquiring nutrition (photo-autotrophy or phago-heterotrophy) may be disadvantaged with increasing temperatures and ocean acidification impacting vital rates, thermal stratification decreasing water column nutrient exchange, and anthropogenic pollution shifting amounts, forms, and proportions of nutrients. These conditions can lead to stoichiometric imbalances that may promote mixoplanktonic species with an increasing likelihood of harmful blooms. Such changes in plankton species composition alters the interconnectivity of oceanic microbes with direct consequences on biogeochemical cycling, trophic dynamics, and ecosystem services. Here, the implications of the mixoplankton paradigm relative to traditional concepts of microbial oceanography in a globally-changing, anthropogenically-impacted world are explored.

In the plankton microbial world, there is competition for resources and winners achieve that status by avoiding death as much as from an inherent faster 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 coexisting microbes occupy outwardly similar, but sometimes subtly different, niches led to various researchers in the 1940's–1960's to question the validity of the then-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 in species that seemed to occupy the same ecological niche. That a number of species can coexist 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 giving them access to different resources in different ways. More recently our understanding of marine

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ecology—specifically how the base of the food web functions—has been questioned.

The origin of most marine concepts has a basis in terrestrial ecology. For example, the aquatic food-chain was originally conceptualized based on the 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 photosynthesizing phytoplankton as the primary producers, the “herbivorous” zooplankton as the primary consumers, followed by carnivorous zooplankton and fish as the secondary consumers, etc. Based upon the concept of functional groupings in botany, the marine organisms have also been assigned functional groups such as phytoplankton, bacterioplankton, zooplankton. Over the last decade, there has been an increasing awareness about the existence of another important functional group within the marine microbial communities—the mixoplankton, 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 reimagining of the marine plankton food-web, and a reinterpretation of “phytoplankton” and “(proto)zooplankton”.

With the ongoing changes in the global climate, plankton diversity and succession, the understanding of the functioning of the marine food-web is 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 radically alters our understanding of how plankton interact, how the dynamics of nutrient cycling may change, and the importance of understanding these dynamics in predicting how the microbial community may change in a future ocean.

From food chains to webs and loops

The importance of plankton as feed for other marine organisms has long been known. Likewise, the functioning of higher trophic levels of the marine ecosystem as a food-web has also been recognized for nearly over a century, such as the North Atlantic herring food-web (Hardy 1924). However, the functioning of the microbial communities has traditionally been viewed to follow a linear food-chain where plant-like phytoplankton form the base. The phytoplankton have been viewed to be consumed by the primary consumers (protozooplankton and mesozooplankton) which are then eaten by small fish, and these in turn provide food for larger fish and higher trophic levels. Thus, even though feeding habits of multiple components of the marine food-web were considered multilayered even by Hardy (1924), no such complexity was considered for the base of the food-web—the prokaryotic and protistan planktonic communities.

In these traditionally conceived food-webs, the microbial interactions were considered as linear food chain systems with

very little focus on bacteria. If considered at all, bacteria were viewed primarily as decomposers of what “rained out” of the surface ocean (e.g., Mann 1988 and references therein) 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 (Pomeroy 1974; Azam et al. 1983). The microbial loop introduced by Pomeroy (1974), and more formally described by Azam et al. (1983), led to the food chain description of microbial components of marine food-webs to be reimagined as a “web,” with the flow of energy and materials mediated at numerous steps by bacteria (Fig. 1a); bacteria were thus recognized to be consumers as well as decomposers. While one could argue that this is a semantic difference, it should be noted that it is an important point as consumption of dissolved organic matter (DOM; see Table 1 for list of abbreviations) by bacteria enables its repackaging and reincorporation. This conceptual change also recognized 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.

The concept of the “microbial loop” represented a major paradigm shift in marine ecology of the 20th century. This conceptual shift was also dependent on acceptance of a greater abundance of bacteria in the ocean than previously recognized, and better quantification of all types of microbes. 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 web, which encompasses not only bacteria, but also viruses, archaea, and heterotrophic protists, rather than unidirectionally decomposing materials, provides pathways by which DOM, largely derived from phytoplankton exudates, is recycled, and ultimately either passed to higher trophic levels or sequestered to deeper oceans in recalcitrant forms (Polimene et al. 2017). The heterotrophic protists, as consumers of bacteria, further contribute to the dissolved organic pool via excretion and to the particulate organic pool via production of fecal material. Thus, the protist predator–bacterial prey interactions, coupled with bacterial nutrient uptake and decomposition activities, modulate not only the amounts and rates of inorganic and organic material available for phytoplankton and bacterial growth, respectively, but also the transfer of energy.

The advancement of methods also included the advent and proliferation of field techniques specifically associated with microbial grazing (e.g., dilution experiments; Landry and Hassett 1982, reviewed by Schmoker et al. 2013; cf. Ferreira et al. 2022) which revealed that microzooplankton, rather

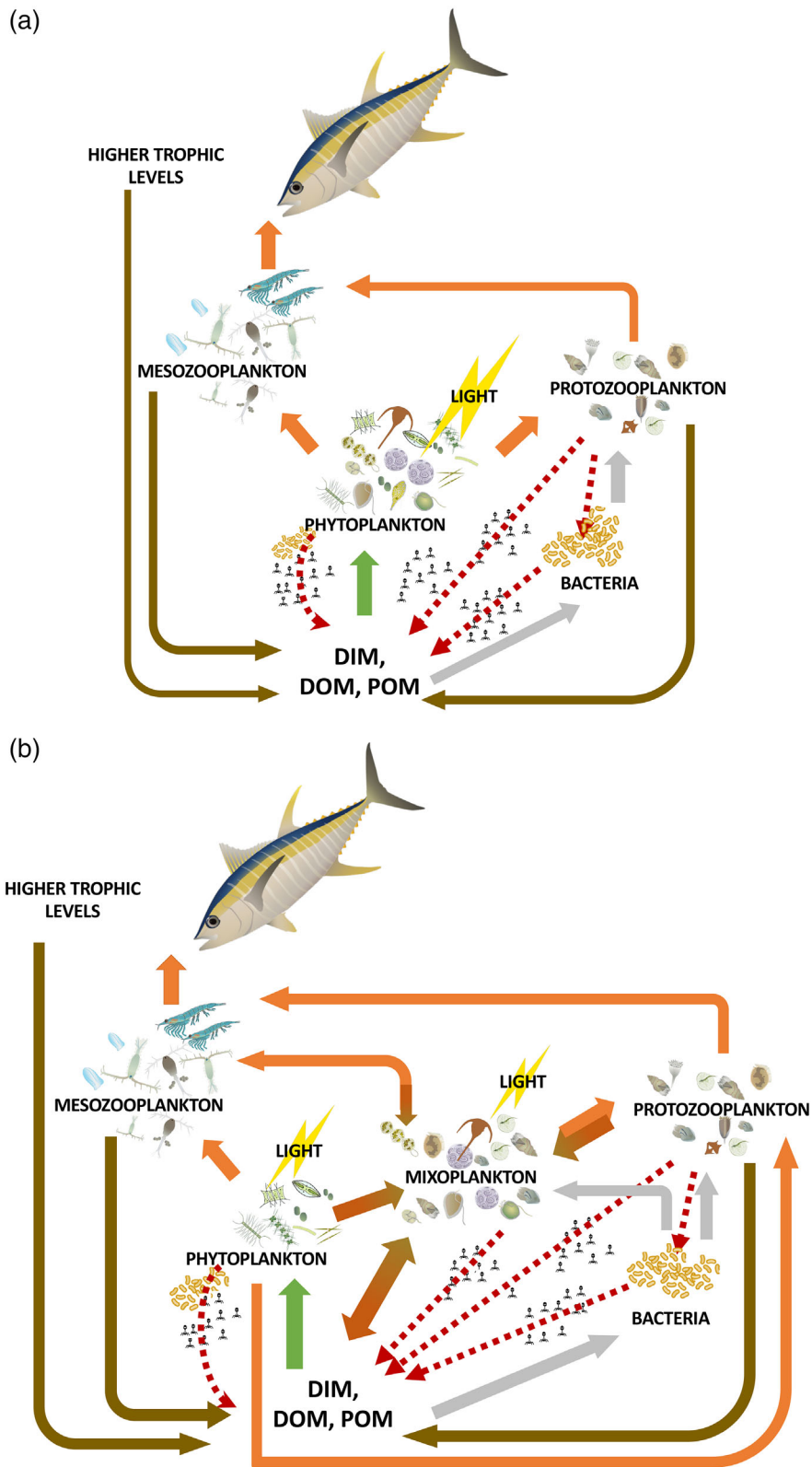


Fig. 1. Conceptual understanding of microbial interactions and the oceanic food web. (a) Concepts before the advent of the mixoplankton paradigm. This scheme envisions phytoplankton as the primary producers using dissolved inorganic matter (DIM; green arrows) and light for carbon fixation. Zooplankton are the consumers with protozooplankton ingesting phytoplankton and bacteria while mesozooplankton ingest phytoplankton and (Figure legend continues on next page.)

than mesozooplankton, were responsible for most of the consumption of primary production. Thus, the acceptance of the role of bacteria evolved, from simple decomposers, as a significant and actively cycling flux of DOM became recognized and understanding of the different forms of DOM improved. Further, it was recognized that this active flux could only be accounted for by an active and abundant bacterial community—a community that could be counted with the advancement of methods (reviewed by Williams and Ducklow 2019).

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 DOM and particulate organic matter (POM; Wilhelm and Suttle 1999; Jiao et al. 2010; Fig. 2a, Table 1). With 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 communities became more prominently recognized. The importance of the viral shunt to the understanding of microbial trophic dynamics was that, depending on the specific compounds released by viral lysis, the cycling of organic—and therefore inorganic—compounds could be modified, as some these compounds could be readily used by the microbial plankton while other compounds were more calcitrant (Weitz and Wilhelm 2012). Viruses were also found to be responsible for lysing some types of harmful algal blooms (HABs) (e.g., Lawrence et al. 2002; Baudoux et al. 2006) and thus the recognition that the abundance of specific “phytoplankton” taxa could be modified through viral interactions (Irigoien et al. 2005). 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 DOM and POM, respectively, for further consumption by bacteria.

From loops and shunts to pumps

The microbial loop concept subsequently 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 debated with the differentiation between the biological pump (BP) and the microbial carbon pump (MCP). The former encapsulates the notion that bacteria mediate the transformation of dissolved organic C (DOC) to more recalcitrant and particulate forms that are subsequently lost to the deep sea via marine snow or sinking fecal pellets, a process considered important in under most oceanic-oligotrophic conditions (e.g., Jiao et al. 2014). 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, 2014).

The balance between the BP and MCP is a function of the production of DOM and its resulting quality (i.e., labile vs semi-labile vs recalcitrant, Jiao et al. 2014). This then impacts the composition of microbial communities (e.g., labile DOM supports primary producers while recalcitrant DOM does not) and therefore the stoichiometric balance of the microbial communities (Polimene et al. 2017). It has also been proposed that viruses can be significant drivers of the BP, 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 BP 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).

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protozooplankton. The mesozooplankton support the higher trophic levels (as prey) and also the phytoplankton and bacterial community (via excretion of DIM and defecation of particulate organic matter [POM], respectively). Orange arrows indicate grazing activities; brown arrows, excretion, and defecation. The microbial loop, depicted using gray arrows, portrays the multi-role of the bacteria where dissolved inorganic and organic matter (DIM, DOM) are taken up by bacteria that are, in turn consumed by protozooplankton; protozooplankton support higher trophic levels and contribute to nutrient recycling through excretion and defecation. The viral shunt process is depicted using dashed red arrows; viruses infect bacteria, phytoplankton and protozooplankton, in turn releasing particulate and dissolved organic matter. **(b)** The revised interactions in the food-web under the mixoplankton paradigm. Same as (a) except recognition of mixoplankton as a functional group engaging in photo-phago-mixotrophic strategies (orange-green gradient arrows). The true “phytoplankton” are now comprised of diatoms and cyanobacteria. The mixoplankton community is comprised of constitutive mixoplankton (CM) that can graze on a wide range of particles, from bacteria to other phytoplankton, zooplankton, 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. Colored arrows as in panel (a). Organismal icons are from the University of Maryland Center for Environmental Science Integration and Application Network.

Table 1. Abbreviations and terminologies used in text.

Abbreviation	Definition
Autotrophy	Nutrition involving the synthesis of complex organic substances using photosynthesis (phototrophy) or chemosynthesis. Typically associated with the use of inorganic nutrients
BP	Biological pump; transport and sequestration of organic carbon from upper (euphotic) ocean to deeper ocean on time scales of months to millennia
CM	Constitutive mixoplankton; protist plankton with an inherent capacity for phototrophy that can also exhibit phagotrophy
DIM	Dissolved inorganic matter; this includes inorganic nitrogen (e.g., nitrate, nitrite, ammonium), carbon, and phosphorus
DOC	Dissolved organic carbon
DOM	Dissolved organic matter; this includes dissolved organic nitrogen (DON), carbon (DOC) and phosphorus (DOP). DOM could be labile, semi-labile, or refractory
eSNCM	Endosymbiotic specialist non-constitutive mixoplankton; SNCM that acquire their capacity for phototrophy through harboring photosynthetic endosymbionts
GNCM	Generalist non-constitutive mixoplankton; NCM that acquire their capacity for phototrophy from general (i.e., nonspecific) phototrophic prey
HAB	Harmful algal blooms
Heterotrophy	Nutrition involving the consumption and interconversions of sources of organic carbon; this includes osmotrophy, phagotrophy
MCP	Microbial carbon pump; bacterially mediated transformation of labile DOC to recalcitrant DOC
Microbial loop	Consumption of DOM by bacteria following consumption of the bacteria by protozooplankton leading to reintroduction of energy into the food-web
Mixoplankton	Plankton protists capable of obtaining nourishment via photo-auto-trophy and phago-hetero-trophy and osmo-hetero-trophy
Mixotrophy	Nutrition involving both autotrophy and heterotrophy
NCM	Non-constitutive mixoplankton; protist plankton that acquire the capability for phototrophy from consumption (via phagotrophy) of phototrophic prey. There are three types, GNCM, pSNCM and eSNCM.
Osmotrophy	A mode of heterotrophy involving the uptake and consumption of DOM, also referred to as osmo-hetero-trophy
Phagotrophy	A mode of heterotrophy involving the engulfment of particles (often whole organisms) into a phagocytic vacuole in which digestion occurs, also referred to as phago-hetero-trophy
Phototrophy	A mode of autotrophy involving the fixation of CO ₂ using energy derived from light, also referred to as photo-auto-trophy
Phytoplankton	Plankton obtaining nourishment via photo-auto-trophy and osmo-hetero-trophy. They are incapable of phagotrophy. These include the eukaryotic diatoms and prokaryotic cyanobacteria
POC	Particulate organic carbon
POM	Particulate organic matter, this includes organic nitrogen, carbon, and phosphorus
Protist	Single-celled eukaryotic organism
Protozooplankton	Protist zooplankton obtaining nourishment via heterotrophy. They cannot engage in phototrophy
pSNCM	Plastidic specialist non-constitutive mixoplankton; SNCM that acquire their capacity for phototrophy from sequestration of photosynthetic apparatus and nuclear material from specific phototrophic prey
SNCM	Specialist non-constitutive mixoplankton; NCM that acquire their capacity for phototrophy from specific phototrophic prey. There are two types, pSNCM and eSNCM
Viral shunt	Release of cellular material through lysis of plankton hosts by marine viruses in the form of DOM and POM

Loops, shunts, and pumps to microbial multitasking

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 experiments, 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 by 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; Burkholder et al. 2008). It now seems, from the perspective of

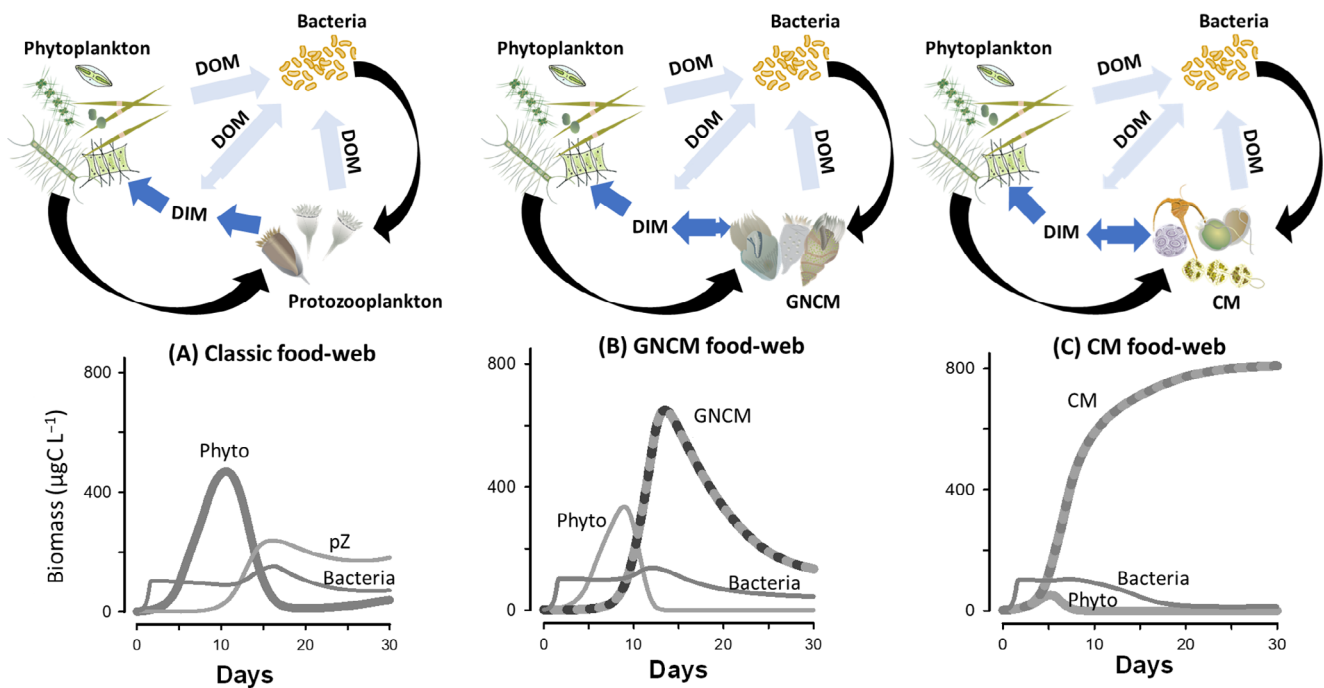


Fig. 2. Three contrasting food web structures (upper panels) and the corresponding temporal patterns (lower panels) of the development of biomass ($\mu\text{gC L}^{-1}$) in the simulated communities over 30 d. (a) The classic food-web paradigm in which phytoplankton (Phyto) and Protozooplankton (pZ) and bacteria (b) are the dominant protist plankton functional types; panel (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 panel (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.

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 multiple reasons, prior to the most recent decade, 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).

The mixoplankton paradigm

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 and cyanobacteria, are also potential grazers, and over one-third of protozooplankton are capable of acquired phototrophy (Stoecker et al. 2009, 2017; Flynn et al. 2013). This has 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 helps to flag 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 (Fig. 1b). They perform the dual role of being primary producers, photosynthesizing with either their own or acquired chloroplasts, as

well as being primary consumers ingesting bacteria, phytoplankton, protozooplankton, and indeed other mixoplankton. These interactions then impact physiological vital rates and quality of release of DOM and/or dissolved inorganic material (DIM) through digestion, excretion, and leakage. While mixoplankton are themselves consumers, due to their wide size range, they can also be prey (or predators) for many primary and secondary consumers (Flynn et al. 2019).

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 (1) constitutive mixoplankton (CM)—those plankton that have an inherent ability to photosynthesize (they have genetic ability to make their own chloroplasts) but that also acquire food through predation; and (2) 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*, *Triplos 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 mixoplankton (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 mixoplankton (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 SNCM (eSNCM), those that harbor photosynthetic endosymbionts (e.g., different rhizarians).

Implications of the mixoplankton paradigm for the food-web and microbial loop

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. 1b). At the simplest level, there are advantages to eating your competitor; phytoflagellates, previously considered as strictly phototrophic, now recognized capable of consuming other algae or bacteria, may gain this advantage (Thingstad et al. 1996; Mitra et al. 2014). Productivity and/or growth may then increase due to the dual channels by which the organism gains its C or nutrients. Essential elements such as C, nitrogen (N) or phosphorus (P) are typically

rich in microbial prey, and therefore upon feeding, the mixoplankton are provided not only an element that may have been otherwise in limiting proportion, but the consumer gains all elements prepackaged 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*, traditionally considered a phytoplankton, nearly doubles its growth rate when allowed to grow as a mixoplankton compared to when no prey were provided and therefore it was forced to survive on phototrophy only (Jeong et al. 2004). Similar observations have been reported for the HAB-forming dinoflagellates *Karlodinium veneficum* and *Karenia brevis*—now known to be of the CM type (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 pure phototrophs, may in reality have an advantage over competitors under dissolved-nutrient-limiting conditions, leading to compounded gains over time (Fig. 2). This is one strategy by which HABs may form (e.g., Glibert and Burkholder 2011). Indeed, most protist HAB species (i.e., excluding the mixotrophic cyanobacterial and diatom HABs) are now recognized to be mixoplanktonic (Mitra and Flynn 2021).

Mixoplankton may selectively feed on prey that have a nutritional composition complementary to themselves, 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). When a macrograzer consumes a mixoplankton, this upgraded trophic benefit may be further transferred (Traboni et al. 2020). If the grazer is itself a mixoplankton, as in the case of the multi-link mixoplankton consortia of a bacterivorous CM cryptophyte—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; Fig. 3). Such advantages afforded to mixoplankton may outweigh their supposedly comparatively poor growth rates in otherwise non-limiting nutrient conditions (e.g., Burkholder et al. 2008). Similarly, in oceanic waters, mixoplanktonic activity may help to explain why oceanic “phytoplankton” have the ability to grow relatively rapidly even though inhabiting oligotrophic waters (Goldman et al. 1979); they may be opportunistic in taking up dissolved nutrients and they may graze on bacteria or cyanobacteria or other prey to

acquire nutrients (Zubkov and Tarran 2008; Hartmann et al. 2012; Mitra et al. 2014).

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 (e.g., Glibert and Burkholder 2011). The toxin may harm or stun the prey, in turn making the prey easier to capture (e.g., 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 and therefore the MCP (Flynn et al. 2021; Thingstad et al. 2021). Further, mixoplanktonic 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; Leles et al. 2021).

Implications of the mixoplankton paradigm for microbial oceanography

That a large proportion of protist plankton are, in fact, mixoplankton (Flynn et al. 2013) suggests that they are able

to thrive in widely varying nutrition conditions from an inorganic-nutrient perspective (Burkholder et al. 2008; Jeong et al. 2010; Glibert and Burkholder 2011; Glibert 2020). Thus, mixoplankton as a functional group are ubiquitous in the ocean and can be found from oligotrophic conditions, where nutrients are limiting, to eutrophic environments, where nutrients may be available but perhaps stoichiometrically imbalanced (Burkholder et al. 2008; Leles et al. 2017, 2019, 2021; 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. 2).

Much is yet to be explored—empirically and in models—with respect to the significance of mixoplankton in ocean biogeochemistry, C and nutrient cycling, and in trophic dynamics. Modeling 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. 2) with incorrect predictions

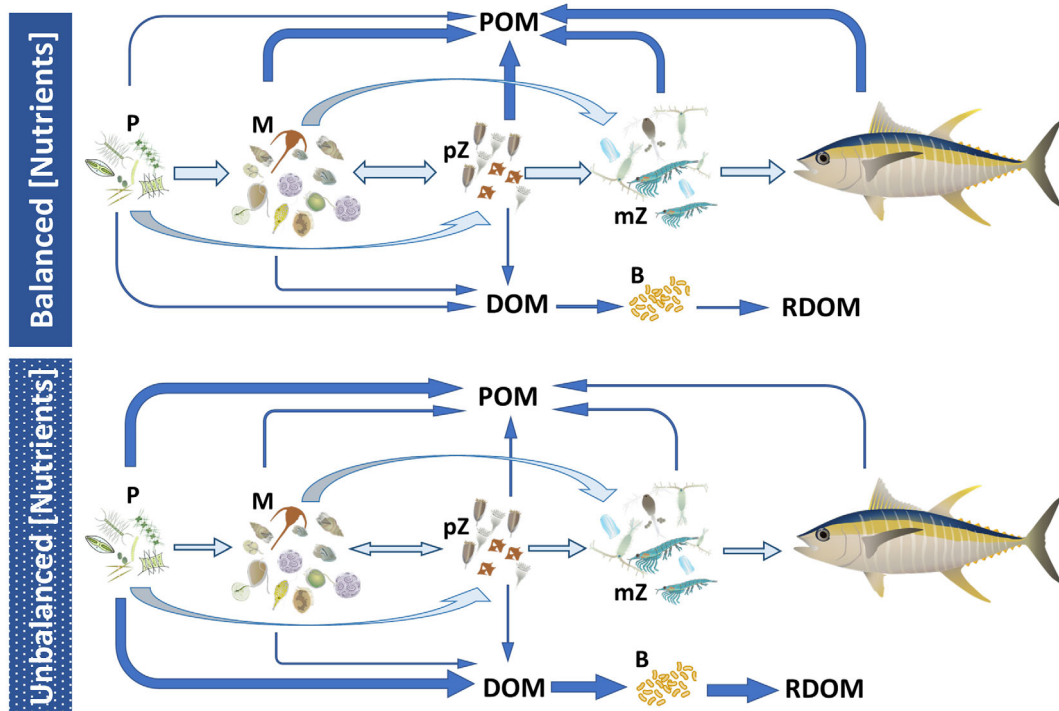


Fig. 3. Conceptual model demonstrating impact of different environment nutrient concentrations on protist plankton stoichiometry and the biological and microbial carbon pumps (BP and MCP) under the mixoplankton paradigm. Modified from Polimene et al. (2017).

associated with the MCP (Mitra et al. 2014; Polimene et al. 2017). 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 world—where waters may be more CO₂ enriched, more eutrophic, and generally warmer (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 disproportionately 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 (Fig. 3). Any enhancement of the MCP will further enhance mixoplankton dominance by enhanced bacterial activity and abundance, in turn increasing food availability for those microbial multitaskers 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; Glibert 2020), 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 (Fig. 3). Meunier et al. (2017) predict that increasing N : P ratios should shift ecosystems toward 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 (Fig. 1b). The inclusion of mixoplankton alters the quality and fate of DOM and POM, 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 (Fig. 3).

Inclusion of mixoplankton interactions in understanding microbial trophic dynamics is not simply a more complex version of the previous conceptual models, it has fundamental differences. 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

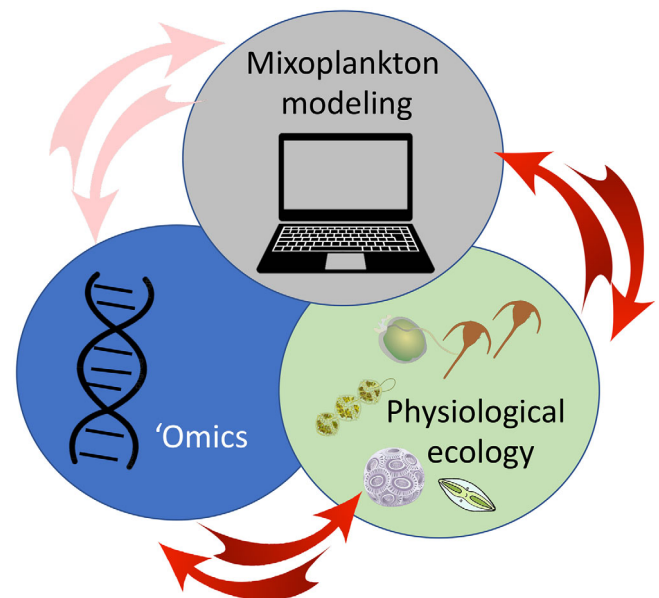


Fig. 4. Cooperation between different scientific approaches is needed in support of mixoplankton research. While ecophysiological studies are relatively easily linked to both molecular and ‘omics’ studies and modeling, linkages from the semi-quantitative science of molecular biology to modeling remains in its infancy. Modified from Mitra et al. (2021).

processes involved, adds tremendous complexity to models. While progress is advancing in characterizing metabolic versatility via genomic data, incorporating such data in models remains problematic (e.g., Caron et al. 1999; Coles and Hood 2016; Santoferrara et al. 2020). For example, 'omics tools do not necessarily provide the rate data required to support simulation and mechanistic models (Mitra et al. 2021; Fig. 4). Experiments are required that explicitly explore the processes of photosynthesis, grazing and nutrient status under varying environmental conditions—including conditions that are outside the traditionally considered “normal” range of temperature, CO₂, nutrients, pH and allied factors. 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. Likewise, there is a need to reappraise extant fieldwork methodologies which typically focus on phototrophic or phagotrophic activities; for example, the traditional dilution experiments do not appear to be suitable for gauging mixoplanktonic activity (Ferreira et al. 2022). Advancing the next generation ecological models will occur as biologically and physiologically meaningful empirical experiments are conducted, as suitable empirical data are generated and as new approaches to incorporate new data streams (including 'omics data) in models become routine (Fig. 4).

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.

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Conflict of Interest

None declared.

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