

Chapter 7

Mixotrophy in Harmful Algal Blooms: By Whom, on Whom, When, Why, and What Next



Kevin J. Flynn, Aditee Mitra, Patricia M. Glibert,
and JoAnn M. Burkholder

7.1 Introduction

The traditional view of the planktonic food web is simplistic: nutrients are consumed by phytoplankton that, in turn, support zooplankton that ultimately support fish. Historically, harmful algal species have been viewed as phototrophic phytoplankton, although with some notable exceptions. This primary producer-centric structure is the foundation of most models that have been used to explore fisheries production, biogeochemical cycling, and climate change. In recent years, however, the importance of mixotrophs increasingly has been recognized, changing scientists' view of these traditional food web interactions (Katechakis et al. 2005; Glibert and Legrand 2006; Burkholder et al. 2008; Jeong et al. 2010; Hansen 2011; Flynn et al. 2013; Mitra et al. 2016). Mixotrophy, the combination of phototrophy and heterotrophy (phago- and/or osmotrophy), enables planktonic protists traditionally labelled as “phytoplankton” or “microzooplankton” to function at multiple trophic levels. Harmful algal blooms (HABs), including both high-biomass bloom formers and toxic species, are now recognized to be not only more prevalent in waters worldwide but also typically mixotrophic (Burkholder et al. 2008). Thus, it is important that concerted efforts are taken to understand mixotrophy as an important nutritional strategy that alters food web dynamics.

K.J. Flynn (✉) • A. Mitra
Wallace Building, Swansea University, Swansea, UK
e-mail: k.j.flynn@swansea.ac.uk

P.M. Glibert
University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge,
MD, USA

J.M. Burkholder
Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC, USA

The processes of mixotrophy can radically change the dynamics and efficiency of activities of the “phytoplankton” and “microzooplankton” (Flynn and Mitra 2009; Mitra and Flynn 2010; Flynn et al. 2013; Mitra et al. 2014b). Mixotrophy includes the concept of osmotrophy, the uptake of organic substrates, and phagotrophy, the ingestion of particulate material, by those organisms normally thought to be dependent on inorganic carbon (C) via photosynthesis. Osmotrophy is ubiquitous among the protists (e.g., Berman and Bronk 2003; Glibert and Legrand 2006); phagotrophy is also much more common than previously recognized and has direct implications for trophic dynamics (namely, grazing). A fundamental revision of the functional taxonomy of aquatic protists has recently been proposed in recognition of the importance of mixotrophy (Mitra et al. 2016). In this chapter we narrowly define mixotrophy as phototrophy with phagotrophy. We emphasize that mixotrophy warrants realistic descriptions within food web models; it represents a more flexible strategy in nutrient acquisition, growth, and production, with far-reaching consequences not only for growth of the mixotrophs but also for their competitors and prey. In essence, mixotrophic organisms alter the flow of nutrients through the planktonic system, potentially upgrading it biochemically and changing the nature of predator-prey interactions in a highly efficient manner. An appreciation of the importance of mixotrophy also affects which nutrient forms should be considered in water management strategies; for example, if mixotrophic HABs are promoted by events such as bacterial growth on organic substances, through ingestion of bacteria, then dissolved organics as well as the usual dissolved inorganic nutrient loads require consideration.

Within the framework of the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) Programme, the understanding of the role of nutrients generally, and of eutrophication more specifically, was highlighted as important areas of research needed to strengthen predictive capabilities for HABs [see Chap. 12, Glibert et al. (2018a)]. The importance of increased understanding about adaptive strategies of HABs was also recognized. When GEOHAB was conceived (GEOHAB 1998, 2001), only a few HAB species had been identified as mixotrophic, and these largely were considered curiosities. In the ensuing decades, it has been recognized that mixotrophic organisms, many of which include HAB species (e.g., Burkholder et al. 2008), are not only much more common than originally thought, but also their physiology is now known to be far more complex than could be described by simply “bolting together” descriptions of the contrasting processes of phototrophy and phagotrophy (Mitra and Flynn 2010). In addition, the central mission of GEOHAB was to strengthen the prediction of HABs through improvements in detection capability and modelling [GEOHAB 2001; see also Chap. 3, Kudela et al. (2018)]. The need for predictive models is increasingly great, particularly when considering climate change, increasing acidification, changes in nutrient input types and ratios, and increasing geographic spread of some HAB species. Yet, for the most part, one of the most important physiological functions of the dominant plankton communities—mixotrophy—is still not being explicitly included within ecosystem food web models. As aquatic ecosystems move toward an increasingly uncertain future, models describing them need to be

mechanistically sound, accurately reflecting food web dynamics, and that requires incorporation of mixotrophs.

Accordingly, a working group funded under the auspices of the Leverhulme Trust of the UK was formed to address such issues. While not formally a GEOHAB endeavour, and not exclusively focused on HABs as the only types of mixotrophs, the advancements made by this working group helped to advance many important questions on nutrient physiology and adaptive strategies posed by GEOHAB. In this chapter we review some of these advancements and pose additional, still-unresolved questions regarding mixotrophic HAB species ecology.

7.2 Mixotrophy Across the Spectrum of Nutrient Supply

Planktonic mixotrophy has long been considered important in oligotrophic habitats, where potentially limiting elements are much more concentrated in microbial prey than in the water column (Jones 1994). More recently it has been recognized that mixotrophy is also important in eutrophic estuaries and coastal marine embayments, where many HAB taxa are mixotrophic (Burkholder et al. 2008). In such environments, mixotrophs respond both directly to nutrient inputs and indirectly through high abundance of bacterial and algal prey that are stimulated by the elevated nutrients. It is worth noting that in so-called eutrophic systems, light can often be limiting and hence limit newly fixed C. Thus, mixotrophy provides a mechanism to exploit alternative routes for the acquisition of C. Another important facet of mixotrophy is that all mixotrophs are not the same; Mitra et al. (2016) divide these protists into those that have a constitutive capacity for mixotrophy and those that acquire phototrophy by acquisition of photosystems from other organisms. The constitutive mixotrophs include HAB species such as small-celled toxigenic taxa (e.g., the dinoflagellate *Karlodinium* and the haptophyte *Prymnesium*), as well as non-HAB species such as the large dinoflagellate *Ceratium*. Among the non-constitutive mixotrophs, some, the plastidic ciliates, are generalists and can steal chloroplasts from a range of prey types, while others are specialists making use of plastids acquired from very few prey sources. These include the nontoxic “red tide” ciliate *Mesodinium/Myrionecta* and the toxigenic dinoflagellate *Dinophysis*, which actually obtains its plastids “3rd hand” from *Mesodinium* (Hansen et al. 2013 and references therein). Another group of non-constitutives that harbours symbiotic phototrophs includes the biogeochemically important foraminifera and radiolaria and also the HAB-forming dinoflagellate, “green” *Noctiluca* [Gomes et al. 2014; Stoecker et al. 2017; see also Chap. 17, Goes et al. (2018)]. These contrasting mixotrophic strategies impart different ecological impacts through the need and removal of prey (perhaps competitors) that could, in turn, affect the operation of other trophic pathways. Mixotrophic HABs may thus be expected to be associated with complex ecological scenarios.

Many different types of harmful microalgal species thrive in eutrophying or eutrophic estuarine and coastal marine habitats, including cyanobacteria, dinoflagellates, haptophyte flagellates, heterokontophytes (diatoms, golden flagellates,

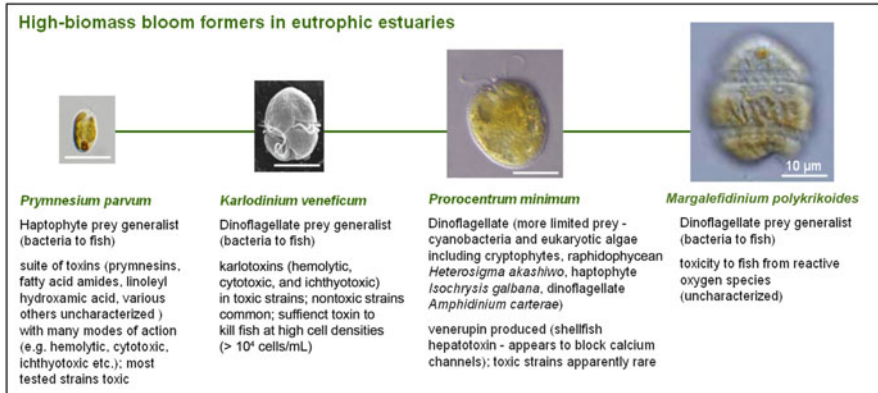


Fig. 7.1 Four examples of toxigenic mixotrophs common in eutrophic estuaries include *Prymnesium parvum*, *Karlodinium veneficum*, *Prorocentrum minimum*, and *Cochlodinium (Margalefidinium) polykrikoides*. Size comparison and other information is from Denardou-Queneherve et al. (1999), Dorantes-Aranda et al. (2010), Henrikson et al. (2010), Van Wagoner et al. (2010), Bertin et al. (2012), Glibert et al. (2012), Rimmel and Hambright (2012), Johnson (2014), and references therein

brown-tide algae, and raphidophycean flagellates) (Burkholder et al. 2008; Graham et al. 2016; Fig. 7.1). In fact, the bulk of eukaryotic planktonic protists commonly classed as phototrophs is actually mixotrophic (Flynn et al. 2013; Mitra et al. 2016). Thus, it appears that most phytoflagellates (heterokontophytes, haptophytes, and cryptophytes) are capable of consuming bacteria and small eukaryotic cells (Unrein et al. 2007; Zubkov and Tarran 2008; Stukel et al. 2011). Dinoflagellates exhibit a wide range in capabilities, from those that express phagotrophy only upon extreme nutrient starvation to exclusively phagotrophic forms. Even the latter organisms may gain at least transitory inorganic C-fixing capacity from ingestion of phototrophs (Raven et al. 2009; Jeong et al. 2010; Hansen 2011) or other mixotrophs (Minnhagen et al. 2011).

Some potentially toxic species appear to have relatively poor affinities (high K_s values) for uptake of inorganic N and P substrates (DIN, dissolved inorganic N; DIP, dissolved inorganic P), and may therefore depend on mixotrophy as a means to supplement their acquisition of dissolved substrates (Smayda 1997). A contrary argument is that because of mixotrophy (phagotrophy being the ancestral state in protists—Mitra et al. 2016), these species did not need to evolve inorganic nutrient transporters with high affinity. Importantly, mixotrophy also may permit growth to be sustained or even accelerated during periods of apparent water-column nutrient deficiency or imbalance (or real nutrient deficiency for non-mixotrophic competitors) when only inorganic forms of dissolved nutrients are considered. The dinoflagellates *Cochlodinium (Margalefidinium) polykrikoides* and *Karenia brevis* exemplify these advantages of mixotrophy. When growing as a phototroph, *C. polykrikoides* has a growth rate of $0.17 \text{ div day}^{-1}$ (Jeong et al. 2004). Yet, when growing as a mixotroph, consuming cryptophytes, the division rate of

C. polykrikoides nearly doubles to $0.34 \text{ div day}^{-1}$ (Jeong et al. 2004). Toxicogenic *Karenia brevis* has been found to graze on the cyanobacterium *Synechococcus* sp., as well as on cryptophytes (Jeong et al. 2005; Adolf et al. 2008; Glibert et al. 2009). In laboratory experiments, Jeong et al. (2005) estimated that up to 5 cells h^{-1} of *Synechococcus* were grazed by mixotrophic *K. brevis*, while Glibert et al. (2009) found that from ~ 1 to $80 \text{ Synechococcus cells h}^{-1}$ were grazed by *K. brevis*, depending on the predator-prey ratio. The growth rate of *K. brevis* increased as the supply of *Synechococcus* increased, indicating that natural variability in this food source may affect growth rates of this HAB species in nature. In contrast, *Prymnesium parvum* (as *P. patelliferum*) and *Chrysochromulina* spp., which ingest prey under light- and nutrient-sufficient conditions, maintain similar growth rates with versus without phagotrophy (Pintner and Provasoli 1968; Larsen et al. 1993; Granéli and Carlsson 1998).

Harmful estuarine and marine algae have been reported to have significant impacts on prey populations and to contribute substantially to elemental demands of the mixotroph, illustrated by the following three examples from eutrophic Chesapeake Bay, USA. In situ grazing rates were estimated for the large dinoflagellate *Akashiwo sanguinea* (as *Gymnodinium sanguineum*; cells $60 \times 45 \mu\text{m}$, biovolume $79,000 \mu\text{m}^3$) grazing on oligotrich ciliates (length $< 20 \mu\text{m}$) (Bockstahler and Coats 1993a, b). Based on integrated station and transect averages, *A. sanguinea* removed 34% and 24%, respectively (medians 17% and 22%, respectively) of the oligotrich ciliate population daily. Daily consumption of ciliates supplied an average of 2.5% (maximum 11.6%) of the grazer C cell $^{-1}$. Mixotrophy also was estimated to supply 4% (maximum, 18.5%) of the grazer cellular N daily and 15% of its N requirements for asexual reproduction. Thus, it was concluded that mixotrophy may help balance the N requirements for *A. sanguinea* and thence confer an advantage over strictly phototrophic algae under N stress conditions (Bockstahler and Coats 1993a, b). Grazing by another large dinoflagellate species, *Ceratium furca* (cells $250 \mu\text{m} \times 25 \mu\text{m}$, biovolume $3400 \mu\text{m}^3$), was estimated in the same ecosystem using fluorescently labelled ciliate prey (Smalley and Coats 2002). Feeding rates ranged from 0 to $0.11 \text{ prey dinoflagellate}^{-1} \text{ h}^{-1}$, and grazing by *C. furca* was estimated to remove an average of 67% of the ciliates *Strombolidium* spp. day $^{-1}$. Not only is such predation important for this mixotroph, but the removal of the ciliates would also have a “knock-on” effect by relieving grazing constraints upon the ciliate’s prey. Phagotrophy in *Karlodinium veneficum* (reported as *Gyrodinium galatheanum* or *Karlodinium micrum*) has been the subject of intensive research focus in Chesapeake Bay. Li et al. (2000) found that *K. veneficum* became phagotrophic at suboptimal light and/or nutrient conditions, and the mean ingested prey was positively correlated with cryptophyte abundance. Adolf et al. (2008) determined that *K. veneficum* blooms in this bay were positively correlated with cryptophyte abundance. Ingestion rates among multiple strains of cultured *K. veneficum* ranged from 0 to $4 \text{ prey dinoflagellate}^{-1} \text{ day}^{-1}$, and cultured toxic strains were capable of consuming an array of cryptophyte species ($31\text{--}421 \mu\text{m}^3 \text{ cell}^{-1}$). The authors hypothesized from these data and supporting studies (e.g., Adolf et al. 2003, 2006) that cryptophyte prey abundance is a key factor that support

blooms of *K. veneficum* in eutrophic habitats. This work built from a previous intensive effort with cultures to examine the balance between phototrophy and heterotrophy during mixotrophic growth of *K. veneficum* on radiolabelled cryptophyte prey (Adolf et al. 2006). Earlier work had suggested that cryptophytes are linked to blooms of other toxigenic dinoflagellates as well (Lewitus et al. 1999; Glasgow et al. 2001). Based upon such studies, it appears that mixotrophy is important in the nutrient acquisition and growth of harmful species and, therefore, should be important in the development and maintenance of their blooms in eutrophic habitats.

While the data support the premise that mixotrophic harmful algae are significant grazers and predators in eutrophic habitats, for most of these species, quantitative data continue to be lacking. Data relating laboratory information to natural field assemblages, and data to enable descriptions of various nutrition acquisition modes of mixotrophs in nature, and the implications of switching between these (such as on growth rates, vertical migration, and toxicity) have yet to be obtained. It should also be emphasized that little is known about the drivers for mixotrophy in constitutive mixotrophs, whether it is C or N (as per the studies mentioned above) or for P or growth cofactors. While rates of contribution of C or N from mixotrophy may appear minor, contributions to mixotroph well-being may be very different. For example, because the relationship between cellular-P and growth rate is strongly nonlinear (Flynn 2008), a relatively low rate of P acquisition from prey ingestion could have an impact on health and growth that extends far beyond the traditional Redfield-based expectations. For the non-constitutive mixotrophs, the ongoing role of mixotrophy is obvious; these organisms rely upon acquired phototrophy and, thus, must feed and conduct photosynthesis.

While most species are free-living, mixotrophy also occurs in some parasitic dinoflagellates with photosynthetic life stages, such as *Blastodinium* spp. (Chatton 1920; Pasternak et al. 1984), *Dissodinium psuedocalani* (Drebes 1969), and *Crepidodinium australe* (Lom et al. 1993), within estuarine and marine waters that include eutrophic habitats. Mixotrophy is apparently operative, although not well studied, among other photosynthetic parasitic dinoflagellates as well (e.g., Gaines and Elbrächter 1987; Coats 1999). The importance of mixotrophy to overall nutrition is unknown for most of these species, but could be significant—for example, Pasternak et al. (1984) reported that the endoparasitic species acquire up to half of the energy needed for their growth through phagotrophy.

While progress is being made in understanding “who is mixotrophic and on whom,” there is less confidence about questions linked to “under what conditions” and “at what rate.” Thus, focus is needed to understand how the nutritional state of the mixotroph and that of its prey alter the rate of feeding and the growth response of the mixotroph. Lundgren et al. (2016) found that in studies of *Prymnesium parvum* grazing on *Rhodomonas salina*, each grown under varying N:P ratios and mixed in varying combinations, mortality rates of prey were higher when prey were N-rich, regardless of the nutritional states of *P. parvum*. Furthermore, growth of the mixotroph was higher when prey were provided in high N:P ratio conditions. Experiments by Lin et al. (2017) have shown that growth rates of initially

exponential- and stationary-phase *Karlodinium veneficum* were enhanced in the presence of prey (*Rhodomonas* sp.) with reciprocal nutrient conditions. Feeding rates (measured as prey death rates) were highest for low-N:P *K. veneficum* initially growing exponentially and mixed with N-rich prey. Maximum feeding rates of low-N:P *K. veneficum* on N-rich prey during exponential growth were ~fourfold higher than feeding rates of high-N:P *K. veneficum* on N-rich prey. Both studies suggest that conditions of elevated N:P in the prey led to higher growth of the mixotroph relative to stoichiometrically balanced or low-N:P conditions in the mixotroph. As noted by Lundgren et al. (2016), it is therefore not surprising that mixotrophs are common in eutrophic waters where stoichiometrically imbalanced conditions prevail, especially high N:P ratios. These complex experiments also underscore the complexity of the natural environments inhabited by these organisms, which often are characterized by sharp gradients in salinity, light, and nutrient concentrations and ratios.

7.3 Mixotrophs and Cellular Nutrient Stoichiometry

The processes of mixotrophy have the potential to radically change the dynamics and efficiency of nutrient assimilation (Flynn and Mitra 2009; Mitra and Flynn 2010; Flynn et al. 2013; Mitra et al. 2014a, b). Mixotrophy is not simply additive or substitutional; rather, it is synergistic (Mitra and Flynn 2010; Mitra et al. 2014b). The synergism of phototrophy and phagotrophy in mixotrophs has important consequences for cell metabolism, especially the maintenance of cell stoichiometry. An advantage for mixotrophs over non-phagotrophic phytoplankton is the provision of additional nutrients (N, P, Fe) from feeding to support primary production, together with a supply of C to supplement photosynthesis under conditions of light limitation (including during the night).

However, perhaps of more importance is that mixotrophs have a stoichiometric advantage over phagotrophic heterotrophs through an ability to enhance retention of nutrients (elements and biochemicals) from their prey. With respiration and additional C losses associated with prey digestion, an organism has to release nutrients to maintain its elemental balance (e.g., C:N:P) (Sterner and Elser 2002; Mitra and Flynn 2005). An ability to decrease that stoichiometrically-linked loss would be of particular value in nutrient-impoverished waters (e.g., mid-oceans and also many temperate waters in summer). Having an additional source of organic C via photosynthesis—that is, being a mixotroph—which can balance C lost through respiration (Putt 1990; Stoecker and Michaels 1991), decreases the need to release surplus N and P. Obtaining data with sufficient temporal resolution and detail to help in the parameterisation of these events is challenging. It requires detailed understanding of both the prey and predator (mixotroph) physiologies when considered alone, and together, conducted under different conditions of light and nutrient abundance. Modelling the interactions presents parallel challenges (Flynn and Mitra 2009), especially when considering contrasts between different mixotroph functional types (Mitra et al. 2016).

7.4 Mixotrophs and the Food Web

The traditional view of the planktonic food web describes consumption of inorganic nutrients by phototrophic phytoplankton, which in turn support zooplankton and ultimately higher trophic levels. Pathways centred on bacteria provide mechanisms for nutrient recycling. This structure lies at the foundation of most models used to explore biogeochemical cycling, the functioning of the “biological pump,” and the impact of climate change on these processes. Mixotrophy radically changes the traditional food web interactions, enabling primary producers to acquire nutrients directly from ingestion of prey such as bacterial and algal competitors and even from their predators.

An alternative conceptualization considers the base of this food web to be strongly supported by protist plankton communities that are mixotrophic—combining phototrophy and phagotrophy within a single cell (Flynn et al. 2013; Mitra et al. 2014b). Such a mixotroph-dominated marine food web construct considers the traditional phytoplankton-zooplankton structure (especially involving diatoms) as predominating only during short, albeit important, periods of the production cycle. These periods are characterized by pioneer species (analogous to r-selected species; Parry 1981) growing in developmental phases of ecosystems (Odum 1969), exemplified by the spring bloom in temperate waters or in upwelling waters (Fig. 7.2). They typically have enhanced illumination and inorganic nutrient availability coupled with an absence of effective grazing control (Irigoien et al. 2005), favouring rapid proliferation of strict phototrophs (e.g., diatoms and coccolithophorids during their strictly phototrophic stages; Rokitta et al. 2011). Such conditions favour the emergence of strict phagotrophs as the dominant protist predators. As these ecosystems mature, however, their nutrient regimes and particulate organic loading change, giving rise to conditions that favour mixotrophy and/or disfavour their competitors (Fig. 7.2). Therefore, during much of the planktonic production cycle, mixotrophs are abundant or dominant. The basis of the new structure suggests that the “strict” phototrophic configuration represents an optimal strategy only in immature r-selecting conditions, exemplified by the spring bloom in temperate waters, or in well-illuminated waters that are highly eutrophic (Fig. 7.2). As the nutrient conditions change (mature) due to algal growth, and with the emergence of a prey community, the assemblage transitions to a “K-selecting” environment and mixotrophy becomes selectively advantageous (Fig. 7.2). This more mature environment is dominated by heterotrophs that have phototrophic capabilities and phototrophs that have heterotrophic capabilities; the diversity of mixotrophic strategies is great (Mitra and Flynn 2010; Flynn et al. 2013) and is broadly split between constitutives and the three types of non-constitutive (acquired phototrophy) forms (Mitra et al. 2016). It is in more mature ecosystems, which often are also warmer during summer, where HABs typically develop.

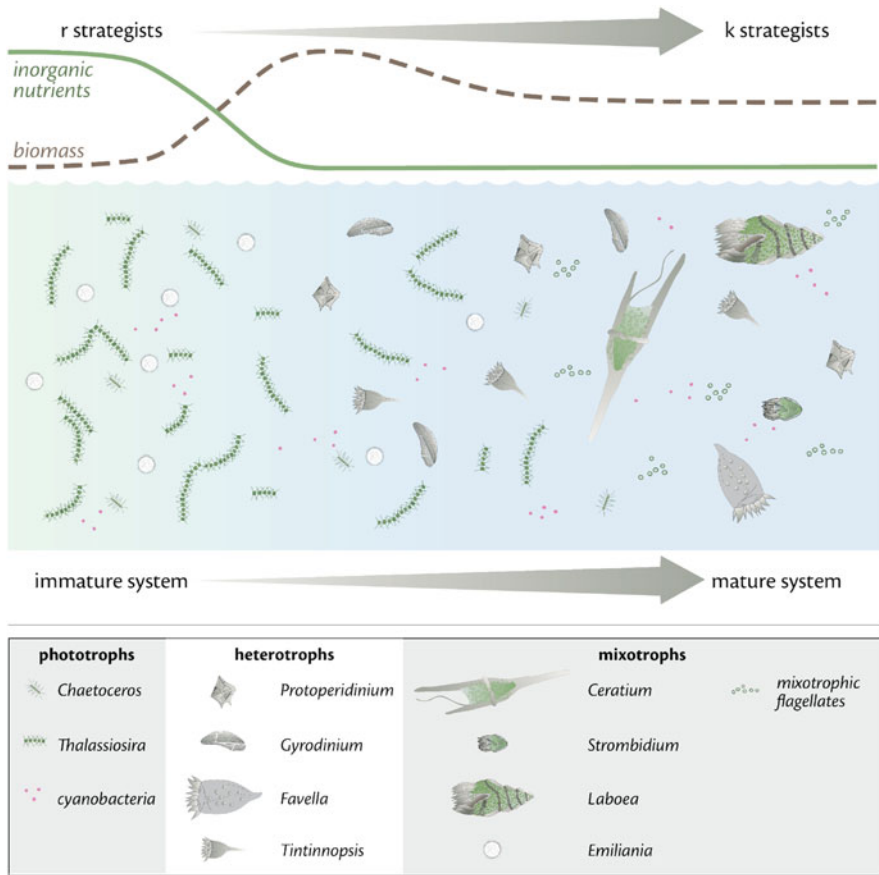


Fig. 7.2 Conceptual diagram of the changes to the planktonic food web over an annual cycle, with transitions between ecosystem states. Transitions between developmental and mature stages of the ecosystem are as indicated; early periods are optimal for strict photoautotrophs, whereas later periods (transition to the more mature state) are suboptimal for strict phototrophs and/or strict phagotrophs and more supportive for mixotrophs. Reproduced from Mitra et al. (2014b) under a creative commons licence

7.5 Inclusion of Mixotrophy in State-of-the-Art Ecosystem Modelling: The Rationale

Models, as simplifications of reality, must balance simplicity with realism and the inevitable complexity present in real biological and ecological systems. Models also depend on parameters which are not always easily measured or available and, as a result, mass fluxes, dynamics, and physiological variables are often not adequately captured. This is so even when considering the relative simplicity of the traditional “phytoplankton–zooplankton” conceptual framework. Ecosystem

modelling has advanced tremendously in the past decade, with increasing sophistication in visualization of large datasets, conceptualization of complex interactions, and formulation of predictive scenarios based on model ensembles. Physiological traits of marine organisms are now being applied in models of emergent marine biodiversity (Follows et al. 2007). Newer-generation, mechanistic population dynamic models and three-dimensional ocean biogeochemical models incorporate phytoplankton functional groups, multiple limiting nutrients, flexible elemental composition, and iron limitation (e.g., Baird and Emsley 1999; Flynn 2001; Moore et al. 2004; Klausmeier et al. 2004; Christian 2005; Le Quéré et al. 2005; McGillicuddy et al. 2010; Ramin et al. 2012; Polimene et al. 2015). Yet, despite clear evidence that mixotrophs are important, conceptual as well as theoretical descriptions of plankton food webs typically give little recognition to the importance of mixotrophs. In part this omission could be viewed as a facet of a continuing failure to describe zooplanktonic processes with the level of fidelity accorded to phytoplankton (Mitra et al. 2014a), and indeed many of the challenges in zooplankton modelling are common with models of protist mixotrophs.

In addition to mixotrophy, much has been learned about organismal acclimation through physiological responses to variable environments—from phytoplankton growth to competition, allelopathy, prey switching and/or prey rejection, and the relationships between these processes and various abiotic factors such as temperature, light, and pH. Models that have incorporated these findings have made progress in predicting phytoplankton dynamics, but they still often depend on poorly characterized physiological relationships. In general, traditional mass-based models (often single nutrient, either N or C) are relatively simple and operate using classic uptake kinetic relationships. Various authors (e.g., Allen and Polimene 2011; Flynn 2010a; Glibert et al. 2013; Kana and Glibert 2016) have recently argued the need for a new generation of models with a stronger foundation based on new and emerging information about the dynamic cellular biology and ecophysiology of phytoplankton [see also Chap. 6, Glibert et al. (2018c)].

In conditions under which mixotrophs dominate (and that, it turns out, includes most conditions), the minimum configuration of a model should consider C:N:P:Chl ratios. Variable elemental stoichiometric models should become the norm. Multielement descriptions, as opposed to fixed Redfield constructs, provide a basis for the development of mechanistic models that contain functional response descriptors with recognized physiological bases (Flynn 2010a; Glibert et al. 2010, 2013). Multi-element descriptions also support bioenergetic descriptions, which may be important for predicting the survival of organisms under unfavourable conditions. There are important implications of variable stoichiometry for food web dynamics (Sterner and Elser 2002; Mitra and Flynn 2005; Glibert et al. 2013). There may be preferential grazing and also alterations in flow of regenerated nutrients, which can create the potential for shifts in dominant bloom species including HABs. Therefore, it is not sufficient to consider only a single currency (C, N, or P), or even pairs of elements, in experiments or in models. Rather, it is the

stoichiometric (im)balance between these that controls phytoplankton physiology, and conceptualization, parameterization, and modelling of this stoichiometric balance in mixotrophy should be prioritized in models of HABs. Multi-stressor studies are also essential; there are very few studies in which multi-stressor impacts have been studied and even fewer in which non-limiting nutrient consumptions are also measured. It is essential to conduct such studies, because what is not removed by today's generation is available for tomorrow's and vice versa. For studies of mixotrophic HABs, this challenge for experiments is extended further because, as with zooplankton modelling (Mitra et al. 2014a), there is the added complexity of sampling the prey and predator. It is also important to correctly describe the growth of the diatoms, as the only major group of protist non-mixotrophs; correctly describing C:N:P:Si in diatom populations sets the basis for nutrient availability and thence plankton succession in the summer (Flynn 2005, 2010a, b).

7.6 Including Mixotrophy in State-of-the-Art Ecosystem Modelling: An Approach

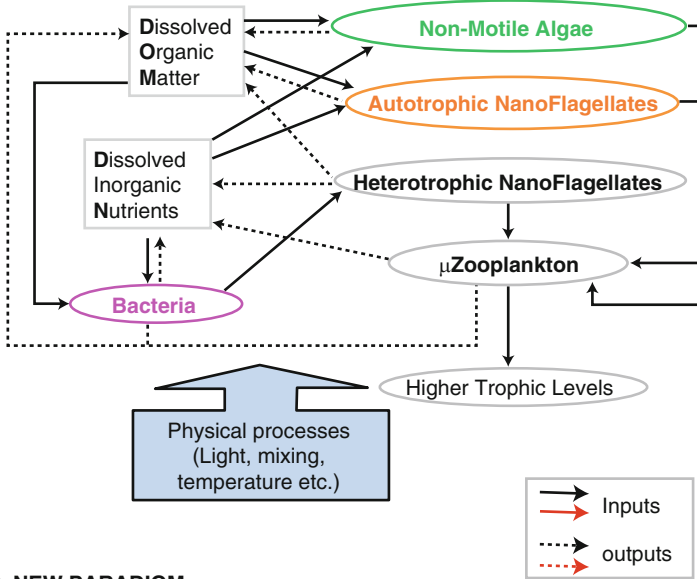
Most plankton food web models continue to be based upon derivations of the classic nutrient-phytoplankton-zooplankton (NPZ) model (Fasham et al. 1990; Plagányi 2007). Within these models, plankton functional types (PFTs) are increasingly used to aid descriptions of processes in biogeochemistry through to fisheries (e.g., end-to-end models; Rose et al. 2010). This has driven an expansion of the classic NPZ model structure (originally N-based) such that “phytoplankton” may be split into groups (e.g., the diatom PFT which require Si or non-diatom PFTs that do not), with each having its own state variables (e.g., Baretta et al. 1996; Flynn 2001; Thingstad et al. 2007). Far less emphasis has been placed on expanding the “zooplankton” component (Mitra et al. 2014a). Even given the current trend of including multiple compartments to represent different groups of PFTs, most existing descriptions of planktonic food web and ecosystem models do not explicitly include mixotrophs. The implicit assumption is that these organisms do not warrant a “box” of their own within these multicomponent ecosystem models. If considered at all, the phototrophic activity of mixotrophs is often assigned to the “phytoplankton” group, while the heterotrophic activity is assigned to the “zooplankton” component, an approach that has been called into question because it fails to capture the synergy of mixotrophic physiology (Mitra and Flynn 2010). Such assumptions stem from two misconceptions. One is that mixotrophy serves a substitutional function in providing C or a nutrient, rather than a *synergistic* function which demands a multi-nutrient description rather than the traditional “single-currency” approach (usually N-based). Secondly, there is a perceived notion that mixotrophy represents some form of inferior middle ground, such that mixotrophs only survive in specific niches (de Castro et al. 2009; Ward et al. 2011). That incorrect assumption typically is

based on assumed lower growth rates of mixotrophs compared to their “specialized” counterparts. Yet, when viewed in the context that *mixotrophs dominate in mature ecosystems*, growth at slower rates (in keeping with fluxes through the ecosystem) would be a *desirable* trait rather than an inferior trait (Flynn 2009).

Recasting plankton models with inclusion of mixotrophs may be approached in many ways. Mitra et al. (2014b) extended the NPZBD (NPZ-bacteria-detritus) approach (Fasham et al. 1990; Fig. 7.3), expressing the new mixotrophic paradigm in a new model structure. A new box is added, the “M” box, which includes all protists that are mixotrophic through a combination of phototrophy and phagotrophy. This box is linked to the other boxes in reflection of the realistically wide range of physiological options; thus, mixotrophs perform phototrophy while grazing on bacteria, phytoplankton, microzooplankton, detritus, and/or other mixotrophs. Including mixotrophs within a simple NPZBD model in this way results in a substantial difference in planktonic trophic dynamics. This is because food web dynamics are not merely a summation of a series of rate processes (and kinetic curves). They are an outcome of both the quantity *and quality* of the substrate (or food) provided, not just the rate at which it is produced. The ecophysiology (nutritional quality) of the component organisms present in the ecosystem affects not only their own growth potential but also the activities of co-occurring organisms: the cellular composition of algae, for instance, has consequences for grazers and their emergent properties, with potential for a positive feedback loop generating ungrazable primary producers (Mitra and Flynn 2006; Glibert et al. 2010, 2013). Thus, for grazers, the nutritional value of the prey, not just its availability, is a key aspect of their population success (Lundgren et al. 2016; Lin et al. 2017). From the population standpoint, rate of growth of the prey population is also important.

A critical issue in modelling mixotrophs, then, is controlling the growth rate as a function of the supply of energy and nutrients presented by different modes of nutrition. The classic way of describing both phytoplankton and zooplankton growth rates in models depicts the maximum growth rate (μ_{\max}) as a reflection of the maximum nutrient acquisition rate (Fasham et al. 1990). Thus, in models of N-limited phytoplankton, μ_{\max} is matched to the maximum rate of N assimilation. For zooplankton, μ_{\max} is a function of the maximum grazing rate (G_{\max}) and constants defining assimilation efficiency (AE) and respiration (Mitra et al. 2014a). In both instances these are shortcuts that have no basis, at all, in reality. Nutrient transport in phytoplankton may exceed that required to support μ_{\max} by an order of magnitude (e.g., McCarthy and Goldman 1979; Goldman and Glibert 1983; Flynn et al. 1999). Likewise, and setting aside the fact that AE is not a constant but, rather, declines with ingestion rate and food quality, the value of G_{\max} greatly exceeds the grazing rate needed to match μ_{\max} (Mitra et al. 2014a). For models of mixotrophs, the mismatch between such simplifying modelling approaches and reality is particularly apparent because of the interactions between phototrophy and phagotrophy. Those interactions are more complex due to the multi-nutrient basis of the metabolic processes involved. Physiological regulation occurs across the spectrum of substrate conditions, and classic “kinetic curves”

a TRADITIONAL PARADIGM



b NEW PARADIGM

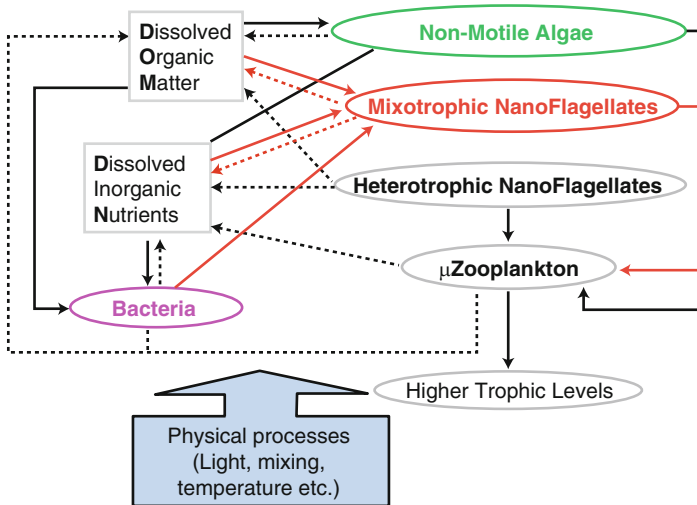


Fig. 7.3 Schematic representing two alternate modelled food web structures. In the traditional paradigm (panel **a**), the physiology of the photoautotrophic flagellates (ANFs) is similar to that of the nonmotile microalgae (NMA), and neither group is mixotrophic. Inorganic nutrients for supporting primary production are regenerated via bacteria, heterotrophic nanoflagellates (HNFs), and phagotrophic microzooplankton (μ Z). In the new paradigm (panel **b**), the ANFs are replaced with mixotrophic nanoflagellates (MNFs), conforming to present understanding of protist physiology (Mitra et al. 2016). The MNFs are capable of consuming bacteria (red-lined black arrow) and, hence, of deriving nutrients for supporting their primary production, directly rather

cannot capture the extent of the resultant physiological regulation that organisms must attain (Glibert et al. 2013; Kana and Glibert 2016). Moreover, the often-used description of predation by reference to half-saturation abundance of prey also gives rise to kinetics that are not consistent with biological reality (Flynn and Mitra 2016). Even the most complex of the aforementioned phytoplankton models, operating within global ecosystem simulations, has been married with grossly oversimplified zooplankton models. With the emergence of mixotrophy as an important facet of plankton ecology, the zooplankton descriptions need to be upgraded not only to enhance generic realism, but because those same grazing descriptions are needed within the mixotroph descriptions themselves.

7.7 Conclusions

In summary, mixotrophic protists are important, often dominant, members of the plankton community (Leles et al. 2017). More often than not, the mixotrophic potential in these protists likely is realized. Mixotrophic microzooplankton are less susceptible to classic swings in predator-prey dynamics since, for the most part, they are not doomed to death upon exhaustion of a given type of prey; instead, they can be sustained for longer periods of time. The trophic and allometric upgrading in mixotrophy provides a route to introduce important biochemicals (notably fatty acids) into what would otherwise be microbial-loop organisms. Through mixotrophs the microbial loop becomes an effective link and not just a sink to the “classic food web,” on stoichiometric and allometric grounds. Lastly and perhaps most importantly, most HABs are mixotrophs; phagotrophs exclude cyanobacteria and diatoms, but those HAB species are osmotrophs (e.g., Burkholder et al. 2008 and references therein).

The last decade or so has been a time of great advancement in our appreciation of mixotrophs—their ubiquitous characteristic, their physiological complexity, and their role in shaping trophic dynamics. Although much is known about which organisms are mixotrophs and how they feed, little is known about how the organisms modulate phototrophic and phagotrophic activities or how the flow of energy and materials through mixotrophic predator-prey interactions are altered under varying nutrient, temperature, light, pH, or $p\text{CO}_2$ conditions—and all these factors are rapidly changing in coastal and oceanic environments with accelerating eutrophication and climate change [Flynn et al. 2015; see also Chap. 4, Glibert et al. (2018b) and Chap. 5, Wells and Karlson (2018)]. This raises the potential for more

Fig. 7.3 (continued) than (for ANFs in panel A) via the activities of HNFs + μZ . *Dashed arrows* indicate functions contributing to nutrient pools (*blue*, inorganic; *brown*, organic). *Heavy black arrows* indicate predatory links. Reproduced from Mitra et al. (2014b) under a creative commons licence

HABs (Burkholder et al. 2008; Glibert et al. 2014). Lacking are critically needed experimental data that systematically assess how primary production, mixotrophy, and N–P–C flow vary under these changing environmental conditions, and the appropriate inclusion of these organismal processes in plankton food web models. Accurate representation of mixotrophy in next-generation models, within the context of the suite of environmental drivers likely to change, will require acquisition of substantial new experimental data that explicitly explore the connectivity between phototrophy and phagotrophy. Because of the critical role of grazing as both a fundamental feature of the zooplanktonic regulation of HAB development (as per traditional food web constructs), and as a contribution to the growth of HAB mixotrophs, enhancing models of predatory activity are of particular importance. Accurate parameterization of mixotrophy should become a high priority in water quality and fisheries models used as aids to regional and/or international policy development. The next-generation international HAB Programme, GlobalHAB [see Chap. 22, Berdalet et al. (2018)], may present opportunities for these greatly needed advancements.

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