LIMNOLOGY AND OCEANOGRAPHY
Letters

ASLO
Open Access

Limnology and Oceanography Letters 3, 2018, 341–356

doi: 10.1002/lol2.10084

CURRENT EVIDENCE

Hidden in plain sight: The importance of cryptic interactions in marine plankton

Nicole C. Millette [a,1,2,3]* *Julia Grosse* [4] *Winifred M. Johnson* [b,5] *Michelle J. Jungbluth* [6] *Elizabeth A. Suter* [7,8]

[1]Mississippi State University, Starkville, Mississippi; [2]Northern Gulf Institute, Mississippi State University, Stennis Space Center, Mississippi; [3]National Oceanic and Atmospheric Administration, Atlantic Oceanographic and Meteorological Laboratory, Miami, Florida; [4]GEOMAR, Helmholtz Centre for Ocean Research Kiel, Biological Oceanography, Kiel, Germany; [5]Woods Hole Oceanographic Institution, Woods Hole, Massachusetts; [6]San Francisco State University, Estuary & Ocean Science Center, Tiburon, California; [7]School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York; [8]Department of Biological Sciences, Wagner College, Staten Island, New York

Scientific Significance Statement

Generalizations in foodweb modeling have helped scientists explain and study interactions within the aquatic environment. Established methods focus on quantifying and qualifying these generalizations, but rapid technological advancements in recent years have revealed that our generalizations of interactions may obscure significant processes within the plankton community. We explore a range of interactions within the planktonic foodweb that are "cryptic" because existing methods are biased against them with the intention of highlighting a range of cryptic interactions and the potential impact of overlooking them in future research. We discuss how including these interactions in biogeochemical and foodweb models alters our understanding of the transfer of carbon and other materials from one species/functional group to another and highlight examples of recent models that have incorporated cryptic interactions.

Abstract

Here, we present a range of interactions, which we term "cryptic interactions." These are interactions that occur throughout the marine planktonic foodweb but are currently largely overlooked by established methods, which mean large-scale data collection for these interactions is limited. Despite this, current evidence suggests some of these interactions may have perceptible impacts on foodweb dynamics and model results. Incorporation of cryptic interactions into models is especially important for those interactions involving the transport of nutrients or energy. Our aim is to highlight a range of cryptic interactions across the plankton foodweb, where they exist, and models that have taken steps to incorporate these interactions. Additionally, it is discussed where additional research and effort is required to continue advancing our understanding of these cryptic interactions. We call for more collaboration between ecologists and modelers in order to incorporate cryptic interactions into biogeochemical and foodweb models.

*Correspondence: nmillette@whoi.edu

Author Contribution Statement: All authors contributed equally to the writing and editing of the paper. NM led and organized the writing and editing processes and had final approval on the completed paper. Authors after NM are listed alphabetically.

Data Availability Statement: There are no data associated with this article

[a]Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts
[b]Present address: Naval Research Laboratory, Center for Bio/Molecular Science and Engineering, Washington, DC

The term cryptic, meaning hidden or obscured, has been used in aquatic ecology to describe both species and processes. Cryptic species are morphologically indistinct organisms that may be classified as the same species, despite their inability to successfully reproduce with each other (Bickford et al. 2007). Cryptic processes have been previously defined as "overlooked" processes (Canfield et al. 2010). Failing to account for these cryptic species and processes can have a large effect on our capacity to properly understand the environment. Here, we further explore crypsis in marine plankton in the form of cryptic interactions.

Similar to the definition for cryptic microbial metabolites used by Durham et al. (2015), herein we define cryptic interactions as interactions that are not detected by traditional or standard sampling and experimental methods. The transfer of energy or materials by cryptic interactions in most models is either falsely attributed to traditional interactions or ignored altogether. Planktonic interactions occur at the base of the foodweb, and thus a misrepresentation of how energy and materials enter and are transferred at this level can have implications for our understanding of biogeochemical cycling and transfer of materials throughout the entire foodweb (Worden et al. 2015; Ward and Follows 2016).

We present known species–species and species–substrate interactions within the plankton community that fit into this definition of cryptic interactions and discuss the impact these interactions have on the flow of nutrients or energy. We focus on cryptic interactions in the planktonic community because the microscopic size of these organisms makes it difficult to directly observe them interacting with each other and with their surrounding environment. As a result, scientists have regularly developed methods to indirectly infer planktonic interactions, such as prey removal experiments (Frost 1972), the dilution method (Landry and Hassett 1982), and using geochemical profiles to measure the presence or absence of products and reactants (Jørgensen 1977). These types of methods require scientists to make a range of assumptions about the interactions being measured. Cryptic interactions arise when these assumptions are violated and/or flawed or the methods obscure potentially vital processes.

Here, we discuss five clear examples of cryptic planktonic interactions that represent a range of exchanges at all levels of the planktonic foodweb. In this review, we consider how accounting for these interactions alters our current understanding of biogeochemical cycles and foodweb dynamics in pelagic marine environments, provide recent examples of foodweb and biogeochemical models that have incorporated some of the interactions we discuss, and examine how to bridge the gap between the data collected on these interactions and what data is needed to incorporate them into models.

Cryptic interactions

Below, we describe five examples of cryptic interactions in the foodweb affecting a variety of plankton, from bacteria to mesozooplankton. These specific interactions were selected because they represent the range of potential interactions that fit our definition of cryptic in different parts of the plankton foodweb; however, they do not represent all the possible types of cryptic interactions. We discuss what makes each example a cryptic interaction, how we can measure the interaction, how prominent the interaction is in ecosystems, and how the interaction alters our current understanding of biogeochemical cycling.

Intracellular carbon partitioning

Oceanic phytoplankton are the main contributors to global primary production and form the base of most marine foodwebs (Field et al. 1998). Primary productivity is typically measured as rates of total carbon (C) fixation, commonly by using dissolved inorganic carbon (DIC) tracers enriched in ^{14}C- (radioactive) or ^{13}C- (stable) isotopes (Cullen 2001). Measurements of total C fixation are a great tool to determine system productivity but they lack information about the intracellular fate of fixed C. External factors, such as the availability of light and nutrients, regulate the internal synthesis of macromolecules (e.g., carbohydrates, amino acids, fatty acids, and nucleic acids) as they differ in their C, N, and P requirements (Arrigo et al. 1999). The interaction between resource availability and intracellular carbon pool partitioning determines phytoplankton growth efficiency, modulates cellular C : N : P ratios, and ultimately regulates the nutritional quality of phytoplankton biomass (Fig. 1; Klausmeier et al. 2004). Measurements of total C fixation alone fail to account for this internal interaction, thus making it cryptic.

Historically, determining in situ changes of specific compounds has been difficult. Recent technological advances, especially in liquid chromatography-isotopic ratio mass spectrometry (LC-IRMS), have made it possible to obtain specific isotope information of a wide range of compounds directly from complex mixtures. ^{13}C-DIC tracer can now be used to follow photosynthetically fixed C into all major biomolecules, which allows scientists to create a cellular C budget on the level of the total macromolecule pool (e.g., total carbohydrates) or on a more detailed individual compound level within that pool (e.g., glucose, fructose, galactose, etc.; Grosse et al. 2017 and references therein). The latter also provides detailed information on changes in essential compounds for higher trophic levels, such as specific amino and fatty acids.

Intracellular carbon partitioning is ubiquitous to all phototrophic organisms. Studies have shown that the sum of C fixation into carbohydrates, amino acids, and fatty acids is comparable to bulk C fixation rates (Suárez and Marañón 2003; Grosse et al. 2017). It was shown that synthesis of different compounds is affected by nutrient availability and can change significantly over short time spans ($<$ 24 h), while bulk C fixation rates take longer to respond to changes in

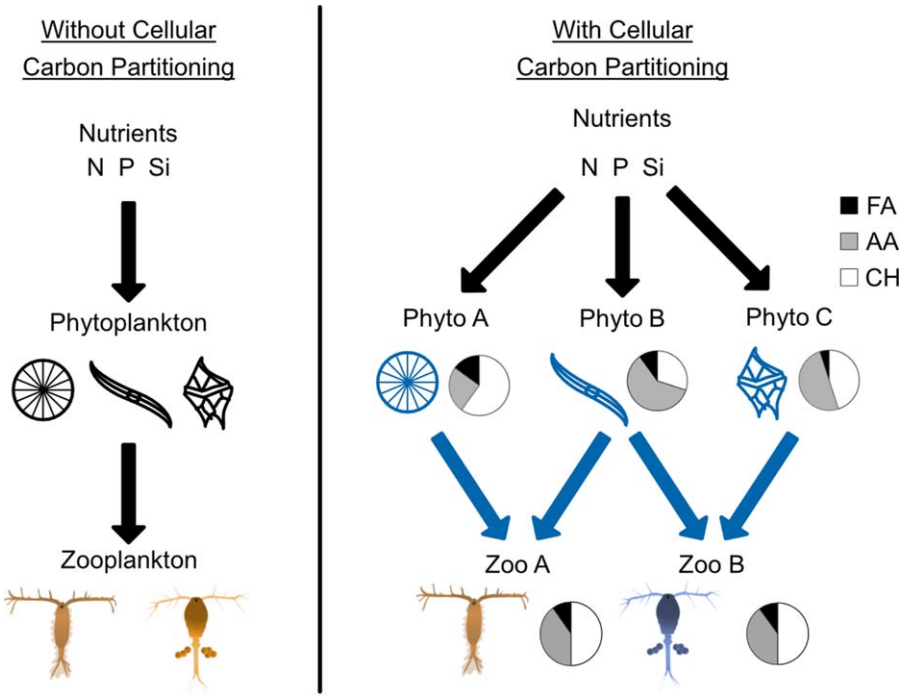

Fig. 1. Photosynthetically fixed carbon is partitioned between different biomolecules depending on nutrient availability (lipids [FA], amino acids [AA] and carbohydrates [CH] have different C : N : P requirements) and affect phytoplankton biomass and community composition. The biochemical composition of phytoplankton determines its food quality for zooplankton, which translates up the food chain and affects trophic transfer efficiency and ultimately the carrying capacity of an ecosystem. Blue indicates fluxes and populations that are affected by considering this process.

nutrient concentrations (Grosse et al. 2017). So far, available datasets cover a small subset of global phytoplankton communities and ecosystems but have already shown that the response in biomolecule composition to changing environmental factors varies between different groups of phytoplankton (e.g., diatoms, dinoflagellates, green algae). It is therefore necessary to survey additional ecosystems (e.g., tropical or polar, oligotrophic or eutrophic) and conduct complementary laboratory studies with individual phytoplankton species to globally quantify responses in biomolecule specific C fluxes under changing abiotic conditions.

Understanding how the environment controls intracellular partitioning of photosynthetically fixed C and its subsequent movement through the foodweb has the potential to provide the basis for explaining ecosystem level differences in C : N : P ratios, food-chain lengths and trophic transfer efficiencies (Fig. 1). For example, in nutrient enriched marine areas such as upwelling regions, trophic transfer efficiencies and the availability of N and P are higher (Stibor and Sommer 2009) and therefore phytoplankton biomass should contain high amounts of essential amino acids and fatty acids. In contrast, in N-limited oligotrophic regions, biomass will contain less amino acids but more storage compounds, providing limited essential amino acids and fatty acids per unit biomass that may cause the low trophic transfer efficiencies encountered in these areas (Stibor and Sommer 2009). Measuring only bulk primary production can cause an overestimation of C-fluxes up the food web. For example, to compensate for low food quality (e.g., high C : P ratios) zooplankton consumes large amounts of phytoplankton biomass but consequently excrete large amounts of this excess C as DOC. This does not just decrease food chain efficiency but also fuels microbial loop processes and redirects C fluxes (DeMott et al. 1998). By understanding how environmental factors, intracellular processes and foodweb constituents are linked, C fluxes, and budgets can be modeled more accurately.

Redox-related cross-feeding

Traditionally, redox processes mediated by microbes in the ocean were revealed simply by the detection of the presence or absence of reactants and products across a redox gradient. For example, the absence of sulfate and appearance of sulfide in a bulk water sample would indicate biological sulfate reduction. However, beginning in the 1970s, evidence suggested that the use of geochemical tracers of these processes revealed an incomplete story; the product of sulfate reduction, sulfide, could be rapidly re-oxidized by sulfide-oxidizing bacteria in anaerobic sediments, masking the detection of both sulfate reduction and sulfide oxidation (Jørgensen 1977). For years, this process was also hypothesized to occur in the water column (Hastings and Emerson 1988), but remained undetectable.

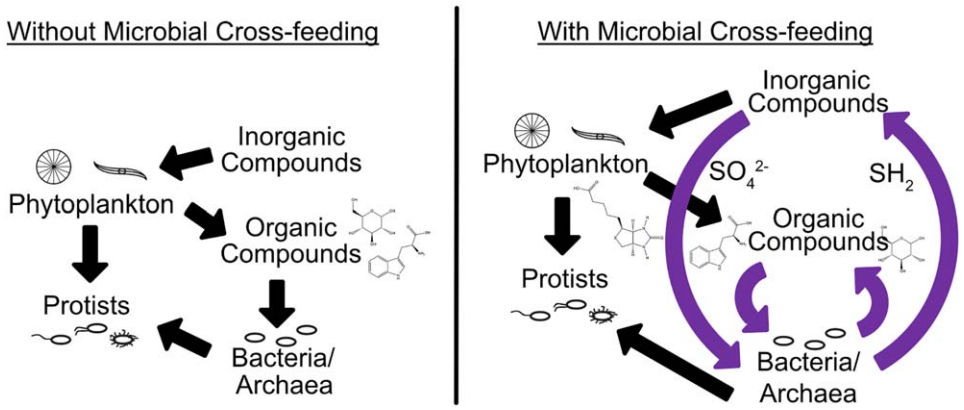

Fig. 2. In the current understanding of the microbial foodweb (left panel), energy flows from inorganic compounds (e.g., nutrients, CO_2) to autotrophs (such as phytoplankton), then enters the microbial loop, in which phytoplankton cells are grazed by protistan grazers or become part of the organic matter pool, either through lysis or enzymatic mineralization. This organic matter can support bacterial production. Microbial biomass can also be grazed by protistan bacterivores. With the incorporation of cryptic interactions (right panel), complexity is added due to the release and recycling of compounds within the bacterial community itself, which may increase rates of particular biogeochemical cycles such as sulfur oxidation or nitrogen reduction. This will have mixed results on biomass pools, depending on which reactions are taking place and their stoichiometric relationship to carbon fixation (chemoautotrophy) and remineralization. Purple indicates fluxes and populations that are affected by considering this process.

By pairing metagenomics with classical geochemical rate measurements, Canfield et al. (2010) showed that "cryptic" sulfur cycling can occur in the marine water column in habitats where oxygen is depleted (i.e., oxygen minimum zones) and where there is no detectable sulfide (Fig. 2). Despite its hidden nature, this biogeochemical process is likely of great importance; sulfide is toxic to most higher organisms and therefore its oxidation is critical for detoxification. Furthermore, the clades of bacteria involved in cryptic sulfur cycling are found worldwide in oxygen-depleted ocean habitats (Wright et al. 2012), suggesting that this interaction is potentially globally important. Despite its potential global presence, the magnitude of cryptic sulfur cycling is virtually unknown. It is not detectable by classic isotopic tracer methods (Johnston et al. 2014), and the direct production of sulfide by sulfate reduction has been measured in only a few discrete studies in the water column (Albert et al. 1995; Canfield et al. 2010). Moreover, Canfield and authors linked the oxidation of sulfide with nitrate respiration. The potential importance of sulfur-linked denitrification is a caveat to the classical model of how nitrogen is lost from the marine environment, and offsets the direct link between organic matter supply and denitrification for some regions of the ocean (Ward 2013). Currently, the quantitative importance of cryptic sulfur cycling for nitrogen loss from the oceans is unknown.

Besides sulfur, other chemical species have been shown to be cryptically cycled in redox gradients. For example, in Aarhus Bay, Denmark, a cryptic sulfur cycle was linked to methane oxidation (Holmkvist et al. 2011). In a meromictic lake, microbial iron oxidizers and reducers were found concomitant with high rates of iron oxidation but no detectable iron oxides, suggesting rapid reduction (Berg et al. 2016). Evidence of carbon substrate sharing between fermenters and denitrifiers was shown in a mixed culture from marine sediments (Hanke et al. 2016). Also in marine sediments, recycling of nitrite contributed to higher-than-expected rates of sulfide oxidation (Rios-Del Toro and Cervantes 2016). These studies highlight the importance of cryptic microbial exchanges in aquatic redox gradients, regions that are important for global scale biogeochemical turnover of nitrogen and sulfur. In addition, the electron donor/acceptor that a microbe uses dramatically alters how much carbon it can fix or remineralize, and thus cryptic exchanges of redox-related species can change microbial carbon biomass estimates (Reed et al. 2014). The complex mechanisms of these interactions will be further revealed as molecular and geochemical tools are improved and future studies jointly apply these tools to better understand these important habitats.

Auxotrophy

In the marine environment, auxotrophy, the requirement by an organism for a specific biomolecule that it cannot synthesize, has been most extensively studied in the context of phytoplankton vitamin auxotrophy which was identified in the 1950s (Droop 1957; reviewed by Croft et al. 2006). However, it may be a more common strategy than has been currently documented. Auxotrophy is thought to be beneficial to an organism because they can gain fitness by obtaining a molecule from the environment and not having to synthesize it themselves. This concept of gaining fitness by exploiting a resource that is produced by another group of organisms in the environment is known as the "Black Queen Hypothesis" (Morris et al. 2012). Auxotrophy can arise as a result of genome streamlining, a process in which cell size and genomic content are reduced to minimize the resources

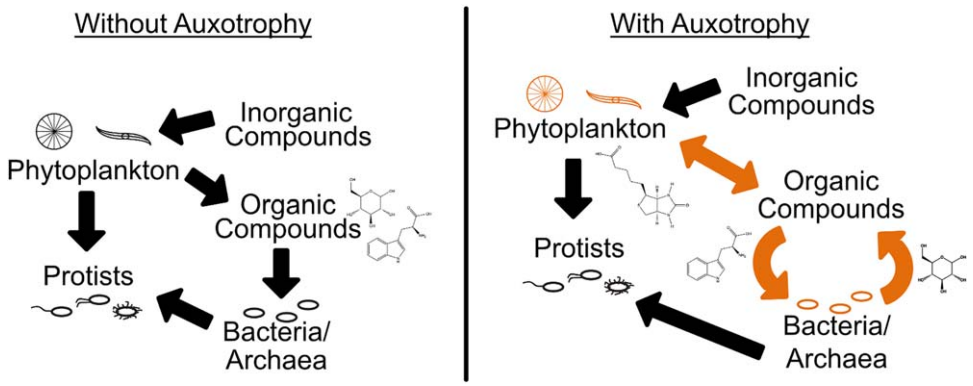

Fig. 3. Without considering auxotrophy (left) there is a direct flow of organic matter from phytoplankton into heterotrophic bacteria, archaea, and protists. Factors such as light, temperature, and nutrient availability control phytoplankton productivity and thus total organic matter availability for heterotrophic communities. When auxotrophy is considered (right) the flux of organic matter between organisms is more complex, with the availability of specific molecules potentially impacting the abundance of organisms that rely on those molecules. Orange indicates fluxes and populations that are affected by considering this process.

required for cell replication (reviewed by Giovannoni et al. 2014). In some cases, genome streamlining can reach such extremes that the organism can no longer function without direct physical attachment to organism that provides the required substrates, resulting in a symbiotic relationship (e.g., Tripp et al. 2010; Thompson et al. 2012).

The most well-known case of auxotrophy in the ocean is that of phytoplankton who cannot synthesize all of the vitamins, such as cobalamin, thiamin, and biotin that they require and thus must obtain them from other microbes. Croft et al. (2006) compiled information on vitamin auxotrophy for 306 species of algae and found that around 50% required cobalamin, 22% thiamin, and 5% biotin. While the prevalence of auxotrophy in marine bacteria has been less extensively documented, the heterotrophic marine bacterium *Candidatus* Pelagibacter ubique (member of the SAR11 clade), lacks a complete biosynthetic pathway for the vitamin thiamin, but can transport a precursor-molecule for thiamin into the cell to complete the synthetic pathway (Carini et al. 2014). In addition, this organism has been demonstrated to be effectively auxotrophic for glycine and serine, with glycine also playing role in regulating primary carbon metabolism in the cell (Tripp et al. 2009). In a survey of bacterial genomes (from any environment) only $\sim$ 1% of the genomes examined had the synthetic pathways for all 20 essential amino acids (Mee and Wang 2012).

While there are relatively few experimentally confirmed examples of free-living auxotrophic organisms in the marine environment, there may be more cases of auxotrophy in marine microbes because a substantial portion of microbial diversity in the ocean has not been extensively studied (Rusch et al. 2007; Sunagawa et al. 2015). For example, in a metagenomic survey around half of the bacterial species detected (by ribotype) were considered novel (Rusch et al. 2007). In addition, organisms with specific requirements are much more challenging to culture and thus most of the microbes that have been studied experimentally in culture have not been auxotrophs (Pande and Kost 2017). Analysis of microbial genome databases suggests that 85% of free-living bacteria may be auxotrophic for at least one compound (D'Souza et al. 2014). This suggests that auxotrophy in marine microbes may be more common than currently documented.

These types of interactions are challenging to identify but they may be crucial to the structuring of microbial communities in the ocean and other environments (Fig. 3). Studies on auxotrophy indicate that metabolic dependencies such as the passive exchange of certain amino acids, particularly those that are synthetically costly, can have a stabilizing effect on microbial communities (Mee et al. 2014) and that stronger metabolic dependencies yield more cooperative microbial communities that increase cell growth (Estrela and Brown 2013). These findings suggest that metabolite exchange, as required by auxotrophic microbes, could result in an overall more productive community. For example, increased availability of a vitamin such as Vitamin B_{12} can result in a shift in community composition away from diatoms and toward nanoflagellates and dinoflagellates that could result in differences in carbon export (Koch et al. 2011). Characterizing the extent that auxotrophy occurs in the ocean and quantifying its impact on microbial community structure, primary productivity, and remineralization is essential to understanding its role in biogeochemical cycles (Fig. 3).

Predator–prey interactions over ontogeny

Changes in prey selection and feeding strategies associated with growth and development in a species can be difficult to identify. The inability to study predatory diets over more than one period of an organism's life have limited our ability to understand the impact of key predators on carbon

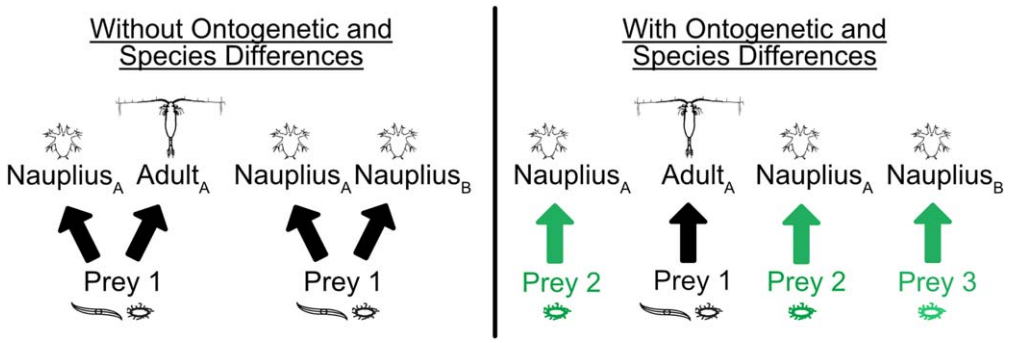

Fig. 4. Copepod grazing studies that ignore ontogenetic differences in feeding between nauplii and adults of the same species assume that they consume the same prey, and that adults consume a greater amount of food than nauplii given their larger body size. In reality, some species switch from omnivory to carnivory over development, and nauplii can have a higher specific ingestion rate than adults. In addition, lumping nauplii of different species into a single group for grazing studies assumes they are all consuming similar items at the same rate. Even in the naupllia stages, feeding can differ across species in important ways. Green indicates fluxes and populations that are affected by considering this process.

transfer in the planktonic foodweb. Most aquatic organisms that increase in size over their developmental period experience changes in diet as a result of changes in feeding appendages and body size (Werner and Gilliam 1984; Hansen et al. 1994). In cases such as in pelagic copepods, ontogenetic diet changes can be drastic, exhibiting variations in optimum prey size by up to an order of magnitude (Frost 1972; Hansen et al. 1994) or differences in the types of prey cleared at maximum rates (Saiz et al. 2014). Most of the following discussion of ontogenetic changes in predator–prey interactions will use examples from the copepod literature; however, ontogenetic shifts in these interactions are common across many holo- and meroplanktonic organisms.

A single species can play very different roles in the foodweb throughout its life history, but the implications of these changes on broader biogeochemical cycles are often overlooked. For example, some predatory adult copepod species (e.g., *Acartia* and *Labidocera*) spend time in their earlier life stages as grazers of phytoplankton and later switch to zooplankton (Neill and Peacock 1980; Conley and Turner 1985). Recent studies suggest that seasonal or prey size-based changes in copepod diets can result in differing quantity and quality of excreted compounds, and as a result can generate shifts in the active bacterial community (Valdés et al. 2017), which has the potential to change the plankton assemblage from the bottom up. Figure 4 illustrates the general concept behind different foodweb connections between developmental stages and species ("Adult A," "Nauplius A," and "Nauplius B") and how alternate prey communities can be affected ("Prey 1," "Prey 2," or "Prey 3") when differences across development or species are considered. However, challenges in identification of early life stages can make it difficult to discern the prey of individual species and how their prey selection evolves over development. As a result, it is often assumed that morphologically similar nauplii consume the same prey (Fig. 4), but nauplii can exhibit species-specific selective feeding behavior and can impact prey communities differently (Jungbluth et al. 2017). Modeling efforts that include key species level interactions in aquatic environments result in a better fit to observed ecosystem dynamics (Boit et al. 2012); therefore it is important to work toward a better understanding of ontogenetic changes in predator–prey interactions and their impacts on biogeochemical cycles. For species in diverse and complex ecosystems, the prevalence and implications of ontogenetic changes in predator–prey interactions are even more poorly understood due primarily to limitations in experimental methods to study them.

Common methods for studying planktonic foodweb interactions include artificially enhancing consumer (usually mesozooplankton) abundance in bottle or mesocosm incubations (Båmstedt et al. 2000), or use of dilution experiments to study microzooplankton grazing rates (Landry and Hassett 1982). These studies take a subtractive approach, measuring the change in prey between initial and final time points, but can be flawed due to bottle effects (Roman and Rublee 1980). Within these studies, different methods to quantify the amount of each prey type or species being consumed have been developed. Commonly used methods include using flow cytometry for identification of different types of autotrophic prey (Cucci et al. 1989), imaging for identification of body shapes (Sieracki et al. 1998), using a Coulter Counter for particle sizing and counting (Roman and Rublee 1980), measuring gut fluorescence for studies of autotrophic prey (Mackas and Bohrer 1976), and applying various DNA-based methods (e.g., Nejstgaard et al. 2008; Craig et al. 2014). Despite the availability of a range of techniques for studying foodweb interactions, major challenges remain that include accurate measurement of species-level differences in ingestion and remineralization rates over development with natural food resources, and the integration of traditional methods with newer technologies.

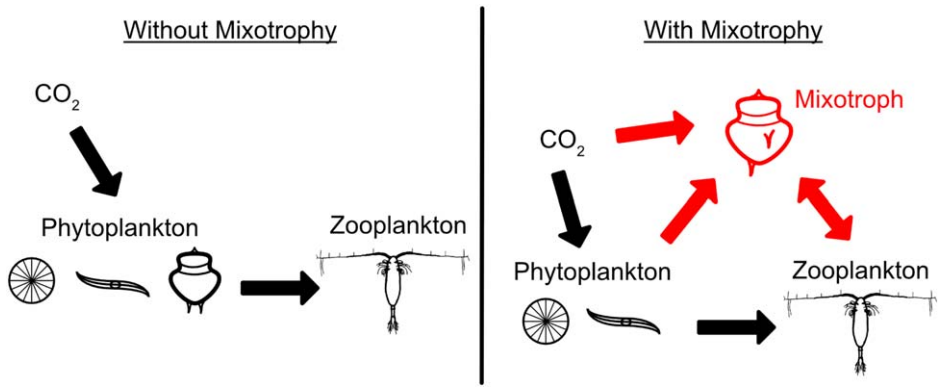

Fig. 5. Mixotrophy creates smooth transitions between using organic or inorganic C pools depending on environmental conditions, blurring the lines between producers and consumers (Worden et al. 2015). The simplified view that organisms with chloroplasts are only capable of photosynthesis and vice versa needs to be revised in order to do this metabolic plasticity justice. An additional functional group of plankton, one that is capable of phototrophy and phagotrophy, needs to be established to reduce the current underestimation of biomass and energy transfer to higher trophic levels (Ward and Follows, 2016). Red indicates fluxes and populations that are affected by considering this process.

Mixotrophy

Aquatic protists are often categorized as either strict heterotrophs or autotrophs, despite increasing evidence that mixotrophs are the default and strict heterotrophs/autotrophs are the exception (Flynn et al. 2013). Mixotrophs are protists that use both phototrophy and phagotrophy to obtain energy and increase their biomass (Stoecker 1998). A majority of protist groups, with the exception of diatoms, express some level of mixotrophy between a gradient of strict heterotrophy and strict autotrophy, with most being primarily a phagotroph or a phototroph that supplements their growth with alternative energy sources (Flynn et al. 2013).

Grazing in the protist community is typically estimated indirectly through prey removal experiments (Frost 1972) or the dilution method (Landry and Hassett 1982). Grazers in these types of experiments are identified by their lack of chloroplasts, while mixotrophs have to be identified as protists that contain both chloroplasts and prey (Sanders and Gast 2012). Therefore, grazing by mixotrophs has to be measured directly through the uptake of fluorescently labeled bacteria (Sherr and Sherr 1993), fluorescently labeled algae (Rublee and Gallegos 1989), or fluorescent microspheres (Steinkamp et al. 1982). Thus, unless a study is specifically focused on quantifying grazing or ingestion rates by mixotrophs, all grazing rates measured by standard methods are usually attributed to pigmentless protists (strict heterotrophs). The miscategorization of mixotrophs as strict autotrophs or heterotrophs has major implications for the way the flow of carbon and nutrients through the planktonic foodweb is understood (Fig. 5). Mixotrophs can increase trophic transfer efficiency by offsetting some of the carbon lost to respiration in secondary and tertiary plankton producers with carbon from photosynthesis (Ward and Follows 2016).

Of the cryptic interactions presented here, mixotrophy is the most researched. Mixotrophy has been studied for decades (Stoecker 1998), modeled in a variety of ways (Stickney et al. 2000; Flynn and Mitra 2009; Ward et al. 2011), and incorporated into recent foodweb models (D'Alelio et al. 2016; Ward and Follows 2016). However, while mixotrophs have been included in recent foodweb models, a majority of new models do not account for it and the ecological data required to accurately model this interaction and ground truth the results are scarce. Currently, a generalized understanding of the relative occurrence of mixotrophs compared to strict autotrophs and heterotrophs is unknown because most studies of mixotrophy focus on the grazing impact of a particular species or groups of protists (Seong et al. 2006). This approach only provides an estimation of the impact of mixotrophy during blooms of particular species.

A few studies conducted in a small range of specific environments have determined the grazing impact that mixotrophs can have on the consumption of bacteria compared to strict heterotrophs. These studies have shown that the consumption of bacteria by mixotrophs can be less than (Sanders et al. 1989), equal to (Unrein et al. 2007), or even more than heterotrophs (Domaizon et al. 2003), depending upon the time of year and study location. However, in order to improve recently developed foodweb models that account for mixotrohps, more data on variability of and grazing impact of mixotrophic assemblages in the environment is needed.

The cryptic interactions discussed here are embedded in the interactions between existing nodes of the marine foodweb. In Fig. 6, foodweb linkages are highlighted that are modulated by cryptic drivers, affecting ecological and biogeochemical interactions at different trophic levels. It will therefore be crucial to use sufficiently detailed models, similar to the Ecopath approach (http://www.ecopath.org) used in D'Alelio et al. (2016), to update the classical view of

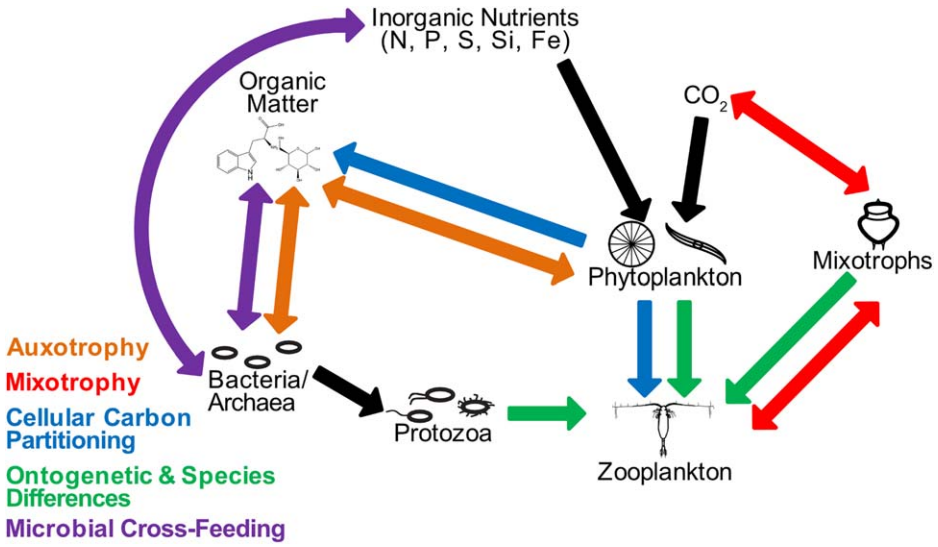

Fig. 6. The cryptic interactions described here affect many areas of the classic marine foodweb. This figure illustrates the material fluxes, populations, and molecular pools that are impacted by the five cryptic interactions discussed (red: mixotrophy; green: ontogenetic and species differences; purple: microbial cross-feeding; orange: auxotrophy; blue: cellular carbon partitioning). In fact, these interactions may have synergistic effects as the regions of the foodweb that they impact overlap. For example, cellular carbon partition in phytoplankton may affect both downstream pools of organic matter utilized in microbial cross-feeding and exchanged in cases of auxotrophy, as well as prey selection based on ontogenetic and species differences.

aquatic foodwebs to include more complex interactions, which is a necessary challenge in order to properly advance our understanding of the transfer of materials and energy through the foodweb.

Methodological advancements reveal cryptic interactions

Understanding energy transfer and elemental cycling in the ocean is among the primary research objectives of oceanographers. However, elucidating microbial interactions that affect the biogeochemical state of the ocean still presents a challenge. Pool size estimates of nutrients, organic compounds, or dissolved gases, for example, have been quantified in many ecosystems alongside fluxes for a variety of chemical compounds. However, there are many chemical compounds, interactions, and fluxes for cryptic interactions that are only measured in selected habitats or snapshots in time (e.g., Unrein et al. 2007; Grosse et al. 2017; Jungbluth et al. 2017). It can be challenging to apply findings from these studies to dissimilar systems, especially if underlying processes and fluxes are difficult to determine. Despite the vastness of the ocean and the expense of ship-time, field campaigns are the primary means of obtaining samples. This is an issue for cryptic interactions among the plankton, which occur on small spatial scales (nm to mm, individual cells to microscopic organism level) or over very short time spans, making the detection of intermediates challenging or impossible. Several key methodological advancements have been made to close gaps in spatial and temporal resolution, identify key-players and interactions between individuals, and measure rates in order to determine fluxes.

In situ platforms such as autonomous underwater vehicles, gliders, Argo floats (Argo Steering Team, 1998), and equipment installations on commercial vessels have had a revolutionizing effect on oceanographic data collection. These tools allow for the monitoring of many physical, chemical, and biological parameters in higher resolution across broader spatial and finer temporal scales and are able to reach places that were previously difficult to access. At the same time, these tools decrease ship-operating costs and provide higher sample frequency and/or resolution that can be made available almost in real-time. Many in situ, sometimes autonomous platforms, have been developed to incorporate genetic and transcriptomic samplers (review see McQuillan and Robidart 2017) or other sensors to measure microbial diversity and activity in situ with higher resolution than more commonly used ship-based sampling (Pachiadaki et al. 2016). In particular, the high resolution of biogeochemical and genetic data obtained through in situ sampling and incubation methods can reveal the presence of cryptic interactions and their seasonality, infer their global distribution, and provide a view of cryptic interactions in the ocean that has not been possible previously.

Genetic and transcriptomic methods have evolved to a point where they have revolutionized our ability to better understand key players in community-level and individual interactions (Shilova et al. 2016), as well as the physiological responses of organisms to stressors (e.g., Todgham and Hofmann 2009, Roncalli et al. 2016). Through methods applying DNA sequencing to predator gut contents, progress has been

made describing the breadth of prey taxa that can be consumed by species (Craig et al. 2014) and identifying unique prey items such as gelatinous zooplankton in the guts of adult calanoid copepods (e.g., Ho et al. 2017). Quantitative estimates of grazing and digestion rates of adult copepods on nauplii or algal prey items using species-specific DNA amplification of gut content has also allowed for a greater understanding of prey preference by different species (Durbin et al 2008; Nejstgaard et al. 2008). Next-generation sequencing enables identification of novel organisms and pathways that were previously unknown (DeVargas et al. 2015; Jungbluth et al. 2016), particularly in the microbial world where culturing and microscopy were historically the only methods to identify species. Now, researchers regularly identify and study cryptic interactions based purely on comparisons of genetic information, however more work is needed to enable use of DNA to rapidly and quantitatively estimate specific interactions in different species and life stages.

Making observations across the scales involved in microbial interactions is challenging because some processes are too rapid to leave evidence of intermediate molecules, even though they may have large implications on the C turnover of an ecosystem (see "Redox-Related Cross-Feeding" section). Microbial interactions take place on nano- or micrometer spatial and temporal scales and thus can be observed using microsensors as tools to determine chemical micro-gradients. However, to elucidate bacterial metabolism, additional methods are required. Method advancements in determining stable isotope labeling of individual compounds as well as nano-SIMS techniques make it now possible to identifying specific compounds utilized by bacteria and plankton, visualize their distribution within the cells and quantify the incorporation of specific molecules into cells (see "Intracellular carbon partitioning" section; Vasquez-Cardenas et al. 2015). Furthermore, advances in high resolution mass spectrometry are allowing us to identify and quantify at the molecular level the low-abundance, rapidly cycling metabolites that underlie microbial interactions such as auxotrophy (e.g., Sañudo-Wilhelmy et al. 2012; Durham et al. 2015; reviewed Kujawinski 2011) (see "Auxotrophy" section).

Although advancements in methodology have helped expose cryptic interactions, each area comes with its own challenge. For example, the high resolution of autonomous platforms and molecular methods provide massive amounts of data, which need to be stored, validated, analyzed, and interpreted (Marx 2013; Chen and Zhang 2014). The cost of obtaining DNA sequences has decreased substantially over the years, while other challenges with genetic and transcriptomic methods remain to be overcome. For example, metabarcoding for quantitative estimates of species abundance or biomass for metazoans is still not optimized (Bucklin et al. 2016). An additional challenge with genetic and transcriptomic methods is that they all require baseline databases and vetting for comparison, and while there are major efforts such as the Barcode of Life and the Census of Marine Life projects, and databases like NCBI and EMBL-EBI databases that are aimed at building these resources, consistent and reliable curation of these resources continues to be a challenge. Finally, microbiologists who utilize high-throughput sequencing methods to describe new life or new biogeochemical processes now need the more traditional field and bench skills to process samples and are often expected to develop computer programming skills to deal with "big data," or have access to a willing computational collaborator.

Furthermore, newly developed methods may take years to be established as routine or necessary. Recently discovered processes are accompanied by sparse field observations, meaning that the natural distribution of many cryptic interactions can remain poorly documented. Investigating cryptic interactions without in situ samplers is labor intensive and the expense of gear such as in situ sampling equipment can further limit the number of studies using advanced methods that would help elucidate cryptic processes. However, it is crucial that scientists ensure, whenever it is appropriate, that they are accounting for and collecting data on cryptic interactions in future studies in order to properly understand the important processes occurring in a system.

Examples of cryptic interactions in recent models

Novel technologies have allowed us to gain more information about cryptic interactions, but incorporating these interactions into our understanding of global processes is a separate effort. We believe that the specific cryptic interactions discussed in this review can be fundamental to the underlying mechanisms of many ecosystems and are critical to both simplified theoretical models, biogeochemical models, and computational foodweb models (Fig. 6). Some of the cryptic interactions we examined have been incorporated into models (discussed below) that provide examples of how certain cryptic interactions can be modeled and their effect of the transfer of materials. Additionally, we consider how some of these models might be improved by accounting for additional cryptic interactions.

There are numerous examples of mixotrophs being modeled. Stickney et al. (2000) expanded on the basic nutrient–phytoplankton–zooplankton–detritus model to include a node for mixotrophy. Flynn and Mitra (2009) created a complex mechanistic model that described how mixotrophs interacted with carbon, nitrogen, and phosphorus. Ward et al. (2011) developed an idealized model that modeled the physiology of different plankton to determine which environmental conditions favored mixotrophs. D'Alelio et al. (2016) produced a mass balance model that computed biomass flow between 63 functional nodes, ten of which were mixotrophic groups or species. Ward and Follows (2016) designed a simplified global model of the plankton foodweb

that removed phytoplankton and zooplankton, modelling plankton as mixotrophs on a spectrum between strict autotrophy and heterotrophy. However, the models by Stickney, Flynn and Mitra, and Ward are examples of modelling mixotrophs rather than incorporating mixotrophs into complex foodweb models. The Ward and Follows model provides an estimation of the role mixotrophs have in the transfer of carbon, but it is theoretical and still needs to be validated with field observations. The D'Alelio model incorporates mixotrophs, uses some real data, and accounts for the different trophic strategies mixotrophs may have under different conditions. Nevertheless, we propose the D'Alelio model could be improved by another cryptic interaction: intracellular carbon partitioning (see "Intracellular Carbon Partitioning" section).

Resource-dependent intracellular carbon partitioning can have a potentially large effect on the amount of new carbon entering the foodweb through primary production. In a mass-balanced model like D'Alelio's, the input of carbon biomass (primary production) has to be balanced by the loss of carbon (respiration, grazing, excretion) (D'Alelio et al. 2016). However recent research has shown that the amount of carbon that is produced by primary production and available to be assimilated by grazers is less than bulk estimates currently used in models when intracellular carbon partitioning is measured (Klausmeier et al. 2004; Grosse et al. 2017). The error associated with overestimating the amount of carbon that is put into a mass-balance model will cascade throughout each functional node and distort the mass balance. For example, with the model, D'Alelio compares two different ecosystem states, bloom, and nonbloom. It is likely that the environmental conditions associated with the different states were different, which would affect the allocation of carbon within autotrophs between the two states (Grosse et al. 2017). In one possible scenario, if phosphate is limiting, then grazers would be feeding on high C : P autotrophs and need to increase respiration to get rid of excess carbon. Thus, decreasing the amount of carbon biomass transferred to higher trophic levels in the model (DeMott et al. 1998).

Reed et al. (2014) developed a model that takes steps to bridge the gap between geochemistry and metagenomics data. Currently, most biogeochemical models simulate fluctuations in the biomass of organismal functional groups, defined by their metabolisms. However, Reed et al. (2014) grouped organisms together based on their functional genes and used relative functional gene abundance rather than biomass. This approach exposed the close coupling between nitrate reduction and sulfur cycling and revealed those parameters that were tightly controlling the activities of different microorganisms (mainly half-saturation constants). Metagenomics has revealed a higher amount of species and functional diversity than what is typically accounted for in models. Incorporating more of the pathways revealed by metagenomics can create more complex biogeochemical models with high predictive capability (Reed et al. 2014). Additionally, this approach to modeling can be used to examine how certain never-before-modeled cryptic interactions, such as sulfur cycling, can alter biogeochemical dynamics.

However, the ability to model additional cryptic interactions is limited by data gathered through laboratory studies and environmental observations (Reed et al. 2014). Through metagenomics, we are discovering new metabolic pathways for which we do not know the reaction stoichiometry or, alternatively, we know a metabolic pathway but not the associated genes. This means there are likely more cryptic interactions similar to sulfur cycling, but more environmental data and laboratory studies are needed before they can be incorporated into a functional gene model.

The cryptic interactions presented in this paper, along with numerous other interactions not mentioned, are important to ecosystem dynamics and deserve a broader incorporation into modeling efforts. The models discussed here provide examples of how some cryptic interactions have been modeled, but they are just starting points. These recent attempts to include cryptic interactions in models are encouraging, but are still the exception to most models, so we urge these efforts to continue.

Future research needs

The importance of each cryptic interaction discussed in this paper is generally established, however, the quantitative relationship of these processes to carbon, nitrogen, or phosphorus biomass pools has not been described in a comprehensive enough manner. Even when they are recognized to be important, it is difficult to incorporate cryptic interactions into models without appropriate data. Microbial ecologists are often quantitative in their description of communities but rarely make parallel rate measurements, which are the real requirements of foodweb models. Furthermore, field measurements of biomass pools and rate processes are not always made under variable environmental conditions or across geochemical gradients so that these measurements may be used in various states of complex models. Even when rate measurements are made, there are doubts about the methods to make these measurements. Bottle effects or the inability to mimic in situ conditions can confound measurements made in incubations (Edgcomb et al. 2016). Recent improvements in collection of these data should continue and dialogue between ecologists and modelers should motivate experimental design by outlining the needs of each party (Flynn 2005).

Generally, the biomass and flow rates of energy between biomass components are the fundamental parameters necessary for an ecosystem model (Table 1). Field measurements must be made using the same units as the model. While this may seem obvious, ecologists tend to measure parameters

Table 1. A list of data we currently have for each cryptic interaction discussed in this paper and a list of data we need in order to incorporate these interactions into models.

Interaction	Data we have	Data we need	Approaches
Redox-related cross-feeding	In some cases, potential rate measurements and concentrations of intermediates. Relative abundances of microbial genes and transcripts.	Verification of processes in situ and measurement of stoichiometry. Absolute quantities of genes and transcripts.	In situ incubation studies Metatranscriptomics Metaproteomics
Intracellular carbon partitioning	Several measurements of nutrient dependent primary production and compound synthesis, limited to temporal coastal systems	Relationship between primary production/nutrient + light availability/ compound production for different groups of phytoplankton	In situ incubation studies Compound specific isotope analysis
Auxotrophy	Knowledge of some cases of auxotrophy	Other cases of auxotrophy, rates of uptake, impact on community structure	Bioinformatics approaches to enhance identification of auxotrophy, isotopic labeling to measure uptake, mass spectrometry to measure organic compound abundances
Mixotrophy	Some measurements of mixotrophic and heterotrophic grazing on prey	Biomass (carbon) of mixotrophs, autotrophs, and heterotrophs, mixotrophic primary production, and mixotrophic and grazing under a range of environmental conditions	Fluorescent labeled algae Fluorescent labeled bacteria Fluorescent microspheres
Ontogenetic predatory-prey	Estimates for some species in some environments (mostly temperate), mostly for adult	Relationship between complex prey communities and strength of grazing/predation preferences, grazing rates and impacts of early life history stages	Bottle incubations Gut content analysis DNA sequencing Next Generation Sequencing

such as relative abundances of organisms, chlorophyll *a* (Chl *a*), or cell concentrations. These parameters are meaningful for ecological understanding but models generally require total carbon, nitrogen, or phosphorus units (Flynn 2005). Carbon : cell or carbon : Chl *a* ratios vary widely depending on species and environmental conditions and are therefore not easily incorporated into models (Sathyendranath et al. 2009). As a result, incorrect or broad assumptions tend to be made in order to estimate carbon content from cell counts. Additionally, while ecologists recognize ecological complexity, most field studies do not include sufficient biogeochemical measurements of all important limiting or co-limiting nutrients that affect the dynamics of the organisms being studied (Flynn 2005). Suggestions for data collection and technical approaches for all of the cryptic interactions discussed here are listed in Table 1.

Metagenomics (or other "omics") data are increasingly being incorporated into models (e.g., Reed et al. 2014; Coles et al. 2017) because they supply rich datasets for the building of model frameworks or testing of geochemical and community assembly hypotheses. However, one drawback is that gene marker abundances from metagenomics can only be reported as relative abundances. Thus, paired measurements such as absolute gene abundance by quantitative PCR or turnover rate measurements of targeted geochemical pools are necessary for incorporation into models. Furthermore, functional gene markers incorporated into models can only be those associated with well-defined processes, leaving many abundant genes of poorly defined function out of the model (Reed et al. 2014).

With sufficient data, models can also be used to determine the importance of incorporating a complex interaction to the overall model performance. For example, the cryptic exchanges of molecules between microbes have been described for sulfur cycling and its importance to the eastern tropical oxygen minimum zones is known (Canfield et al. 2010). Preliminary attempts at modeling this process in the Arabian Sea show that it is sensitive to nitrate concentrations and, when active, significantly reduces total microbial biomass with no significant changes in geochemical profiles (Reed et al. 2014). Thus, with sufficient data on stoichiometric parameters and metabolic potential, models can be used to establish the sensitivity of ocean characteristics to cryptic interactions. In addition, iterative quality-control processes help determine those interactions that are essential to the overall skill of the model and identify which relationships are most important (Van Nes and Scheffer 2005). Ultimately, the quality of the data and level of complexity that goes

into building a model defines the quality and level of complexity of the results that come out of the model, and thus specific models can be tailored to particular questions encompassing cryptic interactions in the plankton. But, without field studies with sufficient spatial and temporal data coverage, no model will be sufficient to describe these processes.

However, modelers do not necessarily need to wait for ecologists to collect empirical data for parameterization before they begin to explore how incorporating cryptic interactions into theoretical models may affect a model's output. For example, Ward and Follows (2016) developed a theoretical model that compared the transfer of carbon within a foodweb with strict autotrophs and heterotrophs to a foodweb with only mixotrophs. This model did not require any detailed quantitative data, but produced results that could be ground-truthed by the current understanding of the foodweb. Importantly, this study highlighted the types of data collection on mixotrophs that were further needed, specifically a more generalized understanding of the portion of mixotrophs within each plankton size group. Studies like Ward and Follows (2016) can help direct the focus of research done by ecologists and provide testable hypotheses for their projects. Moving forward, this back-and-forth approach between ecologists and modelers can be used to rapidly advance our understanding of the role of cryptic interactions in foodwebs.

Conclusions

Simplifications such as "zooplankton consume phytoplankton," "phytoplankton take up inorganic nutrients," "gross primary production determines the amount of carbon available to the foodweb," etc. have helped scientists explain and model general interactions in the aquatic environment. Traditional methods have focused on quantifying and qualifying these generalizations, but rapid advancements in genomics, sensor detection limits, experimental methods, and other technologies in recent years have shown our generalization of interactions within the plankton community may be too simple (Fig. 6). These enhancements in technology have exposed a number of interactions we consider cryptic because bulk sampling efforts and experimental methods are biased against them. Our understanding of these cryptic interactions within the plankton community has expanded to the point where ignoring some of these complexities in favor of generalizations results in large errors. Lack of data on an interaction does not mean that it is unimportant to the flow of material through the foodweb. Further examination of the cryptic interactions presented in this paper requires extra effort, time, and money. But these studies are necessary in order to understand the structure of the foodweb and its resulting effect on the transfer of nutrients and energy.

Ultimately, what is required to incorporate these cryptic interactions into foodweb and biogeochemical models is enhanced cooperation between ecologists and modelers and expanding the research of these interactions to a larger range of systems. As new technologies allow for a better understanding of the full impact of cryptic interactions throughout all aquatic systems, open communication between foodweb modelers and field scientists will insure appropriate data collection to model these interactions. It is important to start building cryptic interaction into models, but ecologists need to be confident they are supplying modelers with the data they need to accomplish this task.

References

Albert, D., C. Taylor, and S. Martens. 1995. Sulfate reduction rates and low molecular weight fatty acid concentrations in the water column and surficial sediments of the Black Sea. Deep-Sea Res. Part I **42**: 1239–1260. doi:10.1016/0967-0637(95)00042-5

Argo Steering Team. 1998. On the design and Implementation of Argo - an initial plan for the global array of profiling floats. International CLIVAR Project Office Report, 21, 32, http://www.argo.ucsd.edu/

Arrigo, K. R., D. H. Robinson, D. L. Worthen, R. B. Dunbar, G. R. DiTullio, M. VanWoert, and M. P. Lizotte. 1999. Phytoplankton community structure and the drawdown of nutrients and CO_2 in the Southern Ocean. Science **283**: 365–367. doi:10.1126/science.283.5400.365.

Båmstedt, U., D. J. Gifford, X. Irigoien, A. Atkinson, and M. R. Roman. 2000. Feeding, p. 297–399. *In* R. Harris, P. H. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley [eds.], ICES Zooplankton Methodology Manual. Academic Press.

Berg, J. S., and others. 2016. Intensive cryptic microbial iron cycling in the low iron water column of the meromictic Lake Cadagno. Environ. Microbiol. **18**: 5288–5302. doi:10.1111/1462-2920.13587

Bickford, D., D. J. Lohman, N. S., Sodhi, P. K. Ng, R. Meier, K. Winker, K. K. Ingram, and I. Das. 2007. Cryptic species as a window on diversity and conservation. Trends Ecol. Evol. **22**: 148–155. doi:10.1016/j.tree.2006.11.004

Boit, A., N. D. Martinez, R. J. Williams, and U. Gaedke. 2012. Mechanistic theory and modelling of complex food-web dynamics in Lake Constance. Ecol. Lett. **15**: 594–602. doi:10.1111/j.1461-0248.2012.01777.x

Bucklin, A., P. K. Lindeque, N. Rodriguez-Ezpeleta, A. Albaina, and M. Lehtiniemi. 2016. Metabarcoding of marine zooplankton: Prospects, progress and pitfalls. J. Plankton Res. **38**: 393–400. doi:10.1093/plankt/fbw023

Canfield, D. E., F. J. Stewart, B. Thamdrup, L. De Brabandere, T. Dalsgaard, E. F. Delong, N. P. Revsbech, and O. Ulloa. 2010. A cryptic sulfur cycle in oxygen minimum zone waters off the Chilean coast. Science **330**: 1375–1378. doi:10.1126/science.1196889

Carini, P., and others. 2014. Discovery of a SAR11 growth requirement for thiamin's pyrimidine precursor and its distribution in the Sargasso Sea. ISME J. **8**: 1727–1738. doi:10.1038/ismej.2014.61

Chen, C. L. P., and C.-Y. Zhang. 2014. Data-intensive applications, challenges, techniques and technologies: A survey on Big Data. Inform. Sciences. **275**: 314–347.

Coles, V. J., and M. R. Stukel, M. T. Brooks, A. Burd et al. 2017. Ocean biogeochemistry modeled with emergent trait-based genomics. Science. **358**: 1149–1154.

Conley, W. J., and J. T. Turner. 1985. Omnivory by the coastal marine copepods *Centropages hamatus* and *Labidocera aestiva*. Mar. Ecol. Prog. Ser. **21**: 113–120. doi: 10.3354/meps021113

Craig, C., W. J. Kimmerer, and C. S. Cohen. 2014. A DNA-based method for investigating feeding by copepod nauplii. J. Plankton Res. **36**: 271–275. doi:10.1093/plankt/fbt104

Croft, M. T., M. J. Warren, and A. G. Smith. 2006. Algae need their vitamins. Eukaryot. Cell **5**: 1175–1183. doi: 10.1128/EC.00097-06

Cucci, T. L., S. E. Shumway, W. S. Brown, and C. R. Newell. 1989. Using phytoplankton and flow cytometry to analyze grazing by marine organisms. Cytometry **10**: 659–669. doi:10.1002/cyto.990100523

Cullen, J. J. 2001. Primary production methods, p. 578–584. *In* J. H. Steele [ed.], Encyclopedia of ocean sciences, 2nd ed. Academic Press.

D'Alelio, D., S. Libralato, T. Wyatt, and M. R. d'Alcala. 2016. Ecological-network models link diversity, structure and function in the plankton food-web. Nature **6**: 21806. dio: 10.1038/srep21806

DeMott, W. R., R. D. Gulati, and K. Siewertsen. 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. Limnol. Oceanogr. **43**: 1147–1161. doi:10.4319/lo.1998.43.6.1147

DeVargas, C., S. Audic, N. Henry, and J. Decelle. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science. **348**. doi:10.1126/science.1261605

Domaizon, I., S. Viboud, and D. Fontvieille. 2003. Taxon-specific and seasonal variations in flagellates grazing on heterotrophic bacteria in the oligotrophic Lake Annecy – importance of mixotrophy. FEMS Microbiol. Ecol. **46**: 317–329. doi:10.1016/S0168-6496(03)00248-4

Droop, M. R. 1957. Auxotrophy and organic compounds in the nutrition of marine phytoplankton. J. Gen. Microbiol. **16**: 286–293. doi:10.1099/00221287-16-1-286

D'Souza, G., S. Waschina, S. Pande, K. Bohl, C. Kaleta, and C. Kost. 2014. Less is more: Selective advantages can explain the prevalent loss of biosynthetic genes in bacteria. Evolution **68**: 2559–2570. doi:10.1111/evo.12468

Durbin, E. G., M. C. Casas, T. A. Rynearson, and D. C. Smith. 2008. Measurement of copepod predation on nauplii using qPCR of the cytochrome oxidase I gene. Mar. Biol. **153**: 699–707. doi:10.1007/s00227-007-0843-5

Durham, B. P., and others. 2015. Cryptic carbon and sulfur cycling between surface ocean plankton. Proc. Natl. Acad. Sci. USA **112**:435–357. doi: 10.1073/pnas.1413137112

Edgcomb, V. P., C. Taylor, M. G. Pachiadaki, S. Honjo, I. Engstrom, and M. Yakimov. 2016. Comparison of Niskin vs. in situ approaches for analysis of gene expression in deep Mediterranean Sea water samples. Deep-Sea Res. Part II **129**: 213–222. doi:10.1016/j.dsr2.2014.10.020

Estrela, S., and S.P. Brown. 2013. Metabolic and demographic feedbacks shape the emergent spatial structure and function of microbial communities. PLoS Comput. Biol. **9**: e1003398. doi:10.1371/journal.pcbi.1003398

Field, C. B., M. J. Behrenfeld, J.T. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. Science **281**: 237–240. doi:10.1126/science.281.5374.237.

Flynn, K. J. 2005. Castles built on sand: Dysfunctionality in plankton models and the inadequacy of dialogue between biologists and modellers. J. Plankton Res. **27**: 1205–1210. doi:10.1093/plankt/fbi099

Flynn, K. J., and A. Mitra. 2009. Building the "perfect beast": Modelling mixotrophic plankton. J. Plankton Res. **31**: 965–992. doi:10.1093/plankt/fbp044.

Flynn, K. J., D. K. Stoecker, A. Mitra, J. A. Raven, P. M. Glibert, P. J. Hansen, E. Graneli, and J. M. Burkholder. 2013. Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. J. Plankton Res. **35**: 3–11. doi: 10.1093/plankt/fbs062

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod Calanus pacificus. Limnol. Oceanogr. **17**: 805–815. doi:10.4319/lo.1972.17.6.0805

Giovannoni, S. J., J. Cameron Thrash, and B. Temperton. 2014. Implications of streamlining theory for microbial ecology. ISME J. **8**: 1–13. doi:10.1038/ismej.2014.60

Grosse, J., P. van Breugel, C. P. D. Brussaard, and H. T. S. Boschker. 2017. A biosynthesis view on nutrient stress in coastal phytoplankton. Limnol. Oceanogr. **62**: 490–506. doi:10.1002/lno.10439.

Hanke, A., J. Berg, T. Hargesheimer, H. E. Tegetmeyer, C. E. Sharp, and M. Strous. 2016. Selective pressure of temperature on competition and cross-feeding within denitrifying and fermentative microbial communities. Front. Microbiol. **6**: 1–14. doi:10.3389/fmicb.2015.01461

Hansen, B., P. K. Bjornsen, and P.J. Hansen. 1994. The size ratio between planktonic predators and their prey. Limnol. Oceanogr. **39**: 395–403. doi:10.4319/lo.1994.39. 2.0395

Hastings, D., and S. Emerson. 1988. Sulfate reduction in the presence of low oxygen levels in the water column of the Cariaco Trench. Limnol. Oceanogr. **33**: 391–396. doi: 10.4319/lo.1988.33.3.0391

Ho, T. W., J. S. Hwang, M. K. Cheung, H. S. Kwan, and C. K. Wong. 2017. DNA-based study of the diet of the marine

calanoid copepod Calanus sinicus. J. Exp. Mar. Biol. Ecol. **494**: 1–9. doi:10.1016/j.jembe.2017.04.004

Holmkvist, L., T. G. Ferdelman, and B. B. Jørgensen. 2011. A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). Geochim. Cosmochim. Acta **75**: 3581–3599. doi:10.1016/j.gca.2011.03.033

Johnston, D. T., B. C. Gill, A. Masterson, E. Beirne, K. L. Casciotti, A. N. Knapp, and W. Berelson. 2014. Placing an upper limit on cryptic marine sulphur cycling. Nature **513**: 530–533. doi:10.1038/nature13698

Jørgensen, B. B. 1977. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol. Oceanogr. **22**: 814–832. doi:10.4319/lo.1977.22.5.0814

Jungbluth, S. P., R. M. Bowers, H. T. Lin, J. P. Cowen, and M. S. Rappé. 2016. Novel microbial assemblages inhabiting crustal fluids within mid-ocean ridge flank subsurface basalt. ISME J. **10**: 2033–2047. doi:10.1038/ismej.2015.248

Jungbluth, M. J., K. E. Selph, P. H. Lenz, and E. Goetze. 2017. Species-specific grazing and significant trophic impacts by two species of copepod nauplii, *Parvocalanus crassirostris* and *Bestiolina similis*. Mar. Ecol. Prog. Ser. **572**: 57–76. doi:10.3354/meps12139

Klausmeier, C. A., E. Litchman, T. Daufresne, and S. A. Levin. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature **429**: 171–174. doi: 10.1038/nature02454.

Koch, F., M. A. Marcoval, C. Panzeca, K. W. Bruland, S. A. Sañudo-Wilhelmy, and C. J. Gobler. 2011. The effect of vitamin B12 on phytoplankton growth and community structure in the Gulf of Alaska. Limnol. Oceanogr. **56**: 1023–1034. doi:10.4319/lo.2011.56.3.1023

Kujawinski, E. B. 2011. The impact of microbial metabolism on marine dissolved organic matter. Ann. Rev. Mar. Sci. **3**: 567–99. doi:10.1146/annurev-marine-120308-081003

Landry, M. R., and R. P. Hassett. 1982. Estimating the grazing impact of marine micro-zooplankton. Mar. Biol. **67**: 283–288. doi:10.1007/BF00397668

Mackas, D., and R. Bohrer. 1976. Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. J. Exp. Mar. Biol. Ecol. **25**: 77–85. doi: 10.1016/0022-0981(76)90077-0

McQuillan, J. S., and Robidart, J. C. 2017. Molecular-biological sensing in aquatic environments: Recent developments and emerging capabilities. Curr. Opin. Biotechnol. **45**: 43–50. doi:10.1016/j.copbio.2016.11.022

Mee, M. T., J. J. Collins, G. M. Church, and H. H. Wang. 2014. Syntrophic exchange in synthetic microbial communities. Proc. Natl. Acad. Sci. USA **111**: E2149–E2156. doi:10.1073/pnas.1405641111

Mee, M. T., and H. H. Wang. 2012. Engineering ecosystems and synthetic ecologies. Mol. Biosyst. **8**: 2470–2483. doi: 10.1039/c2mb25133g

Morris, J. J., R. E. Lenski, and E. R. Zinser. 2012. The black queen hypothesis: Evolution of dependencies through adaptive gene loss. Mar. Biol. **3**: 1–7. doi:10.1128/mBio.00036-12

Neill, W., and A. Peacock. 1980. Breaking the bottleneck: Interactions of invertebrate predators and nutrients in oligotrophic lakes, p. 715–724. *In* W.C. Kerfoot [ed], Evolution and ecology of zooplankton communities. Univ. Press of New England.

Nejstgaard, J. C., M. E. Frischer, P. Simonelli, C. Troedsson, M. Brakel, F. Adiyaman, A. F. Sazhin, and L. F. Artigas. 2008. Quantitative PCR to estimate copepod feeding. Mar. Biol. **153**: 565–577. doi:10.1007/s00227-007-0830-x

Pachiadaki, M. G., C. Taylor, A. Oikonomou, M. M. Yakimov, T., Stoeck, and V. Edgcomb. 2016. *In situ* grazing experiments apply new technology to gain insights into deep-sea microbial food webs. Deep-Sea Res. Part II Top. Stud. Oceanogr. **129**: 223–231. doi:10.1016/j.dsr2.2014.10.019

Pande, S., and C. Kost. 2017. Bacterial unculturability and the formation of intercellular metabolic networks. Trends Microbiol. **25**: 349–361. doi:10.1016/j.tim.2017.02.015

Reed, D. C., C. K. Algar, J. A. Huber, and G. J. Dick. 2014. Gene-centric approach to integrating environmental genomics and biogeochemical models. Proc. Natl. Acad. Sci. USA **111**: 1879–1884. doi:10.1073/pnas.1313713111

Rios-Del Toro, E. E., and F. J. Cervantes. 2016. Coupling between anammox and autotrophic denitrification for simultaneous removal of ammonium and sulfide by enriched marine sediments. Biodegradation **27**: 107–118. doi:10.1007/s10532-016-9759-4

Roman, M. R., and P. A. Rublee. 1980. Containment effects in copepod grazing experiments: A plea to end the black box approach. Limnol. Oceanogr. **25**: 982–990. doi: 10.4319/lo.1980.25.6.0982

Roncalli, V., M. J. Jungbluth, and P. H. Lenz. 2016. Glutathione S-transferase regulation in *Calanus finmarchicus* feeding on the toxic dinoflagellate *Alexandrium fundyense*. PLoS One **11**: e0159563. doi:10.1371/journal.pone.0159563

Rublee, P. A., and C. L. Gallegos. 1989. Use of fluorescently labeled algae (FLA) to estimate microzooplankton grazing. Mar. Ecol. Prog. Ser. **51**: 221–227. doi:10.3354/meps051221

Rusch, D. B., and others. 2007. The Sorcerer II global ocean sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. PLoS One **5**: e77. doi:10.1371/journal.pbio.0050077

Saiz, E., K. Griffell, A. Calbet, and S. Isari. 2014. Feeding rates and prey:predator size ratios of the nauplii and adult females of the marine cyclopoid copepod *Oithona davisae*. Limnol. Oceanogr. **59**: 2077–2088. doi:10.4319/lo.2014.59.6.2077

Sanders, R. W., and R. J. Gast. 2012. Bacterivory by phototrophic picoplankton and nanoplankton in Arctic waters.

FEMS Microbiol. Ecol. **82**: 242–253. doi:10.1111/j.1574-6941.2011.01253.x

Sanders, R. W., K. G. Porter, S. J. Bennett, and A. E. DeBiase. 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol. Oceanogr. **34**: 673–687. doi:10.4319/lo.1989.34.4.0673

Sañudo-Wilhelmy, S. A., and others. 2012. Multiple B-vitamin depletion in large areas of the coastal ocean. Proc. Natl. Acad. Sci. USA **109**: 14041–5. doi:10.1073/pnas.1208755109

Sathyendranath, S., and others. 2009. Carbon-to-chlorophyll ratio and growth rate of phytoplankton in the sea. Mar. Ecol. Prog. Ser. **383**: 73−84. doi:10.3354/meps07998

Seong, K. A., H. J. Jeong, S. Kim, G. H. Kim, and J. H. Kang. 2006. Bacterivory by co-occurring red-tide algae, heterotrophic nanoflagellates, and ciliates. Mar. Ecol. Prog. Ser. **322**: 85–97. doi:10.3354/meps322085

Sherr, E. B., and B. F. Sherr. 1993. Protistan grazing rates via uptake of fluorescently labeled prey, p. 695–701. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology. Lewis Publishers.

Shilova, I. N., J. C. Robidart, E. F. DeLong, and J. P. Zehr. 2016. Genetic diversity affects the daily transcriptional oscillations of marine microbial populations. PLoS One **11**: e0146706. doi:10.1371/journal.pone.0146706

Sieracki, C. K., M. E. Sieracki, and C. S. Yentsch. 1998. An imaging-in-flow system for automated analysis of marine microplankton. Mar. Ecol. Prog. Ser. **168**: 285–296. doi:10.3354/meps168285

Steinkamp, J. A., J. S. Wilson, G. C. Saunders, and C. C. Stewart. 1982. Phagocytosis: Flow cytometric quantitation with fluorescent microspheres. Science **215**: 64–66. doi:10.1126/science.7053559

Stibor, H., and U. Sommer. 2009. Ocean food webs and trophic, p. 96–113. *In* C. M. Duarte, and A. L. Helgueras [eds.]. Marine ecology, developed under the auspices of the UNESCO. Eolss Publishers.

Stickney, H. L., R. R. Hood, and D. K. Stoecker. 2000. The impact of mixotrophy on planktonic marine ecosystems. Ecol. Model. **125**: 203–230. doi:10.1016/S0304-3800(99) 00181-7

Stoecker, D.K. 1998. Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. Eur. J. Protistol. **34**: 281–290. doi:10.1016/S0932-4739(98)80055-2

Suárez, I., and E. Marañón. 2003. Photosynthate allocation in a temperate sea over an annual cycle: The relationship between protein synthesis and phytoplankton physiological state. J. Sea Res. **50**: 285–299. doi:10.1016/j.seares.2003.04.002.

Sunagawa, S., E. Karsenti, C. Bowler, and P. Bork. 2015. Computational eco-systems biology in Tara Oceans: Translating data into knowledge. Mol. Syst. Biol. **11**: 10–12. doi:10.15252/msb.20156272

Thompson, A. W., R. Foster, A. Krupke, B. J. Carter, N. Musat, D. Vaulot, M. M. M. Kuypers, and J. P. Zehr. 2012. Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. Science **337**: 1546–1550. doi:10.1126/science.1222700

Todgham, A. E., and G. E. Hofmann. 2009. Transcriptomic response of sea urchin larvae *Strongylocentrotus purpuratus* to CO_2-driven seawater acidification. J. Exp. Biol. **212**: 2579–2594. doi:10.1242/jeb.032540

Tripp, H. J., S. R. Bench, K. Turk, R. Foster, B. Desany, F. Niazi, J. P. Affourtit, and J. P. Zehr. 2010. Metabolic streamlining in an open-ocean nitrogen-fixing cyanobacterium. Nature **464**: 90–94. doi:10.1038/nature08786

Tripp, H. J., M. S. Schwalbach, M. M. Meyer, J. B. Kitner, R. R. Breaker, and S. J. Giovannoni. 2009. Unique glycine-activated riboswitch linked to glycine-serine auxotrophy in SAR11. Environ. Microbiol. **11**: 230–238. doi:10.1111/j.1462-2920.2008.01758.x

Unrein, F., R. Massana, L. Alonso-Sáez, and J. M. Gasol. 2007. Significant year-round effect of small mixotrophic flagellates on bacterioplankton in an oligotrophic coastal system. Limnol. Oceanogr. **52**: 456–469. doi:10.4319/lo.2007.52.1.0456

Valdés, V. P., C. Fernandez, V. Molina, R. Escribano, and F. Joux. 2017. Dissolved compounds excreted by copepods reshape the active marine bacterioplankton community composition. Front. Mar. Sci. **4**: 343. doi:10.3389/fmars.2017.00343

Van Nes, E. H. and M. Scheffer. 2005. A strategy to improve the contribution of complex simulation models to ecological theory. Ecol. Model. **185**: 153–164. doi:10.1016/j.ecolmodel.2004.12.001

Vasquez-Cardenas, D., and others. 2015. Microbial carbon metabolism associated with electrogenic sulphur oxidation in coastal sediments. ISME J. **9**: 1966–1978. doi:10.1038/ismej.2015.10.

Ward, B. B. 2013. How nitrogen is lost. Science **341**: 352–353. doi:10.1126/science.1240314

Ward, B. A., S. Dutkiewicz, A. D. Barton, and M. J. Follows. 2011. Biophysical aspects of resource acquisition and competition in algal mixotrophs. Am. Nat. **178**: 98–112. doi:10.1086/660284.

Ward, B. A., and M. J. Follows. 2016. Marine mixotrophy increases trophic transfer efficiency, mean organism size, and vertical carbon flux. Proc. Natl. Acad. Sci. USA **113**: 2958–2963. doi:10.1073/pnas.1517118113

Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annu. Rev. Ecol. Syst. **15**: 393–425. doi:10.1146/annurev.es.15.110184.002141

Worden, A. Z., M. J. Follows, S. J. Giovannoni, S. Wilken, A. E. Zimmerman, and P. J. Keeling. 2015. Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. Science **347**: 1257594. doi:10.1126/science.125

Wright, J. J., K. M. Konwar, and S.J. Hallam. 2012. Microbial ecology of expanding oxygen minimum zones. Nat. Rev. Microbiol. **10**: 381–394. doi:10.1038/nrmicro2778

Acknowledgments

The authors would like to thank Dr. Holly Moeller (University of California Santa Barbara), Dr. Jessica Luo (University Corporation for Atmospheric Research, National Center for Atmospheric Research) and two anonymous reviewers for their input on the manuscript. The authors would like to acknowledge the Eco-DAS symposium (NSF Award OCE-1356192) for opportunities and support.

Submitted 17 November 2017
Revised 5 March 2018
Accepted 1 April 2018