

# Mixotrophy stirs up our understanding of marine food webs

David A. Caron<sup>a,1</sup>

Mixotrophy among planktonic eukaryotic organisms is broadly defined as the combined use of photosynthetic and heterotrophic nutrition within a single organism. The conventional view of species as either phototrophic (capable of producing energy and carbon for growth using only inorganic compounds and light) or heterotrophic (wholly dependent on preformed organic material for nutrition) is a preconception rooted in the fact that most terrestrial species can easily be divided into “plants” or “animals.” Indeed, the diversity of terrestrial organisms that combine photosynthetic and heterotrophic nutrition is mostly relegated to a dozen or so genera of carnivorous plants that capture and digest insects and other small creatures for the nutrients they contain, but also capture light energy via photosynthesis. In contrast to life on land, mixotrophic nutrition is widespread among single-celled and multicellular organisms comprising the ocean’s plankton. The ecological significance for the species that possess this behavior has not been lost on biologists who have endeavored to document and understand mixotrophy, but its importance to pelagic food-web structure and function has not yet become entrained into mathematical models of marine ecosystems. In PNAS, Ward and Follows (1) address this disparity, and provide one of the first attempts to incorporate mixotrophic nutrition into biogeochemical models of oceanic food webs. The authors use their model to estimate the impact that mixotrophy has on food-web structure, the efficiency of carbon transfer to higher trophic levels, and the sinking of carbon into the deep ocean.

Mixotrophic nutrition among planktonic species in the ocean occurs throughout many phylogenetic groups and plankton size classes, and takes a variety of forms and strategies (Fig. 1). For example, numerous species from most algal classes in the nanoplankton size class (2–20  $\mu\text{m}$ ) possess the ability to consume minute prey (usually bacteria), although there is great variance in the degree to which different mixotrophic species blend phototrophic and heterotrophic abilities into their nutritional repertoire (Fig. 1 A and B). Some species are nearly completely autotrophic, whereas many others are efficient predators and use photosynthesis only to prolong survival in the absence

of food (2). Predatory phytoflagellates are ubiquitous in the lighted waters of marine and freshwater ecosystems. They generally constitute a significant numerical fraction of the phytoplankton community, and are an important source of bacterial mortality, particularly in oligotrophic environments (3). Many bloom-forming harmful algae are also able to consume prey. It has been speculated that mixotrophic nutrition is a factor that may explain the ability of these algae to dominate phytoplankton communities (4).

Many heterotrophic dinoflagellates and ciliates in the microplankton size class (20–200  $\mu\text{m}$ ) exhibit mixotrophy by conducting kleptochloroplastidy, a process that involves the ingestion and digestion of algal prey, but also retention of the chloroplasts of the prey in a photosynthetically functional state (Fig. 1 D–F). The effectiveness of these species in maintaining the function of their “stolen” chloroplasts varies from the nearly autotrophic ciliate *Mesodinium rubrum* (Fig. 1 D and E) to more-transient associations in which chloroplasts lose function within a few days and must be replaced by the ingestion of additional prey. The nutrition of these mixotrophs complicates the description of food-web structure in pelagic ecosystems, but also provides endless subjects for studying the evolutionary history of organelle acquisition and stabilization in eukaryotes, a vibrant subject of research at this time (5).

Intimate symbiotic associations between heterotrophic planktonic organisms and microalgae represent another form of mixed nutrition in the plankton. Symbiotic associations between ciliates (Fig. 1 C), or many species of Rhizaria (Foraminifera, Acantharia, Radiolaria) (Fig. 1 G and J–L) in the micro- and macroplankton size classes (20  $\mu\text{m}$  to >1 mm) occur in all oceans but are particularly common in tropical and subtropical oceans. Rhizarian skeletal remains in marine sediments are extensively used in paleoclimatology, and the life histories and abundances of many of these species are intimately tied to the algal species that these organisms harbor as endosymbionts within their cytoplasm (6, 7). These large “holobionts” constitute intimate and productive partnerships in which the feeding activity of the host provides energy, whereas carbon and

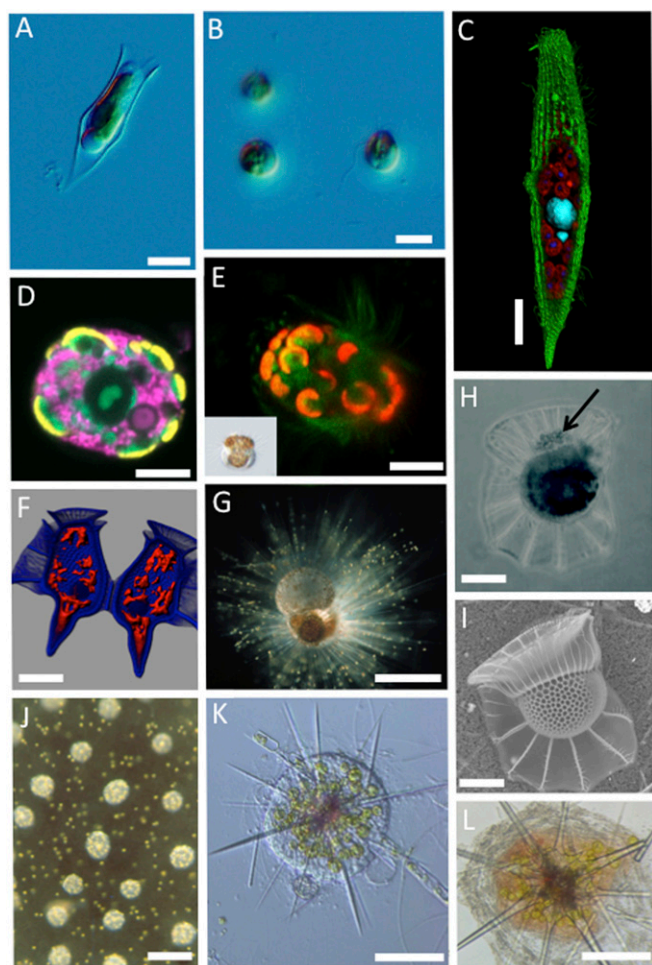
<sup>a</sup>Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371

Author contributions: D.A.C. wrote the paper.

The author declares no conflict of interest.

See companion article on page 2958.

<sup>1</sup>Email: dcaron@usc.edu.



**Fig. 1. Putting faces to the name: examples of mixotrophy by various planktonic organisms.** The ability to consume prey is common among many classes of nanoplanktonic algae in freshwater and marine communities, including the chrysophyte alga, *Dinobryon* sp. (A) and the haptophyte alga, *Prymnesium parvum* (B). (C) The ciliate *Tiarina* sp. (shown in green) containing numerous endosymbiotic algae of the genus *Symbiodinium* (shown in red). The ciliate nuclei are light blue. (D and E) The kleptochloroplastidic ciliate *Mesodinium rubrum* (*Myrionecta rubra*) preys on cryptophyte algae and retains the alga's chloroplasts in a functional state. These photomicrographs show in situ hybridization probes for the *M. rubrum* (pink) and chloroplasts of *Geminigera cryophila* (the latter are green in D, red in E). Inset in (E) is a light micrograph of *Mesodinium* sp. from a plankton tow, containing chloroplasts stolen from its prey, the dinoflagellate *Geminigera* sp. (F) The heterotrophic dinoflagellate, *Dinophysis caudata*, is a kleptochloroplastidic species that feeds on *Mesodinium* spp. and retains its stolen cryptophyte chloroplasts (shown in red). (G) The planktonic foraminifer, *Globigerinoides sacculifer* possesses several hundred to a few thousand endosymbiotic dinoflagellate algae (golden cells). (H and I) A heterotrophic dinoflagellate, *Ornithocercus* sp. harbors ectosymbiotic cyanobacteria. The cyanobacteria are the dark cells in the girdle region in the photomicrograph (arrow in H), I is a scanning electron micrograph of the dinoflagellate. (J) A darkfield image of a colonial radiolarian colony showing central capsules of this large rhizarian (large spheres) and endosymbiotic dinoflagellate algae (golden cells). (K and L) Two unidentified acantharia showing the presence of endosymbiotic algae (colored cells). C and F were photographed using confocal laser scanning fluorescent microscopy. Images courtesy of (C) ref. 19, with permission from Macmillan Publishers Ltd: *The ISME Journal*, copyright 2002; (D) ref. 20, with permission from Macmillan Publishers Ltd: *Nature*, copyright 2007; (E) Matthew Johnson; (F) ref. 8, with permission from AAAS; (I) Karla B. Heidelberg (University of Southern California, Los Angeles, CA). [Scale bars: 5  $\mu$ m (A, B, D, E); 25  $\mu$ m (C, F, H, I); 500  $\mu$ m (G); 200  $\mu$ m (J); 50  $\mu$ m (K and L).]

nutrients from remineralized prey fuel symbiont photosynthesis. In essence, they are functionally analogous to symbiont-bearing corals of tropical ecosystems, possessing the same efficient nutrient utilization and retention as corals. The findings of the recent circumglobal TARA Oceans expedition indicates that we have grossly underestimated the importance of these large, delicate symbioses in the world ocean (8). Additionally, some heterotrophic dinoflagellates in these large plankton size classes harbor ectosymbiotic cyanobacteria attached to their outer cell walls or in specialized compartments constructed by the dinoflagellates (Fig. 1 H and I). These associations place minute, nitrogen-fixing photosynthetic cyanobacteria in intimate contact with large predatory hosts, enabling efficient transfer of fixed nitrogen into larger size classes.

The earliest models of mixotrophy were largely conceptual and focused on the evolutionary significance and ecological advantage conferred by the behavior (9), such as mixotrophic algae that consume bacteria that might otherwise compete with them for growth-limiting nutrients (10). Following these efforts, and with increased awareness of mixotrophy as a nutritional strategy among many planktonic species, modelers began to investigate the consequences of different forms of mixotrophy, as described above (11), including elemental stoichiometric considerations of prey and nutrient availability (12). Subsequent mathematical analyses revealed that the inclusion of mixotrophy in models could affect the equilibrium community structure and improve stability of the biological community (13, 14). This early work envisioned mixotrophs as constitutively photosynthetic organisms (i.e., predatory phytoflagellates). More recent theoretical models have begun to encompass other forms of mixotrophy, such as kleptochloroplastidy (15). Mostly recently, studies have advocated for including mixotrophy in biogeochemical modeling of the ocean's food web, to examine the importance of this behavior for plankton community structure and the flux of sinking particles in the ocean (16).

That is the important step that Ward and Follows have taken in their latest work (1). A previous publication by the authors examined a size-structured global ocean food-web model (albeit without mixotrophy), a break from the highly simplistic nutrient-phytoplankton-zooplankton models that have dominated biogeochemical modeling activities for so long (17). With their present publication (1), Ward and Follows formally recognize that combined photosynthetic-heterotrophic ability throughout the size spectrum of planktonic organisms fundamentally changes the movement of nutrients and energy through the various size classes of organisms in the community. Their model results with and without mixotrophy indicate a threefold increase in the mean size of organisms when mixotrophy is allowed. This is a significant finding because larger average organismal size translates into a greater proportion of carbon sinking out of surface waters. Large organisms contribute disproportionately, compared with small ones, to the vertical flux of particles in the ocean. The overall effect is an enhancement of the "biological carbon pump" (18), a term referring to the biologically mediated sinking of carbon out of surface waters of the ocean. Sinking particles sequester carbon in the deep ocean for periods of hundreds to thousands of years, thereby making it unavailable for remineralization, and return to the atmosphere as carbon dioxide. Because the biological carbon pump works effectively to remove carbon over long time periods, it reduces the contribution of this greenhouse gas to global warming. Ward and Follows (1) show that the outcomes of their model are changed by allowing mixotrophic nutrition by the plankton. The result is more efficient movement of carbon

into larger organisms, and consequently a greater contribution to sinking particle flux.

It has been an unduly long time for the important nutritional strategy of mixotrophy to make it into global-scale biogeochemical modeling, and hopefully this work (1) is only a beginning. Future iterations of the model described by Ward and Follows in PNAS might incorporate some of the fundamentally different types of mixotrophy exhibited by different species, as our knowledge expands regarding their physiological and ecological strategies. As noted above, one size does not fit all when it comes to defining mixotrophy. Nonetheless, even the inclusion of a “generic” form of this behavior in global modeling is a major step forward. Another consideration for future additions to these

models might be the explicit incorporation of bacteria and the fundamental roles that they play in organic matter transformation. The inclusion of bacteria would enable mixotrophy by organisms in the 1.5- and 5- $\mu\text{m}$  size categories of the model (presently not possible because of the lack of prey small enough to be eaten by those species). Like mixotrophy, bacterial activity and their trophic fate are features that have been poorly represented in biogeochemical models, if not completely omitted. Future modifications might continue to yield improvements in the accuracy of predictions obtained from this and other models, providing valuable tools for predicting the response of the ocean’s biological community and elemental cycles in the face of ongoing climate change.

- 1 Ward BA, Follows MJ (2016) Marine mixotrophy increases trophic transfer efficiency, mean organism size, and vertical carbon flux. *Proc Natl Acad Sci USA* 113:2958–2963.
- 2 Jones RI (1994) Mixotrophy in planktonic protists as a spectrum of nutritional strategies. *Mar Microb Food Webs* 8(1):87–96.
- 3 Hartmann M, et al. (2012) Mixotrophic basis of Atlantic oligotrophic ecosystems. *Proc Natl Acad Sci USA* 109(15):5756–5760.
- 4 Burkholder JM, Glibert PM, Skelton HM (2008) Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* 8(1):77–93.
- 5 Keeling PJ, McCutcheon JP, Doolittle WF (2015) Symbiosis becoming permanent: Survival of the luckiest. *Proc Natl Acad Sci USA* 112(33):10101–10103.
- 6 Anderson OR (1983) *Radiolaria* (Springer, New York).
- 7 Hemleben C, Spindler M, Anderson OR (1988) *Modern Planktonic Foraminifera* (Springer, New York).
- 8 de Vargas C, et al.; Tara Oceans Coordinators (2015) Ocean plankton. Eukaryotic plankton diversity in the sunlit ocean. *Science* 348(6237):1261605.
- 9 Stoecker DK (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Eur J Protistol* 34(3):281–290.
- 10 Thingstad TF, et al. (1996) On the strategy of “eating your competitor”: A mathematical analysis of algal mixotrophy. *Ecology* 77(7):2108–2118.
- 11 Stickney HL, Hood RR, Stoecker DK (2000) The impact of mixotrophy on planktonic marine ecosystems. *Ecol Modell* 125(2-3):203–230.
- 12 Troost TA, Kooi BW, Kooijman SALM (2005) Ecological specialization of mixotrophic plankton in a mixed water column. *Am Nat* 166(3):E45–E61.
- 13 Hammer AC, Pitchford JW (2005) The role of mixotrophy in plankton bloom dynamics, and the consequences for productivity. *ICES J Mar Sci* 62(5):833–840.
- 14 Jost C, et al. (2004) The effects of mixotrophy on the stability and dynamics of a simple planktonic food web model. *Theor Popul Biol* 66(1):37–51.
- 15 Mitra A Flynn KJ (2010) Modelling mixotrophy in harmful algal blooms: More or less the sum of the parts? *J Mar Systems* 83(3):158–169.
- 16 Mitra A, et al. (2014) The role of mixotrophic protists in the biological carbon pump. *Biogeosciences* 11(4):995–1005.
- 17 Ward BA, et al. (2012) A size-structured food-web model for the global ocean. *Limnol Oceanogr* 57(6):1877–1891.
- 18 Sigman DM, Hain MP (2012) The biological productivity of the ocean. *Nature Ed Knowl* 3(6):1–16.
- 19 Mordret S, et al. (2015) The symbiotic life of *Symbiodinium* in the open ocean within a new species of calcifying ciliate (*Tiarina* sp.). *ISME J*, 10.1038/ismej.2015.211.
- 20 Johnson MD, et al. (2007) Retention of transcriptionally active cryptophyte nuclei by the ciliate *Myrionecta rubra*. *Nature* 445(7126):426–428.