

New perspectives on the functioning and evolution of photosymbiosis in plankton

Mutualism or parasitism?

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Photosymbiosis is common and widely distributed in plankton and is considered to be beneficial for both partners (mutualism). Such intimate associations involving heterotrophic hosts and microalgal symbionts have been extensively studied in coral reefs, but in the planktonic realm, the ecology and evolution of photosymbioses remain poorly understood. Acantharia (Radiolaria) are ubiquitous and abundant heterotrophic marine protists, many of which host endosymbiotic microalgae. Two types of photosymbiosis involving acantharians have recently been described using molecular techniques: one found in a single acantharian species involving multiple microalgal partners (dinoflagellates and haptophytes), and the other observed in more than 25 acantharian species exclusively living with the haptophyte *Phaeocystis*. Contrary to most benthic and terrestrial mutualistic symbioses, these symbiotic associations share the common feature of involving symbionts that are abundant in their free-living stage. We propose a hypothetical framework that may explain this original mode of symbiosis, and discuss the ecological and evolutionary implications. We suggest that photosymbiosis in Acantharia, and probably in other planktonic hosts, may not be a mutualistic relationship but rather an “inverted parasitism,” from which only hosts seem to benefit by sequestering and exploiting microalgal cells. The relatively small population size of microalgae in hospite would prevent reciprocal evolution that can select uncooperative symbionts, therefore making this horizontally-transmitted association stable over evolutionary time.

The more we learn about the diversity of life and the structure of genomes, the more it appears that much of the evolution of biodiversity is about the manipulation of other species—to gain resources and, in turn, to avoid being manipulated (John Thompson, 1999).

Photosymbiosis, whereby microalgae live within a heterotrophic host organism, is a key evolutionary process that led to the acquisition of photosynthesis in eukaryotes and consequently the emergence of several lineages.¹ In today's oceans,

such symbiotic relationships involve a wide diversity of unicellular and multicellular organisms, particularly in nutrient-deficient tropical and subtropical waters.² Symbiosis encompasses a wide range of associations along a continuum from parasitism, commensalism to mutualism.³ Photosymbiosis is typically considered mutually beneficial for both partners: the symbiont transfers photosynthetically-produced compounds to the host, which in turn provides shelter and nutrients to the symbiont.^{4,5} This intimate association tends to be obligatory for the host, which in most cases must acquire its microalgal symbionts from the environment at each generation (i.e., horizontal transmission). For the symbionts, the degree of reliance on the host remains unclear. The iconic example of marine photosymbiosis occurs in reef ecosystems, where many benthic invertebrates such as corals, giant clams, anemones and sponges, live in symbiosis with members of the dinoflagellate genus *Symbiodinium*.⁶ This symbiosis has been extensively studied as it sustains and drives highly diverse, productive and economically important reef ecosystems.⁷ Photosymbiosis is also common in oceanic plankton, particularly in the large heterotrophic protists Foraminifera and Radiolaria, which are known to host various symbiotic microalgae, such as dinoflagellates, haptophytes and prasinophytes.^{8,9} However, despite the evolutionary significance of symbiosis and the fundamental ecological role of marine microbiota in the biosphere, the diversity, functioning and evolution of photosymbiosis in plankton are still poorly described. To improve our understanding of symbiosis in what is one of the largest ecosystems on earth, we focused on the radiolarian group Acantharia (Fig. 1).

Acantharia are widely distributed throughout the world's oceans and typically outnumber their planktonic counterparts Foraminifera and other Radiolaria in oligotrophic open ocean waters (up to 40 acantharian cells. l⁻¹).¹⁰ Acantharia that are abundant in surface waters generally live in symbiosis with microalgae, whereas their non-symbiotic relatives tend to inhabit deeper waters.^{11,12} In order to unveil the diversity and specificity of this photosymbiotic association at a global scale, we isolated individual symbiont-bearing acantharian cells from different oceanic regions worldwide and subsequently PCR amplified different genetic markers from the host (18S and 28S rDNA) and the symbiotic microalgae (18S rDNA, ITS, 28S rDNA, rbcL and psbA). We thereby identified both partners for

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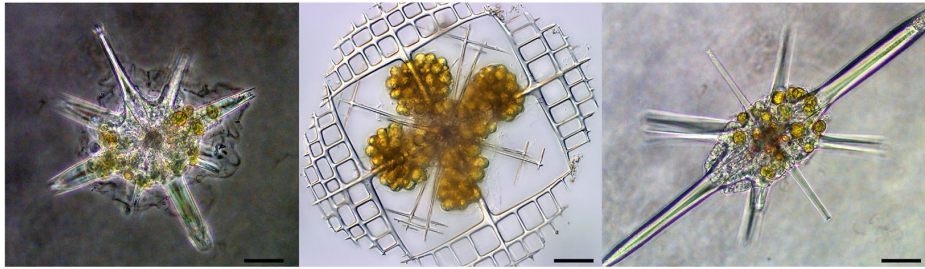


Figure 1. Microscopic pictures of symbiotic Acantharia that harbor 10 to 100 symbiotic microalgae (yellow cells) in their cytoplasm. Scale bars = 20 μ m.

more than 100 distinct host-symbiont associations covering a large taxonomic range within the Acantharia.

From the poles to the tropics, in a sample set including all of the most abundant surface water acantharian species, we quasi-systematically found that the microalgal symbionts belong to the haptophyte genus *Phaeocystis*.¹³ The genetic footprint of *Phaeocystis* symbionts was identical or very similar to known free-living species (e.g., *P. cordata*, *P. antarctica*). The only exception was a single acantharian species from an early-branching clade (*Acanthochiasma* sp) that was found to harbor multiple symbiotic microalgae, including distantly related dinoflagellates (*Heterocapsa* sp, *Pelagodinium* sp, *Azadinium* sp and *Scrippsiella* sp) as well as a haptophyte (*Chrysochromulina* sp).¹⁴ While *Pelagodinium* and *Chrysochromulina* were already known to occur in symbiosis with some species of Foraminifera and Radiolaria,^{8,9} the other microalgae (*Phaeocystis*, *Heterocapsa*, *Scrippsiella* and *Azadinium*) have never been reported to be involved in symbiotic relationships, despite their key role in marine ecosystems. The common ecological feature of the free-living stage of all of these microalgae (except *Pelagodinium*, for which little data are available) is their high abundance and broad distribution in coastal and oceanic waters.^{15,16} Some of these microalgae even periodically form extensive blooms that cause negative effects on the marine food web and human activities.¹⁷

These results are in stark contrast with the vast majority of terrestrial and marine symbiotic associations described to date, including the coastal-benthic photosymbiosis in reef ecosystems. In these classical symbiotic model systems, the symbionts are typically elusive outside the host,¹⁸ and can even be considered as members of the rare biosphere.^{19,20} The symbiont population is therefore mainly found dwelling in the host cells or tissues. By contrast, the symbiotic microalgae of Acantharia appear to essentially thrive in the free-living phase (up to several million cells per liter for *Phaeocystis*),²¹ and only a relatively small pool occurs in symbiosis (10–100 microalgal cells per acantharian host cell).¹³ This original mode of symbiosis may be prevalent in the planktonic realm since previous studies have shown that the very abundant photosynthetic cyanobacteria *Synechococcus* and *Prochlorococcus* can also form symbiotic associations with various protistan hosts in the open ocean.^{22,23} We therefore propose a hypothetical framework illustrating the contrasted modes of photosymbiosis in pelagic and reef ecosystems (Fig. 2), which

has a number of potential ecological and evolutionary implications.

The success of a horizontally-transmitted symbiosis is highly dependent on the encounter rates between partners. Open ocean and reef ecosystems are both characterized by oligotrophic and high-light conditions, but their physical features are very different. The open ocean is a vast, voluminous, turbulent and microbiota-diluted habitat, whereas reefs are densely-populated and essentially restricted to shallow coastal

waters. In oceanic plankton, encounter rates between putative partners can be therefore constrained by their low concentrations, hence making the horizontal transmission a risky step in the symbiotic interaction. In coral reefs, the high concentration of hosts probably facilitates the symbiont transmission, which is viewed as pseudo-vertical.²⁴ Moreover, compared with benthic invertebrates like corals that can live for more than a century,²⁵ unicellular hosts in plankton have very short generation times (typically 3–4 wk),²⁶ imposing a complete and very dynamic reset of the association at each generation. Overall, this suggests that the establishment of an obligate symbiotic association, whereby two free-living partners need to physically interact in the right place and at the right time, is more challenging in the pelagic realm. Forming associations with microalgae that have widespread and extensive free-living populations could thus represent an advantageous ecological strategy for planktonic hosts since it increases the chance to encounter their specific symbionts and favors long-distance colonization.

Different relative sizes of the free-living vs. *in hospite* microalgal populations in pelagic and reef ecosystems would imply that photosymbiosis is shaped by different evolutionary forces in these environments (Fig. 2). In mutualistic symbioses, both host and symbiont may evolve to sustain the partnership, in a so-called coevolving system.²⁷ However, the fact that in pelagic photosymbiosis only a tiny proportion of the symbiont population lives inside the host would dictate that selective pressures generated by interactions with the host would be relatively weak. Selection would mainly act to increase the fitness of the microalgae in the external environment. As a consequence, evolutionary change in the host/holobiont is unlikely to cause evolutionary change in the symbiotic microalga. The evolution of the symbiotic relationship would therefore rely mainly on the adaptive capacity of the host, and potentially also on indirect effects of adaptations of the symbiont in its free-living phase. Conversely, in coral reefs, the main selective environment experienced by the symbionts would be within the host. Host/holobiont evolution would therefore play an influential role in evolution of the microalgal symbiont taxon and vice versa, and both partners can evolve to sustain the symbiosis.

How is pelagic photosymbiosis maintained over time without coevolutionary dynamics? Despite their key ecological roles and wide occurrence in terrestrial and aquatic ecosystems, horizontally-transmitted mutualistic symbioses between unrelated

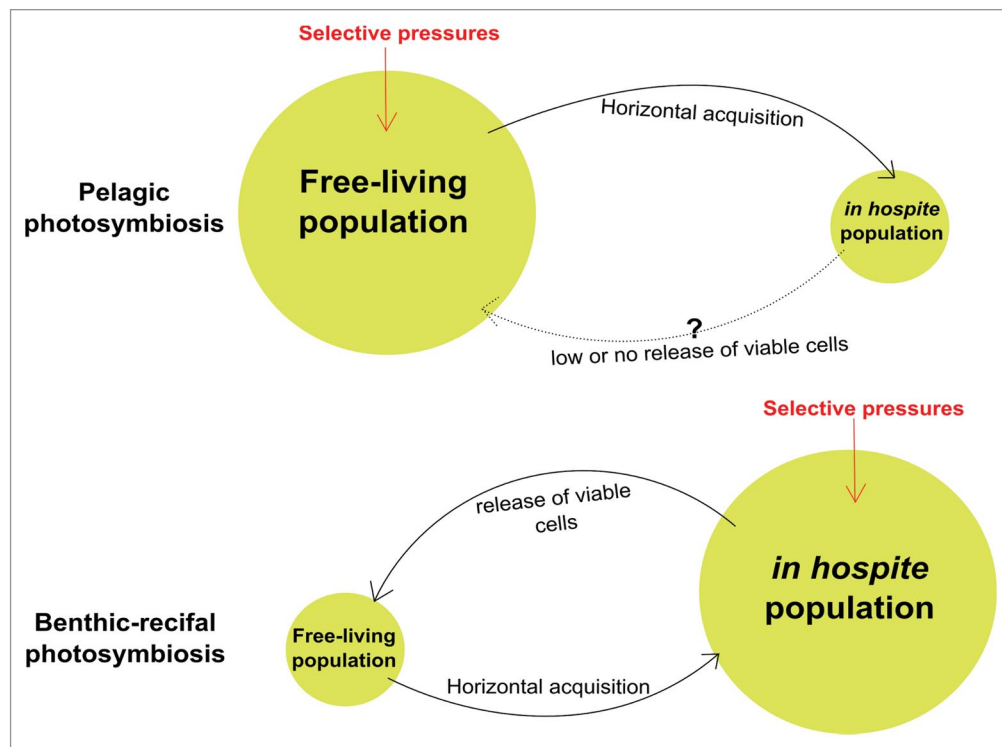


Figure 2. Hypothetical view of the different modes of photosymbiosis in pelagic (top) and reef (bottom) ecosystems. The size of the green circles represents the population size of the symbiotic microalgae in their free-living (left) and symbiotic phase (right). Contrary to benthic-recifal photosymbiosis, evidence is lacking in pelagic ecosystems showing that symbionts can be released from their host and return to the free-living phase.

organisms remain a puzzling paradox for theorists.^{28,29} Such cooperation is doomed to be evolutionarily unstable since natural selection would favor cheats that exploit the association while providing reduced or no services in return. Contrary to the commonly held view, a mathematical model has demonstrated that mutualism can persist and evolve without vertical transmission if the free-living population of the symbiont is large.³⁰ This is the case in the Acantharia and probably in other planktonic taxa living in photosymbiosis. The evolutionary persistence of pelagic photosymbiosis can be explained by the fact that symbiosis-related selective pressures have no or low impact on the small *in hospite* population of symbionts. Thus, the probability that any trait of the symbiont that is unfavorable for the symbiosis is selected is very low.

Contrary to reef symbionts, the extensive free-living phase of planktonic symbiotic microalgae implies that the degree of dependence on their host for their growth and reproduction is very low. It is interesting to note that the reef symbiont *Symbiodinium* has a 18S rDNA gene that evolves six times faster than that of its close pelagic relative *Pelagodinium*,⁹ and it also has a relatively small genome compared with other abundant dinoflagellates like *Heterocapsa*.³¹ This likely reflects the prominence of the *in hospite* stage for reef symbionts and a certain dependence on the host. Both can lead to genome reduction (loss of non-essential genes) and higher genetic drift associated with less effective purifying selection due to decreased frequency of sexual reproduction within the free-living pool. In this respect, the genetic footprint of *Symbiodinium* resembles that of host-dependent

endosymbiotic bacteria.³² By contrast, the genetic repertoire of planktonic symbionts seems to remain intact as it is primarily influenced by the external environment rather than the host cytoplasm, and probably by sexual exchanges occurring within large free-living populations.

It is therefore difficult to assert that the microalgae involved in planktonic symbioses increase their net fitness in symbiosis and ultimately benefit from this asymmetrical association over evolutionary time. We rather argue that only hosts profit from this asymmetrical association by capturing and farming microalgal cells that are otherwise ecologically successful in the environment. This enslavement of microalgae would be possible because of the lack of reciprocal evolution mentioned above. In the Acantharia-*Phaeocystis* symbiosis, the host seems to manipulate and transform the symbiont in order to maximize its photosynthetic capacity (e.g., 10-fold increase of cell volume, presence of numerous large plastids and vacuoles). We suppose that this drastic metamorphosis of *Phaeocystis* symbiont cells precludes a return to the free-living phase, hence representing an evolutionary bottleneck (Fig. 2). We named this phenomenon “cytoklepty” (theft of whole cells) by analogy to kleptoplastidy (theft of organelles).

While the mutualistic nature of the symbiosis has already been questioned in corals^{33,34} and lichens,³⁵ it seems to be clearer that photosymbiosis in Acantharia and other planktonic hosts cannot be considered as mutualism, but rather as an “inverted parasitism,” where the larger partner (the host) is the parasite of the smaller (the symbiont). Our hypotheses based on the results

obtained for the Acantharia need to be validated by characterizing other photosymbiotic associations in oceanic plankton. Investigation of relative population sizes of the free-living and *in hospite* phases of the symbionts, as well as the capacity of symbionts to return to a free-living form, would provide useful information. A better assessment of symbiont fitness (e.g., division rate in the two phases) and costs and benefits for each partner (e.g., nutritional exchange) would help to shed further light on the true nature of these important symbiotic relationships in the ocean.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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