Chapter 11
Photosymbiosis in Marine Pelagic Environments

Fabrice Not, Ian Probert, Catherine Gerikas Ribeiro, Klervi Crenn, Laure Guillou, Christian Jeanthon and Daniel Vaulot

Abstract Photosymbiosis is a symbiotic relationship between two or more organisms, one of which is capable of photosynthesis. Like other forms of symbiosis, photosymbioses can involve the full spectrum of trophic interactions from mutualism through commensalism to parasitism. As in marine benthic environments (e.g., coral reef ecosystems), photosymbiotic associations are frequently encountered in marine pelagic environments and can involve various combinations of microalgae with bacteria, protists, or metazoans. Here, we aim to provide a brief overview of current knowledge on the diversity of the organisms involved in pelagic photosymbioses, their ecological role, and their relevance for the ecosystem. This chapter focuses on mutualistic interactions occurring between photosynthetic protists and bacteria, between two protists and between microalgae and metazoans, as well as on photosymbiotic interactions involving parasitic protists. A section reviewing the most common and recent approaches used to study pelagic photosymbioses and presenting general perspectives in the field concludes the chapter.

11.1 Introduction

While studying the formation of lichens in the 19th century, H.A. de Bary first coined the term "symbiosis" as "the living together of unlike organisms" (de Bary 1879). This definition is broad and technically includes any distinct taxa, from any kingdom of life that are physically in contact and that have an enduring relationship

F. Not (✉) · I. Probert · K. Crenn · L. Guillou · C. Jeanthon · D. Vaulot
UPMC Univ Paris 06, CNRS, UMR 7144, Station Biologique, Sorbonne Universités, Place Georges Teissier, 29680 Roscoff, France
e-mail: not@sb-roscoff.fr

C. Gerikas Ribeiro
Departamento de Oceanografia Biológica, Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brazil

L.J. Stal and M.S. Cretoiu (eds.), *The Marine Microbiome*,
DOI 10.1007/978-3-319-33000-6_11

over multiple generations. Symbiosis therefore includes the full spectrum of trophic interactions, from mutualism through commensalism to parasitism.

Photosymbiosis is a symbiotic relationship between two (or more) organisms, one of which is capable of photosynthesis. Photosynthesis originated in cyanobacteria and has since spread across the eukaryotic tree of life by multiple serial endosymbiotic events, leading to the evolution of multiple lineages of algae, one of which (the Chlorobionta) was at the origin of the 'higher' terrestrial plants. Photosymbiosis has thus been, and still is, a highly relevant evolutionary process, but is also a key ecological interaction for ecosystem functioning both on land and in the ocean (Thompson 1999). Terrestrial plants are involved in many well-known photosymbiotic relationships, both mutualistic (e.g., with nitrogen-fixing bacteria in root nodules) and parasitic (e.g., with the oomycete *Phytophthora infestans* causing the disease known as potato blight). Unicellular algae are also involved in some prominent symbiotic relationships in terrestrial environments, notably in partnership with filamentous fungi in lichens.

The best-known photosymbiotic relationship in the marine environment is the association of cnidarian corals with unicellular algae from the dinoflagellate genus *Symbiodinium*. This photosymbiotic relationship structures and sustains benthic reef ecosystems and has been extensively studied, notably in relation to the negative impact of stresses linked to environmental change ('coral bleaching', e.g., Sampayo et al. 2008). Unicellular algae are involved in mutualistic symbiotic relationships with a number of other benthic hosts in the marine environment, including other cnidarians such as sea anemones, molluscs such as the giant clam *Tridacna*, and acoel flatworms (Bailly et al. 2014). Benthic seaweeds are known to have a number of bacterial, unicellular eukaryotic (='protistan') and macroalgal parasites, and some unicellular algae have been reported to parasitize benthic invertebrates (Trench 1993).

Photosymbiotic associations are also frequently encountered in the marine pelagic environment and can involve various combinations of microalgae with bacteria, protists, or metazoans (Anderson 2012; Decelle et al. 2015; Jephcott et al. 2015; Nowack and Melkonian 2010; Stoecker et al. 2009; Taylor 1982). Despite, the independently recognized key roles of oceanic plankton on the one hand and symbiosis on the other hand, the nature, diversity, and importance of pelagic photosymbioses are still poorly understood. In this chapter, we aim to provide a brief overview of the current knowledge of the diversity of the organisms involved in pelagic photosymbioses and their ecological role and importance in the ecosystem. The review will focus on mutualistic interactions occurring between photosynthetic protists and bacteria, between two protists and between microalgae and metazoans, as well as on photosymbiotic interactions involving parasitic protists. An overview of the most common approaches used to study pelagic photosymbioses and general perspectives in the field will conclude this chapter.

11.2 Symbioses Between Phytoplankton and Cyanobacteria

11.2.1 Symbiotic Nitrogen Fixation

Nitrogen is a major limiting factor in oceanic ecosystems (Moore et al. 2013). Eukaryotes can only obtain nitrogen through the uptake of dissolved forms (mainly nitrates and ammonia), whereas some bacteria and a few archaea have the ability to fix dinitrogen (N_2) and convert it into particulate organic nitrogen. Land plants have developed symbioses with N_2-fixing bacteria such as *Rhizobium* (Franche et al. 2009; Santi et al. 2013) and similar symbioses exist in eukaryotic phytoplankton. The earliest reports were from diatom-diazotroph associations (DDAs), with the cyanobacterial symbionts ('cyanobionts') *Richelia* (Ostenfeld and Schmidt 1902) and *Calothrix* (Lemmerman 1905). More recently, the unicellular N_2-fixing cyanobacteria UCYN-A has been shown to form an unusual symbiosis with a unicellular haptophyte alga (Thompson et al. 2012). Diazotrophic cyanobacteria have also been documented to form symbiotic partnerships with a wide variety of eukaryotic marine organisms, like sponges, ascidians (although N_2 fixation in ascidians can be linked to Rhizobiales, see Erwin et al. 2014), flagellated protists, dinoflagellates, radiolarians, macroalgae, and tintinnids (Carpenter 2002; Foster et al. 2006, and references therein).

11.2.2 Symbioses Between Cyanobacteria and Diatoms

DDAs involve either filamentous heterocystous (e.g., *Calothrix rhizosolenia* and *Richelia intracellularis*) or unicellular (e.g. *Cyanothece* sp.) nitrogen-fixing cyanobacteria (Rai et al. 2002). DDAs are non-obligate endosymbioses between diatoms from several different genera (notably including *Hemiaulus*, *Rhizosolenia*, and *Chaetoceros*) and diazotrophic cyanobacteria. The diatom hosts and the cyanobacterial symbionts can be found free living in the ocean, and horizontal transfer between cells and vertical transmission from host to daughter cell are both common. In diatom–*Richelia* associations, cyanobiont *hetR* sequences from the same host species vary by less than 1 % which suggests a high degree of specificity, probably linked to vertical transmission of the cyanobiont during the host division process (Janson et al. 1999). When in association, the diazotrophs appear to be localized in different regions of the diatom depending on the host species (Foster and O'Mullan 2008). After a long period in isolation, *Calothrix* trichomes start to change their morphological features, indicating host control of cyanobiont characteristics (Foster and O'Mullan 2008).

The metabolic influence of DDA symbioses on the cyanobiont has been observed in recent studies. Foster et al. (2011) estimated that symbiotic *Richelia* fixes up to 651 % more N_2 than required for its own growth. Symbiont genome

reduction can be an evolutionary consequence of long-term nutrient exchanges pointing to an increasing dependency between symbiont and host. Genome streamlining of cyanobionts has been reported for *Richelia* in intracellular association with *Hemiaulus* (Hilton et al. 2013), with genome reduction mainly affecting genes related to nitrogen metabolism: symbionts have a decreased capability to assimilate urea or nitrate (lack of ammonium transporters, nitrate and nitrite reductases and glutamine: 2-oxoglutarate aminotransferase), thus favoring N_2 fixation (Hilton et al. 2013). Diazotrophic cyanobacteria have evolved several mechanisms (both spatial and temporal) to overcome the deleterious effect of oxygen, a photosynthetic by-product, for the nitrogenase enzyme (Berman-Frank et al. 2001; Fay 1992; Thompson and Zehr 2013). In *Richelia intracellularis* in symbiosis with *Rhizosolenia clevei*, nitrogenase is protected by spatial separation, being confined to the heterocysts, the thick-walled, specialized N_2-fixation cells (Janson et al. 1995). In addition to spatial separation, a pronounced day-night periodicity of N_2 fixation was observed for *Richelia-Rhizosolenia* associations at the ALOHA station (Church et al. 2005, Foster and Zehr 2006). Unicellular *Cyanothece* sp. separate temporally the processes of carbon and nitrogen fixation (Reddy et al. 1993), and were found in association with the diatom *Climacodium frauenfeldianum* (Carpenter and Janson 2000).

11.2.3 Symbioses Between Cyanobacteria and Haptophytes

Using HISH-SIMS (halogenated in situ hybridization nanometer-scale secondary ion mass spectrometry) imaging, Thompson et al. (2012) observed a loose cell-surface association between the diazotrophic cyanobacterium UCYN-A and an apparently non-calcifying microalgal host. The host partial 18S rRNA gene sequences were >99 % identical to sequences obtained from sorted picoeukaryotic cells from South Pacific Ocean samples (BIOSOPE T60.34) (Shi et al. 2009) related to sequences of *Braarudosphaera bigelowii* (an atypical coccolithophore that produces pentalith-shaped coccoliths) and the non-calcifying haptophyte *Chrysochromulina parkeae* (Thompson et al. 2012). Using transmission electron microscopy Hagino et al. (2013) observed spheroidal bodies within *B. bigelowii* which were determined to be intracellular cyanobacterial symbionts belonging to the UCYN-A clade. Hagino et al. (2013) suggested that *C. parkeae* might be an alternate life-cycle stage of *B. bigelowii*, the former being an elongate, motile, unicellular organism with non-calcified organic scales (Green and Leadbeater 1972). *B. bigelowii* seems to comprise a set of pseudo-cryptic species, consisting of at least five 18S rRNA genotypes that correspond to morphotypes that differ slightly in size (Hagino et al. 2009). As *B. bigelowii* has a coastal distribution and the haptophyte related to BIOSOPE T60.34 was recovered from an open ocean site (Shi et al. 2009; Thompson et al. 2012), it has been hypothesized that the intracellular UCYN-A symbiosis in *B. bigelowii* was acquired after separation of those coastal/open ocean haptophyte ancestors (Hagino et al. 2013). Adding further

complexity, three clades of UCYN-A, with distinct but overlapping distributions, can be distinguished based on *nifH* sequences (Thompson et al. 2014), forming a monophyletic group with the marine cyanobacteria *Crocosphaera* sp. and *Cyanothece* sp. (Bombar et al. 2014). UCYN-A1 is mostly found in the open ocean (Thompson et al. 2012) and its host is smaller than that of UCYN-A2, which has coastal distribution and whose host is *B. bigelowii* (Hagino et al. 2013). Little is known about the host and spatial distribution of UCYN-A3. A global study by Cabello et al. (2016) has provided evidence that these cyanobacterium-haptophyte symbioses are mandatory for the hosts. UCYN-A cells were reported to transfer up to 95 % of newly fixed nitrogen to their hosts (Thompson et al. 2012). UCYN-A has a reduced genome that lacks the genes involved in carbon fixation, such as those for RuBisCO (ribulose-1,5-bisphosphate carboxylase-oxygenase) (Zehr et al. 2008) and the tricarboxylic acid (TCA) cycle responsible for the biosynthesis of amino acids (Bombar et al. 2014; Tripp et al. 2010). Such modifications in the genome of symbionts are analogous to the situation for cellular organelles with specific metabolic functions such as the chloroplast or the mitochondrion, although there are still no reports on the existence of a "diazoplast" (Thompson and Zehr 2013). Tripp et al. (2010) observed that the reduced genome of UCYN-A (1.44 Mb) structurally resembles those found in most chloroplasts (as well as in some bacteria), which may indicate a similar evolutionary path. In addition, the lack of the oxygen-evolving pathway (Tripp et al. 2010; Zehr et al. 2008) de facto prevents nitrogenase damage.

11.2.4 Symbioses Between Cyanobacteria and Dinoflagellates

Little is known about symbioses between cyanobacteria and dinoflagellates despite the fact that they were first observed more than 100 years ago (Schütt 1895). In most known cases, such as for the dinoflagellates *Ornithocercus* and *Histoneis* (Farnelid et al. 2010), the cyanobacteria are ectosymbionts (i.e., associated to the cell surface) located in the cingulum of the dinoflagellate cell. These cyanobacteria appear to be nitrogen-fixers (Foster et al. 2006), but more than one type can occur in association with a single dinoflagellate cell (Farnelid et al. 2010; Foster et al. 2006). Surprisingly, sequences recovered from dinoflagellate symbionts corresponded to cyanobacteria that are not known to fix nitrogen such as *Prochlorococcus* or to other types of bacteria, suggesting the complexity of the associations between dinoflagellates and bacteria.

11.2.5 Ecological Relevance of Symbioses Involving Diazotrophs

New production in oligotrophic areas is largely dependent on N_2 fixation, since upward nutrient fluxes are limited in these regions. Several studies have highlighted the importance of symbiosis between diazotrophic bacteria and photosynthetic eukaryotes in the marine environment, both in terms of the abundance of the organisms involved and of the impact on overall N_2 fixation (Foster et al. 2009; Goebel et al. 2010; Montoya et al. 2004; Turk et al. 2011). Goebel et al. (2010) and Foster et al. (2007) found high abundances of *Richelia-Hemiaulus* symbiosis in the western equatorial Atlantic under the influence of the Amazon River plume, while during the circumnavigating Malaspina expedition, *Richelia*-diatom associations were mostly found in the South Atlantic Gyre and Indian South Subtropical Gyre (Fernández-Castro et al. 2015). The nitrogen fixed by DDAs may be an important source of nutrients to other, non-diazotrophic planktonic groups. Villareal (1990) reported evidence of release of newly fixed N to the environment in *Rhizosolenia-Richelia* symbiosis under culture conditions. Due to their size and aggregation capability, diatoms sink rapidly. Therefore, DDAs might account for an important part of the downward flux of carbon linked to new production (Scharek et al. 1999), representing an important link between nitrogen and carbon cycles in the ocean (Foster and O'Mullan 2008). Goebel et al. (2010) observed that UCYN-A was the second most abundant diazotrophic organism in tropical Atlantic waters. UCYN-A N_2 fixation was the highest among diazotrophic groups in both coastal and oligotrophic waters of the eastern North Atlantic (Turk et al. 2011). The widespread distribution of UCYN-A cells throughout the tropical and subtropical ocean observed by Cabello et al. (2016) indicates that the symbioses involving these unicellular cyanobacteria may have an important, and thus, far underestimated impact on the nitrogen cycle in these environments. This unicellular cyanobacteria-Prymnesiophyceae association may also be responsible for important contributions to vertical carbon fluxes.

11.3 Symbioses Between Phytoplankton and Heterotrophic Bacteria

11.3.1 Diversity and Dynamics of Microalgal-Bacterial Interactions

Interactions between phytoplankton and heterotrophic bacteria in marine environments are numerous, varied and often complex (Amin et al. 2012; Bell and Mitchell 1972; Ramanan et al. 2015). Some bacteria are loosely associated with algae, while others are associated more closely and colonize algal surfaces (Kaczmarska et al. 2005). Interactions range from obligate to facultative, as well as from mutualistic to

parasitic, and can be mediated by cell-to-cell attachment or through the release of allelopathic compounds (Doucette 1995; Geng and Belas 2010; Seyedsayamdost et al. 2011).

The development of molecular biology tools has facilitated the study of links between phytoplankton and bacteria in natural communities (Grossart et al. 2005; Rooney-Varga et al. 2005) and from culture collections (Abby et al. 2014; Green et al. 2004; Jasti et al. 2005; Sapp et al. 2007). A molecular survey of bacterial diversity from cultures of six diatom genera (*Ditylum*, *Thalassiosira*, *Asterionella*, *Chaetoceros*, *Leptocylindrus*, and *Coscinodiscus*) revealed distinct bacterial phylotypes associated with each genus. Alphaproteobacteria related to the genera *Sulfitobacter*, *Roseobacter*, *Ruegeria*, and *Erythrobacter*, members of the Bacteroidetes and to a lesser extent Betaproteobacteria were the most prominent bacteria in the diatom cultures examined (Schäfer et al. 2002). Of these, members of the *Roseobacter* clade are commonly found in natural assemblages with marine algae, and have been shown to increase in abundance during phytoplankton blooms (Allgaier et al. 2003; Buchan et al. 2014; Mayali et al. 2008). Several molecular microbial surveys using the 16S rRNA gene marker have shown that key bacterial phylogenetic groups such as Bacteroidetes and Alpha- and Gammaproteobacteria actively respond to the decay of algal blooms (Pinhassi and Hagstrom 2000; Pinhassi et al. 2004; Riemann et al. 2000). Succession of bacterial taxa was observed during a bloom of centric diatoms in the North Sea and their occurrence patterns were linked to their capacity to degrade algal-derived organic matter (Teeling et al. 2012). The final phase of the bloom favored the dominance of Bacteroidetes with *Ulvibacter* and *Formosa* during early and mid-stages of the decline, and *Polaribacter* in the final stages. The latter metagenomic analysis demonstrated that the bacterial response to coastal phytoplankton blooms was more dynamic than previously thought and consisted of a succession of different bacterial populations with distinct functional and transporter profiles.

11.3.2 Parasitic Interactions

Bacteria can control microalgal populations by inhibiting growth or by active lysis of algal cells. Reports of algicidal bacteria have mainly focused on bacteria acting against bloom forming algae known to produce toxins that can affect human health (Mayali and Azam 2004; Paul and Pohnert 2011). The most common algicidal bacteria belong to the Gammaproteobacteria (mainly the genera *Alteromonas* and *Pseudoalteromonas*) and the Bacteroidetes (mainly the genera *Cytophaga* and *Saprospira*) (Mayali and Azam 2004). The algicidal activity can be caused either by the release of dissolved algicidal compounds or by the lysis of microalgal cells after attachment. Only few compounds or enzymes responsible for the algicidal effect have been identified. Different levels of specificity have been reported from algicidal bacteria. Selective activity against one algal species and universal activity against all tested species in a given taxon have been reported as well as all

intermediate forms of specificity (Mayali and Azam 2004). Several studies indicate that some algicidal bacteria can kill their algal prey by releasing proteases (Lee et al. 2000; Paul and Pohnert 2011). Other algicidal bacteria directly attach to the microalgal cells in order to lyse them (Furusawa et al. 2003).

11.3.3 Mutualistic Interactions

Mutualistic partnerships between bacteria and marine microalgae based on the exchange of metabolites and nutrients are common (see Cooper and Smith 2015 for a recent review). Identifying chemical compounds involved in these trophic interactions between bacteria and phytoplankton is essential for our understanding of marine elemental cycles. Amin et al. (2009) found that several clades of the gammaproteobacterial genus *Marinobacter* provide an enhanced supply of Fe(III) to the dinoflagellate *Scripsiella trochoidea*, and, in return, the bacterium depends on organic matter produced by the alga. Durham et al. (2015) established a model microbial system in which the marine alphaproteobacterium *Ruegeria pomeroyi* had an obligate trophic dependency on the diatom *Thalassiosira pseudonana* for carbon while the diatom obtained vitamin B_{12} from the bacterium. A transcriptional analysis of cocultures of *T. pseudonana* and *R. pomeroyi* using RNA-seq revealed that many transcripts up-regulated in *R. pomeroyi* were involved in the transport and metabolism of 2,3-dihydroxypropane-1-sulfonate (DHPS), a sulfur compound produced by the diatom with no currently recognized role in marine microbial food webs, but which, like dimethylsulfoniopropionate (DMSP), is produced in large amounts by many marine algae. Amin et al. (2015) combined transcriptomic analysis with microbiological and biochemical experiments to study the mutualistic interactions between the coastal diatom *Pseudonitzschia multiseries* and its associated bacteria. Among 49 bacterial strains isolated from *P. multiseries* cultures, members of the genus *Sulfitobacter* (Rhodobacterales) had the largest positive effect on the growth of the alga. A *Sulfitobacter* species promoted diatom cell division via secretion of the auxin indole-3-acetic acid (IAA), while this bacterium used both diatom-secreted and endogenous tryptophan. This study also detected levels of IAA in five coastal North Pacific sites equivalent to that found in laboratory cocultures and presented transcriptomic evidence from natural samples for multiple IAA biosynthesis pathways. Amin et al. (2015) proposed that tryptophan and IAA are signaling molecules to recognize and sustain beneficial partners. Another study of *Phaeobacter inhibens* BS107, a member of the *Roseobacter* clade, and *Emiliania huxleyi*, a dominant marine phytoplankton found in large algal blooms, revealed that interaction between algae and *Roseobacter* could be mutualistic, antagonistic, or shift between both (Seyedsayamdost et al. 2011). The bacterium initially provided a growth enhancing effect by producing an auxin and an antibiotic that protected the alga from other bacteria. This mutualistic relationship shifted to a pathogenic relationship when the algal senescence signal p-coumaric acid released by aging *E. huxleyi* cells elicited the production by the bacterium of algicidal

compounds termed roseobacticides that increase cell death of *E. huxleyi*. A similar effect was also observed in co-cultures of the dinoflagellate *Prorocentrum minimum* and *Dinoroseobacter shibae*, suggesting that a shift from mutualism to parasitism is a common feature in *Rhodobacterales*-based symbiosis (Wang et al. 2014).

11.4 Mutualistic Photosymbioses Between Eukaryotes

In pelagic environments, photosymbiotic interactions between eukaryotes include relationships that involve microalgae with other protists or with metazoans. Often assumed to be mutually beneficial or commensal because of the presumed trophic exchanges and recycling of nutrients between the host and symbionts, the exact nature of the partnership is often difficult to formally demonstrate. Eukaryotic epibionts (i.e., cells living on the surface of other organisms) are common in benthic environments and are also encountered in pelagic ecosystems, such as the association between the centric diatom *Thalassiosira* sp. and the coccolithophore *Reticulofenestra sessilis* (Decelle et al. 2015; Taylor 1982). However, planktonic photosymbioses between eukaryotes most often involve a photosynthetic symbiont that lives intracellularly within a heterotrophic host (Anderson 2012; Decelle et al. 2015). The most common host taxa in marine plankton are Radiolaria, Foraminifera, ciliates and dinoflagellates (Stoecker et al. 2009). Microalgal symbionts, often collectively referred to as "zooxanthellae," have long been thought to all be rather similar, but recent studies have revealed more diversity in this group.

11.4.1 Radiolarian Hosts

Based on current knowledge, Radiolaria is the most diverse group of planktonic hosts harboring eukaryotic microalgal symbionts. All main radiolarian lineages (Spumellaria, Collodaria, Nassellaria, Acantharia) include numerous species harboring obligate eukaryotic microalgal symbionts (Suzuki and Not 2015). It is assumed that these symbiotic species have to specifically acquire their symbionts from the environment at each host generation (i.e., horizontal transmission). In the Spumellaria, Collodaria, and Nassellaria, the most commonly occurring symbiont appears to be the dinoflagellate *Brandtodinium nutricula* that was first described (as *Zooxanthella nutricula*) over a century ago (Brandt 1881), but which was only recently cultured and morphologically characterized, leading to placement in the new genus *Brandtodinium* (Probert et al. 2014). The exact identity of the microalgal symbionts of the main monophyletic clade of symbiotic Acantharia was recently revealed to be members of the well-known haptophyte genus *Phaeocystis* (Decelle et al. 2012). In apparent contrast to the symbionts of other radiolarians, based on phylogenies performed on the 18S rRNA and D1–D2 region of the 28S rRNA gene sequences, acantharian symbionts have the exact same genetic identity as species

that are abundant in the plankton in their free-living stage, and display a lack of species-level host specificity (e.g., symbiont geography rather than host taxonomy is the main determinant of the association). *Acanthochiasma*, an early branching clade of Acantharia, has been found to simultaneously harbor multiple symbiotic microalgae, including distantly related dinoflagellates (*Heterocapsa* sp., *Pelagodinium* sp., *Azadinium* sp., and *Scrippsiella* sp.) as well as a haptophyte (*Chrysochromulina* sp.) (Decelle et al. 2013).

Acantharia is widely distributed throughout the world's ocean and typically outnumber planktonic Foraminifera and other Radiolaria in oligotrophic open ocean waters. Environmental molecular diversity surveys of protistan communities in pelagic ecosystems have demonstrated the ubiquitous occurrence of radiolarian sequences and notably those of Collodaria (de Vargas et al. 2015; Not et al. 2009). The Collodaria are large, fragile, colony-forming Radiolaria that have been estimated, using in situ imaging tools, to contribute significantly to total oceanic carbon standing stock in the upper 200 m of the water column (Biard et al. 2016). Along with other heterotrophic protists harboring microalgal endosymbionts, their predominance in surface waters of the intertropical ocean is likely linked to their photosymbiotic character, illustrating the significance of acquired phototrophy for global marine ecology (Stoecker et al. 2009).

11.4.2 Foraminiferal Hosts

Only 5 of the nearly 50 species of planktonic Foraminifera described to date harbor microalgal symbionts, yet these five species correspond to 50–90 % of Foraminifera individuals found in surface waters of the tropical and subtropical ocean (Caron et al. 1995; Stoecker et al. 1996). Each host cell can contain up to 20,000 symbionts. These five species, belonging to the genera *Globigerinoides*, *Globigerinella*, and *Orbulina*, form a monophyletic clade within the Foraminifera based on 18S rRNA gene phylogenies and they all possess spines along which symbionts are positioned during the day (Spero 1987). In contrast to benthic Foraminifera that have a wide diversity of microalgal symbionts, all planktonic symbiotic species form associations with the recently described dinoflagellate genus *Pelagodinium* (Siano et al. 2010), which is related to *Symbiodinium* in the order Suessiales. Other microalgal symbionts belonging to the haptophyte genus *Chrysochromulina* have been reported (Gast et al. 2000), but this relationship is less well characterized.

11.4.3 Ciliate Hosts

Symbiotic associations between ciliates and eukaryotic microalgae (e.g., *Paramecium bursaria* and *Chlorella* sp.) are well known and abundant in

freshwater ecosystems (Kodama et al. 2014). In marine environments ciliates preferentially associate with cyanobionts (e.g., *Codonella* sp.) or perform kleptoplastidy (retention of plastids only rather than the whole cell) such as the well-known *Mesodinium rubrum* which sequesters plastids from a cryptophyte algae and can form massive blooms (Johnson and Stoecker 2005), or Oligotrichida ciliates which harbor klepto-chloroplasts from green algae in estuarine environments (Stoecker et al. 1989a). An original pelagic photosymbiosis between a calcifying ciliate host and the dinoflagellate *Symbiodinium* was recently described from surface ocean waters (Mordret et al. 2015). The host is a new ciliate species closely related to *Tiarina fusus* (Colepidae) and phylogenetic analysis of the symbionts revealed that they are novel genotypes of *Symbiodinium*, closely related to clade A, that do not seem to associate with any benthic host. Based on molecular diversity surveys, this symbiotic partnership occurs globally, in particular in nutrient-poor surface waters.

11.4.4 Dinoflagellate Hosts

Photosynthetic dinoflagellates can be symbionts of other large protists (e.g., Foraminifera or Radiolaria), but heterotrophic dinoflagellates can also harbor photosynthetic symbionts. These symbionts are mainly cyanobionts (see above), but in some cases can be eukaryotic microalgae. For instance, the genus *Amphisolenia* has been described to simultaneously harbor both cyanobionts and pelagophyte microalgae (Daugbjerg et al. 2013). The bioluminescent dinoflagellate species *Noctiluca scintillans* lives in symbiosis with a green prasinophyte alga, described from its morphology as *Pedinomonas noctilucae* (Sweeney 1976), and can harbor up to 10,000 symbionts that swim freely within large vacuoles in the host cell. The *Noctiluca–Pedinomonas* association is common in tropical and subtropical areas of southeast Asia, in the Indian Ocean, the Pacific Ocean, and the Red Sea where it regularly forms extensive blooms (called "green tides") reaching densities of up to 5×10^6 cells L^{-1} (Harrison et al. 2011). Diatoms ("dinotoms") and other symbionts of uncertain affiliation can be found in symbiosis with dinoflagellate hosts, but these are less well described (Imanian et al. 2010).

11.4.5 Metazoan Hosts

Endosymbiotic microalgae are also found in association with large multicellular metazoan plankton, such as jellyfish and acoel flatworms. Among the most studied jellyfish, the scyphozoan *Cassiopea* has been described in symbiosis with the dinoflagellate *Symbiodinium microadriaticum*, but the specificity of the relationships between host and symbiont are currently unclear as morphological, biochemical, and physiological differences between strains cultured from different

hosts have been observed (Arai 1997). Other dinoflagellates, namely *Gymnodinium linuchae* and *Scrippsiella vellelae*, have been isolated and described from the scyphozoan *Linuche unguiculata* and the hydrozoan *Vellela vellela*, respectively (Trench and Thinh 2007). *S. vellelae* was redefined as *B. nutricula* and is the same symbiont found in the majority of radiolarians (Probert et al. 2014). All species of pelagic acoel flatworms collected over a 13 year sampling effort in surface waters of the open oceans harbor microalgal endosymbionts (Stoecker et al. 1989b). From this latter study, three types of oceanic flatworms were discriminated: a "bright green" and a "dark brown" acoel presumably belonging to the host genus *Convoluta* and both harboring a green prasinophyte-like symbiont identified based on ultrastructure. The "dark brown" flatworm was mostly observed on the surface of colonial radiolarians and other gelatinous plankton. The third type of acoel was referred to as "golden" and harbors a dinoflagellate symbiont of uncertain taxonomic affiliation. Dinoflagellate endosymbionts of the pelagic acoel *Amphiscolops* sp. were identified as belonging to the genus *Amphidinium* (Lopes and Silveira 1994). Acoel flatworms with algal endosymbionts depend on both autotrophic and heterotrophic nutrition and are a widespread, though sporadic component of the plankton in the upper water column in warm, oceanic waters. Jellyfish harboring photosymbionts are frequently observed in the environment and besides a trophic relationship that is presumably advantageous in oligotrophic environments (i.e., recycling of nutrients between the host and symbionts), the exact role of the photosymbiosis is not well understood. It has been suggested that symbiosis enhances the rate of strobilation, being potentially involved in the host cell cycle (Arai 1997).

11.5 Parasitic Photosymbioses Between Eukaryotes

Parasitism is a non-mutual symbiotic relationship that can be neutral to lethal (i.e., never beneficial) for the host. The parasite has an obligate physical association with its host, at least during a part of its life cycle. Parasitic photosymbioses include heterotrophic protists infecting microalgae and microalgae infecting larger animals. All marine protists involved in parasitic photosymbioses have a similar life cycle that typically includes three stages which allow the parasite to fulfill three essential functions: infection of the host via an actively swimming zoospore, acquisition of energy via a feeding stage (the trophont), and sporulation by a sporocyst which produce zoospores used for propagation (i.e., they are all zoosporic parasites). These parasites are thus horizontally transmitted, meaning that the host is newly infected from the surrounding environment at each generation.

All of these parasites can be classified based on their impact on their host, their localization on their host and their mode of transmission (Lafferty and Kuris 2002, Poulin 2011). Parasites infecting microalgae generally kill their host and these highly virulent parasites are called parasitoids. The impact of microalgal parasites infecting larger animals is generally lower. Beside their negative impact on host

populations, zoospores of parasites are actively grazed by predators and should also be considered as an important trophic resource in marine pelagic systems.

11.5.1 Heterotrophic Parasites Infecting Microalgae

All known protistan parasites of marine microalgae infect either diatoms or dinoflagellates. Protistan parasites infecting other important marine microalgal lineages exist in freshwater, e.g., the perkinsoan *Rastrimonas subtilis* infecting the cryptophyte *Chilomonas paramecium* (Brugerolle 2002), but have never been reported in marine habitats. Parasites of marine diatoms include chytrids, aphelids, stramenopiles (including the genus *Pirsonia*, oomycetes, labyrinthuloids, and hyphochytrids), dinoflagellates, cercozoans, and phytomyxids (for a review see Scholz et al. 2016). Parasites of dinoflagellates include chytrids (different from those infecting diatoms), Syndiniales (Amoebophryidae) and Perkinsozoa (for a review see Jephcott et al. 2015). The trophont of these parasites develops either outside (ectoparasites) or inside (endoparasites) their host. Ectoparasites may partially penetrate inside the host (part of the cytoplasm, and even the mitochondrion, Lepelletier et al. 2014a), but the nucleus always remains outside the host. Ectoparasites of microalgae use different strategies to penetrate their host. Fungi produce a germ tube that penetrates into the dinoflagellate host through gaps between thecal plates (Lepelletier et al. 2014a, b). Most ectoparasites of microalgae, however, are active phagotrophs and feed either by endocytosis, pinocytosis or phagocytosis. The heterokont *Pirsonia* spp. and the cercozoans *Pseudopirsonia mucosa* and *Cryothecomonas longipes* infect diatoms using a pseudopodium-like cytoplasmic strand that either pierces the diatom frustule, generally in the girdle region, or passes through natural orifices of centric diatoms (Schnepf and Kuhn 2000; Schweikert and Schnepf 1997). *Paulsenella* is a dinoflagellate ectoparasite of diatoms that pierces the host plasmalemma by a feeding tube (called the pedoncule) and gradually sucked out the host cytoplasm, resembling drinking through a straw. The prey's cytoplasm is deposited in a food vacuole where it is digested. This mode of endocytosis (called myzocytosis, Schnepf and Deichgräber 1984) is a feeding mode exclusively observed in alveolate parasitoids.

When the nucleus of the parasite enters inside the host, the parasite is considered as an endoparasite. There are several advantages to being an endoparasite. First, the endoparasite remains protected by the external envelope of its host during its whole maturation. Second, an endoparasite more efficiently exploits its host than an ectoparasite. While in the case of ectoparasites, the host nucleus and enough of the cytoplasm can be left to allow the host to survive the infection (Kühn et al. 1996; Schnepf and Melkonian 1990; Schweikert and Schnepf 1997), endoparasites always kill their host and can digest them entirely, including the nucleus. However, endoparasites need to overcome two major difficulties: bypassing the natural defenses of the host to enter the cell and finding a way to leave the cell after maturation. Different strategies are used by endoparasites to enter and develop

safely inside their host. Like ectoparasites, the aphelid *Pseudaphelidium drebessi* (Karpov et al. 2014) and the cercozoan *Cryothecomonas aestivalis* (Drebes et al. 1996) produce a pseudopodium-like structure and squeeze into the interior of the diatom frustule. Inside the diatom frustule, these parasites are in intimate contact with the host plasma membrane, but never pierce it (Schweikert and Schnepf 1997). Apicomplexans and relatives (Syndiniales and Perkinsozoa) use an apical complex derived structure to penetrate their host (Leander and Keeling 2003). The host of *Parvilucifera* spp. is rapidly immobilized at the penetration of the parasite that then feeds by osmotrophy by producing external enzymes that totally digest its host. In contrast, *Amoebophrya ceratii* is an endoparasitic phagotroph that preserves its host alive (swimming in the water column) until the very end of the maturation process.

The intracellular trophont often distorts the host cell. Dinoflagellates infected by *A. ceratii* become much larger than healthy cells (Hanic et al. 2009; Kim et al. 2004). For sporulation, *C. aestivalis* forms slightly amoeboid flagellate spores that are discharged by slipping with their posterior pole foremost through the diatom frustule (Drebes et al. 1996). Oomycetes and chytrids infecting the diatom *Pseudo-nitzschia pungens* (Hanic et al. 2009) produce similarly shaped discharge tubes through the host cell wall. In *A. ceratii* the sporocyst makes a complex evagination to leave its host (Cachon 1964) and once outside the cell becomes an elongated multicellular flagellated structure (the vermiform stage). Within hours, each cell forming this vermiform is released from this multicellular structure and is available to infect a novel host.

Several parasites are known to produce resting stages, e.g., *Pirsonia* spp. (Drebes and Schnepf 1988; Kühn et al. 1996), aphelids (Karpov et al. 2014) or *C. aestivalis* (Drebes et al. 1996). Whether these resting stages are the result of a sexual reproduction is unknown. Mature zoospores will be released via several opercules that will be opened possibly after activation by water-borne signals (Garcés et al. 2013). *A. ceratii* may enter into dormancy in its host resting cyst and new infections are initiated after germination of the host cyst (Chambouvet et al. 2011a). This strategy allows a perfect physical coupling in time and space of the parasite with its host.

11.5.2 Microalgal Parasites Infecting Larger Organisms

Microalgae may be obligatory and/or facultative parasites of marine metazoans in benthic and pelagic ecosystems (Rodriguez et al. 2008). Members of the dinoflagellate genus *Blastodinium* are endocommensal of copepods (Skovgaard et al. 2012). They are directly ingested by their hosts as food particles and once inside the copepod gut, they develop and produce one to several large (several hundreds of microns) trophont or sporocyst individuals surrounded by the same outer mother membrane. Infected copepods are generally smaller, less fit, and less fecund than healthy ones as a consequence of a physical blocking of the alimentary tract in the gut of the animal and competition for food uptake.

11.5.3 Detection of Parasites in Environmental Genetic Surveys

In molecular surveys of plankton diversity, Amoebophryidae, also known as Marine ALVeolate group II or MALV II (López-García et al. 2001), always represent a large fraction of the sequences retrieved from marine habitats, from surface waters to deep hydrothermal vents, but have never been found in freshwater. This family includes a single genus, *Amoebophrya*, with seven species, all described by Cachon (1964). Environmental surveys based on sequencing the SSU rRNA gene have revealed an impressive diversity within this lineage, including more than 40 genetic clusters identified, most composed of several distinct subclusters that potentially correspond to separate species (Guillou et al. 2008). All *Amoebophrya* are parasites, infecting either radiolarians (*A. acanthometrae* and *A. sticholonchae*), ciliates (*A. tintinni*), dinoflagellates (*A. ceratii* and *A. leptodisci*) or other parasites (i.e., the hyperparasites *A. grassei* and *A. rosei*). *A. ceratii* infects most, if not all, phototrophic dinoflagellates (Cachon 1964; Siano et al. 2011). This wide host range may explain the ecological success of this parasitic group. MALV II are predominantly detected in the smallest picoplanktonic size fractions by metabarcoding (Massana et al. 2015) and from environmental DNA rather than RNA (Massana et al. 2015; Not et al. 2009). Environmental genetic surveys are likely to detect preferentially zoospores, which are actively swimming propagules that do not reproduce mitotically. Their viability in marine water is at most a few days, even in culture (Cachon 1964; Coats and Park 2002). One infection potentially releases hundreds of zoospores that, like spermatozoids, have a high nucleus/cytoplasm ratio. The genetic trace of these zoospores is likely detectable long after they die as a part of free environmental genetic material. Not all marine parasites are preferentially detected in the smallest size fractions. *Blastodinium* spp. environmental sequences are more prevalent within the mesoplanktonic fraction (180–2000 μm; de Vargas et al. 2015), leading to the conclusion that these parasites are mainly detected within their hosts. Less destructive methods, such as Fluorescent In Situ Hybridization (FISH) techniques, have been used to confirm that infections of dinoflagellates by *A. ceratii* and of copepods by *Blastodinium* spp. occur from coastal environments to the most oligotrophic areas of the planet (Alves-de-Souza et al. 2011; Siano et al. 2011).

11.6 Methods for Studying Pelagic Symbioses

11.6.1 Microscopy and Related Approaches

Marine pelagic photosymbiotic associations were first discovered soon after light microscopes started to become widely available in the latter half of the 19th century. Only two years after de Bary (1879) coined the term symbiosis, K. Brandt

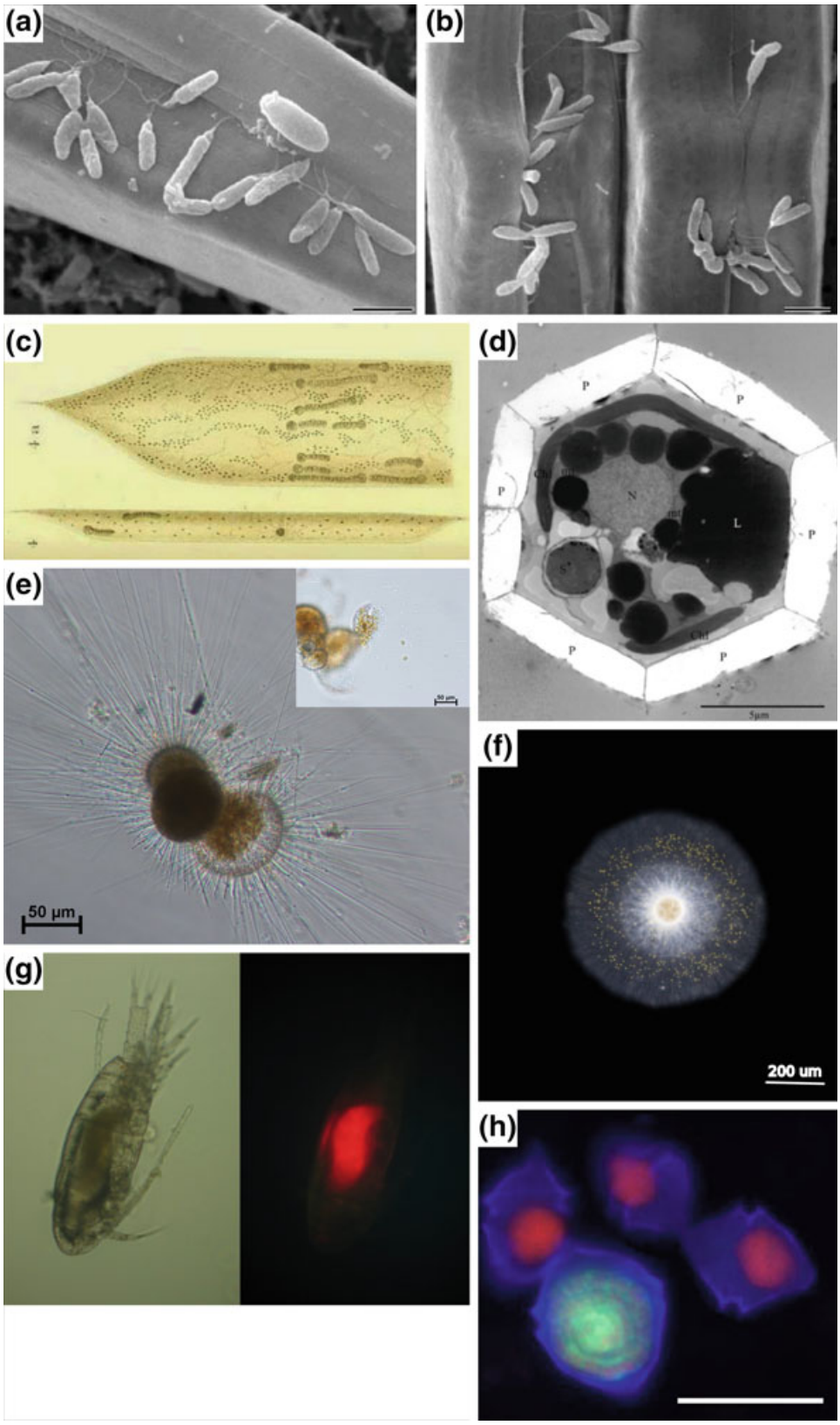
used light microscopy to recognize that the "yellow cells" inside radiolarians, actinian corals, and hydrozoans were in fact symbiotic microalgae (Brandt 1881). Symbiotic associations between diatoms and cyanobacteria were first described from light microscope observations by Karsten (1907). In the second half of the 20th century, increasingly sophisticated microscopy-related methods were employed to discover and describe pelagic symbiotic relationships. The development of transmission electron microscopy in the 1960s allowed more precise localization and sometimes taxonomic identification of symbionts inside host cells (e.g., Hollande and Carré 1974; Taylor 1971). Characterization by electron microscopy of the morphology and/or ultrastructure of symbionts either within the host cell '*in hospite*' (Hagino et al. 2013; Miller et al. 2012; Yuasa et al. 2012) or outside, '*ex hospite*' or 'free living' (Probert et al. 2014), remains central to the study of these associations. When coupled with immuno-labeling, electron microscopy allows precise intracellular localization within host and/or symbiont cells of specific proteins such as nitrogenase (Foster et al. 2006). From the 1980s onwards, epifluorescence microscopy has been widely used to assess the type of pigment present in the symbionts (and thus general taxonomic assignation). For example, fluorescence microscopy permits easy distinction of chlorophyll-containing eukaryotes from phycoerythrin-containing cyanobacteria (Stoecker et al. 1987) or Syndiniales parasites within dinoflagellates based on their specific green fluorescence (Chambouvet et al. 2011a). The subsequent development of molecular probes coupled with fluorescent labels (FISH) using amplification approaches, such as Tyramide Signal Amplification (TSA), necessary in many cases because of the low ribosomal signal of the symbionts or parasites compared to their hosts, allows determination of the taxonomical affiliation of hosts and/or symbionts (e.g., Biegala et al. 2002; Cabello et al. 2016; Chambouvet et al. 2008). Nanoscale secondary ion mass spectrometry (Nano-SIMS; Musat et al. 2012) is a powerful emerging technique which allows assessment of the cellular localization and metabolic fluxes of compounds such as nitrogen or carbon (Foster et al. 2011; Thompson et al. 2012). Flow cytometry allows characterization and physical separation of cells based on their size and fluorescence and has been used, for example, to sort small eukaryotes associated to nitrogen-fixing cyanobacteria in order to determine their taxonomic affiliation (Thompson et al. 2012) or to study pico- and nano-phytoplankton associations with fungi (Lepère et al. 2015).

11.6.2 Ex Situ Laboratory Culture

The successful maintenance of planktonic organisms in ex situ laboratory culture greatly facilitates in depth morphological, genetic and physiological studies. Culturing of organisms that are capable of living in isolation from other species is a challenge in itself (Stewart 2012). The co-culture of organisms involved in symbiotic associations tends to be even more complex. For pelagic photosymbioses, most success to date has come from separating the partners and culturing one

(or more rarely both) as a free-living organism. For mutualistic symbioses involving unicellular photosynthetic organisms as symbionts, separation of the partners by manual micropipetting has been increasingly successful (e.g. Decelle et al. 2012; Probert et al. 2014; Siano et al. 2010). This method involves disintegration (crushing) of the host cell with a micropipette under an inverted microscope to release the symbionts, which are subsequently individually isolated into an appropriate culture medium in which they develop as a culture of the free-living form. Disintegration methods are often used to release microalgal symbionts from corals and anemones, but induction of the release of symbionts by physical (e.g. heat, light, or salinity shock) and/or chemical (e.g. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or menthol) treatment has also been reported for these benthic metazoan hosts (Wang et al. 2012). The viability of the symbionts released using these physical or chemical treatments may vary for a given method between host species (Wang et al. 2012). The fact that microalgal symbionts are typically maintained within host cells in a simplified ('protoplasmic') state with considerably altered phenotype (for example, lack of flagella and/or theca, abnormally large cell size) facilitates single-cell isolation once the cells are released, but can mean that cells are more prone to deleterious physicochemical shock when released from the host. There is undoubtedly considerable scope for better mimicking the physico-chemical environment within the host in order to increase the success of isolation of symbionts, particularly for those with specific physiological capacities such as N_2-fixation. It has been possible to maintain the N_2-fixing cyanobacterial symbiont *Calothrix* in culture outside its host diatom *Chaetoceros* (Foster et al. 2010), but *Richelia* can only be maintained within its host. Neither the host diatom *Rhizosolenia* (Villareal 1990) or the haptophyte hosts of UCYN-A N_2-fixing cyanobacterial symbiont have been cultivated to date (Fig. 11.1).

Co-culturing of host and symbiont (the 'holobiont') is required to conduct experiments to develop a mechanistic understanding of the functioning of symbiotic relationships. Many symbiotic coral species can be maintained in culture for long periods of time with colonies growing by asexual reproduction. However, mastering the sexual reproduction of corals in ex situ culture has proven much more difficult, particularly for species that have separate mating types and that expel (rather than brood) their gametes. Some pelagic organisms harboring microalgal symbionts can be maintained for short periods (up to a few weeks) in laboratory conditions, particularly if kept in circulating seawater aquarium systems that maintain them in suspension. However, the almost total lack of knowledge about asexual and sexual reproduction processes of host organisms from the pelagic environment has prevented successful long-term culture of these organisms. Most heterotrophic hosts are known to undergo sexual reproduction and release aposymbiotic (i.e., without symbiont) gametes at some point in their life cycle, but nothing is known about the processes of gamete recognition, fusion, and subsequent establishment of a daughter generation that would need to reacquire symbionts from the environment (horizontal transmission). The culture of pelagic photosymbiotic holobionts will require considerable advances not only in the technology of culture



◀ **Fig. 11.1** Illustrations of pelagic photosymbioses. **a** and **b** Scanning electron microscopy images of heterotrophic bacteria associated to the diatom *Pseudonitzschia multiseries* (from Kaczmarska et al. 2005, scale bars = 1 µm). **c** Symbiosis between the cyanobacterium *Richelia* and the diatom *Rhizosolenia* drawn from microscopic observations (Karsten 1907). **d** TEM images of the prymnesiophyte algae *Braarudosphaera bigelowii* showing nucleus (N), chloroplasts (Chl), lipid globules (L), pentaliths (P), mitochondria (mt) and cyanobacterial symbiont (S) (from Hagino et al. 2013, under CC BY license). **e** Planktonic Foraminifera in association with its dinoflagellate symbiont *Pelagodinium beii*, insert shows the Foraminifera test broken and symbiotic algae released (small golden dots). **f** One large Radiolaria cell (Collodaria), displaying its dinoflagellate symbionts (*Brandtodinium nutricula*) on the outer part (numerous small golden dots). **g** Left, optical microscopy image of a copepode (*Clausocalanus* type) infected by the microalgae *Blastodinium contortum*, and right, same specimen observed under epifluorescence showing the chlorophyll autofluorescence (*red*) of its algal parasitic endosymbiont. **h** The dinoflagellate species *Heterocapsa triquetra* infected and noninfected cells from a natural sample collected in the Penzé estuary, France. The parasites is detected by a FISH using the ALV01 probe (*green*), the host parasite is stained in red by propidium iodine and the host theca stained in blue by calcofluor (photo credit. C. Alves-de-souza, scale bar: 20 µm)

systems, but also in knowledge of the undoubtedly complex life cycles of these organisms.

The majority of parasites cannot be maintained in culture without their host. Generalist parasites (infecting a large range of hosts) are typically much easier to isolate than specialist parasites (having a narrow host range). For specialists, it is recommended to first establish the host strain in culture from the locality where the parasite will be isolated. The main bottleneck for their cultivation remains the labor intensiveness of their maintenance, as parasites of phytoplankton typically have rapid life cycles and have to be regularly transferred into a fresh host culture (as frequently as twice per week). Some parasites of microalgae, such as *Parvilucifera* spp., can be stored for longer periods at 4 °C and/or cryopreserved (Lepelletier et al. 2014a, b).

11.6.3 Molecular Approaches

The introduction of molecular techniques into plankton research has allowed much better characterization of the nature and diversity of hosts as well as symbionts using marker 'barcode' genes such as 18S or 16S rRNA (e.g., Chambouvet et al. 2011b; Decelle et al. 2012; Thompson et al. 2014) or functional genes linked to the key role of the symbiont such as *nifH* or *hetR* involved in N_2-fixation (Foster and Zehr 2006). In light of the difficulty of culturing pelagic photosymbiotic associations, one big advantage of molecular techniques is that they can usually be employed in culture-independent studies. In recent years, new "omics" approaches (genomics, transcriptomics, and their meta- declinations when dealing with uncultured organisms) have increasingly been employed to study the nature of symbiotic relationships. For example, determination from flow cytometry sorted cells of the genome sequence of the symbiotic cyanobacterium UCYN-A

highlighted the absence of photosystem II in this organism and therefore its inability to fix carbon (Zehr et al. 2008) for which it has to rely on its host (Thompson et al. 2012). Genome sequencing also revealed that *Richelia*, a cyanobacterial symbiont of diatoms, lacks key N metabolism genes (Hilton et al. 2013). Transcriptomic approaches are currently more accessible than full genome sequencing for eukaryotes and these have been used, for example, to identify genes potentially involved in symbiosis or parasitic attack such as those coding for lectins (Balzano et al. 2015; Lu et al. 2014).

Interactions between marine protists and bacteria have been demonstrated using single-cell sorting by flow cytometry and further sequencing SSU rRNA genes of the individual protist and the bacteria physically associated with it (Martinez-Garcia et al. 2012). In particular, the latter pilot study suggested the discovery of novel symbionts, distantly related to Rickettsiales and the candidate divisions ZB3 and TG2, associated with cercozoan, and chrysophyte hosts. Although further studies are required to unequivocally determine whether these newly discovered associations represent parasitic or mutualistic relationships, single-cell sequencing is a promising approach for the analysis of ecological interactions between uncultured protists and bacteria.

11.7 Concluding Remarks

Only in recent years scientists have started to realize the full extent of the critical roles and services provided by symbioses across ecosystems and scales, from molecular to ecological (McFall-Ngai 2008). It has long been recognized that symbiotic interactions exist in the marine pelagic environment, but the pace of discovery has increased in recent years through the application of both classical techniques and novel methodologies such as high-throughput sequencing associated to bioinformatic analysis of interaction networks (Guidi et al. 2016; Lima-Mendez et al. 2015; Thompson et al. 2012; Worden et al. 2015). Several new, ecologically important pelagic photosymbiotic associations have been discovered and at least partially characterized and it would not be surprising to see this trend continue and even intensify in the near future. It is clear that future studies aiming to model nutrient and energy budgets in the ocean must take into account the importance of pelagic symbiotic associations for the input of new nitrogen, as well as for the downward flux of carbon in the water column.

In order to progress toward a holistic understanding of the marine microbiome (Dubilier et al. 2015), it is important to further complement descriptive studies of the nature of photosymbiotic interactions with understanding of the physiological and molecular mechanisms involved. Despite promising developments in culture-independent methods (e.g., single-cell approaches), ex situ culturing, and experimentation remains a critical step to comprehensively understand any biological system. The establishment of new, ecologically relevant, culturable

biological model systems to study pelagic photosymbioses is one of the main challenges facing researchers in this field in coming years.

Acknowledgments The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement n° 311975. This publication reflects the views only of the author, and the European Union cannot be held responsible for any use which may be made of the information contained therein. The authors also acknowledge the ANR HAPAR (ANR-14-CE02-0007), the ANR IMPEKAB (ANR-15-CE02-0011), the CNRS GDRI "Diversity, Evolution and Biotechnology of Marine Algae", the CAPES-COFECUB "PicoBras" project (Te 871-15), and the MICROMAR project funded by a CNRS grant (INSU-EC2CO program). Klervi Crenn received a doctoral grant half funded by Région Bretagne.

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