Zooxanthellae (*Symbiodinium*, Dinophyceae) symbioses on coral reefs

The large three-dimensional structures that make up coral reefs (Figure 1) are primarily the product of calcium carbonate deposition by zooxanthellate scleractinian corals, i.e., stony corals living in symbiosis with dinoflagellate algae of the genus *Symbiodinium* (a.k.a. zooxanthellae) (Figure 2). This photosymbiosis permits fast nutrient cycling in the generally oligotrophic tropical waters.

The zooxanthellae live inside the gastrodermal cells of the coral, translocate photosynthate to their coral host and increase rates of skeletal growth. In this way, a large part of the coral’s energy requirements are met through zooxanthellae photosynthesis. The zooxanthellae receive several essential nutrients from the host and in some cases also protection from external conditions such as damaging light intensities. The coral-*Symbiodinium* symbiosis is obligate; corals will starve and may die if prolonged disruption of the symbiosis occurs. Disruption of the symbiosis is usually expressed as the loss of the single-celled photosymbionts and/or their photosynthetic pigments, and is a common stress response of corals.
Over the past few decades, this phenomenon—called coral bleaching—has been more prominent than before, a pattern which is strongly correlated to increasing sea surface temperatures (SSTs) as a consequence of climate change. These mass bleaching events have caused the loss of a significant portion of the world’s coral reefs. Further, a recent study suggests that ocean acidification caused by high atmospheric CO2 levels leads to coral bleaching under high irradiance conditions, and acts synergistically with high temperature to lower thermal bleaching thresholds. Ocean acidification also reduces coral growth, at least in massive corals. Hence, the increased greenhouse gas levels in our atmosphere stemming from human activity and some jellyfish also host zooxanthellae. In addition, non-cnidarian coral reef invertebrates and a few protists form symbioses with members of the genus Symbiodinium. However, these symbioses have received far less attention and their sensitivity to increased CO2 and temperature are not as well understood.

**Symbiodinium diversity**

Initially, it was believed that the genus Symbiodinium is represented by a single species, *Symbiodinium microadriaticum* Freudenthal. With the advent of molecular tools, however, it has become evident that the genus is unusually diverse and comprises at least eight divergent lineages (i.e. phylogenetic clades A-H), each associated with a range of zooxanthellae types, e.g. C1, B3, etc. Members of four of the eight clades (A-D) are most common with scleractinian corals, while members of clades F and G are rare and those of clades E and H have never been observed in these reef-builders. Indo-Pacific scleractinians mainly associate with Symbiodinium types belonging to clades C and D. In West Atlantic scleractinian corals, however, members of Symbiodinium B are most common, followed by C, A and D. It is becoming clear that much of the genetic diversity encompassed within the genus is matched by physiological diversity, and that the type of zooxanthellae harboured can shape the physiological performance of the coral colony, including its bleaching susceptibility.

**Climate change and coral bleaching**

Corals derive their deep brown colour from the zooxanthellae living within their tissues. Coral bleaching is a term used to describe the loss of the photosymbionts and/or their photosynthetic pigments, leading to a blanched and eventually white appearance of the coral as the calcium carbonate skeleton becomes visible through its now translucent tissues. It is well established that widespread or mass bleaching is caused by higher than usual temperatures in combination with high irradiance, and that bleaching is a response to elevated levels of reactive oxygen species (ROS) in the tissues. There are three key pathways in which temperature and light stress can result in net production of ROS: (1) through damage to the reaction centre protein D1 of photosystem II (PSII) of the zooxanthellae, (2) through the impairment of CO2 fixation mechanisms in the Calvin cycle downstream of PSII, and (3) through the thermal instability and disruption of the thylakoid membranes on which PSII (and PSI) is located. Some of the ROS produced in the photosymbiont diffuse into the host cell. High levels of ROS are also produced in the host mitochondria as a consequence of temperature/light-induced damage to the mitochondrial membrane (reviewed in [18]). It has recently been proposed that ROS activate the host transcription factor NF-kB, which in turn induces apoptosis of the Symbiodinium-containing host cell, either directly or indirectly via the induction of nitric oxide synthase. Nitric oxide can also be produced by the photosymbiont and diffuse into the host cell, thereby inducing the apoptotic pathway.

In addition to apoptosis, a range of other mechanisms have been observed to lead to loss of photosymbionts in cnidarians, and it is not yet clear which of these are the most prevalent during natural bleaching events. Whether different mechanisms occur with different stressors or extent of stress, or whether these occur sequentially or simultaneously and vary between taxa. These include in situ degradation of zooxanthellae, exocytosis, detachment of zooxanthellae-containing host cells, and host cell necrosis.

**Bleaching susceptibility and the role of the zooxanthellae**

Corals vary in their bleaching susceptibility, differences exist between species, but also among and within conspecific populations. In the case of the former, a range of host animal factors, such as tissue thickness and growth form, skeletal light scattering, anti-oxidant levels and photoprotective pigments may be responsible for the differences. In addition to host factors, exposure to high irradiance or high temperature prior to the heat stress during the summer season as well as the type of zooxanthellae harboured have been shown to play important roles in some coral-algal associations. For example, the coral *Acropora millepora* associates with a range of zooxanthellae types on the Great Barrier Reef, but those with types D and C1 are more bleaching tolerant, both in the laboratory and in the field.

Further, individual colonies that changed from dominance by type C2 (while harbouring type D at extremely low densities) to D increased their thermal tolerance limit, a process called symbiont shuffling. Sympatric colonies of the coral *Stylophora pistillata* hosting distinct clade C zooxanthellae types also show...
Symbiodinium associations with non-cnidarians

A range of non-cnidarian invertebrates and protists living on coral reefs host symbionts of the genus *Symbiodinium*. Within the molluscs there are the giant clams (e.g. *Tridacna* or *Hippopus* spp. 39) as well as some nudibranch species (e.g. *Phyllodesmium* spp. 39, 40) that house zooxanthellae. *Symbiodinium* can also be found in a small number of sponge species (Porifera 41), turbellarians (Plathelminthes 42) and Foraminifera 43, 44. Within these reef organisms the symbiosis is either facultative or obligate and symbionts are normally living intracellularly. In nudibranchs they are located in digestive glandular cells 45; in Foraminifera in the ecto- as well as in the endoplasm 46. In contrast, in bivalves like *Tridacna*, *Symbiodinium* is located in the lumen of digestive glandular ducts 39, whereas within the Turbellaria they can be found inside parenchymal cells as well as extracellularly 42. As with reef-building corals, the symbionts photosynthesise *in hospite*, exchange nutrients and gases with the host and reproduce asexually 45-51.

Bleaching has been described from *Tridacna* 52, 53, sponges 34 and foraminifers 35. However, these studies are of a limited nature and investigate very few species, with comparative studies investigating the impact of environmental stressors on these holobionts almost completely lacking. Arguably the best studied non-cnidarian invertebrate-algal symbiosis on coral reefs is that of clams (e.g. 39). Infection of larvae of *Hippopus* and *Tridacna* clams with homologous (from the same host species) or heterologous (from different host species) clonal *Symbiodinium* types followed by a grow-out period in the field, showed that growth and survival differed based on the zooxanthellae type offered 37. However, the symbiont populations were no longer clonal and had become genetically diverse at the end of the grow-out period, making these results somewhat difficult to interpret.

The potential sources of this symbiont genetic diversity in clam hosts include acquisition of new genotypes from the water column during filter feeding, mutation during clonal growth, and sexual reproduction 37. The high diversity of the zooxanthellae populations may be important in allowing the giant clam associations to acclimatise to changing environmental conditions, including variations in solar radiation, temperature, and nutrients. Thus there might be some potential for changing symbionts under bleaching conditions by switching (i.e. uptake of novel zooxanthellae types from the environment 52) or shuffling.

A less known group of solar-powered animals is that of the nudibranchs. Most solar-powered nudibranch species obtain *Symbiodinium* by feeding on corals (Figure 3); instead of digesting the zooxanthellae, the hosts keep the algae intact and photosynthetically active 46, 48, 49. Whether both hosts (coral and nudibranch) share the same symbiont diversity is still under investigation [Burghardt et al., unpublished data]. Using chlorophyll fluorescence data and histological analyses, it has been demonstrated that various species of *Phylodesmium* represent different stages in the evolution of zooxanthellae-nudibranch association. Within the genus there are species without symbionts, others with a short-term symbiotic relationship and a small number of species with a highly efficient symbiosis 56, 57. The efficiency of the symbiosis is correlated with certain morphological adaptations. A preliminary phylogeny of *Phylodesmium* confirms this hypothesis [Wägele et al., unpublished data].

**The way forward**

The recent release of cnidarian genome and partial cnidarian and *Symbiodinium* transcriptome sequences (reviewed in 61, 62) has allowed coral reef microbial symbiosis research to venture into new avenues. This includes the identification of cellular processes underlying the establishment and disruption of the symbiosis through gene expression research 63-66 and comparative genome

![Figure 3. The solar-powered nudibranch Phylodesmium rudmani (left), next to its xeniid coral food (right). The nudibranch mimics its coral host perfectly; the brownish colour in both organisms is due to the presence of zooxanthellae.](image-url)
analyses. Further, the close interaction between hosts and their endosymbionts is evident from two recent studies where lateral gene transfer occurred between microbial symbionts (both bacterial and eukaryotic) and their eukaryotic hosts. The next few years of genomic research are likely to provide a leap forward in our understanding of coral reef microbial symbioses and to reveal examples of lateral gene transfer between zooxanthellae and their invertebrate hosts.

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References


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