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Photosynthetic symbioses in animals

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Abstract

Animals acquire photosynthetically-fixed carbon by forming symbioses with algae and cyanobacteria. These associations are widespread in the phyla Porifera (sponges) and Cnidaria (corals, sea anemones etc.) but otherwise uncommon or absent from animal phyla. It is suggested that one factor contributing to the distribution of animal symbioses is the morphologically-simple body plan of the Porifera and Cnidaria with a large surface area:volume relationship well-suited to light capture by symbiotic algae in their tissues. Photosynthetic products are released from living symbiont cells to the animal host at substantial rates. Research with algal cells freshly isolated from the symbioses suggests that low molecular weight compounds (e.g. maltose, glycerol) are the major release products but further research is required to assess the relevance of these results to the algae in the intact symbiosis. Photosynthesis also poses risks for the animal because environmental perturbations, especially elevated temperature or irradiance, can lead to the production of reactive oxygen species, damage to membranes and proteins, and 'bleaching', including breakdown of the symbiosis. The contribution of non-photochemical quenching and membrane lipid composition of the algae to bleaching susceptibility is assessed. More generally, the development of genomic techniques to help understand the processes underlying the function and breakdown of function in photosynthetic symbioses is advocated.

Key words: Bleaching, *Chlorella*, Cnidaria, coral, metabolite profiling, nutrient release, photosynthesis, *Symbiodinium*, symbiosis, symbiotic algae.

Introduction

Oxygenic photosynthesis has apparently evolved just once, in the lineage that gave rise to all extant cyanobacteria (Cavalier-Smith, 2006). This metabolic capability has, however, been acquired on multiple occasions by eukaryotes through symbiosis either with cyanobacteria or with their unicellular eukaryotic derivatives generically termed 'algae'. Overall, 27 (49%) of the 55 eukaryotic groups identified by Baldauf (2003) have representatives which possess photosynthetic symbionts or their derivatives, the plastids. These include the three major groups of multicellular eukaryotes: the plants, which are derivatives of the most ancient symbiosis between eukaryotes and cyanobacteria; the fungi, many of which are lichenized with algae or cyanobacteria; and the animals. We, the authors, and probably many readers were taught that animals do not photosynthesize. This statement is true in the sense that the lineage giving rise to animals did not possess plastids, but false in the wider sense: many animals photosynthesize through symbiosis with algae or cyanobacteria.

This article on 'photosynthesis in animals' focuses on the central role of photosynthesis in symbioses between algae and animals, by considering three topics: the importance of light capture as a factor limiting the distribution of the symbioses in the animal kingdom; photosynthate transfer to the animal host, especially the underlying mechanisms and significance to the animal host; and risks to the symbiosis posed by photosynthetic reactions in the plastids of symbiotic algae. Two issues are beyond the scope of this article. These are the transient animal associations with photosynthetically-active chloroplasts obtained from algae consumed by the animal, and the significance of symbiotic algae to the animal host beyond the nutritional role of photosynthesis. In particular, some algal symbionts contribute to the nitrogen

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relations of their hosts by acting as a sink for animal metabolic waste, especially ammonia. Furthermore, the algae may recycle nitrogen, i.e. assimilate ammonia into compounds valuable to the host, such as essential amino acids, which are released to the host. Early approaches to explore nitrogen recycling were experimentally flawed (see the discussion in Wang and Douglas, 1998), but recent approaches exploiting stable isotope techniques are yielding firm indications of nitrogen recycling in some symbioses (Tanaka *et al*., 2006).

Distribution of photosynthetic symbioses in animals

Most animals with photosynthetic symbioses are members of two phyla, the Porifera (the sponges) and Cnidaria (including the hydroids, corals, sea anemones, and jellyfish) (Table 1). The dominant algal symbionts in these animals are freshwater chlorophytes of the genus *Chlorella* found, for example, in *Spongilla* sponges and freshwater hydras; dinoflagellates of the genus *Symbiodinium* in benthic marine hosts, including virtually all reef-building corals in the photic zone; and cyanobacteria in many marine sponges. These two animal phyla are morphologically simple and traditionally considered as the basal animal groups.

Animal body plan may contribute to the distribution of photosynthetic symbioses in animals. The body plans of both Porifera and Cnidaria inherently have a large surface area:volume relationship promoting the capture of light to meet the demands of the bulk tissue for photosynthetically-derived carbon. In particular, the Cnidaria are diploblast animals, comprising just two sheets of cells (the outer epidermis and inner gastrodermis) and can be moulded, through natural selection, into a variety of shapes; in his book on sea anemones, Malcolm Shick (1991) aptly terms these animals 'origami animals'. All other animals have the triploblast body plan, i.e. with a third body layer derived from the embryonic mesoderm. The triploblast body plan is 'solid', generally of low surface area per unit volume, and with limited capacity for extensive evaginations suitable for the absorption of light. Consistent with this distinction, photosynthetic symbioses in triploblastic animals is largely restricted to flatworms (turbellarian platyhelminths) constrained, despite their possession of mesoderm, to a large surface area:volume relationship by their lack of circulatory system (Table 1).

Photosynthetic symbioses do, however, occur in a few molluscs, which are large and morphologically complex animals; and molluscan hosts display considerable morphological adaptation for the symbiotic habit, an indication that the basic body form is not well-suited to photosynthesis. The tridacnid molluscs (family Tridacnidae: giant clams) are particularly informative. This group comprises five species, including *Tridacnis gigas* of up to 180 kg weight, and they all bear photosynthetic algal symbionts of the dinoflagellate genus *Symbiodinium*. The tridacnids differ from other bivalve molluscs in that the body is rotated through 180° relative to the hinge, and the siphonal tissue is hypertrophied, such that the 'gape' of the shell reveals the siphonal tissue and mantle of the animal, and not the foot as in other bivalves. Accompanying this radical re-organization of the body plan to accommodate photosynthesis, the digestive tract is transformed into multiple dichotomously branching diverticula bearing *Symbiodinium* cells and projecting towards the light—like branches of a tree (Norton *et al*., 1992). These remarkable morphological adaptations are a consequence of the poor compatibility of photosynthesis and the massive body form characteristic of most triploblastic animals.

These considerations should not, however, be interpreted to mean that photosynthesis poses no morphological conflict for the diploblast Cnidaria. The morphological conflict for Cnidaria is particularly evident in relation to the animals' tentacles, especially of sea anemones and their allies. Most Cnidaria with symbiotic algae are also carnivores, and in most species prey items

Table 1. *Survey of symbioses between animals and photosynthetic symbionts*

Hosts	Symbionts	References
Porifera	*Cyanobacteria* in many marine sponges, *Symbiodinium* in clionid (boring) marine sponges, *Chlorella* in freshwater sponges, e.g. *Spongilla*	Lee *et al*., 2001
Cnidaria	*Symbiodinium* in benthic marine corals, sea anemones etc. *Scripsiella* spp. in pelagic taxa, e.g. *Velella* *Chlorella* in freshwater hydra	Stat *et al*., 2006 Banaszak *et al*., 1993
Platyhelminthes	Various in marine turbellarians, e.g. *Tetraselmis* (prasinophyte) in *Symsagittifera* (=*Convoluta*) *roscoffensis;* *Licmophora* (diatom) in *Convoluta convoluta* *Chlorella* in freshwater turbellarians, e.g. *Dalyella* spp	Parke and Manton, 1967 Apelt and Ax, 1969 Douglas, 1987
Mollusca	Dinoflagellates, usually *Symbiodinium* in marine gastropods and bivalves, including tridacnid clams; *Chlorella* in few freshwater clams, e.g. *Anodonta*	Belda-Baillie *et al*., 2002
Ascidia	Cyanobacteria, usually *Prochloron* in various ascidians	Lewin and Chang, 1989

are trapped by nematocysts on the animals' tentacles. (Nematocysts are organelles which subdue prey by their toxic barbs and adhesive secretions prior to ingestion.) Food capture is promoted by the possession of relatively few, long, mobile tentacles with a high density of nematocysts; and photosynthesis is promoted by large numbers of short tentacles with high densities of symbiotic algal cells. Most of the Cnidaria which obtain nutrition by a combination of feeding and photosynthesis display morphological 'compromise' in relation to their tentacles. The several exceptions are informative.

The remarkable sea anemone *Lebrunia danae* has dimorphic tentacles. One form of its tentacles lacks symbiotic algae and is long and slender, suited for feeding; and the other form is short but branched and densely colonized by *Symbiodinium* (Fig. 1A). Interference between the two tentacle types is avoided in the living animal by specific extension of the feeding tentacles at night, when food is available in the water column, and photosynthetic tentacles by day, for light capture. The morphology of the two tentacles is so different that *L. danae* was originally classified as a member of two different genera.

Other sea anemones have resolved the conflict by the evolution of novel structures for either feeding or photosynthesis. In *Bunodeopsis* species, the tentacles are predominantly feeding structures and the *Symbiodinium* algae are concentrated in vesicles on the body column (Fig. 1B). By day, the vesicles inflate by the accumulation of sea water, so maximizing the surface area for light capture, with the tentacles retracted; and the reverse occurs at night (Day, 1994). *Discosoma* species adopt a different morphological strategy (Fig. 1C). These coralliomorph cnidarians have small, 'stubby' tentacles almost entirely devoid of nematocysts, and food capture is mediated by the lateral expansion of the oral disc (the upper surface of the body column bearing the mouth and tentacles) to form a muscular 'oral veil'. When prey make contact with the oral disc, the oral veil extends rapidly up-and-over the prey, which is essential 'bagged' before transfer whole via the mouth to the gut (Elliott and Cook, 1989).

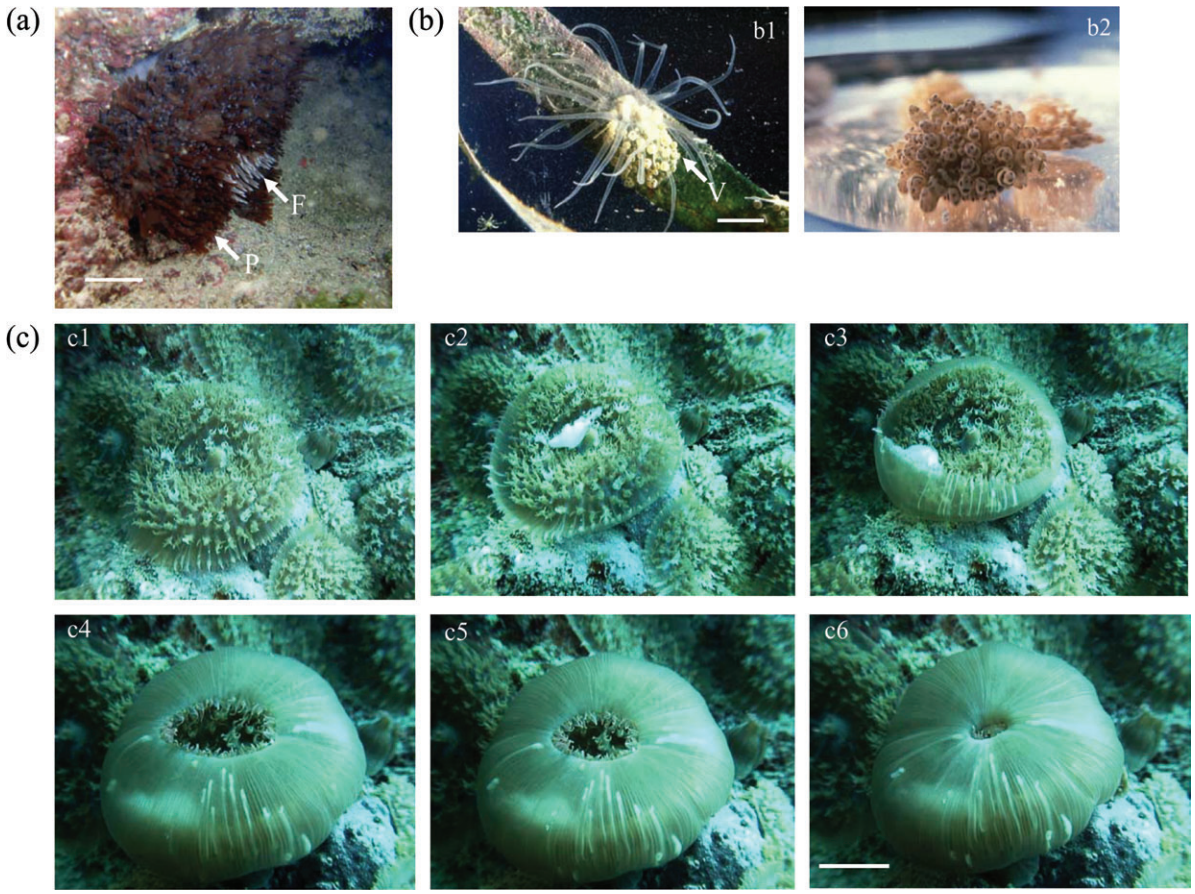

Fig. 1. Morphological adaptations for light capture in sea anemones with photosynthetic symbionts. Scale bars=2.5 cm. (a) Dimorphic tentacles of *Lebrunia danae* (i) photosynthetic tentacles and (ii) colourless feeding tentacles emerging in response to a prey item. (b) Vesicles on the body wall of *Bunodeopsis antilliensis* bearing symbiotic algal cells: at night, when feeding tentacles are extended; and by day when the feeding tentacles are retracted and the vesicles expanded. (Photographs of R Day.) (c) Tentacles on the oral disc of *Discosoma sanctithome* (i), and capture of small fish prey by extension of the oral veil (2–4, see text for details). (Photographs of B Watson.)

These various morphological adaptations are indicative of the selective advantage of symbiosis with algae for their animal hosts. A key basis of this selective advantage is host access to alga-derived photosynthetic carbon, which is considered in the next section.

Host access to photosynthetic carbon fixed by symbiotic algae

Algal cells in symbiosis with animals are photosynthetically competent, fixing carbon dioxide and releasing oxygen at rates (per cell or per unit chlorophyll) at least comparable to algal cells in culture. In most animal hosts, the algal cells are intracellular, bounded by an animal membrane, known as the symbiosomal membrane. As a result, they obtain the carbon dioxide and water for the photosynthetic reaction and all substrates required for synthesis of cellular constituents, including the photosynthetic apparatus, from the host cell. Delivery of substrates has involved biochemical and cellular adaptation by the animal. In particular, marine cnidarians bearing *Symbiodinium*, first, maintain high activity of carbonic anhydrase and, second, have specific transporters for the delivery of bicarbonate ions to the cells bearing the symbiotic algae. These traits provide a partial pressure of CO_2 in the immediate surroundings of the symbiont cells that is high enough to support photosynthetic carbon fixation (Allemand *et al*., 1998).

Radiotracer experiments with ^{14}C-bicarbonate demonstrate that the algae release up to 50% of photosynthetically-fixed carbon to the animal host; and these values may be underestimates because of incomplete penetration of ^{14}C-radioisotope in short-term experiments, and respiratory loss of radioactivity from the animal as CO_2 which is refixed by the algal cells. Photosynthate release is biotrophic (i.e. from living cells) and apparently selective, with the dominant photosynthetic products of the algal cells apparently 'protected' from release.

Much research has focused on the identity of the compounds translocated to the animal tissues; these compounds are known as 'mobile' compounds (Table 2). There has been a strong expectation that the mobile compounds are dominated by one (or a few) compounds of low molecular weight, by analogy with the release of triose phosphate from chloroplasts and single polyols from the algal symbionts in lichens. Early research on the symbiotic *Chlorella* in freshwater hydra appeared to confirm this expectation. When the *Chlorella* cells are isolated from hydra and incubated immediately in simple buffer of pH 4–5 under illumination, they release large amounts of the disaccharide maltose. Metabolic analyses revealed that the maltose is synthesized within 1 min of carbon fixation and derived from a small metabolic pool with high turnover (Cernichiari *et al*., 1969). It was concluded, entirely plausibly, that maltose is the mobile compound of symbiotic *Chlorella*. Consistent with this interpretation, non-symbiotic *Chlorella* do not release photosynthate in response to low pH. Doubt over the relevance of this research with freshly-isolated *Chlorella* has been cast subsequently, with the demonstration that the pH in the immediate environment of *Chlorella* in the symbiosomal space in the symbiosis is >5.5, and would not be expected to induce substantial maltose release (Rands *et al*., 1992).

Studies seeking to identify the processes mediating photosynthate release and the identity of the mobile compounds in symbioses with *Symbiodinium* has also focused on algal cells freshly-isolated from the symbiosis. Photosynthate release from isolated *Symbiodinium* cells is not triggered by manipulation of pH of the medium, but it is induced by the addition of 'a drop of' homogenate of the host tissues (Trench, 1971). The active component in the host homogenate has variously been identified as proteinaceous (Sutton and Hoegh-Guldberg, 1990) and amino acids, including high concentrations of protein amino acids (Gates *et al*., 1995) and micro-molar concentrations of the non-protein amino acid, taurine (Wang and Douglas, 1997), but its identity remains elusive. Its mode of action involves modification of algal metabolism (Grant *et al*., 2006), and it can be mimicked by clotrimazole (1-α-2 chlorotrityl imidazole) (Ritchie *et al*., 1997), raising the possibility that the host factor is an antagonist of calmodulin and may modulate Ca^{2+}-calmodulin-mediated signalling in either the animal or algal cell.

Symbiodinium cells incubated with host homogenate release various low molecular weight compounds derived from photosynthesis, including glycerol, organic acids, glucose, and amino acids, the composition and diversity of released compounds varying among different symbioses and studies (Trench, 1979). The factors contributing to this variation may include homogenate composition, genotype, and metabolic condition of *Symbiodinium* and the protocol used for separation and identification of released compounds. These uncertainties notwithstanding, these data suggest that the mobile compounds of *Symbiodinium* are not derived directly from a single metabolic pool in the alga. In other words, photosynthate release from *Symbiodinium* is likely to be more complex metabolically than from the plastids of plants and symbiotic *Chlorella*.

Just as with the *Chlorella* system, there is doubt over the relevance of the traits of isolated *Symbiodinium* to the intact symbiosis. Perhaps the most telling study was conducted by Ishikura *et al*. (1999) on *Symbiodinium* in the giant clam *Tridacna crocea*. Unlike the symbioses in corals and other Cnidaria, *Symbiodinium* is extracellular in tridacnids, making direct analysis of the photosynthetic compounds released from the algae in symbiosis technically feasible. Ishikura *et al*. (1999) demonstrated that the dominant compound released by the algae in *T. crocea* is

Table 2. *Photosynthetic products released from photosynthetic symbionts in animals*

Host		Symbiont	Experimental Technique	Mobile Compounds	References
Porifera	Marine	Cyanobacteria	^{14}C tracer experiments: isolated algae	Glycerol and a small organic	Wilkinson, 1979
	Fresh water	*Chlorella* sp.	^{14}C tracer experiments: isolated algae with comparison to metabolism of ^{14}C-glucose *in vivo*	Glucose	Wilkinson, 1980
			^{14}C tracer experiments: isolated algae	Glucose	Fischer *et al*., 1989
Cridaria	Coral	*Symbiodinium*	^{14}C tracer experiments: freshly isolated algae with or without host homogenate.	glycerol, sugars, organic acids and amino acids	Muscatine, 1967; Sutton and Hoegh Guldberg, 1990; Gates *et al*., 1995, Grant *et al*., 1997 Hinde, 1988, Lewis and Smith, 1971
			Measurement of respiratory quotients	Glycerol in shallow colonies ($\leqslant$ 5m) and lipid in deeper colonies (30 m)	Gattuso and Jaubert, 1990
			Identification of dinoflagellate (algal)-specific compounds in animal tissue	Polyunsaturated fatty acids	Papina *et al*., 2003
			^{14}C tracer experiments with $NaH^{14}CO_2$ and ^{14}C acetate: intact symbioses	Lipid	Patton *et al*., 1983
			^{14}C tracer experiements with ^{14}C-acetate : isolated algae and intact symbiosis.	Lipid	Patton *et al*., 1977
			^{14}C tracer experiments with ^{14}C-glucose: intact symbiosis.	Lipid	Oku *et al*., 2003
	Anemones	*Symbiodinium*	^{14}C tracer experiments: isolated algae	[d]Glycerol, glucose, alanine and organic acids	Trench, 1971 Lewis and Smith, 1971
			^{14}C tracer experiments with partial uncoupling of photosyntheis and respiration: intact symbiosis and isolated algae	Glycerol	Battey and Patton, 1987
			Metabolite profiling: intact symbiosis	Glucose, succinate/fumarate	Whitehead and Douglas, 2003
			Identification of symbiosis-specific compounds in animal tissue	Triglyceride	Harland *et al*., 1991
			^{14}C tracer studies with ^{14}C-acetate: intact symbiosis and isolated algae	Lipid	Blanquet *et al*., 1979
			Metabolism of $NaH^{14}CO_3$, ^{14}C-acetate and ^{14}C-glucose: intact symbiosis	Glycerol and lipid.	Battay and Patton, 1984
			Metabolism of ^{14}C-aspartate and ^{14}C-glutamate in aposymbiotic and symbiotic anemones	Essential amino acids	Wang and Douglas, 1999
	Zoanthids	*Symbiodinium*	^{14}C tracer experiments: isolated algae	Organic acids, amino acids and sugars	von Holt and Holt, 1968b Lewis and Smith, 1971
	Gorgonians, Jelly-fish and Hydrozoans	*Symbiodinium*	^{14}C tracer experiments: isolated algae using inhibition technique	Glycerol, glucose, alanine	Lewis and Smith, 1971
Fresh water Platyhelminthes	Hydra	*Chilorella Tetraselmis*	^{14}C radiotracer studies: isolated algae	Maltose	Mews, 1980
			^{14}C radiotracer studies: intact symbiosis	Amino acids Particularly alanine	Muscatine *et al*., 1974 Botyle and Smith, 1975
Mollusca	Tridacnid clams	*Symbiodinium*	^{14}C tracer studies: isolated algae	Glycerol	Muscatine, 1967 Streamer *et al*., 1988; Rees *et al*., 1993
			^{14}C tracer studies: intact symbiosis	Glucose	Ishikura *et al*., 1999
			Carbon isotopic [^{13}C] analysis of animal and algal tissue	16:0 and 16:1 fatty acids	Johnston *et al*., 1995
Sacoglossan sea slugs		Chloroplasts from food alga	^{14}C tracer studies on isolated chlorolasts/homogenized tissue	Glucose, alamine, glycolic acid	Gallop, 1974; Trench *et al*., 1973
			^{14}C tracer studies impact symbiosis	Glucose, galactose, mannose, alanine	Trench *et al*., 1974
Chordata	Ascidians	*Prochloron*	^{14}C tracer on isolated algae	Glycolate	Fisher and Trench, 1980

glucose in the symbiosis but glycerol in isolated algal preparations. The implication that photosynthate release induced by host homogenate may be mechanistically different from that in symbiosis, and consequently artefactual, has led to a renewed interest in the study of photosynthate release in the intact symbiosis.

One approach with considerable potential to study the identity of the mobile compounds of symbiotic algae of animals is metabolite profiling (Whitehead and Douglas, 2003). The rationale of this method is that, over a period of one-to-several min , the animal host metabolizes the mobile compounds to a 'signature' panel of products that is also produced when the animal takes up the same compounds from the exogenous medium. In other words, exogenous compounds that yield the 'signature panel' of animal products are likely to be the mobile compounds. Using this approach, candidate mobile compounds for *Symbiodinium* in the sea anemone *Anemonia viridis* have been identified as glucose and the dicarboxylic organic acids succinate and fumarate. As analytical methods for the identification and quantification of metabolites improve, the utility of this method to pinpoint candidate mobile compounds for further investigation will increase.

An important message to emerge from these considerations concerns the experimental approach to study photosynthate release from symbiotic algae. Our understanding of photosynthate release comes principally from research on isolated algae, but insufficient attention has been given to the relevance of the findings to the traits of the algae in symbiosis. Although there is no basis to 'write off' all research with isolated algae as artefact, future research on isolated algal preparations should seek strategies to check the compatibility of results with the interactions in the intact symbiosis.

In comparison to the uncertainty over the mechanisms underlying photosynthate release from symbiotic algae and the identity of the mobile compounds, there is widespread acceptance that photosynthetic products derived from the symbiotic algae are important to the animal host [although even this point is also contested, for example by Goreau (2006)]. Circumstantial evidence includes the apparent absence of alga-free individuals of most animal species capable of forming the symbiosis in natural habitats, and the depressed growth rates of most symbioses incubated in continuous darkness. Classical budget analyses particularly of Muscatine *et al.* (1984) and Davies (1984) indicate that photosynthetic carbon derived from *Symbiodinium* is sufficient to meet much or all the total carbon requirement for basal respiration in several coral species. In some coral species, much of the photosynthetic carbon is allocated to mucus, which is produced in copious amounts and is important in feeding and protection from mechanical damage and pathogens. A significant proportion of the photosynthate received by the animal is not, however, dissipated rapidly (Rinkevich, 1989; Tanaka *et al*., 2006), and this includes photosynthetically-derived carbon in the animal lipid pools (Grotolli *et al*., 2004), which are crucial for gametogenesis and sexual reproduction in corals and other Cnidaria (Arai *et al*., 1993).

One important generality embedded in these considerations is that the algae provide the animal with fixed carbon. The animals cannot 'live on' their algal symbionts alone. They obtain nitrogen, phosphorus, and other elements from a combination of dissolved compounds and, for most taxa, holozoic feeding (Schlicter, 1982; Ferrier, 1991; Grover *et al*., 2006). In this context, the photosynthetic algae have been likened to a source of 'junk food', which meets the immediate energetic demands and other carbon-based requirements of the host, valuable to the animal in environments where other sources of nutrients are in short supply.

The susceptibility of photosynthetic symbioses to environmental stress

Photosynthesis brings to animals major risks as well as nutritional benefits. Photosynthesis can be perturbed by upsetting the balance between the rates of light collection and light use, resulting in the production of reactive oxygen species (ROS) (Asada, 1996). Left unchecked, ROS are damaging to protein function and membrane integrity, and pose a serious threat to both the photosynthetic symbionts and their animal hosts.

Photosynthetic symbioses in animals respond to certain environmental changes by bleaching, literally a loss in colour. Bleaching is caused by the loss of algal pigments or elimination of algal cells from the symbiosis (Brown, 1997). Most research has concerned bleaching in symbioses with *Symbiodinium* and these symbioses may be particularly susceptible to environmental perturbation. Because *Symbiodinium* is the usual symbiont of corals, bleaching is often described as 'coral bleaching', but sea anemones, clams and other hosts of *Symbiodinium* appear to be as susceptible to bleaching as corals. Bleaching can be triggered by multiple factors (Douglas, 2003; Jones, 2004), but most bleaching observed in the field is caused by elevated temperature often acting synergistically with solar radiation (Fitt *et al*., 2001; Lesser and Farrell, 2004). The impacts of bleaching include reduced growth and reproduction, increased susceptibility to disease and mechanical damage, and occasionally death of the host (Szmant and Gassman, 1990; Glynn, 1996; Baird and Marshall, 2002). Mass bleaching events in recent years has led to the mortality of hosts across entire reefs (Hoegh-Guldberg, 1999).

Photosynthetic corals and other tropical symbioses with *Symbiodinium* live close to their upper thermal tolerance limits (Fitt *et al*., 2001). On the basis of projected

increases in sea temperature with climate change, mass bleaching events and die-offs have been predicted to increase in frequency and severity over the coming decades (Hoegh-Guldberg, 1999). Indeed, reef collapse has been predicted by the 2020s for the Indian Ocean (Sheppard, 2003) and globally by 2050s (Hoegh-Guldberg, 1999). As corals are the foundation to coral reef ecosystems, coral mortality caused by thermal bleaching is a first-order threat to biodiversity in tropical and subtropical seas.

A large body of evidence now points to the impairment of algal photosynthesis as a key step in the thermal bleaching of *Symbiodinium* symbioses. Uncertainty, however, still exists over the sequence of events, and several sites have been proposed as the position of the initial lesion in algal photosystems (Fig. 2). Iglesias-Prieto *et al*. (1992) demonstrated a decrease in the efficiency of Photosystem II (PSII) when cultured algae were exposed to temperatures of 34–36 °C, leading to the suggestion that the primary impact of high temperature was to cause a malfunction in the light reactions of photosynthesis. Warner *et al*. (1996) recorded a loss of photosynthetic efficiency in algae in bleached corals subjected to elevated temperatures, making a direct link between temperature-dependent impairment of photosynthesis and coral bleaching. Furthermore, the degradation of the reaction centre D1 protein co-occurred with temperature-dependent loss of PSII activity (Warner *et al*., 1999). The D1 protein has an important structural and functional role in the PSII reaction centre, binding components for charge separation and electron transport. When PSII is excited there is a probability that damage to the D1 protein will occur. Under normal physiological conditions the rate of photodamage does not exceed the capacity to repair the damage, but D1 re-synthesis and replacement may become impaired in algae at elevated temperature, leading to a loss of PSII functional reaction centres (Warner *et al*., 1999).

An alternative viewpoint is provided by Jones *et al*. (1998), who argued that damage to PSII was a downstream impact of impairment of the Calvin–Benson Cycle. Specifically, these authors suggest that a decrease in carboxylation of ribulose 1,5 *bis*phosphate (RuBP) by Rubisco could lead to reduced rates of consumption of products of the photosynthetic electron transport (ATP and NADPH) restricting the rate of flow in the electron transport chain (sink-limitation). These events would result in maximal reduction of the plastoquinone pool, causing damage at PSII through a build-up of excess excitation energy. The net consequence would be the formation of highly reactive triplet states of chlorophyll which react with O_2 to form singlet oxygen ($-O_2^*$) (Smith *et al*., 2005). Singlet oxygen is potentially damaging to proteins; it can trigger the degradation of the D1 protein (Asada, 1996) and also react with components in the light-harvesting antennae leading to bleaching of pigments (Halliwell, 1991).

The Mehler reaction is an alternative sink for electrons. It may help prevent maximal reduction of the PSII quinone receptors at the point where the rate of light-driven transport exceeds the capacity of the temperature-impaired Calvin cycle (Jones *et al*., 1998), but at the cost of producing ROS (Asada, 1999). The Mehler reaction involves reduction of O_2 by PSI to produce superoxide radicals that are rapidly converted within the chloroplast to hydrogen peroxide by superoxide dismutase (Fig. 2). Detoxification of hydrogen peroxide to water then occurs by ascorbate peroxidase (Fig. 2). Both superoxide dismutase and ascorbate peroxidase are present in *Symbiodinium* (Matta and Trench, 1991) and under most conditions the rate of detoxification should exceed the rate of ROS generation. During bleaching, however, ROS could accumulate if the rate of its production exceeds its removal (Smith *et al*., 2005).

Analysis of variable chlorophyll fluorescence kinetics in *Symbiodinium* by Tchernov *et al*. (2004) indicates that elevated temperatures of 32 °C damage thylakoid membranes causing an increase in the rate of electron transport on the acceptor side of PSII with a simultaneous decrease in the maximum quantum yield of photochemistry in the reaction centre. These changes are diagnostic of energetic uncoupling of electron transport, where the transmembrane proton gradient, established by the photochemical reactions in the reaction centres, is dissipated without generating ATP. Thylakoid membranes remain capable of splitting water and photosynthetically-produced O_2 is reduced by the Mehler reaction leading to the accumulation of ROS as described above. Dye tracer experiments conducted by the same authors on algae in culture indicate that the ROS could leak from algal cells (Fig. 2).

Whatever the initial site of damage to algal photosystems, the generation of ROS through temperature-irradiance impairment of photosynthesis presents a source of oxidative stress for the algal and animal partners. Oxidative stress can elicit a wide spectrum of cellular responses (Martindale and Holbrook, 2002), some of which are known to occur in bleaching, for example, exocytosis of algae from host cells (Brown *et al*., 1995), and lysis of both animal and algal cells by elements of necrotic and programmed cell death pathways (Dunn *et al*., 2002; Franklin *et al*., 2004). Little is known about the cellular pathways that mediate cellular responses to oxidative stress in animals with photosynthetic symbionts, although these pathways are well described in model systems (Martindale and Holbrook, 2002).

Recent research of Perez and Weis (2006) on the symbiotic anemone *Aiptasia pallida* provides an indication of the processes involved. Exposure of this symbiosis to heat stress or the photosynthetic inhibitor DCMU

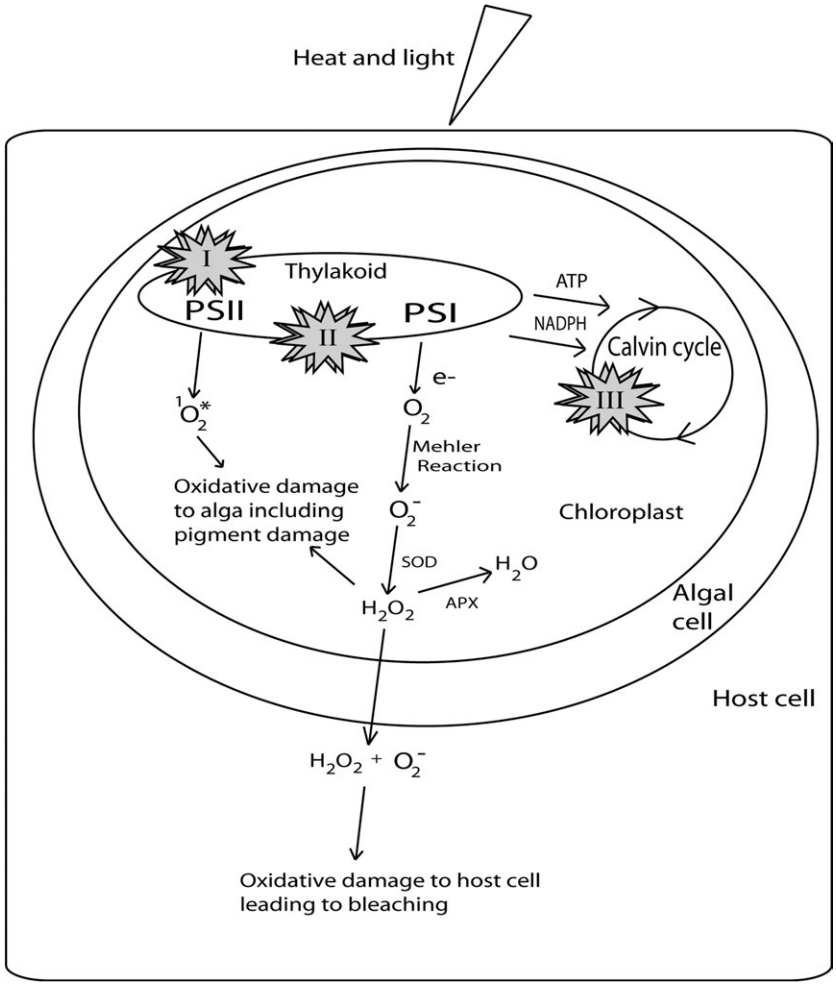

Fig. 2. Three proposed primary impacts of elevated temperature on the photosystems of symbiotic algae in animal hosts, shown as I, II, and III in the figure. (I) Dysfunction of PSII and degradation of the D1 protein. (II) Energetic uncoupling in the thylakoid membranes. (III) Impairment of the Calvin cycle. During bleaching ROS are generated from O_2 via the Mehler reaction and are detoxified by superoxide dismutase (SOD) and ascorbate peroxidase (APX). If the rate of ROS generation exceeds detoxification, then oxidative damage can occur, triggering signalling pathways and cellular responses that underpin bleaching. Singlet oxygen can be generated at damaged PSII reaction centres and in the photosynthetic antennae causing photobleaching of chlorophyll and accessory pigments.

triggers the production of nitric oxide (NO) in the host cell, leading to bleaching of the symbiosis. Incubation of anemones in lipopolysaccharide (LPS) also induces NO in gastrodermal cells of *A. pallida* to similar levels observed with elevated temperature and an inhibitor of phtosystem-II. LPS is known to induce an up-regulation of nitric oxide synthase through the nuclear factor κB (NF-κB)-dependent signalling in mammalian systems, and Perez and Weiss (2006) suggest that ROS released from heat-stressed photosystems in the algal cells might induce NO production in the host via the similar pathways. They specifically hypothesize that NO converts to cytotoxic peroxynitrite on interaction with superoxide in the host cell, leading to cell death and bleaching.

A number of mechanisms have evolved in the animal hosts that protect the algal photosystems against elevated thermal and light stress. For example, corals can retract soft tissues to reduce light capture (Brown *et al*., 1994, 2002), and symbiotic sea anemones and corals possess a large diversity of SOD isoforms, similar to those described in terrestrial plants (Richier *et al*., 2003). Interestingly, corals also possess fluorescent proteins (FPs) which may play a role in ameliorating the impact of high irradiance on algal cells (Salih *et al*., 2000). The protection offered by FPs is reduced at elevated temperatures however, raising doubts about their photoprotective role during thermal bleaching (Dove, 2004).

Algal–animal symbioses vary greatly in their susceptibility to bleaching and much of this variation has been attributed to differences in thermotolerance among algal partners. Single host species associated with different algal genotypes may vary in bleaching susceptibility (Rowan *et al*., 1997; Perez *et al*., 2001). Research on the mechanistic basis of thermal tolerance in the algal cells

has focused primarily on non-photochemical quenching (NPQ), which can dissipate excess excitation energy in temperature-impaired algal photosystems (Warner *et al*., 1996). The xanthophyll cycle is a key NPQ mechanism and it has been described in *Symbiodinium* (Brown *et al*., 1999; Venn *et al*., 2006). Conversion of the pigments diadinoxanthin to diatoxanthin (analogous to the xanthophylls violaxanthin, antheraxanthin, and zeaxanthin in terrestrial plants) diverts excess excitation energy away from PSII reaction centres. Although inhibition of xanthophyll cycling by dithiothreitol (DTT) can lead to increased oxidative damage in corals (Brown *et al*., 2002), doubts over its significance have been raised by the finding that the abundance and cycling of xanthophyll pigments is not greater in bleaching-resistant than bleaching-susceptible corals (Venn *et al*., 2006). Other mechanisms of NPQ described in *Symbiodinum* might contribute to thermal tolerance; they include dissipation of excess excitation energy as heat within the PSII reaction centres (Gorbunov *et al*., 2001), and state 1 to state 2 transitions associated with phosphorylation and the migration of light- harvesting complexes away from PSII (Hill *et al*., 2005).

Experiments of Tchernov *et al*. (2004) suggest that lipid saturation determines the stability of the thylakoid membranes at elevated temperature. As the same authors also found evidence that thermal disruption of the thylakoids is the initial site of damage during bleaching, the membrane lipids of symbiotic algae may determine the bleaching susceptibility of animal–algal symbioses. Certain algal genotypes possess thylakoid membranes with a higher content of the mono-unsaturated fatty acid Δ9-*cis*-octadecenoic acid (18:1) relative to the major polyunsaturated fatty acid Δ6,9,12,15-*cis*-octadecatetraenoic acid (18:4), affording them stability at elevated temperatures.

These findings illustrate a general point: that a variety of processes, from ROS to NO and membrane lipid composition, have all been implicated as determinants of bleaching and variation in susceptibility to bleaching. The task for the future is to establish the relative importance of these different processes and how they interact to mediate the observed bleaching phenomena. This, in turn, will provide a basis for predicting the scale of future bleaching events and their impacts on the coral communities, on which reef ecosystems depend.

Concluding comments

Photosynthesis in animals has been the focus of sustained research for 40–50 years. Despite this, understanding is very limited of both how animals exploit this metabolic capability of their symbiotic algae and how the symbiosis responds to the risks of ROS and photosystem damage. In comparison to other symbioses, notably the rhizobial–legume associations, the photosynthetic symbioses in animals have a major handicap of lacking a genetic 'toolkit' that would enable molecular dissection of the symbiosis based on forward and reverse genetics and gene expression analysis. Some molecular resources are, however, becoming available, notably EST and BAC libraries, with plans for complete genome sequencing, for the algal and animal partners in *Symbiodinium*–cnidarian symbioses. Furthermore, methods for the genetic transformation of *Symbiodinium* and RNAi-mediated suppression of host gene expression have been reported (ten Lohuis and Miller, 1997; Dunn *et al*., 2007), and these deserve sustained investment by the community to promote the development of robust, general protocols for manipulations. These approaches have great potential for identification of genes underpinning the mechanisms by which photosynthesis has been accommodated in animals.

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