

# Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals?

DAVID J. SMITH, DAVID J. SUGGETT and NEIL R. BAKER

*Coral Reef Research Unit, Department of Biological Sciences, University of Essex, Colchester, CO4 3SQ, UK*

## Abstract

The bleaching of corals in response to increases in temperature has resulted in significant coral reef degradation in many tropical marine ecosystems. This bleaching has frequently been attributed to photoinhibition of photosynthetic electron transport and the consequent photodamage to photosystem II (PSII) and the production of damaging reactive oxygen species (ROS) in the zooxanthellae (*Symbiodinium* spp.). However, these events may be because of perturbations of other processes occurring within the zooxanthellae or the host cells, and consequently constitute only secondary responses to temperature increase. The processes involved with the onset of photoinhibition of electron transport, photodamage to PSII and pigment bleaching in coral zooxanthellae are reviewed. Consideration is given to how increases in temperature might lead to perturbations of metabolic processes in the zooxanthellae and/or their host cells, which could trigger events leading to bleaching. It is concluded that production of ROS by the thylakoid photosynthetic apparatus in the zooxanthellae plays a major role in the onset of bleaching resulting from photoinhibition of photosynthesis, although it is not clear which particular ROS are involved. It is suggested that hydrogen peroxide generated in the zooxanthellae may have a signalling role in triggering the mechanisms that result in expulsion of zooxanthellae from corals.

*Keywords:* bleaching, corals, hydrogen peroxide, photoinhibition, photosynthesis, reactive oxygen species, singlet oxygen, zooxanthellae

*Received 7 October 2004; revised version received 3 November 2004; accepted 8 November 2004*

## Introduction

Coral reefs, one of the dominant features of shallow tropical oceans, are extremely biologically diverse and highly productive. It is currently estimated that 0.5 billion people around the globe are dependent on coral reef resources (Wilkinson, 2002), however, this number is expected to double over the next 50 years (Hoegh-Guldberg, 1999; Wilkinson, 2002). The major components of coral reefs are the scleractinian reef building (hermatypic) corals. These corals are also referred to as zooxanthellate corals because of the presence of endosymbiotic zooxanthellae (*Symbiodinium* spp.) within gastrodermal cells of the coral tissue. Zooxanthellae occur at densities of between 0.5 and 5 million/cm<sup>2</sup> of coral and translocate up to 95% of their photosynthate to their coral host (Trench, 1979; Muscatine, 1990). In

return, the coral host provides the zooxanthellae with nitrogenous compounds, phosphates and CO<sub>2</sub> (Davies, 1984). It is this symbiotic relationship, involving the recycling of nutrients and close coupling between trophic levels, that is the key to the ecological success of the scleractinian reef building corals (Falkowski *et al.*, 1984, 1993; Stanley & Swart, 1995; Muller-Parker & D'Elia, 1997). Any factor that reduces the efficiency of this symbiotic relationship will have a major effect on scleractinian corals, and consequently the productivity of coral reefs (Glynn, 1996).

In the past few decades, coral reef ecosystems across the world have suffered unprecedented degradation, which has been attributed primarily to anthropogenic factors (Glynn, 1996; Wilkinson, 2002). Thermal-induced bleaching of hermatypic corals has become a major feature of the degradation of many coral reefs (Brown, 1997; Hoegh-Guldberg, 1999; Fitt *et al.*, 2001; Lesser, 2004). Coral bleaching is a generic term used to describe a loss of pigmentation from corals, and can involve both a loss of photosynthetic pigments from the

Correspondence: Neil R. Baker, fax +44 1026 872592, e-mail: baken@essex.ac.uk

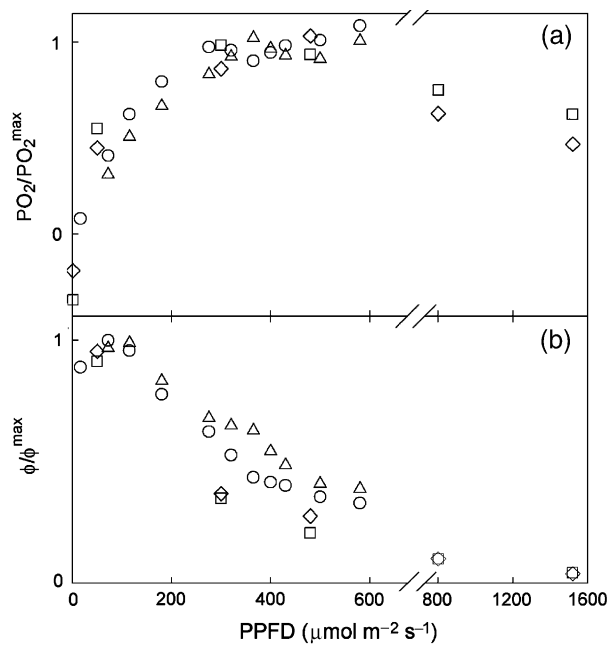
zooxanthellae and a release of zooxanthellae from the coral host (Fitt *et al.*, 2001). Specifically, photoinhibition of photosynthesis and photodamage to photosystem II (PSII) of the zooxanthellae, with the consequent increase in the production of damaging reactive oxygen species (ROS), have been implicated as the cause of thermal bleaching (Brown, 1997; Fitt *et al.*, 2001; Lesser, 2004; Tchernov *et al.*, 2004). Small increases in temperature of less than 2 °C above the average summer maxima can result in coral bleaching (Podesta & Glynn, 2001). This review examines possible mechanisms by which increases in temperature may induce photoinhibition of photosynthetic electron transport in coral zooxanthellae and result in bleaching of the coral. Hypotheses are proposed for mechanisms involved in pigment bleaching in the zooxanthellae and expulsion of the zooxanthellae from the coral host.

#### *Photosynthesis and light acclimation in zooxanthellate corals*

Efficient photosynthetic performance of zooxanthellae is essential for the maintenance and growth of corals. Since the light environment experienced by corals can be highly variable, the zooxanthellae must be capable of acclimating to large changes in light quality and quantity to optimize photosynthetic performance and coral growth (Falkowski & Dubinsky, 1981; Dubinsky *et al.*, 1984; Wyman *et al.*, 1987; Falkowski *et al.*, 1990). The typical response of zooxanthellae photosynthesis to increasing light is shown in Fig. 1. At low light levels, maximum quantum efficiency ( $\phi^{\max}$ ) is achieved when photosynthesis increases linearly with increasing light absorption. With further increases in light intensity, the relationship between photosynthesis and absorbed light becomes nonlinear and the quantum efficiency decreases. These decreases in quantum yield of electron transport can be attributed to a number of processes (Baker *et al.*, 2005). The first is a direct consequence of electron transport components becoming reduced, resulting in a reduction of the probability of the use of excitation energy for electron transport. The other processes are because of the activation of mechanisms that convert absorbed excitation energy to heat. Initially with increasing light, the limiting step in electron transport appears to be the oxidation of plastoquinone by the cytochrome *b<sub>6</sub>f* complex (Genty & Harbinson, 1996). Restriction of electron flux through the cytochrome *b<sub>6</sub>f* complex is under physiological control mediated via a light-induced decrease in the pH of the thylakoid lumen. However, with further light increases and as photosynthesis moves toward light saturation (Fig. 1), photosynthesis becomes increasingly limited by the metabolic capacity to consume ATP and

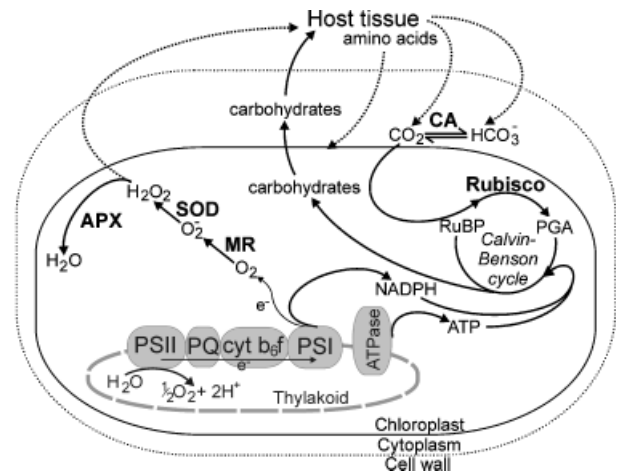
NADPH. This results in a further increase in the proportion of reduced electron transport components thereby decreasing the probability of PSII photochemistry (Baker *et al.*, 2005). Besides CO<sub>2</sub> assimilation, nitrogen metabolism is also likely to be a significant sink for ATP and NADPH in zooxanthellae. The second process causing a decrease in the quantum yield is an increase in the amount and proportion of excitation energy absorbed by the PSII antennae that is diverted directly to heat through a thermal dissipation mechanism. In zooxanthellae this thermal dissipation is associated with the conversion of the xanthophyll diadinoxanthin to diatoxanthin (Falkowski & Raven, 1997; Brown *et al.*, 1999). Xanthophyll conversion decreases the rate at which photons are delivered to PSII reaction centers and thus helps prevent over-reduction of quinone acceptors by effectively reducing the functional absorption cross-section of PSII reaction centers ( $\sigma_{\text{PSII}}$ ). The development of another quenching mechanism associated with thermal dissipation of excitation energy within PSII reaction centers themselves (Finazzi *et al.*, 2004) also appears to play an important role in decreasing the PSII quantum efficiency in corals (Gorbunov *et al.*, 2001). Finally, light-induced state 1–state 2 transitions, associated with phosphorylation and migration of light-harvesting complexes away from PSII, reduce  $\sigma_{\text{PSII}}$  and may also contribute to decreases in the quantum efficiency of photosynthesis (Falkowski & Raven, 1997).

Ultimately, the rate of increase in the proportion of absorbed light released through thermal dissipation may not be sufficient for complete protection of PSII as the photosynthetic rate approaches light saturation and becomes light saturated. In many microalgae, the quinone acceptors rarely become fully reduced except at light intensities considerably above that required for saturation of photosynthesis (Falkowski & Raven, 1997). Maintenance of the quinone acceptors in a partially oxidized state is important to prevent photoinactivation and photodamage to the PSII reaction centers. When PSII is excited, there is a probability that damage to the D1 protein of the reaction center will occur. This probability is low at low excitation levels but increases with increasing light absorption and a decreasing ability to perform photochemistry. Under normal physiological conditions the rate of photodamage does not exceed the capacity to repair the damage. However, under conditions where the PSII quinone acceptors become highly reduced, such as light intensities considerably above that required for saturation of photosynthesis, the rate of damage can considerably exceed the rate of repair. Consequently, there is a loss of functional PSII reaction centers and photosynthesis declines (Fig. 1).



**Fig. 1** Light response of coral zooxanthellae (a) photosynthetic oxygen production ( $\text{PO}_2$ ) and (b) quantum yield of oxygen production ( $\phi$ ). Data are taken from Dubinsky *et al.* (1984) for sun ( $\Delta$ ) and shade ( $\circ$ ) colonies of *Stylophora pistillata* and Shick *et al.* (1995) for shallow ( $\diamond$ ) and deep ( $\square$ ) colonies of *Acropora microphthalma*. Values of  $\text{PO}_2$  reported by Shick *et al.* (1995) were transformed to values of  $\phi$  using measurements of light absorption from *Acropora* colonies located at similar depths but reported by Wyman *et al.* (1987). For comparative purposes,  $\text{PO}_2$  and  $\phi$  for each light response was normalized to the maximum value,  $\text{PO}_2^{\text{max}}$  and  $\phi^{\text{max}}$ , respectively. In the PPFD range 0–100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic  $\text{O}_2$  production increases linearly with increasing PPFD with maximum quantum yield of  $\text{O}_2$  production being achieved. Decreases in the quantum yield then occur with increasing PPFD. Photosynthetic  $\text{O}_2$  production saturates at a PPFD of ca. 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Increases in PPFD above 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  result in a reduction in the light-saturated rate of  $\text{O}_2$  production because of photoinhibition of photosynthesis.

Alternative sinks for electrons, other than  $\text{CO}_2$  assimilation, may provide additional protection mechanisms to help prevent the PSII quinone acceptors from becoming reduced at high light intensities. PSI can directly reduce  $\text{O}_2$  by the Mehler reaction to produce superoxide radicals that are rapidly converted within the chloroplast to hydrogen peroxide by superoxide dismutase (Fig. 2). Hydrogen peroxide is then detoxified to produce water by ascorbate peroxidase, which oxidizes ascorbate to monodehydroascorbate (Asada, 1999). This photosynthetic electron flow, from water at PSII through PSI to produce the ascorbate peroxidase-generated water, is often termed the water–water cycle or Mehler-peroxidase reaction. In order to maintain



**Fig. 2** Schematic representation of possible interactions between the products of photosynthetic electron transport and carbon assimilation in zooxanthellae and links with the host coral. Absorption of light by the light-harvesting pigments associated with PSI and PSII in the thylakoid membrane produces linear electron transport from water via a series of electron carriers (plastoquinone, PQ; cytochrome,  $b_6/f$ ) to NADP, producing NADPH and a  $\text{H}^+$  electrochemical difference across the membrane. Dissipation of this  $\text{H}^+$  electrochemical difference by movement of  $\text{H}^+$  through the ATPase drives the production of ATP. Within the chloroplast stroma, ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco) catalyses the assimilation of  $\text{CO}_2$  with ribulose 1,5-bisphosphate (RuBP) to form phosphoglyceric acid (PGA), which is then used, together with NADPH and ATP, in the Calvin–Benson cycle to regenerate RuBP and produce triose phosphates that are required for carbohydrate synthesis.  $\text{O}_2$  can be directly photoreduced by electron transfer from PSI to produce superoxide ( $\text{O}_2^-$ ) by the Mehler reaction (MR).  $\text{O}_2^-$  is dismutated by superoxide dismutase (SOD) to hydrogen peroxide, which is detoxified to water by ascorbate peroxidase (APX). There are a number of interactions between the zooxanthellae and host cells. Carbohydrates produced from photosynthesis can be transported from the zooxanthellae across the symbiosome. This transport is thought to be stimulated by the production of amino acids within the host tissue. Host tissues supply the zooxanthellae with  $\text{CO}_2$  and  $\text{HCO}_3^-$ . Conversion of  $\text{HCO}_3^-$  to  $\text{CO}_2$  in the zooxanthellae is catalysed by carbonic anhydrase (CA).

operation of this water–water cycle monodehydroascorbate must be converted back to ascorbate. This is achieved by an electron flux from ferredoxin (Asada, 1999). Alternatively,  $\text{O}_2$  can be reduced in photorespiration (Ort & Baker, 2002). However, zooxanthellae possess a carbon concentrating mechanism to maintain a high  $\text{CO}_2$  concentration in the chloroplast (Leggat *et al.*, 2002), which minimizes the probability of oxygen reduction by ribulose 1,5-bisphosphate carboxylase-oxygenase (Rubisco). Consequently, it is unlikely that photorespiration in these microalgae is a significant sink for  $\text{O}_2$  and, by definition, electrons.

Coral zooxanthellae *in hospite* have been shown to respond rapidly to fluctuations in light, in particular diurnal changes. As light intensity increases towards mid-day, the rate of thermal dissipation of absorbed excitation energy increases, as evidenced by decreases in  $F_v/F_m$ , and  $\sigma_{\text{PSII}}$  (Brown *et al.*, 1999; Hoegh-Guldberg & Jones, 1999; Gorbunov *et al.*, 2001; Jones & Hoegh-Guldberg, 2001; Lesser & Gorbunov, 2001; Levy *et al.*, 2004). Both xanthophyll cycling (Ambarsari *et al.*, 1997; Brown *et al.*, 1999) and PSII reaction center quenching (Gorbunov *et al.*, 2001) have been implicated in this protective regulation. Differences in the estimated rates of electron transport from chlorophyll fluorescence parameters and direct measurements of  $\text{O}_2$  evolution during the day (Lesser & Gorbunov, 2001) might suggest the possibility of an increased operation of a water–water cycle at high light intensities. However, making accurate estimates of electron transport rates in the zooxanthellae *in hospite* is fraught with difficulties, particularly in determining the absolute amount of light absorbed by the symbiotic microalgae (Falkowski *et al.*, 1990) and making the simplistic assumption that 50% of absorbed light is distributed to PSII.

Besides the rapid modifications to their photosynthetic apparatus described above, coral zooxanthellae also exhibit a number of much slower (in the order of days) acclimatory responses to changes in their growth light regimes. Although there have only been a limited number of critical studies, increases in growth light intensity appear to result in reductions in the cellular concentrations of light-harvesting pigments and PSI and PSII reaction centers with a concurrent increase in photoprotective carotenoids (Falkowski & Dubinsky, 1981; Iglesias-Prieto *et al.*, 1992; Iglesias-Prieto & Trench, 1994; Maritorea *et al.*, 2002). In the natural environment, photoacclimation of coral zooxanthellae has been shown to be strongly depth dependent, with decreases in  $\sigma_{\text{PSII}}$  (Lesser & Gorbunov, 2001) and increases in the proportion of photosynthetic pigments associated with thermal dissipation of absorbed excitation energy (Shick *et al.*, 1995; Lesser *et al.*, 2000) occurring as the sea surface is approached. Consequently, decreases in  $\phi^{\text{max}}$  and  $F_v/F_m$  are observed towards the sea surface (Falkowski & Dubinsky, 1981; Ralph *et al.*, 1999; Lesser, 2000; Lesser *et al.*, 2000; Warner *et al.*, 2002), suggesting that photoacclimation results from an increase in light dependent, nonphotochemical quenching processes, assuming there is no photoinactivation or damage to PSII occurring.

Clearly, coral zooxanthellae, as would appear to be the case with all microalgae, possess a range of photoacclimatory processes that operate on different time scales to optimize the use of light for photosynthesis.

### Photoinhibition and photodamage

At high light levels zooxanthellae often absorb considerably more light than can be utilized for photosynthesis, thus creating a potentially hazardous situation in which their photosynthetic apparatus can be photodamaged with a consequent loss in photosynthetic productivity. Photodamage can also occur at light intensities below those normally required to saturate photosynthesis when other environmental factors, such as elevated temperature, limit the ability of zooxanthellae to acquire and assimilate  $\text{CO}_2$ . Under conditions where the rate of excitation exceeds the rate of photosynthesis, the mechanisms for rapidly dissipating excitation energy as heat and through photochemistry to electron acceptors other than  $\text{CO}_2$  will operate to minimize the possibility of photoinactivation and damage to PSII reaction centers. However, these photoprotective mechanisms unavoidably result in reductions in photosynthetic efficiency and productivity of the zooxanthellae (Porter *et al.*, 1989; Ambarsari *et al.*, 1997; Brown *et al.*, 1999; Gorbunov *et al.*, 2001; Jones & Hoegh-Guldberg, 2001). When these mechanisms cannot dissipate the excess excitation energy effectively, then photoinactivation and damage to PSII reaction centers will occur.

Photoinactivation of the PSII reaction center can occur by at least two independent mechanisms, associated with either the acceptor or donor side of PSII. Both result in inhibition of electron transfer through PSII and cause degradation and programmed turnover of the D1 protein. Donor side photoinhibition has not yet been demonstrated to occur in cells operating under natural physiological conditions and is unlikely to be a major feature of coral bleaching. Acceptor side inhibition will occur under high light conditions when the plastoquinone pool is fully reduced causing the plastoquinone-binding ( $\text{Q}_B$ ) site on D1 to be empty for lack of unbound oxidized plastoquinone. Acceptor side inhibition can occur in cells when  $\text{CO}_2$  assimilation is restricted. In this state, the primary quinone acceptor ( $\text{Q}_A$ ) may become abnormally doubly reduced on a second turnover of the reaction center to form  $\text{Q}_A^{2-}$ , as  $\text{Q}_A^-$  cannot transfer an electron to the vacant  $\text{Q}_B$ .  $\text{Q}_A^{2-}$  then becomes protonated to form  $\text{Q}_A\text{H}_2$  and is released from the  $\text{Q}_A$ -binding site on the D1 protein. With the  $\text{Q}_A$  site vacated, excitation of the reaction center chlorophyll ( $\text{P}_{680}$ ) will result in the formation of the radical pair,  $\text{P}_{680}^+$  Pheophytin $^-$  (Diner & Babcock, 1996). Recombination of these radicals can result in the formation of the triplet state of  $\text{P}_{680}$ , which reacts with  $\text{O}_2$  to form singlet oxygen. Singlet oxygen is potentially damaging to proteins, and will react with the D1 protein thus

triggering its degradation (Asada, 1996). If the rate of D1 degradation exceeds the rate of synthesis and insertion of new D1, then a loss of D1, and active PSII reaction centers, will occur (Osmond, 1994). Under excess light conditions, D1 content has been found to decrease in coral zooxanthellae (Warner *et al.*, 1999; Lesser & Farrell, 2004).

When cells experience high levels of excess excitation energy, photodamage can occur at sites in the thylakoid membranes, other than D1, as a result of singlet oxygen generation in the light-harvesting antennae. When a chlorophyll molecule absorbs a photon, it enters the singlet excited state and excitation energy is normally rapidly transferred to neighbouring chlorophyll molecules. Under high light conditions the probability for the formation of the chlorophyll triplet state increases. The triplet state is long lived compared with the singlet state and can interact with oxygen to produce singlet oxygen (Owens, 1996). Singlet oxygen will readily react with compounds containing carbon-carbon double bonds, such as carotenoids, chlorophylls and lipids, to form peroxides (Halliwell, 1991). Peroxidation of membrane components results in membrane dysfunction and disturbed organization. Importantly, peroxidation of chlorophylls results in bleaching.

Operation of the Mehler reaction at high rates may prevent maximal reduction of the PSII quinone acceptors but can result in accumulation of damaging ROS (i.e. superoxide and hydrogen peroxide; Fig. 2; Asada, 1999). Superoxide dismutase and ascorbate peroxidase, key enzymes in detoxification of superoxide and hydrogen peroxide, are found in zooxanthellae (Matta & Trench, 1991). Under natural physiological operating conditions, cells have sufficient capacity of the enzymes required to scavenge these ROS and hence they are rapidly detoxified. However, if the rate of ROS generation exceeds the rate of detoxification, then accumulation of the ROS can result in oxidative damage to a range of cell components, for example, lipids, nucleic acids, proteins, and result in severe physiological malfunction (Asada, 1999). Particularly damaging to chloroplast components are hydroxyl radicals, which are produced by interaction of hydrogen peroxide with reductants, such as reduced transition metals generated by reduction of oxidized transition metals by superoxide (Asada, 1999). Increased operation of the Mehler reaction may occur in zooxanthellae when exposed to elevated temperatures since increases in both the rate of ROS production and the level of enzymes involved in ROS scavenging have been observed (Lesser & Shick, 1989; Shick *et al.*, 1995; Lesser, 1996; Downs *et al.*, 2002). Furthermore, addition of exogenous antioxidants to corals exposed to increased temperature alleviated the temperature-in-

duced inhibition of photosynthesis, bleaching and loss of the zooxanthellae from the host (Lesser, 1996, 1997). Consequently, increased ROS production from the Mehler reaction may be an important factor in elevated temperature-induced coral bleaching. It has been suggested that the increase in ROS with increases in temperature may be the result of photosynthetically induced hyperoxic conditions which, in the presence of UV radiation, will result in the generation of ROS (Lesser, 1996, 1997). However, the direct reduction of O<sub>2</sub> by photosynthetic electron transport would seem to be a far more plausible mechanism to explain thermally induced increased production of ROS.

#### *Possible primary causes of photoinhibition*

Recently, it has been suggested that the lipid composition of the thylakoid membranes in coral zooxanthellae determines their susceptibility to bleaching (Tchernov *et al.*, 2004). Analyses of the kinetics of variable chlorophyll fluorescence have suggested that thermal bleaching is associated with an uncoupling of photosynthetic electron transport in the thylakoids of the zooxanthellae, where the proton electrochemical potential difference across the membrane is dissipated without generating ATP (Tchernov *et al.*, 2004). This would result in an increased rate of electron transport and an increased rate of oxygen reduction, as carbon assimilation is restricted by the lack of ATP. The consequent increased rate of ROS production could induce bleaching. Parallel analyses of the thylakoid lipids, from a range of corals with differing temperature sensitivities to bleaching, revealed that thermally tolerant zooxanthellae have a markedly lower content of the major polyunsaturated fatty acid,  $\Delta 6,9,12,15$ -*cis*-octadecatetraenoic acid relative to  $\Delta 9$ -*cis*-octadecatetraenoic acid (Tchernov *et al.*, 2004). Tchernov *et al.* (2004) argue that elevated concentrations of this polyunsaturated fatty acid will enhance thermal stability of membranes and reduce the susceptibility of the lipids to attack by ROS. Although the differences in the level of polyunsaturated lipid content of thylakoids between zooxanthellae in corals would account for differences in the ability of corals to tolerate increases in temperature, it is quite possible that the uncoupling of electron transport from ATP synthesis could result from peroxidative damage to thylakoid components and is not the primary cause of an increased rate of production of ROS.

Besides the possibility that uncoupling of electron transport from ATP synthesis may produce an increase in ROS production and lead to photobleaching, photoinhibition of photosynthesis and the consequent photobleaching are frequently the terminal effects of

environmental stresses, whose primary sites of perturbation are not the thylakoid membrane. Photoinhibition can result from a decrease in the rate of consumption of the products of photosynthetic electron transport (ATP and NADPH) since accumulation of these products will restrict electron transport. Consequently, decreases in processes such as carboxylation of ribulose 1,5-bisphosphate (RuBP) by Rubisco, regeneration of RuBP by the Calvin cycle, supply of CO<sub>2</sub> to the carboxylation sites or the consumption and transport of carbohydrates out of cells can trigger the onset of photoinhibition (see Fig. 2). As such, in the case of high temperature-induced coral bleaching, it is possible that the primary site of perturbation is a metabolic or transport process. Inhibition at one or more of these primary sites will result in a decrease in the rate of utilization of ATP and NADPH. To date, the primary effects of elevated temperatures that lead to coral bleaching have not been unequivocally identified. Clearly, to understand the mechanistic basis of high temperature induced coral bleaching it is important to identify the site(s) within the corals that are perturbed initially by temperature increase. There are a number of possible candidates for the primary site of perturbation other than the thylakoid membrane, which are examined below.

Rubisco is an attractive candidate site, since an increase in growth temperature has been shown to reduce Rubisco activity of *Symbiodinium* in culture (Lesser, 1996; Leggat *et al.*, 2004). Jones *et al.* (1998) speculated from fluorescence analyses that inhibition of carboxylation of RuBP by Rubisco may be the primary effect of temperature increase in the coral *Stylophora pistillata*. It is possible that Rubisco is damaged directly by ROS that are produced from increased rates of the Mehler reaction. In higher plants, decreases in Rubisco activity and content have been shown to occur as a response to stresses that generate ROS, such as exposure to ozone (Heath, 1996) and UVB radiation (Allen *et al.*, 1998). Consequently, it is reasonable to speculate that ROS generated from the Mehler reaction could produce a similar loss of Rubisco. When leaves are exposed to high doses of UVB, large decreases in CO<sub>2</sub> assimilation and Rubisco activity and content are observed in the absence of any significant effect on PSII photochemistry (Allen *et al.*, 1998). This indicates that PSII photoinhibition is not the primary cause of loss of photosynthetic competence (Allen *et al.*, 1998). Furthermore, a loss of Rubisco and CO<sub>2</sub> assimilation but not photosynthetic electron transport (Allen *et al.*, 1997) suggests that increased ROS production via the Mehler reaction may be directly involved in the loss of Rubisco. However, this is unlikely to be the case for ozone-induced inhibition of photosynthesis in leaves since ozone does not penetrate the cells but reacts with

components of the cell wall and plasma membrane to produce ROS (Heath, 1996). It is well established that preferential loss of Rubisco in leaves of higher plants is a characteristic of the onset of senescence (Thayer *et al.*, 1987). Generation of ROS by ozone at the cell surface is likely to trigger a signalling cascade that will induce changes in gene expression and potentially induce programmed cell death, which would involve proteolytic degradation of Rubisco (Apel & Hirt, 2004). It is possible that ROS generation as a result of absorption of UVB or increased rates of Mehler reaction could trigger a similar response in zooxanthellae.

In higher plants, elevated temperatures deactivate Rubisco as a result of inhibition of the Rubisco activase (Crafts-Brandner & Salvucci, 2000). This decrease in Rubisco activation induced by moderate heat stress correlates with reduced rates of photosynthesis and is accompanied by increased levels of RuBP (Law & Crafts-Brandner, 1999; Crafts-Brandner & Law, 2000; Sharkey *et al.*, 2001; Crafts-Brandner & Salvucci, 2002; Salvucci & Crafts-Brandner, 2004a). Such decreases in photosynthesis cannot be attributed to a direct effect of elevated temperature on electron transport or other enzymes in the Calvin cycle, both of which would result in decreases in RuBP. The thermal properties of Rubisco activase can vary depending on the thermal environment of the plant's growth environment; in Antarctic hairgrass Rubisco activation decreased above 30 °C, whereas in the creosote bush, a desert plant, inactivation occurred above 20 °C (Salvucci & Crafts-Brandner, 2004b). Assuming that the characteristics of Rubisco thermal deactivation can vary in a similar way in zooxanthellae, then the photosynthetic responses of different corals to increases in temperature might be expected to exhibit marked differences. However, zooxanthellae contain form II Rubisco (Morse *et al.*, 1995; Rowan *et al.*, 1996), which differs from the form I found in higher plants and green algae and may have very different kinetic and activation properties to form I Rubisco.

As eluded to above, if inhibition of Rubisco activity in zooxanthellae results in accumulation of RuBP, as is the case in higher plants, then inhibition of other enzymes of the Calvin cycle cannot be implicated as a limitation to photosynthesis at elevated temperatures.

In many situations, photosynthesis can be limited by the rate of supply of CO<sub>2</sub> to the sites of carboxylation (see Fig. 2). Rates of uptake of CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> by zooxanthellae have been found to be different when the cells are free living compared with residing in the host (Goiran *et al.*, 1996; Leggat *et al.*, 2002). Other productivity–light response experiments have implicated that the host environment may limit the supply of CO<sub>2</sub> for zooxanthellae (Muscatine *et al.*, 1989a, b; Fitt &

Cook, 2001). It would seem unlikely that temperature increases would result in decreases in the rate of inorganic carbon diffusion into corals. However, active transport systems are required for inorganic carbon uptake into zooxanthellae cells (Weis *et al.*, 1989; Al-Moghrabi *et al.*, 1996; Leggat *et al.*, 1999, 2002), and these may be adversely affected by elevated temperatures. *Symbiodinium* has a carbon concentrating mechanism which increases the concentration of CO<sub>2</sub> around Rubisco (Leggat *et al.*, 1999). Carbonic anhydrase facilitates the diffusive transport of inorganic carbon to the carboxylation sites by concentrating CO<sub>2</sub> within the cells (Fig. 2). Efficient photosynthesis in zooxanthellae is highly dependent upon this CO<sub>2</sub> concentrating mechanism (Weis *et al.*, 1989), because the form II Rubisco in zooxanthellae has a lower specificity for CO<sub>2</sub> relative to O<sub>2</sub> than form I Rubisco of higher plants (Jordan & Ogren, 1981). Consequently, perturbation of carbonic anhydrase activity could result in reduced CO<sub>2</sub> supply to Rubisco and limit photosynthesis (Weis *et al.*, 1989). However, increases in temperature did not modify significantly the affinity of cultured *Symbiodinium* cells for inorganic carbon, suggesting that the carbon concentrating mechanism was not being adversely affected by increases in temperature (Leggat *et al.*, 2004).

A large proportion of the photosynthate produced by coral zooxanthellae is released to the host cells (Dubinsky & Jokiel, 1994; Dubinsky & Berman-Frank, 2001). Consequently, the host cells can be considered a major sink for photosynthate. Restriction of photosynthate transport out of the zooxanthellae would lead to a build up of photosynthate (Fig. 2), which would result in a feedback inhibition of photosynthetic electron transport. Consequently, photoinhibition and photodamage would follow unless photosynthate was broken down. Release of photosynthate from zooxanthellae cells is stimulated by amino acids (Gates *et al.*, 1995), such as taurine (Wang & Douglas, 1997), which are produced and released by the host cells. Perturbation of the production and release of these signalling amino acids from host cells would lead accumulation of photosynthate in the zooxanthellae.

#### *Bleaching because of expulsion of zooxanthellae from host*

Besides loss of photosynthetic pigments from zooxanthellae, bleaching of corals can occur because of loss of zooxanthellae from the hosts (Hoegh-Guldberg & Smith, 1989; Glynn & D'Croz, 1990; Lesser *et al.*, 1990; Gleason & Wellington, 1993; Brown *et al.*, 1995; Fitt & Warner, 1995; Le Tissier & Brown, 1996). In high temperature-stressed corals, zooxanthellae are often ejected from the hosts by exocytosis (Brown, 1997) or

by shedding of host cells containing zooxanthellae (Gates *et al.*, 1992; Fitt *et al.*, 2001). The mechanisms involved in triggering the expulsion of zooxanthellae from the host are not understood. In animal cells, exocytosis is a highly regulated process that can be mediated by a mitogen-activated protein kinase (MAPK) signalling cascade (Coxon *et al.*, 2003). Hydrogen peroxide is known to activate MAPKs (Jonak *et al.*, 2002; Ramachandran *et al.*, 2002) and play a role in redox signalling in animal cells (Chen *et al.*, 2004; Shiva *et al.*, 2004). Receptor systems that sense hydrogen peroxide and trigger cell regulatory responses have been identified in yeast (Toledano *et al.*, 2004). Hydrogen peroxide can interact with the cysteine thiol groups of the receptor proteins producing sulphur oxidation products that have the potential to induce specific cellular responses (Kim *et al.*, 2002). A potential role for hydrogen peroxide as a signalling molecule is supported by the fact that it is a weak oxidant with a relatively long lifetime in biological systems (Liu & Zweier, 2001), it is soluble in both lipid and aqueous environments and capable of diffusing rapidly through cells and across membranes (Halliwell, 1991; Chen *et al.*, 2003), much more quickly than superoxide (Asada, 1999). Consequently, a possible mechanism for loss of zooxanthellae from coral host cells by exocytosis would be that hydrogen peroxide generated from the Mehler reaction diffuses out of the photo-inhibited zooxanthellae cells into the host. In the host, the hydrogen peroxide interacts with specific receptors that stimulate MAPK activity, which then triggers exocytotic ejection of the zooxanthellae cells from the host. Similarly, in corals that lose host cells containing zooxanthellae, it is quite possible that production of hydrogen peroxide by the zooxanthellae may stimulate the host to shed these cells. During bleaching of the symbiotic sea anemone *Aiptasia* sp. (Dunn *et al.*, 2002), and the Caribbean coral *Monastrea faveolata* (Lesser & Farrell, 2004), host cells exhibited characteristics associated with the onset of apoptosis or necrosis, which would result in release of the zooxanthellae.

#### *Concluding remarks*

Thermal bleaching is inherently associated with an accumulation of excitation pressure within PSII and PSI beyond the ability with which photochemical and nonphotochemical pathways operate in zooxanthellae (Jones *et al.*, 1998). Acclimation to higher growth irradiances can alleviate the rate at which excitation pressure accumulates, and thus decrease susceptibility to temperature-induced breakdown of the various photochemical pathways and constituents (Brown

*et al.*, 2002). However, thermal bleaching of many corals is ultimately the result of the destruction of photosynthetic pigments by ROS. Reasons for the increase in ROS production, as a result of temperature increases, have not yet been unequivocally identified. Inhibition of electron transport will result in singlet oxygen production and consequent peroxidation of chlorophylls. However, increased electron transport to oxygen via the Mehler reaction will generate superoxide and hydrogen peroxide, which can result in production of damaging hydroxyl radicals that would also peroxidate chlorophylls. Where bleaching occurs because of expulsion of the zooxanthellae from the host, hydrogen peroxide production by the zooxanthellae may be a signal that triggers a response in the host cell to eject the zooxanthellae or shed the host cell from the coral.

Unequivocal resolution of the mechanisms involved in the induction of coral bleaching will require a holistic approach. This will require simultaneous monitoring of a range of physiological, biochemical and biophysical processes of the zooxanthellae *in hospite* and the interactions between the host and zooxanthellae in order to resolve the kinetics of changes in specific processes that may be implicated in the triggering of bleaching. Although experiments with isolated zooxanthellae cultures can provide useful information on the nature and operation of the photosynthetic apparatus and its susceptibility to photoinhibition, they are unlikely to resolve definitively the factors that induce photoinhibition and ROS production *in hospite*. It will be important to determine in intact corals the identity of the ROS whose increased production accompanies the initial loss of pigments or zooxanthellae. This may be possible by infiltrating corals with dyes and fluorescence probes sensitive to specific ROS (Fryer *et al.*, 2002).

Identification of the underlying mechanisms involved in the thermal triggering of coral bleaching is likely to be complicated in that a number of different mechanisms may be involved in any given coral depending on physiological state and environmental conditions. Also, the mechanisms may differ between different coral species. This may also be the case between individual cells of a specific coral species, since many corals can contain differing zooxanthellae ecotypes or clades (Rowan & Knowlton, 1995; Rowan *et al.*, 1997), which can vary in their ability to tolerate stresses (Toller *et al.*, 2001). Such variability in acclimatory physiology of zooxanthellae to thermal stress may give rise to heterogeneity of the bleaching within individual corals. Imaging of chlorophyll fluorescence parameters could help identify such variability between zooxanthellae cells in individual corals.

## Acknowledgements

We thank Richard Geider, John Raven and Mike Wilson for stimulating and helpful discussions on topics covered in this review. The Natural Environmental Research Council, UK provided financial support for David J. Suggett (NER/A/S/2000/01237).

## References

- Allen DJ, McKee IF, Farage PK *et al.* (1997) Analysis of the limitation to CO<sub>2</sub> assimilation on exposure of leaves of two *Brassica napus* cultivars to UV-B. *Plant Cell and Environment*, **20**, 633–640.
- Allen DJ, Nogués S, Baker NR (1998) Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? *Journal of Experimental Botany*, **49**, 1775–1788.
- Al-Moghrabi S, Goiran C, Allemand D *et al.* (1996) Inorganic carbon uptake for photosynthesis by symbiotic coral/dinoflagellate associations. II. Mechanisms for bicarbonate uptake. *Journal of Experimental Marine Biology and Ecology*, **199**, 227–248.
- Ambarsari I, Brown BE, Barlow RG *et al.* (1997) Fluctuations in algal chlorophyll and carotenoid pigments during solar bleaching in the coral *Goniastrea aspera* at Phuket, Thailand. *Marine Ecology Progress Series*, **159**, 303–307.
- Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, **55**, 373–399.
- Asada K (1996) Radical production and scavenging in the chloroplasts. In: *Photosynthesis and the Environment* (ed. Baker NR), pp. 123–150. Kluwer Academic Press, Dordrecht.
- Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 601–639.
- Baker NR, Ort DR, Harbinson J *et al.* (2005) Sunlight processing: chloroplast to leaf. In: *Photosynthetic Adaptation: Chloroplast to Landscape* (eds Smith W, Vogelmann T, Critchley C), Springer, New York (in press).
- Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs*, **16**, S129–S138.
- Brown BE, Ambarsari I, Warner ME *et al.* (1999) Diurnal changes in photochemical efficiency and xanthophylls concentrations in shallow water coral reefs: evidence for photoinhibition and photoprotection. *Coral Reefs*, **18**, 99–105.
- Brown BE, Downs CA, Dunne RP *et al.* (2002) Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. *Marine Ecology Progress Series*, **242**, 119–129.
- Brown BE, Le Tissier MDA, Bythell JC (1995) Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. *Marine Biology*, **122**, 655–663.
- Chen K, Thomas SR, Albano A *et al.* (2004) Mitochondrial function is required for hydrogen peroxide-induced growth factor receptor transactivation and downstream signalling. *Journal of Biological Chemistry*, **279**, 35079–35086.
- Chen K, Thomas SR, Keaney JF (2003) Beyond LDL oxidation: ROS in vascular signal transduction. *Free Radical Biology and Medicine*, **35**, 117–132.

- Coxon PY, Rane MJ, Uriate S *et al.* (2003) MAPK-activated protein kinase-2 participates in p38 MAPK-dependent and ERK-dependent functions in human neutrophils. *Cellular Signalling*, **15**, 993–1001.
- Crafts-Brandner SJ, Law RD (2000) Effect of heat stress on inhibition and recovery of ribulose-1,5-bisphosphate carboxylase/oxygenase activation state. *Planta*, **212**, 67–74.
- Crafts-Brandner SJ, Salvucci ME (2000) Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO<sub>2</sub>. *Proceedings of the National Academy of Sciences USA*, **97**, 13430–13435.
- Crafts-Brandner SJ, Salvucci ME (2002) Sensitivity of photosynthesis in a C<sub>4</sub> plant, maize, to heat stress. *Plant Physiology*, **129**, 1773–1780.
- Davies PS (1984) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. *Coral Reefs*, **2**, 181–186.
- Diner BA, Babcock GT (1996) Structure, dynamics, and energy conversion efficiency in photosystem II. In: *Oxygenic Photosynthesis: The Light Reactions* (eds Ort DR, Yocum CF), pp. 213–247. Kluwer Academic Publishers, Dordrecht.
- Downs CA, Fauth JE, Halas JC *et al.* (2002) Oxidative stress and seasonal coral bleaching. *Free Radical Biology and Medicine*, **33**, 53–543.
- Dubinsky Z, Berman-Frank I (2001) Uncoupling primary production from population growth in photosynthesising organisms in aquatic ecosystems. *Aquatic Sciences*, **63**, 4–17.
- Dubinsky Z, Falkowski PG, Porter JW *et al.* (1984) Absorption and utilisation of radiant energy by light- and shade-adapted colonies of the hermatypic coral *Stylophora pistillata*. *Proceedings of the Royal Society of London Series B*, **222**, 203–214.
- Dubinsky Z, Jokiel PL (1994) Ratio of energy and nutrient fluxes regulates symbiosis between zooxanthellae and corals. *Pacific Science*, **48**, 313–324.
- Dunn SR, Bythell JC, Le Tissier MDA *et al.* (2002) Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia* sp. *Journal of Experimental Marine Biology and Ecology*, **272**, 29–53.
- Falkowski PG, Dubinsky Z (1981) Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. *Nature*, **289**, 172–174.
- Falkowski PG, Dubinsky Z, Muscatine L *et al.* (1984) Light and the bioenergetics of a symbiotic coral. *Bioscience*, **34**, 705–709.
- Falkowski PG, Dubinsky Z, Muscatine L *et al.* (1993) Population control in symbiotic corals. *Bioscience*, **43**, 606–611.
- Falkowski PG, Jokiel PJ, Kinzie, III RA (1990) Irradiance and corals. In: *Ecosystems of the World 25: Coral Reefs* (ed. Dubinsky Z), pp. 89–107. Elsevier, Amsterdam.
- Falkowski PG, Raven JA (1997) *Aquatic Photosynthesis*. Blackwell Science, Malden, MA.
- Finazzi G, Johnson GN, Dalosto I *et al.* (2004) A zeaxanthin-independent non-photochemical quenching mechanism localized in the photosystem II core complex. *Proceedings of National Academy of Sciences*, **33**, 12375–12380.
- Fitt WK, Brown BE, Warner ME *et al.* (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs*, **20**, 51–65.
- Fitt WK, Cook CB (2001) Photoacclimation and the effect of the symbiotic environment on the photosynthetic response of symbiotic dinoflagellates in the tropical marine hydroid *Myrionema amboinense*. *Journal of Experimental Marine Biology and Ecology*, **256**, 15–31.
- Fitt WK, Warner ME (1995) Bleaching patterns of four species of Caribbean corals. *Biological Bulletin*, **189**, 298–307.
- Fryer MJ, Oxborough K, Mullineaux PM *et al.* (2002) Imaging of photo-oxidative stress responses in leaves. *Journal of Experimental Botany*, **53**, 1249–1254.
- Gates RD, Baghdasarian G, Muscatine L (1992) Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. *Biological Bulletin*, **182**, 324–332.
- Gates RD, Hoegh-Guldberg GO, McFallngai MJ *et al.* (1995) Free amino-acids exhibit anthozoan host factor activity – they induce release of photosynthate from symbiotic dinoflagellates in vitro. *Proceedings of the National Academy of Sciences USA*, **92**, 7430–7434.
- Genty B, Harbinson J (1996) Regulation of light utilization for photosynthetic electron transport. In: *Photosynthesis and the Environment* (ed. Baker NR), pp. 67–99. Kluwer Academic Publishers, Dordrecht.
- Gleason DF, Wellington GM (1993) Ultraviolet radiation and coral bleaching. *Nature*, **365**, 836–838.
- Glynn PW (1996) Coral reef bleaching: facts, hypothesis and implications. *Global Change Biology*, **2**, 495–509.
- Glynn PW, D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El-Nino-coincident coral mortality. *Coral Reefs*, **12**, 1–17.
- Goiran C, Al-Moghrabi S, Allemand D *et al.* (1996) Inorganic carbon uptake for photosynthesis by symbiotic coral/dinoflagellate associations. I. Photosynthetic performances of symbionts and dependence on sea water bicarbonate. *Journal of Experimental Marine Biology and Ecology*, **199**, 207–225.
- Gorbunov MY, Kolber ZS, Lesser MP *et al.* (2001) Photosynthesis and photoprotection in symbiotic corals. *Limnology and Oceanography*, **46**, 75–85.
- Halliwel B (1991) Oxygen radicals: their formation in plant tissues and their role in herbicide damage. In: *Herbicides* (eds Baker NR, Percival MP), pp. 87–129. Elsevier Science Publishers B.V., Amsterdam.
- Heath RL (1996) The modification of photosynthetic capacity induced by ozone exposure. In: *Photosynthesis and the Environment* (ed. Baker NR), pp. 469–476. Kluwer Academic Publishers, Dordrecht.
- Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reef. *Marine and Freshwater Research*, **50**, 839–866.
- Hoegh-Guldberg O, Jones RJ (1999) Photoinhibition and photoprotection in symbiotic dinoflagellates from reef building corals. *Marine Ecology Progress Series*, **183**, 73–86.
- Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. *Journal of Experimental Marine Biology and Ecology*, **129**, 279–303.

- Iglesias-Prieto R, Matta JL, Robins WA *et al.* (1992) Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. *Proceedings of the National Academy of Sciences USA*, **89**, 10302–10305.
- Iglesias-Prieto R, Trench RK (1994) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. *Marine Ecology Progress Series*, **113**, 163–175.
- Jonak C, Ökresz L, Bögre L *et al.* (2002) Complexity, cross talk and integration of plant MAP kinase signalling. *Current Opinion in Plant Biology*, **5**, 415–424.
- Jones RJ, Hoegh-Guldberg O (2001) Diurnal changes in the photochemical efficiency of the symbiotic dinoflagellates (Dinophyceae) of corals: photoprotection, photoinactivation and the relationship to coral bleaching. *Plant Cell and Environment*, **24**, 89–99.
- Jones RJ, Hoegh-Guldberg O, Larkum AWD *et al.* (1998) Temperature-induced bleaching of corals begins with impairment of the CO<sub>2</sub> fixation mechanism in zooxanthellae. *Plant Cell and Environment*, **21**, 1219–1230.
- Jordan DB, Ogren WL (1981) Species variation in the specificity of ribulose biphosphate carboxylase/oxygenase. *Nature*, **291**, 513–515.
- Kim SO, Merchant K, Nudelman R *et al.* (2002) OxyR: a molecular code for redox signaling. *Cell*, **109**, 383–396.
- Law RD, Crafts-Brandner SJ (1999) Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant Physiology*, **120**, 173–181.
- Le Tissier MDA, Brown BE (1996) Dynamics of solar bleaching in the intertidal reef coral *Goniastrea aspera* at Ko Phuket, Thailand. *Marine Ecology Progress Series*, **136**, 235–244.
- Leggat W, Badger MR, Yellowlees D (1999) Evidence for an inorganic carbon-concentrating mechanism in the symbiotic dinoflagellate *Symbiodinium* sp. *Plant Physiology*, **121**, 1247–1255.
- Leggat W, Marendy EM, Braillie B *et al.* (2002) Dinoflagellate symbioses: strategies and adaptations for the acquisition and fixation of inorganic carbon. *Functional Plant Biology*, **29**, 309–322.
- Leggat W, Whitney S, Yellowlees D (2004) Is coral bleaching due to the instability of the zooxanthellae dark reactions? *Symbiosis*, **37**, 137–153.
- Lesser MP (1996) Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnology and Oceanography*, **41**, 271–283.
- Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. *Coral Reefs*, **16**, 187–192.
- Lesser MP (2000) Depth-dependent photoacclimatization to solar radiation in the Caribbean coral *Montastraea faveolata*. *Marine Ecology Progress Series*, **192**, 137–151.
- Lesser MP (2004) Experimental biology of coral reef systems. *Journal of Experimental Marine Biology and Ecology*, **300**, 217–252.
- Lesser MP, Farrell JH (2004) Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs*, **23**, 367–377.
- Lesser MP, Gorbunov MY (2001) Diurnal and bathymetric changes in chlorophyll fluorescence yields of coral reefs measured in situ with a fast repetition rate fluorometer. *Marine Ecology Progress Series*, **212**, 69–77.
- Lesser MP, Mazel CM, Phinney D *et al.* (2000) Light absorption and utilization by colonies of the congeneric hermatypic corals *Montastraea faveolata* and *Montastraea cavernosa*. *Limnology and Oceanography*, **45**, 76–86.
- Lesser MP, Shick JM (1989) Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymatic defense against oxygen toxicity. *Marine Biology*, **102**, 243–255.
- Lesser MP, Stochaj WR, Tapley DW *et al.* (1990) Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. *Coral Reefs*, **8**, 225–232.
- Levy O, Dubinsky Z, Schneider K *et al.* (2004) Diurnal hysteresis in coral photosynthesis. *Marine Ecology Progress Series*, **268**, 105–117.
- Liu XP, Zweier JL (2001) A real-time electrochemical technique for measurement of cellular hydrogen peroxide generation and consumption: evaluation in human polymorphonuclear leukocytes. *Free Radical Biology and Medicine*, **31**, 894–901.
- Maritorena S, Payri C, Claustre H *et al.* (2002) Photoacclimatization in the zooxanthellae of *Pocillopora verrucosa* and comparison with a pelagic algal community. *Oceanologica Acta*, **25**, 125–134.
- Matta JL, Trench RK (1991) The enzymatic response of the symbiotic dinoflagellate *Symbiodinium microadriaticum* (Freudenthal) to growth in vitro under varied oxygen tensions. *Symbiosis*, **11**, 31–45.
- Morse D, Salois P, Markovic P *et al.* (1995) A nuclear-encoded form II RuBisCo in dinoflagellates. *Science*, **268**, 1622–1624.
- Muller-Parker G, D'Elia CF (1997) Interactions between corals and their symbiotic algae. In: *Life and Death of Coral Reefs* (ed. Birkeland C), pp. 96–113. Chapman & Hall, New York.
- Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: *Ecosystems of the World 25: Coral Reefs* (ed. Dubinsky Z), pp. 89–107. Elsevier, Amsterdam.
- Muscatine L, Falkowski PG, Dubinsky YZ *et al.* (1989a) The effect of external nutrient resources on the population-dynamics of zooxanthellae in a reef coral. *Proceedings of the Royal Society of London Series B*, **236**, 311–324.
- Muscatine L, Porter JW, Kaplan IR (1989b) Resource partitioning by coral reefs as determined from stable isotope composition. 1.  $\Delta C^{13}$  of zooxanthellae and animal tissue versus depth. *Marine Biology*, **100**, 185–193.
- Ort DR, Baker NR (2002) A photoprotective role of O<sub>2</sub> as an alternative electron sink in photosynthesis. *Current Opinion in Plant Biology*, **5**, 193–198.
- Osmond CB (1994) What is photoinhibition? Some insights from comparisons of shade and sun plants. In: *Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field* (eds Baker NR, Bowyer JR), pp. 1–24. BIOS Scientific Publishers, Abingdon, UK.
- Owens TG (1996) Processing of excitation energy by antenna pigments. In: *Photosynthesis and the Environment* (ed. Baker NR), pp. 1–23. Kluwer Academic Publishers, Dordrecht.

- Podesta GP, Glynn PW (2001) The 1997–98 El-Nino event in Panama and Galapagos: an update of thermal stress indices relative to coral bleaching. *Bulletin of Marine Science*, **69**, 43–59.
- Porter JW, Fitt WK, Spero HJ *et al.* (1989) Bleaching in reef corals: physiological and stable isotopic responses. *Proceedings of the National Academy of Sciences USA*, **86**, 9342–9346.
- Ralph PJ, Gademann R, Larkum AWD *et al.* (1999) In situ underwater measurements of photosynthetic activity of coral zooxanthellae and other reef dwelling dinoflagellate endosymbionts. *Marine Ecology Progress Series*, **180**, 139–147.
- Ramachandran A, Moellering D, Go YM *et al.* (2002) Activation of a c-Jun N-terminal kinase and apoptosis in endothelial cells mediated by endogenous generation of hydrogen peroxide. *Biological Chemistry*, **383**, 693–701.
- Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proceedings of the National Academy of Sciences USA*, **92**, 2850–2853.
- Rowan R, Knowlton N, Baker A *et al.* (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, **388**, 265–269.
- Rowan R, Whitney SM, Fowler A *et al.* (1996) Rubisco in marine symbiotic dinoflagellates: form II enzymes in eukaryotic encoded in a multigene family. *The Plant Cell*, **8**, 539–553.
- Salvucci ME, Crafts-Brandner SJ (2004a) Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiologia Plantarum*, **120**, 179–186.
- Salvucci ME, Crafts-Brandner SJ (2004b) Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. *Plant Physiology*, **134**, 1460–1470.
- Sharkey TD, Badger MR, von Caemmerer S *et al.* (2001) Increased heat sensitivity of photosynthesis in tobacco plants with reduced Rubisco activase. *Photosynthesis Research*, **67**, 147–156.
- Shick JM, Lesser MP, Dunlap WC *et al.* (1995) Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. *Marine Biology*, **122**, 41–51.
- Shiva S, Moellering D, Ramachandran A *et al.* (2004) Redox signalling: from nitric oxide to oxidised lipids. *Biochemical Society Symposium*, **71**, 107–120.
- Stanley GD, Swart PK (1995) Evolution of the coral-zooxanthella symbiosis during the Triassic: a geochemical approach. *Paleobiology*, **22**, 179–199.
- Tchernov D, Gorbunov MY, de Vargas C *et al.* (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences USA*, **101**, 13531–13535.
- Thayer SS, Choe HT, Tang A *et al.* (1987) Protein turnover during senescence. In: *Plants Senescence: Its Biochemistry and Physiology* (eds Thomson WW, Nothnagel EA, Huffaker RC), pp. 71–80. The American Society of Plant Physiologists, Rockville.
- Toledano MB, Delaunay A, Monceau L *et al.* (2004) Microbial H<sub>2</sub>O<sub>2</sub> sensors as archetypical redox signalling molecules. *Trends in Biochemical Sciences*, **29**, 351–357.
- Toller WW, Rowan R, Knowlton N (2001) Repopulation of zooxanthellae in the Caribbean corals *Monastrea annularis* and *M. faveolata* following experimental and disease associated bleaching. *Biological Bulletin*, **201**, 360–373.
- Trench RK (1979) The cell biology of plant–animal symbiosis. *Annual Reviews of Plant Physiology*, **30**, 485–531.
- Wang JT, Douglas AE (1997) Nutrients, signals, and photosynthate release by symbiotic algae – the impact of taurine on the dinoflagellate alga *Symbiodinium* from the sea anemone *Aiptasia pulchella*. *Plant Physiology*, **114**, 631–636.
- Warner ME, Chilcoat GC, McFarland FK *et al.* (2002) Seasonal fluctuations in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building coral *Monastrea*. *Marine Biology*, **141**, 31–38.
- Warner ME, Fit WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proceedings of the National Academy of Sciences USA*, **96**, 8007–8012.
- Weis VM, Smith GJ, Muscatine L (1989) A “CO<sub>2</sub> supply” mechanism in zooxanthellae cnidarians: role of carbonic anhydrase. *Marine Biology*, **100**, 195–202.
- Wilkinson C (2002) *Status of Coral Reefs of the World: 2002*. Australian Institute of Marine Science, Queensland.
- Wyman KD, Dubinsky Z, Porter JW *et al.* (1987) Light absorption and utilisation among hermatypic corals: a study in Jamaica, West Indies. *Marine Biology*, **96**, 283–292.