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Testing the 'photoinhibition' model of coral bleaching using chemical inhibitors

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ABSTRACT: Explants of the hard coral *Seriatopora hystrix* were exposed to sublethal concentrations of the herbicide diuron DCMU (*N'*-(3,4-dichlorophenyl,-*N*,*N*-dimethylurea)) and the heavy metal copper. Pulse amplitude modulated (PAM) chlorophyll fluorescence techniques were used to assess the effects on the photosynthetic efficiency of the algal symbionts in the tissue (*in symbio*), and chlorophyll fluorescence and counts of symbiotic algae (normalised to surface area) were used to assess the extent of coral bleaching. At 30 µg DCMU l^{-1}, there was a reduction in both the maximum effective quantum yield ($\Delta F/F_m'$) and maximum potential quantum yield (F_v/F_m) of the algal symbionts *in symbio*. Corals subsequently lost their algal symbionts and discoloured (bleached), especially on their upper sunlight-exposed surfaces. At the same DCMU concentration but under low light (5% of growth irradiance), there was a marked reduction in $\Delta F/F_m'$ but only a slight reduction in F_v/F_m and slight loss of algae. Loss of algal symbionts was also noted after a 7 d exposure to concentrations as low as 10 µg DCMU l^{-1} under normal growth irradiance, and after 14 d exposure to 10 µg DCMU l^{-1} under reduced irradiance. Collectively the results indicate that DCMU-induced bleaching is caused by a light-dependent photoinactivation of algal symbionts, and that bleaching occurs when F_v/F_m (measured 2 h after sunset) is reduced to a value of $\leqslant$0.6. Elevated copper concentrations (60 µg Cu l^{-1} for 10 h) also induced a rapid bleaching in *S. hystrix* but without affecting the quantum yield of the algae *in symbio*. Tests with isolated algae indicated that substantially higher concentrations (300 µg Cu l^{-1} for 8 h) were needed to significantly reduce the quantum yield. Thus, copper-induced bleaching occurs without affecting the algal photosynthesis and may be related to effects on the host (animal). It is argued that warm-water bleaching of corals resembles both types of chemically induced bleaching, suggesting the need for an integrated model of coral bleaching involving the effect of temperature on both host (coral) and algal symbionts.

KEY WORDS: Coral · Coral bleaching · Symbiotic dinoflagellate · Diuron · Copper · Antifouling paint · Herbicide

INTRODUCTION

Several models have been proposed for the mechanism of coral bleaching (the dissociation of the coral–algal symbiosis that occurs during heat stress)—for recent reviews see Brown (1997), Hoegh-Guldberg (1999) and Douglas (2003). Gates et al. (1992) noted that a dysfunction in animal (host) cell adhesion occurs during temperature shock, releasing symbiotic algae still within the host-cell membrane. The underlying cause was thought to be a thermotropic phase-transition in the host membrane and a passive influx of Ca^{2+} ions which could then collapse the cytoskeleton and perturb cell adhesion (Gates et al. 1992). At the same time, using chlorophyll fluorescence and oxygen evolution techniques, Iglesias-Prieto et al. (1992) first identified the sensitivity of photosynthesis of cultured symbiotic dinoflagellates to elevated temperature. Their observations, which were later supported by work with the intact coral–algal association (*in symbio*; Fitt & Warner 1995, Warner et al. 1996, Jones et al. 1998, Brown et al. 2002), suggested that a temperature-related weak point in the photophysiology of the algal symbionts is the cause of bleaching.

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Fitt et al. (2001) recently questioned whether the shedding of host cells containing symbiotic algae is prevalent in the field at current sea-surface temperatures and whether this type of response may be associated with experimental laboratory protocols that involve rapid and large changes in temperature. Also, in subsequent studies with the anemone *Aiptasia pallida* and the hard coral *Pocillopora damicornis*, Sawyer & Muscatine (2001) found no evidence for a phase-transition or bleaching induced by a flux of calcium ions, and subsequently suggested that bleaching is caused by a temperature-induced alteration in protein phosphorylation. Most of the recent work on the mechanism of coral bleaching has been concerned with the algal symbionts and their response to light, because of the possibility that coral bleaching is caused by a form of photoinhibition.

Several studies thus examined diel changes in chlorophyll fluorescence in symbiotic dinoflagellates *in symbio* in efforts to establish what occurs under 'normal' conditions *in situ* (Brown et al. 1999, Hoegh-Guldberg & Jones 1999, Ralph et al. 1999, Gorbunov et al. 2001). The studies confirm a range of photoprotective mechanisms including non-photochemical quenching, the operation of a xanthophyll cycle, as well as reduction in the functional absorption cross-section for Photosystem II (PSII). Some slightly unusual night-time changes in chlorophyll fluorescence were identified which were thought to be associated with tissue hypoxia, chlororespiration and state transitions (Jones & Hoegh-Guldberg 2001). However, the overall conclusion of these studies was that the symbiotic dinoflagellates routinely experience photoinhibition similar to other marine photoautotrophs (Brown et al. 1999, Hoegh-Guldberg & Jones 1999, Ralph et al. 1999, Gorbunov et al. 2001)

In the context of these studies, the term 'photoinhibition' was used to reflect light-dependent and often rapidly reversible changes in chlorophyll fluorescence associated with a 'downregulation' of photosynthesis, i.e. to a suite of processes operating in both the short- and long-term to divert excess light away from the PSII reaction centre and prevent damage to the photosynthetic apparatus (for review see Long & Humphries 1994). These processes are mostly associated with events occurring in the chlorophyll antennae (Osmond et al. 1999). Photoinhibition as photodamage of PSII reaction centres is a longer-lasting process, occurring when photoprotective processes are largely exhausted. It is more closely associated with events within the reaction centre complex and associated with an enhanced rate of degradation and turnover of the reaction centre D1 (Osmond et al. 1999). Recently, Osmond et al. (1999) revised the dichotomy of 'dynamic' and 'chronic' photoinhibition in favour of the terms 'photoprotection' and 'photoinactivation' to describe these 2 distinct types of photoinhibition.

Both photoprotection and photoinactivation can operate at the same time, and the balance between the two is dynamic and can be influenced by environmental conditions, especially temperature (Franklin 1994). Synthesizing the information up to that point, and based also on studies with the inhibitor cyanide (Jones & Hoegh-Guldberg 1999), the 'Photoinhibition model' of coral bleaching was proposed (Jones et al. 1998, Hoegh-Guldberg 1999, Jones & Hoegh-Guldberg 2001). The essence of this ecophysiological model is that normal short-term photoprotective downregulation of photosynthesis *in symbio* is gradually replaced with a longer-term photoinactivation during conditions of elevated seawater temperature. Light is a crucial modulating factor associated with the response. This model offers a mechanistic explanation of the known interaction between light and temperature in the coral bleaching response described by earlier experimental work (Jokiel & Coles 1977, Coles & Jokiel 1978). Similarly, the model also offers an explanation for the numerous *in situ* observations that corals preferentially bleach on their upper sunlight-exposed surfaces (for example Harriott 1985, Oliver 1985). The model indicates why the ratio of variable (F_v) to maximal fluorescence (F_m) (correlated to the quantum yield of photosynthesis; Bjorkman & Demmig-Adams 1987) is reduced in corals after bleaching events (Warner et al. 1999, Jones et al. 2000), and why there is a loss of D1 reaction centre protein (the subunit that constitutes half of the heterodimeric reaction centre of PSII) in bleached corals (Warner et al. 1999).

Associated with the model was the suggestion that the initial impairment of photosynthesis in the algae is due to the effect of temperature on enzymes of the Calvin-Benson cycle or to the CO_2 acquisition process, i.e. sink imitation (Jones et al. 1998, Hoegh-Guldberg 1999). Experimental support for this has been provided by work with chemical inhibitors and cold stress (Jones & Hoegh-Guldberg 1999, Saxby et al. 2003). However, arguments have also been made that other sites in photosynthesis are the most susceptible, including the water-splitting apparatus (Iglesias-Prieto 1997) and the D1 protein repair mechanism (Warner et al. 1999).

Oxidative stress has also been implicated as the cause of coral bleaching, originating from observations that symbiotic cnidarians routinely experience elevated photosynthetically produced oxygen within their tissues (Dykens & Shick 1982, 1984). It has been suggested that physiological hyperoxia and exposure to UV radiation act synergistically with elevated temperatures to produce reduced oxygen species (ROS) in the symbiotic dinoflagellates (Lesser et al. 1990, Lesser 1996). Oxidative stress can cause damage to photosyn-

thesis at the D1 protein (Tschiersch & Ohmann 1993) as well as other sites of photosynthesis, including ribulose bisphosphate carboxylase/oxygenase (RUBISCO; Asada & Takahashi 1987), and DNA, proteins and membranes (see Lesser et al. 1990, Lesser 1996, 1997). Support for the involvement of oxidative stress in coral bleaching also comes from work with antioxidant enzymes, which has shown that PSII photochemistry can be improved by their addition (Lesser et al. 1990, Lesser 1996, 1997).

It follows that the thermal sensitivity of symbiotic dinoflagellates *in vivo* and *in symbio* and the photoinhibition model of coral bleaching may have their origins in oxidative stress. However, Dykens & Shick (1982) noted that the activity of the antioxidant enzyme superoxide dismutase (SOD) decreases in the anemone *Anthopleura elegantissima* treated with the photosynthetic inhibitor DCMU (*N'*-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea), or when held in darkness. Thus, paradoxically, once photosynthesis is reduced during heat stress, so should the level of oxidative stress decrease. Lesser (1996) argued that despite a reduction in algal photosynthesis, it does not cease entirely, and that tissues will still be hyperoxic with respect to the surrounding medium. Nevertheless, a decrease in algal photosynthesis, or an increase in host and algal respiration in response to heat stress, should serve to regulate photosynthetically produced ROS production. Nii & Muscatine (1997) offered a possible solution, showing that both symbiotic and aposymbiotic anemones *Aiptasia pulchella* produced superoxide radicals in response to thermal stress, and that neither light nor inhibition of photosynthesis by DCMU affected superoxide production. The implication was that oxidative stress may originate in the host (i.e. an animal response), caused by increased electron leakage from mitochondrial electron transport as a result of temperature-dependent increases in the respiration rate (Nii & Muscatine 1997).

Whether a temperature-related reduction in algal photosynthesis is a cause or an effect of oxidative stress during coral bleaching, or whether the host or the algae are the initial source of the ROS, is presently unknown. The extent of transport of active oxygen species between partners, the exact nature of the transported products, and the extent to which transport in either direction is facilitated by heat-induced changes in membrane structure is also poorly understood. This study has tested the photoinhibition model of coral bleaching with the herbicide DCMU and the heavy metal copper. The work supplements a more widespread examination of the ecotoxicological effects of antifouling paint biocides and herbicides to corals (see Jones et al. 2003 and Jones & Kerswell 2003).

MATERIALS AND METHODS

Experiments were conducted at the Heron Island Research Station (23° 26′ S, 151° 55′ E), in the Capricorn-Bunker group of reefs (Great Barrier Reef, Australia), with small multibranched fragments of *Seriatopora hystrix* (Dana, 1846; family Pocilloporidae) collected from colonies located at the top of the protected reef slope (1 to 2 m depth). The colony collection, handling and manipulation procedures have been described previously (Jones & Kerswell 2003). Before experiments, all fragments were held for at least 6 d in a holding tank receiving a supply of running seawater. Experiments were conducted indoors under artificial light or outdoors under natural sunlight. In experiments outdoors, light levels were reduced using neutral-density shade-cloth. Light levels (photosynthetically active radiation, PAR 400 to 700 nm) were measured using a LI-190SA quantum sensor and recorded at 10 min intervals using a data logger (Dataflow Systems). All seawater used in the tests was filtered to 1 µm (nominal levels) using a polypropylene filter cartridge (see Jones et al. 2003 for details). During all experiments, water temperatures were checked episodically and ranged from 23 to 27°C. DCMU stock solutions were made up in acetone, and in all experiments control corals received an equivalent volume of the carrier solvent. All test containers were aerated and small submersible pumps provided water motion.

Seriatopora hystrix explants were exposed to 30 µg DCMU l^{-1} or control conditions either under the growth irradiance (approximately 50% of surface irradiation, i.e. maximum of ~800 to 1000 µmol quanta m^{-2} s^{-1} PAR) or under reduced irradiance (5% surface irradiation) for 7 d before being transferred to flowing seawater for a 7 d monitoring period under an irradiance of 25% of surface irradiance. All experiments were conducted in glass aquaria that were semi-submerged within a larger 500 l container receiving a supply of running seawater for temperature control (i.e. a water bath). Solutions were replaced every second day. Duplicate tanks were used for each light regime at each treatment (i.e. with or without DCMU) and 5 branches were used in each aquaria. Aquaria were randomly located within the water bath. The chlorophyll fluorescence parameter $\Delta F/F_m'$ (maximum effective quantum yield) was measured at solar noon, and the parameter F_v/F_m (maximum potential quantum yield) was measured 2 h after sunset each day (see below).

At the end of the 14 d experiment, a 3 cm long branch was cut from each of the fragments and the branches were dark-adapted (placed in darkness) for 2 h. The chlorophyll fluorescence parameter F_o was then determined using a DIVING-PAM fluorometer in combination with a mini-fibre-optic probe (2 mm dia-

meter), at 4 points, at 0.5 mm intervals from the tip to the base of the branch and along both the upper and lower surfaces. F_o values were averaged to provide a measure of the fluorescence on the upper surface, the lower surface, the whole branch (i.e. all 8 readings) and at the 4 reference points along the branch from the tip to the base. Chlorophyll florescence was then used as a proxy for the density of symbiotic dinoflagellates remaining in the tissues (see below for chlorophyll fluorescence determination). The explant was then frozen for counts of algal density (see below).

Seriatopora hystrix fragments (n = 10) were exposed to 1 and 10 µg DCMU l^{-1} or control conditions under 50% surface irradiance using the same procedures described above. $\Delta F/F_m'$ and F_v/F_m were measured for the 7 d exposure period as described previously. The corals were then returned to running seawater for a further 7 d before being frozen for counts of algal density.

Seriatopora hystrix fragments were exposed to 10 µg DCMU l^{-1} or control conditions for 14 d under artificial light (100 µmol quanta m^{-2} s^{-1} PAR) provided by cool-white fluorescent lights (12:12 h light:dark [L:D] cycle). $\Delta F/F_m'$ was measured at 12:00 h, and F_v/F_m was measured in the evening (2 h after the lights were turned off each day). Solutions were replaced every other day, and after 14 d the corals were frozen for counts of algal density. Corals were also held in darkness over the duration of the experiment and sub-sampled on Days 7 and 14 for counts of algal density.

To examine the rate at which algae are expelled from *Seriatopora hystrix* fragments in response to chemical inhibitors, a time-course experiment was conducted whereby corals were exposed to relatively high concentrations of DCMU or copper, and the incubation water was episodically tested for the presence of algae using fluorescence techniques. Firstly, 3 *S. hystrix* fragments were exposed to 60 µg Cu l^{-1} for 10 h in individual containers with 200 ml of filtered seawater. Periodically, any expelled algae that collected in the container were concentrated by centrifugation, and counted using a haemacytometer. Known volumes of the algal concentrate were then pipetted into 10 ml of filtered seawater and the sample was shaken for 30 s. A 1 ml subsample was then placed into a 2.5 ml quartz cuvette inside the emitter-detector unit of a WATER-PAM chlorophyll fluorometer. The baseline fluorescence F_o was then determined and the relationship between algal density as (cells ml^{-1}) and F_o (arbitrary units) was established. This relationship was then used to calculate the number of algae expelled from corals exposed to 60 µg l^{-1} Cu or DCMU, or control conditions, during a 10 h incubation in individual glass beakers containing 200 ml of test solution. Containers were stirred using magnetically coupled stir bars and experiments were conducted under artificial lights (Colour 11 fluorescent light, Osram) at 100 µmol quanta m^{-2} s^{-1} PAR. Testing for the presence of expelled algae was conducted after 2, 3, 6, 8, and 10 h. The number of algae expelled was normalised to the surface area of the coral (see below). $\Delta F/F_m'$ of the algae *in symbio* was measured after the 10 h exposure and F_v/F_m was measured after 24 and 48 h in running seawater and after 3 d in flowing seawater; corals were then frozen for counts of algal density.

The effects of elevated copper concentrations were also examined using symbiotic dinoflagellates isolated from the coral *Heliofungia actiniformis* growing on the reef flat at Heron Island. Methodological procedures for toxicity testing with isolated algae, including collection and preparation of algal suspensions, have been described in Jones & Heyward (2003). Briefly, tentacles (n = 3) were homogenised, passed through mesh filters of various sizes, and repeatedly centrifuged and washed to remove the host tissues. Toxicity tests were conducted in 3 ml-capacity glass vials with 2 ml of test medium (control or 7 concentrations of copper in the range 1 to 1000 µg Cu l^{-1}). All vials were placed randomly on illuminated photographic slide viewers to provide illumination of ~30 µmol quanta m^{-2} s^{-1} PAR, and a 20 µl aliquot of concentrated algal suspension was added to give a final density in the vial of 1×10^3 cells ml^{-1}. Incubations were conducted at 24 ± 1°C, and after 10 h $\Delta F/F_m'$ of the algae was measured in a WATER-PAM fluorometer, as described in Jones & Heyward (2003). We used 4 replicate vials at each control or copper concentration.

Coral tissues were stripped from the skeletons with a jet of ~100 ml of re-circulated filtered seawater using a WaterPik™, and the number of symbiotic dinoflagellates in 10 ml aliquots of the homogenate was determined with a haemacytometer (using the techniques outlined in Jones et al. 2003). The density of symbiotic algae was expressed as no. cm^{-2}, and the surface area was determined using the paraffin-wax adhesion technique (Stimson & Kinzie 1991).

Chlorophyll fluorescence measurements *in symbio* were measured using a DIVING-PAM chlorophyll fluorometer (Walz) with a 2 mm fibre-optic cable (see Jones & Kerswell [2003] for procedures and instrument settings). Chlorophyll fluorescence measurements of expelled symbiotic dinoflagellates (*in vitro*) were made with a WATER-PAM chlorophyll fluorometer (Walz).

RESULTS

$\Delta F/F_m'$ in corals exposed to 30 µg DCMU l^{-1} was reduced to ~0.05 under growth irradiance and to ~0.1–0.2 in corals exposed to DCMU under low light (Fig. 1A). When corals were returned to flowing sea-

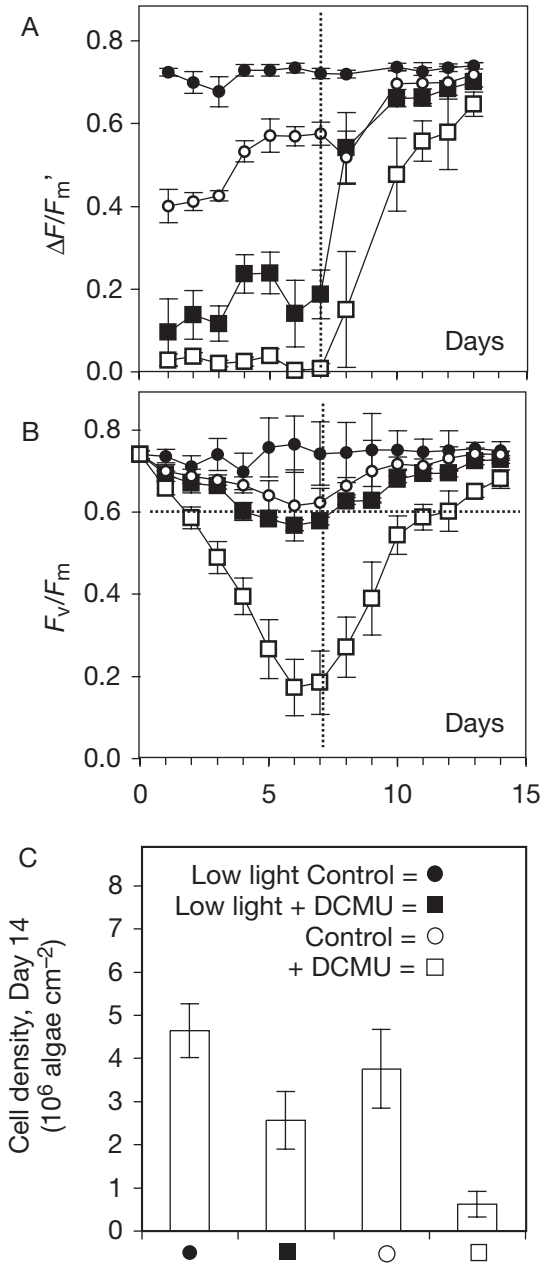

Fig. 1. *Seriatopora hystrix*. (A) Maximum effective quantum yield ($\Delta F/F_m'$) and (B) maximum potential quantum yield (F_v/F_m) of symbiotic dinoflagellates (*in symbio*) in coral exposed to 30 µg DCMU l^{-1} or control conditions under growth irradiance or low light (5% of growth irradiance) for 7 d before return to flowing seawater (vertical dotted line marks return); (C) symbiotic algal cell density at end of 7 d recovery period (Day 14). All data are mean ± SD; n = 10 corals

water under an irradiance of 25% of the surface irradiance, $\Delta F/F_m'$ recovered to ~0.6–0.7 in both treatments by Day 14. In control corals, $\Delta F/F_m'$ ranged between 0.4 and 0.5 in the higher of the 2 light treatments, compared with ~0.7 in the lower light treatment. During the recovery period, $\Delta F/F_m'$ was the same (~0.7) in both treatments at Day 14. F_v/F_m in corals exposed to DCMU in the higher light treatment systematically declined during the exposure period, from 0.7 to <0.2 (Fig. 1B). During the recovery period, F_v/F_m recovered in an initial rapid phase (until t = 10 d), and then in a secondary slower phase until the end of the experiment. In control corals exposed to the higher light treatment and in corals exposed to DCMU under the lower light treatment, F_v/F_m declined from 0.7 to 0.55–0.6, and then returned slowly to 0.7 during the recovery period (Fig. 1B). In control corals held in the lower light treatment, F_v/F_m remained at 0.7 over the duration of the experiment.

Tissue discolouration (bleaching) was observed in corals exposed to 30 µg DCMU l^{-1} in the higher of the 2 light treatments. Bleaching was first perceptible at t = 4 d, and corals continued to discolour until after the first few days of the recovery period. Initially, discolouration was more pronounced on the upper sunlight-exposed surfaces than the lower (shaded) surfaces (see arrows in Fig. 2A). At the end of the recovery period, some of the corals were nearly bone-white, with only a slight residual colouration on the lower shaded surfaces of the branches (Fig. 2B). Colonies exposed to the same DCMU concentrations but under reduced irradiances retained their normal pigmentation (Fig. 2B). At the end of the 14 d recovery period, the density of symbiotic algae in the DCMU-exposed corals was significantly different from the respective control corals for both light treatments, corresponding to a loss of 40 and 85% of the algae respectively (Fig. 1C).

The baseline fluorescence level (F_o, a proxy for symbiotic dinoflagellate density, see Fig. 3A), in the bleached corals (exposed to DCMU in the higher of the 2 light treatments) was ~35% of the level in the normally pigmented control corals incubated under the same light treatment (Fig. 3B). In the bleached corals, F_o on the upper surfaces was <50% of that on the lower surfaces (Fig. 3C), whilst in the normally pigmented corals F_o were directly comparable between surfaces.

$\Delta F/F_m'$ in *Seriatopora hystrix* exposed to 1 and 10 µg DCMU l^{-1} under artificial light (maximum 100 µmol quanta m^{-2} s^{-1} PAR, 12:12 h L:D cycle) was ~0.2 and ~0.6, compared with ~0.75 for control corals over the 14 d duration of the experiment (Fig. 4A inset). F_v/F_m in the 10 µg DCMU l^{-1} treatments decreased slowly to just under 0.6, whilst F_v/F_m in the corals exposed to 1 µg DCMU l^{-1} was similar to that of control corals (Fig. 4A). A slight discolouration of the corals exposed to 10 µg DCMU l^{-1} was noted after about 10 d of treatment, and after 14 d exposure, the density of symbiotic algae in the corals exposed to 10 µg DCMU l^{-1} was about 60% of the density in the control corals (Fig. 4B).

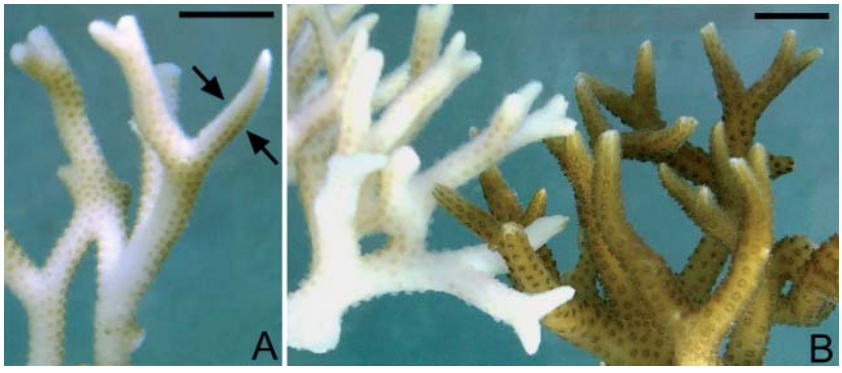

Fig. 2. *Seriatopora hystrix*. (A) Preferential discolouration (loss of symbiotic algae) on upper sunlight-exposed surfaces of branches exposed to 30 µg DCMU l^{-1} for 7 d (arrows). (B) Two colonies exposed to same concentration of diuron but at different irradiance intensities; bleached (left-hand) and normally pigmented (right-hand) colonies were both exposed to 30 µg DCMU l^{-1} for 7 d and then transferred to clean flowing seawater for 7 recovery period (see 'Materials and methods'); the colony that bleached was incubated under normal growth irradiances, whilst the colony that remained normally pigmented experienced the same DCMU concentrations but under reduced irradiances. Scale bars = 1 cm

$\Delta F/F_m'$ in *Seriatopora hystrix* exposed to 10 µg DCMU l^{-1} under normal growth irradiances was ~0.1 over the 7 d exposure, compared to between 0.4 and 0.6 in the control corals (Fig. 5A). F_v/F_m in the 10 µg DCMU l^{-1} treatments decreased to ~0.1 after 1 wk. Corals were returned to flowing seawater under reduced irradiances and a gradual discolouration was noted between Days 7 and 14. Loss of discolouration occurred preferentially on the upper sunlight-exposed surfaces. The density of symbiotic algae in the corals (2×10^6 algae cm^{-2}) was about 60% of the density in the control corals (Fig. 5B) at Day 14.

In *Seriatopora hystrix* exposed to 60 µg Cu l^{-1}, a marked loss of symbiotic algae occurred, turning the incubation water cloudy. This corresponded to a loss of 5×10^5 algae cm^{-2}, or over 1000 × the rate of loss from the control corals or corals exposed to 60 µg DCMU l^{-1}. $\Delta F/F_m'$, measured at the end of the 10 h incubation was <0.05 in the DCMU-exposed corals only, compared to 0.65–0.7 in the copper-exposed and control corals (Fig. 6A). The copper-exposed corals bleached extensively during the 48 h recovery period in clean running seawater. F_v/F_m measured at night-time during the recovery period was the same (~0.7) between treatments (Fig. 6A). At the end of the recovery period, the density of symbiotic algae in the copper-exposed corals was ~30% of the density in the other treatments (Fig. 6C). The F_o baseline fluorescence in the copper-exposed corals was lower than in the control corals (Fig. 3B); however, in contrast to DCMU-induced bleaching, there was no preferential discolouration on the upper surfaces of the corals and no difference in the baseline fluorescence between the surfaces (Fig. 3C).

Tests with isolated algae indicated that substantially higher concentrations (300 µg Cu l^{-1} for 8 h) were needed to significantly reduce the quantum yield (Fig. 7).

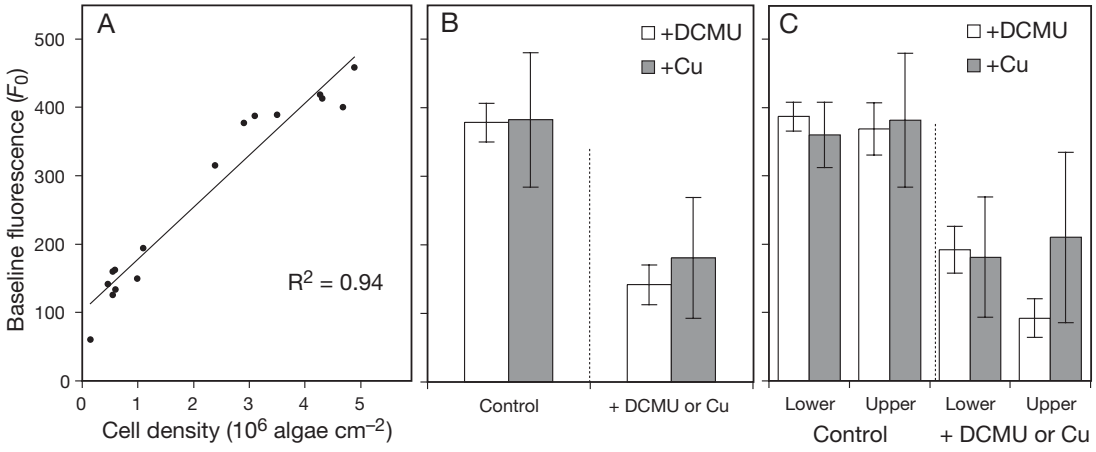

Fig. 3. *Seriatopora hystrix*. (A) Regression curve of mean F_o (baseline) chlorophyll fluorescence (relative units) and symbiotic algal cell density on coral branches; (B) mean F_o (baseline) chlorophyll fluorescence (relative units) of symbiotic dinoflagellates (*in symbio*) per explant; (C) chlorophyll fluorescence for upper and lower surfaces of branches exposed to 30 DCMU µg l^{-1} or control conditions for 7 d and then transferred to flowing seawater for 7 d recovery period (see Fig. 2), or exposed to 60 µg Cu l^{-1} or control conditions for 10 h and then transferred to clean running seawater for 2 d. In (B) and (C), data are mean ± SD of n = 6 corals, for each of which mean of 8 measurements (4 each for lower [shaded] and upper [sunlight-exposed] surfaces of branch) was obtained

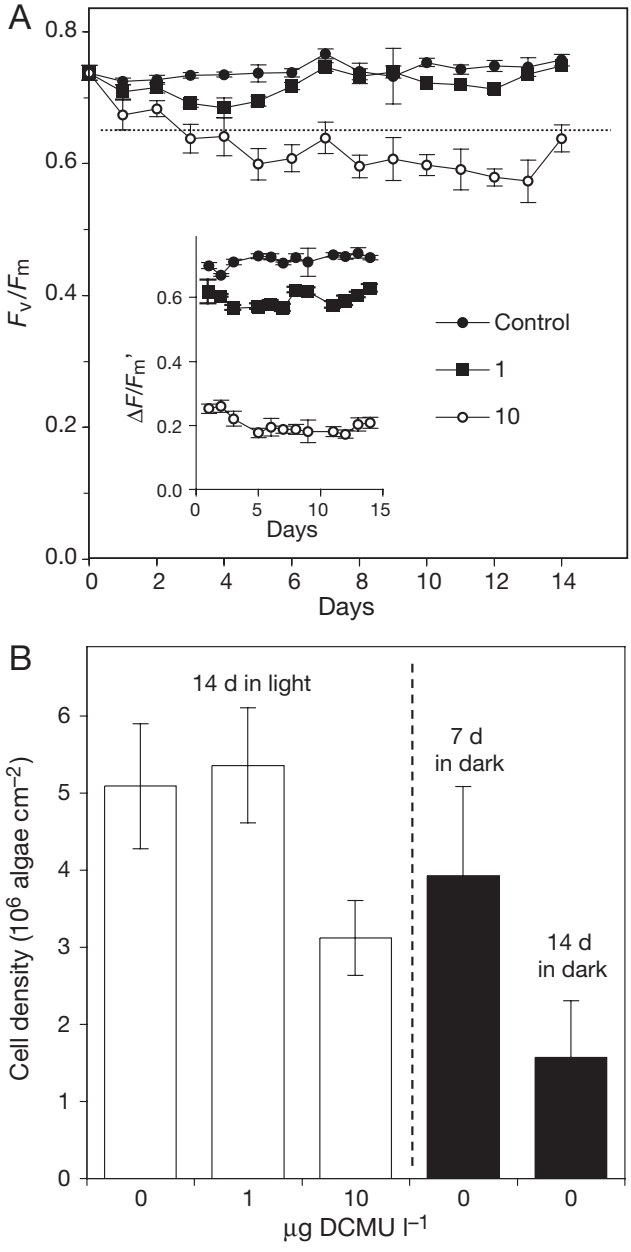

Fig. 4. *Seriatopora hystrix*. (A) F_v/F_m and $\Delta F/F_m'$ (inset) of symbiotic dinoflagellates (*in symbio*) in coral exposed to 1 or 10 µg DCMU l^{-1} or control conditions under artificial light (100 µmol quanta m^{-2} s^{-1} (12:12 h L:D cycle)) for 14 d; (B) symbiotic algal cell density at t = 14 d in control corals (0 µg) and coral exposed to 1 or 10 µg DCMU l^{-1}, or held in darkness for 7 or 14 d. All data are mean ± SD; n = 10 corals

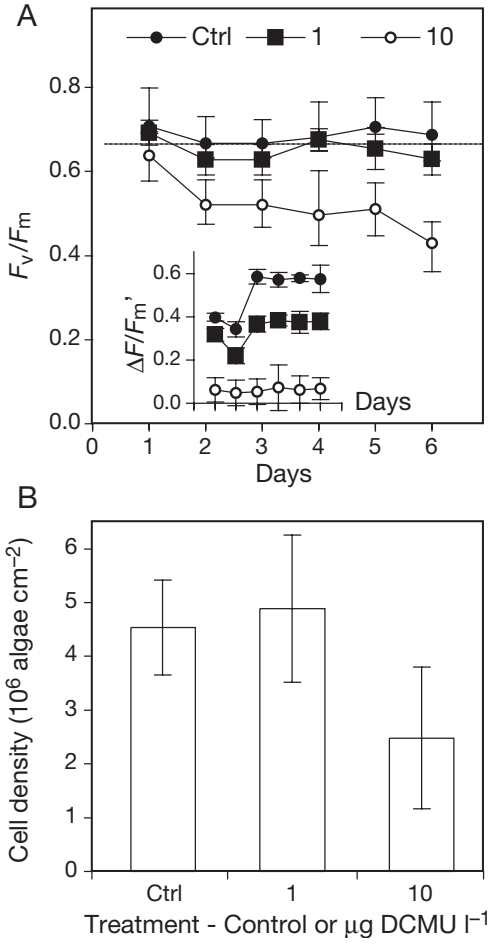

Fig. 5. *Seriatopora hystrix*. (A) F_v/F_m and $\Delta F/F_m'$ (inset) of symbiotic dinoflagellates (*in symbio*) in coral exposed to 1 or 10 µg DCMU l^{-1} or control (Ctrl) under growth irradiance for 7 d; (B) symbiotic algal cell density after a further 7 d recovery period (Day 14). All data are mean ± SD; n = 10 corals

DISCUSSION

Both DCMU and copper can induce bleaching in *Seriatopora hystrix*, but phenomenologically, appear to do so by different processes. DCMU-induced bleaching appears to be a selective process associated with the light-dependent impairment of the photosynthetic capacity of the algal symbionts. Copper-induced bleaching appears to be a rapid process that occurs without affecting photosynthesis and may well be associated with effects on the animal (host). The significance of these 2 different chemically induced patterns of bleaching are discussed with respect to the nature and cause of warm-water bleaching of corals.

DCMU-induced bleaching of coral

DCMU binds to the site of the exchangeable quinone (Q_B) site on the D1 protein, which includes the P680 chlorophyll and the neighbouring phaeophytin (Ph) that form the heart of the reaction centre. When bound, the herbicide blocks forward electron transport beyond the 1-electron reduction of the first stable elec-

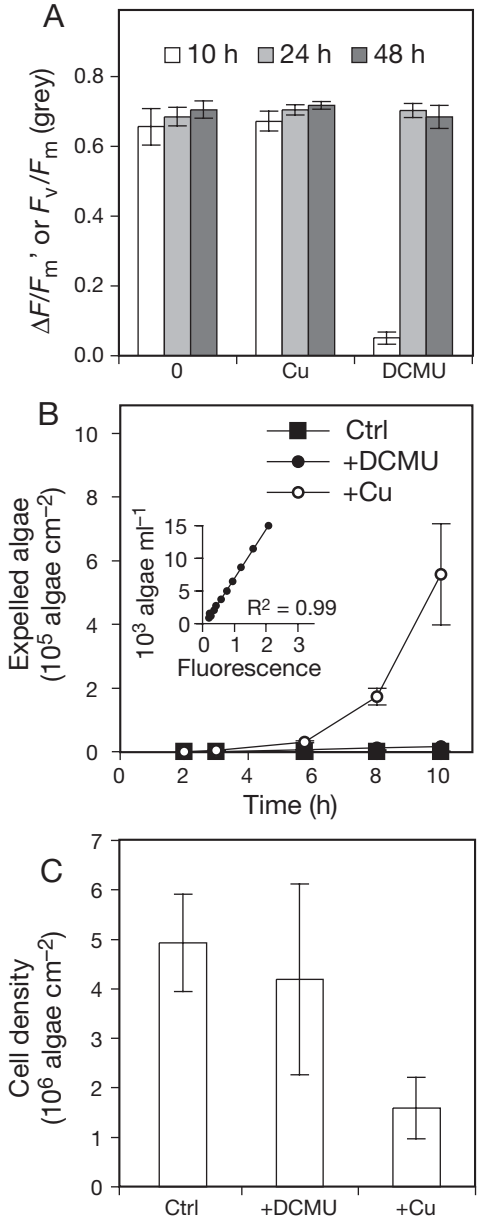

Fig. 6. *Seriatopora hystrix.* (A) $\Delta F/F_m'$ of symbiotic dinoflagellates (*in symbio*) in coral exposed to 60 µg l^{-1} DCMU or copper, or control (Ctrl) conditions under 100 µmol quanta m^{-2} s^{-1} PAR for 10 h, and F_v/F_m 24 and 48 h after being placed in clean flowing seawater. (B) $\times 10^5$ algae expelled cm^{-2} during 10 h exposure period; inset: relationship between chlorophyll fluorescence (arbitrary units, measured using WATER-PAM fluorometer) and concentration of algae ml^{-1} incubation water. (C) Symbiotic algal cell density in control coral and in coral exposed for 10 h to 60 µg l^{-1} DCMU or copper followed by 48 h in clean flowing seawater. All data are mean ± SD; n = 8 corals

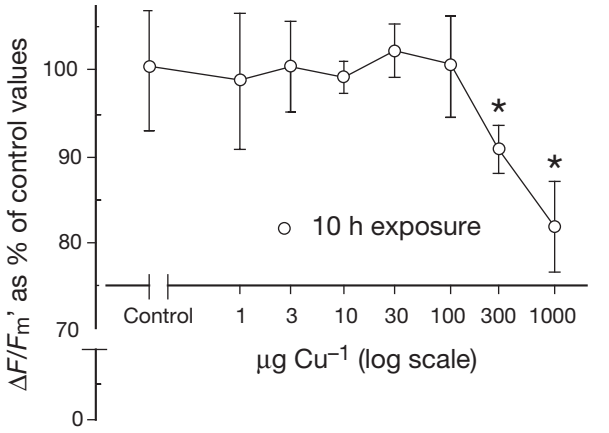

Fig. 7. $\Delta F/F_m'$ (normalized to control values) of symbiotic dinoflagellates *in vitro* isolated from scleractinian coral *Heliofungia actiniformis* and exposed to elevated copper concentrations in the range 1 to 1000 µg l^{-1} in 2 ml glass vials and exposed to 30 µmol quanta m^{-2} s^{-1} PAR for 10 h. Data are mean ± SD; n = 6. *Significant differences ($p < 0.05$, ANOVA) from controls

tron acceptor, the bound quinone Q_A (Bowyer et al. 1991). The currently accepted view is that damage to cells by PSII-binding herbicides occurs from pigment-mediated photogeneration of active oxygen species (Bowyer et al. 1991). When bound, a back reaction in PSII occurs, resulting in the formation of the $P680^+$ Ph^- radical pair. Charge recombination may then favour the generation of the longer-lived triplet chlorophyll state (^{3}chl). The triplet chlorophyll state has sufficient energy to convert molecular oxygen from its more passive triplet to the more reactive singlet oxygen ($^1O_2$) state (Durrant et al. 1990). Singlet oxygen can, in turn, destroy the integrity of the chlorophylls bound to protein, disconnecting them and neighbouring chlorophylls from the antennae systems and protective carotenoids. Under continued light, disconnected chlorophylls may then be involved in photogeneration of more $^1O_2$ in a chain reaction, although at a certain point photoinactivated complexes may also serve as heat-dissipating sinks, protecting neighbouring connected PSII complexes from subsequent damage (Krause 1988, Oquist et al. 1992). Further aspects of herbicide-induced, chlorophyll-mediated oxidative stress are reviewed in Bowyer et al. (1991).

Light is thus a key variable associated with DCMU toxicity and clearly influenced the outcome of the experiments exposing *Seriatopora hystrix* to DCMU. If corals are exposed to elevated DCMU concentrations under reduced irradiances (or low DCMU concentrations at normal growth irradiances), then short-term exposure would probably be similar to a cloud passing overhead (i.e. there would be a temporary, reversible reduction in photosynthetic electron flow and the translocation of fixed carbon products from the algae to the host). However, under normal growth irradiances, there would most likely be photoinactivation of

the algal symbionts and a long-term reduction in the maximum potential quantum yield of photosynthesis (indicated by a suppression of the chlorophyll fluorescence parameter F_v/F_m) synonymous with damage to PSII reaction centres (photoinactivation sensu Osmond et al. 1999). It should be noted that electrons accumulated at the PSII acceptor side during illumination will recombine in PSII in the dark; hence, even if DCMU is bound to the binding site, the reaction centres will remain open and (in a truly dark-adapted state) the chlorophyll fluorescence parameter F_v/F_m should not be able to detect bound DCMU. However, there will be a reduction in F_v/F_m if, as a result of the combination of DCMU dose and irradiance intensity, there has been an appreciable increase in photoinactivation of PSII reaction centres. From an ecotoxicological perspective, DCMU-induced decreases in $\Delta F/F_m$ and F_v/F_m provide different sorts of information. Measurements of $\Delta F/F_m$ are more relevant in assessing the binding of PSII herbicides, but measurements of F_v/F_m are more relevant for assessing light-induced damage as a consequence of herbicide binding. In corals, the dark-adapted yield is a very useful parameter since decreases in F_v/F_m are usually synonymous with the dissociation of the coral–algal symbiosis.

In all experiments with DCMU, when F_v/F_m decreased below a level of ~0.6 the corals began to lose their symbiotic algae and discolour (bleach). Similarly, a correlation between a long-term, sustained reduction in F_v/F_m (i.e. when measured several hours after sunset) and subsequent loss of algae has been reported many times previously, for example in response to cyanide exposure (Jones & Hoegh-Guldberg 1999), high irradiance intensities (Jones & Hoegh-Guldberg 2001), reduced salinity (Kerswell & Jones 2003), cold stress (Saxby et al. 2003), and an effluent from the offshore oil and gas industry (Jones & Heyward 2003). Importantly, loss of symbiotic algae from *Seriatopora hystrix* exposed to 30 µg DCMU l^{-1} was much faster than from corals held in darkness for 14 d. Thus the rapid dissociation of the symbiosis in response to DCMU exposure is not the result of 'passive' loss of symbionts, i.e. a down-regulation of the density of algal cells that have become a net burden to the host, but probably more of an 'active' process, involving either expulsion of photoinactivated algal symbionts or damage to host cells as a result of photoinactivation of the algae.

Given the central role of light in the mode of action of DCMU, it is not surprising that corals preferentially bleached on their upper sunlight-exposed surfaces (see Fig. 2A). This effect was most pronounced when bleaching was first noted, but eventually the lower surfaces discoloured as well, making the corals more uniformly pale (Fig. 2B). Since loss of algae from the corals correlates so closely with a reduction in F_v/F_m (i.e. synonymous with photoinactivation), and present models of the herbicidal action of PSII herbicides indicate that this occurs through the generation of $^1O_2$, it follows that DCMU-induced bleaching of corals is most probably caused by ROS. Whether translocation of singlet oxygen or other active oxygen species from the algae to the host cell occurs is unknown.

Perhaps the most remarkable aspect of the DCMU-induced bleaching reported here is that despite the nearly complete blocking of electron transport and subsequent marked decreases in the quantum yield of the algal symbionts *in symbio* (symptomatic of photoinactivation), and despite rapid export of the algal symbionts from the tissues, there was no mortality or partial mortality in any of the corals. In fact, during the 30 µg DCMU l^{-1} treatments, all polyps were usually expanded and responded to tactile stimulation. Similarly, M. VanOppen (Australian Institute of Marine Science pers. comm.) has noted that exposure of *Seriatopora hystrix* to concentrations as high as 300 µg DCMU l^{-1} for 7 d under 70% sunlight can induce full bleaching without causing any partial or whole-colony mortality in the corals, which also respond when gently touched.

Copper-induced bleaching of coral

Elevated copper concentrations also caused bleaching in *Seriatopora hystrix*, as has been reported in numerous previous studies with hard corals and other invertebrate–dinoflagellate symbioses. For example, discolouration has been reported in *Pocillopora damicornis* and *Montipora verrucosa* exposed to 10 µg Cu l^{-1} for 6 d (Evans 1977), and loss of symbiotic dinoflagellates has been shown in *M. verrucosa* exposed to 30 µg Cu l^{-1} for 1 h (Howard et al. 1986). *Acropora formosa* exposed to >20 µg Cu l^{-1} for 48 h had significantly fewer symbiotic dinoflagellates than the respective control corals, and loss of symbiotic dinoflagellates from *A. formosa* (measured by examining the incubation water) was detected at concentrations as low as 5 and 10 µg Cu l^{-1} (Jones 1997). Loss of symbiotic dinoflagellates has been reported in sea anemones *Anemonia viridis* exposed to 200 µg Cu l^{-1} for 3 to 5 d (Harland & Ngrano 1990), and even from clams *Tridacna crocea* exposed to 60 µg Cu l^{-1} for >10 d (Duquesne & Coll 1995).

Loss of symbiotic dinoflagellates from *Seriatopora hystrix* in response to copper was rapid, occurring in as little as 6 h in a 60 µg Cu l^{-1} solution, and occurred without preferential bleaching on the upper surfaces of the coral. Similar rapid expulsion of algae from copper-exposed corals has been reported in studies where the incubation water has been examined for the presence of algae

(Howard et al. 1986, Jones 1997). Probably the most significant aspect of copper-induced bleaching is that despite being a well-known inhibitor of photosynthesis (primarily affecting both the acceptor and donor sides of PSII; Barón et al. 1995), it is not associated with any significant change in the quantum yield of the algae. Repeated attempts to find the copper concentration needed to reduce $\Delta F/F_m'$ in *S. hystrix* have been confounded by mortality (tissue-sloughing) of the coral before any effects *in symbio* could be measured. *In vitro* tests were therefore used with the symbiotic dinoflagellates isolated from tentacles of the solitary coral *Heliofungia actiniformis* (for which collection, cleaning and preparation of the dinoflagellates is simple—see Jones & Heyward 2003). In this study, a significant reduction in $\Delta F/F_m'$ occurred after 10 h exposure to 300 µg Cu l^{-1} and a more substantial (25%) reduction at 1 mg Cu l^{-1}. These concentrations are substantially higher than those that induced rapid bleaching in *S. hystrix*.

We compared our results with those of other studies. Juneau et al. (2002) reported no effects of copper on *Chlorella vulgaris* at the highest concentration tested (100 µg Cu l^{-1}), but the median effective concentration (EC_{50}) values for a reduction in $\Delta F/F_m'$ were 29 µg Cu l^{-1} for *Chlamydomonas reinhardtii* and 50 µg Cu l^{-1} for *Selenastrom capricornutum*. In seagrasses, Ralph & Burchett (1998) only observed effects on $\Delta F/F_m'$ at very high copper concentrations (1 to 10 mg Cu l^{-1}). In studies with corals, Nystrom et al. (2001) showed that an exposure of 11 µg Cu l^{-1} for 24 h had no effect on oxygen evolution from symbiotic dinoflagellates in *Porites cylindrica*. Grant et al. (2003) showed that exposing the scleractinian coral *Plesiastrea versipora* to ~40 µg Cu l^{-1} for 12 to 36 d had no effect on ^{14}C incorporation in the symbiotic dinoflagellates in the tissue. In other photoautotrophs, a 60% reduction in oxygen evolution (and 30% reduction in F_v/F_m) has been reported in the seaweed *Gracilariopsis longissima* after 7 d exposure to 250 µg Cu l^{-1}, whereas no effects were noted at 60 µg Cu l^{-1} (Brown & Newman 2003).

On the basis of these previous studies and considering the *in vitro* and *in symbio* fluorescence data reported here, it is unlikely that there were any significant effects on algal photosynthesis *in symbio* whilst a rapid loss of algae was occurring from *Seriatopora hystrix*. In contrast to the DCMU experiments, all *S. hystrix* explants exposed to the elevated copper concentrations were strongly retracted within their polyps. Whilst the copper-induced bleaching was a sublethal response, it is clear from this and other studies that, when it occurs, corals are very close to dying. For example, in the studies mentioned previously, Evans (1977) recorded mortality in *Montipora verrucosa* exposed to 100 µg Cu l^{-1} for 6 d. Howard et al. (1986) reported that 50% of *M. verrucosa* explants died after exposure to 30 µg Cu l^{-1} for 5 d and 50 µg Cu l^{-1} for 4 d. Jones (1997) reported high levels of mortality in some tests for *Acropora formosa* fragments exposed to 40 and 80 µg l^{-1} for 48 h, while for *S. hystrix* all corals exposed to 30 µg Cu l^{-1} for 24 h died (R. J. Jones unpubl. data). For the clam *Tridacna crocoea*, mortality occurs within 3 d when exposed to 200 µg Cu l^{-1} (Duquesne & Coll 1995).

Copper is an essential micronutrient, but at higher concentrations has many toxic effects on plant (Fernandes & Henriques 1991) and animal (Gaetke & Chow 2003) systems. In the cytosol, it blocks and reduces – SH enzyme groups and free thiols, disrupting enzyme-active sites and cell division (Stauber & Florence 1985). In organelles, it may interfere with mitochondrial electron transport and ATP production (Viarengo et al. 1981). However, it is well established that damage to membranes is one of the events associated with Cu^{2+}-induced toxicity, and copper ions can oxidize SH-groups of cysteine from membrane proteins forming disulfide bridges between membrane proteins. There is a growing awareness that the toxicity of metals such as copper is associated with the generation of ROS (Stohs & Bagchi 1995). Copper (and iron) can act as catalysts for the generation of ROS such as the hydroxyl radical ($HO^{\cdot}$) by Fenton and Haber-Weiss reactions (Halliwell & Gutteridge 1984), and initiate a self-propagating lipid peroxidation process (Chan et al. 1982) that increases membrane permeability/leakiness. While it is not possible from the information reported here to identify the mechanism of copper-induced bleaching in *Seriatopora hystrix*, one can speculate that it is basically pathological and associated with the effect of copper on the host (animal) cells.

Warm-water bleaching of coral

Overall, the pattern of warm-water bleaching of corals bears similarities with both DCMU-induced and copper-induced bleaching. For example, many studies have indicated that in warm-water bleaching events there is preferential bleaching of the upper sunlight-exposed surfaces of corals (Harriott 1985, Oliver 1985), supporting laboratory-based experiments of an interaction between light and temperature (Jokiel & Coles 1977)[1]. Both laboratory studies and observations of

[1]This is not the case in bacterial bleaching of *Oculina patagonica*, for which few bleached colonies are found in shallow (<1 m) as opposed to deeper water. Manipulative experiments with ultraviolet (UV) screens have indicated that this is probably due to the damaging effects of ultraviolet radiation (UVR) on *Vibrio shiloi*, the causative agent of this form of bleaching (Fine et al. 2002)

natural bleaching events have indicated that bleaching through loss of the symbiotic algae is rapid (i.e. within a matter of days). Several studies have also measured decreases in photosynthetic efficiency in the algal symbionts in naturally bleached corals (Warner et al. 1999, Jones et al. 2000, Fitt et al. 2001), in experimental manipulations of isolated algae, and in the intact association (Iglesias-Prieto et al. 1992, Fitt & Warner 1995, Warner et al. 1996, Jones et al. 1998). There is also clear evidence for enhanced degradation of the D1 protein in bleached corals, synonymous with photodamage (Warner et al. 1999). These diagnostic symptoms of warm-water bleaching are clearly similar to that of DCMU-induced bleaching, and the studies reported here clearly support the photoinhibition model of coral bleaching as outlined in Jones et al. (1998), Hoegh-Guldberg (1999) and Jones & Hoegh-Guldberg (2001).

However, it is evident from laboratory-based experiments that warm-water bleaching of corals is a sublethal response that occurs just prior to mortality (e.g. Jokiel & Coles 1977, Hoegh-Guldberg & Smith 1989). In contrast, complete stoppage of the photosynthetic electron transport using elevated DCMU can initiate a rapid and substantial loss of algae without obviously affecting the host. This aspect of warm-water bleaching is more analogous to the copper-induced bleaching described herein, i.e. possibly associated with the host and possibly pathological.

In an unstressed state, the algae of corals are found at comparatively high densities (see Drew 1972). Crossland & Barnes (1977) noted that the shape of the light-response curve for high-density suspensions of dinoflagellates isolated from *Acropora acuminata* was the same as for the intact symbiosis, suggesting that algae are self-shaded *in symbio* (see also Goiran et al. 1996). If, on the basis of high areal density, the algae *in symbio* are self-shaded, it follows that as a coral loses algae, i.e. bleaching, more light may become available for the remaining algal symbionts. This could theoretically promote further photodamage and thus initiate further loss of algae from the tissues in a 'positive-feedback' response. Such a mechanism may run unchecked until there is either a marked change in the light or temperature regimes (i.e. following a storm or cyclone), or the majority of algal symbionts have been expelled. Such a mechanism should promote high levels of intra-specific and intra-colony variation in the severity of bleaching, given slightly different starting points in the bleaching process. Such variability is often reported during bleaching events. Theoretically, loss of symbiotic algae by a host-mediated process (such as host-cell adhesion dysfunction) could also expose the remaining symbionts to light-shock which, in turn, would result in photodamage and promote further loss of algae, initiating an autocatalytic response. Testing such an integrated model of bleaching (i.e. involving both host and algae partners) could be achieved by combining chlorophyll fluorescence measurements at various temperature and light combinations to initiate different types of bleaching, and/or by the use of chemical inhibitors such as copper and DCMU.

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LITERATURE CITED

Asada K, Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CB, Arntzen CJ (eds) Photoinhibition. Elsevier, Amsterdam, p 228–287

Barón M, Arellano JB, Gorgé JL (1995) Copper and Photosystem II: a controversial relationship. Physiol Plant 94: 174–180

Bjorkman O, Demmig-Adams B (1995) Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. In: Schulze ED, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer-Verlag, Berlin, p 17–47

Bowyer JR, Camilleri P, Vermaas WFJ (1991) Photosystem II and its interaction with herbicides. Top Photosynth 10: 27–85

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16:129–138

Brown BE, Ambasari I, Warner ME, Fitt WK, Dunne RP, Gibb SW, Cummings DG (1999) Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. Coral Reefs 18:99–105

Brown BE, Downs CA, Dunne RP, Gibb SW (2002) Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. Mar Ecol Prog Ser 242:119–129

Brown MT, Newman JE (2003) Physiological responses of *Gracillariopsis longissima* to sublethal copper concentrations. Aquat Toxicol 64:201–213

Chan PC, Peller OG, Kesner L (1982) Copper (II) catalysed lipid-peroxidation in liposomes and erythrocyte-membranes. Lipids 17:331–337

Coles SL, Jokiel PL (1978) Synergistic effects of temperature, salinity and light on the hermatypic coral *Montiporra verrucosa*. Mar Biol 49:187–195

Crossland CJ, Barnes DJ (1977) Gas-exchange studies with the staghorn coral *Acropora acuminata* and its zooxanthellae. Mar Biol 40:185–194

Douglas AE (2003) Coral bleaching—how and why? Mar Pollut Bull 46:385–392

Drew EA (1972) The biology and physiology of algal–invertebrate symbiosis. II. The density of algal cells in a number of hermatypic hard corals and alcyonarians from various depths. J Exp Mar Biol Ecol 9:71–75

Duquesne SJ, Coll JC (1995) Metal accumulation in the clam *Tridacna crocea* under natural and experimental

conditions. Aquat Toxicol (Amst) 32:239–253
Durrant JR, Giorgi LB, Barber J, Klug DR, Porter G (1990) Characterisation of triplet chlorophyll states in isolated photosystem II reaction centres: oxygen quenching as a mechanism for photodamage. Biochim Biophys Acta 1017: 167–175
Dykens JA, Shick JM (1982) Oxygen production by endosymbiotic algae controls superoxide dismutase activity in their animal host. Nature 297:579–580
Dykens JA, Shick JM (1984) Photobiology of the symbiotic sea anemone, *Anthopleura elegantissima*: defenses against photodynamic effects, and seasonal photoacclimatization. Biol Bull (Woods Hole) 167:683–697
Evans EC (1977) Microcosm responses to environmental perturbants. Helgol Wiss Meeresunters 30:178–191
Fernandes JC, Henriques FS (1991) Biochemical, physiological and structural effects of excess copper in plants. Bot Rev 57:246–273
Fine M, Banin E, Israely T, Rosenberg E, Loya Y (2002) Ultraviolet radiation prevents bleaching in the Mediterranean coral *Oculina patagonica*. Mar Ecol Prog Ser 226: 249–254
Fitt WK, Warner ME (1995) Bleaching patterns of four species of Caribbean reef corals. Biol Bull 189:298–307
Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thesholds in tropical corals. Coral Reefs 20:51–65
Franklin LA (1994) The effects of temperature acclimation on the photoinhibitory responses of *Ulva rotundata* Blid. Planta 192:324–331
Gaetke LM, Chow CK (2003) Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicol 189:147–163
Gates R, Baghdasarian G, Muscatine L (1992) Temperature stress causes host-cell detachment in symbiotic cnidarians: implications for coral bleaching. Biol Bull (Woods Hole) 182:324–332
Goiran C, Al-Moghrabi S, Allemand D, Jaubert J (1996) Inorganic carbon uptake for photosynthesis by the symbiotic coral/dinoflagellate association. I. Photosynthetic performances of symbionts and dependence on sea water bicarbonate. J Exp Mar Biol Ecol 199:207–225
Gorbunov MY, Kolber ZS, Lesser MP, Falkowski PG (2001) Photosynthesis and photoprotection in symbiotic corals. Limnol Oceanogr 46:75–85
Grant AJ, Graham K, Frankland S, Hinde R (2003) Effects of copper on algal–host interactions in the symbiotic coral *Plesiastrea versipora*. Plant Physiol Biochem 41:383–390
Halliwell B, Gutteridge JMC (1984) Oxygen toxicity, oxygen transition metals and disease. Biochem J 219:1–14
Harland AD, Ngrano NR (1990) Copper uptake by the sea anemone *Anemonia viridis* and the role of zooxanthellae in metal regulation. Mar Biol 104:297–301
Harriott VJ (1985) Mortality rates of scleractinian corals before and during a mass bleaching event. Mar Ecol Prog Ser 21:81–88
Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. Mar Freshw Res 50:839–866
Hoegh-Guldberg O, Jones RJ (1999) Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Mar Ecol Prog Ser 183:73–78
Hoegh-Guldberg O, Smith GJ (1989) The effects of sudden changes in light, temperature and salinity on the population density and export of zooxanthellae from the reef corals *Seriatopora hystrix* and *Stylophora pistillata*. Mar Ecol Prog Ser 129:279–303
Howard LS, Crosby DG, Alino P (1986) Evaluation of some of the methods for quantitatively assessing the toxicity of heavy metal to corals. Tech Rep Hawaii Inst Mar Biol 37: 452–464
Iglesias-Prieto R (1997) Temperature-dependent inactivation of photosystem II in symbiotic dinoflagellates. Proc 8th Int Coral Reef Symp 2:1313–1318
Iglesias-Prieto R, Matta JL, Robins WA, Trench RK (1992) Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. Proc Natl Acad Sci USA 89:10302–10305
Jokiel PL, Coles SL (1977) Effects of temperature on the mortality and growth of Hawaiian reef corals. Mar Biol 43: 201–208
Jones RJ (1997) Zooxanthellae loss as a bioassay for assessing stress in corals. Mar Ecol Prog Ser 149:163–171
Jones RJ, Heyward A (2003) The effects of produced formation water (PFW), an effluent from the offshore oil and gas industry, on coral and isolated symbiotic dinoflagellates. Mar Freshw Res 54:1–10
Jones RJ, Hoegh-Guldberg O (1999) The effects of cyanide on coral photosynthesis: implications for identifying the cause of coral bleaching and for assessing the environmental effects of cyanide fishing. Mar Ecol Prog Ser 177: 83–91
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 Environ 24:89–99
Jones RJ, Kerswell AP (2003) Phytotoxicity of Photosystem-II (PSII) herbicides to coral. Mar Ecol Prog Ser 261:149–159
Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature induced bleaching of corals begins with impairment to the carbon dioxide fixation mechanism of zooxanthellae. Plant Cell Environ 21:1219–1230
Jones RJ, Ward S, Yang-Amri, Hoegh-Guldberg O (2000) Changes in quantum efficiency of Photosystem II of symbiotic dinoflagellates of corals after heat stress and during the 1998 Great Barrier Reef mass bleaching event. Mar Freshw Res 51:63–71
Jones RJ, Muller J, Haynes D, Schreiber U (2003) Effects of herbicides diuron and atrazine on corals of the Great Barrier Reef, Australia. Mar Ecol Prog Ser 251:153–167
Juneau P, El Berdey A, Popovic R (2002) PAM fluorometry in the determination of the sensitivity of *Chlorella vulgaris*, *Selenastrum capricornutum*, and *Chlamydomonas reinhardtii* to copper. Arch Environ Contam Toxicol 42: 155–164
Kerswell AP, Jones RJ (2003) Effects of hypo-osmosis on the coral *Stylophora pistillata*: nature and cause of 'low-salinity bleaching'. Mar Ecol Prog Ser 253:145–154
Krause GH (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol Plant 74:566–574
Lesser MP (1996) Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol Oceanogr 41:271–283
Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16: 187–192
Lesser MP, Stochaj WR, Tapley DW, Schick JM (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
Long SP, Humphries S (1994) Photoinhibition of photosynthesis in nature. Annu Rev Plant Physiol Plant Mol Biol 45: 633–662

Nii CM, Muscatine L (1997) Oxidative stress in the symbiotic sea anemone *Aiptasia pulchella* (Carlgren, 1943): Contribution of the animal to superoxide ion production at elevated temperature. Biol Bull 192:444–456

Nystrom M, Nordemar I, Tedengren M (2001) Simultaneous and sequential stress from increased temperature and copper on the metabolism of the hermatypic coral *Porites cylindrica*. Mar Biol 138:1225–1231

Oliver J (1985) Recurrent seasonal bleaching and mortality of corals on the Great Barrier Reef. Proc 5th Int Coral Reef Congr 4:201–206

Oquist G, Chow WS, Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. Planta 186:450–460

Osmond CB, Anderson JM, Ball MC, Egerton JJG (1999) Compromising efficiency: the molecular ecology of light resource utilisation in terrestrial plants. In: Scholes C, Baker M (eds) Advances in physiological plant ecology. Blackwell, Oxford, p 1–24

Ralph PJ, Burchett MD (1998) Photosynthetic response of *Halophila ovalis* to heavy metal stress. Environ Pollut 103:91–101

Ralph PJ, Gademann R, Larkum AWD, Schreiber U (1999) *In situ* underwater measurements of photosynthetic activity on coral zooxanthellae and other reef-dwelling dinoflagellate endosymbionts. Mar Ecol Prog Ser 180: 139–147

Sawyer SJ, Muscatine L (2001) Cellular mechanisms underlying temperature-induced bleaching in the tropical sea anemone *Aiptasia pulchella*. J Exp Biol 204:3443–3456

Saxby T, Dennison WC, Hoegh-Guldberg O (2003) Photosynthetic responses of the coral *Montipora digitata* to cold temperature stress. Mar Ecol Prog Ser 248:85–97

Stauber JL, Florence TM (1987) Mechanism of toxicity of ionic copper and copper complexes to algae. Mar Biol 94: 511–519

Stimson J, Kinzie RA (1991) The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J Exp Mar Biol Ecol 153:63–74

Stohs SJ, Bagchi D (1995) Oxidatove mechanisms in the toxicity of metal ions. Free Radic Biol Med 18:321–366

Tschiersch H, Ohmann E (1993) Photoinhibition in *Euglena gracilis*: involvement of reactive oxygen species. Planta 191:316–323

Viarengo A, Pertica M, Mancinelli G, Capell R, Orunesu M (1981) Effects of copper on the uptake of amino acids, on propteon synthesis and on ATP content in different tissues of *Mytilus galloprovincialis*. Mar Environ Res 4:145–152

Warner ME, Fitt WK, Schmidt GW (1996) The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: a novel approach. Plant Cell Environ 19:291–299

Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. Proc Natl Acad Sci USA 96:8007–8012

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