



Photosynthetic responses of
the coral *Montipora digitata* to
cold temperature stress

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**Photosynthetic responses of the coral *Montipora digitata* to
cold temperature stress**

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STATEMENT

The work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and the material has not been submitted, either in whole or in part, for a degree at this or any other University.

Tracey Saxby

April 2001

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ABSTRACT

Coral bleaching events have become more frequent and widespread, largely due to elevated sea surface temperatures. Global climate change could lead to increased variability in sea surface temperatures, hence, the effect of cold temperature stress on corals could become more pronounced. The photosynthetic responses of a cold sensitive coral species, *Montipora digitata*, were investigated in a series of temperature and light experiments. Small replicate coral colonies were exposed to ecologically relevant lower temperatures for varying durations and under different light regimes. In addition, acclimation of coral colonies to repeated temperature stress was examined after a 3 month recovery period. Photosynthetic efficiency was analysed using a Pulse Amplitude Modulated (PAM) fluorometer (F_o , F_m , F_v/F_m) and chlorophyll content and symbiotic dinoflagellate density were analysed with spectrophotometry and microscopy, respectively. Cold temperature stress had a negative impact on *M. digitata* colonies indicated by decreased photosynthetic efficiency (F_v/F_m), loss of symbiotic dinoflagellates and changes in photosynthetic pigment concentrations. Corals in higher light regimes were more susceptible to cold temperature stress. Moderate cold stress resulted in photoacclimatory responses, but severe cold stress resulted in photodamage, bleaching and increased mortality. Cumulative impacts of cold temperature stress are likely, based on photosynthetic responses measured after repeated cold temperature treatments. Responses to cold temperature stress of *M. digitata* appeared similar to those observed following warm temperature stress of *M. digitata* and various other coral species. This has implications in the context of global climate change, as both cold and warm temperature extremes could become more prevalent. While cold temperature stress can directly cause coral bleaching and mortality, it could also increase susceptibility to subsequent stress.

INTRODUCTION

Coral bleaching is a response to extreme environmental conditions, (Fang *et al.* 1997; Hoegh-Guldberg & Jones 1999; Hoegh-Guldberg & Smith 1989b; Jones *et al.* 1998; Yonge & Nicholls 1931) and has been observed following various physical and chemical stresses, both in the laboratory and in the field (Jokiel & Coles 1974; Kleppel *et al.* 1989; Reimer 1971; Yonge & Nicholls 1931). Physical factors include variation in temperature, light and salinity whereas chemical factors include cyanide and herbicides. Bleaching involves the dissociation of the symbiosis between corals and their symbiotic dinoflagellates. There is a loss of pigmentation due to decreased numbers of symbiotic dinoflagellates, a reduction in photosynthetic pigments, or both (Hoegh-Guldberg 1989; Jokiel & Coles 1990; Kleppel *et al.* 1989; Porter *et al.* 1989; Yonge & Nicholls 1931).

The first anecdotal reports of coral bleaching occurred in the 1870's (Brown 1997b; Glynn 1993). Over the last decade, however, reports of coral bleaching have increased in frequency and scale (Brown *et al.* 1994; Gates *et al.* 1992). The majority of reported bleaching events have been correlated with elevated sea surface temperatures (Hoegh-Guldberg & Jones 1999). Several studies indicate that elevated temperatures act to increase the susceptibility of the symbiotic dinoflagellates of corals to photoinhibition, with the resulting damage leading to expulsion from the coral host (Hoegh-Guldberg & Jones 1999; Iglesias-Prieto *et al.* 1992; Lesser *et al.* 1990; Roberts 1990). However, localised spatial variability and differences both within and between species suggests that other factors may also influence coral bleaching (Berkelmans & Willis 1999; Brown 1997b).

The susceptibility of corals to temperature stress has taken on particular significance in the context of global warming, and the occurrence of world wide bleaching events has attracted considerable political, social and scientific comment (Buss & Vaisnys 1993; Glynn 1993; Hoegh-Guldberg 1999; Williams & Bunkley-Williams 1990). Observed temperature responses of corals suggest they are living very close to their upper thermal limits (Jokiel & Coles 1990; Lesser 1997), prompting concern that increasing global temperatures, in conjunction with El Niño Southern Oscillation events, could have a dramatic influence on reef communities. The observation that sea temperatures have increased by almost one degree over the past 100 years

has been suggested as an underlying reason for why corals exist so close to their thermal limit (Hoegh-Guldberg 1999).

Bleaching has also been correlated with decreases in sea surface temperatures (Coles & Jokiel 1977; Gates *et al.* 1992). It has long been observed that lowered temperatures limit the survival and development of coral reefs, with 18°C accepted as the lower temperature threshold for corals (Dana 1843; Vaughan 1918). However, certain species of corals can survive temperatures as low as 11.5°C for several months (Coles & Fadlallah 1991). Nevertheless, a minimum thermal threshold of 18°C still applies for most tropical reef corals. The passage of polar continental air masses have been shown to have rapid cooling effects on shallow water carbonate environments, with chilling and mixing of water bodies augmented by associated strong winds (Roberts *et al.* 1982). Upwelling may also affect open ocean reefs, with temperatures dropping several degrees with the changing of tides (Glynn & Stewart 1973). The greatest daily temperature ranges (commonly 6-14°C) have been recorded in shallow reef flat environments (Brown 1997a; Endean *et al.* 1956; Orr 1933; Wells 1952).

The present study explored the effect of cold stress on the physiology of the coral *Montipora digitata* Studer and its symbiotic dinoflagellates. Changes in key physiological parameters such as the photochemical efficiency (F_v/F_m) were measured, which has been found to be central to responses of phototrophic organisms to elevated thermal stress. Other key parameters that provide an insight into the physiological response of corals to cold stress include the density of symbiotic dinoflagellates and concentrations of their photosynthetic pigments. Initial experiments were conducted to investigate how the duration of exposure affected responses to colder than ambient temperatures. The potential for recovery of photosynthetic efficiency following temperature stress was examined, over short- and long-term periods, and the cumulative impacts of cold temperature stress were determined. The synergistic effects of light and temperature in causing an increased sensitivity to photoinhibition were also investigated.

METHODS

Study Site

Small replicate branches (nubbins) of the scleractinian coral *Montipora digitata* Studer were collected from the southern reef flat at Heron Island, Australia (23°26' 47.5 S, 151°54' 41.2 E) (Plate 1). Coral nubbins (2-3 cm in length) were removed from twenty colonies of *M. digitata* growing on the reef flat using long-nosed pliers. Ten to fifteen nubbins were collected from the upper surfaces of each colony.

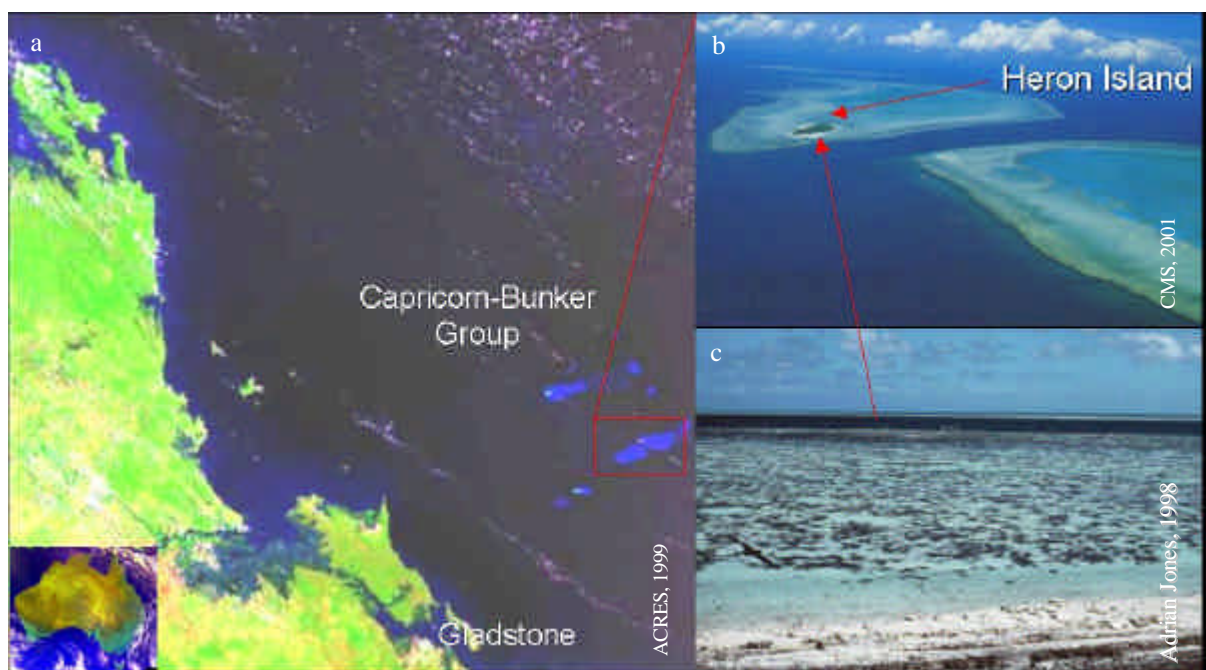


Plate 1: Location of collection site at Heron Island, Australia. a) Satellite picture of Heron Island, b) Aerial photo of Heron Island, c) Southern reef flat at Heron Island.

Following collection, each coral nubbin was mounted in the lids of scintillation vials using non-toxic modelling clay, and placed within aquaria. This technique has been successfully used in a number of physiological studies of reef-building corals (e.g. Hoegh-Guldberg & Jones 1999; Jones *et al.* 1999). Corals were left to adapt to aquaria conditions for 7 d to minimise the influence of stress due to collection and nubbin preparation. Both field and handling controls were measured whenever fresh corals were collected to determine whether handling caused any significant differences in the measured parameters.

Experimental design

Maintenance of experimental aquaria

Four treatment tanks were established and temperatures were maintained to within $\pm 0.5^\circ\text{C}$ of the desired treatment. Each tank consisted of three chambers containing aerated seawater. Two tanks were connected to a water bath (Grant LTD6) that recirculated cooled water around the outside of the chambers. The other two tanks received ambient seawater from the reef flat around the outside of the chambers ($23\text{-}26^\circ\text{C}$ seasonally dependent) (Plate 2). Daily fluctuations were within $\pm 0.5^\circ\text{C}$. The different temperature treatments were conducted on different days, each with a corresponding control treatment. Coral nubbins were maintained at a set orientation throughout the experiment.

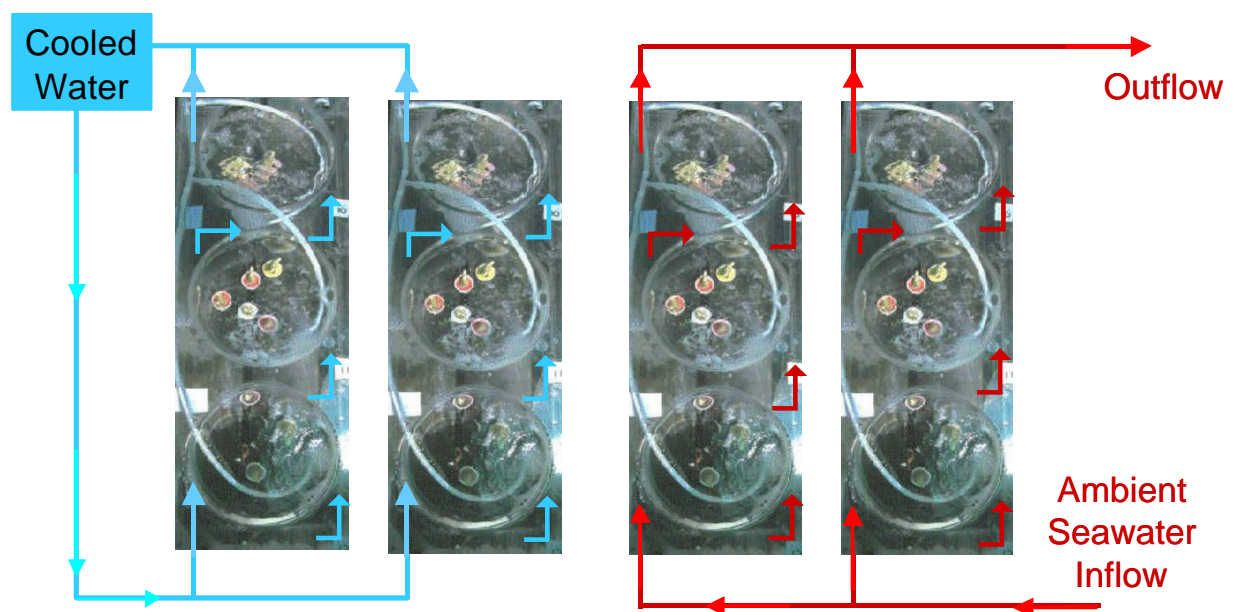


Plate 2: Experimental design showing the four temperature treatment tanks with 3 chambers. The two tanks on the left were maintained at lowered temperatures, while the two on the right were maintained at ambient temperatures. Arrows indicate water flow.

Time course of cold temperature response

Corals were collected in October 2000 and allowed to acclimatise to aquaria conditions at ambient temperatures over a 7 d period. Nubbins were randomly assigned to one of 3 different temperature regimes (12°C , 16°C , 20°C) or an ambient temperature control (23°C). Experiments commenced at 8 am each day and terminated at 2 am the following day. Three replicate nubbins were randomly removed from each treatment at 1h, 3 h, 6 h, 12 h, and 18 h following commencement of the experiment. Photosynthetic efficiency (F_v/F_m) was determined before

returning nubbins to ambient temperatures where they were monitored for a further 3 d at the same time each day. Following the monitoring period nubbins were immediately frozen for further laboratory analysis of chlorophyll concentration and dinoflagellate density (Fig. 1).

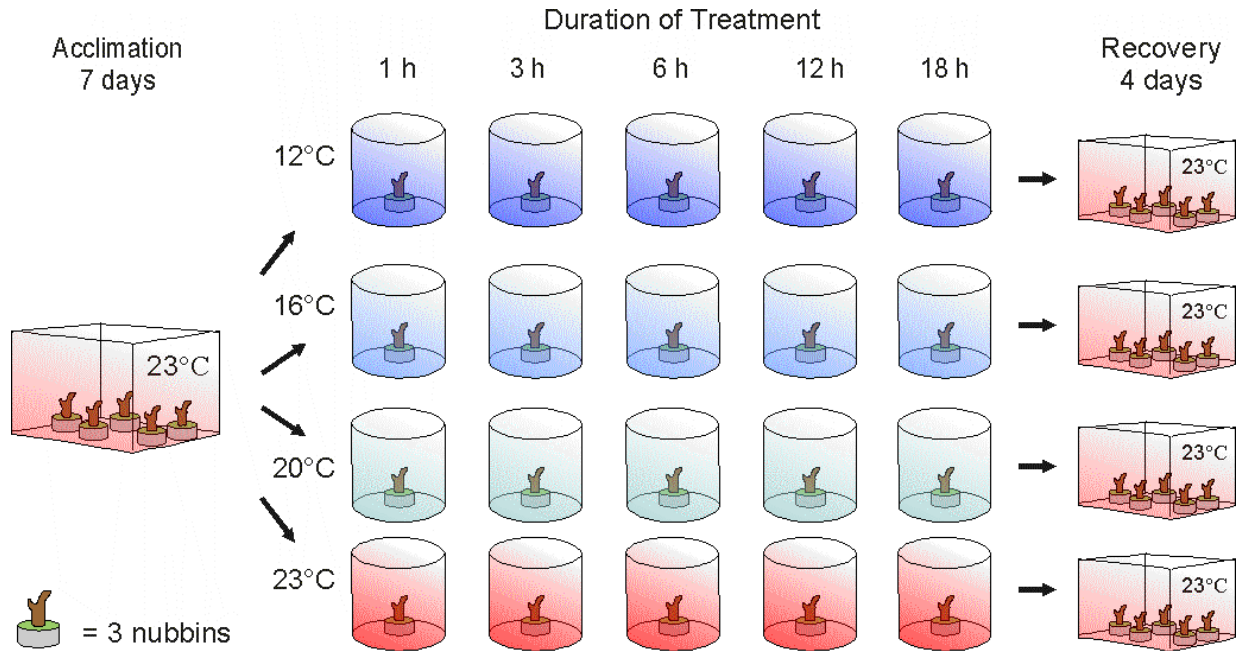


Figure 1: Overview of time course experiment. Replicate coral nubbins (small replicate colonies) were collected from the reef flat and acclimated to aquaria conditions over 7 d. They were then subjected to one of 4 temperature treatments (12°C, 16°C, 20°C, or 23°C) for a period of 1 h, 3 h, 6 h, 12 h, or 18 h. Corals were then transferred to ambient temperatures and monitored over a 4 d period.

Cumulative impacts of cold temperature stress

Corals were collected in October 2000 and allowed to acclimate to aquaria conditions at ambient temperatures over 7 d. Nubbins were then randomly assigned to either a 15°C treatment or an ambient control (23°C). Corals were exposed to these temperature treatments for 6 h from 12 pm to 6 pm. Photosynthetic efficiency (F_v/F_m) was determined before returning nubbins to ambient temperatures where they were monitored for a further 2 d at the same time each day. Nubbins were attached to oyster mesh, following measurement, using scintillation vial lids and cable ties and placed out on the reef flat to recover over a three month period (October to January).

In January 2001 nubbins were retrieved from the reef flat and allowed to acclimate to aquaria for 7 d at ambient temperatures. The nubbins from both the 15°C and control treatment were then subjected to a further treatment at either 15°C or ambient control (26°C) for 6 h from 12 pm to

6 pm. Photosynthetic efficiency (F_v/F_m) was determined before returning nubbins to ambient temperatures where they were monitored for a further 2 d at the same time each day. Following the monitoring period nubbins were immediately frozen for further laboratory analysis of chlorophyll concentration and dinoflagellate density (Fig. 2).

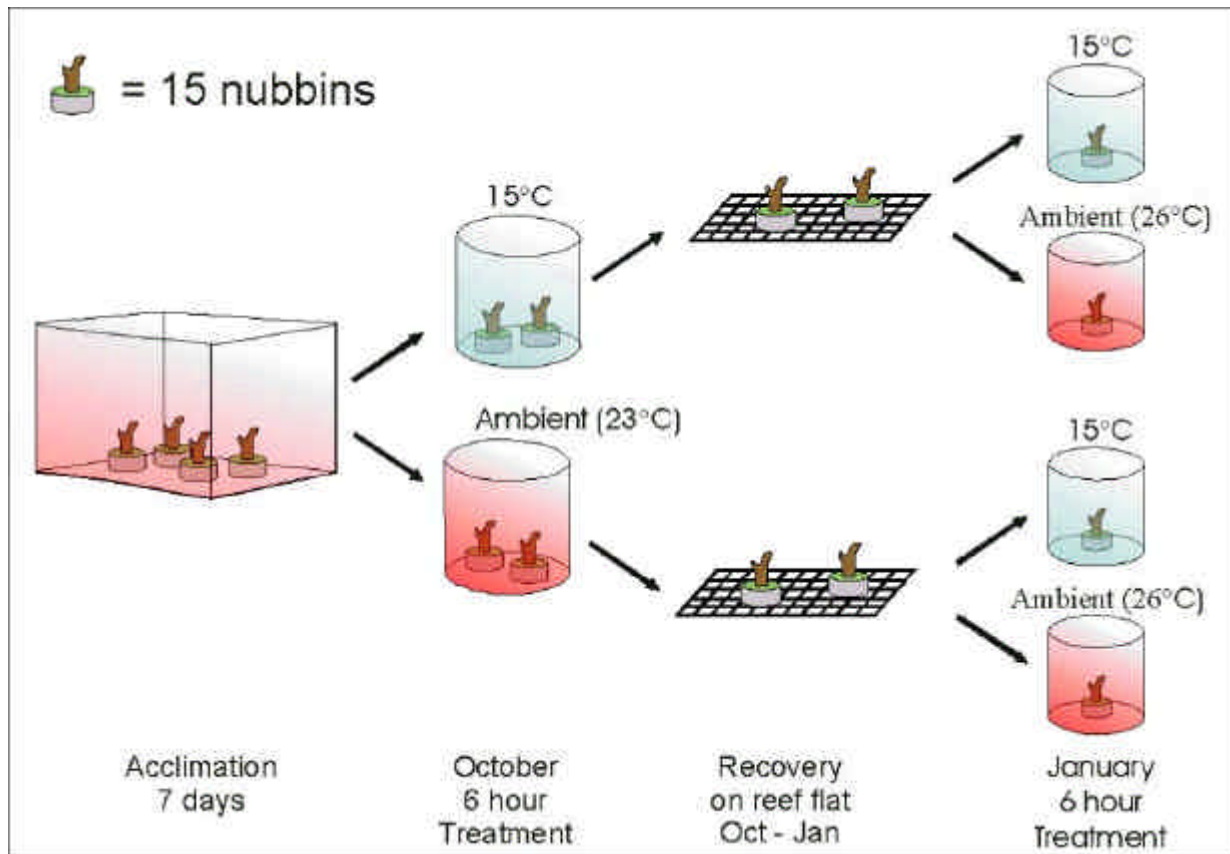


Figure 2: Overview of cumulative impacts experiment. Corals nubbins were collected from the reef flat and allowed to acclimate to aquaria conditions over 7 d. They were then subjected to either a 15°C treatment or 23°C ambient control for 6 h. Corals were then attached to oyster mesh and placed on the reef flat for 3 months. In January, corals were retrieved and further experiments at 15°C and ambient temperatures (26°C) were conducted.

Synergistic effects of light and temperature

Nubbins were collected from the reef flat in January 2001 and allowed to acclimate to aquaria conditions at ambient temperatures over 7 d. Corals were then randomly assigned to one of three temperature treatments (14°C, 20°C, 26°C). Three different irradiance regimes (100%, 50% and 0% light) were used within each temperature treatment. Irradiance was provided by natural sunlight and 100% light was equivalent to $1200-1450 \mu\text{m}^{-2} \text{s}^{-1}$. Corals were exposed to these temperature and light treatments for 6 h from 12 pm to 6 pm. Photosynthetic efficiency (F_v/F_m) was determined before returning nubbins to ambient temperatures where they were monitored for

a further 2 d at the same time each day. Respective light regimes were maintained for the entire period. Following the monitoring period nubbins were immediately frozen for further laboratory analysis of chlorophyll concentration and dinoflagellate density (Fig. 3).

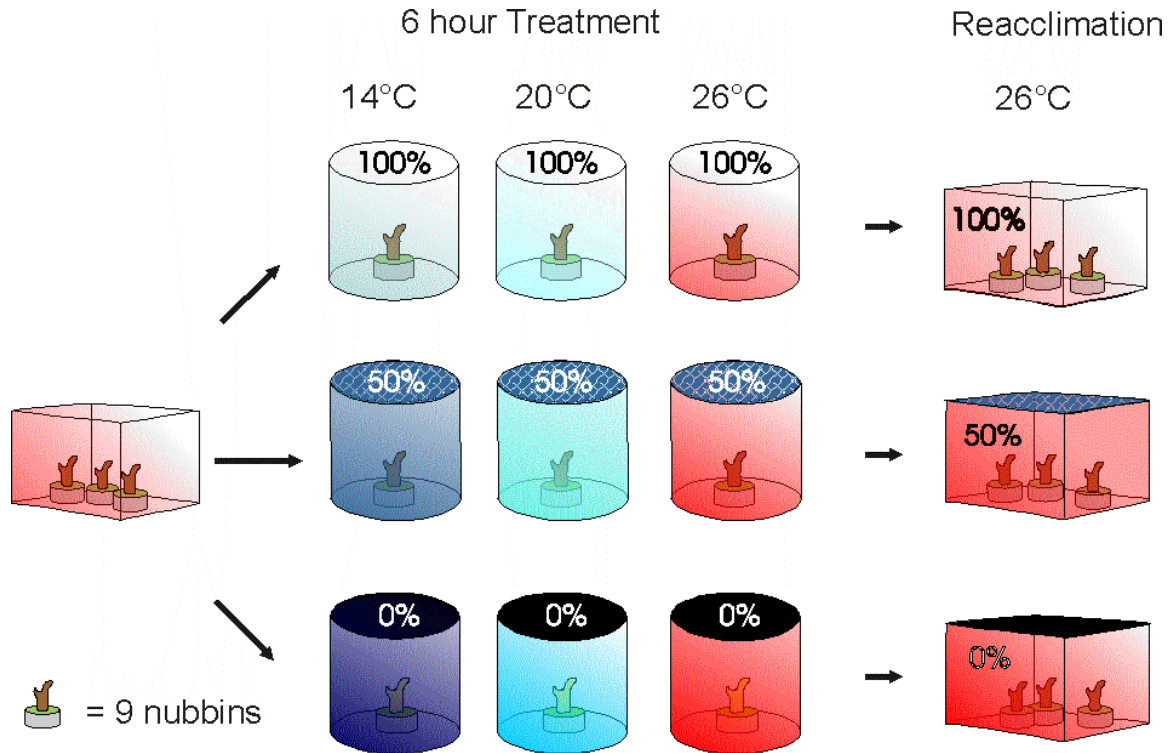


Figure 3: Overview of the synergistic effects of light and temperature experiment. Corals were acclimated for 3 d at ambient temperatures. They were then subjected to one of 3 temperature treatments (14°C, 20°C, or 26°C) for 6 h while maintained under either 100%, 50% or 0% light. Corals were then re-acclimated to ambient temperatures whilst these light regimes were maintained.

Laboratory Analyses

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured using a Pulse Amplitude Modulated (PAM) Fluorometer (DIVING-PAM, Walz, Effeltrich, Germany) (Schreiber *et al.* 1986). The DIVING-PAM was used to measure the minimal (F_o) and maximal (F_m) fluorescence yields. Variable fluorescence (F_v) was calculated as $F_m - F_o$, and maximum potential quantum yield as F_v/F_m . The dark-adapted quantum yield (F_v/F_m , Schreiber 1986), provides a good approximation of the maximum photochemical efficiency of Photosystem II (PSII) (Björkman & Demmig 1987; Öquist *et al.* 1992; Schreiber & Neubauer 1990). Measurements were taken following a dark-adaptation period of 30 min, which allows enough time for relaxation of photoprotection in corals (Jones &

Hoegh-Guldberg 1999). During measurements, the fibre-optic cable of the fluorometer was maintained approximately 1 mm from the coral surface. Measurements were taken at the base of each colony on the side facing directly north during the experimental incubation.

Determination of chlorophyll a content and density of symbiotic dinoflagellates

Coral tissues were stripped from the skeleton with a jet of re-circulated filtered seawater using an oral irrigator (WaterPik™). The resulting slurry was homogenised with a blender for 30 s and the volume of the homogenate was recorded (~100 mL). A 10 mL aliquot of the homogenate slurry was preserved with 1 mL of formalin and the density of symbiotic dinoflagellates was determined by using 8 replicate counts on a haemocytometer. After correcting for homogenate volume and surface area of the coral branch, the densities of symbiotic dinoflagellates were determined and expressed per unit surface area.

Three 10 mL aliquots of homogenate were taken to determine chlorophyll *a* content. Samples were centrifuged for 5 min at 3000 rpm and the supernatant was discarded, leaving an algal pellet. The samples were resuspended with acetone, and placed in a freezer for 24 h. Samples were centrifuged again at 3000 rpm for 5 min and the absorbances of the supernatant were determined at 664 nm, 647 nm, 630 nm, and 750 nm on a spectrophotometer (Pharmacia LKB Ultraspec III) to determine chlorophylls *a*, *b* and *c*, and turbidity, respectively. The absorbances at 664 nm and 750 nm were recorded following acidification with 10% HCl to determine phaeophytin concentration. Concentrations of chlorophyll were calculated using equations of Jeffrey and Humphrey (1975) after correcting for homogenate volume and the surface area of the coral samples.

Determination of surface area

Surface area was determined using melted wax maintained at 65°C in a water bath. Nubbins were dipped in the melted wax for a standard period of time (5 s). When set, the nubbin was weighed then dipped again into the wax (5 s). The difference between the first and second weight allows the surface area to be calculated by comparison with standardised cubes of known surface area (Ward *et al.* 2000).

Statistical analyses

A two-way ANOVA with fixed effects was conducted on STATISTICA® software (StatSoft, Inc. Tulsa USA) to determine differences between treatments for photosynthetic pigment analyses,

counts of symbiotic dinoflagellates, and measurements of photosynthetic efficiency (F_v/F_m). Before analysis Cochran's Test was used to determine the homogeneity of variances, and data were then log transformed when required, resulting in homogeneous or near-homogeneous samples (Eisenhart *et al.* 1947). Square root transformations were applied when the group variances were proportional to the means (Zar 1984). When ANOVA determined a significant difference, Tukey's Post-hoc test was used to attribute differences between specific treatments (Zar 1984).

RESULTS

Time course of response to cold temperature stress

Photosynthetic efficiency

There were no significant differences in F_o , F_m or F_v/F_m between control treatments conducted on three different days ($p > 0.05$). Results from the different control treatments were therefore pooled. This also indicated that the different temperature treatments, while conducted on different days with different light regimes, could be directly compared. There was no immediate effect on F_v/F_m after 1 h exposure to any temperature treatment. However, following 3 h exposure to decreased temperatures F_v/F_m declined by 0.06, 0.15, and 0.22 in the 20°C, 16°C and 12°C treatments, respectively. This trend was maintained with increasing duration of the treatments, with the 12°C treatment showing the greatest decrease in F_v/F_m throughout, followed by the 16°C treatment. While F_v/F_m in the 20°C treatment was lower than the control treatment, this difference was not significant ($p > 0.05$). However, both the 16°C and 12°C treatment were significantly different from the controls after 3 h and 6 h exposure to colder temperatures ($p < 0.05$, $p < 0.001$, respectively). Only the 12°C treatment was significantly different after 12 h and 18 h exposure (Fig. 4).

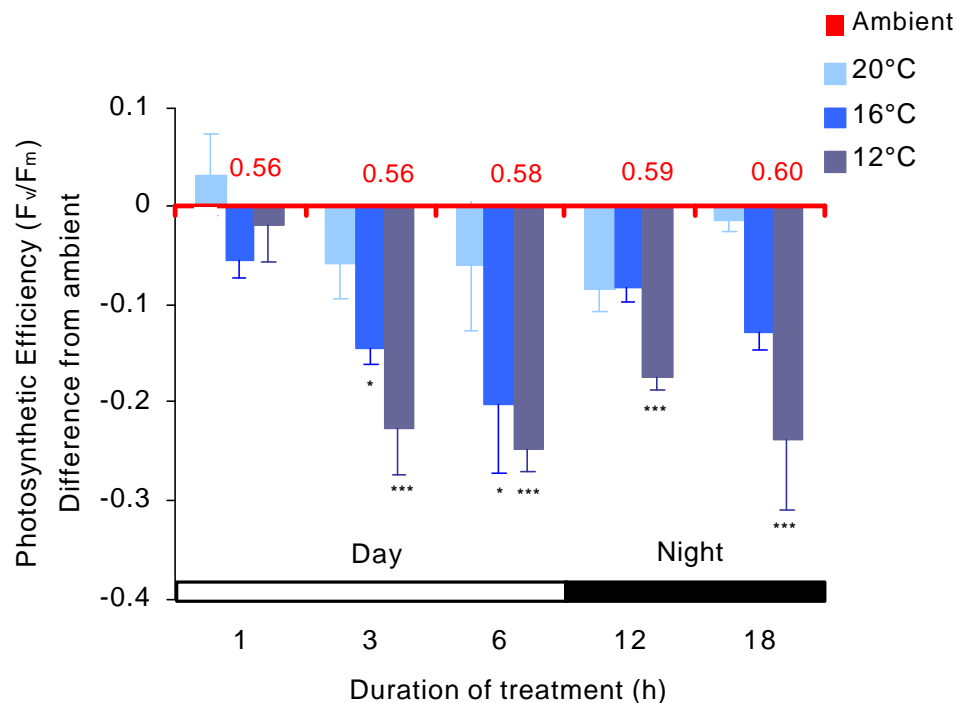


Figure 4: Difference in photosynthetic efficiency (F_v/F_m) of *Montipora digitata* immediately following exposure to one of four temperature treatments (23°C (ambient), 20°C, 16°C, or 12°C) of varying duration (1 h, 3 h, 6 h, 12 h, or 18 h). The bar represents the day/night cycle. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Red numbers on the x-axis are the mean F_v/F_m at ambient temperatures.

There were no significant differences in F_o between different temperature regimes throughout the experiment ($p > 0.05$). F_o was not significantly different between treatments at the 1 h, 3 h and 6 h sampling times. However, after 12 h and 18 h exposure, F_o in the 16°C treatment increased substantially, though not significantly ($p > 0.05$) compared to the 12°C and 23°C treatments. The F_o of the 20°C treatment also increased after 12 h (Fig. 5).

In comparison, F_m showed a significant reduction in both the 12°C and 16°C treatments after 6 h exposure compared to the control ($p < 0.01$ and $p < 0.01$, respectively). F_m remained significantly reduced in the 12°C treatment throughout the experimental period (12 h, $p < 0.01$; 18 h, $p < 0.05$). However in the 16°C treatment, F_m increased significantly after 12 h and 18 h to the levels shown in the 20°C and 23°C treatments, with a significant difference from the 12°C treatments observed ($p < 0.01$) (Fig. 5).

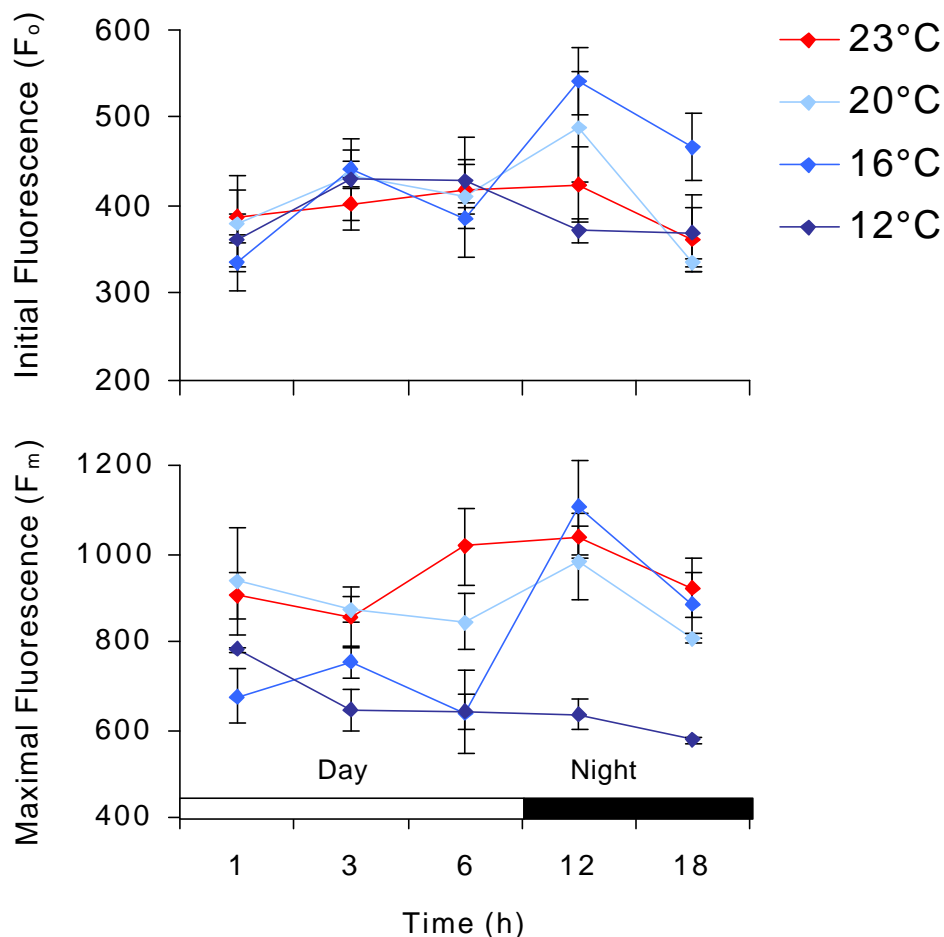


Figure 5: Differences in initial fluorescence (F_o) and maximal fluorescence (F_m) of the coral *Montipora digitata* immediately following exposure to one of four temperature treatments (23°C (ambient), 20°C, 16°C, or 12°C) of varying duration (1 h, 3 h, 6 h, 12 h, or 18 h). The bar represents the day/night cycle.

Recovery of the photosynthetic efficiency of the dinoflagellates of corals (measured using F_v/F_m) also varied depending on treatment temperature and duration of exposure. F_v/F_m in the control corals remained consistent throughout the four day experiment, and was similar to levels measured in corals freshly collected from the field (range 0.56 – 0.62, $n = 20$). Diurnal fluctuations in F_v/F_m were apparent in the controls, with F_v/F_m lowest after 6 h (corresponding to 2 pm) and highest after 18 h (corresponding to 2 am). In the 20°C treatment, F_v/F_m decreased slightly after 3 h, 6 h and 12 h exposure, however there was no apparent decrease after 1 h or 18 h exposure. F_v/F_m then recovered over the next 72 h. A similar trend was observed in the 16°C treatment, with an initial decrease in F_v/F_m , followed by recovery. In the 12°C treatment, F_v/F_m showed the greatest initial decrease after 3 h, 6 h, 12 h, and 18 h exposure. While F_v/F_m did recover in the 3 h and 6 h treatments, there was no recovery of F_v/F_m after 12 h or 18 h exposure at 12°C. This lack of recovery corresponded to severe coral bleaching and/or resulting mortality. It must be noted that recovery was not complete in either the 12°C or the 16°C treatment, as F_v/F_m remained considerably depressed when compared to initial levels (Fig. 6).

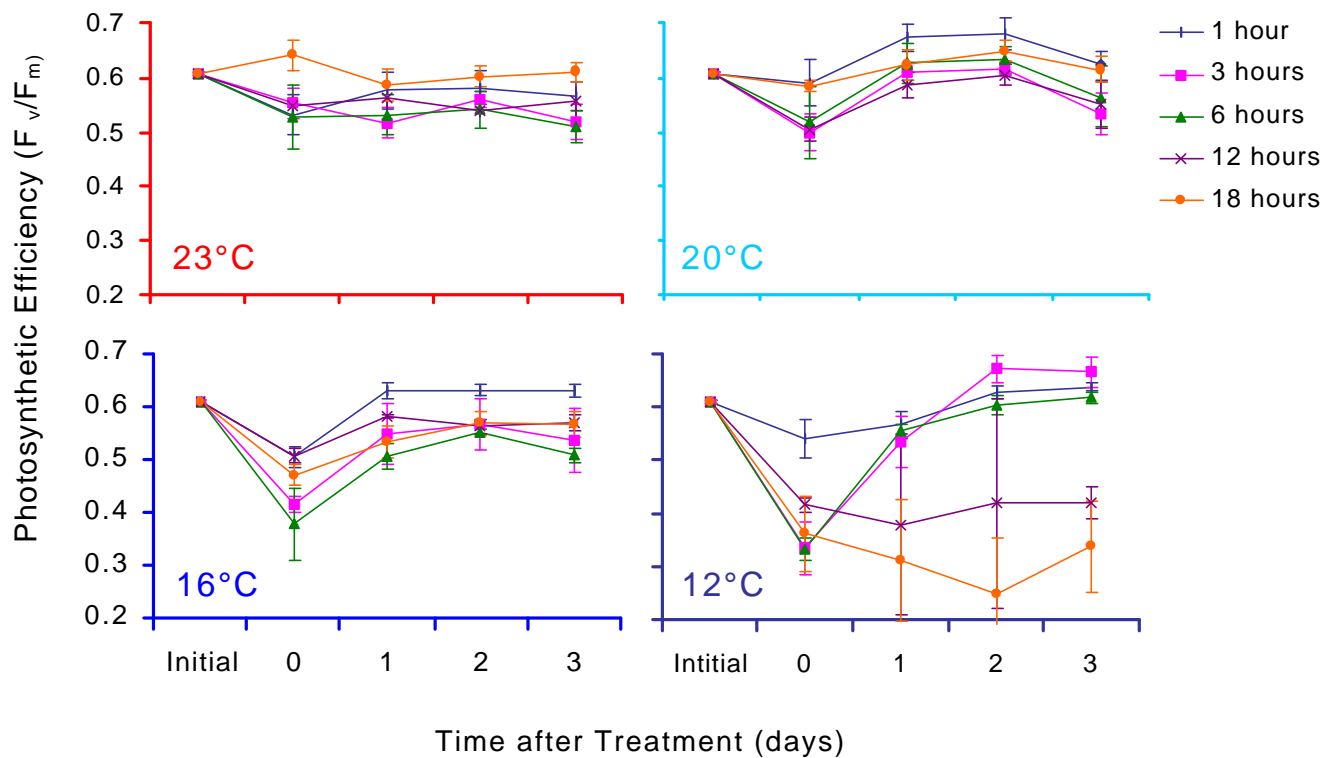


Figure 6: Recovery of photosynthetic efficiency (F_v/F_m) in *Montipora digitata* over a 4 d period following exposure to one of four temperature treatments (23°C (ambient), 20°C, 16°C, or 12°C) of varying duration (1 h, 3 h, 6 h, 12 h, or 18 h).

Concentration of photosynthetic pigments and symbiotic dinoflagellates

The duration of exposure to decreased temperature showed no significant effect ($p > 0.05$) on either the concentration of photosynthetic pigments or the density of symbiotic dinoflagellates. Therefore results were pooled to give an indication of overall trends of these parameters between the four different temperature treatments. The 12°C treatment showed a significant decrease in chlorophyll *a*, *c*, and *a+c* content compared to the other treatments ($p < 0.001$). The 12°C treatment also showed a significant decrease in the density of symbiotic dinoflagellates from the 23°C and 20°C treatments ($p < 0.001$, $p < 0.001$, respectively). There were no significant differences in the chlorophyll *a*: *c* ratio or in phaeophytin content between treatments ($p > 0.05$). However, the 16°C treatment showed a significant increase from the 23°C control in chlorophyll *a* cm^{-2} and chlorophyll *a+c* content, and in chlorophyll *a* content per dinoflagellate ($p < 0.01$, $p < 0.05$, $p < 0.001$, respectively). Chlorophyll *a* content per dinoflagellate was also significantly increased in the 16°C treatment compared to the 12°C treatment ($p < 0.001$) (Table 1).

Table 1: Differences in chlorophyll *a* and chlorophyll *c* parameters and the density of dinoflagellates in the coral *Montipora digitata* following exposure to one of 4 temperature treatments (23°C (ambient), 20°C, 16°C, or 12°C).

Temperature (°C)	Chlorophyll <i>a</i> content (µg cm ⁻²)	Chlorophyll <i>c</i> content (µg cm ⁻²)	Chlorophyll <i>a</i> : <i>c</i>	Chlorophyll <i>a</i> + <i>c</i>	Phaeophytin content (µg cm ⁻²)	Dinoflagellate density x 10 ⁶ (cm ⁻²)	Chlorophyll <i>a</i> content per dinoflagellate
23	3.55 ^a	2.65 ^a	1.37	6.20 ^a	0.27	3.51 ^a	1.08 ^a
20	4.19 ^{ab}	2.87 ^a	1.55	7.06 ^{ab}	0.32	3.64 ^a	1.17 ^a
16	4.71 ^b	3.01 ^a	1.64	7.72 ^b	0.25	3.04 ^{ab}	1.63 ^b
12	2.09 ^c	1.49 ^b	1.60	3.58 ^c	0.20	2.37 ^b	0.93 ^a
F-value	21.6 ^{***}	18.5 ^{***}	1.1	24.8 ^{***}	0.2	9.4 ^{***}	12.4 ^{***}

* p < 0.05; ** p < 0.01; *** p < 0.001. ^{abc} means with different letters are significantly different at p < 0.05.

Cumulative impacts of cold temperature stress

Photosynthetic efficiency

Photosynthetic efficiency (F_v/F_m) of the dinoflagellates in the control corals remained consistent throughout the initial experiment, and was similar to levels measured in freshly collected corals (range 0.66 – 0.7, $n = 10$; see also (Hoegh-Guldberg & Jones 1999 and others). No significant differences in F_o , F_m or F_v/F_m were observed between treatments in the initial cold experiment ($p > 0.05$). However, there was 12% mortality of corals exposed to the initial 15°C treatment during the 3 d recovery period following treatment. No mortality occurred among control corals.

Following re-collection of the experimental corals from the reef flat, photosynthetic efficiency (F_o , F_m and F_v/F_m) of both pre-treated and pre-control corals was similar to the photosynthetic efficiency of freshly collected corals (range 0.62 – 0.68, $n = 10$). However both F_o and F_m showed a significant decrease after the 3 month recovery period compared to values in the initial experiment ($p < 0.001$). As F_o and F_m at both times were similar to field measurements of corals, this is likely due to seasonal differences. Approximately 20% mortality was observed in both the control and treatment corals 3 months after being placed back in the field. This was largely due to uncontrolled factors such as sedimentation and algal overgrowth.

Following the second cold treatment, those corals that received two treatments of 15°C showed a significant decrease in F_v/F_m relative to controls and pre-treated controls ($p < 0.001$ and $p < 0.001$, respectively). In contrast, the F_v/F_m of corals that received only one treatment at 15°C in January was not significantly different from the controls or from the corals receiving two temperature treatments. However, F_v/F_m was substantially decreased in these corals. There was a significant decrease in F_o in the single-treatment corals compared with the controls ($p < 0.001$), while the F_m of the single-treatment corals was significantly different from both the pre-treated controls and the control corals ($p < 0.001$). In comparison, there was a significant decrease in F_m in those corals that received two treatments from both the pre-treated controls and the control corals ($p < 0.001$). No mortality was observed in either the treatment or control corals following the second cold treatment (Table 2).

Table 2: Photosynthetic efficiency (dark adapted F_v/F_m , F_o and F_m), dinoflagellate density and chlorophyll *a* content per dinoflagellate and percentage mortality in the coral *Montipora digitata* following treatment at 15°C or 23°C, measured before and after a recovery period of three months on the reef flat at Heron Island, southern Great Barrier Reef. These parameters are measured again following a secondary treatment at either 15°C or 23°C.

Sample	Photosynthetic efficiency (F_v/F_m)	Initial fluorescence (F_o)	Maximal fluorescence (F_m)	Density of dinoflagellates ($\times 10^6$)	Chlorophyll <i>a</i> content per dinoflagellate	Mortality (%)
Initial Cold Treatment						
Control (23°C)	0.689 ^a	343.3 ^a	1097.9 ^{ad}	2.32 ^{ab}	1.14 ^{ab}	0
Treatment (15°C)	0.61 ^a	342.3 ^a	888.5 ^{be}	1.42 ^a	1.16 ^{ab}	12
3 Month Recovery						
Control	0.659 ^a	234.4 ^b	686.5 ^b	2.38 ^{ab}	1.04 ^{ab}	21
Treatment	0.657 ^a	218.6 ^b	665.6 ^c	2.44 ^{ab}	1.15 ^{ab}	40
Second Cold Treatment						
Treatment (15°C)/Treatment (15°C)	0.607 ^b	424.3 ^{ac}	1081.3 ^{def}	1.35 ^a	0.79 ^a	0
Control (23°C)/Control (26°C)	0.657 ^a	472.2 ^c	1375.3 ^f	2.77 ^{ab}	1.49 ^{ab}	0
Control (23°C)/Treatment (15°C)	0.629 ^{ab}	354.0 ^{ad}	963.0 ^{de}	1.77 ^{ab}	1.33 ^{ab}	0
Treatment (15°C)/Control (26°C)	0.659 ^a	453.7 ^{cd}	1335.7 ^{af}	3.07 ^b	1.6 ^b	0
F-Value	16.0 ^{***}	48.1 ^{***}	39.5 ^{***}	4.0 ^{**}	3.1 [*]	-

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ^{abc} means with different letters are significantly different at $p < 0.05$.

Density of symbiotic dinoflagellates

There was no significant difference in either the density of symbiotic dinoflagellates or the amount of chlorophyll *a* content per dinoflagellate between the control and the treatment corals after the initial treatment ($p > 0.05$). However, there was a substantial decrease in the density of symbiotic dinoflagellates exposed to the 15°C treatment. Over the 3 month recovery period, the density of dinoflagellates in the pre-treated corals recovered to those observed in the controls and in field collected corals ($p > 0.05$). Following the second experiment, those corals that were subjected to two treatments at 15°C showed a significant decrease in the density of dinoflagellates ($p < 0.01$) and chlorophyll *a* content per dinoflagellate ($p < 0.05$) when compared to the controls and the pre-treated controls. While the corals that received only one treatment at 15°C also showed a decrease in these parameters, it was not significantly different from either the controls, the pre-treated controls or the pre-treated treatment corals ($p > 0.05$) (Table 2).

Concentration of photosynthetic pigments

Following the initial treatment at 15°C, there was a substantial decrease in chlorophyll *a*, chlorophyll *c*, and chlorophyll *a+c* content of the treated corals, however this decrease was not significant ($p > 0.05$). There was also a substantial increase in the chlorophyll *a:c* ratio related to a relative decrease in chlorophyll *c* content. There were no significant differences observed in phaeophytin concentration between treatments and controls ($p > 0.05$) (Table 3).

After a three month recovery period, photosynthetic pigments recovered in the pre-treated corals with slightly higher concentrations compared to the controls. Following the second experiment, chlorophyll *a* and chlorophyll *c* content was significantly reduced in the corals subjected to two treatments at 15°C compared to the controls and pre-treated controls ($p < 0.001$). In comparison, the corals subjected to a single treatment did not show as great a decrease in either chlorophyll *a* or *c*. While there was a significant difference in chlorophyll *c* and chlorophyll *a+c* between the single treatment and both the controls and pre-treated controls ($p < 0.05$), the chlorophyll *a* content in the single treatment was only significantly different from the pre-treated controls ($p < 0.001$). The chlorophyll *a:c* ratio was significantly decreased in the corals subjected to two treatments ($p < 0.05$), again indicating a relative decrease in chlorophyll *c*. There was no observed decrease in this ratio in the corals subjected to one treatment only (Table 3).

Table 3: Pigment concentrations of the coral *Montipora digitata* following treatment at 15°C or 23°C, measured before and after a 3 month recovery period on the reef flat at Heron Island, southern Great Barrier Reef. These parameters are measured again following a secondary treatment at either 15°C or 23°C.

Sample	Chlorophyll <i>a</i> ($\mu\text{g cm}^{-2}$)	Chlorophyll <i>c</i> ($\mu\text{g cm}^{-2}$)	Chlorophyll <i>a:c</i>	Chlorophyll <i>a+c</i>	Phaeophytin ($\mu\text{g cm}^{-2}$)
Initial Cold Treatment					
Control (23°C)	2.82 ^{ab}	2.21 ^{ab}	1.33 ^{ab}	5.57 ^{abc}	0
Treatment (15°C)	1.52 ^b	1.01 ^{acd}	1.58 ^{ab}	2.54 ^{bc}	0.13
3 Month Recovery					
Control	3.19 ^{ab}	2.38 ^{ab}	1.33 ^{ab}	5.57 ^{abc}	0
Treatment	3.49 ^{ab}	2.44 ^{ab}	1.44 ^{ab}	5.94 ^{abc}	0.001
Second Cold Treatment					
Treatment (15°C)/Treatment (15°C)	1.45 ^b	1.35 ^{ac}	1.06 ^a	2.8 ^c	0.1
Control (23°C)/Control (26°C)	4.07 ^{acd}	2.77 ^b	1.47 ^{ab}	6.84 ^{ab}	0
Control (23°C)/Treatment (15°C)	2.61 ^{bd}	1.77 ^{ac}	1.50 ^{ab}	4.38 ^c	0.01
Treatment (15°C)/Control (23°C)	5.23 ^c	3.07 ^{bd}	1.72 ^b	8.3 ^a	0
F-value	6.6 ^{***}	5.5 ^{**}	5.1 [*]	6.7 ^{***}	2.8

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ^{abc} means with different letters are significantly different at $p < 0.05$.

Synergistic effects of light and temperature

Photosynthetic efficiency

There were significant differences in photochemical efficiency (F_v/F_m) dependant upon the combination of the temperature and light regime. Photochemical efficiency of dinoflagellates within the corals exposed to 14°C under 100% incident irradiance was significantly decreased (0.46) compared to corals exposed to 20°C (0.53) and 26°C (0.58) ($p < 0.001$ and $p < 0.001$ respectively). The 14°C treatment was also significantly lower (0.53) than the 26°C treatment (0.60) at 50% light ($p < 0.01$). However there were no significant differences between any of the three temperature treatments at 0 % light ($p > 0.05$).

Following exposure to 14°C, photochemical efficiency was also significantly different between the 100% light treatment and corals maintained at 0% and 50% light ($p < 0.05$ and $p < 0.001$, respectively). While photochemical efficiency decreased at higher light intensities in the 20°C treatment, these differences were not significantly different ($p > 0.05$) either within the treatment, or when compared to the control (Fig. 7).

The reductions in photochemical efficiency can be explained by changes in the F_o and F_m of the corals. At 100% light, both the 14°C and 20°C treatments showed a significant increase in F_v/F_m from the control at all light levels ($p < 0.001$ and $p < 0.01$, respectively). At 50% light, the 14°C treatment is also significantly higher than the control ($p < 0.01$). Overall, there is an increase in F_o with decreasing temperature. F_m also varies significantly between different treatments and light regimes. F_m in the 14°C treatment is significantly reduced at 100% light compared to at 50% ($p < 0.01$). At 0% light, F_m in the 26°C control is significantly lower than in the 14°C and 20°C treatments ($p < 0.001$ and $p < 0.05$, respectively). Overall, there is an increase in F_m with decreasing temperature in both the 50% and 0% light treatments, however, in the 100% light treatment F_m decreases again in the 14°C treatment (Fig. 8).

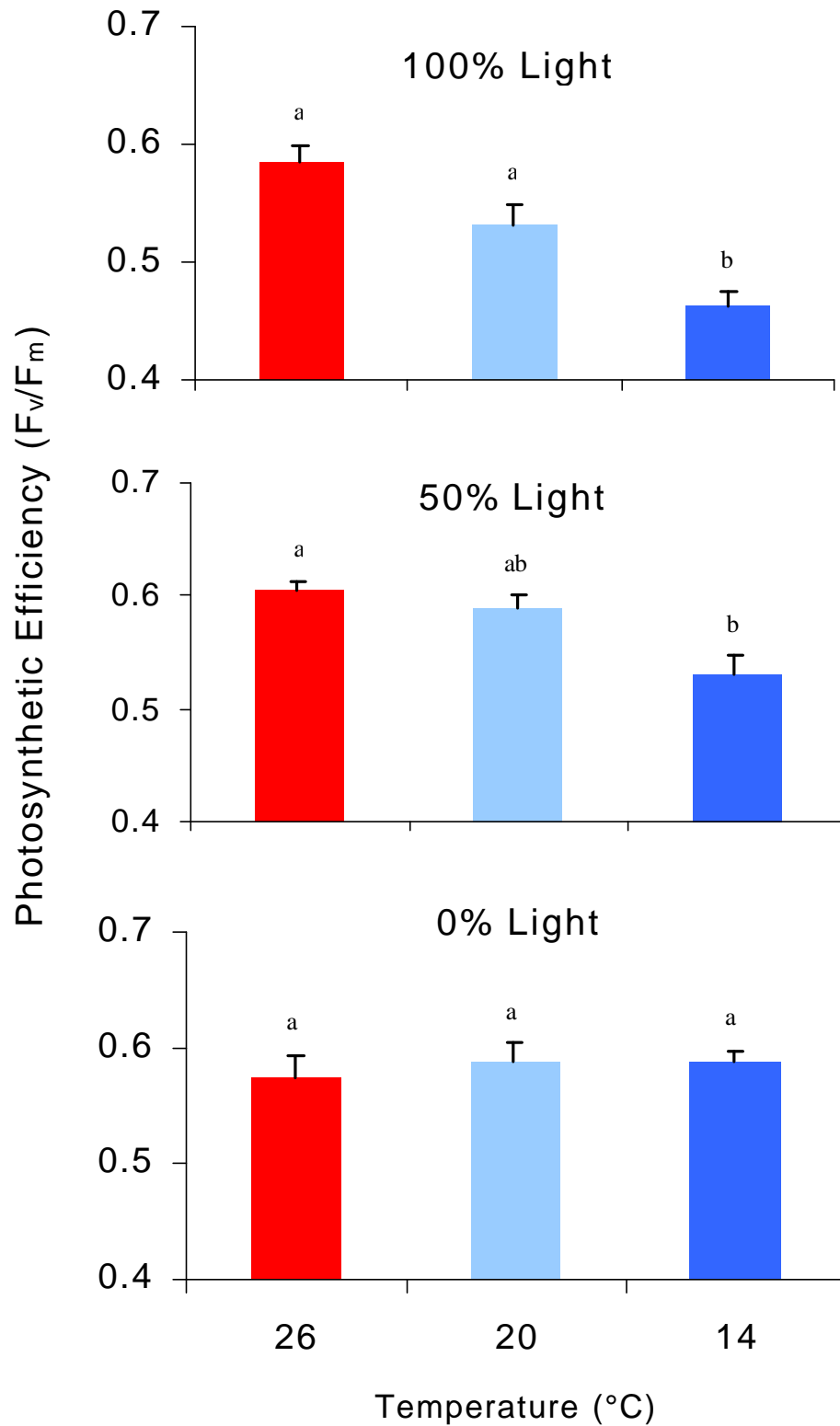


Figure 7: Photosynthetic efficiency (F_v/F_m) of the coral *Montipora digitata* following exposure to different temperature treatments (14°C, 20°C, 26°C) and light regimes (0%, 50%, 100%). ^{abc} means with different letters are significantly different at $p < 0.05$.

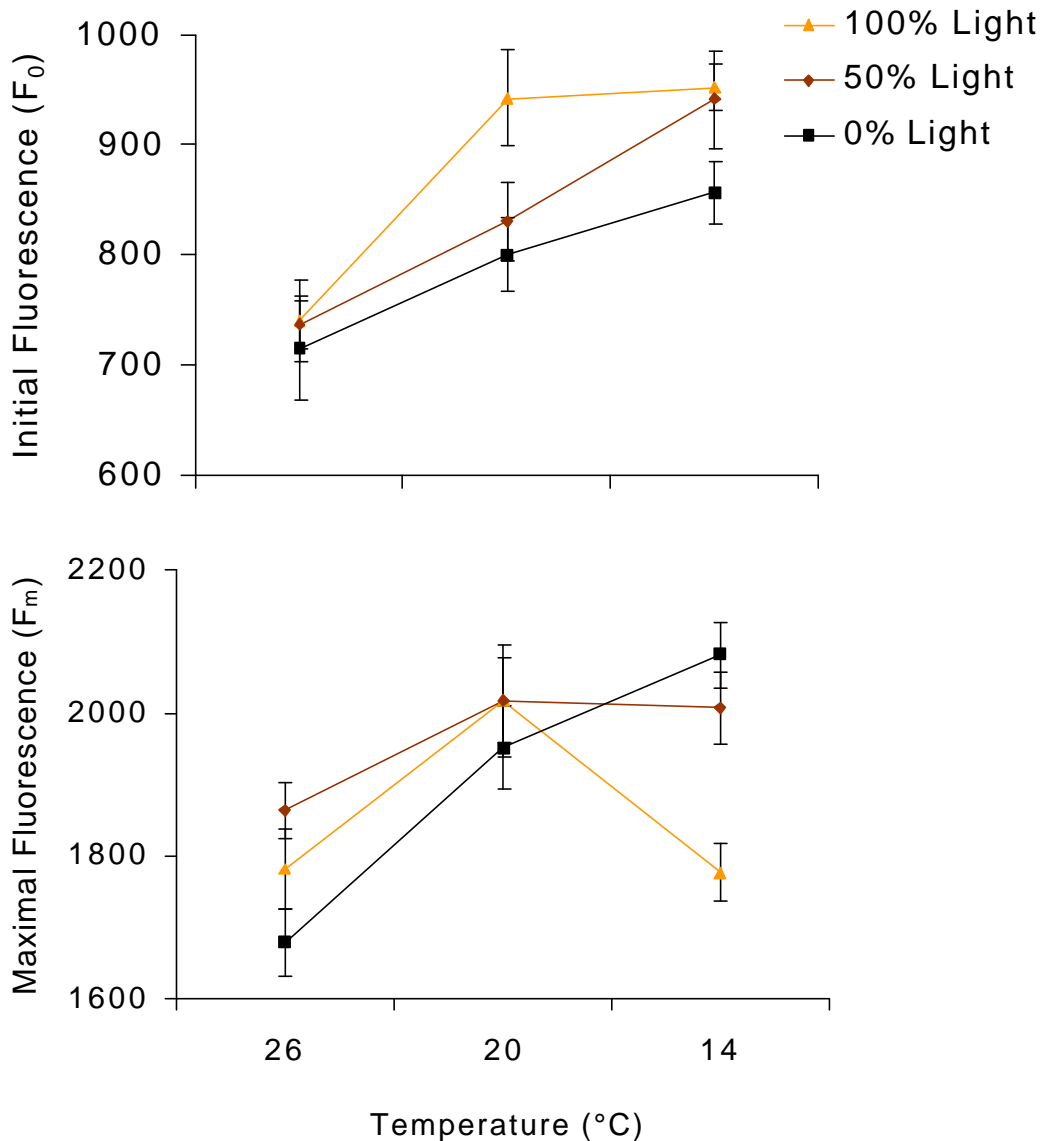


Figure 8: Differences in initial fluorescence (F_0) and maximal fluorescence (F_m) of the coral *Montipora digitata* immediately following exposure to one of three temperature treatments (26 $^{\circ}\text{C}$ (ambient), 20 $^{\circ}\text{C}$, 14 $^{\circ}\text{C}$) while maintained under one of three light regimes (100%, 50% or 0%).

Density of symbiotic dinoflagellates

The density of symbiotic dinoflagellates was significantly reduced in the 14 $^{\circ}\text{C}$ treatment compared to the 20 $^{\circ}\text{C}$ treatment in all light regimes ($p < 0.01$). There was also a significant difference between the 26 $^{\circ}\text{C}$ treatment and the 14 $^{\circ}\text{C}$ treatment at 100% ($p < 0.05$) (Fig. 9).

Chlorophyll *a* content per symbiotic dinoflagellate showed significant increases in the 14 $^{\circ}\text{C}$ treatment at 50% and 100% light, compared to the control treatment ($p < 0.001$ and $p < 0.001$, respectively). Generally, chlorophyll *a* content per dinoflagellate appeared to increase with decreasing temperature at all light levels (Fig. 10).

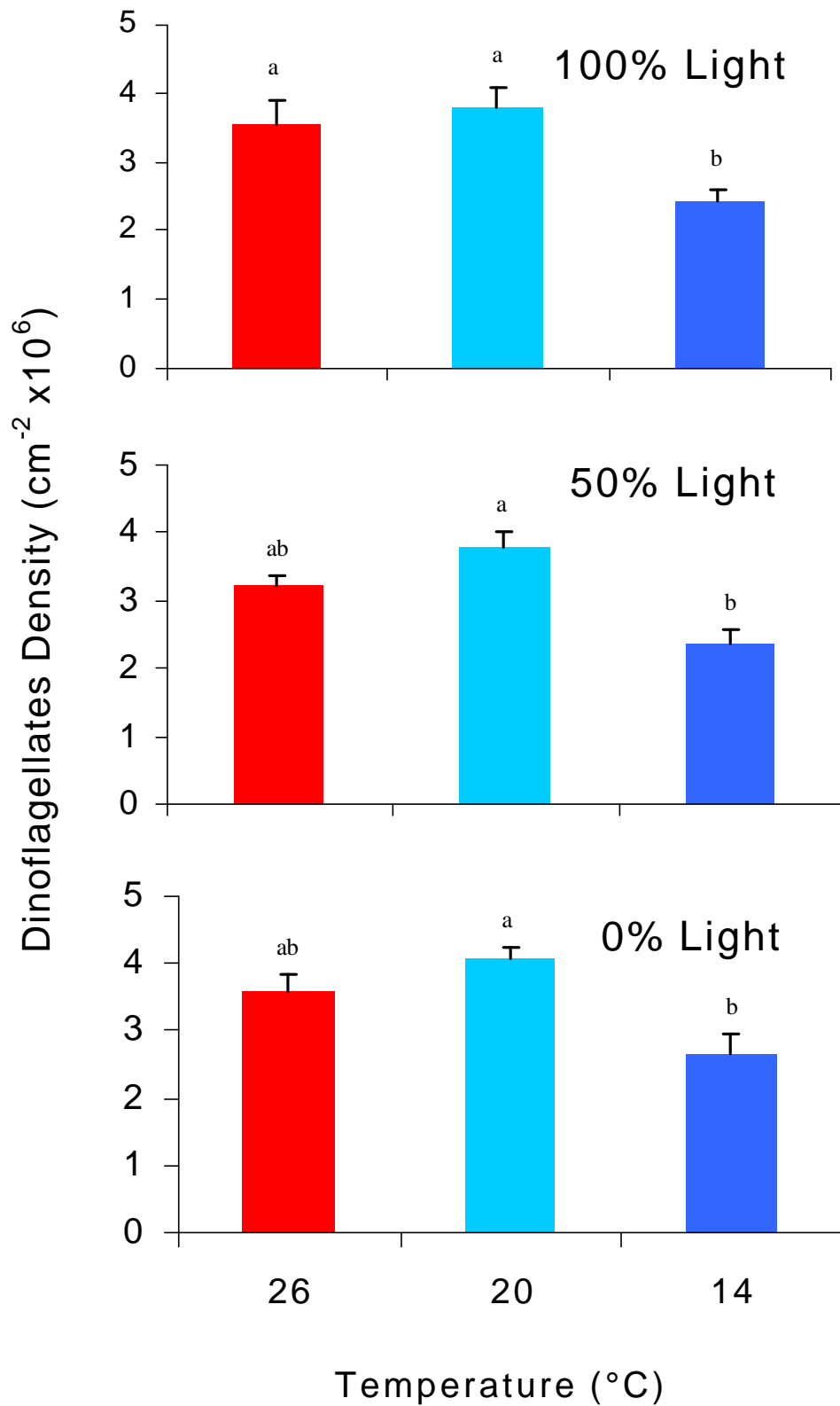


Figure 9: Density of symbiotic dinoflagellates cm⁻² in the coral *Montipora digitata* following exposure to one of three different temperature treatments (14°C, 20°C, 26°C) whilst maintained at one of three light regimes (100%, 50%, 0%). ^{abc} means with different letters are significantly different at p < 0.05.

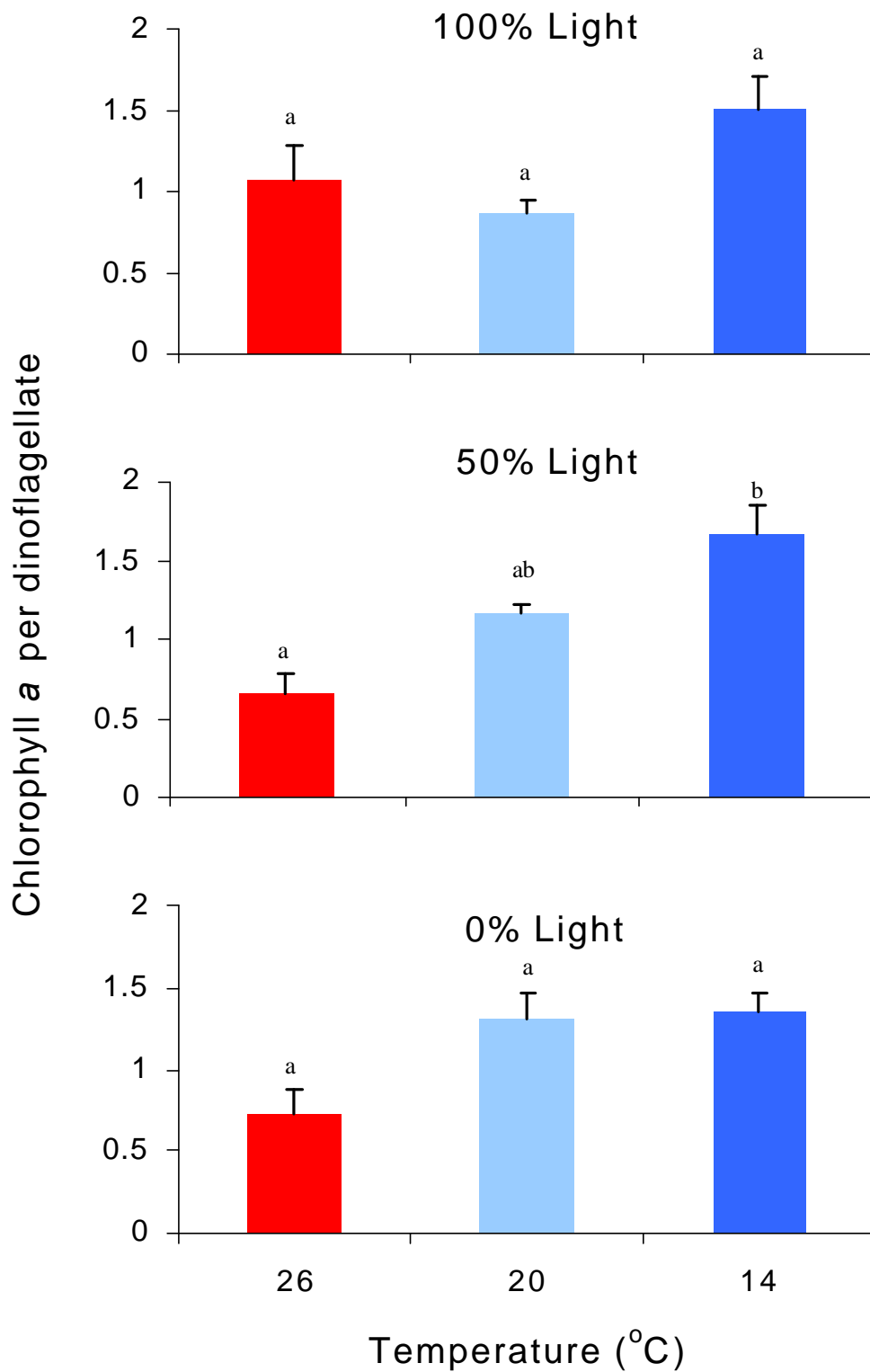


Figure 10: Differences in chlorophyll *a* content per symbiotic dinoflagellates in the coral *Montipora digitata* following exposure to one of three different temperature treatments (14°C, 20°C, 26°C) whilst maintained under one of three light regimes (100%, 50%, 0%). ^{abc} means with different letters are significantly different at $p < 0.05$.

Concentration of photosynthetic pigments

In the 100% light regime, chlorophyll *a* content increased slightly in the 14°C treatment when compared to the 26°C ambient control. Under 50% and 0% light, this trend was more pronounced, with a significant difference observed between the 20°C treatment and the 26°C treatment at 0% and 50% light ($p < 0.05$ and $p < 0.01$, respectively) (Table 4).

There was a significant increase in chlorophyll *c* content in the 20°C treatment compared to the 14°C and 26°C treatments under all light regimes ($p < 0.001$ and $p < 0.001$, respectively). This corresponded with a large decrease in the chlorophyll *a*: *c* ratio in the 20°C treatment under all light regimes, as the amount of chlorophyll *c* increased relative to chlorophyll *a*. The ratio of chlorophyll *a*: *c* appeared to increase with increasing light in the 14°C and 26°C treatments, indicating a relative decrease in chlorophyll *c* content. The overall content of photosynthetic pigments (chlorophyll *a+c*) showed a significant increase in the 20°C treatment at 50% and 0% light compared to the 14°C and 26°C treatments ($p < 0.001$ and $p < 0.001$, respectively). This contrasts with the amount of phaeophytin present, with a significant decrease observed in the 20°C treatment compared to the 14°C and 26°C treatments ($p < 0.01$ and $p < 0.05$). (Table 4).

Table 4: Differences in chlorophyll a and chlorophyll c parameters in the symbiotic dinoflagellates of the coral *Montipora digitata* following treatment at one of 3 temperatures (26°C (ambient), 20°C and 14°C) and 3 light regimes (100%, 50%, and 0%). ^{abc} indicate significant differences between treatments.

Temperature (°C)	Light Intensity (%)	Chlorophyll <i>a</i> (i g cm ⁻²)	Chlorophyll <i>c</i> (i g cm ⁻²)	Chlorophyll <i>a: c</i>	Chlorophyll <i>a+c</i>	Phaeophytin (i g cm ⁻²)
26	100	3.60 ^{ab}	2.56 ^a	4.15 ^a	6.16 ^{ab}	0.35 ^a
	50	2.09 ^a	2.78 ^a	0.93 ^a	4.39 ^a	0.28 ^a
	0	2.48 ^a	2.38 ^a	1.15 ^a	4.87 ^a	0.43 ^a
20	100	3.23 ^{ab}	4.71 ^b	0.71 ^a	7.95 ^{ab}	0.03 ^b
	50	4.41 ^b	4.70 ^b	0.94 ^a	9.11 ^b	0.10 ^b
	0	5.32 ^b	4.85 ^b	1.07 ^a	10.17 ^b	0.13 ^b
14	100	3.83 ^{ab}	1.75 ^a	2.11 ^a	5.59 ^{ab}	0.63 ^a
	50	3.75 ^{ab}	2.01 ^a	1.92 ^a	5.75 ^a	0.47 ^a
	0	3.51 ^{ab}	2.47 ^a	1.46 ^a	5.97 ^a	0.72 ^a
F – values		9.5 ^{***}	62.8 ^{***}	1.1	26.7 ^{***}	48.3 ^{**}

* p < 0.05; ** p < 0.01; *** p < 0.001. ^{abc} means with different letters are significantly different at p < 0.05.

DISCUSSION

The impact of changes to sea temperature has been a major issue in recent literature due to the widespread impacts of mass coral bleaching events (Glynn 1993; Hoegh-Guldberg 1999; Sebens 1994; Wilkinson 1993) and the global importance of climate change. Most previous studies have focused on the effects of increased sea surface water temperatures. While average temperatures are warming in tropical areas due to greenhouse warming (IPCC 2000), the influence of climate change on the overall variability of weather systems like the El Niño Southern Oscillation (ENSO) suggest that there may be periods in which colder than normal temperatures may be experienced. There is little knowledge or understanding of how these colder excursions in sea temperature (e.g. during La Niña periods) will affect marine organisms. This study has explored how reduced temperatures will impact a coral-dinoflagellate symbiosis (*Montipora digitata*).

Synergistic effects of light and temperature

The results of the present study clearly show that exposure to cold-water stress has a negative impact on the physiology of the coral *Montipora digitata*. This is indicated by decreased photosynthetic efficiency, loss of symbiotic dinoflagellates, and changes in concentrations of photosynthetic pigments associated with chlorophyll degradation. The mechanisms of coral bleaching at lowered temperatures are similar to those involved in elevated temperature stress. It is well documented that decreased temperatures intensify photoinhibition in higher plants (Aro *et al.* 1990; Foyer *et al.* 1994; Greer 1990; Greer & Laing 1991; Long *et al.* 1994; Lyons 1973; Smillie *et al.* 1988) due to a reduction in the rate at which the quenching of Photosystem II (PSII) develops (Krause 1992). In the present study decreased temperatures clearly exacerbated the photoinhibitory response of corals at higher light intensities and produced physiological responses similar to those observed in corals exposed to elevated temperatures (Jones *et al.* 1998).

Symbiotic dinoflagellates exposed to cold-water conditions and high light intensity had significant decreases in photochemical efficiency (F_v/F_m) and reduced numbers of viable dinoflagellates compared to the controls. Decreased photochemical efficiency is indicative of photoinhibition (Hoegh-Guldberg & Jones 1999; Schreiber & Bilger 1987) while reduced dinoflagellate density is a response that is typical of general stress among reef-building corals. These responses implicate an increased sensitivity to photoinhibition as a key aspect associated

with coral bleaching following exposure of corals to cold-water stress. Previous studies have also shown increased respiration of the symbiotic dinoflagellates in response to lowered temperatures (Steen & Muscatine 1987), probably due to the direct effects of lower temperature on the activity of enzymes and their substrates.

It is likely that the increased susceptibility of corals to photoinhibition with decreasing temperatures is caused by the impairment of the Calvin-Benson cycle (Hoegh-Guldberg 1999). The rates of enzyme activity are temperature dependent (Hoegh-Guldberg 1995), therefore with decreasing temperature the enzymes catalysing the Calvin-Benson cycle are slowed. The subsequent reduction in photosynthetic electron transport combined with continued high absorption of light energy leads to damage or inactivation of PSII from the production of toxic oxygen species (Lesser 1996; Osmond 1994). The photosystem has a decreased capacity to capture and process photons (Long *et al.* 1994; Osmond 1994), characterised by the accumulation of photochemically inactive PSII reaction centres (Krause 1994). This typically results in a decrease in the overall photosynthetic rate (Richter *et al.* 1990), indicated by increased baseline fluorescence (F_o), which translates as a decreased yield (F_v/F_m), which was observed in the present study.

It has been proposed that the resulting cellular damage to the symbiotic dinoflagellates caused by oxidative stress results in an energetic cost to the coral host, either in terms of decreased translocation of photosynthate, or exposure to highly reactive oxygen radicals (Lesser & Shick 1989). As a result, the dinoflagellates are expelled from the host tissues, probably as a protective mechanism against further oxidative stress (Lesser 1997). This was supported by the present study as the density of symbiotic dinoflagellates was significantly reduced with decreasing temperatures and increasing light (Plate 3).

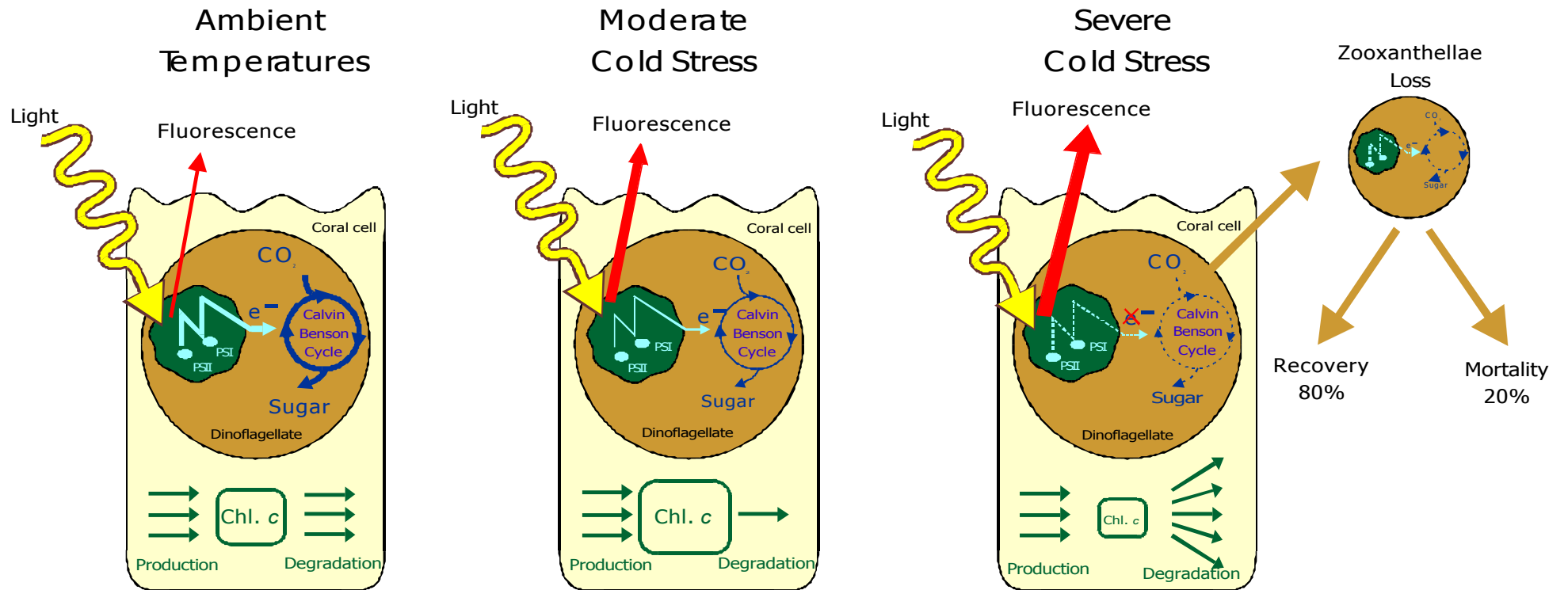


Plate 3: Conceptual model showing the effects of light and cold water stress on the physiological reactions of symbiotic dinoflagellates in the coral *Montipora digitata*.

Chlorophyll *a* content per symbiotic dinoflagellate showed a significant increase in corals subjected to 14°C at 50% and 100% light compared to controls. Increases in the content of chlorophyll *a* present in the endosymbiotic dinoflagellates have previously been recorded following stress-related loss of symbiotic dinoflagellates due to sub-aerial exposure (Le Tissier & Brown 1996), and heat induced bleaching (Brown *et al.* 2000; Jones 1997). This is in contrast to several different studies that have reported decreased concentrations of photosynthetic pigments per dinoflagellate in bleached corals (Hoegh-Guldberg & Smith 1989b; Jokiel & Coles 1990; Kleppel *et al.* 1989; Porter *et al.* 1989; Shing *et al.* 1995). Four possible causes for this increase in both overall chlorophyll *a* content and chlorophyll *a* content per dinoflagellate are discussed: i) confounding effects of chlorophyll breakdown products, ii) mechanism of dinoflagellate loss, iii) nutrient status of the dinoflagellates, iv) effect on enzymatic reactions.

It is suggested that increased chlorophyll *a* could be an artefact of chlorophyll breakdown products, such as phaeophytin and pyropheophytin, following symbiotic dinoflagellate loss (Jones 1997), which have been observed in considerable quantities in bleached tissues of *Goniastrea aspera* (Ambarsari *et al.* 1997). While there were significantly higher concentrations of phaeophytins detected in the tissues of the corals subjected to the 14°C treatment, chlorophyll *a* content has been corrected for these.

It is possible that these observed differences in both chlorophyll *a* and phaeophytin content are related to the mechanism of symbiotic dinoflagellate expulsion. For example, in *Goniastrea aspera*, the mechanism of dinoflagellate loss possibly involved *in situ* degradation, where the algal cell degrades inside the host coral tissue before being expelled (Brown 1997b). This would explain the increased concentrations of both phaeophytin and pyropheophytin within the coral tissues. In the present study, it is proposed that the primary mechanism of symbiotic dinoflagellates expulsion is exocytosis of the algal cell. This mechanism has also been suggested as the preferred mechanism of release in the anemone *Aiptasia pulchella* after exposure to chilling temperatures (Steen & Muscatine 1987).

Alternatively, the observed increase in chlorophyll *a* content could be related to the nutrient status of the algal symbionts (Hoegh-Guldberg & Smith 1989a; Jones 1997). Chlorophyll *a* concentration of endosymbiotic dinoflagellates has been proven to be a reliable indicator of nutrient status (Hoegh-Guldberg & Smith 1989a; Rees 1991), with increased chlorophyll *a*

production observed in corals exposed to increased levels of nutrients (Hoegh-Guldberg & Smith 1989a). Nutrient enrichment also increases the cell-specific density of symbiotic dinoflagellates, as well as increasing division rates (Muscatine *et al.* 1998). Increased division frequencies of dinoflagellates also occur in bleached but recovering corals (Fitt *et al.* 1993; Hoegh-Guldberg & Smith 1989a; Jones & Yellowlees 1997). It is therefore likely that the loss of the symbiotic dinoflagellates from the tissues of affected corals trigger increases in production of chlorophyll *a* and increased algal cell division in response to increased availability of nutrients, either from the degradation of damaged algal cells, or from decreased competition for limiting nutrients such as nitrogen and iron (Ferrier-Pages *et al.* 2001; Hoegh-Guldberg & Smith 1989a).

Finally, the significant increase in both chlorophyll *a* and chlorophyll *c* content in the 20°C treatment compared to the 14°C and 26°C treatments at all light regimes could be explained by the effect of temperature on enzymatic reactions. While the Calvin Benson cycle may be slowed in the 20°C treatment due to a temperature-related decrease in enzyme activity (Hoegh-Guldberg 1995), there was no apparent damage to PSII. This lack of damage is possibly due to a down-regulation of photosynthesis associated with quenching of chlorophyll fluorescence (Brown *et al.* 1999; Bruce *et al.* 1997). This photoprotective mechanism is known as non-photochemical quenching which functions by dissipating excess absorbed light energy in the PSII antenna system as heat (Demmig-Adams 1990). Therefore, to cope with the excess light energy caused by lowered efficiency of the Calvin-Benson cycle, more chlorophyll *c* is produced to act as a quenching mechanism. This is substantiated by the reduction in chlorophyll *a* and *c* turnover (indicated by decreased phaeophytins), and the relative decrease in the chlorophyll *a*: *c* ratio with increasing light in the 20°C treatment, and compared to the 14°C and 26°C treatments, indicating higher relative chlorophyll *c* content (Plate 3). A similar response to lowered temperatures was also observed in corals exposed to a 16°C treatment, with significantly increased concentrations of chlorophyll *a* and chlorophyll *a+c* compared to the ambient control.

Effect of duration of temperature stress

Previous experiments have shown that many coral species can tolerate temperatures of 15°C only for short periods (Roberts *et al.* 1982), and the massive coral *Montastrea annularis* cannot survive water temperatures of 14°C for more than 9 hours (Mayer 1914). However, it has

recently been shown that certain species of corals can survive temperatures as low as 11.5°C for several months (Coles & Fadlallah 1991). In the present study, the duration of the applied cold treatment had a major effect on the photochemical efficiency of the dinoflagellates within treated corals. The observed decrease in the ratio of variable fluorescence to maximal fluorescence was primarily related to a decrease in F_m following exposure to decreased temperatures, indicating increased photoprotection (Jones & Hoegh-Guldberg 2001).

Differences in F_m , and hence F_v/F_m of corals within the control and treatment groups may be due to diurnal fluctuations in photochemical efficiency. Previous studies have shown that marine dinoflagellates have clear diurnal cycles of photochemical efficiency, with F_v/F_m decreasing after dawn to reach a low between midday and early afternoon, then recovering during late afternoon and early evening (Brown *et al.* 1999; Hoegh-Guldberg & Jones 1999; Jones & Hoegh-Guldberg 2001). This pattern is consistent with the substantial increase in F_v/F_m in all treatment temperatures after the 6 h sampling time (2pm). While temperature still has a significant effect on F_v/F_m , its impact is tempered by the natural diel cycle of the symbiotic dinoflagellates. Therefore at the 12 h and 18 h sampling times, (8pm and 2am respectively) F_v/F_m recovered slightly due to the absence of light.

It is important to note that duration had no significant effect on either the density of symbiotic dinoflagellates or photosynthetic pigment concentration in any of the different temperature treatments. Therefore, while the duration of the exposure to cold-water stress affected the short-term response of the corals to stress (i.e. photoprotection), the overall impact on the remained the same. This is similar to another study on the sea anemones *Aiptasia pulchella* and *A. pallida*, which showed that increasing duration of cold stress did not elicit further release of dinoflagellates after a certain threshold was reached (Muscatine *et al.* 1991). In contrast, time did play a major role in the ultimate survival of corals after exposure to cold stress, as mortality only occurred in those corals exposed to 12°C for more than 12 h. This could be related to the effects of cold temperatures on host cells (Watson & Morris 1987). Reduced temperature is known to increase membrane permeability and change the relative rates of reactions (via effects on the kinetic energy of enzymes and substrates), leading to metabolic disorder and greatly reduced rates of cell division (Hoegh-Guldberg 1995). It can therefore be suggested that cold-water bleaching events are analogous to warm-water bleaching events in that severe coral bleaching preceding mortality only occurs once the thermal and temporal limits of a particular coral are exceeded.

Short-term recovery

The partial recovery of photochemical efficiency of symbiotic dinoflagellates in cold-stressed coral occurred over a 24 h period after corals were returned to ambient temperature. However F_v/F_m remained substantially lower than initial levels throughout the 3 d monitoring period. This pattern of short-term recovery has also been observed following exposure of corals to heat stress and cyanide (Jones & Hoegh-Guldberg 1999; Jones *et al.* 2000). It has been suggested that this initial increase in F_v/F_m occurs through the selective expulsion of damaged dinoflagellates (Jones *et al.* 2000), thereby increasing the relative proportion of unaffected algal cells. This is supported by the apparent bleaching that occurred throughout this period in both the 12°C and 16°C treatments, substantiated by the significant loss of symbiotic dinoflagellates and decreased chlorophyll *a* and chlorophyll *c* content. However, while bleaching was greatest in the corals exposed to 12°C for 12 h and 18 h, no recovery of F_v/F_m was observed following treatment. This is probably due to extreme cellular damage caused by irreversible photodamage, resulting in expulsion of damaged algal cells.

In addition to photo-oxidative stress induced by photoinhibition, lowered temperatures appear to have other deleterious physiological effects. In a study by Muscatine *et al.* (1991), the principle mechanism of expulsion of symbiotic dinoflagellates following cold stress involved the sloughing off of intact endodermal cells containing symbiotic dinoflagellates in various stages of degradation. This response was also observed in the present study following exposure of corals to 12°C for 12 h and 18 h periods, but did not occur in corals treated for shorter periods of time. This suggests that there may be a correlation between the type of stress imposed upon the coral, as well as its severity and duration, and the mechanism of coral/algal dissociation. The mechanisms involved may also vary depending on whether the environmental stress principally affects the host cells (Gates *et al.* 1992) or the algal symbionts (Glynn & D'Croz 1990; Iglesias-Prieto *et al.* 1992; Lesser *et al.* 1990).

Long-term recovery

Previous studies of corals exposed to increased temperature stress have indicated that symbiotic dinoflagellates in bleached regions of a colony exhibit reduced photosynthetic activity for up to one year after a bleaching event (Lombardi *et al.* 2000). In the present study, photochemical efficiency, density of symbiotic dinoflagellates and concentration of photosynthetic pigments of corals exposed to a 6 h treatment at 15°C had recovered completely

over the three month period. This is consistent with another study where all bleached coral specimens partially recovered symbiotic dinoflagellate pigment within 1 month, with full recovery occurring within 2 months following exposure to lowered temperatures (Jokiel & Coles 1977). However, while recovery of photochemical efficiency associated with symbiotic dinoflagellates density and chlorophyll *a* content can occur relatively quickly, the implications of the initial bleaching event may affect the corals over a much longer time scale. Symbiotic dinoflagellates typically provide their coral host with organic compounds produced through photosynthesis (Muscatine 1990), providing energy for maintenance, growth and reproduction (Szmant & Gassman 1990). Therefore, the initial loss of symbiotic dinoflagellates, and the observed decrease in photosynthetic capacity can be translated into a significant decrease in cellular growth rates (Lesser, 1996), calcification (Muscatine 1990) and reduced reproductive capacity of corals (Gleason & Wellington 1993; Porter *et al.* 1989; Szmant & Gassman 1990; Ward *et al.* 2000). Other effects include increased respiration rates, and declines in coral protein, lipid and carbohydrate content (Glynn 1990; Jokiel & Coles 1990; Kleppel *et al.* 1989), which can be expected as corals utilise reserve products to support basic metabolism (Szmant & Gassman 1990). The regrowth of tissue biomass and the production of metabolic reserves lost during the period of decreased photosynthetic activity may take significantly longer (Fitt *et al.* 1993).

Acclimation of coral to repeated temperature stress

The potential for thermal acclimation in corals was first suggested following a series of experiments whereby colonies of *Montipora verrucosa* displayed increased survivorship at high temperatures following incubation at 28°C, compared with colonies incubated at lower temperatures (Coles & Jokiel 1978). This is confirmed by general observations that individual coral colonies exposed to high temperature environments can survive at temperatures a few degrees higher than other colonies of the same species that are exposed to lower temperatures (Jokiel & Coles 1990). This highlights the importance of the thermal history of corals with regard to their susceptibility to bleaching events (Marshall & Baird 2000). In the present study, no observed acclimation was apparent in response to repeated temperature stress. Instead, the pre-treated corals appeared to be slightly more susceptible to cold stress, shown by decreased F_v/F_m , reduced densities of dinoflagellates and decreased chlorophyll content. This decreased ability of the pre-treated coral to cope with secondary cold treatment may be associated with a decrease in metabolic reserves as a result of incomplete recovery or other elements such as sub-chronically damaged cell organelles and proteins that eventually fail

under a recurring stress. Therefore the pre-treated corals are not as “fit” as those that received only one cold treatment in January. This is an important area that should also be pursued with respect to all stress and coral reef studies, especially with regard to increased temperature stress, as the ability of corals to survive predicted changes to the global environment over the next century will depend on their physiological mechanisms of acclimatisation (Gates & Edmunds 1999).

Alternatively, it is possible that the time of year that the two experiments were conducted may affect the response of photochemical efficiency of the corals. A study by Berkelmans and Willis (1999) showed significant differences in the upper thermal limits of 3 different coral species on a seasonal basis. Therefore, it is likely that corals would also show differences in lower thermal thresholds between the seasons. This might account for differences in F_v/F_m response, symbiotic dinoflagellate density, chlorophyll concentrations and mortality rates between the two experiments.

Another factor that may indicate that the pre-treated corals are still recovering is the slight increase in all photosynthetic parameters in the pre-treated corals, compared to the control corals. Following a bleaching event, the mitotic index of the symbiotic dinoflagellates often increases significantly (Fitt *et al.* 1993; Hoegh-Guldberg & Smith 1989a; Jones & Yellowlees 1997). This is most likely due to the increased availability of nutrients, space and other resources (Hoegh-Guldberg & Smith 1989a). This often results in an overcompensation, where the density of dinoflagellates, and hence the concentration of photosynthetic pigments, exceeds those found in non-stressed corals in order to restore the reserves of protein, lipid and carbohydrate lost during the previous bleaching event. (Muscatine & Pool 1979). Once these reserves are restored, the number of dinoflagellates return to the levels found in ‘normal’ corals.

CONCLUSIONS

The present study has explored the impact of cold stress on a symbiotic coral species on the southern Great Barrier Reef. Reducing the sea temperature to below that normally experienced by *Montipora digitata* led to the advent of physiological stress, both in its tissues and in the symbiotic dinoflagellates. Lowered temperatures increased the sensitivity of the endosymbiotic dinoflagellates of *Montipora digitata* to photoinhibition. The observed effects appeared to be similar to those observed in warm water bleaching of corals, indicated by loss of algal symbionts, and long-term reduction in photosynthetic efficiency (Hoegh-Guldberg 1999). There is also no observed acclimation of the coral to repeated temperature stress, instead pre-treated corals appeared more susceptible to decreased temperatures due to an incomplete recovery. This has serious implications in the context of global climate change, as a reduction in the intervals between bleaching events (both warm- and cold-water) decreases the recovery time available for affected individuals. This in turn may increase the susceptibility of corals to a bleaching event when they would otherwise remain unaffected. Therefore the cumulative impacts of sequential El Niño and La Niña events could potentially result in decreased coral cover, reduced species diversity, and changes in community composition.

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APPENDIX



Plate 1: Overview of experimental setup, showing the four temperature regulated tanks with three chambers per tank.



Plate 2: Impacts of cold-water stress on the coral *Montipora digitata*. The four nubbins show the different extremes of bleaching, ranging from healthy, to bleached, to dead.