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The influence of irradiance on tolerance to high and low temperature stress exhibited by *Symbiodinium* in the coral, *Pocillopora damicornis*, from the high-latitude reef of Lord Howe Island

Laura C. Wicks,[a,1] Ross Hill,[b] and Simon K. Davy[a,*]

[a] Centre for Marine Environmental & Economic Research, School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand

[b] Plant Functional Biology and Climate Change Cluster, University of Technology, Sydney, Australia

Abstract

The coral *Pocillopora damicornis* hosts genetically distinct and novel types of dinoflagellate symbionts at the high-latitude site of Lord Howe Island (LHI), yet why these novel types exist at this marginal site is unknown. In this study, it was determined whether one of the novel *Symbiodinium* types at LHI is physiologically adapted for this high-latitude site, where water temperatures annually range from 18°C to 26°C. Low and high short-term thermal bleaching thresholds of the coral–symbiont partnership were measured as 14°C and 30°C. Photochemical sensitivity to temperature (15°C and 29°C) and light treatments (100% and 40% of sunlight), measured as effective quantum yield and fast induction curves, were determined over a 72-h period. A greater effect on the photochemical reactions of LHI *P. damicornis* symbionts was recorded in response to a 3°C temperature increase from annual maxima than a 3°C temperature decrease from annual minima. Corals did not bleach when temperature was reduced to 15°C for 72 h; in contrast, a 92% decline in photochemical efficiency was recorded in the 29°C treatment ($\Delta F : F'_m < 0.05$), compared to 35% loss in the control (20°C). For the first time, a Pulse Efficiency Analyser fluorometer was used to assess the effect of reduced temperature on symbionts, showing a reduced rate of Q_A reduction, further enhanced by high light levels. This type of *Symbiodinium* at LHI may be specialized for cooler and more variable temperatures, so contributing to the success of corals at this marginal location.

The success of coral reefs results from the symbiotic relationship between scleractinian corals and dinoflagellates of the genus *Symbiodinium*. The host relies on the dinoflagellate symbionts for survival, because they release substantial amounts of photosynthate to the host, and aid the recycling and conservation of nitrogen (Muscatine 1990). In return, the symbionts reside within the host tissue and the host provides carbon dioxide for the photosynthetic reaction and substrates (e.g., nitrogen and phosphorus) required for synthesis of cellular constituents, including the photosynthetic apparatus.

Symbionts exposed to stress (e.g., high or low temperature outside of the optimal range) can sustain photosynthetic damage when light capture exceeds the rate of light utilization (Jones et al. 1998; Saxby et al. 2003). In response to increased temperatures, excess absorbed light energy leads to the production of reactive oxygen species, which can disrupt cellular components and induce physiological malfunction, and lead to expulsion of the symbiont from the host (reviewed in Weis 2008). Excessive irradiance or thermal stress can lead to permanent damage, due to inhibition of the photosystem II (PSII) reaction centers (degradation of the D1 protein [Warner et al. 1999; Hill and Ralph 2006]) and associated light-harvesting complexes (Takahashi et al. 2008), energetic uncoupling in the thylakoid membranes (Tchernov et al. 2004; Hill et al. 2009) or inhibition of the dark reactions (Jones et al. 1998). However, few studies have characterized the effect of cold temperature stress on PSII of *Symbiodinium*. On the southern Great Barrier Reef (GBR), loss of photochemical efficiency was recorded after 12 h exposure to 12°C (Saxby et al. 2003) and bleaching at 13.3°C was observed in situ (Hoegh-Guldberg et al. 2005), with winter water temperatures usually averaging 21°C (Ulstrup et al. 2006).

A common technique used to characterize the photosynthetic condition of coral symbionts during bleaching events is the measure of chlorophyll *a* fluorescence. Pulse Amplitude Modulated (PAM) fluorometry is the most common (Jones et al. 1998; Warner et al. 1999; Jones and Hoegh-Guldberg 2001), although measures of fast induction curves (FICs) using other fluorometers (such as Plant Efficiency Analysers and Double Modulation Fluorometers) are becoming more widespread (Hill et al. 2004*a*; Ulstrup et al. 2005; Hill and Ralph 2006). These instruments allow for nondestructive and rapid measures of photosynthetic condition on the same sample over time in both laboratory and field settings (Beer et al. 1998; Ralph et al. 1999; Hill and Ralph 2005). While PAM fluorometers provide information on the photosynthetic efficiency of PSII, FIC measurements provide a detailed insight into the movement of electrons from the donor-side to the acceptor-side of PSII (Govindjee 1995; Strasser et al. 1995; Hill et al. 2004*a*). Changes in the amplitude of steps and shape of FICs have been used to identify the site in

* Corresponding author: simon.davy@vuw.ac.nz

[1] Present address: The Centre for Marine Biodiversity and Biotechnology, Heriot Watt University, Edinburgh, United Kingdom

Table 1. LHI environmental variables (recorded in 2007), and coral and *Symbiodinium* diversity (Wicks et al. 2010).

Parameter	LHI	Sylphs Hole	Lagoon
Annual min. temp. (°C)	18	—	—
Annual max. temp. (°C)	26	—	—
Turbidity (FTU)	—	1.4	2.4
Coral spp. (Harriott et al. 1995)	83	—	—
Coral cover (%; Harriott et al. 1995)	2.7–52.4%	31.7%	—
Symbiodinium types (ITS) by host:			
Acropora spp.	C100, C102, C1c, C3w	C1c, C3w	C3
Pocillopora sp.	C100, C103, C108	C103	C100
Goniastraea sp.	C108	—	C1c, C3w
Favites sp.	C1	—	C1
Montastraea sp.	C1	—	—
Stylophora sp.	C1, C3gg, C1bb	—	—

corals exposed to stressful conditions (Hill et al. 2004*a*; Ulstrup et al. 2005; Hill and Ralph 2006).

The photosynthetic efficiency and sensitivity to stress of the dinoflagellates is a function of both their environment and the diversity within the genus *Symbiodinium* (Iglesias-Prieto et al. 2004). The genus is highly diverse, with nine clades (A–I) and numerous subclades identified (LaJeunesse 2001; Coffroth and Santos 2005; Pochon and Gates 2010). As a reflection of the high genetic diversity of *Symbiodinium*, considerable physiological diversity exists at both cladal and subcladal levels, in terms of tolerance to irradiance and temperature (Iglesias-Prieto et al. 2004; Robison and Warner 2006; Hennige et al. 2009). Despite evidence that these symbiotic dinoflagellates play an important role in determining the environmental tolerance and distribution of the holobiont (host and symbiont), and potentially its survival in response to environmental stress, little is known of the physiology of *Symbiodinium* spp. that live in marginal environments. These environments are defined as those that are near or beyond the normal limits of reef distribution, close to the environmental thresholds for coral survival (Kleypas et al. 1999).

Marginal reefs and communities frequently occur at high latitudes, where they are generally subject to wide ranges in seasonal temperatures. For instance, temperatures at Lord Howe Island (LHI), Australia, annually range from 18°C to 26°C (Table 1) compared to the central GBR's annual range of 24–29°C (Ulstrup et al. 2006). High-latitude corals also experience low annual minimum temperatures (lowest 13.3°C, Iki Island, Japan; Yamano et al. 2001) and relatively lower irradiance levels than in the tropics (photosynthetically active radiation [PAR] in the winter months is ~ 40% lower at 30° N or S, compared to 10° N or S under the same environmental conditions [Campbell and Aarup 1989]).

In the limited information available, it has been shown that the specific partnerships between host corals and their symbionts at temperate or high-latitude reef sites differ from their tropical counterparts (LaJeunesse and Trench 2000; Rodriguez-Lanetty et al. 2001). For example, the coral *Plesiastrea versipora* hosted clade B *Symbiodinium* in temperate southeastern Australia (35° S), whereas in tropical Australian locations it harbored clade C (Rodriguez-Lanetty et al. 2001). In a recent study at the high-latitude site of LHI, Australia, many corals contain different ITS2 (internal transcribed spacer 2) types of clade C *Symbiodinium* to those found in the same coral species on the GBR (Table 1; LaJeunesse et al. 2003).

LHI (31°33′S, 159°05′E) is situated > 1000 km from the southern GBR, and is home to the southernmost coral reef in the world. Despite the high-latitude conditions and geographic isolation of the Island, both symbiont and coral diversity are high (Table 1; Harriott et al. 1995). Of particular note, *Pocillopora damicornis* hosts three novel C types (C100, C103, C118) at LHI (Table 1), but is known only to associate with types closely related to C1 (such as C1c, which differs by one base-pair), and D1 elsewhere in the Indo-Pacific (LaJeunesse et al. 2003, 2008; Sampayo et al. 2007). These novel types at LHI are environmentally specialized, with only one type found in deeper and turbid waters (C103). It is unclear why these novel types exist at LHI, and the potential for physiological variability in these symbiont types is unknown. Because it is projected that almost all reef locations are likely to become marginalized in a future of rapid climate change, studies of the adaptive or acclimative capacity of corals in an already marginal environment will be crucial in determining the future of coral reef ecosystems (Guinotte et al. 2003).

In this study, we aim to determine whether the novel *Symbiodinium* types at Lord Howe Island are physiologically adapted for this high-latitude site and so help explain the success of this marginal coral reef. We focused on responses to temperature and light intensity, and hypothesized that high-latitude *Symbiodinium* types would be able to withstand a greater range of temperatures than their tropical counterparts on the GBR. By elucidating the relationship between *Symbiodinium* physiology and biogeography, we aim to further our understanding of the mechanisms involved in local adaptation to thermal and solar conditions, and the persistence of coral reefs at marginal sites.

Methods

Sampling—In September 2008, colonies of the scleractinian coral *P. damicornis* were collected from 2 m to 3 m deep (during low tide) at two lagoon sites at LHI (31°33′S 159°05′E); the turbid site of Sylphs Hole (2.4 Formazine

Turbidity Units [FTU] compared to 1.4 FTU at other lagoon sites; Table 1) and off Lagoon Beach in the central lagoon. Colonies were collected from two sites to increase the chance of sampling multiple symbiont types. A previous study found symbiont C103 predominated in *P. damicornis* colonies at Sylphs Hole, whilst C100 was dominant in other lagoon sites (Wicks et al. 2010; see Table 1 for summary of findings) In the present study, postexperiment genetic analysis revealed C100 was predominantly sampled (n = 9 of 10, 1 C103 identified). Water temperatures at time of collection ranged between 19°C and 22°C. Colonies were collected from the central lagoon and maintained in indoor tanks (< 100 μmol photons m^{-2} s^{-1}) at ambient lagoon temperature (19–20°C) for 2 d to supply samples with a period of recovery from any stress experienced prior to or during collection (Hill and Ralph 2005, 2006, 2008).

Experimental procedure—All experiments were set-up in direct sunlight close to the shore at LHI, with 60-liter tanks angled to all receive the same duration and intensity of sunlight. Portable air pumps aerated and circulated the water, and 50% of the water was changed every 2 h to eliminate the effect of solar heating; this ensured that the water temperature did not deviate by more than ± 1°C from the experimental temperature.

To determine upper and lower bleaching thresholds of *P. damicornis* at LHI, corals from the lagoon were exposed to five different temperatures (14°C, 15°C, 20°C, 29°C, and 30°C) for a 62-h period, beginning at 06:00 h (n = 6/ treatment). Sunrise was at 06:00 h, with sunset at 18:00 h. Coral nubbins were placed in the tanks at 06:00 h on the first day at ambient lagoon temperature (19.8°C) and the temperature ramped to reach the required temperature over 6 h. It should be noted that the rate of ramping was rapid due to experimental and time limitations at this remote field site and, thus, the experiments act as a proxy for determining temperature stress reactions.

All temperature treatments were run simultaneously, with one tank per treatment due to experimental limitations. Temperatures in the tanks were maintained using a chiller (Aqua Medic HL-260CA) for the 14°C and 15°C treatments, and two heating elements (Jager 150 W; Eheim) for the 29°C and 30°C treatments. In the control treatment (20°C), temperatures were maintained by regular water changes with lagoon water. Temperatures were recorded each minute throughout the experiment using Hobo pendant loggers (Onset Computer; UA-002-08). Measurements of effective quantum yield ($\Delta F : F'_m$; *see* Physiological measurements) were taken using a submersible Pulse Amplitude Modulated fluorometer (Diving-PAM; Walz) every 2 h between 06:00 h and 20:00 h for the 62-h period. The intensity of PAR was logged every minute by a submersible light meter (Odyssey).

Once the upper and lower thermal thresholds during a rapid temperature change had been established (14°C and 30°C), four new colonies per site were divided into six nubbins and exposed to six different temperature and light combinations for 72 h: 15°C, 20°C (control), and 29°C each at 'high light' (full light at 30-cm depth, peak irradiance ~ 1900 μmol photons m^{-2} s^{-1}) and 'low light' (60% neutral density filter [Lee Filters], peak irradiance ~ 800 μmol photons m^{-2} s^{-1} at midday). Treatment light intensities were relevant to measures made by a submersible light meter (Licor LI-1000) at 2–3 m deep at 12:00 h for the highest light intensity. The light treatment that applied 40% of the full irradiance is relevant to cloudy conditions, or more turbid waters. Although both light levels are high compared to other studies (Ulstrup et al. 2006; Hennige et al. 2009) and are likely to exceed minimum saturating irradiance (Hill et al. 2004*b*; Ulstrup et al. 2008), the 'low light' still represents a relatively low light level compared to what the corals would experience in the field on a cloudless day. The low and high temperatures were chosen because they were ~ 1°C clear of the determined upper and lower bleaching thresholds of 14°C and 30°C.

FICs and $\Delta F : F'_m$ (*see* Physiological measurements) were taken every 3 h starting from dawn (05:30 h) until after dark (21:00 h) on each of the 3 d of the experiment. Where samples appeared dead, assessed using fluorescence signals and tissue loss, they were removed and preserved for symbiont genetic analysis. In these cases (< 5% of samples), physiological measures could not be taken.

Physiological measurements—Photosynthetic efficiency of symbionts was measured with Diving-PAM and Plant Efficiency Analyser (PEA) chlorophyll fluorometers. The Diving-PAM applied 3 μs pulses of weak (< 0.15 μmol photons m^{-2} s^{-1}) light from a red light-emitting diode (peak emission at 650 nm) as the measuring light, for detection of minimum fluorescence. Saturation pulses of white light (> 4500 μmol photons m^{-2} s^{-1}) allowed for the detection of maximum fluorescence. Chlorophyll fluorescence was detected at wavelengths above 710 nm (*see* Schreiber 2004). Effective quantum yield of PSII ($\Delta F : F'_m$) was measured on samples when in a light-acclimated state and maximum quantum yield ($F_v : F_m$) when dark-acclimated at predawn and after dark (Schreiber 2004). In corals, a decline in $\Delta F : F'_m$ precedes coral bleaching (Jones and Hoegh-Guldberg 2001), and $\Delta F : F'_m$ varies both diurnally and seasonally (Gorbunov et al. 2001; Jones and Hoegh-Guldberg 2001; Hill and Ralph 2005). Evidence of down-regulation can be found from diel experiments where $F_v : F_m$ recovers by the evening or following morning; photo-damage is implied when this recovery takes longer to occur (several days; Jones and Hoegh-Guldberg 2001).

The PEA measures polyphasic fluorescence-induction kinetics to achieve detailed information on the photochemical state of PSII, as well as the plastoquinone pool (Hill et al. 2004*a*). FICs have been employed to assess the effect of bleaching conditions on symbiont photosynthesis in terms of the condition of PSII and the redox state of the primary (Q_A) and secondary (Q_B) electron acceptors (Hill et al. 2004*a*; Hill and Ralph 2006), as well as changes in these mechanisms diurnally and seasonally (Hill and Ralph 2005). Prior to each PEA measurement, dark adaptation was conducted for 5 min to allow substantial re-oxidation of the primary electron acceptor (Q_A; Hill and Ralph 2006), with maximum excitation irradiance set at 3200 μmol photons m^{-2} s^{-1}. For the first 2 ms of saturation the

fluorescence signal was measured every 10 μs, followed by 1 ms sampling up to 1 s, and then 100 ms sampling up to 5 s. All curves were normalized to the fluorescence yield at 0.05 ms (F_o) to eliminate any changes linked to a possible reduction in chlorophyll concentration (or symbiont density) due to stress (Iglesias-Prieto 1995; Hill et al. 2004*a*). When plotted on a $\log_{10}$ time scale, the FICs followed the O–J–I–P steps of the 'Kautsky' curve. The 'O' step (F_o, the origin) occurred at 0.05 ms, the J step (intermediate) at 1 ms, the I step (second intermediate) at 100 ms and the P step (F_m, maximum) at 2 s (Hill et al. 2004*a*).

Genetic analysis—Prior to, and following the treatments, the coral tissue was removed from each of the different fragments with an airbrush. The tissue was processed and stored as outlined in Loh et al. (2001), and the DNA extracted using a DNeasy plant kit (Qiagen) according to the manufacturer's instructions. The ITS2 region of the ribosomal DNA was amplified with the primers ITSintfor and ITS2clamp (LaJeunesse et al. 2003). Amplified ITS2 fragments were separated using 30–60% denaturing gradient gel electrophoresis gels (DCode system; Bio-Rad) during a 14 h run at 100V, after which gels were stained with SYBR green (Invitrogen). Profiles were compared with symbiont profiles previously identified in *P. damicornis* at Lord Howe Island (Wicks et al. 2010).

Data analysis—Repeated measures ANOVA (rmANOVA) was used to determine whether the fluorescence parameters ($F_v:F_m$, $\Delta F:F'_m$ and O, J, I, and P steps) differed significantly ($\alpha = 0.05$) between temperature and light treatments in the 3-d period (19 time points). Because no significant differences were observed in physiological parameters between site of sample collection (rmANOVA, $p = 0.4$–0.9), samples were pooled for further analysis. rmANOVA also tested for significant differences in $\Delta F:F'_m$ between temperature treatments in the bleaching threshold test. Assumptions of normality and of homogeneity of variances were tested using the Kolmogorov–Smirvov's and Levene's test, respectively. If these assumptions were not met, arcsine transformations were performed. Where rmANOVA determined a significant difference, Box's test of equality of covariance matrices was used to test the homogeneity of variances between treatments. The assumption of sphericity was tested, and where assumptions were not met, the Greenhouse–Geisser adjustment was performed. Tukey's multiple comparisons were used to identify significant differences between individual temperature treatments. Each physiological parameter was analyzed separately, with temperature and light as between-subject factors. Time was analyzed as a within-subject factor because photochemical measures were repeated on the same colony. All analyses were performed using SPSS software version 16.0.0.

Results

Symbiont identification—All coral colonies ($n = 10$) collected from the lagoon harbored *Symbiodinium* type C100, while three of four colonies collected from the more turbid site of Sylphs Hole also harbored this type; the fourth colony from Sylphs Hole contained type C103.

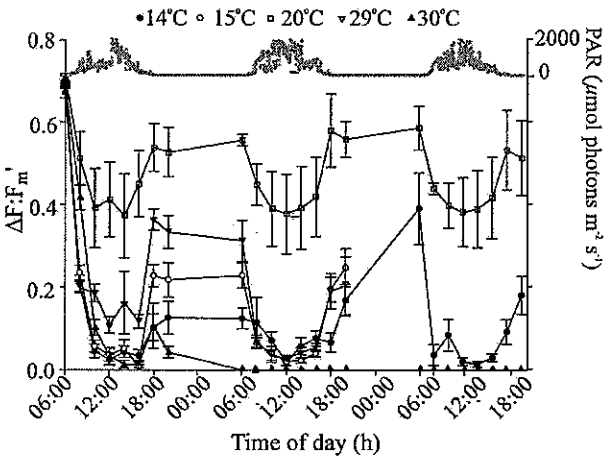

Fig. 1. $\Delta F:F'_m$ in *Pocillopora damicornis* nubbins over a 60-h treatment period at five temperature treatments. PAR (μmol photons m^{-2} s^{-1}) on right axis (gray line). Averages (± SE) shown ($n = 6$).

Bleaching thresholds—In the bleaching threshold experiment, all *P. damicornis* samples contained C100. The effective quantum yield of PSII ($\Delta F:F'_m$) declined from 06:00 h to 12:00 h in all temperature treatments, with significantly higher values for the control nubbins than the nubbins subject to high (29°C and 30°C) or low (14°C and 15°C) temperatures ($p < 0.001$; Fig. 1). After 06:00 h on day 2 (24 h after the start of the treatments), all nubbins ($n = 6$) exposed to 30°C had bleached or died. Mean $F_v:F_m$ (± SE) of nubbins exposed to 29°C after 72 h was 0.2 ± 0.01, with one nubbin bleached. Three of the six nubbins exposed to 14°C had $F_v:F_m < 0.1$ after 24 h and 48 h, with $F_v:F_m$ of the three remaining nubbins ~ 0.25. The 29°C and 15°C treatments exhibited significantly higher $F_v:F_m$ values than 30°C and 14°C throughout ($p < 0.001$; Fig. 1); for example, after 24 h, mean $\Delta F:F'_m$ (± SE) in the control treatment was 0.46 (± 0.02), significantly higher than the 29°C and 15°C treatments ($\Delta F:F'_m = 0.31 \pm 0.04$ and 0.22 ± 0.03, respectively), and four-times $\Delta F:F'_m$ (± SE) in the 14°C treatment (0.12 ± 0.02, all nubbins at 30°C dead).

Photochemical efficiency—Once the bleaching thresholds of 14°C and 30°C were established, a longer term experiment on photochemical efficiency in response to temperature and light treatments was performed. Maximum ($F_v:F_m$), effective quantum yield ($\Delta F:F'_m$) and FICs were measured every 3 h from predawn to postdusk in nubbins exposed to three temperature treatments (29°C, 20°C, 15°C), each at two light levels (100% and 40% full light, referred to as high and low light) over 72 h. Maximum quantum yield measures were possible at predawn and postdusk only, when nubbins had been dark-adapted. Significant differences in each of the parameters measured between temperatures and light

Table 2. *p*-values of the rmANOVA analyses that tested for changes in the maximum ($F_v:F_m$) and effective ($\Delta F:F'_m$) quantum yields, and the O, J, I, and P steps over the 72-h experiment for three temperature treatments (29°C, 20°C, and 15°C) at two light levels (high and low) of *Pocillopora damicornis* from Lord Howe Island.

Variables	$\Delta F:F'_m$	$F_v:F_m$	O	J	I	P
Within subjects						
Time	<0.001*	<0.001*	<0.001*	<0.01	<0.001	<0.001
Time×temp	<0.001*	<0.01*	<0.001*	0.09	0.058	0.062
Time×light	<0.001*	<0.001*	<0.01*	<0.05	<0.05	<0.05
Time×temp×light	0.339	0.458	0.292	0.384	0.391	0.924
Between subjects						
Temp	<0.001*	<0.001*	0.229	<0.01*	<0.001*	<0.001*
Light	<0.001*	<0.001*	<0.05*	<0.001*	<0.001*	<0.01*
Temp×light	0.517	0.938	0.735	0.326	0.510	0.561
	15, 20>29	20>29>15	—	15, 20>29	15, 20>29	15, 20>29

* Indicates significance at $p<0.05$.

treatments are displayed in Table 2. No significant differences in $F_v:F_m$, O, J, I, and P were recorded between site of sample collection ($p = 0.4$–0.9).

For each day of the experiment, maximum $F_v:F_m$ was recorded at 06:00 h, with $\Delta F:F'_m$ then decreasing diurnally from 09:00 h, reaching a minimum at 15:00 h following the 12:00-h peak in sunlight intensity (Fig. 2). In the afternoon, $\Delta F:F'_m$ recovered as light intensity decreased. Overnight, $F_v:F_m$ did not significantly change (ANOVA, $p < 0.01$). Significant differences in $\Delta F:F'_m$ were recorded between both light and temperature treatments, in addition to the additive effect of both factors (rmANOVA, $p < 0.001$; refer to Table 2). $\Delta F:F'_m$ was significantly higher over the 72 h in low light than in high light for all treatments (rmANOVA, $p < 0.001$; Table 2). Figure 2b shows that after 72 h at low light, $\Delta F:F'_m$ maintained 86%, 65%, and 21% of initial readings at 20°C, 15°C, and 29°C temperature treatments, respectively, compared to 65%, 26%, and 8% in the high-light treatment. Assessing the effect of temperature in both light treatments, coral nubbins in the 20°C temperature treatment maintained the highest $\Delta F:F'_m$ over the 72-h period, which was significantly greater than in the 29°C and 15°C treatments (rmANOVA, $p < 0.001$; Table 2). The difference in $\Delta F:F'_m$ between temperature treatments varied over time at both light levels; the highest was recorded in the 20°C low-light treatment (0.58 ± 0.03), closely followed by 20°C high light and 15°C low light (0.43 ± 0.03 and 0.42 ± 0.04, respectively). Lowest mean $\Delta F:F'_m$ overall was recorded in the 29°C high-light treatment, which remained at < 0.05 for the final 21 h of the experiment.

Fast induction kinetics—Figure 3a–f shows the FICs of *P. damicornis* for a series of thermal bleaching treatments at two light levels (100% and 40% of natural daylight, high and low light, respectively). For each treatment, a selection of FICs normalized to F_o is shown from the 72-h period (T = 0, 9, 24, 33, 48, 72 h). At each time point in all

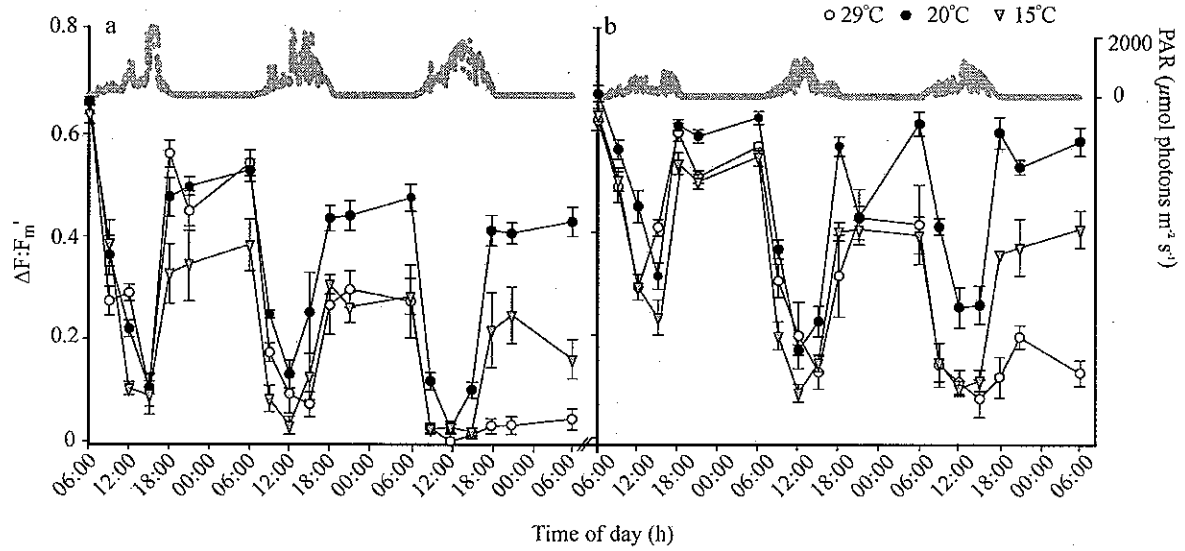

Fig. 2. $\Delta F:F'_m$ in *Pocillopora damicornis* nubbins over 72-h temperature treatment period at (a) high light and (b) low light. Averages (± SE) shown ($n = 4$). PAR (Δmol photons m^{-2} s^{-1}) on right axis (gray line).

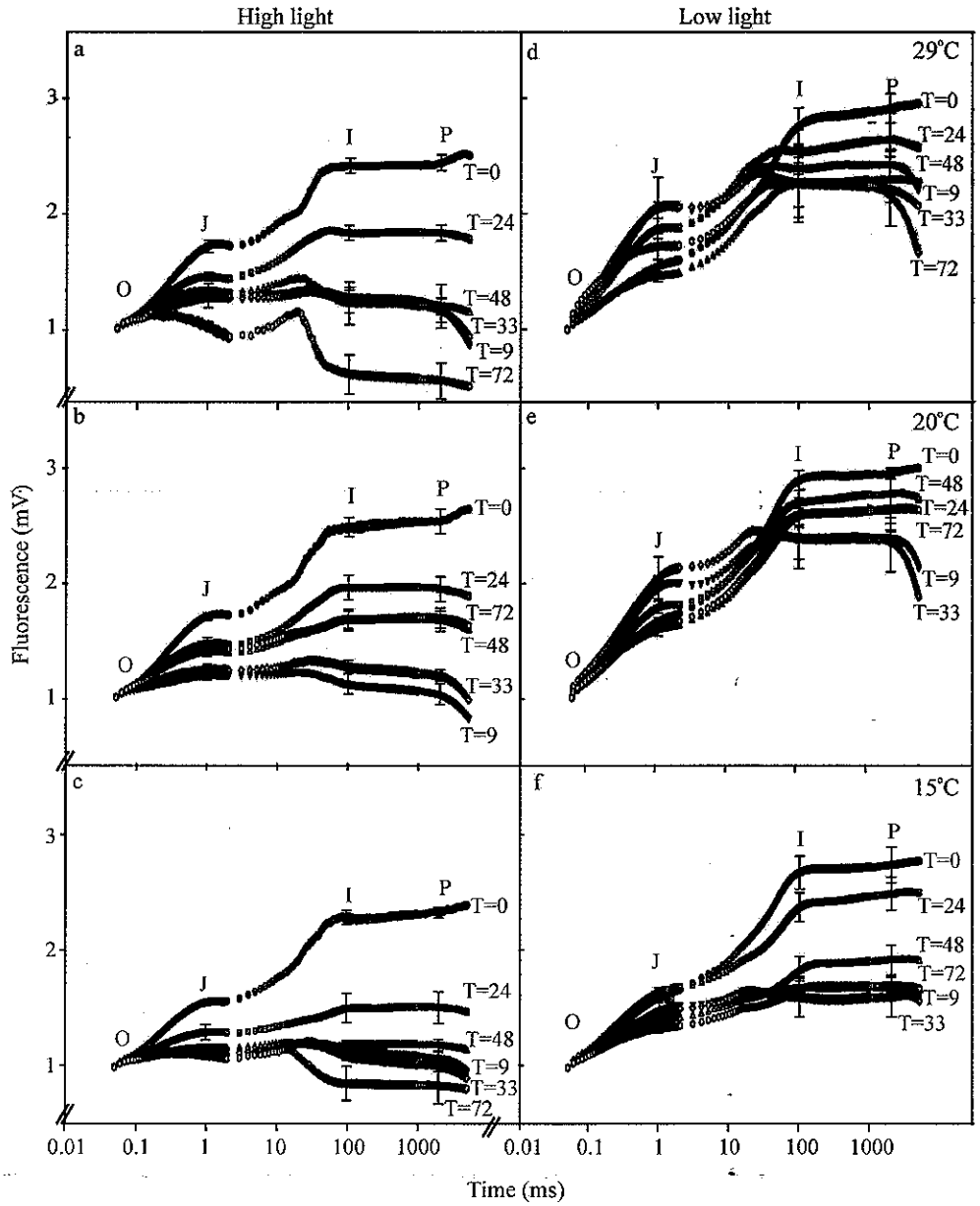

Fig. 3. FICs of thermal treatments of *Pocillopora damicornis* at six time points over a 72-h period at (a–c) high light and (d–f) low light. Treatment temperatures were (a, d) 29°C, (b, e) 20°C, and (c, f) 15°C. Curves are normalized to F_o and are plotted on a $\log_{10}$ time scale. Average curves are shown (n = 6–8), with SE at J, I, and P steps (marked).

temperature treatments, the FIC amplitude of nubbins subjected to high light was lower than that of corals subjected to low light. In the low-light treatment at all temperatures, the P step remained high at 24 h (i.e., dawn on day 2), despite falling at 9 h (15:00 h on day 1). After 24 h in low light, the P step declined in the 29°C and 15°C treatments; however, in the 20°C treatment it remained high. In the high-light treatment, the P step declined over time in all temperature treatments. Least variability between FICs over time was recorded in the 20°C and 29°C low-light treatments, with greatest variability in the 29°C high-light treatment.

The mean amplitudes of the O, J, I, and P steps over the 72-h period are shown in Fig. 4a–f. In all treatments, the amplitude of steps varied diurnally, declining from a maximum at 06:00 h to a minimum at 15:00 h (following maximum PAR at 12:00 h). In all steps, the amplitudes were significantly lower at high light than low light in all temperature treatments over the 72 h (rmANOVA $p < 0.01$; Table 2). Magnitude of the J, I, and P steps was significantly

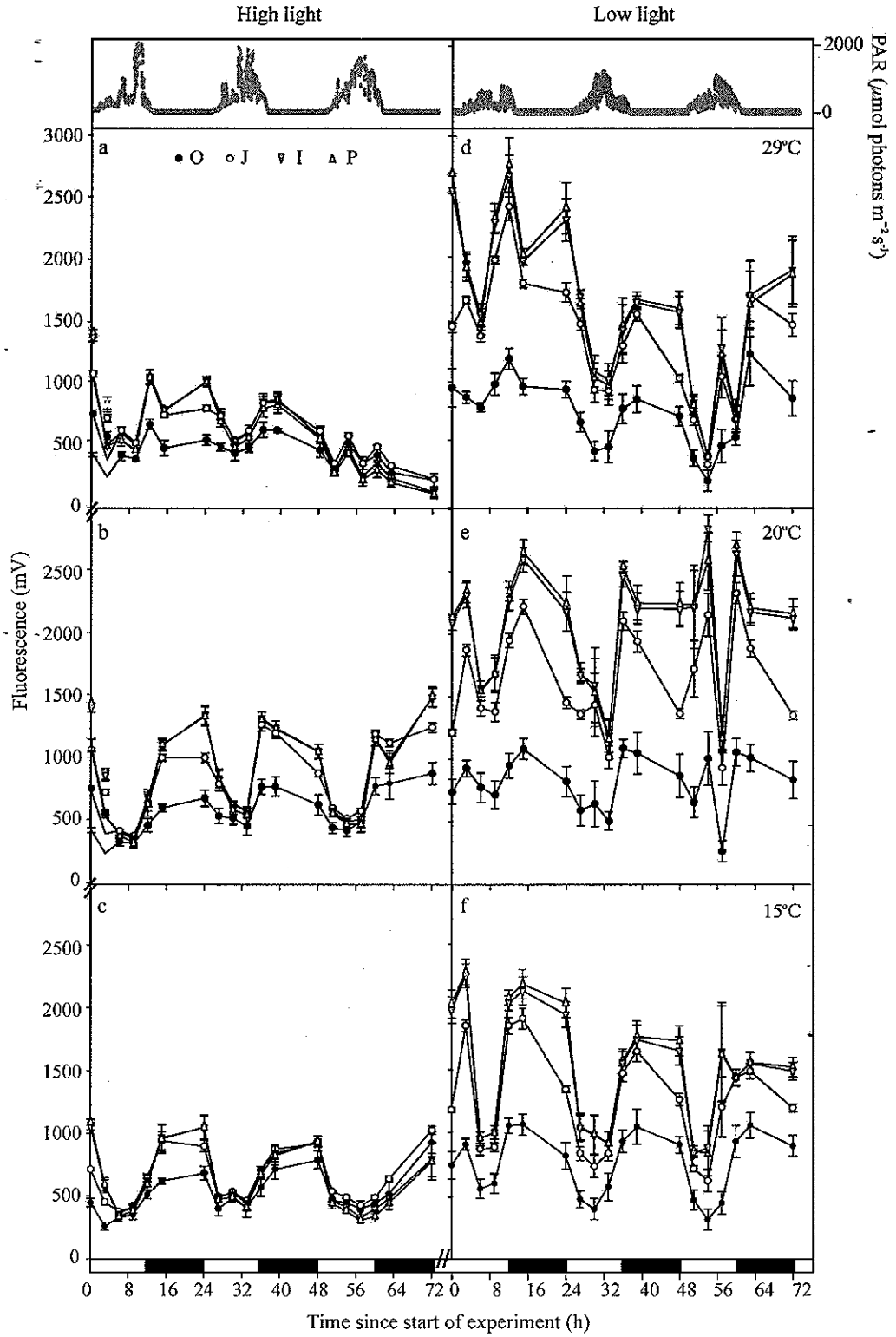

Fig. 4. Amplitude of the O, J, I, and P steps along FICs in *P. damicornis* over a 72-h period at (a–c) high light and (d–f) low light. Treatment temperatures were (a, d) 29°C, (b, e) 20°C, and (c, f) 15°C. Average values (n = 7) from all nubbins and SE shown. PAR (μmol photons m^{-2} s^{-1}) on right axis.

higher in the 20°C treatment than the 29°C or 15°C treatments (rmANOVA, $p < 0.05$), with O significantly variable between temperature treatments when considered over time. No significant interactive effect of temperature and light was observed in any of the steps (rmANOVA, $p > 0.3$; Table 2).

Diurnal changes in the steps varied between temperature treatments at both light levels. In the 20°C treatments at both light levels, each step had increased in amplitude by 18:00 h, to return to predawn measurements by 21:00 h. Similarly, in the 15°C treatments, amplitudes of each step at dusk were comparable to predawn measures, following the decline associated with the high natural light intensity from 12:00 h to 15:00 h. However, at 29°C in both light levels, amplitudes of each peak did not return to their predawn maxima, and were significantly lower at 72 h than at 0 h.

After 72 h, differences between the magnitude of each of the steps in relation to light and temperature treatments were apparent, with values significantly lower in nubbins subject to high light than low light (one-way ANOVA, $p < 0.05$). In low light, the O step was not significantly different from initial readings after 72 h in all temperature treatments. However, over 72 h in high light the O step had increased significantly in the 20°C and 15°C treatments (by 101% and 100%, respectively), but declined in the 29°C treatment by 55% (rmANOVA, $p < 0.001$). Over 72 h at low light, percent change in J (normalized to F_o) in the 29°C treatment was not significantly different to that of J in the 15°C treatment (1% increase in both treatments); however, at high light, percent change in the 29°C treatment was significantly greater than the 20°C or 15°C treatments (67% decline vs. 67% and 42% increase, respectively; $p < 0.001$). For the I and P steps, magnitude and variability were greater in low light than high light, but similar responses to temperature treatments existed for both light treatments over the 72 h: in the 29°C treatment at both light levels I and P declined over 72 h (low light: 26% and 3% decline, respectively, high light: 86% and 88% decline, respectively) and in the 15°C treatment the decline was significantly less (low light, I and P both 25%; high light, I and P 24% and 28%, respectively, $p < 0.01$). However, in the 20°C treatment, I and P actually increased after 72 h in high light (by 37% and 34%, respectively), but there was no significant change in the low-light treatment.

Discussion

Our results demonstrate a greater tolerance of the dinoflagellate symbiont, *Symbiodinium* C100, of *P. damicornis* at LHI, to a 3°C temperature decrease than a 3°C temperature increase from annual temperature range (18–26°C; Table 1), in terms of its photo-physiological responses. These responses are specific to the rapid increase in temperature applied in these experiments. It should be noted that if the temperature ramp and experimental duration was extended over a longer period of time (such as weeks, rather than days), different bleaching responses and thresholds may have been observed. An additive effect of light and temperature stress was demonstrated on the coral symbionts of *P. damicornis* on LHI, with high light and high temperatures having the greatest effect on symbiont PSII photochemical efficiency. The results suggest that the presence of novel types of clade C *Symbiodinium* at LHI may be explained by physiological differences between the LHI symbionts and those found at lower latitudes, with LHI *Symbiodinium* types perhaps specialized for cooler and variable temperatures, so contributing to the success of corals at this marginal location.

Physiology of Lord Howe Island Symbiodinium—*P. damicornis* at LHI has been observed to host three novel symbiont types, C103, C100, and C118, with their local scale distribution relating to environmental conditions (Table 1). Contrastingly, *P. damicornis* across the whole GBR range hosts *Symbiodinium* type C1 and closely related types (such as C1c, which differs by one base-pair from C1 [LaJeunesse et al. 2003; Ulstrup et al. 2006]). Ulstrup et al. (2006) tested the bleaching sensitivity of *P. damicornis* associating with C1 and D1 (ITS1 types) across a latitudinal range, from the southern to central GBR. They found that the decline in photochemical efficiency with increased temperature and bleaching susceptibility corresponded more strongly with latitude than with symbiont type, suggesting their temperature history was of great importance in determining thermal sensitivity. Likewise, the present study suggests a potential physiological adaptation of LHI *Symbiodinium* to the cool conditions, perhaps due to their temperature history. The mere presence of distinct symbionts associated with *P. damicornis* at LHI suggests the partnerships may be adapted to the high-latitude thermally variable environment. Additionally, the fact that C1c is harbored by other host species at LHI suggests that C1c may not be the optimal symbiont for *P. damicornis* in this variable environment. It should be noted that the study of Ulstrup et al. (2006) used a peak irradiance of 300 μmol photons m^{-2} s^{-1} and, thus, a direct comparison of results may not be possible.

Photochemical responses of LHI Symbiodinium *to low temperature*—Symbiotic algae in corals at LHI are seasonally exposed to extremes of temperature, annually ranging from 18°C to 26°C (Table 1). The well-known inverse relationship of photosynthetic quantum yield with sunlight intensity (Hoegh-Guldberg and Jones 1999; Gorbunov et al. 2001; Hill and Ralph 2005) was observed for all *P. damicornis* nubbins in all treatments. Cold temperature stress in the present study caused a decline in effective quantum yield, particularly evident in the first 24 h of the experiment at both light levels. It is well-documented that decreased temperatures intensify photo-inhibition in higher plants (Smilie et al. 1988) due to a reduction in the rate at which the quenching of PSII occurs (Krause 1992). However, few studies have documented the effect of cold temperature stress on corals, despite knowledge of increased temperatures causing a breakdown of the symbiotic relationship (Lesser 1996; Thornhill et al. 2006).

Early evidence showed that while some corals are adapted for cooler conditions (Roberts et al. 1982), others are unable to survive severe declines in temperature (Saxby et al. 2003; Hoegh-Guldberg et al. 2005). Cold temperature

stress causing light-dependent photo-inhibition of *Symbiodinium* has been documented in the coral *Montipora digitata*, following short-term cold exposure (Saxby et al. 2003). Following exposure to 12°C and 14°C in symbionts usually subject to temperatures of 23–26°C, irreversible photo-damage was reported, evident in chronically depressed photochemical efficiency several hours after temperatures were returned to their original state. These findings parallel the present study, with photochemical efficiency depressed following cold temperature exposure. However, the LHI symbionts here were still able to photosynthesize at 63% of original capacity after 72 h in low light, suggesting a degree of tolerance to cold stress. Symbiont-type specific reactions to cold stress has been observed in cultured *Symbiodinium*, with clade B2 from the temperate western Atlantic found to be physiologically cold tolerant and able to rapidly recover from prolonged exposure to low temperatures (10°C), unlike *Symbiodinium* types from tropical environments (A3, B1, and C2; Thornhill et al. 2008). The cold-tolerance of B2 was suggested to contribute to its distribution in temperate habitats (Thornhill et al. 2008); a pattern that may be emulated by the symbionts present at LHI. Although recovery was not recorded in the present study, the reduced effect of cold temperatures after 32 h suggest this *Symbiodinium* type can tolerate cold conditions and, thus, may explain why this type is present in corals at LHI.

Despite knowledge on the effect of cold stress on the symbiotic relationship in corals, little is known of the effect to the photosynthetic apparatus of *Symbiodinium* cells in response to cold temperature stress. The present study used a PEA fluorometer to determine the effect of temperature stress on the condition of PSII and the redox state of the primary (Q_A) and secondary (Q_B) electron acceptors in symbionts, a method previously used to show the site of impact of high temperature stress on coral symbionts (Hill et al. 2004*a*; Hill and Ralph 2006). Our results suggest that cold temperatures have a similar site of impact to increased temperatures, with a decrease in the J step along FICs indicating a decline in the rate of Q_A reduction and the reduced capacity for Q_A to transport electrons through the electron transport chain to Q_B, due to photo-inhibition (Chylla and Whitmarsh 1990; Hill and Ralph 2006). A primary cause for this response could be an increase in the proportion of inactive PSII centers, whereby Q_B cannot oxidize Q_A^-, and as a consequence the photochemical capacity of PSII declines (Hill and Ralph 2006). The cold-induced reduction in photochemical efficiency, specifically the capacity to transport electrons, may also be linked to the effect of cold temperatures on rates of reactions, via the effect on the kinetic energy of enzymes and substrates (Saxby et al. 2003), including the Calvin–Benson cycle (Nobel 1991). Similar mechanisms may be a result of cold temperature stress; however, further study would be required.

Whilst the responses to cold temperature stress documented in the present study are similar to those reported for increased thermal stress (both in the present study and multiple comparable studies [Hill and Ralph 2006; Robison and Warner 2006]), the effect of a 3°C temperature decrease on photochemical efficiency and FICs, appears to be less than that of a 3°C temperature increase. The experimental temperatures chosen were 1°C above and below the low and high bleaching threshold temperatures, respectively. Although we only measured physiological effects to the symbiotic algae, it should also be noted that the thermal tolerance of the animal host is likely to play a role in determining the susceptibility of the coral to temperature changes (Baird et al. 2009).

Interactive effect of altered temperature and high irradiance—Most bleaching observed in the field is caused by elevated temperatures combined with high solar radiation, with excessive light causing the impairment of PSII by a loss of functional PSII centers (Lesser and Farrell 2004). The present study found an additive effect of elevated irradiance with both increased and lowered temperatures, causing a decline in photochemical efficiency. An additive effect of low temperature and high irradiance was demonstrated by Saxby et al. (2003), whereby exposure of corals to 14°C in low light had little effect on photochemical efficiency while corals exposed to 14°C and full sunlight were heavily affected. Variation in the photochemical responses to light intensities and temperatures are also evident from the FICs in the present study. A greater decline in the J step is evident in high-light treatments compared to low light, which equates to a decrease in the rate in Q_A reduction, and an increase in nonphoto-chemical quenching (Hill and Ralph 2005). Diurnal patterns in the FIC in the present study are similar to those observed by Hill and Ralph (2005); however, a lack of daily recovery overnight is evident in the high and low temperature treatments, particular in high light, suggesting that photodamage has occurred. The lack of a morning rise in the P step, particularly in the high-light treatments and irrespective of temperature, provides further evidence of photoinhibitory damage to PSII.

Ecological implications—Although many high-latitude corals can withstand seasonal variations in sea temperature of > 12°C (Kleypas et al. 1999), small excursions above and below geographically associated maxima or thresholds lead to stress in tropical and high-latitude corals (Hoegh-Guldberg 1999). Corals thriving in marginal environments must be physiologically adapted to cope with the extreme or variable conditions. At LHI, the flexibility of *P. damicornis* to associate with a range of symbiont types as opposed to the fidelity to *Symbiodinium* C1c in the Indo-Pacific previously reported, and its ability to cope with low temperatures by hosting thermally resilient symbionts, may help to explain the success of this coral at this marginal location. However, the detrimental effect of a 3°C increase above the summer maximum suggests that *P. damicornis* may be on the boundary limits of its survival, and that future warming may lead to coral bleaching events. Indeed, up to 90% of corals (including *P. damicornis*) at two sites in LHI lagoon recently experienced bleaching when sea temperatures exceeded 28°C during calm and clear weather conditions in February 2010, with moderate bleaching at other sites (P. Harrison, pers. comm.). It appears that rapid

increases in sea surface temperature beyond the normal range are enough to cause bleaching, and possibly death, even in corals adapted to thermally variable environments. Further, geographically and genetically isolated reefs such as LHI are especially vulnerable to climate change, with infrequent long-distance dispersal (Noreen et al. 2009) likely to be insufficient to enable recovery from fatal bleaching events.

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