PHOTOACCLIMATORY AND PHOTOPROTECTIVE RESPONSES TO COLD VERSUS HEAT STRESS IN HIGH LATITUDE REEF CORALS[1]

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Corals at the world's southernmost coral reef of Lord Howe Island (LHI) experience large temperature and light fluctuations and need to deal with periods of cold temperature (<18°C), but few studies have investigated how corals are able to cope with these conditions. Our study characterized the response of key photophysiological parameters, as well as photoacclimatory and photoprotective pigments (chlorophylls, xanthophylls, and β-carotene), to short-term (5-d) cold stress (~15°C; 7°C below control) in three LHI coral species hosting distinct *Symbiodinium* ITS2 types, and compared the coral–symbiont response to that under elevated temperature (~29°C; 7°C above control). Under cold stress, *Stylophora* sp. hosting *Symbiodinium* C118 showed the strongest effects with regard to losses of photochemical performance and symbionts. *Pocillopora damicornis* hosting *Symbiodinium* C100/C118 showed less severe bleaching responses to reduced temperature than to elevated temperature, while *Porites heronensis* hosting *Symbiodinium* C111* withstood both reduced and elevated temperature. Under cold stress, photoprotection in the form of xanthophyll de-epoxidation increased in unbleached *P. heronensis* (by 178%) and bleached *Stylophora* sp. (by 225%), while under heat stress this parameter increased in unbleached *P. heronensis* (by 182%) and in bleached *P. damicornis* (by 286%). The xanthophyll pool size was stable in all species at all temperatures. Our comparative study demonstrates high variability in the bleaching vulnerability of these coral species to low and high thermal extremes and shows that this variability is not solely determined by the ability to activate xanthophyll de-epoxidation.

Key index words: Acropora yongei; coral bleaching; Lord Howe Island; photosynthesis; *Pocillopora damicornis*; *Porites heronensis*; *Stylophora*; *Symbiodinium*; xanthophyll de-epoxidation

Abbreviations: $^1O_2$, singlet oxygen; chl *a*, chlorophyll *a*; chl c_2, chlorophyll c_2; COI, mitochondrial cytochrome *c* oxidase subunit I; Dd, diadinoxanthin; DDE, diadinoxanthin de-epoxidase; DEP, diatoxanthin epoxidase; DGGE, denaturing gradient gel electrophoresis; Dt/[Dt+Dd], xanthophyll de-epoxidation; Dt, diatoxanthin; FSW, filtered seawater; F_v/F_m, maximum quantum yield of PSII; HPLC, high-pressure liquid chromatography; ITS2, internal transcribed spacer region 2; LHI, Lord Howe Island; NPQ, nonphotochemical quenching; O_2^-, superoxide anion; ORF, hypervariable mitochondrial open reading frame; PCR, polymerase chain reaction; Pd, *Pocillopora damicornis*; Ph, *Porites heronensis*; PSII,

photosystem II; PSP, pool (concentration) of photosynthetic pigments; Q_A, primary electron acceptor; Q_B, secondary electron acceptor; Q_m, excitation pressure over PSII; rmANOVA, Repeated-measures analysis of variance; ROS, Reactive oxygen species; RUBISCO, ribulose-1,5-bisphosphate carboxylase oxygenase; S.E., standard error; SD, *Symbiodinium* cell density; S, *Stylophora* sp.; T_a, initial annealing temperature in polymerase chain reaction; ZEP, zeaxanthin epoxidase; β-car, β-carotene; $\Delta F/F_m'$, effective quantum yield of PSII

Reef-building corals associate with symbiotic dinoflagellates of the genus *Symbiodinium.* This symbiotic relationship is sensitive to environmental stress, exhibiting coral bleaching when stressors cause substantial degradation or loss of dinoflagellate symbionts and/or their pigments (Falkowski and Dubinsky 1981, Hoegh-Guldberg 1999). Thermal stress associated with elevated temperature anomalies is the principal cause for episodes of mass coral bleaching throughout the global distribution of reefs (Hoegh-Guldberg 1999, Lesser 2011). However, coral bleaching has also been documented under subnormal temperatures at a range of locations (Hoegh-Guldberg 1999), including the Arabian Gulf (Coles and Fadlallah 1991), southern Great Barrier Reef (Hoegh-Guldberg and Fine 2004, Hoegh-Guldberg et al. 2005), Gulf of California (LaJeunesse et al. 2010), and Florida Keys (Kemp et al. 2011). While the physiological and cellular impacts of increased temperature have been thoroughly studied (reviewed in Weis 2008), only a few studies have addressed the effects of decreased temperature on coral-*Symbiodinium* associations (Saxby et al. 2003, Hoegh-Guldberg and Fine 2004, Hoegh-Guldberg et al. 2005, Thornhill et al. 2008, LaJeunesse et al. 2010, Kemp et al. 2011, Roth et al. 2012). Saxby et al. (2003) and Kemp et al. (2011) found that the physiological response to cold stress is similar to that to heat stress and includes the loss of symbiont cells, photosynthetic pigments (PSP), and photosynthetic capacity. Roth et al. (2012) suggested that this response to chilling might only be similar to heating in the early stages. The underlying cellular and biochemical mechanisms of cold-water vulnerability remain elusive, though they might include lower RUBISCO enzyme activity (Saxby et al. 2003) or impaired thylakoid membrane fluidity (discussed in Thornhill et al. 2008, Kemp et al. 2011) under reduced temperatures. Similarly, while the photoprotective responses of corals and their symbionts to heat stress have been well studied (reviewed in Smith et al. 2005, Baird et al. 2009), photoacclimation and photoprotection during cold stress have received little attention, and there have been few comparative studies of heat and cold stress (Roth et al. 2012).

At Lord Howe Island (LHI, 31°33′ S, 159°05′ E), the world's southernmost coral reef, the long-term averages of sea surface temperature for each month are between 19°C and 24°C (monthly climatology: www.coralreefwatch.noaa.gov), with a minimum point measurement of 14.5°C and a maximum of 28°C recorded in the past 10 years (www.data.aims.gov.au). LHI coral communities are highly diverse, with at least 83 temperate, subtropical, and tropical species (Veron and Done 1979, Harriott et al. 1995) hosting at least 15 distinct *Symbiodinium* types, 12 of which have been proposed as being endemic, including C100, C111, and C118 (Wicks et al. 2010a). It has been shown that the combination of corals and their dinoflagellate and microbial symbionts (i.e., the holobiont) influence bleaching propensity (Sampayo et al. 2008, Fitt et al. 2009, Fisher et al. 2012) and postbleaching mortality of the host (Sampayo et al. 2008, Fitt et al. 2009). For example, *Symbiodinium* type determined bleaching susceptibility of the reef-building coral *Pocillopora damicornis* during a cold-water period in the Gulf of California; corals hosting *Symbiodinium* type C1b-c bleached, while those hosting *Symbiodinium* type D1 remained mostly unaffected (LaJeunesse et al. 2010).

The symbiotic breakdown caused by thermal stress involves a number of cellular changes within the holobiont whose sequential order is still being elucidated. Early responses within the host include apoptotic cell death in the gastrodermis (Ainsworth et al. 2008, Paxton et al. 2013) and a reduction of the coral epithelium which may precede cellular changes in the symbiont (Ainsworth et al. 2008). Furthermore, integrity, ultrastructure, and gene expression in the host's mitochondria change independently from *Symbiodinium* cell integrity (Dunn et al. 2012). The suppressed expression of the mitochondrial gene cytochrome c may result in reduced scavenging of reactive oxygen species (ROS), so increasing the overall levels of ROS within the holobiont (Dunn et al. 2012). Within the chloroplast of symbionts, it has been proposed that thermal stress directly affects photosystem II (PSII; Warner et al. 1999), the thylakoid membranes (Iglesias-Prieto et al. 1992, Tchernov et al. 2004, Díaz-Almeyda et al. 2011), and the Calvin Cycle (Jones et al. 1998, Leggat et al. 2002, 2004), promoting electron accumulation in the chloroplast and associated overproduction of ROS (reviewed in Venn et al. 2008, Weis 2008). Further, it has been suggested that excess ROS damage host and symbiont DNA, proteins, and lipids, so accelerating the rate of damage to the photosystem and resulting in the breakdown of the symbiosis (reviewed in Venn et al. 2008, Weis 2008). However, recent studies also show that the initial production of ROS may not increase photodamage per se, but rather inhibit the repair of photodamaged components of PSII, such as the major PSII core protein D1 (Takahashi et al. 2004, 2009) and

light-harvesting proteins (Takahashi et al. 2008). If so, small amounts of ROS could promote photoprotective steps: i.e., the down-regulation of linear electron transport could prevent the uncontrolled generation of excess ROS in the chloroplast (reviewed in Takahashi and Badger 2011).

ROS-mediated inhibition of protein synthesis is attenuated by an array of ROS-scavenging compounds, such as β-carotene quenching singlet oxygen within the chloroplast ($^1O_2$; Asada 2006), and by mechanisms alleviating the excess production of ROS, such as thermal dissipation of excitation energy. Other mechanisms alleviating excess ROS production include carbon fixation and alternative electron pathways such as cyclic electron flow and photorespiration in the light and chlororespiration in the dark (reviewed in Takahashi and Badger 2011, Murata et al. 2012).

Thermal dissipation is a nonphotochemical quenching (NPQ) process that allows the elimination of excessively absorbed energy at the light-harvesting complexes of PSII as heat, thus avoiding overexcitation of the photosystem (reviewed in Niyogi 1999). In dinoflagellates such as *Symbiodinium* spp., thermal dissipation relies on the conversion of the structural epoxy-xanthophyll diadinoxanthin (Dd) to the photoprotective epoxy-free diatoxanthin (Dt, xanthophyll de-epoxidation), catalyzed by diadinoxanthin de-epoxidase (DDE), which is equivalent to the conversion of violaxanthin to zeaxanthin catalyzed by violaxanthin de-epoxidase in vascular plants. In both systems, epoxidation is primarily activated by exposure to high light and subsequent acidification of the thylakoid lumen caused by light-driven photosynthetic transport. However, in contrast to vascular plants, in Dd-containing organisms DDE can be activated by a weak acidification of the thylakoid lumen that may be generated by chlororespiration in the dark or low light (reviewed in Goss and Jakob 2010). The ability of *Symbiodinium* to maintain Dd de-epoxidation in the dark has been demonstrated by Middlebrook et al. (2010), who showed that 75% of the xanthophyll pool was in the de-epoxidated state even after 70 min of dark acclimation, in the reef-building coral *Acropora formosa*. Reversal from the photoprotective to the light-harvesting state is catalyzed by the enzyme Dt epoxidase (DEP) in dinoflagellates and by zeaxanthin epoxidase (ZEP) in vascular plants. In contrast to ZEP, DEP is inhibited by a proton gradient, which is necessary to ensure Dt accumulation in conditions of high light, because DDE and DEP have highly similar rate constants (reviewed in Goss and Jakob 2010).

While several studies have addressed xanthophyll de-epoxidation and/or the role of β-carotene in *Symbiodinium* cells (e.g., Brown et al. 1999, Ulstrup et al. 2008, Hennige et al. 2009), and its protective role in conditions of high light (Ambarsari et al. 1997, Krämer et al. 2012) and high temperature (e.g., Dove et al. 2006, Abrego et al. 2008, Middlebrook

et al. 2008), only one study has investigated xanthophyll de-epoxidation during cold-water stress (21°C; Roth et al. 2012). Comparable detailed studies from corals at high latitude reefs are missing. In particular, it is not known whether corals that live close to their lower thermal thresholds (Kleypas et al. 1999) use photoacclimatory and/or photoprotective strategies to deal with cold exposure (<18°C), whether in the short or long term. We therefore investigated the photophysiological response of three typical LHI holobionts (*Porites heronensis* C111*, *Stylophora* sp. C118 and *P. damicornis* C100/C118) to short-term cold stress. In particular, this study explored the dynamics of photoacclimatory and photoprotective pigments (chlorophylls, xanthophylls, and β-carotene) in the corals' dinoflagellate symbionts, and the possibility that the activation of thermal dissipation in the form of xanthophyll de-epoxidation might be a key strategy for withstanding bleaching at low temperature; the response was compared with that at elevated temperature.

METHODS

Sampling. In October 2010, fragments of three coral species (Table 1) were collected from the lagoon site of Sylphs Hole, LHI, Australia (31°31′ 249″ S, 159°03′ 261″ E). Corals were identified as *P. damicornis* ($n = 3$), *Stylophora* sp. ($n = 2$) and *P. heronensis* ($n = 4$). Sample size (n) refers to the number of colonies. In addition, fragments of *P. damicornis* ($n = 5$) were sampled from the lagoon reef site of Stephens Hole (31°31′ 937″ S, 159°03′ 251″ E). We sampled *P. damicornis* from Sylphs Hole and Stephen's Hole to increase the chance of sampling different symbiont types, as this coral species has been shown to host *Symbiodinium* internal transcribed spacer 2 (ITS2) types C100, C103, and C118 at LHI (Wicks et al. 2010a,b). All coral species were identified based on morphological characteristics described in Veron and Stafford-Smith (2000). Because *Stylophora* sp. shared morphological characteristics common to both pocilloporid species, they were additionally identified using the molecular markers *COI* (mitochondrial cytochrome *c* oxidase subunit I), as outlined in Folmer et al. (1994), and ORF (hypervariable mitochondrial open reading frame) as described in Flot et al. (2008). All samples were taken from a depth of 0.5–3 m during low tide. Fragments were obtained from the top of the colony to ensure that they had received a similar light regime. Sea temperatures at Sylphs Hole at the time of collection and experimentation (Austral spring) within the lagoon peaked at 22°C at a depth of 2.5–5.5 m (AIMS data center; www.data.aims.gov.au). Irradiance in the lagoon, at a depth of 0.5–3 m, peaked at ~1,750 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} as measured with a submersible HOBO pendant data logger (ENVCO, Auckland, New Zealand) at noon. The HOBO pendant data logger was calibrated with a Li-Cor LI-189 photometer (Li-Cor, Lincoln, Nebraska, USA).

Experimental procedure. Coral explants of 1–2 cm length were immediately prepared from fragments following collection (25 explants per colony). One explant was prepared immediately for *Symbiodinium* genotyping (see below). Eight explants per colony were placed in one of three aerated 50 L tanks that were supplied with unfiltered seawater from the LHI lagoon at regular intervals (approximately every 90 min during daylight hours) and acclimatized to these conditions for 4 d. During both acclimation and subsequent

TABLE 1. Coral species and corresponding *Symbiodinium* ITS2 types identified by PCR-DGGE and subsequent sequencing.

Species	Family	*Symbiodinium* ITS2 type	Closest GenBank match (identity, %)	GenBank accession no.
Porites heronensis	Poritidae	C111*	HM222438 (279/283, 99%)	KF194186
Pocillopora damicornis	Pocilloporidae	C100	HM222433 (283/283, 100%)	KF194189
		C118	HM222440 (283/283, 100%)	KF194190
Stylophora sp.	Pocilloporidae	C118	HM222440 (283/283, 100%)	KF194191

temperature treatment, light intensity was reduced using shade cloth, and temperature and natural light within tanks were monitored using a submersible HOBO pendant logger. During acclimation, F_v/F_m was monitored daily (data not shown) and stabilization of this parameter suggested that corals had acclimated adequately. Within each of the 50 L treatment tanks, there were eight explants from each of four *P. heronensis* colonies ($n = 4$), each of two *Stylophora* sp. colonies ($n = 2$) and each of eight *P. damicornis* colonies ($n = 8$).

After acclimation, coral explants were exposed to temperatures averaging: (i) $22 \pm 0.1°C$ (mean $\pm$ S.E.; control), (ii) $15 \pm 0.1°C$ ($-7°C$ below control) and (iii) $29 \pm 0.1°C$ ($+7°C$ above control). Treatment temperatures were based on previous bleaching experiments conducted by Wicks et al. (2010b) at the same location. Furthermore, temperatures exceeding 27°C elicited coral bleaching at LHI (Harrison et al. 2011). The chilling/heating to respective treatment temperatures was started at 06:00 h in the morning of Day 1. Treatment temperatures were attained on Day 1 at 09:10 h (29°C) and 17:00 h (15°C). The experiment was terminated at 18:00 h on Day 5. Between 13:00 h and 14:00 h on Days 0 (i.e., the day prior to the start of thermal ramping), 2, and 5, one coral explant *per* coral colony was flash frozen in liquid nitrogen. Frozen samples were stored at $-80°C$ until pigment analysis.

Symbiodinium genotyping. One explant of each coral colony was airbrushed, and *Symbiodinium* cells isolated through repeated washes in 0.22 μm filtered seawater (FSW) and preserved in sodium chloride saturated 20% dimethyl sulfoxide buffer for subsequent genotyping. Genomic DNA was isolated following the procedure of Stat et al. (2009), and isolated DNA was stored in Tris-ethylenediaminetetraacetic acid (10 mM, pH 8.0). The ITS2 of the ribosomal DNA was amplified by polymerase chain reaction (PCR) using the primers itsD (Pochon et al. 2001) and Its2CLAMP, using a touchdown protocol modified after LaJeunesse (2002). The initial annealing temperature (T_a) started at 65°C. T_a was then decreased every two cycles by 1°C and held at the final T_a of 55°C for an additional 15 cycles. Amplicons were separated using denaturing gradient gel electrophoresis (DGGE, BioRad DCode system) as performed in Pontasch et al. (2014), and then DNA from distinct and prominent bands was eluted overnight in 20 μL sterile water, re-amplified, and sequenced in forward and reverse directions at Macrogen, South Korea. The consensus sequence was analyzed by BLAST on GenBank and compared to sequences available on Geosymbio (Franklin et al. 2012).

Chlorophyll fluorescence. Chlorophyll fluorescence of PSII was measured using a diving pulse amplitude modulated fluorometer (Walz, Effeltrich, Germany). Maximum quantum yield of PSII (F_v/F_m) was recorded each day 30 min before sunrise (06:00 h) and 30 min after sunset (18:00 h). Effective quantum yield of PSII ($\Delta F/F_m'$) was measured between 12:00 h and 12:45 h noon. Excitation pressure over PSII (Q_m) (Iglesias-Prieto et al. 2004) was calculated as in Fisher et al. (2012):

$$Q_m = 1 - [(\Delta F/F_m')\text{at noon}/(F_v/F_m)\text{at dawn of Day0}]$$

Sample preparation and analysis. Coral fragments were airbrushed in 0.22 μm FSW at 4°C. Two subsamples of the tissue slurry were removed and frozen at $-80°C$ for later determination of *Symbiodinium* cell density. The remaining tissue slurry was processed immediately for pigment identification and quantification. To determine *Symbiodinium* cell density, cells were counted in 10 replicate counts using a haemocytometer and standardized to cells $\cdot$ cm^{-2} coral surface area, as determined using the paraffin wax method (Stimson and Kinzie 1991).

All steps of pigment extraction were performed at 4°C and pigment extracts were kept in the dark. The coral/algal homogenate was centrifuged at 4,000g for 15 min. Supernatant was discarded, 3 mL of high-pressure liquid chromatography (HPLC)-grade methanol was added, and the sample was vortexed and extracted for 10 min. The extract was then centrifuged at 4,000g for 3 min. The extract was carefully removed in order to not disturb the algal pellet and the extraction repeated with new methanol. Extractions were repeated five times to ensure that all pigments were successfully extracted, and the extracts were combined and filtered through a 0.22 μm cellulose acetate filter prior to pigment analysis. A subsample of the extract was analyzed by HPLC using an Agilent Technologies 1200 Series HPLC system (Agilent Technologies, Santa Clara, California, United States), in three replicate measurements (Zapata et al. 2000, Dove et al. 2006). Pigments were quantified by comparison to known concentrations of chlorophyll *a* (chl *a*), chlorophyll c_2 (chl c_2), peridinin, diadinoxanthin (Dd), diatoxanthin (Dt), and β-carotene (β-car). These standards were obtained from DHI, Denmark. Pigment concentrations were normalized to cell number and chlorophyll *a*.

Statistical analysis. SPSS software was used for statistical analysis. Repeated-measures analysis of variance (rmANOVA) and post hoc pairwise comparison with Bonferroni adjustment were performed to determine significant changes in photophysiological parameters, symbiont density, and pigment parameters. If assumptions of normality (Kolmogorov–Smirnov test) and assumptions of homogeneity of variance (Levene's test) were violated, data were arcsine (F_v/F_m, Q_m) or log (pigments) transformed. When assumptions of equality of the variances between repeated measures were not met (Mauchly's test of sphericity), the Greenhouse-Geisser correction is reported. All parameters were analyzed for differences among treatments or between species on Day 0, using univariate ANOVA with post hoc Tukey's HSD comparison. Only statistically significant results are reported in the text.

RESULTS

Genetic identification of Symbiodinium. *Pocillopora heronensis* hosted a symbiont type that showed 99% sequence identity to type C111 (Wicks et al. 2010a) and differed by two base-pair mismatches and one base-pair deletion within the ITS2 region, and one

base-pair mismatch in the 5.8S rRNA coding region that flanks the ITS2 region. This type is hereafter referred to as C111* (Table 1). *Pocillopora damicornis* hosted a mixture of *Symbiodinium* types C100 and C118 (Wicks et al. 2010a). *Stylophora* sp. hosted *Symbiodinium* type C118.

Maximum quantum yield of PSII. On Day 0, F_v/F_m measured at 18:00 h was 0.682 ± 0.01 (mean $\pm$ S.E.) in *P. heronensis,* 0.667 ± 0.01 in *P. damicornis,* and 0.670 ± 0.01 in *Stylophora* sp.

Over a period of 5 d, coral species responded differently to a given temperature (significant Time × Temperature × Species interaction, Table 2). While F_v/F_m was relatively stable at the control temperature, this parameter declined over time in most coral associations at low and high temperature, though to differing degrees between species, and with very different responses to low and high temperature within species (Fig. 1; Table 3). In the cold treatment (15°C), F_v/F_m ultimately declined by 19.7% in *P. heronensis*, 35.6% in *P. damicornis* and 79.8% in *Stylophora* sp. (Day 5 vs. Day 0). In comparison, at high temperature (29°C), F_v/F_m declined by 29.2% in *P. heronensis*, 86.2% in *P. damicornis*, and 22% in *Stylophora* sp. over the 5 d experiment (Day 5 vs. Day 0).

Population density of symbionts. On Day 0, symbiont densities (mean $\pm$ S.E.) were $2.68 \times 10^6 \pm 0.19 \times 10^6$ cells · cm^{-2} in *P. heronensis,* $1.87 \times 10^6 \pm 0.01 \times 10^6$ cells · cm^{-2} in *P. damicornis,* and $2.08 \times 10^6 \pm 0.02 \times 10^6$ cells · cm^{-2} in *Stylophora* sp. Univariate ANOVA ($F_{2,38} = 11.403$, $P < 0.001$) with post hoc Tukey's HSD comparison ($P < 0.001$) revealed that symbiont density was significantly higher in *P. heronensis* than in *P. damicornis*.

While at the control temperature symbiont density was stable over time in all corals, in the cold treatment it significantly decreased in *P. damicornis* and *Stylophora* sp. but not in *P. heronensis* (Fig. 2, A–C). *Pocillopora damicornis* lost 59% of its symbionts by the end of the experiment, while *Stylophora* sp. eventually lost 68% of its symbionts over this same period (Day 5 vs. Day 0). In these two coral species, the decline resulted in significantly lower symbiont densities at the end of the experiment at 15°C than at 22°C or 29°C (Bonferroni: $P < 0.05$ for both comparisons between treatments in both species). In comparison, at elevated temperature, symbiont density was stable in two of the three species over the duration of the experiment—*P. heronensis* and *Stylophora* sp.—but in *P. damicornis* it declined even more sharply than it did in the cold (92% decline Day 5 vs. Day 0; Fig. 2B). In this latter species, symbiont density was significantly lower after 5 d exposure to 29°C than after exposure to 15°C or 22°C (Bonferroni: $P < 0.001$ for both 29°C vs. 15°C and 29°C vs. 22°C).

Photosynthetic pigments. On Day 0, the concentration (mean $\pm$ S.E.) of PSP (pool of chl *a*, chl c_2, peridinin) was 18.0 ± 1.10 pg · cell^{-1} in *Symbiodinium* C111* hosted by *P. heronensis,* 9.2 ± 0.34 pg · cell^{-1} in C100/C118 hosted by *P. damicornis,* and 7.5 ± 0.27 pg · cell^{-1} in C118 hosted by *Stylophora* sp. (Fig. 2, D–F). Univariate ANOVA ($F_{2,39} = 56.042$, $P < 0.001$) with post hoc Tukey's HSD comparison ($P < 0.001$) revealed that the concentration of pigments in *Symbiodinium* C111* in *P. heronensis* was significantly higher than for the other *Symbiodinium* types/associations.

At control and cold temperatures, the PSP pool size and concentrations of specific PSPs (chl *a*, chl c_2, and peridinin) were stable with time in all *Symbiodinium* types (Bonferroni: $P > 0.05$ for all comparisons of days over time; Fig. 2, D–F; Table 4). Similarly, at elevated temperature, the overall PSP

TABLE 2. Results of rmANOVA analysis for the parameters: photochemical maximum quantum yield of PSII (F_v/F_m); *Symbiodinium* density (SD); concentration of photosynthetic pigments (PSP) per cell; xanthophyll de-epoxidation (Dt/Dt+Dd); concentration of diatoxanthin (Dt) and diadinoxanthin (Dd) per chlorophyll *a* (chl *a*); concentration of diatoxanthin (Dt) per chlorophyll *a* (chl *a*); and excitation pressure over PSII (Q_m).

Variables	F_v/F_m*	SD ·cm^{-2}*	PSP ·cell^{-1}*	Dt/(Dd+Dt)	(Dd+Dt)/chl *a**	Dt/chl *a*	Q_n*
Time	**<0.001**	**<0.001**	0.057	**<0.001**	**0.015**	**<0.001**	**<0.001**
	$F_{3.2} = 95.1$	$F_{1.3} = 20.1$	$F_{1.2} = 3.6$	$F_{2.31} = 50.0$	$F_{1.2} = 6.0$	$F_{2.31} = 18.7$	$F_{3.2} = 80.8$
Time × Temperature	**0.001**	**0.011**	0.160	**0.001**	0.314	**0.021**	**<0.001**
	$F_{6.5} = 15.9$	$F_{2.5} = 4.5$	$F_{2.5} = 2.3$	$F_{4.62} = 5.6$	$F_{2.4} = 1.2$	$F_{4.62} = 3.1$	$F_{6.4} = 6.2$
Time × Species	**<0.001**	**0.006**	0.070	**0.009**	0.338	**0.008**	**<0.001**
	$F_{6.5} = 7.4$	$F_{2.5} = 5.2$	$F_{2.5} = 2.7$	$F_{4.62} = 3.8$	$F_{2.4} = 1.1$	$F_{4.62} = 3.8$	$F_{6.4} = 9.8$
Time × Temperature × Species	**<0.001**	**<0.001**	0.133	**<0.001**	0.279	**0.003**	**<0.001**
	$F_{13} = 9.5$	$F_{5.1} = 7$	$F_{4.9} = 1.8$	$F_{8.62} = 6.9$	$F_{4.8} = 1.3$	$F_{8.62} = 3.3$	$F_{12.8} = 5.2$
Error (Time)	106.9	39.3	39.6	31	38.1	31	105.7
Temperature	**<0.001**	**0.003**	0.053	**0.002**	0.683	**0.035**	**<0.001**
	$F_2 = 20.7$	$F_2 = 7.0$	$F_2 = 3.2$	$F_2 = 7.8$	$F_2 = 0.4$	$F_2 = 3.7$	$F_2 = 47.9$
Species	0.06	**<0.001**	**<0.001**	**<0.001**	0.366	**0.003**	**<0.001**
	$F_2 = 3.1$	$F_2 = 19.7$	$F_2 = 46.2$	$F_2 = 10.1$	$F_2 = 1.0$	$F_2 = 6.8$	$F_2 = 42.9$
Temperature × Species	**<0.001**	**0.002**	0.185	0.326	0.846	0.655	**<0.001**
	$F_4 = 12.5$	$F_4 = 5.6$	$F_4 = 1.7$	$F_4 = 1.2$	$F_4 = 0.3$	$F_4 = 0.6$	$F_4 = 12.4$
Error	33	32	32	32	32	32	33

*Greenhouse-Geisser correction is reported.

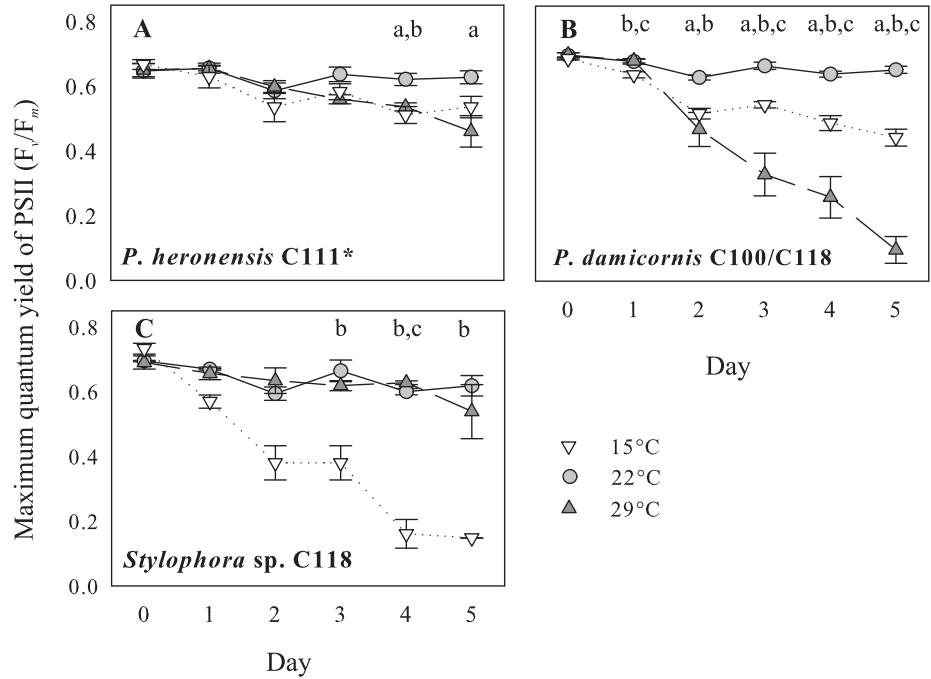Bold numbers highlight significant differences ($p \leq 0.05$).

FIG. 1. Effect of temperature over a 5 d period on maximum quantum yield of PSII (F_v/F_m) in (A) *Porites heronensis* hosting *Symbiodinium* C111*, $n = 4$, (B) *Pocillopora damicornis* hosting C100/C118, $n = 8$, and (C) *Stylophora* sp. hosting C118, $n = 2$. Values are means ± S.E. Significant differences are reported for (a) 22°C versus 29°C, (b) 22°C versus 15°C, and (c) 15°C versus 29°C at the level of $P < 0.05$ (rmANOVA and pairwise comparison with Bonferroni correction).

TABLE 3. Results of rmANOVA analysis (effect of Time × Temperature) when each coral–symbiont association is analyzed separately. The parameters are maximum quantum yield of PSII (F_v/F_m); *Symbiodinium* density (SD); pool of photosynthetic pigments (PSP); xanthophyll de-epoxidation (Dt/(Dt+Dd)); pool of diadinoxanthin (Dd) and diatoxanthin (Dt) per chlorophyll *a* (chl *a*); Dt per chl *a*; and excitation pressure over PSII (Q_m).

Parameter	*Porites heronensis* C111*	*Pocillopora damicornis* C100/C118	*Stylophora* sp. C118
F_v/F_m			
$F_{(df, Error\ df)}$	$5.1_{(4.3, 19.5)}$[†]	$30.2_{(5.5, 57.6)}$[†]	$12.5_{(5, 7.4)}$[†]
P	0.005	<0.001	0.002
SD			
$F_{(df, Error\ df)}$	ns	$5.3_{(4, 38)}$	$8_{(4, 4)}$
P	ns	0.002	0.035
PSP			
$F_{(df, Error\ df)}$	ns	$5.3_{(4, 38)}$	ns
P	ns	0.002	ns
Dt/(Dt+Dd)			
$F_{(df, Error\ df)}$	$8.5_{(4, 16)}$	$13.3_{(4, 38)}$	ns
P	0.001	< 0.001	ns
(Dt+Dd)/chl *a*			
$F_{(df, Error\ df)}$	ns	ns	ns
P	ns	ns	ns
Dt/chl *a*			
$F_{(df, Error\ df)}$	$7.3_{(4, 16)}$	$6.2_{(4, 38)}$	$7_{(4, 4)}$
P	0.001	0.001	0.043
Q_m			
$F_{(df, Error\ df)}$	$6.3_{(10, 10)}$	$21_{(5.7, 59.4)}$[†]	ns
P	0.004	<0.001	ns

[†]Greenhouse-Geisser correction reported.
Only significant effects ($p \leq 0.05$) are shown.
df = degrees of freedom; ns = not significant on the level $P > 0.05$.

pool size remained relatively constant in *P. heronensis* and *Stylophora* sp. (Fig. 2, D and F, Table 3), though in C111* in *P. heronensis* the chl *a* pool size

had declined by 25.3% at the end of the experiment (Bonferroni: $P < 0.001$; Table 4), so that on Day 5, the concentration of chl *a* was significantly lower at 29°C than at both 15°C and 22°C (Bonferroni: $P < 0.05$ for both comparisons). Moreover, the PSP pool size at elevated temperature declined in C100/C118 in *P. damicornis* (Fig. 2E), so that by Day 5 it was significantly lower than at both 22°C and 15°C (Bonferroni: $P < 0.01$ for 29°C vs. 22°C and $P < 0.05$ for 29°C vs. 15°C, respectively). This decline was associated with a 74.4% loss of chl *a*, 73.3% loss of chl c_2, and 53% loss of peridinin, as outlined in Table 4 (Day 5 vs. Day 0).

Xanthophyll de-epoxidation and β-carotene. On Day 0, xanthophyll de-epoxidation (Dt/[Dt+Dd]) was 0.25 ± 0.015 (mean ± S.E.) in *Symbiodinium* C111* hosted by *P. heronensis*, 0.22 ± 0.013 in C100/C118 hosted by *P. damicornis*, and 0.25 ± 0.025 in C118 hosted by *Stylophora* sp. (Fig. 3, A–C).

Over the 5 d period at the control temperature, xanthophyll de-epoxidation remained stable in *P. damicornis* (Bonferroni: $P > 0.05$) but not in *Stylophora* sp. and *P. heronensis*, where it increased and decreased, respectively (Bonferroni: $P < 0.001$, Day 5 vs. Day 0; and $P < 0.05$, Day 5 vs. Day 2, respectively). Nevertheless, after 5 d in the cold, *Symbiodinium* C118 in *Stylophora* sp. increased xanthophyll de-epoxidation over time at least 2-fold (Fig. 3C). *Symbiodinium* C111* in *P. heronensis* significantly increased xanthophyll de-epoxidation 1.8-fold over time, to 0.43 ± 0.013, resulting in a significantly higher rate than at the control temperature at the same time (0.24 ± 0.023; Fig. 3A; Table 3). In comparison, at high temperature, both C111* in *P. heronensis* and C100/C118 in *P. damicornis* enhanced
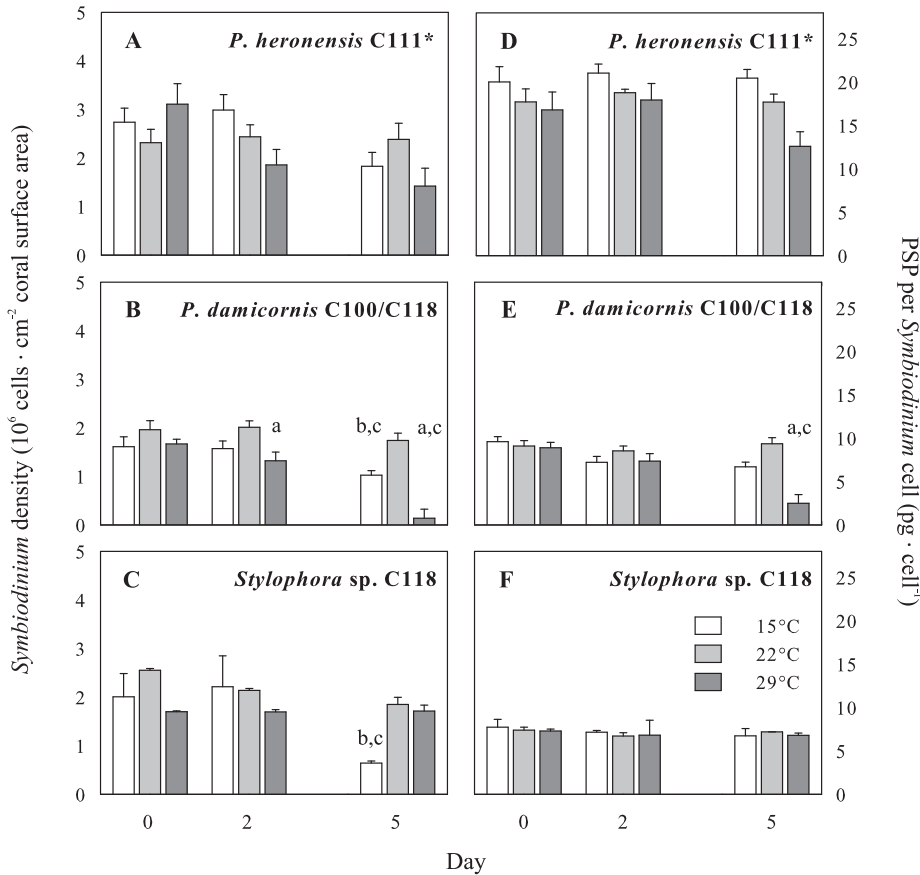
Fig. 2. *Symbiodinium* cell density in million cells per cm² coral surface area (A–C) and pool of photosynthetic pigments (PSP, comprising chl *a*, chl *c₂*, and peridinin) (D–F) in pg ·cell⁻¹ for (A, D) *Porites heronensis* hosting *Symbiodinium* C111*, *n* = 4; (B, E) *Pocillopora damicornis* hosting C100/C118, *n* = 8; and (C, F) *Stylophora* sp. hosting C118, *n* = 2. Presented are mean values ± S.E. Significant differences are reported for (a) 22°C versus 29°C, (b) 22°C versus 15°C, and (c) 15°C versus 29°C at the level of *P* < 0.05 (rmANOVA and pairwise comparison with Bonferroni correction).

TABLE 4. Concentration of light-harvesting pigments chlorophyll *a* (chl *a*), chlorophyll *c₂* (chl *c₂*), peridinin (pg cell⁻¹) on Days 0, 2, and 5 of the temperature treatment for *Symbiodinium* C111* in *Porites heronensis* (**Ph**), C100/C118 in *Pocillopora damicornis* (**Pd**), and C118 in *Stylophora* sp. (**S**).

	chl *a*			chl *c₂*			Peridinin		
	0	2	5	0	2	5	0	2	5
Ph									
C111*									
15°C	11.0 ± 0.9	11.3 ± 0.6	11.3 ± 0.4	2.7 ± 0.2	2.8 ± 0.1	2.6 ± 0.3	6.4 ± 0.7	6.9 ± 0.4	6.5 ± 0.4
22°C	9.8 ± 0.9	10.2 ± 0.2	9.6 ± 0.4	2.3 ± 1.2	2.5 ± 0.1	2.4 ± 0.2	5.7 ± 0.5	6.1 ± 0.2	5.7 ± 0.4
29°C	9.1 ± 1.1	9.8 ± 1.0	**6.8 ± 1.0**[a,b]	2.3 ± 0.3	2.4 ± 0.3	1.6 ± 0.3	5.5 ± 0.6	5.8 ± 0.6	4.2 ± 0.5
Pd									
C100/C118									
15°C	5.2 ± 0.4	3.9 ± 0.4	3.5 ± 0.3	1.5 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	2.9 ± 0.2	2.1 ± 0.2	2.0 ± 0.2
22°C	5.0 ± 0.4	4.7 ± 0.3	5.0 ± 0.3	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	2.8 ± 0.2	2.4 ± 0.1	2.8 ± 0.2
29°C	4.7 ± 0.4	3.9 ± 0.5	**1.2 ± 0.5**[a,b]	1.5 ± 0.1	1.3 ± 0.1	**0.4 ± 0.1**[a,b]	2.7 ± 0.2	2.2 ± 0.3	**1.3 ± 0.5**[a,b]
S									
C118									
15°C	4.6 ± 0.5	4.2 ± 0.1	3.9 ± 0.5	1.2 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	2.0 ± 0.3	1.9 ± 0.1	1.8 ± 0.3
22°C	4.4 ± 0.2	3.8 ± 0.3	4.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.0	1.9 ± 0.1
29°C	4.4 ± 0.1	3.8 ± 1.2	4.0 ± 0.2	1.0 ± 0.0	1.2 ± 0.1	1.0 ± 0.0	1.9 ± 0.1	1.9 ± 0.4	1.8 ± 0.1

Significant differences (*P* < 0.05) from values at 15°C ([a]) and 22°C ([b]) based on rmANOVA analysis with post hoc Bonferroni corrected pairwise comparison, are highlighted in bold.

their rates of xanthophyll de-epoxidation to 0.43 ± 0.021 and 0.55 ± 0.030, respectively (Fig. 3, A and C), resulting in significantly higher rates at the end of the experiment than at both the control and cold temperatures (Bonferroni: *P* < 0.001 for

both comparisons, 29°C vs. 15°C and 29°C vs. 22°C).

Light intensity (μmol photons · m⁻² · s⁻¹) was lowest on Day 1 and highest on Day 2 and averaged 1,095 ± 119 on Day 0, 561 ± 79 on Day 1,
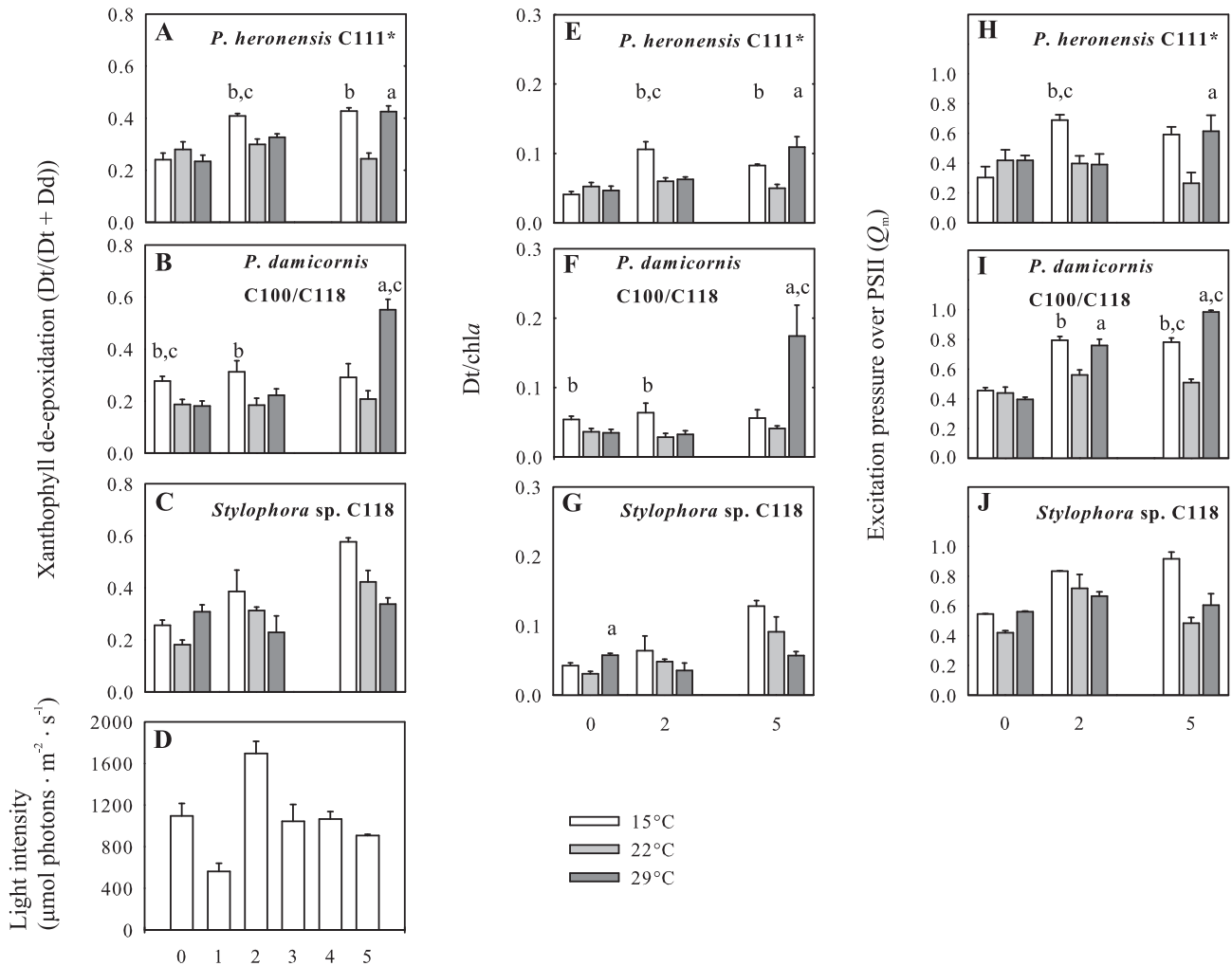
FIG. 3. Xanthophyll de-epoxidation (A–C), light intensity (D), concentration of diatoxanthin per chlorophyll *a* (Dt/chl *a*) (E–G) and excitation pressure over PSII, Q_m (H–J) over a 5 d period in (A, E, H) *Porites heronensis* hosting *Symbiodinium* C111*, $n = 4$; (B, F, I) *Pocillopora damicornis* hosting C100/C118, $n = 8$; and (C, G, J) *Stylophora* sp. hosting C118, $n = 2$. Light intensities are means ± S.E. between 12:00 h and 13:00 h, when chlorophyll fluorescence was measured. All other values are means ± S.E. Significant differences are reported for (a) 22°C versus 29°C, (b) 22°C versus 15°C, and (c) 15°C versus 29°C at the level of $P < 0.05$ (rmANOVA and pairwise comparison with Bonferroni correction).

1,694 ± 117 on Day 2, 1,042 ± 162 on Day 3, 1,064 ± 71 on Day 4, and 906 ± 12 on Day 5, between 12:00 h and 13:00 h (Fig. 3D).

The concentration of diatoxanthin relative to chl *a* (Dt/chl *a*) was similar between coral species at the beginning of the experiment (ANOVA: $P > 0.05$; Fig. 3, E–G). Over the duration of the experiment, control values of Dt/chl *a* were stable in *P. heronensis* and *P. damicornis* (Bonferroni: $P > 0.05$ for both species), but increased in *Stylophora* sp. (Bonferroni: $P < 0.01$, Day 5 vs. Day 0). In the cold treatment, Dt/chl *a* increased over time in *P. heronensis* and *Stylophora* sp. (Bonferroni: $P < 0.01$, Day 5 vs. Day 0 for both species), while at high temperature, this parameter increased in *P. heronensis* (Bonferroni: $P < 0.01$, Day 5 vs. Day 0) and *P. damicornis* (Bonferroni: $P < 0.001$, Day 5 vs. Day 0). When Dt/chl *a* was compared between treatments on Day 5, it was significantly higher at both the high and low temperatures than at the control temperature in *P. heronensis* (Fig. 3E). In contrast, in *P. damicornis*, Dt/chl *a* at high temperature was significantly greater than at both the low and control temperatures (Fig. 3F).

Xanthophyll pool size relative to chl *a* ((Dt + Dd)/chl *a*) was similar between species and remained constant over time in all corals at all temperatures (ANOVA and rmANOVA: $P > 0.05$ for all comparisons; Table 5).

At the beginning of the experiment, *P. heronensis* had a higher concentration of β-carotene relative to chl *a* than *P. damicornis*, as revealed by univariate ANOVA ($F_{2,39} = 8.498$, $P = 0.001$) with post hoc Tukey's HSD comparison ($P < 0.05$). This parameter did not vary over time in any species or thermal treatment (rmANOVA: $P > 0.05$; Table 5).

Excitation pressure over PSII. On Day 0, excitation pressure (Q_m) averaged 0.38 ± 0.04 (mean $\pm$ S.E.) in *P. heronensis*, 0.42 ± 0.01 in *P. damicornis,* and 0.51 ± 0.03 in *Stylophora* sp. (Fig. 3, H–J) and was significantly higher in *Stylophora* sp. than in *P. heronensis* (Tukey HSD: $P < 0.05$).

The temporal response to a given temperature was distinct between corals (significant Time $\times$ Temperature $\times$ Species interaction, Table 2). On Days 0, 2, and 5, Q_m was stable in all corals at the control temperature (Bonferroni: $P > 0.05$ for all comparisons of days over time in all species), but over this period at both the low and high temperatures, all corals except *Stylophora* sp. responded significantly (Fig. 3, H–J, Table 3). Specifically, on Day 2, Q_m was elevated at cold temperature relative to the control in *P. heronensis* (Bonferroni: $P = 0.01$) and *P. damicornis* ($P < 0.01$), and this elevation persisted until the end of the experiment in the latter species ($P < 0.001$). Similarly, Q_m was elevated by high temperature in *P. damicornis* ($P = 0.01$) on Day 2 and in *P. heronensis* ($P < 0.05$) and *P. damicornis* ($P < 0.001$) on Day 5. Of note, *P. damicornis* responded more strongly to the high temperature than the cold one on Day 5 (Bonferroni: $P < 0.001$).

DISCUSSION

Lord Howe corals exhibit highly variable responses to thermal stress. When compared with tropical sites, sea temperatures and light penetration at high latitude sites, such as LHI, are seasonally lower and annually more variable (Kleypas et al. 1999). It is not known whether corals that live close to their lower thermal thresholds (Kleypas et al. 1999) use photoacclimatory and/or photoprotective strategies to deal with cold exposure, whether in the short or long term. The plasticity of light-harvesting (chlorophyll *a*, c_2, and peridinin) and photoprotective pigments (xanthophylls and β-carotene) at cold temperatures has received little attention, and there have been few comparative studies of heat and cold stress (Wicks et al. 2010b, Roth et al. 2012). Therefore, this study addressed the plasticity of light-harvesting and photoprotective pigments in symbiotic dinoflagellates which might be a key parameter for understanding the ability of corals to deal with environmental change at high latitudes, and in particular to cope with lower temperatures. This issue is particularly important given that, with global warming, climate variability is projected to increase and more frequent cold temperature anomalies are expected to occur (Urban et al. 2000, Abram et al. 2008).

This study demonstrates that corals on the high latitude coral reef of LHI exhibit highly variable responses to short-term cold stress (~7°C below the control temperature) and short-term heat stress (~7°C above the control temperature, ~4°C above the bleaching threshold predicted by Coral Watch at www.coralreefwatch.noaa.gov). *Porites heronensis* hosting *Symbiodinium* C111* did not bleach and showed similar photophysiological responses to both cold and heat stress. *Pocillopora damicornis* hosting *Symbiodinium* C100/C118 bleached less severely when exposed to cold stress than heat stress, corroborating results obtained for the same coral species at the same location (Wicks et al. 2010b). Indeed, these authors suggested that *P. damicornis* at Lord Howe might be specialized for cooler temperatures, and this study supports this hypothesis. That *P. damicornis* was the more bleaching-susceptible coral at increased temperature was consistent with previous field observations that showed that Pocilloporidae were among the more bleaching-susceptible corals during the LHI coral bleaching event of March 2010, when seawater temperatures exceeded 27°C (Harrison et al. 2011). Finally, *Stylophora* sp. hosting *Symbiodinium* C118 bleached under cold stress but

TABLE 5. Concentration of photoprotective pigments β – carotene/chl *a*, and the xanthophylls diadinoxanthin (Dd) and diatoxanthin (Dt)/chl *a* on Days 0, 2, and 5 of the temperature treatment for *Symbiodinium* C111* in *Porites heronensis* (**Ph**), C100/C118 in *Pocillopora damicornis* (**Pd**), and C118 in *Stylophora* sp. (**S**).

	β – carotene/chl *a*			(Dd + Dt)/chl *a*		
	0	2	5	0	2	5
Ph						
C111*						
15°C	0.057 ± 0.002	0.062 ± 0.004	0.048 ± 0.004	0.17 ± 0.003	0.21 ± 0.002	0.19 ± 0.003
22°C	0.075 ± 0.017	0.057 ± 0.004	0.060 ± 0.001	0.19 ± 0.002	0.20 ± 0.005	0.20 ± 0.005
29°C	0.060 ± 0.002	0.054 ± 0.003	0.093 ± 0.031	0.20 ± 0.007	0.19 ± 0.005	0.26 ± 0.043
Pd						
C100/C118						
15°C	0.040 ± 0.007	0.033 ± 0.009	0.026 ± 0.006	0.19 ± 0.007	0.19 ± 0.016	0.18 ± 0.014
22°C	0.045 ± 0.003	0.028 ± 0.007	0.038 ± 0.007	0.19 ± 0.012	0.15 ± 0.010	0.19 ± 0.010
29°C	0.038 ± 0.006	0.025 ± 0.006	0.031 ± 0.013	0.18 ± 0.009	0.14 ± 0.008	0.30 ± 0.063
S						
C118						
15°C	0.043 ± 0.000	0.034 ± 0.001	0.043 ± 0.003	0.17 ± 0.003	0.16 ± 0.020	0.20 ± 0.008
22°C	0.041 ± 0.001	0.031 ± 0.003	0.046 ± 0.004	0.17 ± 0.002	0.15 ± 0.004	0.21 ± 0.029
29°C	0.041 ± 0.013	0.021 ± 0.007	0.038 ± 0.005	0.19 ± 0.007	0.15 ± 0.005	0.17 ± 0.005

not under heat stress. In a comparable study, Roth et al. (2012) found that, in the branching coral *Acropora yongei*, cold stress induces acute stress in both symbionts and coral host, though heat stress is more harmful in the long-term. Unfortunately, due to experimental constraints imposed by the remoteness of our study site, we could not perform a long-term experiment here, so we cannot predict how the three species of corals and their symbionts would perform when exposed to a more prolonged period of cold (>5 d). However, as with heat stress, acute cold stress may reduce symbiont density (Steen and Muscatine 1987, Saxby et al. 2003, Kemp et al. 2011) and photosynthetic capacity (Kemp et al. 2011), with potential negative consequences for the host, such as reduced growth or impaired reproductive capacity. As such, projected periods of cold sea surface temperature will likely put additional pressures on the stability of coral reef communities that also have to cope with warming oceans in the long term.

In our study, bleaching resistance and performance of PSII photochemistry under cold and heat stress were not consistently correlated with symbiont properties such as enhanced use of xanthophyll de-epoxidation. Under cold stress, symbionts in both bleaching-resistant *P. heronensis* and bleached *Stylophora* sp. increased xanthophyll de-epoxidation, while this capacity remained unchanged in symbionts of bleaching-susceptible *P. damicornis*. While enhanced xanthophyll de-epoxidation might act photoprotectively to prevent cold-water bleaching in *P. heronensis*, this mechanism was not sufficient to avoid bleaching in *Stylophora* sp. Similarly, under heat stress, symbionts in both bleaching-resistant *P. heronensis* and bleached *P. damicornis* increased xanthophyll de-epoxidation, while this parameter remained unchanged in symbionts of unbleached *Stylophora* sp. A similar lack of correlation between xanthophyll de-epoxidation and bleaching susceptibility was demonstrated previously in heat-stressed *Symbiodinium* cells (Venn et al. 2006). Collectively, these results demonstrate that the susceptibility of a coral to cold stress is not determined by its capacity for xanthophyll de-epoxidation, consistent with previously reported results for heat-stressed corals. This highlights the complex physiological responses involved in cold and heat stress.

Mode of bleaching. Although coral bleaching varied in intensity, a similar mode of bleaching across species and temperatures was observed: symbiont cells were expelled before a significant reduction in the total pool of PSPs per symbiont cell could be observed. These results agree with earlier findings of symbiont loss due to cold stress in the sea anemone *Aiptasia pulchella* (Steen and Muscatine 1987), and the corals *Montipora digitata* (Saxby et al. 2003), *Montastraea faveolata*, *Siderastrea siderea* (Kemp et al. 2011), and *A. yongei* (Roth et al. 2012), and heat stress in the corals *Stylophora pistillata* and *Seriatopora*

hystrix (Hoegh-Guldberg and Smith 1989). The observed mode of bleaching contrasts with findings in cold-stressed *Porites astreoides* (Kemp et al. 2011), as well as heat-stressed *Acropora digitifera* (Takahashi et al. 2004) and *Montipora monasteriata* (Dove et al. 2006), that reduced their cell-specific pigment concentration but not their *Symbiodinium* cell density. It has been suggested before that the rate of heating/chilling (slow vs. rapid) influences the cellular mechanisms of bleaching, i.e., in situ degradation, host cell apoptosis, and necrosis (Krueger et al. 2015). Therefore, our different findings may result from different heating and hence bleaching rates; due to technical constraints in the field, a fairly fast rate of heating/chilling was used in this study. Symbiont densities declined in response to cold stress in *Stylophora* sp. without concurrent pigment loss in its symbionts. In contrast, the mixed population of C100/C118 in *P. damicornis* suffered dramatically reduced cell numbers and concentrations of all major PSPs when exposed to short-term cold stress and, to a greater extent, when exposed to short-term heat stress. Thermally induced concurrent loss of symbiont cells and pigments has been described before (Kleppel et al. 1989) and might exacerbate the process of bleaching that was observed in *P. damicornis*: the reduction of cells and pigments increases light scattering from the underlying coral skeleton and enhances the internal light intensity (Enríquez et al. 2005).

Chlorophyll fluorescence and photoprotection by xanthophyll de-epoxidation. Although it has been shown that symbionts expelled from *P. damicornis* due to thermal stress can be fully photosynthetically functional (Ralph et al. 2001), a decline in photosynthetic performance in remaining symbionts, which can be estimated by chlorophyll fluorescence of PSII, has been shown to occur during coral bleaching (Hoegh-Guldberg 1999). In this study, in both pocilloporid species, the loss of symbiont cells both under cold stress (*P. damicornis* and *Stylophora* sp.) and heat stress (*P. damicornis*) was accompanied by a pronounced decline in maximum quantum efficiency of PSII (F_v/F_m); the decline in the cold was fastest and steepest in *Stylophora* sp. Possible underlying causes for the declines in F_v/F_m under cold and heat stress are (i) enhanced Dt accumulation and thermal dissipation, which might be sustained in the dark by a trans-thylakoidal ΔpH gradient, possibly generated by chlororespiration (Hill and Ralph 2005, Reynolds et al. 2008); and/or (ii) the inactivation of PSII reaction centers, likely coupled with ROS-mediated inhibition of their repair and the inability to transfer electrons past PSII (Tyystjärvi 2008, Takahashi and Badger 2011). Wicks et al. (2010b) showed that both high and low temperature had a similar site of impact on PSII and the redox state of the primary (Q_A) and secondary (Q_B) electron acceptors in *Symbiodinium* cells in *P. damicornis* from Lord Howe. Specifically, perhaps as a result of inactivation of

PSII centers, the rate of Q_A reduction and the capacity to transport electrons from Q_A through Q_B, and consequently the photochemical capacity of PSII, declined under both cold and heat stress, though the impact was less severe in the cold. The excitation pressure over PSII (Q_m) is a parameter that is influenced by NPQ processes. Q_m is also dependent on the light intensity, which varied over the course of our experiment, with the lowest light intensities on Day 1 and highest light intensities on Day 2. Despite these variations in light intensity, Q_m dynamics over time can be compared between treatments because all corals had the same light history. In this study, all corals, with the exception of *Stylophora* sp, had higher Q_m values after 5 d at both 15°C and 29°C than at the control temperature. The increase of Q_m was due to a decrease in the effective quantum yield ($\Delta F/F_m'$) at peak solar radiation, which has been described in previous studies (e.g., Hoegh-Guldberg and Jones 1999, Gorbunov et al. 2001). This drop in $\Delta F/F_m'$ at noon has been linked to PSII core rearrangement or degradation (photoinactivation; Niyogi 1999), as well as increased NPQ associated with increased xanthophyll de-epoxidation (Brown et al. 1999, Warner and Berry-Lowe 2006). In algae that contain Dd and Dt, NPQ associated with thermal dissipation seems to be directly correlated with the concentration of Dt (Goss and Jakob 2010). *Porites heronensis* consistently increased xanthophyll de-epoxidation and Dt/chl *a* with Q_m at both low and high temperatures, suggesting that at both temperatures NPQ is efficiently activated through xanthophyll de-epoxidation, while *Stylophora* sp. increased xanthophyll de-epoxidation and Dt/chl *a* at 15°C and 22°C but not at 29°C, indicating increased thermal dissipation at lower temperatures only. This latter response is especially interesting, as it suggests a greater need for photoprotection under cold stress, and greater resilience to warmer temperatures, in *Stylophora* sp. than in the other species. Alternatively, *Stylophora* sp. might use different protective mechanisms, so negating the necessity of NPQ at high temperatures. Greater resilience to high temperature in this species is corroborated by declines in the photochemical efficiency of PSII (Fig. 1C) and symbiont density (Fig. 2C) under cold stress and maintenance of these parameters at high temperature. In our study, differences in xanthophyll de-epoxidation and Dt/chl *a* among treatments on Day 0 in *P. damicornis* (higher values for both parameters at 15°C than at 22°C and 29°C, see Fig. 3, B and F) might be due to the sampling regime (corals at 15°C had been sampled last and therefore were exposed at least 15 min longer to peak solar irradiation, corresponding to a light intensity (μmol photons · m^{-2} · s^{-1}) of ~1,095 on Day 0, 1,694 on Day 2, and 906 on Day 5). However, this sampling regime was maintained throughout the experiment, so we believe that comparisons to the control over time are meaningful. At 15°C, Q_m

but not xanthophyll de-epoxidation or Dt/chl *a* was increased over time relative to the control in *P. damicornis*, while at 29°C, xanthophyll de-epoxidation, Dt/chl *a*, and Q_m were enhanced. These results suggest that, at low temperatures, alternative quenching mechanisms are activated in these species. These may include reaction center inactivation due to PSII-core rearrangement or degradation of their symbionts, as has been observed in heat-stressed corals (Takahashi et al. 2004).

The quantum efficiency of electron transport past PSII is affected by NPQ mechanisms, such as thermal dissipation, and also by the ability to transfer electrons to acceptors downstream of PSII (Ort and Baker 2002). The latter is affected by the availability of electron sinks such as CO_2 and O_2 (Badger et al. 2000, Ort and Baker 2002). At sub- or supra-optimal temperatures, the affinity of ribulose-1,5-bisphosphate carboxylase oxygenase (RUBISCO), the key-enzyme of the Calvin-Benson cycle, for CO_2 decreases due to altered kinetic properties of the enzyme. Saxby et al. (2003) suggested that cold-induced impairment of the Calvin-Benson cycle might underlie reduced photosynthetic electron transport and subsequent overreductions of the light reactions. A similar model has been proposed for heat-stressed symbiotic dinoflagellates (Jones et al. 1998). Recently, different capabilities of carbon fixation at elevated temperature have been demonstrated for symbionts of type/clade C1 and D (Baker et al. 2013). Under thermal extremes, the electron pressure over PSII may accumulate because light energy is continuously absorbed but not processed adequately. Downstream of PSII, electrons may alternatively be processed by (i) the reduction of O_2 by the Mehler reaction, which generates O_2^- and other ROS in subsequent reduction processes as part of the water–water cycle (Asada 1999); or (ii) the reduction of O_2 in the photorespiratory pathway (Ort and Baker 2002). RUBISCO catalyzes the initial oxygenation in the photorespiratory cycle and its efficiency is temperature-dependent, i.e., while low temperatures lead to a decreased affinity for both CO_2 and O_2 uptake, high temperatures decrease the affinity of RUBISCO for CO_2 and increase the affinity for O_2. Hence, at low temperatures photorespiration might be less important as an electron sink than at high temperatures, and the Mehler reaction might be more efficient in reducing O_2 (Badger et al. 2000, Ort and Baker 2002). What is more, Mehler O_2 uptake might be facilitated by higher O_2 solubility at cold temperatures. With ongoing cold stress, increased reduction of O_2 could cause an imbalance of ROS and detoxifying enzymes (Lesser 2011), and hence cellular damage and/or the inhibition of the repair of photodamaged PSII reaction centers (Takahashi and Badger 2011). This may ultimately result in reduced photochemical efficiency and increased Q_m at cold temperatures, as observed in *P. damicornis* in this study.

Compared with other species, *P. heronensis* and its symbionts had a higher baseline concentration of β-carotene relative to chlorophyll *a*. This might contribute to the observed bleaching resistance at both low and high temperatures in this species, because in marine organisms β-carotene is one of the major antioxidants responsible for the quenching of singlet oxygen ($^1O_2$; Ben-Amotz et al. 1989). Removal of excessive $^1O_2$ by β-carotene might suppress ROS-mediated inhibition of protein synthesis, as has been proposed for α-tocopherol, and promote the repair of reaction centers (Murata et al. 2012). However, levels of β-carotene relative to chlorophyll *a* did not increase over time at high or low temperature, either in *P. heronensis* or in *P. damicornis*, agreeing with findings in *Goniastrea aspera* when naturally bleached by high temperature (Ambarsari et al. 1997), and findings in *Porites porites*, *Porites astreoides* (both *Symbiodinium* clade A), Montastraea franksi, *Favia fragum* (both *Symbiodinium* clade B), and Montastraea cavernosa (*Symbiodinium* clade C) when experimentally exposed to elevated temperature and irradiation (Venn et al. 2006). In contrast to these results, however, Hill et al. (2012) found that the proportion of β-carotene relative to chlorophyll *a* in *A. millepora* and *P. damicornis* was correlated to bleaching sensitivity, implicating enhanced detoxification of potentially harmful ROS. Still, it seems that an increased level of β-carotene is not a common major response to high temperature.

CONCLUSION

This study investigated the impact of cold stress versus heat stress on coral species at the southern end of their distribution, focusing on key photophysiological parameters of their symbiotic algae. While overall, the physiological impacts of cold stress were very similar to those observed under heat stress (loss of photochemical efficiency and loss of symbionts), we found host species-specific differences that did not depend on symbiont-specific properties. In particular, photoprotection in the form of xanthophyll de-epoxidation was not clearly linked to resistance to low temperature. Although the underlying cellular and biochemical mechanisms that determine resilience or susceptibility of the holobiont to low temperature appear to be as complex as those observed at high temperature, they are unlikely to be identical. To predict a coral's resilience to low temperature, key cellular adaptation mechanisms need to be identified. Such adaptation mechanisms might include the maintenance of enzyme activity, protein turnover or thylakoid membrane fluidity in their symbiotic algae under periods of cold stress, and should be the focus of future work.

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