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Repeated and Prolonged Temperature Anomalies Negate Symbiodiniaceae Genera Shuffling in the Coral *Platygyra verweyi* (Scleractinia; Merulinidae)

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Kuo-Wei Kao, Shashank Keshavmurthy, Cing-Hsin Tsao, Jih-Terng Wang, and Chaolun Allen Chen (2018) With climate change, global average sea surface temperatures are expected to increase by 1.0-3.7°C by the end of this century. Even a 1.0°C increase in seawater temperature from local long-term summer maxima lasting for weeks to months results in bleaching and/or mortality in reef-building corals. Studies on coral resistance mechanisms have proposed a correlation between shuffling of different Symbiodiniaceae genera (changing the dominant Symbiodiniaceae genera) and putative thermal tolerance in corals. Although it was suggested that some corals can increase their tolerance by 1.0-1.5°C through shuffling to thermally tolerant *Durusdinium trenchii* (formerly D1a), the effects of accumulated thermal stress due to prolonged high temperatures on the survival of corals that have shuffled have not been investigated. We show herein that prolonged exposure to high temperature (> 10.43-degree heating weeks) can drastically reduce coral survival rate even after it has shuffled to stress-tolerant Symbiodiniaceae genera. Our study suggests that there is a limit to the capacity of for shuffling, and hence is likely to lose its efficacy in the future as repeated and prolonged thermal stress events become more frequent and pronounced.

Key words: Climate change, Seawater temperature fluctuations, Degree of heating weeks, Reciprocal transplantation, Kenting-Taiwan.

BACKGROUND

In response to rising CO_2 emissions, up to 90% of global coral reefs may suffer from annual bleaching by 2055 (IPCC 2013; van Hooidonk et al. 2014; Hoegh-Guldberg 1999; Frieler et al. 2012). In recent years, the frequent bleaching of corals worldwide has indicated that adaptation is not keeping up with environmental changes (Hoegh-Guldberg et al. 2007; Camp et al. 2016). In order to monitor and predict global coral bleaching events, data on degree heating weeks (DHW; °C-weeks) are often based on regional satellite temperature records against long-term summer maxima (Liu et al. 2003; Howells et al. 2013; Ridgway et al. 2016). Typically, a reef site with a DHW value of 4°C-weeks experiences significant coral bleaching, whereas a value of 8°C-weeks may cause widespread coral

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bleaching accompanied by mortality (Liu et al. 2003). Recently, DHW has been used to reflect the accumulated thermal stress on corals under experimentally manipulated conditions and assess thermal tolerance (Schoepf et al. 2015).

Thermal tolerance in scleractinian corals, depending on the environmental conditions, is the result of a combination of the coral host and Symbiodiniaceae genera resistance mechanisms. There is evidence indicating an advantage for corals in overcoming stress when they associate with *Durusdinium* species. For example, corals are more thermally tolerant when associated with symbiont *D. trenchii* compared to conspecifics associated with *Cladocopium* C3 (Silverstein et al. 2015; Keshavmurthy et al. 2012). Generally, species in the genus *Durusdinium* are considered to be heat tolerant and species in the genus *Cladocopium* as stress sensitive, with the exception of some *Cladocopium* species (*e.g*. *in-hospite Cladocopium* C15), which are relatively stress tolerant (Fisher et al. 2012). The proposed shuffling mechanism involves changes in the relative abundances of different symbiont types within the coral host depending on the temperature (Cunning et al. 2015b); this allows "background" genera of *Symbiodiniaceae* to become dominant. A transition from thermally sensitive to thermally tolerant dominant symbionts offers a greater likelihood of corals surviving thermally induced bleaching (Bay et al. 2016). This acclimatization mechanism, although with its limits, is one of the strategies that may help corals survive the effects of global warming in the near future (Berkelmans and van Oppen 2006; Palumbi et al. 2014).

In an earlier study, we gave evidence that coral species compositions change with long-term exposure to high temperatures and concurrent associations with tolerant *Durusdinium* spp. (Keshavmurthy et al. 2014); however, this does not mean that Symbiodiniaceae genera are driving thermal tolerance. Many studies have proposed that the potential for symbiont shuffling during coral acclimatization helps corals survive the effects of climate change (Silverstein et al. 2015; Bay et al. 2016; Berkelmans and van Oppen 2006; Palumbi et al. 2014). However, studies have also shown that, after shuffling their Symbiodiniaceae genera, corals revert back to a relationship with their pre-stress symbionts if conditions change (see LaJeunesse et al. 2010). In this study, we show that shuffling between Symbiodiniacceae genera may not always benefit corals.

We used a field-based study to demonstrate the influence of different temperature patterns on the behavior of a coral species, *Platygyra verweyi*, associated with different Symbiodiniceae genera and conclude that shuffling between sensitive and tolerant Symbiodiniaceae genera is insufficient for corals to survive repeated and prolonged thermal stress. We conducted two sets of *in situ* reciprocal transplant experiments (RTEs) - one in 2014 (2014RTE) and the other in 2015 (2015RTE) using cores of the coral *P. verweyi* collected from sites proximal to a nuclear power plant outlet and a nuclear power plant inlet, and Wanlitung in Kenting National Park, Taiwan (Fig. 1a). We calculated DHW values for each experimental group from *in situ* temperature records at each site and assessed Symbiodiniaceae genera compositions to test our hypothesis that prolonged thermal stress will influence the survival of a coral host even after shuffling to a stress-tolerant Symbiodiniaceae genus. In Kenting National Park, *P. verweyi* was associated with *Durusdinium glynii* (ITS type D1), *D. trenchii* (ITS type D1a), *Cladocopium* C3 (ITS2 type C3) and *Cladocopium* C3cc (ITS2 type C3cc), either specifically or in combination depending on the location (Keshavmurthy et al. 2012).

MATERIALS AND METHODS

Experimental design

The coral species used in this study, *Platygyra verweyi*, is a massive coral species inhabiting shallow waters (2-4 m) at Kenting National Park. Individual colonies can host each symbiont alone or in combination with another type. In this study, all *Cladocopium* sp.-dominated colonies associated with both *Cladocopium* C3 and *Cladocopium* Ccc and all *Durusdinium* sp.-dominated colonies associated with both *D. glynii* and *D. trenchii*.

Three sites were included in this study, Nuclear Power Plant Outlet (OL), Nuclear Power Plant inlet (IL), and Wanlitung (WLT), in Nanwan, south Taiwan (Fig. 1a). Among the three sites, OL (21°55'54.4"N, 120°44'42.7"E) and IL (21°57'20.3"N, 120°45'14.2"E) are located within Nanwan in Kenting National Park, Taiwan. A recent long-term (2007 to 2010, and 2013) seawater temperature data set obtained from the deposited underwater data loggers shows that the average summer (June to August) daily seawater temperature at OL (29.31 ± 1.36°C) is approximately 1°C higher than at IL (28.14 ±

1.18°C). Due to the tidally induced upwelling in Nanwan (Lee et al. 1997), the maximum daily seawater temperature fluctuation at OL and IL can be more than 8°C in summer (Fig. 1b). The third site, WLT (21°59'41.0"N, 120°42'19.6"E), is located on the west coast of Kenting National Park with average summer (June to August) seawater temperature (28.99 ± 0.74°C) similar to OL while the intervals of extreme temperature events (≥ 30°C) and the daily seawater temperature fluctuations are shorter and less extreme than OL (Fig. 1b, c).

In the first reciprocal transplant experiment conducted in 2014 (2014RTE, Fig. 1a), two sets of reciprocal transplant experiments (RTE) were carried out - one between OL and Wanlitung WLT, and the other between OL and IL - in Kenting National Park, Taiwan in 2014. On March 2014, 25 mm-dimeter cores from 5 colonies of *P. verweyi* from each study site (OL: 21 cores/colony, WLT: 16 cores/colony, IL: 10 cores/colony) at a depth of 1 to 2 m were sampled underwater using a pneumatic drill and placed in Ziploc bags underwater before being transferred to a wet lab at the National Museum of Marine Biology & Aquarium (NMMBA). The difference in sample size between OL and the other two sites (WLT and IL) was due to mortality of tagged mother colonies in WLT and small population size of *P. verweyi* at IL. Coral cores were maintained in indoor seawater tanks with constantly filtered seawater input. For RTEs set between the OL and WLT, coral cores from each site were randomly assigned to racks (a native group and a transplant group). Each rack contained 40 cores (5 colonies × 8 replicates). For another set between OL and IL, each rack contained 25 cores (5 colonies × 5 replicates). In the case of the native group of OL, the same rack was used for both sets of experiments. Fewer replicates were used in the RTE set between OL and IL due to the small size of *P. verweyi* colonies at IL. Coral cores were attached to PVC adapters with underwater epoxy to be fixed onto the acrylic rack and were transferred back to their original, respective sampling sites at similar depths (1-2 m) as sampled colonies for 1.5-month recovery to ensure coral health before being transplanted. On April 2014, all the racks were retrieved and transported to NMMBA and stained with Alizarin Red S (Sigma-Aldrich, USA) 20 mg/L, 24 hrs (LeGore et al. 1989) for the analysis of skeleton growth. Subsequently, all the racks were put back

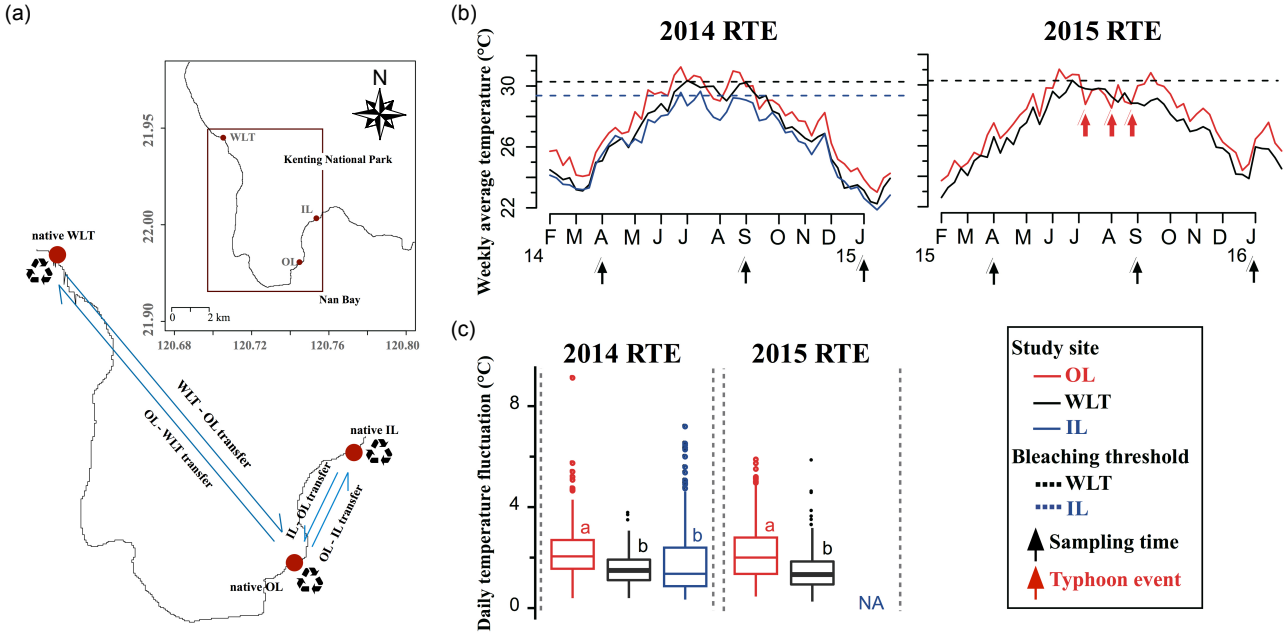

Fig. 1. Reciprocal transplant experiment (RTE) designs and temperature regimes at each study site. (a) Study sites in Kenting National Park. Blue arrows represent the 2014RTE and 2015RTE. (b) Weekly average temperatures recorded through time. (c) Daily seawater temperature fluctuations (°C) at each site. NA = no data. Different lowercase letters indicate significant differences in daily temperature ranges among sites (Kruskal-Wallis test, Dunn's post-hoc test, Bonferroni adjusted *p* values at $\alpha = 0.05$). Bars within each box represent the median with boundaries representing the 25 to 75th percentiles. Whisker boundaries represent the 1.5x interquartile range and black dots represent outliers with values beyond that range. OL, nuclear power plant outlet; IL, nuclear power plant inlet; WLT, Wanlitung.

to the study sites with treatment groups being reciprocally transplanted. Sampling was conducted every 4 months, with 5 coral cores (1 cores from each colony) from each rack being retrieved each time. The sampled cores were cut into three parts. Tissue from the first part was removed by scraping the surface and stored in 95% Ethanol for DNA extraction. The second part was wrapped in aluminium foil and stored at -20°C for chlorophyll concentration measurement and total symbiont density count, and the last part was snap-frozen in liquid nitrogen and stored for protein analysis. Monthly maintenance of the racks underwater was performed by cleaning the macroalgae attached to coral cores and the racks components to minimize any competition effect.

The second reciprocal transplant experiment of *Platygyra verweyi* was conducted in 2015 (2015RTE, Fig. 1a) between OL and WLT with a sample size (n = 30 colonies from each site). IL was not included as one of the study sites in 2015RTE because not enough *P. verweyi* colonies were found to make a balanced design. Following identical procedures as 2014RTE, thirty colonies were sampled from OL and WLT in March 2015 and were reciprocally transplanted in April 2015 after one- month of recovery.

Coral cores of each group were retrieved (April, September, and January), subsampled, and preserved for DNA extraction (in 95% Ethanol), qPCR, and physiological parameters analysis.

Seawater temperature

The seawater temperature was recorded *in situ* at 30-minute intervals using data loggers (HOBO; Pendant™, USA) deployed underwater near the transplant racks (1-2 m) at each study site. The raw temperature data were transformed into Degree Heating Week (DHW) (IPCC 2013; Liu et al. 2003) to assess both the intensity and duration of the thermal stress for each experimental group. Although this indicator is typically used to reflect large-scale bleaching monitoring, its application to experimental manipulation has also been used to assess the cumulative thermal stress on heat-treated corals over daily scales (Schoepf et al. 2015). To calculate DHW, first, the maximum monthly mean temperature (MMM) was obtained from historical long-term data (recorded from 2007-2010 and 2013). Second, the weekly mean temperature during the RTEs for each study site was calculated. Finally, the weekly mean temperature was subtracted from MMM to get the temperature anomalies and those anomalies that were at least 1.0°C above MMM were summed, within the past 12-week windows, to obtain DHWs using following equation:

$$DHW_{WLT\text{-}OL\ transfer} = \sum [(T_{transfer} - MMM_{native}) \geq 1°C]$$

In the equation, $T_{transfer}$ is the weekly mean temperature at OL and MMM_{native} is the MMM of WLT, to calculate DHW for WLT-OL transfer.

The projections of DHW in Nanwan, which is included in one of the 1° × 1° resolution grid reef cells locating at southern Taiwan, were obtained from van Hooidonk et al. (2014).

Symbiont community dynamics

DNA extraction

DNA extraction was carried out using a modified salting-out method (Ferrara et al. 2006). Coral tissue (30 mg) was cut and incubated overnight at 56°C with 200 μL lysis buffer (1M Tris 25 mL, 0.5M EDTA pH8 10 mL, 20% SDS 10 mL, 5M NaCl 2 mL, ddH_2O 53 mL) and 10 μL proteinase E (10 mg/mL). 7M NaCl (210 μL) was added to the tissue, centrifuged (6000 g, 30 sec), and transferred into B/T Genomic DNA Mini Column (Viogene, Taiwan). After a series washing with cold (-20°C) 70% ETOH and centrifugation, the column was dried at 37°C for 15 mins and finally, the DNA was eluted with 50 μL of preheated (65°C) 1X TE buffer and was isolated from the column after centrifugation (15000 g, 3 min). The concentrations of genomic DNA were determined using NanoDrop 2000 (Thermal Scientific, USA).

Denaturing gradient gel electrophoresis

The initial and final subclades (types) of the symbionts in the native and transplant groups in 2014RTE and 2015RTE were randomly selected and identified by amplifying the internal transcribed spacer 2 (ITS2) region of DNA samples with primer sets ITSintfor2 5'GAATTGCAGA ACTCCGTG-3' and ITS2clamp 5'CGCCCGCCGC GCCCCGCGC CCGTCCCGCCG CCCCCGCCC GGGATCCATA TGCTTAAGTT CAGCGGGT-3'. A touch-down PCR (LaJeunesse 2002) program was used: 92°C for 3 min followed by 20 cycles of 30 s at 92°C was carried out. Annealing temperature was set at 62°C and then decreased by 0.5°C in each cycle to a final temperature of 52°C, 30 secs at 72°C to ensure specificity. Denaturing gradient gels

from 45% to 80% were used for electrophoresis under 115v for 15 hours using a CBS Scientific system (Del Mar, CA, USA). The gel was stained with SYBR Gold (Invitrogen, USA). Prominent bands were excised and amplified for sequencing. All sequences were aligned and compared with GioSymbio database (Franklin et al. 2012).

Real-time quantitative PCR

The copy numbers of symbiont *Cladocopium* sp. and *Durusdinium* sp. in *P. verweyi* samples were detected under LightCycler® 480 Instrument II (Roche, Switzerland) with the protocol modified from Mieog et al. (2007). Each 10 μL qPCR reaction consisted of 5 μL of 1x SYBR Fast Master Mix, 0.5 μL of UF primer (2 nM/μL), 0.5 μL of CR or DR primer (2 nM/μL), 7.5 μL of ddH_2O, and 2.5 μL of DNA templates (equal to 1 ng of genomic DNA) with primer sets, ITS1 *Cladocopium* sp.-specific reverse primer (CR) 5-AAGCATCCCTCACAGCCAAA-3, *Durusdinium* sp.-specific reverse primer (DR) 5-CACCGTAGTGGTTCACGTGTAATAG-3, and universal forward primer (UF) 5-AAGGAGAAGTCGTAACAAGGTTTCC-3 (Ulstrup and van Oppen 2003). In each run, each sample was run in triplicate (technical replicates), and no-template control (NTC) was also run in triplicate with ddH_2O to inspect any contamination in the reagent.

Coral growth

Tissue coverage growth

Top-view photos of all the coral cores on the rack were photographed *in situ* with a scale, and the surface area covered by the coral tissue of each core was estimated using ImageJ software (1.48v, USA). Data were transformed into the relative percentage change of tissue coverage from the initial sampling (April 2014).

Skeleton growth

At the end of the experiment, all the retrieved coral cores were sliced and airbrushed to remove the remaining tissue, and the length of the newly accreted skeletons perpendicularly above the Alizarin Red S stain mark from 6-10 septa were measured under a microscope (SZ61 Olympus, Japan); the results were averaged to determine the skeleton growth for each nubbin.

Skeleton growth was not measured in 2015RTE because no cores were retrieved for this group. The protocol for the analysis of physiological parameters is given in the supplementary file.

Statistical analysis

All the statistical analyses in this study were performed in R version 3.1.1. (R Core Team 2014). Differences in daily mean seawater temperatures and daily seawater temperature fluctuations between sites were tested using Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni adjusted *p*-value. Data were presented as the mean ± standard deviation (S.D.). Relative symbiont abundances of each sample were assigned into categories, *Cladocopium* sp. or *Durusdinium* sp. dominant (*i.e.* either *Cladocopium* or *Dururdinium* ≥ 90% relative abundance) and *Cladocopium* sp. + *Dururdinium* sp. (10% < both *Cladocopium* and *Durusdinium* < 90% relative abundance) and differences in symbiont community distributions were tested using Fisher's exact test. For photochemical efficiency and each physiological parameter, differences between each transfer group and its native group were tested using Student *t*-test. Data were box-cox transformed (Box and Cox 1964) if they failed to meet normality and/or homogeneity of variance assumptions. Wilcoxon rank sum test was performed on raw data if statistical assumptions were violated. All data were presented as the mean ± standard deviation (S.D.). For tissue coverage growth and skeleton growth in 2014RTE, differences between groups for each transplantation sets (WLT↔OL or OL↔IL) were tested using one-way ANOVA followed by Tukey's post hoc test with Bonferroni adjusted *p*-value. For 2015RTE tissue coverage growth, differences between groups were tested using one-way ANOVA followed by Tukey's post hoc test with Bonferroni adjusted *p*-value while the origin versus location effect was tested using two-way ANOVA.

RESULTS

Seawater temperature

The maximum monthly mean for 3 sites were: MMM_{OL} = 29.63°C; MMM_{WLT} = 29.28°C; MMM_{IL} = 28.37°C. In the 2014RTE, the average daily summer (June to August) seawater temperature at the OL (30.11 ± 1.07°C) was higher than that

at WLT (29.59 ± 0.67°C; Dunn's post-hoc test, $p < 0.001$) and the IL (28.61 ± 0.96°C; $p < 0.001$), and the weekly average temperature at the OL repeatedly exceeded *P. verweyi*'s bleaching threshold at WLT (30.28°C) and the IL (29.37°C; Fig. 1a). The daily seawater temperature fluctuation at the OL (2.23 ± 1.00°C; Fig. 1b) also differed from that at WLT (1.53 ± 0.58°C; $p < 0.001$) and the IL (1.80 ± 1.30°C; $p < 0.001$) during the summer, reaching a maximum of 9.12°C. In the 2015RTE, the average summer daily seawater temperature at the OL (29.77 ± 1.12°C) differed from that at WLT (29.52 ± 0.52°C; Wilcoxon rank-sum test, W = 5097, $p < 0.05$; Fig. 1b) and the daily seawater temperature fluctuation at the OL (2.38 ± 1.04°C) differed from that at WLT (1.57 ± 0.70°C; Wilcoxon rank-sum test, W = 655557, $p < 0.001$; Fig. 1c).

Degree heating weeks

The prolonged thermal stress patterns observed during this study allowed us to predict *P. verweyi* survival in future warming scenarios. From DHW projections of the simulation model, the 1° × 1° grid reef cell containing Nanwan Bay was predicted to exceed 6°C-weeks at least twice in 2020-2030 under the RCP8.5 scenario (van Hooidonk et al. 2014), and to exceed 8°C-weeks at least twice in the same time interval (2020-2030; Table 1a). In the 2015RTE, DHW = 5.68°C-weeks (for coral cores transplanted from WLT to the OL), represented a "shuffle and survive" scenario similar to what can be expected at Nanwan Bay before 2020 (Table 1a, b), while in the 2014RTE, DHW = 10.43°C-weeks (for coral cores transplanted from WLT to the OL) represented a "shuffle but likely not to survive" scenario, which may become a common and frequent occurrence after 2030 (Table 1a, b). Data fitting considered the potential DHW threshold, which determines the survival of corals, *i.e.*, DHW = 10.43°C-weeks could cause severe mortality, and in the present study, transplanted coral cores had already experienced a DHW of > 8.0°C-weeks.

Table 1. Predicted degree heating weeks (DHW in °C-weeks) by year. (a) Future predictions for DHWs at Nanwan Bay under emission scenarios based on IPCC AR5. **2x**-DHW conditions that may occur at least twice within a given time interval. **10x**-DHW conditions that may occur every year within a given time interval. Bold texts represent worst-case scenarios. (b) Maximum DHW during the experiment and the percent of *Symbiodinium* shuffled in *Platygyra verweyi* transplanted to the nuclear power plant outlet (OL), and the subsequent coral core mortality rate. For the 2014 reciprocal transplant experiment (2014RTE), we only report the DHW for coral cores transplanted from Wanlitung (WLT) to the OL (WLT-OL transfer), since all coral cores transplanted from the nuclear power plant inlet (IL) to the OL (IL-OL transfer) died

(a)

RCP	CO_2 emissions	DHW > 6	DHW > 8
2.6 2x	Low	2010-20	2020-30
4.5 2x	Median	2020-30	2030-40
6 2x	Median	2020-30	2040-50
8.5 2x	**High**	**2020-30**	**2020-30**
2.6 10x	Low	2030-40	2040-50
4.5 10x	Median	2050-60	2050-60
6 10x	Median	2060-70	2070-80
8.5 10x	**High**	**2040-50**	**2050-60**

Representative Concentration Pathway based on IPCC AR5 climate models.

(b)

	2015RTE	2014RTE
Maximum DHW	5.68	10.43
Shuffling	73%	40%
Mortality	0%	75%

Reciprocal transplantation experiment - 2014

All coral cores transplanted from WLT to the OL (WLT-OL transfer) and those transplanted from the IL to OL (IL-OL transfer; n = 5) in the 2014RTE were initially associated with the dominant symbiont *Cladocopium* sp. and background *Durusdinium* spp. (Fig. 2a, b; Table S1). In contrast, OL corals were dominated by *Durusdinium* spp., and maintained the same dominant symbiont species even after being moved to the more moderate WLT and IL site.

Thermal stress was first observed in both groups as a reduction in the photochemical efficiency (Fig. 3; Tables S2, S3) in early July 2014 when repeated seawater temperature anomalies occurred at the OL (Fig. 1b) causing the DHW to reach 4.41°C-weeks for WLT-OL transfer and 8.00°C-weeks for IL-OL transfer (Fig. 2a, b). The DHW continued to rise to maximum values of 10.43°C-weeks for WLT-OL transfer and 21.30°C-weeks for IL-OL transfer by early September 2014 (Fig. 2a, b), when both groups exhibited significant bleaching resulting in decreased symbiont cell densities and changes in other physiological parameters (Fig. 3; Tables S2, S3). Concurrently, *Durusdinium* spp. became dominant in 40% (n = 2) of WLT-OL transfer coral cores, *Cladocopium* + *Durusdinium* became co-dominant in 80% (n = 4) of IL-OL transfer colonies, and most colonies showed increased symbiont *Durusdinium* cell densities (10^4-10^5 cells cm^{-2}; Fig. 2a, b; Table S4), despite no or few *Durusdinium* cells being detected initially. For both WLT-OL transfer and IL-OL transfer, distributions of symbiont communities differed from their initial compositions (Fisher's exact test, $p < 0.01$). However, only one colony of WLT-OL transfer survived after the thermal stress dissipated. In total, WLT-OL transfer and IL-OL transfer coral cores experienced 90% mortality (Fig. 2a, b; Table 2). *Cladocopium* remained dominant in all colonies

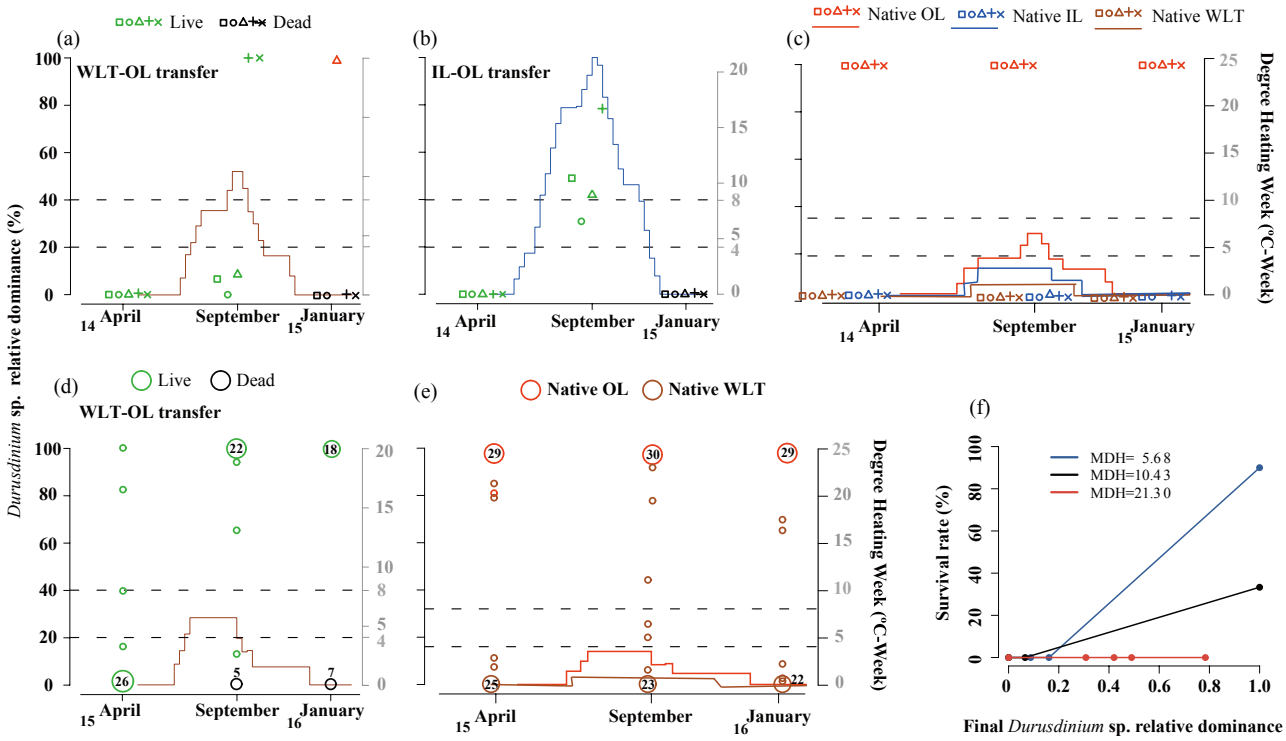

Fig. 2. *Durusdinium* sp. dominance (%; symbols) and degree heating week (DHW in °C-weeks; shaded lines) of each group through time. (a) Transplanted group from Wanlitung (WLT) to the nuclear power plant outlet (OL) (WLT-OL transfer); (b) transplanted group from the nuclear power plant inlet (IL) to OL (IL-OL transfer); and (c) native group from the OL to OL (native OL), native group from the IL to IL (native IL), and native group from WLT to WLT (native WLT) for the 2014 reciprocal transplant experiment (2014RTE). (d) WLT-OL transfer and (e) native OL and native WLT for the 2015RTE. Each symbol in the data represents a single *Platygyra verweyi* colony. For 2015RTE data, colonies with similar dominance were merged into larger groups with sample numbers shown above the circle. Circles represent symbiont type D dominance of each colony at its last sampling time. (f) Survival rates at WLT-OL transfer and IL-OL transfer for different final *Durusdinium* sp. dominance values under each maximum DHW (based on 5 colonies in 2014RTE). Horizontal lines are 4 and 8 DHW.

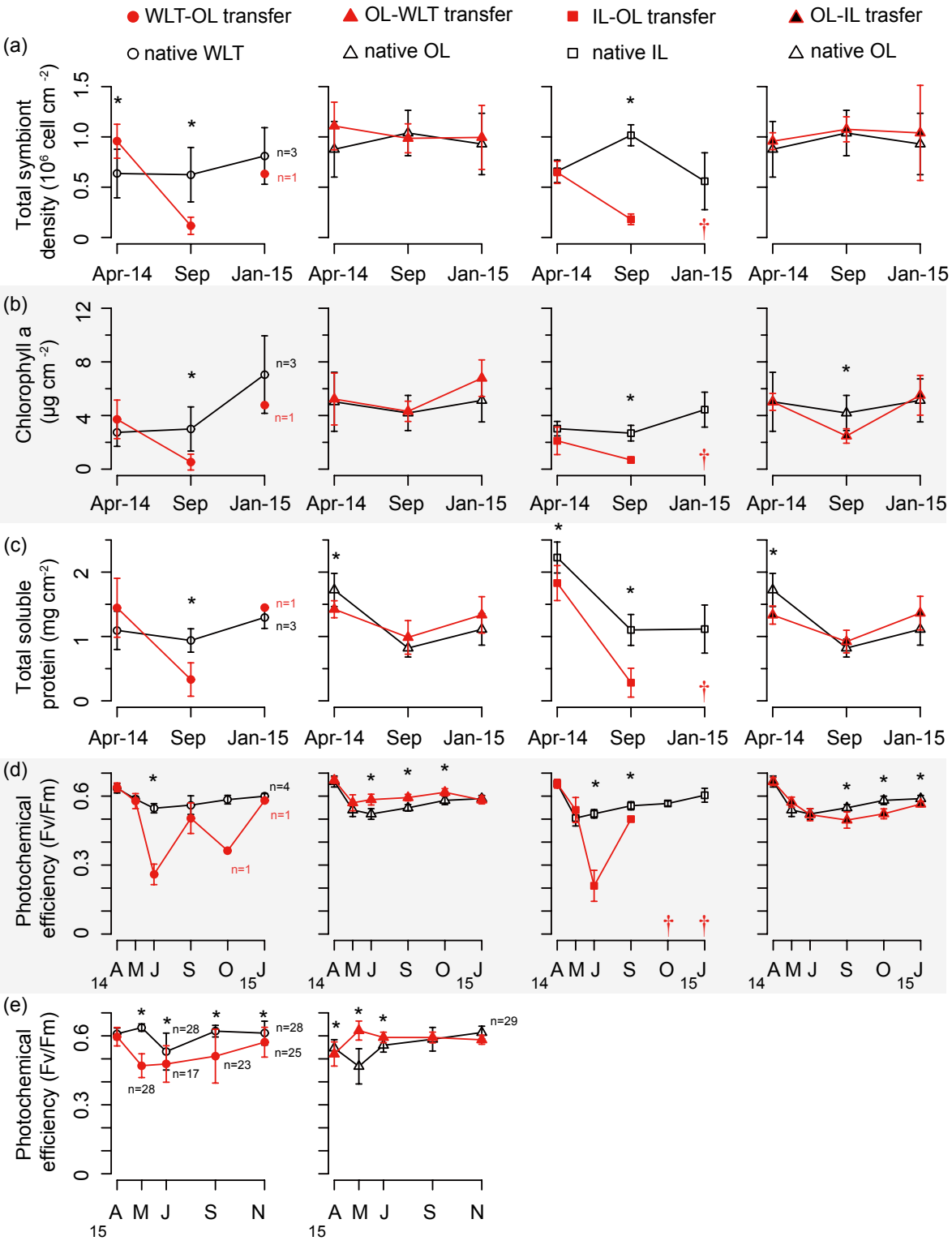

Fig. 3. Physiological parameters of experiment groups located at NPP-OL at each sampling time. (a) Total symbiont cell densities. (b) Chlorophyll *a* concentrations per cm^2. (c) Total soluble protein concentrations. (d) Dark-adapted photochemical efficiency measured in 2014RTE. (*n* = 5 colonies for each group per sampling time unless stated otherwise). (e) Photochemical efficiency measured in 2015RTE. (*n* = 30 colonies for each group per sampling time unless stated otherwise). All data are presented as mean ± SD. Asterisks represent a significant difference of total symbiont density between each transplant group and the native group on each month. †All the samples were dead.

of the native group at WLT (native WLT) and the IL (native IL) throughout the 2014RTE (Fig. 2c; Table S1), and *Durusdinium* remained dominant in all colonies of the native group at the OL (native OL) (Fig. 2c; Table S1).

Reciprocal transplantation experiment - 2015

WLT-OL transfer coral cores in the 2015RTE were initially dominated by *Cladocopium* sp. (n = 26; Fig. 2d). Signs of bleaching were observed in coral cores of WLT-OL transfer with a decreasing photochemical efficiency (Fig. 3; Tables S6, S7) in early July, when seawater temperature anomalies occurring at the OL caused the DHW to reach 5.68°C-weeks for WLT-OL transfer (Figs. 1b; 2d). The prevalence of *Durusdinium* sp. increased in September as a result of 73% (n = 22) of WLT-OL transfer colonies shuffling from *Cladocopium* sp. to *Durusdinium* spp. dominance (Fig. 2c). However, as the seawater temperature at the OL subsequently decreased due to several typhoon events (Fig. 1b), the DHWs of WLT-OL transfer did not further increase. The mortality of WLT-OL transfer colonies was only 23% after the thermal stress had dissipated (Fig. 2d; Table 2). In the end, the Symbiodiniaceae genera composition of WLT-OL transfer colonies differed from their initial composition (Fisher's exact test; $p < 0.001$). All native WLT colonies retained their *Cladocopium* sp. dominance throughout the 2015RTE (Fig. 2e; Table S1), and all native OL colonies retained their *Durusdinium* spp. dominance (Fig. 2e; Table S1).

Even though coral cores transplanted to the OL in both the 2014RTE and 2015RTE showed increasing relative abundances of *Durusdinium* spp., distinct mortality rates appeared (90% for the 2014RTE vs. 23% for the 2015RTE, Table x) after

Table 2. Information on the coral cores used in the transplant experiment. Live cores versus total cores and final survival rate at each sampling time in; (a) 2014 reciprocal transplantation and (b) 2015 reciprocal transplantation experiment, respectively

(a)

	Live cores/Total cores (Survival rate)			
Group	2014Apr	2014Sep	2015Jan	Cores retrieved per sampling time
native OL	40/40(100%)	34/34(100%)[a]	29/29(100%)	5
OL-WLT transfer	40/40(100%)	35/35(100%)	30/30(100%)	5
OL-IL transfer	25/25(100%)	20/20(100%)	15/15(100%)	5
native WLT	36/36(100%)	31/31(100%)	19/25(76%)*[c]	5
WLT-OL transfer	36/36(100%)	28/31(90.3%)	4/26(15.4%)	5
native IL	25/25(100%)	20/20(100%)	15/15(100%)	5
IL-OL transfer	25/25(100%)	16/18(88.9%)[b]	0/13(0%)	5

[a]1 core was missing. [b]2 cores were missing. [c]1 core was missing.

(b)

	Live cores/Total cores (Survival rate)		
Group	2015Apr	2015Sep	2016Jan
native OL	30/30(100%)	30/30(100%)	30/30(100%)
OL-WLT transfer	30/30(100%)	30/30(100%)	30/30(100%)
native WLT	30/30(100%)	30/30(100%)	28/30(93.3%)
WLT-OL transfer	30/30(100%)	30/30(100%)	23/30(76.7%)

different DHW levels (with maxima of 10.43 and 21.30°C-weeks for the 2014RTE vs. 5.68°C-weeks for the 2015RTE; Fig. 2). Our results during the 2014RTE and 2015RTE showed that higher maximum DHW values occurred on *P. verweyi* cores during bleaching, lowering their survival rate despite their having shuffled to *Durusdinium* spp. (Fig. 2f).

Coral growth

Transplantation not only influenced the survival of coral cores in a non-native environment, but also affected their growth (skeletal accretion and tissue growth) over time (Fig. 4). Coral cores from the OL associated with *Durusdinium* spp. were able to grow better in the non-native environment at WLT and exhibited equal to higher tissue expansion rates (2-fold higher) than those with *Cladocopium* sp. (native WLT).

DISCUSSION

Our observation that shuffling toward *Durusdinium* spp. under thermal stress agrees with previous studies that corals dominated by stress-resistant *Durusdinium* sp. have higher thermal tolerance than those associated with *Cladocopium* sp. (Silverstein et al. 2015; Berkelmans et al. 2006) (Fig. 2d). However, high mortality still occurred in those colonies that shuffled their clades in the 2014RTE (Fig 2a, b). One study suggested that shuffling to *Durusdinium* species may enhance the thermal tolerance of coral by 1.0-1.5°C (Berkelmans et al. 2006). In our study, IL-OL transfer coral cores were exposed to *in situ* average daily temperatures of ≥ 1.5°C above the bleaching threshold during one-third of the summer days at the OL. However, temperatures only simultaneously rose higher than 1.5°C above the bleaching threshold at WLT on one day. This is because, in WLT, the daily seawater temperature fluctuations are shorter and less extreme than in OL (Fig. 1b, c).

Furthermore, a recent study suggested that a pre-stress *Durusdinium* sp.: *Cladocopium* sp. (D: C) ratio of < 0.003 limits the ability of corals to survive bleaching after shuffling (Berkelmans et al. 2006). Our study did show a *Durusdinium*

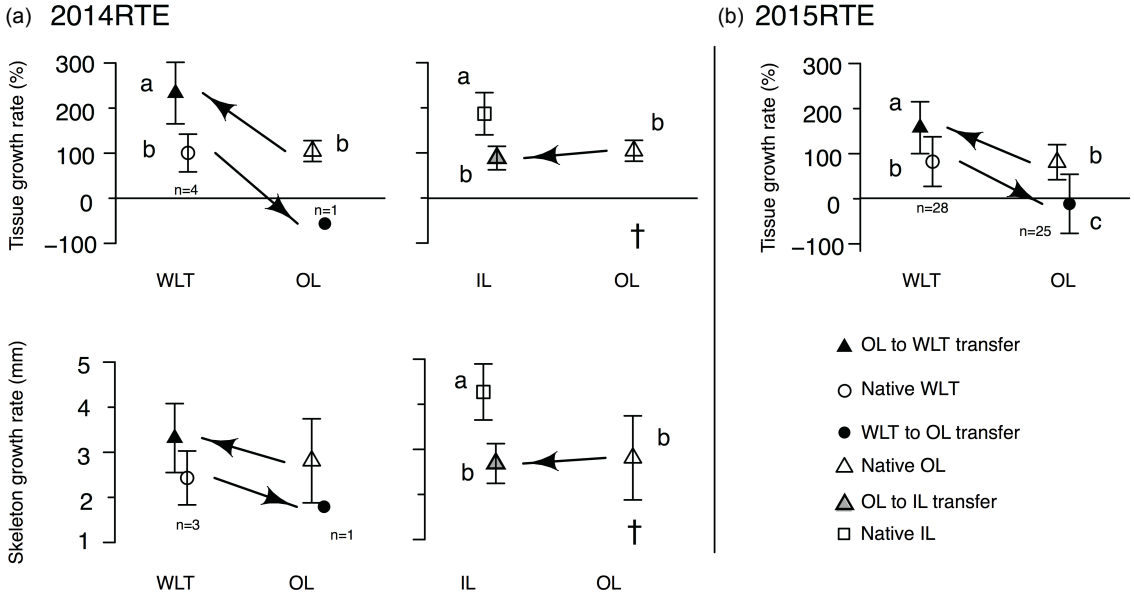

Fig. 4. Tissue coverage growth and skeletal growth of each experiment group in the 2014 reciprocal transplantation experiment (2014RTE) and 2015RTE. (a) (top) Tissue coverage growth and (bottom) skeletal growth in the 2014RTE (n = 5 colonies in each group at each sampling time). (b) Tissue coverage growth in the 2015RTE. Arrows indicate the direction of transplantation. Data were measured at the end of each RTE relative to initial conditions (n = 30 colonies for each group at each sampling time unless stated otherwise). Different lowercase letters indicate a significant difference between groups (two-way ANOVA, Tukey's post-hoc test, Bonferroni adjusted p values at α = 0.05). For tissue coverage growth in the 2015RTE, there was an origin effect (F = 62.163, p < 0.001) and location effect (F = 67.68, p < 0.001), but no origin × location interaction (F = 0.695, p = 0.406). No statistics for the origin versus location effect were conducted in the 2014RTE because of a lack of sufficient sample size caused by mortality. †All samples were dead. OL-WLT transfer, group transplanted from the nuclear power plant outlet (OL) to Wanlitung (WLT); OL-IL transfer, group transplanted from the OL to the nuclear power plant inlet (IL).

sp.: *Cladocopium* sp. (D: C) ratio of < 0.003 in bleached coral cores in both RTEs; however, there were many more coral cores in the 2015RTE that survived with a *Durusdinium* sp.: *Cladocopium* sp. ratio of less than the threshold. This could have been due to differences in the duration of exposure to severe heat stress (Ridgway et al. 2016) between the years, or different thresholds among different species.

Additionally, there was no sign of bleaching or mortality throughout the experiment in native OL colonies, even though native OL coral cores experienced DHW values of up to 6.37°C-weeks (Fig. S1). This could have been the result of long-term acclimatization of corals to the constant thermal effluent at the OL. *Platygyra verweyi* populations in Kenting National Park showed no differences in genetic structure among different locations (according to mitochondrial and nuclear markers in a previous study (Keshavmurthy et al. 2012), or among three locations in this study by microsatellite markers, Table S8), suggesting long-term acclimatization due to a combination of mechanisms including host mediation and its associated Symbiodiniaceae genera.

For more than 30 years (the time since the power plant was established), it seems that *P. verveyi* has adapted to prolonged elevated thermal stress and daily temperature variations (in the case of the native OL corals) and little genetic differentiation (between locations, Keshavmurthy et al. 2012); this could be a developmental acclimatization response, either on the part of the host or through acquisition of a particularly thermally tolerant *Durusdinium* sp.

Nevertheless, degree heating week simulations indicated that there might only be a narrow time gap between the survival (DHW > 6°C-weeks) and death (DHW > 8°C-weeks) of corals even when shuffling occurs after bleaching. The predictions here thus provide insights into the possible fate of corals in Kenting National Park, Taiwan. Based on IPCC predictions for sea temperature changes, under different emission scenarios based on IPCC AR5.2x (Table 1) (IPCC 2013), if the present trends in carbon emissions continue, by 2030 it may be very difficult for corals (in this case stress-resistant *P. verweyi*) to survive. When associated with *Durusdinium* spp., according to our predictions, this coral has a 90% survival rate under a DHW of < 6.0°C-weeks, while at DHWs of >8.0°C-weeks, survival rates decrease to < 30% (Table 2). By the year 2030, DHW trends will be prolonged with values above 8.0°C-weeks occurring with greater frequency, and coral mortality will increase dramatically.

Moreover, transplant groups showed no significant physiological differences (indicated by symbiont photochemical efficiency, see Fig. 3) compared to the controls in May 2014. WLT-OL transfer corals cores showed more significant physiological difference than native WLT coral cores in 2015 May and ended up with high survival rates (see Fig. 3). It appears that the extent of accumulated thermal stress throughout whole summer may be more significant in determining the final survival rate than the minor warm up before July. We performed the same pre-treatment for 2014RTE and 2015RTE and both encountered similar patterns of rising DHW before July (onset of bleaching, Fig. 2), which would have eliminated the potential stress on transplant groups in the beginning of the experiment, if there was any.

Also, the growth of coral cores from the OL (associated with *Durusdinium* sp.) in a non-native environment (WLT) was higher than that of their native (native OL) counterparts (Figs. 2; 4), indicating *Durusdinium* sp.'s tenacity (Silverstein et al. 2017). On the other hand, although WLT and IL coral cores were associated with *Cladocopium* sp. (which is known to contribute to growth, see Stat and Gates 2011), they grew less or did not survive when facing high temperatures in the non-native OL. This phenomenon might be attributed to a difference in the host performance itself and/or a difference in energy contributions of associated symbionts when present in a more favorable environment. While previous studies showed a negative trend in terms of the growth of corals associated with *Durusdinium* sp. (Jones and Berkelmans 2010; Little et al. 2004; Mieog et al. 2009; Cunning et al. 2015a), one study (Yuyama and Higuchi 2014) did show higher growth rates in *Acropora millepora* juveniles inoculated with *Durusdinium* sp. under laboratory condition for 2 months, and herein we showed this phenomenon in natural conditions with *P. verweyi*. This difference could be due to different species of genera *Cladocopium* and *Durusdinium* associated with different corals.

CONCLUSIONS

As temperature anomalies become increasingly frequent and severe, tropical coral species already living at their tolerance limits will bear the brunt of climate change (Hoegh-Guldberg

1999; Hoegh-Guldberg et al. 2007). Coral reefs are already experiencing such severe events. For example, in 2016, the Great Barrier Reef suffered large-scale coral bleaching that affected up to 95% of the reef between Papua New Guinea and Cairns (Hughes et al. 2017). A recent study (Hughes et al. 2018) looking at bleaching records at 100 globally disturbed reefs locations from 1980-2016 has shown that the seawater temperatures are warmer now compared to events three decades ago and coral bleaching has become more frequent, increasing the likelihood of annual bleaching in the coming decades. Also, the time between recurrent events is increasingly short to allow a full recovery. An increase in average global temperature to 1.5-2.0°C will contribute to coral reef degradation. Our study species, a massive coral considered a tolerant species (Loya et al. 2001), did not survive when the DHW exceeded its threshold limit, even when shuffling occurred. Irrespective of temperature tolerance thresholds of corals in the future, given the fact that we are facing continuous change in the climate through carbon emissions, symbiont shuffling might not be sufficient to withstand frequent and prolonged seawater temperature anomalies. Because we examined only one resistant coral species, results of this study cannot be generalized to all coral species around the world. However, the recent bleaching of the GBR should serve as a warning. Many coral species may not survive if the DHW increases to 10.0°C-weeks by 2050. The immediate concern is the loss of coral species altered functionality in the future. To overcome this problem, it is necessary to manage the carbon emissions to keep sea water temperatures from increasing above 2°C. Efforts are already being made to develop coral stocks with enhanced stress tolerance through an acceleration of naturally occurring processes as part of an assisted evolution approach (van Oppen et al. 2017), but we still do not know if such efforts will be able to keep up with rapid climate changes. Hence, it is necessary to urgently determine efficient ways to limit the global warming rate; otherwise, the prediction (Hoegh-Guldberg 1999) that reef systems could collapse by the end of this century may be true.

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Authors' contributions: K-WK, SK, J-TW, and CAC designed the study; K-WK and C-HT conducted the experimental procedures, and K-WK and SK conducted the physiological analyses. K-WK, SK, and C-HT analysed the data. K-WK, SK, J-TW, and CAC wrote and finalized the manuscript.

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Supplementary Materials

Fig. S1. Degree Heating Week values at native OL, WLT and IL site through time (February 2014 through January 2016) spanning 2014RTE and 2015RTE. (download)

Table S1. *Durusdinium* sp. dominance in total symbiont community in each group by time in 2014RTE. NA represents both symbiont *Cladocopium* sp. and *Durusdinium* sp. were undetected from a single colony. (download)

Table S2. Paired t-test results of photochemical efficiency and other physiological parameters in 2014RTE. *p*-values in boldface highlight the significant differences between times. (download)

Table S3. Student's *t*-test results of photochemical efficiency through time in 2014RTE. No statistics were performed for WLT-OL transfer and IL-OL transfer after October due to insufficient sample number caused by mortality. (download)

Table S4. Symbiont cell densities of *Cladocopium* sp. and *Durusdinium* sp. in 2014RTE. Values were estimated by multiplying total symbiont cell densities obtained from cell counting by types C: D ratio obtaining from qPCR. (download)

Table S5. *Durusdinium* sp. in total symbiont community in each group by time in A) 2014RTE and B) 2015RTE. NA represents both symbiont types within C and D were undetected from a single coral core. (download)

Table S6. Paired *t*-test results of photochemical efficiency in 2015RTE. *p*-values in boldface highlight the significant differences between times. (download)

Table S7. Wilcoxon rank sum test result of photochemical efficiency through time in 2015RTE. (download)

Table S8. Characteristic of eight microsatellite loci for 60 colonies of *P. verweyi* between collected at NPP Outlet and Wanlitung. (download)