Marine Pollution Bulletin 162 (2021) 111812

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Thermal tolerance of the hermatypic coral *Acropora tenuis* elucidated by RGB analysis and expression of heat shock proteins in coral and symbiotic dinoflagellates

Rin Shitaoka [a], Hiroshi Ishibashi [b,c], Ichiro Takeuchi [b,c,*]

[a] Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan
[b] Graduate School of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan
[c] Center of Advanced Technology for the Environment, Graduate School of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan

ARTICLE INFO

Keywords:
Acropora tenuis
Coral colour
Heat shock protein (HSP)
Hermatypic coral
Thermal tolerance

ABSTRACT

Increased seawater temperature has resulted in mass coral bleaching events globally. *Acropora tenuis*, the dominant hermatypic coral species in southern Japan, was exposed to four temperature treatments [28 °C, 30 °C, 32 °C, and >32 (=33.3 °C)] for 7 d. The coral colour was converted to R (red), G (green), and B (blue) values, each ranging from 0 (darkest) to 255 (brightest). RGB values exposed to 28 °C and 30 °C decreased slightly, whereas those exposed to 32 °C increased significantly after day 3–6, and those exposed to 33.3 °C changed to white within 2 d. Quantitative RT-PCR analysis revealed no significant changes in heat shock proteins in *Acropora* and symbiotic dinoflagellates at 28 °C and 30 °C after a 7 d exposure. Our findings revealed that 30 °C, higher than the mean temperature of the warmest month in southern Japan, was an inhabitable temperature for *A. tenuis*.

1. Introduction

Since 1998, increased seawater temperatures during heatwaves have killed sensitive corals and/or promoted the expulsion of symbiotic dinoflagellates (*Symbiodinium* and related genera) from coral tissues, resulting in mass bleaching (Morrison et al., 2019). Although most coral populations depleted by bleaching can recover, the interval between bleaching events has considerably decreased from 25 to 30 years in the 1980s to only 6 years since 2010 (Hughes et al., 2018a). The time between recurrent events is decreasing; hence, mature coral assemblages have less time to fully recover, which generally takes 10 to 15 years for the fastest growing species (Hughes et al., 2018a). In addition, due to the increase in seawater temperatures caused by climate change, coral reefs are exposed to an array of interdependent threats, including overfishing, coastal development, agricultural runoff, and shipping activities (Burke et al., 2011). Agricultural runoff and use of anthropogenic chemicals including herbicides, have also been reported to promote coral bleaching (see Wooldridge, 2009; Negri et al., 2011; Takeuchi et al., 2020).

During 2015 to 2016, record high seawater temperatures triggered coral bleaching on a pan-tropical scale, which was the third global-scale bleaching event since mass bleaching was first documented in the 1980s (see Hughes et al., 2017). Hughes et al. (2018b) reported that the 2016 bleaching event triggered an unprecedented loss of coral in the northern third of the Great Barrier Reef (GBR), Australia. This resulted in 50.3% loss of the coral cover on reef crests between March and November 2016, and to a lesser extent in the central third, whereas almost no heat-stress mortality occurred in corals in the southern third of the GBR. Coral bleaching has also been recorded in the area spanning the Sekisei Lagoon near the western end (Nakamura, 2017; Muko et al., 2019) to Sesoko Island, north-west of Okinawa Island (Nishiguchi et al., 2018; Sakai et al., 2019; Singh et al., 2019), along the Ryukyu Archipelago in southern Japan during the summer of 2016.

Jokiel (2004) estimated that the bleaching threshold temperature based on the long-term mean summer maximum sea surface temperature (SST) was 27–32 °C globally, except for 35–36 °C in the Arabian Gulf. The SSTs obtained by satellite data have been used to identify the real extent of coral reef bleaching (Strong et al., 1997). An SST increase of 1 °C above the mean in the month that recorded the maximum temperature induces coral bleaching (see Strong et al., 1997; Jokiel, 2004). The degree heating week (DHW) is an index of the accumulation of temperature anomalies exceeding the monthly maximum mean SST for a given region (see Kayanne, 2017). DHW values >4.0 °C-week are

* Corresponding author at: Graduate School of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan.
E-mail address: takeuchi@agr.ehime-u.ac.jp (I. Takeuchi).

https://doi.org/10.1016/j.marpolbul.2020.111812
Received 24 March 2020; Received in revised form 21 October 2020; Accepted 26 October 2020
Available online 16 November 2020

thought to lead to coral bleaching, and those >8.0 °C-week are thought to lead to widespread bleaching. The analysis by Kayanne (2017) indicated that the temperature of the warmest month, based on NOAA OI SST v2, was 29.22 °C for Ishigaki, 28.92 °C for Okinawa, and 28.53 °C for Amami in the Ryukyu Archipelago, southern Japan. Singh et al. (2019) also defined DHW between 4 °C-week and 8 °C-week as moderate thermal anomalies for corals around Sesoko Island, Okinawa. These thermal anomalies correspond to 30–31 °C owing to continuous temperature increases of 1–2 °C during the 4 weeks of the warmest month.

The substantial geographic variation in bleaching thresholds was thought to be the result of ongoing evolution of temperature tolerance (Hughes et al., 2003). Recently, the upper thermal tolerance of *Acropora* was reported to be strongly influenced by the difference in the types of symbiotic dinoflagellates (Howells et al., 2012). Symbiotic dinoflagellates from *Acropora* in warm environments of the GBR exhibit greater photosynthetic performance at high temperatures than the same dinoflagellates from *Acropora* in cooler environments (Howells et al., 2012; Jurriaans and Hoogenboom, 2019). This leads to a thermal difference in coral bleaching based on locality. Compared to the massive morphological type of hermatypic coral, including *Porites*, the branching type, including *Acropora*, display a lower tolerance for high temperatures (see Loya et al., 2001; Nakamura, 2017; Nishiguchi et al., 2018).

In the present study, we exposed the apical branches of *Acropora tenuis* to four 7 d temperature treatments, 28 °C, 30 °C, 32 °C, and >32 °C, which covered the range of seawater temperatures in the warmest month of the Okinawa Island area (see Kayanne, 2017). *Acropora tenuis* is a common hermatypic coral species in the Indo–Pacific region (Hoeksema and Cairns, 2020a). The succession of coral colour was monitored daily throughout the experiment. Heat shock proteins (HSPs) are molecular chaperones involved in maintaining regular cellular functions and play a crucial role in protein homeostasis. The HSPs are classified into several families based on their molecular mass (kDa), including small HSPs, HSP40, HSP60, HSP70, HSP90, and HSP110 (Santoro, 2000). HSP families in symbiotic *Symbiodinium*, that are associated with cnidarians such as coral and sea anemones, modulate gene expression levels in response to different environmental stresses, including elevated temperature and salinity (Császár et al., 2009; Kenkel et al., 2011; Rosic et al., 2011; Bonesso et al., 2017; Ellison et al., 2017). Our previous study demonstrated that the expression level of *A. tenuis HSP90* is significantly downregulated after a 7 d exposure to both 1.0 μg/L and 10 μg/L of Irgarol 1051, which is originally PSII binding herbicide, and is also used in the new generation of anti-fouling paints (Ishibashi et al., 2018). Moreover, the expression of *HSP70* and *HSP90* in symbiotic dinoflagellates is upregulated after a 7 d exposure to both 20 ng/L and 200 ng/L of Irgarol 1051 (Kamei et al., 2020). In the present study, we analysed the expression of *HSP* families in *A. tenuis* and its symbiotic dinoflagellates after a 7 d temperature exposure, to elucidate the function of HSPs under thermal stress.

2. Materials and methods

A colony of cultured *A. tenuis* was purchased from Umino-tane Inc. (Yomitan-son, Okinawa, Japan) and transported to the laboratory of Ehime University at Matsuyama, Ehime, central Japan. The original cultured *A. tenuis* was collected from the coast of Okinawa Island. The coral colony was acclimated in a 72 L aquarium filled with artificial seawater [29 °C, same as the mean SST of the warmest month for Okinawa Island (see Kayanne, 2017)] and kept in a temperate incubator (IS-2000, Advantec Toyo Co., Ltd., Chiyoda-ku, Tokyo, Japan), measuring W 900 × D 550 × H 1035 mm, for approximately 2 weeks. The aquarium was connected to a protein skimmer (Prizm Skimmer, Red Sea Fish Pharm Ltd., Eilat, Israel) and aquarium filter (Tetra Auto OneTouch Filter AT-60, Spectrum Brands Japan Co. Ltd., Yokohama, Kanagawa, Japan). Artificial seawater (hereafter, referred to as seawater) prepared from LIVE sea salt (Delphis Inc., Itami, Hyogo, Japan) was used in the present study because the coastal waters of Japan, including Okinawa Island, reportedly contain various contaminant chemicals, including PS II herbicides (see Okamura et al., 2003; Takeuchi et al., 2004; Murai et al., 2005; Sheikh et al., 2009). An LED light source (PowerShot; Kotobuki Co., Ltd., Matsubara, Osaka, Japan) was installed, as described by Hirayama et al. (2017). Using a diagonal cutting plier, the tip of each branch-like part (approximately 1.0–1.5 cm in length) of the colony was neatly cut to minimise damage to the coral, and then secondarily acclimated for an additional 2 days in the same aquarium under similar conditions, as described by Hirayama et al. (2017).

Exposure experiments were conducted over a period of 7 d. Kayanne (2017) reported that the SST of the warmest month on Okinawa Island is 28.92 °C. Four different temperature treatments, i.e. 28 °C, 30 °C, 32 °C, and >32 °C, were designed to cover the temperature range. Each temperature treatment was set up in a temperate incubator (IS-2000). Although we attempted to set up a 34 °C treatment for the highest temperature in the incubator, this temperature could not be stably maintained. Thus, the highest temperature treatment was >32 °C. The seawater temperature in a single Petri dish (approximately 80 cm^3) in the incubators was recorded at 1 h intervals throughout the experiment using a small data logger (TidbiT v2, Onset Computer Corporation, Bourne, MA, USA). The average seawater temperature throughout the experiment was 28.2 ± 1.0 °C for the 28 °C treatment, 30.1 ± 0.3 °C for the 30 °C treatment, 32.1 ± 0.4 °C for the 32 °C treatment, and 33.3 ± 0.6 °C for the >32 °C treatment.

In each temperature treatment, eight Petri dishes (approximately 80 cm^3), each with one coral (approximately 1.0–1.5 cm in length) per dish, were placed in the incubator around the Petri dish containing the data logger for recording temperature. The incubator was maintained at a 12 h:12 h light: dark photoperiod illuminated by the LED light. Photosynthetic photon flux density at the centre of the outer side of the Petri dish cover was set at 50 μmol m^{-2} s^{-1} using a LI-250A light sensor (LI-COR, Inc., Lincoln, NE, USA), which was the same as that in our previous studies on hermatypic corals (Hirayama et al., 2017; Ishibashi et al., 2018; Kamei et al., 2020; Takeuchi et al., 2020).

To determine the colour, *A. tenuis* were photographed with a colour chart daily at the same time (12:00–13:00), based on our previous methods (Hirayama et al., 2017; Ishibashi et al., 2018; Kamei et al., 2020), using a digital single-lens reflex camera (Nikon df, Nikon Corporation, Chiyoda-ku, Tokyo, Japan). The main settings for photography were for light sensitivity (ISO 100), shutter speed (1/125 s), aperture (f10), and manual flash mode.

The NEF (RAW) files were then subjected to RGB value analysis. Each R, G, B value ranged from 0 (darkest) to 255 (brightest); R = G = B = 0 represents completely black, and R = G = B = 255 represents completely white. The analysed area, which corresponded to the coral apical branch surface, was converted into RGB values using Adobe Photoshop CC™ (Adobe Systems Incorporated, San Jose, CA, USA) based on the published study by Hirayama et al. (2017). The RGB values of the corals were corrected according to the red colour of the colour chart.

The corals were moved to new Petri dishes with fresh seawater after photographing on days 2, 4, and 6. The Petri dishes and other glass devices used for the present experiment were washed and cleaned using acetone, hexane, HCl, tap water, and distilled water, as described in our previous ecotoxicity study on amphipod crustaceans (Aono and Takeuchi, 2008) and studies on hermatypic corals (see Hirayama et al., 2017; Kamei et al., 2020; Takeuchi et al., 2020).

After the 7 d thermal treatment, corals were harvested in RNA*later* solution (Qiagen K.K., Chuo-ku, Tokyo, Japan), and stored at −80 °C until total RNA isolation. Total RNA was extracted from the coral tissue using the RNeasy Mini Kit (Qiagen). Briefly, 1 μg of total RNA was reverse transcribed into cDNA using the ReverTra Ace qPCR RT Kit (Toyobo Co., Ltd., Osaka, Japan). Quantitative real-time PCR was performed using the Fast SYBR Green Master Mix and StepOne™ Real-Time PCR System (Life Technologies Japan Ltd., Minato-ku, Tokyo, Japan), as

described in our previous studies (Ishibashi et al., 2018; Kamei et al., 2020). Primers for target genes, including *A. tenuis HSP90* (*atHSP90*) and *Symbiodinium HSPs* (*symHSP90*, *symHSP70*, and *symHSP40*); reference genes, including *A. tenuis β-actin* (*atβ-actin*) and *elongation factor* (*atEF*); and *Symbiodinium ribosomal protein S4* (*symRp-S4*), *S-adenosyl-L-methionine synthetase* (*symSAM*), and *Cyclophin* (*symCyc*), are shown in Table 1. Data were normalised to *atEF* mRNA for *A. tenuis* and to *symRp-S4* mRNA for *Symbiodinium*. All analyses were performed in triplicate for each gene in each sample.

Statistical analyses were performed using GraphPad Prism ver. 7.0 (GraphPad Software, San Diego, CA, USA). All data were analysed for the assumption of homogeneity of variance using Bartlett's test. The differences in the RGB values and mRNA expression levels among the thermal-treatment groups were assessed using parametric one-way analysis of variance, followed by Dunnett's multiple comparisons tests. Differences were considered statistically significant at $p < 0.05$.

3. Results

The RGB values of *A. tenuis* in the lower two treatments (28 °C and 30 °C) decreased slightly throughout the 7 d experiment by 9.4% to 10.7% in R values, 11.8% to 12.0% in G values, and 16.4% to 21.6% in B values, respectively. Those in the higher two treatments indicated an opposite increasing trend, i.e. moving to the white end of the spectrum (Figs. 1, 2). The RGB values in the 32 °C treatment slightly decreased, and then generally increased from around day 4; RGB values on day 7 increased 3.1% to 9.8% compared to those on day 0. Significant differences from the 28 °C treatment were recognised on day 3 for R, day 5 for G, and day 6 for B ($p < 0.05$). Compared to day 0, the RGB values of corals in the >32 °C treatment increased by approximately 2.7% to 12.1% on day 1, 27.6% to 102.0% on day 2, and 37.0% to 155.1% on day 3. Significant differences from the 28 °C treatment ($p < 0.05$) were recognised for all RGB values from day 1 to the end of the experiment. In the >32 °C treatment, the release of symbiotic dinoflagellates from host corals at the bottom of the Petri dish was observed on day 2 (Fig. 3).

In the 32 °C and > 32 °C treatments, the mRNA expression levels of target genes could not be analysed in corals or *Symbiodinium*, because all corals were bleached in the >32 °C treatment, and the expression levels of the reference genes in the 32 °C treatment were lower than those in the 28 °C or 30 °C treatments ($p = 0.029$ to 0.138).

In corals, the mRNA expression levels of *atHSP90* in the 30 °C treatment (1.26-fold increase) were higher than those in the 28 °C treatment, but no significant difference ($p = 0.130$) was observed between these groups (Fig. 4). In *Symbiodinium*, no significant effects were observed in the mRNA expression levels of *symHSP90* ($p = 0.840$) and *symHSP70* ($p = 0.718$) between the 28 °C and 30 °C treatments (Fig. 4).

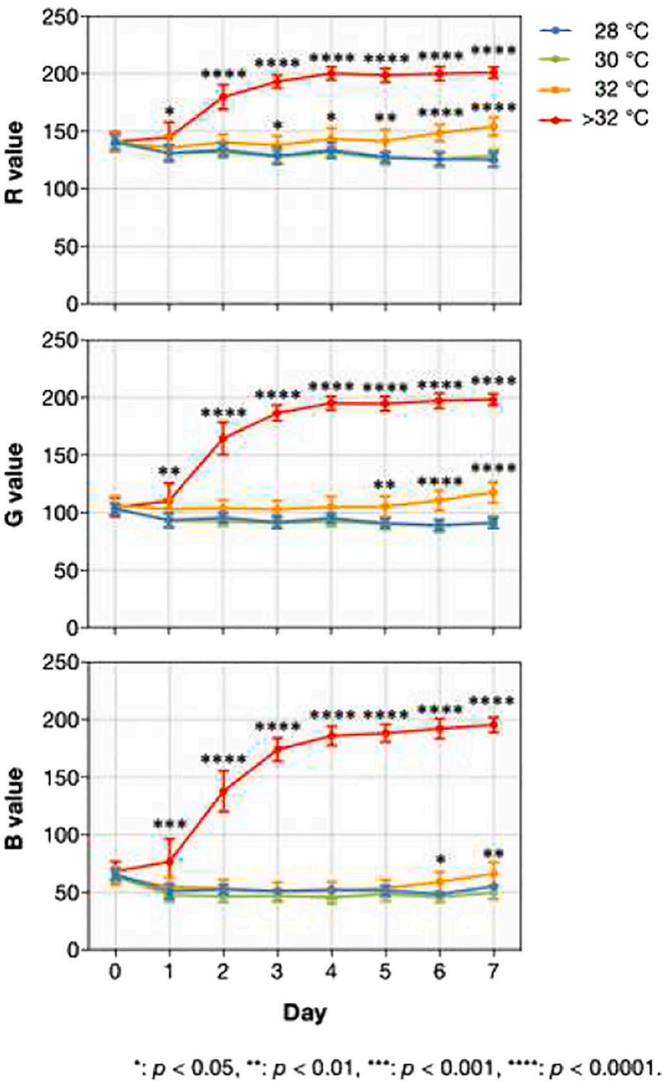

Fig. 1. Succession of RGB values in *Acropora tenuis* in the four temperature treatments. Vertical lines indicate standard deviations ($n = 8$).

However, the mRNA expression level of *symHSP40* in the 30 °C treatment (0.59-fold decrease) was lower than that in the 28 °C treatment, but no significant difference ($p = 0.076$) was observed (Fig. 4).

Table 1
Genes, GenBank accession numbers, and primer sequences in the qRT-PCR assay.

Organism	Gene name	Abbreviation	GenBank accession no.	5′-3′ sequence (upper, sense; lower, antisense)	Reference
Coral (*Acropora tenuis*)	*Heat shock proteins 90*	*atHSP90*	DC999945	AAAACCCTTGTGGATGCG GACCTTCAACCGAAAAGTGC	Ishibashi et al. (2018)
	β-actin	*atβ-actin*	BJ999688	CCTTCAATTCACCAGCCATGT CGAGGCGTACAAAGACAACACA	Yuyama et al. (2012)
	Elongation factor	*atEF*	Q1HR88	TTTGCGCCTGCAATGCT CAGACTTTCATGGTGCATTTCAA	Yuyama et al. (2012)
Symbiodinium	*Heat shock proteins 90*	*symHSP90*	EH038163.1	GAGGATCTGCCACTGAACATCTC GCGAACATCTCCAAGCACTTC	Rosic et al. (2011)
	Heat shock proteins 70	*symHSP70*	EH038080.1	CAGATGAGGCCGTGGCTTAT GGGAGTCACATCCAACAGCAA	Rosic et al. (2011)
	Heat shock proteins 40	*symHSP40*	EH035912.1	GCGAAAATTTCACTGCGAGACT GGTCGGACAACTTCATTTAGTGGTA	Rosic et al. (2011)
	Ribosomal protein S4	*symRp-S4*	EH036413.1	CCGCACAAACTGCGTGAGT CGCTGCATGACGATCATCTT	Rosic et al. (2011)
	S-adenosyl-L-methionine synthetase	*symSAM*	EH036622.1	GCCTACATTTGCCGACAGATG AATGGCTTGGCAACACCAAT	Rosic et al. (2011)
	Cyclophin	*symCyc*	EH037450	ATGTGCCAGGGTGGAGACTT CCTGTGTGCTTCAGGGTGAA	Rosic et al. (2011)

Fig. 2. Photographs of *Acropora tenuis* in the four temperature treatments from day 0 to day 7. Still images were taken from the JPEG images saved simultaneously with the RAW files used for the RGB value analysis. The bar indicates 1.0 cm.

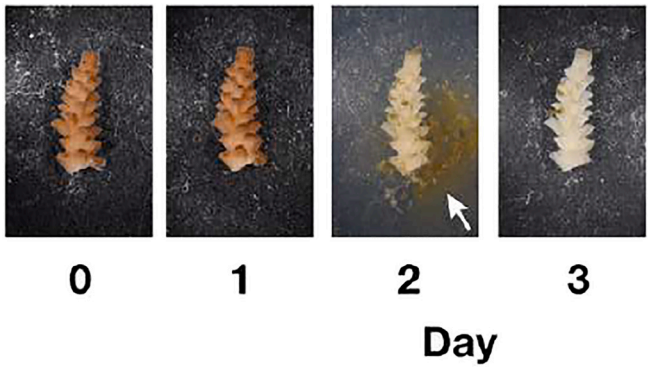

Fig. 3. Photographs of *Acropora tenuis* in the >32 °C treatment from day 0 to day 3. Still images were taken from the JPEG images saved simultaneously with the RAW files used for the RGB value analysis. The arrow indicates the release of symbiotic dinoflagellates.

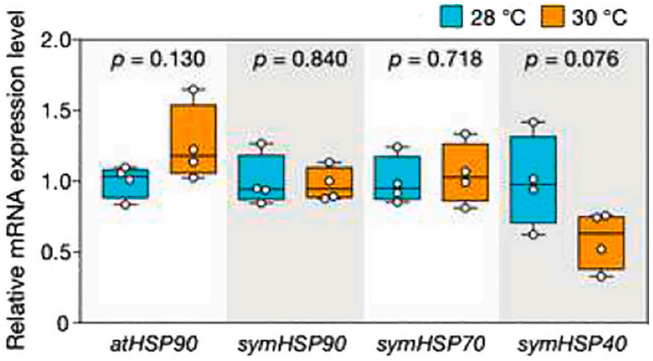

Fig. 4. Expression levels of *Acropora tenuis HSP90* (*atHSP90*) and *Symbiodinium HSP90* (*symHSP90*), *symHSP70*, and *symHSP40* genes after treatment at 28 °C and 30 °C for 7 d. Boxes represent upper and lower quantiles, whiskers represent minimum and maximum values, and black lines represent the medians ($n = 4$ per treatment group).

4. Discussion

The thermal stress that drives bleaching differs among morphological types and genera of hermatypic corals in southern Japan. The branched or table-like type of hermatypic corals, including *Acropora*, were less tolerant to high temperature than the massive type of coral, including *Porites* (see Loya et al., 2001; Nakamura, 2017; Nishiguchi et al., 2018). Loya et al. (2001) reported that, during the 1998 bleaching event on Sesoko Island in southern Japan, branched *Acropora* and pocilloporid corals were severely affected, but the coverage and abundance of massive types of *Porites*, namely, *Porites lutea* and *Porites lobata*, increased after the bleaching event. Nishiguchi et al. (2018) reported that during the summer of 2016, *Acropora cytherea* was gradually bleached, whereas *Porites* sp. retained their natural colour on Sesoko Island during this time. The average seawater temperature from the end of July to early September was 29.9 °C at their study site. Nakamura (2017) surveyed the bleaching status of 11 species of major hermatypic corals, including *Acropora*, *Pocillopora*, *Seriatopora*, *Stylophora*, *Favites*, and *Porites*; from 3 to 12 September 2016 over the entire Sekisei Lagoon near the western end of southern Japan. His survey showed that the bleaching of 10 species, including *Acropora*, was >98%, whereas massive *Porites* exhibited approximately 60% bleaching. Singh et al. (2019) compared the population dynamics of adult *Acropora* spp. 6 months before and after the 2016 bleaching events around Sesoko Island; and found that, of the five monitored stations, all colonies of *Acropora* spp. were bleached at one station, which was the only station where the DHW was above 4 °C-weeks.

Fujise et al. (2014) reared colonies of *Acropora selago* and *Acropora muricata* collected from Ishigaki Island, Okinawa, under nonthermal

stress (27 °C) for 5 d to moderate heat-stress conditions (30 °C) for 6 d via stepwise increases for 2 d. Although *Symbiodinium* densities in both species of *Acropora* did not show any significant differences between sampling days, degraded *Symbiodinium* cells from coral predominated at 30 °C, and the proportion of expelled normal *Symbiodinium* cells exhibited a lower maximum potential quantum yield (F_v/F_m), an indicator of photosynthetic performance, at 30 °C. Thus, 30 °C was found to be a moderate heat-stress temperature in several recent studies conducted in Okinawa, southern Japan (see Fujise et al., 2014; Yorifuji et al., 2017; Singh et al., 2019). This temperature is also considered to induce moderate heat stress in *Acropora palmata*, a Caribbean species (Portune et al., 2010).

In contrast to the above field and laboratory studies conducted at Okinawa (see Fujise et al., 2014; Yorifuji et al., 2017; Singh et al., 2019), the present study clearly showed that the colour of *A. tenuis* in the 28 °C and 30 °C treatments was stable throughout our 7 d experiment. The release of dinoflagellates was observed only in the >32 °C treatment. Moreover, the coral *HSP90* and dinoflagellate *HSP90*, *HSP70*, and *HSP40* in these two treatments were stable and not up- or down-regulated after the 7 d exposure.

Yorifuji et al. (2017) reared juveniles after the metamorphosis of *A. tenuis* collected from Aka Island, Okinawa, which had settled on tiles under three temperature treatments for 2 or 4 months during the summer of 2012 and 2013. The treatments comprised ambient temperature (22.66–30.82 °C in 2012 and 27.01–30.93 °C in 2013), moderate heat-stress temperature (30 °C), and high heat-stress temperature (31 °C in 2012 and 32 °C in 2013) conditions. The moderate heat-stress temperature of 30 °C was 1 °C above the monthly mean in the month that recorded the maximum temperature in Ishigaki to Amami in the Ryukyu Archipelago, southern Japan (see Kayanne, 2017). In the 2012 experiment, *A. tenuis* in the 30 °C treatment survived for up to 1.5 years with a 37.0% survival at 4 months, while the survival rates were 24.4% under ambient conditions and 14.0% under 31 °C conditions at 4 months (Yorifuji et al., 2017). In the 2013 experiment, the survival rate of juvenile *A. tenuis* was 15.2% in the ambient treatment, and 51.3% in the 30 °C treatment and 3.4% in the 32 °C treatment at 2 months (Yorifuji et al., 2017). In both experiments, the survival rate was maximum at 30 °C, which was considered to represent a moderate heat-stress temperature by Yorifuji et al. (2017). Bell et al. (2017) reported an experiment with juvenile polyps of *Acropora digitifera* from Sesoko Island, Okinawa, attached to plates and reared under four temperature treatments (20 °C, 22 °C, 27 °C, and 31 °C) for 3 weeks. The juveniles at 31 °C exhibited the highest calcification rate, which was significantly different from that exhibited in the 27 °C treatment. *Acropora digitifera* is also a common hermatypic coral species in the Indo–Pacific region (Hoeksema and Cairns, 2020b). Baird et al. (2006) reported that of the *A. muricata* larvae collected from Okinawa, 30% of those in the 26 °C and 32 °C treatments survived after 198 h, whereas all larvae in the 36 °C treatment died within 40 h. These findings on *Acropora*, especially *A. tenuis* and *A. digitifera* from Okinawa, together with our results indicate that 30–31 °C is an inhabitable environmental temperature, similar to the <30 °C treatment in the current study.

Several previous studies have demonstrated that elevated temperature, especially >31 °C has the potential to modulate the expression levels of *HSP*s, such as *HSP90* and *HSP70*, in corals and *Symbiodinium*, suggesting that HSPs, as molecular chaperones, have vital cytoprotective properties (see Császár et al., 2009; Kenkel et al., 2011, 2013; Leggat et al., 2011; Rosic et al., 2011; Bonesso et al., 2017; Lee et al., 2018). Leggat et al. (2011) collected *Acropora aspera* from the GBR and subjected them to experiments involving elevated temperatures. After 5 days of acclimation, the rearing tanks were heated by raising the temperature by approximately 1 °C per day for 6 days before they were held at the specified temperature for an additional 2 days. Seawater temperatures increased from a midday temperature of approximately 27 °C to a midday value of 34 °C over the 8 days of the experiments (Leggat et al., 2011). In the corals, the expression of both *HSP70* and *HSP90* was significantly upregulated from day 7 and day 5, respectively, when exposed to increased temperatures, whereas in the dinoflagellates, only *HSP70* was significantly upregulated on day 5 (Leggat et al., 2011). Lee et al. (2018) studied the gene expression of *A. muricata* collected from Kochi Prefecture, Shikoku Island, southern Japan during winter (~16 °C) and summer (~27 °C). They reported that *HSP70* transcription was upregulated when corals collected in the winter were subjected to 33 °C for 24 h.

In conclusion, the present study showed that the RGB values of *A. tenuis* exposed to 28–30 °C for 7 d decreased slightly, whereas those exposed to 32 °C for 7 d moved to the white end of the spectrum, and the expression of *HSPs* in *A. tenuis* and its symbiotic dinoflagellates kept at 28–30 °C for 7 d was stable. The results of the above-mentioned studies in combination with our findings showed that a seawater temperature of 30 °C is a normally inhabitable environmental temperature for *Acropora*, at least for *A. tenuis* and *A. digitifera*, along the coast of southern Japan. The seawater temperature of 30 °C is 1 °C above the monthly mean in the month that recorded the maximum temperature in southern Japan. Further detailed study on the thermal tolerance of *Acropora* spp. over a longer period corresponding >4.0 °C-week in DHW combined with the analysis of physiology of symbiotic dinoflagellates would contribute to the conservation of *Acropora* in a warming environment.

CRediT authorship contribution statement

Rin Shitaoka: Conceptualization, Methodology, Investigation, Writing - original draft. **Hiroshi Ishibashi:** Methodology, Investigation, Data curation, Formal analysis. **Ichiro Takeuchi:** Conceptualization, Methodology, Writing - review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by a research grant from the Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (KAKENHI; Grant no. 18H03387).

References

Aono, A., Takeuchi, I., 2008. Effects of tributyltin at concentrations below ambient levels in seawater on *Caprella danilevskii* (Crustacea: Amphipoda: Caprellidae). Mar. Pollut. Bull. 57, 515–523.

Baird, A.H., Gilmour, J.P., Kamiki, T.M., Nonaka, M., Pratchett, M.S., Yamamoto, H.H., Yamasaki, H., 2006. Temperature tolerance of symbiotic and non-symbiotic coral larvae. In: Proceedings of 10th International Coral Reef Symposium, Okinawa, Japan. June 28–July 2, 2004, pp. 38–42.

Bell, T., Nishida, K., Ishikawa, K., Suzuki, A., Nakamura, T., Sakai, K., Ohno, Y., Iguchi, A., Yokoyama, Y., 2017. Temperature-controlled culture experiments with primary polyps of coral *Acropora digitifera*: calcification rate variations and skeletal Sr/Ca, Mg/Ca, and Na/Ca ratios. Palaeogeogr. Palaeoclimatol. Palaeoecol. 484, 129–135.

Bonesso, J.L., Leggat, W., Ainsworth, T.D., 2017. Exposure to elevated sea-surface temperatures below the bleaching threshold impairs coral recovery and regeneration following injury. PeerJ 5, e3719. https://doi.org/10.7717/peerj.3719.

Burke, L., Reytar, K., Spalding, M., Perry, A., 2011. Reefs at Risk Revisited. World Resources Institute, Washington, DC, USA p. i–x + 1-114.

Császár, N.B.M., Seneca, F.O., van Oppen, M.J.H., 2009. Variation in antioxidant gene expression in the scleractinian coral *Acropora millepora* under laboratory thermal stress. Mar. Ecol. Prog. Ser. 392, 93–102.

Ellison, M.A., Ferrier, M.D., Carney, S.L., 2017. Salinity stress results in differential Hsp70 expression in the *Exaiptasia pallida* and *Symbiodinium* symbiosis. Mar. Environ. Res. 132, 63–67.

Fujise, L., Yamashita, H., Suzuki, G., Sasaki, K., Liao, L.M., Koike, K., 2014. Moderate thermal stress causes active and immediate expulsion of photosynthetically damaged zooxanthellae (*Symbiodinium*) from corals. PLoS One 9 (12), e114321. https://doi.org/10.1371/journal.pone.0114321.

Hirayama, K., Takayama, K., Haruta, S., Ishibashi, H., Takeuchi, I., 2017. Effect of low concentrations of Irgarol 1051 on RGB (R, red; G, green; B, blue) colour values of the hard-coral *Acropora tenuis*. Mar. Pollut. Bull. 124, 678–686.

Hoeksema, B.W., Cairns, S., 2020a. World list of scleractinia. *Acropora tenuis* (Dana, 1846). Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=207105 (on 2020-03-10).

Hoeksema, B.W., Cairns, S., 2020b. World list of scleractinia. *Acropora digitifera* (Dana, 1846). Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=207045 (on 2020-03-10).

Howells, E.J., Beltran, V.H., Larsen, N.W., Bay, L.K., Willis, B.L., van Oppen, M.J.H., 2012. Coral thermal tolerance shaped by local adaptation of photosymbionts. Nat. Clim. Chang. 2, 116–120.

Hughes, T.P., Baird, A.H., Bellwood, D.R., Card, M., Connolly, S.R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J.B.C., Kleypas, J., Lough, J.M., Marshall, P., Norström, M., Palumbi, S.R., Pandolfi, J.M., Rosen, B., Roughgarden, J., 2003. Climate change, human impacts, and the resilience of coral reefs. Science 301, 929–933.

Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., Bridge, T.C., Butler, I.R., Byrne, M., Cantin, N.E., Comeau, S., Connolly, S.R., Cumming, G.S., Dalton, S.J., Diaz-Pulido, G., Eakin, C.M., Figueira, W.F., Gilmour, J.P., Harrison, H. B., Heron, S.F., Hoey, A.S., Hobbs, J.-P.A., Hoogenboom, M.O., Kennedy, E.V., Kuo, C.-Y., Lough, J.M., Lowe, R.J., Liu, G., McCulloch, M.T., Malcolm, H.A., McWilliam, M.J., Pandolfi, J.M., Pears, R.J., Pratchett, M.S., Schoepf, V., Simpson, T., Skirving, W.J., Sommer, B., Torda, G., Wachenfeld, D.R., Willis, B.L., Wilson, S.K., 2017. Global warming and recurrent mass bleaching of corals. Nature 543, 373–377.

Hughes, T.P., Anderson, K.D., Connolly, S.R., Heron, S.F., Kerry, J.T., Lough, J.M., Baird, A.H., Baum, J.K., Berumen, M.L., Bridge, T.C., Claar, D.C., Eakin, C.M., Gilmour, J.P., Graham, N.A.J., Harrison, H., Hobbs, J.-P.A., Hoey, A.S., Hoogenboom, M., Lowe, R.J., McCulloch, M.T., Pandolfi, J.M., Pratchett, M., Schoepf, V., Torda, G., Wilson, S.K., 2018a. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science 359, 80–83.

Hughes, T.P., Kerry, J.T., Baird, A.H., Connolly, S.R., Dietzel, A., Eakin, C.M., Heron, S. F., Hoey, A.S., Hoogenboom, M.O., Liu, G., McWilliam, M.J., Pears, R.J., Pratchett, M.S., Skirving, W.J., Stella, J.S., Torda, G., 2018b. Global warming transforms coral reef assemblages. Nature 556, 492–496.

Ishibashi, H., Minamide, S., Takeuchi, I., 2018. Identification and characterization of heat shock protein (HSP90) in the hard coral *Acropora tenuis* in response to Irgarol 1051. Mar. Pollut. Bull. 133, 773–780.

Jokiel, P.L., 2004. Temperature stress and coral bleaching. In: Rosenberg, E., Loya, Y. (Eds.), Coral Health and Disease. Springer, Berlin, Germany, pp. 401–425.

Jurriaans, S., Hoogenboom, M.O., 2019. Thermal performance of scleractinian corals along a latitudinal gradient on the great barrier reef. Phil. Trans. R. Soc. B 374, 20180546. https://doi.org/10.1098/rstb.2018.0546.

Kamei, M., Takayama, K., Ishibashi, H., Takeuchi, I., 2020. Effects of ecologically relevant concentrations of Irgarol 1051 in tropical to subtropical coastal seawater on hermatypic coral *Acropora tenuis* and its symbiotic dinoflagellates. Mar. Pollut. Bull. 150, 110734.

Kayanne, H., 2017. Validation of degree heating weeks as a coral bleaching index in the northwestern Pacific. Coral Reefs 36, 63–70.

Kenkel, C.D., Aglyamova, G., Alamaru, A., Bhagooli, R., Capper, R., Cunning, R., deVillers, A., Haslun, J.A., Hédouin, L., Keshavmurthy, S., Kuehl, K.A., Mahmoud, H., McGinty, E.S., Montoya-Maya, P.H., Palmer, C.V., Pantile, R., Sánchez, J.A., Schils, T., Silverstein, R.N., Squiers, L.B., Tang, P.-C., Goulet, T.L., Matz, M.V., 2011. Development of gene expression markers of acute heat-light stress in reef-building corals of the genus *Porites*. PLoS One 6, e26914. https://doi.org/10.1371/journal.pone.0026914.

Kenkel, C.D., Meyer, E., Matz, M.V., 2013. Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments. Mol. Ecol. 22, 4322–4334.

Lee, S.T., Keshavmurthy, S., Fontana, S., Takuma, M., Chou, W.-H., Chen, C.A., 2018. Transcriptomic response in *Acropora muricata* under acute temperature stress follows preconditioned seasonal temperature fluctuations. BMC Res Notes 11, 119.

Leggat, W., Seneca, F., Wasmund, K., Ukani, L., Yellowlees, D., Ainsworth, T.D., 2011. Differential responses of the coral host and their algal symbiont to thermal stress. PLoS One 6, e26687. https://doi.org/10.1371/journal.pone.0026687.

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., van Woesik, R., 2001. Coral bleaching: the winners and the losers. Ecol. Lett. 4, 122–131.

Morrison, T.H., Hughes, T.P., Adger, W.N., Brown, K., Barnett, J., Lemos, M.C., 2019. Save reefs to rescue all ecosystems. Nature 573, 333–336.

Muko, S., Suzuki, G., Saito, M., Nakamura, T., Nadaoka, K., 2019. Transitions in coral communities over 17 years in the Sekisei Lagoon and adjacent reef areas in Okinawa, Japan. Ecol. Res. 34, 524–534.

Murai, R., Takahashi, S., Tanabe, S., Takeuchi, I., 2005. Status of butyltin pollution along the coasts of Western Japan in 2001, 11 years after partial restrictions on the usage of tributyltin. Mar. Pollut. Bull. 51, 940–949.

Nakamura, T., 2017. Mass coral bleaching event in Sekisei lagoon observed in the summer of 2016. J. Japan. Coral Reef Soc. 19, 29–40.

Negri, A.P., Flores, F., Röthig, T., Uthicke, S., 2011. Herbicides increase the vulnerability of corals to rising sea surface temperature. Limnol. Oceanogr. 56, 471–485.

Nishiguchi, S., Wada, N., Yamashiro, H., Ishibashi, H., Takeuchi, I., 2018. Continuous recordings of the coral bleaching process on Sesoko Island, Okinawa, Japan, over about 50 days using an underwater camera equipped with a lens wiper. Mar. Pollut. Bull. 131, 422–427.

Okamura, H., Aoyama, I., Ono, Y., Nishida, T., 2003. Antifouling herbicides in the coastal waters of western Japan. Mar. Pollut. Bull. 47, 59–67.

Portune, K.J., Voolstra, C.R., Medina, M., Szmant, A.M., 2010. Development and heat stress-induced transcriptomic changes during embryogenesis of the scleractinian coral *Acropora palmata*. Mar. Genomics 3, 51–62.

Rosic, N.N., Pernice, M., Dove, S., Dunn, S., Hoegh-Guldberg, O., 2011. Gene expression profiles of cytosolic heat shock proteins Hsp70 and Hsp90 from symbiotic dinoflagellates in response to thermal stress: possible implications for coral bleaching. Cell Stress Chaperones 16, 69–80.

Sakai, K., Singh, T., Iguchi, A., 2019. Bleaching and post-bleaching mortality of *Acropora* corals on a heat-susceptible reef in 2016. PeerJ 7, e8138. https://doi.org/10.7717/peerj.8138.

Santoro, M.G., 2000. Heat shock factors and the control of the stress response. Biochem. Pharmacol. 59, 55–63.

Sheikh, M.A., Higuchi, T., Fujimura, H., Imo, T.S., Miyagi, T., Oomori, T., 2009. Contamination and impacts of new antifouling biocide Irgarol-1051 on subtropical coral reef waters. Internat. J. Environ. Sci. Technol. 6, 353–358.

Singh, T., Iijima, M., Yasumoto, K., Sakai, K., 2019. Effects of moderate thermal anomalies on *Acropora* corals around Sesoko Island, Okinawa. PLoS One 14, e0210795. https://doi.org/10.1371/journal.pone.0210795.

Strong, A.E., Barrientos, C.S., Duda, C., Sapper, J., 1997. Improved satellite techniques for monitoring coral reef bleaching. In: Proceeding of 8th International Coral Reef Symposium 2, pp. 1495–1498.

Takeuchi, I., Takahashi, S., Tanabe, S., Miyazaki, N., 2004. Butyltin concentrations along the Japanese coast from 1997 to 1999 monitored by *Caprella* spp. (Crustacea: Amphipoda). Mar. Env. Res. 57, 397–414.

Takeuchi, I., Takaichi, D., Katsumata, M., Ishibashi, H., 2020. Succession of delayed fluorescence correlated with coral bleaching in the hermatypic coral *Acropora tenuis*. Mar. Pollut. Bull. 154, 111008.

Wooldridge, S.A., 2009. Water quality and coral bleaching thresholds: Formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. Mar. Pollut. Bull. 58, 745–751.

Yorifuji, M., Harii, S., Nakamura, R., Fudo, M., 2017. Shift of symbiont communities in *Acropora tenuis* juveniles under heat stress. PeerJ 5, e4055. https://doi.org/10.7717/peerj.4055.

Yuyama, I., Ito, Y., Watanabe, T., Hidaka, M., Suzuki, Y., Nishida, M., 2012. Differential gene expression in juvenile polyps of the coral *Acropora tenuis* exposed to thermal and chemical stresses. J. Exp. Mar. Biol. Ecol. 430–431, 17–24.