

# 1 Natural thermal stress-hardening of corals through 2 cold temperature pulses in the Thai Andaman Sea

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4 Marlene Wall<sup>1,2</sup>, Talisa Doering<sup>1,3</sup>, Nina Pohl<sup>1</sup>, Lalita Putchim<sup>4</sup>, Tipwimon Ratanawongwan<sup>5</sup>, Anna Roik<sup>2,6</sup>

5  
6 <sup>1</sup>GEOMAR, Helmholtz Centre for Ocean Research, Kiel, Germany

7 <sup>2</sup>Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany

8 <sup>3</sup>School of BioSciences, University of Melbourne, Parkville, VIC, Australia

9 <sup>4</sup>Marine and Coastal Resources Research Center, Upper Eastern Gulf of Thailand, Songklong, Bangpakong,  
10 Chachoengsao 24130

11 <sup>5</sup>Marine and Coastal Resources Research Center Lower Andaman Sea, Sikao District, Trang 92150, Thailand

12 <sup>6</sup>Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Oldenburg,  
13 Germany

14  
15 Keywords

16 thermal variability, coral, bleaching, short-term acute heat stress, thermal resistance, thermal tolerance, plasticity,  
17 environmental history, large amplitude internal waves, solitons, Thailand, Andaman Sea

## 18 Abstract

19  
20 Thermal variability can render corals stress resistant through a phenomenon coined as “stress-hardening  
21 induced by environmental priming”. Fluctuations that involve high temperature peaks have been  
22 commonly investigated, however, the effects of a stress-hardening stimulus generated by cold-water  
23 pulses has rarely been studied. Offshore island reefs in the Andaman Sea offer an ideal natural setting  
24 to study these effects, as cooling water of internal waves induce strong variability with peak intensity  
25 in January to June and absence in August to November. While western island shores are exposed to  
26 this stimulus, eastern shores remain sheltered. This study examined (1) whether corals from exposed  
27 reefs were more heat stress resistant compared to stimulus-sheltered conspecifics and (2) whether this  
28 trait can last in the absence of the stimulus. We quantified the thermal stress resistance in two  
29 ecologically important coral species, *Pocillopora* sp. and *Porites* sp., from the two island shores, during  
30 the two seasons. Coral bleaching intensity and photosynthetic efficiency of algal symbionts were  
31 measured as response variables after a short-term heat stress assay (24-48 h, 34 °C) to assess thermal  
32 stress resistance. Stress responses of all stimulus-exposed corals were either undetectable (during the  
33 season of stimulus presence) or very weak (during stimulus absence), while corals from the stimulus-  
34 sheltered shore responded strongly to heat stress irrespective of the season. Hence, thermal resistance  
35 was overall greater in corals originating from the stimulus-exposed shore, but it was slightly diminished  
36 during the season of stimulus absence, emphasizing the relevance of stimulus recurrence in maintaining  
37 the resistance trait. We exemplify that the stimulus of fluctuating low temperature pulses successfully  
38 induced stress-hardening in corals. This suggests that priming stimuli do not necessarily need to  
39 transgress certain upper thermal thresholds, but can also touch on lower thresholds to be effective. Even  
40 more, we argue that cooling pulses might represent a safer stress-hardening regime, since warming-  
41 stress accumulation can be avoided. More research is required to obtain a better understanding of

42 environmental priming, but current findings should encourage the development of artificial stress-  
43 hardening approaches to enhance coral resistance in reef restoration efforts.

## 44 Introduction

45  
46 Reef-building corals live near their thermal limits, so that the growing thermal stress caused by ocean  
47 warming poses the most pressing threat to the existence of many coral species and the tropical reef  
48 ecosystems (Hoegh-Guldberg 1999). Thermal anomalies that culminate during long-lasting heat-waves  
49 (Sully et al. 2019) impose intense stress on corals, which triggers “coral bleaching”, i.e., the loss of  
50 symbiotic algae from the coral holobionts that is apparent through the paling of the coral tissues (Glynn  
51 1991). Bleached corals are exposed to starvation, as the nutrient exchange between the host and  
52 dinoflagellate symbionts is disrupted (Brown 1997; Rådecker et al. 2021). As a result, coral bleaching  
53 events have already been fatal to vast portions of coral populations worldwide, resulting in significant  
54 losses of the coral reef ecosystem (Hughes et al. 2018).

55 Tropical corals have adapted to mostly stable thermal conditions of the tropical waters which feature  
56 only little seasonal change (Kleypas et al. 1999). Consequently, they do not cope well with even slight  
57 increases in temperature. However, observations of coral reef habitats that feature comparably high  
58 thermal (and other) fluctuations provide a glimpse into the remarkable plasticity of certain individuals  
59 or populations. Such studies have demonstrated that corals, pre-exposed to challenging conditions, can  
60 feature a higher thermal resistance, especially in terms of their thermal threshold and/or show a higher  
61 ability to recover after (thermal) stress compared to their counterparts living in more stable  
62 environments nearby. Subsequently, this phenomenon has been coined “environmental priming” or  
63 “environmental memory” (Brown et al. 2002; Rivest et al. 2017; Hackerott et al. 2021; Martell 2023).  
64 Most of these observations originate from reefs that experience strong environmental variability, e.g.,  
65 inshore reef habitats (Kenkel and Matz 2016), reef flats and tidal pools that are temporarily exposed to  
66 extreme conditions (Oliver and Palumbi 2011a; Schoepf et al. 2015), sites exposed to internal waves or  
67 other upwelling events (Doering et al. 2021; Buerger et al. 2015), or reef-adjacent habitats, such as  
68 lagoons and mangroves (Camp et al. 2016, 2017). Also, temporal stress events, such as moderate heat  
69 waves (Bellantuono et al. 2012b; Ainsworth et al. 2016; Fox et al. 2021) or consecutive bleaching events  
70 (Maynard et al. 2008; Guest et al. 2012; Penin et al. 2013), have been observed to be associated with  
71 greater stress resistance and/or a faster recovery of corals following such events, where mechanisms  
72 underlying adaptation and/or acclimation can be suspected.

73 The phenomenon of stress resistance gain is not unique to corals. Stress-hardening through  
74 environmental priming relies on the phenotypic and physiological plasticity of an organism (Hilker et  
75 al. 2016) and has long been observed and studied across many taxa of the tree of life, most prominently  
76 in plants (Nicotra et al. 2010; Tanou et al. 2012; Li et al. 2014). Past environmental challenges can  
77 “prime” organisms to respond to future stressors more efficiently and/or rapidly. In contrast to the  
78 mechanisms of adaptation, which arise from genetic variation and selection dynamics over several  
79 generations, the effect of environmental priming can occur within one generation and in the same  
80 individual (Whitman and Agrawal 2009; Foo and Byrne 2016). For long-lived coral species with  
81 relatively long generation times and, hence, naturally slower adaptive processes, rapid acclimation  
82 through plasticity can become life-saving (Palumbi et al. 2014). As such, environmental priming could  
83 fundamentally increase the odds for corals to successfully resist rapid ocean warming.

84 Today, reefs that exhibit high thermal variability have become attractive sites to obtain stress tolerant  
85 corals to study the mechanisms underpinning plasticity and thermal resistance (Oliver and Palumbi  
86 2011b; Ziegler et al. 2017; Hackerott et al. 2021; Majerova et al. 2021). Furthermore, study of these

87 stress resistant phenotypes can assist the development of interventions to enhance coral stress tolerance  
88 (Doering et al. 2021; Epstein et al. 2019; Howells et al. 2021). Most recently, *ex situ* experiments aiming  
89 to develop stress-hardening procedures for the laboratory have gained traction. Such studies have shown  
90 that artificial preconditioning treatments applying fluctuating temperatures in artificial aquarium  
91 environments, can improve thermal stress resistance of corals (Hawkins and Warner 2017; Majerova et  
92 al. 2021; Alexander et al. 2022; DeMerlis et al. 2022). However, findings are still equivocal, as several  
93 other studies did not report any stress-hardening effects (Putnam and Edmunds 2011; Klepac and  
94 Barshis 2020; Alexander et al. 2022; Schoepf et al. 2022). Obtaining an understanding of the greater  
95 detail of these phenomena is of urgent relevance. Hence, important questions concerning the “dosage”  
96 of a priming stimulus, including the exposure duration and the regime of the priming conditions which  
97 is required to achieve the desired effect, remain to be answered (Brown et al. 2023; Martell 2023).  
98 To shed light on some of the questions, this study took advantage of the environmentally and seasonally  
99 dynamic reef sites located in the Andaman Sea in Thailand, where island shores are seasonally exposed  
100 to a stimulus of thermal variability induced by large amplitude internal waves (Osborne and Burch  
101 1980). Internal waves are oceanographic features that are ubiquitous in the world's oceans and, contrary  
102 to surface waves, travel deep along strong density gradients (Jackson et al. 2012). Particularly in the  
103 Thai Andaman Sea, western reef sites are exposed to the internal waves and experience the strongest  
104 impacts. Internal waves induce variations of temperature (i.e., negative anomalies with minima at 26 to  
105 24 °C), pH, salinity and other environmental variables during their peak season of wave intensity that  
106 is from January to June. They can impose tremendous stress on corals, but can provide protective  
107 cooling during natural marine heat waves (Wall et al. 2015; Wyatt et al. 2019). In contrast, reefs on the  
108 east shores of the islands are sheltered from these impacts, fostering stable environmental conditions  
109 (Schmidt et al. 2012; Wall et al. 2012). During the second half of the year, impacts of internal waves  
110 are typically minor or absent on the western shores, which allows the study of coral thermal stress  
111 resistance in presence and absence of the priming stimulus. While a majority of studies have focused  
112 on coral stress-hardening by exposing them to high temperature regimes under constant or variable  
113 conditions (Middlebrook et al. 2008; Bellantuono et al. 2012b; Putnam and Gates 2015; Hackerott et  
114 al. 2021; Martell 2023), internal wave sites provide the opportunity to study the effect of high variability  
115 conditions that do not involve high but rather low thermal pulses.  
116 A previous study has established that internal wave impact, in the Andaman Sea region, was linked to  
117 higher coral thermal stress resistance, specifically in *Porites* sp. corals studied during the season of high  
118 internal wave intensity (Buerger et al. 2015). We followed up on these previous findings and  
119 investigated whether this stress-hardening effect reported for *Porites* sp. is a species-specific  
120 phenomenon or can be also found in other coral species. Second, we investigated whether a greater  
121 thermal resistance of corals originating from the stimulus-exposed reefs is a persistent or a transient  
122 trait, which occurs only during the season when environmental variability is at its peak. To address  
123 these questions, we employed high-throughput short-term heat stress assays (duration: 24 - 48h, peak  
124 of 34 °C) (Doering et al. 2021; Evensen et al. 2021) to assess the thermal stress responses in two coral  
125 species, *Pocillopora* sp. and *Porites* sp., from a stimulus-exposed and a sheltered site during the two  
126 seasons.

## 127 Materials and Methods

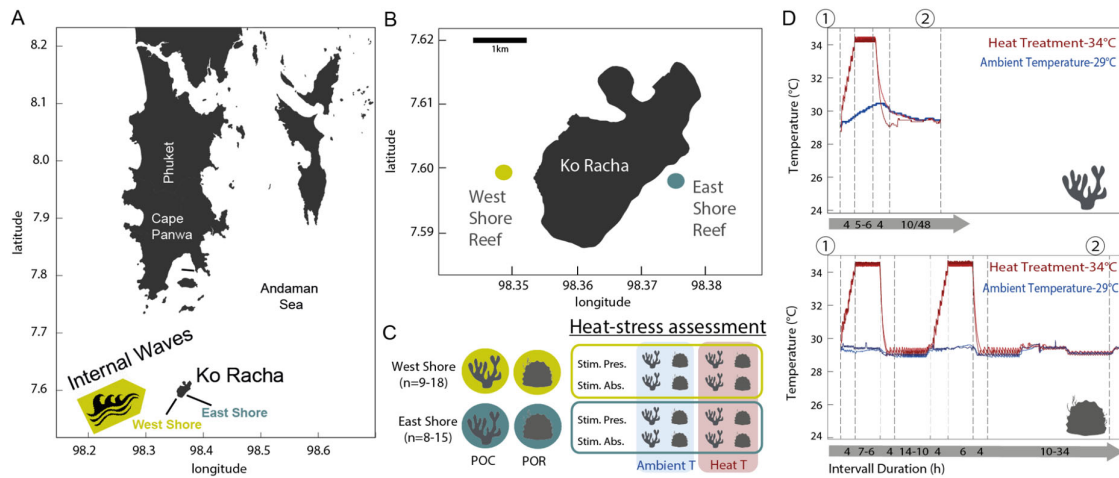
### 128 Study sites and coral collection

129 Study sites were located at Racha Island in the Andaman Sea off the coast of Thailand, both at 15 m  
130 water depth (Figure 1 A-B). A reef on the western shore was chosen (7.595530°N, 98.354320°E, Figure

131 1 B) where internal wave forcing induced environmental variability through frequent upwelling of deep,  
132 cool, and nutrient rich water onto the shelf (Wall et al. 2012; Schmidt et al. 2016). A reef on the eastern  
133 shore, sheltered from the internal wave stimulus, was chosen to represent a low variability reef  
134 (7.598910°N, 98.373100°E, Figure 1 B). Temperature fluctuations were monitored *in situ* as a proxy  
135 for internal wave impact and environmental variability. Temperature loggers (HOBO Pendant  
136 Temperature/Light 8K Data Logger, Onset, USA) were deployed at the study sites one month before  
137 heat stress assays were performed. In each study site, visually healthy coral colonies of *Pocillopora* sp.  
138 and *Porites* sp. were permanently tagged to assess their thermal resistance levels during the two seasons  
139 (n=8 to n=18, Figure 1 C, Table S1). These two coral species are cosmopolitan reef-builders in Thailand  
140 and within the entire Indo-Pacific region (Brown and Phongsuwan 2012; Schmidt et al. 2012; Jain et  
141 al. 2023). Coral fragments were collected at the end of April 2018, an episode of strongest internal wave  
142 impact, and at the end of October (*Porites* sp.) and November (*Pocillopora* sp.), during the absence of  
143 internal wave stimulus. Two fragments (*Porites* sp.:  $\varnothing \sim 6$  cm; *Pocillopora* sp.: length  $\sim 5$  cm) per  
144 colony were collected using a chisel and a hammer (Table S1).

### 145 Short-term heat stress assays

146 Collected fragments were instantly transported to the Phuket Marine Biological Center (Phuket,  
147 Thailand) where they were maintained in two 500 L flow-through tanks with a flow rate of  $2.8 \pm 1.31$   
148 L/min until the start of each heat stress assay. Another 500 L source tank constantly supplied both flow-  
149 through tanks with 5  $\mu$ m-filtered seawater from the reef adjacent to the research center. Its temperature  
150 was held at constant  $29.43 \pm 0.32$  °C using a temperature-controlling device including a chiller and a  
151 heater (Titanium Heater 100 W, Schego, Germany; Temperature Switch TS 125, HTRONIC, Germany;  
152 Aqua Medic Titan 1500 Chiller, Germany). LED lights (135 W, Hydra Fiftytwo HD LED, Aqua  
153 Illumination, USA) mimicked the average light conditions of the sampling sites (Text S1).  
154 For each heat stress assay (Figure 1 D), two 40 L experimental tanks were set up inside each of the 500  
155 L flow-through tanks that were used as temperature-controlling water baths (Table S2). The seawater  
156 of all four experimental tanks was supplied by daily, manual 50% water changes from the source-tank.  
157 Each experimental tank was equipped with a temperature-controlling device, one heater, air supply, a  
158 small current pump and a temperature logger (Temperature Switch TS 125, HTRONIC, Germany;  
159 Titanium Heater 100 W, Schego, Germany; Koralia nano 900 L/h, Hydor, Italy; HOBO Pendant  
160 Temperature/Light 8K Data Logger, Onset, USA). Two coral fragments per coral colony were randomly  
161 distributed among the four tanks “34°C” ( $N = 2$ ) and “29°C” ( $N = 2$ ), resulting in one fragment per  
162 colony per treatment. The 34°C-treatment was established over the course of one day by ramping  
163 temperatures from 29°C to 34°C for 4 h, holding at 34°C for 5 h or 6 h (*Pocillopora* sp.) or for 6 h or 7  
164 h (*Porites* sp.), and decreasing temperatures to 29 °C within 4 h. After the heat exposure, corals were  
165 maintained at ambient temperatures for 10 h until the next day. While *Pocillopora* sp. fragments were  
166 subjected to the short-term heat exposure once, resulting in a 24 h experiment, *Porites* sp. corals were  
167 exposed to the treatment over two consecutive days resulting in a duration of 72 h (Figure 1 D).



168

169 **Figure 1 Study sites, experimental design, and temperature profiles of short-term heat stress**  
 170 **assays.** (A) The study area was located at Ko Racha in the Andaman Sea off the coast of Thailand, a  
 171 region that is exposed to large amplitude internal waves (light green arrow). These deep waves cause  
 172 high thermal (and environmental) variability at western reef sites (light green), while the eastern  
 173 shores (dark green) remain sheltered. (B) Two reef sites were chosen, on each shore side. (C)  
 174 Fragments of colonies (n = replicate numbers) of *Pocillopora* sp. (POC) and *Porites* spp. (POR)  
 175 were collected for heat tolerance assessment during two seasons - the season of stimulus presence and peak  
 176 of internal wave frequency and intensity (Stim. Pres.) and peak of internal wave frequency and  
 177 intensity in April and the season of stimulus and internal wave absence (Stim. Abs.) in  
 178 October/November. Fragments were subjected to a short-term heat stress assay exposing them to a  
 179 heat pulse treatment of 34 °C (Heat T). A control group was maintained at ambient temperature of 29  
 180 °C (Ambient T). (D) Temperature profiles were customized for each coral species, accounting for  
 181 their taxon-specific temperature sensitivity. Pocilloporid fragments were challenged with a single heat  
 182 stress pulse over one day (upper panel, branching coral icon), while *Porites* sp. fragments required  
 183 two heat stress pulses over two days to show a heat stress response (lower panel massive coral icon).  
 184 Measurement timepoints at the start (1) and the end (2) of each experiment are indicated.

## 185 Coral stress response variables

186 We measured two variables that assessed the stress response of each fragment before and after each  
 187 heat stress assay (timepoints (1) and (2) in Figure 1 D). Tissue coloration, a proxy for microalgal  
 188 symbiont cell density in coral tissues and therefore an indicator of holobiont health and coral bleaching  
 189 severity, was assessed using a “bleaching score”. The coloration of each individual fragment was  
 190 visually categorized on the scale from 1 (bleached, pale tissues) to 6 (healthy, dark tissues) using a coral  
 191 bleaching chart (Siebeck et al. 2006). A minimum and maximum score was recorded per fragment and  
 192 averaged. Photosynthetic efficiency of microalgal symbionts was determined by measuring effective  
 193 quantum efficiency ( $\text{yield } \Phi \text{ PSII} = (F_m' - F) / F_m' = \Delta F / F_m'$ , Genty et al. 1989) of electron transport  
 194 using a pulse amplitude-modulated fluorometer (Diving-PAM, Walz, Germany).

## 195 Statistical analyses

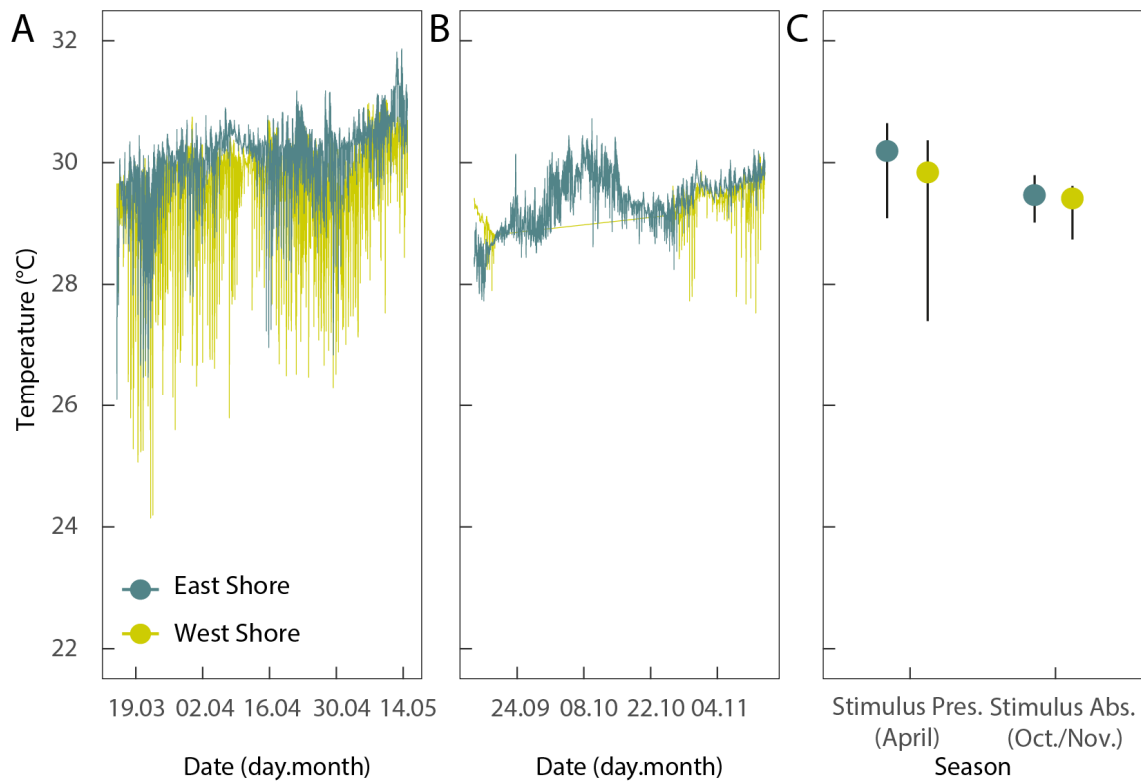
196  $\Delta$ -values of each stress response variable (end – start of each experimental part) were calculated to  
 197 represent the change or the variable over time. Based on these  $\Delta$ -values, effect sizes were estimated

198 using *dabestR* v0.2.3 6 (Ho et al. 2019). Effects of the high temperature treatment (“34 °C” vs. “29 °C”)  
199 were compared between the sites of origin (“West | High variability site” and “East | Low variability  
200 site”) and between the seasons (“Season of stimulus presence” and “Season of stimulus absence”).  
201 Statistical significance was tested in *R* (R Core Team 2013) using linear mixed effect models (*nlme* v4  
202 3.1-148 and *lme4* v1.1-23 package). Where applicable, coral colony genotype was used as a random  
203 factor.

## 204 Results

### 205 Environmental variability of the study sites

206 Temperature was recorded as a proxy for internal wave forcing and provided a measure of  
207 environmental variability on the study sites. The temperature profiles revealed that the intensities of  
208 internal waves were seasonal (Figure 2). Strong and mainly negative temperature anomalies occurred  
209 during March to April, which provided the strongest stimulus for environmental priming with a diurnal  
210 amplitude ranging between 0.7 - 5.4 °C (average amplitude of 3.0 °C ± 1.0 SD) and minimal temperature  
211 values as low as 24 °C (Figure 2 A, C). The impact of internal waves dwindled in September to  
212 November, when the anomalies decreased (Figure 2 B, C). Importantly, temperature anomalies driving  
213 the environmental variability were more frequent and intense on the exposed west shore of Ko Racha  
214 compared to the sheltered east shore (Figure 2 C). During the season when the stimulus of internal  
215 waves was present, the differences were largest between both island sides, east and west (Figure 2 A).  
216 Once the stimulus faded during the second half of the year, conditions on both island sides became more  
217 similar (Figure 2 B).  
218



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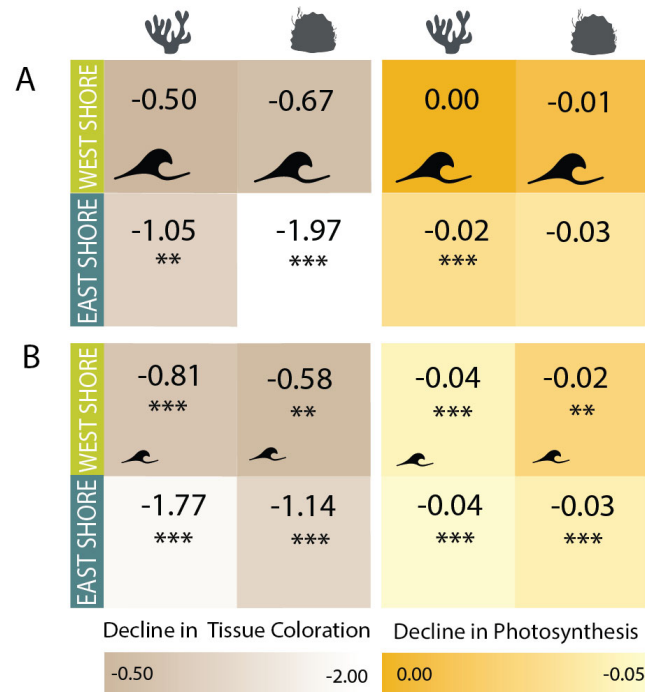
220 **Figure 2 Seasonal difference of environmental variability at the study sites.** Temperature records  
221 served as a proxy for environmental variability in the study sites. This variability was generated by  
222 large amplitude internal waves, a potential stimulus for coral stress-hardening. (A) A time series  
223 depicts that internal waves on the western shores (light green) induced strong temperature anomalies  
224 during March to April. The eastern shore (dark green) remained mostly sheltered from this stimulus.  
225 (B) Fairly constant temperatures, with almost similar dynamics on both island shores, were  
226 characteristic for the second part of the year (October to November), the season of stimulus absence.  
227 During this time, the western and eastern shores featured more similar conditions. (C) The median  
228 temperatures are indicated by circles and the average positive and negative diurnal anomalies from the  
229 median are displayed as whiskers for both island shores and seasons.

### 230 Stress responses to short-term heat stress assays

231

232 Overall, the bleaching score and photosynthetic efficiency data indicated that stress levels after the  
233 short-term heat stress assay were highest in corals from the eastern, stimulus-sheltered site, as reflected  
234 in the lighter color tones in the heatmap, where effect sizes are visualized (Figure 3). This becomes  
235 clear, as the largest and significant declines in the two variables, tissue coloration and photosynthesis,  
236 were recorded in east shore corals irrespective of the season. Corals from the west shore did not show  
237 any significant signs of stress when tested during stimulus presence (Figure 3A), but significant declines  
238 of the two variables were noted, when corals were tested during stimulus absence (Figure 3B). Here  
239 effect sizes of the heat stress treatment were fairly small ( $< 0.9$ ) for tissue coloration, indicating a mild  
240 stress response. In comparison, the large stress responses measured for the east shore corals were in the  
241 effect size range of 1.05 - 1.97 (for the tissue coloration). Interestingly, the decline in photosynthesis of  
242 the west-shore corals during stimulus absence was comparable to the decline of photosynthesis in corals  
243 from the east shore.

244



245

246 **Figure 3 Summary of thermal stress levels of corals under experimental heat exposure compared**  
 247 **between their sites of origin and seasons.** Stress levels of corals assessed in short-term heat stress  
 248 assays during the (A) season of stimulus presence (April) and the (B) season of stimulus absence  
 249 (November) are visualized. Lighter tones indicate higher stress levels. Colors represent the effect sizes  
 250 determined using Cohen's d metric as the mean differences of measurements between the heat treatment  
 251 and ambient control group of the heat stress assays. Negative values and lighter color tones indicate  
 252 decreases of the bleaching score (brown tones) and the decline in photosynthetic efficiency (yellow  
 253 tones) as a result of heat stress. Significant effects are marked as  $p < 0.001$ \*\*\*,  $< 0.01$ \*\* ,  $< 0.05$ \* as  
 254 obtained from the post hoc tests of generalized linear mixed models. Dark green= eastern sheltered  
 255 shore; light green = western exposed shore; the size of the wave icon indicates the magnitude of internal  
 256 wave impact on the reef as a stress-hardening stimulus.

257

### 258 Bleaching responses

259 Across the seasons the bleaching score of corals from the eastern sheltered reef strongly declined under  
 260 the acute heat exposure during the heat stress assay, as indicated by significant loss of tissue coloration  
 261 (29 °C group vs. 34 °C group,  $p < 0.001$ , Figure 4 A-D, Tables S3-4 and S6). Negative effect sizes were  
 262 largest in these east-shore corals, i.e., -1 to -2, which was mostly 2 to 4-fold larger, compared to those  
 263 of corals from the western reef (i.e., effect sizes of 0 to -0.8). In contrast, the stress responses of  
 264 westshore corals differed between the seasons. Overall, their bleaching score did not decline in response  
 265 to heat stress, when assessment was conducted during the season of stimulus presence (Figure 4 A, C,  
 266 Table S6). However, during the second half of the year (i.e., stimulus absence season), tissue coloration  
 267 of corals from the western shore slightly declined under experimental heat exposure with rather small,  
 268 but measurable, differences between the heat and ambient temperature control group (Figure 4 B, D,  
 269 Table S6). A small but significant decrease of the bleaching score was recorded ( $p < 0.001$ , Figure 3 B,



270 D). Further, a small-scale decline in the bleaching score was recorded for *Porites* sp. assessed during  
271 both seasons with effect sizes were around  $\sim 0.6$  (*n.s.* under stimulus presence and  $p < 0.01$  under  
272 stimulus absence, Figure 4 C-D, Table S4, S6).

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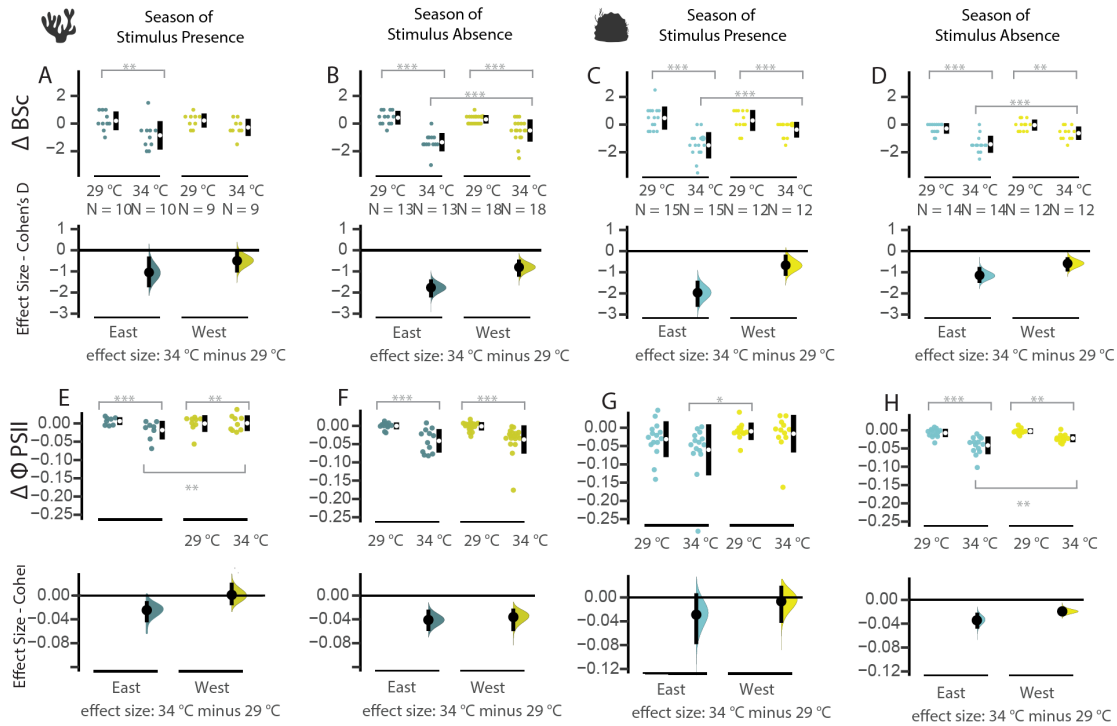
#### 274 Declines in photosynthetic efficiency

275 Across the two seasons, photosynthetic efficiency decreased significantly after the heat stress exposure  
276 in almost all coral fragments after heat exposure (29 °C group vs. 34 °C group,  $p < 0.001$ , Figure 4 E-  
277 H, Tables S3, S5, S8), except for *Porites* sp. assessed during the season of stimulus presence (*n.s.*,  
278 Figure 4 G, Table S8). Despite a significant response of almost all corals, the declines of photosynthetic  
279 efficiency measured by effect size were larger in corals from the eastern reef, i.e., -0.02 to -0.03,  
280 compared to corals from the western reef, i.e., mostly 0 or -0.01 (e.g.,  $p < 0.01$ , Figure E and H).

281

282

283



284

285 **Figure 4 Symbiont loss and photosynthetic efficiency change of corals under experimental heat**  
 286 **exposure compared between their sites of origin and seasons.** Estimation plots compare the effects  
 287 of acute experimental heat exposure (“29°C” vs. “34°C”) on (A - D) the loss of coral tissue coloration  
 288 (bleaching score = “BSc”) and (E - H) the photosynthetic efficiency of coral microalgal symbionts  
 289 (quantum efficiency of electron transport =  $\Delta\Phi$  PSII). Data is shown for the corals *Pocillopora* sp.  
 290 (“branching” coral icon) and *Porites* sp. (“massive” coral icon) during the two seasons of stress-  
 291 hardening stimulus presence and absence. The data is presented as a  $\Delta$ -value of the measured  
 292 variables (i.e., the decline of values between the start and end of stress exposure). Negative values  
 293 indicate the loss of pigments and microalgal symbiont cells from the coral tissues or the decline of  
 294 photosynthetic efficiency as a result of heat stress. Swarm plots show raw data points (first and third  
 295 row) and Cumming Estimation plots (second and fourth row) depict the effect sizes as the mean  
 296 differences between the experimental groups using Cohen’s d and a 95% confidence interval.  
 297 Significant differences between the stress responses within the groups are indicated by connecting  
 298 lines ( $p < 0.001$ \*\*\*,  $< 0.01$ \*\* ,  $< 0.05$ \* obtained from the post hoc tests of generalized linear mixed  
 299 models). Vertical error bars = 95% CI;  $N$  = individuals per treatment group.

## 300 Discussion

301 This study investigated the dynamics of coral thermal resistance in relation to a seasonally changing  
 302 priming stimulus of internal waves in the Thai Andaman Sea. Our data revealed that colonies from two  
 303 coral species, *Porites* sp. and *Pocillopora* sp., that were exposed to high environmental variability  
 304 generated by internal waves, were mostly immune to acute heat stress treatments. In contrast, their  
 305 conspecifics from sheltered shores with low environmental variability demonstrated thermal sensitivity  
 306 under these treatments. Importantly, we showed that stress-hardening through environmental variability  
 307 can exist under a stimulus comprising low temperature pulses (down to  $\sim 26.5^\circ\text{C}$  and minima of  $24^\circ\text{C}$ )

308 that fluctuate at large amplitudes ( $\sim 3^{\circ}\text{C}$  ranging between  $0.7 - 5.4^{\circ}\text{C}$ ). Secondly, we found that the  
309 thermal resistance of corals from exposed reefs persisted throughout the year in presence and in absence  
310 of the internal wave stimulus. However, thermal stress resistance levels appeared to fade slightly during  
311 the season when the stimulus was absent. Surprisingly, the dynamics of thermal stress resistance were  
312 very similar in both coral species, *Pocillopora* sp. and *Porites* sp. despite representing a naturally  
313 thermo-sensitive and a robust coral ecotype, respectively (Brown and Phongsuwan 2012; Schmidt et al.  
314 2012; Jain et al. 2023). In the following we discuss these new insights about environmental priming,  
315 while considering the regional context of the Thai Andaman Sea, and with regard to designing efficient  
316 preconditioning treatments that can enhance coral thermal resistance for coral reef conservation.

317 Pulses of cooler temperatures and large amplitudes of variation provide a stress-hardening  
318 stimulus for corals in the Thai Andaman Sea

319 Various organisms, including corals (Rivest et al. 2017; Hackerott et al. 2021)), are known to be more  
320 stress-tolerant, when previously exposed to environmental variability (Nicotra et al. 2010; Li et al. 2014;  
321 Hilker et al. 2016; Hilker and Schmülling 2019). However, the underlying environmental drivers that  
322 induce environmental variability and generate such “stress-hardening regimes” in coral reefs differ  
323 between habitat types and reef locations. Since temperature is a major determinant of coral reef  
324 distribution and warming poses a threat to corals (Hoegh-Guldberg 1999; Kleypas et al. 1999), it has  
325 been commonly used as a proxy to characterize reefs and quantify their environmental variability  
326 (Leichter et al. 1996; Oliver and Palumbi 2011a; Wall et al. 2012; Kenkel et al. 2015). In this regard,  
327 the most commonly investigated stimuli for stress hardening were elevated and/or fluctuating  
328 temperatures that are a feature of shallow reef flats, tidal pool sites or lagoon-type habitats (Palumbi et  
329 al. 2014; Camp et al. 2016, 2017). In these locations, corals experience temperature conditions that  
330 often exceed the local bleaching thresholds during midday which provide “training periods” for more  
331 severe heat wave conditions. It seems intuitive that corals exposed to such conditions, occurring in short  
332 term-intervals, “learn” to cope with the environmental stress of elevated temperatures that would  
333 usually lead to massive bleaching events.

334 Notably, the environmental variability regime in our study sites was induced by internal waves and can  
335 be so far considered a unique scenario, as it differs from the other locations where environmental  
336 priming has been typically investigated. In our study area, reef sites on the western shores of the islands  
337 were exposed to the physical forces of internal waves, which create the remarkable difference in  
338 environmental conditions between the exposed, western reef sites and the sheltered, eastern sites  
339 (Schmidt et al. 2012; Wall et al. 2012, 2015). Internal waves in the Andaman Sea transport cooler waters  
340 from the depths to the reefs, significantly increasing the amplitude of temperature variation on western  
341 island shores. Other than in reef flat or tidal pool sites where others have investigated environmental  
342 priming, internal waves at our study sites provided stimuli of lower rather than higher temperatures.  
343 Irrespective of this thermal difference, we report that corals exposed to internal waves were able to cope  
344 better with short-term acute heat stress conditions than corals living without the stimulus of internal  
345 waves. We propose that the specific amplitude of variability ( $\sim 3\text{-}5^{\circ}\text{C}$ ) in our west-shore study sites and  
346 the lowest temperatures, likely reaching lower thermal threshold (minima of  $24^{\circ}\text{C}$ ), might be equally  
347 relevant for environmental priming, as established for fluctuating temperatures that temporarily  
348 transgress upper thermal thresholds (Oliver and Palumbi 2011a).

349 Indeed, short pulses of cold water have been shown to induce an acute stress response in corals, but  
350 corals could more easily acclimatize to the cooling treatment in comparison to the heat treatment where  
351 coral health slowly declined (Roth et al. 2012). In another study, corals performed slightly better under  
352 a cooler but “sublethal” temperature compared to the ambient corals, being able to build up more mass  
353 and energy reserves (Nielsen et al. 2020). Based on these reports and our new insights, we propose that

354 the effects of low temperature pulses deserve to be further investigated, as they could offer a stress-  
355 hardening regime that might emerge as more efficient than the application of high temperature pulses.  
356 This could entail accumulation of heat stress when thermal thresholds are exceeded, leading to a  
357 negative effect.

358 To date, findings supporting the effect of environmental priming regimes on coral thermal tolerance are  
359 equivocal. While most studies indicate that a “challenging” thermal history or preconditioning regime  
360 (of thermal variability or elevated baseline temperature) enhances thermal tolerance of corals  
361 (McClanahan et al. 2005; Bellantuono et al. 2012b; Palumbi et al. 2014; Buerger et al. 2015; Schoepf  
362 et al. 2015; Kenkel and Matz 2016; DeMerlis et al. 2022; Brown et al. 2023), some report neutral or  
363 negative results, including cases where corals ended up less stress resistant compared to the control  
364 group (Putnam and Edmunds 2011; Camp et al. 2016; Schoepf et al. 2019; Henley et al. 2022). It has  
365 been suggested that such preconditioning treatments must have exerted too much stress on the corals  
366 with the consequence of having drained their energy reserves, hence did not contribute to stress-  
367 hardening but rather had a contrary effect (Hackerott et al. 2021; Wong et al. 2021). As such, regimes  
368 of variability at elevated temperatures can be difficult to implement. We often have a limited  
369 understanding of the thermal performance curve of corals, in particular in regard to their species- and  
370 location-specific thermal optimum, as well as to their upper critical temperature (Sinclair et al. 2016;  
371 Hillebrand et al. 2020). This impedes the determination of an effective environmental regime that can  
372 achieve a positive effect of stress-hardening. Considering our knowledge about the effects of cooler  
373 temperature on corals and the results in our study, we conclude that cold-stress could be an effective  
374 tool to stress-harden corals, as it can successfully trigger metabolic flexibility without the effect of stress  
375 accumulation through depletion of energy reserves or taxing coral symbionts.

#### 376 Seasonality of the stimulus and the durability of the environmental priming effect

377 Internal wave activity is seasonal in the shallow reef habitat of the Andaman Sea (Schmidt et al. 2012;  
378 Wall et al. 2012). During the first part of the year internal waves expose corals to cold, deep water at  
379 regular intervals. At that time, the western shore is usually hit by the waves and thus the stimulus is at  
380 its peak, creating the largest environmental differences between the western and the sheltered, eastern  
381 shore. Later in the year, internal wave impact dwindles and consequently the environmental conditions  
382 on both island shores, east and west, become very similar. We took advantage of this seasonality in the  
383 Andaman Sea region to explore the effects of the presence and absence of a variable stimulus. To date,  
384 the persistence of a stress-hardening effect in corals has hardly been considered in great detail and  
385 remains to be investigated (Klepac and Barshis 2022). Our results showed that the positive effect on  
386 thermal resistance had prevailed even in the absence of the stimulus. However, we observed a slight,  
387 but measurable decline of stress resistance during the season of stimulus absence. This speaks for the  
388 case that the effect of stress-hardening is lasting, but could slowly fade in the complete absence of the  
389 priming stimulus. Our observation aligns with the finding that bleaching thresholds of corals decreased  
390 seasonally, e.g., during the cooler winter season, when thermal challenges of the summer time were  
391 absent (Berkelmans and Willis 1999). Similarly, this has been the case for one Caribbean coral species  
392 (Scheufen et al. 2017), however, the same study found that other coral species did not follow this  
393 seasonality. The latter agrees with several other cases which have shown that corals maintained their  
394 stress resistance levels after transplantation from a high variability reef to an aquarium or site with more  
395 stable conditions (Morikawa and Palumbi 2019; Schoepf et al. 2019; Marhoefer et al. 2021). Also, the  
396 effect of various thermal preconditioning treatments (variable and stable) had a measurable effect on  
397 coral thermal tolerance four months later (Drury et al. 2022). Notably, Morikawa and Palumbi (2020)  
398 have observed the permanence of stress resistance across coral taxa in their coral nursery hosting  
399 resilient corals from high variability sites for two consecutive years.

#### 400 Disentangling covariates of variable environmental priming regimes

401 Reef sites that are exposed to internal waves have an important advantage over the typically investigated  
402 intertidal and lagoon-type coral habitats. They represent a reef habitat with all typical physicochemical  
403 features of an ocean-facing reef slope and can be compared to similar reefs that are sheltered from the  
404 impact of internal waves. This provides a setting where the effects of reef site-specific characteristics  
405 are accounted for and effects induced by the variability regime can be studied in isolation. In contrast,  
406 tidal pools and lagoons are habitats that are fundamentally different from the typical coral reef. Extreme  
407 light intensities and elevated salinities are characteristic for these shallow sites (Yates et al. 2014). Yet,  
408 these sites are often compared to control sites located in a proper reef slope habitat, which does not  
409 allow to disentangle the sole effect of the variability experienced in these sites. Still, it is important to  
410 consider that corals in our study were not solely challenged by temperature fluctuations caused by the  
411 internal waves. The deep-water brought into the reefs by internal waves is also typically rich in  
412 inorganic nutrients and particulate matter. Both can be either beneficial or challenging for corals and  
413 microalgal symbionts (Risk 2014). On one hand, the increase in nutrient sources could be valuable for  
414 corals and contribute to their resilience (Ferrier-Pagès et al. 2000; Meunier et al. 2022). On the other  
415 hand, particle loads may reduce light penetration and reduce photosynthetic output of microalgal  
416 symbionts (Anthony et al. 2007). In addition, sediment particles typically threaten to smother corals  
417 (Tuttle et al. 2020) that will need to spend energy on mucus production to free their tissues from these  
418 sediments. Depending on the amount of nutrients introduced by internal waves and the requirements of  
419 the corals, increases in inorganic nutrients and particulates can lead to a nutrient imbalance that can  
420 threaten the intricate balance between host and algal symbiont (Wiedenmann et al. 2012; Rådecker et  
421 al. 2015; Morris et al. 2019). Similarly, a slightly lower pH and oxygen-depleted seawater carried by  
422 internal waves (Schmidt et al. 2012; Wall et al. 2012) may pose a stressor to corals and challenge their  
423 performance (Chan and Connolly 2013; Alderdice et al. 2021). To better understand how co-variation  
424 of these variables influences coral physiology and stress-hardening in the Andaman Sea, holistic  
425 surveys will be needed that assess and consider a diversity of physico-chemical variables. However,  
426 this is not only important when studying internal wave sites. Co-fluctuating variables exist in all types  
427 of high variability reef sites, including tidal pools, reef flats, and lagoons in proximity to seagrass or  
428 mangroves (Ruiz-Jones and Palumbi 2015; Camp et al. 2016). To explain some of the ambiguous  
429 findings of coral stress-hardening studies, future research will need to explore the effects of co-varying  
430 variables, as they may play a role in modulating the effects of stress-hardening.

431

#### 432 Considerations for the design of efficient stress-hardening regimes

433 Studies of stress-hardening in corals through thermal variability regimes have sparked the idea of  
434 instrumentalizing this phenomenon to improve coral thermal stress resistance during climate change. It  
435 is anticipated that simulation of a stress-hardening stimulus can be used to enhance thermal tolerance  
436 of corals for the purpose of conservation and restoration of coral reefs (Middlebrook et al. 2008;  
437 Bellantuono et al. 2012b). The phenomenon, however, is still poorly understood and some findings  
438 remain equivocal. While many studies have reported positive effects of a variable environment on the  
439 stress tolerance in corals (Doering et al. 2021; Oliver and Palumbi 2011b; Buerger et al. 2015; Wong et  
440 al. 2021; DeMerlis et al. 2022; Brown et al. 2023), a few have not reported any improvements or rather  
441 observed declines in stress tolerance. Negative reports are likely due to stress-buildup during the  
442 preconditioning process (Hackerott et al. 2021), which can occur when a variability regime becomes  
443 too challenging (Putnam and Edmunds 2011; Camp et al. 2016; Schoepf et al. 2019; Klepac and Barshis  
444 2020; Henley et al. 2022). Also, dynamic interaction of all covariates present in the respective study  
445 sites can act as confounding factors and influence the outcomes of preconditioning (as laid out in the

446 chapter above), but most importantly, the “priming dosage” will be decisive for the success of the  
447 method. Fine-scale differences in the amplitude and frequency of variation employed (Klepac and  
448 Barshis 2022; Brown et al. 2023), the average temperature in the preconditioning regime, as well as the  
449 duration of the exposure (Bellantuono et al. 2012a; Hackerott et al. 2021; Martell 2023) deserve careful  
450 consideration. In some studies that have failed to observe a positive effect, environmental variability in  
451 the reef sites or treatments might have been too small in comparison to the ambient regime in order to  
452 elicit a measurable effect on corals. For instance, the variability ranges of the study sites in (Camp et al.  
453 2016), only differed by  $\sim 1 - 2^{\circ}\text{C}$ , which might be too small of a difference to pinpoint any effect (Rivest  
454 et al. 2017). Only a few efforts so far have set out to systematically identify optimal priming regimes.  
455 Early surveys and experiments have found that heat tolerance was correlated with the magnitude of  
456 variability, as corals from the tidal pool with the highest variability appeared to be most resistant to heat  
457 (Palumbi et al. 2014). Several recent study designs have allowed us to gain insights at a higher resolution  
458 and have found that an intermediate variability regime might likely be the most effective for stress-  
459 hardening of corals. For instance, corals living in sites of intermediate variability on Heron Island in the  
460 Great Barrier Reef (Brown et al. 2023) or in the moderately variable pools of the well-known study  
461 sites in American Samoa (Klepac and Barshis 2022), have outperformed conspecifics that had  
462 experienced lower or higher variability. Most recent findings suggest that exposure to thermal  
463 variability at a rather low average mean temperature, or involving cooling rather than heat pulses, could  
464 be more efficient than variability at a higher average temperature, as it has led to better stress-hardening  
465 results in corals (Drury et al. 2022). Future studies will be needed to further refine our understanding of  
466 how environmental priming regimes work, which will lead to the design of efficient preconditioning  
467 protocols.

468 On a last note, it still remains to be elucidated whether and at which cost(s) stress-hardened corals  
469 acclimate to perform well under challenging environmental regimes. Trade-offs will be an important  
470 aspect of future investigations. Conservation and restoration efforts that aim to apply preconditioning  
471 strategies to stress-harden corals will need to evaluate whether the gain in thermal resistance is related  
472 to any critical trade-offs. At our study sites in the Andaman Sea, resistance of west-shore corals might  
473 be coupled with a lower reef framework building capacity that was reported from these sites earlier  
474 (Schmidt et al. 2012; Wall et al. 2012). This calls for detailed investigations into the calcification  
475 capacity of these resistant corals. Recent study focussing on trade-offs (Wong et al. 2021) have found  
476 that corals from high variability sites or long-term high-temperature treatments, had either a lower  
477 metabolic capacity, lower growth rates, or lower reproductive potential compared to the control groups  
478 from stable or ambient habitats or treatments. Overall, efforts aimed at increasing thermal tolerance of  
479 corals will need a holistic approach to the subject. For the development of new interventions, it will be  
480 essential to carefully assess cost-benefits and evaluate each new method and its potential ecological  
481 consequences.

## 482 Conclusion

483 We showed that two coral species that occupy different ecological niches were receptive to the same  
484 environmental priming of cooling pulses, which improved their thermal stress resistance to acute short-  
485 term heat stress. A cold-water priming pulse can induce stress-hardening effectively. It might be a safer  
486 option compared to the implementation of high temperature peaks in variability regimes used as  
487 preconditioning treatments as heat-stress accumulation is avoided. Our study also showed that a  
488 temporary priming exposure can induce a stress-hardening effect, which, however, is likely to fade in  
489 longer absence of the stimulus, suggesting that a reapplication of a preconditioning treatment will be  
490 necessary. Most importantly, the ideal dosage and length of thermal variability exposure in a  
491 preconditioning treatment will need to be determined. Eventually, the enhancement of stress resistance

492 traits is likely to come at the cost of other traits. Therefore, research into the trade-offs that accompany  
493 thermal resistance gain in corals will be crucial in order to understand the capacity and limitations of  
494 corals to resist future thermal stress.

495

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508

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513

## 514 Authors’ contributions

515 AR conceived the study. AR and TD designed the experiment. TD, AR, MW conducted coral  
516 experiments. AR, MW, LP, and TR performed coral collection. TD, NP, MW, AR performed the data  
517 analysis. AR generated data visualization. AR, MW, TD wrote and edited the manuscript. Field  
518 facilities and logistics were provided by LP, TR and the PMBC team. The authors read and approved  
519 the final manuscript.

520

## 521 Declarations

522 The authors declare that they have no competing interests.

523

## 524 Corresponding author

525 Correspondence to: [anna.roik@hifmb.de](mailto:anna.roik@hifmb.de)

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