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DNA methylation dynamics in response to acute thermal exposure reveal the influence of heating, dose and recovery in the staghorn coral *Acropora cervicornis*

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ABSTRACT

Survival in the Anthropocene is dictated by the ability of organisms to respond to increasing environmental change. For thermally sensitive organisms, understanding the mechanisms that govern their response to environmental perturbation, and the plasticity therein, is key to predicting survival. Epigenetic mechanisms, such as DNA methylation, underlie phenotypic plasticity, regulating gene function in response to the environment. However, how the methylome responds to sublethal thermal exposure, the temporal dynamics of the response, and whether this is dose-dependent are poorly understood. Corals are sessile and have a narrow thermal envelope, making them an ideal model to explore epigenetic mechanisms underlying rapid responses to thermal variation. We exposed fragments of the staghorn coral *Acropora cervicornis* to +3 °C and +5 °C of heating for 1 and 2 days and compared the DNA methylation profiles of each dose to the ambient treatment immediately following the exposure and one week later. We present the first study to demonstrate the alteration of the methylome after acute sublethal thermal exposure in *A. cervicornis*. We observed a rapid response to thermal exposure in as little as 1 day, and no differences among doses applied immediately following heating. After a week-long recovery period, the DNA methylation profiles of heated corals no longer differed from those of the ambient treatment, suggesting the wash out of DNA methylation marks. This demonstrates the dynamic nature of the methylome, capable of rapidly changing to meet environmental conditions, though marks from acute exposure may not be retained in the long term.

1. Introduction

In the Anthropocene, ecosystems are under threat, leaving us to question whether adaptive timescales match the pace of our rapidly changing climate (Malhi et al., 2020; Martin et al., 2023). Organisms affronted with inhospitable conditions can either move or change their physiology to survive (Brandon, 2014). Adaptive physiological responses are accomplished via phenotypic plasticity, genetic evolution or both (Aitken et al., 2008; Zhu et al., 2012). Physiological changes are mediated in part by epigenetic mechanisms, which can ultimately lead to adaptation (Skinner, 2015). Given the severity and pace of climate change effects on ecosystems, adaptive epigenetic mechanisms and their temporal dynamics are being explored to better predict the stress tolerance in at-risk species under the increasing pressure (Carneiro and Lyko, 2020; Hofmann, 2017; Yen et al., 2024).

Epigenetic modifications include DNA methylation, histone post-translational modifications, and small RNAs. These mechanisms interact synergistically in response to environmental signals, modulating the epigenome which in turn influences downstream gene expression and regulation in a reversible manner (Eirin-Lopez and Putnam, 2019; Feil and Fraga, 2012; Miryeganeh, 2021). For example, epigenetic modifications upstream of genes, or within actively expressed genes have been found to influence the initiation of transcription and alter or inhibit gene expression in some organisms (Adrian-Kalchhauser

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et al., 2020). Importantly, environmentally triggered epigenetic marks will be reinforced under predictable environmental conditions, providing the basis for a rapid acclimatization process in the form of cellular stress memory, which may therefore enable the foundation for rapid adaptation (Oberkofler et al., 2021).

Recent work has highlighted the influence of the environment on epigenetic mechanisms that can induce rapid changes. If environmentally induced changes are retained, environmental memory is the result, which can contribute to longer-term acclimatization and even heritable changes across generations (i.e., adaptation). Environmental stress memory refers to the process in which a memory of abiotic stressors is developed (Hackerott et al., 2021; Hilker and Schmülling, 2019). Memory of environmental change appears to be related to changes in genome function, rather than genetic mutations, at the level of both of gene expression (Bellantuono et al., 2012; Majerová and Drury, 2022; Mirbahai and Chipman, 2014) and epigenetic mechanisms (Beer et al., 2014; Dion et al., 2022; Hackerott et al., 2023; Lämke and Bäurle, 2017; Putnam et al., 2016; Rodríguez-Casariego et al., 2018), with evidence of heritable epigenetic marks from some stressors (Fitz-James and Cavalli, 2022; Liew et al., 2020; Putnam et al., 2020; Putnam and Gates, 2015). While several mechanisms have been identified in relation to environmental memory, the doses required to elicit changes in such mechanisms, how rapidly those changes can occur, and then be either lost or "remembered" are largely unknown, particularly in non-model organisms.

Currently, the most widely studied epigenetic mechanism in most taxa is DNA methylation (Eirin-Lopez and Putnam, 2019; Hofmann, 2017). This modification primarily occurs on Cytosine bases adjacent to a Guanine (i.e., CpG sites), and the ways in which the environment influences DNA methylation dynamics are currently an active area of research. In invertebrates, intragenic methylation is generally positively correlated with expression (Gavery and Roberts, 2014; Wang et al., 2014), particularly with the stable activation of housekeeping genes (Gavery and Roberts, 2014). However, this relationship is not strictly linear. Recent studies have shown substantial transcriptional differences between cell types with similar methylation landscapes (de Mendoza et al., 2020; Gatzmann et al., 2018; Harris et al., 2019). It is debated whether the mosaic of the DNA methylome in invertebrate organisms serves to regulate genes or transposable elements (i.e., gene body methylation) via the recruitment of proteins that reduce expression, or by inhibiting/promoting binding of transcription factors to DNA (Moore et al., 2013), or whether DNA methylation acts as genomic protection from parasitism (Dimond and Roberts, 2016; Regev et al., 1998; Ying et al., 2022). Transposons have been found to be the target sites of DNA methylation and thus it has been suggested that DNA methylation at CpG sites may have evolved as genomic protection (Ying et al., 2022).

Major questions remain in our ability to gauge the potential for epigenetic mechanisms to mitigate the effects of climate change: (1) what are the temporal dynamics of epigenetic modifications? and (2) how does the dose of stress influence the outcome? Knowing how fast the mechanisms can operate and how much stress is required to evoke changes is necessary to determine whether these responses will be on par with the pace of warming.

Coral reefs are perhaps the best example of an ecosystem in peril. Occupying a small global footprint (Lyons et al., 2024), they support the livelihoods of more than 1 billion people on Earth (Sing Wong et al., 2022). Yet, they continue to face significant decline from anthropogenic climate change and other stressors (Klein et al., 2024). Scleractinian corals (*hereto* forth, called corals) form the physical framework of reefs. Due to the importance of coral reefs, a large body of research has focused on understanding coral resilience and the mechanisms that underlie resilience (Hackerott et al., 2023; Hughes et al., 2010; Putnam et al., 2017). Corals are sessile, long-lived species with ages on the scale of decades to centuries and are regularly subjected to high environmental variability in salinity (Coles and Jokiel, 1992), temperature (Carilli et al., 2012; Schoepf et al., 2015), light (Edmunds et al., 2018), and other abiotic factors across multiple timescales (Carilli et al., 2012; Koweek et al., 2015). There is strong evidence for coral environmental stress memory across timescales ranging from days to decades (Bellantuono et al., 2011; Brown et al., 2014; Hackerott et al., 2021 and references therein; Majerová et al., 2021; Martell, 2023; Middlebrook et al., 2008). However, the mechanisms allowing corals to rapidly respond to rapid environmental variation are not fully understood.

DNA methylation in corals appears to be related to maintaining transcriptional homeostasis (Liew et al., 2018). Given that corals are holobionts, organismal consortia living in symbiosis from multiple parasitism events throughout extant coral evolutionary history (Bhattacharya et al., 2016; Stanley, 2003), it is possible that DNA methylation could serve to both regulate genes related to maintaining homeostasis and provide genomic protection. It has also been proposed that absence of germline methylation enables random variation that contributes to phenotypic plasticity (Roberts and Gavery, 2012). Changes in the coral methylome have been attributed to environmental nutrient and thermal regimes, ocean acidification, seasonal cycles, and with acclimation to novel environments (Dimond and Roberts, 2016, 2020; Hackerott et al., 2023; Putnam et al., 2016; Rodríguez-Casariego et al., 2018, 2022, 2024). Consistent with other invertebrates, coral genome methylation is bimodal, displaying hypermethylation of housekeeping genes, and hypomethylation of inducible genes (Dimond and Roberts, 2016). The latter appears to be consistent with differential gene expression in response to temperature and ocean acidification stress in some coral species (Dimond and Roberts, 2016). Accordingly, the weak DNA methylation observed in genes that are reactive to environmental perturbations supports the role of this epigenetic modification mediating transcriptional plasticity in response to environmental changes (Dimond and Roberts, 2016; Dixon et al., 2018).

Much of the work on coral epigenetic mechanisms has broadly focused on early life stage environmental priming (e.g., Liew et al., 2018; Putnam et al., 2016) or inherited epigenetic marks as the fodder for rapid transgenerational acclimatization (e.g., Dimond and Roberts, 2020; Liew et al., 2020). While the link between environmentally driven changes in DNA methylation and phenotypic plasticity in corals seems now to be evident (Hackerott et al., 2023), questions remain as to how this epigenetic modification is shaped by the environment and whether and how epigenetic changes on finer timescales might influence organismal performance within an individual's lifetime. More precisely, the role of stress intensity and duration in triggering and reinforcing DNA methylation modifications is still unknown, particularly when it comes to the presence of thresholds above which modifications in the epigenome may last longer or even become permanent (i.e., cellular memory). The present manuscript aims to address these outstanding questions by investigating the influence of thermal exposure dose on DNA methylation, along with the temporal dynamics (i.e., washout vs. retention) of the induced marks.

For that purpose, the DNA methylation profiles of corals exposed to a range of thermal stress doses ($n = 5$) were examined over a short timescale, both immediately after exposure (1 and 2 days), and following an 8-day recovery period at ambient temperature. The proportion of DNA methylation states following acute thermal exposure were quantified, along with the frequency of methylation and demethylation transitions. Acute heating induced changes in DNA methylation profiles which were dose-independent and wash out of these changes occurred on the order of a week.

2. Materials and methods

2.1. Sample collection & exposure

Samples of the staghorn coral, *Acropora cervicornis*, were collected from a nearshore coral nursery in Broward County, Florida established in 2007 (Larson, 2010). The donor colonies of all genotypes were located within nearshore hardbottom and Inner Florida Reef Tract environments

within the bounds of Broward County, and they were confirmed to be distinct genotypes by microsatellite analysis (Larson, 2010). All coral colonies had been in the nursery for at least 5 years at between 2 and 5 m depths on trees or modules before they were collected, providing a common garden setting. Previous work on these genotypes found significant differences between genets in growth rates in the nursery (U et al., 2023) along with growth and survival after outplanting (Goergen and Gilliam, 2018). A full phenotypic description of the present work on these genotypes is found in Martell (2023).

Eight genotypes (C-J) of corals were fragmented into ramets (independent genetic clones of each genotype; *sensu* Cohen, 2018), allowed to heal for 2 months, and collected for experiments. Two ramets of each genotype were distributed to each treatment tank randomly and held at ambient temperature (26 °C) until the exposure began. During the exposure period, ramets of each genotype either remained at ambient temperature (control treatment) or were exposed to low (29 °C) or high (31 °C) temperatures for 1 or 2 days in a factorial design, resulting in five doses along a stress continuum (Fig. 1, Table 1).

Treatment temperatures were chosen based on the reduction of the ratio of gross photosynthesis to respiration of the same genotypes at 29 °C and 31 °C, indicating they were sublethal but influenced metabolism (Martell and Zimmerman, 2021), while staying below 32 °C, the annual bleaching threshold for the region (Glynn and D'Croz, 1990). Bleaching thresholds are estimated by NOAA's Coral Reef Watch program to be 1 °C above the maximum monthly mean sea surface temperature. *In situ* temperature was measured every minute using HOBO loggers (Onset Corp.) in all tanks for the entire experimental period and used to calculate heat accumulation above the average maximum monthly mean for April and May of 25.8 °C (NOAA Coral Reef Watch, 2017) in degree heating days (°C·days), as in Leggat et al. (2022).

Corals were sampled at the conclusion of the 2-day exposure period (post heating, Timepoint 1, $n = 40$: 1 ramet per genotype × 8 genotypes per treatment × 5 treatments) and after an 8-day recovery period in a common tank held at ambient temperature. During the acute heating period, corals in the treatment with the greatest exposure (i.e., 31 °C for 2 days) began to pale and two of these corals died during the recovery period, indicating the 9 DHD treatment was lethal. Because the intent was to assess the effect of sublethal stress on DNA methylation patterns, corals in this treatment were excluded from analysis in the second timepoint (post recovery, Timepoint 2, $n = 32$: 1 ramet per genotype × 8 genotypes per treatment × 4 treatments). There was no heat accumulation during the recovery period (Timepoint 2), therefore the treatments were distinguished by the heat accumulation at the conclusion of the thermal exposure period (Timepoint 1; Fig. 1). At each timepoint, coral fragments were removed from treatments, immediately frozen at −80 °C, and an apical piece was preserved in RNALater at −4 °C for 24 h, then stored at −80 °C until DNA was extracted.

Table 1
Treatment conditions for acute thermal priming of *Acropora cervicornis* from Martell (2023; see also Fig. 1B). The table includes treatment name (e.g., Ambient, A), mean temperature ± 2SE (Temperature, °C), duration of the heating period (Duration, d), and heat accumulation (i.e., thermal dose) in degree heating days (DHD, °C·d) for the heating period. Heat accumulation was defined as any duration wherein temperature > NOAA's local maximum monthly mean + 1 °C. The maximum monthly mean temperature for April and May 2017 in Broward County, Florida was 25.8 °C. All fragments were exposed to 0.49 °C·d of thermal stress in the field before collection. For clarity, this value was not added to the heat accumulation doses below.

Treatment	Temperature (°C)	Heating Duration (d)	Heat Accumulation (°C·d)
A	25.9 ± 0.20	0	0
2 DHD	28.8 ± 0.06	1	2
4 DHD	28.9 ± 0.04	2	4
5 DHD	31.3 ± 0.04	1	5
9 DHD	31.3 ± 0.08	2	9

2.2. DNA extraction

Samples were thawed, washed twice with 500 µL 1· PBS and transferred to a grinding tube (VWR) with 425 µL DNA Shield (Zymo). Samples were ground with a pestle until completely powdered, then vortexed for at least 5 s. Once all powder was suspended, tubes were centrifuged at 14,500 rpm for 1 min. Genomic DNA was extracted from 200 µL supernatant using the Quick-DNA Miniprep Plus kit according to the Biofluids and Cells Protocol provided by the manufacturer (Zymo), with a vacufuge step after washing at 30 °C for 2 min. DNA quality was assessed by spectrophotometric ratios (260/280 nm > ~1.8) and gel electrophoresis. DNA concentration was determined using the Qubit dsDNA broad range kit (Thermofisher).

2.3. Methylation sensitive amplification polymorphism (MSAP)

In brief, MSAP is a modification of the Amplified Fragment Length Polymorphism (AFLP) protocol that detects DNA methylation using two restriction enzymes, *Hpa*II and *Msp*I, which recognize the same 5′-CCGG-3′ site but differ in their sensitivity to cytosine methylation. *Hpa*II cuts only when the external cytosine is unmethylated, whereas *Msp*I cleaves regardless of external cytosine methylation but does not cut when the internal cytosine is fully methylated (Reyna-López et al., 1997). Thus, by comparing banding patterns from independent digestions using both enzymes on the same DNA, four methylation states per loci can be inferred: non-methylated (*Hpa*II+ & *Msp*I+), hemi-methylated (*Hpa*II+ & *Msp*I-), internally methylated (*Hpa*II- & *Msp*I+) and hypermethylated/mutated (*Hpa*II- & *Msp*I-). Although MSAP effectively detects genome-wide DNA methylation variation and allows affordable epigenetic analysis between experimental groups, it is restricted to CCGG sites, does not provide the genomic context of these epigenetic

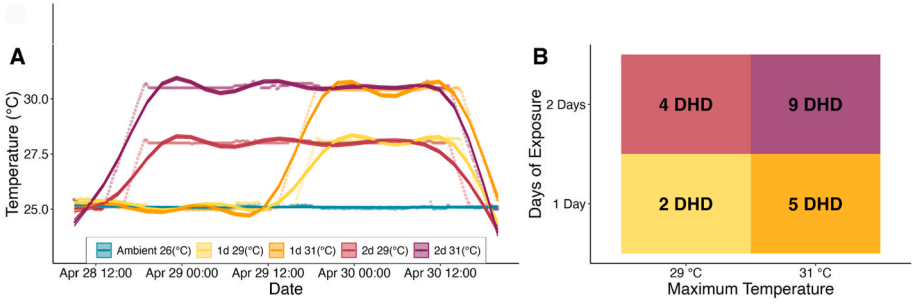

Fig. 1. Experimental Design: (A) The experimental temperature series during the priming period, depicting five temperature dose treatments, and (B) the heat accumulation above ambient (control), displayed in experimental degree heating days (DHD, °C·d). Temperature doses are displayed by colors (teal: ambient or 0 DHD, yellow: 2 DHD, orange: 5 DHD, red: 4 DHD, purple: 9 DHD). In panel A, temperature measurements, averaged over 10-min intervals, are shown by the points while the lines depict smooth curves from generalized additive models.

alterations and is sensitive to DNA quality and experimental conditions, which may affect reproducibility. Nonetheless, it remains a valuable tool for studying epigenetic variation in non-model organisms lacking a reference genome, where whole-genome bisulfite sequencing is otherwise impractical.

Coral DNA samples were prepared for Methylation-Sensitive Amplification Polymorphism (MSAP) according to Reyna-López et al. (1997) with the modifications developed for *Acropora cervicornis* corals by Hackerott et al. (2023). Samples were standardized to 36.5 ng/μL DNA and 5.5 μL aliquots of the standardized samples were used to characterize DNA methylation patterns. Standardized samples were prepared for digestion-ligation with *Eco*RI/*Hpa*II or *Eco*RI/*Msp*I restriction enzymes (Table 2). The digestion-ligation product was diluted with 90 μL molecular grade water and amplified in two pre-selective PCR reactions with *Eco*RI + X and *Hpa*II/*Msp*I + X primers and 4 μL of digestion-ligation product. Gel electrophoresis was used to examine the products to ensure that the digestion-ligation and pre-selective PCR reactions were effective (see MSAP Protocol Optimization in the Supplemental Information, SI).

The pre-selective PCR product was diluted with 90 μL molecular grade water and 2 μL was further amplified with four fluorescently labeled selective primer-pair combinations (Tables S1 and S2, SI: Selection of Selective Primer Combinations): A1, A2 (*Hpa*II/*Msp*I + AXX), B1, and B2 (*Hpa*II/*Msp*I + TXX), in four separate selective PCR reactions (Table 2). Selective PCR products were multiplexed with 65 % HEX (A1 or B1), 25 % FAM (A2 or B2), and 10 % molecular grade water. Samples were then prepared for fragment analysis using 2 μL multiplexed mixture, 1 μL MapMarker 1000-Rox (Bioventures), and 9.5 μL Hi-Di Formamide (Thermofisher). The final mixture was analyzed on an ABI Prism 310 at Florida International University's DNA Core Facility.

2.4. Determination of methylation states

2.4.1. Peak calling

Restriction profiles were generated for each primer combination using GeneMapper v.3.7 software (Applied Biosystems, Foster City, CA, United States), considering signals above 50 Relative Fluorescent Units (RFU) for 6-HEX and 6-FAM, and a peak width of 2 base pairs (bp) within the 100 to 1000 bp range. To ensure reproducibility, 10 % of the analyzed samples were replicated across the entire protocol. Epialleles were deduced for each locus and sample from the resulting binary matrix using the R-package *msap* (Pérez-Figueroa, 2013) based on the presence or absence of *Eco*RI-*Hpa*II and *Eco*RI-*Msp*I bands, resulting in four possible methylation states: Non-Methylated (NMT, 1/1), Hemi-Methylated (HMM, 1/0), Internal Cytosine Methylated (ICM, 0/1), or Hypermethylated/Lack of Target (HPM, 0/0); Table S3).

Table 2
MSAP primer and adapter sequences used in each PCR step. The PCR steps included the digestion-ligation, pre-selective combination A or B, and selective A1, A2, B1 or B2. The selective primers were fluorescently labeled with HEX or FAM.

Step/Combo	Oligo Name	Sequence (5′ to 3′)	Label
Digestion-Ligation	*Eco*RI Adaptor Fwd	CTCGTAGACTGCGTACC	
	*Eco*RI Adaptor Rv	AATTGGTACGCAGTCTAC	
	*Hpa*II/*Msp*I Adaptor Fwd	CGTTCTAGACTCATC	
	*Hpa*II/*Msp*I Adaptor Rv	GACGATGAGTCTAGAA	
Pre-Selective combo A	Pre *Eco*RI + C	GACTGCGTACCAATTCC	
	Pre *Msp*I-*Hpa*II + A	GATGAGTCTAGAACGGA	
Pre-Selective combo B	Pre *Eco*RI + A	GACTGCGTACCAATTCA	
	Pre *Msp*I-*Hpa*II + T	GATGAGTCTAGAACGGT	
A1	*Msp*I-*Hpa*II-ATC	GATGAGTCTAGAACGGATC	HEX
	*Msp*I-*Hpa*II-ACA	GATGAGTCTAGAACGGACA	HEX
A2	*Msp*I-*Hpa*II-ACT	GATGAGTCTAGAACGGACT	FAM
	*Msp*I-*Hpa*II-AAG	GATGAGTCTAGAACGGAAG	FAM
B1	*Msp*I-*Hpa*II-TCA	GATGAGTCTAGAACGGTCA	HEX
	*Msp*I-*Hpa*II-TAG	GATGAGTCTAGAACGGTAG	HEX
B2	*Msp*I-*Hpa*II-TTG	GATGAGTCTAGAACGGTTG	FAM
	*Msp*I-*Hpa*II-TCT	GATGAGTCTAGAACGGTCT	FAM

2.4.2. Clarification of hypermethylation

To clarify whether 0/0 represented Hypermethylation (HPM) or Lack of Target due to genetic variation, we followed a similar approach to Fulneček and Kovařík (2014). First, the *msap* command was run to classify cases of no bands (i.e., 0/0) as uninformative (NA). Next, each locus with NAs was examined across all samples within a genotype (i.e., at both timepoints and all treatments) to determine whether the locus was polymorphic. The presence of any methylation state other than NA (i.e., polymorphism) indicated that the target sequence was indeed present within that genome and was therefore reclassified as HPM for that genotype (Table S3). If the locus consistently was NA within a genotype (i.e. monomorphic in 100 % of the cohort), the locus remained classed as Lack of Target and uninformative (i.e., NA) for that genotype and was excluded in downstream analyses. Each genotype was examined individually to clarify hypermethylation and the clarified methylation states were retained.

2.4.3. Methylation profiles

Of the total number of loci identified, those determined to be susceptible to methylation (MSL: methylation-susceptible loci; Table S3) were retained (Herrera and Bazaga, 2010). MSL were further filtered to retain loci that were polymorphic as described in Pérez-Figueroa (2013). Finally, loci that contained cases of "Lack of Target" for any genotype (described above in 2.4.2) were removed from downstream analysis to focus on epigenetic variation rather than genetic variation. The multivariate DNA methylation profile was defined as the pattern of each categorical methylation state (e.g., u = NMT, h = HMM, i = ICM, or f = HPM) across each retained loci (i.e., clarified polymorphic MSL) for a particular sample.

In addition to determining categorical methylation states, the *msap* function also includes an initial analysis pipeline fully described in Pérez-Figueroa (2013). These preliminary analyses utilize two binary datasets: the matrix of polymorphic MSL (1: locus is in a methylated state, 0: locus is in the non-methylated state) and the matrix of non-methylated loci (NML) following AFLP genetic analysis (1: the locus is present in the genome, 0: the locus absent in the genome; Pérez-Figueroa, 2013). Analysis of Molecular Variance tests (AMOVAs) compared both MSL and NML profiles across genotypes (Excoffier et al., 1992) and these were also visualized with Principal Components Analysis (PCA). Mantel tests were used to evaluate the correlation between genetic (NML) and epigenetic (MSL) matrices (Mantel, 1967). Other than these preliminary analyses and PCAs, all main results and figures presented here are derived from the categorical dataset of polymorphic MSL after the clarification of loci as HPM (described above).

2.5. Comparison of DNA methylation profiles

First, broad patterns of DNA methylation were characterized (see section 2.5.1) across all thermal doses (DHD: 0, 2, 4, 5, and 9) and timepoints (T1 and T2). Additionally, differences in multivariate DNA methylation profiles were evaluated across the broad independent variables of the study: treatment (control or heated), timepoint (T1 and T2), and genotype (genotypes C-J). To evaluate the initial and lasting influence of heat exposure (section 2.5.2), DNA methylation profiles were then compared across two levels of heat exposure treatments: 1) treatment (control or heated) or 2) heated dose (DHD: 2, 4, 5, and 9). This evaluation of heat response was conducted at each timepoint, immediately after heat exposure (T1) and following 8 days of recovery (T2). Finally, two approaches were used to describe temperature-induced differences in DNA methylation within each genotype (section 2.5.3). First, the degree of difference in DNA methylation profiles between heated and control treatment clonemates (i.e., ramets of the same

genotype) were quantified and compared across heated doses and timepoints. Additionally, shifts in methylation states were quantified between heated and control treatment clonemates and, again, compared across thermal doses and timepoints.

2.5.1. Broad DNA methylation patterns

The proportion of each methylation state (i.e., NMT, HMM, ICM, or HPM) across retained polymorphic MSL was quantified for each sample. The proportion, or frequency of each state, was then averaged within thermal doses and timepoints to characterize broad changes in methylation frequency. To compare DNA methylation profiles across all genotypes, treatments, and timepoints, permutational analysis of variance (PERMANOVA) and dispersion (PERMDISP) were performed using the *vegan* package in R (Oksanen et al., 2022) with the Gower distance for categorical methylation states (*cluster* package, Maechler et al., 2023). In the PERMANOVA model, each variable of interest (treatment, timepoint, and genotype) was included as a predictor along with the interactions between treatment × timepoint and treatment × genotype. Significantly different methylation profiles were further compared using PERMDISP to determine whether differences were driven by centroid location or group dispersion.

2.5.2. DNA methylation response to heating

To determine if acute thermal exposure induced alterations of the coral methylome, DNA methylation profiles were compared between the control and heated (pooled) treatments at the conclusion of the acute heat exposure (Timepoint 1). Like the analysis pipeline described above, PERMANOVA and PERMDISP analyses were conducted, with heat treatment (heated vs. control) as the predictor of interest, and the PERMANOVA stratified by genotype. To assess the dose-dependence of the DNA methylation response to acute heating, the analysis pipeline was repeated on only heated samples to compare the DNA methylation profiles across thermal doses.

A Discriminant Analysis of Principal Components (DAPC) was performed to visualize differences between treatments and thermal doses, setting the thermal doses as *a priori* clusters (k = 5: DHD 0, 2, 4, 5, and 9) following Miller et al. (2020). The number of Principal Components to retain was set at k-1 based on Thia (2023). To evaluate the persistence of temperature-induced DNA methylation responses over time, the analysis described above (PERMANOVA, PERMDISP, and DAPC) was repeated for Timepoint 2, following the 8-day recovery period at ambient conditions.

2.5.3. Genotype-accounted DNA methylation responses

Due to the effect of genotype on DNA methylation patterns, two approaches were conducted to further evaluate heat-induced methylation responses by comparing the DNA methylation profiles of heated corals to those of the respective control coral (i.e., the same genotype). The purpose was to more accurately describe the difference triggered from heat exposure by using the ambient control coral as the baseline DNA methylation profile for each specific genotype. These "genotype-accounted" changes in DNA methylation were first quantified for the multivariate DNA methylation profiles. A distance matrix was calculated (Gower dissimilarity of the categorical variables treatment, genotype and timepoint) to quantify the pairwise "shifts" in the DNA methylation profiles between each sample (Hackerott et al., 2023). The pairwise dissimilarity matrix was filtered to retain the pairwise distances of interest. Specifically, the contrasts between each heated coral and the corresponding ambient control of the same genotype and within the same timepoint were retained (T1 $n = 8$ genotypes × 4 thermal doses = 32; T2 $n = 8$ genotypes × 3 thermal doses = 24, without 9 DHD). The normality of the resulting dataset of genotype-specific dissimilarities was confirmed by Shapiro-Wilk test (Shapiro and Wilk, 1965) and visually via histogram. To assess the dose-dependence and temporal dynamics of DNA methylation while accounting for genotype-specific baselines, the degree of dissimilarity was compared between timepoints and treatments via two-way ANOVA. Additionally, the slopes of dissimilarity over time were compared between temperature doses using least-squared means (*emmeans::lstrends*) of a linear model (dissimilarity ~ dose * timepoint). Independent ramets of all eight genotypes were represented in each treatment, so that linear models represented the average treatment dissimilarity. A negative slope would indicate a treatment converging (i.e., becoming more similar) with the control, while a positive slope would indicate the treatment becoming more different from the control. Finally, the dissimilarity of DNA methylation profiles was compared across heat doses within each timepoint with linear models and ANOVAs to further evaluate the dose-dependence of heat-induced DNA methylation shifts.

Next, the genotype-accounted differences in DNA methylation profiles were further characterized based on the type of shift from the baseline profile. This shift from baseline was calculated for each loci using the control coral of the same genotype as the corresponding baseline state. First, each methylation state was converted into a methylation "level," where non-methylated loci had 0 % methylation, hypermethylated loci had 100 %, and loci with either hemi-methylation or internal cytosine methylation were both considered as 50 % methylated. To determine if heated corals experienced a gain ($\Delta = +100$ % methylation), partial gain ($\Delta = +50$ % methylation), partial loss ($\Delta = -50$ % methylation), or full loss ($\Delta = -100$ % methylation) of DNA methylation, the methylation state of each loci in the control coral was subtracted from each of the heated corals, within each respective genotype (Table S3). When the heated and control corals were identical, there was no difference in methylation level. These matrices of methylation changes were calculated for each sample and the frequency of each methylation shift was quantified for each thermal dose and timepoint.

3. Results

3.1. Determination of methylation states

3.1.1. Peak calling results

Selective primer sets A1 and A2 (*Hpa*II/*Msp*I + AXX; Table 2) resulted in the amplification of a total of 257 and 362 loci, respectively. Similarly, selective primer sets B1 and B2 (*Hpa*II/*Msp*I + TXX; Table 2) resulted in 262 and 290 loci, respectively. The initial determination of methylation states (i.e., where the case of "no bands" was considered as uninformative) identified 656 Methylation-Susceptible Loci (MSL) across all primers and 324 (49 % of total MSL) were polymorphic. DNA methylation varied by genotype (pooled across treatments and timepoints, Initial MSL AMOVA, $p = 0.002$, Table S4A; Fig. S1A left) and analysis of Non-Methylated Loci (NML = 432) confirmed that the genotypes were genetically distinct (Initial NML AMOVA, $p < 0.0001$, Table S4A, Fig. S1B left). Additionally, epigenetic (MSL) and genetic (NML) variation were correlated (Initial Mantel, $p = 0.01$, Table S4A).

3.1.2. Clarification of hypermethylation

In the process of clarifying instances of hypermethylation or lack of target, considering a lack of band as hypermethylation resulted in 506 additional MSL being identified, for a total of 1162 MSL. Yet, a similar total number of loci (359, 31 % of total MSL) were polymorphic. As expected, clarifying lack of bands as hypermethylation increased the overall proportions of the full methylation state (Table S5). Like the previous analysis (section 3.1.1), DNA methylation profiles varied by genotype (Clarification MSL AMOVA, $p < 0.0001$, Table S4B; Fig. S1A right), and epigenetic and genetic variation were marginally correlated (Clarification Mantel, $p = 0.10$, Table S4B). There were fewer NML ($n = 8$) with these analysis parameters and consequently, analysis of Non-Methylated Loci did not detect genetic differences between genotypes (Clarification NML AMOVA, $p = 0.62$, Table S4B, Fig. S1B right), likely due to less statistical power. After hypermethylation was clarified and NAs were removed from the dataset, a total of 226 polymorphic MSL

remained. Of the original NAs, 26.33 % were imputed as hypermethylated (HPM). The average proportions of methylation across samples (i.e., genotypes) by treatment were consistent with previous studies on the same species (Hackerott et al., 2023; Rodríguez-Casariego et al., 2018; SI, Fig. S2).

3.2. Comparison of DNA methylation profiles

3.2.1. Broad DNA methylation patterns

DNA methylation profiles differed across genotypes, with 21 % of the variation in methylation profiles explained by genotype (Overall: genotype PERMANOVA $p = 0.001$, Table 3). The preliminary pooled analyses across genotypes also identified a significant effect of genotype on methylation sensitive loci (Initial: MSL AMOVA $p = 0.002$, Table S4A; Secondary Clarification: MSL AMOVA $p < 0.0001$, Table S4B), further supporting the influence of genotype on methylation. The differences across genotypes were due to mean (i.e., centroid) differences between groups (PERMANOVA $p < 0.05$, Table 3) rather than differences in group dispersion (Overall: genotype PERMDISP $p > 0.05$, Table 4). The influence of heating treatments also differed between timepoints (Overall: treatment × timepoint PERMANOVA $p = 0.02$, Table 3), therefore each timepoint was further analyzed individually.

3.2.2. DNA methylation response to heating

When analyses were performed by timepoint, there was an effect of treatment at Timepoint 1, but not at Timepoint 2 (Timepoint 1 All: treatment $p = 0.03$, Timepoint 2 All: treatment $p = 0.39$, Table 3). The heat-induced methylation profiles did not differ across the range of thermal doses at Timepoint 1 (Timepoint 1 Heated: dose PERMANOVA $p = 0.97$, Table 3; Timepoint 1 Heated; dose PERMDSIP $p = 0.81$ Table 4; Fig. 2A). After the recovery period, there was no longer a difference between heated and control DNA methylation profiles nor between thermal doses (Timepoint 2 All: treatment $p = 0.39$, Timepoint 2 Heated: dose $p = 0.54$, Table 3, Fig. 2B). However, there was a difference in dispersion, that is, how much they varied, between heated and control treatments (Timepoint 2 All; treatment PERMDSIP $p = 0.04$, Table 4). Overall, no difference in DNA methylation profiles was detected across thermal doses, and the heat-induced shift in DNA methylation (pooled heated vs. control) which was observed at Timepoint 1 was lost after recovery.

After the thermal exposure, the first and second components of the DAPC (LD1 and LD2) explained 86.41 % and 8.47 % of the variance between treatments, respectively (Fig. 2A). The heated treatments separated from the control treatment along the LD1 axis, yet no clear separation among heated treatments was apparent (Fig. 2A), consistent with the PERMANOVA results where a dose-independent effect of heating was detected (Timepoint 1 All: treatment PERMANOVA $p = 0.03$, Timepoint 1 Heated: dose PERMANOVA $p = 0.97$, Table 3). Following recovery, the DNA methylation profiles of the heated treatments were more dispersed than those of the control treatment (Fig. 2B), particularly across the LD1 axis (which explained 64.8 % of the variation), consistent with the PERMDISP results (Timepoint 2 All; treatment PERMDSIP $p = 0.04$, Table 4).

3.2.3. Genotype-accounted DNA methylation response

The degree of dissimilarity in DNA methylation profiles between the heated and control treatments declined between Timepoint 1 and 2 (time ANOVA $p = 0.004$, Table 5A.1, Fig. 3A). The average dissimilarity in DNA methylation profiles was higher immediately following the acute heat exposure and dissimilarity decreased (*i.e.,* heated treatments were more like the respective control) after the 8-day recovery period (negative slope estimates in Table 5A.2, Fig. 3A). There was not a difference in the degree of dissimilarity between thermal doses (dose ANOVA $p = 0.43$, Table 5A.1), and the change in dissimilarity over time did not differ between doses (dose × time ANOVA $p = 0.64$, Table 5A.1; pairwise comparisons of slopes all $p > 0.05$, Table 5A.3). Additionally, the dissimilarity of DNA methylation profiles did not differ between doses within each timepoint (Timepoint 1 dose ANOVA $p = 0.99$, Timepoint 2 dose ANOVA $p = 0.42$, Table 5B). These genotype-accounted results support those from the PERMANOVA (Table 3) and DAPC analyses (Fig. 2), where the heat-induced shift in DNA methylation was lost over time and patterns were consistent across thermal doses.

At Timepoint 1, the most common change in DNA methylation induced by acute heat exposure was a partial loss, which occurred in 23.3–25.8 % of loci (Figs. 3B and 4A). A partial gain of methylation was the second most common, occurring in 15.3–18.1 % of loci. A complete

Table 3
PERMANOVA comparisons of DNA methylation profiles between genotypes across treatments and between timepoints. Values include the Comparison, Degrees of Freedom (*df*), Sum of Squares (*Sum Sq.*), R squared (R^2), F-statistic (*F*), and adjusted p-value (*p*). Models including all samples and comparing profiles between heated and control treatments (i.e., Treatment effect) are noted as "All". Models including only samples from the heated treatments and comparing profiles across temperature doses (i.e., Dose effect) are noted as "Heated". Significant *p*-values at $\alpha < 0.05$ are bolded.

Comparison	Predictor	*df*	*Sum Sq.*	R^2	*F*	*p*
Overall	Treatment	1	0.125	0.01	1.213	0.26
	Timepoint	1	0.131	0.02	1.277	0.22
	Genotype	7	1.817	0.21	2.529	**0.001**
	Treatment × Timepoint	1	0.282	0.03	2.750	**0.02**
	Treatment × Genotype	7	0.753	0.09	1.049	0.38
	Residuals	54	5.542	0.64		
	Total	71	8.65	1		
Timepoint 1 All	Treatment	1	0.313	0.06	2.642	**0.03**
	Residuals	38	4.495	0.94		
	Total	39	4.808	1		
Timepoint 1 Heated	Dose	3	0.156	0.04	0.439	0.97
	Residuals	28	3.328	0.96		
	Total	31	3.485	1		
Timepoint 2 All	Treatment	1	0.103	0.03	0.856	0.39
	Residuals	30	3.617	0.97		
	Total	31	3.72	1		
Timepoint 2 Heated	Dose	2	0.216	0.07	0.811	0.54
	Residuals	21	2.798	0.93		
	Total	23	3.015	1		

Table 4
PERMDISP comparisons of DNA methylation profiles between genotypes (overall model) and treatments (All: pooled treatments, Heated: thermal doses) at each timepoint, with Degrees of Freedom (*df*), Sum of Squares (*Sum Sq.*), Mean Square (*Mean Sq.*), F-statistic (*F*), and adjusted p-value (*p*). Models including all samples and comparing profiles between heated and control treatments (i.e., Treatment effect) are noted as "All". Models including only samples from the heated treatments and comparing profiles across temperature doses (i.e., Dose effect) are noted as "Heated". Significant *p*-values at $\alpha < 0.05$ are bolded.

Comparison	Predictor	*df*	*Sum Sq.*	*Mean Sq.*	*F*	*p*
Overall	Genotype	7	0.01	0.001	0.154	0.99
	Residuals	64	0.588	0.009		
Timepoint 1 All	Treatment	1	0.003	0.003	0.426	0.52
	Residuals	38	0.272	0.007		
Timepoint 1 Heated	Dose	3	0.007	0.002	0.322	0.81
	Residuals	28	0.193	0.007		
Timepoint 2 All	Treatment	1	0.032	0.032	4.399	**0.04**
	Residuals	30	0.216	0.007		
Timepoint 2 Heated	Dose	2	0.019	0.009	1.006	0.38
	Residuals	21	0.196	0.009		

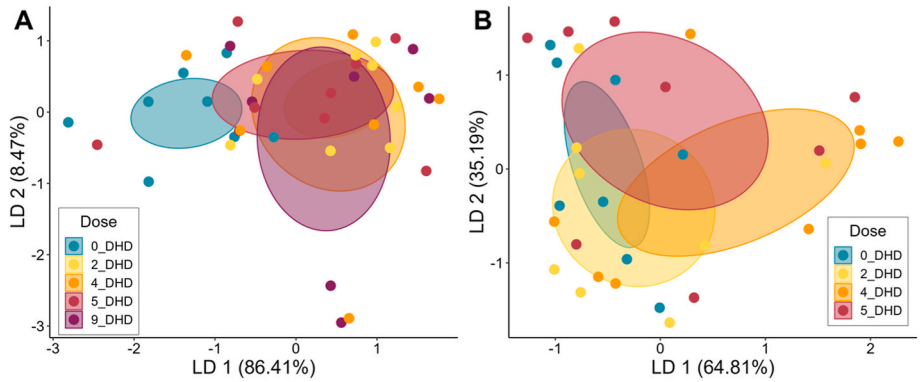

Fig. 2. A Discriminant Analysis of Principal Components (DAPC) of each temperature dose treatment after the thermal exposure period (A, Timepoint 1) and a subsequent 8-day recovery period (B, Timepoint 2). Each point represents the DNA methylation profile of a single sample, which are colored by temperature dose (teal: ambient, 0 DHD, yellow: 2 DHD, orange: 4 DHD, red: 5 DHD, purple: 9 DHD). Ellipses represent 95 % confidence intervals around each treatment group.

Table 5
Model results comparing dissimilarity of DNA methylation profiles of each heated dose relative to the ambient control A) over time and B) within each timepoint. For each ANOVA model (A.1, B.1, B.2), Degrees of Freedom (*df*), Sum of Squares (*Sum Sq.*), Mean Square (*Mean Sq.*), F-statistic (*F*), and p-values (*p*) are shown for each predictor of dissimilarity. For heat doses measured in both timepoints (2, 4, and 5 DHD), slopes over time were estimated (A.2), and compared between doses (A.3). Each estimate (*Estimate*, A.2) or difference in slope (*Estimate*, A.3), as well as Standard Errors (*SE*) and Degrees of Freedom (*df*) are included. Estimates of slope (A.2) include lower and upper 95 % confidence intervals (*Lower CI*, *Upper CI*) and pairwise contrasts (*Contrast*, A.3) include t ratios (*t ratio*) and p-values (*p*). Significant *p*-values at $\alpha < 0.05$ are bolded.

A.) Dissimilarity over time

A.1. ANOVA

Predictor	*df*	*Sum Sq.*	*Mean Sq.*	*F*	*p*
Dose	3	0.042	0.014	0.949	0.43
Time	1	0.131	0.131	8.946	**0.004**
Dose × Time	2	0.013	0.007	0.449	0.64
Residuals	49	0.716	0.015		

A.2. Slopes ~ Time

Dose	*Estimate*	*SE*	*df*	*Lower CI*	*Upper CI*
2 DHD	−0.147	0.060	49	−0.268	−0.025
4 DHD	−0.066	0.060	49	−0.187	0.056
5 DHD	−0.101	0.060	49	−0.222	0.021

A.3. Contrasts

Contrast	*Estimate*	*SE*	*df*	*t ratio*	*p*
2 DHD vs. 4 DHD	−0.081	0.086	49	−0.945	0.62
2 DHD vs. 5 DHD	−0.046	0.086	49	−0.537	0.85
4 DHD vs. 5 DHD	0.035	0.086	49	0.408	0.91

B) Doses within timepoints

B.1. Timepoint 1

Predictor	*df*	*Sum Sq.*	*Mean Sq.*	*F*	*p*
Dose	3	0.002	0.001	0.042	0.99
Residuals	28	0.417	0.015		

B.2. Timepoint 2

Predictor	*df*	*Sum Sq.*	*Mean Sq.*	*F*	*p*
Dose	2	0.026	0.013	0.898	0.42
Residuals	21	0.299	0.014		

loss or gain of methylation was uncommon, ranging from 2.0 to 3.0 % and 1.4–1.9 %, respectively. At Timepoint 2, more loci of corals in the heated treatments exhibited the same methylation state as those in the control treatment (60.5–66.6 %) compared to during Timepoint 1 (54.6–55.9 %, Figs. 3C and 4B), supporting the increase in similarity between Timepoint 1 and Timepoint 2 (Fig. 4).

4. Discussion

4.1. A rapid, but fleeting response of the DNA methylome to heating

Significant changes in DNA methylation profiles in *A. cervicornis* were observed within just 1 and 2 days of heating at 3–5 °C above the ambient temperature. To our knowledge, a response of the coral DNA methylome this rapid had not been previously reported. This rapid response at the doses applied here demonstrates DNA methylation sensitivity in corals, with the potential for patterns to be altered on hourly to daily timescales in response to changes in temperature. Previous work on the great star coral, *Montastraea cavernosa*, identified significant alteration of DNA methylation profiles from 26 days of laboratory heating (i.e., a 6 °C increase over 12 days and a 14-day hold at the bleaching temperature of 32°C; Rodríguez-Casariego et al., 2022). Alteration of gene expression has been observed within 90 min of a +2 °C exposure in the congener *Acropora hyacinthus*, with transcription factors being upregulated within 60–240 min (Traylor-Knowles et al., 2017).

There are examples of rapid epigenetic changes in response to environmental factors in the literature in other taxa. Heating led to global variation in DNA methylation 6 h post-heatshock in *Brassica napus* plants (Li et al., 2016). DNA methylation profiles were altered in response to temperature and hypoxia stress in salmon after 3 days (Beemelmanns et al., 2021) and in brown trout fed a salty diet after 2 days (Morán et al., 2013). Given the potential role of DNA methylation in gene regulation in invertebrates, where it is primarily associated with gene activation (Liew et al., 2018; Roberts and Gavery, 2012), a rapid response of the methylome, in coordination with other epigenetic mechanisms including histone modifications and small RNAs, would be necessary to provide a timely downstream gene response. In response to environmental stress, differential methylation patterns can modulate physiological processes linked to fitness, such as metabolism, reproduction, or stress resilience (Putnam et al., 2016; Dimond and Roberts, 2020). While MSAP does not provide gene-specific resolution, it remains a valuable tool for detecting genome-wide epigenetic variation in non-model organisms, offering insights into potential adaptive responses (Reyna-López et al., 1997).

In addition to this intrinsic limitation of the MSAP protocol, our results revealed a high proportion of non-canonical methylated marks (i.e., hemi-methylated sites), which are typically rare in metazoans. Whole-genome bisulfite sequencing has demonstrated that CpG methylation is the predominant target of DNA methylation (Ying et al., 2022), though other researchers have identified that non-canonical

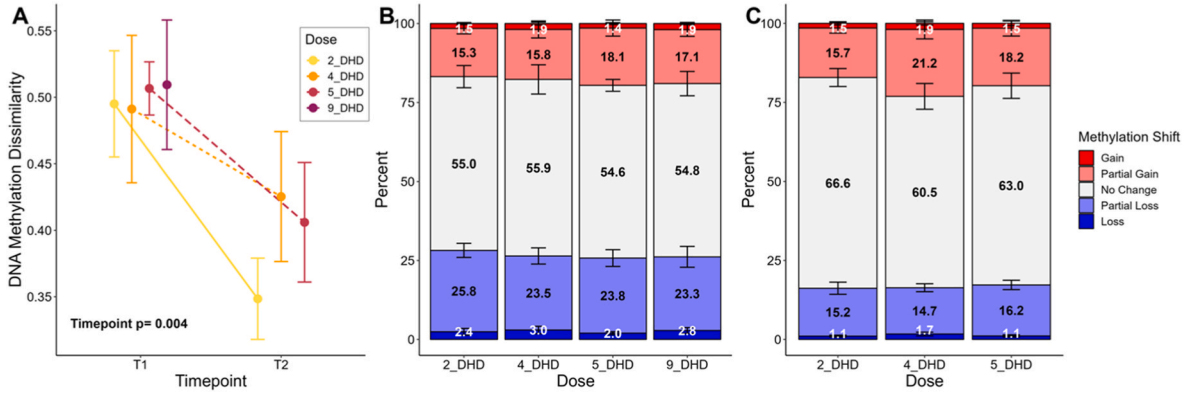

Fig. 3. A) The dissimilarity in DNA methylation profiles for each heated treatment relative to the ambient treatment from Timepoint 1 to Timepoint 2. Points represent averages for each temperature dose (colored by dose) and error bars show standard error. Dissimilarity decreased significantly over time (i.e., washout, Table 5A). B-C) The range of shifts in DNA methylation state (%) relative to the control across temperature doses at Timepoint 1 and Timepoint 2, respectively. Values indicate average proportions of each methylation shift, where losses are shown in shades of blue (full: darker, partial: lighter), and gains are shown in shades of red. Instances of no changes relative to the corresponding control are shown in grey. Error bars show standard error.

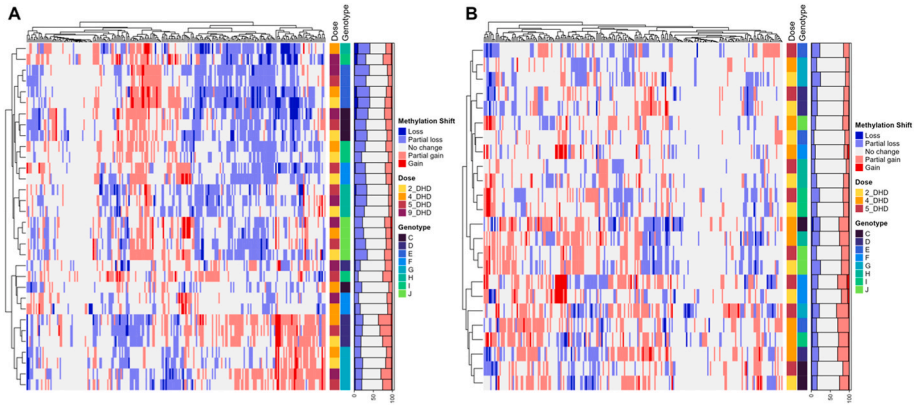

Fig. 4. The temporal dynamics of DNA methylation, showing the transitions in methylation state of methylation sensitive loci ($n = 226$) relative to the ambient (control) treatment at Timepoint 1 (A) and Timepoint 2 (B). Heatmap colors represent the methylation shift: Light grey indicates no change in methylation state between heated and control treatments. Dark blue indicates complete loss of methylation from HPM to NMT, and pale blue indicates a partial loss from HPM to HMM/ICM, or HMM/ICM to NMT (Table S3B). Red colors indicate a gain in methylation at a given locus, with pale red indicating a partial gain either from NMT to ICM/HMM, or ICM/HMM to HPM and dark red indicating a complete methylation gain from NMT to HPM. The stacked bar plot indicates the proportion of methylation state changes across loci for each individual. Doses and genotypes are indicated by colors along the right margin of each figure. Samples (y) and loci (x) are clustered by similarity dendrograms along both axes.

targets other than CpG (i.e., CHH or CHG) can also be the object of DNA methylation in corals (Yu et al., 2024). The reasons why these patterns are seemingly different in MSAP data are elusive to us, though similar results have been consistently observed across independent experiments (e.g., Rodríguez-Casariego et al., 2020; Hackerott et al., 2023). One possibility is that some of these non-canonical methylated loci reside within repetitive DNA regions, which are often collapsed or underrepresented in genome assemblies, making them difficult to detect by bisulfite sequencing. Another possible explanation would be that the hemi-methylation MSAP signal may reflect allelic or cell-type-specific mosaic methylation, producing amplification products in PCR-based MSAP assays but remaining undetectable in bisulfite datasets due to averaging across many molecules or insufficient sequencing coverage.

Corals are sessile organisms that contend with high levels of environmental variability on multiple scales, such as tidally driven hypersalinity and warming (e.g., Smith and Birkeland, 2007), nutrient fluxes at upwelling locations (e.g., Riegl et al., 2019) and seasonal variation (e.g., Scheufen et al., 2017). Moreover, coral reef microhabitats often have temperature fluctuations on the order of meter scales (Cyronak et al., 2020). The sensitivity of the coral methylome to brief temperature perturbation in this study supports a link between epigenetic mechanisms and either coral stress or fitness, given the high degree of variability in reef environments. While this work was performed in artificial conditions (i.e., in the laboratory), there is evidence indicating the coral methylome is responsive to a variety of environmental changes including temperature, outside of the laboratory. Changes in coral DNA methylation in response to ocean acidification (Liew et al., 2018; Putnam et al., 2016), increased nutrients (Hackerott et al., 2023; Rodríguez-Casariego et al., 2018), and seasonal temperature (Rodríguez-Casariego et al., 2020) have been observed outside of the laboratory.

Along with the rapid changes in the methylome, we observed a significant effect of genotype on DNA methylation (genotype $R^2 = 0.21$, $p = 0.001$, Table 3), supporting previous findings that genetic background is critical for understanding variation in DNA methylation. Local genetic variation has a significant influence on epigenetic variation, and genetic and epigenetic patterns of variation in invertebrates appear to be strongly linked (Bogan and Yi, 2024). The inclusion of multiple genotypes as the replicate in the experimental design was chosen intentionally, with the goal of identifying major DNA methylation patterns in response to thermal doses across a broader genetic diversity that was representative of the nursery and natural populations (Burggren, 2015).

Indeed, genotype has been observed to play a significant role in coral DNA methylation (Dimond and Roberts, 2020; Hackerott et al., 2023; Rodríguez-Casariego et al., 2020). The variance introduced by the inclusion of several genotypes in this study underscores the importance of accounting for genetic differences to avoid interpreting differences in DNA methylation as an effect of treatment (e.g., section 2.5.3 of this study).

We found evidence of methylation plasticity, which can modify phenotypes (Anastasiadi et al., 2021), and influence fitness within a single generation (Dixon et al., 2018). A reciprocal transplant on congener *Acropora millepora* found altered methylation states that resembled local corals in transplants with enhanced fitness, compared with those that did not resemble local corals, supporting the role of DNA methylation in influencing gene expression flexibility across environments and populations (Dixon et al., 2018). However, whether those mechanisms confer enhanced fitness or are maladaptive depends on the stability of the phenotype, whether it is matched to the environment and the response to future conditions (Anastasiadi et al., 2021; Venney et al., 2023).

4.2. Temporal dynamics of DNA methylation

Temporary changes to the DNA methylome occurred with heating, but many of these marks were not retained, resulting in methylome changes in heated treatments converging on the ambient treatment methylome over time (i.e., wash out). The majority of MSL remained unchanged relative to the ambient treatment in Timepoint 1 and Timepoint 2 (Fig. 4), and most methylation state changes were a partial loss or gain in methylation (Fig. 4), from non-methylated to hemi-methylated, for example. Additionally, dissimilarity in methylation profiles decreased between Timepoint 1 and 2, indicative of DNA methylation wash out occurring within a week. To our knowledge, no studies have focused on the timing of coral DNA methylation wash out, highlighting a research area for future work.

Differences in DNA methylation profiles from the ambient treatment were reduced in all treatments after the week-long recovery period and there was a "return to ambient" of the methylome after heating (Fig. 3A). The retention of epigenetic marks has a metabolic cost for animals (Venney et al., 2023), and thus plasticity and consequently rapid acclimatization via the methylome is energetically costly. The ability to rapidly respond to environmental changes on hourly to daily timescales is most certainly a fitness advantage to organisms in variable environments. Perhaps transient epigenetic marks offer a "happy medium", wherein the rapid, temporary response enables some acclimatory fitness benefit, while conserving the cellular energy expenditure required to retain the marks. In addition, we noted changes in the methylome of the ambient treatment over time. Future work should be aimed at understanding the dynamics of the coral methylome in the absence of stress (e.g., circadian cycles).

4.3. No influence of dose in response to acute heating

Previous work on the same samples identified a hormetic effect of thermal dose on coral bleaching physiology (i.e., symbiont density; Martell, 2023). Here, we found no difference in DNA methylation between thermal doses, suggesting DNA methylation was not specific to thermal dose, at least in an acute context. The lack of dose-dependence supports a threshold response of DNA methylation to increased temperature. Threshold responses have been observed in other molecular mechanisms in corals. For example, gene expression has been linked to apoptosis (programmed cell death) in response to sub-bleaching temperatures (Ainsworth et al., 2011), and corals have displayed thermal acclimation of gene expression (Kvitt et al., 2016). During these gene expression studies, significant expression differences in both anti- and pro-apoptosis genes were observed on the order of hours to days, like the exposures used here. The lack of a dose-dependent response further underscores the indirect relationship between the epigenome and phenotype (Angers et al., 2010).

The heating rates used in this study were outside of those commonly experienced by these corals in the nursery or on their natal reefs, despite being well within what corals routinely encounter on other reefs. Perhaps the heating rates used in this study overwhelmed the potential for a dose response. The levels of heat accumulation (i.e., degree heating days) used in our study occur on the Florida Reef Tract, albeit with a different pattern being most common in the natural environment (i.e., longer durations with lower magnitude changes).

To date, few studies have examined the influence of thermal dose on DNA methylation in invertebrates (Head, 2014). A recent study on the sea urchin *Arbacia punctulata* has shown significant dose-dependent increases in global DNA methylation in their gonadal tissues in response to higher temperatures (Rahman et al., 2024). In contrast, DNA methylation in the sea cucumber *Apostichopus japonicus* exhibited a complex response to acute heat stress, increasing at aestivation temperatures but decreasing during sublethal exposures (Yang et al., 2023). More work is needed to understand how dose influences DNA methylation, its retention and the consequences on survival.

5. Conclusions

To our knowledge, this is the first study to examine the influence of dose on DNA methylation and its consequent wash out dynamics after acute heat exposure in reef-building corals. We used a novel method to clarify hypermethylation from "lack of target", thus retaining a greater number of informative loci, which was made possible by the experimental design (i.e., all genotypes present in all treatments and timepoints). Additionally, we presented normalized methylation state transitions relative to the ambient treatment to visualize the response over hundreds of MSL across time, revealing the highly dynamic nature of the methylome over daily timescales. Notably, heating, but not dose, altered the DNA methylation profile, and these responses were transient after the 8-day recovery period. These results underscore the rapid and dynamic nature of DNA methylation in response to environmental change.

A more comprehensive mechanistic understanding of the influence of epigenetic mechanisms under rapid warming is needed to accurately predict whether these mechanisms might offer some benefit in the context of climate change. We recommend this work be replicated with a larger sample size and the use of whole-genome bisulfite sequencing to reveal the genes and pathways involved in the temporal dynamics of DNA methylation under warming. Marine heatwaves have increased in duration over the last half century and are predicted to continue becoming longer in duration, rather than greater in magnitude (Li and Donner, 2022). Future work should be aimed at investigating DNA methylation in response to longer duration, lower magnitude heat exposures, to better understand the response of the methylome to warming in an ecologically relevant context (Martell and Donner, 2025).

CRediT authorship contribution statement

Harmony A. Martell: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Serena N. Hackerott:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sabrina Mansoor:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Daniel García Souto:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Formal analysis, Data curation. **Jose M. Eirin-Lopez:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Data accessibility statement

All data, protocols and scripts used for analysis in this study are available at https://github.com/eelabfiu/CSHORE_acute/.

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Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2025.104338.

Data availability

We have included all data and scripts at https://github.com/eelabfiu/CSHORE_acute/.

References

Adrian-Kalchhauser, I., Sultan, S.E., Shama, L.N.S., Spence-Jones, H., Tiso, S., Valsecchi, C.I.K., Weissing, F.J., 2020. Understanding "Non-genetic" inheritance: insights from molecular-evolutionary crosstalk. Trends Ecol. Evol. 35 (12), 1078–1089. https://doi.org/10.1016/j.tree.2020.08.011.

Ainsworth, T.D., Wasmund, K., Ukani, L., Seneca, F., Yellowlees, D., Miller, D., Leggat, W., 2011. Defining the tipping point. A complex cellular life/death balance in corals in response to stress. Sci. Rep. 1 (1), 160. https://doi.org/10.1038/srep00160.

Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T., Curtis-McLane, S., 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolut. Appl. 1 (1), 95–111. https://doi.org/10.1111/j.1752-4571.2007.00013.x.

Anastasiadi, D., Venney, C.J., Bernatchez, L., Wellenreuther, M., 2021. Epigenetic inheritance and reproductive mode in plants and animals. Trends Ecol. Evol. 36 (12), 1124–1140. https://doi.org/10.1016/j.tree.2021.08.006.

Angers, B., Castonguay, E., Massicotte, R., 2010. Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after. Mol. Ecol. 19 (7), 1283–1295. https://doi.org/10.1111/j.1365-294X.2010.04580.x.

Beemelmanns, A., Ribas, L., Anastasiadi, D., Moraleda-Prados, J., Zanuzzo, F.S., Rise, M. L., Gamperl, A.K., 2021. DNA methylation dynamics in Atlantic salmon (*Salmo salar*) challenged with high temperature and moderate hypoxia. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.604878.

Beer, K.D., Wurtmann, E.J., Pinel, N., Baliga, N.S., 2014. Model organisms retain an "Ecological Memory" of complex ecologically relevant environmental variation. Appl. Environ. Microbiol. 80 (6), 1821–1831. https://doi.org/10.1128/AEM.03280-13.

Bellantuono, A.J., Granados-Cifuentes, C., Miller, D.J., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., 2012. Coral thermal tolerance: tuning gene expression to resist thermal stress. PLoS One 7 (11). https://doi.org/10.1371/journal.pone.0050685.

Bellantuono, A.J., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., 2011. Resistance to thermal stress in corals without changes in symbiont composition. Proc. R. Soc. A B 279 (1731), 1100–1107. https://doi.org/10.1098/rspb.2011.1780.

Bhattacharya, D., Agrawal, S., Aranda, M., Baumgarten, S., Belcaid, M., Drake, J.L., Erwin, D., Foret, S., Gates, R.D., Gruber, D.F., Kamel, B., Lesser, M.P., Levy, O., Liew, Y.J., MacManes, M., Mass, T., Medina, M., Mehr, S., Meyer, E., et al., 2016. Comparative genomics explains the evolutionary success of reef-forming corals. eLife 5, e13288. https://doi.org/10.7554/eLife.13288.

Bogan, S.N., Yi, S.V., 2024. Potential role of DNA methylation as a driver of plastic responses to the environment across cells, organisms, and populations. Genome Biol. Evol. 16 (2), evae022. https://doi.org/10.1093/gbe/evae022.

Brandon, R.N., 2014. Adaptation and Environment. Princeton University Press. https://doi.org/10.1002/ajpa.1330860114.

Brown, B.E., Dunne, R.P., Edwards, A.J., Sweet, M.J., Phongsuwan, N., 2014. Decadal environmental 'memory' in a reef coral? Mar. Biol. 162 (2), 479–483. https://doi.org/10.1007/s00227-014-2596-2.

Burggren, W.W., 2015. Dynamics of epigenetic phenomena: intergenerational and intragenerational phenotype 'washout.'. J. Exp. Biol. 218 (1), 80–87. https://doi.org/10.1242/jeb.107318.

Carilli, J., Donner, S.D., Hartmann, A.C., 2012. Historical temperature variability affects coral response to heat stress. PLoS One 7 (3). https://doi.org/10.1371/journal.pone.0034418.

Carneiro, V.C., Lyko, F., 2020. Rapid epigenetic adaptation in animals and its role in invasiveness. Integr. Comp. Biol. 60 (2), 267–274. https://doi.org/10.1093/icb/icaa023.

Cohen, A.A., 2018. Aging across the tree of life: the importance of a comparative perspective for the use of animal models in aging. Biochim. Biophys. Acta Mol. Basis Dis. 1864 (9), 2680–2689. https://doi.org/10.1016/j.bbadis.2017.05.028. Part A.

Coles, S.L., Jokiel, P.L., 1992. Effects of salinity on coral reefs. In: Pollution in Tropical Aquatic Systems. CRC Press. https://doi.org/10.1201/9781351075879-6.

Cyronak, T., Takeshita, Y., Courtney, T.A., DeCarlo, E.H., Eyre, B.D., Kline, D.I., Martz, T., Page, H., Price, N.N., Smith, J., Stoltenberg, L., Tresguerres, M., Andersson, A.J., 2020. Diel temperature and pH variability scale with depth across diverse coral reef habitats. Limnol. Oceanogr. Lett. 5 (2), 193–203. https://doi.org/10.1002/lol2.10129.

de Mendoza, A., Lister, R., Bogdanovic, O., 2020. Evolution of DNA methylome diversity in eukaryotes. J. Mol. Biol. 432 (6), 1687–1705. https://doi.org/10.1016/j.jmb.2019.11.003.

Dimond, J.L., Roberts, S.B., 2016. Germline DNA methylation in reef corals: patterns and potential roles in response to environmental change. Mol. Ecol. 25 (8), 1895–1904. https://doi.org/10.1111/mec.13414.

Dimond, J.L., Roberts, S.B., 2020. Convergence of DNA methylation profiles of the Reef coral *Porites astreoides* in a novel environment. Front. Mar. Sci. 6. https://doi.org/10.3389/fmars.2019.00792.

Dion, A., Muñoz, P.T., Franklin, T.B., 2022. Epigenetic mechanisms impacted by chronic stress across the rodent lifespan. Neurobiol. Stress 17, 100434. https://doi.org/10.1016/j.ynstr.2022.100434.

Dixon, G., Liao, Y., Bay, L.K., Matz, M.V., 2018. Role of gene body methylation in acclimatization and adaptation in a basal metazoan. Proc. Natl. Acad. Sci. 115 (52), 13342–13346. https://doi.org/10.1073/pnas.1813749115.

Edmunds, P.J., Tsounis, G., Boulon, R., Bramanti, L., 2018. Long-term variation in light intensity on a coral reef. Coral Reefs 37 (3), 955–965. https://doi.org/10.1007/s00338-018-1721-y.

Eirin-Lopez, J.M., Putnam, H.M., 2019. Marine environmental epigenetics. Ann. Rev. Mar. Sci 11, 335–368. https://doi.org/10.1146/annurev-marine-010318-095114.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131 (2), 479–491. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1205020/.

Feil, R., Fraga, M.F., 2012. Epigenetics and the environment: emerging patterns and implications. Nat. Rev. Genet. 13 (2), 97–109. https://doi.org/10.1038/nrg3142.

Fitz-James, M.H., Cavalli, G., 2022. Molecular mechanisms of transgenerational epigenetic inheritance. Nat. Rev. Genet. 23 (6), 325–341. https://doi.org/10.1038/s41576-021-00438-5.

Fulneček, J., Kovařík, A., 2014. How to interpret methylation sensitive amplified polymorphism (MSAP) profiles? BMC Genet. 15 (1), 2. https://doi.org/10.1186/1471-2156-15-2.

Gatzmann, F., Falckenhayn, C., Gutekunst, J., Hanna, K., Raddatz, G., Carneiro, V.C., Lyko, F., 2018. The methylome of the marbled crayfish links gene body methylation to stable expression of poorly accessible genes. Epigenetics Chromatin 11 (1), 57. https://doi.org/10.1186/s13072-018-0229-6.

Gavery, M.R., Roberts, S.B., 2014. A context dependent role for DNA methylation in bivalves. Briefings Funct. Genomics 13 (3), 217–222. https://doi.org/10.1093/bfgp/elt054.

Glynn, P.W., D'Croz, L., 1990. Experimental evidence for high temperature stress as the cause of El Niño-coincident coral mortality. Coral Reefs 8 (4), 181–191. https://doi.org/10.1007/BF00265009.

Goergen, E.A., Gilliam, D.S., 2018. Outplanting technique, host genotype, and site affect the initial success of outplanted *Acropora cervicornis*. PeerJ 6, e4433. https://doi.org/10.7717/peerj.4433.

Hackerott, S., Martell, H.A., Eirin-Lopez, J.M., 2021. Coral environmental memory: causes, mechanisms, and consequences for future reefs. Trends Ecol. Evol. 36 (11), 1011–1023. https://doi.org/10.1016/j.tree.2021.06.014.

Hackerott, S., Virdis, F., Flood, P.J., Souto, D.G., Paez, W., Eirin-Lopez, J.M., 2023. Relationships between phenotypic plasticity and epigenetic variation in two Caribbean acropora corals. Mol. Ecol. 32 (17), 4814–4828. https://doi.org/10.1111/mec.17072.

Harris, K.D., Lloyd, J.P.B., Domb, K., Zilberman, D., Zemach, A., 2019. DNA methylation is maintained with high fidelity in the honey bee germline and exhibits global non-functional fluctuations during somatic development. Epigenetics Chromatin 12 (1), 62. https://doi.org/10.1186/s13072-019-0307-4.

Head, J.A., 2014. Patterns of DNA methylation in animals: an ecotoxicological perspective. Integr. Comp. Biol. 54 (1), 77–86. https://doi.org/10.1093/icb/icu025.
Herrera, C.M., Bazaga, P., 2010. Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet *Viola cazorlensis*. New Phytol. 187 (3), 867–876. https://doi.org/10.1111/j.1469-8137.2010.03298.x.
Hilker, M., Schmülling, T., 2019. Stress priming, memory, and signalling in plants. Plant Cell Environ. 42 (3), 753–761. https://doi.org/10.1111/pce.13526.
Hofmann, G.E., 2017. Ecological epigenetics in marine metazoans. Front. Mar. Sci. 4. https://doi.org/10.3389/fmars.2017.00004.
Hughes, T.P., Graham, N.A.J., Jackson, J.B.C., Mumby, P.J., Steneck, R.S., 2010. Rising to the challenge of sustaining coral reef resilience. Trends Ecol. Evol. 25 (11). https://doi.org/10.1016/j.tree.2010.07.011.
Klein, S.G., Roch, C., Duarte, C.M., 2024. Systematic review of the uncertainty of coral reef futures under climate change. Nat. Commun. 15 (1), 2224. https://doi.org/10.1038/s41467-024-46255-2.
Koweek, D.A., Dunbar, R.B., Monismith, S.G., Mucciarone, D.A., Woodson, C.B., Samuel, L., 2015. High-resolution physical and biogeochemical variability from a shallow back reef on Ofu, American Samoa: an end-member perspective. Coral Reefs 34 (3), 979–991. https://doi.org/10.1007/s00338-015-1308-9.
Kvitt, H., Rosenfeld, H., Tchernov, D., 2016. The regulation of thermal stress induced apoptosis in corals reveals high similarities in gene expression and function to higher animals. Sci. Rep. 6 (1), 30359. https://doi.org/10.1038/srep30359.
Lämke, J., Bäurle, I., 2017. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol. 18 (1), 124. https://doi.org/10.1186/s13059-017-1263-6.
Larson, E.A., 2010. Establishment of an *Acropora cervicornis* (Staghorn Coral) nursery: an evaluation of survivorship and growth. https://nsuworks.nova.edu/cgi/viewcontent.cgi?article=1184&context=occ_stuetd&httpsredir=1&referer=/.
Leggat, W., Heron, S.F., Fordyce, A., Suggett, D.J., Ainsworth, T.D., 2022. Experiment degree heating week (eDHW) as a novel metric to reconcile and validate past and future global coral bleaching studies. J. Environ. Manag. 301, 113919. https://doi.org/10.1016/j.jenvman.2021.113919.
Li, J., Huang, Q., Sun, M., Zhang, T., Li, H., Chen, B., Xu, K., Gao, G., Li, F., Yan, G., Qiao, J., Cai, Y., Wu, X., 2016. Global DNA methylation variations after short-term heat shock treatment in cultured microspores of *Brassica napus* cv. topas. Sci. Rep. 6 (1), 38401. https://doi.org/10.1038/srep38401.
Li, X., Donner, S.D., 2022. Lengthening of warm periods increased the intensity of warm-season marine heatwaves over the past 4 decades. Clim. Dyn. 59 (9), 2643–2654. https://doi.org/10.1007/s00382-022-06227-y.
Liew, Y.J., Howells, E.J., Wang, X., Michell, C.T., Burt, J.A., Idaghdour, Y., Aranda, M., 2020. Intergenerational epigenetic inheritance in reef-building corals. Nat. Clim. Change 10 (3). https://doi.org/10.1038/s41558-019-0687-2.
Liew, Y.J., Zoccola, D., Li, Y., Tambutté, E., Venn, A.A., Michell, C.T., Cui, G., Deutekom, E.S., Kaandorp, J.A., Voolstra, C.R., Forêt, S., Allemand, D., Tambutté, S., Aranda, M., 2018. Epigenome-associated phenotypic acclimatization to ocean acidification in a reef-building coral. Sci. Adv. 4 (6). https://doi.org/10.1126/sciadv.aar8028.
Lyons, M.B., Murray, N.J., Kennedy, E.V., Kovacs, E.M., Castro-Sanguino, C., Phinn, S.R., Acevedo, R.B., Alvarez, A.O., Say, C., Tudman, P., Markey, K., Roe, M., Canto, R.F., Fox, H.E., Bambic, B., Lieb, Z., Asner, G.P., Martin, P.M., Knapp, D.E., et al., 2024. New global area estimates for coral reefs from high-resolution mapping. Cell Rep. Sustain. 1 (2), 100015. https://doi.org/10.1016/j.crsus.2024.100015.
Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., Hornik, K., 2023. Cluster: cluster analysis basics and extensions. R package version 2 (1), 6. https://CRAN.R-project.org/package=cluster.
Majerová, E., Carey, F.C., Drury, C., Gates, R.D., 2021. Preconditioning improves bleaching tolerance in the reef-building coral *Pocillopora acuta* through modulations in the programmed cell death pathways. Mol. Ecol. 30 (14), 3560–3574. https://doi.org/10.1111/mec.15988.
Majerová, E., Drury, C., 2022. Thermal preconditioning in a reef-building coral alleviates oxidative damage through a BI-1-mediated antioxidant response. Front. Mar. Sci. 9. https://www.frontiersin.org/articles/10.3389/fmars.2022.971332.
Malhi, Y., Franklin, J., Seddon, N., Solan, M., Turner, M.G., Field, C.B., Knowlton, N., 2020. Climate change and ecosystems: threats, opportunities and solutions. Phil. Trans. Biol. Sci. 375 (1794), 20190104. https://doi.org/10.1098/rstb.2019.0104.
Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27 (2), 209–220. https://aacrjournals.org/cancerres/article/27/2_Part_1/209/476508/The-Detection-of-Disease-Clustering-and-a.
Martell, H.A., 2023. Thermal priming and bleaching hormesis in the staghorn coral, *Acropora cervicornis* (Lamarck 1816). J. Exp. Mar. Biol. Ecol. 560, 151820. https://doi.org/10.1016/j.jembe.2022.151820.
Martell, H.A., Donner, S.D., 2025. Headwinds to understanding stress response physiology: a systematic review reveals mismatch between real and simulated marine heatwaves on coral reefs. Clim. Change Ecol., 100094 https://doi.org/10.1016/j.ecochg.2025.100094.
Martell, H.A., Zimmerman, R.C., 2021. Heating rate modulates the metabolic response of the staghorn coral *Acropora cervicornis* (Lamarck, 1816). Mar. Biol. 168 (6), 83. https://doi.org/10.1007/s00227-021-03847-6.
Martin, R.A., da Silva, C.R.B., Moore, M.P., Diamond, S.E., 2023. When will a changing climate outpace adaptive evolution? WIREs Clim. Change 14 (6), e852. https://doi.org/10.1002/wcc.852.
Middlebrook, R., Hoegh-Guldberg, O., Leggat, W., 2008. The effect of thermal history on the susceptibility of reef-building corals to thermal stress. J. Exp. Biol. 211 (7), 1050–1056. https://doi.org/10.1242/jeb.013284.
Miller, J.M., Cullingham, C.I., Peery, R.M., 2020. The influence of a priori grouping on inference of genetic clusters: simulation study and literature review of the DAPC method. Heredity 125 (5), 269–280. https://doi.org/10.1038/s41437-020-0348-2.
Mirbahai, L., Chipman, J.K., 2014. Epigenetic memory of environmental organisms: a reflection of lifetime stressor exposures. Mutat. Res., Genet. Toxicol. Environ. Mutagen. 764–765, 10–17. https://doi.org/10.1016/j.mrgentox.2013.10.003.
Miryeganeh, M., 2021. Plants' epigenetic mechanisms and abiotic stress. Genes 12 (8), 1106. https://doi.org/10.3390/genes12081106.
Moore, L.D., Le, T., Fan, G., 2013. DNA methylation and its basic function. Neuropsychopharmacology 38 (1), 23–38. https://doi.org/10.1038/npp.2012.112.
Morán, P., Marco-Rius, F., Megías, M., Covelo-Soto, L., Pérez-Figueroa, A., 2013. Environmental induced methylation changes associated with seawater adaptation in brown trout. Aquaculture 392–395, 77–83. https://doi.org/10.1016/j.aquaculture.2013.02.006.
NOAA Coral Reef Watch, 2017. NOAA coral reef watch version 3.0 daily global 5-km satellite virtual station time series data for Southeast Florida, Mar. 12, 2013-Mar. 11, 2014. College Park, Maryland, USA: NOAA Coral Reef Watch. https://coralreefwatch.noaa.gov/vs/data.php. (Accessed 5 February 2018).
Oberkofler, V., Pratx, L., Bäurle, I., 2021. Epigenetic regulation of abiotic stress memory: maintaining the good things while they last. Curr. Opin. Plant Biol. 61, 102007. https://doi.org/10.1016/j.pbi.2021.102007.
Oksanen, J., et al., 2022. *Vegan*: community ecology package. https://cran.r-project.org/package=vegan.
Pérez-Figueroa, A., 2013. *Msap*: a tool for the statistical analysis of methylation-sensitive amplified polymorphism data. Molecular Ecol. Resour. 13 (3), 522–527. https://doi.org/10.1111/1755-0998.12064.
Putnam, H.M., Barott, K.L., Ainsworth, T.D., Gates, R.D., 2017. The vulnerability and resilience of reef-building corals. Curr. Biol.: CB 27 (11), R528–R540. https://doi.org/10.1016/j.cub.2017.04.047.
Putnam, H.M., Davidson, J.M., Gates, R.D., 2016. Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. Evolut. Appl. 9 (9), 1165–1178. https://doi.org/10.1111/eva.12408.
Putnam, H.M., Gates, R.D., 2015. Preconditioning in the reef-building coral *Pocillopora damicornis* and the potential for trans-generational acclimatization in coral larvae under future climate change conditions. J. Exp. Biol. 218 (15), 2365–2372. https://doi.org/10.1242/jeb.123018.
Putnam, H.M., Ritson-Williams, R., Cruz, J.A., Davidson, J.M., Gates, R.D., 2020. Environmentally-induced parental or developmental conditioning influences coral offspring ecological performance. Sci. Rep. 10 (1), 13664. https://doi.org/10.1038/s41598-020-70605-x.
Rahman, M.S., Billah, M.M., Rangel, V., Cantu, E., 2024. Elevated temperature triggers increase in global DNA methylation, 5-methylcytosine expression levels, apoptosis and NOx levels in the gonads of Atlantic sea urchin. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 269, 110899. https://doi.org/10.1016/j.cbpb.2023.110899.
Regev, A., Lamb, M., Jablonka, E., 1998. The role of DNA methylation in invertebrates: developmental regulation or genome defense? Mol. Biol. Evol. 15 (7), 880. https://doi.org/10.1093/oxfordjournals.molbev.a025992.
Reyna-López, G.E., Simpson, J., Ruiz-Herrera, J., 1997. Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphisms. Mol. Gen. Genet. MGG 253 (6), 703–710. https://doi.org/10.1007/s004380050374.
Riegl, B., Glynn, P.W., Banks, S., Keith, I., Rivera, F., Vera-Zambrano, M., D'Angelo, C., Wiedenmann, J., 2019. Heat attenuation and nutrient delivery by localized upwelling avoided coral bleaching mortality in northern Galapagos during 2015/2016 ENSO. Coral Reefs 38 (4), 773–785. https://doi.org/10.1007/s00338-019-01787-8.
Roberts, S.B., Gavery, M.R., 2012. Is there a relationship between DNA methylation and phenotypic plasticity in invertebrates? Front. Physiol. 2. https://doi.org/10.3389/fphys.2011.00116.
Rodríguez-Casariego, J.A., Cunning, R., Baker, A.C., Eirin-Lopez, J.M., 2022. Symbiont shuffling induces differential DNA methylation responses to thermal stress in the coral *Montastraea cavernosa*. Mol. Ecol. 31 (2), 588–602. https://doi.org/10.1111/mec.16246.
Rodríguez-Casariego, J.A., Ladd, M.C., Shantz, A.A., Lopes, C., Cheema, M.S., Kim, B., Roberts, S.B., Fourqurean, J.W., Ausio, J., Burkepile, D.E., Eirin-Lopez, J.M., 2018. Coral epigenetic responses to nutrient stress: Histone H2A.X phosphorylation dynamics and DNA methylation in the staghorn coral *Acropora cervicornis*. Ecol. Evol. 8 (23), 12193–12207. https://doi.org/10.1002/ece3.4678.
Rodríguez-Casariego, J.A., Mercado-Molina, A.E., Garcia-Souto, D., Ortiz-Rivera, I.M., Lopes, C., Baums, I.B., Sabat, A.M., Eirin-Lopez, J.M., 2020. Genome-wide DNA methylation analysis reveals a conserved epigenetic response to seasonal environmental variation in the staghorn Coral Acropora cervicornis. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.560424.
Rodríguez-Casariego, J.A., Mercado-Molina, A., Lemos, L.S., Quinete, N.S., Bellantuono, A., Rodriguez-Lanetty, M., Sabat, A., Eirin-Lopez, J.M., 2024. Multi-omic characterization of mechanisms contributing to rapid phenotypic plasticity in the coral *Acropora cervicornis* under divergent environments. Coral Reefs 43 (1), 53–66. https://doi.org/10.1007/s00338-023-02446-9.
Scheufen, T., Iglesias-Prieto, R., Enríquez, S., 2017. Changes in the number of symbionts and symbiodinium cell pigmentation modulate differentially coral light absorption and photosynthetic performance. Front. Mar. Sci. 4. https://doi.org/10.3389/fmars.2017.00309.
Schoepf, V., Stat, M., Falter, J.L., McCulloch, M.T., 2015. Limits to the thermal tolerance of corals adapted to a highly fluctuating, naturally extreme temperature environment. Sci. Rep. 5, 17639. https://doi.org/10.1038/srep17639.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52 (3–4), 591–611. https://doi.org/10.1093/biomet/52.3-4.591.

Sing Wong, A., Vrontos, S., Taylor, M.L., 2022. An assessment of people living by coral reefs over space and time. Glob. Change Biol. 28 (23), 7139–7153. https://doi.org/10.1111/gcb.16391.

Skinner, M.K., 2015. Environmental epigenetics and a unified theory of the molecular aspects of evolution: a neo-lamarckian concept that facilitates neo-darwinian evolution. Genome Biol. Evol. 7 (5), 1296–1302. https://doi.org/10.1093/gbe/evv073.

Smith, L.W., Birkeland, C., 2007. Effects of intermittent flow and irradiance level on back reef *Porites* corals at elevated seawater temperatures. J. Exp. Mar. Biol. Ecol. 341 (2), 282–294. https://doi.org/10.1016/j.jembe.2006.10.053.

Stanley, G.D., 2003. The evolution of modern corals and their early history. Earth Sci. Rev. 60 (3), 195–225. https://doi.org/10.1016/S0012-8252(02)00104-6.

Thia, J.A., 2023. Guidelines for standardizing the application of discriminant analysis of principal components to genotype data. Molecular Ecol. Resour. 23 (3), 523–538. https://doi.org/10.1111/1755-0998.13706.

Traylor-Knowles, N., Rose, N.H., Palumbi, S.R., 2017. The cell specificity of gene expression in the response to heat stress in corals. J. Exp. Biol. 220 (10), 1837–1845. https://doi.org/10.1242/jeb.155275.

U, A.S.Y., Yang, C., Martell, H.A., 2023. The influence of fragment type, time, and coral host genotype on early growth of nursery reared *Acropora cervicornis*. Restor. Ecol. 31 (4), e13879. https://doi.org/10.1111/rec.13879.

Venney, C.J., Anastasiadi, D., Wellenreuther, M., Bernatchez, L., 2023. The evolutionary complexities of DNA methylation in animals: from plasticity to genetic evolution. Genome Biol. Evol. 15 (12), evad216. https://doi.org/10.1093/gbe/evad216.

Wang, X., Li, Q., Lian, J., Li, L., Jin, L., Cai, H., Xu, F., Qi, H., Zhang, L., Wu, F., Meng, J., Que, H., Fang, X., Guo, X., Zhang, G., 2014. Genome-wide and single-base resolution DNA methylomes of the Pacific oyster *Crassostrea gigas* provide insight into the evolution of invertebrate CpG methylation. BMC Genom. 15 (1), 1119. https://doi.org/10.1186/1471-2164-15-1119.

Yang, Y., Sun, Y., Zhou, X., Xu, X., Wang, W., Feng, Y., Li, Z., Yang, J., Liu, X., Li, B., Sun, G., 2023. Genome-wide analysis of DNA methylation reveals significant impact of two typical high temperature stress on biological functions in *Apostichopus japonicus*. Aquacult. Rep. 29, 101522. https://doi.org/10.1016/j.aqrep.2023.101522.

Yen, E.C., Gilbert, J.D., Balard, A., Afonso, I.O., Fairweather, K., Newlands, D., Lopes, A., Correia, S.M., Taxonera, A., Rossiter, S.J., Martín-Durán, J.M., Eizaguirre, C., 2024. DNA methylation carries signatures of sublethal effects under thermal stress in loggerhead Sea turtles. Evolut. Appl. 17 (9), e70013. https://doi.org/10.1111/eva.70013.

Ying, H., Hayward, D.C., Klimovich, A., Bosch, T.C.G., Baldassarre, L., Neeman, T., Forêt, S., Huttley, G., Reitzel, A.M., Fraune, S., Ball, E.E., Miller, D.J., 2022. The role of DNA methylation in genome defense in Cnidaria and other invertebrates. Mol. Biol. Evol. 39 (2), msac018. https://doi.org/10.1093/molbev/msac018.

Yu, X., Yu, K., Chen, B., Liao, Z., Liang, J., Qin, Z., Gao, X., 2024. Metabolic and immune costs balance during natural acclimation of corals in fluctuating environments. Mar. Environ. Res. 193, 106284. https://doi.org/10.1016/j.marenvres.2023.106284.

Zhu, Y., Dong, A., Shen, W.-H., 2012. Histone variants and chromatin assembly in plant abiotic stress responses. Biochimica et Biophysica Acta (BBA) - Gene Regulat. Mechan. 1819 (3), 343–348. https://doi.org/10.1016/j.bbagrm.2011.07.012.