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Relationships between phenotypic plasticity and epigenetic variation in two Caribbean *Acropora* corals

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Abstract

The plastic ability for a range of phenotypes to be exhibited by the same genotype allows organisms to respond to environmental variation and may modulate fitness in novel environments. Differing capacities for phenotypic plasticity within a population, apparent as genotype by environment interactions (GxE), can therefore have both ecological and evolutionary implications. Epigenetic gene regulation alters gene function in response to environmental cues without changes to the underlying genetic sequence and likely mediates phenotypic variation. DNA methylation is currently the most well described epigenetic mechanism and is related to transcriptional homeostasis in invertebrates. However, evidence quantitatively linking variation in DNA methylation with that of phenotype is lacking in some taxa, including reef-building corals. In this study, spatial and seasonal environmental variation in Bonaire, Caribbean Netherlands was utilized to assess relationships between physiology and DNA methylation profiles within genetic clones across different genotypes of *Acropora cervicornis* and *A. palmata* corals. The physiology of both species was highly influenced by environmental variation compared to the effect of genotype. GxE effects on phenotype were only apparent in *A. cervicornis*. DNA methylation in both species differed between genotypes and seasons and epigenetic variation was significantly related to coral physiological metrics. Furthermore, plastic shifts in physiology across seasons were significantly positively correlated with shifts in DNA methylation profiles in both species. These results highlight the dynamic influence of environmental conditions and genetic constraints on the physiology of two important Caribbean coral species. Additionally, this study provides quantitative support for the role of epigenetic DNA methylation in mediating phenotypic plasticity in invertebrates.

KEYWORDS
Acropora cervicornis, *Acropora palmata*, coral restoration, DNA methylation, genotype by environment interactions, seasonal acclimatization

1 | INTRODUCTION

Phenotypic plasticity refers to the capacity for a single genotype to exhibit a range of phenotypes in response to environmental cues (Bradshaw, 1965). As natural selection occurs at the level of the phenotype, differing potentials for phenotypic plasticity within a population could have evolutionary implications (Stearns, 1989). Plasticity may be considered adaptive if the new phenotype results in higher fitness (e.g. growth, survival or reproduction), yet maladaptive mismatches between phenotype and environment, as well

as neutral effects on fitness, are also possible (Murren et al., 2015). Variation in the capacity for phenotypic plasticity within a population can be apparent as a "genotype by environment interaction" (GxE), where the strength of the relationship between a phenotypic trait and an environmental gradient (i.e. reaction norm) varies across genotypes (de Leon et al., 2016).

The plasticity of phenotypic traits is underpinned by the capacity for plasticity in gene function (Aubin-Horth & Renn, 2009). The concept of reaction norm can also be extended to molecular mechanisms (e.g. gene expression) displaying variation across environmental conditions both within and between genotypes (Manuck, 2010). Such variation is influenced by epigenetic mechanisms regulating gene function and perpetuating altered gene activity states without changes to the DNA sequence (Cavalli & Heard, 2019). These mechanisms, including DNA methylation, histone post-translational modifications (PTMs), changes in nucleosome composition, and non-coding RNA activity, are responsive to environmental cues (Eirin-Lopez & Putnam, 2019), linking the environment with an organism's phenotype and likely mediating phenotypic plasticity (Norouzitallab et al., 2019). Importantly, epigenetically-regulated plasticity also has the potential to influence evolution as environmentally-induced epigenetic modifications can be heritable (Ashe et al., 2021).

A comprehensive understanding of the potential for phenotypic plasticity, as well as the regulatory molecular mechanisms, can help improve predictions of species responses to global change, both within and across generations. Phenotypic plasticity may be particularly critical for long-lived and sessile organisms that must cope with environmental variation without relocating (Stotz et al., 2021). In fact, the influence of phenotypic plasticity on the acclimatory and adaptive potential of critical foundational species such as seagrasses and corals has been of recent interest, especially given their vulnerability to rapid climate change (Pazzaglia et al., 2021; Putnam, 2021). Many coral species rely on asexual fragmentation in addition to sexual reproduction, and their clonality makes these organisms an interesting model in which to study phenotypic plasticity and genotype by environment interactions. Phenotypic plasticity has been well-described in reef-building corals (Bruno & Edmunds, 1997; Foster, 1979; Todd, 2008) and more recent work has begun to highlight the transcriptional underpinnings of this phenomenon (Drury et al., 2022; Kenkel & Matz, 2016; Rivera et al., 2021). Yet, the study of the influence of epigenetic mechanisms on coral phenotypic plasticity is still in its infancy.

DNA methylation is presently the most well-studied epigenetic mechanism (Eirin-Lopez & Putnam, 2019; Hofmann, 2017). DNA methylation typically occurs on cytosine bases next to guanine (i.e. "CpG sites") which are primarily found within gene bodies in invertebrates (Yi, 2017). Invertebrate genomes tend to be sparsely methylated in "mosaic" patterns, where a subset of genes are methylated (Šrut, 2021). Unlike the gene-silencing role of DNA methylation in vertebrates, the function of gene body methylation in invertebrates is likely homeostatic, with constitutively expressed, "housekeeping" genes tending to be more highly methylated (Zilberman, 2017). In fact, such a bimodal pattern of DNA methylation has been found in the genomes of multiple reef-building coral species and genes responsive to environmental conditions are more weakly methylated (Dimond & Roberts, 2016; Dixon et al., 2014). Furthermore, gene body methylation reduces transcriptional noise as well as spurious transcription in corals (Liew et al., 2018), supporting the role of this epigenetic modification in maintaining transcriptional homeostasis. Changes in DNA methylation have been associated with phenotypic responses in corals exposed to ocean acidification conditions (Liew et al., 2018; Putnam et al., 2016) as well as during acclimatization to novel environments (Dixon et al., 2018). However, quantitative assessments of the contribution of epigenetic variation to that of coral phenotype are lacking (Roberts & Gavery, 2012).

The goal of the present study is to further investigate connections between phenotypic plasticity and epigenetic variation using two important Caribbean corals, *Acropora cervicornis* and *A. palmata*, the staghorn coral and elkhorn coral, as model systems. Coral physiology and DNA methylation profiles are predicted to be influenced by both genetic and environmental factors. Additionally, it is hypothesized that plasticity in physiological metrics will be associated with similar shifts in DNA methylation profiles. To evaluate these hypotheses, genetic clones of *A. cervicornis* and *A. palmata* corals were monitored across naturally occurring spatial and seasonal environmental variation at four coral nursery sites in Bonaire, Caribbean Netherlands throughout one seasonal cycle.

2 | MATERIALS AND METHODS

2.1 | Study sites and environmental monitoring

The four Reef Renewal Foundation Bonaire (RRFB) coral nursery sites were selected to cover a range of environmental conditions, including two urban-impacted sites near the capital city and two more isolated sites. The two sites predicted to be more anthropogenically impacted were Buddy's Reef (BD), located in front of the Buddy Dive Resort, and Something Special (SS), a dive site near the capital city of Kralendijk. The two more isolated sites included one at Klein Bonaire (KL), the small uninhabited island off the west coast of the main island and Oil Slick Leap (OL), a dive site north of the capital city (Figure S1a).

Throughout the 1-year study, water temperature and conductivity were monitored every 10 min with Odyssey data loggers (Dataflow Systems Ltd). Water quality was monitored via bi-weekly water samples collected within 0.5 m of the coral nursery trees. Samples remained frozen until analysis at the Florida International University CAChE Nutrient Analysis Core Facility to measure total nitrogen and phosphorus (NELAC Certified Analyses, Certificate# E76930-16).

2.2 | Coral sampling

Coral fragments used for this study were from RRFB nursery stock (Table S1) and hung with monofilament line on fiberglass coral trees suspended in the water column at 3.5–5.5 m depth. Each species was

represented at three of the four study sites, constrained to the distribution of these species across existing RRFB nursery infrastructure. Within a species, genotypes were equally distributed across respective study sites. The four genotypes of *Acropora cervicornis* were each represented at three study sites: BD, SS and KL. The three genotypes of *A. palmata* were each represented at BD, SS and OL. The coral genotypes utilized in the study were selected by RRFB staff to cover a range of expected performance levels represented in their propagation stock (Figure S1b,c, Text S1).

Coral samples were collected at four seasonal timepoints: fall (24–25 September 2019), winter (16–18 December 2019), spring (delayed due to COVID-19 restrictions to 8–16 June 2020) and summer (17–20 August 2020). At each timepoint, a ~10 cm sample was collected from five replicate fragments of *A. cervicornis* (total *n*: 240) and three replicate fragments of *A. palmata* (total *n*: 108). The sample size of *A. palmata* was limited by stock availability. After the removal of apical polyps, half of each sample was frozen for physiological analyses and the other half was preserved in DNA/RNA Shield (Zymo Research) and frozen for DNA extraction (Figure S1d). Each coral fragment was only sampled once throughout the study and no additional pruning occurred during the study period. Coral samples were transported to Florida International University for subsequent analysis (CITES Export permits: BES/2019/004, BES/2019/005, and BQ/2021/001).

2.3 | Coral phenotype

Frozen coral samples were airbrushed with 1× phosphate buffered saline (PBS) on ice to remove all tissue from the skeleton. Tissue slurries were homogenized with a VWR® 200 Homogenizer for 30 s on ice, then vortexed for 30 s prior to aliquoting for downstream analyses. Two replicate 1 mL aliquots were prepared and centrifuged for 3 min at 13,000 *g* at 4°C to separate coral host and algal symbiont cells. All physiological parameters were standardized to total slurry volume and surface area measured via wax dipping with paraffin wax (Veal et al., 2010).

The symbiont pellet from one aliquot was retained to quantify chlorophyll concentrations. Chlorophyll was extracted with 100% acetone for 24 h in the dark. Extracts were vortexed for 15 s then centrifuged for 3 min at 18,000 *g* at 4°C to pellet debris. 175 µL of each extract was added in triplicate to Greiner Bio-One UV-Star™ microplates and the absorbance was measured with a Accuris SmartReader 96 plate reader at 630, 663 and 750 nm wavelengths. The concentration of chlorophyll-a and c2 (µg/mL) was quantified using the equations developed for dinoflagellates, adjusting for pathlength (Jeffrey & Humphrey, 1975; Warren, 2008).

To quantify total protein in both the coral host and symbiont fractions, the coral host supernatant of the second aliquot was transferred to a new tube and the symbiont pellet was resuspended with 1 mL PBS. Protein concentration was measured for each sample in triplicate with the Pierce™ BCA Protein Assay Kit following the manufacturer's instructions for the microplate protocol. Protein concentrations (µg/mL) were calculated from measurements of absorbance at a wavelength of 562 nm using the cubic polynomial equation determined from the standard curve.

Ash free dry weight (AFDW), or biomass, was measured in both coral host and symbiont fractions (Fitt et al., 2000). A 5 mL aliquot of homogenized tissue slurry was centrifuged at 1300 *g* for 3 min at 4°C to separate coral host and algal symbiont cells. 4 mL of the coral host supernatant was transferred to an aluminium pan that had previously burned in a muffle furnace to remove any organic material. After discarding the remaining supernatant, the symbiont pellet was resuspended with 1 mL PBS, and the resuspension was transferred to a pre-burned aluminium pan. Samples were dried at 80°C for 24 h in a drying oven, weighed and then burned at 450°C in a muffle furnace for 4 h. AFDW was measured as the final, burned, weight subtracted from the initial, dried, weight, standardized to surface area (mg/cm^2).

2.4 | DNA methylation

Genomic DNA was extracted from preserved coral samples using a commercially available kit (Zymo Research Quick-DNA Miniprep) with the addition of a 2 min vacufuge (Eppendorf Vacufuge Plus) between the final wash and elution steps to increase extraction purity. DNA quality was assessed by spectrophotometric analysis (NanoVue) and gel electrophoresis. DNA concentration was determined with the Qubit™ dsDNA broad range assay and standardized to 36.4 ng/µL. Global patterns of DNA methylation were characterized using the methylation sensitive amplified polymorphism (MSAP) method (Reyna-López et al., 1997). The MSAP method targets the 5′-CCGG-3′ motif along the genome, with specific locations determined by the additional bases of the pre-selective and selective PCR primers used (Yaish et al., 2014). Rather than single nucleotide or gene-level changes in DNA methylation, MSAP describes the patterns of four categorical methylation states across the loci identified by the selective PCR primer sequences (Pérez-Figueroa, 2013). As DNA sequencing is not required, this method is useful for characterizing differences in DNA methylation in organisms for which a reference genome is not yet available (Beal et al., 2022; Paige Beal et al., 2021). Additionally, MSAP is one of the methods most feasibly applied to ecologically relevant studies with larger sample sizes that aim to describe changes in DNA methylation profiles across the genome (e.g. rather than measurements of "total" methylation) (Eirin-Lopez & Putnam, 2019). Briefly, genomic DNA was digested in parallel reactions with restriction enzymes *Eco*RI and either *Hpa*II or *Msp*I, which are isoschizomers with different sensitivities to DNA methylation. Following the ligation of adapters to the digested DNA, fragments were amplified through two rounds (pre-selective and selective) of PCR and analysed with an ABI Prism 310 Genetic Analyser (Text S1, Table 1).

2.5 | Data analysis

Logistical limitations during the beginning of the COVID-19 pandemic resulted in the loss (flooding) of one temperature and

TABLE 1 MSAP adapter and primer sequences.

Step	Oligo type	Sequence
Digestion-ligation	*Eco*RI forward adapter	CTCGTAGACTGCGTACC
	*Eco*RI reverse adapter	AATTGGTACGCAGTCTAC
	*Hpa*II/*Msp*I forward adpt.	CGTTCTAGACTCATC
	*Hpa*II/*Msp*I reverse adpt.	GACGATGAGTCTAGAA
Pre-selective PCR A	*Eco*RI + A	GACTGCGTACCAATTCA
	*Hpa*II/*Msp*I + T	GATGAGTCTAGAACGGT
Pre-selective PCR B	*Eco*RI + C	GACTGCGTACCAATTCC
	*Hpa*II/*Msp*I + A	GATGAGTCTAGAACGGA
Selective PCR C1	*Hpa*II/*Msp*I + TTG (*FAM)	GATGAGTCTAGAACGGTTG
	*Hpa*II/*Msp*I + TCT (*FAM)	GATGAGTCTAGAACGGTCT
Selective PCR C2	*Hpa*II/*Msp*I + TCA (*HEX)	GATGAGTCTAGAACGGTCA
	*Hpa*II/*Msp*I + TAG (*HEX)	GATGAGTCTAGAACGGTAG
Selective PCR C4	*Hpa*II/*Msp*I + ATC (*HEX)	GATGAGTCTAGAACGGATC
	*Hpa*II/*Msp*I + ACA (*HEX)	GATGAGTCTAGAACGGACA

Note: The 5′–3′ sequences for all adapters and primers utilized in each step: digestion-ligation, pre-selective PCR A or B, and selective PCR C1, C2 or C4. Each selective PCR primer was fluorescently labelled with either FAM or HEX dyes, noted with the asterisk.

conductivity logger as well as a gap in data logging during the spring of 2020. Therefore, only measurements from dates when data was available for all sites were used for statistical analyses to characterize spatial and seasonal (Fall: September–November, Winter: December–February, Spring: March–May, Summer: June–August) differences in temperature and conductivity (80 days for all four sites and 196 days on the three sites with *A. cervicornis* corals (BD, SS and KL)). The influences of site and season, including the interaction, on water temperature and conductivity (daily average and standard deviation), as well as nutrients (total nitrogen (N), total phosphorus (P), and the ratio of N:P) were evaluated with generalized linear models (*glm* function). For each environmental parameter, models with Gaussian, Gamma, and Inverse-Gaussian distributions were compared with Akaike Information Criterion (AIC). From the best fit model, significant effects of each predictor variable as well as pairwise comparisons within each significant predictor were evaluated with analysis of variance (ANOVA, *car* package in R, version 3.1-1) and estimated marginal means (*emmeans* package in R, version 1.6.2-1), respectively. Instances of nitrogen enrichment were identified as measurements above the Redfield ratio (16:1), expected under nutrient balance (Redfield, 1958), as well as the threshold of nutrient imbalance where phosphorus becomes limiting (22:1) (Rosset et al., 2017). The proportions of measurements above these thresholds were then compared between sites and seasons (*prop. test* function).

Coral phenotype was characterized by the multivariate analysis of log(x + 1)-transformed host and symbiont protein and biomass, and symbiont chlorophyll-a and c2 concentrations within each species using the *vegan* package in R (version 2.5-6) with Euclidean distances. Differences in physiology between genotypes, sites, and timepoints were assessed with permutational multivariate analysis of variance (PERMANOVA) and dispersion (PERMDISP) (*adonis* and *betadisper* functions, respectively) (Anderson & Walsh, 2013). Pairwise differences between groups within significant factors were identified with post-hoc Tukey HSD and pairwise PERMANOVA (*pairwiseAdonis* version 0.4, (Arbizu, 2020)) analyses. Variance partitioning and redundancy analyses (RDA) were used to quantify the proportion of variance in coral physiology that can be explained by each genetic (genotype) or environmental (site or season) factor individually, while controlling for the contribution of the other two variables. The influence of genotype, site and season on each individual metric of coral physiology was evaluated with linear regression followed by analysis of variance (ANOVA). Physiological responses that were not normally distributed were log(x + 1)-transformed prior to regression. The effect size of each predictor in the model (genotype, site, season, genotype * site, genotype * season) was calculated as Eta squared (η^2) (Cohen, 1973) (*effectsize* package in R, version 0.8.3) and pairwise differences between levels within significant predictors were assessed with estimated marginal means.

Following DNA fragment analysis, a binary matrix indicating fragment presence (1) or absence (0) across loci for both enzymatic reactions (*Hpa*II or *Msp*I) was obtained by peak calling across each sample and primer combination using GeneMapper v.3.7 (Applied Biosystems, Foster City, CA, EE USA) for each species individually. Only peaks within the 50–1000 bp range, above 20 Relative Fluorescent Units (RFUs), and represented in more than 15% of the sample population within each species were retained. The methylation states across each loci were scored as non-methylated (NMT), if both *Hpa*II and *Msp*I bands were present for a given loci (1/1), hemimethylated (HMM), if only the *Hpa*II product is present (1/0), or methylated at the internal cytosine (ICM) if only the *Msp*I product is present (0/1) for each sample using the *msap* package (version 1.1.9) (Pérez-Figueroa, 2013). The cases where no fragments were present in either reaction (0/0) could indicate either hypermethylation (HPM) or the absence of the target due

to genetic variation. However, as our study design compared genetic clones within 4–3 distinct genotypes (of *A. cervicornis* and *A. palmata*, respectively) that were represented across sites and seasonal sampling periods, we propose that these cases were primarily full methylation and considered them as such for statistical analysis (Rodríguez-Casariego et al., 2020). Only polymorphic, methylation-susceptible loci (MSL) were utilized for further analysis (Herrera & Bazaga, 2010).

The influence of genetic (coral genotype) and environmental (site or season) variation on DNA methylation profiles (i.e. patterns of methylation states across analysed loci) was assessed within each species with PERMANOVA and PERMDISP on the Gower distance given the categorical nature of the methylation states (*cluster* package, version 2.1.0). Pairwise differences between groups within significant factors (genotype and season) were identified following the same methods as in the analysis of physiological data. The proportions of variance in DNA methylation that can be explained by coral genotype and seasonal period were quantified with variance partitioning and distance-based redundancy analyses (dbRDA). Additionally, discriminant analysis of principal components (DAPC) was performed to further evaluate differences in DNA methylation profiles between genotypes and seasons as well as to identify the loci most contributing to these differences for each species (Jombart et al., 2010). Within the DAPC, clusters (*k*) were set a priori based on significant pairwise differences identified through post-hoc Tukey HSD and pairwise PERMANOVA analyses (Miller et al., 2020). The number of PC's retained was set as *k*–1 (Thia, 2022) and 2 discriminant axes were retained in each analysis. The most influential loci were identified as those with loading scores in the 90th percentile for the two discriminant axes. These loci were utilized for subsequent analyses assessing connections between coral physiological and epigenetic variation.

Relationships between coral physiology and DNA methylation were identified by performing distance-based redundancy analyses (dbRDA) on the variation of DNA methylation profiles in each species and including each $\log(x+1)$-transformed physiological metric as predictor variables. To further evaluate how coral phenotypic plasticity is related to epigenetic variation, the correlation between the degree of plasticity in physiology and DNA methylation was quantified for each species. First, centroid locations for each sample set (i.e. particular combination of genotype, site, and timepoint) were identified with the *betadisper* function for multivariate physiology and DNA methylation profiles, independently. Next, coordinates of the first two PCoA axes were extracted and Euclidean distances were calculated between all pairs of centroids within each dataset (Barott et al., 2021). Only meaningful comparisons (i.e. pairs of the same genotype either within the same site or within the same timepoint) were retained for analysis. Distances were further filtered to focus on seasonal plasticity (i.e. distances between pairs of the same genotype at the same site, across seasons) due to the lack of differences in DNA methylation profiles between sites. The correlation (Spearman's Rank) between pairwise centroid distances of coral physiology and those of DNA methylation was then calculated for each species.

3 | RESULTS

3.1 | Environmental conditions

Daily average temperature varied significantly across seasons (Table S2A.1,A.2), with the highest temperatures in the fall, followed by summer, and lowest temperatures in the winter (Figure 1b, Tables S2A.2 and S3A). Additionally, the daily standard deviation of temperature was higher in the summer compared with the fall and winter (Figure S2a, Tables S2A.2 and S3A). Daily average temperature did not significantly differ between study sites (Figure 1b, Table S2A.1,A.2). The daily standard deviation of temperature was lower at OL compared with all other study sites (Figure S2a, Tables S2A.1 and S3B). The daily average and standard deviation of conductivity also differed across both sites and seasons, with a significant interaction between the effect of site and season (Figure 1c, Table S2B.1,B.2). Nitrogen, phosphorus and the ratio of nitrogen to phosphorus (N:P) did not differ significantly between sites or seasons (Table S2C). However, seasonal and spatial patterns in nutrient enrichment were evident (Figure 1d, Figure S2c,d). The proportion of measurements where N:P exceeded the Redfield ratio (16:1) (i.e. instances of nitrogen enrichment) differed marginally across seasons ($p=.054$, *z-test*) with 70.83% and 50.0% in the fall and winter compared with 33.33% and 38.10% in the spring and summer, respectively. Additionally, a higher proportion of measurements exceeded the threshold of nutrient imbalance where phosphorus becomes limiting (22:1) (Rosset et al., 2017) at BD and SS (47.82% and 43.48%, respectively) compared with the two more isolated sites of KL (17.39%) and OL (19.05%) (Site $p=.048$, *z-test*).

3.2 | Coral physiological phenotypes

The physiology of *A. cervicornis* differed between genotype, site and seasonal timepoint (Figure 2a, Table 2). Additionally, the genetic effect on physiology was influenced by both environmental factors (Table 2). Coral genotype, site, and season together explained 48.4% of the variance in physiology of *A. cervicornis* (RDA $p=.001$), with a majority of that being explained by the environmental variables of season and site (Figure 2a). The physiology of *A. palmata* was also influenced by coral genotype and environmental variation (Figure 2b, Table 2), which explained 45.21% of the variance in the physiology of this species (RDA $p=.001$). However, the physiology of *A. palmata* was primarily explained by season (Figure 2b) and no genotype by environment interactions were present (Table 2). For both species, coral physiology of samples collected during the fall and winter timepoints differed from those collected during the spring and summer (Table S4).

Similar trends were apparent in the univariate analysis of each physiological metric (Figures S3–S8, Tables S5–S8). In *A. cervicornis*, each metric (biomass and protein of host and symbiont and chlorophyll-a and c2) was significantly influenced by coral genotype, site, and season expect the biomass of the symbiont which was not influenced by coral genotype (Table S5). Genotype by environment interactions were detected in all metrics except the biomass and protein

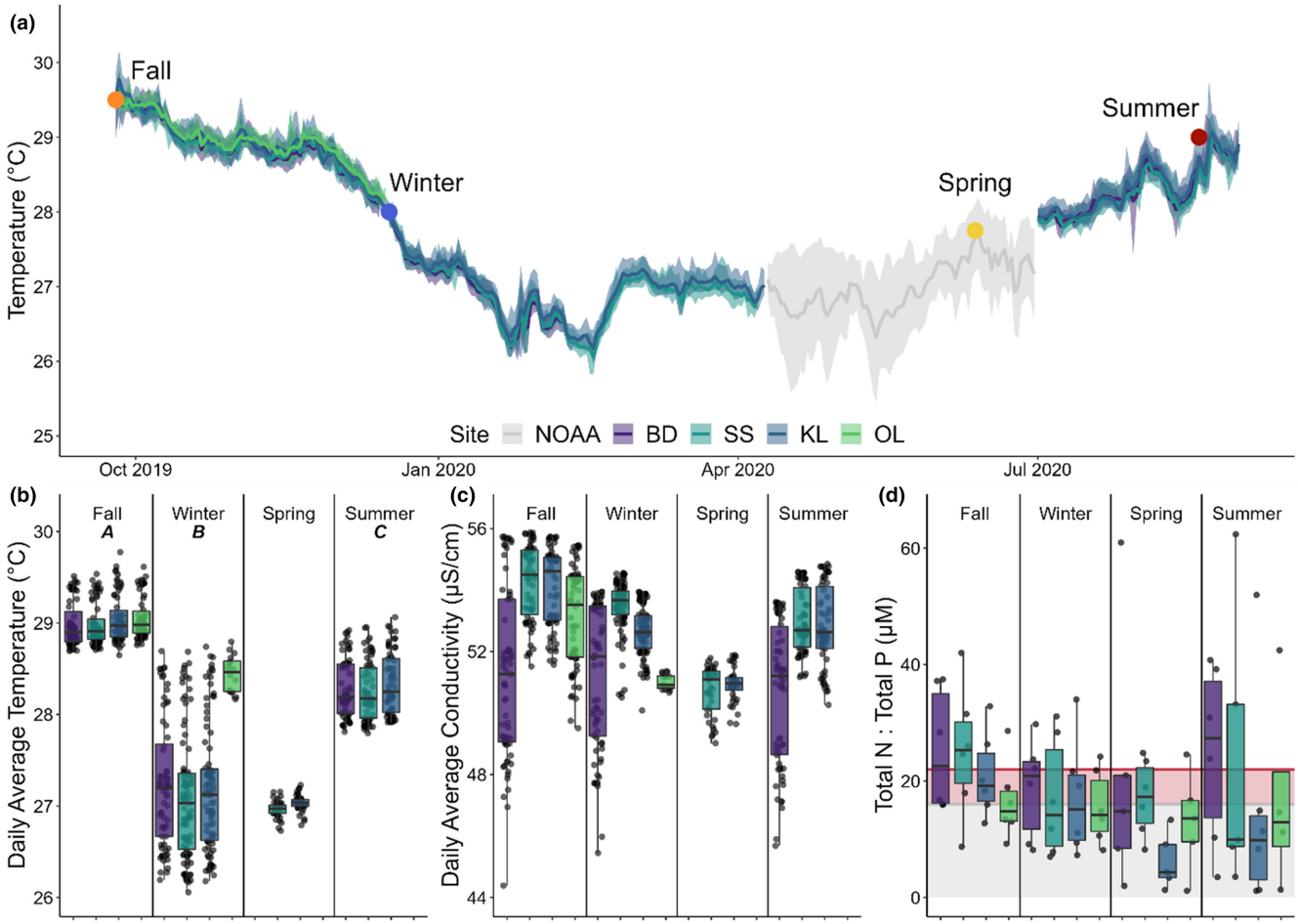

FIGURE 1 Environmental conditions across sites and seasons. (a) Water temperature (°C) for all sites across the study period. Daily averages are shown by the solid lines and daily minimums and maximums by the shaded regions. Sampling timepoints are noted with points, including fall (orange), winter (blue), spring (yellow) and summer (red). Sea surface temperature from the ABC islands (Aruba, Bonaire, Curacao) NOAA Regional Virtual Station (minimum, maximum and average of the two) is included during the interval when in situ water temperature was not logged (NOAA Coral Reef Watch, 2019, updated daily). Daily average (b) temperature (°C) and (c) conductivity (µS/cm) for all sites in each season when data was logged. Individual data points are shown behind the summary boxplots. Significant pairwise differences in average daily temperature are noted by capital letters (Table S3A). (d) Total nitrogen (µM) to phosphorus (µM) ratio for all sites in each season. Individual data points are shown behind the summary boxplots. The Redfield ratio of 16:1 (N:P) is noted with the horizontal grey line (Redfield, 1958). The threshold for phosphorus starvation of 22:1 is noted with the horizontal red line (Rosset et al., 2017). Ratios at or below 16:1 are shown in the grey shaded region and ratios between the Redfield ratio and threshold for phosphorus starvation are shown in the red shaded region.

of the symbiont (Figures S3–S5, Table S5). In contrast in *A. palmata*, only the biomass and protein of the symbiont were influenced by coral genotype (Table S7). Besides the biomass of the symbiont, all other metrics were more strongly influenced by environmental factors, especially by season (Figures S6–S8, Table S7). Additionally, no physiological metric was affected by genotype by environment interactions in this species.

3.3 | DNA methylation profiles show similar trends to coral physiology

In *A. cervicornis*, 99 methylation-susceptible loci (MSL) were identified (Primer C1: 82, Primer C2: 9, Primer C4: 8) and 91 of those were polymorphic. Within *A. palmata* samples, 73 MSL (Primer C1: 38, Primer C2: 12, Primer C3: 23), including 68 polymorphic loci, were identified. For both species, the most common methylation state was full methylation (HPM), followed by hemi-methylation (HMM) and internal cytosine methylation (ICM) (Figure 3a,b).

Within each species, DNA methylation profiles varied between genotypes as well as seasons (Table 3 and Table S9). Genotype by environment interactions only influenced DNA methylation profiles in *A. cervicornis* (specifically Genotype × Site with a marginal ($p < .1$) effect of Genotype × Season) but not those of *A. palmata* (Table 3), similar to the effects on coral physiology in each species (Table 2). Also similar to multivariate coral physiology, DNA methylation profiles in the fall and winter timepoints differed from those in the spring and summer (Table S9A.2,B.2). Seasonal timepoints were therefore grouped into "cooling" (fall and winter) and "warming" (spring and summer) periods for the subsequent DAPC and heatmap analyses (Figure 3c,d and Figure S10). While variation in DNA methylation profiles of *A. cervicornis* were significantly related to both genetic

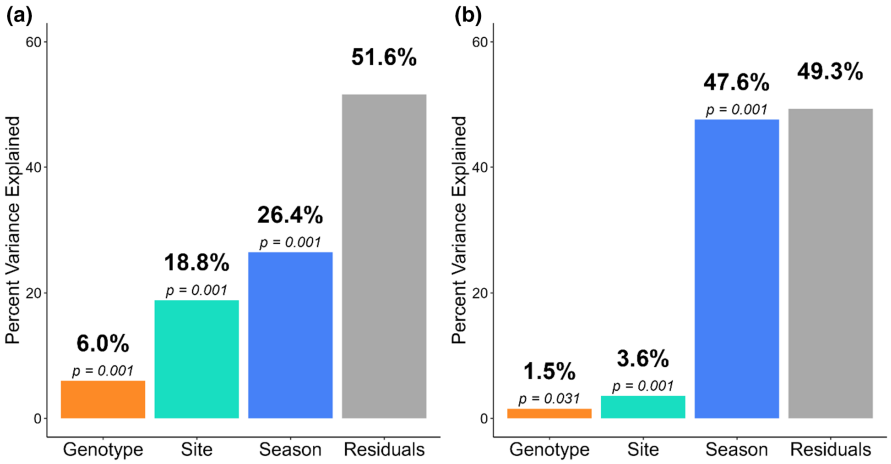

FIGURE 2 Variance partitioning of coral physiology of (a) *Acropora cervicornis* and (b) *A. palmata*. The percentage of variance in the multivariate coral physiology explained by coral genotype (orange), site (teal), season (blue), along with residual unexplained variance (grey), for each species. *p*-values of the influence of each predictor while controlling for the effects of the other variables (partial RDA) are shown.

TABLE 2 Multivariate analysis of variance in coral physiology.

	Acropora cervicornis		*Acropora palmata*	
	PERMANOVA	PERMDISP	PERMANOVA	PERMDISP
Genotype	**.001**	**.024**	**.020**	.360
Site	**.001**	.741	**.002**	.227
Season	**.001**	.151	**.001**	.914
Genotype × Site	**.041**	NA	.182	NA
Genotype × Season	**.039**	NA	.095	NA

Note: *p*-values are displayed for PERMANOVA and PERMDISP and *p*-values less than alpha of .05 are in bold.

and seasonal factors (Table 3, RDA $p = .001$ for each), a higher amount of variance was explained by season (variance partitioning: 7.61%) compared to genotype (3.19%). This relative importance of factors influencing DNA methylation profiles in this species was corroborated by the distribution of seasonal followed by genetic groups along the first and second DAPC axes, respectively (Figure 3c), as well as the clustering of samples on the heatmap (Figure S10a). In contrast, variation in the DNA methylation profiles of *A. palmata* was explained more by genotype (variance partitioning: 15.50%) compared to season (9.61%) (Figure 3d and Figure S10b, RDA $p = .001$ for each). Based on the DAPC analysis, 41 (45.05%) of the polymorphic methylation susceptible loci (MSL) were most influential (90th percentile of DAPC axis 1 and 2 loading scores) to differences in DNA methylation profiles across genotypes and seasons in *A. cervicornis* (Figure S9a,b). In *A. palmata*, 50% ($n = 34$) of the MSL were identified as the most influential (Figure S9c,d).

Variation in DNA methylation state of the most influential loci was significantly related to coral physiology in both species (RDA $p = .001$ for each). In *A. cervicornis*, the distance-based RDA model including each physiological metric explained 10.84% of the variation in DNA methylation profiles, with each metric significantly contributing to the constrained variance, except for the biomass of the host (Figure 4a, Table 4). Similarly, 17.24% of the variance in DNA methylation profiles of *A. palmata* was explained by the model, and the biomass of the symbiont was the only metric that did not significantly contribute (Figure 4b, Table 4). Furthermore, the degree of seasonal phenotypic plasticity in both *A. cervicornis* and *A. palmata* was significantly correlated with the degree of epigenetic plasticity (i.e. distances between the centroids of sample sets of the same genotype and site, across seasons) (Figure 4c,d).

4 | DISCUSSION

4.1 | Spatial and seasonal trends

The sampling timepoints and study sites were selected to encompass both seasonal and spatial environmental variation in Bonaire and record the physiological and epigenetic features of *A. cervicornis* and *A. palmata* corals. Indeed, water temperature, conductivity and nutrients varied within the context of the present study. The predicted seasonal fluctuation in sea water temperature (Bak et al., 2005) was apparent during the period recorded in this study, with warmest temperatures occurring in the fall (Figure 1, Tables S2 and S3). As the study sites were of similar depths and fore-reef habitats, site-specific differences in temperature were not expected. Conductivity (average and standard deviation) varied across both sites and seasons. While a significant interactive effect between site and season prevented post-hoc pairwise comparisons, the conductivity at Buddy's Reef tended to be lower and more variable compared to the other sites (Figure 1 and Figure S2). This site is directly in front of the Buddy Dive Resort dive shop and potentially receives increased freshwater influx from activities associated with the resort such as rinsing dive gear and boats. Terrestrial runoff and groundwater flow

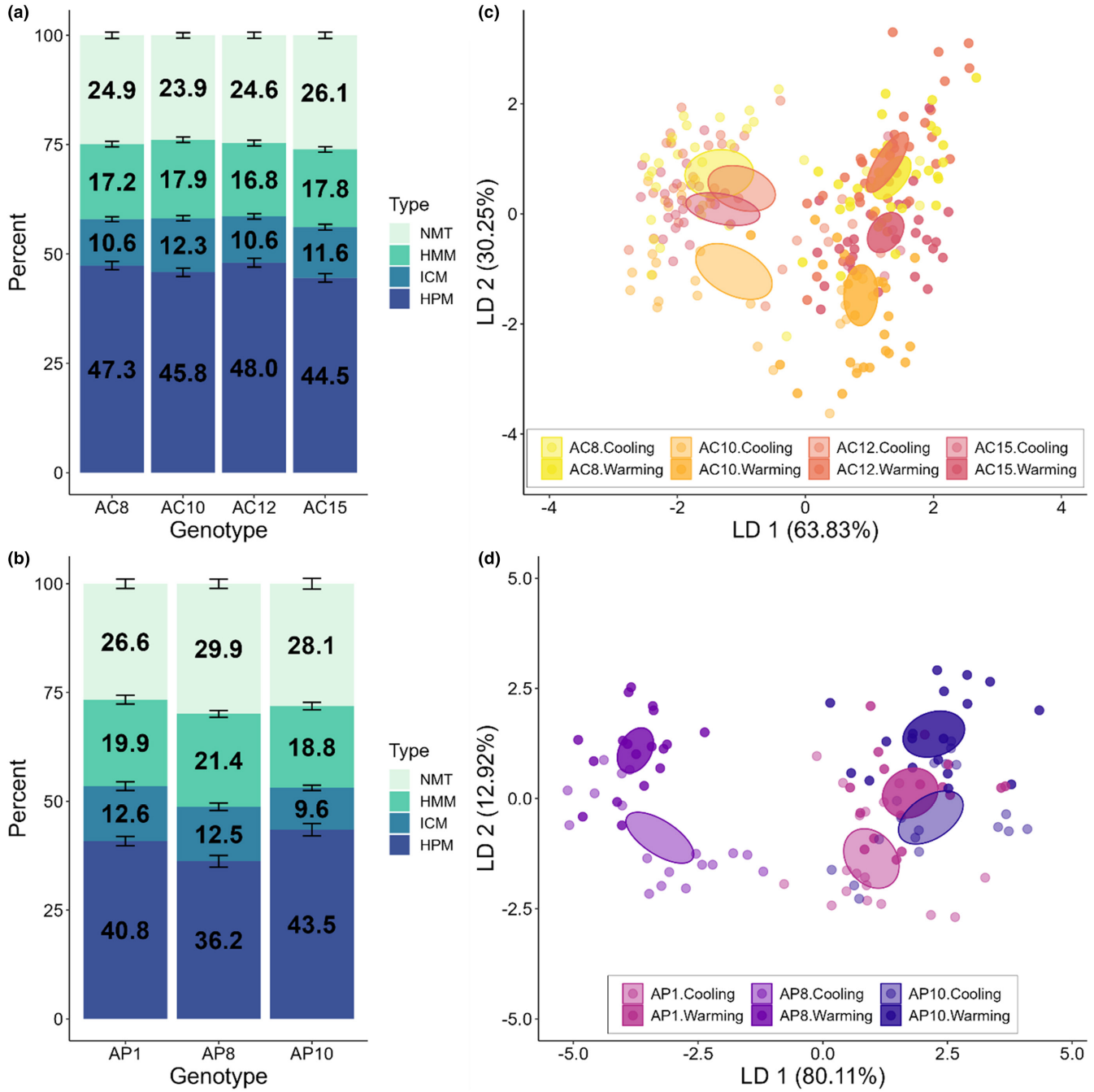

FIGURE 3 DNA methylation states across genotypes and seasons for *Acropora cervicornis* (a and c) and *A. palmata* (b and d). (a, b) The percentage of each DNA methylation type (NMT, non-methylated; HMM, hemi-methylated; ICM, methylated at an internal cytosine; or HPM, hypermethylation) identified across polymorphic, methylation-susceptible loci. Percentages are averaged across all samples for each genotype (i.e. all sites and seasons). Error bars show the standard error. (c, d) Discriminant Analysis of Principal Components (DAPC) of DNA methylation profiles with groups assigned a priori based on significant differences identified between genotypes and seasonal periods (Table 3 and Table S9). Points are coloured by genotype within each species. Within a colour, more transparent circles represent the "cooling" period (fall and winter), while more opaque circles represent the "warming" period (spring and summer). Ellipses represent the 95% confidence interval of each genotype by period group.

were also predicted to contribute to nutrient enrichment at these near-shore coral nursery sites, especially at the locations closer to population centers (Slijkerman et al., 2014) and particularly during the rainy season in the fall and winter (Rivera-Milán et al., 2018). We did not find significant differences in the concentration of nitrogen (N) and phosphorus (P) nor the ratio of N:P between seasons or sites (Figure 1 and Figure S2, Table S2), which may be due, in part, to low statistical power given the frequency of water sample collection (bi-monthly). However, there were seasonal and spatial trends in nutrient enrichment. During the rainy season, 50%–70.83% (winter, fall, respectively) of water sample measurements exceeded the Redfield ratio of 16:1 compared to 33.33%–38.10% of measurements in the

TABLE 3 Multivariate analysis of variance in coral DNA methylation.

	Acropora cervicornis		*Acropora palmata*	
	PERMANOVA	PERMDISP	PERMANOVA	PERMDISP
Genotype	**.001**	.106	**.001**	.208
Site	.498	NA	.055	NA
Season	**.001**	**<.001**	**.001**	.738
Genotype × Site	**.039**	NA	.171	NA
Genotype × Season	.071	NA	.964	NA

Note: *p*-values are displayed for PERMANOVA and PERMDISP and *p*-values less than alpha of .05 are in bold.

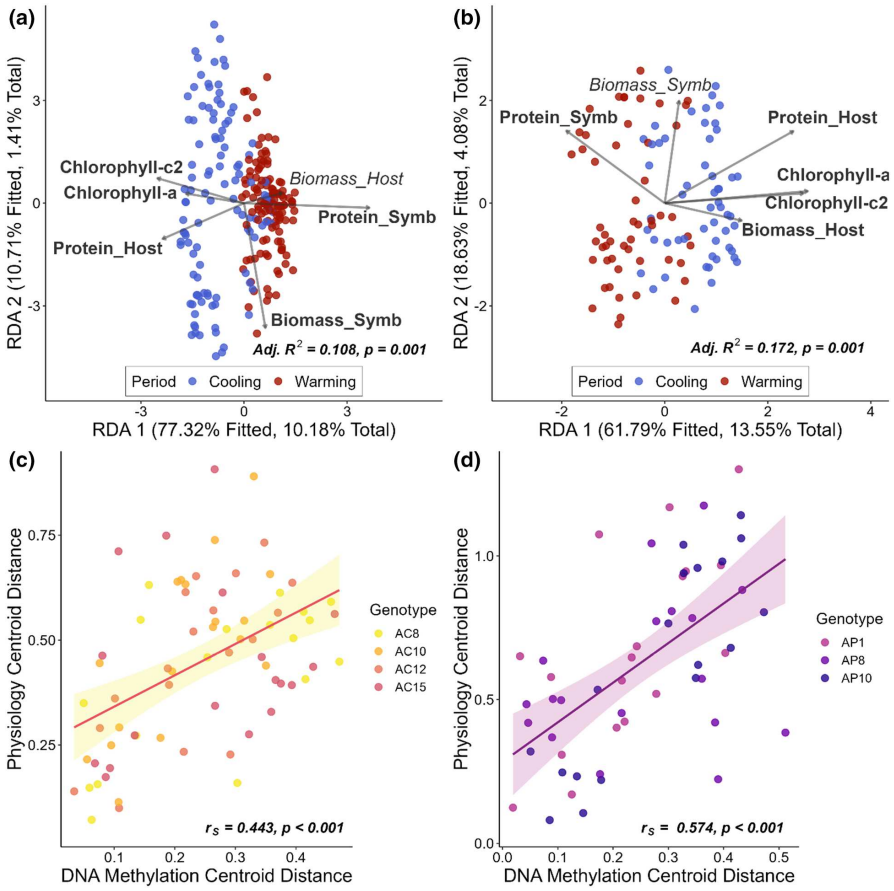

FIGURE 4 Relationship between coral physiology and DNA methylation. (a, b) Distance-based redundancy analysis (dbRDA) of the variation in DNA methylation profiles of the most informative loci (Figure S9) for each species [(a): *Acropora cervicornis* and (b): *A. palmata*] explained by each physiological metric. Each point represents the DNA methylation profile of one sample and points are coloured by seasonal periods ("Cooling": fall and winter, shown in blue; "Warming": Spring and Summer, shown in red). Adjusted R^2 and *p*-value (*anova.cca* function) of the full dbRDA model are shown. Physiological metrics significantly related to variation in DNA methylation are shown in bold (Table 4). (c, d) Correlation between seasonal phenotypic and epigenetic plasticity of (c) *A. cervicornis* and (d) *A. palmata*. Each axis represents the Euclidean distance between the centroids of sample sets (combination of genotype and sites) between pairs of seasonal timepoints for the DNA methylation profiles of the most informative loci (*x*-axis) and multivariate physiology (*y*-axis). Each point represents these respective distances for each relevant sample set and points are coloured by coral genotype. The Spearman's Rank correlation coefficient (r_S) and *p*-value are shown (*cor.test* function). The linear relationship is shown by the solid line with the 95% confidence interval shown by the shaded region.

dry season (spring, summer, respectively). Furthermore, the proportion of measurements exceeding the threshold where phosphorus becomes limiting (Rosset et al., 2017) differed across sites, with higher proportions occurring at the study sites predicted to be more impacted by local stressors (Buddy's Reef and Something Special) compared with the two more isolated study sites. Together, these results demonstrate that spatial and seasonal variation in environmental conditions was present during the study period, which could have implications for the stability of coral symbioses, thereby influencing coral metabolism and stress susceptibility as well as overall coral physiology (Jurriaans & Hoogenboom, 2020; Morris et al., 2019; Sawall et al., 2022; Wiedenmann et al., 2012).

TABLE 4 Distance-based redundancy analysis (dbRDA) of relationships between coral physiology and DNA methylation profiles.

	Acropora cervicornis	*Acropora palmata*
Protein Host (μg/cm^2)	**.001**	**.001**
Protein Symbiont (μg/cm^2)	**.001**	**.001**
Biomass Host (mg/cm^2)	.724	**.003**
Biomass Symbiont (mg/cm^2)	**.002**	.221
Chlorophyll-a (μg/cm^2)	**.020**	**.018**
Chlorophyll-c2 (μg/cm^2)	**.018**	**.013**

Note: *p*-values resulting from the ANOVA-like permutation test (*anova.cca* function, by="terms") of the relationship between each physiological metric and the variation in DNA methylation profiles of *A. cervicornis* and *A. palmata*. *p*-values less than alpha of .05 are in bold.

4.2 | Phenotypic plasticity and genotype by environment interactions

While variation in growth, reproductive success, and stress tolerance across genotypes of *A. cervicornis* and *A. palmata* (Baums et al., 2013; Lohr & Patterson, 2017; Muller et al., 2021) demonstrate genetic constraints on coral fitness, phenotypic plasticity has also been reported in both species (Durante et al., 2019; Kuffner et al., 2017; Million et al., 2022). Indeed, *A. cervicornis* and *A. palmata* corals studied in the present work demonstrated environmentally induced plasticity, with environmental factors more strongly influencing the physiology of both species compared with the effect of coral genotype (Figure 2). Seasonal and spatial environmental variability have similarly been found to be strong drivers of the performance of multiple coral species (Thornhill et al., 2011). For example, between 52% and 83% of the variance in coral phenotype of four Indo-Pacific coral species was explained by spatial and seasonal variation in water quality parameters (Browne et al., 2015), which is comparable with the importance of spatial and seasonal environmental drivers (i.e. explaining ~45%–50% of the variance) on coral phenotype in this study. The protein of the host and symbiont, along with chlorophyll concentrations, were particularly influenced by environmental factors (especially season) in both *A. cervicornis* (Figures S4 and S5) and *A. palmata* (Figures S7 and S8), as evidenced by comparatively larger effect sizes (Tables S5 and S7). Furthermore, seasonal trends in physiological metrics were similar to those previously reported for these species (Fitt et al., 2000; Thornhill et al., 2011). For example, the biomass of the host peaked in the spring and remained higher in the summer compared with the fall and winter in *A. cervicornis* (Figure S3, Table S6). This peak may have occurred sooner in *A. palmata* as the biomass of the host differed between fall and winter timepoints in this species (Figure S6, Table S8). Additionally, similar to Fitt et al. (2000), chlorophyll concentrations (both a and c2) of *A. palmata* peaked in winter and declined into the spring and summer (Figure S8, Table S8). While significant interaction terms prevented the same pairwise statistical comparisons (Table S5), similar seasonal trends of chlorophyll concentrations are apparent in *A. cervicornis* (Figure S5). Overall, the spatial and seasonal trends in coral physiology observed in both *A. cervicornis* and *A. palmata* indicate the capacity for phenotypic plasticity across environmental variation, especially throughout seasons.

The significant interactive effects of genotype × site and genotype × season observed to influence coral physiology in *A. cervicornis* (Table 2 and Table S5) corroborate recent reports of genotype by environment interactions (GxE) in the phenotype of this species. Reciprocal transplant experiments across the Florida Reef Tract found that GxE affected the growth, morphology, bleaching response and survival of *A. cervicornis* (Drury et al., 2017; Drury & Lirman, 2021; Million et al., 2022). In the present work, GxE influenced the biomass and protein of the host, as well as both chlorophyll-a and -c2 in *A. cervicornis* (Table S5). The presence of GxE indicates variation in the capacity for phenotypic plasticity across genotypes of *A. cervicornis*. This has important implications for the restoration of this species as the relative performance of a genet (i.e. individuals of a particular genotype (Heyward & Collins, 1985)) at one site may not be indicative of the performance to be expected under different environmental conditions. Interestingly, morphological plasticity has been found to be adaptive and associated with higher growth rates and survival (Million et al., 2022). Therefore, quantitative measurements of phenotypic plasticity (e.g. measures of the variation of a trait across environmental gradients rather than the mean value of a trait in a single environment) may be important to incorporate into predictions of the performance of *A. cervicornis* genets across environments and under continued climate change. Genotype by environment interactions have also been reported in the bleaching severity, but not growth, of four genotypes of *A. palmata* corals outplanted to patch and fore reef habitats in the Florida Keys (Pausch et al., 2018). Yet, we found no evidence of either spatial or seasonal GxE influencing the multivariate or univariate physiology of the genotypes of *A. palmata* studied on Bonaire (Table 2 and Table S7), which may suggest limited evolutionary capacity (Sirovy et al., 2021). However, this lack of observed GxE could also be due, at least in part, to the focus of the present study on just three genotypes. Unfortunately, the nursery stock for this species was limited, particularly to provide the number of replicates within each genet required for the desired degree of spatial and seasonal sampling. Additionally, the environmental variation experienced by *A. cervicornis* and *A. palmata* corals in this study may have differed between species due to the difference of the third nursery location for each species (KL for *A. cervicornis* and OL for *A. palmata*), which was a limitation from utilizing existing RRFB nursery infrastructure. Therefore, additional research including more distinct genotypes of *A. palmata* across additional sites may be necessary to better describe the potential influence of GxE on the phenotype of this species. Moreover, Pausch et al. (2018) demonstrated that the presence of GxE can vary between performance metrics (e.g. growth vs bleaching severity). Additional metrics of overall fitness including growth, thermal tolerance and reproduction may therefore be important to incorporate in future studies of GxE in these coral species, particularly for the aim of informing restoration practices.

4.3 | DNA methylation across genetic and environmental variation

The average frequencies of each DNA methylation state found across genotypes of *A. cervicornis* and *A. palmata* (Figure 3a,b) were comparable with those previously identified using the MSAP method in *A. cervicornis* (Rodríguez-Casariego et al., 2020). Additionally, intra-specific epigenetic variation was present across genotypes of *A. cervicornis* and *A. palmata* analysed in this study (Figure 3 and Figure S10). Given the contingency of DNA methylation upon the presence or absence of a CpG site in an organism's genome sequence (Gertz et al., 2011), this aligned with our predictions of differences in DNA methylation between genotypes, as well as previous evidence of the genetic influence on DNA methylation profiles in *A. palmata* (Durante et al., 2019).

Contrary to expectations, patterns of DNA methylation did not differ across study sites in either *A. cervicornis* or *A. palmata*. While this could be due to a lack of biologically meaningful differences in environmental conditions between the study sites, particularly due to their relatively close proximity, spatial environmental variation was sufficient to result in differences in coral physiology between at least some of the sites in this study. We suggest that this lack of spatial variance in DNA methylation patterns may provide evidence that corals within this study had previously become acclimated to their nursery sites, especially given the relatively long acclimation period prior to the study (average: ~4 years, range: 3 months–7 years). For instance, the alterations in gene function mediating acclimation may no longer be required once homeostasis is achieved, which may result in the reversion of epigenetic changes that would have been detectable earlier in the acclimation process. Indeed, the differential DNA methylation documented in other coral species through acclimation to novel environments was during relatively shorter-term (3 month–1 year) transplantation experiments (Dimond & Roberts, 2020; Dixon et al., 2018). Yet, further work is needed to better describe the temporal dynamics of DNA methylation throughout acclimatization, especially across multiple years.

The difference in DNA methylation profiles between seasonal periods observed in this study provide support for the role of DNA methylation in seasonal acclimatization in Caribbean Acroporid corals. This relationship was especially pronounced in *A. cervicornis* with patterns of DNA methylation clearly separating along the first DAPC axis for all genets included in this study (Figure 3). Similarly, a conserved response of DNA methylation to seasonal environmental variation was reported in *A. cervicornis* corals in Puerto Rico, which outweighed any effect of transplantation site or depth (Rodríguez-Casariego et al., 2020). Corals must cope with repeated and fairly predictable changes in environmental conditions throughout seasonal cycles. The ability to rapidly acclimatize, within a matter of months, across seasonal variation is particularly important for these long-lived, sessile organisms (Scheufen et al., 2017). It follows that environmentally inducible and reversible epigenetic modifications likely play an important role in the cyclic phenotypic plasticity required for seasonal acclimatization (Eirin-Lopez & Putnam, 2019; McCaw et al., 2020; Suarez-Ulloa et al., 2019).

While patterns of DNA methylation were significantly influenced by both genetic and environmental factors, the majority of variation in DNA methylation of each species was unexplained by the predictive variables included in this study. However, our results align with the few comparable studies that have reported residual variance in DNA methylation. For example, a majority of the variation in DNA methylation in *A. palmata* remained unexplained (median >75%) by genotype, location of sample along the colony and phenotypic bleaching condition (Durante et al., 2019). Additionally, approximately 85%–90% of the variance in DNA methylation within genets of *Montastraea cavernosa* corals was not explained by either temperature treatment or algal symbiont composition (Rodriguez-Casariego et al., 2022). It has been proposed that high variability in DNA methylation may be due to "spontaneous epimutations" that add stochasticity when methylation marks are not faithfully transmitted across cell divisions (Durante et al., 2019). Additionally, variation in DNA methylation may be due to the pooling of different tissue types within the DNA extraction (Trigg et al., 2022), which also can include cells and coral polyps at different stages of development and asexual division. Further research utilizing cell or tissue-specific analyses of DNA methylation would be required to evaluate the contribution of these additional factors to the variation of DNA methylation in corals.

4.4 | Epigenetic drivers of phenotypic plasticity

The significant relationships between physiological metrics and variation in DNA methylation profiles in both *A. cervicornis* and *A. palmata* identified in this study (Figure 4, Table 4) add to the growing body of evidence quantitatively linking epigenetic and phenotypic variation in corals. Differential DNA methylation has been associated with phenotypic responses of corals to experimental conditions (Liew et al., 2018; Putnam et al., 2016), throughout acclimatization (Dixon et al., 2018), and as well as during heating events on reef habitats (Durante et al., 2019). Yet, to our knowledge, only two previous studies have assessed quantitative relationships between coral phenotype and DNA methylation (Dixon et al., 2018; Durante et al., 2019). Following a reciprocal transplant, shifts in DNA methylation profiles of *A. millepora* to be more similar to "local" corals were significantly correlated to metrics of physiological fitness, especially weight gain (Dixon et al., 2018). Additionally, variation in the bleaching response of *A. palmata* across clonemates (i.e. ramets of the same genotype) was related to differential DNA methylation (Durante et al., 2019). In the present work, variation in DNA methylation profiles of *A. cervicornis* and *A. palmata* were significantly related to the protein of the host and symbiont, chlorophyll-a, chlorophyll-c2 and either the biomass of the host (*A. palmata*) or the symbiont (*A. cervicornis*) (Figure 4, Table 4). These relationships are likely driven by changes in both DNA methylation profiles and coral physiology across seasons, especially given the differences between "cooling" (fall and winter) and "warming" (spring and summer) periods that were documented in both features (Figure 3, Figures S10 and S3–S8). Furthermore,

given the well-documented seasonality of coral physiology (Fitt et al., 2000; Scheufen et al., 2017) along with the strong seasonal trends in DNA methylation reported in *A. cervicornis* (Rodríguez-Casariego et al., 2020), we predicted that seasonal phenotypic plasticity would be associated with corresponding epigenetic shifts. More specifically, we expected a positive correlation between the degree of plasticity (i.e. pairwise distances between same-genotype and same-site comparisons across timepoints) in coral physiology and DNA methylation profiles. Such a relationship was evident for both *A. cervicornis* and *A. palmata* (Figure 4). These correlations between the variation in physiology and DNA methylation in both species support the role of epigenetic mechanisms contributing to coral phenotype and phenotypic plasticity.

Although the mechanistic underpinnings of this relationship are not yet well described, lower levels of gene-body methylation have been suggested to provide transcriptional opportunities and therefore increase capacity for phenotypic plasticity in invertebrate taxa (Roberts & Gavery, 2012). In fact, inverse relationships between gene expression and gene body methylation have been described in corals, with less methylation occurring on environmentally-responsive genes (Dimond & Roberts, 2016; Dixon et al., 2018). Additionally, DNA methylation levels in the environmentally-responsive *P. damicornis* were initially lower than those of the more resistant *M. capitata* (Putnam et al., 2016). Although these studies utilized different methodologies, together their results suggest that organisms with more methylated genomes could have a lower potential for inducible transcriptional responses to environmental variation. Gene-body methylation is also related to alternative splicing in some invertebrates, where exons included in gene transcripts were more highly methylated (Bogan et al., 2023; Flores et al., 2012; Song et al., 2017). A reduction in both transcriptional variation and cryptic transcription was related with higher DNA methylation in *S. pistillata* corals (Liew et al., 2018), suggesting that low methylation may provide opportunities for transcription at alternative start sites. Differential methylation may therefore influence exon inclusion and help control the production of splice variants that may lead to phenotypic variation. As such, differing capacities for epigenetic variation and gene regulation likely contribute to the range of phenotypic plasticity within a population (i.e. GxE). We were interested to find that detectable genotype by environment interactions seemed to correspond between coral physiology and DNA methylation in the present study. More specifically, GxE influenced both the phenotype and DNA methylation profiles of *A. cervicornis*, while such interactions were not apparent in the phenotypic or epigenetic variation of *A. palmata*. However, further study, including a larger number of both genotypes and environmental conditions (e.g. sites), is required to quantitatively determine if GxE in coral phenotype is related to epigenetic GxE.

It is possible that coral physiology may differ in controlled coral nurseries compared to on the reef substrate, particularly when comparing wild corals to those in land-based nurseries as previously demonstrated in *A. palmata* (Gantt et al., 2023). Additionally, the seasonal dynamics of chlorophyll content and biomass of *A. cervicornis* were influenced by depth comparing corals sampled at 1–3 m and 13 m (Fitt et al., 2000). However, the setting of coral nursery trees was selected to better facilitate interspecific comparisons and elucidate relationships between coral phenotype and DNA methylation across quantifiable differences in environmental conditions, without the potentially more variable and elusive factors contributing to coral performance within natural reef environments. Additionally, overall seasonal trends in physiological parameters, such as reduced chlorophyll and biomass in the summer than winter, were still present in *A. cervicornis* at both 1–3 m and 13 m depths (Fitt et al., 2000). Furthermore, DNA methylation patterns of *A. cervicornis* were not significantly different between 5 and 15 m depth, even with replicate ramets of each genet present at each depth (Rodríguez-Casariego et al., 2020). We therefore anticipate our findings of the seasonal trends within, as well as relationships between, both coral phenotype and DNA methylation to be applicable to *A. cervicornis* and *A. palmata* in their naturally occurring depth ranges. However, future work should evaluate the relative influences that may be attributed to genetic, epigenetic and environmental drivers of coral performance within the context of the increased variability of reef habitats.

5 | CONCLUSIONS

This study provides evidence of phenotypic plasticity in *A. cervicornis* and *A. palmata* corals, which is significantly related to variation in epigenetic DNA methylation. These results have important conservation implications as *A. cervicornis* and *A. palmata* are critically endangered and the most targeted species for restoration in the Caribbean region (Young et al., 2012). Restoration efforts have prioritized maintaining genetic diversity while also identifying genets that display desirable traits such as higher productivity or stress tolerance (Baums, 2008; Cunning et al., 2021; Shearer et al., 2009). However, the influence of GxE on the physiology of *A. cervicornis* indicates that genotype-specific predictions of fitness may not be accurate for all environments where corals may be restored, and that environmentally induced phenotypic shifts will vary between genets. Metrics of phenotypic plasticity may therefore be more important to incorporate into conservation decisions rather than measures of physiological traits within a static environment (Million et al., 2022). Additionally, the quantitative links between variation in coral physiology and DNA methylation profiles identified in this study provide insights into the role of epigenetic mechanisms mediating phenotypic plasticity in invertebrates. However, many additional epigenetic mechanisms, including histone PTMs, changes in nucleosome composition, and noncoding RNA activity, remain poorly studied in these taxa and should be examined in future work to better understand their influence on environmentally responsive gene regulation and phenotypic plasticity.

AUTHOR CONTRIBUTIONS

SH, JE-L, and FV designed the work. SH and FV performed the fieldwork. SH and WP performed the lab experiments. SH, PF, DG-S and WP analysed the data. SH and JE-L provided funding. JE-L and FV provided supplies. SH wrote the manuscript with input from all contributing authors. All authors contributed to and approved the submitted version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any known conflicts of interest.

DATA AVAILABILITY STATEMENT

Datasets utilized in the study which characterize environmental conditions, coral physiology, and msap loci, along with the associated metadata and data analysis R script can be found in the Github repository https://github.com/eelabfiu/BonaireY1.

BENEFIT-SHARING STATEMENT

Benefits Generated: A research collaboration was developed with Reef Renewal Foundation Bonaire (RRFB). All data has been shared with RRFB and is also publicly available as described above. The research provides important insights into drivers of phenotypic plasticity and mechanisms of seasonal acclimatization in two critically endangered Caribbean coral species which can contribute to their conservation and restoration. The research also addresses concerns regarding water quality monitoring in Bonaire, Caribbean Netherlands. Environmental data has therefore also been shared with Stichting Nationale Parken Bonaire (STINAPA Bonaire) national marine park managers.

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