Supplementary Methods and Data

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Supplemental Methods

S1 : Experimental Conditions

Target pCO_2 gases were formulated by mixing compressed CO_2 with compressed CO_2-free air (low-OA treatment) or with compressed air (moderate- and high-OA treatments) using solenoid-valve-controlled mass flow controllers (Aalborg mass flow controllers, Model GFC17, precision = 0.1mL/min) at flow-rates proportional to the target pCO_2 conditions. The CO_2-free air used in the low-OA treatment was generated by scrubbing CO_2 from compressed air with a Parker Balston FT-IR Purge Gas Generator. The mixed gases were then bubbled into the experimental seawater treatments with 45-cm flexible microporous tubes at rates sufficient for the pCO_2 of aquaria seawater to equilibrate with the pCO_2 of the mixed gases. Filtered seawater was introduced to each aquarium at a flow rate of 150 mL min^{-1}. Temperature of all experimental tanks was maintained at 17°C with 1/4HP chillers (Aqua Euro USA, precision = 0.1°C). Seawater temperature of all aquaria was slowly increased to 19.5°C (Figure S1.2) between days 39-51 of the experiment in an effort to stimulate gonad development for a companion experiment. It should be noted that this is a small temperature shift relative to what these oysters experience seasonally in their native habitats (Figure S1.1). Oysters were fed 1% Shellfish Diet 1800® twice daily following best practices outlined in (Helm and Bourne, 2004).

Temperature, pH, and salinity of all tanks were measured three times per week (M,W, F) for the duration of the experiment. Seawater pH was measured with an Accumet solid state pH electrode (precision = 1mV) and adjusted to the total scale, salinity was measured using a YSI 3200 conductivity probe (precision = 0.1 ppt), and temperature was measured using a NIST-standardized glass thermometer (precision = 0.1 °C). To empirically correct for the liquid junction bias of the pH electrode, we obtained the slope of the calibration using NBS buffers (pH 7.01 and pH 10.01) and adjusted the intercept using Dickson seawater certified reference material. Seawater samples were collected every two weeks from each tank for analysis of dissolved inorganic carbon (DIC) and total alkalinity (TA) on a VINDTA 3C coupled alkalinity gram titration and coulometric DIC analyzer system. In brief, samples were collected in 250 ml

borosilicate glass bottles and immediately poisoned with 100 ul saturated $HgCl_2$ solution, then refrigerated until analyzed. DIC, TA, salinity, and temperature were used to calculate calcite saturation state, pH, CO_3^{2-}, HCO_3^-, aqueous CO_2, and pCO_2 of each sample using CO_2SYS version 2.1 (Pierrot et al., 2011), using the seawater pH scale with K1 and K_2 values from (Roy et al., 1993), a $KHSO_4$ value from (Dickson, 1990), and a $[B]_T$ value from (Lee et al., 2010).

S2 : Calcification Rate

Net calcification rate was calculated for oysters surviving to either 50 or 80 days (n = 35) by buoyantly weighing oysters prior to exposure (BW_1) and on day 33 or 34 of the exposure (BW_2) following the methods of Ries et al. (2009). Buoyant weight was measured in a 27.65 liter tank (48 cm long, 24 cm wide and 24 cm deep) filled with seawater from the flow-through system and was maintained at treatment temperature by an Aqua Euro USA Model MC-1/4HP aquarium chiller. Buoyant weight was measured by completely submerging the oyster on a flat platform suspended from a bottom-loading scale (Cole Parmer Symmetry S-PT 413E, precision = 0.001 g). Care was taken to ensure no bubbles were trapped inside oyster shells. Oysters were weighed three times in the weighing basket, removing the oyster from the basket between each measurement. If replicate measurements varied by more than 0.01 g, oysters were re-weighed. A standard of known weight was weighed every 20 oysters to ensure that no drift was occurring in the scale.

To establish a relationship between buoyant weight and dry weight for the purpose of estimating net calcification rate, shells of oysters sampled for tissue within four days of a buoyant weight measurement were soaked in 10% ethanol to remove salts, dried, and weighed. The dry weight was then regressed against the buoyant weight measurement to establish an empirical dry-buoyant weight relationship (Figure S2.1):

$$DryWgt_{BWi}(mg) = 1.87 * BW_i - 2.74$$

This empirical relationship was then used to calculate dry shell weight at each buoyant weight time point via linear regression.

Calculated dry weights were then used to calculate daily calcification rate:

$$CalcificationRate(\%) = \frac{(DryWgt_{BW2} - DryWgt_{BW1})}{n} * \frac{100}{DryWgt_{BW1}};$$

where dry weight ($DryWgt_{BWi}$) was calculated for each individual using the buoyant weight pre-exposure (BW1) and 33-34 days into the exposure (BW2) and *n* was the number of days between the two measurements. Lastly, the average daily change in dry weight was divided by initial dry weight to standardize calcification rate for allometric effects, and multiplied by 100 to convert that fraction into a percent.

S3 : DNA methylation processing and quantification

Raw sequences were trimmed to remove adapters and low quality sequences by removing 10 bp from both 5' and 3' ends of each fragment using the command *trim_galore* in the program TrimGalore! (Martin, 2011) with the *--clip_r1*, *--clip_r2*, *--three_prime_clip_R1*, and *--three_prime_clip_R2* flags set to 10, and the remaining flags left as the defaults. Quality of sequences was assessed with FastQC (Leggett et al., 2013) within the *trim_galore* command using the *--fastqc_args* flag. Next, a bisulfite converted version of the *C. virginica* genome (NCBI Accession GCA_002022765.4) was prepared with the *bismark_genome_preparation* command in Bismark (Krueger and Andrews, 2011) and the --bowtie2 flag. The trimmed sequences were then aligned to the prepared reference using the *bismark* command with *--non-directionality* specified and the alignment score set to *-score_min L,0,-0.8*. Duplicates were removed from the data using the command *deduplicate_bismark*. All cytosines in the genome with coverage were extracted from the aligned deduplicated data for each individual using the command *bismark_methylation_extractor* with default settings. Next, a coverage report for all CpG associated cytosines within the genome was generated for each individual with the command *coverage2cytosine* using the output from the previous step. This report contains all cytosine loci (even those with no coverage) located within a CpG motif and includes separate columns for methylated vs. unmethylated coverage. Files were then processed using a standard methylKit pipeline, which included normalization of methylation counts among samples, destranding CpGs loci, and filtering loci that did not have at least 5x coverage for each sample (Akalin et al., 2012). Here, destranding involves combining the cytosine calls within a single CpG from either strand (i.e., top or bottom) and the proportion methylation was determined by dividing the number of methylated cytosine calls by the total coverage (regardless of methylation

status). Before proceeding to downstream analyses samples were first visualized in a PCA to identify and remove outlier samples.

S4 : Gene expression processing and quantification

RNA reads were first trimmed to remove adapters and for quality control using Trimmomatic (Bolger et al., 2014), implemented in the dDocent pipeline (v.2.2.20; Puritz et al., 2014) and following recommendations by Puritz et al. (2014). This was done using the *dDocent* command with default settings, which included removing adapters and performing a quality control step that trimmed the leading and trailing bases with phred quality scores < 20, with additional trimming of 5bp windows with mean phred quality scores < 10 (see github repository for complete description). Reads were then aligned to the *C. virginica* genome (Accession: GCA_002022765.4) using a two-step mapping approach with the program STAR (v.2.7.0; Dobin et al., 2013). In the first step, a preliminary alignment to the reference genome was performed for each sample to identify novel splice junctions. In the second step, reads were realigned, but with all the splice junctions discovered from step one included along with the reference genome. Both steps were executed with the command *STAR* using default settings, with the exception that the number of alignments retained was adjusted by setting both the *--outFilterMatchNminOverLread* and *--outFilterScoreMinOverLread* flags to 0.17, and increasing the number of discoverable sequence junctions by setting the flag *--limitSjdbInsertNsj* to 1500000. In the second step, the *--sjdbFileChrStartEnd* flag was also included to specify the inclusion of splice junctions identified in step one. This two-step alignment process has been shown to improve mapping quality (Dobin et al., 2013).

Expression levels for each gene were quantified for each sample with the program RSEM (Li and Dewey, 2011) using the *rsem-calculate-expression* command with *--star* flag, default settings, the bam files generated from the mapping step with STAR, and the same *C. virginica* gene annotations used during mapping (Accession: GCA_002022765.4). Importantly, RSEM takes a probabilistic approach to transcript quantification, which allows for the retention and fractional assignment of multi-mapping reads. Similar to the DNA methylation data, outlier samples were evaluated and removed based on a visualization approach using a PCA.

S5 : Genome-wide DNA methylation and gene expression analyses

The gene expression in the PCA was based on the post-filtered expression level of genes (in columns) for each individual (in rows). Gene expression level was calculated as counts per million using the *cpm* function from the R package edgeR (Robinson et al., 2010) and log2 transformed (i.e., log2-cpm). The DNA methylation PCA was based on all CpG loci located within gene bodies with at least 5x total coverage (in columns) for each individual (in rows). A PERMANOVA to test the null hypothesis of no effect of treatment, time, or their interaction on global gene expression and DNA methylation patterns. The PERMANOVA was based on the Manhattan distance using the *adonis* function in the R package *vegan (v2.5-5; Dixon, 2003)*

The discriminant analysis of principal components (DAPC) was performed with the R package adegenet (Jombart et al., 2010), using the same transcriptomic and methylomic datasets described above for the PCA. The function *dapc* was used to generate a discriminant function that maximized differences between treatments on day 9 samples, then we predicted where samples from day 80 should fall along the discriminant function using the *predict* function to determine if genome-wide variation due to OA was maintained through time.

S6 : Differential molecular response analysis

Differential gene expression amongst treatments was evaluated using a generalized linear model approach implemented in the R package *limma* (Ritchie et al., 2015) using treatment, time, and their interaction as fixed effects. Expression data was first TMM normalized using the *calcNormFactors* function and transformed into log2 counts per million (log2-cpm) using the *voomWithQualityWeights* function (Smyth et al., 2005). Finally, the *geneDuplication* function was used to account for tank as a potential experimental block effect (Oshlack et al., 2007). Site was not considered in this analysis given that it did not have a significant effect on either the phenotypic or genome-wide responses. Genes with FDR ≤ 0.05 and absolute value of $\log_2$ fold ≥ 2 were considered differentially expressed.

Differentially methylated loci (DML) were identified using the R package methylKit

(Akalin et al., 2012). Only CpGs with coverage ≥ 5 for all samples were considered. Differential methylation was performed using a logistic regression approach implemented in methylKit with the functions *calculateDiffMeth* with the overdispersion argument set to "MN" and the default 'slim' method to correct p-values and the function *getMethylDiff* with a differential methylation threshold set to 50% and a q-value threshold set to 0.01.

S7 : Gene co-expression network analysis (WGCNA)

A weigheted gene co-expression network analysis was performed to identify genes that exhibit similar expression patterns among individual oysters using the R package WGCNA (Langfelder and Horvath, 2008). First, a gene dissimilarity matrix was generated based on the log2-cpm gene expression data using first the *adjacency* function followed by the *TOMsimilarity* function in WGCNA. This step estimates the level of dissimilarity between each gene by considering expression across all individuals. Next, genes were hierarchically clustered based on dissimilarity using the function *hclust* and the 'Ward.D2' method for clustering (Murtagh and Legendre, 2014). Modules were determined using the *cutreeDynamic* function with a minimum gene membership threshold of 30. An eigenvalue for module expression (i.e., the first principle component value for each individual) was calculated for each module using *moduleEigengenes*. Lastly, linear regression was used to determine the association between the expression of each module (i.e., the eigenvalue of gene expression) and either mean gene methylation (calculated as the mean methylation of all CpGs among all genes within a module) or EPF response (i.e., ΔpH).

S8 : Functional enrichment analysis

A functional enrichment test was conducted with GO-MWU, a rank-based gene enrichment method developed by (Wright et al., 2015), to identify gene ontology (GO) categories enriched among genes that are differentially regulated or methylated between treatments at each time point. We performed this analysis separately for each time point using the $\log_2$-fold change in gene expression and the difference in mean methylation among treatments. Mean methylation was calculated as the mean among all CpG loci within a gene across all individuals within a particular treatment and time point. Only genes with at least 5

CpG loci were considered for the analysis to ensure mean methylation estimates were based on genes where we had at least moderate CpGs coverage. Importantly, GO-MWU can handle a variety of differentiation metrics (e.g., $\log_2$-fold change in expression) and considers all genes, not just those that are significantly differentially expressed or methylated. This enables detection of GO categories enriched with responsive genes even when there is limited evidence of individually differentially expressed or methylated genes. GO-MWU scripts and the gene ontology database were downloaded from the GO-MWU github repository (https://github.com/z0on/GO_MWU).

Inputs for the GO-MWU analysis include two gene list tables created using the Genebanks IDs from the *C. virginica* genome available on NCBI (Accession: GCA_002022765.4) along with a measure of difference (i.e., $\log_2$-fold change or methylation difference) and a table of GO terms containing a list of all available Genebank IDs and their associated gene ontology (GO) terms. Details on how the latter file was created are outlined by Johnson et al. (2020) and can be found on the associated Github repository (See data availability section). The GO-MWU analysis was performed for both gene expression and methylation tables separately using the *goStats* function in R with default settings and using the gene ontology database provided by the GO-MWU repository. The analysis first clusters highly similar GO categories by combining categories that shared at least 75% of the same genes. After clustering, a Mann-Whitney U test was performed to identify GO categories that were enriched with either up-regulated or down-regulated (or hyper- or hypo-methylated) genes. This analysis was run separately for GO categories associated with molecular function, biological process, and cellular components. A 10% FDR correction (GO-MWU default) was used to adjust for multiple comparisons.

Results Section 1: Experimental Design, Water Chemistry, and Field Data

Table S1.1 Experimental water chemistry summary

Measured water chemistry including temperature (°C), salinity (PSU), and pH (total scale) were measured Mondays, Wednesdays, and Fridays of each week of the experiment. Dissolved carbon (DIC) and total alkalinity (TA) were measured every two weeks. Calculated water chemistry including, calculated pH (SW scale), pCO_2, bicarbonate ion concentration ($[HCO_3^-]$), carbonate ion concentration ($[CO_3^{2-}]$), dissolved CO_2 ($CO_{2,SW}$), calcite saturation state ($\Omega_{calcite}$), and atmospheric pCO_2. Note, whenever possible DIC and TA were used to calculate the complete carbonate chemistry, but measured pH was used in the calculation when either DIC or TA was not available. 'Acc' refers to the 33-day acclimation period.

Measured Parameter		Acc.	Exposure Period					
			Day 9			Day 80		
			Control	OA 1000	OA 2800	Control	OA 1000	OA 2800
N (Temp, salinity, pH)		198	36	36	36	186	186	186
Temperature	(celsius)	17.0	17.4	17.3	17.3	18.3	18.4	18.4
	SEM	0.03	0.08	0.08	0.08	0.08	0.08	0.08
Salinity	(psu)	29.91	30.27	30.28	30.28	31.03	31.06	31.06
	SEM	0.03	0.03	0.03	0.03	0.03	0.03	0.03
pH	(total scale)	7.93	7.99	7.72	7.31	8.00	7.74	7.27
	SEM	0.00	0.01	0.01	0.01	0.01	0.01	0.01
N (DIC and AT)		23	12	9	9	32	32	35
DIC	(µmol/kg)	1904.22	1901.92	1991.29	2161.02	1989.83	2066.89	2215.22
	SEM	3.06	5.69	12.62	11.15	3.53	3.97	11.22
TA	(µmol/kg)	2043.58	2050.38	2069.06	2101.63	2146.79	2142.99	2175.36
	SEM	4.43	7.20	5.59	11.04	1.69	3.79	4.11
Calculated Parameters								
N		25	12	12	12	32	32	36
pH	(SW scale)	7.90	7.90	7.72	7.26	7.89	7.66	7.29
	SEM	0.01	0.02	0.03	0.02	0.01	0.02	0.03
pCO_2	(uatm)	547.3	546.0	879.8	2705.7	579.1	1050.4	2728.6
	SEM	20.2	22.3	57.8	107.2	16.5	47.5	128.0
HCO_3^-	(µmol/kg)	1771.2	1768.0	1866.4	2017.5	1847.5	1952.9	2083.2
	SEM	4.7	6.9	13.4	10.5	4.8	4.7	10.1
CO_3^{2-}	(µmol/kg)	111.0	114.6	80.1	30.0	122.2	77.6	37.0
	SEM	2.9	3.7	3.7	1.0	2.0	2.2	3.2
CO_2 (SW)	(µmol/kg)	19.83	19.34	31.28	95.93	20.17	36.36	94.85
	SEM	0.72	0.80	1.97	3.71	0.57	1.57	4.30
$\Omega_{calcite}$		2.74	2.82	1.97	0.74	3.00	1.91	0.91
	SEM	0.07	0.09	0.09	0.02	0.05	0.05	0.08
pCO_2 (gas)	(ppm)	557.8	556.9	897.2	2759.7	591.0	1072.4	2785.0
	SEM	20.6	22.7	59.0	109.5	16.9	48.6	130.9

Figure S1.1 Field site water chemistry

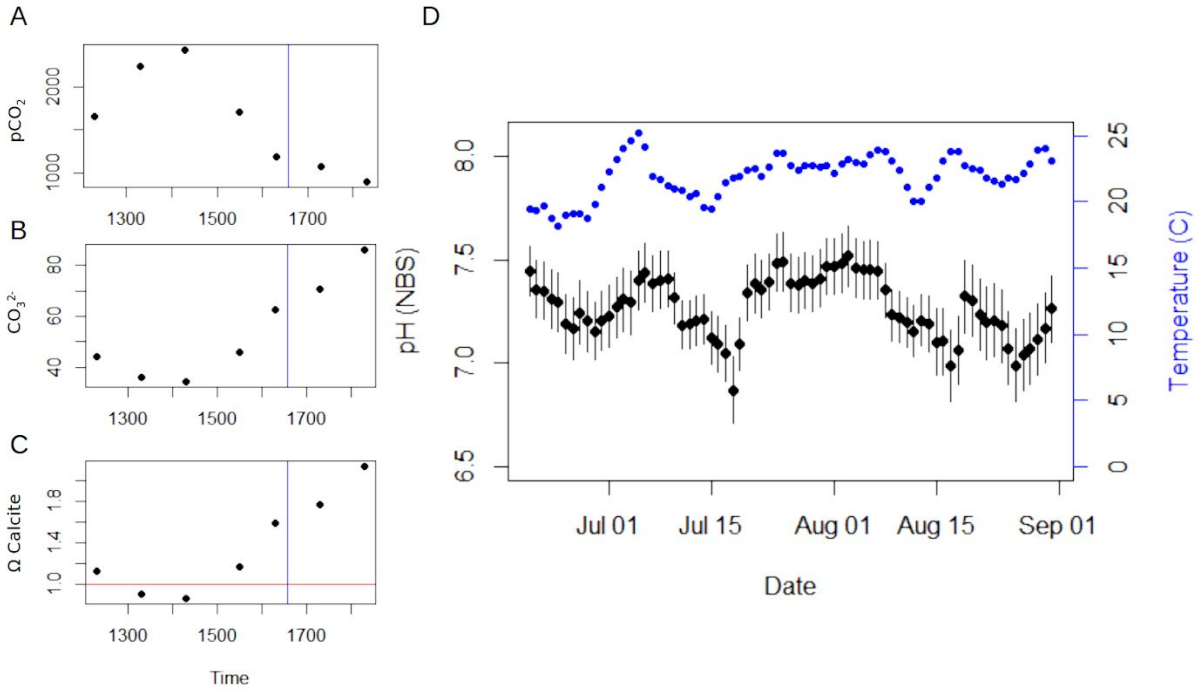


Figure S1.1 | Field site water chemistry. Carbonate chemistry and temperature from collection site 3 (42.681764, -70.813498) including a detailed tidal cycle measurement in summer 2016 (A-C) and daily average pH and temperature over 2.5 months in summer 2017 (D). In summer 2016, seven samples were collected for alkalinity and DIC starting approximately 3 hours prior and continuing until 3 hours post low tide. Blue lines represent the low tide for the nearest NOAA buoy, which had an approximately 2 hour lag compared to the collection site. Measured salinity, temperature, alkalinity and DIC were used in CO_2Sys to determine the complete carbonate chemistry including, pCO_2 (A), carbonate (B), and calcite saturation state (C). The red line indicates the critical calcite saturation state. In panel D, daily mean pH (black diamonds, mean±SE) and temperature (blue circles) over the course of summer 2017.

Figure S1.2 Mean triweekly water chemistry

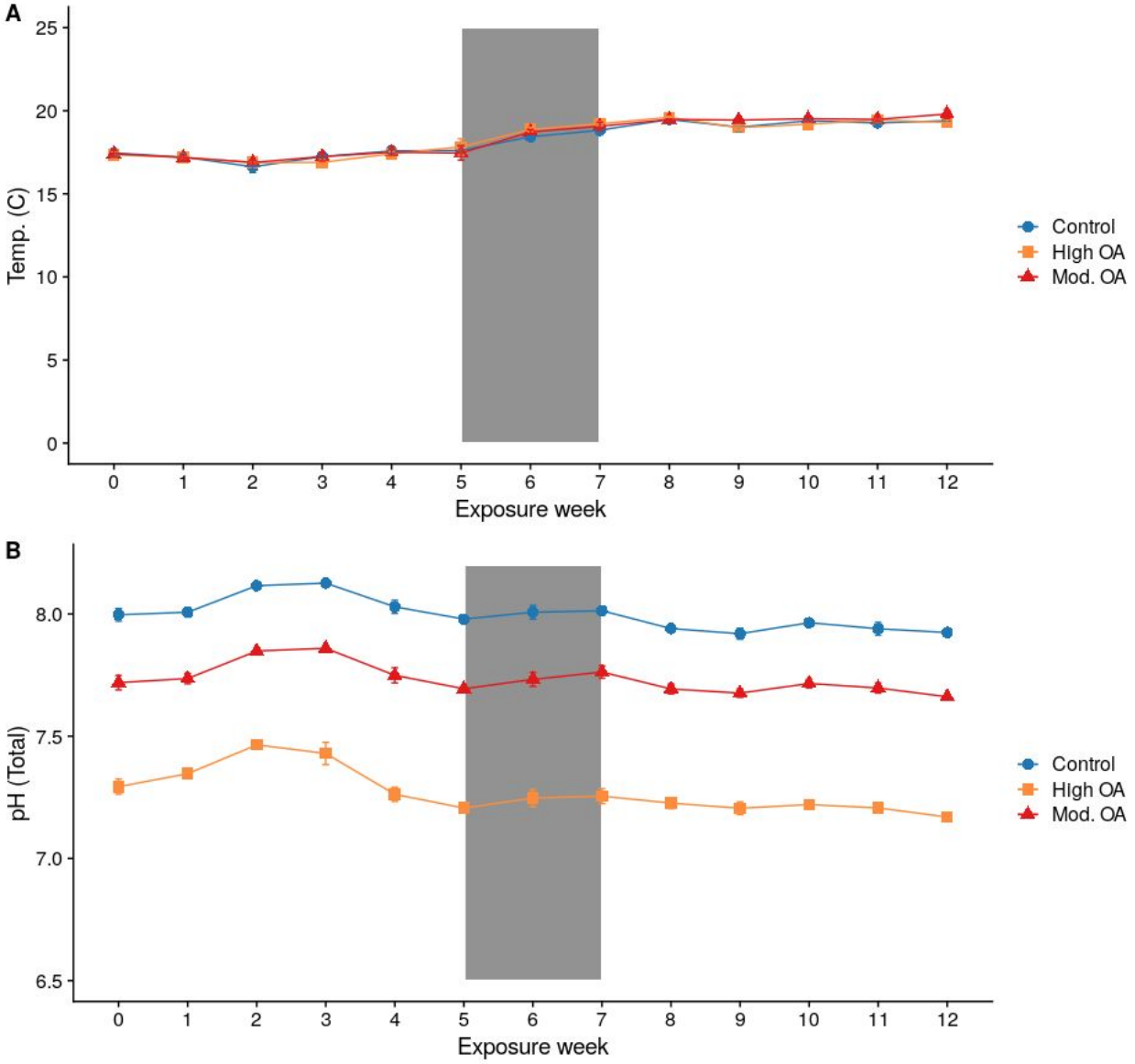
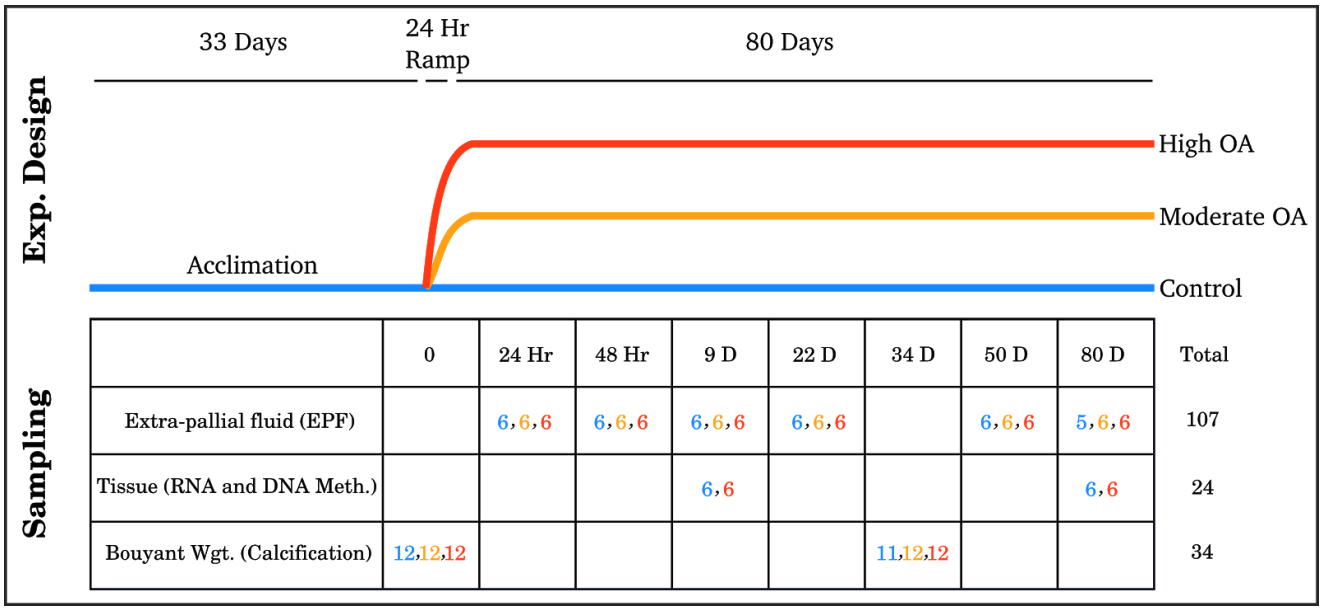


Figure S1.2 | Mean weekly water chemistry. Mean temperature **(A)** and seawater pH (total scale) **(B)** during the experimental exposure. Lines represent means across the 6 replicate tanks per treatment with vertical bars showing the 95% CI. Grey box highlights the ~12 day period where the temperature was increased by 2.5 degrees C.

Figure S1.3 Schematic of experimental design



Sampling

	0	24 Hr	48 Hr	9 D	22 D	34 D	50 D	80 D	Total
Extra-pallial fluid (EPF)		6,6,6	6,6,6	6,6,6	6,6,6		6,6,6	5,6,6	107
Tissue (RNA and DNA Meth.)				6,6				6,6	24
Bouyant Wgt. (Calcification)	12,12,12					11,12,12			34

Figure S1.3 | Experimental design schematic. Bottom table shows sample sizes for extra-pallial fluid, tissue collections, and buoyant weights by time point and treatment (color).

Results Section 2: Phenotype Data

Figure S2.1 Buoyant weight compared to dry weight

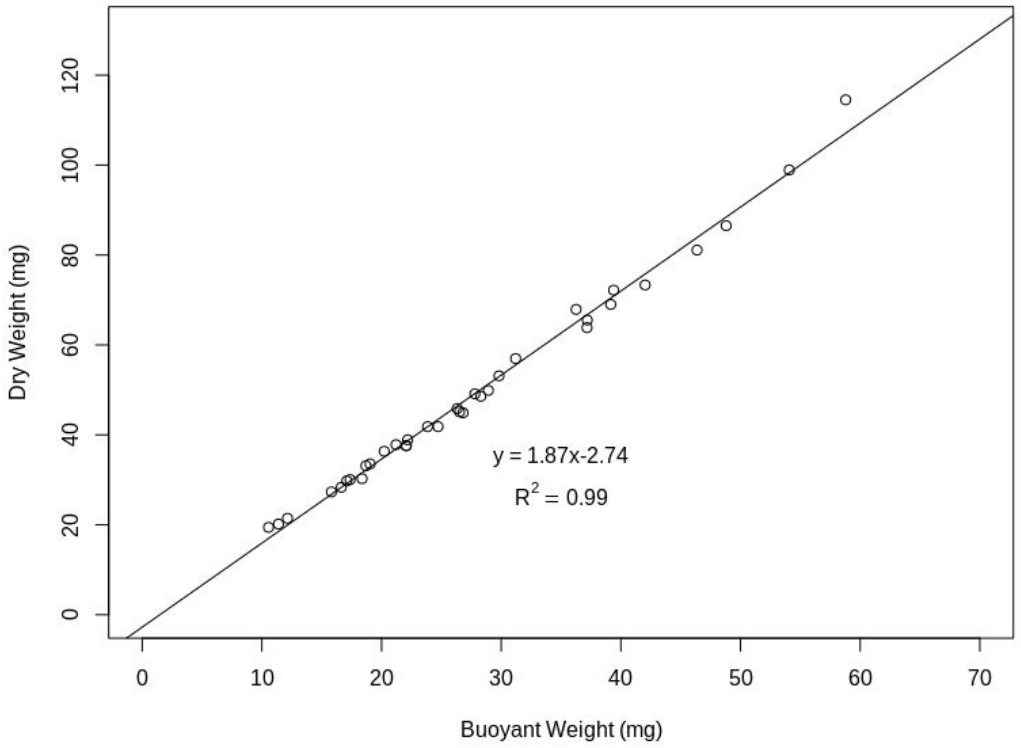


Figure S2.1 | Buoyant weight compared to dry weight. Buoyant and dry weights for oysters from oysters collected during the first and second buoyant weight measuring timepoints. Linear regression was used to evaluate the relationship between the two variables, estimate the slope and intercept, and determine the degree of correlation.

Results Section 3: DNA Methylation Data

Table S3.1 DNA Methylation mapping summary

Trimming and quality control performed using Trim_Galore! with automated adapter detection and clipping 10bp from both 5' and 3' ends of each read. Mapping was performed in bismark with the bowtie2 mapper using a score_min setting of L0,0,-0.8.

Step	**Total**	**Per Sample**			
		Mean	**SD**	**Min**	**Max**
Sequencing Reads (millions)	1409.8	59.99	8.43	37.6	71.6
Reads Mapped (millions)	622.4	25.93	3.88	17.1	31.9
Reads Mapped (percent)	NA	43.35%	0.02%	39.40%	46.00%
Reads Mapped After deduplication (millions)	566.3	23.60	4.41	10.2	28.7

Table S3.2 DNA Methylation CpG by genomic feature summary

Counts based on an estimate of the total number of CpGs in the oyster genome (Accession: GCA_002022765.4). The threshold represents minimum per sample coverage (>1, >=5, >=10) for CpG inclusion. Based on 23 samples after removing one individual due to poor sequencing.

	All CpG Destranded	**Sequenced CpG covered (>=1x)**	**Sequenced CpGs (>=1x per sample)**	**Sequenced CpGs (>=5x per sample)**	**Sequenced CpGs (>=10x per sample)**
Num. of CpGs	14,458,703	12,765,452	932,973	403,976	294,911

Table S3.3 Differentially methylated CpGs (DMLs) among treatments

Summary table of differentially methylated CpGs using the logistic regression approach in methylKit. These include the 2 loci where treatment had a significant effect and the 83 loci which were significant by treatment on either day 9 or 80. The table includes information about CpG position in the genome and associated gene annotations. Attached as a separate excel file.

Figure S3.1 Gene by CpG coverage

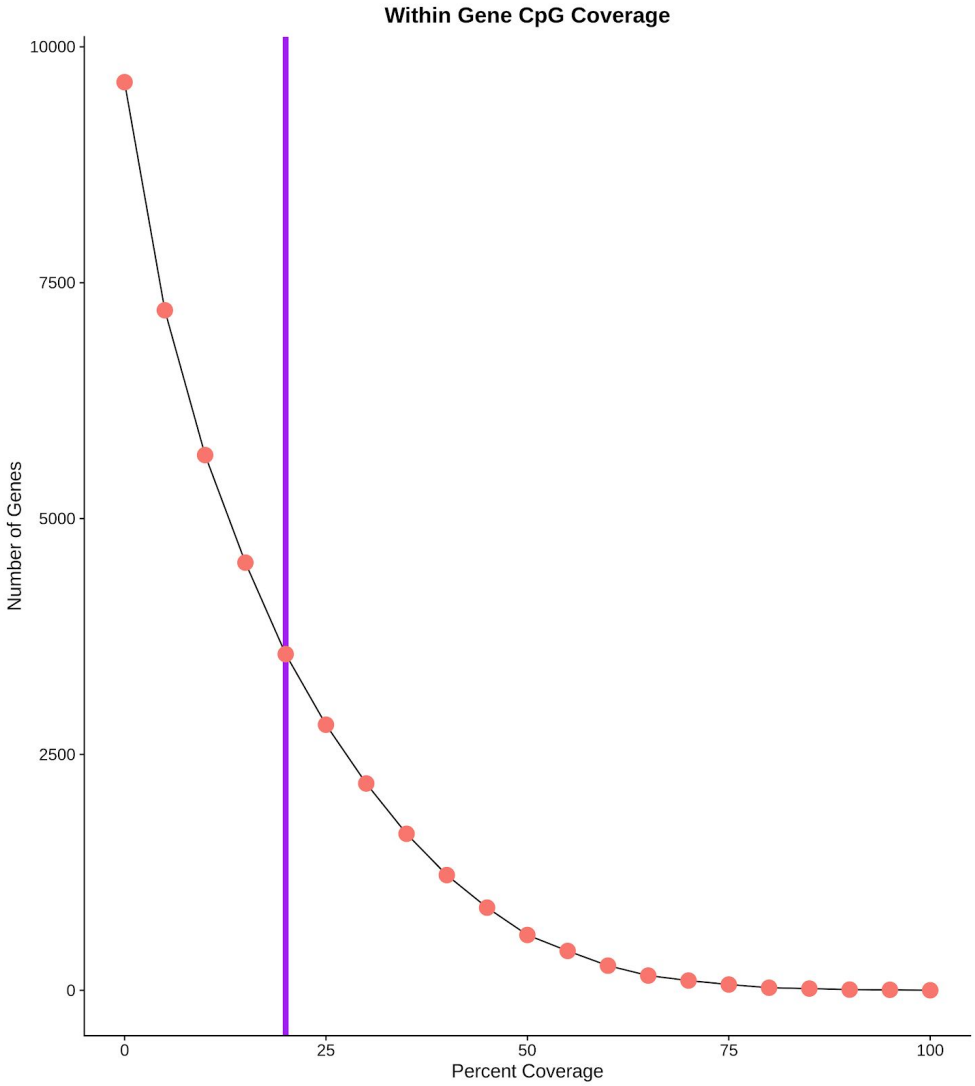


Figure S3.1 | Gene by CpG Coverage. Orange circles show the number of genes that have a minimum percent of CpGs covered within a gene for a range of percent thresholds. The purple line indicates the 20% minimum coverage threshold used.

Figure S3.2 Gene summary PCAs

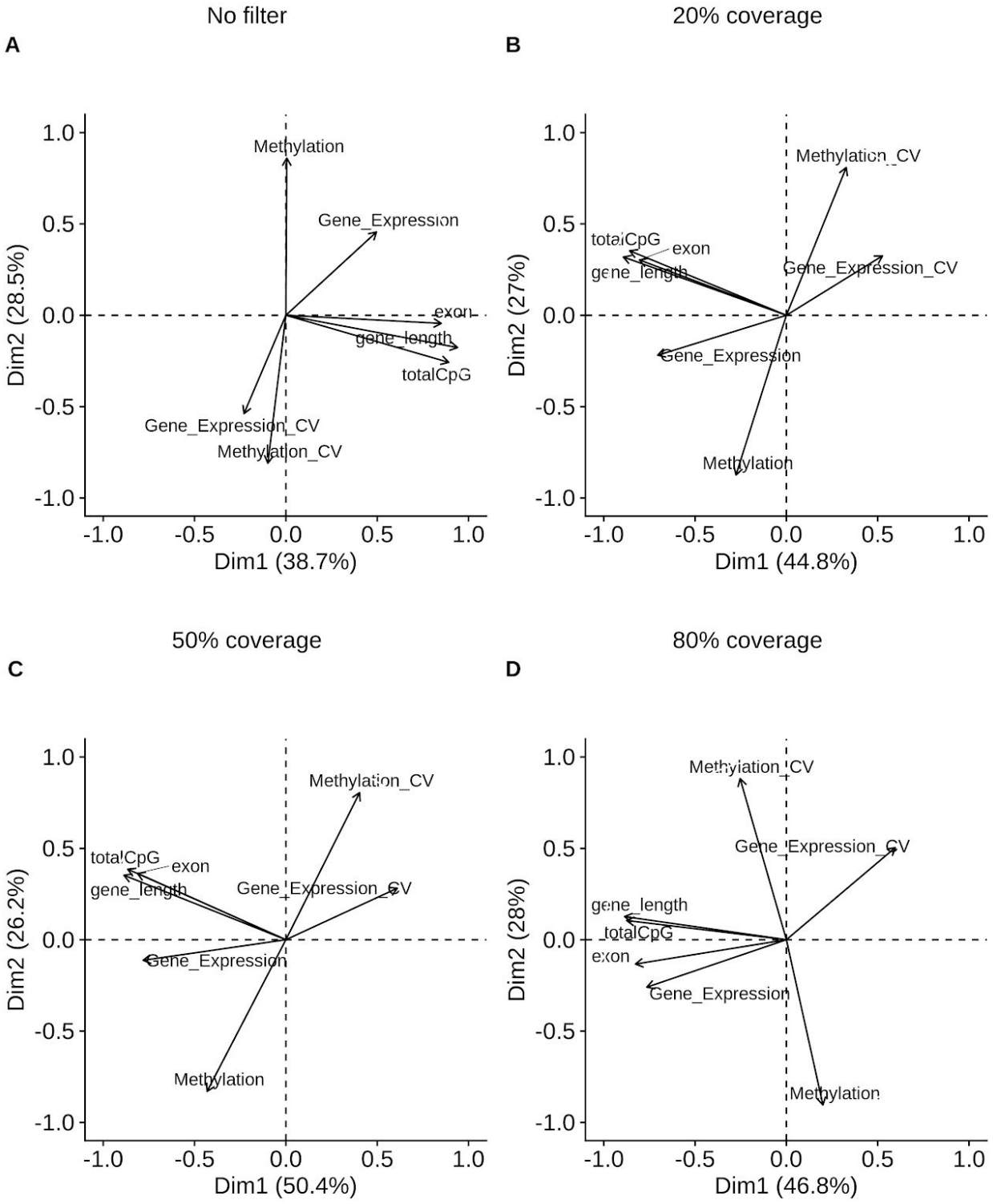


Figure S3.2 | Gene Summary PCAs for all minimum thresholds. First two principal components from a principle component analysis that included gene level summary variables for various attributes, expression, and methylation based on **(A)** no filter, **(B)** 20%, **(C)** 50%, **(D)** and 80% coverage thresholds. Thresholds based on the percent of CpGs with the minimum sequence coverage (i.e., 20% coverage only includes genes with at least 20% CpG sequence coverage in the PCA).Variable loadings plotted as arrows, with the length of the arrow corresponding to the relative contribution to PC variance.

Figure S3.3 Principle component contributions

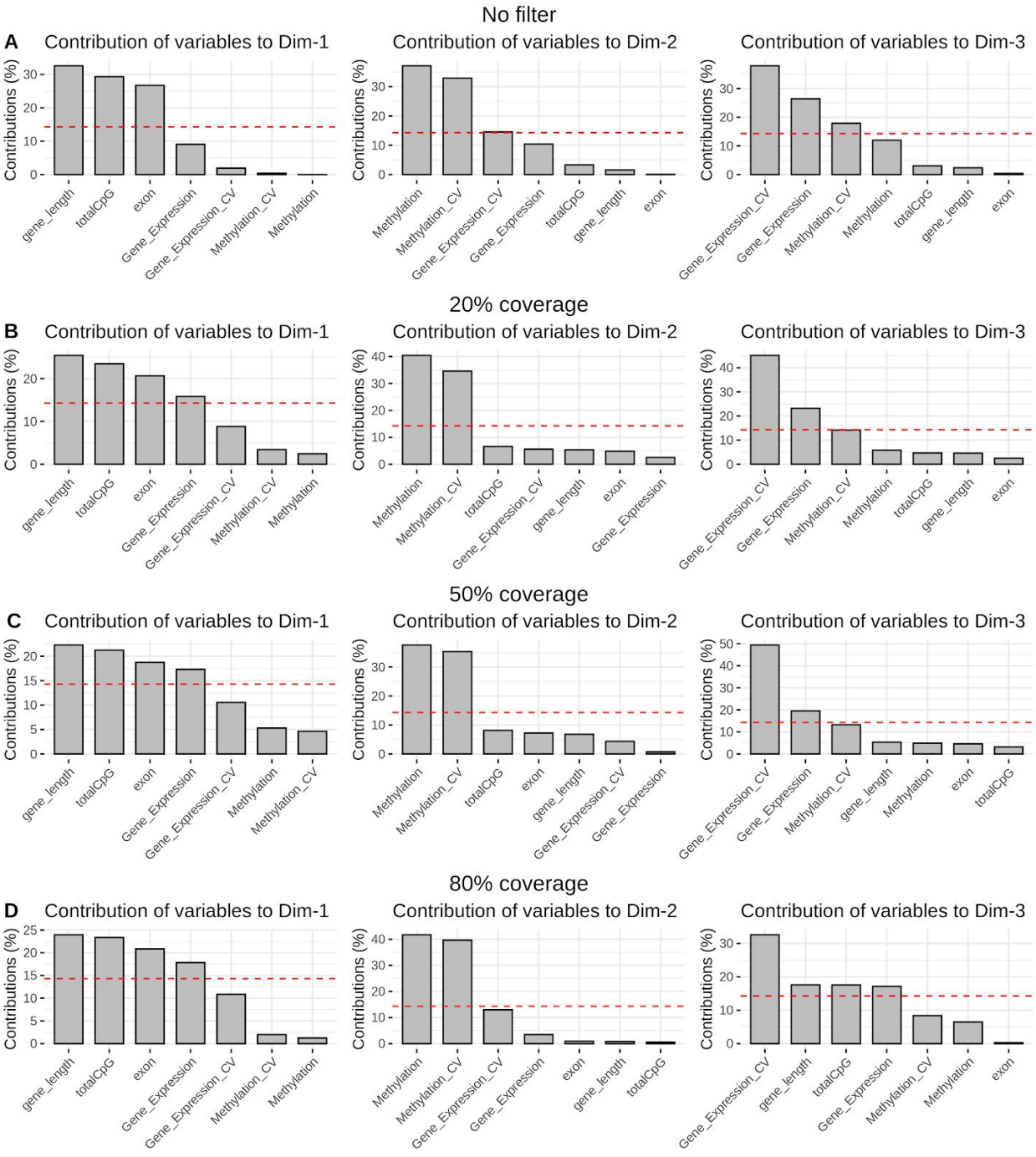


Figure S3.3 | Principle component contributions. Relative contribution (percent) for each variables (loading) for the first (column 1), second (column 2), and third (column3) principal components of the PCA for all genes **(A)**, genes with coverage for at least 20% **(B)**, 50% **(C)**, and 80% **(D)** of all CpGs. The dotted redlines indicate the default 15% contribution significance threshold for individual variables.

Figure S3.4 Venn Diagram of OA-induced DML among mantle and gonadal tissue in *C. virginica*

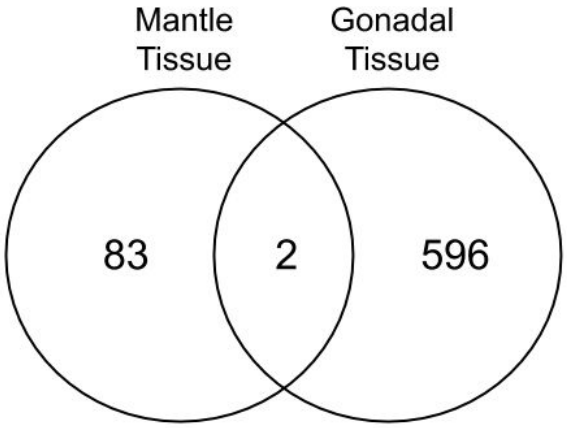


Figure S3.4 | Venn Diagram of OA-induced DML among mantle and gonadal tissue in *C. virginica.* OA-induced DML in the gonadal tissue were from Venkataraman et al. (2020) and downloaded from: https://github.com/epigeneticstoocean/paper-gonad-meth.

Results Section 4: Transcriptomic Data

Table S4.1 Per sample read and mapping summary for RNAseq data

Trimming and quality control performed using a custom pipeline implemented in dDocent and using trimmomatic. Mapping performed using the aligner STAR with the 2pass procedure. Statistics are calculated over samples (n = 24).

	MEAN	**SD**	**MIN**	**MAX**
Sequencing Reads (per sample)	39,750,000	2,670,000	36,150,000	45,050,000
Sequencer Read Quality	39.0	0.1	38.8	39.1
Number Read After Trimming and QC	27,930,000	2,260,000	24,390,000	32,740,000
STAR - Unique_reads	20,540,000	1,770,000	18,200,000	24,630,000
STAR - Unique_Percent	73.5%	1.4%	69.0%	75.3%
STAR - Multi_reads	3,600,000	280,000	3,130,000	4,130,000
STAR - Multi_Percent	12.9%	0.8%	12.0%	14.8%

Table S4.2 Gene expression quantification summary

Mean gene expression counts and number of putative genes before and after filtering (n = 23). Filtering included removing genes that did not contain at least 1 transcript per million in at least 5 (out of 6) samples in at least one treatment and time level.

Gene Quantification Method	Mean	SD	Min	Max	Number of genes
Total	13,456,348	1,271,404	11,592,273	16,427,649	37,098
After Filtering	13,320,344	1,256,664	11,474,534	16,257,768	20,387

Table S4.3 Target list of genes associated with biomineralization in marine calcifiers from the literature

(A) Full summaries of all gene names and locations in reference to the oyster genome (NCBI BioProject ID: PRJNA594029) and **(B)** summary of literature sources. Attached as a separate excel file.

Figure S4.1 General expression of biomineralization genes (pt 1)

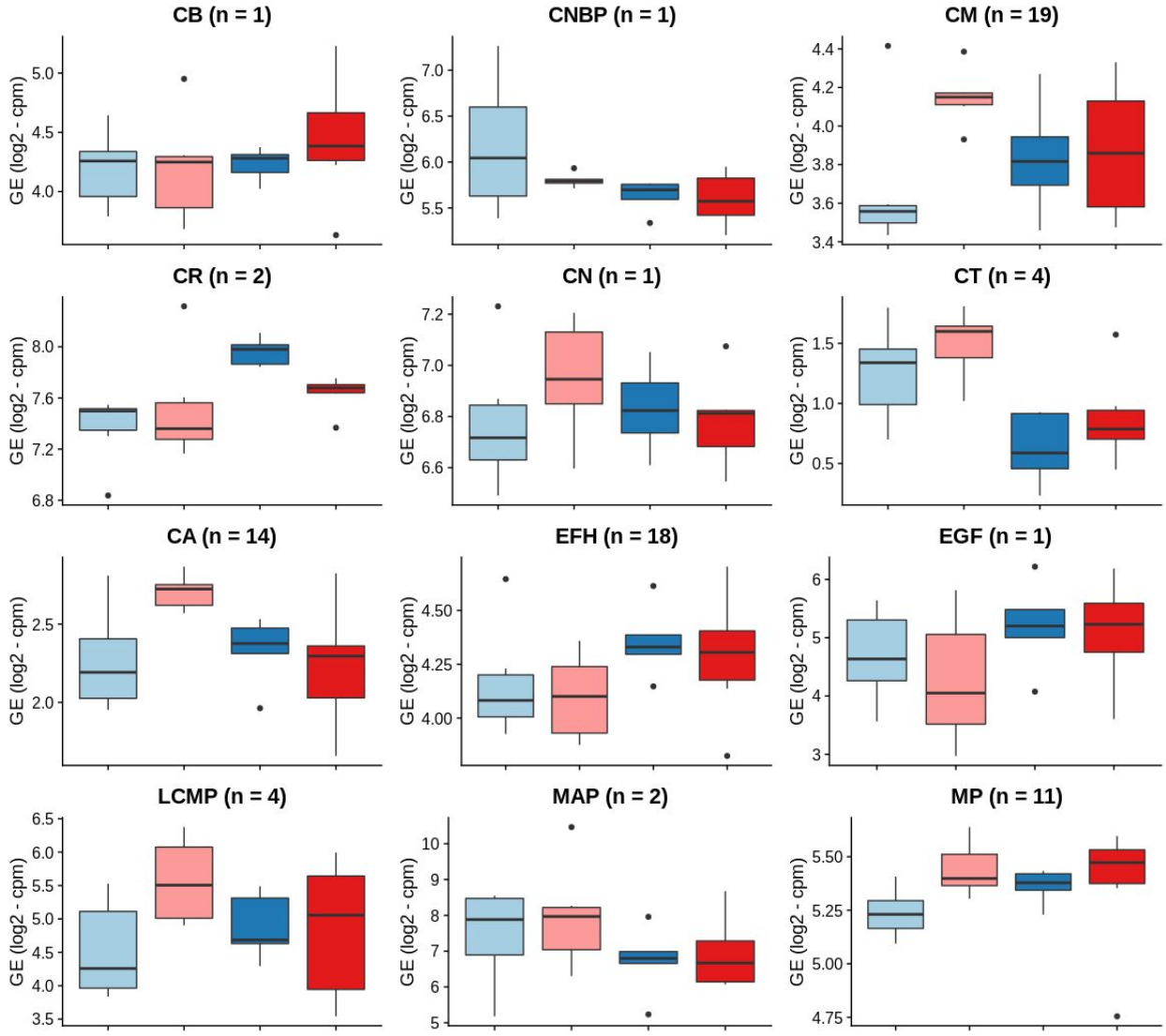


Figure 4.1 General expression of biomineralization genes (pt 2)

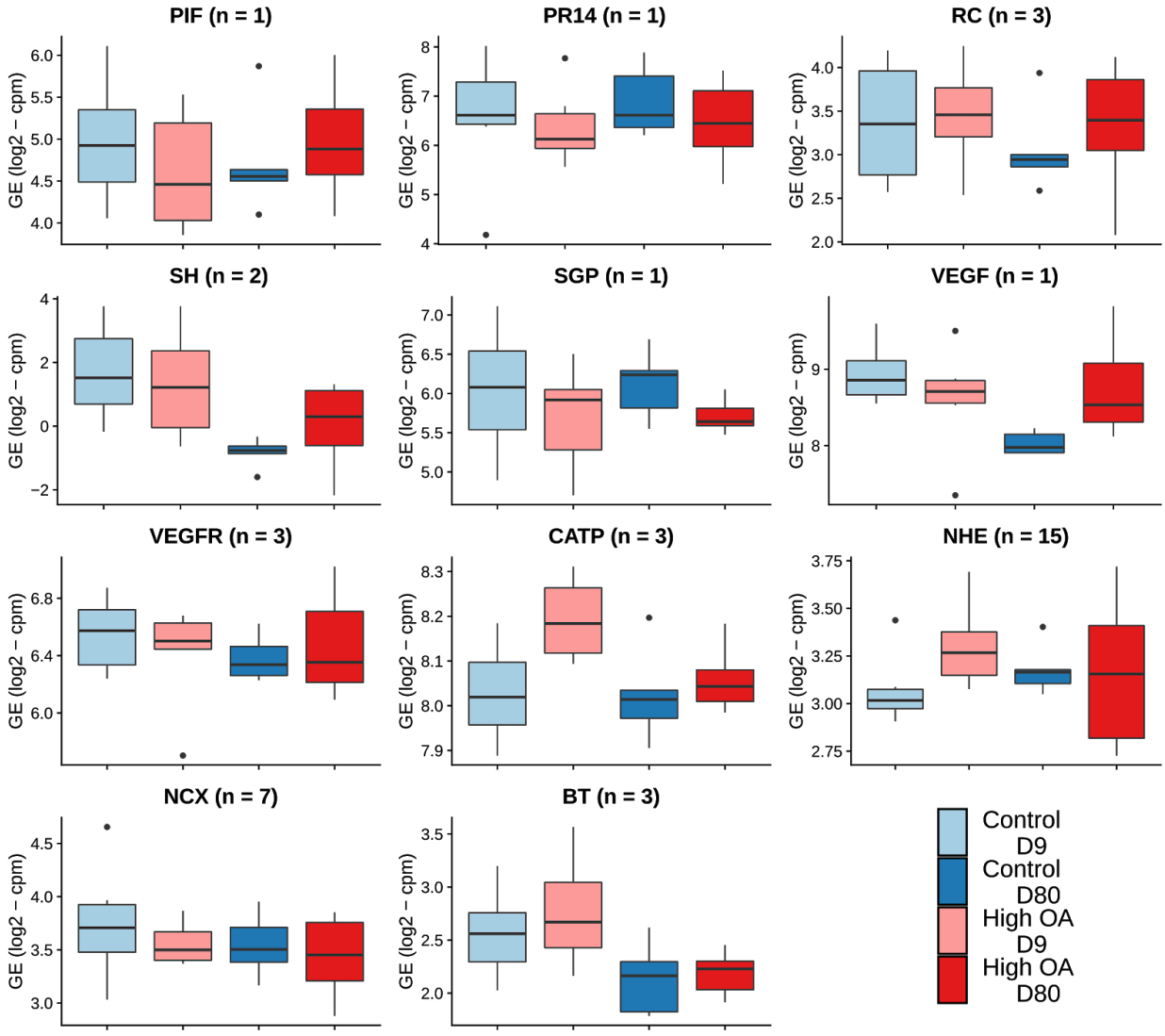


Figure S4.1 | Gene expression of biomineralization associated genes. Boxplots based on mean expression per individual for major biomineralization genes identified from the literature (see Table 4.3 for complete list of abbreviations; n refers to the number of copies for each putative gene in the *C. virginica* genome). For each gene none of the fixed effects (treatment, time, or their interaction) were significant.

Figure S4.2 Density plot of expression in all genes vs. biomineralization target list

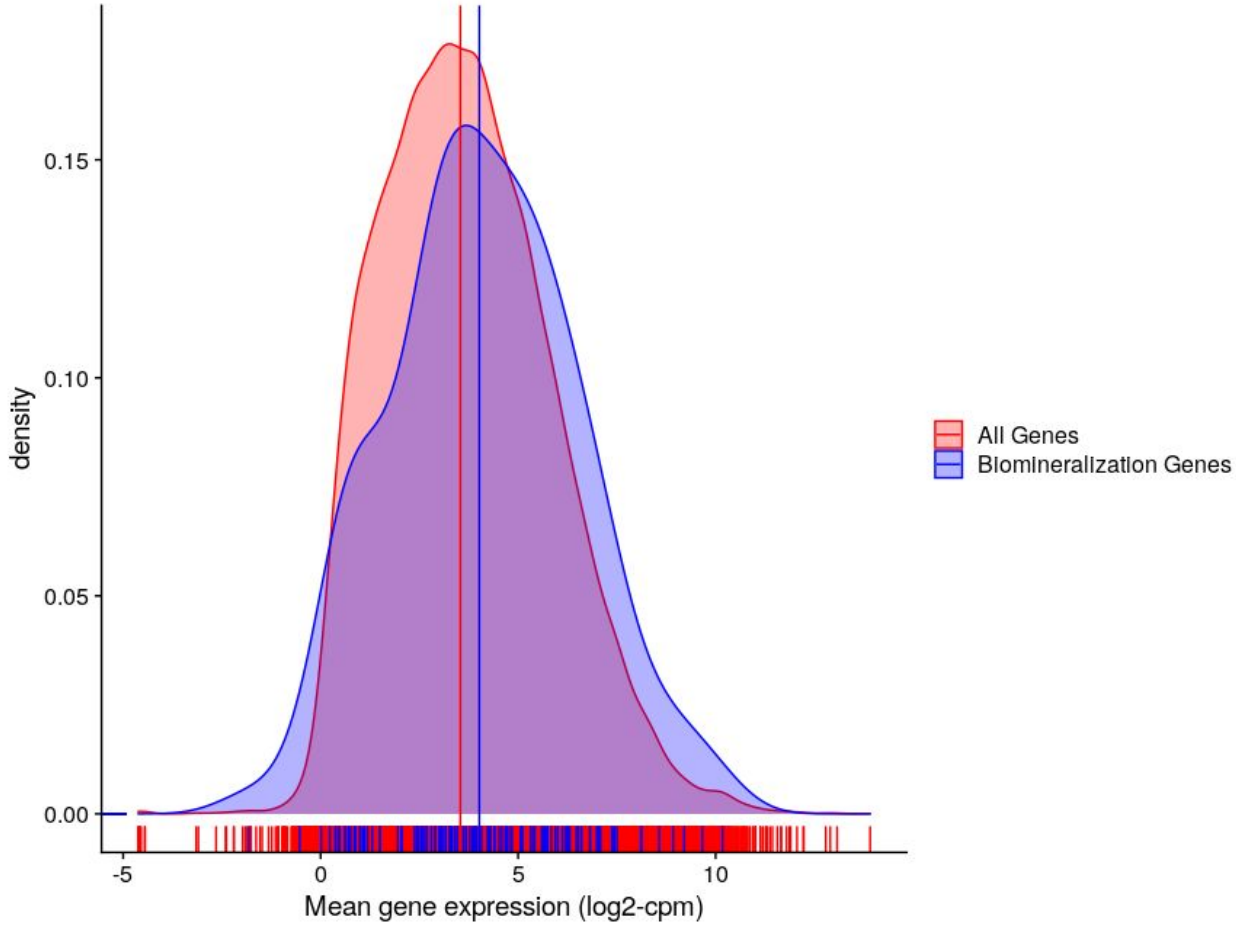


Figure S4.2 | Density plot of expression in all genes vs. biomineralization genes. Dark vertical lines indicate the median expression for all genes (red, n = 20387) and biomineralization genes (blue, n = 119); these represent statistically different medians (P = 0.02719, Mann Whitney U rank sum).

Results Section 5: Comparative

Table S5.1 WGCNA module summary

(A) Summary of the 52 different co-expression gene modules generated with WGCNA, including number of genes in the module, eigen expression, percent methylation, proportion of CpG with coverage, and model statistics (P and R2 values) for each comparison. Values in bold indicate significant effects. The asterisk (*) indicate the modules used for Figure 7. (**B-E**) Gene list summaries of the three top module candidates. Attached as a separate excel file.

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