Supplementary Data

Table of Contents

Supplemental Section 1: Transcriptomic Data

Table S1.1 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 S1.2 Gene expression quantification summary.

Mean gene expression counts and number of putative genes before and after filtering (n = 24). 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 S1.3 Target list of genes associated with biomineralization in marine calcifiers from the literature.

Table of biomineralization genes collected from the literature. The table contains gene names and locations in reference to the oyster genome (NCBI BioProject ID: PRJNA594029). In addition, it contains some basic summary information about the expression of these genes within our data. Attached as a separate excel file.

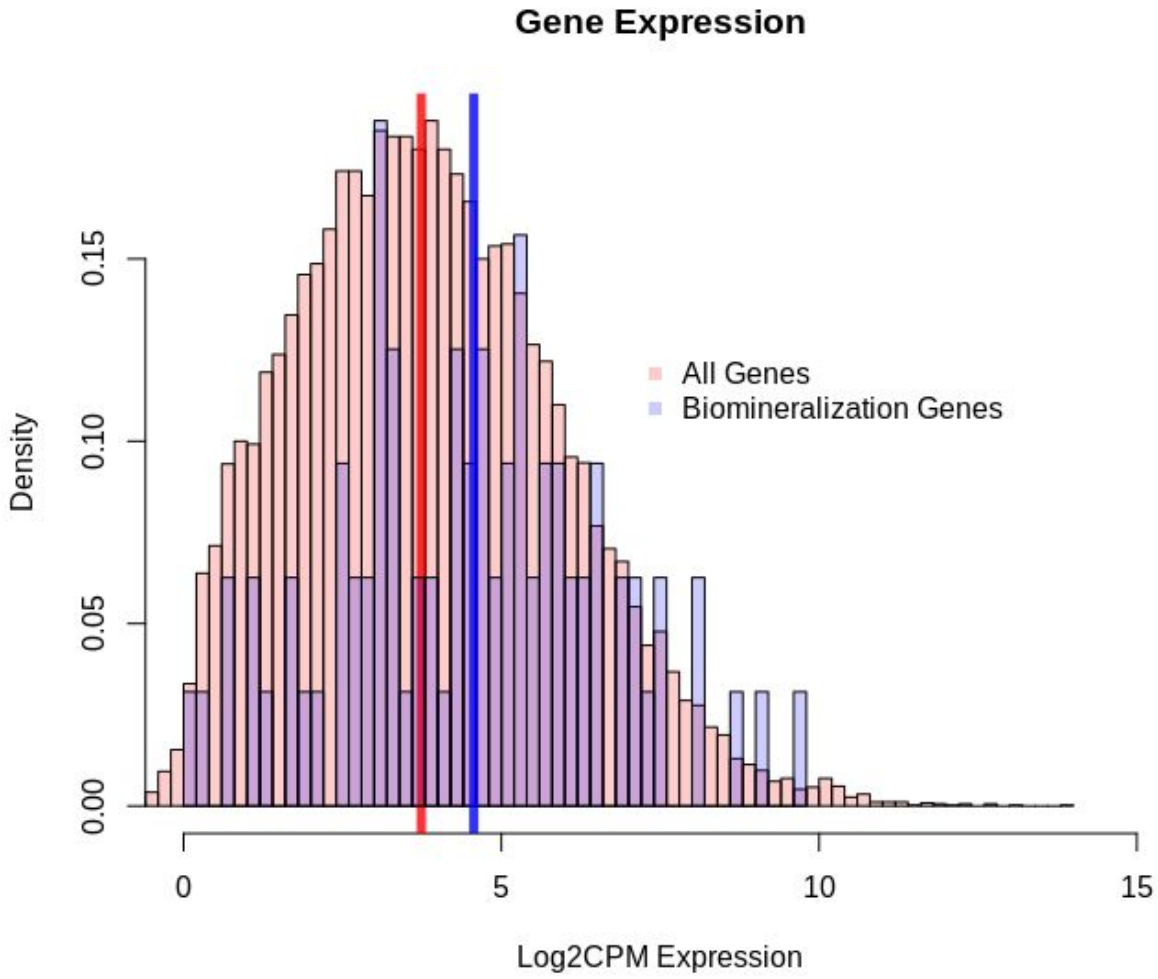


Figure S1.1 - Density Plot of Expression in All Genes vs. Biomineralization Target List

Dark bars indicate the median expression for all genes (red, n = 20387) and biomineralization genes (blue, n = 90), these represent statistically different medians ($P = 0.002$, Mann Whitney U rank sum).

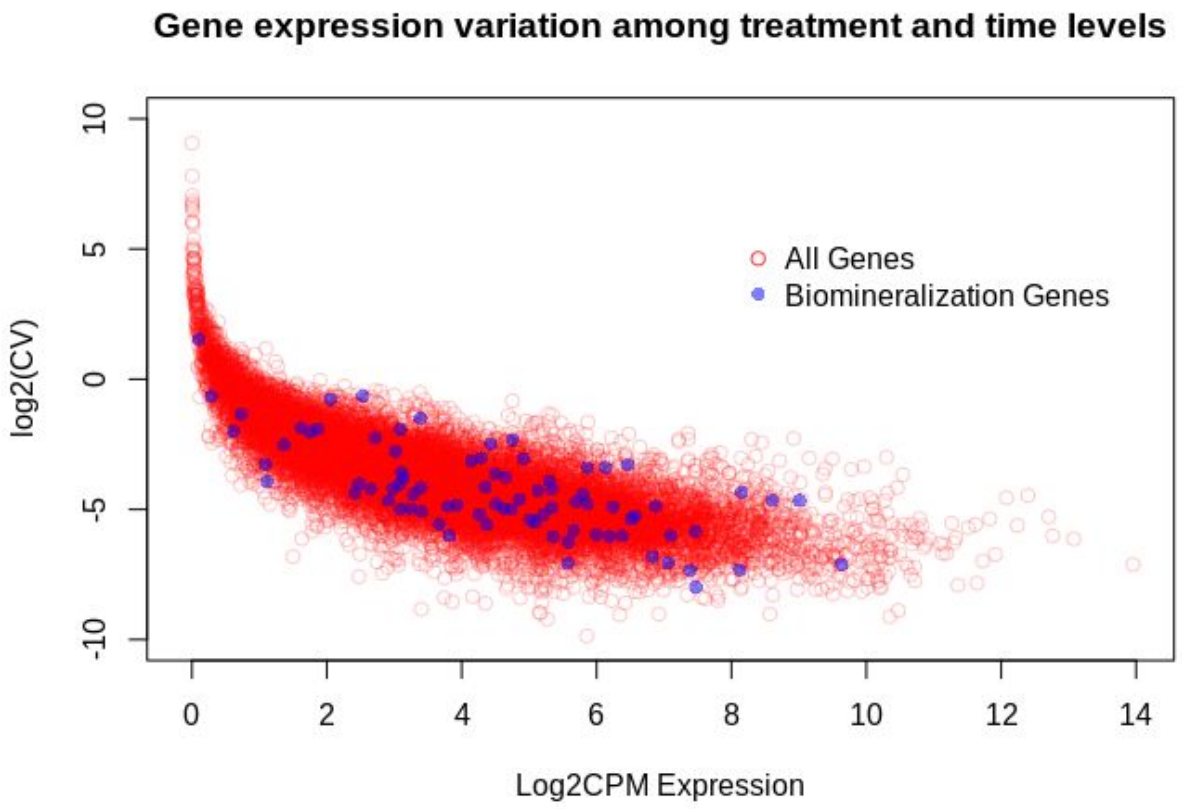


Figure S1.2- Expression Variation in All Genes vs. Biomineralization Target Among Treatment Levels

Coefficient of variation (CV) was calculated based on the mean log2-cpm expression among all treatment and time combination for all genes (red, n = 20387) and the target biomineralization genes (blue, n = 90).

Supplemental Section 2: DNA Methylation Data

Table S2.1 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 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 S2.2 DNA Methylation CpG by genomic feature summary.

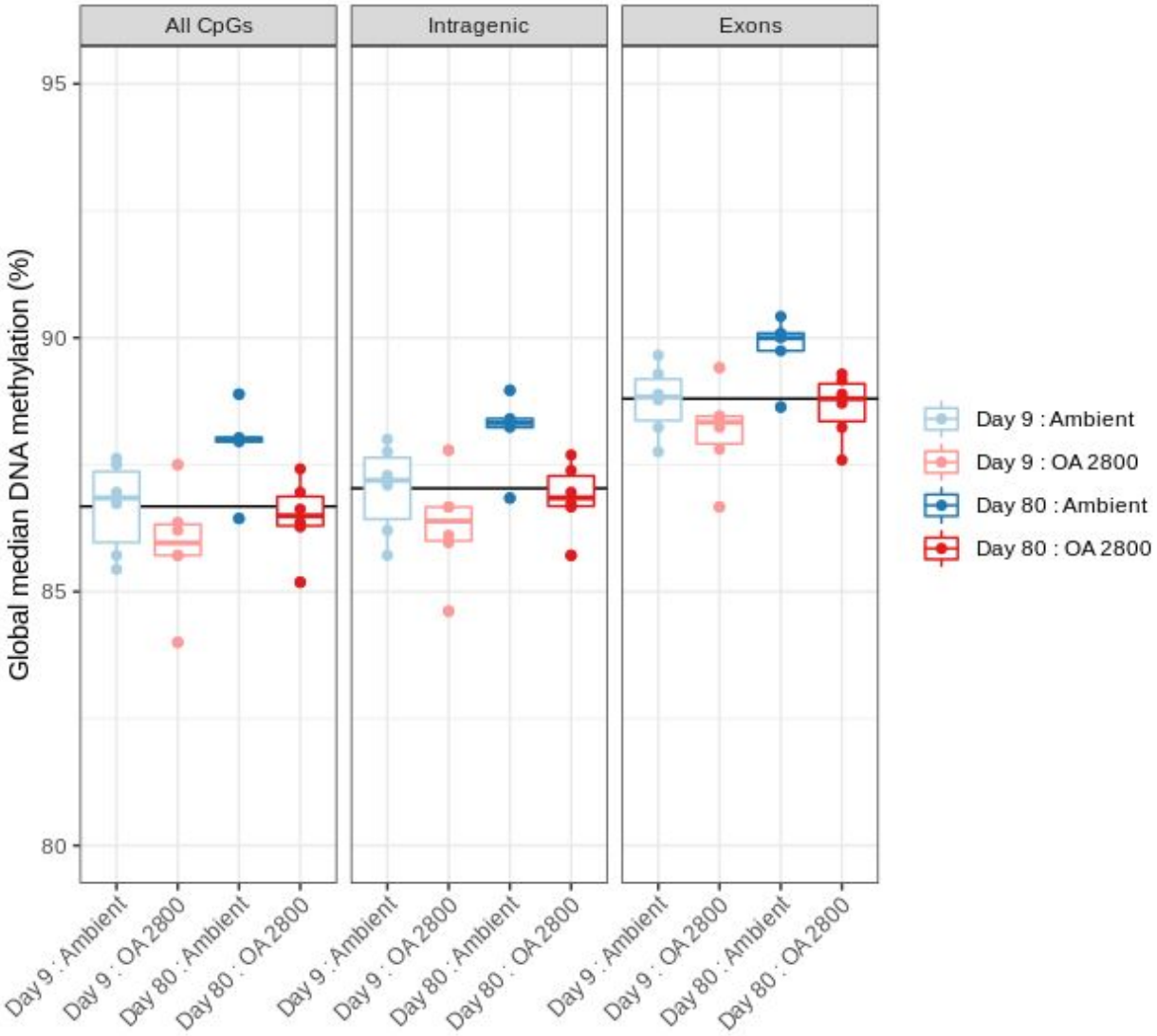
Counts based on an estimate of the total number of CpGs in the oyster genome (Accession: GCA_002022765.4), separated out into major genomic features. The threshold represent minimum per sample coverages (>1, >=5, >=10) for CpG inclusion. * Based on 23 samples after removing one individual due to poor 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	427,988	294,911

Table S2.3 Differentially methylated CpGs (DMLs) among treatments.

Summary table of CpGs found to be differentially methylated from our bayesian binomial mixed effects model. These include the 6 loci where treatment had a significant effect and the 116 loci which were significant by treatment at either day 9 or 80. (attached as a separate excel file).

Figure S2.1 Global Methylation Across Features



Global median methylation among genomic feature based on individual CpGs after 5x per sample filtering. Black lines indicate the mean for all individual methylation medians for each feature. An ANOVA was used to evaluate significant differences among global methylation median among time and treatment levels for each feature. Significant effects of treatment (P_{All} = 0.018, P_{GB} = 0.017, P_{Exon} = 0.016) and time (P_{All} = 0.04, P_{GB} = 0.032, P_{Exon} = 0.029) but not their interaction was found at each feature level.

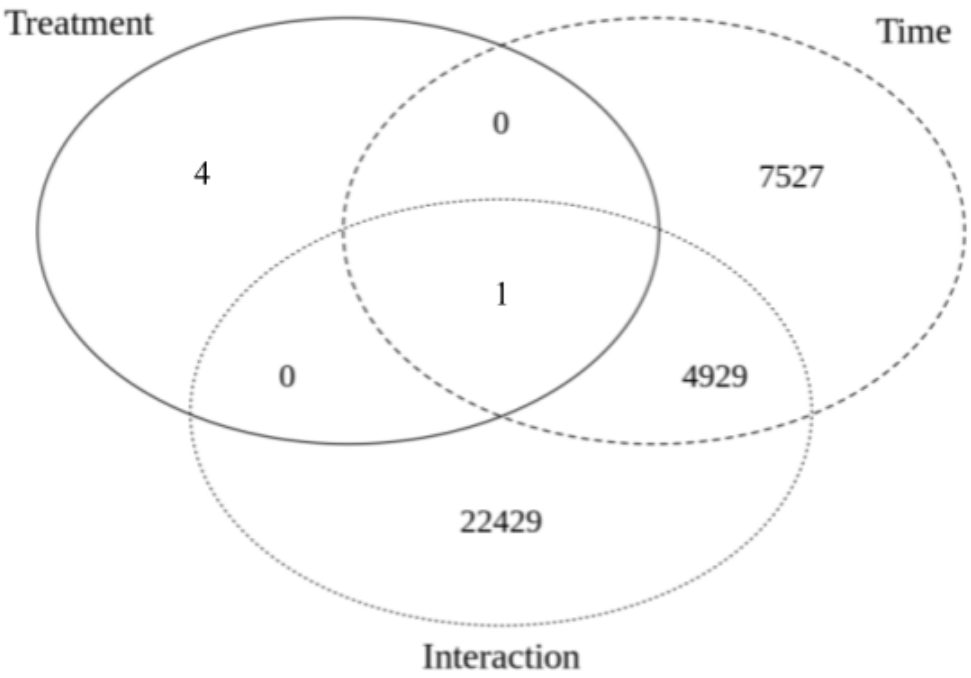


Figure S2.2 Venn diagram of differentially methylated loci in (DMLs)

Differentially methylated loci within gene bodies (n = 397,063, coverage >= 5 per sample) identified with the binomial mixed model by treatment, time, and the interaction between treatment and time.

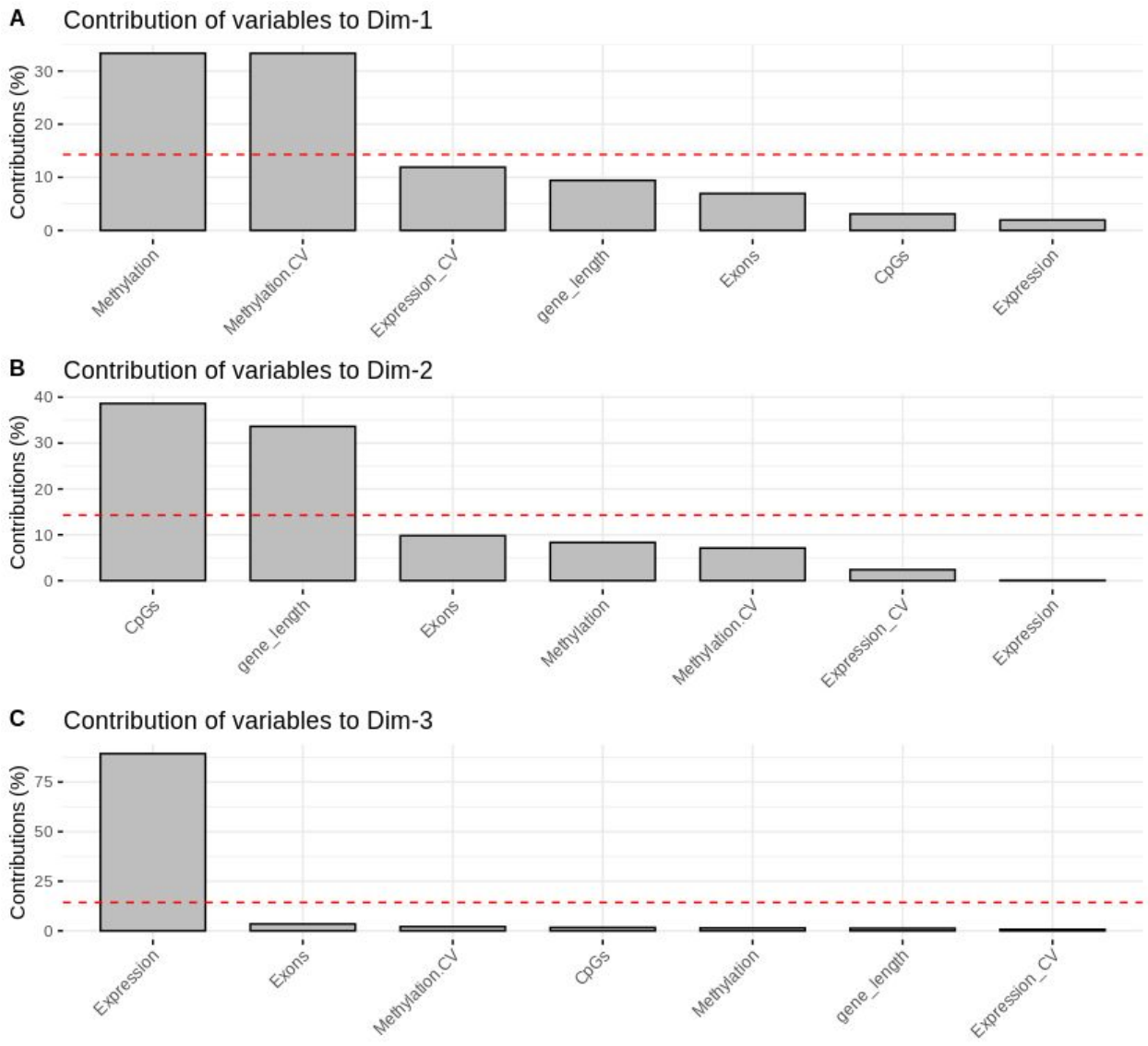


Figure S2.3 PCA Summary - Principle Components

Relative contribution (percent) for each variables (loading) for the first (A), second (B), and third (C) principal components of the PCA. The dotted redlines indicates the default 15% contribution significance threshold for individual variables.

Supplemental Section 3: Phenotype Data

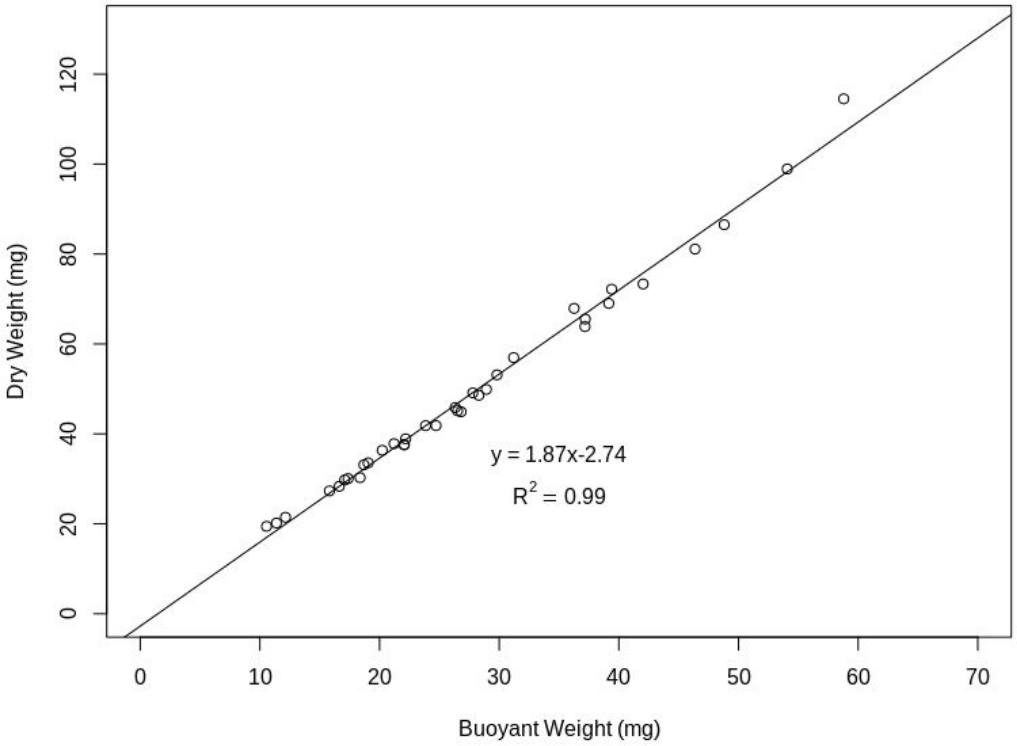


Figure S3.1 Buoyant weight compared to dry weight.

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.

Supplemental Section 4: Water Chemistry and Field Data

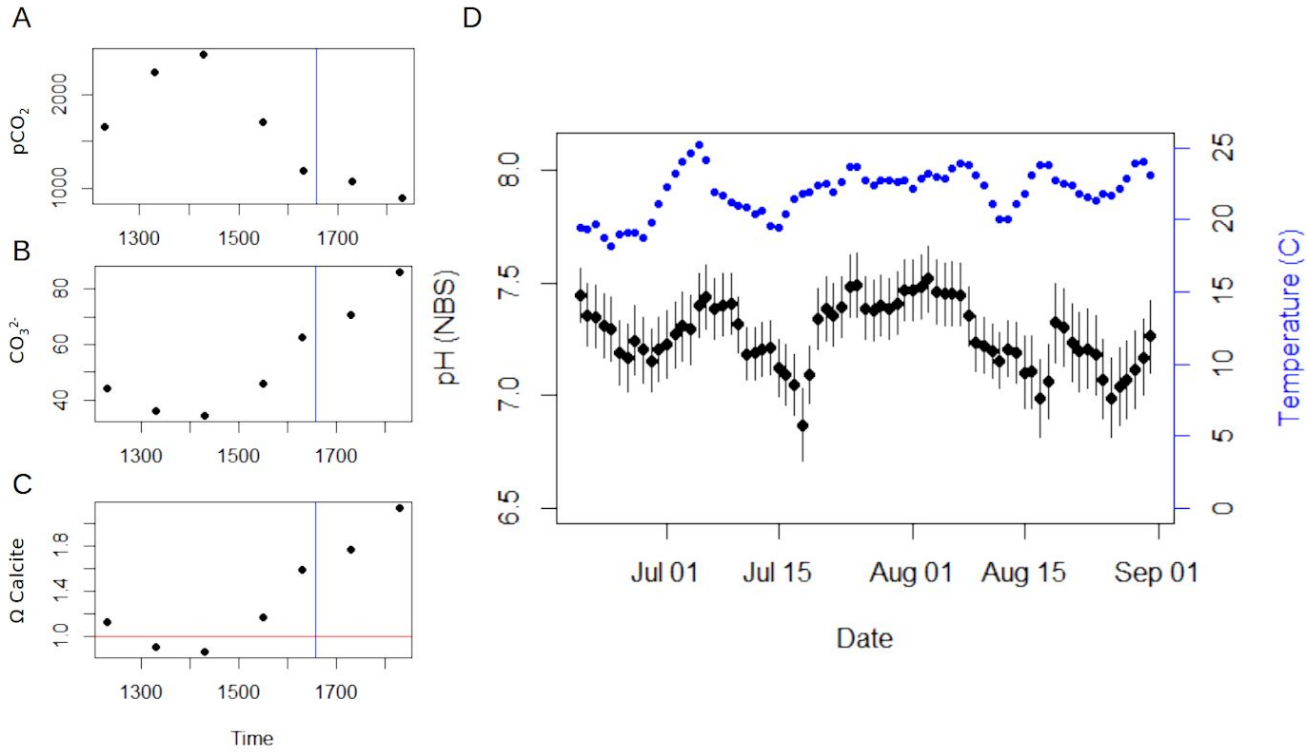


Figure S4.1 Field site water chemistry.

Carbonate chemistry and temperature from collection site 3 (42.681764, -70.813498) including, a detailed tidal cycle measurement (A-C) and daily average pH and temperature summary (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±SD) and temperature (blue circles) over the course of summer 2017.

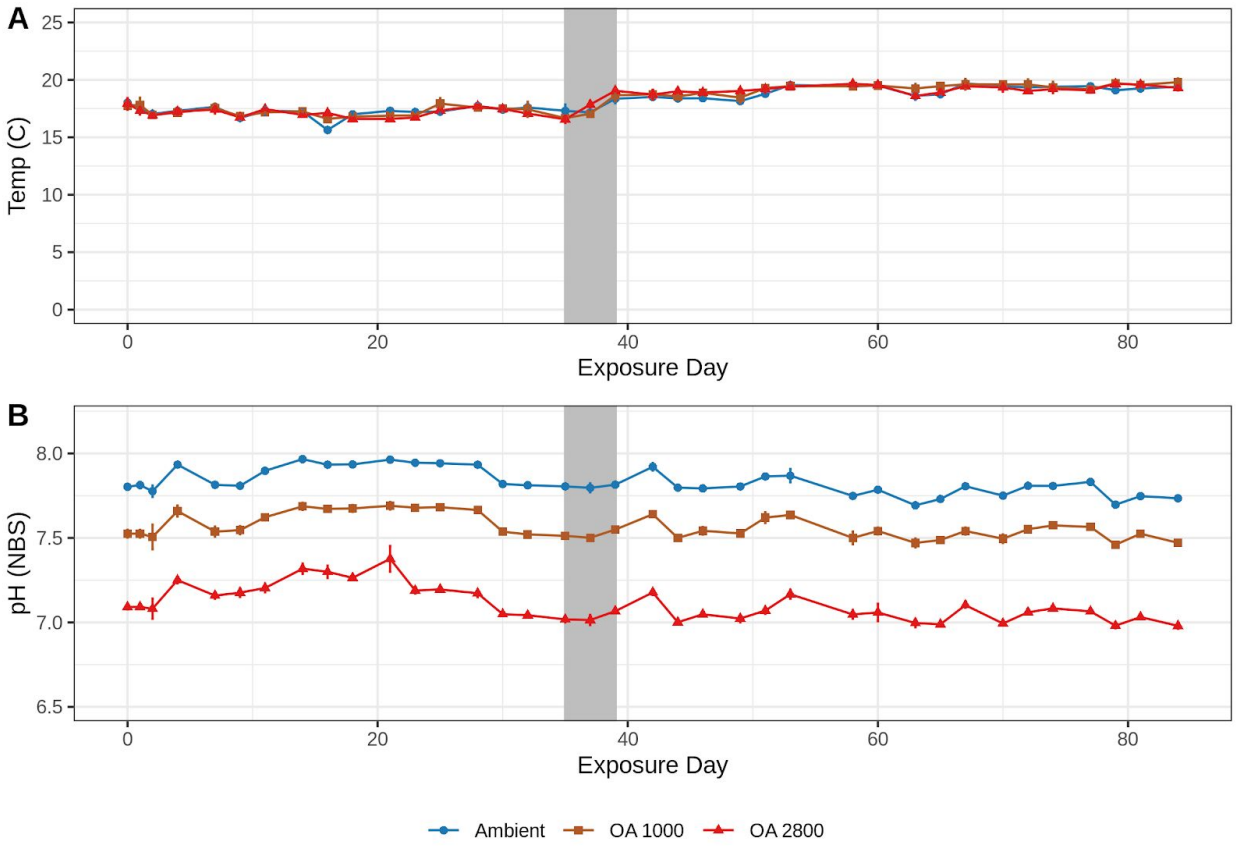


Figure S4.2 Mean triweekly water chemistry.

Mean temperature (A) and pH (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 ~5 day period where the temperature was increased by 1.5 degrees C.