Figure 1.

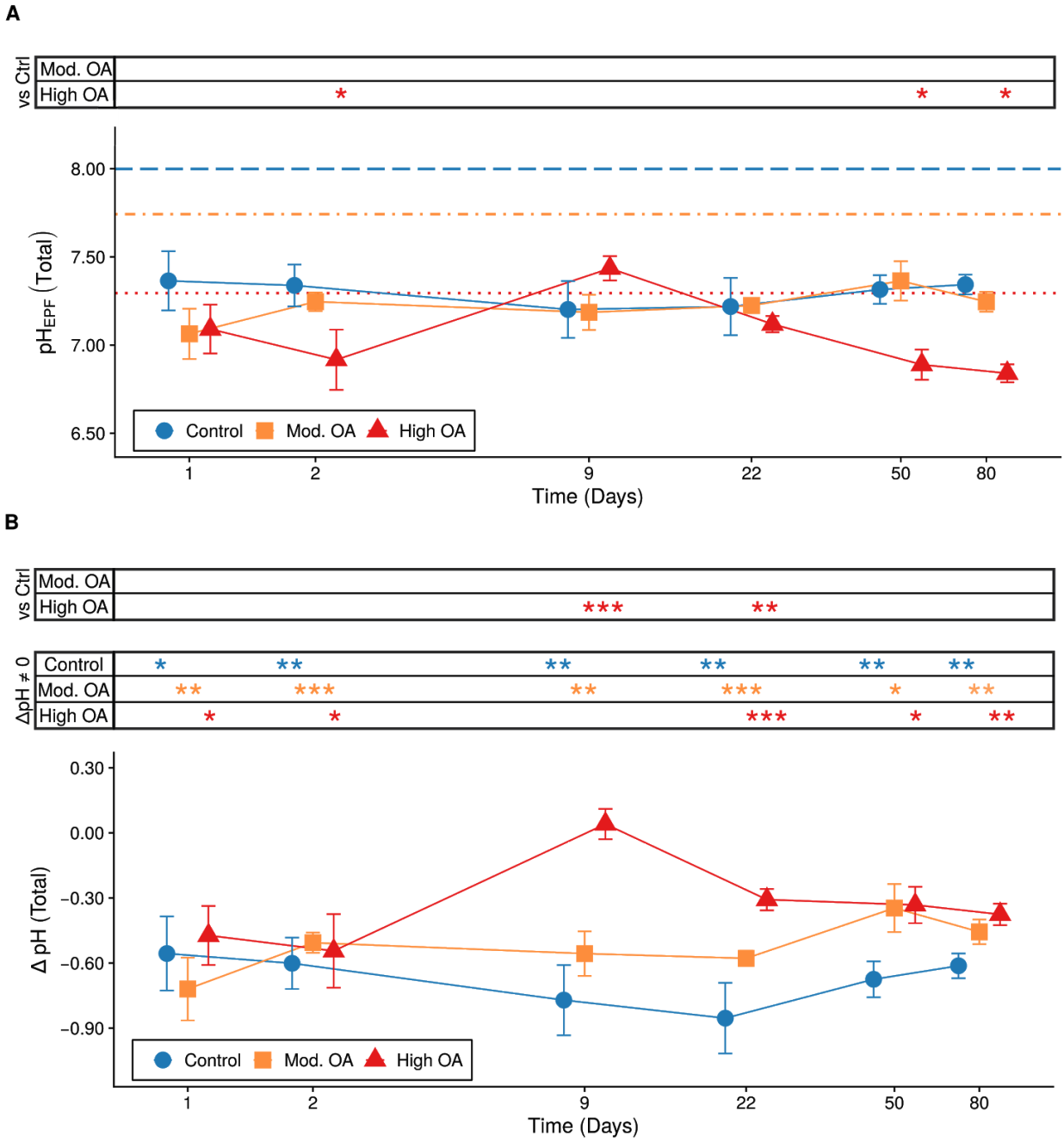
A
vs Ctrl
Mod. OA
High OA
8.00
7.50
7.00
6.50
pH_EPF (Total)
Control
Mod. OA
High OA
1
2
9
22
50
80
Time (Days)
B
vs Ctrl
Mod. OA
High OA
ΔpH ≠ 0
Control
Mod. OA
High OA
0.30
0.00
−0.30
−0.60
−0.90
Δ pH (Total)
Control
Mod. OA
High OA
1
2
9
22
50
80
Time (Days)

Fig. 1 | Extrapallial fluid pH over the 80 day experiment. **(A)** pH_{EPF} (total scale) and **(B)** ΔpH (pH_{EPF} - $pH_{seawater}$) across time with standard error bars. **(A)** Colored lines represent each treatment level averaged over the duration of the exposure (dashed: control, dot-dashed: moderate OA, dotted: high OA) and symbols at the top of the graph indicate if the pH_{EPF} in one of the OA treatments ('Mod. OA', 1000 uatm; 'High OA', 2800 µatm) was significantly different from the pH_{EPF} in the control treatment ('Control', 580 µatm) for each time point (e.g., H_0: Control pH_{EPF} = High OA pH_{EPF}). **(B)** Symbols at the top of the graph indicate significant post hoc tests. The 'vs. Ctrl' comparisons indicate time points where ΔpH in one or both of the OA treatments was significantly different from the ΔpH in the control treatment (e.g., H_0: Control ΔpH = High OA ΔpH), while 'ΔpH ≠ 0' comparisons indicate time points where ΔpH in one of the treatments was significantly different from 0 (e.g., H_0: Control ΔpH = 0). The latter comparison is equivalent to evaluating whether pH_{EPF} of a particular treatment is significantly different from its respective $pH_{seawater}$. Statistical significance is denoted by asterisks (*** = $P < 0.001$,** = $P < 0.01$, * = $P < 0.05$). Treatment points within time points were staggered along the x-axis to improve visualization.

Figure 2.

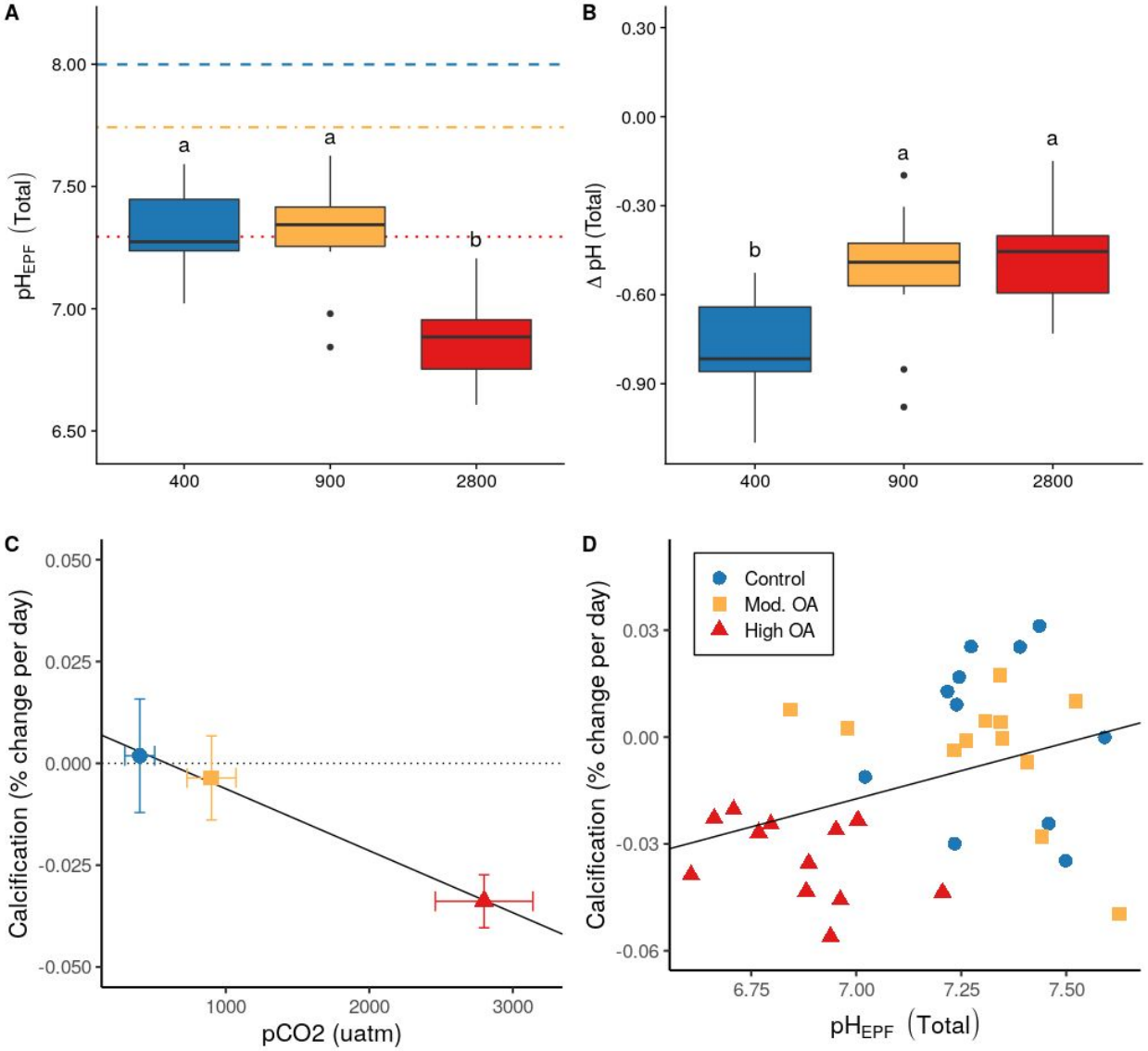
A
8.00
7.50
7.00
6.50
pH_EPF (Total)
a
a
b
400
900
2800
B
0.30
0.00
-0.30
-0.60
-0.90
Δ pH (Total)
b
a
a
400
900
2800
C
0.050
0.025
0.000
-0.025
-0.050
Calcification (% change per day)
1000
2000
3000
pCO2 (uatm)
D
Control
Mod. OA
High OA
0.03
0.00
-0.03
-0.06
Calcification (% change per day)
6.75
7.00
7.25
7.50
pH_EPF (Total)

Fig. 2 | Long term trends in EPF pH, ΔpH and calcification. **(A)** Long-term average pH_{EPF} (total scale) by treatment; horizontal lines indicate mean seawater pH for the three treatments (dashed: control, dot-dashed: moderate OA, dotted: high OA). (**B)** Long-term average ΔpH (pH_{EPF} - $pH_{seawater}$; total scale) by treatment. (**C**) Percent daily calcification rate (%-change in shell mass/day) by treatment (*slope* = -1.751e-05, $P < 0.0001$, $R^2 = 0.409$), dotted line indicates calcification rate of zero percent and solid black line represents fitted regression. (**D**) Calcification rate versus pH_{EPF} (*slope* = 0.032, $P = 0.028$, $R^2 = 0.112$); solid black line represents fitted regression. pH_{EPF} measured on day 50 or 80 (n = 35). Letters on first two panels (**A** and **B**) represent levels of significance based on post hoc testing, while bars on (**C**) represent standard errors.

Figure 3.

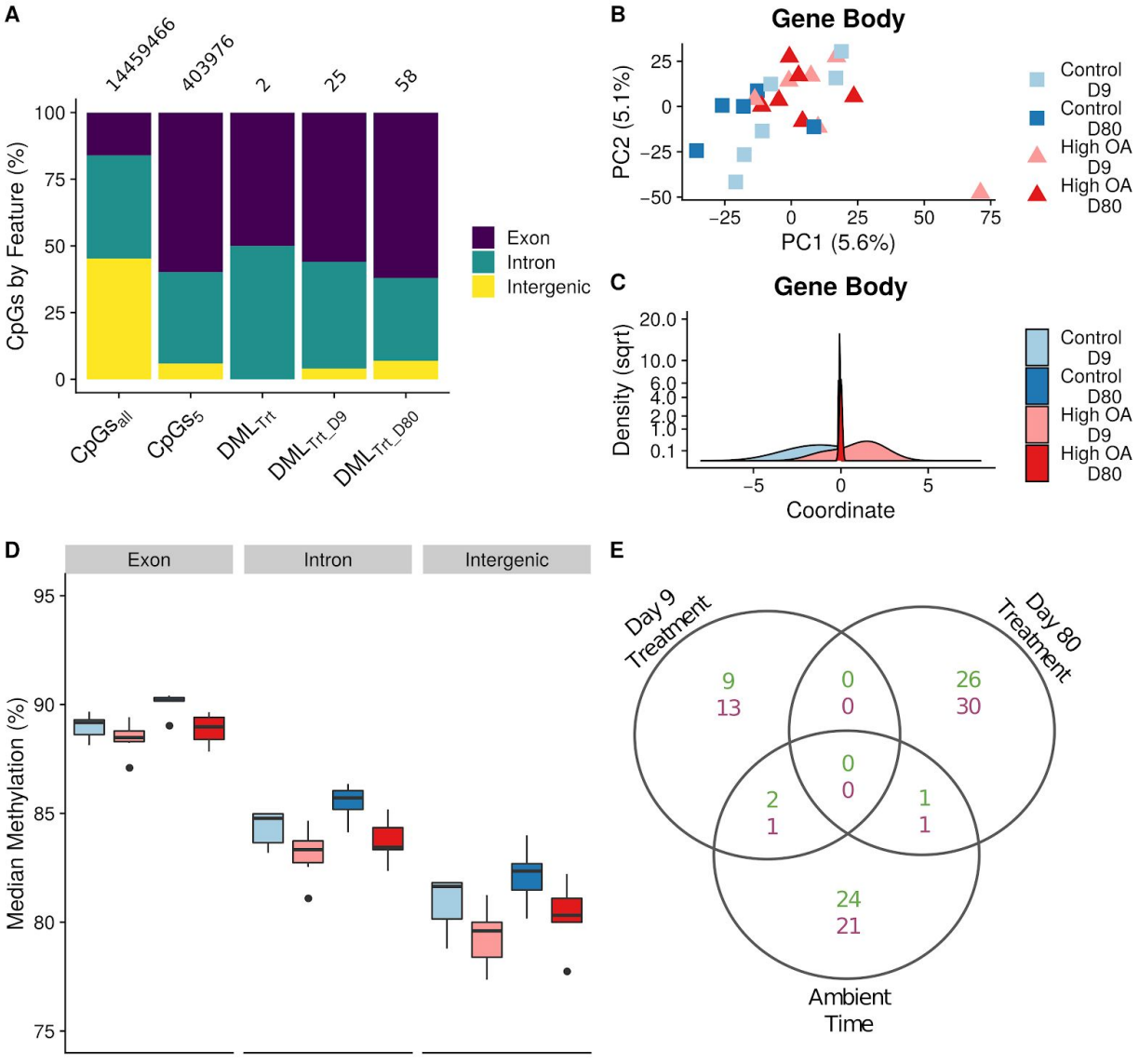
A
14459466
403976
2
25
58
CpGs by Feature (%)
100
75
50
25
0
CpGsall
CpGs5
DMLTrt
DMLTrt_D9
DMLTrt_D80
Exon
Intron
Intergenic
B
Gene Body
PC2 (5.1%)
PC1 (5.6%)
Control D9
Control D80
High OA D9
High OA D80
C
Gene Body
Density (sqrt)
Coordinate
D
Exon
Intron
Intergenic
Median Methylation (%)
E
Day 9 Treatment
Day 80 Treatment
Ambient Time
9
13
0
0
26
30
0
0
2
1
1
1
24
21

Figure 3 | DNA methylation responses to OA. (A) Proportion of CpGs by feature across data subsets. $CpGs_{all}$: all CpGs in genome; $CpGs_5$: CpGs with at least 5X coverage for each individual; DML_{Trt}: differentially methylated loci (DMLs) between control and OA treatments across both time points; DML_{Trt_09} and DML_{Trt_80}: DMLs between treatments on day 9 and 80, respectively. Numbers above the bars represent the total number of CpGs for each group. **(B)** Boxplot of median global methylation for each sample by genome feature ($P_{Trt} < 0.0001$, $P_{Time} = 0.001$, $P_{Feature} < 0.0001$)**. (C)** Plot of the first two principal components from a principal components analysis ($P_{Trt} = 0.027$, $P_{Time} = 0.235$, $P_{TimexTrt} = 0.364$), and (**D**) density plot of the discriminant values from a DAPC based on gene body methylation (exons and introns). Discriminant values were determined by a function that maximally discriminates between treatment using samples from day 9, then the discriminant value for each sample from day 80 was predicted using the same discriminant function. **(E)** Venn diagram of DMLs among treatments for each day (Day 9 Treatment and Day 80 Treatment) and among time points in the control treatment (Control Time). Overlapping regions indicate DMLs shared among comparisons. Hypermethylated DMLs in green (top number), hypomethylated DMLs in purple (bottom number).

Figure 4.

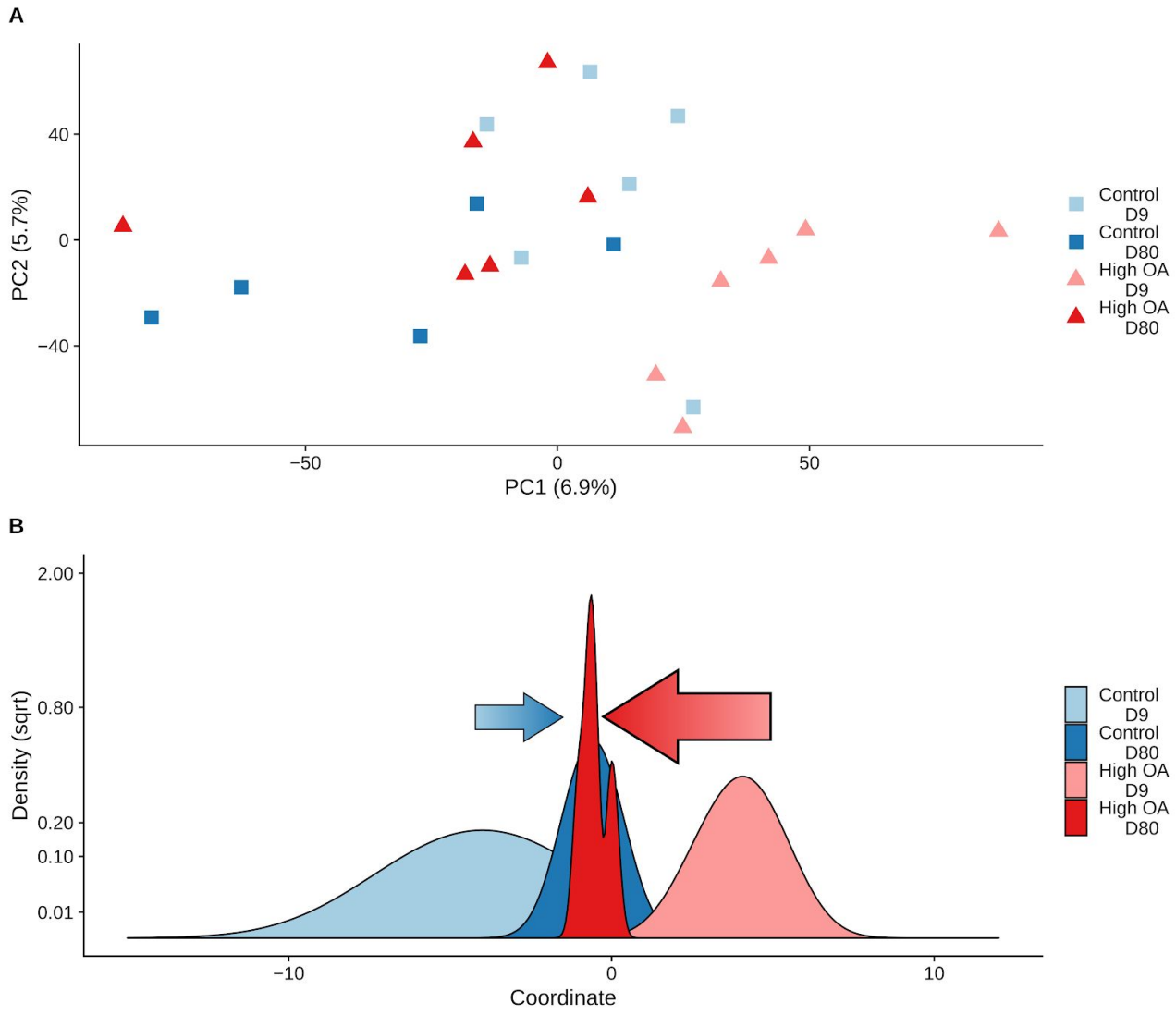
A
PC2 (5.7%)
40
0
−40
−50
0
50
PC1 (6.9%)
Control D9
Control D80
High OA D9
High OA D80
B
Density (sqrt)
2.00
0.80
0.20
0.10
0.01
−10
0
10
Coordinate
Control D9
Control D80
High OA D9
High OA D80

Figure 4 | Global transcriptomic responses to OA. (A) Plot of the first two principal components from a principal components analysis ($P_{Trt} = 0.037$, $P_{Time} < 0.001$, $P_{TimexTrt} = 0.214$)**. (B)** Density plot of the discriminant values from a DAPC. Discriminant values were determined by using the DAPC package to estimate a function that maximally discriminates between treatment using samples from day 9, then the discriminant value for each sample from day 80 was predicted using the same discriminant function. Arrows indicate direction and degree of movement of expression patterns for control (blue) or OA (red) treatments along the discriminant function from day 9 to day 80. Both plots are based on log2-cpm gene expression.

Figure 5.

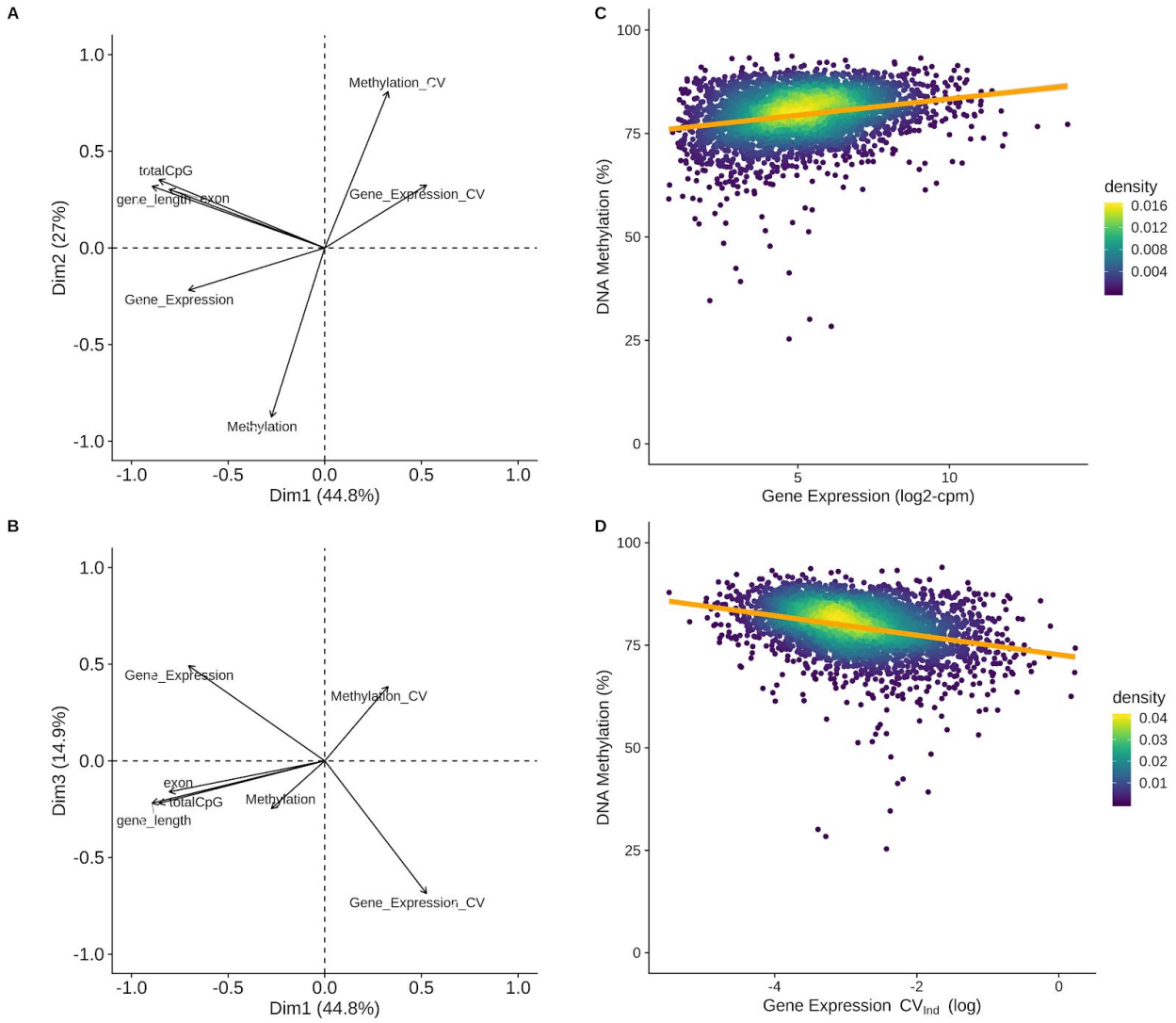
A
Methylation_CV
totalCpG
gene_length
exon
Gene_Expression_CV
Gene_Expression
Methylation
Dim2 (27%)
Dim1 (44.8%)
B
Gene_Expression
Methylation_CV
exon
totalCpG
gene_length
Methylation
Gene_Expression_CV
Dim3 (14.9%)
Dim1 (44.8%)
C
DNA Methylation (%)
Gene Expression (log2-cpm)
density
0.016
0.012
0.008
0.004
D
DNA Methylation (%)
Gene Expression CV$_{Ind}$ (log)
density
0.04
0.03
0.02
0.01

Figure 5 | DNA methylation and gene expression correlations. (A) First two principal components and **(B)** the first and third components from a principle component analysis that included gene level summary variables for various attributes, expression, and methylation. Variable loadings plotted as arrows, with the length of the arrow corresponding to the relative contribution to PC variance. Significant loadings on the first two PCs included: mean methylation level (Methylation), the coefficient of variance of mean methylation levels among treatments (Methylation_CV), gene expression (Gene_Expression), the number of exons (exon), the number of CpG dinucleotides (totalCpGs), and the gene length in base pairs (gene_length). The CV of gene expression (Expression_CV) was not significant for the first two PCs but was the primary contributor to the third PC (see S3.3). **(C-D)** Plot of gene level DNA methylation against either **(C)** gene expression or the **(D)** gene expression CV among individuals based samples collected in the control treatment at day 9 (Gene Expression - $R_2 = 0.0624$, $P < 0.0001$; Gene Expression CV_{Ind} - $R_2 = 0.0658$, $P < 0.0001$). Only genes with coverage for at least 20% of all CpGs within the gene were included (n=3,604).

Figure 6.

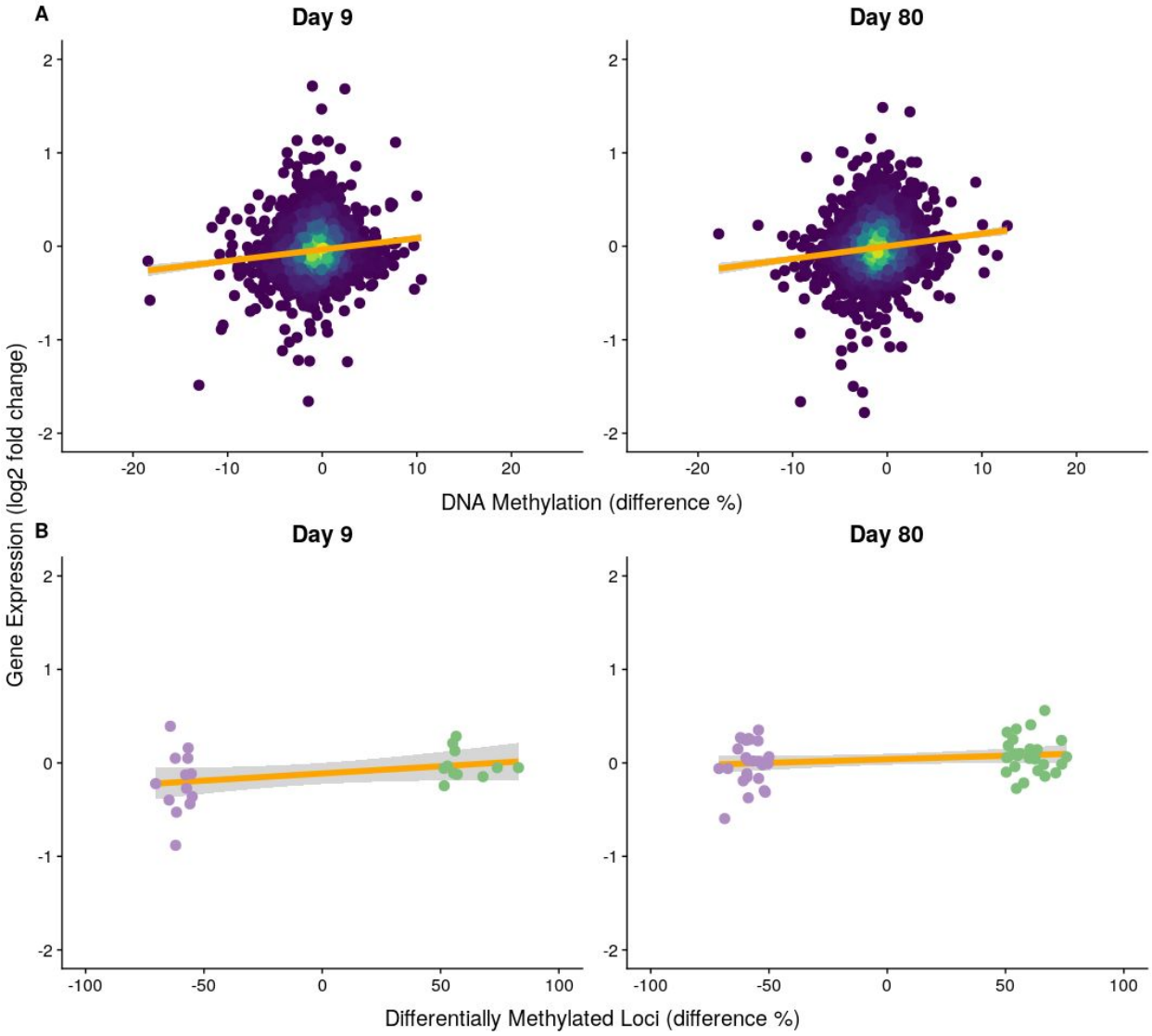
A
Day 9
Day 80
Gene Expression (log2 fold change)
DNA Methylation (difference %)
B
Day 9
Day 80
Gene Expression (log2 fold change)
Differentially Methylated Loci (difference %)

Figure 6 | Gene expression change in response to OA as a function of DNA methylation change. Log2-fold change in gene expression compared to % difference in **(A)** mean gene methylation among treatments (Day 9 - slope = 0.0121, $P < 0.0001$, $R^2 = 0.012$; Day 80 - slope = 0.0137, $P < 0.0001$, $R^2 = 0.014$) and **(B)** significantly differentially methylated loci (DML) among treatments (Day 9 - slope = 0.0005, $P = 0.0005$, $R^2 = 0.406$; Day 80 - slope = 0.00054, $P = 0.0004$, $R^2 = 0.276$). Orange line represents the fitted linear model. Colors in **(B)** correspond to DMLs that were significantly hyper- (green) and hypomethylated (purple) in the OA treatment.

Figure 7.

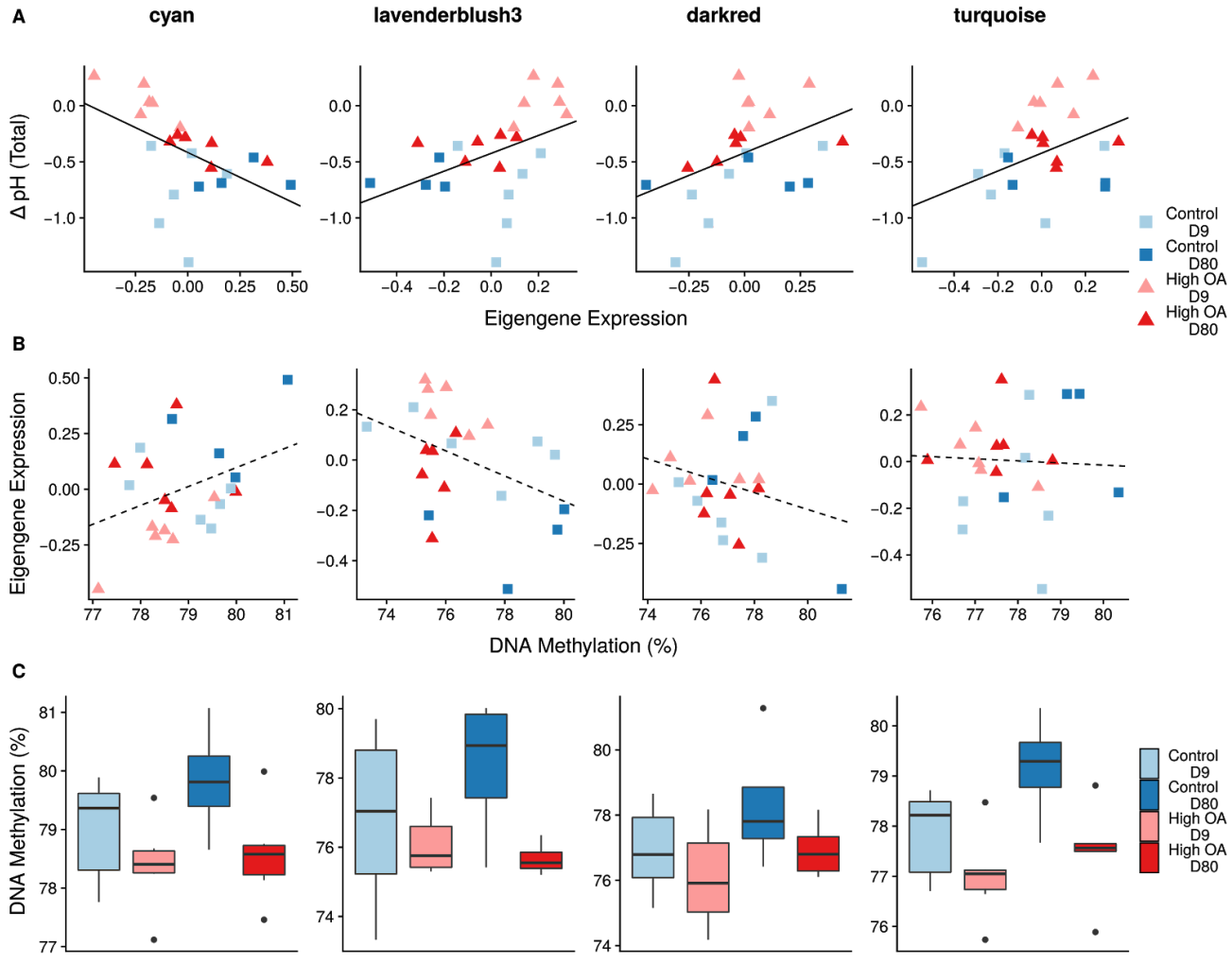
A
cyan
lavenderblush3
darkred
turquoise
Δ pH (Total)
Eigengene Expression
B
Eigengene Expression
DNA Methylation (%)
C
DNA Methylation (%)
Control D9
Control D80
High OA D9
High OA D80

Figure 7 | Co-expression and DNA methylation responses to OA. Four of the top five modules associated with oyster ΔpH (colors are randomly assigned by the WGCNA package and have no meaning). Scatterplots of **(A)** ΔpH by eigengene expression (P_{cyan} = 0.021, $P_{lavenderblush3}$ = 0.044, $P_{darkred}$ = 0.037, $P_{turquoise}$ = 0.042), **(B)** eigengene expression by mean module methylation (P_{cyan} = 0.087, $P_{lavenderblush3}$ = 0.052, $P_{darkred}$ = 0.264, $P_{turquoise}$ = 0.833), and boxplot of **(C)** mean module methylation by treatment and time point. For **(C)** there was a significant effect of treatment in three of the four modules (P_{cyan} = 0.035, $P_{lavenderblush3}$ = 0.038, $P_{darkred}$ = 0.123, $P_{turquoise}$ = 0.013), but neither time point or their interaction was significant for any of the modules. Mean module methylation was calculated as the mean methylation for all CpGs for each gene within a module. Solid lines indicate a significant relationship between explanatory (x-axis) and response variable (y-axis) using a linear model, while dotted lines indicate non significant trends among variables.