Figure 1. **A**. Metagene profile of analyzed individuals from 2kb upstream of the Transcription Start Site (TSS) to 2 kb downstream of the Transcription End Sites (TES) **B**. Mean methylation profiles across genomic context centered on the *D. labrax* genome in freshwater- (FW) and seawater-acclimated fish (SW). All genomic regions were scaled to the same length.
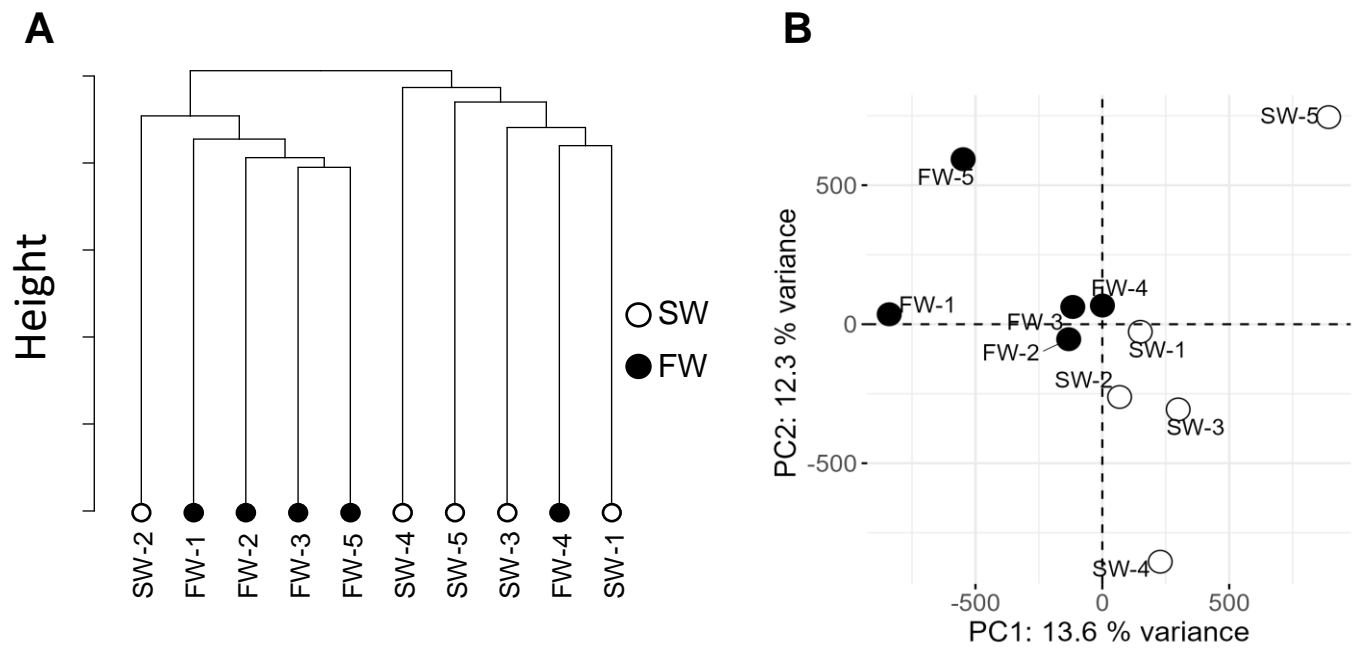
Figure 2. Cluster analysis based on CpG methylation profiles in 5 freshwater-exposed (FW, black discs) and 5 seawater-exposed *D. labrax* (SW, white discs). **A**. Hierarchical clustering of samples was performed using Ward's method based on Pearson's correlation distance for cytosine CpG methylation. **B**. Principal Component Analysis (PCA) showing (PC1xPC2) coordination plane.
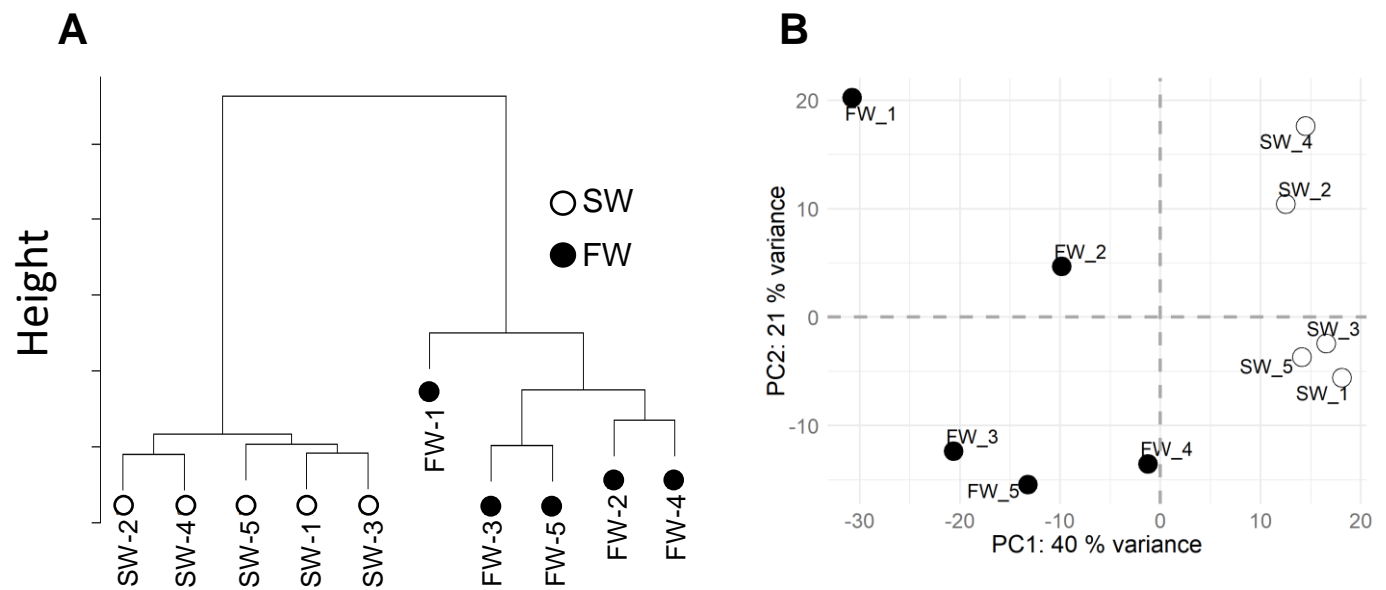

Figure 3. Cluster analysis based on gene expression levels in 5 freshwater-exposed (FW, black discs) and 5 seawater-exposed *D. labrax* (SW, white discs). **A.** Hierarchical clustering of samples was performed using Ward's method based on Pearson's correlation distance for gene expression levels. **B**. Principal Component Analysis (PCA) showing (PC1xPC2) coordination plane.
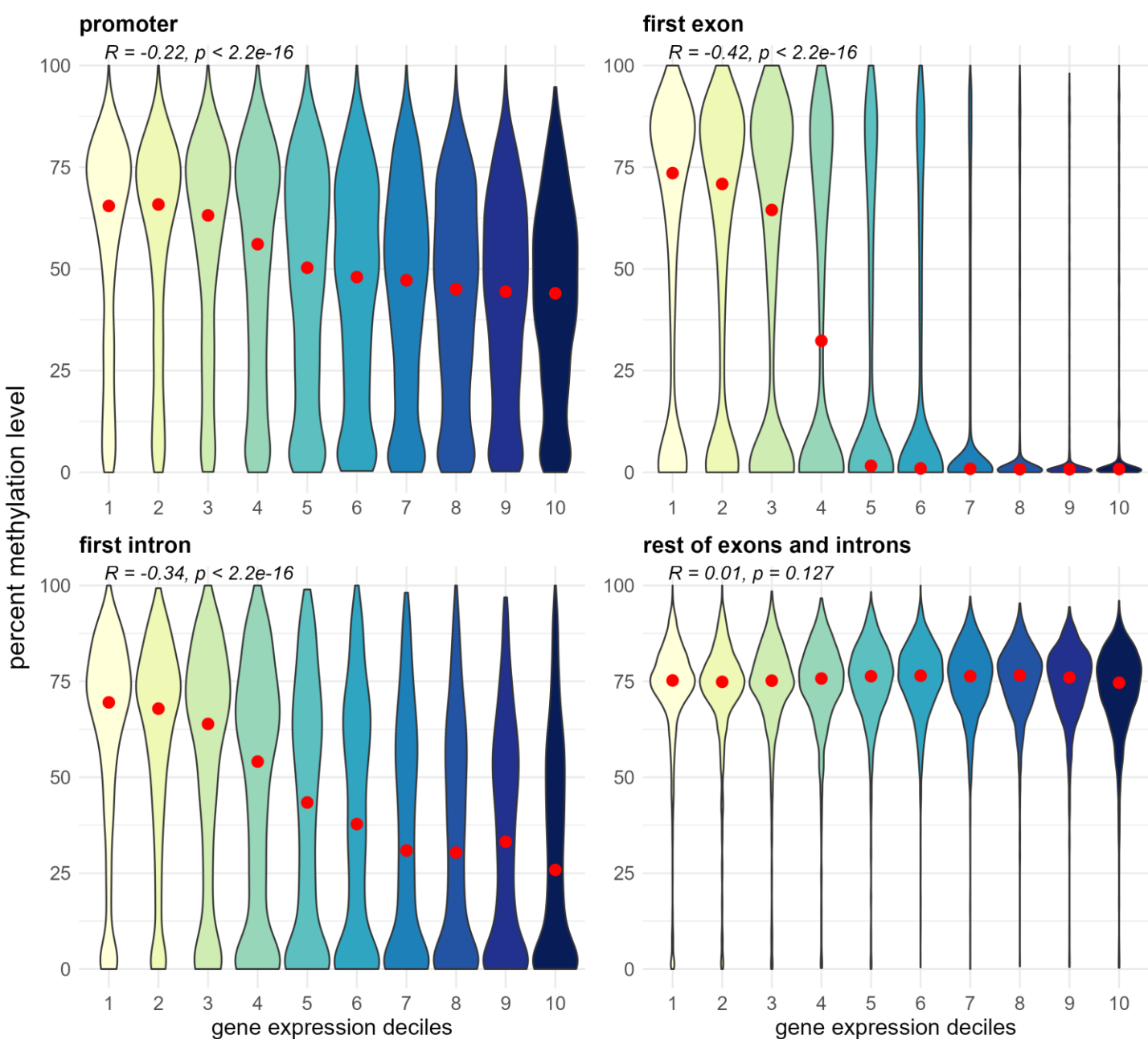
Figure 4. DNA methylation levels (in %) in different genomic context for different gene expression levels. Violin plots showing DNA methylation in promoter, first exon, first intron and gene body at different gene expression levels (divided into deciles based on increasing ranking of gene expression measured as log2-transformed normalized counts from DEseq2). Central red dots represent the median of the distribution. Correlations between DNA methylation and gene expression were measured using Spearman's rank correlation coefficient.
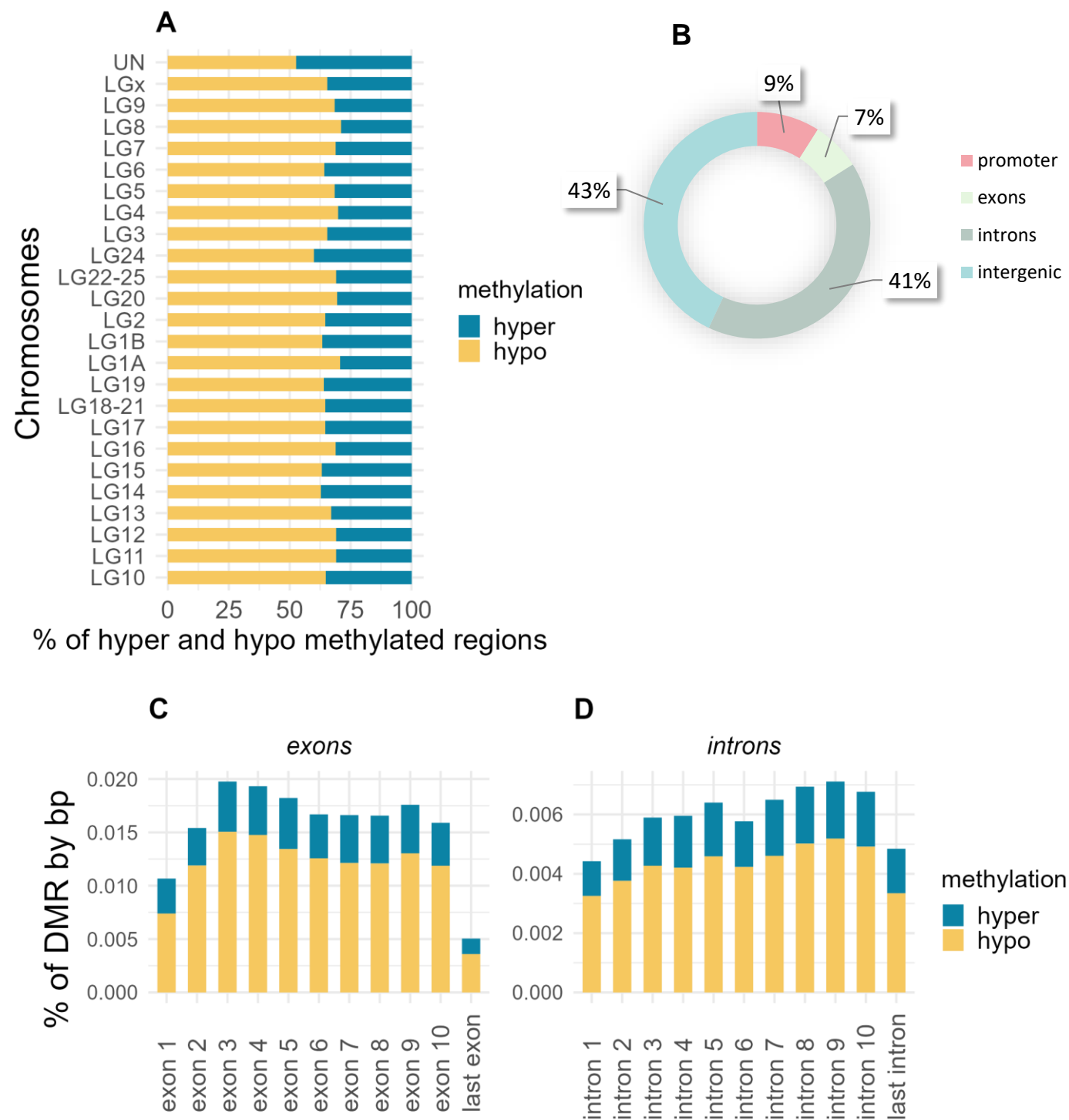
Figure 5. Genome-wide profile of CpG methylation. **A**. Chromosomal distribution of hyper- and hypo- methylated regions in freshwater- compared to seawater exposed *D. labrax*. p-value < 0.05 and methylation difference ≥ 25 %. **B**. Distribution of the differentially methylated regions (DMRs) in different genomic context. **C.** and **D**. Percentage of DMRs per exon and intron position normalized by the total size (in bp) of exons and introns respectively.
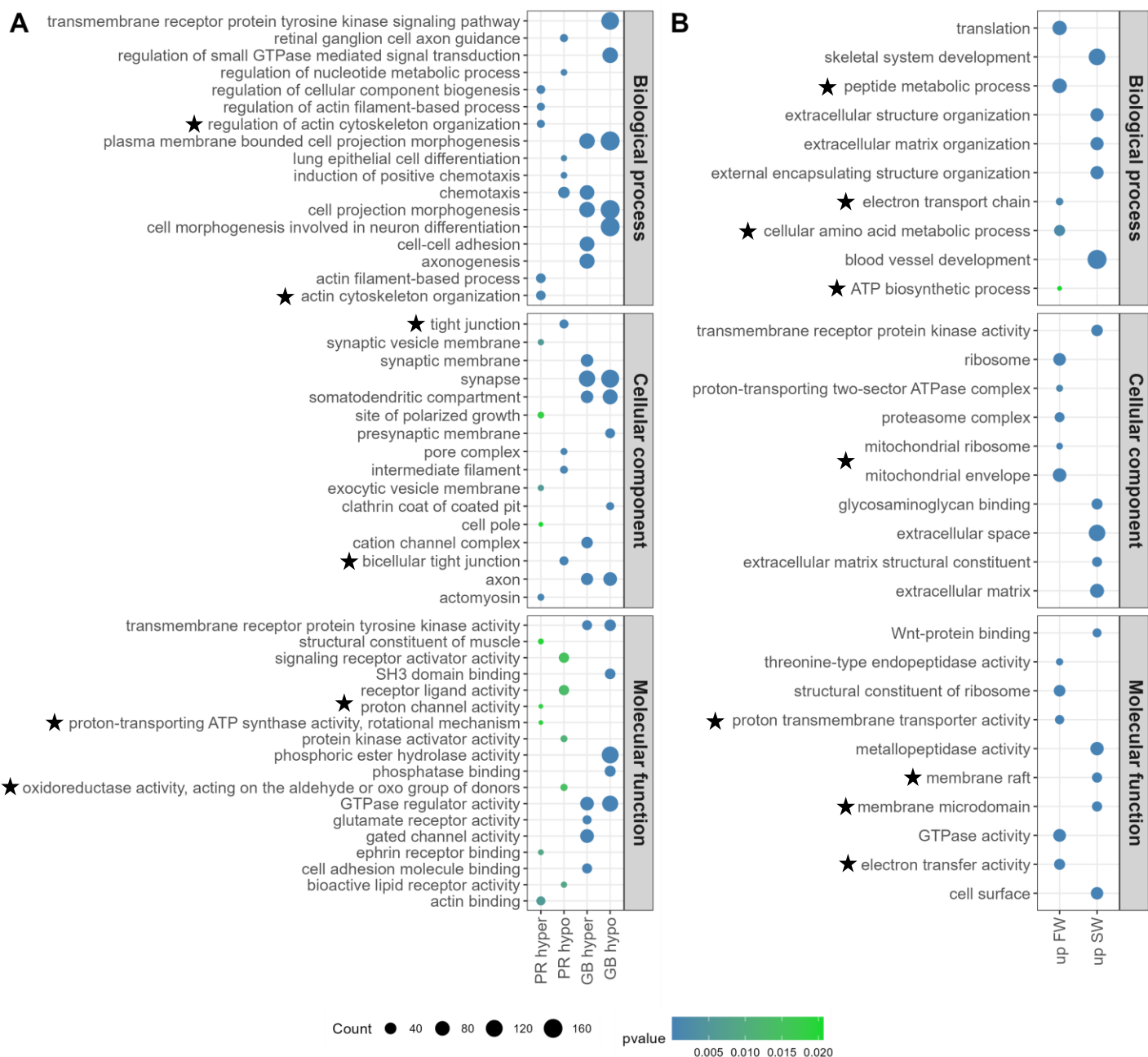
Figure 6. Enrichment analysis of genetic ontology (GO) terms. The 5 most significant GO terms for each group are represented. **A.** DNA methylome. **B.** RNA transcriptome. FW : fresh water; GB : gene body; PR : promoter; SW : seawater. The size of the dots represent the ratio of genes associated with the GO term and the colors indicate the p-values (threshold set at 0.05). Discussed GO terms are indicated by stars.
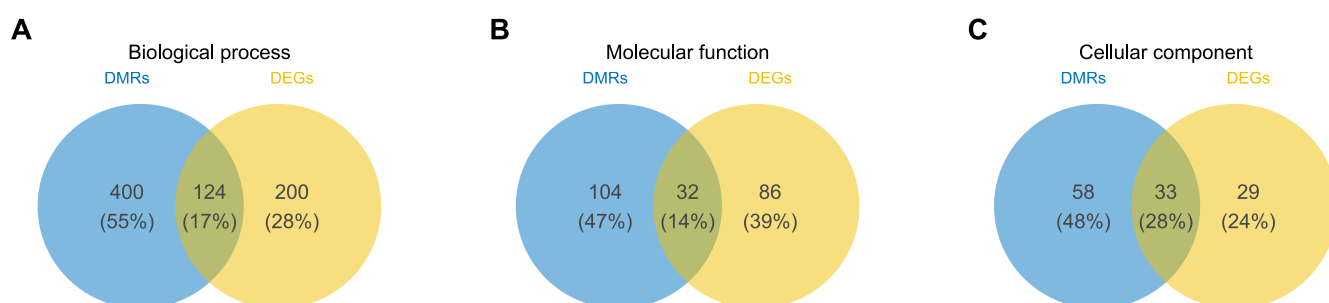

Figure 7. Venn analysis of methylome and transcriptome showing significantly overrepresented (p < 0.05) GO terms related to DEGs and DMRs (in genes and promoters) in FW *vs* SW conditions. **A.** Biological process. **B.** Molecular function. **C.** Cellular component. DMRs: differentially methylated regions ; DEGs: differentially expressed genes.
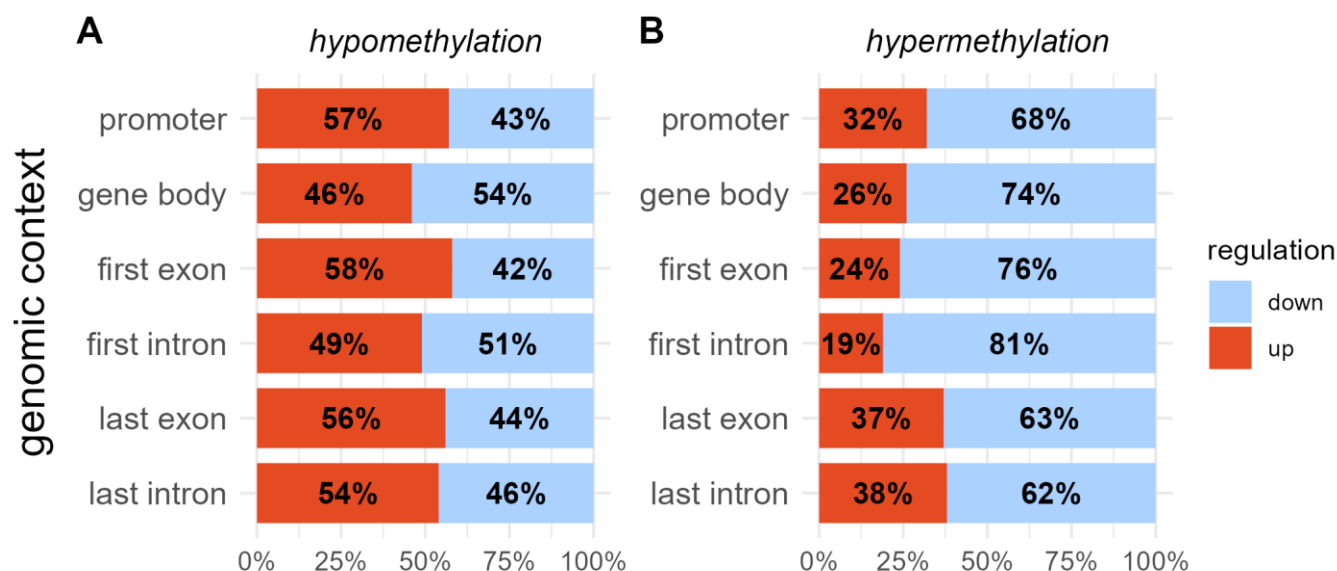
Figure 8. Comparison between freshwater (FW)-triggered gene expression changes and methylation changes in different genomic contexts. **A**: Proportion of down- and upregulated genes that are hypomethylated. **B**: Proportion of down- and upregulated genes that are hypermethylated. Blue color = downregulation in FW and red color = upregulation in FW.

Figure 9. Enrichment plot of the KEGG pathways enrichment analysis of differentially expressed and differentially methylated genes in promoters, first exons or first introns. The 5 KEGG pathways of each dataset displaying the most significant p-values have been represented. The proportion of clusters in the pie chart was determined by the proportion of genes in a specific category. The size of each circle represents the number of genes involved in each pathways (p-value < 0.05).

Table 1. List of selected differentially expressed and methylated genes

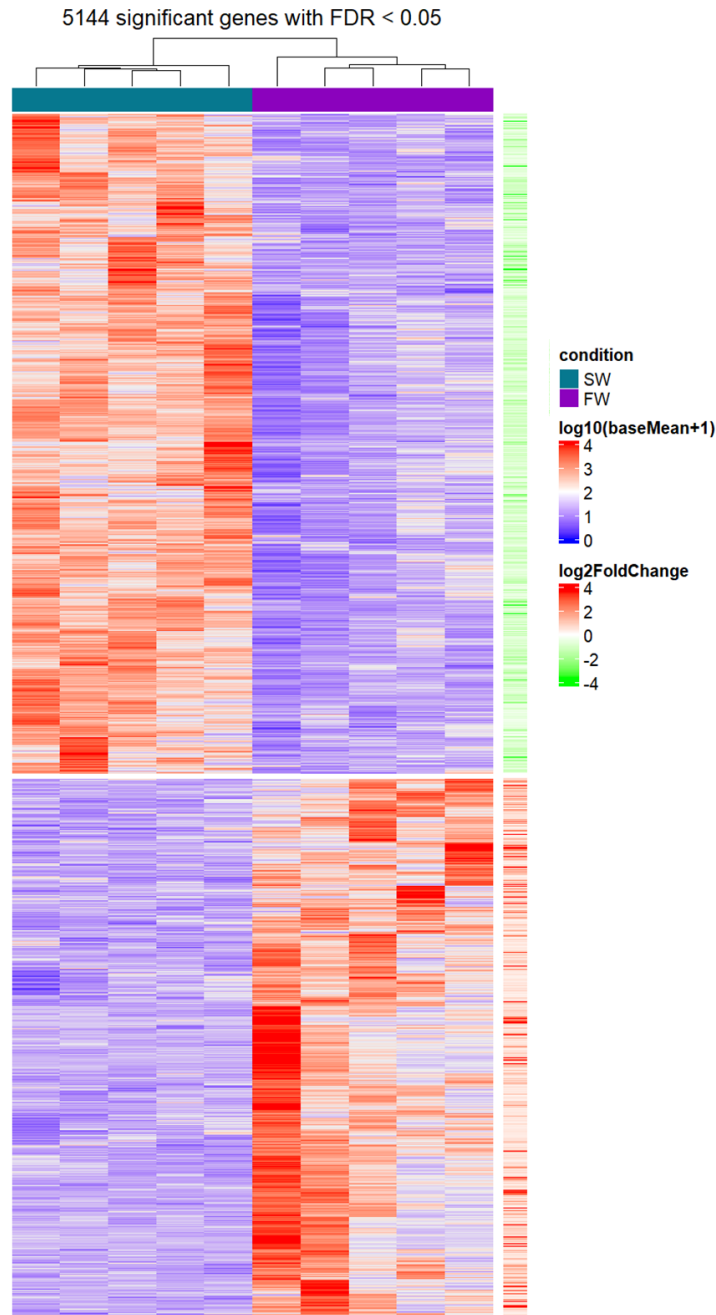| Gene ID	Gene symbol	Gene expression (pval)	Methylation pattern (pval)	Position	Functions
DLAgn_00025050	*dnmt3a*	repressed (2.26E-3)	hypo (7.49E-3)	GB	DNA methylation
DLAgn_00069860	*dnmt3a*	repressed (2.87E-2)	hyper (8.39E-3)	GB	DNA methylation
DLAgn_00083540	*prlr*	induced (2.70E-2)	hypo (7.70E-5)	PR/GB	hormone receptor
DLAgn_00045010	*nr3c1 (gr)*	repressed (6.73E-4)	hypo (2.14E-4)	GB	hormone receptor
DLAgn_00137410	*atp1a1 (nka α1a)*	induced (1.92E-3)	hypo (5.93E-3)	PR/GB	ion transporter
DLAgn_00058440	*atp1a3b (nka α3)*	induced (3.35E-3)	hypo (3.94E-2)	PR	ion transporter
DLAgn_00174550	*atp2a2 (serca)*	repressed (3.84E-2)	hyper (2.30E-3)	PR/GB	ion transporter
DLAgn_00018050	*atp6v1b2 (vha)*	induced (2.93E-2)	hypo (3.54E-3)	GB	ion transporter
DLAgn_00070690	*atp6v1d (vha)*	induced (3.35E-4)	hypo (4.02E-2)	PR	ion transporter
DLAgn_00165220	*atp6v1e1b (vha)*	repressed (6.39E-3)	hypo (6.52E-3)	GB	ion transporter
DLAgn_00172160	*cftr*	repressed (3.52E-2)	hyper (1.58E-2)	GB	ion transporter
DLAgn_00005770	*clcn2*	repressed (7.48E-5)	hypo (4.39E-2)	GB	ion transporter
DLAgn_00028970	*clcn2*	induced (4.32E-10)	hyper (7.39E-3)	GB	ion transporter
DLAgn_00144740	*clcn3*	induced (6.18E-3)	hypo (2.12E-2)	GB	ion transporter
DLAgn_00027240	*kcnh5*	induced (1.38E-2)	hypo (1.33E-2)	GB	ion transporter
DLAgn_00019070	*kcnk5*	induced (1.44E-4)	hypo (1.45E-2)	PR	ion transporter
DLAgn_00102830	*kcnma1*	repressed (1.06E-3)	hypo (2.08E-2)	GB	ion transporter
DLAgn_00082860	*kcnt1*	induced (3.40E-2)	hypo (4.76E-2)	GB	ion transporter
DLAgn_00157760	*rhcg*	repressed (1.77E-2)	hypo (3.64E-2)	PR/GB	ion transporter
DLAgn_00214830	*scn4a (na+ channel)*	repressed (1.56E-3)	hypo (3.53E-3)	GB	ion transporter
DLAgn_00080120	*slc12a2 (nkcc1)*	repressed (5.46E-13)	hyper (9.42E-4)	GB	ion transporter
DLAgn_00082210	*slc4a4a (nbc)*	repressed (5.29E-14)	hypo (2.51E-3)	GB	ion transporter
DLAgn_00044830	*slc9a6 (nhe6)*	induced (4.71E-3)	hyper (3.84E-2)	GB	ion transporter
DLAgn_00076860	*slc9a9 (nhe9)*	induced (1.99E-2)	hyper (2.54E-2)	GB	ion transporter
DLAgn_00117370	*aqp3a*	induced (1.15E-3)	hypo (1.38E-2)	PR/GB	water channel
DLAgn_00063040	*ptk2*	repressed (3.95E-3)	hypo (2.54E-4)	GB	transcription factor
DLAgn_00202150	*ptk2*	repressed (1.59E-19)	hyper (3.87E-2)	GB	transcription factor
DLAgn_00119940	*ostf1*	induced (7.75E-6)	hypo (4.48E-3)	GB	transcription factor
DLAgn_00088630	*cldn19*	repressed (5.41E-7)	hyper (2.94E-2)	GB	tight junction
DLAgn_00036050	*cldn4*	induced (5.73E-3)	hypo (1.63E-3)	PR	tight junction
DLAgn_00036080	*cldn4*	induced (3.82E-7)	hypo (9.15E-3)	PR	tight junction
DLAgn_00040400	*cldn7b*	induced (2.66E-3)	hypo (7.26E-4)	PR/GB	tight junction
DLAgn_00035230	*cldn8*	induced (3.99E-7)	hypo (1.57E-2)	PR/GB	tight junction
DLAgn_00035240	*cldn8*	induced (3.27E-2)	hypo (1.57E-2)	PR	tight junction
DLAgn_00036040	*cldna*	induced (6.95E-7)	hypo (1.63E-3)	PR/GB	tight junction
DLAgn_00044120	*cldnb*	induced (9.16E-5)	hypo (8.77E-3)	PR	tight junction
DLAgn_00143220	*cldnd*	induced (2.15E-5)	hypo (7.34E-3)	GB	tight junction
DLAgn_00087290	*tjp2*	induced (1.57E-2)	hypo (2.85E-3)	GB	tight junction
DLAgn_00124300	*tjp2*	repressed (4.59E-2)	hypo (4.74E-3)	GB	tight junction

Figure 1S. Heatmap of significant differentially expressed genes across all samples with adjusted p-value < 0.05. Each row represents a differentially expressed gene between salinities arranged by ascending order of fold change from top to bottom. The red and purple colors indicate the log10 (baseMean+1) values. On the right, the colors indicate log2FC for downregulated genes (upper part of the figure, in light green) or upregulated genes (bottom of the figure, in red). Samples were visually clustered using hierarchical clustering. Dark green (top of the figure) = 5 SW individuals and purple = 5 FW individuals.