Altered spawning seasons of Atlantic salmon broodstock genetically and epigenetically influence cell cycle and lipid-mediated regulations in their offspring

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Abstract

Manipulating spawning seasons of Atlantic salmon (*Salmo salar*) is a common practice to facilitate year-round harvesting in salmon aquaculture. This process involves adjusting water temperature and light regime to control female broodstock maturation. However, recent studies have indicated that altered spawning seasons can significantly affect the nutritional status and growth performance of the offspring. Therefore, gaining a deeper understanding of the biological regulations influenced by these alterations is crucial to enhance the growth performance of fish over multiple generations.

In this study, we investigated omics data from four different spawning seasons achieved through recirculating aquaculture systems (RAS) and sea-pen-based approaches. In addition to the normal spawning season in November (sea-pen), three altered seasons were designated: off-season (five-month advance, RAS), early season (two-month advance, sea-pen), and late season (two-month delay, sea-pen). We conducted comprehensive gene expression and DNA methylation analysis on liver samples collected from the start-feeding larvae of the next generation.

Our results revealed distinct gene expression and DNA methylation patterns associated with the altered spawning seasons. Specifically, offspring from RAS-based off-season exhibited altered lipid-mediated regulation, while those from sea-pen-based early and late seasons showed changes in cellular processes, particularly in cell cycle regulation when compared to the normal season. The consequences of our findings are significant for nutritional studies, potentially providing information for developing valuable tools for assessing growth potential and optimizing production strategies in aquaculture.

Keywords

Broodstodk, Spawning season, Intergenerational, Nutrition, Omics, DNA methylation, Cell cycle, Lipid metabolism

Introduction

The Atlantic salmon (*Salmo salar*) follows an anadromous life cycle, beginning in freshwater, migrating to seawater for most of its life, and returning to freshwater to spawn. In salmon aquaculture, the timing of spawning is adjusted to ensure year-round harvesting availability. This manipulation involves controlling abiotic factors, such as water temperature and photoperiod, to

influence the spawning timing (Pankhurst and King 2010; Taranger et al. 2010). Additionally, apart from the traditional open sea-based cages, a land-based approach utilising recirculating aquaculture systems (RAS) is employed to more accurately replicate the salmon's natural lifecycle, including both saltwater gonad maturation and final freshwater maturation periods (Good and Davidson 2016). Despite the ongoing and successful practices of spawning season alterations in salmon aquaculture, recent studies have reported that these practices can impact the nutritional status of broodstock, leading to negative effects on the growth performance in the next generation (Skjaerven et al. 2020; Skjærven et al. 2022).

While various nutrients influence the health condition and growth performance of Atlantic salmon (Council 2011; Hamre et al. 2016; Hemre et al. 2016), altered spawning seasons have appeared to affect the status of multiple metabolites and nutrients including micronutrients, amino acids, and lipid classes (Skjærven et al. 2022; Skjaerven et al. 2020). These nutrients are also closely associated with vital biological functions necessary for growth, such as one carbon (1C) metabolism, the citric acid cycle, and the Cahill cycle. Multiple key micronutrients, including vitamin B12, folate, vitamin B6, and methionine, are integrated with the 1C metabolism (Danchin, Sekowska, and You 2020; Lyon et al. 2020), which regulates cellular methylation potential through S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), critical for various cellular processes (Ducker and Rabinowitz 2017). The citric acid cycle enables the catabolism of carbohydrates, fats, and amino acids, releasing stored energy crucial for growth and development (Akram 2014), while the Cahill cycle facilitates the transport of amino groups and carbons between muscle and liver (Felig 1973), both playing essential roles in various developmental processes. Amino acids are essential for growth and tissue development, in addition to being the building blocks of proteins (Wu 2010), while lipids serve as major energy reserves and contribute to the formation of cell membranes, also supporting various developmental processes (Fahy et al. 2011).

Beyond the importance of nutrients, understanding genetic influences provides invaluable insights from a molecular biology perspective. Recent studies have revealed that alterations in micronutrient levels within the feed can have genetic effects on various biological pathways in salmon, including lipid metabolism, protein synthesis, and post-transcriptional regulation (Saito et al. 2021; Saito et al. 2023). Moreover, an additional layer of information comes from the analysis of DNA methylation, which supports to reveal important epigenetic regulations. DNA methylation, as an epigenetic regulatory process, influences various cellular mechanisms, including cell stability, differentiation, and development, potentially in response to environmental stimuli. (Moore, Le, and Fan 2013; Amenyah et al. 2020). Since DNA methylation is inherited during cell replication, it can result in long-term changes in epigenetic regulation (Goll and Bestor 2005; Moore, Le, and Fan 2013).

The present study investigates the genetic and epigenetic effects on offspring resulting from four different spawning seasons. These seasons include the natural sea-pen-based spawning in November, an accelerated RAS-based spawning occurring five months earlier, and two variations of sea-pen-based spawning: one with a two-month advance and another with a two-month delay relative to the natural spawning season. The main analysis of the study focuses on omics analysis of liver samples collected from start-feeding larvae at the end of the endogenous feeding phase. The liver serves as a key target organ for various biological reactions crucial to growth, and using start-feeding larvae helps eliminate the influences of exogenous feeding. To uncover both genetic and epigenetic impacts comprehensively, we employed RNA-sequencing (RNA-seq) for gene expression and reduced-representation bisulfite sequencing (RRBS) for DNA methylation analysis. Our findings offers potential usages of gene expression and DNA methylation data as powerful tools for assessing current and future growth performance, which can significantly benefit a wide range of nutritional programming studies.

Methods and Materials

Ethical considerations

The experiment adhered to the ARRIVE guidelines for design and reporting. Broodstock and offspring used in the study were obtained from AquaGen's commercial production facility of Atlantic salmon at their breeding station in Kyrksæterøra, Norway.

The conditions and protocols applied in this experiment were identical to those used in commercial production, including certain confidential information protected under commercial interests. Throughout the sampling procedures for broodstock and larvae, anesthetics were administered by the supplier's instructions and compliance with both Norwegian and European legislation on animal research.

Formal approval from the Norwegian Animal Research Authority (NARA) was not required for this experiment. This exemption is because the experimental conditions fall under practices recognized as commercial animal husbandry, thereby exempting them from the European Convention on the protection of animals used for scientific purposes (2010/63/EU), specifically under Article 5d. Additionally, the Norwegian ethics board also approved the experiment by the Norwegian regulation on animal experimentation, § 2, 5a, d, for activities classified as "non-experimental husbandry (agriculture or aquaculture)" and "procedures in normal/common breeding and husbandry".

Experimental design and sampling

As the details of experimental design and sampling methods have been described elsewhere (Skjærven et al. 2022; Skjaerven et al. 2020), we only present summarized descriptions here. The study utilized broodstock and offspring obtained from AquaGen's breeding station in Kyrksæterøra,

118 Norway. The female broodstock, sourced from the 15th generation, covered four spawning seasons:
119 off-season, early, normal, and late seasons.

120 The off-season broodstock were reared in a land-based recirculating aquaculture system
121 (RAS) in brackish (12‰ salinity), while the broodstock from the early, normal, and late seasons
122 were raised in three open sea-based net pens with ambient photoperiod and temperatures. All
123 broodstock were fed to satiation with broodstock feed (EWOS Breed 3500) until transferred to
124 freshwater. In freshwater, the normal season broodstock matured naturally under ambient
125 photoperiod and temperatures, whereas artificial controls were applied to regulate maturation for
126 the other spawning seasons. Specifically, while the normal season broodstock were maintained at
127 6 °C with an 8-hour daylight cycle upon transfer to freshwater tanks in August, the early season
128 broodstock were maintained by following the protocol outlined by Naeve and colleagues (Naeve et
129 al. 2018). Details about the temperature and photoperiod used to postpone maturation in the off-
130 season and late season groups are proprietary information owned by AquaGen AS. The off-season
131 and early season broodstock were subjected to five- and two-month advance maturation,
132 respectively, while the late season broodstock were subjected to a two-month delay. The fish
133 received no further feeding after the transfer to freshwater. The maturation process in freshwater
134 lasted for 109, 163, 120, and 166 days for off-season, early, normal, and late season broodstock,
135 respectively, before fertilization to obtain the next generation (Supplementary Table S1).

136 From each spawning group, five females from separate tanks were sacrificed using Benzoak
137 (200 mg/L, ACD Pharmaceuticals AS). After stripping, each female's growth measures, including
138 body weights (n=5 per spawning season, randomly selected), were recorded. Liver samples were
139 dissected and flash frozen in liquid N2 for nutrient analysis. All oocytes were fertilised with
140 cryopreserved sperm from two pooled males. Newly fertilized eggs (2 d°) were flash-frozen in
141 liquid N2 for nutrient analysis (Supplementary Table S1).

142 At the end of the endogenous feeding phase (start-feeding larvae 979–994 d°;
143 Supplementary Table S1), 36 larvae from each broodstock were randomly selected, euthanised with
144 an overdose of buffered tricane methanesulfonate (MS222, Pharmaq, Norway), and body weights
145 (n=180 per spawning season) were recorded. Additionally, liver samples were dissected from larvae
146 and flash frozen in liquid N2 for RNA and DNA analysis.

147 Growth measure and nutrient analysis

148 We collected the data on body weights for broodstock and larvae from previous studies (Skjærven
149 et al. 2022; Skjaerven et al. 2020). In addition, we obtained the data on selected nutrients and
150 metabolites from the same studies, specifically vitamin B6 & B12, folate, S-adenosylmethionine
151 (SAM), S-adenosylhomocysteine (SAH), cholesterol, the sum of six different lipids, glutamate, L-
152 serine, L-lysine, L-alanine, L-glutamine, urea, and B-alanine (Skjærven et al. 2022; Skjaerven et al.
153 2020). To analyse the data from all four spawning seasons, we performed ANOVA followed by
154 Tukey's HSD test.

155 DNA and RNA extraction

156 RNA extraction was carried out using the BioRobot EZ1 and EZ1 RNA Universal Tissue kit
157 (Qiagen), followed by DNase treatment using the Ambion DNA-free DNA removal kit (Invitrogen,
158 USA) according to their respective protocols. The RNA quantity was assessed using the NanoDrop
159 ND-1000 Spectrophotometer (Nanodrop Technologies) and the Agilent 2100 Bioanalyzer with the
160 RNA 6000 Nano LabChip kit (Agilent Technologies). For DNA isolation, the DNeasy Blood &
161 Tissue Kit (Qiagen, Cat. No. #69506) was used as per the manufacturer's protocol. Liver samples
162 underwent RNase A treatment (provided by the Qiagen kit, 50ng/µL, 10 min at room temperature)
163 followed by proteinase K treatment (New England Biolabs, #8102S 20µg/µL, 1.5 h at 55°C). DNA
164 was eluted in Milli Q water. DNA quantification was performed using the Qubit High Sensitivity

165 Assay (Life Technologies #Q32854). Detailed methods for both DNA and RNA extraction were
166 described elsewhere (Saito et al. 2021).

167 Library preparation of high throughput sequencing

168 Liver samples were sequenced at two different sequencing facilities for specific analyses. For RNA-
169 seq, the liver samples were processed at the DeepSeq sequencing facility at Nord University, Bodø,
170 Norway, and the library preparation was conducted using the NEBNext Ultra II Directional RNA
171 Library Prep Kit for Illumina (New England Biolabs). Sequencing was performed on the
172 NextSeq500 machine (Illumina). For RRBS, the liver samples were processed at the CeMM
173 Biomedical Sequencing Facility, Vienna, Austria. The genomic DNA was extracted, digested by
174 MspI, and bisulfite-converted before library preparation. The RRBS libraries were sequenced on
175 Illumina HiSeq 3000/4000 instruments. Detailed methods of the library preparation for RNA-seq
176 and RRBS were described elsewhere (Saito et al. 2021).

177 Atlantic salmon genome and genomic annotation

178 The reference genome (ICSASG version 2) and RefSeq data (version 100) for gene annotation were
179 downloaded from the NCBI web site (https://www.ncbi.nlm.nih.gov/assembly/GCF_000233375.1).
180 In cases where gene symbols were either outdated or unavailable from RefSeq data (version 100),
181 gene symbols from a newer version of RefSeq data (version 102) and UniProt (Bateman et al. 2022)
182 were used. The genome regions were annotated into intron, exon, three promoter regions, and
183 flanking regions. Promoter regions were divided into three categories based on their distance from
184 transcription start sites: P250 (1-250 bp), P1K (251-1000 bp), and P5K (1001-5000 bp). Flanking
185 regions were defined as 5000 upstream from P5K and 10000 downstream from the 5' end of the
186 gene.

Pre-processing of high throughput sequencing

For both RNA-seq and RRBS data, the same pre-processing procedures were followed as described in a previous study (Saito et al. 2021). This involved trimming the reads using Cutadapt (Martin 2011) and Trim Galore! (Barbraham Institute), aligning the trimmed reads to the reference genome using STAR (Dobin et al. 2013) for RNA-seq and Bismark/Bowtie 1 (Krueger and Andrews 2011; Langmead et al. 2009) for RRBS with their default parameters. The mapped RNA-seq reads were quantified using featureCounts (Liao, Smyth, and Shi 2014), while the mapped RRBS reads were processed by Bismark (Krueger and Andrews 2011) for methylation calling and CpG site extraction. The samples were divided into four spawning season groups for further analysis: off-season, early, normal, and late seasons, and clustering analysis was performed using the factoextra package (https://CRAN.R-project.org/package=factoextra). RNA-seq counts were subjected to a variance stabilizing transformation (VST) using DESeq2 (Love, Huber, and Anders 2014) before principal component analysis (PCA).

Raw reading data were uploaded to the repository of the Sequence Read Archive (SRA) on the NCBI web site and available under the accession numbers PRJNA680425 and PRJNA642998 for RNA-seq and RRBS, respectively.

Differential gene expression analysis

Differentially expressed genes (DEGs) were identified using DESeq2 (Love, Huber, and Anders 2014) with an adjusted p-value cut-off of less than 0.05. P-values were adjusted by the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995). From the candidate DEGs, those with absolute log fold changes (LFCs) greater than or equal to 1.2 were selected as the final set of DEGs by using the lfcThreshold argument of the results function of DESeq2 (Love, Huber, and Anders 2014). Shrunken LFCs values, calculated by the normal shrinkage method provided by DESeq2 (Love, Huber, and Anders 2014), were used for both heatmaps showing functional annotation

211 results and scatter plots showing merged results between DNA methylation and gene expression
212 differences.

213 Functional annotation with KEGG

214 Over-representation analysis (ORA) was performed on the Kyoto Encyclopedia of Genes and
215 Genomes (KEGG) database (Kanehisa and Goto 2000a) using clusterProfiler (Yu et al. 2012). Gene
216 lists of DEGs identified from the three pair-wise comparisons against the normal season were used
217 as input, and enriched pathways were determined based on adjusted p-values less than 0.05. P-
218 values were adjusted by the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995).

219 Methylation rate analysis

220 Mapped RRBS reads were filtered by methylKit (Akalin et al. 2012), discarding reads with
221 coverage less than or equal to 10 and above the 99.9th percentile. Additional RRBS data from two
222 other studies were obtained for post-smolt and harvesting stages (Saito et al. 2021; Saito et al. 2023)
223 and underwent the same pre-processing procedures to compare the distributions of methylation
224 rates. Raw data of these RRBS sequencing data can be found in SRA on the NCBI site under the
225 accession numbers of PRJNA680423 and PRJNA628740 for post-smolt and harvesting stages,
226 respectively.

227 Differential methylation analysis

228 Differentially methylated CpG sites (DMCs) were identified by methylKit (Akalin et al. 2012), and
229 Q-values were calculated using the sliding linear model (SLIM) method (Wang, Tuominen, and Tsai
230 2011). CpGs were identified as DMCs when Q-values were less than 0.01 and methylation
231 differences were greater than or equal to 15%.

232 To filter out DMCs that were strongly affected by altered spawning seasons, we used the
233 counts of DMCs per gene in two promoter regions: P250 and P1K. Genes were sorted by the count

234 of DMCs in a descendant order, separately for P250 and P1K, and genes that contained at least three
235 DMCs were selected for further analysis.

236 **Bioinformatics analysis**

237 In-house R and Python scripts with Snakemake (Koster and Rahmann 2012) were utilized for high-
238 throughput sequence analysis, basic statistical analysis, and figure generation.
239

240 # Results

241 **Altered spawning seasons impacted the weight and nutritional status of**
242 **offspring**

243 The present study examines the effects on gene expression and DNA methylation patterns in the
244 liver of offspring hatched from four different spawning seasons: an off-season based on a
245 recirculating aquaculture system (RAS) and three sea-pen based seasons (early, normal, and late)
246 (Fig. 1a). Off-season spawning occurred in June, early season in September, and late season in
247 January, relative to the normal spawning months of November. (see detailed dates and degree days
248 in Supplementary Table S1). The growth performance and nutritional status of the broodstock and
249 offspring have already been assessed and discussed elsewhere (Skjærven et al. 2022; Skjaerven et
250 al. 2020). This section provides a summary of previous research, emphasizing the weight and the
251 status of selected nutrients, even though the primary focus of the present study is on omics analysis.

252 Broodstock weights showed no significant differences at spawning (upper part of Fig. 1b),
253 but offspring weights at the larvae stage (979 and 994 degree days) showed noticeable variation
254 (lower part of Fig. 1b). Specifically, off-season and early season larvae had lower weights compared
255 to normal and late seasons (Supplementary Table S2). This trend was consistent with the

observations in egg sizes, where eggs from off-season and early-season exhibited smaller sizes compared to the other two seasons (see the number of eggs per litter in Supplementary Table S1).

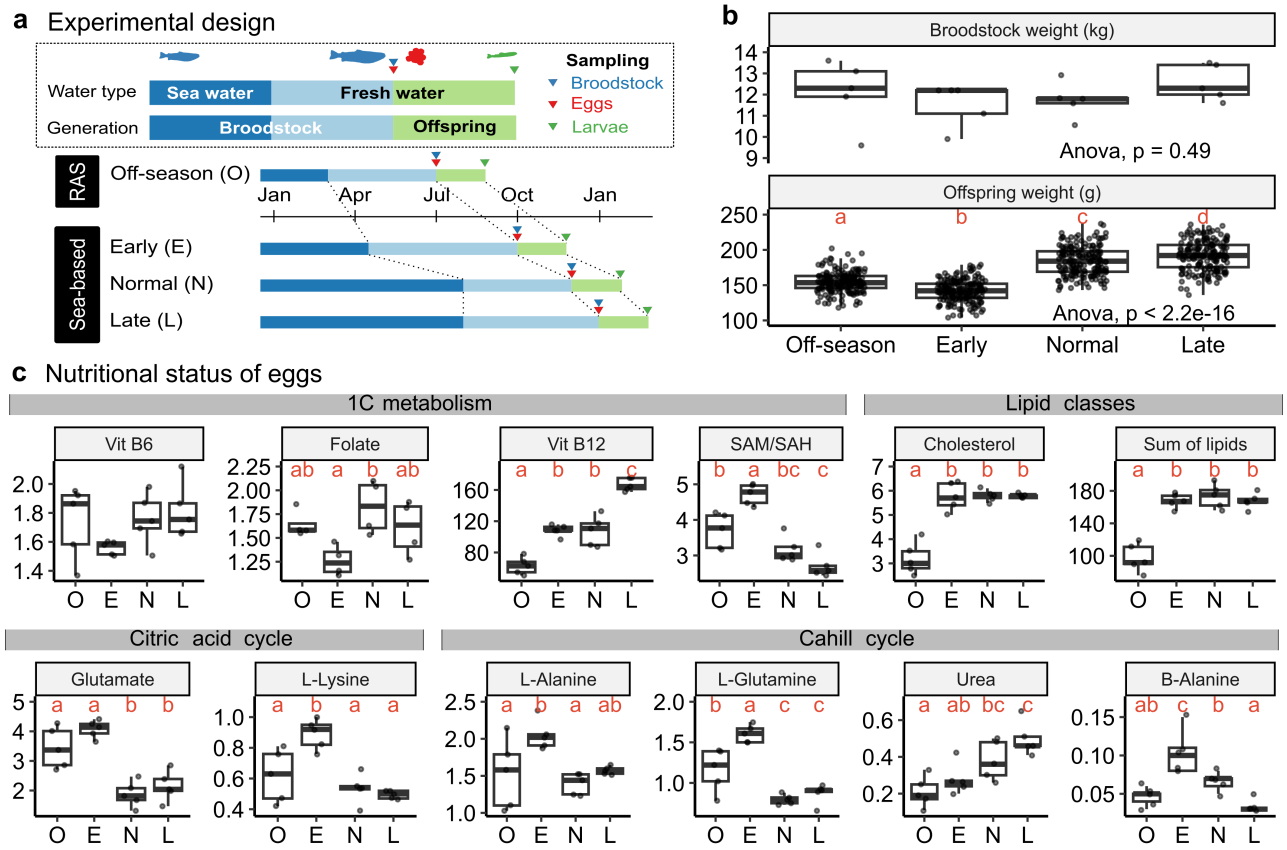


Fig. 1. Experimental design, body weights, and nutritional status across spawning seasons.

a) Schematic diagram illustrating four distinct spawning seasons: off-season (O), early (E), normal (N), and late (L). The off-season was conducted in RAS, while others were in a sea-based environment. Broodstock were transferred from seawater (blue bars) to freshwater (light blue bars) before spawning. Offspring were raised in freshwater until sampling (green bars). Triangles indicate sampling points: broodstock (blue), eggs (red), and larvae (green). **b)** Boxplots showing body weights of broodstock (n=5) and larvae (n=180). Red letters represent Tukey's HSD compact letter display after ANOVA tests ($p<0.05$). **c)** Boxplots of 12 nutrients and metabolites in egg samples. Nutrients and metabolites are categorised into four groups: 1C metabolism, lipid classes, citric acid cycle, and Cahill cycle. Units: 1C metabolism group (Vitamin B6: mg/kg ww, Folate: mg/kg ww, Vitamin B12: µg/kg ww); remaining groups: lipid classes (mg/g ww), citric acid cycle (µmol/g ww), Cahill cycle (µmol/g ww). Red letters represent Tukey's HSD compact letter display after ANOVA tests ($p<0.05$).

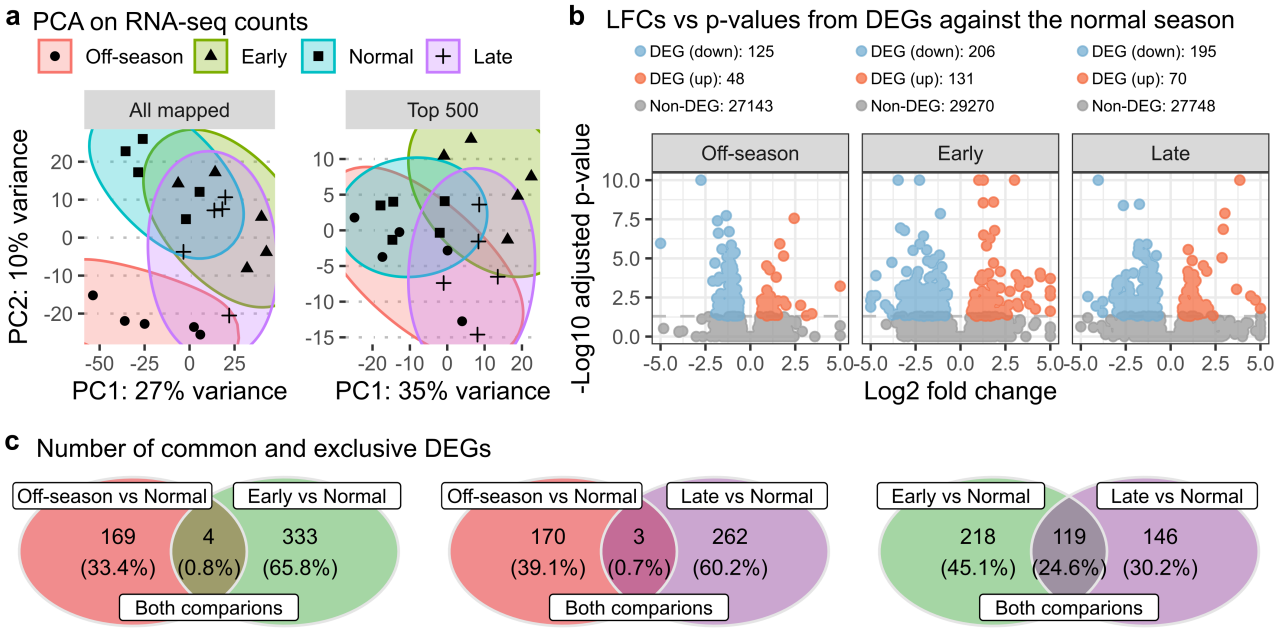
Altered spawning seasons widely impacted the nutritional status of offspring eggs, particularly regarding 1C metabolism, lipid classes, citric acid cycle, and Cahill cycle (Skjærven et al. 2022; Skjaerven et al. 2020), but with inconsistent patterns across nutrients and metabolites (summarised in Fig. 1c and Supplementary Tables S3-S6). Previous studies also reported that

altered spawning seasons affected the nutritional status of the broodstock in addition to their offpsring (Skjærven et al. 2022; Skjaerven et al. 2020). Thus, altered spawning seasons significantly influenced the nutritional status of broodstock without impacting their weights, subsequently affecting offspring nutritional status and growth potential of their offspring.

Early and late spawning seasons exhibited similar gene expression patterns when compared to the normal season

We performed gene expression analysis on 20 liver samples divided into four groups (n=5) to investigate the impact of different spawning seasons to the offspring when compared to the normal season (detailed statistics for each sample are provided in Supplementary Table S7).

Principal component analysis (PCA) differentiated the off-season from the early and normal seasons when considering all mapped genes (left part of Fig. 2a). However, when focusing on the top 500 genes with high variances, the off-season moderately deviated from the early season but not notably from the normal season (right part of Fig. 2a). In both cases, the late season overlapped with all other seasons.



292 **Fig. 2.** Gene expression analysis comparing altered spawning seasons to the normal season.

293 **a)** PCA plots displaying clustering patterns of four spawning seasons - off-season (red), normal (green), early (blue),
294 and late (purple) - using 20 RNA-seq samples. "All mapped" plot includes 45,153 genes, while "Top 500" includes top
295 500 genes with high variance. **b)** Volcano plots showing differential expression analysis results for three pairwise
296 comparisons: off-season vs. normal (Off-season), early vs. normal (Early), and late vs. normal (Late). Dots represent
297 down-regulated DEGs (blue), up-regulated DEGs (red), and non-DEGs (grey). The counts of DEG and non-DEGs were
298 provided in the legend. **c)** Venn diagrams illustrating overlaps of DEGs identified between two comparisons, indicating
299 common DEGs as well as those exclusive to a single comparison.
300

301 In three pairwise comparisons for differential expression analysis against the normal season
302 (off-season vs. normal, early vs. normal, and late vs. normal), we identified 173, 337, and 265
303 differentially expressed genes (DEGs), respectively (Fig. 2b). All comparisons revealed a higher
304 number of down-regulated genes than up-regulated genes. Additionally, Venn diagrams indicated
305 that the DEGs from the off-season vs. normal comparison had very few overlapping genes (<1%)
306 with those from the other two comparisons (depicted in the first and second diagrams in Fig. 2c).
307 On the other hand, early vs. normal and late vs. normal comparisons shared approximately 25% of
308 the DEGs (depicted in the third diagram in Fig. 2c). These results suggest that the expression
309 pattern of the off-season differed from both the early and late seasons when compared to the normal
310 season.

311 Altered spawning seasons affected various biological pathways related to
312 metabolism, cellular processes, and organismal systems

313 To explore the impact of altered spawning seasons on biological pathways, we conducted functional
314 analysis on DEGs using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Over-
315 representation analysis (ORA) identified a total of 11 enriched KEGG pathways, which were
316 classified into three functional categories: metabolism, cellular processes, and organismal systems,
317 when comparing the three altered spawning seasons against the normal season (Fig. 3).
318

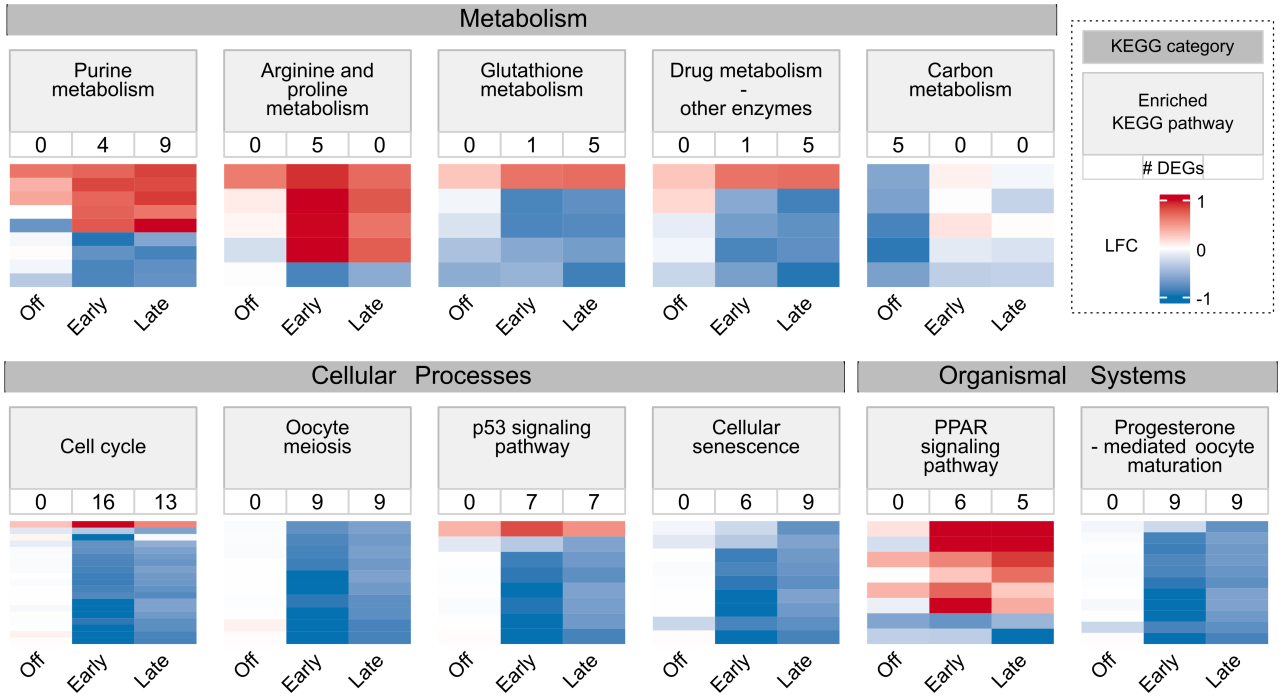


Fig. 3. Enriched KEGG pathways for DEGs identified in three pair-wise comparisons.

Heatmaps showing log fold changes (LFCs) of genes associated with 11 KEGG pathways enriched through over-representation analysis. LFCs underwent normal shrinkage transformation. Genes were included if identified as DEGs in at least one of the three comparisons: off-season vs normal (Off), early vs normal (Early), and late vs normal (Late). The number below each pathway represents DEG count. KEGG pathways are categorized into metabolism, cellular processes, and organismal systems groups.

The off-season vs. normal comparison identified only one enriched pathway, carbon metabolism (Supplementary Table S8). All five DEGs associated with carbon metabolism showed down-regulation (Fig. 3).

The early vs. normal comparison revealed six enriched pathways: one in metabolism, three in cellular processes, and two in organismal systems (Supplementary Table S9). Metabolism-related pathways exhibited both up- and down-regulation, while most genes in cellular processes showed significant down-regulation. In organismal systems, PPAR signalling showed up-regulation, while progesterone-mediated oocyte maturation showed down-regulation (Fig. 3).

The late vs. normal comparison identified nine enriched pathways: three in metabolism, four in cellular processes, and two in organismal systems (Supplementary Table S10). Remarkably, this

337 comparison shared five enriched pathways with the early vs. normal comparison, indicating similar
338 impacts on biological pathways between them. Moreover, these pathways displayed consistent
339 expression patterns in terms of up- and down-regulation between early and late seasons. Notably,
340 genes associated with cellular processes exhibited strong down-regulation for both seasons (Fig. 3).

341 In summary, the off-season appeared to down-regulate carbon metabolism, while the early
342 and late seasons primarily had strong suppressive effects on pathways associated with cellular
343 processes.

344 Altered spawning seasons had an impact on the expression of genes in a similar 345 manner

346 To further investigate the genes commonly affected by multiple spawning seasons, we used
347 overlapping DEGs (represented as intersections of Venn diagrams in Fig. 2c) by applying two
348 filtering steps: selecting DEGs found in at least two comparisons and choosing the top five DEGs
349 from each comparison based on adjusted p-values (Supplementary Table S11). This filtering
350 approach revealed a total of 13 genes (Table 1). Among them, two genes (gene IDs: 106612264 and
351 106601246) lacked corresponding gene names and symbols. All genes, except *pc* and *pitpnm2*,
352 consistently displayed up- or down-regulation across all spawning seasons, suggesting that all
353 altered spawning seasons had a similar effect on this specific set of genes when compared against
354 the normal season (Table 1).
355
356 **Table 1**. List of top five DEGs identified in at least two pairwise comparisons.
357

Pattern[1]	Gene ID	Symbol	LFC[2]			Gene name[3]	Function
			Off-season	Early	Late		
All Down	106563634	*pcyt1b*	-1.9 *	-1.1	-1.6 *	choline-phosphate cytidylyltransferase B	Metabolism
	106612672	*bmp5*	-1.2 *	-1.2 *	-0.7	bone morphogenetic protein 5-like	Signalling
	106585803	*rhobtb4*	-0.3	-1.1 *	-0.9 *	Rho related BTB domain containing 4	Ubiquitin mediated proteolysis

	106608456	*prox3*	-0.2	-2.3 *	-1.7 *	prospero homeobox 3	Lymphangiogenesis
	106584736	*tcf12*	-0.7	-3.3 *	-2.3 *	transcription factor 12	Myogenic regulatory
Up & Down	106592454	*pc*	-1.7 *	1.5 *	0.9	pyruvate carboxylase, mitochondrial-like	Pyruvate metabolism, TCA cycle, redox
	106568312	*pitpnm2*	0.6	-2.1 *	-2.6 *	membrane-associated phosphatidylinositol transfer protein 2-like	Cell signalling
All Up	106612264	-	5.2 *	4.1	6.1 *	Uncharacterized	-
	106601246	-	1.8 *	2.2 *	1.5	Uncharacterized	-
	106613828	*crim1*	0.3	3.0 *	3.0 *	cysteine-rich motor neuron 1 protein-like	CNS development, Bone morphogenic and growth factor
	106566973	*krt8*	0.1	1.3 *	1.0 *	keratin, type II cytoskeletal 8	Liver development
	106583637	*klf15*	2.3	4.5 *	3.8 *	Krueppel-like factor 15	Metabolism
	106569008	*cyp7a1*	1.4	3.1 *	2.9 *	cytochrome P450 7A1	Metabolism

[1]All Down: All three comparisons showed down-regulation, Down+Up: Comparisons showed both down- and up-regulation. All Up: All three comparisons showed up-regulation. [2]Log fold changes of pairwise comparison against the normal season. *: significant LFC (adjusted p-value<0.05). [3]When gene symbols were unavailable, we utilised the gene symbols from either the latest NCBI version or UniProt as substitutes.

These genes can be classified into three categories by relevant biological functions: metabolism (*pcyt1b*, *pc*, *klf15*, and *cyp7a1*) (Kanehisa and Goto 2000b; Cappel et al. 2019; Takashima et al. 2010), tissue development (*prox3*, *crim1*, and *krt8*) (Del Giacco, Pistocchi, and Ghilardi 2010; Scott et al. 2007; Kolle et al. 2000; Ku et al. 2007), and information processing, which can be further classified into three sub categories as signalling (*bmp5* and *pitpnm2*) (Kanehisa and Goto 2000b; Garner et al. 2012), transcription (*tcf12*) (Hu, Olson, and Kingston 1992), and post-translation (*rhobtb4*) (Kanehisa and Goto 2000b). As these categories are typically associated with growth, all three altered spawning seasons affected a certain set of genes associated with growth regulation in a similar manner, despite PCA plots (Fig. 2a) and Venn diagrams (Fig. 2c) showing a distinct gene expression pattern of the off-season.

Overall methylation rates increased until the harvesting stage within and around gene bodies, except near transcription start sites

We conducted DNA methylation analysis on 20 liver samples from larvae across four different spawning seasons (n=5; detailed statistics in Supplementary Table S12) to investigate the epigenetic effects of altered spawning seasons on DNA methylation.

Before analysing the effects of altered spawning seasons, we used RRBS data from two independent studies on liver samples to compare two developmental stages: post-smolt (approximately 31 weeks) and harvest (around 45 weeks) (Saito et al. 2021; Saito et al. 2023), against our RRBS data at the larvae stage. The post-smolt samples were divided into three groups with different 1C metabolism nutrient levels (Ctrl, 1C+, and 1C++) according to their experimental diets, while the harvest samples were divided into three groups with varying micronutrient levels (L1, L2, and L3) based on their experimental diets. We compared these six groups from two developmental stages with our larvae stage samples, which were collected across four spawning seasons (Fig. 4a).

Notably, the average methylation rates exhibited a consistent increase from larvae to post-smolt to harvest stages: 83.7%, 83.9%, and 84.9% for larvae, post-smolt, and harvest stages, respectively (Kruskal-Wallis, p-value < 0.001, black horizontal lines in Fig. 4a). Within the larvae group, the average rate of the normal group was higher than that of the off-season group, but the difference was not significant. Within the post-smolt group, the Ctrl group showed a significantly lower methylation rate than the other two groups (pair-wise Wilcoxon test with the Holm correction, p-values < 0.05; Supplementary Table S13). The Ctrl group was given much lower methionine along with B-vitamins as the 1C metabolism nutrients compared to other groups (1C+ and 1C++) (Saito et al. 2023).

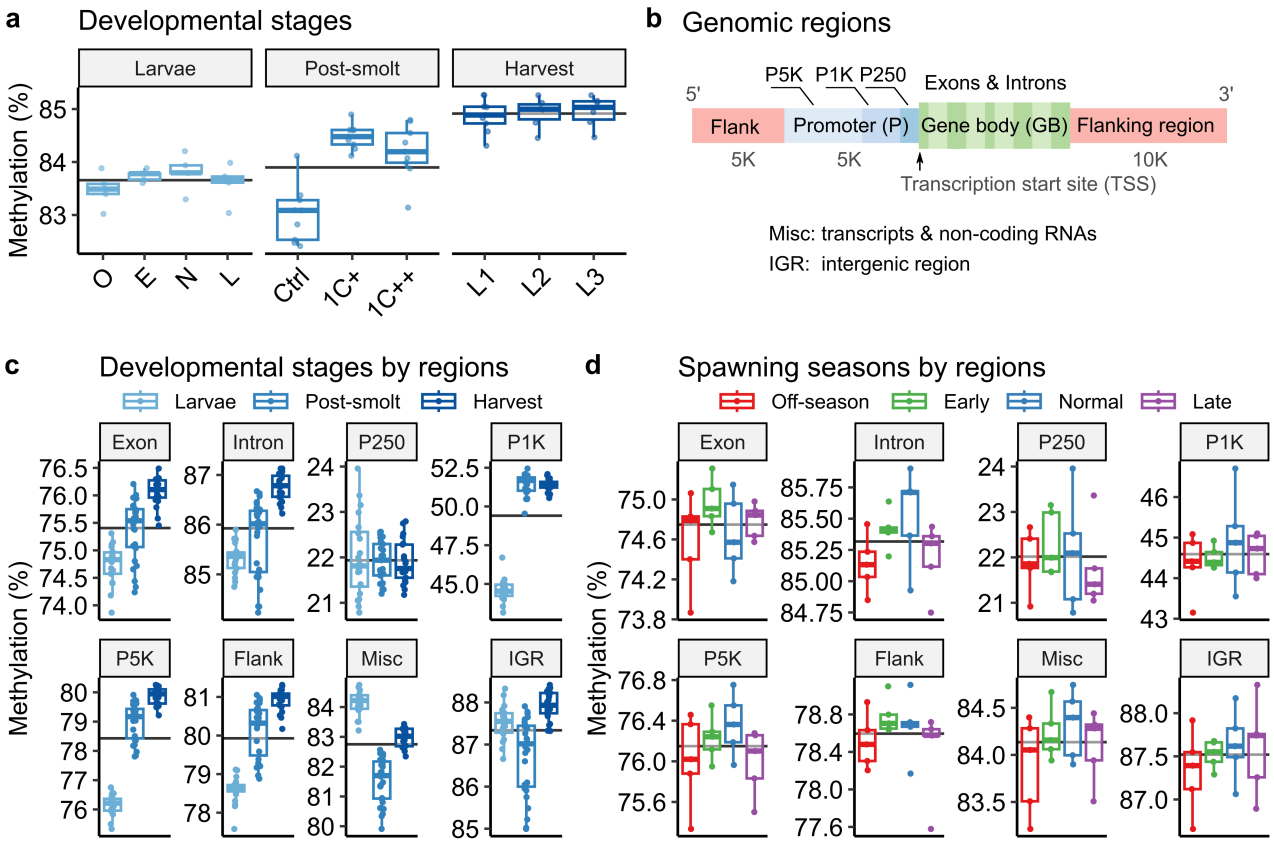


Fig. 4. Distribution of methylated CpG sites across genomic regions.

Box plots showing methylation data from the present study (larvae, light blue) and two other studies: post-smolt (blue) and harvest (dark blue). In the larvae stage, O, E, N, L indicate off-season, early, normal, and late seasons, respectively. While the post-smolt stage contains three groups with different 1C-metabolism nutrient levels (Ctrl, 1C+, and 1C++), the harvest stage contains three groups with different micronutrient levels (L1, L2, and L3) in their diets. Horizontal lines indicate the average rates for each group. **a)** Box plots showing average methylation rates of the samples from three developmental stages. **b)** Genomic region diagram illustrating three regions: flanking regions (Flank, red), promoters (P, blue), and gene bodies (GB, green). The promoter region is divided into three subregions: P250, P1K, and P5K. The gene body includes exons and introns. **c)** Box plots showing average methylation rates of the samples from three developmental stages across eight genomic regions. **d)** Box plots showing average methylation rates of the samples from four spawning seasons across eight genomic regions.

To examine regional DNA methylation patterns, CpG sites were categorised into three genomic regions based on their positions relative to nearby genes: gene body (GB; including exons and introns), promoter (P; including P250, P1K, and P5K), flanking regions, miscellaneous regions (Mics, including various transcripts and non-coding RNA), and intergenic regions (IGR) (Fig. 4b; see Methods and Materials for definitions).

The trend of the rate increment became more noticeable when the rates were examined separately by different genomic regions (Fig. 4c). In most regions, including gene bodies, promoter regions except for P250, and flanking regions, the samples from the harvest stage exhibited significantly higher average rates, followed by the post-smolt samples, and then the larvae samples (pair-wise Wilcoxon test with the Holm correction, p-values < 0.05; Supplementary Table S14). Specifically, within the two promoter regions (P1K and P5K), both the post-smolt and harvest stages showed a significant increase in methylation rates compared to the larvae stage. Moreover, methylation rates were generally lower in P250 (~22%) and P1K (~49%) compared to other regions (75% ~ 88%).

This comparative investigation suggests a potential increase in methylation rates as salmon progress in growth, at least until the harvesting stage, while also noting that methylation rates near transcription start sites (TSSs) tend to remain lower and more stable than in other genomic regions.

The off-season showed non-significant but consistently lower methylation rates compared to the normal and early seasons

To investigate the regional shift of the methylation rates among four spawning seasons, we applied the same methods used for the comparison analysis between developmental stages. Unlike the result of the developmental stages, spawning seasons exhibited no significant differences in methylation rates when separated by genomic regions (Fig. 4d; pair-wise Wilcoxon test with the Holm correction in Supplementary Table S15). Despite the non-significant differences, the average rates of the off-season samples were lower than those of the normal and early seasons in all regions (Supplementary Table S16). Nonetheless, the average rates of the off-season were higher than those of the late season in the two promoter regions (P250 and P5K) and flanking regions.

Differential methylation patterns indicated higher dynamic regulation in promoter regions compared to other regions

We conducted six pairwise comparisons to identify CpG sites with differential methylation (Fig. 5a). Unlike the analysis of the RNA-seq data, we considered all six possible pair-wise comparisons to capture a comprehensive pattern of methylation differences with our RRBS data. Remarkably, all comparisons consistently demonstrated similar characteristics, for both the number of differentially methylated CpGs (DMCs) and the balanced ratio of hypo- and hyper-methylation (Fig. 5a).

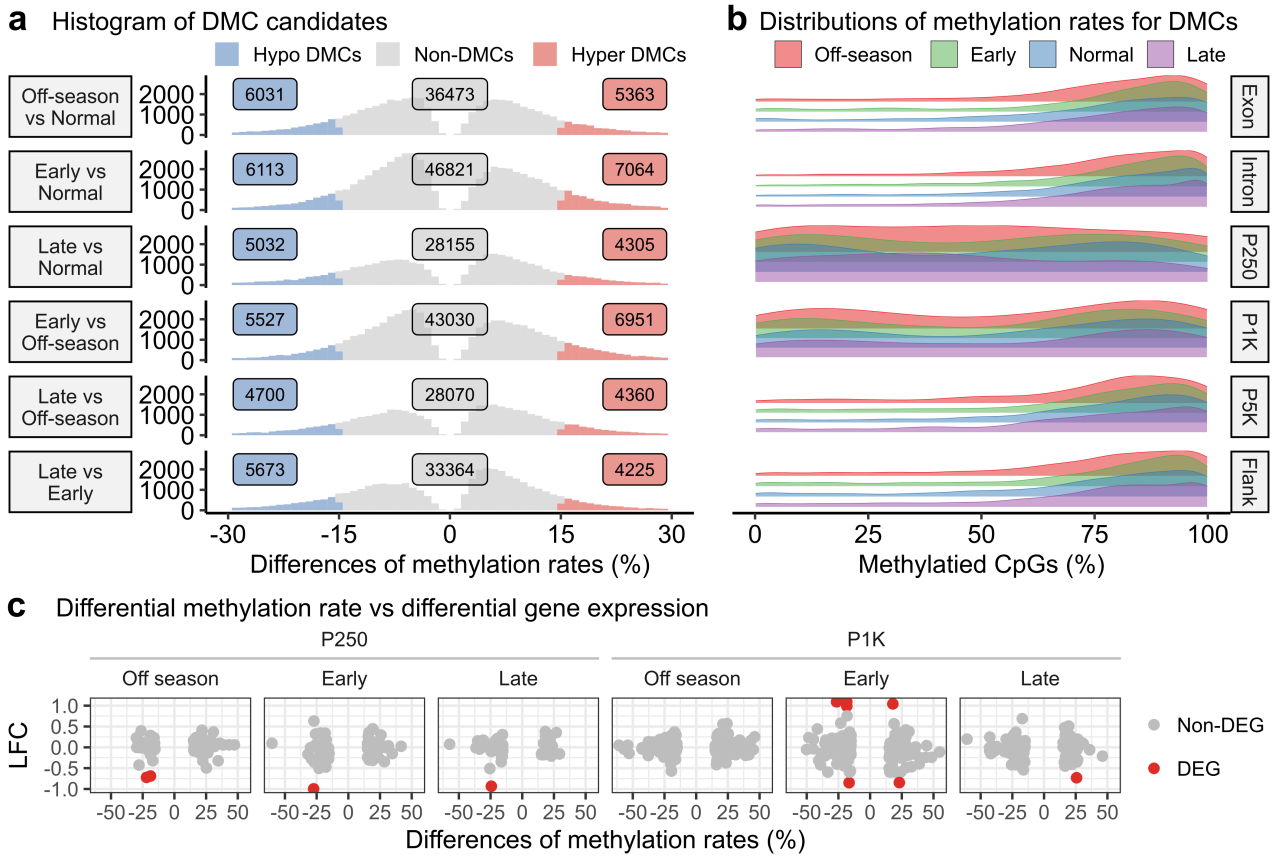


Fig. 5. DNA methylation differences and correlations with gene expression.

a) Histograms displaying distributions of methylation rate differences in six pairwise comparisons. Hypo-methylated DMCs in blue, hyper-methylated DMCs in red, non-DMCs in grey. All CpG sites in plots showed significant methylation differences. **b)** Ridge density plots illustrating distribution patterns of methylated CpG sites across four spawning seasons: off-season (red), early (green), normal (blue), and late (purple). CpG sites were selected if at least one site identified as DMC in any of six comparisons. **c)** Scatter plots showing the relationship between methylation rate differences and log fold changes (LFCs) of gene expression. DMCs were selected from three comparisons: off-season vs. normal (Off-season), early vs. normal (Early), and late vs. normal (Late), and also selected based on two regions, P250 and P1K. Corresponding genes were linked to these DMCs chosen, and their LFCs were transformed by

normal shrinkage. Data points are color-coded to indicate DEGs in red and non-DEGs in grey.

Further investigation focused on a set of 10,308 common DMCs, identified in at least one comparison. Distributions of methylation rates for these common DMCs exhibited a distinct left-skewed pattern across the exon, intron, P5K, and flanking regions (Fig. 5b). This observation strongly suggested that methylation differences resulting from altered spawning seasons primarily occurred within regions that were already highly methylated. In contrast, the P250 and P1K regions displayed nearly uniform methylation rates across a range of 0-100%, indicating significant methylation differences occurred irrespective of the underlying methylation rates. These findings imply a potentially greater level of dynamic regulation of DNA methylation in P250 and P1K compared to the other regions.

Filtering multiple DMCs identified key genes affected by spawning season alterations

We examined genes with multiple DMCs in three pairwise comparisons against the normal season. Since most genes had only a single DMC, especially in promoters (~75% in P250 and ~70% in P1K; Supplementary Figure S1), we avoided identifying differentially methylated regions (DMRs) using a sliding window approach. Instead, we filtered out genes strongly supported by multiple DMCs based on specific criteria (see Methods and Materials), resulting in a total of 11 genes with multiple DMCs on their promoters (Table 2; see the details in Supplementary Table S17).

Table 2. List of genes with multiple DMCs in their promoter region.

Pattern[1]	Gene ID	Symbol	DMC[2]			Gene name	Function
			Off-season	Early	Late		
All hypo	106563027	*slc31a1*	Hypo (3) *	-	Hypo (3) *	solute carrier family 31 member 1	Transporter
Hyper & Hypo	106567512	*stk32a*	-	Hypo (2)	Hypo (2), * Hyper (1)	serine/threonine-protein kinase	Signalling

						32A	
	106607889	*inf2*	Hypo (1)	Hyper (3) *	-	inverted formin-2-like	Actin filament polymerization
	106584059	*scp-2*	Hyper (4) *	Hypo (1)	Hyper (1)	sterol carrier protein 2-like	Lipid transfer, transporter
	106579168	*klhl11*	Hypo (1), Hyper (2) *	-	-	kelch-like protein 11	Ubiquitination
All hyper	100196480	*ctl2b*	Hyper (3)	Hyper (7) *	Hyper (2)	CTLA-2-beta	Unknown
	106573279	-	-	Hyper (1)	Hyper (3) *	Uncharacterized	-
	106583651	*helz2*	Hyper (4) *	-	-	helicase with zinc finger domain 2-like	Transcriptional coactivator
	106581656	*lrrfip2*	Hyper (3) *	-	-	leucine-rich repeat flightless-interacting protein 2-like	Signalling
	106609256	*prrx2*	Hyper (3) *	-	-	paired mesoderm homeobox protein 2-like	Transcription
	106576160	*ttll12*	-	Hyper (3) *	-	tubulin tyrosine ligase-like family, member 12	Post-translational modification

[1]All hypo: All three comparisons showed hypo-methylated DMCs, Hyper & Hypo: Comparisons showed both hypo- and hyper-methylated DMCs. All hyper: All three comparisons showed hyper-methylated DMCs. [2]Either hyper or hypo-methylated DMC against the normal season. The number in brackets represents the number of DMCs. *: genes selected by filtering of multiple DMCs.

All 11 genes were identified by a single comparison except for *slc31a1*, which was identified in two comparisons (off-season vs. normal and late vs. normal). Most genes (9 out of 11) exhibited either hypo-methylated or hyper-methylated DMCs, but not both, within a single comparison (Table 2). Furthermore, approximately 60% of the genes (7 out of 11) consistently displayed either hypo-methylation or hyper-methylation even across multiple comparisons ('All hypo' and 'All hyper' in the first column named ´Pattern´ of Table 2, respectively).

Literature analysis associated these genes with two biological functions: development (*inf2*) and information processing, which can be further classified into four sub categories as transporter (*slc31a1* and *scp-2*) (Zhou and Gitschier 1997; Stolowich et al. 2002), signalling (*stk32a* and *lrrfip2*) (Alliance of Genome Resources 2022; Bateman et al. 2022), post-translation (*klhl11* and

495 *ttll12*) (Bateman et al. 2022; Brants et al. 2012), and transcription (*helz2* and *prrx2*) (Tomaru et al.
496 2006; Norris et al. 2000). However, *ctl2b* and an uncharacterised gene (Gene ID: 106573279) had
497 no clear associations with specific biological functions. Although the DMCs on these genes were
498 potentially linked to the underlying gene expression, none of the 11 genes were identified as DEGs
499 in our differential gene expression analysis.

500 Altered spawning seasons influenced gene expression and DNA methylation of 501 cell cycle-related genes

502 To investigate potential associations between DNA methylation and gene expression, we combined
503 DEGs and DMCs from three pairwise comparisons against the normal season (off-season vs.
504 normal, early vs. normal, and late vs. normal) in P250 and P1K. This resulted in a total of seven
505 DEGs that contain a total of 11 DMCs in their promoters (Fig. 5c, Table 3, Supplementary Table
506 S18).

507 In addition to the significant results, we expanded the list of seven DEGs with additional
508 information from all pair-wise comparisons (additional entries were marked with parentheses in
509 Table 3). Among the identified genes, three (*caprin-1*, *kifc1*, and *aurkb*) exhibited consistent
510 regulations of DMCs and DEGs compared to the normal season. Specifically, *caprin-1* and *kifc1*
511 had hypo-methylated CpG sites, while *aurkb* had hyper-methylated CpG sites; all three genes
512 showed down-regulated gene expression (Table 3). Notably, these three genes (*caprin-1*, *kifc1*, and
513 *aurkb*) were associated with cell cycle regulation (Grill et al. 2004; Walczak, Verma, and Mitchison
514 1997; Wheatley et al. 2001), while the remaining genes were linked to metabolism (*cyp8b1*,
515 *adrenodoxin*, and *lpin1*) (Kanehisa and Goto 2000a; Hansson and Wikvall 1982; Grinberg et al.
516 2000) and transport (*slc43a1a*) (Babu et al. 2003). These findings indicate that altered spawning
517 seasons potentially had genetic and epigenetic effects on several genes associated with cell cycle

518 regulation.

519

520 **Table 3**. List of DEGs that contain DMCs in their promoter region.

521

Gene ID	Symbol	DMC[1]			DEG[2]			Gene name	Function
		Off-season	Early	Late	Off-season	Early	Late		
106562317	*caprin-1*	Hypo	(Hypo)	(Hypo)	Down	(Down)	(Down)	caprin-1	Cell cycle
106599887	*cyp8b1*	Hypo	-	-	Down	-	-	5-beta-cholestane-3-alpha,7-alpha-diol 12-alpha-hydroxylase	Metabolism, cytochrome P450
106588407	*kifc1*	-	Hypo	Hypo	-	Down	Down	carboxy-terminal kinesin 2	Cell cycle, Meiosis
106582038	*adrenodoxin*	-	Hypo		-	Down	-	adrenodoxin	Metabolism, cytochrome P450
106604665	*slc43a1a*	-	Hypo	-	-	Up	(Up)	solute carrier family 43 member 1a	Transporter
106561604	*aurkb*	(Hyper)	Hyper	Hyper	-	Down	Down	aurora kinase B	Cell cycle
106570052	*lpin1*	-	Hyper	-	-	Up	-	phosphatidate phosphatase LPIN1	Metabolism

522

523 [1]Either hypo- or hyper-methylated DMC against the normal season. Parentheses indicate that the site was identified as
524 DMC, but the corresponding genes were not identified as stringent DEG.[2]Either up- or down-regulation against the
525 normal season. Parentheses indicate that down- or up-regulation was determined by non-stringent DEGs.

526

527 # Discussion

528 In the present study, we investigated the influence of altered spawning seasons on gene expression
529 and DNA methylation in offspring. Previous studies emphasized the significant impacts of these
530 changes on the nutritional status of both broodstock and their offspring, with no observed effects on
531 the body weights of broodstock (Skjærven et al. 2022; Skjaerven et al. 2020). Our primary aim was
532 to reveal the genetic and epigenetic effects resulting from observed variations by utilizing liver
533 samples from larvae at the first feeding stage.

534 **What insights do our omics analyses provide?**

535 Gene expression analysis revealed distinct patterns associated with altered spawning seasons.
536 Specifically, early and late seasons exhibited similar gene expression patterns when compared to the
537 normal season. The differential expression of genes associated with metabolism, cellular processes,
538 and organismal systems indicates that spawning seasons can have wide-ranging effects on
539 biological pathways related to growth and development. Furthermore, the analysis with overlapped
540 DEGs revealed that all three altered spawning seasons similarly affected genes associated with
541 metabolism, tissue development, and information processing. Hence, while early and late seasons
542 influenced a wide range of biological pathways in a similar manner, all three seasons, including the
543 off-season, also similarly affected a certain set of genes, especially those strongly differentiated
544 from the normal season.

545 DNA methylation analysis provided valuable insights into the epigenetic effects of different
546 spawning seasons. Altered spawning seasons led to increased methylation rates for the early and
547 normal seasons. Notably, these shifts primarily occurred in regions that were already highly
548 methylated, but the rates were altered independently of the underlying methylation rates in promoter
549 regions around transcription start sites. In addition, our findings with different developmental stages
550 imply that methylation rates could serve as a measure of growth levels. However, more research is
551 needed to understand the mechanism of methylation rate shifting and potential applications in
552 aquaculture.

553 Several genes with multiple DMCs exhibited consistent hypo- or hyper-methylation patterns
554 across multiple spawning seasons in promoter regions, suggesting a common epigenetic response to
555 altered spawning seasons. These genes were associated with key biological functions, such as
556 development and information processing, potentially indicating their role in growth performance.
557 Although both gene expression and DNA methylation analyses resulted in similar biological

558 functions impacted by altered spawning seasons, they showed little overlap between DEGs and
559 genes with DMCs. This could be attributed to regulatory differences between gene expression and
560 DNA methylation within the same biological pathway, or it might be a limitation caused by the
561 restriction enzyme used in RRBS.

562 The observed correlations between DNA methylation and gene expression were weaker than
563 anticipated in our liver samples. Notably, our findings revealed inconsistent correspondence
564 between hypo-methylation and active gene expression and between hyper-methylation and gene
565 suppression. These results suggest that the regulation of DNA methylation is more intricate than
566 commonly assumed as it is known that high promoter CpG methylation reduces gene expression,
567 while low promoter CpG methylation allows for active gene expression (Lanata, Chung, and
568 Criswell 2018). Within promoter regions, not only DNA methylation but several other factors,
569 including underlying gene expression levels, transcription factor binding sites, and chromatin
570 remodelling, likely play a role in regulating actual gene expression.

571 What are the significant biological differences observed in offspring?

572 **Off-season vs. normal:** Off-season spawning in June, facilitated through a RAS, resulted in
573 Atlantic salmon offspring with lower larval weights compared to the normal and late seasons
574 (Skjærven et al. 2022; Skjaerven et al. 2020). Offspring eggs also exhibited reduced levels of
575 vitamin B12 and lipid classes relative to normal and late seasons (Skjærven et al. 2022; Skjaerven et
576 al. 2020). Gene expression analysis indicated significant down-regulation of genes associated with
577 central carbon metabolism (CCM). CCM involves several biological pathways, including the citric
578 acid cycle, and acts as a major source of energy for growth and development. Moreover, lipids are
579 interconnected with CCM through the utilisation of acetyl-CoA as a central metabolite (Westfall and
580 Levin 2018). Among the six genes with multiple DMCs from the off-season vs. normal comparison,
581 sterol carrier protein2-like (*scp-2*) contained four hyper-methylated DMCs in its promoter. The *scp-*

582 *2* gene encodes a transfer protein that plays a key role in intracellular lipid transport (Stolowich et
583 al. 2002). Overall, RAS-based off-season spawning appeared to impact lipid-mediated regulations
584 both genetically and epigenetically. This negative impact on lipid-related mechanisms potentially
585 accounted for impaired growth performance observed in body weight as our previous research
586 indicated the genetic and epigenetic importance of lipid regulation with acetyl-CoA for growth
587 performance (Saito et al. 2021).

588 **Early season vs. normal season:** Offspring resulting from early season spawning in
589 September also exhibited lower weights at the larval stage compared to those from normal and late
590 seasons. However, the nutritional status of offspring eggs displayed higher levels of SAM/SAH,
591 lysine, glutamine, and alanine (Skjærven et al. 2022; Skjaerven et al. 2020). Gene expression
592 analysis revealed significant down-regulation of genes associated with cell cycle regulation, while
593 genes involved in metabolism showed both down- and up-regulation. Among the five genes that
594 exhibited differential expression and had at least one DMC in their promoters, two were associated
595 with cell-cycle regulation. Specifically, the carboxy-terminal kinesin 2 (*kifc1*) gene promotes
596 mitotic spindle assembly (Walczak, Verma, and Mitchison 1997), and the aurora kinase B (*aurkb*)
597 gene is involved in mitotic progression (Wheatley et al. 2001). The *kifc1* gene was hypomethylated,
598 while the *aurkb* gene was hypermethylated. Consequently, the early spawning season had an impact
599 on cell-cycle regulation both genetically and epigenetically. However, it is challenging to
600 hypothesise that this negative impact on cell-cycle regulation was linked to impaired growth
601 performance because similar effects were observed for the late season.

602 **Late season vs. normal season:** Late season spawning in January showed that offspring
603 weights were almost as close, but slightly heavier than those from the normal season. The
604 nutritional status of offspring eggs also exhibited similar levels of various nutrients and metabolites
605 to the normal season, except for higher vitamin B12 and lower B-alanine (Skjærven et al. 2022).
606 Gene expression patterns in the late season were comparable to those observed in the early season,

607 showing significant down-regulation of genes related to cellular processes, especially cell cycle
608 regulation. Two genes, *kifc1* and *aurkb*, showed differential expression and had at least one DMC in
609 their promoters, and both were associated with cell-cycle regulation. Hence, the late season showed
610 similar growth performance and nutritional status to the normal season, while the gene expression
611 pattern was similar to the early season.

612 **Three altered spawning seasons vs. normal season:** Although strong similarities were
613 observed in gene expression patterns between early and late spawning seasons, certain genes
614 exhibited common regulation in both gene expression and DNA methylation across all three altered
615 spawning seasons. Among the four DEGs identified in the off-season, choline-phosphate
616 cytidylyltransferase B (*pcyt1b*) and bone morphogenetic protein 5-like (*bmp5*) showed concordant
617 down-regulation across all three altered spawning seasons. The *pcyt1b* gene encodes an enzyme
618 involved in phosphatidylcholine biosynthesis, playing essential roles in multiple metabolic
619 pathways (Bateman et al. 2022), while the *bmp5* gene is involved in signalling (Kanehisa and Goto
620 2000a). Additionally, all DMCs found in the promoter of the cytotoxic T lymphocyte-associated
621 protein 2 beta (*ctl2b*) gene were hyper-methylated across all three altered spawning seasons. The
622 function of *ctl2b* remains unknown, but it shares similarities with genes encoding cysteine
623 proteinase, an enzyme involved in protein breakdown within cells (Denizot et al. 1989).
624 Furthermore, among genes that were both differentially expressed and had at least one DMC in their
625 promoters, the *caprin-1* gene exhibited consistent regulation, with all DMCs being hypo-methylated
626 and its expression being suppressed. The *caprin-1* gene is associated with cell cycle regulation,
627 playing a crucial role in cellular activation or proliferation (Grill et al. 2004). This indicates that the
628 off-season also influenced cell cycle regulation to a certain degree.

Why are our findings important in the context of aquaculture and nutritional studies?

Our study unveiled the significant effects of altered spawning season on offspring gene expression and DNA methylation patterns in Atlantic salmon. These findings provide valuable insights into assessing current and future growth potential, which can be challenging to determine using only nutritional status and growth performance measures. Understanding the genetic and epigenetic responses to altered spawning seasons will help develop strategies to optimize growth and production in aquaculture practices. Additionally, our study can be linked to the potential impact of increasing global ocean temperatures on the spawning of aquatic vertebrates, as temperature is a key abiotic factor influencing spawning seasons. Even though genetic and epigenetic profiles can be species- and even tissue-specific, the approaches we demonstrated here could be applied to other fish species whose spawning seasons are artificially altered in aquaculture practices. Nonetheless, further studies are needed to elucidate the fundamental effects caused by spawning season alterations across various species.

Conclusions

The present study emphasises the substantial impact of altering spawning seasons on gene expression and DNA methylation patterns in Atlantic salmon offspring. Our observations indicate that RAS-based off-season spawning exerts an influence on lipid-mediated regulations in offspring, while sea-pen based early and late seasons affect the regulation of the offspring's cellular processes, especially cell cycle regulation. These effects potentially play a crucial role in shaping both current and future growth performance.

These findings have important implications for aquaculture and nutritional studies, as they provide valuable tools for assessing growth potential and optimizing production strategies. Future research focusing on tissue and developmental stage-specific DNA methylation maps will enhance the accuracy of growth performance estimation and provide further insights into the genetic and epigenetic responses to altered spawning conditions.

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Author contributions

K.H.S conceived and designed the research. M.E. and M.M. conducted and designed the feeding trial. J.M.O.F. and C.B. prepared RNA-seq and RRBS data. T.S., M.E., and K.H.S. analysed and interpreted data. T.S. drafted the manuscript, and K.H.S revised it. The authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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