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Climate change-related stressors in aquaculture: modulation of gill microbiota and transcriptome in Atlantic salmon

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The aquaculture industry is not sufficiently prepared to deal with the impacts of climate change. Increasing temperatures, intensified marine heat waves, and more frequent jellyfish blooms threaten production. Gill disorders in farmed Atlantic salmon (*Salmo salar*) have become one of the most significant challenges for the industry. Few studies have explored the interactions between fish mucosal microbiomes and scyphozoans within a climate change context. This study explored how increased temperature and limited oxygen availability interact with the salmon gill microbiome and gill gene expression after jellyfish (*Aurelia aurita*) exposure. Gill microbiota changes were determined by ONT MinION sequencing of the V1-V3 hypervariable region of the 16S rRNA gene and gill transcriptomic responses were assessed by total RNA sequencing with Illumina. Alpha diversity Shannon index was significantly higher in fish exposed to increased temperature, regardless of exposure to jellyfish. In addition, LEfSe analysis identified a significant increase in the abundance of *Streptococcus* and *Staphylococcus*, which were identified as biomarkers in all groups exposed to increased temperatures. At the transcriptomic level, principal component analysis showed that group separation was mainly driven by the increased temperature variable, which was responsible for the largest number of differentially expressed transcripts. Pathway analysis revealed that the different experimental conditions significantly impacted the expression of transcripts related to glycosylation, immune response and tissue development and integrity. Although the jellyfish exposure did not significantly affect the gill transcriptomic response, the combination with the other environmental stressors induced further changes related to haemostasis, protein modification and cell migration. Overall, the results provide important evidence regarding how combined stressors may affect Atlantic salmon's gill mucosal health and highlight key genes and microbial taxa to be explored in more detail to understand the mechanisms behind climate-change effects on aquaculture.

KEYWORDS
16S rRNA sequencing, aquaculture, jellyfish, metataxonomics, RNA sequencing, Salmo salar

1 Introduction

Gill disorders constitute an important challenge in Atlantic salmon (*Salmo salar*) aquaculture, affecting fish welfare and the economics of production (Herrero et al., 2018). Many gill health issues in farmed salmon are caused by a mix of infectious and non-infectious agents, such as Complex Gill Disease (CGD) (Boerlage et al., 2020; Amlie et al., 2025; Vidal et al., 2025). This syndrome presents a wide range of clinical and histological gill disease manifestations with probable multifactorial aetiology. CGD limits gill function and, as a result, affects fish health and performance (Boerlage et al., 2020). Environmental factors can further exacerbate gill health problems, adding another layer of complexity to their management (Mougin and Joyce, 2023). Fish gills are essential for several physiological functions, such as gaseous exchange, osmoregulation, excretion of nitrogenous waste, pH regulation, immune function, and hormone production (Evans et al., 2005; Chen et al., 2023). They are in constant contact with the marine environment, making them susceptible to infection and damage from external agents such as parasites, viruses, and bacteria (Boerlage et al., 2020; Clinton et al., 2024). This makes the gills at risk of stress and trauma and at the same time, it also makes them an excellent indicator organ for measuring the impact of external stress on fish health.

Over the last decade, it has been shown that gill microbiota might play a crucial role in maintaining the hosts' health. Microbiota acts as a primary defence barrier, influencing immune responses, and participating in essential physiological processes such as nitrogen metabolism and osmotic regulation (Gomez et al., 2013; Clinton et al., 2024). Gill microbiota is hypothesized to function as a barrier against pathogens by acting as a protective microbial community that competes with harmful microorganisms for space and resources (Elsheshtawy et al., 2021). The microbial diversity and composition of gill microbiota can therefore serve as a measure to indicate changes in the health status of fish with factors such as loss of diversity or increase in abundance of specific bacteria (e.g. *Candidatus Branchiomonas cysticola*, *Tenacibaculum maritimum* or *Tenacibaculum dicentrarchi*) reflecting health alterations (Boerlage et al., 2020; Clinton et al., 2024; Lagadec et al., 2026). Bacterial communities are influenced by both host-specific and environmental factors, which further render them as a good target for monitoring and improving fish health and water quality in aquaculture systems (Mougin and Joyce, 2023).

Over the past decades, salmon productivity has been affected by many climate change-related stressors, and despite the constant innovation in husbandry practices, production systems, and feeds, aquaculture development is not properly prepared for the impacts of climate change (Falconer et al., 2022). Extreme weather events, increasing air and sea temperatures, rising sea levels, and changes in salinity and dissolved oxygen availability are all becoming more frequent, thereby pushing the physiological limits of aquatic organisms (Ytteborg et al., 2023). In particular, temperature plays a key role in the physiology of Atlantic salmon, where optimal physiological function was observed at 12.7 °C (Korus et al., 2024). Moreover, temperatures above 17-20 °C could lead to a decline in feed intake and growth, increasing stress and mortality risk (Korus et al., 2024). Within this context, the proliferation of jellyfish has been linked to climate change since higher temperatures facilitate increased asexual reproduction, resulting in more frequent blooms (Wang et al., 2025; Pigeon et al., 2026). The toxic stinging nematocysts of Cnidarians have a direct effect on fish when blooms aggregate around aquaculture farms and pass through the mesh of cages whole or broken in parts. As a result, nematocytes can induce direct damage to the fish skin or gills and indirect damage through de-oxygenation of the surrounding water by the subsequent accumulation and decomposition of cnidarian biomass, exacerbating respiratory distress by consuming dissolved oxygen and thus, creating hypoxic conditions (Clinton et al., 2021). Particularly, *Aurelia aurita*, a jellyfish species described as one of the most cosmopolitan jellyfish in our oceans (including Norwegian waters), can lead to various forms of fish damage, such as gill and skin lesions, respiratory and osmoregulatory stress, as well as presenting the risk of secondary infections, which increase fish mortality rates during blooms (Baxter et al., 2011; Bosch-Belmar et al., 2020).

As climate change alters water temperatures, physiological stressors that influence gill microbial structure can be exacerbated (Pratte et al., 2018), potentially impairing gene expression (Kelly and Salinas, 2017; Jonsson, 2023). Thus, in this study we hypothesize that multiple climate change-related environmental stressors could distinctly alter the gill mucosal microbiota composition and gill gene expression, and that subsequent exposure to *A. aurita* could intensify these alterations and impact gill health.

2 Materials and methods

2.1 Ethics statement

All fish handling and manipulations in the study adhered to the guidelines and protocols of the European Union Directive 2010/63/EU, and the trial was approved by the Norwegian Food Safety Authority (FOTS ID 29728).

2.2 Experimental setup and sampling

The experiment was performed at the Fish Health Laboratory of the Aquaculture Research Station (Tromsø, Norway). Atlantic salmon (*Salmo salar*) smolts (~50 g) were purchased from a nearby smolt producer (Norway Royal Salmon, Dåfjord, Tromsø, Norway) and were acclimated for four weeks in 1800 L tanks in a flow-through system at 12-13 °C with continuous illumination (LD 24:00). During this period, fish were fed to apparent satiation with a commercial diet (Nutra Olympic, Skretting). Ten days prior to the trial, 600 fish were transferred to the experimental units and distributed into six 500-L tanks at a rate of 100 fish per tank. Each tank was then exposed to one of three environmental conditions for 26 days (Figure 1). In the first condition (Control "C" group), fish from two tanks were maintained at 12 °C throughout the trial. This is considered within the optimal growth range for smolts and thus served as the control group. The second group simulated a condition where fish were exposed to varied temperatures to simulate fluctuating temperatures on farms, including an

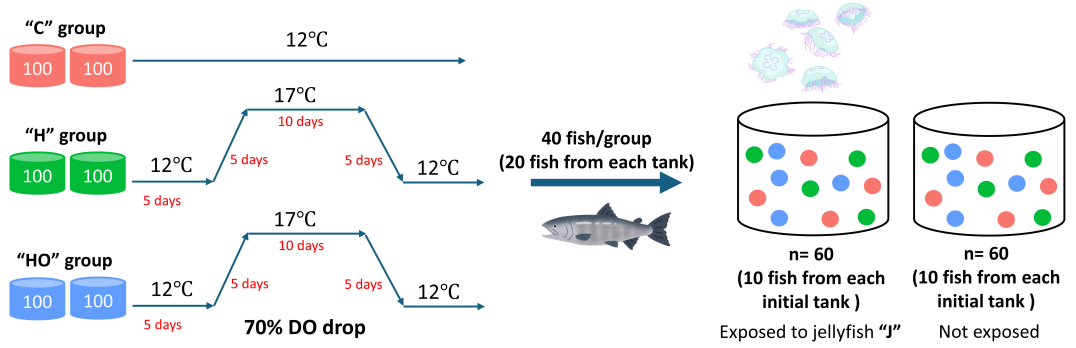

FIGURE 1
Experimental setup. Experimental conditions (treatments) where fish were kept and monitored for 26 days: Red tanks represent the control ("C") group maintained at 12 °C, green tanks represent the increased temperature (heat "H") group, which was maintained at 12 °C, gradually increasing up to 17 °C and then progressively lowered again to 12 °C, and blue tanks represent the increased temperature plus limited oxygen ("HO") group, whose temperature changes were identical to those in the "H" group, with the additional challenge of a decreased percentage of available oxygen at 17 °C. Numbers within the tanks indicate the number of fish. For the Jellyfish trial, 40 fish per experimental condition (20 fish per tank) were distributed into two tanks (each tank finally containing 10 fish from the initial 6 tanks: 20 fish per experimental condition). One tank (n=60) was exposed to macerated jellyfish (J) and the other served as a control group.

extended period of elevated temperature. For this second group, fish from two tanks were initially maintained for five days at 12 °C with a gradual increase of temperature during five days up to 17 °C. We used 17 °C as the peak, as this is in the upper range of temperatures when salmon growth starts to be affected (Jensen et al., 1989) and temperature scenarios partly reflect the observations documented in Norwegian salmon farms (Burke et al., 2020). This higher temperature was maintained for 10 days, followed by a progressive decline to 12 °C over 5 days. Fish exposed to this condition are referred to as the increased temperature (heat) "H" group. The rate of temperature increase, and decrease may not fully reflect natural environmental conditions but was selected as a practical and reproducible experimental model. This temperature regime has been previously applied in a climate change simulation of a marine heatwave that we conducted with Atlantic cod, a marine species also farmed in Norway (Cohen-Rengifo et al., 2025). In the third condition, temperature changes were identical to those experienced by the H group, with the additional challenge of a decreased percentage of available oxygen. Dissolved oxygen was reduced to 70% once the water temperature reached 17 °C and was maintained at this level throughout the exposure period, with oxygen levels continuously logged and monitored. Prior to the trial, a pilot study at the station was conducted to ensure that this level could be maintained throughout the experiment. This condition simulated a mild event of oxygen drop in the fish cage when an Atlantic salmon farm can only supplement a moderate amount of oxygen. Fish from the two tanks exposed to the combined conditions of increased temperature and limited oxygen were described as "HO" group. Beyond logistical constraints, we did not include a group exposed to low oxygen alone, as our aim was to investigate the effects of combined stressors under elevated temperature conditions. This scenario more closely reflects events that can occur in the field but are often not well documented in experimental studies.

After the 26 day period, when all groups reached 12 °C, a subset of 120 fish (40 fish per condition/20 fish per tank) was tagged using visible implant elastomers (Northwest Maritime Technology, WA, USA) to identify origin tanks and moved to the challenge unit where they were kept in two 500 L tanks for 4 days to acclimate prior to use in a common-garden exposure trial at 12 °C (n = 60 fish per tank; 20 fish from each condition; 10 fish per experimental tank). The average weight and length of the fish at this stage were 112.2 (± 21.4 SEM) grams and 21.7 (± 1.2 SEM) centimetres, respectively. Prior to the exposure trial, jellyfish (*Aurelia aurita*) were collected from the waters close to the city of Tromsø and were kept frozen at -40 °C until the exposure day. The jellyfish were macerated for around 1 min using a handheld mixer. Macerated jellyfish were used as the exposure model to ensure that fish were subjected to a consistent and controlled level of jellyfish-derived material across treatments. While exposure to live or intact jellyfish may better reflect natural ecological conditions, this approach introduces substantial variability in dose and contact. Our previous *in vitro* studies demonstrated that salmonid gill cells retain a clear biological response to macerated jellyfish, supporting its relevance as a standardised and biologically meaningful exposure model (Helland-Riise et al., 2025). The exposure protocol was as follows: In one tank (tank "J", containing groups C_J, H_J, HO_J), water flow to the tank was closed, macerated jellyfish were added (20 mL of macerated jellyfish/L of water), and the fish were exposed for three hours. During this period, oxygen was supplemented to maintain above 90% saturation. After the exposure period, the water was replaced 100% within 15 minutes. The exact same procedure was applied to a second control tank, except that there was no addition of the macerated jellyfish (groups C, H, HO). The fish were allowed to recover for 72 hours (Figure 1). After jellyfish exposure, 10 tagged fish per previous treatment and tank (C, H, HO and C_J, H_J, HO_J) were killed by an overdose of anaesthetic (Benzoak vet, 200mg/L, EuroPharma, Leknes, Norway). After euthanasia, fish were immediately inspected for gill condition and sampled as described below. Gill scoring was performed using a standard protocol in Atlantic salmon aquaculture (Taylor et al., 2009) consisting of a visual scoring system entailing a macroscopic examination of all gill arches for gill lesions. The severity of the lesions ranges from 1 (no lesion) to 5 (severe lesions). Gill mucus samples were collected by rolling a sterile cotton swab along the

right gill arch surface and were placed into 1.5 mL Eppendorf tubes and immediately stored at -80 °C until DNA extraction. A portion of the left gill filaments was collected and placed into 1 mL of RNAlater in 1.5 mL Eppendorf tubes and stored for 24 h at 20 °C before freezing at -20 °C.

2.3 DNA extraction

For microbiota samples (10 fish/treatment/tank: C, H, HO and C_J, H_J, HO_J), swabs were incubated with 150 µL of Tris-HCl 10 mM, pH 8 containing 10 µg/mL of lysozyme (Sigma, Darmstadt, Germany) for 15 min at 37 °C. Next, swabs were removed from tubes, and DNA extraction was performed using the High Pure PCR Template Preparation Kit (Roche) according to the manufacturer's instructions. DNA quantification was performed with PicoGreen™ (Life Technologies, Carlsbad, CA, USA), and extracted DNA was stored at -20 °C until sequencing, following the extraction kit's recommendations. Individual samples were used for analysis; no pooling was performed.

2.4 RNA extraction

Total RNA was isolated from the gills using the Agencourt RNAdvance™ Tissue Total RNA Purification Kit (Beckman Coulter Inc., CA, USA) and using the Biomek 4000 automated workbench station (Beckman Coulter, Inc., CA, USA). RNA concentration and purity were assessed using a NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). RNA quality was further evaluated using an Agilent 2100 Bioanalyzer (Agilent, California, USA). All samples were above 8.0 RIN (RNA Integrity Number), except four samples with RIN < 6 which were excluded.

2.5 16S rRNA PCR amplification and purification

Two consecutive 16S rRNA PCR reactions were performed in a reaction volume of 25 µL with 10 ng of input DNA targeting the V1-V3 hypervariable region of the 16S rRNA gene ('27F'AGAGTTTGATCMTGGCTCAG and '519R'GW ATTACCGCGGCKGCTG). While full-length 16S rRNA sequencing is considered the standard ONT approach, recent studies suggest that shorter long-read amplicons may provide important advantages depending on the biological system under study. In particular, it has been previously demonstrated that the V1–V3 region can constitute a reliable alternative to full-length V1–V9 sequencing when genus level community characterization is the primary objective (Domingo-Bretón et al., 2024). Although full-length 16S rRNA provided improved species-level assignments, the authors observed reduced primer hybridization efficiency and under-representation of specific taxa, whereas V1–V3 generated more consistent amplification across sample types (water and microbiomes) and yielded microbial community profiles comparable to those obtained with longer amplicons.

Amplification conditions were: 95 °C for 1 min, 30 cycles of 95 °C for 20 s, 56 °C for 30 s, and 65 °C for 2 min, and a final extension step of 65 °C for 5 min for the first PCR, and 95 °C for 1 min, 25 cycles of 95 °C for 20 s, 56 °C for 30 s, and 65 °C for 2 min, with a final extension step of 65 °C for 5 min for the second PCR reaction, which used 2.5 µL of the first PCR product as template. We have used this two-step amplification as a strategy to improve amplification success of low-biomass samples (Villette et al., 2021). Amplicons were purified using AMPure XP magnetic beads (Beckman Coulter) following the manufacturer's protocol to remove primers, primer dimers, and unincorporated nucleotides. Purified products were verified by 1% agarose gel and stored at −20 °C until used for sequencing.

2.6 ONT MinION sequencing and bioinformatics pipeline

The amplified V1-V3 16S rRNA gene variable regions were sequenced using the ONT MinION device and the native barcoding kit 96 (SQK-NBD112.96) version NBA_9137_v112_revH_01Dec2021, according to the manufacturer's instructions. After library preparation, the sample was quantified using PicoGreen™, and approximately 5 ng were loaded into the MinION device. Libraries were sequenced using an FLO-MIN106 flow cell and demultiplexed using the MinKNOW v23.07.15. The total run time was 18 hours for each library, and, when needed, flow cells were washed according to the Flow Cell Wash Kit (EXP-WSH004) instructions. The sequencing raw data is available in NCBI Sequence Read Archive, Bioproject PRJNA1221282, sample accession numbers SAMN46736498-SAMN46736557.

After sequencing, the basecalling of the samples was performed with Guppy v5.1.12, using the default parameters (FAST). The resulting FASTQ reads were pre-processed using Porechop v0.2.4 for removing sequencing adapters, NanoFilt v2.8.0 for filtering reads below 450 base pairs (bp) and above 600 bp, and yacrd v0.6.2 for chimera detection and removal. Sequences were assigned as distinct amplicon sequence variants (ASVs) and subsequently mapped for taxonomy assignment with Minimap2 v2.17-r941, using SILVA v138.1 as the reference database.

2.7 RNA sequencing and bioinformatics pipeline

RNA samples were sent to the Norwegian Sequencing Centre (OUS, Norway) for RNA sequencing. RNA-seq libraries were created using the strand-specific TruSeq RNA Library Prep Kit (Illumina, CA, USA) according to the manufacturer's instructions. Libraries were then combined and sequenced in half a lane of a NovaSeq S4 flowcell using 150 bp paired end reads. The sequencing raw data is available in NCBI Sequence Read Archive, Bioproject PRJNA1234164, sample accession numbers SAMN47291655-SAMN47291770. Four RNA samples from the HO_J group did not meet the quality requirements (RIN < 6) and were not sequenced.

Raw sequence data were pre-processed using BBDuk to remove/trim adaptor sequences, and low-quality reads. Next, cleaned data was aligned against the *Salmo salar* Ssal V3.1.110 genome (ENSEMBL) using HISAT2 v.2.2.1. Stringtie v.2.1.5 was used to estimate the number of reads (raw count data) aligned with the reference genes in the Ssal V3.1.110 GTF annotation. Raw count

data were initially explored and analysed using the *SARTools* (v1.7.4) R (v4.1.1) package.

2.8 Statistical analysis and visualization

For the 16S rRNA gene sequencing data, samples were analysed using the R package *phyloseq* v1.41.1 through which species richness estimates, alpha diversity indexes, phylum abundance bar charts, and NMDS plots were created. Differences in richness and diversity indexes (Shannon) and microbial abundance were determined by the Kruskal-Wallis' test, followed by Dunn's post-test, with a significance threshold of $p < 0.05$. Beta diversity across groups was tested with permutation multivariate analysis of variance (PERMANOVA) using the non-parametric method *adonis* (10, 000 random permutations) from the *Vegan* R package. Non-metric multidimensional scaling (NMDS) was used to visualize sample distribution. Concerning taxa abundance, differential heat trees were produced using the *Metacoder* R package. To test the significant differences in abundance between the same taxa in the different experimental conditions, a Wilcoxon rank-sum test followed by a Benjamini-Hochberg (FDR) correction was applied, and the p-value threshold was set to 0.05 ($p < 0.05$).

A linear discriminant analysis (LDA) effect size (LEfSe) at the genus level was used to identify significant differences among groups (described here as microbial biomarkers) using the Bioconductor R package *microbiomeMarker*. In this work, microbial biomarkers are defined as taxa that act as an indicator of change in the microbial community structure due to biotic or abiotic conditions. These biomarkers were identified through LEfSe, ensuring that they were not only present in significant amounts but also statistically significant in differentiating among groups. Briefly, LEfSe is an algorithm for high-dimensional biomarker discovery and explanation that identifies genomic features (taxa) characterizing the differences between two or more experimental conditions. To find differentially abundant features that are also consistent with biologically meaningful categories, it strongly emphasizes statistical significance, biological consistency, and effect relevance (Segata et al., 2011). Dysbiosis scores were calculated using the *dysbiosisR* R package which calculates the median variation for a sample compared to a reference sample set. The standard protocol defined by the package authors was followed (Lloyd-Price et al., 2019). Briefly, the reference sample set was the control group, not exposed to environmental stressors or jellyfish exposure. The distance matrix used was the Bray-Curtis dissimilarity matrix. To identify highly divergent samples (dysbiotic) the threshold was set at the 90^{th} percentile of the score in control samples. Thus, microbial configurations of dysbiotic samples will have less than 10% probability of occurring in control conditions.

For RNA-seq data analysis, differentially expressed transcripts (DET) from all group comparisons against the C group were retrieved using the *DESeq2* R package. Individual expression values of 686 DET (*padj* < 0.05, mean counts > 20, log2 fold change > 1) were used to construct a principal component analysis (PCA) using the plotPCA function of the *DESeq2* R package. Unique and shared DET were analysed using the R package *UpSetR*. DET for each comparison were analysed by Fisher test-based over-representation analyses for gene ontology-biological process (GO-BP) terms using ShinyGO v0.81. Statistical significance was accepted at FDR < 0.05. GO-BP dotplots and the transcript heatmap were plotted using the *ggplot2* R package. Correlation analysis was calculated by pairwise Spearman correlation coefficients. Correlations were performed between bacterial taxa (abundance > 1%) and selected DET related to mucus and glycosylation. Data were normalized using centred log-ratio (clr) transformation and the Spearman correlation coefficient (r), p values, and Benjamini–Hochberg adjusted p values (FDR) were calculated using the cor-test function of the *corrplot* R package. Significant gene-taxa correlations were considered at FDR < 0.001, r > |0.5|. Correlation networks were constructed using Cytoscape v3.8.

3 Results

3.1 Biometric data and gill scoring

At the end of the exposure trial experimental fish had a mean weight of 112.3 g (± 21.37 SD) and a mean length of 21.7 cm (± 1.14 SD). No mortality was observed during the trial. Length, weight and condition factor did not show great variation among groups, with the H and HO groups showing slightly lower weight and condition factor but not body length than the other groups (Table 1). Gill scoring revealed only a few gross lesions particularly in the C_J group. However, the gross appearance of the gills was generally good, with no significant differences among groups.

3.2 Sequencing results

The MinION sequencing and processing of 60 samples yielded 978, 441 taxonomically assigned reads at a mean of 16, 307 reads per sample (Supplementary File 1A). A total of 15, 091 ASV were retrieved, belonging to 987 genera.

RNA sequencing of 56 samples yielded approximately 3 billion raw reads (mean of 27 million reads/sample). After reads were quality filtered and mapped against the Atlantic salmon genome, the mean percentage of aligned reads was 50.4%. (Supplementary File 1B).

3.3 Gill mucosal microbial composition

Alpha diversity Shannon index significantly differed among groups, where lower diversity was detected in the C and C_J groups (Figure 2A). Regarding beta diversity, microbial community structure differed significantly between treatment groups (PERMANOVA $P = 0.001$, $F = 9.540$, $R2 = 0.488$), with the treatment explaining 48.8% of the variance in community composition. A non-metric multidimensional scaling (NMDS) model of the data was conducted to visualize dissimilarity between the samples. The NMDS plot showed a clear separation between the microbial structure of the experimental C and C_J groups from the other groups (Figure 2B).

TABLE 1 Fish biometric data and gill scores at the sampling time.

Group	C	C_J	H	H_J	HO	HO_J	P value
Length (cm)	22.5 ± 1.27	22.19 ± 1.07	21.56 ± 1.11	21.56 ± 0.82	21.06 ± 0.92	21.58 ± 1.37	0.0648
Weight (g)	127.7 ± 25.99^{a}	121.9 ± 19.25^{ab}	99.97 ± 16.62^{c}	107.1 ± 18.93^{bc}	103.2 ± 16.52^{c}	114.0 ± 17.94^{abc}	0.0165
CF	1.11 ± 0.08^{a}	1.11 ± 0.09^{a}	0.99 ± 0.12^{b}	1.06 ± 0.11^{ab}	1.09 ± 0.09^{a}	1.13 ± 0.05^{a}	0.0462
Gill score	1.1 ± 0.32	1.4 ± 0.69	1.2 ± 0.42	1.0 ± 0.00	1.0 ± 0.00	1.2 ± 0.41	0.194

Data represents the mean ± SD of n = 10 fish/group. Different letters indicate significant differences among groups. CF, condition factor = 100 x (weight/length^{3}). P values: ANOVA + Holm-Sidak test.

At an overall scale, gill microbiota composition and differences among experimental groups is shown in Figure 3 and Supplementary File 2. Three phyla, *Bacteroidota*, *Firmicutes*, and *Actinobacteriota* accounted for 27%, 29%, and 38%, respectively, of the total mean relative abundance in all samples, and showed significant changes among the experimental groups. At a closer look, *Actinobacteriota* was the most abundant phylum in the C and C_J groups, and its total relative abundance ranged between 65% in the C_J group and 19.6% in the HO_J group. *Firmicutes* was the most abundant phylum in the H, H_J, HO, and HO_J groups, ranging between 40.7% in the HO_J group and 11.4% in the C_J group. Similar to *Firmicutes*, *Bacteroidota*, was the most abundant phylum in the H, H_J, HO, and HO_J groups, ranging between 33.1% in the HO_J group and 17.2% in the C_J groups (Supplementary File 3A).

At the genus level, LEfSe analysis identified a significant increase in the abundance of *Streptococcus* and *Staphylococcus* in the H, H_J, HO, and HO_J groups (Figure 4), accounting for a mean total relative abundance of 17.7% and 14%, respectively. In contrast, for the C and C_J groups, the mean total relative abundance accounted for 4.5% and 3.8%, respectively. No specific biomarkers were found for the jellyfish-exposed groups. Information about individual and grouped abundance of taxa can be found in Supplementary File 3A. Despite the described differences, no significant dysbiosis was detected (Supplementary File 3B).

3.4 Gill RNA-seq analysis

The DESeq2-analysis revealed 686 differentially expressed transcripts (DET) with mean counts > 20 and log2 fold change > |1| between the C group and the C_J, H, H_J, HO, or HO_J groups (Supplementary File 4). Principal component analysis (PCA) showed a clear separation between the C and C_J groups, and the H, H_J, HO, and HO_J groups (Figure 5A). An UpSetR plot was used to visualize the overlap between DET across the experimental group's comparisons (C_J vs C, H vs C, H_J vs C, HO vs C, and HO_J vs C). Certain transcripts were unique to individual experimental groups, while others were shared (Figure 5B). Jellyfish exposure did not cause a significant change in transcript expression when temperature was maintained constant, with only 7 downregulated transcripts detected when compared to the control group. Groups exposed to increased temperatures showed between 341 to 420 upregulated transcripts with 255 transcripts shared in all H groups. When the oxygen variable is added (HO group), 52 new transcripts were upregulated, and oxygen plus jellyfish (HO_J) induced the upregulation of 59 new transcripts (Figure 5B).

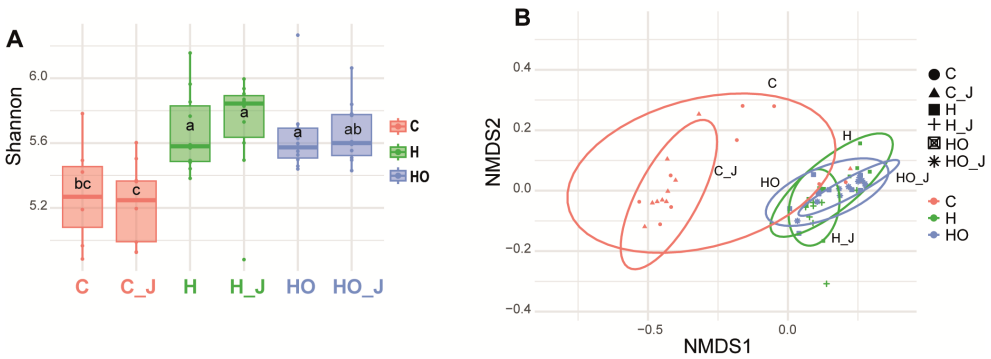

FIGURE 2
Gill microbiota alpha and beta diversity. **A)** Boxplot representing the Shannon diversity index of all groups (n = 10/group). Boxes represent the interquartile range (IQR) between the first and third quartiles, and the horizontal line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles. Samples with a relative abundance exceeding those values are represented as points outside the boxes. Different letters indicate significant differences among the groups (Kruskal–Wallis + Dunn's post-test, $p < 0.05$). **B)** NMDS ordination plot based on Bray-Curtis distance matrixes of normalized sample counts showing community differences among samples. The C and C_J groups are showing a clear separation from the rest of the experimental groups. Red represents the Control treatment, where fish were maintained at 12 °C before the jellyfish exposure trial; circles represent samples from the non-exposed tank, and triangles represent samples from the tank exposed to macerated jellyfish. Green represents the H treatment, where fish were maintained at 12 °C, with the temperature gradually increasing up to 17 °C and then progressively lowered to 12 °C. Squares represent samples from the non-exposed tank, and crosses represent samples from the tank exposed to macerated jellyfish. Blue represents the HO treatment, where fish were kept at the same temperature conditions as group H, additionally decreasing the percentage of dissolved oxygen to 70% when 17 °C was reached. Green squares represent samples from the non-exposed tank, and asterisks represent samples from the tank exposed to macerated jellyfish.

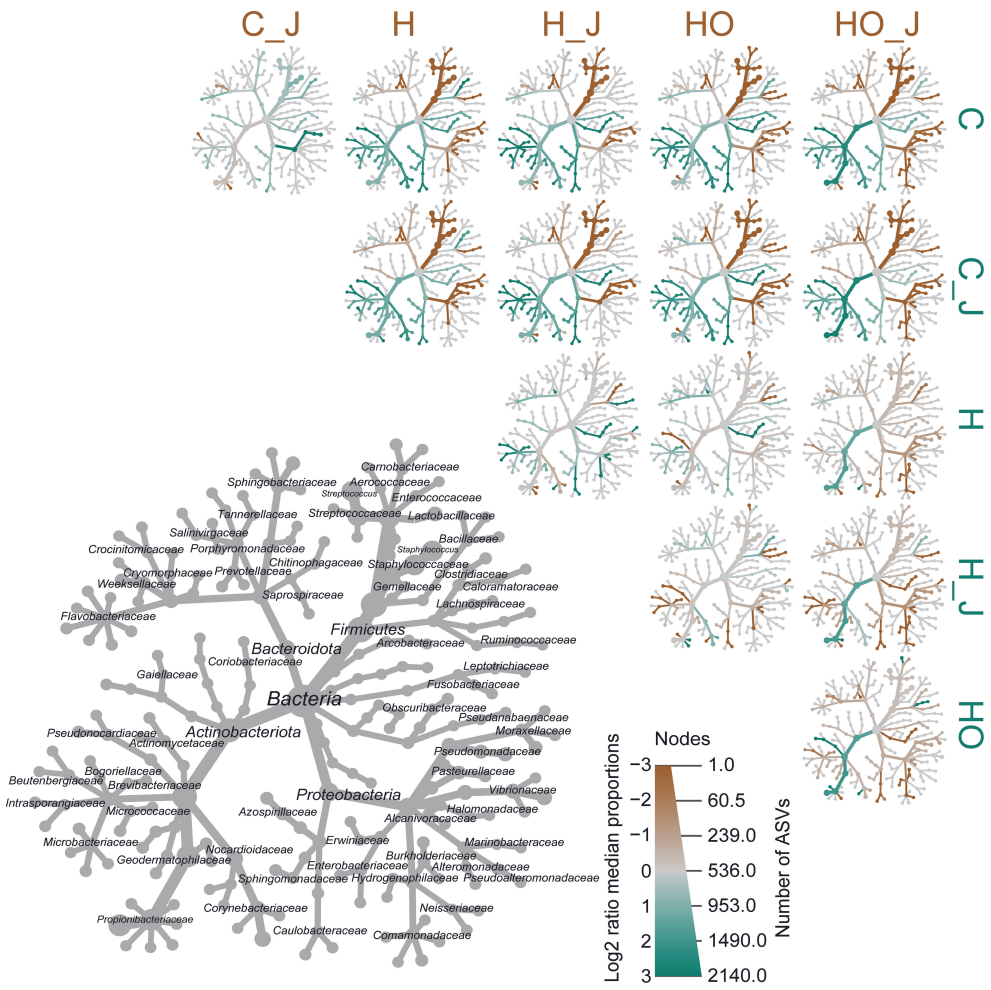

FIGURE 3
Metacoder heat-tree overview of gill microbiota composition and differences among experimental groups. The grey tree on the lower left is a key for the smaller unlabelled trees. The colour of each taxon represents the log2 ratio of median proportions of reads observed in each group. Only significant differences are coloured, determined using a Wilcox rank-sum test followed by a Benjamini-Hochberg (FDR) correction for multiple comparisons. Taxa coloured green are enriched in the groups shown in the row, and those coloured brown are enriched in the groups shown in the column. C, H and HO stand for the different treatments to which fish were submitted before the exposure trial, and J stands for the groups submitted to the jellyfish exposure.

Interestingly, out of the 686 DET, 271 are long non-coding RNAs, and 47 transposable elements (Supplementary File 4).

Next, gene ontology biological pathways (GOBP) enrichment analysis was performed for each set of upregulated or downregulated DET in each of the comparisons with the control group (Supplementary File 5). When upregulated DET were analysed, several pathways related to glycosylation were enriched, particularly in groups exposed to increased temperatures and jellyfish (H_J and HO_J). Pathways related to protein modification and signalling were enriched in the HO_J group, while pathways related to response to stimuli were found enriched in the H_J group. All groups exposed to increased temperatures showed enrichment in pathways related to skin development or differentiation (Figure 6).

Among the downregulated DET, enriched GO-BP terms were more group-specific, highlighting lipid metabolism in the H group, apoptosis and protein phosphorylation in the J group, immune response and tissue development in the H_J group, and haemostasis and cell motility in the HO_J group (Figure 7).

Considering the pathway enrichment analysis results, a subset of DET involved in pathways related to glycosylation, haemostasis, immune response and tissue development/integrity was selected to study in more detail. Nine transcripts for glycosylation enzymes and mucins were significantly upregulated and were detected in almost all groups subjected to increased temperatures. Haemostasis-related genes (e.g. hemopexin or plasminogen) appeared mainly downregulated in groups H and HO_J. Immune response-related transcripts were more diverse, but some clear patterns could be observed. For example, there was a general downregulation of complement genes (e.g. *c2, c3, c4b*) in all groups subjected to increased temperatures. Noteworthy, the immune-suppressing gene *socs3* was consistently upregulated in groups subjected to increased temperatures and jellyfish exposure. Regarding tissue development/integrity, several collagen genes were found downregulated in the H, HO and H_J groups, while several keratins were significantly upregulated in all groups subjected to increased temperatures (Figure 8). None of these genes were differentially regulated in the J group.

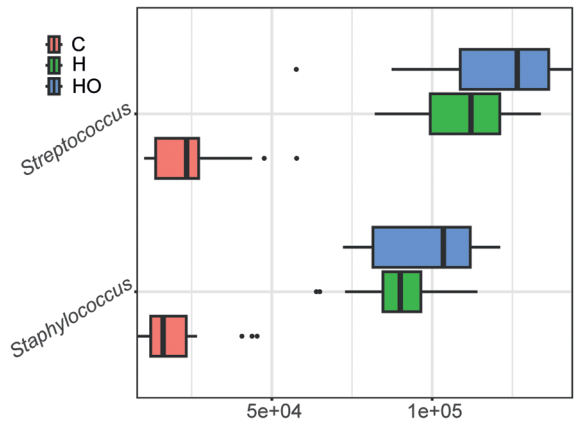

FIGURE 4
Gill microbiota biomarkers. Linear discriminant effect size analysis performed at the genus level representing the significant bacterial biomarkers for each treatment and their abundance in normalized counts (n = 60). Boxes represent the interquartile range (IQR) between the first and third quartiles, and the vertical line inside the box defines the median. Whiskers represent the lowest and highest values within 1.5 times the IQR from the first and third quartiles. Samples with a relative abundance exceeding those values are represented as points outside the boxes. C, H and HO stand for the different treatments fish were submitted to, regardless of the jellyfish exposure. Red represents the Control (C) group and Control exposed to jellyfish (C_J) groups, which were maintained at 12 °C. Green represents the increased temperature (H) and increased temperature exposed to Jellyfish (H_J) groups, which were maintained at 12 °C, gradually increasing up to 17 °C and then progressively lowered again to 12 °C. Blue represents the increased temperature plus limited oxygen (HO) and increased temperature plus limited oxygen exposed to jellyfish (HO_J) groups, which were maintained at the same temperature conditions as the H group, but in addition, the percentage of dissolved oxygen was dropped to 70% at the 17 °C step.

3.5 Host-microbiota correlation network

Considering that mucus structure and glycosylation are important factors affecting or affected by microbiota and the observed changes in glycosylation and mucus barrier transcripts, a correlation analysis was performed between the most abundant bacteria detected in the gill microbiota (> 1%) and the expression of the nine differentially expressed glycosyltransferases or mucin genes (Figure 9; Supplementary File 6). *Skermanella* and *Corynebacterium* were negatively correlated with most of the studied transcripts, whereas *Lactobacillus* and the transferase *galnt8* presented a separate positive correlation. Of interest, the galactosyltransferases *b3galt1* and *b3galt2* were positively correlated with the microbial biomarkers for increased temperature, *Streptococcus* and *Staphylococcus*, although with moderate Spearman r values (between 0.54 and 0.61).

4 Discussion

Climate change exposes marine organisms to multiple, concurrently acting stressors that pose a serious threat to aquatic health and productivity. These stressors often occur concurrently and can interact with the magnitude of the impact depending on factors such as the species and life stage (Reid et al., 2019). Compounding stressors, like increased temperature and decreased oxygen, not only affect the physiology and dynamics of marine species but are also driving the proliferation of jellyfish or algae, affecting fish health directly and indirectly via physical interactions and by modifying the composition of the surrounding microbial environment (Tinta et al., 2019; Bosch-Belmar et al., 2020). As a conse-

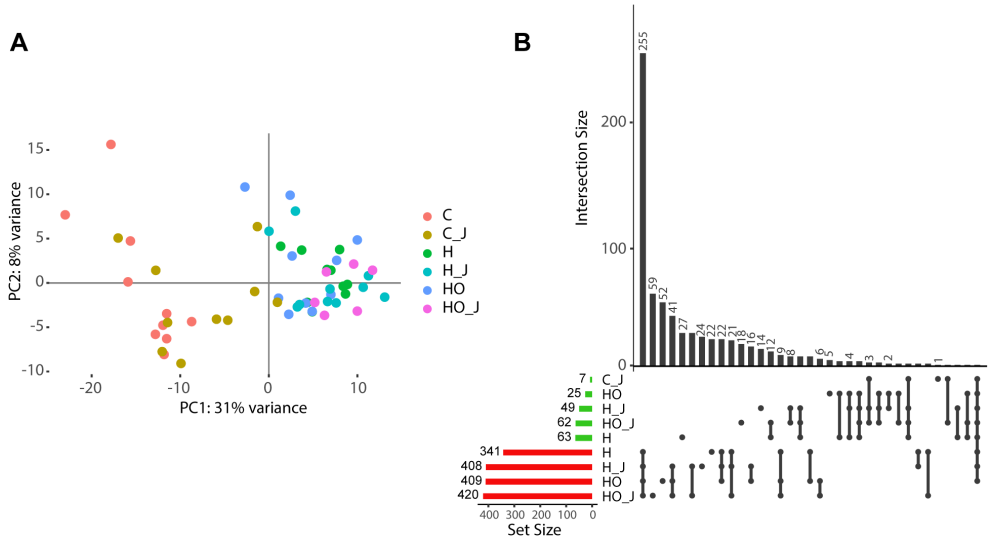

FIGURE 5
(A) Principal component analysis (PCA) of the samples included in the RNA sequencing study (n = 56) based on the differentially expressed (DE) transcripts. Different coloured dots indicate a particular individual in the experimental groups. **(B)** UpSetR intersection plot showing the number of unique and shared differentially expressed transcripts between the C group and the rest of the experimental groups. Green and red indicate downregulated and upregulated transcripts, respectively, when compared to the control group. C, H and HO stand for the different treatments to which fish were submitted before the exposure trial, and J stands for the groups submitted to the jellyfish exposure.

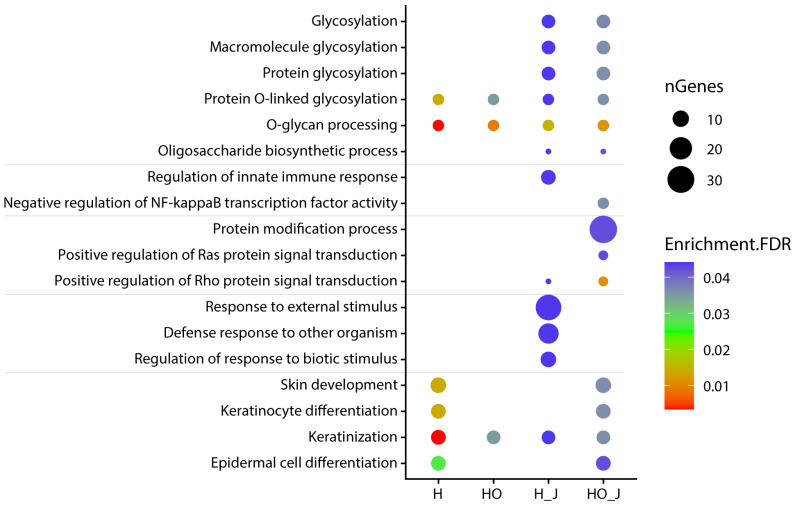

FIGURE 6
Dotplot pathway enrichment map showing the significantly overrepresented pathways (FDR < 0.05) when considering all upregulated transcripts in the different comparisons with the control group. The size of the dots represents the number of unique genes related to a pathway and the colour scale indicates the FDR value. C, H and HO stand for the different treatments to which fish were submitted before the exposure trial, and J stands for the groups submitted to the jellyfish exposure. Grey horizontal lines separate pathways by related categories. Redundant pathways (similar categories and containing the same genes) were omitted for clarity. The full list of pathways per comparison can be found in Supplementary File 5.

quence, fish microbial balance could be disrupted (Clinton et al., 2021). Previous research suggested that, apart from gill damage, jellyfish could act as vectors for the spread of bacteria in fish (Ferguson et al., 2010). However, with our experimental setup, jellyfish exposure did not cause a significant shift in the gill microbial populations. It is possible that the experimental design impaired the potential negative impacts of *A. aurita*, including early activation of nematocytes prior to fish exposure through induction of "stinging" during the preparation and storage (Karabulut et al., 2022). It would be worth exploring whether longer exposure times, multiple exposures, higher jellyfish concentrations, or exposure to freshly caught live jellyfish affect the microbial balance in Atlantic salmon mucosa. While jellyfish are a recognized threat, our data suggest that under conditions of elevated temperature and reduced oxygen, the microbial response is primarily governed by these abiotic factors, with jellyfish playing a secondary or conditional role.

Concerning microbiome composition, the most abundant phyla present in our samples, *Actinobacteriota*, *Bacteroidota*, and *Firmicutes* have been previously described as commonly present in the gill mucosal microbiota of Atlantic salmon (Bledsoe et al., 2022). However, the phylum *Proteobacteria* is usually described as the most abundant, with relative abundances ranging approximately from 70% to 40% of total in gill mucus (Slinger et al., 2021a), whereas in our study, the total mean relative abundance across all samples was 5.7%. This lower *Proteobacteria* abundance could be attributed to several factors, such as water temperature, dissolved oxygen, and season (Diwan et al., 2023). Nonetheless, the identified phyla align with the populations expected in healthy salmon gills. Considering the experimental variables, the increased temperature condition induced a significant decrease of *Actinobacteriota*, and an increase of *Bacteroidota*, and *Firmicutes* when compared to control fish not exposed to temperature changes. However, at lower taxonomic level the most significant differences were detected within the phylum *Firmicutes*.

At the genus level, *Streptococcus* and *Staphylococcus* (*Firmicutes*) were identified as microbial biomarkers for the groups with increased temperatures (H), regardless of the limited oxygen (HO) or jellyfish exposure (J) variables. The presence of these bacteria in salmon gills has been previously described (Slinger et al., 2021b) and contains pathogenic isolates that have been related to individuals with compromised health, presenting skin abscesses, haemorrhages around the eye, and ulcerative disorders (Romalde et al., 2008). Previous research has described *Streptococcus* as an emerging pathogen for salmon, as certain species within this genus, such as *S. phocae*, are the causative agents of streptococcosis in this species (Romalde et al., 2008). In the same line, high levels of *Staphylococcus* in salmon gills have been associated with other pathologies such as amoebic gill disease (AGD), suggesting its potential role in disease development (Slinger et al., 2021b). In addition, it has been reported that temperature changes can influence the epidemiology and virulence of bacterial pathogens in aquatic systems. For example, *Streptococcus agalactiae* has shown temperature-dependent virulence, with higher temperatures enhancing mortality and modulating the expression of genes associated with pathogenicity (Kayansamruaj et al., 2014; Wang et al., 2016). Moreover, transcriptomic and proteomic analyses have reported that higher temperatures can influence bacterial metabolism, posing a threat to fish health (Wang et al., 2016; Tavares et al., 2018). The current approach does not allow to reach species or strain level. Therefore, future studies should be directed to unveil the pathogenicity of the detected bacteria. Still, no significant pathological changes were observed during gill assessment.

Of note, even if the gill scores did not present significant differences among groups, it is worth mentioning that the assessment of gill damage upon jellyfish exposure remains an understudied area since there is no record of a standardized gill scoring system, unlike the established method for AGD (Taylor et al., 2009), which is often used to assess jellyfish-induced damage. However,

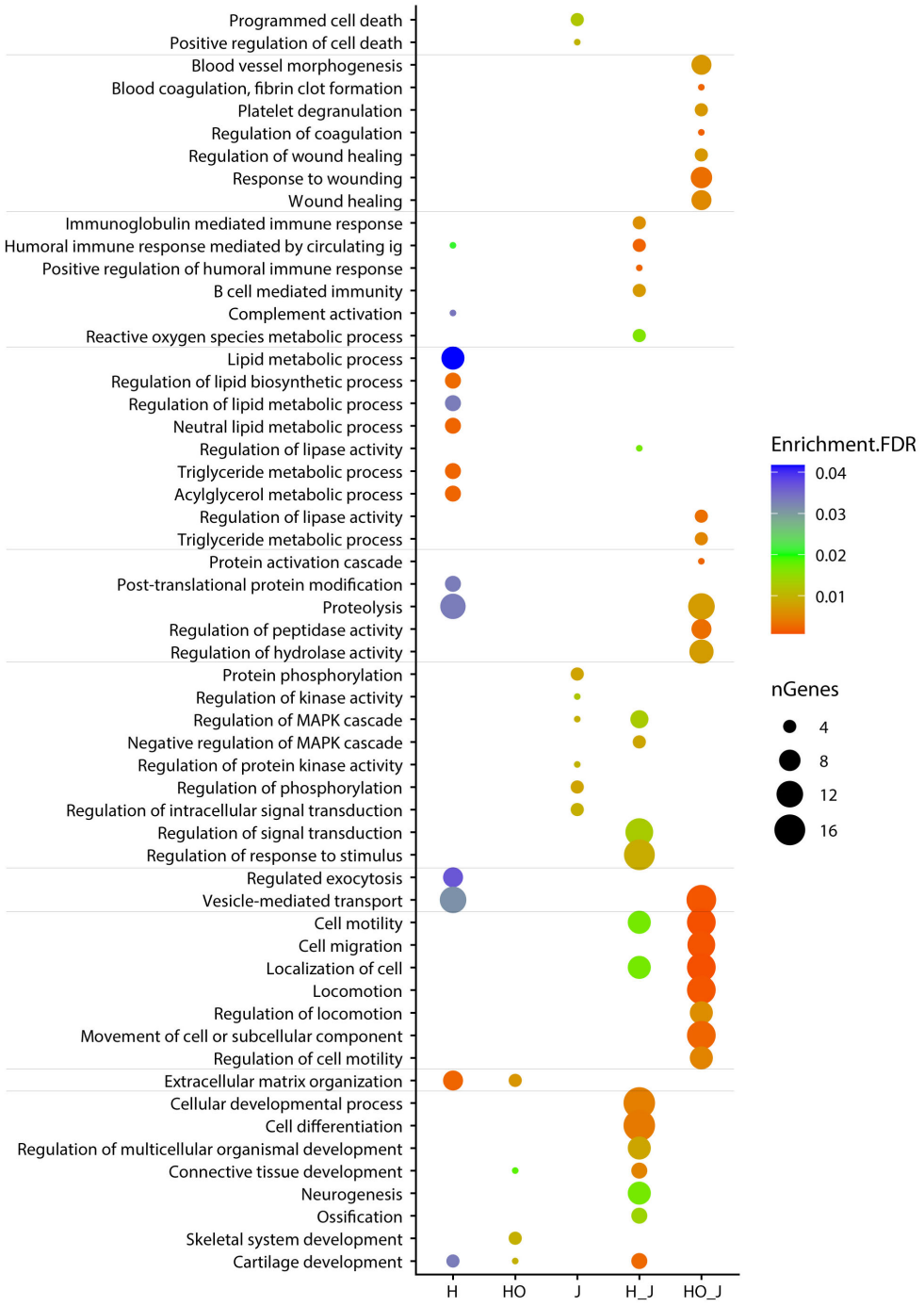

FIGURE 7
Dotplot pathway enrichment map showing the significantly overrepresented pathways (FDR < 0.05) when considering all downregulated transcripts in the different comparisons with the control group. The size of the dots represents the number of unique genes related to a pathway and the colour scale indicates the FDR value. C, H and HO stand for the different treatments to which fish were submitted before the exposure trial, and J stands for the groups submitted to the jellyfish exposure. Grey horizontal lines separate pathways by related categories. Redundant pathways (similar categories and containing the same genes) were omitted for clarity. The full list of pathways per comparison can be found in Supplementary File 5.

AGD scoring is not entirely suitable since the pathologies observed are different. Still, some progress has been made in scoring gill damage upon jellyfish exposure. Given the increase in jellyfish bloom events, there is a need for a standardized and even species-specific gross gill scoring system that would help to improve the accuracy of describing jellyfish impacts, facilitate comparisons across experimental groups, as well as to understand regeneration potential and recovery periods of the gills after an attack.

At the gill transcriptome level, the majority of differences observed among experimental groups were due to increased temperature as a common factor. Overall, the affected pathways identified from these differential comparisons align with previous

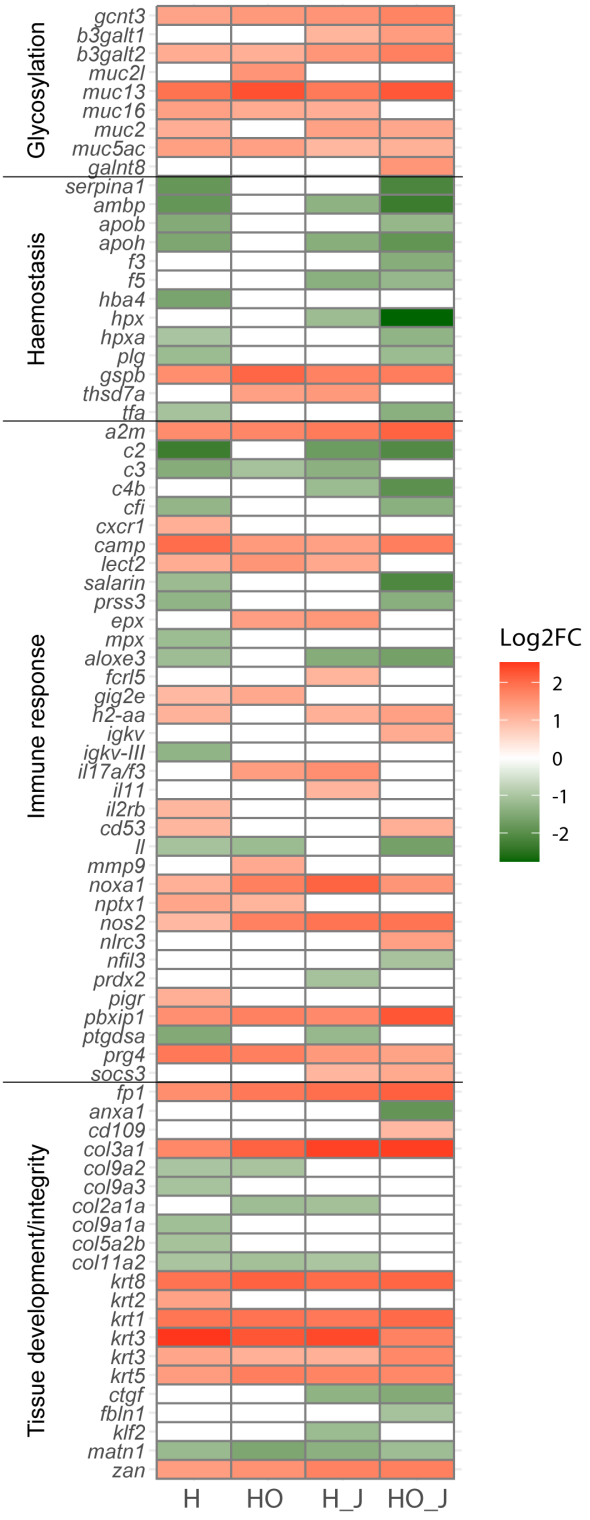

FIGURE 8
Heatmap depicting the log2 fold change (FC) of the selected transcripts involved in pathways related to glycosylation, haemostasis, immune response and tissue development/integrity. Transcripts that were significantly up- or downregulated (padj < 0.05) by the different treatments when compared to the control group appear in shades of red or green, respectively. C, H and HO stand for the different treatments to which fish were submitted before the exposure trial, and J stands for the groups submitted to the jellyfish exposure.

research, which shows that the co-occurrence of multiple environmental stressors can affect pathways such as glycosylation and protein modification (Milisav, 2011; Jin et al., 2015; Benktander et al., 2021; Bergstrom and Xia, 2022). The mucus layer structure and glycosylation patterns are key factors for homeostasis, host-microbiota interactions and infection (Bergstrom and Xia, 2022). Glycosylation patterns are highly dynamic and depend on different factors. Specifically, in Atlantic salmon skin, these patterns showed to be highly dependent on temperature, which also affected gene expression patterns and bacterial binding (Thomsson et al., 2025). In gills, Atlantic salmon smolts present larger, more complex, and diverse mucin glycans than in skin, and their structure is significantly affected in pathogenic conditions like amoebic gill disease (Benktander et al., 2020). Mucus glycosylation patterns had been correlated with changes in presence and abundance of microbial taxa during heat stress conditions in different species (Lee et al., 2016; Yang et al., 2022; Wu et al., 2023). However, this has been mainly studied at the level of sugar composition rather than expression of specific galactosyltransferase genes (Lee et al., 2016; Yang et al., 2022). The current results show that, when fish are subjected to elevated temperature events, a consistent upregulation of several glycosyltransferase genes (*gcnt3*, *b3galt1*, *b3galt2*, *galnt8*) involved in different steps of mucin glycosylation was observed, and their expression levels were significantly correlated with the abundance of different bacteria. While correlation alone cannot infer causality, our findings emphasize the importance of future mechanistic studies to characterize climate change–induced alterations in gill mucin glycosylation and to directly determine their impact on microbial binding dynamics.

The different conditions tested affected different sets of genes, with jellyfish alone not inducing significant changes. However, the combination of jellyfish exposure with other environmental stressors revealed new pathways not detected only with temperature or with temperature plus oxygen changes. More than 25 genes related to response to stimulus were upregulated in the H_J group, while pathways such as cell differentiation and cell development were downregulated. These expression patterns align with previously reported studies in Atlantic salmon post-smolts exposed to elevated temperatures, which showed a strong overall response to stimulus through the upregulation of genes related to heat shock, oxidative stress, apoptosis, and immune pathways (Beemelmanns et al., 2021). At the same time, high temperature stress has been linked to the downregulation of genes associated with transcription, translation, and cytoskeleton organization, which are crucial to cell differentiation and development (Nuez-Ortín et al., 2018). This downregulation of developmental pathways in the H_J group could represent a trade-off in which cellular resources are redirected from differentiation and growth to stress reduction and repair. A previous study by Fast et al. reported that exposure to jellyfish toxins triggered a stress response, characterized by the upregulation of genes involved in healing response, inflammation, and redox balance, directly implicating pathways for cellular response to stimulus and tissue development/integrity (Fast et al., 2025).

On the other hand, the HO_J group showed a downregulation of numerous genes involved in haemostasis and cell motility/

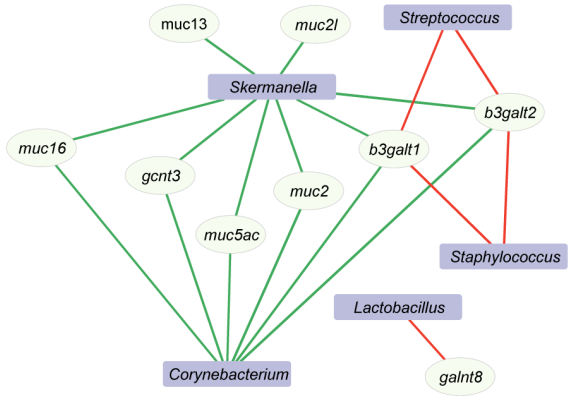

FIGURE 9
Correlation network showing significant positive (red lines) and negative (green lines) correlations (Spearman, FDR < 0.001, r > | 0.5|) between most abundant bacteria (>1% relative abundance) and differentially expressed transcripts related to mucus production and glycosylation.

migration. Besides elevated temperatures, moderate or chronic hypoxic conditions in Atlantic salmon alter the gill transcriptome, affecting immune gene expression and reducing resistance to bacterial pathogens, suggesting that hypoxia can disrupt gill homeostasis and repair capacity (Krasnov et al., 2021). In addition, in a study conducted by Emam et al. that included environmental stressors such as hypoxic conditions, gill expression patterns were associated with pathways such as tissue remodelling and stress responses (Emam et al., 2022). Furthermore, it is known that exposure to jellyfish toxins induces a strong upregulation of genes involved in inflammation, tissue healing, and redox balance, but also implicates pathways for tissue remodelling (Fast et al., 2025), which could involve haemostatic and cell migratory processes as part of the repair response. Similarly, severe gill injuries from hydrogen peroxide treatment showed upregulation of genes involved in healing processes and DNA repair, while modulating immune genes, suggesting that external stressors can activate cellular repair mechanisms that depend on cell motility and haemostasis to restore tissue integrity (Veltman et al., 2025).

Concerning tissue integrity, a number of collagen genes were downregulated in groups with elevated temperatures, except in the HO_J group, while at the same time, several keratins were upregulated in all H groups. It has already been reported that increased temperature triggers coordinated molecular responses in salmonids that involve extracellular matrix (ECM) and collagen pathways, structural components, and immune systems across organs, including skin, liver, and heart (Jørgensen et al., 2014; Beemelmanns et al., 2021; Ignatz et al., 2022). For example, Atlantic salmon skin maintained at 16 °C triggered an upregulation of stress and heat-shock genes alongside structural remodelling, such as epidermal thinning, indicating temperature-sensitive ECM dynamics (Cerda-Celis et al., 2025). Similarly, elevated liver temperatures (18–20 °C) strongly induce Serpinh1 (*HSP47*), a collagen-specific chaperone, while warm-acclimated hearts also show collagen I accumulation supporting connective tissue remodelling (Jørgensen et al., 2014; Beemelmanns et al., 2021). Based on the current results and the described previous findings, it could be stated that increased temperatures, especially at sub-optimal temperature levels close to the thermal threshold, consistently modulate collagen (ECM), keratin and immune pathways across tissues, indicating these gene networks are central to the heat-stress responses.

It is worth mentioning the significant percentage of long non-coding RNAs found regulated by the increased temperature variable, in combination or not with oxygen drop and jellyfish, and their known role in fish responses to elevated temperatures (Yan et al., 2023). Recent research highlights that lncRNAs are involved in multiple physiological processes in fish, such as growth, immunity and stress adaptation, acting through transcriptional mechanisms (Deng et al., 2022). In particular, transcriptomic analyses in rainbow trout (*Oncorhynchus mykiss)* under heat stress have identified key lncRNAs co-expressed with heat shock proteins, immune-related genes, and metabolic pathways, suggesting their involvement in coordinating systemic stress responses (Zhou et al., 2022). lncRNAs could play a crucial role as molecular regulators linking elevated temperatures to developmental, metabolic, and immune regulation, with possible implications for aquaculture resilience under climate change. In salmon, this has not been deeply explored, but future studies should focus on the set of lncRNA identified in the current work and their response to environmental stressors (e.g., fluctuating temperatures, compound stressors) and co-expression with other genes to determine regulatory frameworks.

Overall, the integrative approach employed in this study provides a deeper understanding of how the gill microbiota and transcriptome of Atlantic salmon gill respond to different climate-change related stressors. The alterations in the gill microbiome and transcriptome were mainly driven by the increased temperature variable. Exposure to a higher temperature wave induced a significant increase in *Streptococcus* and *Staphylococcus* and significant changes in host gene expression that could indicate changes in mucin glycosylation, haemostasis, immune response and tissue integrity. Although jellyfish exposure alone did not show major effects, when combined with the other environmental stressors, specific responses related to haemostasis, tissue integrity and cell motility or response to stimulus were detected. These results provide valuable datasets and highlight specific genes and microbial taxa that can help future studies directed to functionally elucidate how combined stressors influence the multiple barriers underpinning salmon gill mucosal health. Thus, future work should be directed to better characterize the bacterial changes at species or strain levels and to functionally evaluate the correlation of these bacteria with host physiological changes. Understanding the effects of multiple stressors on farmed salmon fish health is essential for preparing the sector to different impacts from climate change.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was approved by Norwegian Food Safety Authority (FOTS ID 29728). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ST-R: Writing – review & editing, Writing – original draft, Formal analysis, Visualization, Data curation, Investigation. EY: Writing – review & editing, Funding acquisition, Project administration, Investigation, Conceptualization. L-HJ: Conceptualization, Writing – review & editing, Funding acquisition. AS-B: Writing – review & editing, Project administration, Supervision, Funding acquisition, Resources. JP-S: Supervision, Writing – review & editing, Funding acquisition, Project administration, Resources. CL: Writing – original draft, Project administration, Data curation, Resources, Investigation, Conceptualization, Writing – review & editing, Funding acquisition, Supervision. MP: Writing – original draft, Writing – review & editing, Investigation, Supervision, Data curation, Visualization.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY FILE 1

Sample information and ID nomenclature used in the study, including sequencing metrics for the 16S rRNA analysis **(A)** and the RNA-seq analysis **(B)**. C, Control, J, jellyfish exposure, H, increased temperature, HO, increased temperature + oxygen drop.

SUPPLEMENTARY FILE 2

Gill microbiota composition. Stacked bar chart representing the relative abundance of bacterial phyla in the different experimental groups. The Kruskal–Wallis test (post-test Dunn's, P-value < 0.05) showed significant differences among groups for the phyla *Actinobacteria*, *Bacteroidota* and *Firmicutes*. Differences are indicated by different letters which correspond to pairwise comparisons within each phylum among groups.

SUPPLEMENTARY FILE 3

Sample information on the percentage of total abundance at the phylum, family and genus level. The least abundant taxa are grouped in the category Others **(A)**. Dysbiosis scores based on Bray–Curtis dissimilarities to control microbial structures **(B)**.

SUPPLEMENTARY FILE 4

DESeq2 results for the comparison of the experimental groups against the control group. The different comparisons can be found in the different tabs. Only significant results (padj < 0.05, baseMean > 20, log2FoldChange > |1|) are shown. The Control (C) group and Control exposed to jellyfish (C_J) groups were maintained at 12 °C. The increased temperature (H) and increased

temperature exposed to Jellyfish (H_J) groups were maintained at 12 °C, gradually increasing up to 17 °C and then progressively lowered again to 12 ° C. The increased temperature plus limited oxygen (HO) and increased temperature plus limited oxygen exposed to jellyfish (HO_J) groups were maintained at the same temperature conditions as the H group, but in addition, the percentage of dissolved oxygen was dropped to 70% at the 17 °C step.

SUPPLEMENTARY FILE 5

Gene ontology-biological process (GO-BP) enrichment analysis results for the differentially expressed transcripts between the experimental groups against the control group. The different analyses, separated by up- and downregulated transcripts, can be found in the different tabs. Only significant results (FDR < 0.05, nGenes > 2) are shown. The Control (C) group and Control exposed to jellyfish (C_J) groups were maintained at 12 °C. The increased temperature (H) and increased temperature exposed to Jellyfish (H_J) groups were maintained at 12 °C, gradually increasing up to 17 °C and then progressively lowered again to 12 °C. The increased temperature plus limited oxygen (HO) and increased temperature plus limited oxygen exposed to jellyfish (HO_J) groups were maintained at the same temperature conditions as the H group, but in addition, the percentage of dissolved oxygen was dropped to 70% at the 17 °C step.

SUPPLEMENTARY FILE 6

Correlation analysis results between the most abundant bacteria taxa (> 1%) and selected differentially expressed transcripts related to mucus production and glycosylation. The correlation coefficient **(r)** was calculated for the non-parametric Spearman correlation (two-tailed). A correlation was considered significant when FDR < 0.001 and r > |0.5|. Non-significant comparisons are marked in grey text.

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