Repurposing Host-Derived RNA-seq Data for Microbial Taxonomic and Functional Profiling in Aquaculture Settings

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Abstract

Aquaculture plays a key role in global food security, yet disease outbreaks remain a major challenge. While host transcriptomic response have been widely studied, the role of microbial communities in disease risk in aquaculture remains understudied.

Here, we conducted a metatranscriptomic analysis of gill microbiomes in Atlantic salmon (*Salmo salar*) by repurposing publicly available RNA-seq data originally generated for host transcriptomics. These poly(A)-selected RNA-seq datasets, though primarily targeting eukaryotic mRNA, retained a detectable signal from microbial transcripts, allowing retrospective analysis of microbial patterns prior to a disease

outbreak. Taxonomic profiles generated by Bracken and MATAM showed strong concordance across dominant genera, confirming that host RNA-seq datasets retain sufficient microbial signal for downstream classification. In addition to tank-dependent microbial variation, our presence-absence analysis revealed survival-associated microbiome shifts. Several taxa, including *Sedimentibacter*, *Fusarium*, and *Demequina* were more frequetly observed in died individuals, whereas *Micrococcaceae, Pseudomonas, and Burkholderiaceae* were enriched in survivors. were enriched in survivors.

Functional gene analysis revealed differential transcriptional responses within the same microbial taxa between survivors and non-survivors, particularly in metabolic adaptation, stress response, and host–microbe interactions. These results suggest that host-derived RNA-seq datasets, though not originally designed for microbial analysis, offer new opportunities to investigate early microbial signals and environmental factors associated with disease susceptibility in aquaculture.

Keywords: Metatranscriptomics, Aquaculture, Gill microbiome, Functional profiling, *Salmo salar*

Short Summary: This study shows that host RNA-seq data can be repurposed to reveal early microbial changes and functional shifts associated with disease outcomes in Atlantic salmon aquaculture.

69

70

71 **Introduction**

72 Aquaculture is a promising solution for meeting the growing global demand for food and nutrition (Little,
73 Newton and Beveridge 2016; Boyd, McNevin and Davis 2022). Despite advancements in intensive
74 breeding systems that have improved production efficiency, infectious diseases remain a major
75 challenge, because of high fish densities in captive environments (Leung and Bates 2013; Assefa and
76 Abunna 2018; Bouwmeester *et al.* 2021; Ferreira *et al.* 2021; Mougin and Joyce 2022). Disease
77 outbreaks not only cause substantial economic losses and food waste (Stentiford *et al.* 2017) but also
78 affect wild fish populations and surrounding ecosystems as aquaculture environments can serve as
79 reservoirs for pathogens (Diana 2009; Colombo *et al.* 2016; Hamza *et al.* 2017; Bouwmeester *et al.* 2021;
80 Glidden *et al.* 2021).

81

82 The frequent occurrence of infectious diseases in aquaculture has led to the widespread use of
83 antibiotics. Although the use of antibiotics in aquaculture is subject to regulation in many countries,
84 particularly in Europe, substantial application has been reported in numerous major aquaculture-
85 producing nations, especially in Asia and South America (Lulijwa, Rupia and Alfaro 2020). While this
86 approach offers short-term efficacy, it contributes to the emergence of antibiotic-resistant bacteria, posing
87 challenges to long-term disease management and ecosystem health (Defoirdt, Sorgeloos and Bossier
88 2011; Muziasari *et al.* 2016; Santos and Ramos 2018; Topp *et al.* 2018; Haro-Moreno *et al.* 2020; Lulijwa,
89 Rupia and Alfaro 2020). Vaccination programs have also been implemented for several pathogens (Park
90 2009; Rodger 2016; Mkulo *et al.* 2024). However, a comprehensive understanding of pathogen diversity
91 and microbial interactions in aquaculture remains elusive, particularly regarding the complexity and
92 functional dynamics of the aquatic microbiome, which plays a crucial role in host health, immune
93 regulation, and pathogen resistance (Rajeev *et al.* 2021; Canellas *et al.* 2022; Chen *et al.* 2022; Samsing
94 and Barnes 2024).

95

96 Recent advances in high-throughput sequencing, particularly metatranscriptomics, have enabled the
97 functional characterization of microbiomes in real-time, providing deeper insights into microbial activity
98 and host-microbe interactions (Jiang *et al.* 2016; Ojala *et al.* 2023). Unlike traditional microbiome
99 analyses that rely on taxonomic profiling, metatranscriptomics allows for the identification of microbial
100 functional signatures, potentially serving as early warning indicators before clinical symptoms emerge.
101 Integrating this approach into aquaculture disease monitoring could facilitate a shift from reactive disease
102 management to proactive risk assessment and control, reducing dependence on antibiotics and
103 improving biosecurity (Jorth *et al.* 2014; Hua *et al.* 2015; Welti-Chanes *et al.* 2020; Massimino *et al.* 2021)

104 In the winter of 2021, a severe outbreak of infectious skin disease occurred in an Atlantic salmon (*Salmo*
105 *salar*) breeding facility in Western Norway, leading to high mortality rates and revealing the need for early
106 disease detection strategies. Current disease investigations in aquaculture are largely conducted post-
107 outbreak, yet microbial and immunological alterations may occur before clinical symptoms appear (de
108 Bruijn *et al.* 2018). While most transcriptomic studies in aquaculture focus on host immune responses,
109 an alternative and complementary approach is to examine microbial community function as a predictor
110 of disease susceptibility (Yilmaz *et al.* 2022). The gill microbiome, in particular, plays a key role in
111 pathogen defense and host homeostasis (Morshed and Lee 2023), yet their functional activity prior to
112 disease outbreaks remains largely unexplored.

113 In this study, we repurposed a publicly available RNA-seq dataset originally generated for host
114 transcriptomic analyses of Atlantic salmon gill biopsies collected prior to a disease outbreak. At the time
115 of sampling, fish exhibited no visible disease symptoms, allowing retrospective investigation of microbial
116 signatures preceding disease emergence. Despite the use of poly(A)-selected libraries, we successfully
117 retrieved both rRNA and mRNA transcripts from microbial taxa. By comparing microbial taxonomic
118 profiles and transcriptional patterns between survivors and non-survivors, we identified taxa- and
119 transcript-level differences that may reflect early microbial responses associated with disease

susceptibility. Our findings demonstrate the potential of host-derived RNA-seq data as an underutilized resource for microbial ecological analysis in aquaculture systems.

Materials and Methods

Metatranscriptome dataset

The metatranscriptome dataset analyzed in this study was derived from Atlantic salmon gill samples and is publicly available at the European Nucleotide Archive (ENA) under the project number PRJEB47441. These data originated from 109 full-sib families of the Mowi (Mowi Genetics AS) strain, sampled in November 2019. This study did not involve direct handling of fish; instead, it retrospectively analyzed metatranscriptome data to investigate microbial dynamics prior to disease onset. A comprehensive description of the fish population is provided in Gjerde et al. (Gjerde *et al.* 2024) while details on metatranscriptome data generation are outlined in Grønvold et al. (Grønvold *et al.* 2024). The original experiment was performed according to EU regulations concerning the protection of experimental animals (Directive 2010/63/EU). Appropriate measures were taken to minimize pain and discomfort. The experiment was approved by the Norwegian Food and Safety Authority (FOTS id. number 25658) (Gjerde *et al.* 2024).

Fish Rearing and Sampling Timeline

Fish were initially maintained under continuous light (LL) from hatching until they reached approximately 50g. They were then exposed to a 6-week 12:12 photoperiod (12 hours light, 12 hours dark) before returning to LL for 8 weeks, reaching 100g, the standard size for seawater transfer.

144 In early February 2021, fish were vaccinated with Alpha Ject micro 6 (PHARMAQ®) against *Aeromonas*
145 *salonicida, Listonella anguillarum O1* and *O2a, Vibrio salmonicida, Moritella viscosa, and IPN virus Sp*.
146 In June 2021, one week before seawater transfer, gill biopsies were collected for RNA sequencing at a
147 time when fish showed no visible disease symptoms.

148 On June 23, 2021, fish were transported to the ARST seawater facility. Approximately eight weeks after
149 transfer, signs of winter ulcers began to appear, and mortality increased throughout the autumn. Despite
150 the removal of affected individuals during trait recordings in early November, the prevalence of winter
151 ulcers persisted into winter. Over the following 10 months, fish were monitored for survival and growth.

152

153

154 **Data Selection**

155 To ensure a robust and unbiased investigation into microbial communities associated with health
156 outcomes, sample selection was based on multiple biological and experimental constraints. The dataset
157 included 15 surviving fish, alive at five months post-transfer, and 13 non-surviving fish, which died within
158 40 days post-transfer (**Table 1**). Sex was evenly distributed, with 13 males and 15 females, to account
159 for potential sex-specific microbial differences. Fish were reared in two separate tanks, C1 and C2,
160 allowing the assessment of environmental effects. The died group consisted exclusively of fish with visible
161 wounds, ensuring a consistent disease phenotype, while the survived group included only wound-free
162 individuals, providing a clear contrast (**Figure 1**). While the selection aimed for a balanced representation
163 across conditions, final sample availability was constrained by the accessibility of metatranscriptome data
164 and the biological conditions of the cohort.

165

166 **Metatranscriptome data preparation**

167 Publicly available RNA-seq data were retrieved in FastQ format from the European Nucleotide Archive
168 (ENA, project PRJEB47441). The dataset consists of gill metatranscriptome from Atlantic salmon, with

169 sequencing performed using a paired-end (PE) approach (2×150 bp) generating approximately 20 million
170 reads per sample. Quality assessment of raw reads was conducted using FastQC (v0.12.0). Adapter
171 sequences, low-quality bases, and short reads were removed using fastp (Chen *et al.* 2018)(v0.23.4)
172 with default parameters. Filtered reads were then mapped to the Atlantic salmon reference genome
173 (Stenløkk 2023) (Ssal_v3.1, GCA_905237065.2) using bwa-mem2 (v2.2.1). Reads that failed to align
174 with the host genome were extracted and retained for microbiome analysis.

175 The RNA-seq libraries were prepared using a poly(A) selection method to enrich for mRNA, rather than
176 actively depleting ribosomal RNA (rRNA). While poly(A) selection primarily targets eukaryotic mRNA,
177 certain bacteria and some prokaryotic organisms possess polyadenylated mRNA at low frequencies,
178 which may result in the retention of microbial transcripts. Additionally, host-derived poly(A) transcripts
179 from Atlantic salmon may be present at a consistent proportion across samples.

180 For preprocessing, adapter trimming and quality filtering were performed using fastp (v0.23.4), followed
181 by host genome mapping against the Atlantic salmon reference genome (Ssal_v3.1,
182 GCA_905237065.2). Unmapped reads were retained for microbiome analysis, under the assumption that
183 they predominantly represent microbial sequences. However, given the poly(A) selection strategy, it is
184 possible that a proportion of these unmapped reads include artifacts from host transcripts or residual
185 polyadenylated bacterial transcripts.

186 Although this potential artifact is present, it is expected to be uniformly distributed across all samples
187 rather than selectively biased toward a specific group (e.g., survived vs. died fish). Therefore, while this
188 factor must be considered when interpreting metatranscriptomic results, the relative comparisons
189 between survival groups remain valid, as any systematic bias introduced by poly(A) selection would be
190 consistent across conditions.

191

192 **Taxonomic Classification and Analysis**

193 For taxonomic classification, Kraken2 (Wood, Lu and Langmead 2019) (v2.14) and Bracken (v3.0) were
194 utilized. Kraken2 was executed using a custom-built database (GTDB_plus) (Parks *et al.* 2022). Following
195 Kraken2 classification, Bracken was used to estimate species taxonomic read count at different
196 taxonomic levels. Each sample was processed iteratively using paired-end FASTQ files. The GTDB_plus
197 database was employed for Kraken2 classification. This extensive database integrates GTDB
198 representative genomes (Release 2.20) and NCBI RefSeq standard libraries, including viral, fungi, plant,
199 protozoa, and human sequences for contamination screening. The database utilizes a hybrid taxonomy,
200 with GTDB taxonomy for prokaryotes and NCBI taxonomy for other taxa. Entries with tax_id greater than
201 4,000,000 originate from GTDB, while lower taxonomic IDs correspond to NCBI classifications. For rRNA-
202 based microbial community analysis, MATAM (1.6.1) was employed to assemble 16S/23S rRNA reads.
203 Non-host sequence reads were aligned against the SILVA 138.1 database.

204 **Taxonomic read count Analysis**

205 Bracken output files were processed in R to obtain family-level taxonomic read count data. The data were
206 merged with sample metadata and cleaned to remove unwanted characters and taxa absent in all
207 samples. A threshold-based filtering approach was applied to merge low taxonomic read count taxa (<1%
208 max taxonomic read count) into an "Other" category. Hominidae sequences were excluded. Read counts
209 were normalized by dividing by the total read count per sample and multiplying by 200,000. This serves
210 as a scaling method to allow comparability across samples while preserving relative taxonomic read
211 count within each sample. Alpha diversity indices (Shannon and Simpson) were computed using the
212 vegan package. Statistical differences in diversity indices were tested using the Kruskal-Wallis test. Beta
213 diversity was assessed using Bray-Curtis and Jaccard dissimilarity matrices, and statistical significance
214 was determined using PERMANOVA (Adonis test) (Tang, Chen and Alekseyenko 2016). SIMPER
215 analysis was conducted to identify taxa contributing to beta diversity differences. Barnard's exact test
216 was applied to binary presence-absence data to evaluate the association between taxa and fish health
217 status.

218

219

220 **Gene Expression Quantification**

221 Metatranscriptome assembly was performed using MEGAHIT (v1.2.9). For transcript quantification,
222 Salmon (v0.8.1) was used. The analysis was conducted using an index built for the dataset. Paired-end
223 reads were processed with automatic library type detection, validation of mappings. A p-adjusted value
224 < 0.05 was considered statistically significant for the differential expression analysis using DESeq2 (Love,
225 Huber and Anders 2014). Functional annotation was conducted on DRAM (Shaffer *et al.* 2020) on K-
226 base (Arkin *et al.* 2018).

227

228 **FASTQ Statistics Analysis**

229 Contig lengths were computed using a custom script. The N50 statistic was determined by sorting contig
230 lengths in descending order and identifying the length where the cumulative sum reached half of the total
231 length. Mean and standard error was also calculated.

232

233 The scripts used in this study are available at
234 https://github.com/mariesaitou/paper_2025-/tree/main/metaT.

235

236

237 **Results**

238 **Assessment of Poly(A) selection bias and validation of taxonomic classification**

239 We obtained an average of 537,995 ± 289,590 unmapped reads from a total of 11,328,436 ± 1,190,386
240 reads from the 28 samples. The observed distribution of poly(A) and poly(T) tail lengths in unmapped
241 reads indicates a selective retention of polyadenylated sequences (**Figure S1**). Shorter poly(A) tails (e.g.,

242 4–8 nucleotides) were detected at high frequencies, while longer tails (≥12 nucleotides) were less
243 common but consistently present above theoretical expectations. This suggests that bacterial transcripts
244 with short poly(A) tails were retained, aligning with the known role of bacterial polyadenylation in mRNA
245 degradation and turnover rather than transcript stabilization.

246

247 To assess the effectiveness of microbial taxonomic assignments in the poly(A)-selected RNA-seq
248 dataset, we first compared results from Bracken (the top-observed taxa are shown in **Table 2AB**),

249

250 We evaluated the effectiveness of microbial taxonomic assignments in the poly(A)-selected RNA-seq
251 dataset by comparing Bracken, which classifies reads based on total transcript counts , with MATAM,
252 which reconstructs 16S/23S rRNA reads for taxonomic profiling. MATAM identified 54 genera in total,
253 while its coverage was limited, suggesting that a small fraction of microbial rRNA transcripts may have
254 escaped the poly(A) selection process. The detected rRNA sequences were primarily corresponded to
255 the most transcriptionally dominant bacterial taxa identified by Bracken (**Table 2**). **Figure S2** compares
256 Bracken and MATAM estimates for genera ranked highest in transcript quantification. The strong
257 correlation observed (Spearman's ρ ranging from 0.48 to 0.96; *e.g., Brevundimonas*, ρ = 0.94, p = 2.4e-
258 12; *Sphingomonas,* ρ = 0.96, p = 0) suggests that Bracken provides reliable taxonomic resolution despite
259 potential biases from poly(A) selection. Given Bracken's broader taxonomic coverage and its ability to
260 classify both rRNA and mRNA-derived sequences, we opted to use it for downstream analyses. Given
261 that bacterial transcripts were successfully detected despite poly(A) selection, we proceeded with
262 taxonomic classification to profile microbial communities and assess their association with fish health.

263

264

265 **Overview of Microbial Community Composition and Environmental Influence**

266 Taxonomic classification was performed using Kraken and Bracken. At the species level, taxonomic read
267 counts were relatively low, leading to unstable classification results. Therefore, initial quantitative
268 analyses were conducted at the family level, followed by a deeper examination of notable taxa at the
269 genus level. The composition of metatranscriptomic taxa exhibited individual variation among fish
270 samples; however, when grouped by conditions, the overall community structure remained relatively
271 stable (**Figure 2A**).

272 Across all samples, the top ten most transcriptionally represented families accounted for 79.4% of the
273 total taxonomically assigned reads (**Table 2A**). Among these, Micrococcaceae was the dominant family,
274 contributing 20.34% of the total reads, followed by f__UBA9655 (17.18%) and f__UKL13-3 (12.89%).
275 Other notable families included Vampirovibrionaceae (7.23%) and Burkholderiaceae (6.12%), indicating
276 their prevalence in the microbial community. Micrococcaceae includes some opportunistic pathogens
277 that may be involved in fish infections. Their presence in aquaculture environments suggests a possible
278 role in disease dynamics. Families f__UBA9655 and f__UKL13-3 remain insufficiently characterized, with
279 unknown physiological functions, but they have been frequently detected in aquaculture environments,
280 suggesting a potential ecological role. Since they are metagenome-only classifications, their functional
281 significance might be underestimated. Burkholderiaceae comprises highly adaptable environmental
282 bacteria with multifunctional roles. Some species exhibit pathogenic traits, while others show
283 antimicrobial properties (Morya, Salvachúa and Thakur 2020). Their frequent presence in aquatic
284 environments highlights their potential role in host-pathogen interactions and microbiome stability.
285 Enterobacteriaceae includes many pathogenic species known to cause infections in humans and
286 animals, and their presence in fish samples could indicate potential health risks (Janda and Abbott 2021).
287 Pseudomonadaceae is associated with fish diseases, including Aeromonas infections, and is linked to
288 water quality degradation and host immune suppression, potentially serving as an indicator of disease
289 risk. Moraxellaceae, frequently detected in aquatic environments, may serve as an indicator of

290 environmental pollution and water quality stress factors. These families will be further examined at the
291 genus level in subsequent analyses.

292

293 Alpha diversity was assessed using the Shannon and Simpson indices, and their relationships with sex,
294 tank, and survival status were examined using Kruskal-Wallis tests. The results showed that neither sex
295 nor survival status significantly influenced Shannon diversity ($p = 0.39$ and $p = 0.39$, respectively).
296 However, tank identity exhibited a highly significant effect on Shannon diversity ($p = 0.00049$), suggesting
297 that environmental conditions played a dominant role in shaping microbial diversity. Similar trends were
298 observed for the Simpson index, with tank identity again showing a significant effect ($p = 0.00041$), while
299 sex and survival status had no significant impact ($p = 0.24$ and $p = 0.27$, respectively) (**Figure 2B**). Tank
300 C1 exhibited higher overall diversity and a more balanced community structure compared to C2, where
301 a smaller number of dominant taxa contributed disproportionately to the community composition. This
302 pattern aligns with the observations in **Figure 2A**, further supporting the notion that tank-specific
303 environmental factors shaped microbial diversity.

304

305 Despite the observed differences in microbial diversity between tanks, survival analysis suggested that
306 these environmental differences did not directly influence mortality rates. Chi-squared tests using the
307 total fish record (637 fish, recorded in November 2021 with all the information) for survival status across
308 tanks and sex categories yielded non-significant results ($p = 0.51$ for tank, $p = 0.38$ for sex), indicating
309 that while tank identity influenced microbial community composition, it did not translate into a detectable
310 effect on survival outcomes.

311

312 To identify taxa contributing to the observed differences in diversity between tanks, SIMPER analysis
313 highlighted a subset of taxa with the lowest adjusted p-values ($padj < 0.05$). A total of 207 families were
314 identified as contributing to tank-specific microbial differentiation. Notably, 85 of these belonged to
315 functionally uncharacterized metagenomic lineages, including f__UBA2385, f__UBA2392, and

316 f__UBA2428. The prevalence of these uncharacterized taxa suggests that they may play a critical but
317 currently unknown role in shaping microbial community structure and ecological dynamics across tanks.

318

319 Beta diversity analysis was conducted using Bray-Curtis and Jaccard distance matrices, followed by
320 PERMANOVA tests to assess the effects of sex, tank, and survival status. The Bray-Curtis-based
321 analysis indicated that sex had no significant effect on microbial composition ($p = 0.49$), while tank identity
322 exerted a strong influence ($p = 0.001$), confirming the results from alpha diversity analysis. Survival status
323 had no significant effect ($p = 0.53$).

324 The Jaccard distance-based analysis further supported these findings, with tank identity showing no
325 significant impact ($p = 0.59$) and sex remaining non-significant ($p = 0.14$). However, survival status
326 exhibited a marginally significant effect ($p = 0.025$), suggesting that while overall microbial diversity was
327 not strongly linked to survival, specific microbial taxa might still contribute to differences between
328 survivors and non-survivors. However, the effect size was relatively small, as indicated by the low R^2
329 value (0.059) from the PERMANOVA analysis.

330

331 To visualize these patterns, non-metric multidimensional scaling (NMDS) was initially attempted, but even
332 with $k = 10$ dimensions, the stress value remained extremely high (2.3285), suggesting that the underlying
333 data structure was too complex for effective dimensionality reduction. Instead, a principal component
334 analysis (PCA) was performed, which revealed clustering patterns by survival status. The PCA results
335 showed that PC1 accounted for 39.57% of the variance, while PC2 accounted for 8.3%, providing a more
336 informative representation compared to NMDS (MDS1: 18.03%, MDS2: 9.83%) (**Figure 3A**).
337 Nonetheless, PCA did not fully capture the variation in microbial composition, highlighting the complexity
338 of the dataset.

339

340 **Survival-Associated Taxa Based on Presence-Absence Analysis**

341 Given that the Jaccard-based analysis indicated a significant survival effect, we hypothesized that
342 presence-absence patterns of certain taxa, rather than their relative taxonomic read count, may be key
343 determinants of disease outcomes. To test this, we calculated the proportion of individuals within each
344 survival group that tested positive for each taxon and visualized the differences using histograms (**Figure**
345 **3B**). Wilcoxon rank-sum tests were conducted to assess differences in taxon presence between groups.
346 A significant effect was observed for tank identity ($p = 4.89e-10$), reinforcing its role in shaping microbial
347 community structure. However, sex showed no significant effect ($p = 0.077$), while survival status
348 exhibited a strong effect ($p = 4.53e-05$), further supporting the idea that specific taxa are
349 disproportionately associated with mortality.

350 To quantify the asymmetry in taxon prevalence between groups, we examined the distribution of
351 presence-absence differences. A skewness analysis indicated a strong right-tailed distribution (skew =
352 11.615, $p < 2.2e-16$), confirming that taxa more commonly observed in deceased individuals were nearly
353 twice as numerous as those enriched in survivors (698 vs. 360 taxa). This was further supported by a
354 Kolmogorov-Smirnov test ($D = 1$, $p < 2.2e-16$), indicating a significant divergence in distribution.

355 To further investigate taxa associated with survival status, a presence-absence analysis was conducted
356 using Barnard's exact test. However, traditional multiple testing correction methods, such as the
357 Benjamini-Hochberg procedure, were overly stringent ($\pi0$ estimate = 0.90), as the method assumes a
358 high proportion of null hypotheses. This conservative approach led to no significant taxa remaining after
359 adjustment. Specifically, the high $\pi0$ estimate indicated that the majority of tests were expected to reflect
360 true null hypotheses, causing the correction to be excessively stringent and excluding potential true
361 positives. To address this, an Empirical Bayes approach implemented in the ashR package (Stephens
362 2017) was applied, refining the detection of survival-associated taxa and reducing the number of
363 candidate taxa from 32 to 16 for further, genus-level downstream analysis (**Table 3A**).

364 Among the taxa with survival associations, several groups emerged, including a substantial proportion of
365 uncharacterized lineages (e.g., f__UBA4486, f__UBA9219). Additionally, some well-characterized taxa
366 were identified, including Aerococcaceae and Sedimentibacteraceae, a potential indicator of water quality
367 conditions (Fan *et al.* 2023). Nectriaceae, a fungal family associated with infection risks (Ulaszewski *et*
368 *al.* 2025), and Peduoviridae, a bacterial viruses family whose genome incorporates into the bacterial
369 genome (Wang, Xie and Huang 2024), potentially influencing microbiome stability (**Figure 4**).

370

371 Building on the family-level findings, we next examined microbial composition at the genus level to further
372 characterize taxa with potential functional or ecological relevance. At the genus level, some highly
373 represented taxa were identified. *Arthrobacter_D* (Micrococcaceae) was the dominant genus, accounting
374 for 19.87% of the total reads (**Table 2B**). This was followed by several uncharacterized genera, including
375 *g__UKL13-3, g__UBA9655*, and another unidentified *g__UBA9655*, which remain insufficiently
376 understood but were consistently present at high taxonomic read count. Among the well-characterized
377 genera, some stood out due to their potential roles in fish health and aquaculture environments.
378 *Pseudomonas_E* (Pseudomonadaceae), for instance, is known to be associated with waterborne
379 infections, making it a potential risk factor for fish disease (Rahman *et al.* 2024). *Serratia*
380 (Enterobacteriaceae), an opportunistic pathogen in humans and animals, was also notable due to its
381 presence in the aquatic environment (Mahlen 2011). Similarly, *Escherichia* (Enterobacteriaceae), while
382 often a commensal gut bacterium, includes pathogenic strains such as *E. coli*, which could potentially
383 impact fish health (Mumbo *et al.* 2023).

384 To further investigate the microbial differences between tanks, Mann-Whitney U tests were performed at
385 the genus level, comparing Tank C1 and C2. Genera that showed significant differences (adjusted $p <$
386 0.05 using BH correction) included taxa associated with environmental and metabolic functions (**Table**
387 **4**). Methylophilaceae (*CABHOJ01*) was one such genus, known for its role in one-carbon metabolism,
388 particularly in methanol or methylated compound cycling (Taubert *et al.* 2019). Ktedonobacteraceae
389 (*JALYTT01)*, generally a soil-associated bacterium (Gschwend *et al.* 2021) was also detected,

390 suggesting a possible role in organic matter degradation within the aquatic environment. Another
391 particularly interesting genus was Burkholderiaceae_A (*Kinetoplastibacterium*), which can be found as
392 an endosymbiont of protozoa (Alves *et al.* 2013). The detection of this genus in fish-associated microbial
393 communities raises interesting questions about potential host-protozoan interactions and whether certain
394 protozoa might be influencing microbial community structures in the tanks. Among the host-associated
395 taxa, Microbacteriaceae (*Microbacterium*) is able to thrive in both environmental and host-associated
396 niches, potentially playing a role in organic matter processing or host-microbe interactions (Wippel *et al.*
397 2021). Acetobacteraceae (*BOG-930*), generally associated with wine and vinegar fermentation, was also
398 present (Komagata, Iino and Yamada 2014), which might indicate shifts in fermentation-based metabolic
399 processes or pH fluctuations in the tank environment.

400 Next, genera associated with survival outcomes were examined. Barnard's exact test was applied, but
401 due to the stringent nature of multiple testing corrections, no taxa remained statistically significant after
402 Benjamini-Hochberg adjustment. However, using nominal *p*-values, several genera were observed to
403 differ between survivors and non-survivors. *Demequina*, which is often identified in aquaculture species
404 (Pedrosa-Gerasmio *et al.* 2020), was more detected in the non-surviving group, suggesting that its
405 presence may be linked to disease susceptibility. Similarly, *Fusarium*, a fungal genus that includes
406 opportunistic pathogens, was more frequently observed in non-surviving fish, indicating its potential
407 involvement in disease progression or secondary infections. *Sedimentibacter*, an anaerobic bacterium
408 associated with organic matter degradation, was also enriched in non-surviving fish, which could reflect
409 differences in microbial community structure that associated with host health deterioration (Huang *et al.*
410 2016) (**Table 3B, Figure 4**).

411

412 **Transcriptional Signals Reveal Functional Differences Between Survival Groups**

413 Sequence reads from all samples were merged, and sequences corresponding to vertebrates (Atlantic
414 salmon and human) were removed before contig assembly. The assembled contigs had the following

415 characteristics: a total of 16,037 contigs with 7,255,640 bases, an N50 value of 434, and a mean contig
416 length of 452.43 (SE= 4.34) base pairs.

417

418 In line with the earlier taxonomic analysis, standard differential gene expression analysis using DESeq2
419 did not identify any genes that survived Benjamini-Hochberg (BH) correction for significance between the
420 survived and died groups. This is likely due to the stringent multiple testing threshold for such a dataset.
421 To address this, we reanalyzed the data by converting gene presence/absence into a binary format and
422 applied Barnard's test. This approach identified 31 differentially observed genes with nominal $P < 0.05$,
423 which were functionally annotated using DRAM (especially Pfam returned the best comprehensive
424 annotation to this dataset). To determine which microorganisms were responsible for expressing these
425 genes, we performed a BLAST search, revealing that *Achromobacter, Escherichia coli, Pseudomonas,*
426 *Stenotrophomonas,* and *Serratia* exhibited highly polarized expression patterns across the survived and
427 died groups, where a transcript was frequently absent in one group while being widely present in the
428 other. As previously observed, these bacterial taxa were commonly observed across all groups (**Table**
429 **2B**).

430

431 **Figure 5** illustrates transcript read counts (TPM) for a subset of differentially observed microbial genes,
432 while the complete list of genes is provided in **Table 5.** In *Achromobacter,* the died group exhibited
433 increased expression of genes related to sugar transporters, aldehyde dehydrogenase, and bacterial
434 extracellular solute-binding proteins, whereas the survived group showed increased expression of genes
435 associated with *TonB-dependent receptors, AcrB/AcrD/AcrF efflux systems*, and proline dehydrogenase,
436 suggesting a shift in metabolic strategies. Similarly, in *Escherichia coli*, the died group showed an
437 upregulation of peroxidase genes, indicative of a potential response to oxidative stress, whereas in the
438 survived group, genes encoding *phage minor tail protein U* were more abundant, implying active
439 bacteriophage interactions. For Pseudomonas, the died group showed increased expression of DNA

topoisomerase, a gene involved in DNA replication and repair, while the survived group exhibited higher expression of RadC-like JAB domain, another gene potentially linked to DNA repair, though its precise functional role remains unclear. In *Stenotrophomonas*, the died group expressed genes belonging to the POT family (peptide transporters), while the survived group exhibited increased expression of transketolase and pyrimidine binding proteins, suggesting a potential shift in metabolic preferences (Patil *et al.* 2021). For Serratia, the survived group displayed increased expression of multiple genes, including *SecY (membrane protein transport), His Kinase A domain (signal transduction), Flavodoxin-like proteins (redox reactions), and Holliday junction DNA helicase (DNA repair and recombination mechanisms)*, indicating possible functional advantages under the fish-survival-associated conditions (Iguchi *et al.* 2014).

Notably, *Achromobacter phage* was more frequently observed in the survived group, suggesting a potential association between phage activity and bacterial dynamics under different host conditions (Hoyle *et al.* 2018). These findings indicate that bacterial communities exhibit distinct transcriptional responses depending on host status, with potential implications for disease progression, microbial adaptation, and host-microbe interactions.

Discussion

Metatranscriptome as a Tool for Microbial Ecology and Disease Risk Assessment in Aquaculture

This study conducted a metatranscriptomic analysis using RNA-seq data collected from Atlantic salmon gills before a disease outbreak in aquaculture. The goal was to determine whether microbial transcriptional profiles could provide insights into environmental conditions and potential health risks, as well as to understand the microbial dynamics before disease emergence. While our study demonstrates that microbial taxonomic and functional signals can be extracted from poly(A)-selected RNA-seq data, we acknowledge that this approach is inherently limited by selective retention of transcripts. Microorganisms lacking polyadenylated transcripts may be underrepresented or entirely absent from the

465 dataset, potentially excluding key indicator taxa. Although we reasoned that the poly(A) selection bias is
466 systematic across samples, it does not fully eliminate the concern that certain microbial groups—
467 especially those with critical ecological roles—might be disproportionately filtered out. Thus, our findings
468 should be interpreted as reflecting patterns among the subset of microbial taxa that are detectable within
469 this data structure, rather than as a comprehensive view of the microbiome. Future studies integrating
470 rRNA-based or total RNA approaches will be crucial to validate and expand on these insights.

471 Through this retrospective metatranscriptomic analysis, we observed microbial changes before disease
472 outbreaks in aquaculture. By leveraging RNA-seq data collected before clinical disease emergence,
473 microbial shifts associated with survival outcomes were detected. In particular, presence-absence
474 analysis revealed taxa that were associated with mortality risk, suggesting that community shifts are not
475 solely driven by statistical changes in relative taxonomic read count. The right-tailed distribution of
476 presence-absence differences, with taxa more frequently found in non-surviving fish nearly doubling
477 those enriched in survivors, suggests the potential contribution of specific microbial members to disease
478 susceptibility. Furthermore, numerous characterized bacterial taxa (e.g., f__UBA9655, f__UKL13-3) were
479 detected, emphasizing the need for further biological investigation of these insufficiently understood
480 microbial lineages(Zamkovaya *et al.* 2021). While tracking individual microbial dynamics remains
481 complex, our findings suggest that monitoring functionally relevant taxa could provide insights into
482 disease susceptibility and aquaculture ecosystem(Leray *et al.* 2021). In the following sections, we discuss
483 these findings, focusing on the relationship between microbial composition and survival outcomes,
484 functional shifts in microbial transcriptional activity, and the role of environmental factors in shaping
485 microbial diversity.
486

487 Our study demonstrates that publicly available poly(A)-selected RNA-seq datasets, originally generated
488 for host transcriptomics, can be repurposed for microbial metatranscriptomic analysis. While poly(A)
489 selection introduces constraints, most notably the exclusion of rRNA-derived taxonomic markers, it also
490 enables the capture of actively transcribed microbial mRNA, providing functional insights that
491 complement traditional 16S rRNA-based profiling. The strong correlation observed between Bracken-

492 based abundance estimates and MATAM-based rRNA profiles suggests that despite the absence of
493 rRNA, meaningful taxonomic classification can still be achieved when analyzing microbial mRNA.

494 One important consideration is that poly(A) selection may differentially retain microbial transcripts
495 depending on the extent of polyadenylation (Hajnsdorf and Kaberdin 2018). While some bacterial
496 transcripts are naturally polyadenylated as part of RNA degradation pathways, others may be
497 underrepresented due to the lack of poly(A) tails(Mofayezi *et al.* 2024). This potential bias should be
498 acknowledged when interpreting taxonomic and functional profiles derived from poly(A)-selected
499 datasets. However, given that any such bias is likely to be systematic across all samples, comparative
500 analyses, such as those between surviving and non-surviving fish, remain valid.

501 One important limitation is the lack of 16S rRNA data, which makes it difficult to directly confirm taxonomic
502 identities using standard phylogenetic approaches. Although this does not prevent meaningful microbial
503 profiling, it does require careful attention to the methods used. Future studies aiming to maximize
504 microbial resolution in aquaculture metatranscriptomics may benefit from hybrid approaches that
505 integrate poly(A)-selected RNA-seq with targeted rRNA sequencing or metagenomic methods.

506 Despite these limitations, our findings highlight the potential of repurposing host RNA-seq datasets for
507 microbial analysis, particularly in retrospective studies where dedicated metatranscriptomic data may not
508 be available. Given the increasing availability of RNA-seq datasets across diverse aquaculture settings,
509 further exploration of poly(A)-selected metatranscriptomics could provide valuable insights into microbial
510 dynamics and their role in water environment and disease susceptibility.

511

512 **Environmental Influence on Microbial Community Structure**

513 An unexpected key finding of this study was that microbial diversity and community composition varied
514 between tanks, despite the absence of intentional environmental modifications. This raises a question:
515 what factors might have contributed to the observed differences? One possibility is that unmonitored
516 environmental variations played a role in shaping microbial community structure. In closed aquaculture

517 systems, small-scale differences in water flow, temperature microgradients, microbial seeding effects, or
518 organic matter accumulation can drive shifts in microbial communities (Testerman *et al.* 2022). Previous
519 research has shown that biofilm development on tank surfaces and infrastructure can serve as reservoirs
520 for microbial colonization, influencing the microbial load in the surrounding water and subsequently
521 affecting the fish microbiome (Dang and Lovell 2016; Dahle *et al.* 2020, 2023) (Dang and Lovell 2016;
522 Dahle *et al.* 2023). Differences in microbial succession during early rearing stages, even if tanks started
523 with the same water source, could have resulted in distinct community structures over time. While it was
524 impossible to pinpoint specific environmental drivers using current datasets, the observed tank-
525 dependent microbial differentiation suggests that environmental heterogeneity, even in controlled
526 aquaculture settings, plays a substantial role in shaping microbial community structure. This highlights
527 the need for longitudinal environmental monitoring, including water physicochemical properties and
528 microbial colonization dynamics, to fully understand the mechanisms underlying these patterns. Also,
529 despite these differences, no direct association between tank identity and fish survival rates was
530 detected. This suggests that the compositional shifts in microbial communities did not translate into
531 differential mortality rates in this case or that the key drivers of mortality were independent of the observed
532 inter-tank variations.

533

534

535 **Taxa and Transcripts Associated With Host Disease Outcome**

536 Beyond environmental influences, our results suggest that the presence or absence of specific microbial
537 taxa was associated with survival outcomes. The enrichment of opportunistic pathogens such as
538 *Pseudomonas* and *Aeromonas* in died fish raises concerns about their potential role in disease
539 progression. These bacteria are known to exploit compromised immune defenses, which may indicate
540 that their increased prevalence reflects a state of physiological stress or immunosuppression in affected
541 fish. Fish wounds serve as entry points for such opportunistic pathogens, facilitating their colonization
542 and potentially exacerbating disease progression (Joseph *et al.* 1979; Hal and El-Barbary 2020; Wang

543 *et al.* 2022). Conversely, taxa such as Burkholderiaceae and Achromobacter-associated phages were
544 more frequently detected in survivors. Burkholderiaceae comprises metabolically versatile bacteria that
545 have been implicated in both pathogenic and beneficial interactions, raising the possibility that some
546 members of this family may contribute to microbial stability or even exert protective effects against
547 pathogens (Carrión *et al.* 2018). The presence of bacteriophages in survivors suggests that phage-
548 mediated microbial regulation may influence disease resistance by modulating bacterial populations
549 within the host-associated microbiome(Ranveer *et al.* 2024). While these associations provide intriguing
550 insights, it remains unclear whether these taxa actively influenced disease outcomes or merely reflected
551 underlying health conditions. Further experimental infection models and functional assays will be
552 necessary to better understand the role of specific microbial taxa.

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555

556 **Future Directions and Ecological Implications**

557 While this study provides valuable insights, several methodological challenges must be considered when
558 applying metatranscriptomics to aquaculture microbiome research. One of the primary limitations is the
559 reliance on host RNA-seq datasets that have undergone rRNA depletion, which removes 16S rRNA, the
560 conventional marker for microbial taxonomic classification. Although previous studies have demonstrated
561 that mRNA-based taxonomic profiling can approximate rRNA-based methods (Stewart, Ottesen and
562 DeLong 2010; Ranjan *et al.* 2021; Zhang *et al.* 2021), differences in transcript taxonomic read count and
563 turnover rates may introduce biases in microbial community representation. To solidify this limitation,
564 future follow-up studies could integrate rRNA-based sequencing with metatranscriptomics to provide a
565 more comprehensive assessment of microbial community dynamics.
566

567 Though these sequences were excluded from downstream microbial analysis, we observed major
568 histocompatibility complex (MHC) sequences that were absent in the reference genome but reported in

569 NCBI. Their presence suggests that conventional reference genome-based mapping may fail to detect
570 certain variants, particularly in the MHC region, due to its extreme diversity. This issue is widely
571 recognized across vertebrates, including humans and other model organisms, where highly polymorphic
572 immune-related loci often escape detection in standard genome-wide analyses (Okada *et al.* 2015; He,
573 Minias and Dunn 2021; Luo *et al.* 2021; Heimeier *et al.* 2024). Parallel selection on MHC regions has
574 been observed in aquaculture populations of Atlantic salmon, including this Western Norway aquaculture
575 population (Buso *et al.* 2024), suggesting that such variation may be relevant to disease resistance.
576 Although investigating host genomic variation is beyond the scope of this study, it is likely that additional
577 MHC diversity exists within this population. Future studies focusing on host-pathogen interactions may
578 benefit from targeted sequencing approaches to fully capture non-reference MHC alleles and their
579 potential role in host immune response.

580

581 While we identified microbial signatures associated with survival outcomes, it remains unclear whether
582 these shifts occurred as a direct cause of disease susceptibility or as a secondary consequence of host
583 physiology. To address this, future research should prioritize longitudinal studies that track microbial
584 dynamics over time, from early life stages through disease outbreaks, to identify robust microbial
585 biomarkers for disease prediction. Experimental validation through controlled infection trials also could
586 provide mechanistic insights into the role of specific microbial taxa in disease emergence and
587 transmission.

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592 **Tables and Figures**

593

594 **Table 1. Summary of individual-level metadata used in this study.**

595 This table lists sex, tank identity, survival outcome, and skin lesion status for each fish included in the
596 analysis. It also includes raw and unmapped RNA-seq read counts used for downstream
597 metatranscriptomic profiling. The skin condition column indicates whether the fish exhibited visible
598 wounds at the time of sampling. The Unmapped column represents the number of unmapped RNA-seq
599 reads, which potentially contain non-host microbial sequences, while the Raw column indicates the total
600 number of raw sequencing reads per sample. Samples were selected to ensure representation across
601 key biological variables while maximizing available high-quality metatranscriptome data.

				Table1 Sample info				
ID	ENA_ID	Sex	Tank	Status	Skin	Mapped_Reads	Unmapped_Reads	Ratio
ns_1	Plate22H2	Female	C1	not survived	wounds	21,843,548	92,009	0.00421
ns_10	Plate16F11	Female	C2	not survived	wounds	25,731,837	74,096	0.00288
ns_11	Plate18H4	Male	C2	not survived	wounds	26,733,388	51,610	0.00193
ns_12	Plate20D7	Male	C2	not survived	wounds	20,369,628	254,381	0.01249
ns_13	Plate19H1	Female	C2	not survived	wounds	26,574,632	43,839	0.00165
ns_2	Plate22B6	Female	C1	not survived	wounds	20,556,179	92,061	0.00448
ns_3	Plate24C8	Female	C1	not survived	wounds	25,453,351	805,946	0.03166
ns_4	Plate25A11	Male	C1	not survived	wounds	21,969,245	951,660	0.04332
ns_5	Plate19C6	Female	C2	not survived	wounds	21,485,507	387,453	0.01803
ns_6	Plate20D6	Male	C2	not survived	wounds	31,966,933	43,896	0.00137
ns_7	Plate21C2	Male	C2	not survived	wounds	24,339,317	130,984	0.00538
ns_8	Plate17A12	Male	C2	not survived	wounds	28,818,781	64,005	0.00222
ns_9	Plate17F12	Female	C2	not survived	wounds	30,032,409	97,111	0.00323
s_1	Plate21C10	Female	C1	survived	no-wounds	23,609,879	36,054	0.00153
s_10	Plate20H3	Male	C2	survived	no-wounds	21,128,196	104,443	0.00494
s_11	Plate22D4	Male	C1	survived	no-wounds	25,073,829	144,122	0.00575
s_12	Plate21E10	Male	C1	survived	no-wounds	22,723,648	200,530	0.00882
s_13	Plate16D5	Female	C2	survived	no-wounds	23,069,585	66,850	0.00290
s_14	Plate18E4	Female	C2	survived	no-wounds	22,873,120	46,668	0.00204
s_15	Plate17H5	Male	C2	survived	no-wounds	27,028,680	58,394	0.00216
s_2	Plate21E5	Female	C1	survived	no-wounds	28,229,183	75,661	0.00268
s_3	Plate24G6	Male	C1	survived	no-wounds	25,547,504	242,683	0.00950
s_4	Plate22A10	Male	C1	survived	no-wounds	26,715,029	128,125	0.00480
s_5	Plate18E12	Female	C2	survived	no-wounds	23,150,040	84,397	0.00365
s_6	Plate19E12	Female	C2	survived	no-wounds	26,662,780	115,416	0.00433
s_7	Plate25H1	Female	C1	survived	no-wounds	27,517,530	500,383	0.01818
s_8	Plate19F2	Male	C2	survived	no-wounds	24,271,043	280,739	0.01157
s_9	Plate24C3	Female	C1	survived	no-wounds	25,564,010	106,096	0.00415

602

603 **Table 2. Top transcriptionally represented microbial taxa in Atlantic salmon gill**
604 **metatranscriptomes.** (A) Top 20 most transcriptionally abundant microbial families across all 28
605 samples. (B) Top 20 most transcriptionally abundant microbial genera across all 28 samples. Taxonomic
606 abundance was estimated using Bracken based on Kraken2 assignments.

Table 2A

Family	Read Percentage
Micrococcaceae	20.34
f__UBA9655	17.18
f__UKL13-3	12.89
Vampirovibrionaceae	7.23
Burkholderiaceae	6.12
Caulobacteraceae	3.87
Enterobacteriaceae	3.59
Cellulosilyticaceae	3.33
Pseudomonadaceae	2.63
Moraxellaceae	2.23
Xanthomonadaceae	1.56
Lachnospiraceae	1.49
DSM-18226	1.12
Sphingomonadaceae	1.05
Xanthobacteraceae	0.87
Rhodocyclaceae	0.70
Nostocaceae	0.65
Rhizobiaceae	0.63
Beijerinckiaceae	0.58
Nitrincolaceae	0.48

Table 2B

Genus	Family	Read Percentage
Arthrobacter_D	Micrococcaceae	19.9
CAISCU01	f__UKL13-3	13.0
JACPNY01	f__UBA9655	8.8
g__UBA9655	f__UBA9655	8.2
Vampirovibrio	Vampirovibrionaceae	7.3
Brevundimonas	Caulobacteraceae	3.6
Epulopiscium_A	Cellulosilyticaceae	3.4
Pseudomonas_E	Pseudomonadaceae	2.3
Acinetobacter	Moraxellaceae	2.1
Serratia	Enterobacteriaceae	2.0
Comamonas	Burkholderiaceae	1.8
Achromobacter	Burkholderiaceae	1.7
Stenotrophomonas	Xanthomonadaceae	1.4
Escherichia	Enterobacteriaceae	1.2
Neobacillus	DSM-18226	1.1
Wujia	Lachnospiraceae	1.1
Sphingomonas	Sphingomonadaceae	0.8
Nostoc_B	Nostocaceae	0.6
Agrobacterium	Rhizobiaceae	0.5
Methylobacterium	Beijerinckiaceae	0.5

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608

609 **Table 3. Microbial genera showing differences in relative transcript representation between Tank C1 and Tank C2.**

610 Genus-level comparisons were conducted using Mann–Whitney U tests. P-values were adjusted with the Benjamini-Hochberg method. Only

611 genera with adjusted $p < 0.05$ are shown.

612

		Table 3			
Genus	**p_value**	**p_adjusted**	**Family**	**Relative_reads_TankC1**	**Relative_reads_TankC2**
AGPOR5	0.0007304	0.0246	f__WGA-3G	4	0
CABHOJ01	0.00066066	0.0246	Methylophilaceae	6	0
CANMOZ01	0.00051346	0.0246	DSM-6294	11	2
Caedimonas	0.00068318	0.0246	Caedimonadaceae	40	5
JACDDX01	0.00063682	0.0246	GWC2-71-9	53	5
JACPNY01	0.00013181	0.0246	f__UBA9655	74	5
JALYTT01	0.00026971	0.0246	Ktedonobacteraceae	5	0
Kinetoplastibacterium	0.00018121	0.0246	Burkholderiaceae_A	12198	21481
Microbacterium	0.00027474	0.0246	Microbacteriaceae	2	0
Sporocytophaga	0.00050941	0.0246	Cytophagaceae	20	2
UBA1952	0.00038959	0.0246	Chitinophagaceae	1091	143
g__JASGCL01	0.00056538	0.0246	f__JASGCL01	17	3
g__UBA9655	0.00054034	0.0246	f__UBA9655	107	5
g__JAOIVE01	0.0011377	0.0357	f__JAOIVE01	121	14
Bin55	0.00128023	0.0377	Azotimanducaceae	75	6
BOG-930	0.00152158	0.0422	Acetobacteraceae	11292	20107

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Table 4. Microbial taxa associated with survival status in Atlantic salmon.

Taxa were identified based on presence-absence analysis using Barnard's exact test. Only taxa with nominal $p < 0.05$ are shown. Includes both characterized and unclassified microbial lineages.

Table 4A

Taxon_name_family		Positive_Not Survived	Positive_Survived	Negative_Not Survived	Negative_Survived	Rate_not_survived	Rate_survived	Difference	Barnard_p	lfdr	svalue	PosteriorMean
Aerococcaceae		8	0	5	15	0.62	0	0.62	0.000	0.023	0.007	0.609
Alcanivoracaceae		6	0	7	15	0.46	0	0.46	0.004	0.037	0.008	0.560
Demequinaceae		1	7	12	8	0.08	0.47	-0.39	0.005	0.038	0.008	0.556
Gorgyraeaceae		9	3	4	12	0.69	0.2	0.49	0.005	0.039	0.008	0.554
Kineococcaceae		8	3	5	12	0.62	0.2	0.42	0.007	0.041	0.009	0.548
Nectriaceae		8	2	5	13	0.62	0.13	0.48	0.007	0.041	0.009	0.548
Peduoviridae		12	7	1	8	0.92	0.47	0.46	0.008	0.043	0.009	0.543
Sedimentibacteraceae		8	15	5	0	0.62	1	-0.38	0.012	0.047	0.010	0.531
f__CAG-698		10	5	3	10	0.77	0.33	0.44	0.013	0.047	0.010	0.529
f__JAAEKA01		6	13	7	2	0.46	0.87	-0.41	0.016	0.049	0.011	0.525
f__JABDET01		7	2	6	13	0.54	0.13	0.41	0.016	0.049	0.011	0.525
f__JAJDZL01		7	2	6	13	0.54	0.13	0.41	0.016	0.049	0.011	0.525
f__JAMCZE01		1	6	12	9	0.08	0.4	-0.32	0.016	0.049	0.012	0.524
f__SZAS-18		4	11	9	4	0.31	0.73	-0.43	0.016	0.049	0.012	0.524
f__UBA4486		9	4	4	11	0.69	0.27	0.43	0.016	0.049	0.013	0.524
f__UBA9219		9	5	4	10	0.69	0.33	0.36	0.016	0.049	0.013	0.524

Table 4B

Taxon_name_family	Taxon_name_genus	Positive_Not Survived	Positive_Survived	Negative_Not Survived	Negative_Survived	Rate_not_survived	Rate_survived	Difference	Barnard_p
Sedimentibacteraceae	*Sedimentibacter*	6	1	7	14	0.46	0.07	0.39	0.01
Demequinaceae	*Demequina*	10	5	3	10	0.77	0.33	0.44	0.01
f__JAAEKA01	*g__JAAEKA01*	0	5	13	10	0.00	0.33	-0.33	0.01
f__JAJDZL01	*g__JAJDZL01*	7	2	6	13	0.54	0.13	0.41	0.01
Nectriaceae	*Fusarium*	9	4	4	11	0.69	0.27	0.43	0.02
Peduoviridae	*uncharacterized*	5	12	8	3	0.38	0.8	-0.42	0.02
Alcanivoracaceae	*Isoalcanivorax*	1	6	12	9	0.08	0.4	-0.32	0.03
Aerococcaceae	*WM01*	3	0	10	15	0.23	0	0.23	0.04
f__UBA4486	*uncharacterized*	8	4	5	11	0.62	0.27	0.35	0.05

629 **Table 5 Differentially observed microbial transcripts between surviving and non-surviving Atlantic salmon.** Transcripts were identified

630 using binary presence-absence calls and Barnard's test, with gene annotations provided by DRAM and Pfam. Taxonomic origin was

631 determined using BLAST alignment. Only transcripts with nominal $p < 0.05$ are included.

Sequence	Not Survived_positive	Not Survived_negative	Survived_positive	Survived_negative	Taxa	pfam_hits_description	pfam_ID
k141_2253_1	5	8	1	14	*Achromobacter*	Sugar transporter	PF00083.27
k141_633_1	6	7	1	14	*Achromobacter*	Aldehyde dehydrogenase	PF00171.25
k141_1992_1	6	7	1	14	*Achromobacter*	Bacterial extracellular solute-binding	PF00496.25
k141_13760_1	0	13	8	7	*Achromobacter*	TonB-dependent Receptor	PF07715.18
k141_1120_1	0	13	5	10	*Achromobacter*	AcrB/AcrD/AcrF	PF00873.22
k141_4255_2	0	13	6	9	*Achromobacter*	Proline dehydrogenase	PF01619.21
k141_2562_1	5	8	1	14	*Escherichia coli*	Peroxidase	PF00141.26
k141_3771_1	1	12	7	8	*Escherichia coli*	Phage minor tail protein U	PF06141.14
k141_6116_1	7	6	3	12	*Pseudomonas*	DNA topoisomerase	PF01131.23
k141_10189_1	0	13	6	9	*Pseudomonas*	RadC-like JAB domain	PF04002.18
k141_14202_1	5	8	0	15	*Stenotrophomonas*	POT family	PF00854.24
k141_5394_1	0	13	5	10	*Stenotrophomonas*	Transketolase, pyrimidine binding	PF02779.27
k141_14429_1	0	13	5	10	*Serratia*	SecY	PF00344.23
k141_1963_1	0	13	5	10	*Serratia*	His Kinase A domain	PF00512.28
k141_10334_1	0	13	6	9	*Serratia*	Flavodoxin like	PF02525.20
k141_129_1	0	13	5	10	*Serratia*	Holliday junction DNA helicase	PF05496.15
k141_10700_1	1	12	7	8	*Achromobacter*	CobW/HypB/UreG, nucleotide-binding domain	PF02492.22
k141_12698_1	0	13	5	10	*Achromobacter*	FecR protein	PF04773.16
k141_15084_1	1	12	6	9	*Achromobacter*	AcrB/AcrD/AcrF family	PF00873.22
k141_15128_1	1	12	7	8	*Achromobacter*	Phosphate acetyl/butaryl transferase	PF01515.22
k141_3419_2	4	9	10	5	*Achromobacter*	Oxidoreductase FAD-binding domain	PF00970.27
k141_362_2	5	8	11	4	*Achromobacter*	ABC transporter	PF00005.30
k141_10013_29	3	10	9	6	*Achromobacter phage*	3'-5' exoribonuclease Rv2179c-like domain	PF16473.8
k141_13338_1	0	13	6	9	*Escherichia coli*	UDP-glucose/GDP-mannose dehydrogenase family, central domain	PF00984.22
k141_13126_1	0	13	5	10	*Escherichia coli*	Protein of unknown function	PF13116.9
k141_8152_2	4	9	12	3	*Escherichia coli*	IS1 transposase	PF03400.16
k141_15759_1	1	12	6	9	*Pseudomonas*	LysR substrate binding domain	PF03466.23
k141_265_1	0	13	5	10	*Pseudomonas*	Monomeric isocitrate dehydrogenase	PF03971.17
k141_4916_2	5	8	1	14	*Pseudomonas*	Transmembrane secretion effector	PF05977.16
k141_12843_1	5	8	1	14	*Serratia*	AAA lid domain	PF17871.4
k141_5692_1	0	13	5	10	*Serratia*	FCD domain	PF07729.15

Figure 1 Disease representation in Atlantic Salmon A. A Died fish with a severe ulcerative lesion on the lateral body surface (highlighted in yellow) B. A survived fish with no visible external lesions.

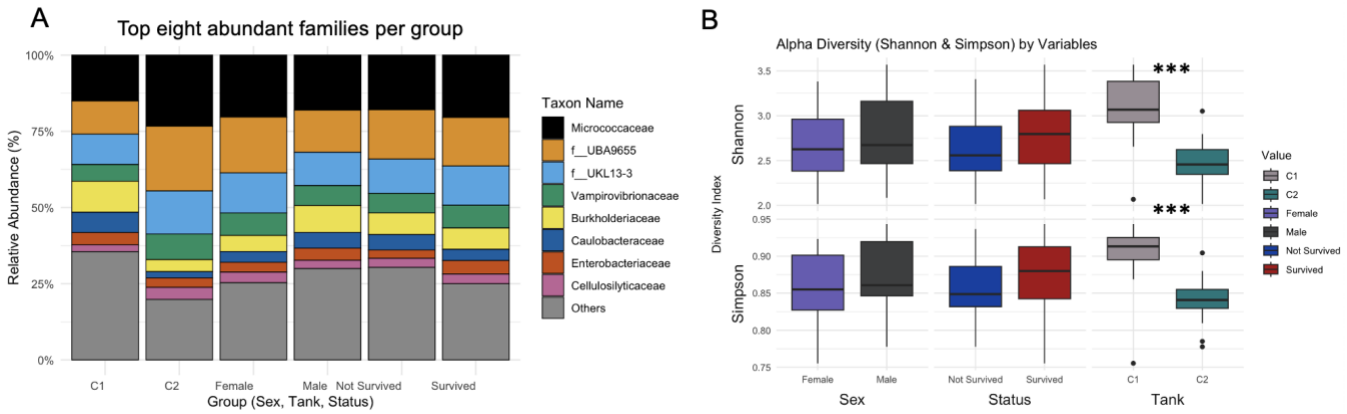


Figure 2: Taxonomic Composition and Alpha Diversity by Tank, Sex, and Survival Status

(A) The relative abundance (taxonomic read count) of microbial families aggregated by tank (C1, C2), sex (female, male), and survival status (died, survived). (B) Alpha Diversity Boxplots: Shannon (top) and Simpson (bottom) diversity indices comparing microbial diversity across sex, survival status, and tank conditions.

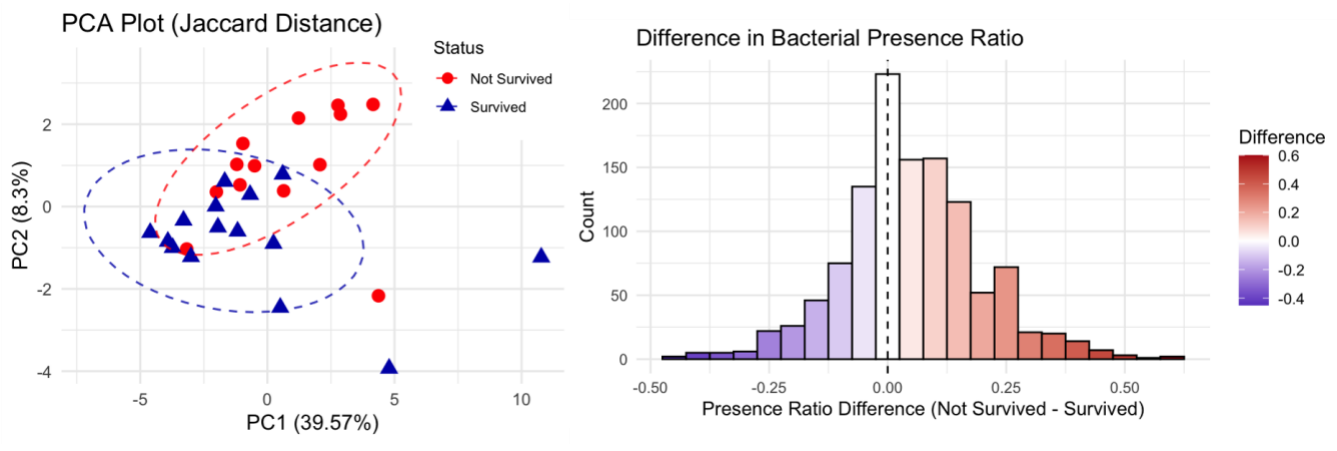


Figure 3 A Principal Component Analysis (PCA) of Microbial Community Structure

This figure presents a PCA based on Jaccard distance, colored by survival status. PC1 (39.57% variance) and PC2 (8.3% variance) capture the major differences in microbial composition.

histogram of Presence-Absence Differences Between Surviving and Non-Surviving Groups

This histogram visualizes the distribution of microbial taxa based on their presence or absence in surviving vs. non-surviving fish. B. A histogram. X-axis: Difference in presence proportion between the two groups (negative values indicate taxa more common in survivors, positive values indicate taxa more common in non-survivors). Y-axis: Number of taxa.

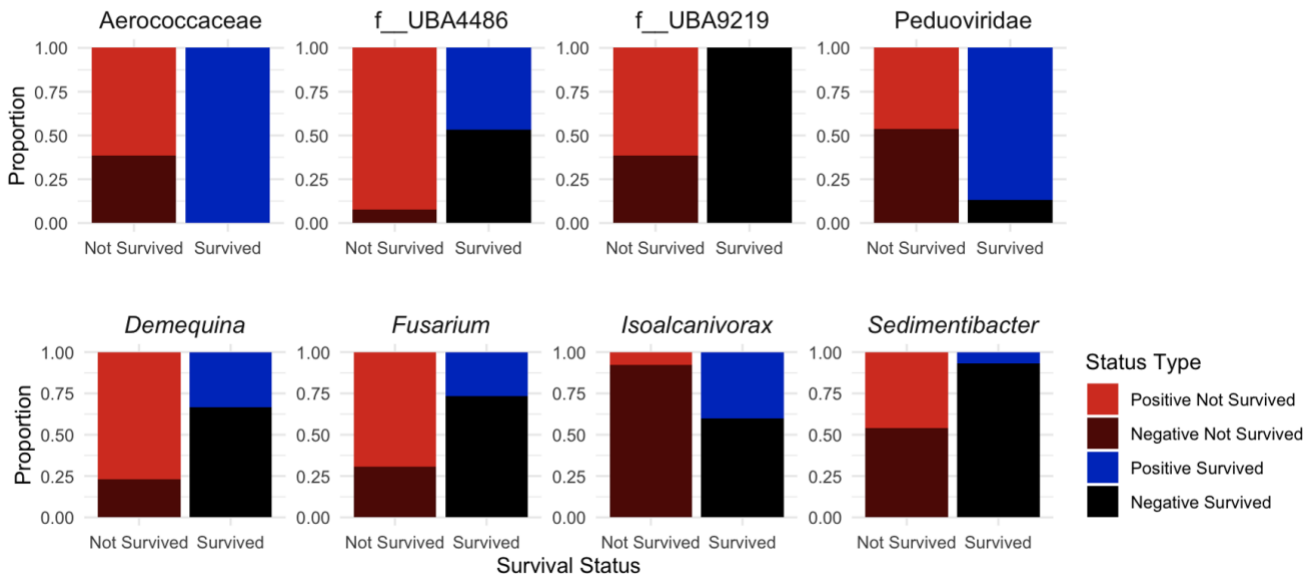


Figure 4: Proportion of fish with and without detection of taxa that differed between survivors and non-survivors

This figure displays the relative proportions of microbial genera and family significantly associated with survival outcomes in Atlantic salmon. Each bar represents the presence (blue/red) or absence (black/dark brown) of specific genera in surviving and non-surviving fish.

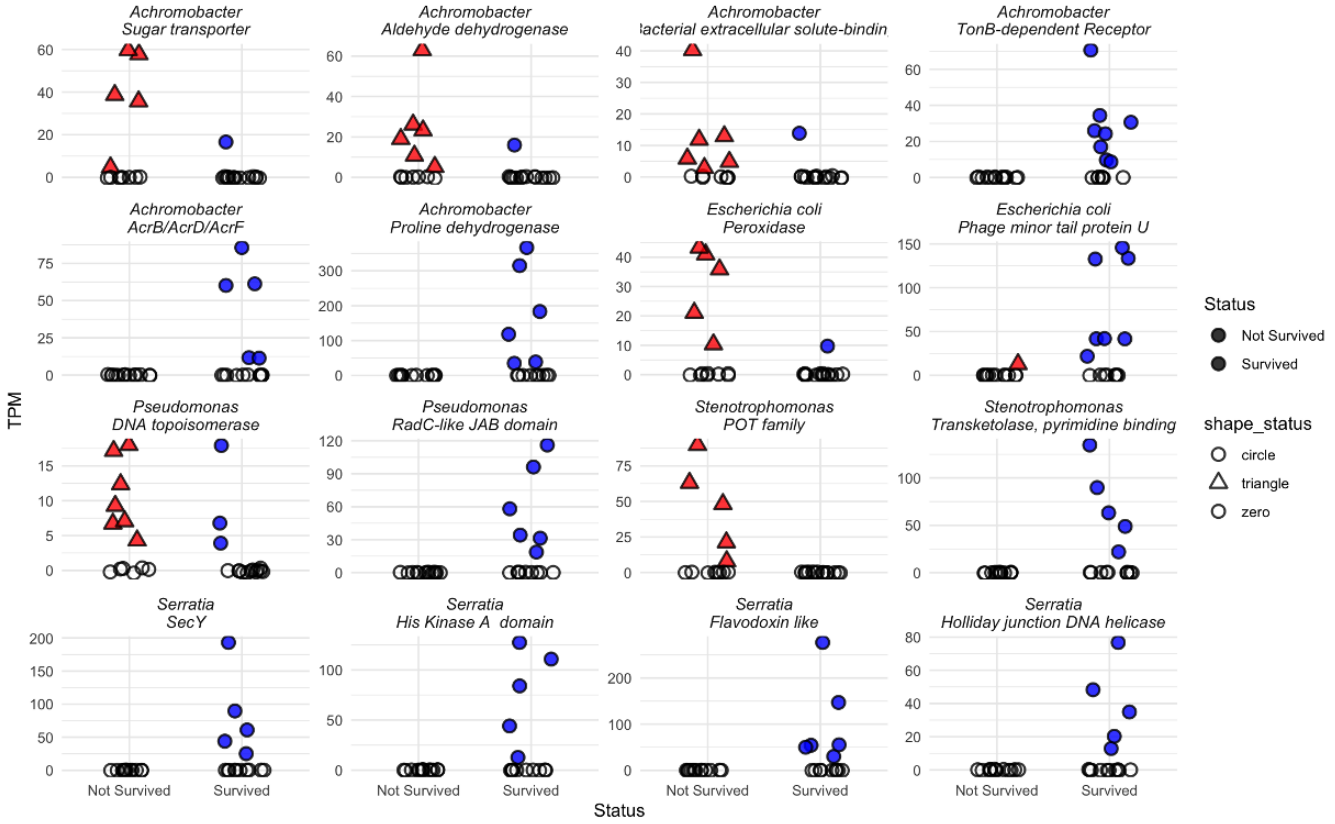
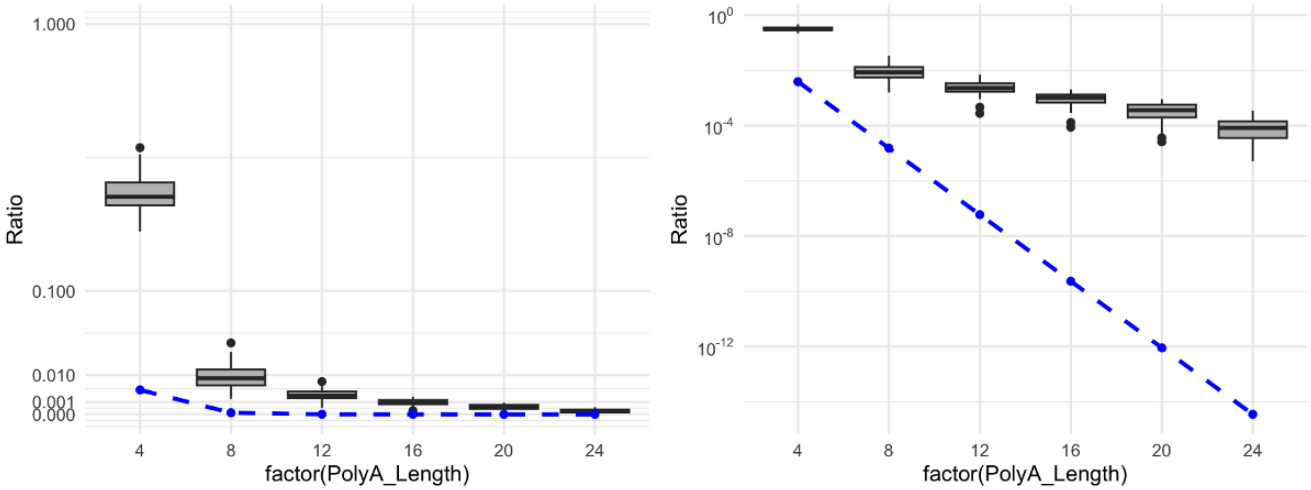


Figure 5: TPM Distribution of Differentially Expressed Genes by Survival Status

This figure illustrates the TPM distributions of differentially observed genes in microbial taxa between surviving and non-surviving Atlantic salmon.X-axis: Survival status (Died vs. Survived). Y-axis: Read count for each gene. Shapes indicate gene expression levels: Triangles: Genes with elevated expression in non-surviving fish. Circles: Genes with elevated expression in surviving fish. Open circles: Zero read count (not detected).



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681 **Figure S1. Retention of poly(A) tail sequences in RNA sequencing data.**

682 Boxplots showing the proportion of reads containing poly(A) tails of varying lengths (4 to 24 nucleotides).

683 The left panel presents the data on a square root-transformed scale, while the right panel uses a log10-

684 transformed scale. The blue dashed line represents the theoretical probability of observing random

685 poly(A) sequences.

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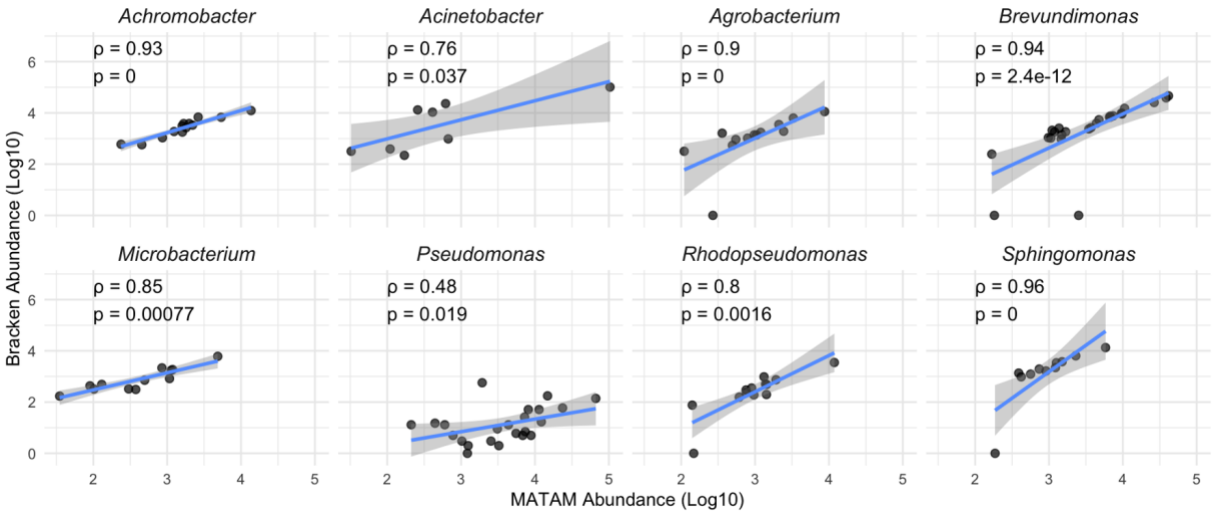

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690 **Figure S2. Correlation between microbial abundance estimates from MATAM and Bracken.**
691 Scatter plots illustrating the correlation between taxonomic abundance estimates obtained using MATAM
692 and Bracken for the top eight most abundant genera. Each panel represents a different bacterial genus,
693 with log10-transformed abundance values on both axes. The blue regression line with a shaded
694 confidence interval (95%) represents the linear trend. Spearman's correlation coefficient (ρ) and
695 corresponding *p*-values are displayed for each genus.

696

697

Author contributions (CRediT)

Akira Harding: Conceptualization, Validation, Formal analysis, Investigation

Celian Diblasi: Conceptualization, Methodology, Investigation, Writing – Original Draft

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David Hazlerigg: Resources, Funding acquisition

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Arturo Vera Ponce De Leon: Conceptualization, Methodology, Supervision

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Marie Saitou: Conceptualization, Investigation, Writing – Review & Editing, Supervision

Conflicts of interest

The authors declare no competing interests.

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