Title:

Gut microbial assembly among freshwater Atlantic salmon reared in a natural stream system during a simulated farm escape and introgression event

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Abstract

Intestinal microbial communities are influenced by a confluence of ecological forces. Understanding the dynamics between environment, microbiota and host is essential to gain insights into microbial community assembly processes. However, few studies systematically assess the contribution of different environmental sources to gut microbial community composition. We used a common garden experiment to determine the roles of biotic, abiotic and stochastic processes shaping gut microbial communities in Atlantic salmon (*Salmo salar*) in a natural river during a simulated 10-month farm escape scenario. Most of the taxa found in the salmon intestine originated from macroinvertebrates (the potential food source) rather than the water column, indicating that diet is an important factor in community assembly. The contribution of food sources to the fish gut community was lowest in winter and increased over March and May, reflecting seasonality in fish appetite. Previous work in salmon has hinted at a role for maternal effects in driving inter-generational sharing of microbial taxa. Our results suggest a possible host and/or maternal genetic effect affecting inter-individual differences in gut microbial community composition, whereby distinct assemblages were noted between farmed, wild and hybrid fish. Neutral modelling estimated that the majority (86%) of taxa present in the gut are transient. Overall, our data highlight the significance of both deterministic and stochastic drivers influencing the seasonal fluctuations of gut microbial communities in young Atlantic Salmon and hint at potential genetic or maternal effects on fish microbiota. These findings greatly enhance our understanding of the complex interactions between hosts, their living environment and associated microbiota.

Keywords

Hybrid Salmonids, Neutral Modelling, Core Microbiome, Microbial Source Tracking, Host Environment Microbiome Interaction, Freshwater Macroinvertebrate Microbiome

Introduction

Host-associated microbiota play a vital role for host health (Di Maiuta et al., 2013; Ray et al., 2012) and development (Bates et al., 2006; Llewellyn et al., 2014; Sommer & Bäckhed, 2013). Intestinal bacteria, for example, are known to facilitate digestion of otherwise inaccessible feeds (Di Maiuta et al., 2013; Ray et al., 2012), stimulate the immune system (Stagaman et al., 2017), protect the host from pathogens due to competitive exclusion (Lawley & Walker, 2013) and may even influence host-behaviour (Cusick et al., 2021; Davis et al., 2016).

The expansion of the aquaculture industry has led to an increased interest in manipulating gut microbiota to improve fish welfare and nutritional absorption capacity (Egerton et al., 2018; Perry et al., 2020). However, to induce desired microbial traits one must understand the underlying processes of microbial community assembly and its temporal development (Dittmann et al., 2017). In theory, fish acquire their intestinal microbiota from the surrounding environment, e.g., by swallowing water or due to bacteria attached to food items (Hansen & Olafsen, 1999). However, recent research indicates the possibility of maternal transmission of bacteria during birth (Rasmussen et al., 2023). As the individual matures, its gut microbial community composition is shaped by a confluence of ecological forces, which interact but can be grouped into two main factors: deterministic/selective and stochastic/neutral (Chase & Leibold, 2009; Hubbell, 2005; Stegen et al., 2012). Deterministic factors create specific conditions and selective pressures that favour the growth and colonisation of certain microbial taxa, leading to the establishment of a unique gut microbial community in each individual. Deterministic factors include host-specific factors such as genetics (Smith et al., 2015), immune response (Kelly & Salinas, 2017) and physiology (Dehler et al., 2017) as well as environmental factors such as food source and food availability (Gajardo et al., 2017; Li et al., 2022; Ringø et al., 2016), parasite presence (Llewellyn et al., 2017; Schaal et al., 2022), temperature (Ghosh et al., 2022; Kokou et al., 2018), pH (Sylvain et al., 2016) and other microbes (Coyte et al., 2015; Kokou et al., 2019). Stochastic processes, on the other hand, are not guided by

specific host or environmental factors but are rather influenced by random events, such as dispersal and ecological drift (Hanson et al., 2012; Vellend, 2010). Dispersal is a process where microorganisms are introduced to the gut from external sources, such as the environment or other individuals. Ecological drift refers to random events of microbial birth, death and replacement and can lead to variability in the gut microbial community even in the absence of strong selective pressures. In fish, neutral community assembly can explain a substantial amount of observable differences in gut microbial community structure (Burns et al., 2016; Heys et al., 2020). Despite advances in microbiome research, there are still substantial knowledge gaps regarding the dynamics of gut microbial communities over time and the origin of microbial taxa within these communities. One key unanswered question is whether the microbial taxa that are detectable in the gut are established, long-term residents, or if they are transient, externally sourced and passing through the intestine without establishing a lasting presence. To understand the dynamics of microbial taxa in the gut it is essential to investigate their sources and to determine how they contribute to the composition and variability of the gut microbiome.

Most studies investigate microbial assembly processes using laboratory models or artificial systems. Yet, insights must also be acquired from natural environments (Cusick et al., 2021) because these reflect the harsh and complex conditions by which host organisms actually live (Friberg et al., 2019). Atlantic salmon is one of the ecological and economical most important fish species worldwide and is extensively researched to examine various aspects of fish biology, aquaculture practices and ecosystem dynamics (Aas et al., 2010; Houston & Macqueen, 2019). One significant research focus revolves around the ramifications of farmed escapes from aquaculture facilities, which pose a serious threat to wild populations (Forseth et al., 2017; Thorstad et al., 2008). These escapes can have detrimental effects on wild fish due to competition for limited habitat and food resources, as well as the potential for genetic interactions through interbreeding with wild individuals (Jonsson & Jonsson, 2006; McGinnity et al., 2003; Reed et al., 2015). Most Atlantic salmon populations have an anadromous life cycle. After hatching in spring, the majority of Atlantic salmon remain in their

freshwater habitat for two years, before migrating to the ocean where they undergo most of their somatic growth (Hoar, 1988). During their juvenile phase, Atlantic Salmon face seasonal variations in food supply (e.g., macroinvertebrate type and quantity) and environmental conditions (e.g., temperature, oxygen concentration and water pH) that might directly or indirectly affect the structure of gut bacteria. Understanding the role of environmental factors determining gut microbial community composition in the wild will enable better predictions of the impact of future environmental changes on fish health in both natural and aquaculture populations. For example, changes in food availability or rising water temperatures due to global warming will likely perturb microbial communities, with direct consequences for fish survival and welfare (Harvell et al., 2002).

In the present study, we take advantage of a large-scale common garden experimental setup in the wild to investigate gut microbial community assembly and development in Atlantic salmon. In a natural river environment, we examined the gut microbiome of juvenile Atlantic salmon sampled over a 10-month period. We evaluated the role of different drivers of gut microbial assembly including abiotic variables, host genetics and water- and feed-associated microbiota. Furthermore, we used source tracking analysis to understand how the composition and abundance of environmental bacteria influences the gut microbiome throughout different seasons. In addition, we utilized abundance-occupancy distributions to estimate the importance of stochastic colonisation processes in the assembly of the gut microbial community and to determine potentially important core taxa. By exploring the intricate interplay between stochastic and deterministic factors driving community assembly, our study offers a comprehensive perspective on the ecological succession of the wild gut microbial community of juvenile Atlantic salmon.

Materials and Methods

Study area and sampling

Atlantic salmon were bred at the Marine Institute in Furnace, Newport, Co. Mayo, Ireland (53°55'22"N 9°34'18"W) located at the Burrishoole river system and consisted of four genetic groups: domesticated farmed fish (F) from the "Fanad MOWI" strain, native wild fish (W) from the Burrishoole river system and their reciprocal hybrids (denoted hybrid farmed female (HFF) and hybrid wild female (HWF, Figure 1a, b). In April 2018 at the swim-up stage, prior to the commencement of exogenous feeding fry were introduced into a section of the Srahrevagh river in the Burrishoole catchment. The experiment river consists of approximately 7520m^2 of high-quality Atlantic salmon habitat. It is contained at its upper end by a series of large waterfalls and at its lower end by a fish trap capable of capturing all life cycle stages from egg to adult. A detailed description of the system is reported in (McGinnity et al., 1997, 2003; Perry et al., 2021).

A total of 80 fish were captured in the Srahrevagh River across a 10-month period in 2019: January (n=16); March (n=14); May (n=8); June (n=13); July (n=11); November (n=18). Fish were caught via electrofishing and transported in buckets filled with oxygenated river water to the Marine Institute Newport Research Station for processing. The feeding status of all fish was unknown. All fish were euthanized by an anaesthetic overdose of methane tricaine sulphonate (MS-222, 80ml/l, FVG, Ireland) and their fork length (mm) and wet weight (g) measured. The intestines of sampled fish were dissected aseptically via an incision along the fish's ventral side. The pyloric caecum was removed, cut into pieces, put into sterile cryotubes and immediately placed on dry ice. An overview of samples taken is shown in Table S 1.

In order to identify free-living water bacteria that might serve as a dispersal source for fish gut communities, three water samples were collected at each sampling timepoint at locations at the bottom (bot), middle (mid) and top of the study section of the Srahrevagh river (Figure 1c). The

water was collected in sterile water bottles (1.5 L). Within one hour of collection the water was filtered in a sterile environment using 0.2µm filters (Whatman, Chicago, IL, USA) at the Marine Institute Newport Research Station. After filtration the filter papers were placed into cryotubes, immediately placed on dry ice and stored at -80°C.

To evaluate the potential contribution of prey organisms to gut microbial community composition, a sample of the macroinvertebrate community was collected at each fish sampling timepoint. Macroinvertebrate samples were retrieved from the Srahrevagh river using a surber sampler (Surber, 1937). The surber sampler was placed in three different sections on the riverbed allowing three macroinvertebrate sample replicates to be obtained at each of the three sampling sites. Larger stones were overturned and wiped to collect attached invertebrates and the riverbed was agitated for three minutes allowing macroinvertebrates to flow into the collection net. Samples were collected in an upstream direction (bottom, middle and top). All macroinvertebrates present, were sorted into common taxa on the riverbank. Macroinvertebrates of the same order (two of each replicate) were pooled into sterile cryotubes on each sampling occasion. The cryotubes were immediately stored on dry ice in the field until samples were taken back to the Marine Institute Newport Research Station and stored in the -80 freezer. We limited the analysis to the five most abundant taxonomic orders of macroinvertebrates in the experimental river: Mayfly (Ephemeroptera), Stonefly (Plecoptera), Fly (Diptera), Caddis fly (Trichoptera) and Beetles (Coleoptera).

All gut, water and macroinvertebrate samples were transported on dry ice to the University of Glasgow for subsequent microbial profiling.

A suite of environmental parameters in the Burrishoole catchment and the Srahrevagh river are measured continuously as part of an ongoing LTER (long-term ecological research) program of monitoring. Parameters that were used in this study include water temperature, water level and water discharge, dissolved oxygen (DO) and conductivity.

To determine the sex and genetic origin (farmed, wild or hybrid provenance) of each fish in the common garden river experiment, fin clips were taken and preserved in absolute ethanol for subsequent genetic profiling and parentage assignment. Parentage analysis was conducted at the University College Cork using a three-panel multiplex PCR which amplified 10 microsatellites loci (for details see Perry et al., 2021).

The study was carried out under the Health Products Regulatory Authority (HPRA) licence number AE19130-P056 in Ireland.

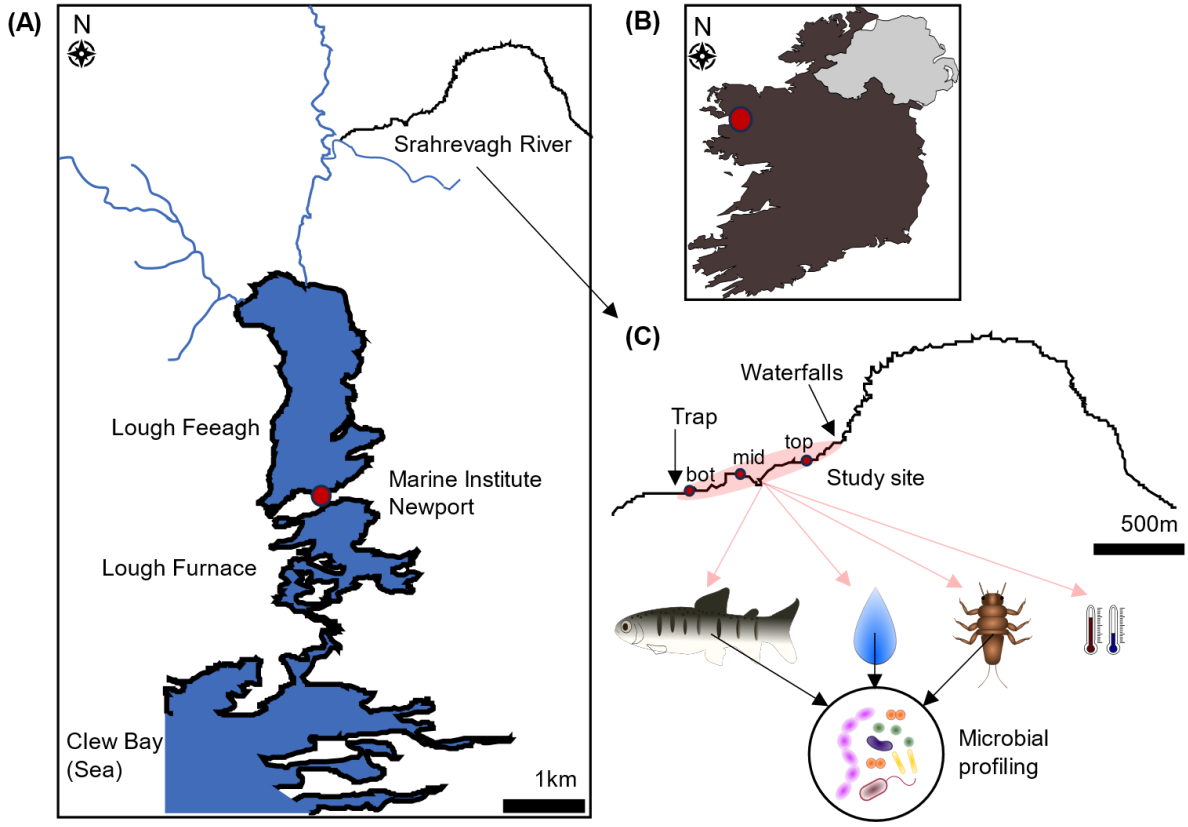

Figure 1: Map highlighting the location of the Marine Institute Research Station in Newport and the experiment river (Shrarevagh river) located within the Burrishoole river system (A) in Western Ireland (B). (C) shows the study site within a section of the experiment river. The study site is contained on its lower end by a trap and its upper end by waterfalls. Atlantic Salmon of four genetic origins (farmed, wild and their reciprocal hybrids) were introduced into the experimental river to simulate a farm escape and introgression event. Environmental parameters, including water temperature, dissolved oxygen, conductivity and river discharge were continuously measured over the course of the 10-month experiment. At each sampling timepoint replicates of water and macroinvertebrate samples were taken at three sections of the river, marked as bot (bottom), mid (middle) and top. Atlantic Salmon were caught via electrofishing across the whole section of the study site. Microbial profiling of Atlantic salmon intestines, water column and macroinvertebrates were conducted at the University of Glasgow.

Microbial DNA extraction and NGS library preparation

DNA extraction and NGS library preparation protocols used were based on methods established and summarized in Kazlauskaite et al., (2021) and Schaal et al., (2022). The frozen gut tissue (100mg) and filter papers were cut up into pieces using sterilized equipment and DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol (Claassen et al., 2013). The pooled macroinvertebrates were crushed with a sterile pestle before DNA extraction. Extracted DNA was amplified using primers targeting the V1 hypervariable 16S rDNA region (Gajardo et al., 2016). V1 was chosen over V4 because the primers are less liable to cross-hybridisation with salmon DNA (Heys et al., 2020; Werner et al., 2012). Amplification of the target region was achieved using tagged barcodes 27F and 338R at a concentration of 1pM for each primer. PCR included an initial denaturation step at 95°C for 10min; 30 cycles at 95°C for 30s, 55°C for 30s and 72°C for 30s; and a final elongation step of 72°C for 10min. First-round PCR products were then used for a subsequent second round of PCR, in which external multiplex identifiers (barcodes) were added. Cycle number was reduced to eight and reaction conditions were identical as to mentioned before. All primer sequences are detailed in (Schaal et al., 2022). Second round amplicons were gel-purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA) and quantified using a Qubit fluorometer (Thermo Fisher Scientific, USA). Final amplicons were pooled equimolarly at a concentration of 10nM and paired-end sequencing was carried out using a NovaSeq 6000 system provided by NovoGene.

Bioinformatic pipeline

Sequence analysis was performed with our bioinformatic pipeline as described previously by Kazlauskaite et al. (2021) and Schaal et al. (2022).

Quality filtering and trimming (>Q33 Phred score) was performed on all sequence reads of the target region using the Sickle (v.1.2) software (Joshi & Fass, 2011). Read error correction was carried out using the BayesHammer module within the SPAdes (v.2.5.0) software to obtain high-quality

assemblies (Nikolenko et al., 2013). Paired-end reads were merged (overlap length 50bp) using PANDAseq (v.2.11) with the simple Bayesian read merging algorithm (Masella et al., 2012; Schirmer et al., 2016). Thereafter, merged reads were dereplicated, sorted, and chimaeras and singletons were removed by using VSEARCH (v.2.3.4; Rognes et al., 2016). Sequences were decontaminated against the last assembled version of *Salmo salar* genome using DeconSeq (v.0.4.3; Schmieder and Edwards, 2011) and overlapped reads were clustered into operational taxonomic units (OTUs) using VSEARCH at 97% sequence identity. Naïve Bayesian classifiers, implemented in QIIME2 (Bolyen et al., 2019; Pedregosa et al., 2011) were used to classify OTUs against the Silva 138 database (Quast et al., 2012). Phylogenetic trees were generated using FastTree (Price et al., 2010).

Statistical analysis

All data were analysed in R (R Core Team, 2022) using the packages PhyloSeq (McMurdie & Holmes, 2013), microeco (Liu et al., 2021), metacoder (Foster et al., 2017) and vegan (Oksanen, 2007). OTUs that were not assigned to the kingdoms of Bacteria and Archaea were removed, as were sequences assigned as chloroplast or mitochondria. To limit sample depth effects on diversity measurements, samples were rarefied to 10000 reads. Alpha diversity was assessed via Chao1 richness and the Shannon's index of entropy. Generalised linear models were used to assess the significance of predictor variables. Akaike Information Criterion (AIC) was used to determine the best-fit model. Beta-diversity measures were visualised by principal coordinates analysis (PCoA) plots using Bray-Curtis and weighted UniFrac distance measures. Permutational multivariate analysis of variance (PERMANOVA) was used to test the effects of sampling date (month), sex and genetic origin on the intestinal microbial communities among individual fish.

Distance-based redundancy analysis (dbRDA)

We used distance-based redundancy analysis (dbRDA) to determine how much of the variation in intestinal or environmental microbial communities could be explained by external environmental factors. Environmental variables were log10 transformed to improve comparability of canonical

coefficients (Buttigieg & Ramette, 2014). Candidate predictors tested were host-specific factors such as fish length or weight and environmental factors such as water temperature, water level and river discharge, dissolved oxygen (DO) and conductivity. Environmental factors were used to predict seasonal differences in the bacterial communities collected from the water column. To estimate the perturbation of aquatic bacteria due to flooding events, the average river discharge was calculated as the seven-day mean prior to sampling timepoints.

Source tracking analysis

Fast expectation-maximization for microbial source tracking (FEAST, Shenhav et al., 2019) was used to analyse the contribution and the relative importance of fish feed (macroinvertebrates) and planktonic water bacteria to intestinal microbial community composition of the individual fish. The tool estimates the contribution of different source environments to a microbial community, referred to as the sink. It also identifies the fraction of the sink attributed to other unidentified origins, known as the unknown source. Mixing proportions were calculated for each individual fish by using five macroinvertebrate samples (each from one order) and three water samples (reflecting top, mid and bot locations within the study site). These eight “sources” were sampled at the same timepoint as the respective fish.

Abundance-occupancy analysis and neutral model fitting

We employed a Shade-Stopnisek abundancy-occupancy analysis to identify potential ‘core’ OTUs in the intestinal microbiome of Atlantic salmon (Shade & Stopnisek, 2019), which uses abundance-occupancy distributions fitted to Sloan's neutral model (Sloan et al., 2006). To determine the core OTUs, we ranked the OTUs based on their occupancy (the frequency of their occurrence in the samples) and weighted them by their abundance. Only OTUs present in all sampling timepoints were considered core. In addition, a potential core OTU had to pass a core inclusion threshold. Therefore, we quantified the contribution of the core subset of taxa to beta diversity using the Bray-Curtis resemblance. To determine the core inclusion threshold, we used a minimum percentage increase in

beta diversity of 4% to identify the point at which further increases would offer marginal returns in explanatory value. Important to note is that the inclusion threshold percentage depends on the study design and must be chosen accordingly. To estimate the importance of neutral processes in the assembly of the intestinal microbiome, we applied the Sloan neutral model. The model assumes that community composition dynamics are primarily driven by random processes such as ecological drift and dispersal rather than by species-specific interactions or adaptations. OTUs that occur more frequently than expected are interpreted as potentially having additional factors influencing their abundance, such as microbe-microbe interactions, niche differentiation or host filtering. In this study we refer to OTUs that occur more frequently as expected as being positively selected. Conversely, if certain OTUs occur less frequently than expected, it may suggest that they are subject to competitive exclusion, environmental filtering or other processes that limit their abundance. To fit the occupancy of OTUs and their mean relative abundances across the metacommunity to the model, we used the R code of Burns et al., 2016.

Differential abundance testing

We used analysis of compositions of microbiomes with bias correction (ANCOM-BC; Lin & Peddada, 2020) to identify differentially abundant taxa between environments. ANCOM-BC was recently recommended as a very robust method for accurately determining differentially abundant taxa (Nearing et al., 2022). The method is based on a log-linear model that accounts for sampling fractions across samples and deals with the sparse compositionality of microbiome data. In addition, ANCOM-BC can deal with different scenarios for zero-counts. Here, we did not correct for structural zeros since we assumed that the presence of taxa was not unique to a certain sampling timepoint. The iteration convergence tolerance for the expectation-maximization algorithm was kept at its default value of $1e^{-5}$. Significance was determined by using Benjamini-Hochberg corrected p-values (Benjamini & Hochberg, 1995).

In addition, we used random forest analysis implemented in the microeco package (Liu et al., 2021), to highlight the relative abundance of core OTUs grouped on genera with respect to the sampling month. The aim was to determine if seasonality in gut microbial community composition persists in core OTUs and to identify which positively selected core OTUs exhibit seasonal patterns (Beck & Foster, 2014; White et al., 2009; Yatsunenko et al., 2012). MeanDecreaseGini was used to determine the importance of differentially expressed taxa per sampling month. P-values were adjusted for multiple comparisons using the Benjamini–Hochberg method (Benjamini & Hochberg, 1995).

Results

Distinct compositions of gut, water, and macroinvertebrate microbial communities

The microbial community compositions of gut, water and macroinvertebrate samples differed significantly ($F=20.67$; $R^2=0.21$; $p=0.001$; Figure 2a). Water communities showed significantly more diversity than gut and macroinvertebrate communities (Figure 2b, c). ANCOM-BC detected 513 taxa on genus level that were differentially abundant between at least two bacterial environments (gut, macroinvertebrate, water). *Schlegellela*, *Corynebacterium*, *Staphylococcus* and *Acidovorax* were most abundant in fish guts, whereas Flavobacterium, *Sphingorhabdus*, *Novosphingobium* and *Methylotenera* were dominant in macroinvertebrates (Figure 2d). 108 genera showed negative log abundances in water samples but were positively enriched in gut communities. Biggest log fold changes between the gut and water environment were observed for *Schlegella* (W= -16.48, p<0.0001), *Corynebacterium* (W= -15.01, p<0.0001), *Staphylococcus* (W= -14.08, p<0.0001), *Delftia* (W= -16.85, p<0.0001) and *Acidovorax* (W= -13.26, p<0.0001). Many genera belonging to the phylum of Firmicutes e.g., *Mycoplasma*, *Clostridium sensu stricto, Bacillus* and several taxa belonging to the order of *Lactobacillus* were also positively enriched in salmon guts.

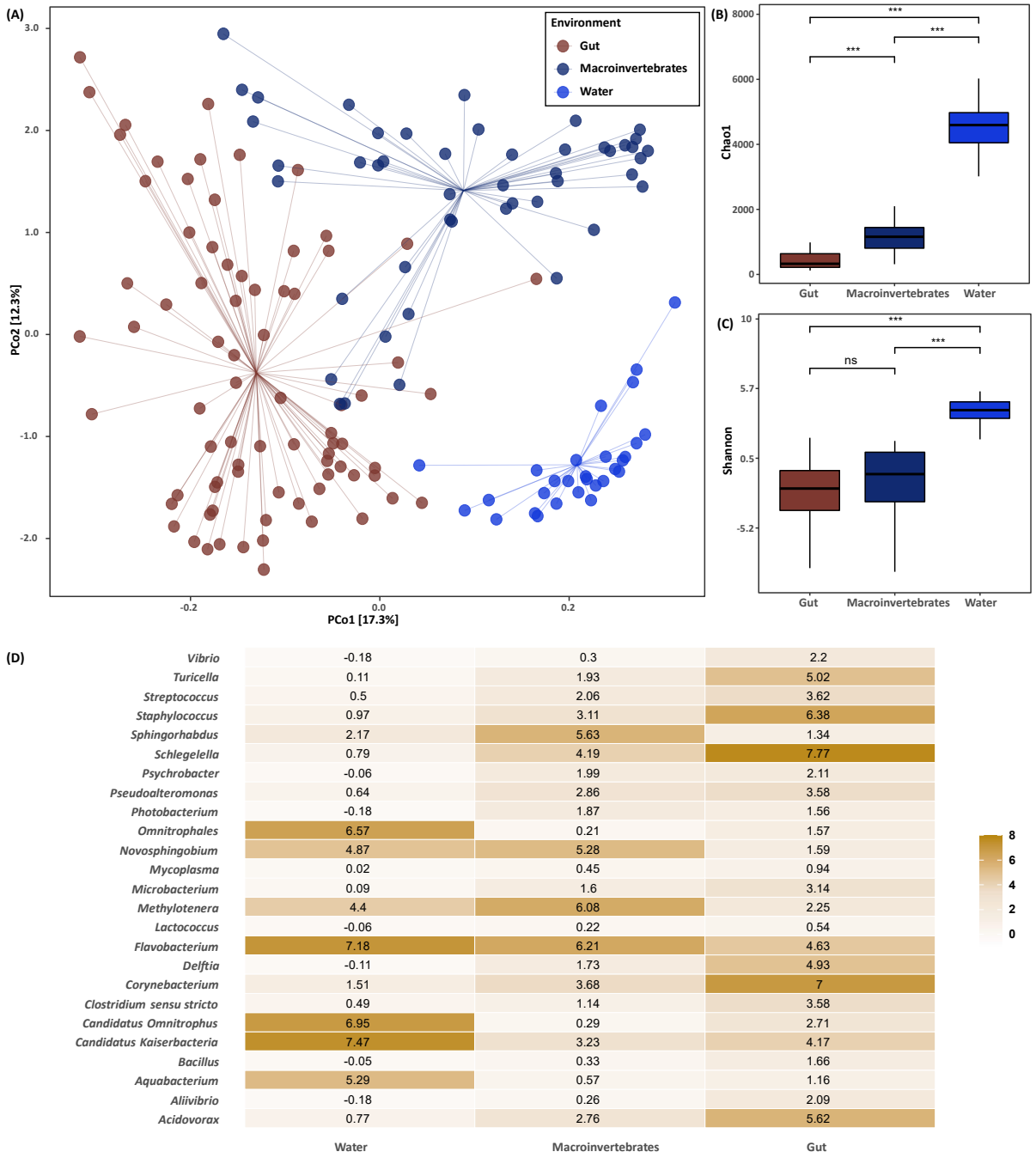

Figure 2: Principal coordinate analysis (PCoA) of bacterial communities from gut, water and macroinvertebrate samples. (B) Chao1 richness of bacterial communities collected from gut, macroinvertebrate and water environments. (C) Shannon diversity index of bacterial communities collected from different environments. Significance was determined by pairwise t-testing. Significance codes: ***<0.001; ns=not significant. (D) Bias-corrected log observed abundances, calculated by analysis of compositions of microbiomes with bias correction (ANCOM-BC). The heatmap shows 25 differentially abundant taxa on genus level. In total, 513 genera were significantly differentially abundant between at least two environments. Values and respective colour schemes depict bias-corrected log observed abundances.

Influences of seasonality and host genetic origin on gut microbial communities

Gut microbial community composition of Atlantic salmon juveniles varied considerably among sampling months (Figure 3a). However, monthly clusters did not align sequentially from January to November and instead appeared to be randomly separated by PCoA ordination. Interestingly, PCoA ordination clustered fish samples from the wild and hybrid wild female origin in March, May and November, indicating a potential genetic and/or maternal effect on gut microbial community structure. However, it is important to note that our sampling design lacks statistical power in terms of the number of fish per sampling time point, specifically in relation to their genetic origin (see Table S 1). Therefore, results indicating a genetic effect associated with the origin of the fish (farm, wild or hybrid) must be treated with care. PERMANOVA indicated statistical support for the differences observed in PCoA ordination. Sampling month (16.4%), genetic origin (5.4%) and their interaction term (20.5%) explained almost half of the observable variation. Fish sex had no significant effect on gut microbial community composition, and 56.5% of the variation remained unexplained (Table S 2). Pairwise testing revealed that the microbial community composition significantly differed between all sampling months (Table S 3). For genetic origin, we found that the microbial communities in wild fish differed significantly from those of farmed fish ($F=2.121$, $p=0.01$) and hybrid farmed female ($F=1.858$, $p= 0.01$) fish but not to hybrid wild female fish ($F=0.897$, $p=0.66$, Table S 4). However, these significant differences might only be present in certain sampling months. Unfortunately, we couldn't elaborate on this further due to the formerly mentioned flaws in the our sampling design.

Fish gut microbiomes showed their highest average diversity in January (Figure 3b, c). Alpha diversity measures were lowest in March and May and increased again over the summer months. The results from the generalised linear models showed that only the sampling time had a significant effect on alpha diversity measures. No significant effects on alpha diversity measures were found for genetic origin or sex (Table S 5,

Table S **6**).

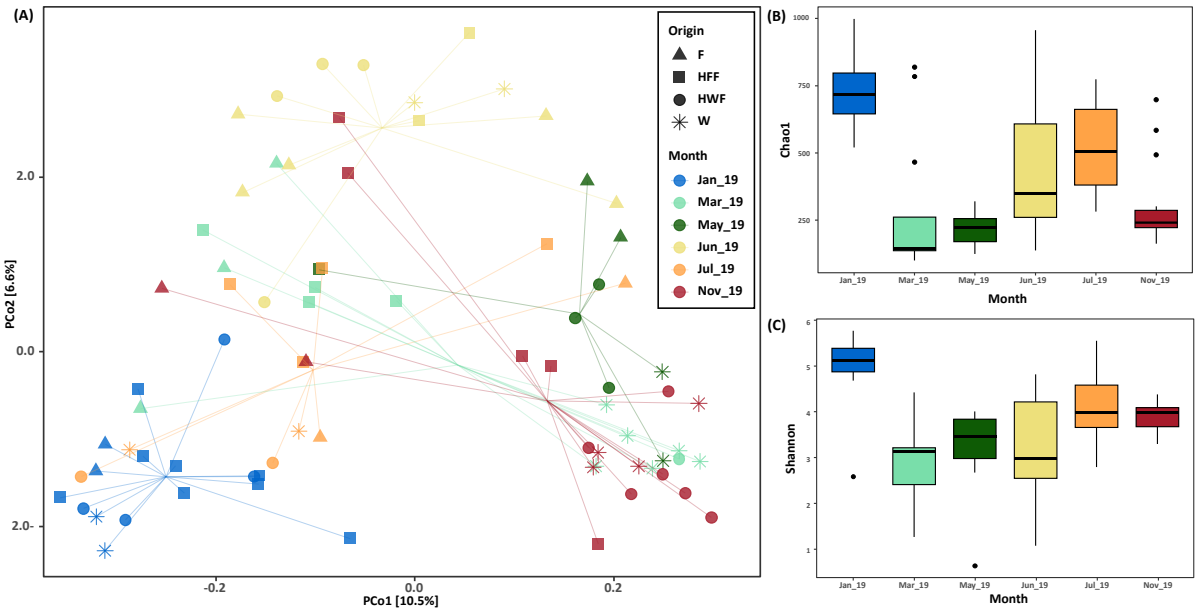

Figure 3: Alpha and beta diversity of Atlantic salmon gut microbiomes obtained from samples collected in the river environment. (A) Principal coordinate analysis (PCoA) of gut samples grouped by sampling month. Each data point represents an individual sample. Shapes represent the genetic background of fish (F=Farmed, HFF=Hybrid Farmed Female, HWF=Hybrid Wild Female, W=Wild). Percentages in parenthesis indicate the amount of variation shown on each axis. Lines mark the centroids of each group. Distance matrix was calculated based on Bray-Curtis dissimilarities. (B) Chao1 richness for gut samples grouped by sampling month. (C) Shannon diversity index for gut samples grouped by sampling month.

Environmental and host-specific factors correlate with gut microbial community composition

We used distance-based redundancy analysis (dbRDA) to attempt to explain monthly differences in gut microbial community composition in terms of environmental (e.g., seasonal) and host-specific (e.g., host developmental stage) factors. Distance-based linear models revealed that water temperature ($F=2.56$; $R^2=0.03$) and fish age ($F=3.58$; $R^2=0.04$) were significant predictors of gut microbial community patterns (Table S 7). However, inspection of Figure 4a, together with the statistical evaluation of the model suggests that the model only explains the community differences between a subset sampling timepoints. The combination of temperature and fish age only explained around 7.3% of the total variation, which indicates that in our study the direct effect of water temperature and host age on gut bacteria might be minor.

Temperature was lowest for the January sampling event at around 6°C (Figure 4b). Intermediate temperatures were detected in March, May, June and November (app. 9°C, 10°C, 12°C and 8°C, respectively) and highest in July (app. 16°C). We observed increased frequencies of high river flow rates during spring and autumn (Figure S 1). March had the highest mean discharge rate (747(l/s)) followed by November (729(l/s)) and January (570(l/s)). Lower discharge rates were recorded in May (78(l/s)), June (88(l/s)) and July (128(l/s)). Observed conductivity was highest in May (0.14(mS/cm) and June (0.13(mS/cm)). Other sampling timepoints showed lower conductivity (0.09(mS/cm), Figure S 1). Dissolved oxygen in the Srahrevagh river was negatively correlated with temperature and showed corresponding patterns over time (Figure 4c).

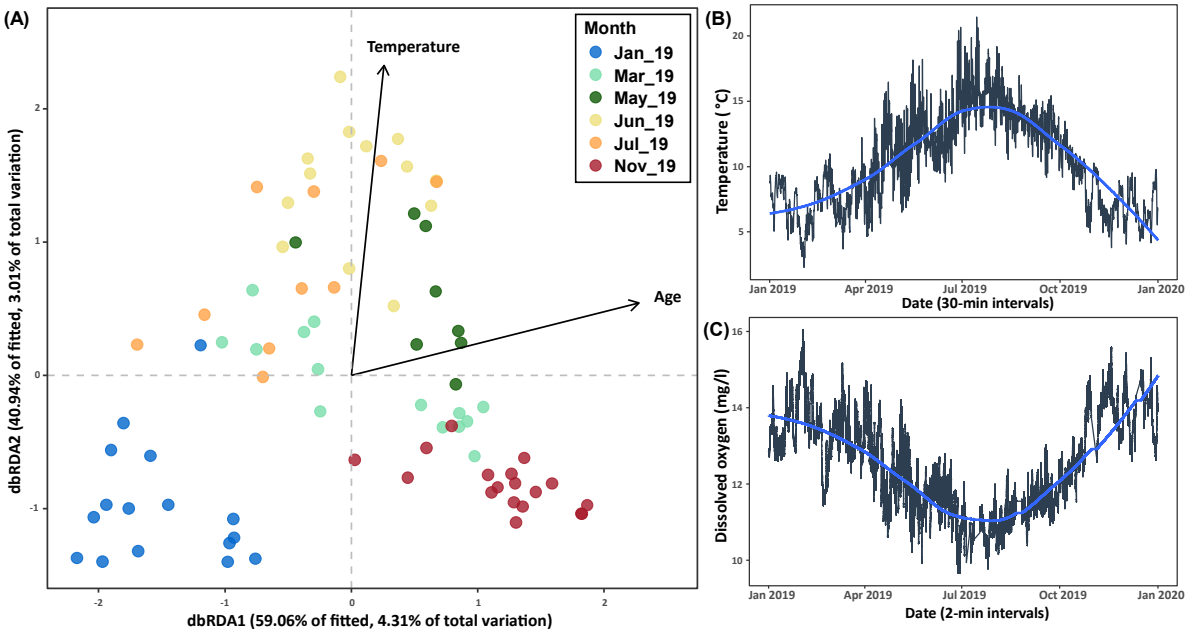

Figure 4: Distance-based Redundancy Analysis (dbRDA) shows correlations between gut microbial community composition and temperature, fish age and dissolved oxygen (A). Relative position of gut samples in the bi-plot is based on Bray-Curtis dissimilarities. Vectors indicate the weight and direction of those environmental and host-specific factors that were best predictors of gut bacterial composition as suggested by the results of the distance-based linear model. The dbRDA axes describe the percentage of the fitted or total variation explained by each axis while being constrained to account for group differences. (B) Water temperature [°C] of the Srahrevagh river in 2019 measured in 30-minute intervals. (C) Dissolved oxygen [mg/l] of the Srahrevagh river in 2019 measured in 2-minute intervals. Blue lines depict mean values fitted with loess regression.

The role of environmental bacteria in shaping gut microbial communities

Microbial communities in the water column showed a pronounced seasonal pattern ($F=10.34$; $R^2=0.81$; $p=0.001$). DbRDA analysis revealed that water temperature ($F=13.09$; $R^2=0.29$; $p=0.001$), conductivity ($F=11.34$; $R^2=0.25$; $p=0.001$), dissolved oxygen ($F=4.52$; $R^2=0.10$; $p=0.009$) and river discharge ($F=5.60$; $R^2=0.08$; $p=0.024$) were all significant predictors of the seasonal changes in bacterial community composition in the water column (Figure 5a). Microbial communities derived from macroinvertebrate samples were clustered by their origin, but also showed temporal trends within groups (Figure 5b). PERMANOVA supports this observation. Macroinvertebrate origin (taxonomic order) explained 30.6% of the observable variation in macroinvertebrate community composition and sampling month explained 14.8%, with 54.5% of variation remaining unexplained. Pairwise testing revealed that all macroinvertebrate orders showed significant differences in microbial community composition except Ephemeroptera and Plecoptera ($F=1.05$; $R^2=0.09$; $p=0.37$), which both showed seasonal differences (Table S 8).

Source tracking analysis revealed monthly variations in mixing proportions in gut microbial communities (Figure 5c). In January, bacteria from the water body contributed approximately 36% of intestinal genera, whereas taxa from the potential food sources (macroinvertebrates) contributed about 21%. The macroinvertebrate contribution steadily increased over the following sampling months, to around 50% in June, July and November. The contribution of taxa which couldn't be associated with either water or food sources declined after May (53%) reaching its lowest percentage in November (23%). Water source contributions were lowest in June at about 8% and highest in January (36%) and November (23%). Within food sources, macroinvertebrates from the order Diptera were the most dominant source of bacteria for gut communities. Microbial taxa derived from Diptera were almost completely absent in November, when Ephemeroptera became the largest source contributor. Similar observations were made for March. Source contributions for individual fish are shown in Figure S 2.

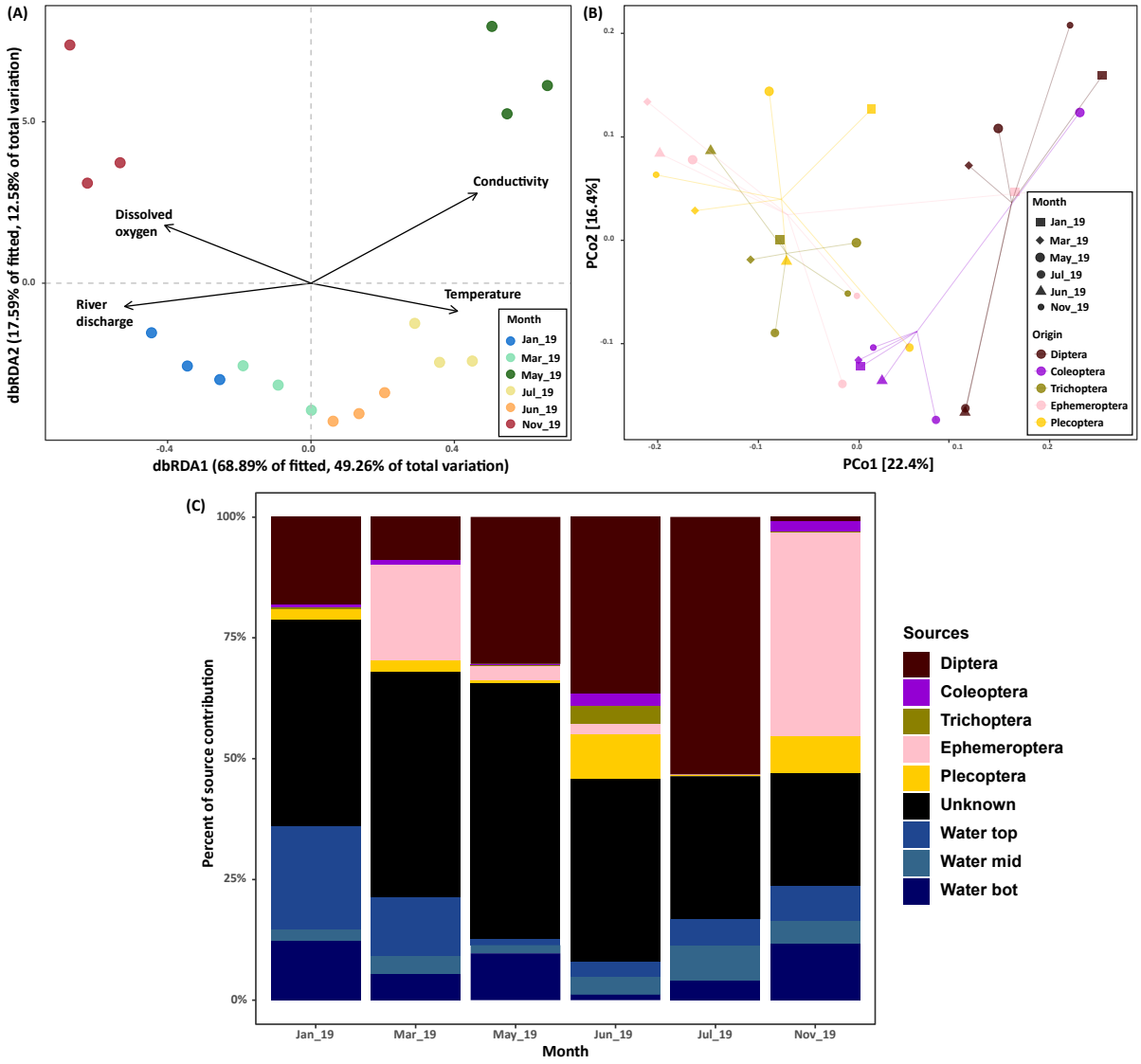

Figure 5: (A) Distance based Redundancy Analysis (dbRDA) shows correlations between microbial community composition of the water column and temperature, dissolved oxygen, conductivity and river flow rate (discharge rate). (B) Principal coordinate analysis (PCoA) of microbial communities from macroinvertebrate samples grouped by origin. (C) Fast expectation-maximization for microbial source tracking (FEAST) estimations of average microbial source contributions for Atlantic salmon gut communities per sampling month. Mixing proportions were calculated by using taxa counts on genus level. Sources contain five different macroinvertebrate orders (potential food source) and three different water samples, collected from the top, the middle (mid) and the bottom (bot) section of the Srahrevagh river (see Figure 1). Source samples were collected at the same sampling day as the fish gut samples.

Potential core taxa of juvenile Atlantic Salmon intestinal microbiomes

Our neutral model estimated that 6821/7911 (86.22%) of OTUs detected in our study were randomly assembled in fish intestines, whereas 987 (12.47%) occurred more frequently than expected by the neutral model (Figure 6a, b). The model assigned 117 OTUs as ‘core’ OTUs, of which 78 occurred

significantly more frequently than expected by the neutral model, 17 were considered neutral, and 22 OTUs occurred less frequently than expected.

After grouping the 117 core OTUs by genus, we discovered that certain genera appeared in multiple sections of the neutral model (Figure S 3): these genera were observed more frequently than expected, less frequently than expected, or with the same frequency as expected. *Corynebacterium* and *Pseudoalteromonas* were the only genera that appeared in all three sections of the neutral model. Additionally, OTUs associated with *Pseudomonas*, *Acidovorax*, *Turicella*, *Schlegellea*, and *Xanthobacteriaceae* were present in two sections of the neutral model.

We used a taxonomic heat tree to illustrate the distribution of the 78 core OTUs that did occur more frequently than expected in the salmon gut (Figure S 4). Those OTUs were dominated by Proteobacteria (85.7%), followed by Firmicutes (10%) and Actinobacteriota (3.5%). At genus level we identified 25 different taxa. Here, *Variovorax* (18.4%), *Pseudomonas* (11.2), *Staphylococcus* (6.7%), *Delftia* (5.8%), *Pseudoalteromonas* (4.4%) and *Schlegella* (4.4%) were the most dominant contributors to the deterministically selected core taxa. Other notable genera included *Acinetobacter* (2.5%), *Streptococcus* (1.8%), *Carnobacterium* (1.3%) and *Fibrobacter* (0.9%). A further 16 OTUs could not be assigned to a specific genus by the reference database, most of those OTUs belonged to the *Comamonadaceae* family.

When restricted to just core taxa we found that 17 out of 25 genera were differentially abundant in at least one sampling month. Interestingly, genera *Pseudomonas*, *Streptoccus*, *Carnobacterium*, *Acientobacter*, *Bosea*, *Methylothenera*, *Gallionella* and *Sphingomonas* showed no significant seasonal differences in their relative abundance (Figure 6c).

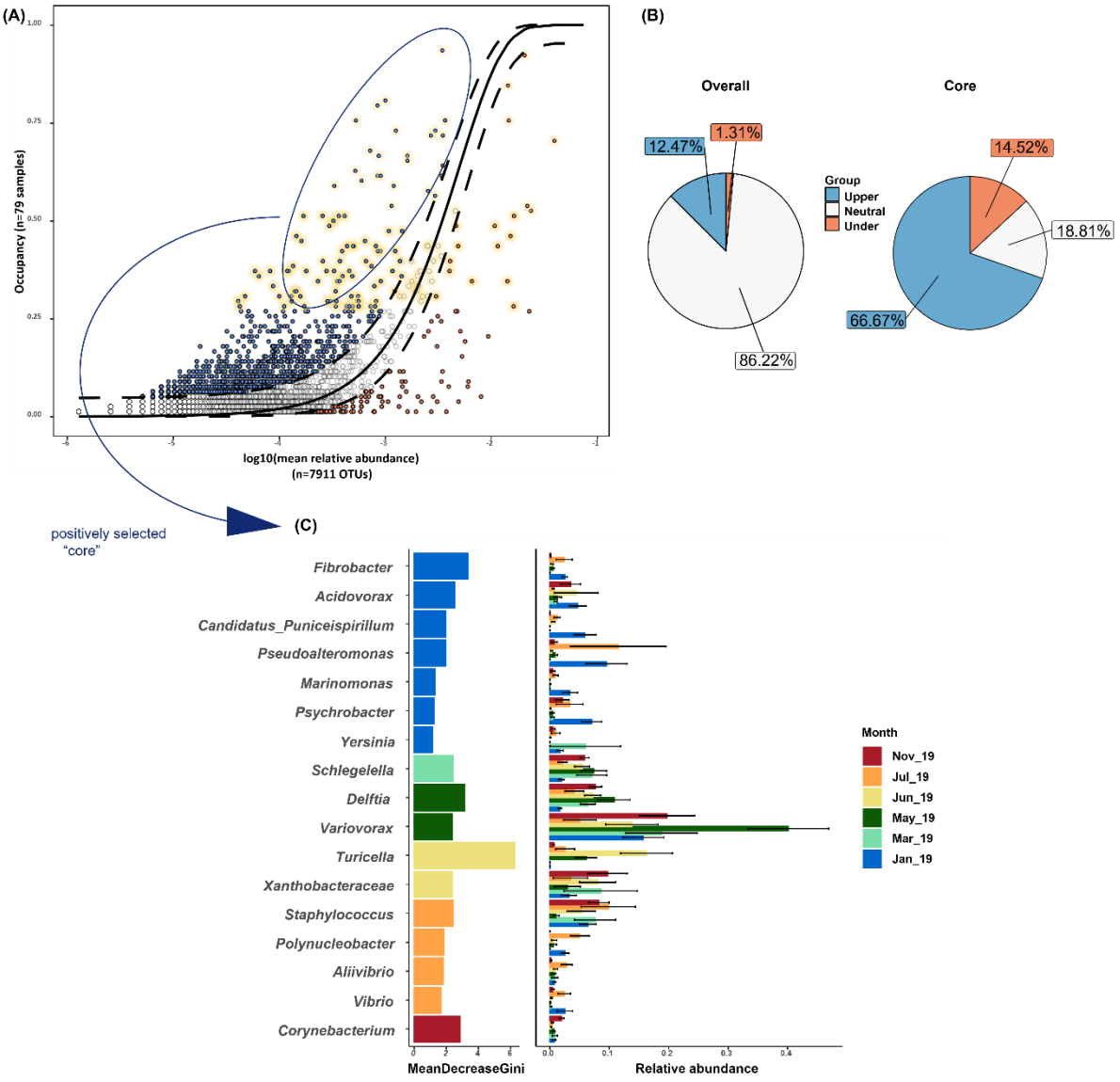

Figure 6: (A)Abundance-occupancy distribution of OTUs from Atlantic salmon intestines sampled at six sampling timepoints over a period of 10 months. Each point represents an OTU. The black line represents the fit of the neutral model, and the dashed lines represents 95% confidence intervals around the model prediction. OTUs that occur more frequently than predicted by the model are shown above (upper) the interval and are marked in blue. OTUs that occur less frequently than predicted are shown below (under) the interval and are marked in orange. OTUs that fit the neutral model are marked in white. OTUs that are classified as upper and under are likely to be deterministically selected by the intestinal environment. Yellow glowing OTUs represent core OTUs and were estimated by abundance–occurrence relationships and their contribution to Bray-Curtis similarity according to (Shade & Stopnisek, 2019). Core OTUs that appear more frequently than expected by the neutral model were used for further analysis (roughly highlighted by the blue ellipsis). (B) Pie plots of percentages of OTUs that fit the neutral model (white), occur more frequently than predicted (blue) or less frequently than predicted (orange). Second pie plot depicts percentages for the core OTUs highlighted by the yellow glow in the abundance-occupancy plot. (C) Differential abundance analysis of deterministically selected core OTUs grouped on genus level. 17 out of 25 genera were differently abundant between at least two sampling months. Mean Decrease Gini indicator represents the importance of each genus in distinguishing between sampling months.

Discussion

We assessed the role of host specific and environmental factors, as well as stochastic processes, in shaping the gut microbial development of Atlantic salmon living in a natural river. We found that bacterial community compositions collected from water and macroinvertebrate samples were significantly different from each other and the salmon intestine. This aligns with findings that gut microbial communities in fish are shaped by the environmental filter conditioned by the gut ecosystem (Cheaib et al., 2020; Kim et al., 2021). In theory, most microbes found in the gut originate from the surrounding environment. However, low-abundance environmental bacteria can further evolve to be more prolific colonisers of a fish's intestine, whereas highly abundant environmental bacteria might lack the capability of surviving in the gut environment (López Nadal et al., 2020; Robinson et al., 2018). We found that certain taxa of Firmicutes, such as *Mycoplasma*, *Clostridium sensu stricto*, *Bacillus*, as well as several taxa belonging to the order *Lactobacillus*, were present in very low abundance in environmental samples. However, the same taxa were significantly enriched in fish intestines, indicating their capability to thrive and multiply in the fish gut environment.

Gut microbial community composition varied significantly between all sampling months. Fish caught in January had, on average, a significantly more diverse gut microbiome than fish caught in the other months. Microbial richness was lowest in spring and autumn, with intermediate levels in summer. The magnitude of the observed difference in diversity between January and the other months was a rather surprising find. In theory, the reduction of feeding in winter should limit the amount of different ecological niches in the gut, leading to less diversity rather than more. However, based on the findings of the FEAST analysis we noticed that more bacteria from the water column were present in the gut in January compared to other sampling months. Given the high microbial diversity of the water column, this carryover might explain the high gut alpha diversity in January. Bray-Curtis distances between sampling months did not follow a sequential pattern, and Bray-Curtis distances within sampling months did not increase gradually over time. The results suggest that Atlantic

478 salmon juveniles' gut microbial community composition is highly dynamic and undergoes
479 considerable changes over time. This indicates that the factors driving the changes in the gut
480 microbiome may vary from one month to another and that multiple factors may be involved.

481 Potential deterministic processes could be seasonal temperature, genetic differences between hosts
482 and diet volume or composition. Whilst temperature is a probable cause of differences in gut
483 microbial community composition between sampling months, genetic background is a potential
484 cause of inter-individual differences within a sampling timepoint. Dietary differences might drive
485 monthly differences as well as inter-individual differences. In our study, we observed that water
486 temperature accounted for approximately 3% of the total variation in gut microbial community
487 composition. Although it was a statistically significant predictor, we determined that the overall
488 impact of temperature on the gut microbiome was relatively minor. Fish are poikilothermic, and
489 each bacterial species has an optimum growing temperature determined by their thermodynamic
490 limitations (Corkrey et al., 2012). Hence, environmental temperature variations might lead to
491 microbial abundance changes. Indeed some studies have suggested that elevated temperature can
492 drive overabundance of pathogenic *Vibrionaceae* in some freshwater systems (Suzzi et al., 2023).
493 PERMANOVA results also suggest that a fish's genetic origin impacts gut microbial communities.
494 However, given that we had an uneven and non-constant distribution of the genetic origins across
495 our sampling months, we do not believe we can confidently say that there are effects of both month
496 and genetic origin.

497 If gut microbial community composition is indeed driven by host genetics, it would not be the first
498 such example in fish. Genetic impacts on fish gut microbiomes have been observed among
499 genetically divergent populations of sticklebacks (Smith et al., 2015), guppies (Sullam et al., 2015)
500 and very recently in Chinook salmon (*Oncorhynchus tshawytscha*, Ziab et al., 2023). In our system,
501 genetic background might impact gut microbial communities in several ways. First, due to
502 differences in feeding habits between farmed and wild fish. Farmed fish are from lineages that have

been fed *ad libitum* in a sheltered environment for multiple generations. Due to this metabolic obligation farmed fish might start feeding earlier in the year and also stay active later in the year than their wild counterparts. A second possibility could be that genetic differences between farmed and wild fish create different selective pressures in the gut environment, possibly in respect to variation among fish of different provenances in immune response genes (de Eyto et al., 2007, 2011). Consequently, this genetic variation may lead to the proliferation of different bacterial species. However, we also cannot discount the possibility of maternal effects, whereby microbes may be transferred during oviposition. Close co-diversification between adult salmon linages and Mycoplasma strains observed recently would be consistent with this hypothesis (Rasmussen et al., 2023). To assess the implications of genetic effects on gut microbial communities it is important to understand if differences in microbial communities between farmed, wild and hybrid fish persist over time and if those changes correlate with variations in host metabolism or disease susceptibility. This knowledge is not only relevant for assessing the impact of introgression events to the fitness of salmonid populations but also for developing targeted strategies to promote fish health and mitigate potential negative effects.

Diet is one of the most important drivers of gut microbial community composition (Gajardo et al., 2017; Zarkasi et al., 2016). Intestinal microorganisms feed on food items that the host cannot digest, producing many host-beneficial metabolites in the process (Koh et al., 2016; Ríos-Covián et al., 2016). A change of diet composition or volume might therefore have major implications for gut bacteria growth and consequently for fish physiology and health. It is known that fish have reduced appetite in the winter months (Volkoff & Rønnestad, 2020). Hence, it is unsurprising that we found a distinct cluster of winter samples in our PCoA. FEAST analysis showed that the contribution percentages of food-derived bacteria were found to be the lowest in January, which is in agreement with a decrease in appetite of juvenile salmon during the winter season (Volkoff & Rønnestad, 2020). We found traces of bacterial taxa from all five macroinvertebrate groups in our fish, but Diptera (Fly) and Ephemeroptera (Mayfly) seemed the favoured food source for salmon in our study based on

529 microbiome sharing. Most of the taxa found in fish guts originated from the macroinvertebrate
530 order Diptera. In March and especially in November, those contribution percentages shifted to
531 Ephemeroptera, which suggests a change in diet or simply a seasonal change in macroinvertebrate
532 abundance. Our results match nicely with the stomach count analysis conducted by de Eyto et al.,
533 2020 who also found Diptera and Ephemeroptera to be the most important food sources for juvenile
534 Atlantic salmon in our experimental river. Despite not being the main topic of this manuscript, it is
535 still worth noting that the investigated macroinvertebrates themselves seem to have an order-
536 specific microbiome. As with most host associated intestinal microbiomes it is likely that diet is of
537 major influence for the microbial structure in macroinvertebrates (Kroetsch et al., 2020). Depending
538 on their life stage aquatic insects feed on algae or leaf litter but may also prey on other
539 macroinvertebrates when older. As adults, certain Diptera species, commonly known as midges, also
540 interact with terrestrial livestock by feeding on their blood (Walker, 2001). It would be interesting to
541 investigate a potential carryover from bacteria associated with terrestrial animals (skin or faeces)
542 through the macroinvertebrate as intermediate host into salmon. In this context, there might be a
543 special interest to investigate the potential carryover of antimicrobial resistance genes from
544 agriculture facilities into the aquatic environment.

545 An important aspect in determining the impact of dietary shifts on host health is whether the
546 bacteria introduced through food consumption persist in the gut or if they are merely transient. Our
547 abundance-occupancy analyses allowed further exploration of this question. The model implied that
548 the majority of OTUs (86%) were neutrally assembled, suggesting that the presence of those OTUs in
549 fish guts is determined by ecological drift or random dispersal from environmental sources. This high
550 percentage is consistent with previous studies, which have also shown that the majority of taxa in
551 salmon microbial communities are neutrally assembled (Burns et al., 2016; Heys et al., 2020).
552 However, it is worth noting that the neutral model approach used in our study has been subject to
553 criticism for potentially overestimating the significance of stochastic processes in shaping
554 community outcomes (Ning et al., 2019, 2020). To identify potentially important taxa, we employed

555 an abundance-occupancy threshold. All taxa that passed the threshold were considered 'core', but
556 we remind readers that 'core' taxa should be treated as study-specific. We identified 78 OTUs as
557 being positively selected core microbiota. These core microorganisms can be considered versatile
558 species that can adapt to the gut environment and persist across individual fish and over time.
559 However, no OTU was present in all our samples, revealing inter-individual variations among hosts.
560 Most positively-selected core genera displayed significant differences in their relative abundance
561 across sampling timepoints, suggesting a pronounced seasonality in the composition of core
562 microbial communities in the intestines of juvenile Atlantic salmon and strong links to seasonally
563 influenced diet. As mentioned in the beginning of this discussion, some taxa were positively enriched
564 in the gut compared to the environment. Out of these taxa our model identified positively selected
565 core OTUs associated with *Turicella*, *Staphylocccus*, *Schlegella*, *Pseudoalteromonas*, *Delftia*,
566 *Aliivibrio*, *Axicodovorax* and *Psychrobacter*. *Mycoplasma* a genus that was found to be an important
567 member in other studies of Atlantic salmon intestines, especially in the marine phase (Cheaib et al.,
568 2021; Heys et al., 2020; Llewellyn et al., 2016; Rasmussen et al., 2021, 2023), was only observed in
569 small numbers of fish in our study. However, all OTUs associated with *Mycoplasma* were predicted
570 to be more frequently abundant than estimated by our neutral model, indicating deterministic
571 selection. It is possible that this genus establishes predominance over the course of Atlantic salmon
572 development (Llewellyn et al., 2016). Whilst characterising core microbiota, we found several other
573 similarities to previous studies that have characterised core members of gut microbial communities
574 in juvenile Atlantic salmon sampled in freshwater. For instance, *Corynebacterium*, several Firmicutes
575 and Vibrionales are often reported as core contributors of Atlantic salmon gut microbial
576 communities (Gajardo et al., 2016; Uren Webster et al., 2018). The model used in this study also
577 classified OTUs associated to bacteria of marine origin (*Marinomonas* and *Pseudoalteromonas*) as
578 core microbiota. These bacteria are known to be highly abundant in marine fish (Schaal et al., 2022)
579 and as mentioned previously, their presence in juvenile freshwater fish could possibly be due to
580 maternal transmission during birth. However, knowledge about maternal effects on microbiota in

581 oviparous fish is lacking. It is crucial to recognise that even though certain OTUs may be classified as
582 neutral, indicating a higher probability of having a short presence in the gut, they can still play a
583 significant role in shaping the overall community structure. This is because these OTUs may interact
584 with the resident microbiota, influencing their composition and function. The question remains how
585 those taxa influence residence bacteria and under what (environmental) circumstances those taxa
586 can incorporate themselves into the resident community. Understanding this process would be
587 especially beneficial to improve the applicability of pre and probiotic treatments.

588 # Conclusion

589 This study provides a comprehensive overview about the factors governing gut microbial succession
590 in Atlantic salmon utilising a large-scale common garden experiment undertaken in the wild. The
591 results suggest that gut microbial community composition is highly dynamic and undergoes
592 considerable changes over time, driven by both deterministic and stochastic processes. We found
593 distinct microbial profiles of water, macroinvertebrate and intestine communities, which indicates
594 that microbial communities in fish are shaped by the environmental filter conditioned by the gut
595 ecosystem. Macroinvertebrates, the potential food source, had an order-specific microbiome,
596 whereas bacteria associated with the water column showed a temporal pattern. Microbial taxa
597 associated with macroinvertebrates were more abundant in the gut than bacteria associated with
598 the water column, indicating that diet is an important factor in gut community assembly. We
599 estimated that most detectable OTUs in our study were assembled stochastically. Deterministic
600 factors impacting gut microbial community composition, like temperature and fish age had a
601 significant influence on the fish's microbiome but their overall importance was estimated to be
602 minor. Monthly differences in gut microbial community composition also persisted in
603 deterministically selected core taxa. Our results also suggest that there might be an additive host
604 genetic effect determining differences observed between farmed and wild fish, thereby promoting
605 inter-individual differences in gut microbial community composition within sampling months.

Previous work in salmon has hinted at a role for maternal effects in driving inter-generational sharing of microbial taxa and our study, although lacking in statistical power, points in that direction. Future work could explore such a genetic and/or maternally determined relationship. Meanwhile, we hope this study should serve as important benchmark for rigorously analysing natural fish gut microbial assembly in their proper trophic and environmental context.

Author Contributions

P.S organised sampling, conducted laboratory work, analysed the data and drafted the manuscript. B.C programmed the bioinformatic pipelines and gave statistical advice. J.K, K.P, L.R and C.H organised sampling, conducted laboratory work and gave statistical advice. All authors were involved in the conception and design of the experiment and contributed to data interpretation and editing of the final draft of the manuscript. P.McG and M.L managed the project.

Acknowledgements

This research has been carried out with the support of the Marine Institute's Newport Research Section and Aquaculture Section, contributing to all field, hatchery, laboratory and logistic support. Special thanks also to Fintan Egan, Elvira de Eyto, Mary Dillane, Alan Drumm, Jamie Coughlan, Floriane Leseur, Sarah Ryan, Ronan Grealis and Catherine Waters.

Funding Information

PS, JK, KP, ER and PMcG were supported by the Science Foundation Ireland grant award (grant no. 16/BBSRC/3316). PS was also granted the Covid extension award "Higher Education Authority Covid-19 Cost extension (Call Three). This research was further supported in part by research grants from the Biotechnology and Biological Science Research Council (grant no. BB/T016280/1, BB/P001203/1 & BB/N024028/1) and by the Science Foundation Ireland, the Marine Institute Ireland and the Department for the Economy, Northern Ireland, under the Investigators Program (grant no. SFI/15/IA/3028). JK is currently supported by Science Foundation Ireland / Ireland Research council grant, under the Pathway programme (grant no. SFI-IRC/21/PATH-S/9671). The funding bodies did not influence data interpretation or in writing of the manuscript.

Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

The raw 16S rRNA gene sequence files and metadata are deposited at the NCBI SRA database under the BioProject PRJNA977248. The scripts and codes generated during the current study are available from the corresponding author on reasonable request.

Benefit Sharing

Benefits from this research accrue from the sharing of our data and results on public databases, as described in the data availability section. The present work strongly benefitted from a collaboration between the University of Glasgow, Scotland, the Marine Institute Newport, Ireland and the University College Cork, Ireland.

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Additional Files

Supplementary Figures

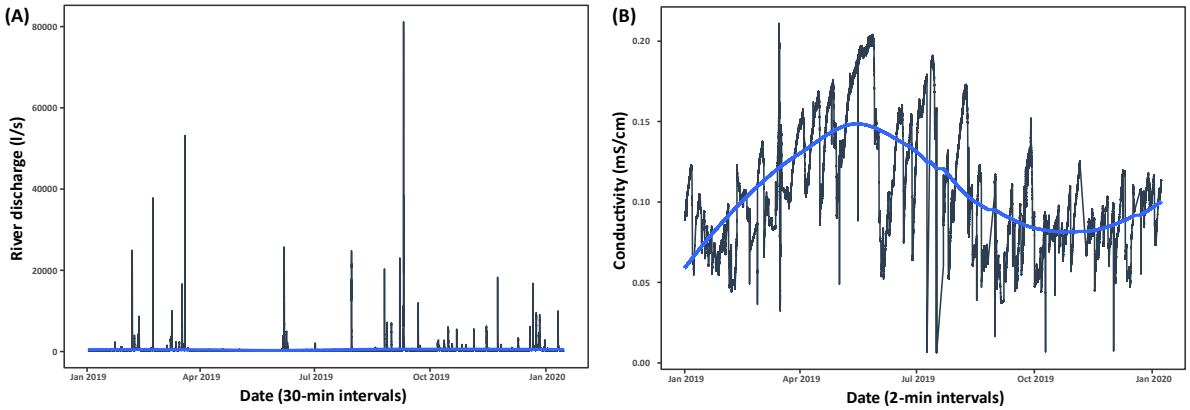


Figure S 1: (A) Mean river discharge (l/s) of the Srahrevagh river in 2019 measured in 30-minute intervals. (B) Mean conductivity (mS/cm) of the Srahrevagh river in 2019 measured in 2-minute intervals. Blue lines depict mean values fitted with loess regression.

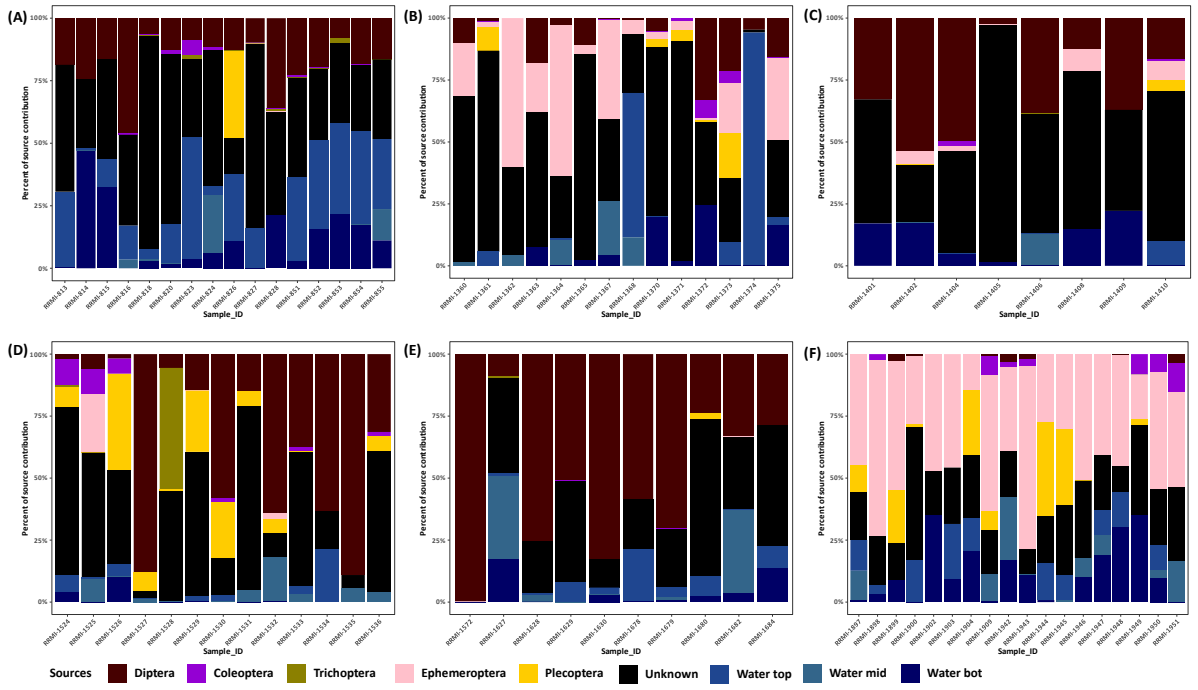


Figure S 2: Fast expectation-maximization for microbial source tracking (FEAST) estimations of microbial source contributions for Atlantic salmon gut communities. Each bar represents one individual sample. Each plot represents one sampling month. (A) January, (B) March, (C) May, (D) June, (E) July, (F) November. Mixing proportions were calculated by using taxa counts on genus level. Sources contain five different macroinvertebrates (feed samples) and three different water samples, collected from the top, the middle (mid) and the bottom (bot) of the experimental study area in the Srahrevagh river. Source samples were collected at the same sampling day as fish guts.

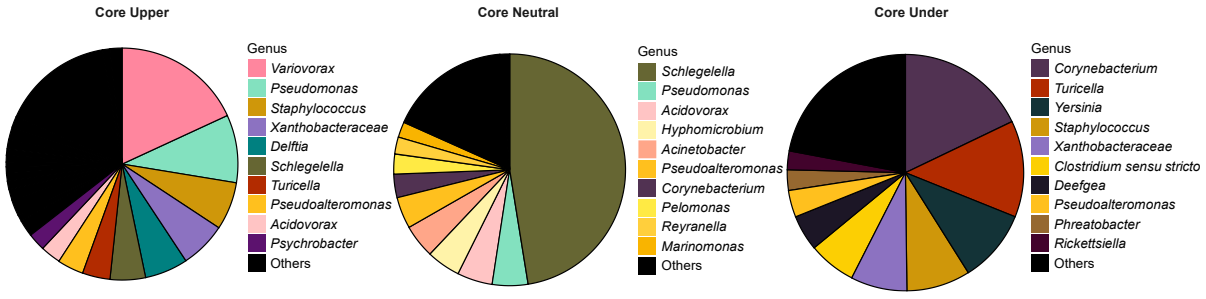
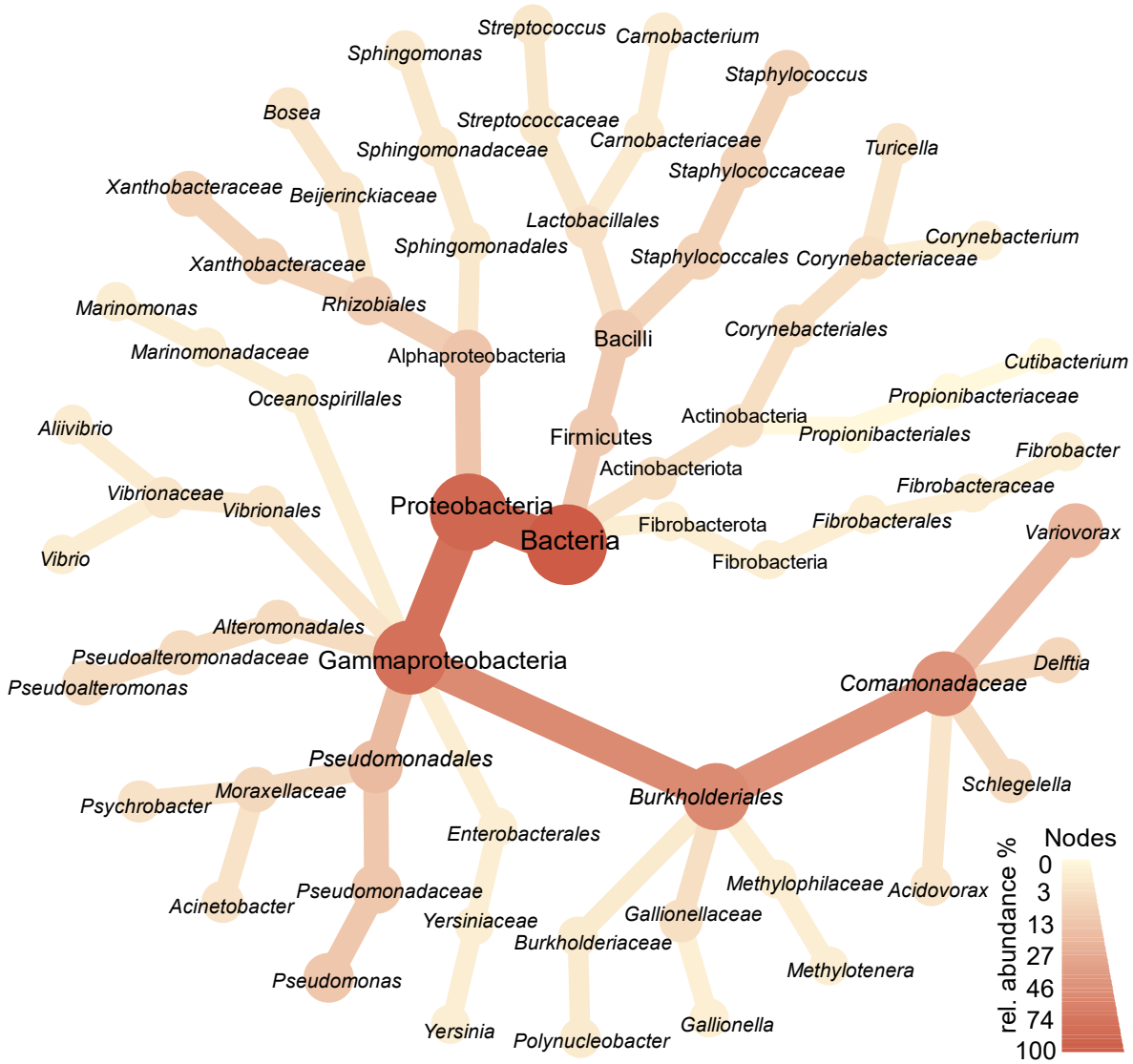


Figure S 3: Pie plots of ten most abundant core genera as determined by the abundance-occupancy model. The model was used to determine core OTUs, which had to surpass a specific core inclusion threshold to be included in the analysis. The core OTUs were further divided into three categories: Core upper, which were positively selected by the intestinal environment and appeared more frequently than expected by a neutral model; Core neutral, which occurred as frequently as predicted by the neutral model; and Core under, which appeared less frequently than expected by the neutral model. For the plots OTUs were grouped on genus level.



983

984 Figure S 4: Taxonomic heat map of deterministically selected core OTUs as determined by the
985 abundance-occupancy model from Figure 6. Node size and colour reflect the mean relative
986 abundance of taxa per taxonomic rank.

987 ## Supplementary Tables

988 **Table S 1: Overview of sampled fish during the 10-month experiment. 80 fish were sampled across**
989 **six sampling timepoints. Fish age is depicted in months. Number of samples taken is shown for**
990 **each sex per origin per month. Respective mean length (mm) and weight (g) measurements are**
991 **included. Brackets show standard deviations. F=Farmed, HFF=Hybrid Farmed Female, HWF=Hybrid**
992 **Wild Female, W=Wild.**

Month	Age (Months)	Origin	Male (N)	Female (N)	Length (mm)	Weight (g)
Jan_19 (N=16)	9	F	1	1	61.91 (±1.85)	2.39 (±0.33)
		HFF	4	4	62.76 (±4.13)	2.36 (±0.49)
		HWF	3	1	55.35 (±7.52)	1.67 (±0.79)
		W	0	2	56.62 (±14.07)	1.78 (±1.27)
Mar_19	11	F	1	2	66.45 (±6.32)	2.82 (±0.82)

(N=14)		HFF	2	2	63.88 (±7.23)	2.50 (±0.88)
		HWF	1	0	53.38	1.28
		W	3	3	53.29 (±4.77)	1.37 (±0.44)
May_19 (N=8)	13	F	2	0	80.00 (±1.41)	4.9 (±0.13)
		HFF	1	0	78.00	4.87
		HWF	1	2	73.67 (±1.53)	3.76 (±0.22)
		W	2	0	69.50 (±3.54)	2.92 (±0.28)
Jun_19 (N=13)	14	F	1	4	90.80 (±7.36)	7.91 (±2.50)
		HFF	0	2	85.67 (±10.07)	5.91 (±2.23)
		HWF	4	0	89.50 (±5.45)	6.57 (±0.60)
		W	1	1	73.00 (±4.24)	4.24 (±0.01)
Jul_19 (N=11)	15	F	1	1	107.50 (±2.12)	13.55 (±1.51)
		HFF	1	3	94.33 (±11.5)	8.36 (±2.93)
		HWF	1	1	87.50 (±3.54)	6.34 (±0.66)
		W	2	1	79.00 (±1.41)	5.21 (±0.04)
Nov_19 (N=18)	19	F	1	1	112.50 (±6.36)	14.59 (±2.41)
		HFF	5	0	111.00 (±14.71)	16.25 (±6.65)
		HWF	3	4	103.43 (±8.73)	11.85 (±2.14)
		W	2	2	102.25 (±6.34)	10.72 (±2.35)

Table S 2: PERMANOVA results to assess the effects of genetic origin, sampling month and sex on the composition of the Atlantic salmon gut microbiome in the river habitat. Significance code: *<0.001**

Group	Df	SumOfSqs	R2	F	Pr(>F)	Signif.
Origin	3	1.690	0.054	1.707	0.001	***
Month	5	5.098	0.164	3.089	0.001	***
Sex	1	0.278	0.009	0.843	0.791	
Origin:Month	15	6.358	0.205	1.284	0.001	***
Residual	53	17.495	0.565			
Total	77	30.922	1			

Table S 3: PERMANOVA testing pairwise comparisons of gut microbial samples grouped by their sampling timepoint (month) in the river habitat. Significance codes: **<0.01; *<0.05.

Groups	measure	F	R2	p.value	p.adjusted	Signif.
Jan_19 vs Mar_19	bray	3.627	0.115	0.001	0.002	**
Jan_19 vs May_19	bray	4.299	0.163	0.001	0.002	**
Jan_19 vs Jun_19	bray	4.856	0.152	0.001	0.002	**
Jan_19 vs Jul_19	bray	1.885	0.073	0.003	0.004	**
Jan_19 vs Nov_19	bray	5.469	0.150	0.001	0.002	**
Mar_19 vs May_19	bray	1.808	0.083	0.008	0.009	**
Mar_19 vs Jun_19	bray	2.706	0.098	0.001	0.002	**
Mar_19 vs Jul_19	bray	1.772	0.075	0.004	0.005	**
Mar_19 vs Nov_19	bray	1.661	0.054	0.018	0.018	*

May_19 vs Jun_19	bray	2.360	0.110	0.001	0.002	**
May_19 vs Jul_19	bray	2.044	0.113	0.004	0.005	**
May_19 vs Nov_19	bray	2.385	0.094	0.001	0.002	**
Jun_19 vs Jul_19	bray	2.183	0.094	0.001	0.002	**
Jun_19 vs Nov_19	bray	4.201	0.130	0.001	0.002	**
Jul_19 vs Nov_19	bray	2.930	0.105	0.001	0.002	**

Table S 4: PERMANOVA testing pairwise comparisons of gut microbial samples grouped by their associated genetic origin in the river habitat. F=Farmed, HFF=Hybrid Farmed Female, HWF=Hybrid Wild Female, W=Wild. Significance codes: *<0.05.

Groups	measure	F	R2	p.value	p.adjusted	Signif.
HWF vs HFF	bray	1.247	0.028	0.076	0.114	
HWF vs W	bray	0.897	0.024	0.662	0.662	
HWF vs F	bray	1.545	0.043	0.036	0.072	
HFF vs W	bray	1.858	0.044	0.004	0.012	*
HFF vs F	bray	1.002	0.025	0.446	0.535	
W vs F	bray	2.121	0.062	0.002	0.012	*

Table S 5: Generalised linear model results to assess the effects of sampling time on Chao1 richness. AIC: 203.45. AIC served as indicator to determine the best-fit model. Significance codes: *<0.001; **<0.01**

	Estimate	Std. Error	t value	Pr(>|t|)	Signif.
(Intercept)	722.760	47.480	15.222	< 2e-16	***
Mar_19	-451.100	69.500	-6.490	0.000	***
May_19	-504.340	82.240	-6.133	0.000	***
Jun_19	-264.220	69.500	-3.801	0.000	***
Jul_19	-219.440	79.130	-2.773	0.007	**
Nov_19	-430.420	65.260	-6.596	0.000	***

Table S 6: Generalised linear model results to assess the effects of sampling time on Shannon diversity. AIC: 1060.9. AIC served as indicator to determine the best-fit model. Significance codes: *<0.001; **<0.01**

	Estimate	Std. Error	t value	Pr(>|t|)	Signif.
(Intercept)	5.017	0.209	24.035	< 2e-16	***
Mar_19	-2.084	0.306	-6.820	0.000	***
May_19	-1.893	0.362	-5.236	0.000	***
Jun_19	-1.762	0.306	-5.767	0.000	***
Jul_19	-0.941	0.348	-2.704	0.009	**
Nov_19	-1.124	0.287	-3.918	0.000	***

Table S 7: Distanced-based linear model showing the best environmental and host specific predictors of gut microbial community composition in Atlantic Salmon parr in a river habitat. Model assessed the marginal effects of the predictors ("by margin"). Significance code: *<0.001**

Predictor	Df	SumOfSqs	R2	F	Pr(>F)	Signif.
Temperature	1	0.972	0.031	2.556	1.00E-04	***
Age	1	1.364	0.044	3.587	4.00E-04	***
Residual	75	28.903	0.885			
Total	78	31.221	0.926			
			1			

Table S 8: PERMANOVA testing pairwise comparisons of macroinvertebrate samples grouped by their origin. Significance codes: **<0.01; *<0.05.

Groups	measure	F	R2	p.value	p.adjusted	Signif.
Diptera vs Coleoptera	wei_unifrac	2.695	0.212	0.019	0.024	*
Diptera vs Trichoptera	wei_unifrac	4.427	0.307	0.001	0.007	**
Diptera vs Ephemeroptera	wei_unifrac	2.992	0.230	0.004	0.007	**
Diptera vs Plecoptera	wei_unifrac	3.365	0.252	0.005	0.007	**
Coleoptera vs Trichoptera	wei_unifrac	2.675	0.211	0.004	0.007	**
Coleoptera vs Ephemeroptera	wei_unifrac	2.458	0.197	0.004	0.007	**
Coleoptera vs Plecoptera	wei_unifrac	3.551	0.262	0.002	0.007	**
Trichoptera vs Ephemeroptera	wei_unifrac	2.010	0.167	0.032	0.036	*
Trichoptera vs Plecoptera	wei_unifrac	2.596	0.206	0.003	0.007	**
Ephemeroptera vs Plecoptera	wei_unifrac	1.055	0.095	0.369	0.369	