Early Life Bacterial Succession Under Different Diet Regime Atlantic Salmon (Salmo salar L.)

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Research

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Abstract

Backgound: The present study investigated the effect of different lipid source in the feed on the colonization and the bacterial succession in early life stages (fertilized eggs until 93 days post first feeding) of *S. salar*. The two diets used in this study, FD (fish oil based diet) and VD (vegetable oil based diet), were formulated to cover the fish nutritional requirements and except the lipid source the components were identical between them.

Hindgut samples collected at 0, 35, 65 and 93 days post first feeding (dpff). Moreover, fertilized eggs, yolk sac larvae, rearing water and feed were also sampled in order to assess a possible contribution of their microbiota to the colonization of the gut. To analyze the composition of the bacterial communities, the Illumina MiSeq platform was used.

Results: *S. salar* growth variables (mean wet weight and total length) did not differ significantly during the experiment ($p > 0.05$) across replicate tanks and between dietary treatments. The analysis of the 16S rDNA sequencing data revealed a total of 4548 unique OTUs, affiliated in 21 bacterial phyla. Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes were the dominant bacterial phyla. 13 OTUs were shared among all *S. salar* samples independent of life stage and diet treatment. Similarity percentages analysis (SIMPER) based on Bray–Curtis distance, showed that the average dissimilarity among the groups of the same life stages was 76.0%, whereas the average dissimilarity within groups of the same dietary treatment was 78.5% (FD) and 83.6% (VD).

Conclusion: Feeding on either fish oil or vegetable oil-based diets, did not result in significant differences in the intestinal microbiota. The composition of gut microbiota did not differ significantly between the two dietary treatments, but changed with age, and each stage was characterized by different dominant bacteria. These OTUs are related to species that provide different functions and have been isolated from a variety of environments. Finally, this study revealed the occurrence of a core microbiota independent of the studied life stages and diet during the early life stages of Atlantic salmon.

Introduction

Atlantic salmon (*Salmo salar* L.) is a carnivorous fish species, with significant economic value in European aquaculture, and Norway is a main producer worldwide [1]. Atlantic salmon has a complex life cycle, with anadromous migrations pattern, that is associated with morphological and physiological changes [2]. Their intestinal microbiota is affected significant by these changes [3, 4], and recent findings have revealed a stage-associated gut bacteria in *S. salar* individuals from the early life in freshwater to the adult stages in seawater[5].

Shifts in the intestinal microbial communities across development have also been reported for other fish species [4, 6–9]. These findings suggest that gut microbiota are affected by environmental sources (feed and the rearing environment), with stage specific selection pressures inside the gut. Moreover, many

studies have reported the crucial effect of the feed on the gut bacterial community composition in fish species (10, 11, 12).

Fishmeal and fish oil have been the main ingredients in diets for carnivorous fish species, providing fed fish the necessary proteins and lipids for high growth performance and resulting in a nutritionally rich final product (13,1 4). Due to the declining availability of fishmeal and fish oil their contents in feed is reduced [15] and substituted by a variety of alternative feed ingredients. Consequently, it is important to evaluate the impact of these new diets with lower fish-meal and -oil contents on the composition of the gut microbial communities for reared fish species (for a review see 16).

The effect of fish-meal and -oil replacement with alternative protein and lipid sources on the gut bacterial communities of *S. salar* have been evaluated previously [e.g. 17–21], and have in some cases revealed changes associated with intestinal disorders. These studies, however, have focused on juveniles and adult stages and on alternative protein sources. The effect on the gut microbiota during the very early stages of feeding, with diets without fish -meal/-oil remains unexplored. However, recent findings from a dietary experiment in diploid and triploid *S. salar* populations have revealed that early dietary interventions can improve the utilization of the new fish-ingredient free formulated diets [22].

The objective of the present study was to characterize the bacterial community assembly and succession in early stages of *S. salar* population fed diets with and without fish oil. We also characterized bacterial communities of the rearing environment to determine their contribution in the early colonization and the succession of the fish intestines. To the best of our knowledge, this is the first study reporting the presence of a core gut bacterial community in *S. salar* during its early life stages, independent of diet and taking into consideration the epibiotas of fertilized eggs and yolk sac larvae.

Materials And Methods

Experimental design and sampling

The experiment was conducted at the Ervik hatchery (Frøya, Norway) as described previously in Jin et al. [23]. Briefly, a fast-growing S. salar aquaculture strain was cultivated from fertilized eggs until 93 days post first feeding (dpff). When yolk sac absorption was observed (D0), *S. salar* individuals were randomly distributed from the initial stock tank (ST) into four tanks and fed two diets with different dietary lipid sources (2 dietary treatments x 2 replicate tanks x 200 individuals). The two diets, FD (fish oil diet) and VD (vegetable oil diet), were formulated to cover the fish nutritional requirements and except the lipid source, the rest components were identical (Table S1).

Hindgut and rearing water sampling was performed at 0, 35, 65 and 93 dpff from all tanks. Ten fish were sacrificed from each tank during sampling (130 individuals in total) by immersion in 40 mg/L Benzocaine (BENZOAK VET, ACD Pharmaceuticals AS, Oslo, Norway). Hindguts were removed by aseptic dissection, rinsed with ultra-pure water and stored at -80 °C until analysis. Moreover, ten whole fertilized eggs (EG) and whole yolk sac larvae (YS) were sampled in order to assess a possible contribution of their

microbiota to early colonization of the gut. Duplicate samples of rearing water microbes (100 ml/tank) were filtered onto 0.2 µm filters (GTTP, Millipore, USA). Approximately 0.25 g of the diets were collected for microbiota analysis.

DNA extraction and Sequencing

Microbial DNA was isolated from all the three types of samples (hindgut, water and diets) by using the QIAGEN QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol "DNA Purification from Tissues". Bacterial communities were characterized by Illumina 16S rDNA amplicon sequencing. To reduce the number of samples in the amplicon library, DNA extracts from 5 individual guts were pooled, resulting in two pooled gut samples from each time point/fish tank. The DNA from the rearing water samples were also pooled, resulting in 1 water sample per tank (STW – initial stock tank, FW – rearing water FD treatment and VW – rearing water VD treatment).

The primer pair S-D-Bact-0341-b-S-17 and S-D-Bact-115 0785-a-A-21 [24] was used to amplify the V3-V4 regions of the 16S rRNA gene. A total of 37 samples (representing 30 pooled fish samples, 5 pooled water samples and 2 feed samples) was used in the final amplicon library. Both sequencing and PCR amplifications were performed according to Dowd et al. [25] at the MRDNA Ltd. (Shallowater, TX, USA) sequencing facilities on a MiSeq Illumina instrument using paired end reads (2 × 300 bp). Briefly, one-step Polymerase Chain Reaction (PCR) of 30 cycles was applied using HotStarTaq Plus Master Mix Kit (Qiagen, Hilden, Germany). Reaction times and cycling conditions were 94 °C for 3 minutes, followed by 28 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds and 72 °C for 1 minute, with a final elongation step at 72 °C for 5 minutes. After amplification, the resulted PCR products were checked in 2% agarose gel to verify the success of amplification and the relative intensity of bands. Then, the PCR products were pooled together in equal proportions based on their molecular weight and DNA concentrations and purified using calibrated Ampure XP beads. Subsequently, the pooled purified pcr product was used to generate the sequencing libraries by following Illumina TruSeq DNA library preparation protocol.

Data analysis

Sequencing raw data were processed with the MOTHUR platform (version 118 1.38) [26,27] and the operational taxonomic units (OTUs) were classified by the SILVA Incremental Aligner (SINA) [28] following the methodology described in Nikouli et al. [29]. Identification of closest relative of each OTU was performed with Blast search (http://blast.ncbi.nlm.nih. gov). Raw sequence data from this study have been submitted to the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/) with BioProject accession number PRJNA520982. Statistical analysis and graphical illustrations were performed using the Palaeontological STudies (PAST) software [30] and the R Studio platform [31].

Results

Fish growth

The initial mean weight (D0) was 0.23 ± 0.03 g (± SD) and the final mean weight (D93) was 4.58 ± 1.74 g for FD and 4.54 ± 1.78 g for VD treatments (Table S2). The initial total mean length (D0) was 29.9 ± 1.6 cm which increased to 76.0 ± 8.9 cm and 73.8 ± 9.2 cm for the diets FD and VD, respectively, at D93 (Table S2). At none of the sampling points the mean wet weight or total length of *S. salar* differed significantly across replicate tanks or between dietary treatments (FD & VD) ($p > 0.05$; Fig. S1).

Bacterial diversity

The analysis of the 16S rDNA sequencing data revealed a total of 4548 unique OTUs, with the rarefaction curves (Fig. S2) and the Chao1 index (Table S3) indicating a satisfactory sequencing depth for the majority of the samples. The diversity was considerably higher for rearing water (STW, FW, VW) than gut and diet samples, both in terms of OTU richness (Table 1) and evenness (Table S3).

Table 1

Illumina results of 16S rRNA gene diversity reported in in all sample categories. Life stages of *S. salar* (EG, YS, D0, D35F, D35V, D65F, D65V, D93F, D93V), rearing water (VW, FW, STW) and feed (VD, FD) samples. N: Number of biological replicates analyzed. D: Day. OTUs: Operational Taxonomic Units

Samples		Reads	Observed OTUs richness	No. of the Most Dominant OTUs (Cumulative Relative Dominance ≥ 80%)	Most Abundant OTU(% of total reads) and Closest Relative (≥ 97%)
Code	Type/treatment				
EG	Fertilized eggs	22151 ± 7168.6 N = 2	172 ± 99.7	16	SOTU0011 (23,9%) - *Methylotenera versatilis*
YS	Yolk sac	14382 ± 3186.2 N = 2	87 ± 0.7	10	SOTU0013 (19,4%) - *Delftia acidovorans*
D0	Hindgut	21081 ± 1712.6 N = 2	132 ± 26.2	14	SOUT0009 (32,3%) - *Iodobacter fluviatilis*
D35F		7658 ± 5011.0 N = 4	121 ± 64.8	46	SOTU0017 (9,3%) - *Pseudomonas viridiflava*
D65F		2735 ± 1660.5 N = 4	110 ± 29.9	56	SOTU0070 (7,9%) - *Janthinobacterium agaricidamnosum*
D93F		2003 ± 637.1 N = 4	93 ± 6.4	51	SOTU0005 (11,5%) - *Cloacibacterium normanense*
D35V		25175 ± 27875.9 N = 4	135 ± 46.2	33	SOTU0005 (10,4%) - *Cloacibacterium normanense*
D65V		4812 ± 1975.0 N = 3	132 ± 11.7	37	SOTU0005 (11,1%) - *Cloacibacterium normanense*

Samples		Reads	Observed OTUs richness	No. of the Most Dominant OTUs (Cumulative Relative Dominance ≥ 80%)	Most Abundant OTU(% of total reads) and Closest Relative (≥ 97%)
Code	Type/treatment				
D93V		1170 ± 608.3 N = 4	79 ± 25.3	46	SOTU0004 (7,0%) - *Weissella cibaria*
FD	Fish oil diet	21022 N = 1	259	7	SOTU0004 (38,6%) - *Weissella cibaria*
VD	Vegetable oil diet	20699 N = 1	216	8	SOTU0004 (37,8%) - *Weissella cibaria*
STW	Initial stock tank	53280 N = 1	2422	259	SOTU0001 (9,4%) - *Polynucleobacter necessarius*
FW	Rearing water-Fish oil diet treatment	76806 ± 11852.5 N = 2	1683 ± 183.8	52	SOTU0001 (14,5%) - *Polynucleobacter necessarius*
VW	Rearing water-Vegetable oil diet treatment	53618 ± 8553.9 N = 2	1100 ± 137.2	35	SOTU0001 (20,8%) - *Polynucleobacter necessarius*

Taxonomic classification showed the presence of 21 bacterial phyla (Fig. 1, Fig. S4). OTUs that were not classified to known bacterial phyla were only 3.0% of the relative abundance and are assigned as "Bacteria_unclassified". Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes were the dominant bacterial phyla in the dataset. The remaining 18 phyla (Planctomycetes, Verrucomicrobia, Patescibacteria, Dependentiae, Acidobacteria, Gemmatimonadetes, Fusobacteria, Cyanobacteria, Deinococcus-Thermus, Fibrobacteres, Armatimonadetes, Nitrospirae, Spirochaetes, Elusimicrobia, Omnitrophicaeota, Tenericutes, Chloroflexi and Kiritimatiellaeota) were present with relative abundance ≤ 2%.

Similarities between Microbial Communities

Statistical analysis revealed no significant differences (Tukey's test, $p > 0.05$, Tab. S4) in the bacterial community composition of the *S. salar* samples between the first ontogenetic stages (EG, YS, D0). However, EG and D0 samples differed significantly from those taken during the feeding period (D35 - D93) in both dietary treatments, with stage D35V as the only exception. YS bacterial communities

differed significantly ($p < 0.05$) with the bacterial communities only at D93 in both dietary treatments (FD & VD). The gut microbiota of the *S. salar* juveniles did not reveal significant differences between the two dietary treatments for the different stages ($p > 0.05$), again with sample D35V as the only exception (Tab. S4).

Ordination of the bacterial community composition of *S. salar* guts, based on a Bray–Curtis distance matrix (Fig. 2), showed a clear separation between bacterial communities in gut and bacterial communities of the rearing environment (WST, VW, FW, FD, VD). Moreover, the bacterial communities of *S. salar* samples were more similar with respect to life stages than to the diet treatments (Fig. 2, Fig. S4).

Similarity percentages analysis (SIMPER) based on Bray–Curtis distance, showed that the average dissimilarity among the groups of the same life stages was 76.0%, whereas the average dissimilarity within groups of the same dietary treatment was 78.5% (FD) and 83.6%(VD).

Common and Unique OTUs

Overall, only 2.3% of the OTUs were found in all categories of samples (the rearing water, the pre-, after first feeding guts and the diets). 75.4% of OTUs occurred only in water samples (Fig. 3). From the 1004 OUTs detected in total in *S. salar* samples, 423 OTUs (9.3% of the OTUs) were unique in that type of samples. The majority of them (343 OUTs) were unique in *S. salar* samples at the active feeding stages, whereas 13 OTUs were shared among all samples independent of life stage and diet treatment.

S. salar microbiota

Comparing *S. salar* microbiota between ontogenetic stages, fertilized eggs (EG) had the highest observed and estimated (Chao1) OTU richness (172 ± 100 and 222 ± 114, respectively). At the yolk sac stage (YS), the OTU richness decreased to 87 ± 0.7 and increased again at first feeding (D0). After that, OTU richness was on the same level until D93 when it decreased (Table 1, Fig. S5).

Proteobacteria was the dominant bacterial phylum in *S. salar* samples, mainly due to *γ*- and β-Proteobacteria (Fig. S6). *β*-Proteobacteria was the dominant subphylum in *S. salar* samples before first feeding (EG, YS, D0), with representatives mainly from the Burkholderiaceae and Chitinibacteraceae families (Fig. S7). However, in fertilized eggs (EG), OTUs representing *β*-Proteobacteriales were classified only at class level (44.1% of the total reads). *γ*-Proteobacteria dominated the period with active feeding in both dietary treatments (D35F, D65F, D93F, D35V, D65V and D93V), with Pseudomonadaceae, Xanthomonadaceae, Vibrionaceae, Enterobacteriaceae, Moraxellaceae and Aeromonadaceae as the most abundant families. However, their relative abundances differed between the two dietary treatments (Fig. S8). Actinobacteria, the dominant bacterial phylum at the late stages (D35V and D65V) in vegetable oil dietary treatment, was due to the high relative abundance of mainly Propionibacteriales,

Corynebacteriales and Micrococcales representatives. The presence of Firmicutes and Bacteroidetes in *S. salar* samples was due to the classes Bacilli and Bacteroidia.

Microbial communities in Diets and rearing water

The bacterial communities in feed samples (FD, VD), consisted almost exclusively of Firmicutes (relative abundance of 84.2 and 82.1% in FD and VD, respectively, Fig. 1). The Firmicutes were affiliated to the Lactobacillaceae (38.5 and 36.6% in FD and VD respectively) and Leuconostocaceae families (37.9 and 38.8% in FD and VD, respectively). The rearing water samples (VW, FW, WST) contained mainly Proteobacteria, Actinobacteria and Bacteroidetes species, with Burkholderiaceae (*β*-Proteobacteria), Sporichthyaceae (Actinobacteria) and Chitinophagaceae (Bacteroidetes) as the most abundant families (Fig. 1). In contrast to the experimental diets, Firmicutes in water samples were detected in relative abundance $\leq 1\%$.

Discussion

In the present study, we characterize the gut bacterial communities of *S. salar* populations during early development (13 weeks of feeding) and fed two diets with different lipid source (FD, VD). Moreover, we characterized the bacterial communities from the rearing environment (rearing water and feeds) and the epibiotas of fertilized eggs and yolk sac larvae to determine their contribution in the bacterial colonization and succession of the gut. Previous studies suggest that the bacterial communities of the rearing environment, mainly from the rearing water and the feed, are important sources for community assembly of the intestinal microbiota of fish [12, 32–37]. For example, Schmidt et al. [19], reported a significant effect on intestinal microbial communities in postsmolt *S. salar* following replacement of dietary fishmeal with plant ingredients. However, the results in the present study suggest that substitution of fish oil by vegetable oils did not significantly affect the composition of intestinal microbial communities in the same host species.

The results of the present study indicate little relationship between the epibiotic, gut and water bacterial communities, whereas the life stage appeared to be the main factor affecting the structure of gut microbiota. These results are in agreement with previous findings from Llewellyn et al. [3], who studied 96 wild-caught individuals of *S. salar* with different age and habitats, and observed grouping of their intestinal bacterial communities based on the lifecycle stage. In addition, Lokesh et al. [5], reported stage specific microbial enrichment in intestinal mucosa of *S. salar* (samples from embryonic stages up to 80-week post hatch). Similar stage specific signatures have also been reported across development in *Sparus aurata* [38], *Danio rerio* [8] and *Gadus morhua* [7] supporting further that the life stage seems to be the primary force shaping gut microbiota in juveniles' stages of fish. The change in microbiota with life stage can be due to both host-microbe (e.g. development in morphology and immune system) and microbe-microbe interactions (mutualism, competitions and antagonism). The significance of these factors are, however, still not known.

Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes were the dominant bacterial phyla detected in *S. salar* samples for both dietary treatments in our study. This bacterial phylla seem to characterize the bacterial communities in individuals of Atlantic salmon (*S. salar*) at the freshwater life cycle stages [3]. These bacterial phyla are also commonly found in the gut bacterial communities of both saltwater and freshwater fish species [3 – 9, 16–21, 29, 32–42]

Despite the fact that the two experimental feeds contained almost exclusively Firmicutes, the increase in relative abundance of Firmicutes in *S. salar* samples after the onset of feeding was not solely due to feed specific OTUs. It should also be noted that 26.4% of the bacterial representatives detected on fertilized eggs (EG) were not detected in the water of the incubation tank (WST). This support the view that the microbial communities of fish eggs may be vertically transmitted from their parents or horizontally from their breading tank [38, 43].

In agreement with previous studies [5, 19, 38], the observed species richness in water samples was always an order of magnitude higher than the richness of the host samples. Bacterial communities in rearing water did not show major shifts during the experiment. OTU0001 dominated at all time points, with closest relative the bacterial species *Polynucleobacter necessaries*. This species is commonly found in freshwater samples and it can contribute to the catabolism of urea and reduction of nitrate [44]. The dominant bacterial species in *S. salar* samples are related with bacterial species from various habitats. The dominant OTU on fertilized eggs (OTU0011) was classified within the *Methylotenera* genus (β-Proteobacteria) and has previously been detected in fertilized salmon eggs by Lokesh et al. [5]. This genus consists of methylotrophic species that use methylamine as sole carbon, energy and nitrogen source [45]. The dominant OTU at the YS stage (OTU0013), seems to be related with *Delftia acidovorans* (*β*-Proteobacteria). Species of the genus *Delftia* are obligate anaerobes, organotrophic and non-fermentative organisms (46). They have previously been detected in the gut of healthy individuals of *Epinephelus coioides* [47], *Oncorhynchus mykiss* [48] and *Sparus aurata* [29, 40] and *S. salar* [49].

Just before onset on feeding (D0), the dominant OTU (OTU0009) showed similarities with the species *Iodobacter fluviatilis* of the Chitinibacteraceae (*β*-Proteobacteria) family. Species of this genus have been recorded mainly in sediment and water samples [50–52]. Their presence on fish skin (*Oncorhynchus mykiss* and *Salmo trutta*) has been associated with skin lesions [53]. However, the species has previously been detected in high relative abundance in healthy *Coreius guichenoti* individuals [54] whereas the present study reports the presence of this bacterial species in *S. salar* gut microbiota for the first time.

After first feeding, although not statistically significant, differences were found between the bacterial communities in gut, each stage was characterized by different dominant OTUs. Moreover, gut bacterial communities differed also between dietary treatments regarding their dominant bacterial species (OTU). Chitinibacteraceae, the dominant bacterial family on D0, (with relative abundance 32.3%), was detected in ~ 50x lower relative abundance (≤ 0.6%) in the rest of the samples. At D35F and D65F, the dominant OTUs (OTU0017 and OTU0070, classified as *Pseudomonas viridiflava* and *Janthinobacterium agaricidamnosum*, respectively), are described as plant [55–58] and mushroom pathogens [59–60].

According to recent findings, *Janthinobacterium lividum* (*β*-Proteobacteria) produce antimicrobial activity against multidrug resistant bacteria of clinical and environmental origin, such as Enterococci and Enterobacteriaceae [61]. Its presence in the gastrointestinal bacterial communities of S. salar, may have probiotic activity.

At D35 and D65 samples from the VD dietary treatment were dominated by OTU0005, with closest relative *Cloacibacterium normanense* (Bacteroidetes). This OTU was also dominant at D93F. According to the literature, this species is frequently present in sewage treatment plants [62,63] where it contributes in decomposition of complex organic compounds [64]. Similar processes may take place in the intestinal system of *S. salar* at D35V, D65V and D93F. The dominant OTU at D93V (OTU0004), also dominant in the provided feed (FD, VD), was affiliated with *Weissella cibaria* (Firmicutes). This bacterial species belongs to the lactic acid bacteria, and has antimicrobial activity in the intestinal system of other fish species [65]. Other *Weissella* spp. have been found in gut of *Oncorynchus mykiss* [66] and *S. salar* [7–69]. It is worth noting that beside OTU0004, also OUT0013 and OTU0017 are associated with probiotic bacterial species (detected in all time points studied here, from EG to D93, independently of the dietary treatment, FD & VD). This observation suggests a co-evolutionary relationship of these bacterial species with the host studied here (*S. salar*), and a possible specialized function in the hosts intestinal system.

Conclusions

The present study investigated the effect of different lipid source in the feed on the colonization and the bacterial succession in early life stages of an aquaculture strain of *S. salar*, from fertilized eggs until 93 days dpff. We demonstrated that feeding on either fish oil or vegetable oil-based diets, did not result in differences in the intestinal microbiota. Our results complement those of other research groups [3, 5] supporting that developmental stage and not the habitat and diet type determine the gut microbiota in *S. salar*. The composition of gut microbiota did not differ significantly between the two dietary treatments but changed with age, and each stage was characterized by different dominant bacteria. These OTUs are related to species that provide different functions and have been isolated from a variety of environments. Finally, this study revealed the occurrence of a core microbiota independent of the studied life stages and diet.

Declarations

Competing interests

The authors declare that they have no competing interests.

Author’s contributions

methodology, E.N., K.A.K., I.B., Y.O. and O.V.; formal analysis, E.N.; data curation, E.N. and K.A.K; writing—original draft preparation, E.N. and K.A.K.; writing—review and editing, E.N., K.A.K., Y.J., Y.O., I.B. and O.V.;

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Ethics approval and consent to participate

The study was carried out within the Norwegian animal welfare act guidelines, in accordance with EU regulation (EC Directive 2010/63/ EU), approved by the Animal Ethics and Welfare Committee of the Norwegian University of Science and Technology (case number 16/10070).

Consent for publication

All authors approved the final version submitted and consent to its publication

Availability of data and materials

Raw sequence data from this study have been submitted to the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/) with BioProject accession number PRJNA520982.

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Figures

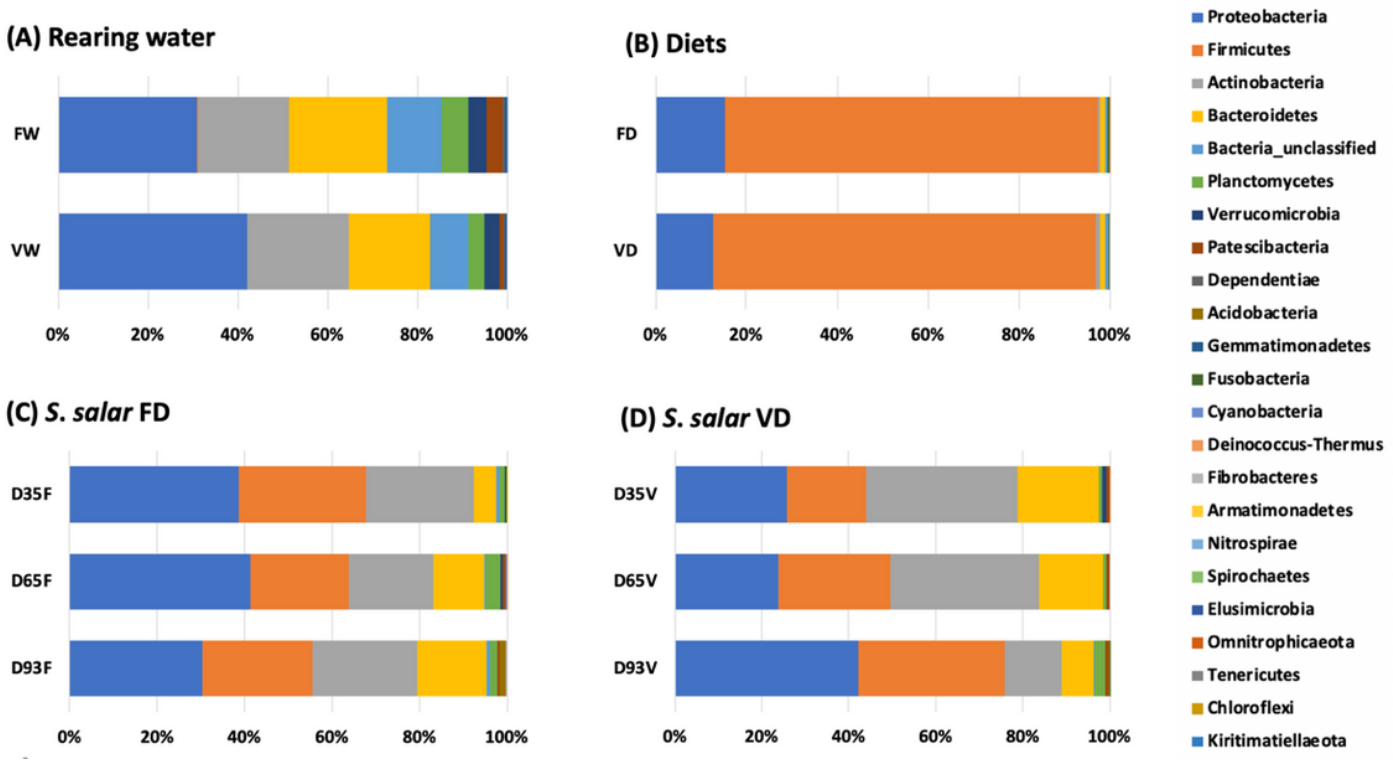

Figure 1

Phylum composition of microbiota from rearing water (A), diets (B) and hindgut of S. salar samples in FD (C) and VD (D) dietary treatments, at D35, D65 and D93 post first feeding Figure 1 Sample figure title. A

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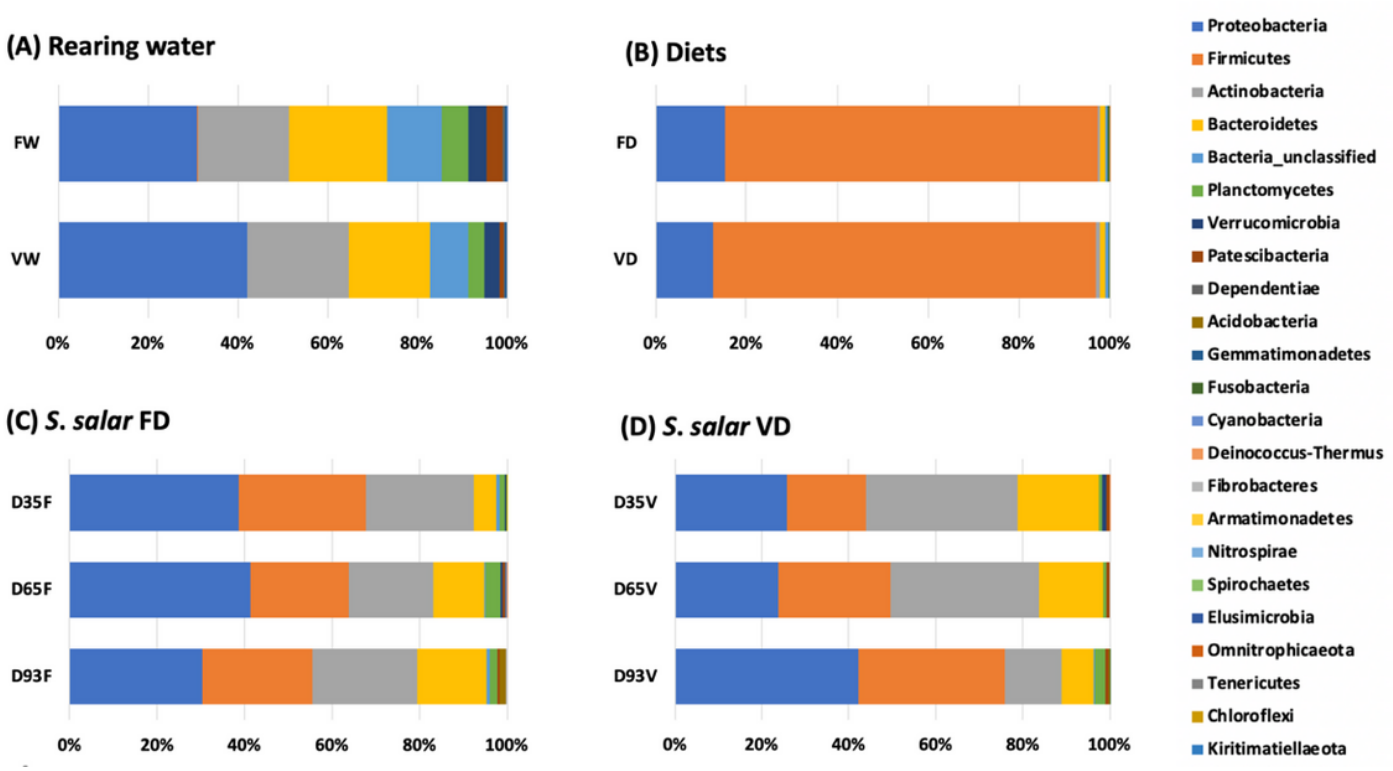

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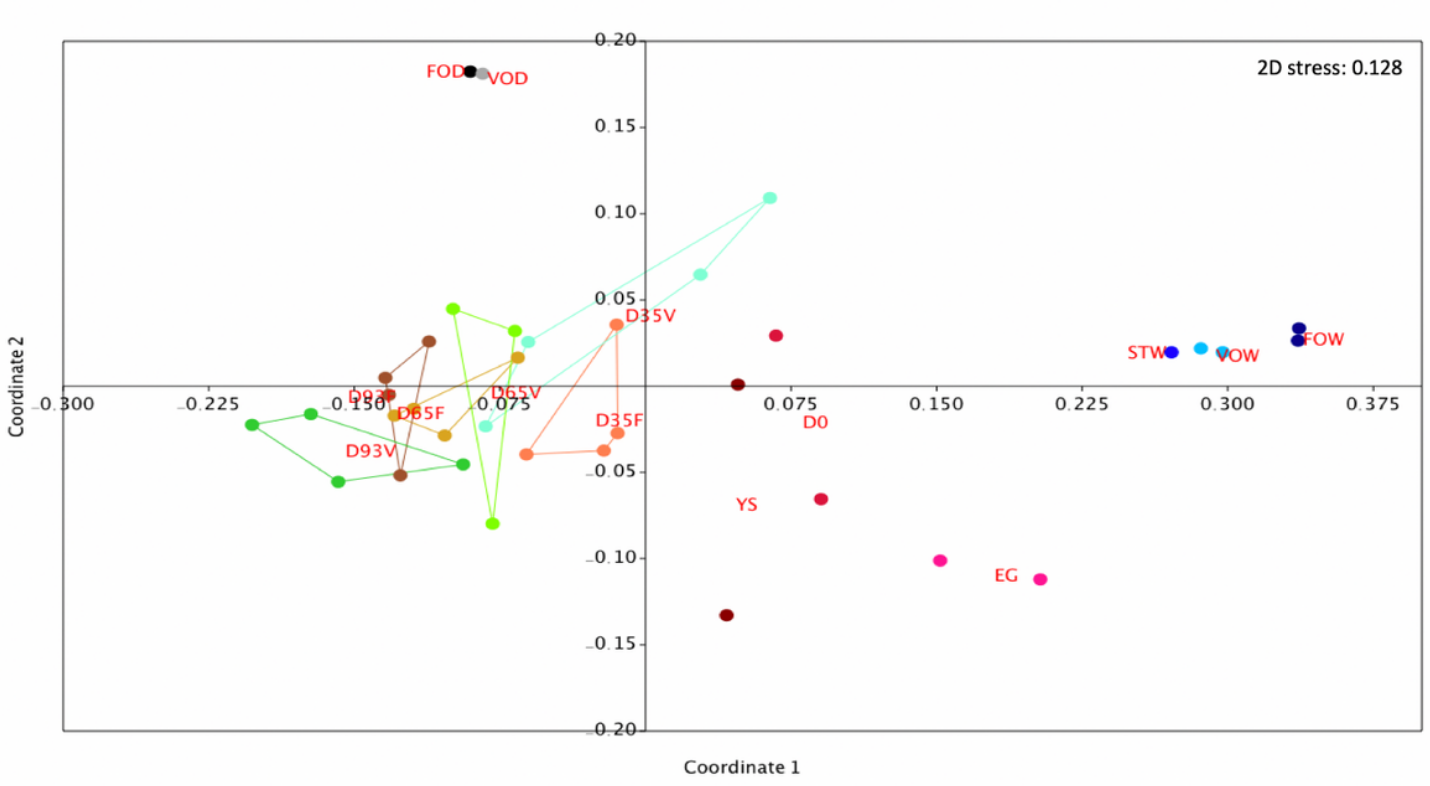
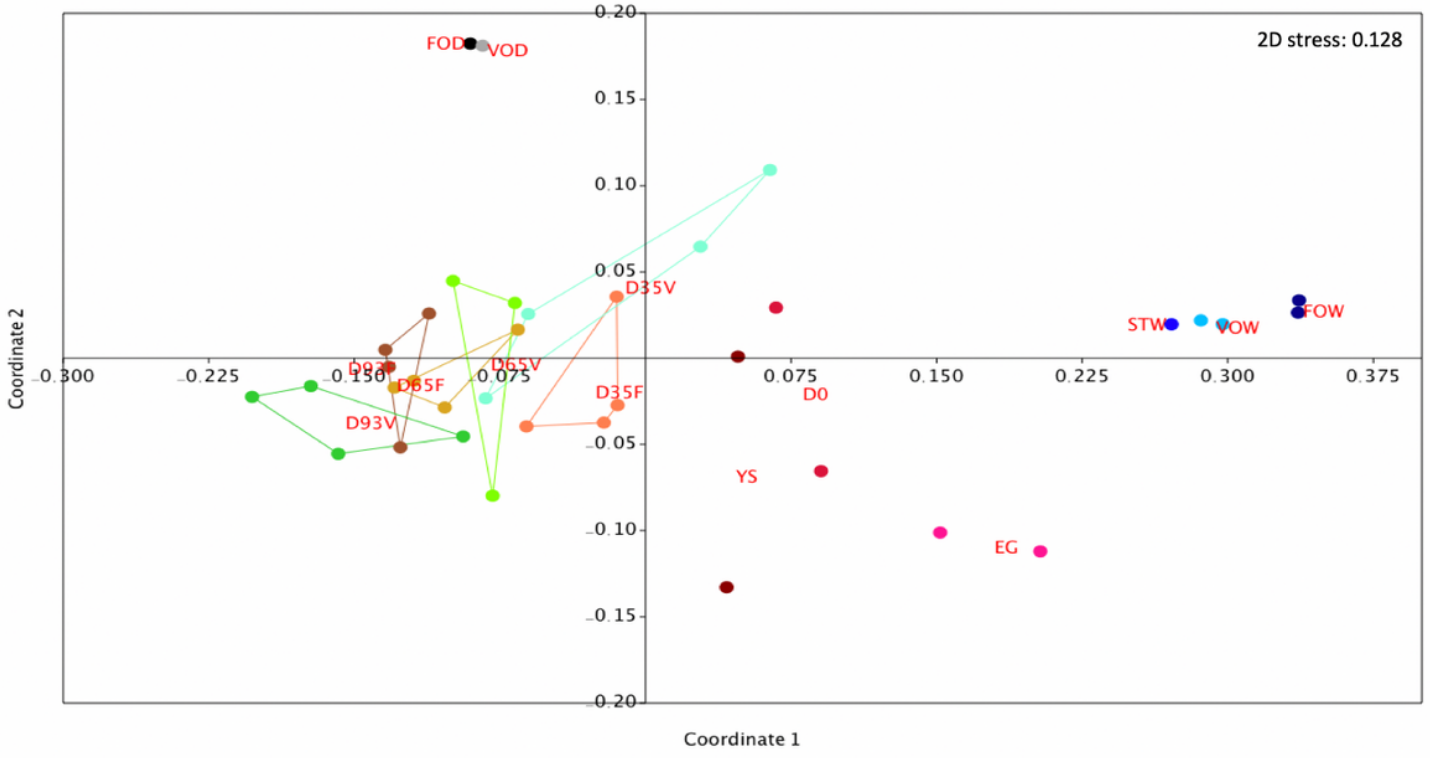


Figure 2

Non-metric multidimensional scaling (nMDS) plot for all the bacterial communities of all sample categories based on Bray-Curtis distances. D: day. Life stages of S. salar: EG, YS, D0, D35F, D35V, D65F, D65V, D93F and D93. Rearing water: VW, FW, STW. Feed: VD and FD



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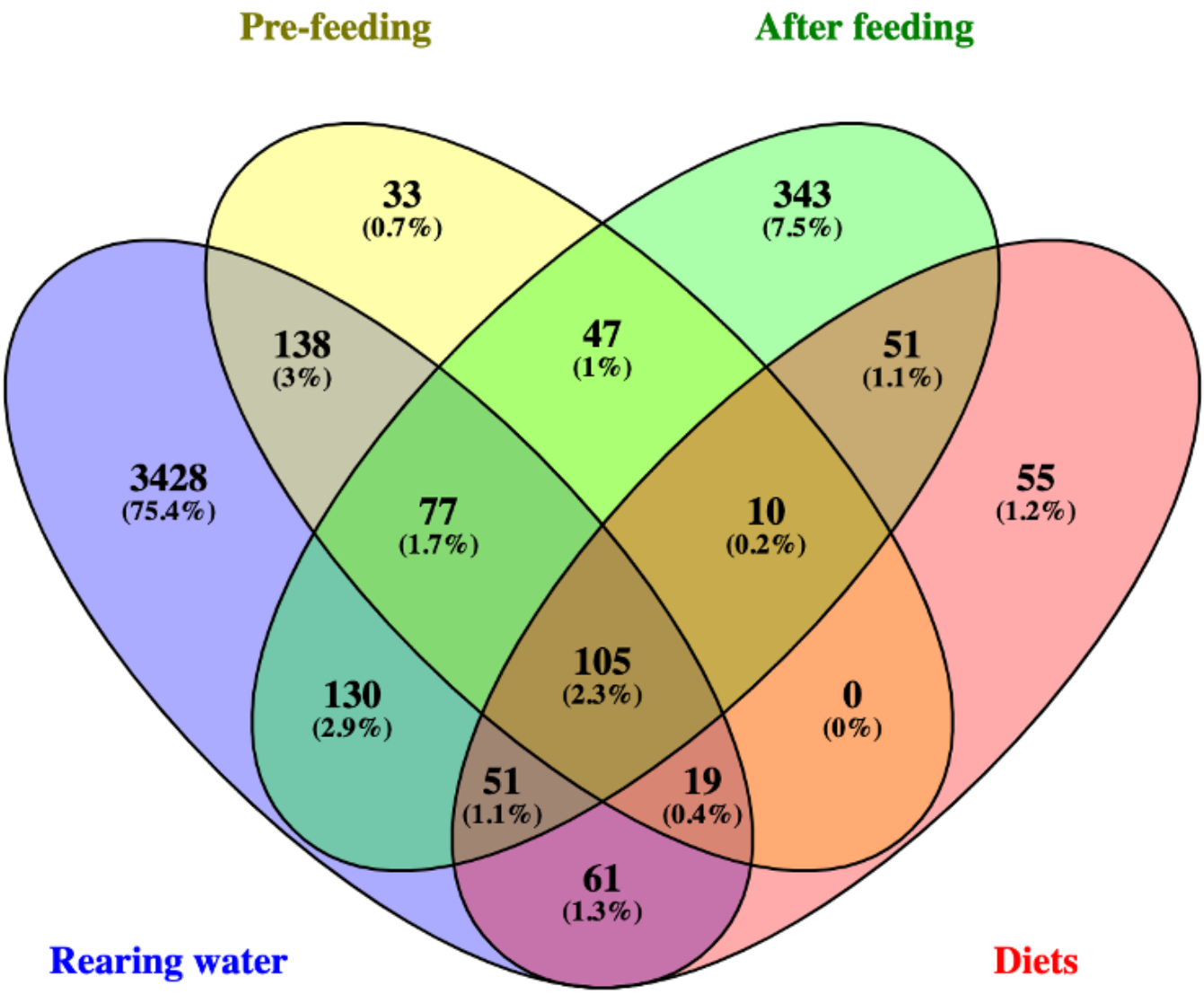
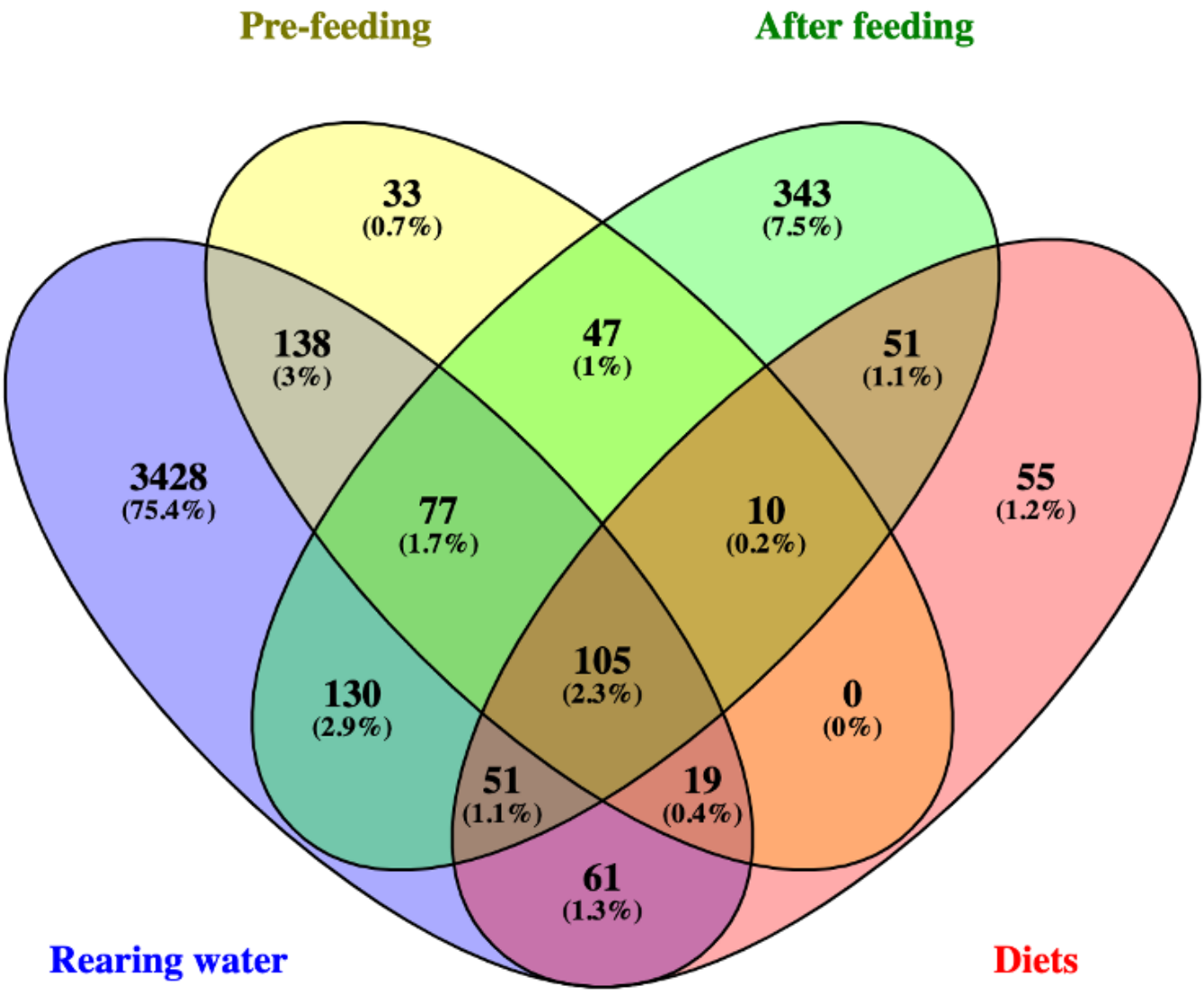


Figure 3

Venn diagram demonstrating the number of the shared and unique operational taxonomic units (OTU) and their percentage of the total library, between S. salar samples (before the first-feeding stage, after the first-feeding stage), diets (FD & VD) and rearing water samples.



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