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2 **Succession of embryonic and intestinal bacterial communities of**  
3 **Atlantic salmon**

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17 **Running Title:** Atlantic salmon microbiota at different life stages

18

19 **Abstract**

20 Host-associated microbiota undergoes continuous transition to achieve a stable  
21 community, and these modifications are immediately initiated from the birth of the  
22 host. In the present study, the succession of early life (eyed egg, embryo, and  
23 hatchling stages) and intestinal (the whole intestine at the early freshwater stages and  
24 the distal intestine at the late freshwater and seawater stages) bacterial communities of  
25 Atlantic salmon (*Salmo salar*; a prominent farmed fish) were studied using a 16S  
26 rRNA gene (V3 region) amplicon sequencing technique.

27 Stage-specific bacterial community compositions and the progressive transitions of  
28 the communities were evident in both the early life and the intestine. The embryonic  
29 communities were relatively less diverse, but after hatching the diversity increased  
30 significantly. A marked transition of the intestinal communities also occurred during  
31 the development. The most abundant functional pathways associated with the  
32 different stages were not affected by the transition of the community composition  
33 A perceptible transition in the community composition occurred during the  
34 development of Atlantic salmon. The transition generally did not alter the core  
35 functions of the community. Hatching and transfer to seawater are the key events that  
36 affect the bacterial diversity and community composition. The contribution of host-  
37 derived factors and environment in shaping the bacterial communities need to be  
38 confirmed through further studies.

39

40 **Keywords:** Fish, Atlantic salmon, developmental stages, bacterial communities,  
41 intestine, 16S rRNA gene

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43

## 44 **Introduction**

45 All animals are born into a microbe-rich environment, and the host establishes a  
46 symbiotic relationship with its microbial community. Such symbiotic relationships  
47 play vital roles in the physiological functions of the host [1-4]. The unstable and  
48 compositionally variable microbiota associated with early life undergoes continuous  
49 transitions (from the first few days to the first few years of life) to achieve a  
50 compositional profile resembling that of adults [5-8]. Although the transition from the  
51 infant to the adult microbiome of humans is relatively well documented, the transition  
52 during the ontogeny of fish and the establishment of their microbial communities are  
53 relatively less explored. Studies on the microbiota of larval Atlantic cod (*Gadus*  
54 *morhua*) and killifish (*Kryptolebias marmoratus*) [9, 10], and the intestinal microbial  
55 communities during the ontogeny of zebrafish (*Danio rerio*) [11, 12] have shed light  
56 on the functional importance and transformation of the early life communities in fish .  
57 Furthermore, the transition of the bacterial composition during the ontogeny of wild  
58 Atlantic salmon belonging to different cohorts was described by Llewellyn *et al.* [13].  
59 However, the general pattern of transition of the microbial communities in fish could  
60 be better explored if these fish belonged to a single cohort and were maintained under  
61 controlled conditions.

62

63 Atlantic salmon is an anadromous fish of high commercial value. In aquaculture  
64 production systems, embryos and larvae maintained in freshwater are immediately  
65 offered feeds after yolk sac absorption (~7-8 weeks post-hatching) and further reared  
66 in freshwater until these fish become smolts (a developmental stage that enables the  
67 fish to adapt to its physiological needs in seawater). Subsequently, the fish are  
68 transferred to seawater where they grow into adults. These ontogenic events are likely

69 to impact the microbiome of fish. In the present study, we employed a 16S rRNA  
70 gene-based phylotyping technique to assess the transition of the bacterial community  
71 at the embryonic stages of Atlantic salmon, and in the intestine of the fish from first  
72 feeding stage to the 80-week post-hatch stage. Additionally, we predicted the  
73 functional potential of the bacterial communities associated with different life stages  
74 of the fish.

75

## 76 **Results**

77 The sequences were clustered into 1442 OTUs, and the rarefied data, with a depth of  
78 2400 sequences/sample, were used to calculate the alpha and beta diversity indices  
79 (Additional file 2: Fig. S1a, b). The succession of the ontogeny-associated microbiota  
80 of the 4 groups is described in the present study.

81

### 82 *Hatching reflects a shift in the diversity and composition of the microbiota*

83 The bacterial communities of the early developmental stages were examined. The  
84 diversity indices (Shannon index and PD Whole tree) of the communities of HL were  
85 significantly higher ( $p < 0.05$ , Fig. 2a) compared with the EE and EBH communities.  
86 The evenness associated with the three stages did not significantly vary ( $p > 0.05$ , Fig.  
87 2a). The community compositions of the early developmental stages were  
88 significantly different (Figs. 2b, c; Additional file 6: Table S3a;  $p < 0.01$ ,  $R > 0.6$ , based  
89 on weighted and unweighted UniFrac distances).

90

91 *Proteobacteria* were significantly abundant in the EBH (Fig. 2f), although the results  
92 obtained for the EE (including *Methylothenera* and *Methylophilus*) and HL stages  
93 (*Undibacterium*) also showed the most dominant OTUs under this phylum (Fig. 2d,

94 e). The proportion of *Proteobacteria* decreased from the EBH to the HL (Fig. 2d),  
95 whereas those of *Actinobacteria*, *Tenericutes*, *Firmicutes*, *Bacteroidetes*,  
96 *Deinococcus-Thermus*, *Spirochaetes* (also identified as biomarkers, Fig. 2f) increased.  
97 *Deltaproteobacteria* in the EE and *Betaproteobacteria* in the EBH were observed as  
98 significantly abundant classes under the phylum *Proteobacteria*. The members of the  
99 orders *Methylophilales* (*Betaproteobacteria*) and *Myxococcales* (*Deltaproteobacteria*)  
100 were abundant in the EE, whereas most of the OTUs under the order *Burkholderiales*  
101 were significantly abundant in either the EBH or the HL (Fig. 2f). The OTUs  
102 belonging to the orders *Pseudomonadales*, *Alteromonadales*, *Virbionales*,  
103 *Rhizobiales*, *Caulobacterales*, *Sphingomonadales*, *Actinomycetales*, *Bacillales*,  
104 *Lactobacillales*, *Spingobacteriales*, *Mycoplasmatales*, *Spirochaetales*, and *Thermales*  
105 were significantly abundant in the HL. Two classes of *Proteobacteria* were  
106 significantly abundant in the HL: *Alpha-* and *Gammaproteobacteria*. Furthermore, all  
107 OTUs under the above-mentioned classes, except one OTU belonging to the  
108 *Rhizobacter*, were significantly abundant in the HL (Fig. 2f). The significantly  
109 abundant OTUs under each phylum and their effect sizes are listed in Additional file  
110 7: Table S4a.

111

112 ***Successional changes in the diversity and composition of the intestinal bacterial***  
113 ***community of fish at the early freshwater stages***

114 The alpha diversity indices of the communities associated with the intestine of fish at  
115 the early freshwater stages did not significantly vary (Fig. 3a). The intestinal bacterial  
116 communities of the fish at the early freshwater stages were significantly different  
117 (Fig. 3c; Additional file 6: Table S3b;  $p < 0.01$ ,  $R > 0.5$ ; based on weighted UniFrac  
118 distances 7, 8, 10 vs. 12 wph).

119 *Proteobacteria* was the dominant phylum in all the stages (Fig. 3d). However, as the  
120 fish were growing the changes were evident from the significantly abundant OTUs  
121 associated with the stages (Fig. 3f). The phylum *Proteobacteria* was significantly  
122 abundant at 8 wph, primarily reflecting the abundance of the OTUs of the order  
123 *Pseudomonadales*, whereas *Vibrionales*, *Alteromonadales* and the families and genera  
124 under these orders were significantly abundant at 10 wph. The significantly abundant  
125 OTUs belonging to *Comamonadaceae* under *Burkholderiales* made  
126 *Betaproteobacteria* a significant feature at 12 wph, whereas the OTUs of  
127 *Oxalobacteriaceae*, belonging to *Betaproteobacteria*, were significantly abundant at 7  
128 wph. *Alphaproteobacteria* was significantly abundant at 7 wph, comprising the OTUs  
129 belonging to *Sphingomonadales* and *Methylobacteriaceae*. However,  
130 *Caulobacteriales* (*Alphaproteobacteria*) and its members were significantly abundant  
131 at 12 wph (Fig. 3f). The phyla *Actinobacteria* and *Deinococcus-Thermus* were  
132 significantly abundant at 7 wph (Fig. 3f). *Bacteroidetes* was significantly abundant at  
133 12 wph, primarily reflecting the significant abundances of the *Flavobacterial* lineage,  
134 whereas the class *Sphingobacteria* (*Bacteroidetes*) was significantly abundant at 7  
135 wph. *Firmicutes* and most of the members of this phylum, particularly the OTUs  
136 belonging to the class *Bacilli*, were significantly abundant at 12 wph. Additional file  
137 7: Table S4b lists the significantly different OTUs and their effect sizes under each  
138 phylum.

139

140 ***Successional changes in the diversity and composition of the distal intestinal***  
141 ***community of fish at the late freshwater stages***

142 The Shannon index of the bacterial communities at 20 and 44 wph were significantly  
143 different (Fig. 4a). However, the richness (PD whole tree) and evenness (Simpson's

144 evenness) of the communities, when considered individually, did not significantly  
145 vary (Fig. 4a). The fish at the late freshwater stages had significantly different [Fig.  
146 4b, c; Additional file 6: Table S3c;  $p < 0.01$ ,  $R > 0.8$ , based on unweighted (20 vs. 44  
147 wph) and weighted UniFrac distances (20 vs. 44, 62 wph)] bacterial communities.  
148 *Firmicutes* was the most dominant phylum in the distal intestine at 20, 44 and 62 wph  
149 (Fig. 4d). In addition, 2 OTUs with taxonomy prediction confidence estimates  $< 0.5$   
150 (hence excluded from the LEfSe analysis) belonging to the phylum *Firmicutes*  
151 (indicated using \*, Fig. 4d; including the genus *Laceyella*, Fig. 4e) were also  
152 predominant in this group of fish. The phylum *Firmicutes* and the OTUs under this  
153 group, *Lactobacillales* and *Bacillales*, comprising the class *Bacilli*, were significantly  
154 abundant at 20 wph (Fig. 4f). The class *Clostridia*, however, was significantly  
155 abundant at 62 wph (primarily reflecting one OTU belonging to *Anaerofilum*). Other  
156 OTUs belonging to *Peptostreptococcaceae* and some unassigned OTUs under  
157 *Clostridiales* were significantly abundant at 44 wph (Fig. 4f). While the phylum  
158 *Tenericutes* and its members were significantly abundant at 62 wph, the phylum  
159 *Bacteroidetes* and its members were significantly abundant at 20 wph. At the phylum  
160 level, *Proteobacteria* was not a significant feature of any of the stages. However, the  
161 classes under this group (*Alpha-*, *Beta-* and *Gammaproteobacteria*) were significant  
162 features at 20 wph (Fig. 4f). Interestingly, at the order level, the significantly  
163 abundant features belonged to different stages, including *Rhizobiales* (20 wph) and  
164 *Caulobacteriales* (62 wph) of *Alphaproteobacteria*, *Pseudomonadales* (20 wph),  
165 *Enterobacteriales* (20 wph), *Vibrionales* (44 wph) and *Aeromonadales* (62 wph) of  
166 *Gammaproteobacteria* (Fig. 4f). Additional file 7: Table S4c lists the significantly  
167 different OTUs and their effect sizes under each phylum.  
168

169 *Successional changes in the diversity and composition of the distal intestinal*  
170 *community of seawater fish*

171 The Shannon indices of the communities associated with the distal intestine of the  
172 Atlantic salmon in seawater (65, 68 and 80 wph stages) were significantly different  
173 (Shannon index; Fig. 5a,  $p < 0.05$ ). The bacterial community compositions of fish at  
174 the seawater stages were significantly different (Fig. 5b, c; Additional file 6: Table  
175 S3d;  $p < 0.01$ ,  $R < 0.6$ , based on unweighted and weighted UniFrac distances).  
176 *Firmicutes*\* was the dominant phylum in the distal intestine of the fish in seawater,  
177 particularly at 65 and 80 wph (Fig. 5d). The 2 OTUs (with low taxonomic assignment  
178 confidence,  $< 0.5$ ) belonging to the genus *Laceyella* (phylum *Firmicutes*) were also  
179 predominant at 65, 68 and 80 wph (Fig. 5e). The phylum *Spirochaetes* was also  
180 predominant in the distal intestine at 68 wph. *Actinobacteria*, *Tenericutes* and  
181 *Firmicutes* were the significantly abundant phyla at 65 wph. *Spirochaetes* and  
182 *Bacteroidetes* were the significant phyla at 68 and 80 wph, respectively. Under  
183 *Firmicutes*, one OTU belonging to *Weissella* was a feature of the 80 wph, making  
184 *Lactobacillales* a significant feature at 80 wph. Although at 65 wph more significantly  
185 abundant taxonomic biomarkers were observed for the phylum *Proteobacteria*,  
186 phylum-level significant abundance was not detected. The classes  
187 *Alphaproteobacteria*, *Epsilonproteobacteria* and their members were significantly  
188 abundant at 65 wph. Under *Proteobacteria*, the orders *Alteromonadales*,  
189 *Pseudomonadales* and 2 OTUs belonging to the genus *Vibrio* were significantly  
190 abundant at 65 wph (Fig. 5f). Under *Pseudomonadales*, 2 OTUs of *Psychrobacter* and  
191 *Pseudomonas* were the significantly abundant features at 80 wph (Fig. 5f). Additional  
192 file 7: Table S4d lists the significantly different OTUs and their effect sizes under  
193 each phylum.



194 The bacterial compositional shift at the phylum-level along all the stages sampled is  
195 shown in Fig. 6. The prominence of *Proteobacteria* decreased slowly, and when the  
196 fish was in seawater *Firmicutes* and *Spirochaetes* surpassed *Proteobacteria* in  
197 dominance.

198

199 *Comparison of the communities associated with the hatchlings and the intestine of*  
200 *fish at the early freshwater stage*

201 The alpha diversity indices of the communities at the HL and 7 wph stages did not  
202 significantly vary (Additional file 2: Fig. S2a). However, the bacterial community  
203 compositions at the HL and 7 wph stages were significantly different (Additional file  
204 2: Fig. S2c; Additional file 6: Table S3e –  $p < 0.01$ ,  $R > 0.6$ ; based on weighted UniFrac  
205 distances).

206

207 The dominant OTUs of the 2 stages (Additional file 2: Fig. S2d, e) and their phylum  
208 level biomarkers (Additional file 2: Fig. S2f) indicate differences in the bacterial  
209 communities. *Actinobacteria*, *Tenericutes* and *Spirochaetes*, and their members were  
210 significantly abundant in the HL, whereas, *Fusobacteria*.ph and *Firmicutes*, and their  
211 members were significant features at 7 wph (Additional file 2: Fig. S2f).

212 *Proteobacteria* was not identified as a biomarker, but the classes *Beta*- and  
213 *Gammaproteobacteria* and the associated OTUs were significantly abundant in the  
214 HL and at 7 wph. The effect sizes of the respective features are provided in Additional  
215 file 7: Table S4e.

216

217 *Comparison of the communities associated with the whole and distal intestine of*  
218 *fish at the freshwater stage*

219 The Shannon index and evenness of the bacterial communities at 20 wph were  
220 significantly lower ( $p < 0.05$ ) compared to those at 12 wph (Additional file 3: Fig.  
221 S3a). However, the richness (PD whole tree) associated with the two stages was  
222 similar. The bacterial community compositions of the two stages were significantly  
223 different (Additional file 3: Fig. S3c; Additional file 6: Table S3e;  $p < 0.01$ ,  $R > 0.8$ ,  
224 based on the weighted UniFrac distances).

225

226 *Firmicutes* and *Proteobacteria* were the dominant phyla in the two stages examined  
227 (Additional file 3: Fig. S3d). The phylum *Actinobacteria*, *Fusobacteria.ph*,  
228 *Bacteroidetes* and *Proteobacteria* were significantly abundant at 12 wph (Additional  
229 file 3: Fig. S3f), whereas *Tenericutes*, *Spirochaetes* and *Firmicutes* were significantly  
230 abundant at 20 wph. Under *Firmicutes*, the class *Clostridia* and its members were  
231 significantly abundant at 12 wph, whereas *Bacilli* were significantly abundant at 20  
232 wph (Additional file 3: Fig. S3f). Under *Bacilli*, 4 OTUs belonging to *Lactobacillus*,  
233 *Streptococcus*, *Vagococcus* and *Filibacter* were the significant features at 12 wph.  
234 The effect sizes of the respective features are provided in Additional file 7: Table S4f.

235

### 236 *Comparison of the communities associated with the distal intestine of freshwater* 237 *and seawater fish*

238 There were no significant differences in the diversity indices (Additional file 3: Figs.  
239 S4a, b, c; Additional file 6: Table S3e;  $p < 0.01$ ,  $R < 0.6$ ) associated with 62 and 65 wph.  
240 *Firmicutes*, *Tenericutes* and *Proteobacteria* were the dominant phyla at the two stages  
241 examined (Additional file 3: Fig. S4d). *Bacteroidetes* and *Firmicutes* were abundant at  
242 62 wph (freshwater), whereas *Proteobacteria* was significantly abundant at 65 wph  
243 (seawater) (Additional file 3: Fig. S4f). Some members of *Proteobacteria* namely,

244 *Caulobacterales*, *Burkholderiales* and *Pseudomonadaceae* were the abundant features  
245 at 62 wph (Additional file 3: Fig. S4f). The OTUs under *Firmicutes*, including  
246 *Clostridiales*, *Bacillales*, *Streptococcus* and *Leuconostocaceae*, were the significant  
247 features at 65 wph. The effect sizes of the respective features are provided in  
248 Additional file 7: Table S4g.

249

### 250 *Presumptive functions of the communities at different stages*

251 The presumptive functional pathways associated with the microbiota at different  
252 stages were analysed to identify the stage-specific significant functional potential of  
253 these bacteria. The NSTI (Nearest Sequenced Taxon Index) scores (Langille *et al.*,  
254 2013) corresponding to each of the predictions are provided in Additional file3: Fig.  
255 S5. Additional file 8: Table S5a lists the five most abundant KEGG modules and  
256 differentially abundant features ( $p < 0.01$  and effect size  $> 0.75$ ) at each stage. The  
257 functional potential of the community of the HL was significantly different from those  
258 of the EE and EBH (Fig. 7, Additional file 9: Table S6a;  $p < 0.01$ ,  $R > 0.85$ ). The  
259 functions associated with the communities of the fish at the early freshwater and the  
260 seawater stages were not significantly different (Fig. 7, Additional file 9: Table S6b,  
261 d). The functional potential of the distal intestinal community of the fish at the early  
262 freshwater stages (20 wph) was significantly different from that of the fish at the late  
263 freshwater stages (44 and 62 wph, Fig. 7, Additional file 9: Table S6c;  $p < 0.01$ ,  
264  $R > 0.6$ ).

265

266 The seven pathways that were significantly abundant across stages were branched-  
267 chain amino acid transport system, peptides/nickel transport system, riboflavin

268 biosynthesis, multiple sugar transport system, pentose phosphate pathway, phosphate  
269 transport system and glycolysis.

270

## 271 *Discussion*

272 The present study profiled the bacterial communities of Atlantic salmon to examine  
273 the progressive transition of these fish communities during the early embryonic stages  
274 (EE, EBH and HL), in the intestine during the early freshwater stages (7, 8, 10, 12  
275 wph), in the distal intestine of the late freshwater stages (20, 44, 62 wph), and in the  
276 distal intestine of the seawater stages (65, 68 and 80 wph). Shifts in the predicted  
277 functional content of the communities are also discussed. The gut microbiota of the  
278 aquacultured species (grass carp, *Ctenopharyngodon idella*; Chinese perch, *Siniperca*  
279 *chuatsi*; and southern catfish, *Silurus meridionalis*) from the same regional pool are  
280 reported to be similar as well as developmental stage-dependent, and they are distinct  
281 when compared with the bacteria in water [14]. It is plausible that the similar  
282 deterministic processes also regulate the succession in the bacterial communities of  
283 the Atlantic salmon.

284

285 Fish eggs are colonized by diverse microbial communities [15, 16]. In the present  
286 study, the bacterial community associated with the whole organism was examined up  
287 to the hatching stage. The alpha diversity indices at the embryonic stages (egg  
288 surface) were the lowest compared with the hatched larvae. The predominant OTUs  
289 associated with the embryonic stages of Atlantic salmon belonged to *Methylothera*  
290 and *Undibacterium*. *Vibrio fischeri* and *Leucothrix mucor* were abundant on cod eggs,  
291 whereas *Moraxella* and *Alcaligenes* were abundant on halibut eggs. In addition,  
292 microbiota of cod larvae was highly distinct from those of their environment and live

293 feed [9, 15, 16]. Taken together, these findings suggest that the early life communities  
294 are species- and stage-specific.

295

296 The transition from eyed eggs (EE) to those prior to hatching (EBH) was  
297 characterised based on changes, particularly at the genus level: *Methylothenera* and  
298 *Methylophilus* were dominant in the EE, whereas *Undibacterium* was dominant in the  
299 EBH and HL. These communities are likely to be egg surface-specific [17, 16, 18],  
300 and the mechanisms causing such shifts are not clear yet, although neutral and non-  
301 neutral assembly models have been proposed for zebrafish [19]. As zebrafish ages, the  
302 assembly of the associated bacterial community is not decided according to chance  
303 and dispersal, but through microbial interactions, active dispersal, or host selection  
304 [19]. The hatchling-associated community was significantly diverse (phylogenetically  
305 different) compared with the communities prior to hatching. Hatching is a critical  
306 process because the sterile embryo contacts the microbe-rich environment [16, 20]  
307 when the immune system of the organism is still immature [21]. These diverse  
308 community members might aid the host in defence against pathogens [22, 16, 23].  
309 From the hatching stage onward, major mucosal organs, such as the gills, skin and  
310 gut, become functionally active [24], and the specific phylotypes that colonize these  
311 microenvironments might play key roles in the normal development of these organs  
312 [25-27, 23, 16]. In addition, at this stage, oxygen uptake changes from cutaneous to  
313 pharyngeal [28], and this development could affect the community composition.  
314 These ontogenic changes might contribute to the HL-associated diverse bacterial  
315 community.

316

317 After the formation of the gut, i.e., 7 weeks after hatching, the bacterial community  
318 associated with the whole intestine was assessed. The alpha diversity indices of the  
319 intestinal microbiota at 7 wph (prior to first feeding) and the stages after feeding (8,  
320 10 and 12 wph) did not significantly vary. Feeding led to a transition of the rainbow  
321 trout larval intestine from a *Bacteroidetes*-dominant to a *Firmicutes*- and  
322 *Proteobacteria*-dominant community [27]. The observations in the present study  
323 suggest that feeding causes a phylum-level shift to *Proteobacteria* (at 8 wph) and  
324 *Bacteroidetes* (as a result of the *Flavobacterial* lineage, at 12wph), and *Firmicutes*  
325 (primarily reflecting the genus *Weissella*, at 12 wph).

326

327 The distal intestine was clearly distinguishable at 20 wph; therefore, the bacterial  
328 community associated with this intestinal region was analysed from this time point.  
329 The significant decrease in the alpha diversity index (20 vs. 44 wph) could reflect the  
330 less diverse community at 44 wph and the overrepresentation of *Spirochaetes* in the  
331 distal intestinal microbiota associated with this stage. Similar to the findings in the  
332 present study, *Spirochaetes* are highly abundant in other carnivorous fish, including  
333 mahi-mahi (*Coryphaena hippurus*) and great barracuda (*Sphyraena barracuda*) [29].  
334 In the present study, the phylum *Firmicutes* was significantly abundant at 20 wph.  
335 The genera *Weissella*, *Laceyella*\* and *Anaerofilum* were the predominant contributors  
336 to the significant abundance of *Firmicutes*. Rainbow trout, also presents a high  
337 abundance of *Firmicutes*, with OTUs belonging to Bacilli as the predominant type  
338 [30]. This observation is similar to the findings in the present study. In contrast,  
339 members of *Bacilli* were not abundant in the gut of the cyprinids common carp  
340 (*Cyprinus carpio*) and zebrafish (*Danio rerio*) [31, 32], indicating the importance of  
341 *Firmicutes* in salmonids. Furthermore, the phylum *Tenericutes* became significantly

342 abundant just prior to the transfer of these fish to seawater. *Tenericutes* are highly  
343 abundant in salmon (both in freshwater and seawater) [33, 13] and trout intestines  
344 [34].  
345  
346 *Firmicutes* were significantly abundant soon after the fish were transferred to  
347 seawater, and the OTUs belonging to *Laceyella*\* remained predominant. In addition,  
348 the OTUs belonging to *Spirochaetes*, *Proteobacteria* and *Tenericutes* were also  
349 prominent. The dominance of *Spirochaetes* at 44 wph and the significant abundance  
350 of the phylum at 68 wph suggest an important role of this taxon in the gut microbiota  
351 of carnivorous fish. During the seawater stages, the alpha diversity index of the distal  
352 intestinal community significantly decreased with time. The lower alpha diversity  
353 indices and the overabundance of the few phylotypes in the microbiota of the intestine  
354 [13] and skin [35] of adult Atlantic salmon and rainbow trout intestine [34] have been  
355 previously documented. Changes in the phylum *Tenericutes* (mainly *Mycoplasma*  
356 spp.) during development were minimal in the present study. Although *Tenericutes*  
357 were part of the microbiota at the early developmental stages and were significantly  
358 abundant in the HL and the distal intestine at 62 and 65 wph, the proportion of this  
359 phylum (20% at 62 wph) was much less compared with the study by Holben *et al.*  
360 [33], who reported 70-90% *Tenericutes* in most of their samples. Another study on the  
361 transition in the community composition of the wild Atlantic salmon by Llewellyn *et*  
362 *al.* [16] showed that the proportion of *Mycoplasma* spp. increased consistently with  
363 development and it was most abundant in the seawater fish. In addition, previous  
364 reports on the abundance of *Mycoplasma* spp. in the intestine are contrasting;  
365 Llewellyn *et al.* [16] and Holben *et al.* [33] found an over dominance, whereas  
366 Zarkasi *et al.* [36, 37] detected only sporadic occurrence of the species. These

367 discrepancies could be because of the genetic background or the geographical  
368 locations of the fish sampled.

369

370 We also examined the diversity and significantly abundant phyla associated with the  
371 whole animal, whole intestine, and distal intestine of the fish in freshwater and  
372 seawater by conducting the following comparisons; HL vs. 7, 12 vs. 20, and 62 vs. 65  
373 wph. These comparisons revealed significant differences in the diversity indices and  
374 the composition of the communities, which was even reflected at the phylum level.

375 *Firmicutes* were significantly abundant in the whole and distal intestine of the fish in  
376 freshwater but not in the distal intestine of the fish in seawater. *Proteobacteria*,  
377 however, were significantly abundant in the whole and distal intestine of the fish in  
378 seawater. These results indicate that the *Proteobacteria*-rich community in the early  
379 intestine changes to a *Firmicutes*-rich distal intestinal community in freshwater.

380 However, when the fish were introduced into seawater, *Proteobacteria* regained  
381 significant abundance. This transition to a *Proteobacteria*-rich community when the  
382 fish enters seawater has been previously recorded in fish skin microbiota [35, 38]. A  
383 meta-analysis also revealed the differences in the gut bacterial community  
384 compositions of freshwater and the seawater fishes [39].

385

### 386 *The presumptive functional pathways of the bacterial communities of Atlantic* 387 *salmon*

388 The mean weighted NSTI scores for the communities at different stages ranged from  
389  $0.043 \pm 0.006$  to  $0.295 \pm 0.037$ . In general, the early stages had lower NSTI values  
390 compared with the stages from 44 wph onward. This finding indicates that the  
391 metagenomes of the communities associated with the distal intestine were predicted



392 based on higher taxonomic levels, which make these data less accurate. The five core  
393 functions of the bacteria associated with Atlantic salmon included biosynthetic  
394 (riboflavin) and transport pathways. These functions were associated with all stages of  
395 development and did not vary, despite differences in the community composition,  
396 indicating their importance throughout development. The core metabolic functional  
397 potential of bacteria can be similar, even when there are differences in the  
398 phylogenetic content [40]. However, the functional pathways that were significantly  
399 represented in the EE and HL and in the intestine at 20 wph indicate the specific  
400 needs of the associated bacteria or host. These pathways included biosynthesis of  
401 pantothenate, biotin, ADP-L-glycero-D-manno-heptose, heme, methionine and ketone  
402 body. The significance of these functional pathways in relation to the physiological  
403 needs of the fish should be explored further.

404

#### 405 *Conclusion*

406 The present study examined the transition of the embryonic and intestinal bacterial  
407 communities of Atlantic salmon. Stage-specific microbial signatures were evident at  
408 the phylum level. *Proteobacteria* was the most abundant phylum in eggs, and its  
409 abundance decreased in the hatchlings. The diversity of the hatchling-associated  
410 community increased, reflecting the significant abundance of *Actinobacteria*,  
411 *Firmicutes*, *Tenericutes*, *Spirochaetes* and *Deinococcus-Thermus*. In the intestine of  
412 the fish at the early freshwater stages, the phylum *Proteobacteria* was dominant.  
413 Although *Firmicutes* and *Bacteroidetes* subsequently became the significantly  
414 abundant phyla, only the dominance of *Firmicutes* was evident in the distal intestine  
415 of the fish at the late freshwater stages. After the fish were in seawater,  
416 *Proteobacteria* again became the significantly abundant phylum. However,

417 *Firmicutes*, *Spirochaetes*, *Tenericutes* and *Actinobacteria* were the significantly  
418 abundant phyla as the fish adapted to its life in seawater. The functional redundancy  
419 of the taxonomically dissimilar communities associated with the different stages are  
420 likely related to the specific needs of the associated bacterial communities or host.

421

## 422 **Methods**

### 423 *Biological material*

424 Samples (n=10) from selected life stages (up to smolts) of the fish were procured  
425 from a local hatchery (Cermaq AS, Hopen, Bodø, Norway). The smolts were  
426 transported to the research station at Nord University and further reared in a seawater  
427 facility at the station. More information is provided in Additional Methods.

428

### 429 *Sampling*

430 The fish were euthanized prior to sampling. The samples from the successive  
431 developmental stages were classified in 4 groups as follows: i) the whole organism  
432 (early developmental stages: eyed egg stage, EE; embryo before hatching, EBH; and  
433 hatched larvae, HL); ii) the whole intestine of the fish at early freshwater stages (7, 8,  
434 10 and 12 weeks post hatch, wph); iii) the distal intestine of fish at the late freshwater  
435 stage (20, 44 and 62 wph); and iv) the distal intestine of fish at the seawater stage (65,  
436 68 and 80 wph) (Fig. 1). Further details are provided in Additional file 1: Methods  
437 and Additional file 4: Table S1.

438

439 *DNA extraction, preparation of the sequencing libraries (V3-V4 region), library*  
440 *quantification and sequencing*

441 DNA from the samples was extracted using the QIAamp Fast DNA Stool Mini Kit  
442 (Qiagen, Nydalen, Sweden). The samples were processed according to the  
443 manufacturer's protocol, with few modifications as detailed in the Additional file 1:  
444 Methods.

445 A paired end, dual index protocol was adopted to amplify and prepare the 16S rRNA  
446 gene (V3-V4 regions) sequencing libraries [41]. The PCR reactions were performed  
447 in a 25  $\mu$ l reaction volume containing 12.5  $\mu$ l of Kapa HiFi Hot Start PCR Ready Mix  
448 (KAPA Biosystems, Woburn, USA), 2.5  $\mu$ l of each forward and reverse primer (300  
449 nM), and 7.5  $\mu$ l of DNA and water. The thermocycling conditions included initial  
450 denaturation at 95°C, followed by 35 cycles of 98°C-30s, 58°C-30s and 72°C. The  
451 final extension was performed at 72°C for 2min. The PCR products (sequencing  
452 libraries) were run on agarose gel and purified, and the libraries were quantified and  
453 pooled at equimolar (2 nM) concentrations prior to sequencing (see Additional file 1:  
454 Methods).

455

#### 456 *Data analysis*

457 UPARSE [42] was used for quality filtering and OTU clustering. Forward reads  
458 comprising the V3 region (see Additional file 1: Methods) of the 16S rRNA gene  
459 were quality filtered, truncated to 200 bp, dereplicated, and abundance sorted, and  
460 reads with less than 10 sequences were discarded. OTUs were clustered at a 97%  
461 similarity level, and chimeric sequences were removed using UCHIME [43]. The  
462 reads were subsequently mapped to OTUs after searching the reads as a query against  
463 the OTU representative sequences. Taxonomic ranks were assigned to the OTUs  
464 using the UTAX algorithm ([http://www.drive5.com/usearch/manual/utax\\_algo.html](http://www.drive5.com/usearch/manual/utax_algo.html)).  
465 OTU tables were prepared and split into the 4 study groups (as described in the

466 section Sampling), and comparisons of the bacterial communities in the 4 groups  
467 (whole organism, EE, EBH and HL; whole intestine at 7, 8,10 and 12 wph; freshwater  
468 distal intestine at 20, 44 and 62 wph; and seawater distal intestine at 65, 68 and 80  
469 wph) were performed separately. To explore the intergroup changes in the diversity  
470 and abundances of the associated microbiota, we conducted three additional  
471 comparisons: whole organism vs. intestine (HL and 7 wph), intestine vs. distal  
472 intestine (12 and 20 wph), and freshwater distal intestine vs. seawater distal intestine  
473 (62 and 65 wph). The read statistics of the sequences are provided in Additional file 5:  
474 Table S2. For each of the 4 groups the diversity indices were calculated, and the  
475 differential abundance analyses were performed separately on the 4 groups using  
476 QIIME [44] and LEfSe [45], respectively. The PCoA plot and cladogram showing the  
477 differential abundances were created using phyloseq [46] and GraPhlAn [47],  
478 respectively. Presumptive metabolic potential was computed using PICRUSt [48], and  
479 the resulting gene abundance data were profiled into metabolic pathways using  
480 HUMAnN [49], with the default settings. Subsequently, the KEGG modules were  
481 analysed using STAMP [50], and the Bray-Curtis dissimilarities were plotted using  
482 phyloseq (see Additional file 1: Methods).

483

#### 484 *List of abbreviations*

485 16S rRNA, 16S ribosomal RNA; EBH, embryo before hatching; EE, eyed egg stage;  
486 FOTS, Forsøksdyrforvatningen tilsyns- og søknadssystem HL, hatched larvae;  
487 HUMAnN, HMP unified metabolic analysis network KEGG, Kyoto encyclopedia of  
488 genes and genomes; LEfSe, linear discriminant analysis effect size NSTI, nearest  
489 sequenced taxon index OTU, operational taxonomic unit; PCoA, principal coordinate  
490 analysis PCR, Polymerase chain reaction; PD Whole tree, phylogenetic diversity

491 whole tree; PICRUSt: Phylotypic investigation of communities by reconstruction of  
492 unobserved states QIIME: Quantitative insights into microbial ecology; V3 and V4  
493 regions, hypervariable regions 3 and 4; wph, weeks post hatch

494

#### 495 **Declarations**

#### 496 *Ethics approval*

497 This study was conducted according to the guidelines of the Norwegian Food Safety  
498 Authority (FOTS ID: 7899).

499

#### 500 **Availability of data and material**

501 Sample metadata, read statistics, statistical analyses results and additional methods  
502 and figures are provided as Additional files. Please contact author for further data  
503 request.

504

#### 505 **Competing interests**

506 The authors declare that they have no competing interests.

507

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511

#### 512 **Author contributions**

513 JL and VK conceived the study. JL performed the experiments and data analysis,  
514 wrote and redressed the manuscript. VK scrutinized the data, read and redressed the

515 manuscript. DS, JF and TM discussed the experimental design, read and critically  
516 edited the manuscript.

517

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523

524

525

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686

687 **Figure legends**

688 **Fig. 1.** Ontogenetic timeline of salmon depicting the successive developmental stages  
689 that were targeted in the present study: early developmental stages; early freshwater  
690 stages; late freshwater stages; seawater stages. wph: weeks post hatching.

691 **Fig. 2.** Plots showing the comparisons of microbiota associated with the early  
692 developmental stages (EE, EBH, and HL) of Atlantic salmon. Stage-specific colour  
693 coding was used for Figures a, b, c, and f. (a) Alpha diversity indices (Shannon index,  
694 PD whole tree, and Simpson evenness) of the bacterial communities and (b, c)  
695 UniFrac distances-based PCoA. The mean relative abundance of the 10 most  
696 abundant OTUs at the (d) phylum and (e) genus levels. The OTUs are coloured  
697 according to their taxonomic classification, and OTUs without any assignment are  
698 shown in grey. (f) Cladogram showing the significantly abundant taxonomic groups in  
699 each of the stages, identified based on the LEfSe ( $p < 0.05$  and effect size  $> 3.5$ ).

700 **Fig. 3.** Plots showing the comparisons of the microbiota associated with the whole  
701 intestine of Atlantic salmon in freshwater (7, 8, 10 and 12 wph). Stage-specific colour  
702 coding is used for Figures a, b, c, and f. (a) Alpha diversity indices (Shannon index,  
703 PD whole tree, and Simpson evenness) of the bacterial communities and (b, c)  
704 UniFrac distances-based PCoA. The mean relative abundance of the 10 most  
705 abundant OTUs at the (d) phylum and (e) genus levels. OTUs are coloured according  
706 to their taxonomic classification, and the OTUs without any assignment are shown in  
707 grey. (f) Cladogram showing the significantly abundant taxonomic groups in each of  
708 the stages, identified based on the LEfSe ( $p < 0.05$  and effect size  $> 3.5$ ).

709 **Fig. 4.** Plots showing the comparison of the microbiota associated with the distal  
710 intestine of Atlantic salmon in freshwater (20, 44 and 62 wph).

711 Stage-specific colour coding is used for Figures a, b, c, and f. (a) Alpha diversity  
712 indices (Shannon index, PD whole tree, and Simpson evenness) of the bacterial  
713 communities and (b, c) UniFrac distances-based PCoA. (d) The mean relative  
714 abundance of the 10 most abundant OTUs at the (d) phylum and (e) genus levels.  
715 OTUs are coloured according to their taxonomic classification, and the OTUs without  
716 any assignment are shown in grey. (f) Cladogram showing the significantly abundant  
717 taxonomic groups in each of the stages, identified based on the LEfSe ( $p < 0.05$  and  
718 effect size  $> 3.5$ ).

719 **Fig. 5.** Plots showing the comparison of the microbiota associated with the distal  
720 intestine of Atlantic salmon in seawater (65, 68 and 80 wph). Stage-specific colour  
721 coding is used for Figures a, b, c, and f. (a) Alpha diversity indices (Shannon index,  
722 PD whole tree, and Simpson evenness) of the bacterial communities and (b, c)  
723 UniFrac distances-based PCoA. (d) The mean relative abundance of the 10 most  
724 abundant OTUs at the (d) phylum and (e) genus levels. OTUs are coloured according  
725 to their taxonomic classification, and the OTUs without any assignment are shown in  
726 grey. (f) Cladogram showing the significantly abundant taxonomic groups in each of  
727 the stages, identified based on the LEfSe ( $p < 0.05$  and effect size  $> 3.5$ ).

728 **Fig. 6.** Overview of the phylum-level shifts in the bacterial communities at the  
729 different life stages of Atlantic salmon originating from a single cohort. \* indicates  
730 that phyla with taxonomy assignment confidence below 0.5.

731 **Fig. 7.** Comparison of the functional pathways associated with different life stages.  
732 Bray-Curtis dissimilarities based on the abundances of different functional pathways  
733 are shown using PCoA biplot. Stage-specific colour coding is used for the figures. (a)  
734 Early developmental stages, (b) whole intestine, (c) distal intestine (freshwater) and  
735 (d) distal intestine (seawater). The seven most abundant features of each stage (with

736 an average abundance >2.5%) are shown using yellow spheres. The size of the  
737 spheres is indicative of the relative abundances of the features and is named  
738 alphabetically (A to E, in the order of decreasing abundance; see Additional file 8:  
739 Table S5a). Significantly abundant features ( $p < 0.05$  and effect size  $> 0.75$ ) belonging  
740 to the respective stages are also represented using yellow spheres, labelled  
741 alphabetically from F, in order of decreasing abundance. The names of the features  
742 are provided in Additional file 8: Table S5a.  
743

744 **Additional files**

745 **Additional file 1:** Additional methods

746 **Additional file 2:** Fig. S1. Rarefaction curves based on the alpha diversity measure

747 (PD Whole tree), for individual samples (a) and each stage (b). The curves indicate

748 that a sequence number of 2400/sample is sufficient to capture most of the alpha

749 diversity present in the samples as the curves become asymptotic at this depth.

750 **Additional file 2:** Fig. S2. Plots showing the comparisons between the HL and 7wph.

751 A stage-specific colour coding is used for figures a, b, c, f. (a) Alpha diversity indices

752 (Shannon index, PD whole tree, Simpson's evenness) of the bacterial communities

753 and (b, c) UniFrac distances-based PCoA. Mean relative abundance of the 10 most

754 abundant OTUs and their (d) phylum-level and (e) genus-level taxonomic ranks.

755 Taxonomic classification-specific color coding is used in figures d, e, and the OTUs

756 without any assignment are shown in grey. (f) Cladogram showing the significantly

757 abundant taxonomic members in the different stages.

758 **Additional file 3:** Fig. S3. Plots showing the comparisons between the stages 12 and

759 20wph. A stage-specific colour coding is used for figures a, b, c, f. (a) Alpha diversity

760 indices (Shannon index, PD whole tree, Simpson's evenness) of the bacterial

761 communities and (b, c) UniFrac distances-based PCoA. Mean relative abundance of

762 the 10 most abundant OTUs and their (d) phylum-level and (e) genus-level taxonomic

763 ranks. Taxonomic classification-specific color coding is used in figures d, e, and the

764 OTUs without any assignment are shown in grey. (f) Cladogram showing the

765 significantly abundant taxonomic members in the different stages.

766 **Additional file 3:** Fig. S4. Plots showing the comparison between stages 62wph -

767 freshwater and 65wph - seawater. A stage-specific colour coding is used for figures a,

768 b, c, f. (a) Alpha diversity indices (Shannon index, PD whole tree, Simpson's

769 evenness) of the bacterial communities and (b, c) UniFrac distances-based PCoA.  
770 Mean relative abundance of the 10 most abundant OTUs and their (d) phylum-level  
771 and (e) genus-level taxonomic ranks. Taxonomic classification-specific color coding  
772 is used in figures d, e, and the OTUs lacking taxonomy assignment are shown in grey.  
773 (f) Cladogram showing the significantly abundant taxonomic groups in each of the  
774 stages.

775 **Additional file 3:** Fig. S5. Mean weighted Nearest Sequenced Taxon Index (NSTI) for  
776 the predicted metagenomes of the microbiota associated with the different stages. The  
777 NSTI scores were around 0.1 until the 20wph followed by a pronounced increase at  
778 44wph and the values remained higher than 0.15 for the succeeding stages sampled,  
779 indicating increasing dissimilarity between the metagenome and available reference  
780 genomes.

781 **Additional file 4:** Table S1: Sample metadata

782 **Additional file 5:** Table S2: Read statistics concerning different samples

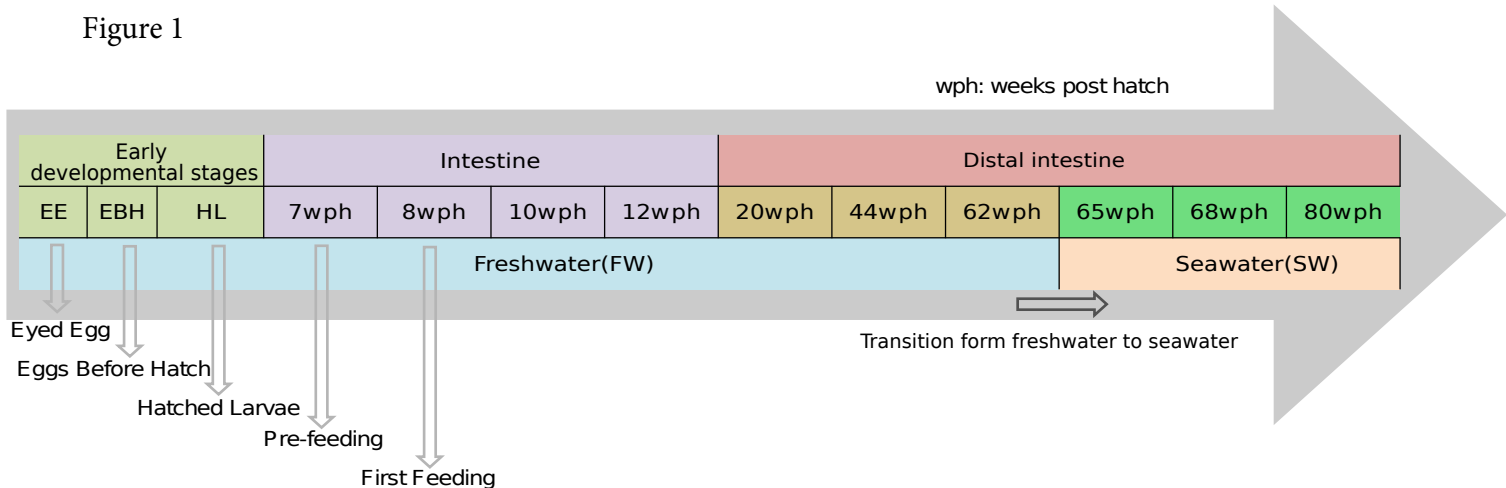
783 **Additional file 6:** Table S3. ANOSIM comparisons and the corresponding p and R  
784 values for each of the comparisons

785 **Additional file 7:** Table S4. The list of taxonomic features belonging to different groups  
786 with their corresponding p values and the LDA effect size

787 **Additional file 8:** Table S5a. List of the 5 most abundance (>2.5%) KEGG modules  
788 that associated with different stages of development; Table S5b. List of KEGG modules  
789 that were significantly associated with different groups within each of the stages of  
790 development. Features passing the p-value filter 0.05 and the effect size filter 0.75 are  
791 listed

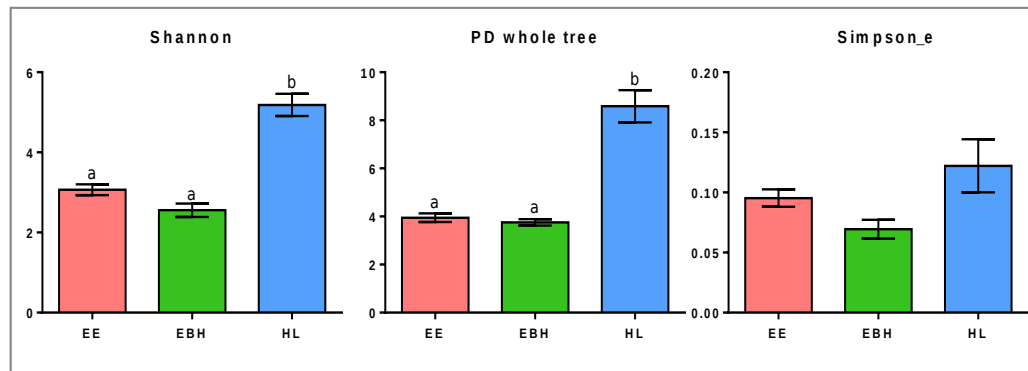
792 **Additional file 9:** Table S6. ANOSIM comparisons and the corresponding p and R  
793 values for each of the comparisons

Figure 1

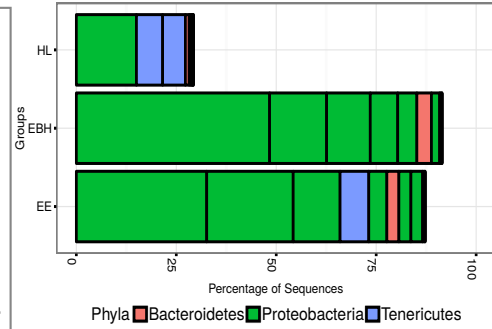




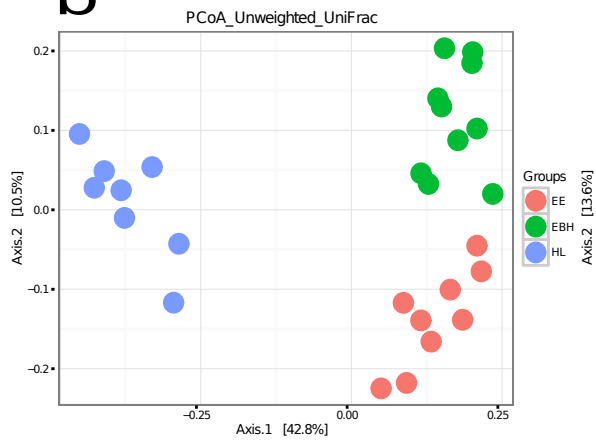
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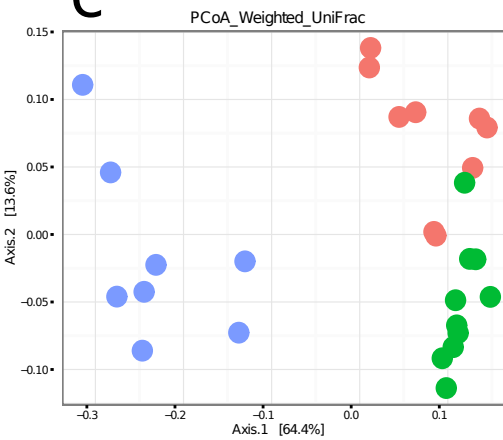
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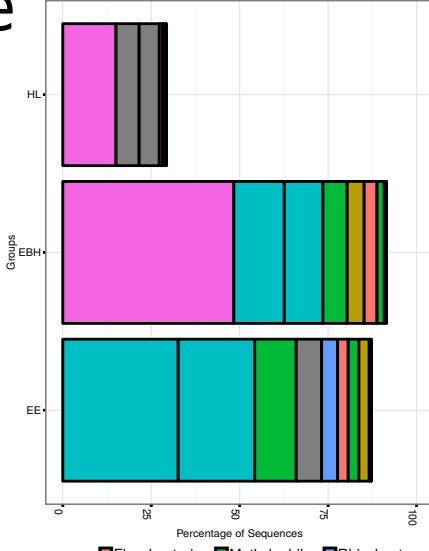
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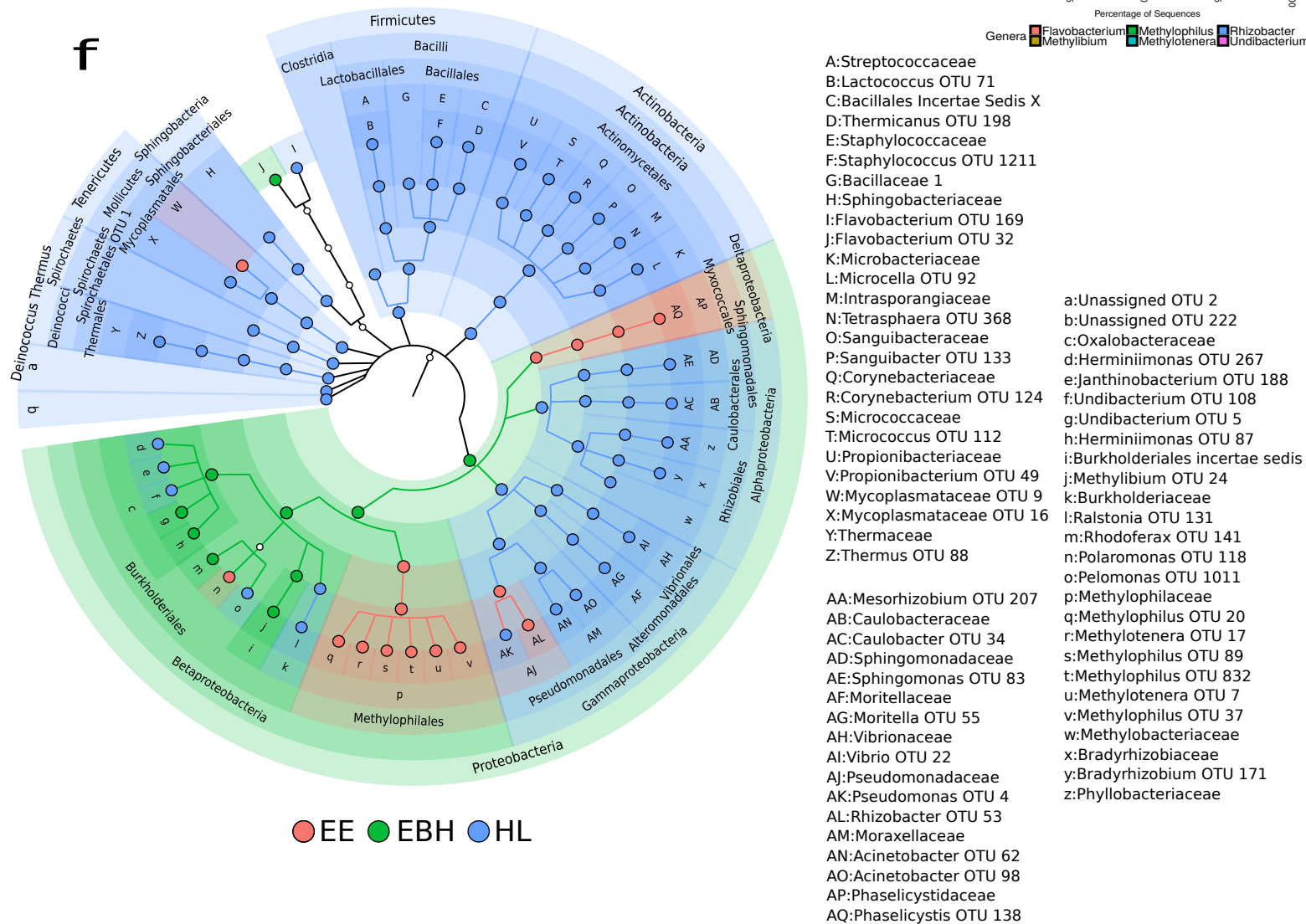
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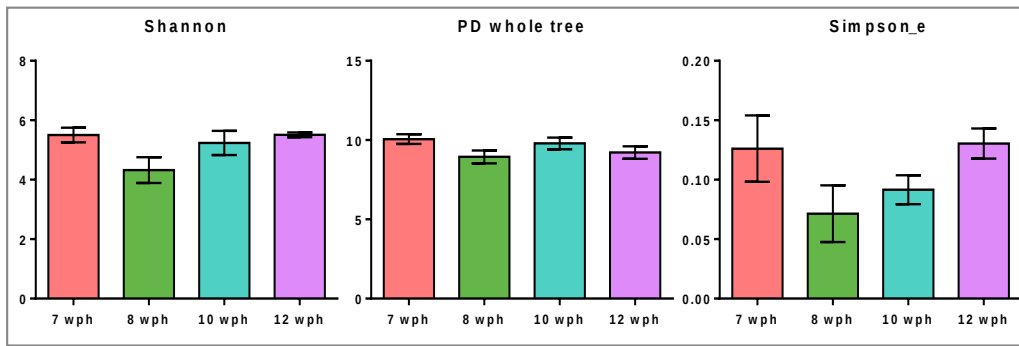


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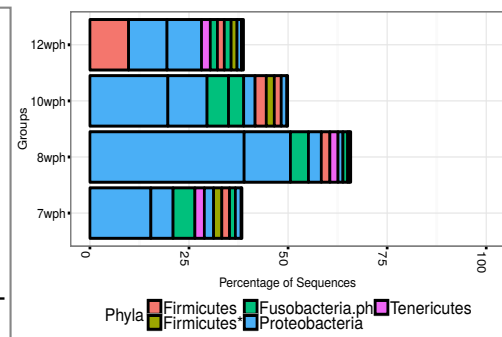


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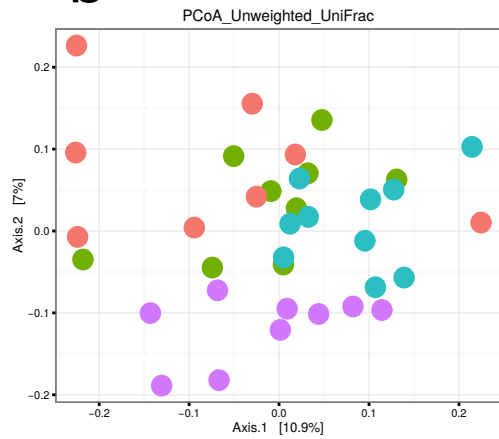
Figure 3



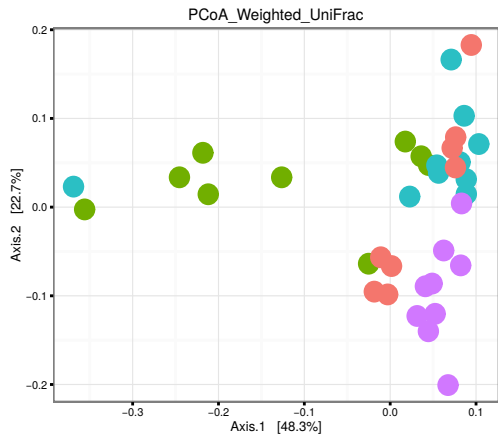
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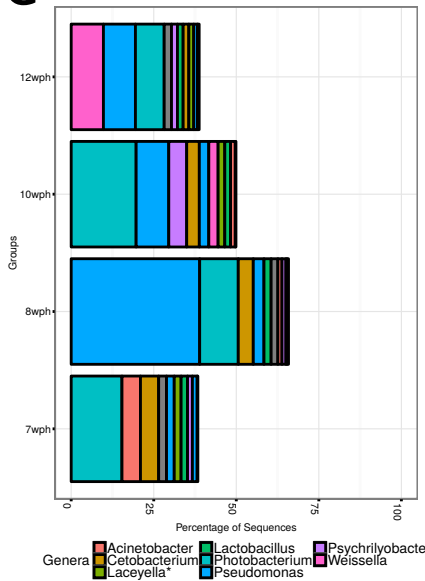
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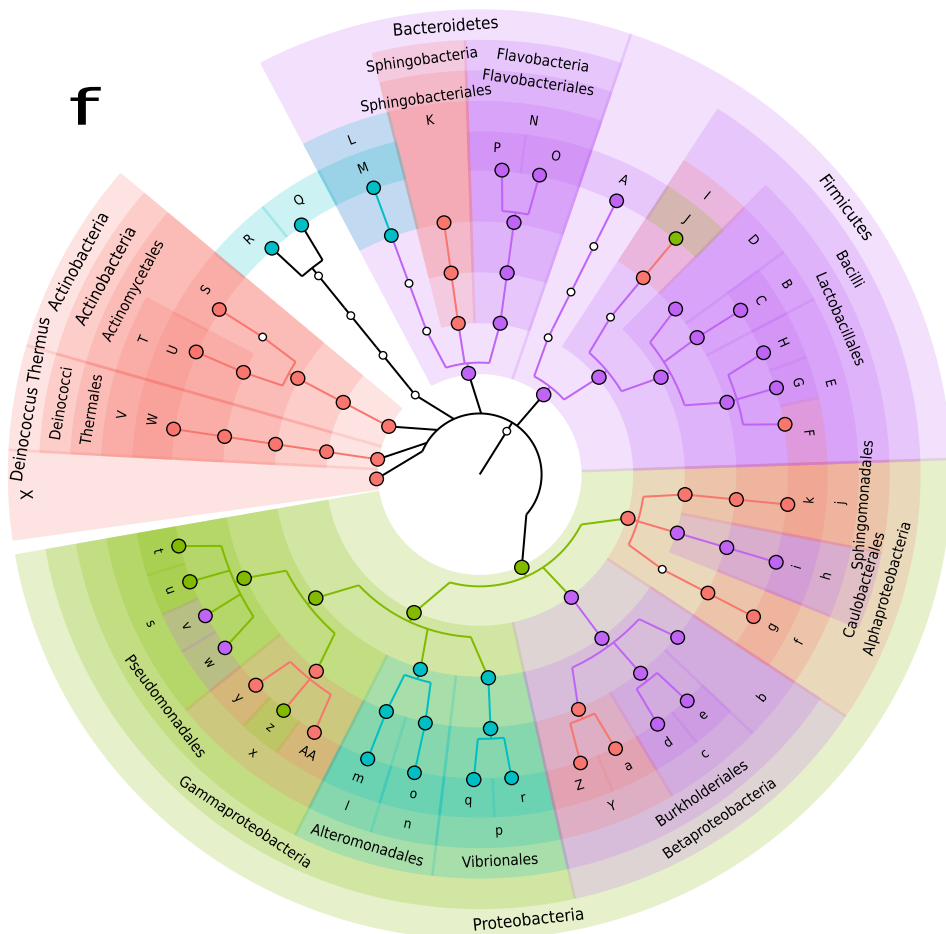
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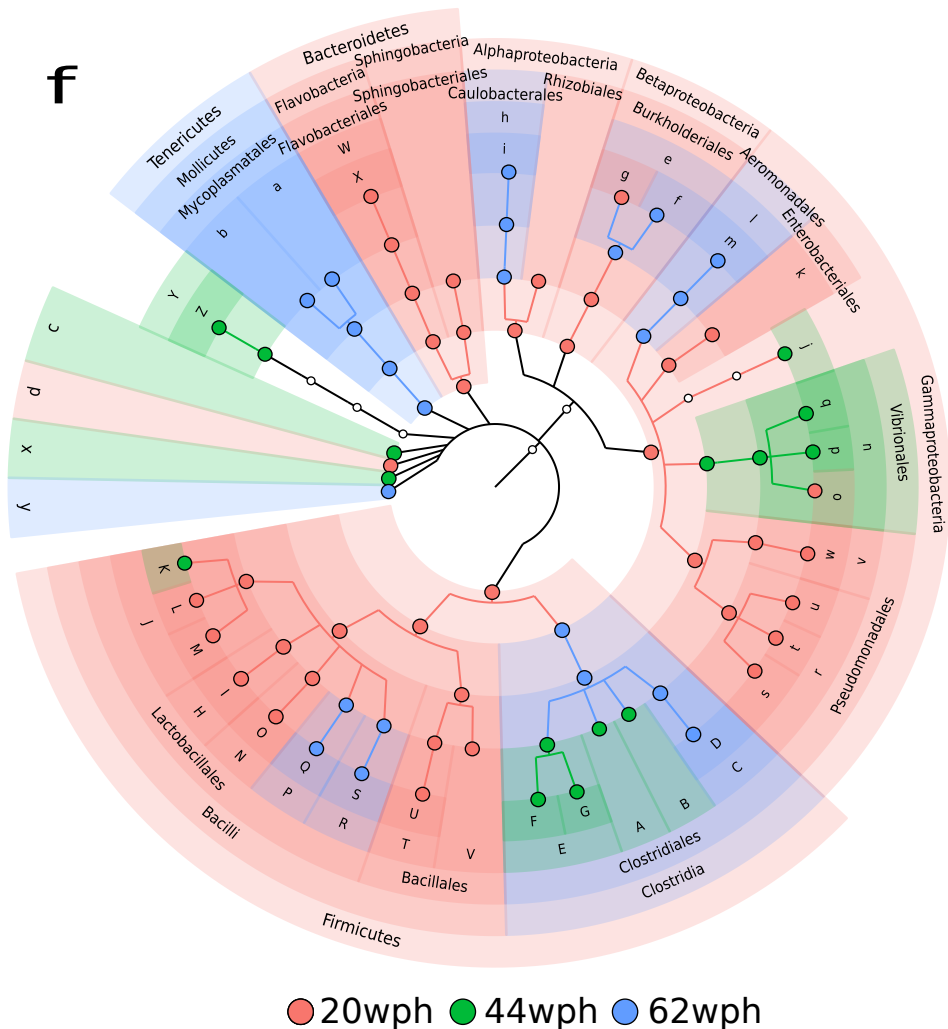
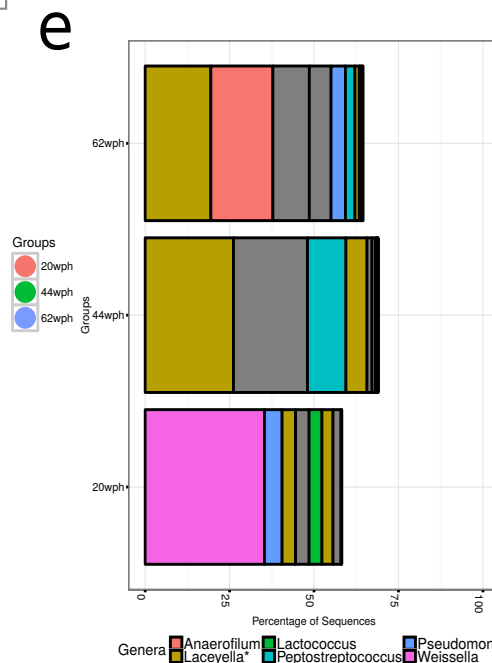
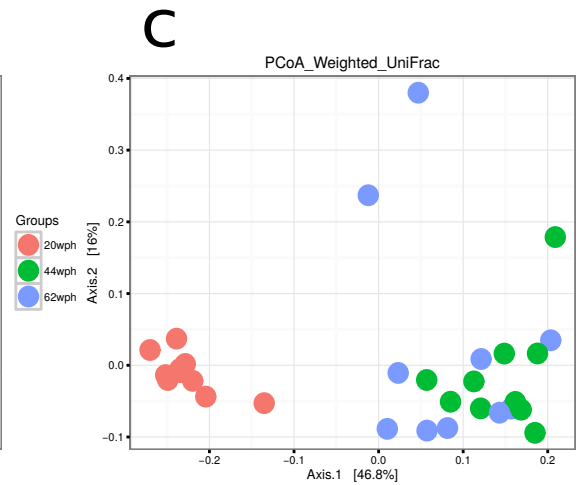
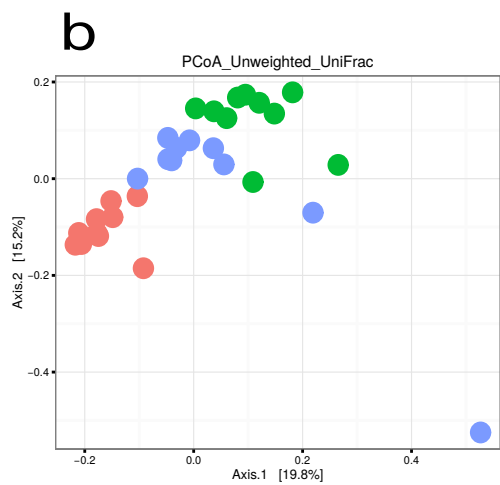
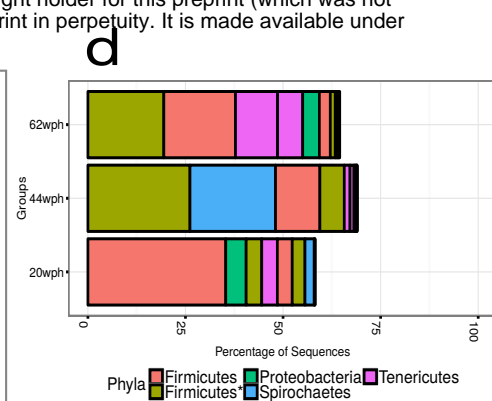
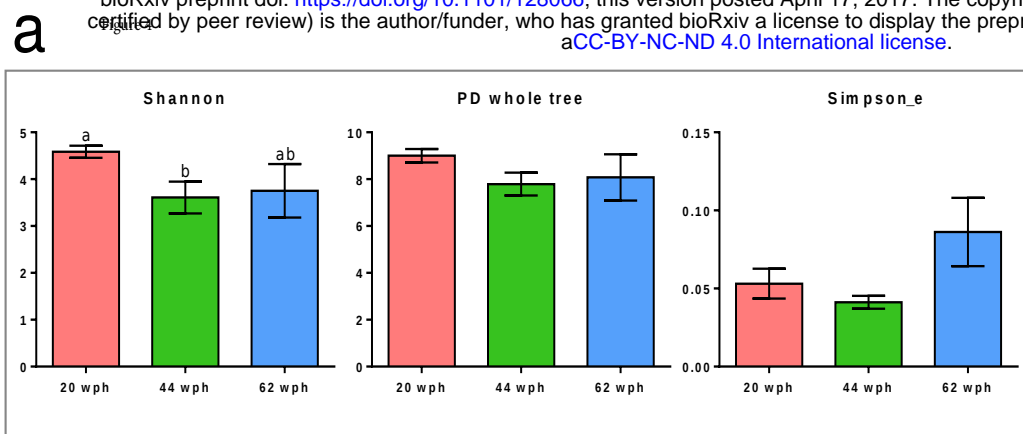
f



● 7wph ● 8wph  
● 10wph ● 12wph

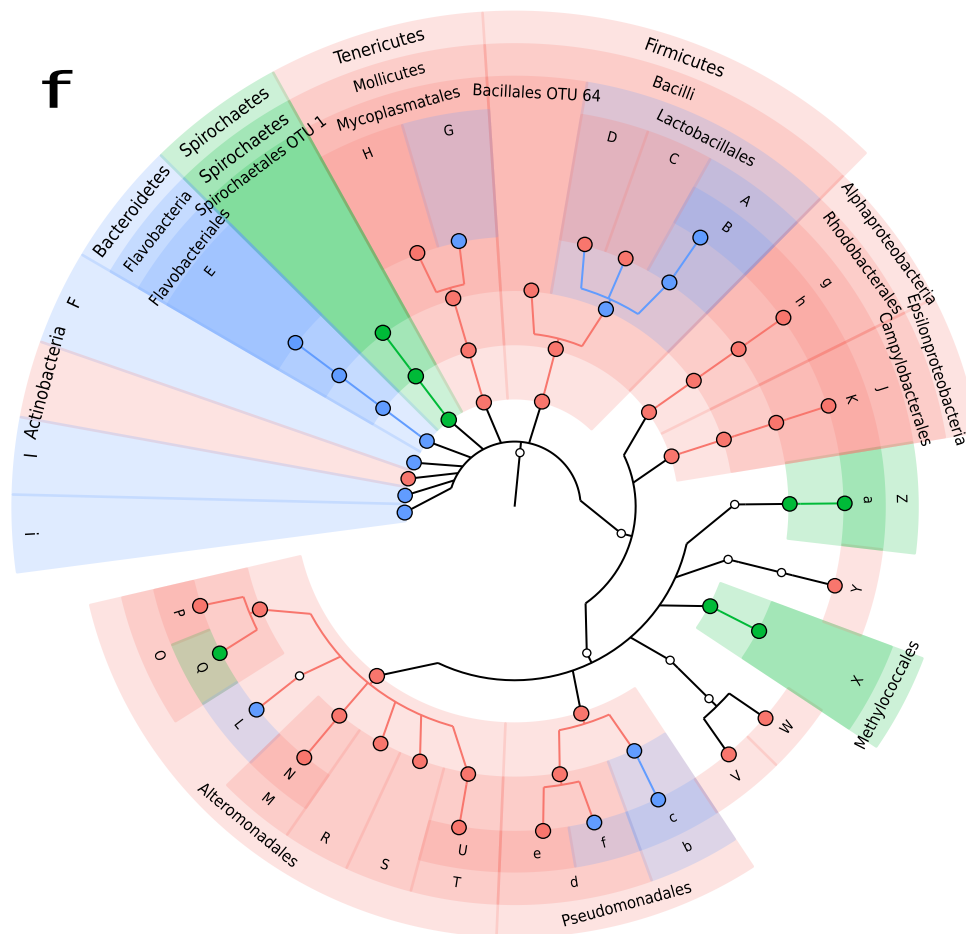
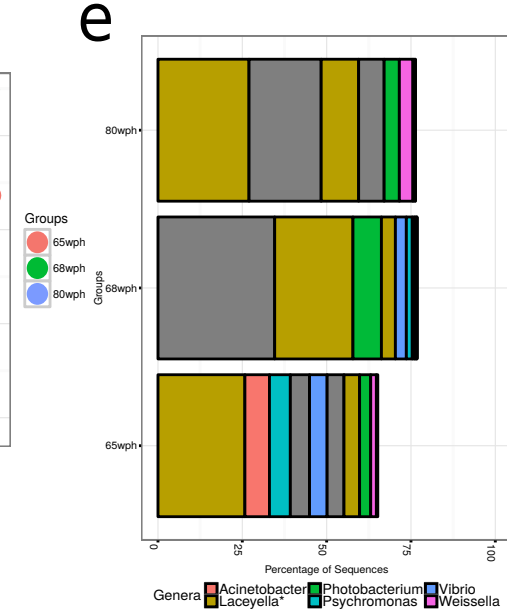
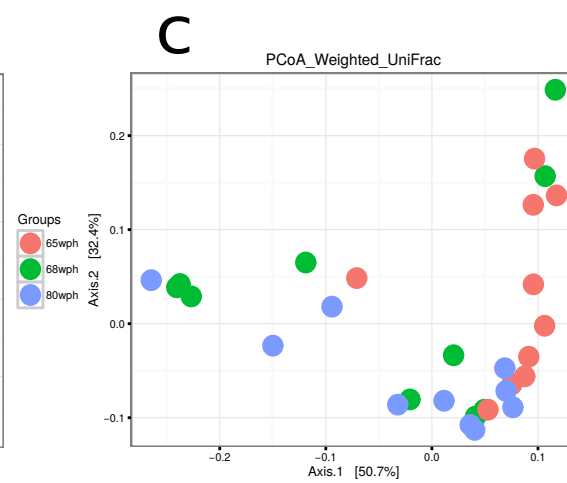
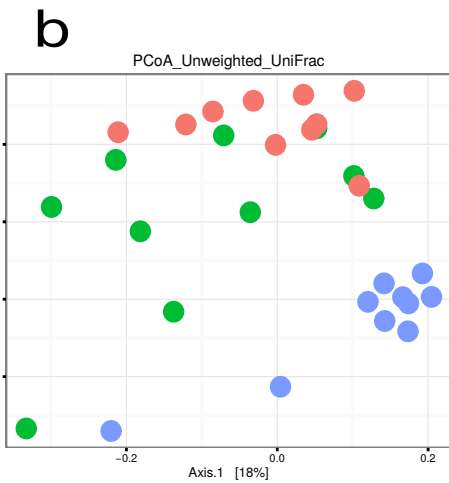
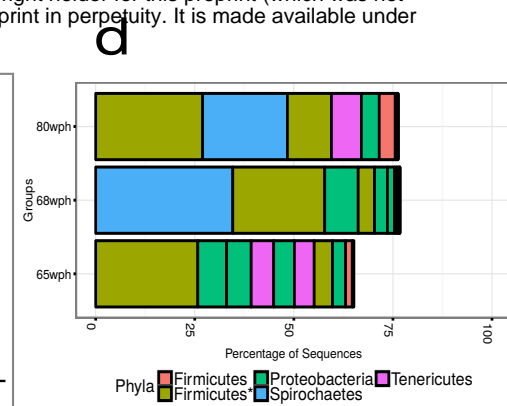
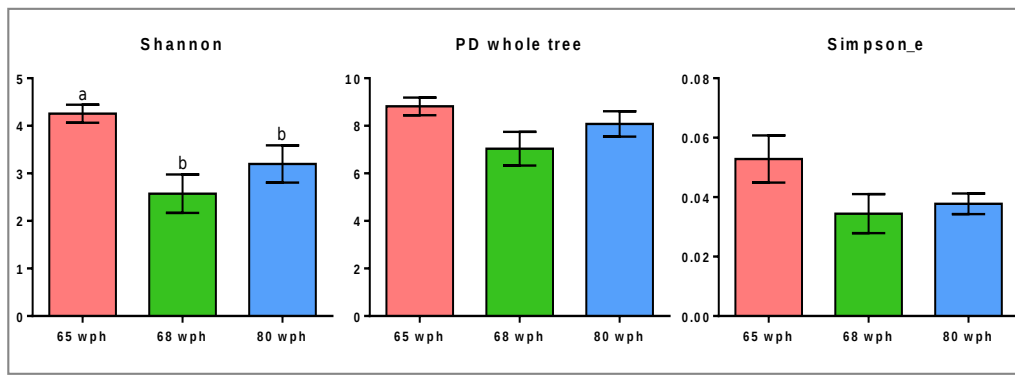
- A:Peptostreptococcus OTU 15  
 B:Leuconostocaceae  
 C:Weissella OTU 14  
 D:Lactobacillaceae  
 E:Streptococcaceae  
 F:Lactococcus OTU 71  
 G:Streptococcus OTU 59  
 H:Lactococcus OTU 40  
 I:Staphylococcaceae  
 J:Staphylococcus OTU 101  
 K:Sphingobacteriaceae  
 L:Marinilabiaceae  
 M:Alkaliflexus OTU 191  
 N:Flavobacteriaceae  
 O:Flavobacterium OTU 68  
 P:Elizabethkingia OTU 41  
 Q:Psychriyobacter OTU 30  
 R:Cetobacterium OTU 39  
 S:Micrococcus OTU 112  
 T:Propionibacteriaceae  
 U:Propionibacterium OTU 49  
 V:Thermaceae  
 W:Thermus OTU 88  
 X:Unassigned OTU 2  
 Y:Oxalobacteraceae  
 Z:Duganella OTU 456
- a:Naxibacter OTU 360  
 b:Burkholderiales incertae sedis  
 c:Comamonadaceae  
 d:Delftia OTU 45  
 e:Pelomonas OTU 1011  
 f:Methylobacteriaceae  
 g:Methylobacterium OTU 100  
 h:Caulobacteraceae  
 i:Caulobacter OTU 34  
 j:Sphingomonadaceae  
 k:Sphingomonas OTU 83  
 l:Moritellaceae  
 m:Moritella OTU 57  
 n:Psychromonadaceae  
 o:Psychromonas OTU 63  
 p:Vibrionaceae  
 q:Photobacterium OTU 10  
 r:Photobacterium OTU 180  
 s:Pseudomonadaceae  
 t:Pseudomonas OTU 44  
 u:Pseudomonas OTU 4  
 v:Pseudomonas OTU 47  
 w:Pseudomonas OTU 84  
 x:Moraxellaceae  
 y:Acinetobacter OTU 62  
 z:Alkanindiges OTU 327

AA:Acinetobacter OTU 98



- A: Clostridiales Incertae Sedis XI OTU 52  
 B: Clostridiales Incertae Sedis XI OTU 42  
 C: Ruminococcaceae  
 D: Anaerofilum OTU 3  
 E: Peptostreptococcaceae  
 F: Peptostreptococcus OTU 15  
 G: Peptostreptococcaceae incertae sedis OTU 21  
 H: Leuconostocaceae  
 I: Weissella OTU 14  
 J: Lactobacillaceae  
 K: Lactobacillus OTU 26  
 L: Lactobacillus OTU 77  
 M: Lactobacillus OTU 29  
 N: Streptococcaceae  
 O: Lactococcus OTU 40  
 P: Enterococcaceae  
 Q: Vagococcus OTU 31  
 R: Carnobacteriaceae  
 S: Carnobacterium OTU 8  
 T: Staphylococcaceae  
 U: Staphylococcus OTU 101  
 V: Bacillaceae 1  
 W: Flavobacteriaceae  
 X: Elizabethkingia OTU 41  
 Y: Corynebacteriaceae  
 Z: Corynebacterium OTU 67
- a: Mycoplasmataceae OTU 9  
 b: Mycoplasmataceae OTU 16  
 c: Unassigned OTU 2  
 d: Unassigned OTU 54  
 e: Comamonadaceae  
 f: Diaphorobacter OTU 91  
 g: Delftia OTU 45  
 h: Caulobacteraceae  
 i: Caulobacter OTU 34  
 j: Moritella OTU 55  
 k: Enterobacteriaceae  
 l: Aeromonadaceae  
 m: Aeromonas OTU 539  
 n: Vibrionaceae  
 o: Vibrio OTU 22  
 p: Photobacterium OTU 10  
 q: Aliivibrio OTU 449  
 r: Pseudomonadaceae  
 s: Pseudomonas OTU 44  
 t: Pseudomonas OTU 4  
 u: Pseudomonas OTU 47  
 v: Moraxellaceae  
 w: Acinetobacter OTU 62  
 x: Unassigned OTU 6  
 y: Unassigned OTU 25

Figure 3



- A:Leuconostocaceae  
B:Weissella OTU 14  
C:Lactobacillaceae  
D:Streptococcaceae  
E:Flavobacteriaceae  
F:Unassigned OTU 243  
G:Mycoplasmataceae OTU 9  
H:Mycoplasmataceae OTU 16  
I:Unassigned OTU 311  
J:Campylobacteraceae  
K:Arcobacter OTU 116  
L:Pseudoalteromonas OTU 50  
M:Moritellaceae  
N:Moritella OTU 55  
O:Psychromonadaceae  
P:Psychromonas OTU 36  
Q:Psychromonas OTU 63  
R:Colwelliaceae  
S:Moritellaceae OTU 113  
T:Ferrimonadaceae  
U:Ferrimonas OTU 615  
V:Vibrio OTU 22  
W:Vibrio OTU 12  
X:Methylococcaceae OTU 43  
Y:Dokdonella OTU 1958  
Z:Oceanospirillaceae
- a:Oleispira OTU 76  
b:Pseudomonadaceae  
c:Pseudomonas OTU 4  
d:Moraxellaceae  
e:Acinetobacter OTU 23  
f:Psychrobacter OTU 35  
g:Rhodobacteraceae  
h:Roseovarius OTU 74  
i:Unassigned OTU 6

● 65wph ● 68wph ● 80wph

Figure 6

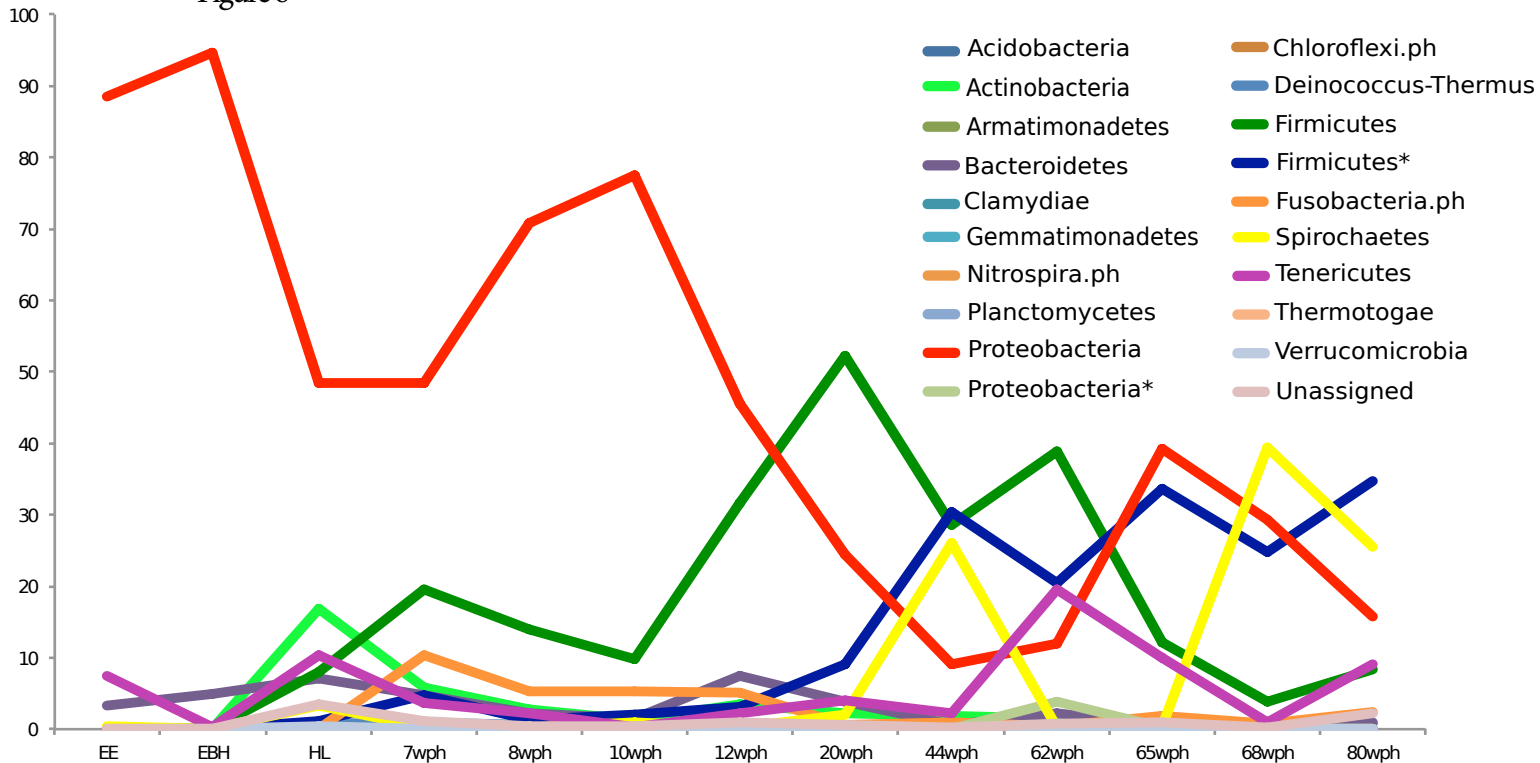


Figure 7

