Environmental plasticity and colonisation history in the Atlantic salmon microbiome: a translocation experiment

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Abstract

Microbial communities associated with the gut and the skin are strongly influenced by environmental factors, and can rapidly adapt to change. Historical processes may also affect the microbiome. In particular, variation in microbial colonisation in early life has the potential to induce lasting effects on microbial assemblages. However, little is known about the relative extent of microbiome plasticity or the importance of historical colonisation effects following environmental change, especially for non-mammalian species. To investigate this we performed a reciprocal translocation of Atlantic salmon between captive and semi-natural conditions. Wild and hatchery-reared fry were transferred to three common garden experimental environments for six weeks: standard hatchery conditions, hatchery conditions with an enriched diet, and simulated wild conditions. We characterised the faecal and skin microbiome of individual fish before and after the environmental translocation, using a BACI (before-after-control-impact) design. We found evidence of extensive plasticity in both gut and skin microbiota, with the greatest changes in alpha and beta diversity associated with the largest

changes in environment and diet. Microbiome richness and diversity were entirely determined by environment, with no detectable historical effects of fish origin. Microbiome structure was also strongly influenced by current environmental conditions but, for the first time in fish, we also found evidence of colonisation history, including a number of OTUs characteristic of captive rearing. These results may have important implications for host adaptation to local selective pressures, and also highlight how conditions during early life can have a long-term influence on the microbiome and, potentially, host health.

Keywords

Microbiota; *Salmo salar;* plasticity; priority effects

Introduction

Microbiome structure is determined by a complex series of delicately balanced interactions with the host, the environment and amongst microbiota (Suzuki 2017; Vellend 2016; Walter & Ley 2011). Unlike the host genome, the metagenome is very dynamic and readily influenced by host-specific and environmental changes (Chen et al. 2018; Davenport et al. 2017). Host-associated microbial communities are able to rapidly respond to local selective pressures due to their short generation times, rapid mutation rates, large population sizes and high levels of phenotypic plasticity and intra-community gene flow (Walter & Ley 2011). Given the critical and wide-ranging influence of the microbiome on host health and fitness (Davenport et al. 2017; Koskella et al. 2017), extensive metagenomic plasticity may also enhance host adaptive phenotypic plasticity (Alberdi et al. 2016; Suzuki 2017). This may play a vital role in driving host adaptation to environmental change, and may even contribute to population-level divergence and local adaptation (Alberdi et al. 2016; Walter &

50 Ley 2011). For example, intestinal microbiota can enhance the digestion of novel food sources and
51 the metabolism of dietary toxins, increase drought and thermal tolerance, and increase resistance to
52 local pathogens (Alberdi et al. 2016; Chevalier et al. 2015; Macke et al. 2017).

53 While microbial communities often show extensive plasticity in response to environmental
54 change, historical processes may potentially also affect the microbiome. The order and timing in
55 which microorganisms arrive in the community can impact microbiome structure, even under
56 otherwise identical environmental conditions (Sprockett et al. 2018; Vellend 2016). Existing
57 colonisers may restrict and/or modify the ecological niches available to subsequent arrivals,
58 influencing their establishment success (Fukami 2015). For example, environmental stress or
59 antibiotic treatment which disturbs the microbiome may allow resistant taxa to flourish in the
60 absence of wider competition, delaying the restoration of the original complex community (Foster et
61 al. 2017; Yassour et al. 2016). During the early stages of microbiome colonisation and establishment,
62 microbial composition is particularly dynamic and readily influenced by variation in the surrounding
63 environment and microbial seeding communities (Korpela et al. 2018; Stephens et al. 2016).
64 Therefore historical effects on microbiome structure due to environmental variation during early life
65 may be particularly important (Gensollen et al. 2016b; Sprockett et al. 2018). Early life stress can
66 have a lasting detrimental effect on the microbiome and health outcomes in mammalian hosts
67 (Foster et al. 2017), but the possibility of conditioning the microbiome during early life to improve
68 lasting host health and disease resistance could also have huge therapeutic benefit (Borre et al.
69 2014). However, overall, little is known about the extent to which historical effects may shape the
70 microbiome, especially for non-mammalian species.

71 Atlantic salmon (*Salmo salar*) is one of the most commercially important fish species and is also a
72 keystone species for freshwater habitats (Griffiths et al. 2011). Populations of Atlantic salmon display
73 local adaptation, and are often threatened in their natural range (Garcia de Leaniz et al. 2007). The
74 Atlantic salmon gut and skin microbiome is known to be strongly influenced by environmental

conditions, especially diet, salinity and season, as well as developmental stage (Dehler et al. 2017; Gajardo et al. 2016a; Llewellyn et al. 2016; Lokesh & Kiron 2016; Zarkasi et al. 2014), and there is also substantial microbiome variation between salmon populations, especially between wild and hatchery-reared fish, reflecting diet and environmental differences (Uren Webster et al. 2018). This suggests there may be considerable scope for microbiome plasticity in response to environmental variation, but may also indicate the potential for lasting historical effects reflecting colonisation history and early life experience, and makes Atlantic salmon an ideal species in which to examine these factors. Understanding the scope for plasticity and historical effects is essential for the management of this species both in aquaculture and the wild. For example, microbiome plasticity and/or historical effects may influence acclimation to environmental challenges, act as a driver of local adaptation, or provide a potential mechanism for improving disease resistance in aquaculture. However, little is known about the scope for plasticity following environmental change, and/or whether historical colonisation effects may continue to influence microbiome diversity and structure. Therefore, to investigate this, we used a reciprocal translocation experiment of salmon fry between hatchery and natural conditions and a BACI (before-after-control-impact) experimental design to test for gut and skin microbiome plasticity related with development and environment/diet, as well as potential lasting historical signatures of origin.

Materials and Methods

Reciprocal translocation experiment

Sixty wild Atlantic salmon fry (~6 months post hatch) were captured using electrofishing from the Aber Bran, a tributary of the river Usk (Wales; lat: 51.954, long: -3.477) and transported a short distance (~8 km) to the NRW Cynrig Fish Culture Unit (Brecon, Wales). Sixty Atlantic salmon fry (6 months post hatch) were also obtained from the hatchery; these fish originated from a 3:3

100 male:female cross between wild-caught parents from the river Taff (Wales) that had been
101 maintained under hatchery conditions. Hatchery fish were adipose fin clipped to differentiate them
102 from wild fish, a procedure that does not cause adverse effects (Roberts et al. 2014). All wild and
103 hatchery fish were measured (fork length) and photographed using a Canon DS126151 400D EOS
104 digital camera, using a 18-55 mm lens. A sample of skin-associated mucus was collected by swabbing
105 the left side of each fish back and forth along the entire length of the lateral line five times using
106 Epicentre Catch-All™ Sample Collection Swabs (Cambio, Cambridge, UK), and gut samples were
107 collected by gently pressing the abdomen of each fish and swabbing the expelled faeces. Duplicate
108 50 ml water samples were also collected from the river and hatchery. All samples were stored at -
109 80°C prior to DNA extraction.

110 Twenty wild and 20 hatchery individuals were randomly assigned to each of the three
111 experimental environments (hatchery, enriched diet, natural) using a common garden design (Figure
112 1). The first experimental group was maintained in standard hatchery conditions. Fish were housed in
113 a 500 L black plastic tank supplied with aerated, flow-through, filtered river water (~10 L/min) and
114 fed a standard commercial Salmonid feed (Skretting) at a rate of 3% body weight/day. The second
115 group were housed in identical conditions to the first group, but were fed the standard hatchery diet
116 enriched with daily addition of 5 g of an invertebrate mix (33% natural invertebrates harvested from
117 the Afon Cynrig, 33% bloodworm and 33% daphnia (both JMC Aquatics, Sheffield, UK)). The third
118 experimental group consisted of near natural conditions, whereby salmon were transferred to a 30m
119 leat of the Afon Cynrig, another tributary of the river Usk (lat: 51.928, long: -3.358). The leat was
120 isolated by upstream and downstream fry screens to prevent fish movement, contained natural
121 substrate, was fed by natural river water, and received no dietary supplementation. The
122 experimental treatments lasted for six weeks, after which fish were recaptured, and sampled for a
123 second time using an identical procedure to the one described for the first sampling point
124 (photograph, fork length, weight, skin swab, faecal swab). Duplicate water samples were collected

125 from the hatchery tank, enriched tank and leat. Water temperature in each of the experimental
126 tanks and the leat ranged from 13.5-16.5 °C during the experiment reflecting ambient conditions.
127 Photographs were used to visually match individual fish at the first and second sampling points,
128 allowing us to conduct a matched before-and-after microbiome analysis. Each fish was identified
129 based on the number, shape and spacing of its parr marks, as juvenile salmonids can be identified
130 based on a unique pattern of pigmentation (Donnelly & Whoriskey 1993; Garcia de Leaniz et al.
131 1994). All matches were independently corroborated by two researchers, and eight individuals from
132 each of the six experimental groups (wild to hatchery; hatchery to hatchery; wild to enriched;
133 hatchery to enriched; wild to natural; hatchery to natural) were positively identified at both time-
134 points and used for further analyses. Specific growth rate ((Ln($length_2$)-Ln($length_1$))/time x100;
135 (Hopkins 1992)) and Fulton's condition factor ((weight/$length^3$) x 100; (Froese 2006) were calculated
136 for each individual fish.
137
138 *16S rRNA amplicon sequencing and bioinformatics*
139 Microbiome analysis was performed for the skin and gut samples at the two time-points for 48
140 individual fish, as well as five water samples in duplicate. DNA was extracted from the skin mucus
141 and faecal samples using the MoBio PowerSoil® DNA Isolation Kit (Qiagen) according to the
142 manufacturer's instructions, with an additional incubation step of 10 minutes at 65°C prior to bead
143 beating. Water samples were centrifuged at 5000xg for 1 hour at 4°C and the DNA was extracted
144 from the pellet using the same method. 16S library preparation was performed as described
145 previously (Uren Webster et al. 2018) amplifying the V4 hypervariable region of the bacterial 16S
146 gene using the primers 341F and 785R, and libraries were sequenced across two lanes of an Illumina
147 MiSeq.
148 Quality filtering of raw sequence reads (removal of adaptor sequences and poor quality bases
149 from the 3' end (Q<20)) was performed using Trimmomatic (Bolger et al. 2014), before analysis using

150 mothur v1.39 (Kozich et al. 2013) and Qiime v1.9 (Caporaso et al. 2010). Forward and reverse reads
151 were concatenated, filtered to retain amplicons of the target size range, then aligned to the Silva
152 seed reference database (version 128)(Quast et al. 2013). Further noise reduction of contigs with
153 high quality alignments was performed using mothur's pre-clustering algorithm, then potential
154 chimeras were identified and removed using UCHIME (Edgar et al. 2011) within mothur. Taxonomic
155 classification of contigs was performed using the Silva reference taxonomy (Quast et al. 2013) and
156 mitochondrial, eukaryote and chloroplast sequences were removed. Contigs were clustered into
157 operational taxonomic units (OTUs) based on 97% overall sequence similarity. Singleton OTUs were
158 removed from the dataset, and all gut, skin and water samples were subsampled to an equal depth
159 of 19,389 reads. Microbial alpha diversity analysis was performed based on Chao1 richness and
160 Shannon diversity, while structural analysis (microbial beta diversity) was based on community
161 distance matrices calculated using the Bray-Curtis dissimilarity index (Bray & Curtis 1957), which
162 accounts for OTU presence/absence and relative abundance. Functional analysis of community
163 structure was performed based on metagenomic predictions made using PICRUSt (Langille et al.
164 2013). The OTU table produced using mothur was converted to a compatible biom format based on
165 Greengenes reference taxonomy v13_8 (DeSantis et al. 2006) using biom convert (McDonald et al.
166 2012). PICRUSt was then used to normalise OTUs, predict metagenomes based on KEGG orthologs
167 and collapse functional predictions to KEGG pathway level. Further visualisation and statistical
168 analysis based on KEGG pathways was then performed in STAMP v2.1.3 (Parks & Beiko 2010).
169
170 *Statistical analysis*
171 All statistical analyses were performed using R (v3.4.3; (R_Core_Team 2014)). To investigate
172 differences in the specific growth rate (SGR) of individually identified fish during the course of the
173 translocation experiment, and in final condition factor (K), linear models including the fixed factors
174 environment (hatchery, enriched, natural), origin (hatchery, wild), and their interaction, were

175 constructed. The best fitting models were selected based on Akaike Information Criterion (AIC)
176 values using the *step* function (Table S1).

177 To identify initial differences in alpha diversity (Chao1 richness and Shannon diversity) at the pre-
178 translocation sampling point, linear models were constructed including origin (hatchery/wild) and
179 fish size (length) as fixed factors. The effects of experimental environment
180 (hatchery/enriched/natural), origin, an environment:origin interaction and SGR on the specific
181 change in alpha diversity for matched individuals during the course of the translocation experiment
182 were also examined. Finally, linear models were constructed to investigate the effect of
183 environment, origin, an environment:origin interaction and size (length), as well as the pre-sampling
184 alpha diversity value for matched individual fish, on final (post-translocation) alpha diversity values
185 (Chao1 richness and Shannon diversity). In each case reduced linear models were selected based on
186 a lowest AIC value using bi-directional stepwise simplification (Table S1). In addition, paired t-tests
187 were performed to further investigate the relationship between pre- and post-translocation
188 measures of alpha diversity for each individually matched fish.

189 Microbiome structure (beta diversity), based on Bray-Curtis distance, was visualised using non-
190 metric multidimensional scaling (NMDS) ordination, performed within mothur then plotted using
191 ggplot2 (Wickham 2009). Multivariate statistical analysis of community separation (PERMANOVA)
192 was performed using Adonis in the Vegan package (Oksanen et al. 2017). Initial (pre-translocation)
193 differences in microbiome structure were investigated, including origin (wild/hatchery) and fish size
194 (length) as fixed factors in the model. For the post-translocation sampling point, the effects of
195 environment (hatchery/enriched/natural), origin, an environment:origin interaction and fish size on
196 final microbiome structure were tested. The specific change in gut and skin community structure
197 (Bray-Curtis distance) over time for individually-matched fish was also investigated using the model
198 (ΔBC ~ environment + origin + environment:origin + SGR). As before, the full models were reduced
199 using stepwise simplification (Table S1).

200 Statistical analysis of OTU abundance was performed using DeSeq2 (Love et al. 2014), using
201 rarefied data as recommended for microbiome libraries with large deviance in total library size
202 between samples (Weiss et al. 2017). For the gut and skin separately, the effect of origin on initial
203 (pre-translocation) OTU abundance was examined, while the effect of environment and origin on
204 final (post-translocation) OTU abundance was identified using a multifactorial design including the
205 main effects of environment, origin and their interaction. Within the DesSeq2 models, independent
206 filtering of low coverage OTUS was applied, optimising power for identification of differentially
207 abundant OTUs at a threshold of alpha=0.05. Default settings were applied for outlier detection and
208 moderation of OTU level dispersion estimates. OTU abundance was considered significantly different
209 at FDR <0.05.
210
211
212 Results
213 *Specific growth rate and condition*
214 There was a significant effect of both environment (hatchery, enriched, natural) and origin
215 (hatchery or wild) on the specific growth rate for pre/post matched individual fish, and a significant
216 interaction between factors (*Environment* $F_{2,42}$ =55.62, P < 0.001; *Origin* $F_{1,42}$ =53.18, P <0.001;
217 *Environment:Origin* $F_{2,42}$ =5.02, P=0.01; Figure S1). Specific growth rate was highest in the natural
218 environment, and fish originating from the hatchery also showed a significantly higher growth rate
219 than wild origin fish in both the hatchery and enriched groups. However, in the natural group there
220 was no difference in growth rate between wild- and hatchery-origin fish. There was no significant
221 effect of environment or origin on final condition index (*Environment* $F_{2,42}$ =2.41, P= 0.10; *Origin* $F_{1,42}$
222 =0.02, P =0.88; *Environment:Origin* $F_{2,42}$ =2.10, P=0.14).
223
224

225 *Microbial alpha diversity*

226 Water microbial richness, but not diversity, was initially higher in the river compared to the
227 hatchery (Chao1: $t_{2,3}$=26.28, P=0.012; Shannon: $t_{1.03}$=2.95, P=0.203), while at the second sampling
228 point both water richness and diversity were significantly higher in the natural environment (leat)
229 than in the hatchery and enriched tanks (Chao1: $F_{1.38}$=11.41, P=0.025; Shannon: $F_{2,3}$=18.67, P=0.020).

230 Gut microbial richness was initially significantly higher in wild than hatchery fish before the
231 translocation, but there was no difference in gut Shannon diversity (Chao1: $F_{1,46}$= 62.85, P< 0.001;
232 Shannon: $F_{1,46}$= 1.12, P= 0.295; Figure 2A). Overall, across all matched fish, gut microbial richness and
233 diversity significantly declined during the course of the translocation experiment (Chao1: t_{47}= 5.40,
234 P< 0.001; Shannon: t_{47}= 7.08, P<0.001; Figure 2C-H). In addition, the degree of individual-level change
235 in gut Chao1 richness, but not Shannon diversity, was significantly affected by both environment and
236 origin (Chao1: *Environment* $F_{2,44}$= 3.46, P=0.040; *Origin*- $F_{1,44}$= 41.90, P<0.001; Shannon: *Origin* $F_{1,46}$=
237 1.31, P= 0.259). Hatchery-origin fish fed an enriched diet showed a smaller reduction in gut microbial
238 richness compared to those maintained in the same environment (hatchery to hatchery group),
239 whilst those translocated to a natural environment actually experienced a small increase in gut
240 richness. All wild-origin fish, which had higher initial gut richness, showed a greater decline in gut
241 richness during the course of the translocation experiment compared to hatchery fish. Wild fish that
242 experienced the largest change in environment and diet (wild to hatchery group) experienced a
243 greater reduction in gut richness compared to those in the enriched and, especially, the natural
244 environment groups. After the translocation, final gut Chao1 richness was strongly influenced by
245 environment with no detectable effect of origin, size or the initial microbial richness for matched
246 individuals (*Environment* $F_{2,44}$= 12.77, P< 0.001, *Length* $F_{1,44}$= 2.72, P= 0.10; Figure 2B). Fish
247 maintained in natural conditions had higher gut richness than in both the hatchery and enriched
248 groups, while an enriched diet also significantly increased gut Chao1 richness compared to that in the

249 hatchery group. There was no significant effect of environment, origin or size on final gut Shannon
250 diversity (*Environment* $F_{2,44}$= 1.77, P= 0.182, *Length* $F_{1,44}$= 3.18, P= 0.081).
251 For the skin microbiome, there was no initial difference in richness or diversity between wild and
252 hatchery fish (Chao1: *Origin* $F_{1,43}$= 3.64, P= 0.063, *Length* $F_{1,43}$= 0.19, P= 0.65, *Origin:Length* $F_{1,43}$=
253 2.72, P= 0.11; Shannon: *Origin* $F_{1,45}$= 0.54, P= 0.466). Across all fish, there was a small reduction in
254 skin diversity but not richness over time (Chao1: t_{46}= -0.39, P= 0.70, Shannon: t_{46}= -2.26, P= 0.03), but
255 environment had a significant effect on the degree of individual-level change in richness and diversity
256 during the course of the translocation experiment (Chao1: *Environment* $F_{2,43}$ =4.38, P= 0.019; *Origin*
257 $F_{1,43}$ =3.61, P=0.064; Shannon: *Environment* $F_{2,43}$= 7.64, P=0.001; *SGR* $F_{1,43}$= 3.28, P=0.077; Figure S2).
258 Post translocation, there was also a significant effect of environment on final skin Chao1 richness and
259 Shannon diversity (Chao1: *Environment* $F_{2,45}$= 4.55, P=0.016; Shannon: *Environment* $F_{2,43}$= 7.89, P=
260 0.001; *Pre-Shannon* $F_{1,43}$= 3.47, P= 0.07; Figure S2). In each case, fish from the natural environment
261 had higher richness/diversity than in both the hatchery and enriched groups. There was no
262 detectable effect of origin, size or the pre-translocation microbial richness/diversity for matched
263 individuals on measures of final alpha diversity in any case.
264
265 *Microbial beta diversity*
266 Before translocation, there was a significant difference in the structure of both the gut and the
267 skin microbiome between wild and hatchery fish, and fish size also had a significant effect on beta
268 diversity (Gut: *Origin* $F_{1,45}$=3.35, P=0.016, *Length* $F_{1,45}$=2.85, P=0.032; Skin: *Origin* $F_{1,44}$=2.55, P=0.025,
269 *Length* $F_{1,44}$=2.15, P=0.050; Figure 3). Overall, during the course of the translocation experiment
270 there was a large change in gut and skin microbiome structure across all fish, but environment and
271 origin significantly affected the degree of structural change (Bray-Curtis distance for individually
272 pre/post matched fish) that occurred for both the gut and skin microbiome (Gut: *Environment*
273 $F_{2,42}$=3.46, P=0.041; *Origin* $F_{1,42}$=5.57, P=0.023; *Environment:Origin* $F_{2,42}$=9.62, P<0.001; Skin:

274 *Environment* $F_{2,43}$=2.05, P=0.041, *Origin* $F_{1,43}$=21.57, P<0.001; Figure S3). The smallest change in
275 community structure occurred for hatchery fish maintained in the same conditions and fed the same
276 diet, while the largest change was found in fish which experienced the greatest environmental and
277 dietary change (i.e. wild-hatchery and hatchery-wild). After translocation, final skin and gut
278 microbiome structure was strongly affected by environment as well as fish origin and fish size (Gut:
279 *Environment* $F_{2,41}$=6.41, P=0.001; *Origin* $F_{1,41}$=2.07, P=0.029; *Length* $F_{1,41}$=2.16, P=0.024;
280 *Environment:Origin* $F_{2,41}$=1.23, P=0.197; Skin: *Environment* $F_{2,40}$=13.20, P=0.001; *Origin* $F_{1,40}$=9.56,
281 P=0.001; *Length* $F_{1,40}$=0.49, P=0.774; *Environment:Origin* $F_{2,40}$=0.97, P=0.427).
282
283 *OTU abundance*
284 Before translocation, a total of 260 gut OTUs were significantly differentially abundant (FDR
285 <0.05) between wild and hatchery fish (Table S2). The vast majority of them (236; 91%) were more
286 abundant in wild fish but, notably, the OTUs which were more abundant in hatchery fish were
287 predominantly Lactobacillales (14/24; 58%). Hatchery fish were dominated by several *Lactobacillus*
288 sp., as well as an unclassified OTU from the order Bacillales. Wild fish were dominated by a similar
289 unclassified OTU from the order Bacillales (93% sequence similarity to that in hatchery fish), as well
290 as several OTUs from the family Enterobacteriaceae and from the Candidate division (CK.1C4.19)
291 (Figure 4a, Figure S4a). In contrast, for the skin there were fewer initial differences in microbiome
292 composition between wild and hatchery origin fish. Only 14 differentially abundant OTUs were
293 identified, but these included an unclassified OTU from the family Rhodobiaceae which was amongst
294 the most abundant OTUs in hatchery fish (Figure 4b, Figure S4b).
295 After translocation, in the gut a total of 88, 126 and 136 OTUs were differentially abundant
296 between the hatchery-enriched, hatchery-natural and enriched-natural experimental groups,
297 respectively (Table S2). In particular, the unclassified Bacillales OTU initially most abundant in wild
298 fish was present at significantly higher levels in fish in the natural environment, while the

299 phylogenetically similar unclassified Bacillales OTU initially dominant in hatchery fish was present at
300 significantly higher levels in fish from both the hatchery and enriched environments. Additionally,
301 fish from the hatchery environment harboured greater numbers of several *Lactobacillus* sp.,
302 *Mycoplasma* sp. and *Rhizobium* sp., while salmon fed the enriched diet displayed increased numbers
303 of *Deefga* sp. and an OTU from the family Aeromonadaceae (Figure 4a, Figure S4a). In the natural
304 environment group there were significantly lower levels of seven OTUs from the family
305 Brevinemataceae and increased abundance of several OTUS from the family Enterobacteriaceae
306 compared to fish from the hatchery and enriched groups. In addition to the effects of experimental
307 environment, a total of 72 gut OTUs showed a significant effect of fish origin (Table S2). Thirty two
308 OTUs from the family Brevinemataceae were all present at higher levels in hatchery-origin fish,
309 together with some of the most dominant gut community members including *Mycoplasma* sp. and
310 *Lactobacillus* sp., while OTUs more abundant in wild-origin fish included several OTUS from the
311 family Enterobacteriaceae.

312 In the skin, a total of 14, 70 and 58 OTUs were differentially abundant between the hatchery-
313 enriched, hatchery-natural and enriched-natural experimental groups, respectively (Figure 4b, Figure
314 S4b, Table S3). Notably, the unclassified OTU from the family Rhodobiaceae, that was initially more
315 abundant in hatchery fish before the translocation, was present at significantly higher levels in both
316 the hatchery and enriched environment groups. This was also the only skin OTU significantly affected
317 by fish origin, and was more abundant in hatchery-origin fish across all experimental environments.

318

319 *Community function*

320 Before translocation, there were pronounced differences in predicted metagenomic function
321 between wild and hatchery origin fish, in both the skin and the gut microbiome. In the gut there
322 were 128 (39%) significantly differentially enriched KEGG pathways (FDR <0.05), with 188 (57%) in

323 the skin. These included some of the most highly represented KEGG pathways such as 'ABC
324 Transporters', 'Purine metabolism', 'Two-component system', and 'Secretion System'.
325 After the translocation, in the gut 107 (32%) KEGG pathways were significantly differentially
326 represented amongst experimental environments. Amongst the most represented KEGG pathways,
327 these included 'DNA repair and recombination proteins', 'Ribosome', 'Purine Metabolism' and
328 'Pyrimidine Metabolism' (Figure S5a). In the skin microbiome, 103 (31%) KEGG pathways were
329 differentially enriched amongst environmental treatments, including 'Secretion system' and
330 'Replication, recombination and repair' (Figure S5b). There was also a lasting effect of origin on seven
331 (2.1%) gut KEGG pathways, and 57 (17 %) skin KEGG pathways (Figure S5).
332
333
334 Discussion
335 Overall, we found evidence of extensive plasticity in the Atlantic salmon gut and skin microbiome
336 associated with environmental change and development, but crucially we also identified some lasting
337 effects of early life experience on microbial structure and function. Our results, demonstrating that
338 both environmental plasticity and historical colonisation effects determine fish microbiome
339 assemblage, are also likely to have important implications for host health and fitness.
340
341 *Change in the gut and skin microbiome over time*
342 Using a powerful BACI approach, we identified a fundamental change in the gut microbiome over
343 time. For each individually matched fish, initial gut alpha diversity had no detectable effect on final
344 alpha diversity value after translocation and, overall, OTU richness and diversity both significantly
345 declined over time. Gut community structure and function also clearly changed over time, including a
346 general reduction in Actinobacteria OTUs and a notable increased abundance of OTUs from the
347 family Brevinemataceae. These differences in diversity and structure are likely to reflect

348 developmental changes in these juvenile salmon, as well as seasonal change across the six week
349 experimental period. With maturation, the teleost gut microbiome tends to become less diverse and
350 increasingly specialised and stable, reflecting a stronger influence of host-specific, microbial
351 interactions and active dispersal (Burns et al. 2016; Stephens et al. 2016). For the skin, there were
352 less pronounced changes in alpha diversity over time, but considerable changes in microbiome
353 structure including a general reduction in Alphaproteobacteria, especially Enterobacteriaceae, and
354 increased abundance of Betaproteobacteria, as well as Actinobacteria and Flavobacteria. These
355 results suggest that temporal dynamics in the skin and gut microbiome differ. Little is known about
356 developmental changes in the teleost skin microbiome, but seasonality is known to be an important
357 determinant of fish and amphibian skin microbiota (Larsen et al. 2015; Longo et al. 2015).
358
359 *Microbiome plasticity in response to diet and environmental change*
360 Before translocation, there was a clear distinction between the gut and skin microbiomes of
361 hatchery- and wild-origin fish, consistent with our previous results showing differences in the
362 diversity and structure of juvenile wild and hatchery-reared Atlantic salmon microbiomes (Uren
363 Webster et al. 2018). Wild salmon had a richer and more diverse gut microbiome, with abundant
364 Enterobacteriaceae compared to the dominance of Firmicutes (especially Lactobacillales) in hatchery
365 fish, while the skin of wild and hatchery fish was dominated by different groups of Proteobacteria.
366 However, following translocation, we found that microbiome structure, function and, especially,
367 diversity was largely determined by environment, rather than fish origin and early-life experience.
368 This suggests that, at a given time, the salmon microbiome is highly dependent on the current diet
369 and environmental conditions that a fish is exposed to, and shows considerable plasticity following
370 environmental change.
371 Salmon exposed to simulated wild conditions in the leat showed significantly higher microbial
372 richness in the skin and, especially, the gut microbiome compared to that of fish in the hatchery

373 environment. This is likely due to a higher degree of dietary diversity and variation compared to that
374 of the artificial hatchery diet (Dehler et al. 2017; Orlov et al. 2006), with the higher growth rates
375 observed under natural conditions also indicating that there was a seasonally rich and plentiful
376 natural diet available to these fish. In addition, we found that microbial richness in the surrounding
377 water was significantly higher in the leat than for the hatchery and enriched tanks, which is also likely
378 to influence microbiome richness in both the gut and the skin (Boutin et al. 2013; Giatsis et al. 2015;
379 Uren Webster et al. 2018). By enriching the standard hatchery diet with invertebrates, we were able
380 to examine the effects of increased dietary diversity on the microbiome in isolation from other
381 environmental variables. We found that gut and skin microbial richness increased with dietary
382 enrichment, but to a much lesser extent than that observed in the natural environment which also
383 encompassed wider environmental change. Furthermore, these pronounced effects of environment
384 occurred regardless of fish origin, and despite the pronounced initial differences between wild and
385 hatchery origin fish, demonstrating a capacity for extensive plasticity in microbial richness following
386 environmental and dietary change.

387 Environment also had a marked effect on gut and skin microbiome structure, with a clear
388 distinction between fish from all three experimental environments. This is consistent with a strong
389 influence of diet (e.g. Gajardo et al. 2016a; Schmidt et al. 2016) as well as other environmental
390 variables (e.g. Burns et al. 2016; Llewellyn et al. 2016; Smith et al. 2015) on gut microbiome structure
391 in Atlantic salmon and other fish species. Evidence from fish in the enriched group shows that dietary
392 change also specifically influences skin microbiome structure and function, although to a lesser
393 extent than for the gut. Overall, our results highlight that there is considerable plasticity in the skin
394 and gut microbiome structure in response to environmental change, regardless of fish origin and in
395 spite of considerable initial differences between the wild and hatchery fish. Notably, fish subject to
396 the largest change in diet and environmental conditions experienced the greatest change in
397 microbiome structure, while hatchery fish maintained in hatchery conditions experienced the least.

398 Additionally, specific microbiome taxonomic composition was particularly distinct between fish in
399 the natural experimental group, and those in the hatchery and enriched diet group, with a number of
400 taxa that appeared to be distinctive of wild or hatchery conditions. In the gut we identified two
401 highly abundant 'wild-type' and 'hatchery-type' OTUs within the order Bacillales (93% sequence
402 similarity) that were consistently favoured by natural or hatchery conditions, before and after the
403 translocation experiment. Multiple OTUs from the family Brevinemataceae became more abundant
404 with time, but strikingly only in fish translocated to the hatchery and enriched environments.
405 Brevinemataceae abundance is known to be variable in fish gut (Gajardo et al. 2016b; Pratte et al.
406 2018), and our results suggest that this taxa, besides being dependent on developmental stage, is
407 strongly favoured by an artificial diet and/or other captive conditions and is signatory of hatchery
408 environments. *Mycoplasma* sp. and several *Lactobacillus* sp. were also consistently enhanced in the
409 hatchery-based environment, while several OTUs from family Enterobacteriaceae were much more
410 abundant under natural conditions. This is consistent with what we found previously in wild and
411 hatchery fish (Uren Webster et al. 2018), and highlights the potential for mutual exclusion between
412 Enterobacteriaceae and *Lactobacillus* sp. similar to that in human gut (Sprockett et al. 2018).
413 Similarly, in the skin microbiome, we identified several OTUs that appeared to be specifically
414 enhanced in captive conditions. These included an OTU from the family Rhodobiaceae, *Burkholderia*
415 sp. and *Pseudomonas* sp., which were initially elevated in hatchery-origin fish and following
416 translocation to the hatchery and enriched environments as well. However, compared to the gut,
417 there appeared to be a less pronounced effect of experimental wild and hatchery conditions on the
418 structural composition of skin microbial communities and fewer differentially abundant OTUs
419 between groups. This could be because the skin microbiome is less strongly influenced by dietary
420 change than the gut, and also likely reflects the fact that the hatchery/enriched groups were supplied
421 with filtered river water with a similar microbial profile to that of the natural environment.
422

423 *Historical colonisation effects in the salmon microbiome*

424 Fish origin had no discernable lasting effects on skin or gut microbial alpha diversity, which
425 showed extensive plasticity and was apparently entirely determined by environment and
426 developmental stage. However, we did identify persistent effects of fish origin on microbiome
427 structure and function.

428 Hatchery conditions, in particular, left signatures in the gut and skin microbiome assembly
429 regardless of environmental change, including the relative abundance of some of the most dominant
430 community members, and in overall community structure. A number of OTUs were more abundant
431 in hatchery-origin fish regardless of the environment they were translocated to. In the skin
432 microbiome, a single very abundant OTU from the family Rhodobiaceae was initially far more
433 prevalent, and remained more prevalent, in hatchery-origin fish translocated to each of the
434 environmental groups. This OTU was also strongly enhanced by the hatchery and enriched
435 conditions, but remained very rare in fish never exposed to hatchery conditions (wild to natural
436 group). Several gut OTUs showed similar persistent patterns in hatchery fish, including some of the
437 most dominant community members *Mycoplasma* sp. and *Lactobacillus* sp. However, the most
438 striking effect of origin in the gut microbiome concerned the differential emergence of OTUs from
439 the family Brevinemataceae. Although Brevinemataceae were rare before translocation, they
440 became significantly more abundant in hatchery-origin fish across all experimental groups, even in
441 fish translocated to natural conditions. This strongly suggests that early-life experience of captivity
442 promotes Brevinemataceae emergence in the gut, regardless of later environmental conditions.

443 These effects of fish origin on community structure likely reflect differential microbial colonisation
444 history. Priority effects describe how the order and timing of microbial arrival alters the availability of
445 ecological niches for successive colonisers, through niche pre-emption or modification (Fukami 2015;
446 Sprockett et al. 2018; Walter & Ley 2011). Fundamentally, within richer microbial communities, such
447 as those found associated with the wild fish before translocation, higher competition is likely to

448 reduce the chance of certain taxa becoming over-dominant (Fukami 2015; Hibbing et al. 2010). It
449 could be that lasting historical signatures were more prevalent in hatchery-origin fish because
450 reduced microbial competition allowed taxa favoured by hatchery conditions to flourish and
451 establish dominance during early development. Large populations of established, dominant bacteria
452 are likely to be able to outcompete later arrivals, and may also have an increased potential to adapt
453 to environmental change, due to a higher probability of mutation and gene flow (Howe et al. 2015;
454 Sprockett et al. 2018; Walter & Ley 2011). This may account for the continued presence of dominant
455 OTUs, including *Mycoplasma* sp. and *Lactobacillus* sp., in hatchery-origin fish following translocation
456 to the natural environment, but a lack of similar persistent, signatory OTUs in wild-origin fish. The
457 preferential emergence of Brevinemataceae OTUs in hatchery-origin fish could be explained by niche
458 modification, whereby previous colonisers in the hatchery gut provide favourable nutrients or
459 conditions for Brevinemataceae growth, and/or conditions in the wild fish gut inhibit their successful
460 colonisation (Fukami 2015).

461 In addition to priority effects due to microbial colonisation history, it is likely that host-specific
462 differences between the wild and hatchery salmon populations may also contribute to the observed
463 lasting signatures of origin on microbiome structure. These could include differences in the genetic
464 background of the two fish populations. Host genotype is known to influence microbiome diversity
465 and structure, although to a lesser extent than environmental factors, with the interaction between
466 microbiota and the immune system likely to represent the predominant selective mechanism (Burns
467 et al. 2017; Gensollen et al. 2016a; Stagaman et al. 2017; Uren Webster et al. 2018). Additionally, the
468 two fish populations are likely to have experienced very different environmental conditions and
469 pathogens in the wild and captivity, shaping the development of their adaptive immune system and,
470 thus, the nature of selective processes influencing microbiota assembly (Foster et al. 2017; Gensollen
471 et al. 2016a). Further research is required to establish the relative contributions of host genetic and

472 environmentally-driven epigenetic influences on lasting signatures of origin in the fish microbiome,
473 relative to historical microbial colonisation effects.
474
475 *Perspective*
476 Overall, we show that the scope for environmental plasticity in the fish gut and skin microbiome is
477 extensive, where degree of change in microbial community assembly closely mirrors the scale of
478 environmental change. In addition, we also show, for the first time in fish, clear evidence that
479 conditions experienced in early life can have a lasting influence on microbiome structure and
480 function, likely primarily due to the influence of microbial colonisation history through priority
481 effects. These results could have a range of important implications for evolutionary ecology,
482 conservation and management. For example, extensive metagenomic plasticity could potentially
483 contribute to host phenotypic plasticity, enhancing capacity to adapt to environmental challenges,
484 such as climate change, emergent pathogens and pollution. Historical colonisation effects, potentially
485 associated with an advantageous host phenotype in a given environment, could represent a
486 mechanism contributing to local adaptation, or even phenotypic mismatch in hatchery-released fish
487 used to supplement natural populations. In addition, our results demonstrate how common hatchery
488 husbandry processes which alter microbial colonisation and succession, such as antimicrobial
489 treatments, could have a lasting impact on the fish microbiome and, potentially, also on host health.
490 However, they also highlight the possibility of conditioning the microbiome, for example through
491 diet, to improve disease resistance in farmed fish or reduce phenotypic mismatch in hatchery-
492 released fish. Future research establishing causal links to host phenotype will be essential to
493 establish the relative importance of microbiome plasticity and historical colonisation effects on host
494 fitness.
495
496

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Data Accessibility Statement

All Illumina sequence reads are will be available from the European Nucleotide Archive under the accession number PRJEB30953.

Author Contributions

CGL, TUW, PG and SC designed the study; TUW, DRB and GC performed the experiment; TUW and DRB analysed the data; TUW, CGL and SC wrote the manuscript. All authors contributed to the final version of the manuscript.

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702 Figure Legends

703 **Figure 1.** Before-after-control-impact (BACI) design. Hatchery- and wild-origin salmon fry were
704 translocated to three experimental environments, employing a common garden design. Individual
705 fish were matched at the pre- and post-translocation sampling based on unique pigmentation marks.
706

707 **Figure 2.** Faecal Chao1 richness in each group A) before (n=24) and B) after the experiment (n=8), red
708 shading indicates hatchery origin and green shading indicates wild origin fish. C-H) Change in faecal
709 Chao1 richness for all matched fish during the course of the experiment. Each dashed line represents
710 an individual fish, with coloured lines displaying the average for each group and grey shading
711 indicating 95% confidence intervals.
712

713 **Figure 3.** Non-metric multidimensional scaling (NMDS) ordination of microbial community structure
714 based on Bray-Curtis distances, for all samples before and after the translocation experiment.
715

716 **Figure 4.** Relative abundance of the 25 most abundant OTUs in (A) faeces and (B) skin microbial
717 communities before and after the translocation experiment. Each bar represents an individual fish.
718 Origin; hatchery (H) and wild (W). Experimental environment; hatchery (H), enriched (E) and natural
719 (N).

Figure 1

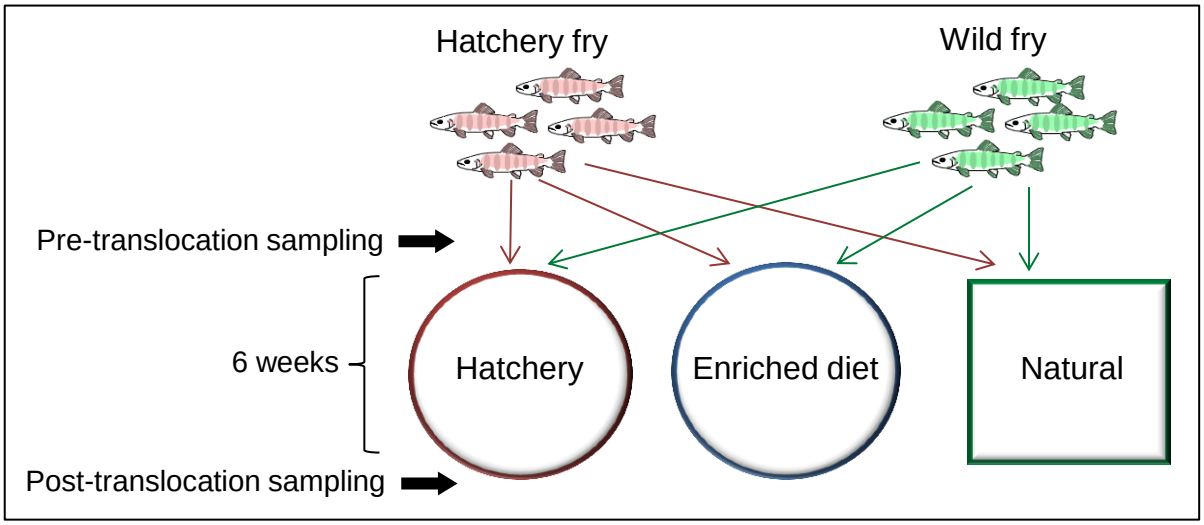
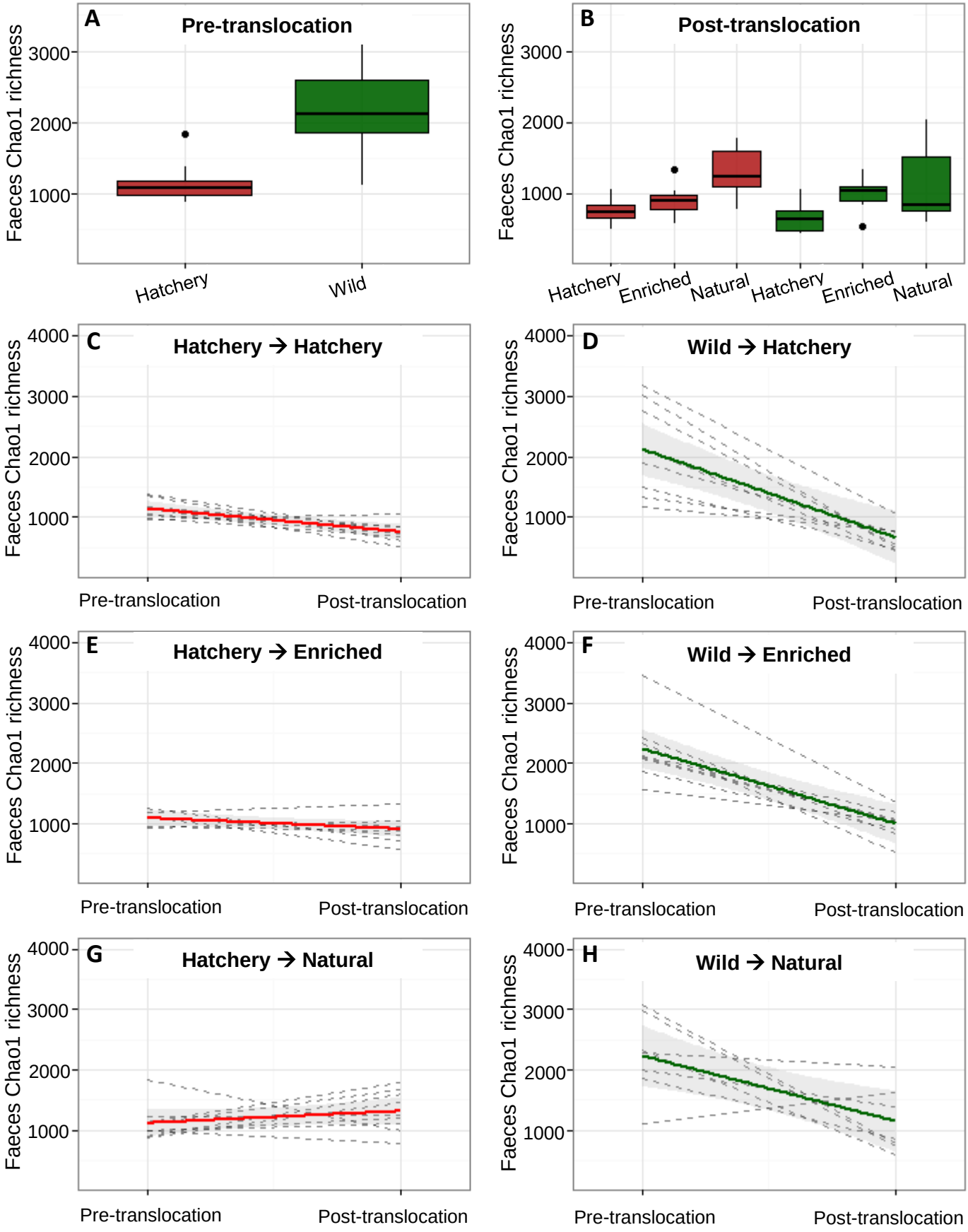

Hatchery fry
Wild fry
Pre-translocation sampling
6 weeks
Hatchery
Enriched diet
Natural
Post-translocation sampling

Figure 2


A
Pre-translocation
Faeces Chao1 richness
3000
2000
1000
Hatchery
Wild
B
Post-translocation
Faeces Chao1 richness
3000
2000
1000
Hatchery
Enriched
Natural
Hatchery
Enriched
Natural
C
Hatchery → Hatchery
Faeces Chao1 richness
4000
3000
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1000
Pre-translocation
Post-translocation
D
Wild → Hatchery
Faeces Chao1 richness
4000
3000
2000
1000
Pre-translocation
Post-translocation
E
Hatchery → Enriched
Faeces Chao1 richness
4000
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Pre-translocation
Post-translocation
F
Wild → Enriched
Faeces Chao1 richness
4000
3000
2000
1000
Pre-translocation
Post-translocation
G
Hatchery → Natural
Faeces Chao1 richness
4000
3000
2000
1000
Pre-translocation
Post-translocation
H
Wild → Natural
Faeces Chao1 richness
4000
3000
2000
1000
Pre-translocation
Post-translocation

Figure 3

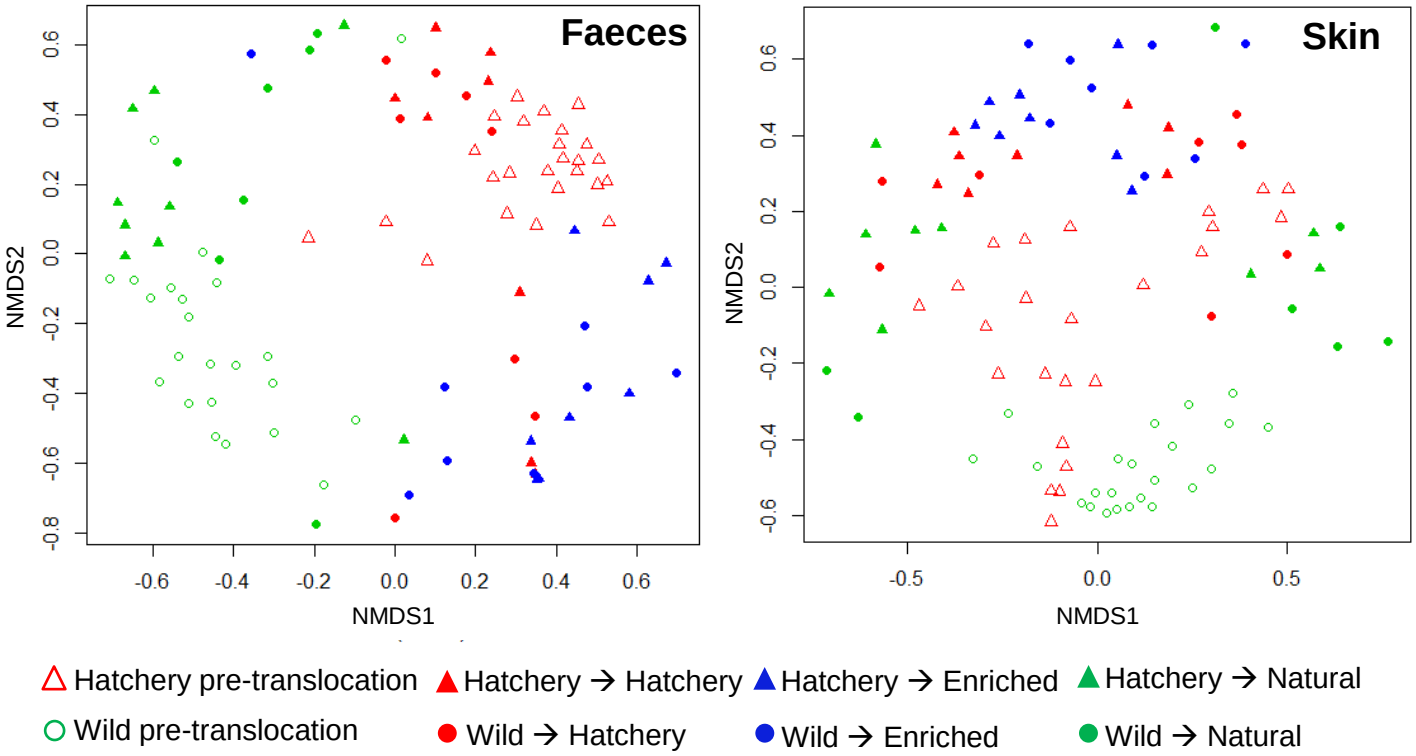
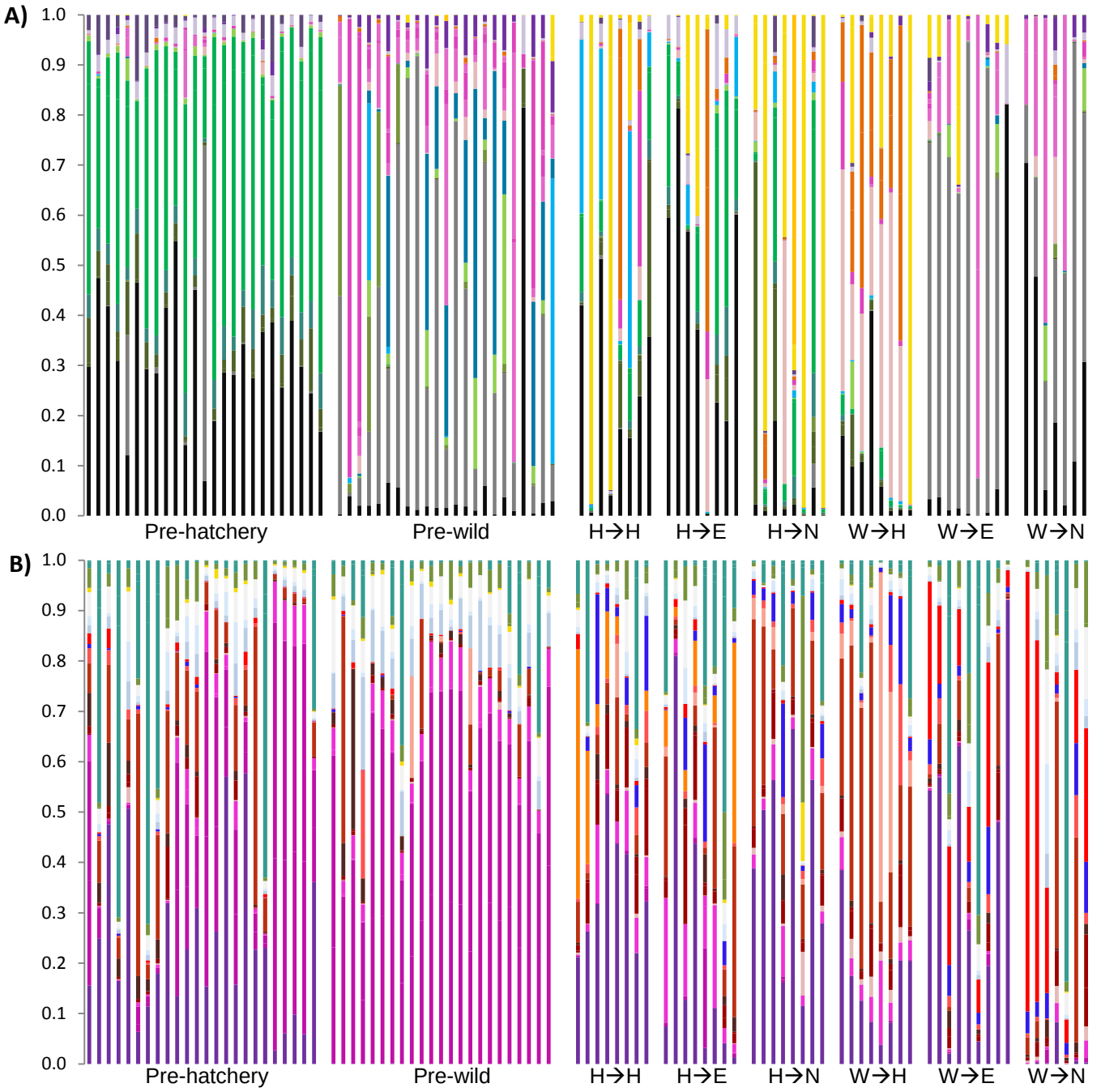

Faeces
Skin
NMDS1
NMDS2
Hatchery pre-translocation
Hatchery → Hatchery
Hatchery → Enriched
Hatchery → Natural
Wild pre-translocation
Wild → Hatchery
Wild → Enriched
Wild → Natural

Figure 4



FIRMICUTES
Clostridia
Clostridiales
- *Clostridium* sp.
- *Staphylococcus* sp.

Bacillales
- *Bacillales*
- *Bacillales*

Bacilli
Lactobacillales
- *Lactobacillus* sp.
- *Carnobacterium* sp.
- *Streptococcus* sp.

PROTEOBACTERIA
Alphaproteobacteria
- *Alphaproteobacteria*

Rhizobiales
- *Rhizobium* sp.

Rhodobacterales
- *Rhodobacter* sp.

Gammaproteobacteria
Enterobacteriales
- *Enterobacteriaceae*

Aeromonadales
- *Aeromonadaceae*

Pseudomonadales
- *Acinetobacter* sp.
- *Pseudomonas* sp.

Betaproteobacteria
Neisseriales
- *Deefgea* sp.
- *Neisseriaceae* sp.

Burkholderiales
- *Burkholderia* sp.
- *Janthinobacterium* sp.
- *Rhodoferax* sp.
- *Achromobacter* sp.

SPIROCHAETES
Brevinematae
Brevinematales
- *Brevinemataceae*

BACTEROIDETES
Flavobacteria
Flavobacteriales
- *Chryseobacterium* sp.

TENERICUTES
- *CK-1C4-19*

Mollicutes
Mycoplasmatales
- *Mycoplasma* sp.

ACTINOBACTERIA
Actinomycetales
- *Propionibacteria* sp.
- *Micrococcus* sp.
- *Corynebacterium* sp.