

1 **Within-species variation in the gut microbiome of fish is driven by the interaction of**
2 **light intensity and genetic background**

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26 **Abstract**

27 Gut microbiome diversity and functions are jointly shaped by the host's genetic background
28 and environmental conditions, but the consequences of this interaction are still unclear.
29 Unravelling the effect of the interaction between evolution and environment on the gut
30 microbiome is particularly relevant considering the unprecedented level of human-driven
31 disruption on the ecological and evolutionary trajectories of species. Here, we aimed to
32 evaluate whether size-selective mortality influences the gut microbiome of medaka (*Oryzias*
33 *latipes*), how environment conditions modulate the effect of the genetic background of medaka
34 on their microbiota, and the association between microbiome diversity and medaka fitness. To
35 do so, we studied two lineages of medaka that were raised under antagonistic size-selective
36 regimes for 10 generations (i.e. the largest or the smallest breeders were removed to mimic
37 fishing-like or natural mortality). In pond mesocosms, the two lineages were subjected to
38 contrasting population density and light intensity (i.e. used as a proxy of primary production,
39 hence resource availability). We observed significant differences in the gut microbiome
40 composition and richness between the two lines, and this effect was mediated by light intensity.
41 Indeed, the bacterial richness of fishing-like medaka (small-breeder line) was reduced by 34%
42 under low-light conditions compared to high-light conditions, while it remained unchanged in
43 natural mortality-selected medaka (large-breeder line). However, the observed changes in
44 bacterial richness did not correlate with changes in growth rate or body condition, possibly due
45 to functional redundancy among the microbial taxa residing in the gut. Given the growing
46 evidence about the gut microbiomes importance to host health, more in-depth studies are
47 required to fully understand the role of the microbiome in size-selected organisms and the
48 possible ecosystem-level consequences.

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50 **Key words** bacterial communities, 16S rRNA gene sequencing, harvest-induced evolution,
51 environment

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Introduction

54 Over the past decade, the expansion of scientific literature investigating the gut microbiome
55 has been largely motivated by increasing evidence of the microbiome's role in health
56 maintenance. Studies have highlighted the variety of pathways through which the gut
57 microbiome can play this role – from influencing nutrient uptake and metabolism to regulating
58 immune responses and pathogen susceptibility (Hanning and Diaz-Sanchez 2015, Moran et al.
59 2019). Gut-associated microbiomes can also incur the consequences of anthropogenic factors
60 such as climate warming (Sepulveda and Moeller 2020), urbanisation (Teyssier et al. 2018,
61 Trosvik et al. 2018, Sonnenburg and Sonnenburg 2019), and environmental pollution (Fouladi
62 et al. 2020, Varg et al. 2021). These factors may increase the chances of imbalances in the gut
63 microbiome (dysbiosis) with potential implications for individual host fitness. A notable
64 example is the effect of increased temperature leading to reduced gut bacterial diversity in the
65 common lizard (*Zootoca vivipara*), also potentially associated with a reduction in survival
66 (Bestion et al. 2017). In teleosts (bony fish), diet composition and quality of surrounding waters
67 have been both linked with variation in the gut microbiome composition (Talwar et al. 2018).
68 Effects of diet composition and water quality can potentially be compounded by the impact of
69 human activities (such as selective fisheries and habitat disruption). Understanding the changes
70 in the gut microbiome and their potential influences on host health and survival is essential to
71 fully understand mechanisms driving changes in impacted populations.

72 It is now well recognised that the gut microbial community (i.e. broadly defined here
73 by its taxonomic composition and diversity) is driven by host's genetic background (Spor et al.
74 2011, Smith et al. 2015, Leopold and Busby 2020) and environmental factors such as

75 population density and diet (Bolnick et al. 2014, Eckert et al. 2020, Mogouong et al. 2020).
76 This holds true in fish (Talwar et al. 2018, Kim et al. 2021). However, host's genotypes and
77 environmental conditions are often in interaction, making it challenging to disentangle their
78 respective effects on the microbiome. So far, the roles of the individual genotype and the
79 environment in shaping gut microbiome communities have been mainly studied independently
80 (but see Navarrete et al. 2012), and we lack assessments of genotype-by-environment effects
81 (Spor et al. 2011, Talwar et al. 2018, Piazzon et al. 2020). Yet, such knowledge may be vital
82 for pinpointing the factors driving variation in gut microbiome assemblages.

83 Size-selective harvesting of wild populations by humans is among the most impactful
84 disturbance factors, resulting in very fast rates of evolutionary change (Sanderson et al. 2022).
85 Evolutionary changes driven by size-selective harvesting (van Wijk et al. 2012, Uusi-Heikkilä
86 et al. 2017) have, in turn, the potential to reshuffle trophic interactions within food webs, with
87 individuals from heavily harvested populations tending to display narrower diets (Hočevar and
88 Kuparienen 2021). Simultaneously, reduced population density due to harvesting also increases
89 resource availability, hence the quality of environmental conditions. Thus, size-selective
90 harvesting provides an ideal context to explore genotype-by-environment interactions on the
91 gut microbiome, and deserves more extensive research, especially in the context of fisheries.

92 Here, we used a replicated pond mesocosm experiment to test at what extent changes
93 in the composition and diversity of the gut microbiota of medaka (*Oryzias latipes*) are driven
94 by the interaction between evolutionary responses to size-selective harvesting and
95 environmental conditions. Native to East Asian countries, the medaka is a small cyprinodont
96 fish (adult length = 32 mm) that has a short generation time and is easily reared in the
97 laboratory, making it an ideal species for selection experiments (Ruzzante and Doyle 1993,
98 Renneville et al. 2020, Bouffet-Halle et al. 2021). The species is omnivorous with an animal-
99 based diet preference, but can also feed on diatoms and filamentous algae (Edeline et al. 2016).

100 We used two lines of medaka originating from a ten-generation size selection experiment,
101 carried out under controlled laboratory conditions. The selection procedure consisted of
102 mimicking either fishing mortality where only small-bodied individuals were allowed to
103 reproduce (small-breeder SB line), or a more natural mortality regime rather favoring the
104 reproduction of large-bodied individuals (large-breeder LB line) (Reneville et al. 2020, Le
105 Rouzic et al. 2020). As we have previously reported, the LB and SB lines evolved opposite
106 life-history traits and behaviors: small-breeder medaka grew slower, matured earlier and were
107 less efficient foragers than the large-breeder medaka (Diaz Pauli et al. 2019, Evangelista et al.
108 2021).

109 We assessed how the genetic background of the two medaka lines interacted with light
110 intensity and medaka population density to shape medaka's gut microbiome composition and
111 diversity. Based on life-history and foraging traits divergence between the two lines (Diaz Pauli
112 et al. 2019, Evangelista et al. 2020, Evangelista et al. 2021), and that these traits are key drivers
113 of gut microbiome variations in teleost fish (Talwar et al. 2018), we hypothesized that the gut
114 microbial community would diverge between SB and LB medaka. We further hypothesized
115 that gut microbiome differences between the two lines would be more pronounced under
116 suboptimal conditions, i.e. when access to food resources is limited (Reese and Dunn 2018,
117 Varg et al. 2021). Finally, because microbiome diversity could be important for host fitness,
118 we evaluated whether fitness proxies (i.e. body growth rate and body condition) were
119 associated with variations in microbiome diversity (Bolnick et al. 2014).

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Methods

122 **Size-dependent selection and fish rearing**

123 The two medaka lines were size-selected over 10 generations under identical laboratory
124 conditions to ensure that differences between lines were genetically-induced. Specifically,

125 medaka were kept in 3-L tank at similar density (14 – 17 fish per tank), and at the same
126 temperature (26°C) and photoperiod (14 h Light / 10 h Dark). They were fed *ad libitum* with a
127 mixed diet of dry food and living brine shrimp *Artemia salina* and/or *Turbatrix aceti*. These
128 standardized environmental conditions ensured that phenotypic differences among the selected
129 lines reflected a genetically-based, evolutionary divergence in response to size-selective
130 harvesting alone (Le Rouzic et al. 2020, Renneville et al. 2020).

131 The selection procedure consisted in removing the largest or the smallest breeders, thus
132 producing two lines with distinct life-history strategies: the small-breeder line where only
133 small-bodied individuals were allowed to reproduce (resulting in slower growth rate and earlier
134 maturation), and the large-breeder lines (resulting in faster growth and delayed maturation).
135 Specifically, size selection was both family- and individual-based. At 60 day-post-hatching
136 (dph), families with the largest (large-breeder line) or smallest (small-breeder line) average
137 standard body length (SL) were kept. Within these families, at 75 dph, the largest-bodied
138 (large-breeder line) or the smallest-bodied (small-breeder line) individuals were used as
139 breeders for the next generation (further details available in Renneville et al. 2020). On average,
140 at 75 dph, SL was 20.7 mm in small breeders and 22.0 mm in large breeders (a 5.7 %
141 difference), and the probability of being mature was 91.7% in small breeders and 77 % in large
142 breeders (a 18.0 % difference) (Renneville et al. 2020).

143 In June 2017, experimental populations were created using fish from the eleventh
144 generation. Specifically, for each line, 180 mature fish (initial standard body length: mean \pm
145 SD; SL_i in small-breeder = 18.9 mm \pm 1.4; SL_i in large-breeder = 19.4 mm \pm 1.4; ANOVA: $F_{1,358}$
146 $= 13.70$, $P < 0.001$) from distinct families were selected to generate 24 experimental
147 populations with limited inbreeding (mean kinship coefficient = 0.23 \pm 0.1 and 0.17 \pm 0.1 SE
148 in LB and SB lines, respectively; further details available in Le Rouzic et al. 2020). Selected
149 fish were anaesthetized with MS-222 and marked using visible implant elastomer (VIE;

150 Northwest Marine Technology, Shaw Island, WA, USA) to render each fish individually
151 identifiable and to allow the calculation of fitness-related traits. Fish from the same
152 experimental population were pooled in a 3 L tank and maintained at the laboratory until the
153 beginning of the experiment when they were released into an outdoor mesocosm.

154

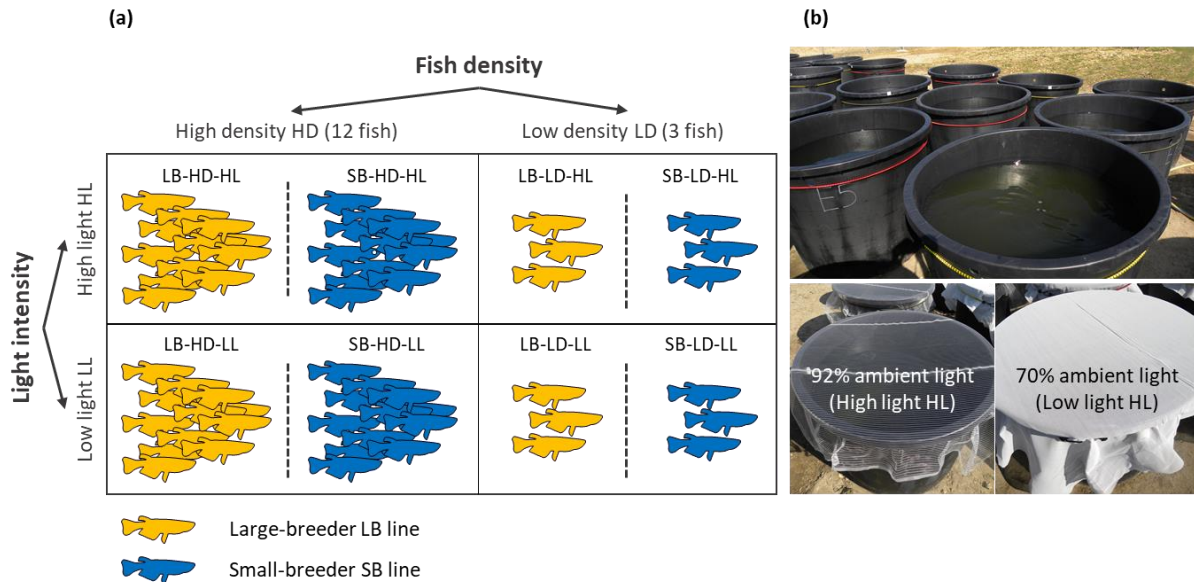
155 **Outdoor mesocosm experiment**

156 The outdoor experiment was conducted at the CEREEP-Ecotron Ile de France (Saint-Pierre-
157 les-Nemours, France; cereep.bio.ens.psl.eu) using 48 mesocosms (500 L, 0.8 m deep, 1.0 m
158 diameter) arranged in 5 blocks. All mesocosms were filled simultaneously from 4 to 6 April
159 2017 with a mix of dechlorinated tap water (100 L) and oligotrophic water from a local pond
160 (300 L). The pond water was pre-filtered through 150 μm mesh to remove large benthic
161 invertebrates, zooplankton and debris. The mesocosms were supplied with 2 L of mature
162 sediment mixture including benthic invertebrates (mainly Ephemeroptera and Chironomidae
163 larvae, Planorbidae, Hydrachnidia, Nematoda and Ostracoda) and 2 L of homogenized mixture
164 of zooplankton (Copepoda and Cladocera) collected from local ponds. In each mesocosm, two
165 floating shelters made of wool threads (30 cm length) provided spawning substrate and two
166 floating brushes made of plastic threads provided protection for larvae. Each mesocosm was
167 then covered with a shading net (see details below) and given 3 months to mature before fish
168 were introduced. On 12 June, all mesocosms were enriched with 2 mL of a liquid mixture of
169 $0.32 \mu\text{g P L}^{-1}$ as KH_2PO_4 and $6.32 \mu\text{g N L}^{-1}$ as NaNO_3 to favor primary production.

170 On 4 July 2017, large- and small-breeder fish were released into the outdoor mesocosms
171 under contrasting environmental conditions. Specifically, we applied two densities (high
172 density, HD: 12 fish per mesocosm or $3.2 \text{ mg fish L}^{-1} \pm 0.3 \text{ SD}$; low density, LD: 3 fish per
173 mesocosm or $0.9 \text{ mg fish L}^{-1} \pm 0.1 \text{ SD}$; female-biased sex ratio of 2:1) and two light intensities
174 using shade nets with different mesh size that allowed the passage of 92% (high light, HL) and

175 70% of ambient light (low light, LL). Light supply was used to modulate primary production
176 while avoiding too high growth of filamentous algae. This factorial design resulted in a total
177 of 8 treatment combinations (Fig. 1), each with 6 replicates.

178



179

180 **Figure 1 (a)** Design of the mesocosm experiment used to test the effects of Line \times Density and
181 Line \times Light intensity on fish gut microbiome. Fish from the Large-breeder (LB) line are in
182 orange and fish from the Small-breeder (SB) line are in blue. (b) Pictures of the outdoor
183 mesocosms (upper picture) and shade nets used to manipulate light intensity (lower pictures).
184

185 Gut microbiome sampling and fitness trait measurements

186 On 22 September 2017, marked fish were recaptured with hand nets (survival rate = 92%). A
187 total of 126 marked fish were randomly and homogeneously sub-sampled among the
188 mesocosms (number of fish per treatment: mean \pm SD = 15.8 ± 0.9 , min = 14, max = 17;
189 number of fish per mesocosm: mean \pm SD = 2.6 ± 0.6 , min = 1, max = 4). After 24 hours
190 fasting, each selected fish was measured for final standard length ($SL_f \pm 1$ mm), weighed (W_f
191 ± 1 mg), euthanized using MS-222 and dissected using disposable laboratory-grade razor
192 blades. The whole intestine (including potential remaining content because of small size) was
193 sampled and flash-frozen in liquid nitrogen for up to 5 hours, then stored in a -80°C freezer

194 until DNA extraction. To limit contaminations during dissection, working environment and
195 dissection tools were sterilised between each individual.

196 Body condition of each selected individual was calculated using the residuals of the
197 relationship between $\log_{10}W_f$ and $\log_{10}SL_f$. The somatic growth rate (mm month^{-1}) of each
198 selected fish was calculated as follows:

$$199 \quad \text{Growthrate} = (SL_f - SL_i)/t$$

200 where SL_f and SL_i are the final and initial standard length, and t is the duration of the
201 experiment (3 months).

202

203 **DNA extraction and sequencing**

204 After defrosting at room temperature, bacterial DNA from medaka gut samples was extracted
205 using the DNeasy PowerSoil kit (Qiagen, Germany) according to the manufacturer's
206 instructions. The quantity and quality of purified DNA was checked using a NanoDrop
207 spectrophotometer (Thermo Fisher Scientific, USA). Library preparation for Illumina
208 sequencing was carried out according to the dual indexing protocol described by Fadrosh et al.
209 (2014). This protocol uses the 319F and 806R primer set to amplify the V3-V4 region of the
210 16S rRNA gene. DNA sequencing was done on an Illumina MiSeq apparatus in 300 bp PE
211 mode. The DNA sequencing was carried out at the Norwegian Sequencing Centre (NSC), and
212 sequence demultiplexing was done using the custom NSC "demultiplexer" software
213 (https://github.com/nsc-norway/triple_index-demultiplexing/tree/master/src), which also
214 removes barcode sequences and heterogeneity spacers. Among the 126 samples, seven
215 displayed amplification failure, and one was removed from the dataset due to mislabelling.

216

217 **Bioinformatics analysis**

218 Further sequence data processing was performed using the Divisive Amplicon Denoising
219 Algorithm as implemented in the dada2 v1.16 R-package (Callahan et al. 2016). Taxonomic
220 classification of amplicon sequence variants (ASVs) was carried out using the Ribosomal
221 Database Project v16 training set (Wang et al. 2007). Using the R package phyloseq (v.1.40.0,
222 McMurdie and Holmes 2013), we further filtered the data in order to remove any contaminant
223 or artefactual sequences. First, ASVs with a Phylum-level assignment probability < 0.80 and
224 those classified as chloroplast DNA were discarded from the dataset. Second, we excluded all
225 ASVs with a total abundance lower than 0.005% of the dataset's total abundance as they are
226 most likely sequencing errors (Bokulich et al. 2013). Finally, samples with a total sequence
227 reads abundance of < 5000 reads were removed from the dataset ($n = 15$). The final dataset
228 consisted of 103 samples, comprising 627 ASVs for a total of 3,591,039 sequence reads.
229 Sequencing depth ranged from a minimum of 5588 to 85470 reads per sample, with a mean of
230 34864 reads per sample. Between-sample differences in library sequencing depth were
231 standardized to the median sequencing depth (Appendix 1).

232

233 **Statistical analyses**

234 All statistical analyses were run with R v.4.2.1 (R Development Core Team, 2022) and with
235 the Family level as taxonomic resolution because it was the best taxonomic level for
236 discriminating (Appendix 2). Using phyloseq, we visualised gut microbiome composition
237 using non-metric multidimensional scaling (NMDS) plots with Bray-Curtis distances. We used
238 a PERMANOVA to test for differences in community composition according to Line \times Density
239 and Line \times Light intensity. This was carried out using the *adonis* function in the vegan package
240 (v.2.6.4, Oksanen et al. 2020), by implementing Bray-Curtis dissimilarities based on Hellinger
241 transformed data and 999 permutations. Statistical tests indicated that there was no deviation
242 from multivariate dispersion ($P > 0.302$; *betadisper* function from vegan). Based on these

243 community composition analyses, we agglomerated the data to family level and visualized the
244 relative abundance of normalized data according to the Line treatment. Significant effects of
245 Line or Line \times Environment on gut microbiome community composition were further
246 investigated using differential abundance analysis based on the linear discriminant analysis
247 (LDA) on effect size (LEfSe) method. LEfSe was implemented in the microeco package
248 (v.0.12.1, Liu et al. 2021) using a non-parametric Kruskal-Wallis test to detect differences in
249 Family abundance (bootstrap test number = 100, significance threshold = 0.01).

250 Gut microbiome diversity was estimated using the first three Hill numbers (qD ; Chiu
251 and Chao 2014, Alberdi and Gilbert 2019a) calculated using the R package hilldiv (v.1.5.1,
252 Alberdi and Gilbert 2019b): $q = 0$ (species richness), $q = 1$ (the exponential of Shannon's
253 entropy index) and $q = 2$ (the inverse of the Simpson's diversity index). Linear models were
254 used to test the effect of Line \times Density and Line \times Light intensity on each Hill number. When
255 significant, the interactions were further investigated using post hoc Tukey's pairwise
256 comparison using the emmeans package (v.1.8.1.1, Lenth 2021). Finally, Spearman
257 correlations (adjusted for multiple testing using false discovery rate (fdr) correction) were used
258 to test for associations between bacterial richness and diversity (i.e. the first three Hill numbers)
259 and medaka fitness traits (i.e. body condition and somatic growth rate) using the *corr.test*
260 function from the psych package (v.2.2.9, Revelle 2021).

261

262 Results

263 Genotype-driven variation in gut microbiome

264 The two medaka lines had a distinct gut microbial community (PERMANOVA: $F = 2.25$, $P =$
265 0.010 , $R^2 = 0.021$; Fig. 2a). Overall, 5 families (i.e. *Aeromonadaceae*, *Neisseriaceae*,
266 *Family_II*, *Rhodobacteraceae* and unspecified *Cyanobacteria*) dominated the gut microbiome
267 of all samples, comprising together 62% and 59% of the total bacterial abundance of LB and

268 SB medaka lines, respectively (Fig. 2c). Of these families, only *Aeromonadaceae* was
 269 significantly more abundant in LB than in SB medaka (LEfSe; $P < 0.001$; Fig. 2d), while the
 270 abundance of the four other families did not significantly differ between the two line.
 271 Differential abundance analyses also showed that *Oxalobacteraceae*, *Verrucomicrobiales*
 272 (*unspecified Family*) and *Desulfovibrionaceae* were significantly more abundant in the gut of
 273 LB than SB medaka ($P = 0.006$, $P = 0.004$, $P = 0.008$, respectively). In contrast, the gut
 274 microbiome of SB medaka had more *Microbacteriaceae* than that of LB ones ($P = 0.009$; Fig.
 275 2d).

276 **Table 1** Analysis-of-variance table derived from the linear models used to assess the effect of
 277 size-selected line, fish density and light intensity on gut microbiome diversity ($q=0$ D, $q=1$ D and
 278 $q=2$ D). Significant P values are highlighted in bold.
 279

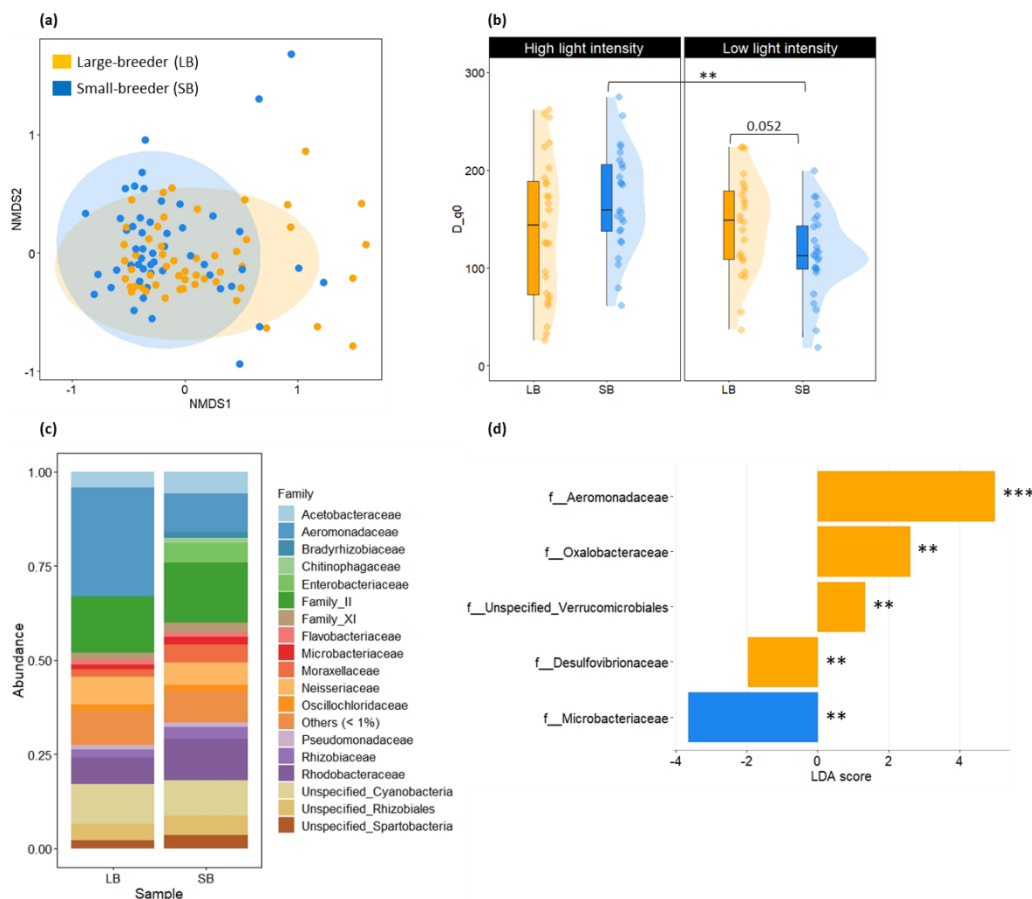
Responses	Predictors	Sum of Sq.	F _{df}	P
$q=0$ D	Intercept	338340	111.04 ₁	< 0.001
	Line	21006	6.89 ₁	0.010
	Density	1187	0.39 ₁	0.534
	Light intensity	148	0.05 ₁	0.826
	Line × Density	11306	3.71 ₁	0.057
	Line × Light intensity	22238	7.30 ₁	0.008
	Residuals	295573	— ₉₇	—
$q=1$ D	Line	1424	3.66 ₁	0.059
	Density	196	0.50 ₁	0.479
	Light intensity	173	0.45 ₁	0.506
	Line × Density	531	1.37 ₁	0.245
	Line × Light intensity	126	0.32 ₁	0.570
	Residuals	37732	— ₉₇	—
$q=2$ D	Line	408.2	3.40 ₁	0.068
	Density	5.7	0.05 ₁	0.828
	Light intensity	1.4	0.01 ₁	0.915
	Line × Density	105.4	0.88 ₁	0.351
	Line × Light intensity	0.0	0.00 ₁	0.995
	Residuals	116642.1	— ₉₇	—

280

281

282 **Gene-environment interaction-driven variation in gut microbiome**

283 The interactions between medaka line and the environment (i.e. Line \times Density and Line \times
 284 Light intensity) had no significant effect on gut microbiome composition (PERMANOVA: F
 285 = 1.41, $P = 0.131$, $R^2 = 0.013$ and $F = 1.29$, $P = 0.166$, $R^2 = 0.012$, respectively). In contrast,
 286 gut microbial richness was modulated by the interaction between the medaka line and the
 287 environment (Table 1). Specifically, SB medaka had a higher bacterial richness (when $q = 0$)
 288 in the high-light intensity compared to the low-light treatment (Line \times Light: Tukey post hoc:
 289 $q = 0$ D_{SB-HL} vs. $q = 0$ D_{SB-LL} : $t_{97} = 3.59$, $P < 0.001$; mean $q = 0$ $D \pm SE$: 169 ± 11 and 114 ± 8 ,
 290 respectively), while bacterial richness of LB medaka did not change with light variation (Fig.
 291 1b; $q = 0$ $D_{LB-HL} = 141 \pm 14$, $q = 0$ $D_{LB-LL} = 145 \pm 10$). Additionally, LB medaka seemed to have a
 292 higher bacterial species richness than SB medaka, but only in the low-light treatment (Line \times
 293 Light: Tukey post hoc: $q = 0$ D_{LB-LL} vs. $q = 0$ D_{SB-LL} : $t_{97} = 1.97$, $P = 0.052$). Other metrics of gut
 294 microbiome diversity (when $q = 1$ or 2) were not influenced by the interaction between Line
 295 and environmental conditions (Table 1).



297 **Figure 2 (a)** NMDS ordination (NMDS stress = 0.16) of variation in bacterial community
298 composition of fish from the Large-breeder (LB; orange dots) and Small-breeder (SB; blue dots)
299 lines. Data represents ordination based on Bray-Curtis distances among the 103 fish individuals.
300 **(b)** Raincloud plot showing the light-intensity effect of Line on bacterial richness ($q = 0$ D). Dots
301 represent the fish (n = 103), boxplots and half violin plots illustrate the probability density of the
302 data. **(c)** Gut microbial taxonomic composition (Family level) of fish according to its selective
303 background (Large-breeder line, LB; Small-breeder line SB). **(d)** Linear discriminant analysis
304 (LDA) effect sizes representing the five ASVs (family level “F”) that significantly differ in
305 abundance between the Large-breeder (orange) and Small-breeder (blue) lines. Significance
306 differences ($P < 0.01$) between the two lines are depicted with asterisks ($*** P < 0.001$, $** P <$
307 0.01).
308

309 **Lack of correlation between microbiome and host fitness proxies**

310 No significant correlation was observed between gut microbiome diversity (estimated using
311 the first three Hill numbers) and both medaka’s body condition and somatic growth (Spearman
312 correlations: adjusted $P > 0.374$ for all; Appendix 3).

313

314 **Discussion**

315 Due to its pivotal role for host fitness and health, there has been a growing research interest in
316 the factors driving gut microbiome variation in animal species. Yet, studies focusing on the
317 genotype-by-environment effects on the gut microbiome remain, to our knowledge, very
318 limited (Piazzon et al. 2020). Using size-selected medaka lines (large-breeder LB and small-
319 breeder SB) in a pond mesocosm experiment, we found that the gut microbial community
320 composition differed between the two lines. In addition, the microbiome richness of SB medaka
321 was influenced by light intensity, while that of LB remained unchanged regardless of the
322 environmental conditions. Together, this is consistent with our prediction that evolutionary
323 changes due to size-selective harvesting have the potential to shape the gut microbiome
324 assemblage within harvested populations. Our results also suggest that the interaction between
325 the genetic background of medaka (i.e. the selected line) and the environmental conditions is
326 important. However, contrary to our prediction, variation in microbiome diversity was not
327 associated with any of the measured fitness-related traits.

328 Our findings confirm observations from literature showing that genotype could drive
329 gut microbiome composition among fish groups (Sevellec et al. 2018, Small et al. 2019, Smith
330 et al. 2015). In our case, the largest difference in relative reads abundance was found for the
331 *Aeromonadaceae* family which was almost 3 times more abundant in LB than in SB medaka
332 (28.9% and 10.1%, respectively). Bacteria from the *Aeromonas* genus have been shown to be
333 pathogenic for numerous fish species (Tomás 2012, Wang et al. 2018). Our previous findings
334 from the same pond experiment did not indicate any difference in survival probability and
335 somatic growth rate among introduced adults of the LB and SB lines (although LB medaka
336 produced more offspring that grew faster), but suggested that adult and juvenile LB medaka
337 foraged more overall (Evangelista et al. 2021). They were especially foraging more on benthic
338 prey hidden in the sediments than the SB medaka (Evangelista et al. 2021). Although further
339 investigation is required to back-up this hypothesis, increased abundance of *Aeromonadaceae*
340 in LB might reflect distinct foraging strategies between the two lines, rather than changes in
341 health conditions as *Aeromonadaceae* are known to be facultative aerobes and are mainly found
342 in anoxic sediments (Tomás 2012, Laviad and Halpern 2016). Additionally, some *Aeromonas*
343 display cellulolytic activity, which can be useful for the digestion of plant-based diet (Li et al.
344 2016). One could hypothesize that a higher proportion of *Aeromonadaceae* in LB medaka
345 could be associated with a more omnivorous feeding habit compared to SB medaka (Liu et al.
346 2016). In addition, *Verrucomicrobia* found in greater quantities in LB would have a potential
347 role as polysaccharide degraders in fresh water (Fuerst 2019). Altogether, our results suggest
348 that even if the gut microbiome composition between the two lines differs, the mere description
349 of microbiome diversity and composition is not sufficient. In fact, more targeted diet
350 manipulation experiments between lines would be required in order to clearly identify whether
351 adaptation to size selection could directly affect the gut microbiome, or indirectly through
352 changes in diet.

353 Under low-light intensity, the gut microbiome of SB medaka showed a 34% decrease
354 of bacterial richness compared to the high-light intensity treatment, and also presented a
355 somewhat lower richness than in LB medaka, though this difference was not significant ($P =$
356 0.052). This suggests that the gut microbiome diversity of fish selected for earlier maturation
357 and slower growth rate (as is often the case with size-selective harvesting) is reduced when
358 environmental conditions are not optimal. The underlying mechanisms of such genotype-by-
359 environment effects are hard to pinpoint and could arise from changes in the SB medaka
360 themselves (e.g. light-induced changes in medaka behaviour or physiology that ultimately alter
361 the gut microbiome), or environmental changes such as light-induced changes in diet or in the
362 bacterial composition of water). But as we did not sequence the microbiome from the water
363 used in the experimental ponds, we are not able to assess the extent to which potential co-
364 amplification of bacterial taxa from the environment (Talwar et al. 2018) could interfere with
365 the composition of the gut communities, and thus bias our results. On the other hand,
366 acquisition of gut bacteria through the water in fish must be common, suggesting that the
367 magnitude of such bias should be rather small. It is also worth noting that studies suggest that
368 there is no association between the gut microbiome of fish and that of the surrounding waters
369 (Schmidt et al. 2015, Wang et al. 2018).

370 Whether high microbial diversity matters for the host remains a central question in
371 microbiome studies. For instance, Bolnick et al. (2014) found a positive effect of the gut
372 microbial diversity on the body condition of laboratory-reared stickleback (*Gasterosteus*
373 *aculeatus*), but no association between variation in microbial diversity and condition of wild
374 stickleback. In our experiment, gut microbiome diversity was not associated with fitness-
375 related traits of medaka, perhaps because low diversity does not entail the loss of essential
376 microbiome-mediated functions. Therefore, the lack of associations could simply reflect our
377 yet limited understanding about the taxonomic identity and functional role of gut bacteria in

378 non-model organisms. Thus, changes in bacterial diversity might be associated with either
379 positive or negative impact for the host according to the degree of decoupling between
380 taxonomic identity, functional role and the environmental context (Bolnick et al. 2014).
381 Overall, our results highlight the fact that our perception about gut microbiome benefits is also
382 probably biased by data based on a very limited range of species (Hammer et al. 2019).
383 Nonetheless, variation in the microbiome can impact the digestive capacity and body condition,
384 and this diversity may act as an underlying mechanism for phenomic plasticity of the host
385 (Alberdi et al. 2016). How changes in bacterial diversity translate into functional changes will
386 require further investigation.

387 Our study reveals that the gut microbiome of fish can be influenced by interactions
388 between their genetic background and the environment. Studying genotype-by-environment
389 effects on the gut microbiome may bring new perspectives into the role of microbiomes in eco-
390 evolutionary dynamics, as changes in gut microbial communities could translate into changes
391 in ecosystem functioning and services (Graham et al. 2016, Dutton et al. 2021), including
392 fisheries productivity (Gallo et al. 2020, Diwan et al. 2021). As the demand for fish for human
393 consumption is increasing, we also claim that more research is needed to enhance our
394 understanding of the possible effects of fisheries-induced evolution on the gut microbiome.
395 Comparison of the gut microbiome of fish in relation to different management strategies (e.g.
396 sustainable versus size-selective fisheries versus protected areas) may reveal important
397 mechanisms influencing populations' adaptability and resilience, and thus help restoring highly
398 impacted fish stocks (Gallo et al. 2020). It is also important for future studies to reveal the
399 functional consequence of changes in gut microbiome (Tarnecki et al. 2017), especially
400 functions directly responsible for fish behaviour and fitness so that the target preservation of
401 highly beneficial gut microbiomes within harvested populations could be incorporated as part
402 of more sustainable fisheries practices.

403

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409

410

Data, script, code, and supplementary information availability

411 Data and R codes that support the findings of this study are hosted in the Figshare repository
412 (<https://figshare.com/s/d5235f25f3d7b15a0e47>). SRA accession for sequences data is
413 PRJNA929943

414

415

Conflict of interest disclosure

416 The authors declare that they comply with the PCI rule of having no financial conflicts of
417 interest in relation to the content of the article. The authors declare the non-financial conflict
418 of interest: SK is a recommender for PCI Ecology.

419

420

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428

429

Ethics

430 The experiment was approved by the Darwin Ethical committee (case file #Ce5/2010/041)

431 from the French Ministry of Education, Higher Education and Research.

432

433

Author's contributions

434 CE conceived and coordinated the study with input from SK, EM and PT; CE, JD and BDP

435 collected the samples; SK and EM carried out the molecular analyses; PT carried out the

436 bioinformatic analyses; CE analyzed the data and wrote the initial draft of the manuscript with

437 input from SK, PT and EM; All authors contributed to revisions and approved the final version

438 of the manuscript.

439

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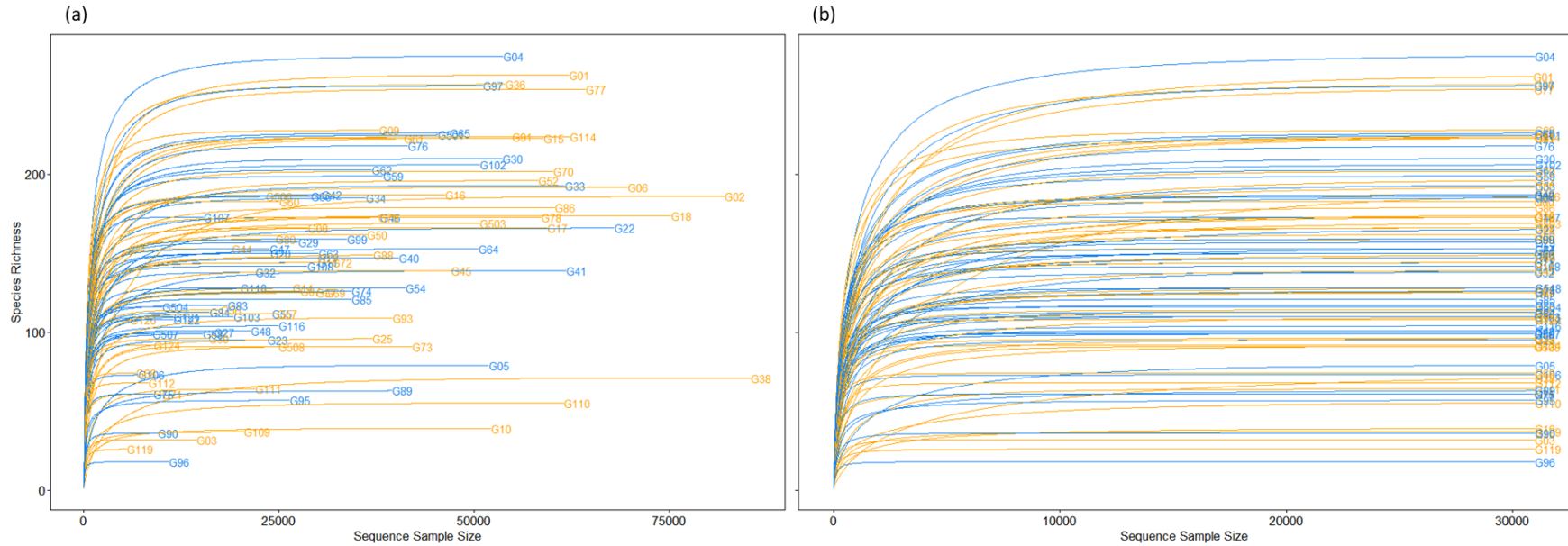
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Appendix 1: Rarefaction curves



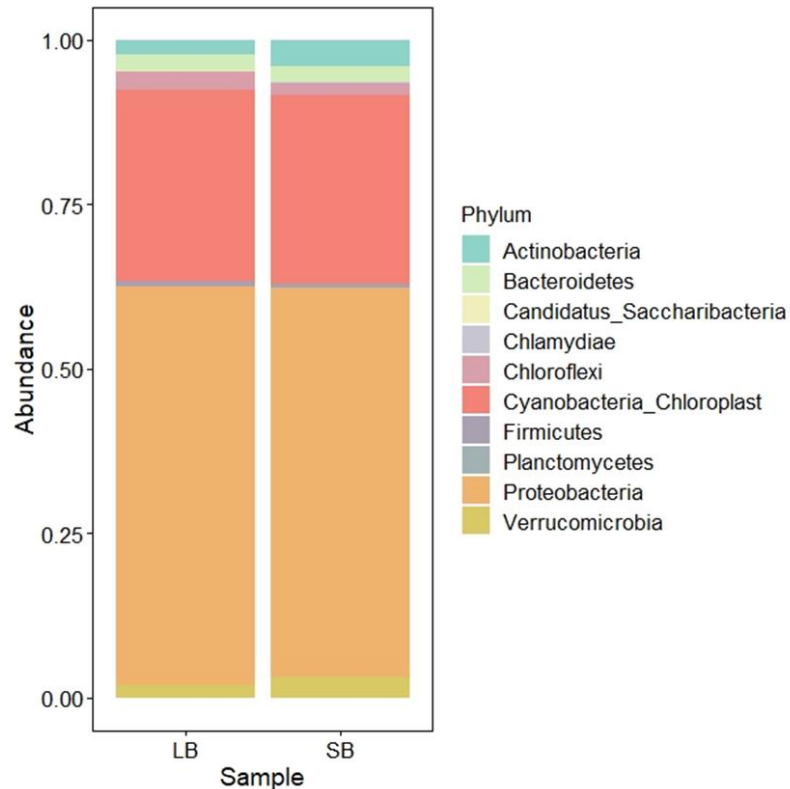
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Figure S1 Rarefaction curves showing the number of ASV with increasing number of reads (Sequence sample size) **(a)** before and **(b)** after standardization to the median sequencing depth. Fish from the Large-breeder (LB) line are in orange (n = 52) and fish from the Small-breeder (SB) line are in blue (n = 51).

642

Appendix 2: Relative abundance of bacterial phyla in gut samples

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644

645 **Figure S2** Gut microbial taxonomic composition (Phylum level) of fish according to its
646 selective background (Large-breeder line, LB; Small-breeder line SB). As reported in other
647 fish studies (Tanercki et al. 2017), dominant phylum of the medaka gut microbiome included
648 Proteobacteria (60.5% and 59.2% of reads in LB and SB medaka, respectively), followed in a
649 lesser percentage by Bacteroidetes (2.5 and 2.4%, respectively), Verrucomicrobia (2.0 and 3.2,
650 respectively) and Actinobacteria (2.2 and 3.9%, respectively). Bacterial communities were also
651 characterized by large relative abundance of Cyanobacteria (29.2% and 28.8%, respectively),
652 while Firmicutes represented only 0.7% and 0.5% of reads, respectively.

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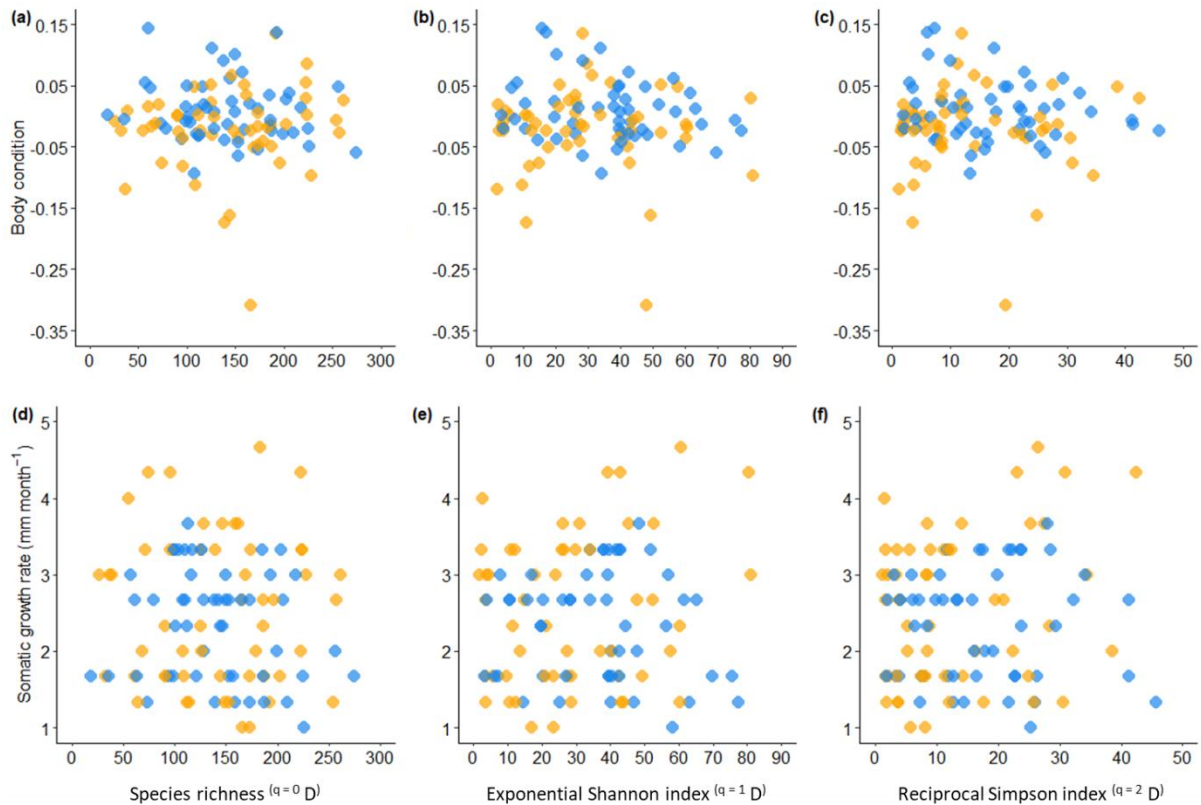
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664 **Appendix 3: Correlations between medaka fitness trait and gut microbiome diversity**

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666

667 **Figure S3** Correlations between gut microbial diversity estimated using the first three Hill
668 numbers (qD ; species richness, exponential Shannon index and Reciprocal Simpson index) and
669 (a-c) body condition estimated as the residuals of the relationship between $\log_{10}W_f$ and \log_{10}
670 SL_f , and (d-f) somatic growth rate [mm month⁻¹]) of fish from the Large-breeder (LB; orange
671 dots, n = 52) and Small-breeder (SB; blue dots, n = 51) lines. None of the correlations were
672 significant.

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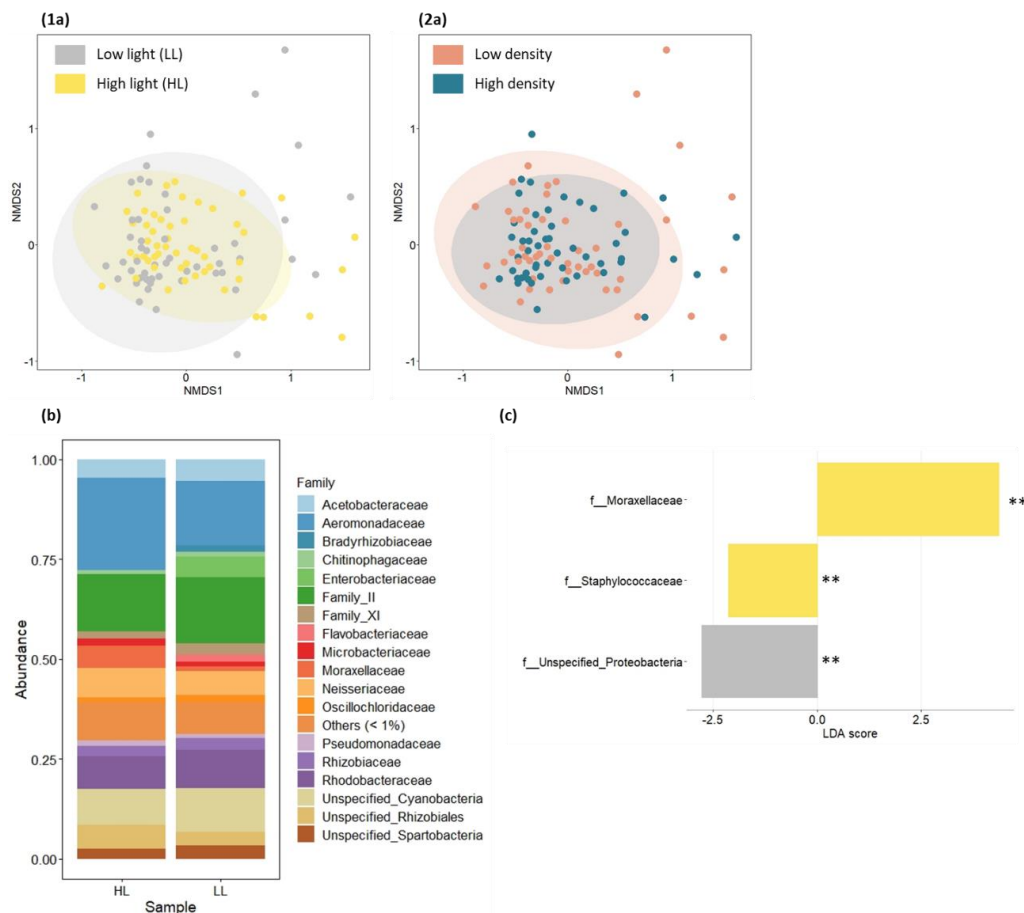
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686 Appendix 4: Variation in gut microbiome according to the environment

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688 PERMANOVA showed a significant effect of light intensity on the gut microbial community
689 ($F = 1.80$, $P = 0.036$, $R^2 = 0.017$; Fig. S4.1a), but no effect of fish density ($F = 1.38$, $P = 0.125$,
690 $R^2 = 0.013$; Fig. S4.2a). The relative abundance of *Moraxellaceae* and *Staphylococcaceae* were
691 more abundant in the gut of medaka living in the high-light, than in the low-light intensity
692 treatment (LEfSe; $P = 0.005$ and $P = 0.001$, respectively). The opposite pattern was observed
693 when considering unspecified *Proteobacteria* ($P = 0.004$), while the abundance of other
694 families did not significantly differ between the two lines (Fig. S4.b-c).



695

696 **Figure S4 (a)** NMDS ordination (NMDS stress = 0.16) of variation in bacterial community
697 composition in (1) low or high light intensity (grey and yellow dots, respectively), and (2) low
698 or high fish density (red and blue dots, respectively). Data represent ordination based on Bray-
699 Curtis distances among the 103 fish individuals. **(b)** Relative abundance of bacterial family
700 according to light intensity conditions (high light, HL; low light, LL) **(d)** Plot of the 15 most
701 important ASVs (family level “f”), evaluated according to the mean decrease in Gini impurity
702 as determined by the random forest classifier. Significance differences ($P > 0.01$) between low
703 (grey bar) and high (yellow bars) light intensity are depicted with asterisks ($*** P < 0.001$).