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

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Abstract

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

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

Introduction

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

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

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

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

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

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

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

Methods

Size-dependent selection and fish rearing

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

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

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

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

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

Outdoor mesocosm experiment

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

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

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

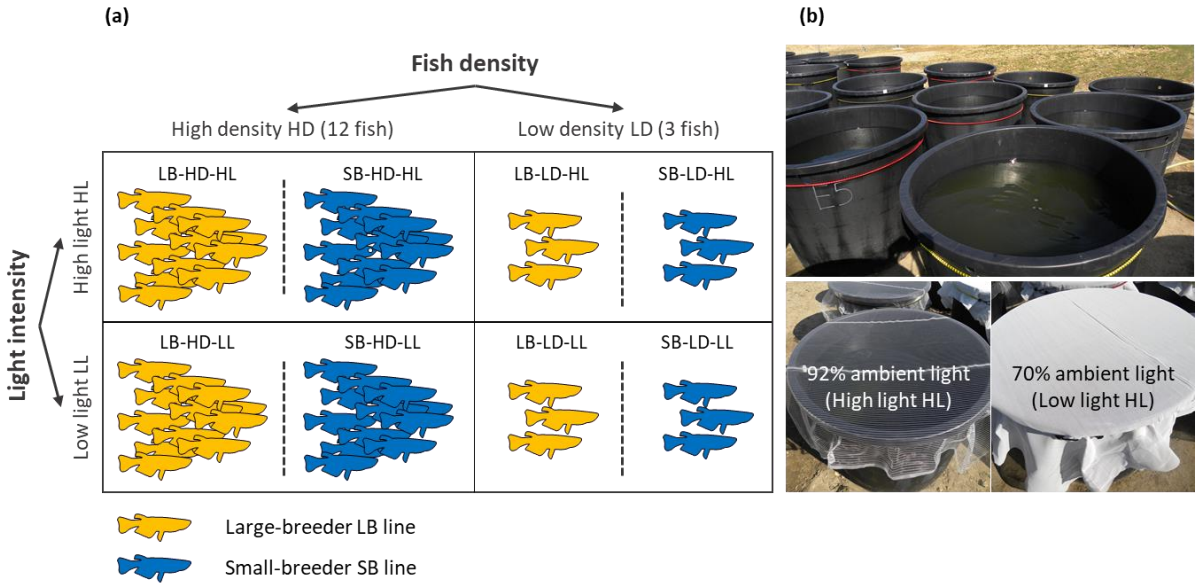

Figure 1 (a) Design of the mesocosm experiment used to test the effects of Line × Density and Line × Light intensity on fish gut microbiome. Fish from the Large-breeder (LB) line are in orange and fish from the Small-breeder (SB) line are in blue. **(b)** Pictures of the outdoor mesocosms (upper picture)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

Gut microbiome sampling and fitness trait measurements

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

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

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

$$Growthrate = \left(SL_f - SL_i\right)/t$$

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

DNA extraction and sequencing

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

Bioinformatics analysis

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

Statistical analyses

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

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

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

Results

Genotype-driven variation in gut microbiome

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

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

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

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	—$_{97}$	—

Gene-environment interaction-driven variation in gut microbiome

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

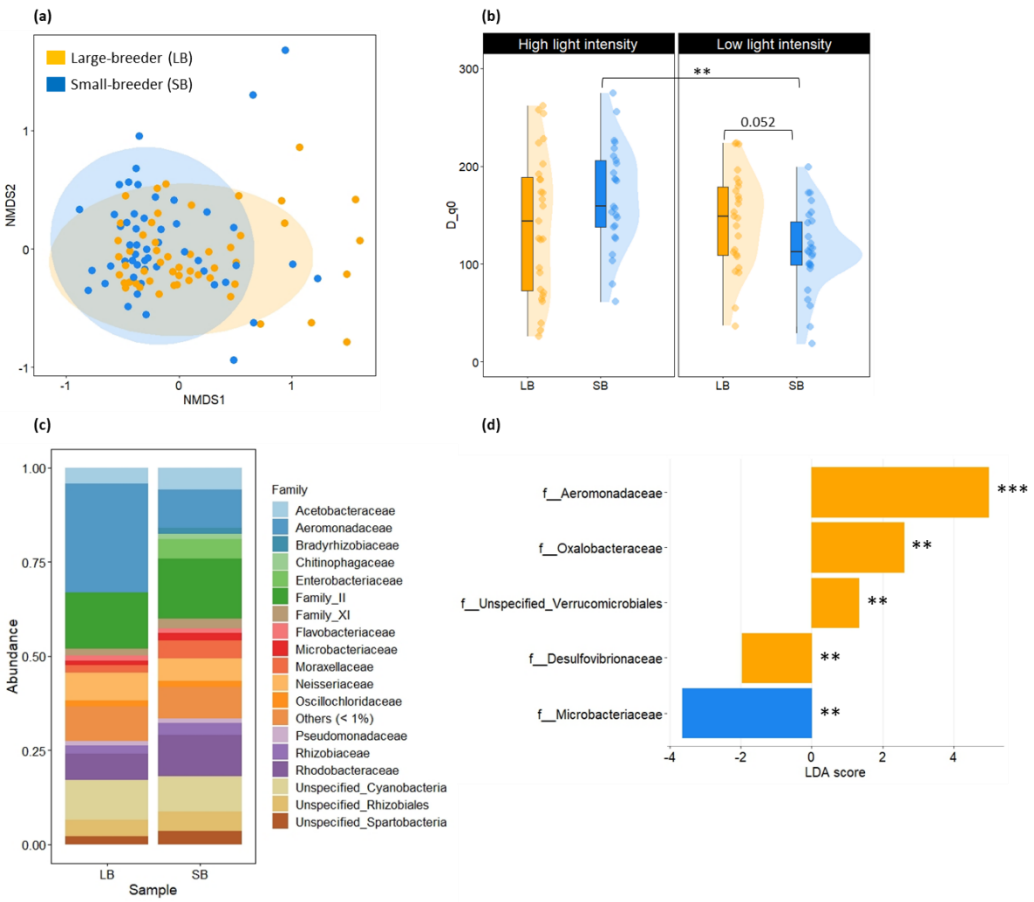

Figure 2 (a) NMDS ordination (NMDS stress = 0.16) of variation in bacterial community composition of fish from the Large-breeder (LB; orange dots) and Small-breeder (SB; blue dots) lines. Data represents ordination based on Bray-Curtis distances among the 103 fish individuals. **(b)** Raincloud plot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

Lack of correlation between microbiome and host fitness proxies

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

Discussion

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

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

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

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

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

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

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Data, script, code, and supplementary information availability

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

Conflict of interest disclosure

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

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Ethics

The experiment was approved by the Darwin Ethical committee (case file #Ce5/2010/041) from the French Ministry of Education, Higher Education and Research.

Author's contributions

CE conceived and coordinated the study with input from SK, EM and PT; CE, JD and BDP collected the samples; SK and EM carried out the molecular analyses; PT carried out the bioinformatic analyses; CE analyzed the data and wrote the initial draft of the manuscript with input from SK, PT and EM; All authors contributed to revisions and approved the final version of the manuscript.

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Appendix 1: Rarefaction curves

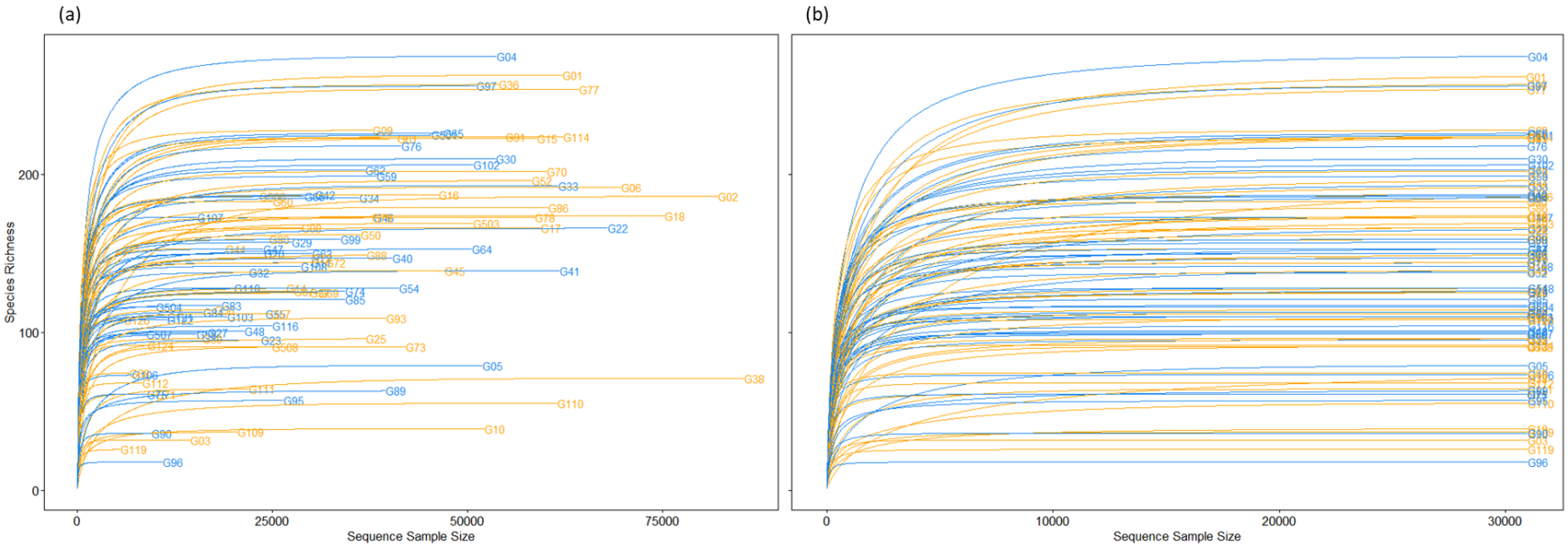

Figure S1 Rarefaction curves showing the number of ASV with increasing number of reads (Sequence sample size) **(a)** before and **(b)** after standardization to the median sequencing depth. Fish from the Large-breeder (LB) line are in orange (n = 52) and fish from the Small-breeder (SB) line are in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

Appendix 2: Relative abundance of bacterial phyla in gut samples

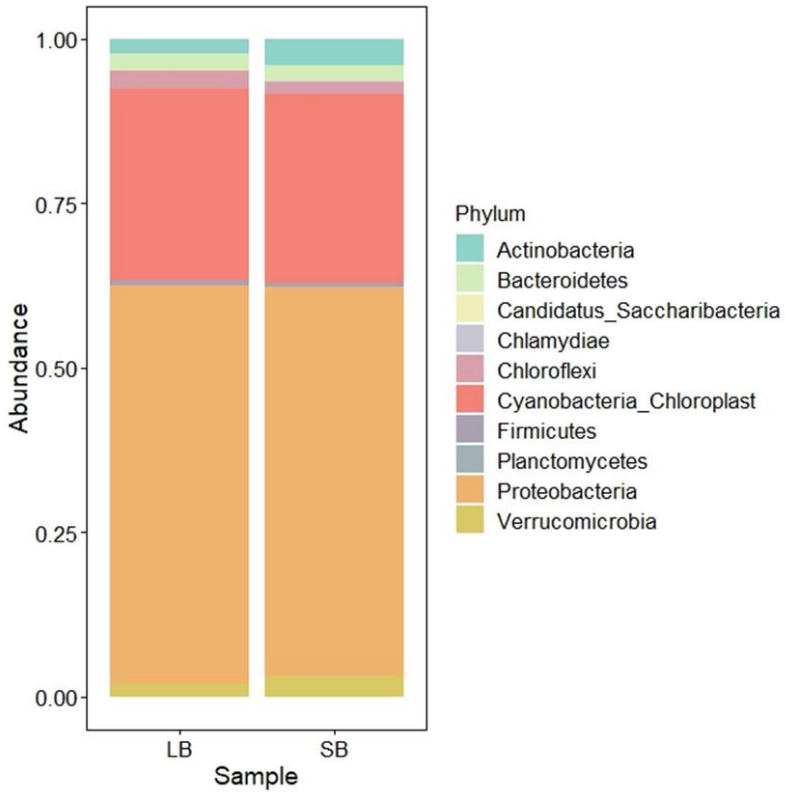

Figure S2 Gut microbial taxonomic composition (Phylum level) of fish according to its selective background (Large-breeder line, LB; Small-breeder line SB). As reported in other fish studies (Tanercki et al. 2017), dominant phylum of the medaka gut microbiome included Proteobacteria (60.5% and 59.2% of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

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

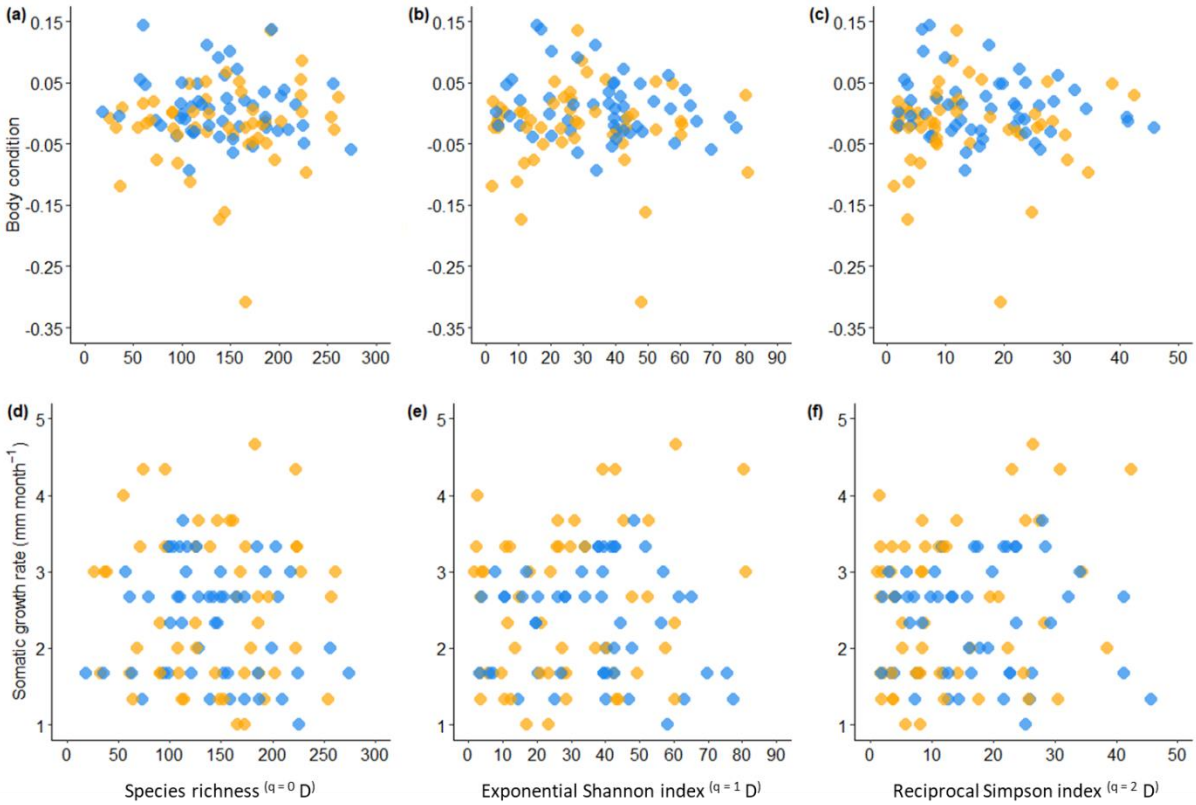

Figure S3 Correlations between gut microbial diversity estimated using the first three Hill numbers (^{q}D; species richness, exponential Shannon index and Reciprocal Simpson index) and **(a-c)** body condition estimated as the residuals of the relationship between $\log_{10}W_f$ and $\log_{10}SL_f$,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

Appendix 4: Variation in gut microbiome according to the environment

PERMANOVA showed a significant effect of light intensity on the gut microbial community (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, R^2 = 0.013; Fig. S4.2a). The relative abundance of *Moraxellaceae* and *Staphylococcaceae* were more abundant in the gut of medaka living in the high-light, than in the low-light intensity treatment (LEfSe; P = 0.005 and P = 0.001, respectively). The opposite pattern was observed when considering unspecified *Proteobacteria* (P = 0.004), while the abundance of other families did not significantly differ between the two lines (Fig. S4.b-c).

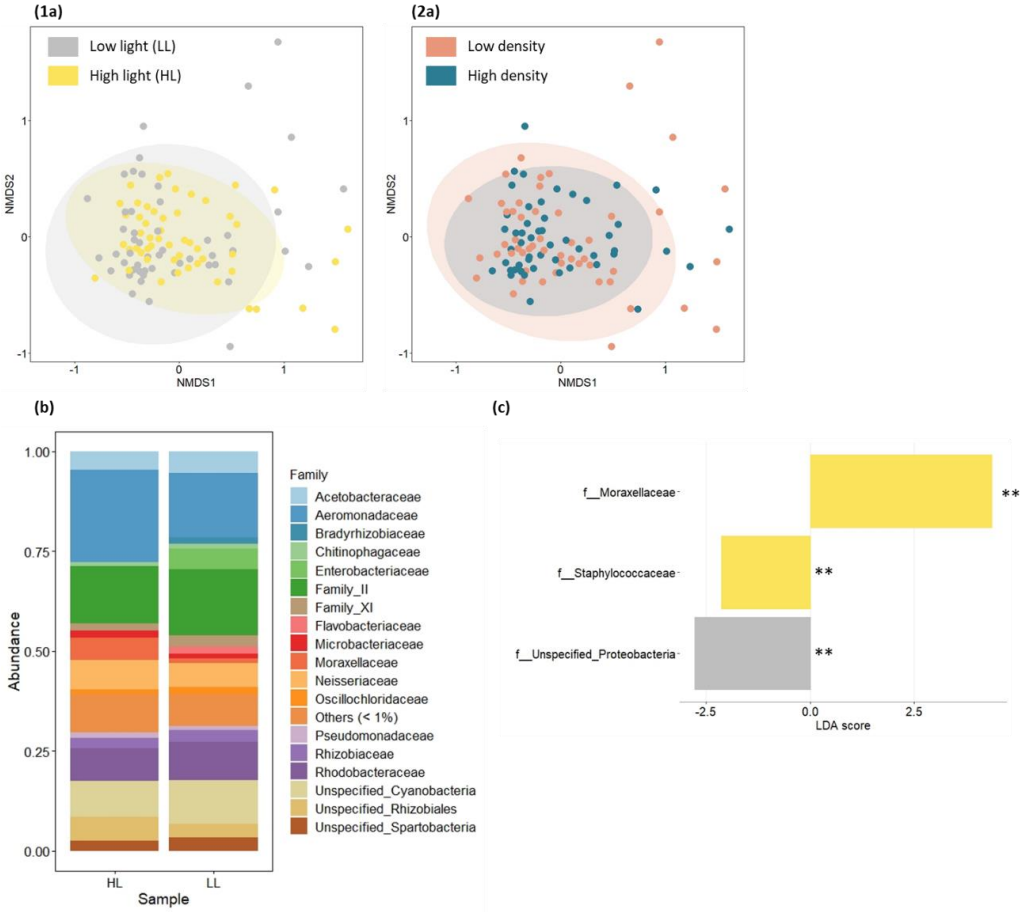

Figure S4 (a) NMDS ordination (NMDS stress = 0.16) of variation in bacterial community composition in (1) low or high light intensity (grey and yellow dots, respectively), and (2) low or high fish density (red and blue dots, respectively). Data represent ordination based on Bray-Curtis distances among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