Monitoring disease and antibiotic treatment in the skin microbiota of farmed seabass fingerlings

Daniela Rosado (de.frosado@gmail.com)
CIBIO-InBIO https://orcid.org/0000-0001-9312-3648
Marcos Pérez-Losada
CIBIO-InBIO
Ricardo Severino
Piscicultura Vale da Lama, Sapal do Vale da Lama
Raquel Xavier
CIBIO-InBIO

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Abstract

The microbiota of fish skin is the primary barrier against disease; however, it is highly dynamic being modulated by several factors. In fish aquaculture, disease outbreaks occur mainly during early-life stages with high associated losses. Antibiotic treatments sometimes remain as the best option to control bacterial diseases, despite many reported negative impacts of its use on fish and associated microbiota. Notwithstanding, studies monitoring the effects of disease and antibiotic treatment on the microbiota of fingerlings are scarce. We used a 16S rRNA metataxonomic approach to assess the impact of a mixed infection with *Photobacterium damselae* ssp. *piscicida* and *Vibrio harveyi* and subsequent antibiotic treatment with flumequine, on the skin microbiota of farmed seabass *Dicentrarchus labrax* fingerlings. Both disease and antibiotic treatment led to a significant increase in bacterial diversity and core microbial communities and impacted microbial structure. Dysbiosis was confirmed by changes in the abundance of potential pathogenic and opportunistic bacterial taxa. Skin bacterial metabolic function was also significantly affected by flumequine administration, suggesting a detriment to fish skin health. Our results add to an increasing body of literature, showing how fish microbiome response to disease and antibiotics is not easily predicted.

Introduction

Commensal microbiota are an essential part of the immune response of animals, including fish, with a crucial role in disease prevention [1, 2]. Perturbations to the homeostasis of the commensal organisms is termed dysbiosis and can occur through three not mutually exclusive events linked to the occurrence of diseases [3]: i) pathobiont expansion, ii) reduced diversity, iii) and loss of beneficial microbes. Most of the microbiome research in vertebrates is focused on the gut microbiota due to their recognized role in sustaining the gut-brain axis and in disease outcome [4]. However, in the case of fish, because pathogens are ubiquitous in the aquatic environment, fish skin and associated mucus are considered the primary barrier against diseases, with increasing numbers of studies focusing on the skin microbiota [e.g., 5–7]. Evidence shows that fish skin bacteria are highly dynamic, with composition and diversity being sensitive to both biotic [e.g., ontogeny, 8] and abiotic factors [e.g., infection, 9].

Although aquaculture is the fastest growing food-producing industry, disease outbreaks due to pathogenic bacteria are one of the biggest constraints for its sustainability [10]. The impact of disease on the microbiota of several fish species has been assessed, albeit being directed mainly at adult populations [e.g., skin and gill, 5–7, 9; gut and stomach, 11, 12]. It is well established that disease often leads to dysbiosis, through a decrease in bacterial diversity and/or increases in the abundance of pathogenic taxa other than the main etiological agent of disease [e.g., 5, 13]. Frequently, dysbiosis was also seen to lead to changes in predicted microbial function [e.g., 6, 12, 14]. Although disease and mortality incidences in aquaculture are higher on fry or young fingerlings, the impact of disease on the microbiota during these early life stages is still poorly known [10]. Nevertheless, existing reports show that diversity, structure and potential function of the microbiotas of early-life stages are also affected by disease [skin, 13; gut, 15; gut, 16]. The European seabass *Dicentrarchus labrax* is susceptible to several bacterial pathogens, two of the most threatening being *Photobacterium damselae* and *Vibrio spp.* [17]. Both bacteria genera are usually reported from the skin of healthy fish, and dysbiosis is usually accompanied by increases in their abundances [e.g., European seabass, Rosado et al. submitted, 7; perl gentian grouper, 18]. These pathogens cause photobacteriosis and vibriosis and infections can be systemic, affecting multiple organs [19, 20].

Although vaccines are available against major diseases, procedures are costly and cause substantial stress and mortality, and they mostly confer short term immunity [21]. For these reasons, antibiotics are still widely used to

control disease in aquaculture. Several studies have reported the negative impacts of antibiotic use on fish, which include behavioral changes [22], microbial diversity decrease [7, 22, 23], increased susceptibility to secondary infections [24], changes in predicted microbial function [23] and mortality [25]. Flumequine is a fluoroquinolone antibiotic active against Gram-negative bacteria and widely used in aquaculture [26]. Depending on water temperature, it can persist in fish skin and muscle up to 4-14 days since the last administration [27]. To the best of our knowledge, the impact of flumequine in the microbiota of fish has never been assessed.

Here, we used a metataxonomic approach [28] to characterize the skin microbiota of the seabass *Dicentrarchus labrax* fingerlings before, during and after a natural disease outbreak of *Photobacterium damselae* ssp. *piscicida* and *Vibrio harveyi*, and subsequent antibiotic treatment with flumequine. Our goal was to describe changes in composition, structure and potential function of the skin microbiota caused by disease and subsequent antibiotic treatment. We predict that: i) disease will cause dysbiosis through a decrease in the microbial diversity and core microbiota, while antibiotic treatment will have the opposite effect; and ii) both disease and antibiotic treatment will lead to changes in the most abundant bacterial taxa and differences in microbial structure.

Methods

Experimental design, sampling and preparation

Fish were reared in an open water circulation system in a semi-intensive farming facility, where water is supplied from the Alvor Estuary (Southern Portugal). Sampled fish belonged to the same age cohort and were sampled from the same rearing pond. Fish were 6 months old on the first sampling date, weighing on average 57 g, and 7 months old on the last sampling date, weighing on average 70 g. Individuals were randomly caught using a fishing pole, and skin samples were taken using tubed sterile swabs (Medical Wire & Equipment, UK). We swabbed the right upper lateral part of the fish skin from head to tail. Swabs were immediately stored at -20 °C until transported on dry ice to the CIBIO-InBIO laboratory where they were kept at -80 °C until processed.

Individuals were collected once a week between August 23 and September 13, 2016, encompassing four different sampling dates corresponding to four different fish health states: healthy (N=30), diseased (N=30), treatment (N=30) and recovery (N=15) (Figure 1). During the “healthy” state (sampled on August 23), all individuals were considered healthy due to lack of visible disease symptoms. The second sampling point occurred on August 30, and on August 31 fish began to die. Hence, samples collected on August 30 were categorized as “diseased” (although still asymptomatic). Treatment with flumequine antibiotic was initiated on August 31, being administered at 35 g/ton of fish through commercial feed until September 6. Bacterial isolates from the liver, kidney and spleen of diseased fish were collected prior to the start of antibiotic treatment and pathogens were identified via PCR by a commercial company (Acuipharma, Spain). PCR amplification showed that the etiological agents of infection were *Photobacterium damselae* ssp. *piscicida* and *Vibrio harveyi*. Fish were again sampled on the last day of antibiotic administration (September 6) and these samples were categorized as “treatment”. A final sampling point occurred on September 13 when fish were asymptomatic, corresponding to the “recovery” state.

Total DNA from 105 fish samples and 6 controls (extraction kit negative controls) was extracted using the PowerSoil DNA Isolation Kit (QIAGEN, Netherlands), following the manufacturer’s protocol. Extraction kit negative controls were pooled into one single sample. DNA extractions were shipped in dry ice to the University of Michigan Medical School (USA) for amplification and sequencing on a single run of the Illumina MiSeq platform according to the protocol of [29]. Each sample plus 4 PCR blanks and 4 identical mock communities (ZymoBIOMICS Microbial

Community DNA Standard) were amplified and sequenced for the V4 hyper-variable region of the 16S rRNA gene (~ 250 bp).

In total, 3,389,081 16S rRNA sequences were retrieved for the skin of the seabass fingerlings, and the number of sequences per sample ranged from 12,891 to 48,211. ASVs present in negative controls (extraction kit and PCR) were removed from downstream analysis. After removal of contaminants and non-bacterial sequences, 6,163 ASVs (3,300,989 sequences) were assigned to the skin microbiota of the seabass fingerlings. Diversity and bacterial abundances of the mock communities corresponded to those described by the manufacturer. Microbial taxa showing a mean relative proportion ≥ 1% were considered as part of the most abundant taxa in the microbiota.

Data and statistical analysis

Raw FASTQ files were denoised using the DADA2 pipeline in R [30]. We estimated a midpoint rooted tree of ASVs using the Quantitative Insights Into Microbial Ecology 2 package (QIIME2; release 2020.11). We constructed a table containing amplicon sequence variants (ASVs) and made taxonomic inferences against the SILVA (138 release) reference database [31]. We normalized ASV abundances using the negative binomial distribution, which accounts for library size differences and biological variability [32]. The core microbiota was assessed at the ASV level for each health state (i.e., sampling date) separately. An ASV was considered as part of the core microbiota if present in 100% of the samples from each state.

Microbial alpha-diversity was calculated using Shannon and Faith's phylogenetic (PD) diversity indices as implemented in the R phyloseq package [33]. Additionally, Pielou's evenness was calculated as implemented in the R microbiome package [34]. Microbial structure (beta-diversity) was estimated using phylogenetic UniFrac (weighted and unweighted) and Bray-Curtis distances. We assessed variation in microbial diversity and structure using Kruskal-Wallis and PERMANOVA [*adonis* function of the R vegan package, 35], respectively. Dissimilarity between samples was visually assessed through a principal coordinates analysis (PCoA) and dendrograms. A heatmap was built to depict changes in the abundance of the most abundant phyla and genera (≥1% of all reads). All analyses were performed in R-studio v1.2.500.

Predicted bacterial metabolic functions were estimated using the metagenomic Phylogenetic Investigation of Communities by Reconstruction of Unobserved States software (PICRUSt2) embedded in QIIME2 [36], applying a weighted nearest sequenced taxon index (NSTI) cutoff of 0.03. Predicted metagenomes were collapsed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway metadata [37]. We used linear discriminant analysis (LDA) in LEfSe to identify differentially abundant metabolic pathways in the skin microbiota of the seabass fingerlings using state as a class, a *P*-value cut-off of 0.05 and a LDA effect size cut-off of 2 [38].

Results

Microbial diversity and composition

Alpha-diversity estimates varied significantly between states ($P \leq 3^{-3}$, Table 1), with the exception of the PD index between treatment and recovery states, and the Pielou's evenness between healthy and recovery states (P = 0.2, Table 1). There was a significant increase in diversity and evenness from healthy to diseased states as well as from

diseased to treatment states (Figure 2). Between treatment and recovery states, a significant decrease in diversity and evenness occurred (Figure 2).

There were a total of 49 bacterial phyla and 926 bacterial genera identified across all samples. Of those, 6 phyla and 32 genera were found in high abundance in at least one of the health states (Figure 3, Online Source 1). The abundance of these taxa varied with the onset of disease and antibiotic treatment (Figure 3, Online Source 1). It is noteworthy that the abundance of *Photobacterium* (identified as *P. damselae*) in the skin remained stable between healthy and disease states, having decreased with antibiotic treatment and then again in recovery (Figure 3, Figure 4, Online Source 1). On the other hand, the abundance of *Vibrio* increased from healthy to the disease state, having decreased with antibiotic treatment and increased again in recovery (Figure 3, Figure 4, Online Source 1).

Of the 74 ASVs recovered from the core microbiota across the four states, 6 were present in the healthy state, 20 in the diseased state, 30 in the treatment state and 67 in the recovery state (Online Source 2). This corresponded to 0.4, 0.7, 1 and 4% of the total ASVs found in the healthy, diseased, treatment and recovery states, respectively. There were 2 unique core ASVs in the diseased and treatment states each, while 38 were unique to the recovery state (Online Source 2). Of the 2 unique core ASVs recovered from the microbiota of diseased fish, one was identified as *Photobacterium damselae* (Online Source 2). Additionally, there were 2 ASVs belonging to *Vibrio* that were part of the core microbiota during the diseased state, but not during the healthy or treatment states. In the recovery state, those two and four other *Vibrio* ASVs were part of the core microbiota of the skin.

Beta-diversity estimates show significant differences between states ($p \leq 6^{-3}$, Table 1), except for the UniFrac weighted distance between healthy and recovery states (P = 0.1, Table 1). On the other hand, differences between states showed relatively low dispersal (R < 0.3, Table 1). Analyses of the PCoA of Bray-Curtis distances showed that samples from the healthy, treatment and recovery states clustered separately, while no apparent structural cluster of samples between states was observed from the UniFrac distances (PCoA, Figure 2). Moreover, the dendrogram constructed from the UniFrac unweighted distance showed two main clusters separating healthy fish from the remainder samples. Within the latter cluster, treatment and recovery states were more closely related (Figure 2). Dendrograms constructed from the UniFrac unweighted and Bray-Curtis distances showed no structure between the health states (Online Source 3).

Microbial predicted function

There were a total of 478 predicted KEEG pathways in the skin microbiota of the seabass. Linear discriminant analysis of the metagenomic predictions performed in LEfSe showed that 3, 5 and 7 different pathways were significantly enriched in the healthy, treatment and recovery states, respectively (Figure 5). Interestingly, there were no significantly enriched pathways in the diseased state. On a broad level, all enriched pathways were related to either biosynthesis (67% in healthy, 57% in recovery) or to degradation/utilization/assimilation (33% in healthy, 100% in treatment, 43% in recovery) categories (Figure 5). Specifically, enriched metabolic pathways in the healthy state were related to amino acid degradation and fatty acid and lipid biosynthesis; in the treatment state enriched pathways were related to carbohydrate degradation and secondary metabolite degradation; and, finally, in the recovery state enriched pathways were related to carbohydrate degradation, vitamin biosynthesis, fatty acid biosynthesis, purine nucleotide biosynthesis and sugar derivative degradation (Figure 5).

Discussion

We characterized for the first time the effects of bacterial infection with *Photobacterium damselae* ssp. *piscicida* and *Vibrio harveyi* and treatment with flumequine in the skin microbiota of seabass fingerlings. Most of our predictions were confirmed with one important exception; contrary to our expectations and most of the previous literature, both core microbiota and microbial diversity increased with the onset of disease. However, dysbiosis was accompanied by an increase in the abundance of potential pathogenic and opportunistic taxa.

Disease effects in skin microbiota of seabass fingerlings: healthy vs *diseased states*

Impacts on microbial diversity, richness and evenness caused by infection by bacterial pathogens and parasites have been described in some fish [in the skin of rainbow trout infected with *Ichthyophthirius multifilis*, 14; gut of Asian seabass infected with *Tenacibaculum singaporense,* 16; skin of Atlantic salmon infected with *Lepeophtheirus salmonis,* 6; gut of grass carp with enteric infection, 12; gut of brown trout infected with *Tetracapsuloides bryosalmonae,* 15; skin of orbicular batfish infected with *Tenacibaculum maritimum,* 13; gut and stomach of rainbow trout infected with *Caligus lacustri,* 11; and skin of adult seabass infected with *Photobacterium damselae,* 7]. Dysbiosis was reported on the vast majority of these studies through decreases in fish microbial diversity and increases in pathobionts. Even though an increase in diversity was observed in the present study, the direction of the changes in the abundance of key microbial taxa indicates dysbiosis occurred in the skin microbiome of seabass fingerlings. In the present study, *Vibrio* (that encompasses one of the etiological agents of infection), and two other unidentified genera belonging to families with opportunistic taxa, Flavobacteriaceae and Vibrionaceae [39], increased their abundance in the diseased state. Another genus that increased in abundance in diseased fish was *Aureispira,* previously found to be highly abundant in the intestinal microbiota of grouper juveniles after iridovirus infections [40]. Similar increases in bacterial diversity after disease have been already described in other fish [e.g., 6, 12, 14, 15], indicating that changes in microbial diversity cannot be readily expected and growth or decline of specific taxa easily predicted.

Alterations to the core microbiota in response to infection were also previously reported in the skin of adult European seabass infected with *Photobacterium damselae* [7], and in the yellowtail kingfish infected with with enteritis [5]. In the present study, a *Photobacterium damselae* ASV was 100% prevalent in the diseased state. However, its mean abundance remained unaltered between the healthy and disease states, suggesting the skin was only indirectly affected by this pathogen. These results are in line with our previous study showing that infection caused by *P. damselae* can lead to dysbiosis of skin microbiota of farmed seabass despite no increase in abundance [7]. Similar results were also obtained by Legrand et al. [5], who described skin and gill dysbiosis in the yellowtail kingfish during enteritis, a gut disease.

Microbial structure of the skin microbiota was also significantly affected by disease. Although samples of diseased fish were collected on the day prior to disease onset, only a few samples from the diseased state (7 out of 30) clustered within the healthy group, confirming that significant taxonomic changes had occurred in most individuals analyzed. These results suggest that some properties of the skin mucous that allowed certain phylogenetically related taxa to thrive in the skin may have been altered by disease, consequently affecting resident microbiota. However, despite the increase in microbial diversity and changes in structure driven by disease, microbial metabolic functions remained unaltered. This suggests that the increase in diversity observed between healthy and diseased states was due to colonization by bacteria with similar functions [1].

Flumequine effects in skin microbiota of seabass fingerlings: diseased vs treatment states

In the present study, microbial diversity was observed to increase on the 8th day of treatment with flumequine. However, as expected, administration of flumequine resulted in a decrease in abundance of both etiological agents of disease in this study. This is unsurprising given the reported sensitivity of both species to this antibiotic [41]. Importantly, this treatment led to an increase of potentially harmful Flavobacteriaceae [39]. Interestingly, the genus *Alteromonas,* which has been shown to exhibit antibacterial activity against fish pathogens, including *Photobacterium damselae* and several *Vibrio* spp. [42], and resistance against amoxicillin, erythromycin and gentamicin [43], increased in abundance during antibiotic treatment. Microbial disruptions have been reported after oxytetracycline and rifampicin treatment in microbiota of adult fish [e.g., 7, 22–24], and after streptomycin, ciprofloxacin or oxytetracycline treatment on earlier life fish stages [e.g., zebrafish larvae, 25, 44]. Although an increase in fish microbial diversity caused by antibiotic administration is less common, it has been reported before [e.g., 7, 44]. Indeed, in the studies where diversity decreased after antibiotic exposure, there were no pre-existing health conditions. On the other hand, in this study as well as in Rosado et al. [7], disease had occurred, and in the study by López Nadal et al. [44], fish were immersed with the anti-nutritional compound saponin before antibiotic treatment. This suggests pre-existing disease/microbiota disruption and antibiotics may have a compound effect on microbial diversity.

Significant changes in the potential function of the skin microbiota were detected after antibiotic treatment. Specifically, the degradation of carbohydrates and secondary metabolites were significantly enriched during antibiotic treatment. However, the production of carbohydrates and secondary metabolites is linked to the protective role of the microbiota [e.g., 1, 45]. For example, carbohydrates are directly related to specific cell-cell adhesion, modulating microbial binding to the mucus [46]. It is suggested that carbohydrate synthesis by the human microbiota helps establish symbiosis with microbial commensals and aids pathogenic evasion [45]. Furthermore, production of secondary metabolites is one of the mechanisms by which commensal microbiota fight against pathogens [1]. These results suggest a microbial response to antibiotics, which may ultimately have a negative effect on fish immunity.

Recovery of the skin microbiota in seabass fingerlings: healthy vs recovery states

Previous studies reported that short-term recovery of the microbiota of fish after antibiotic treatment does not lead to the diversity levels observed in the healthy state (e.g., 1-week recovery, [24]). In the present study, with the exception of Pielou's evenness, diversity significantly increased between healthy and recovery states. Importantly, the abundance of *Vibrio* increased in the recovery state, indicating microbial balance may not have been fully obtained.

Microbial structure was also significantly different between the recovery and healthy states. However, closely related microbial structuring was found in fish from treatment and recovery states. Almost half of the enriched metabolic pathways during the recovery state were related to the same categories of pathways enriched during the treatment state (carbohydrate and secondary metabolite degradation), with significant differences from the healthy state. To the best of our knowledge, only the study by Brumlow et al. [47] has effectively measured the effects of 3-day antibiotic treatments (Tetracycline and Rifampicin) in the biochemical profile of the skin of *Gambusia affinis*. In this study the authors also report changes in community composition relative to pre-treatment, after 8 day recovery. However, unlike the present results, where significant changes to microbial function were predicted, the results of

Brumlow et al. [47] indicated that the biochemical functions of microbiota were mostly reestablished after the 8 day recovery. Flumequine is a highly persistent antibiotic and it can take several weeks to be fully depleted from the blood and tissues of fish [48, 49]. This antibiotic has a slower depletion rate in the skin than in muscle or liver, and can be present in the skin 20 days after oral administration [50]. Although a longer time frame would be necessary to evaluate whether full functional recovery of the skin microbiota does occur, our results highlight the high susceptibility of skin microbiota to antibiotic exposure.

Conclusions

Homeostasis of the microbial communities in the mucosal surfaces of fish is central to control pathogen abundances [2]. Here, we describe a dysbiosis episode caused by a disease outbreak induced by *Photobacterium damselae* ssp. *piscicida* and *Vibrio harveyi,* and subsequent antibiotic treatment with flumequine. Although, overall, antibiotic treatment appeared to have a greater impact on the skin microbiota when compared with the infection, this could be a result of a cumulative effect of both disease and antibiotic treatment. Moreover, the microbial profile of the fish in the recovery state differed from that of the fish in the healthy state, as well as their predicted metabolic function. The results from this study highlight that the response of fish commensal bacteria to disease and antibiotics are complex and not easily predicted.

Declarations

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Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material

The raw sequences are available at NCBI Sequence Read Archive (SRA) database within the BioProject ID XXXX.

Code availability

Not applicable.

Author's constributions

DR, RX and RS designed the research. RS collected the samples. DR performed laboratory work and analyzed the results. MP-L contributed to the statistical analysis. RX and MP-L supervised and provided intellectual content. All authors reviewed the manuscript. The author(s) read and approved the final manuscript.

Ethics approval

Animals in this study were reared in a commercial fish farm located in the estuarine environment of the Alvor Estuary (Portimão), southern Portugal. Fish were handled by the fish farm employees and samples were taken non-invasively. According to the Portuguese legislation DL Nº 113/2013 our work does not involve animal experimentation and therefore is exempted from the need of ethical approval.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Kelly C, Salinas I (2017) Under pressure: Interactions between commensal microbiota and the teleost immune system. Front. Immunol. 8:1
2. Trivedi B (2012) Microbiome: the surface brigade. Nature 492:S60–S61
3. Petersen C, Round JL (2014) Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol 16:1024–1033. https://doi.org/10.1111/cmi.12308
4. Osadchiy V, Martin CR, Mayer EA (2019) The Gut–Brain Axis and the Microbiome: Mechanisms and Clinical Implications. Clin. Gastroenterol. Hepatol. 17:322–332
5. Legrand TPRA, Catalano SR, Wos-Oxley ML, et al (2018) The Inner Workings of the Outer Surface: Skin and Gill Microbiota as Indicators of Changing Gut Health in Yellowtail Kingfish. Front Microbiol 8:2664. https://doi.org/10.3389/fmicb.2017.02664
6. Llewellyn MS, Leadbeater S, Garcia C, et al (2017) Parasitism perturbs the mucosal microbiome of Atlantic Salmon. Sci Rep 7:1–10. https://doi.org/10.1038/srep43465
7. Rosado D, Xavier R, Severino R, et al (2019) Effects of disease, antibiotic treatment and recovery trajectory on the microbiome of farmed seabass (Dicentrarchus labrax). Sci Rep 9:1–11. https://doi.org/10.1038/s41598-019-55314-4
8. Rosado D, Pérez-Losada M, Pereira A, et al (2021) Effects of aging on the skin and gill microbiota of farmed seabass and seabream. Anim Microbiome 3:. https://doi.org/10.1186/s42523-020-00072-2
9. Reid KM, Patel S, Robinson AJ, et al (2017) Salmonid alphavirus infection causes skin dysbiosis in Atlantic salmon (Salmo salar L.) post-smolts. PLoS One 12:e0172856. https://doi.org/10.1371/journal.pone.0172856
10. Borges N, Keller-Costa T, Sanches-Fernandes GMM, et al (2020) Bacteriome Structure, Function, and Probiotics in Fish Larviculture: The Good, the Bad, and the Gaps. Annu Rev Anim Biosci 9:423–452. https://doi.org/10.1146/annurev-animal-062920

11. Parshukov AN, Kashinskaya EN, Simonov EP, et al (2019) Variations of the intestinal gut microbiota of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), depending on the infection status of the fish. J Appl Microbiol 127:379–395. https://doi.org/10.1111/jam.14302
12. Tran NT, Zhang J, Xiong F, et al (2018) Altered gut microbiota associated with intestinal disease in grass carp (Ctenopharyngodon idellus). World J Microbiol Biotechnol 34:71. https://doi.org/10.1007/s11274-018-2447-2
13. Le Luyer J, Schull Q, Auffret P, et al (2021) Dual RNAseq Highlights the Kinetics of Skin Microbiome and Fish Host Responsiveness to Bacterial Infection. https://doi.org/10.21203/rs.3.rs-108248/v1
14. Zhang X, Ding L, Yu Y, et al (2018) The Change of Teleost Skin Commensal Microbiota Is Associated With Skin Mucosal Transcriptomic Responses During Parasitic Infection by Ichthyophthirius multifillis. Front Immunol 9:2972. https://doi.org/10.3389/fimmu.2018.02972
15. Vasemägi A, Visse M, Kisand V (2017) Effect of Environmental Factors and an Emerging Parasitic Disease on Gut Microbiome of Wild Salmonid Fish. mSphere 2:418–435. https://doi.org/10.1128/msphere.00418-17
16. Miyake S, Soh M, Nursyafiq Azman M, et al (2020) Insights into the microbiome of farmed Asian sea bass (Lates calcarifer) with symptoms of tenacibaculosis and description of Tenacibaculum singaporense sp. nov. Antonie Van Leeuwenhoek 113:737–752. https://doi.org/10.1007/s10482-020-01391-9
17. FAO (2016) FAO Fisheries & Aquaculture - Cultured Aquatic Species Information Programme - Dicentrarchus labrax (Linnaeus, 1758). http://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax/en. Accessed 12 Feb 2021
18. Deng Y, Zhang Y, Chen H, et al (2020) Gut–Liver Immune Response and Gut Microbiota Profiling Reveal the Pathogenic Mechanisms of Vibrio harveyi in Pearl Gentian Grouper (Epinephelus lanceolatus ♂ × E. fuscoguttatus ♀). Front Immunol 11:1. https://doi.org/10.3389/fimmu.2020.607754
19. Essam HM, Abdellrazeq GS, Tayel SI, et al (2016) Pathogenesis of Photobacterium damselae subspecies infections in sea bass and sea bream. Microb Pathog 99:41–50. https://doi.org/10.1016/j.micpath.2016.08.003
20. Mohamad N, Amal MNA, Yasin ISM, et al (2019) Vibriosis in cultured marine fishes: a review. Aquaculture 512:734289. https://doi.org/10.1016/j.aquaculture.2019.734289
21. Bakopoulos V, Volpatti D, Gusmani L, et al (2003) Vaccination trials of sea bass, Dicentrarchus labrax (L.), against Photobacterium damsela subsp. piscicida, using novel vaccine mixtures. J Fish Dis 26:77–90. https://doi.org/10.1046/j.1365-2761.2003.00438.x
22. Almeida AR, Alves M, Domingues I, Henriques I (2019) The impact of antibiotic exposure in water and zebrafish gut microbiomes: A 16S rRNA gene-based metagenomic analysis. Ecotoxicol Environ Saf 186:109771. https://doi.org/10.1016/j.ecoenv.2019.109771
23. Almeida AR, Tacão M, Machado AL, et al (2019) Long-term effects of oxytetracycline exposure in zebrafish: A multi-level perspective. Chemosphere 222:333–344. https://doi.org/10.1016/j.chemosphere.2019.01.147
24. Carlson JM, Leonard AB, Hyde ER, et al (2017) Microbiome disruption and recovery in the fish Gambusia affinis following exposure to broad-spectrum antibiotic. Infect Drug Resist 10:143–154. https://doi.org/10.2147/IDR.S129055
25. Pindling S, Azulai D, Zheng B, et al (2018) Dysbiosis and early mortality in zebrafish larvae exposed to subclinical concentrations of streptomycin. FEMS Microbiol Lett 365:188. https://doi.org/10.1093/femsle/fny188

26. De Liguoro M, Maraj S, Merlanti R (2019) Transgenerational toxicity of flumequine over four generations of Daphnia magna. Ecotoxicol Environ Saf 169:814–821. https://doi.org/10.1016/j.ecoenv.2018.11.077
27. FAO (1997) Residues of some veterinary drugs in animals and foods
28. Marchesi JR, Ravel J (2015) The vocabulary of microbiome research: a proposal. Microbiome 3:31. https://doi.org/10.1186/s40168-015-0094-5
29. Kozich JJ, Westcott SL, Baxter NT, et al (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Appl Environ Microbiol 79:5112–5120. https://doi.org/10.1128/AEM.01043-13
30. Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869
31. Quast C, Pruesse E, Yilmaz P, et al (2013) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219
32. McMurdie PJ, Holmes S (2014) Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Comput Biol 10:e1003531. https://doi.org/10.1371/journal.pcbi.1003531
33. McMurdie PJ, Holmes S (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8:e61217. https://doi.org/10.1371/journal.pone.0061217
34. Lahti L, Shetty S (2018) Introduction to the microbiome R package
35. Oksanen J, Kindt R, Legendre P, et al (2008) The vegan package: community ecology package, version 1.13-1
36. Douglas GM, Maffei VJ, Zaneveld J, et al (2019) PICRUSt2: An improved and extensible approach for metagenome inference. bioRxiv 672295
37. Kanehisa M, Sato Y, Furumichi M, et al (2019) New approach for understanding genome variations in KEGG. Nucleic Acids Res 47:D590–D595. https://doi.org/10.1093/nar/gky962
38. Segata N, Izard J, Waldron L, et al (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:R60. https://doi.org/10.1186/gb-2011-12-6-r60
39. Austin B, Austin DA (2016) Bacterial fish pathogens, 6th ed. Springer, Stirling, UK
40. Joe JTX, Tseng YC, Wu JL, Lu MW (2021) The alteration of intestinal microbiota profile and immune response in epinephelus coioides during pathogen infection. Life 11:1–18. https://doi.org/10.3390/life11020099
41. Laganà P, Caruso G, Minutoli E, et al (2011) Susceptibility to antibiotics of Vibrio spp. and Photobacterium damsela ssp. piscicida strains isolated from Italian aquaculture farms. New Microbiol 34:53–63
42. Stevens J, Jackson R, Olson J (2016) Bacteria associated with lionfish (Pterois volitans/miles complex) exhibit antibacterial activity against known fish pathogens. Mar Ecol Prog Ser 558:167–180. https://doi.org/10.3354/meps11789
43. Wu J, Mao C, Deng Y, et al (2019) Diversity and abundance of antibiotic resistance of bacteria during the seedling period in marine fish cage-culture areas of Hainan, China. Mar Pollut Bull 141:343–349. https://doi.org/10.1016/j.marpolbul.2019.02.069
44. López Nadal A, Peggs D, Wiegertjes GF, Brugman S (2018) Exposure to Antibiotics Affects Saponin Immersion-Induced Immune Stimulation and Shift in Microbial Composition in Zebrafish Larvae. Front Microbiol 9:2588. https://doi.org/10.3389/fmicb.2018.02588
45. Hooper L V., Gordon JI (2001) Glycans as legislators of host-microbial interactions: spanning the spectrum from symbiosis to pathogenicity. Glycobiology 11:1R-10R. https://doi.org/10.1093/glycob/11.2.1R

46. Brandley BK, Schnaar RL (1986) Cell-Surface Carbohydrates in Cell Recognition and Response. J Leukoc Biol 40:97–111. https://doi.org/10.1002/jlb.40.1.97

47. Brumlow CE, Luna RA, Hollister EB, et al (2019) Biochemical but not compositional recovery of skin mucosal microbiome communities after disruption. Infect Drug Resist 12:399–416. https://doi.org/10.2147/IDR.S185992

48. Hansen M, Ingebrigtsen K, Hayton W, Horsberg T (2001) Disposition of 14C-flumequine in eel Anguilla anguilla, turbot Scophthalmus maximus and halibut Hippoglossus hippoglossus after oral and intravenous administration. Dis Aquat Organ 47:183–191. https://doi.org/10.3354/dao047183

49. Sohlberg S, Ingebrigtsen K, Hansen M, et al (2002) Flumequine in Atlantic salmon Salmo salar: disposition in fish held in sea water versus fresh water. Dis Aquat Organ 49:39–44. https://doi.org/10.3354/dao049039

50. Malvisi J, Della Rocca G, Anfossi P, Giorgetti G (1997) Tissue distribution and depletion of flumequine after in-feed administration in sea bream (Sparus aurata). Aquaculture 157:197–204. https://doi.org/10.1016/S0044-8486(97)00160-9

Table

Table 1. Microbial diversity comparisons for the skin of the seabass *Dicentrarchus labrax* across all samples and between the four different states. For each Kruskall-Walis test (alpha-diversity) we report the chi-squared value (overall) and significance (P value, overall and pairwise) and for each PERMANOVA test (beta-diversity) we report the R2 statistics (overall) and significance (P value, overall and pairwise). Significant associations are indicated in bold.

Metric	Overall	Healthy/Diseased	Diseased/Treatment	Treatment/Recovery	Healthy/Recovery
Shannon	**66 (3^{-14})**	**4^{-7}**	**2^{-6}**	**1^{-6}**	**4^{-7}**
PD	**55 (9^{-12})**	**6^{-7}**	**3^{-3}**	0.2	**3^{-9}**
Pielou's	**53 (2^{-11})**	**3^{-3}**	**8^{-7}**	**1^{-7}**	0.2
UniFrac weighted	**0.3 (9^{-5})**	**7^{-2}**	**6^{-3}**	**6^{-3}**	0.1
UniFrac unweighted	**0.1 (9^{-5})**	**6^{-3}**	**6^{-3}**	**6^{-3}**	**6^{-3}**
Bray-Curtis	**0.3 (9^{-5})**	**2^{-3}**	**1^{-3}**	**1^{-3}**	**1^{-3}**

Figures

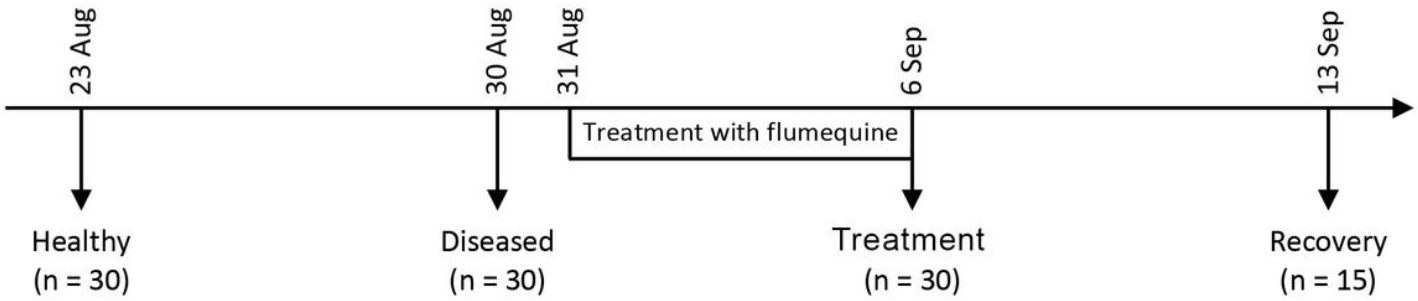

Figure 1

Sampling Scheme

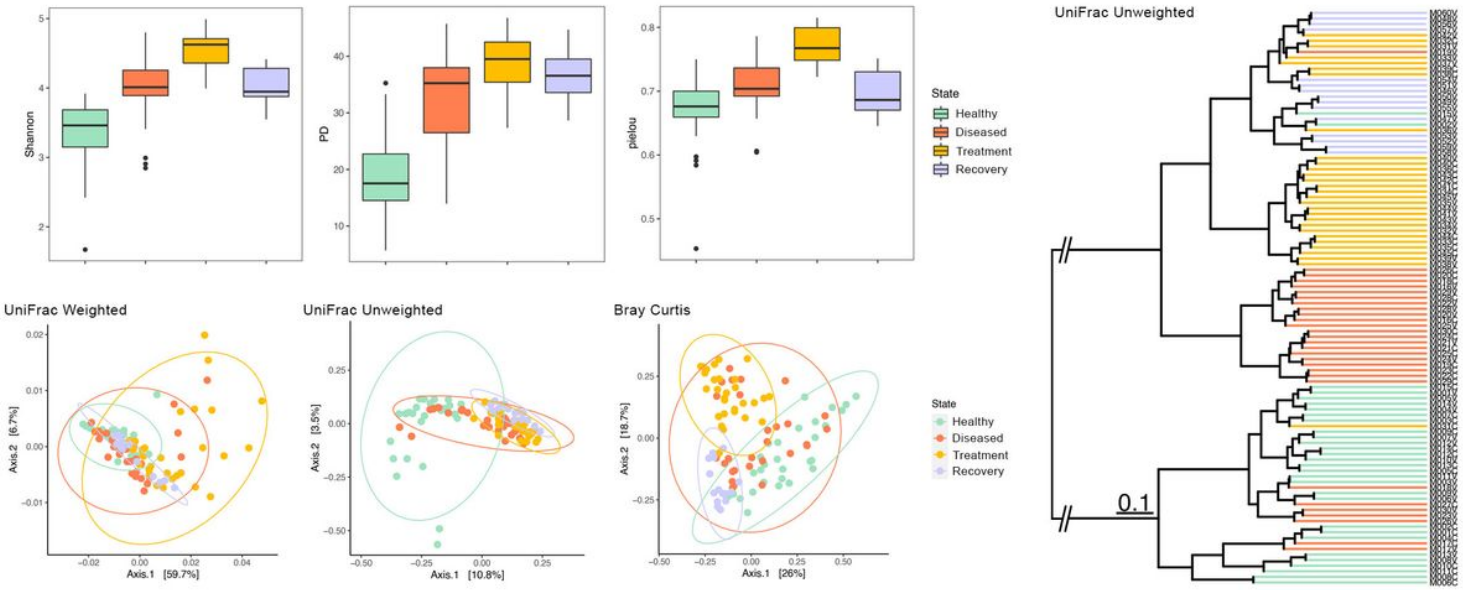

Figure 2

Diversity Graphs

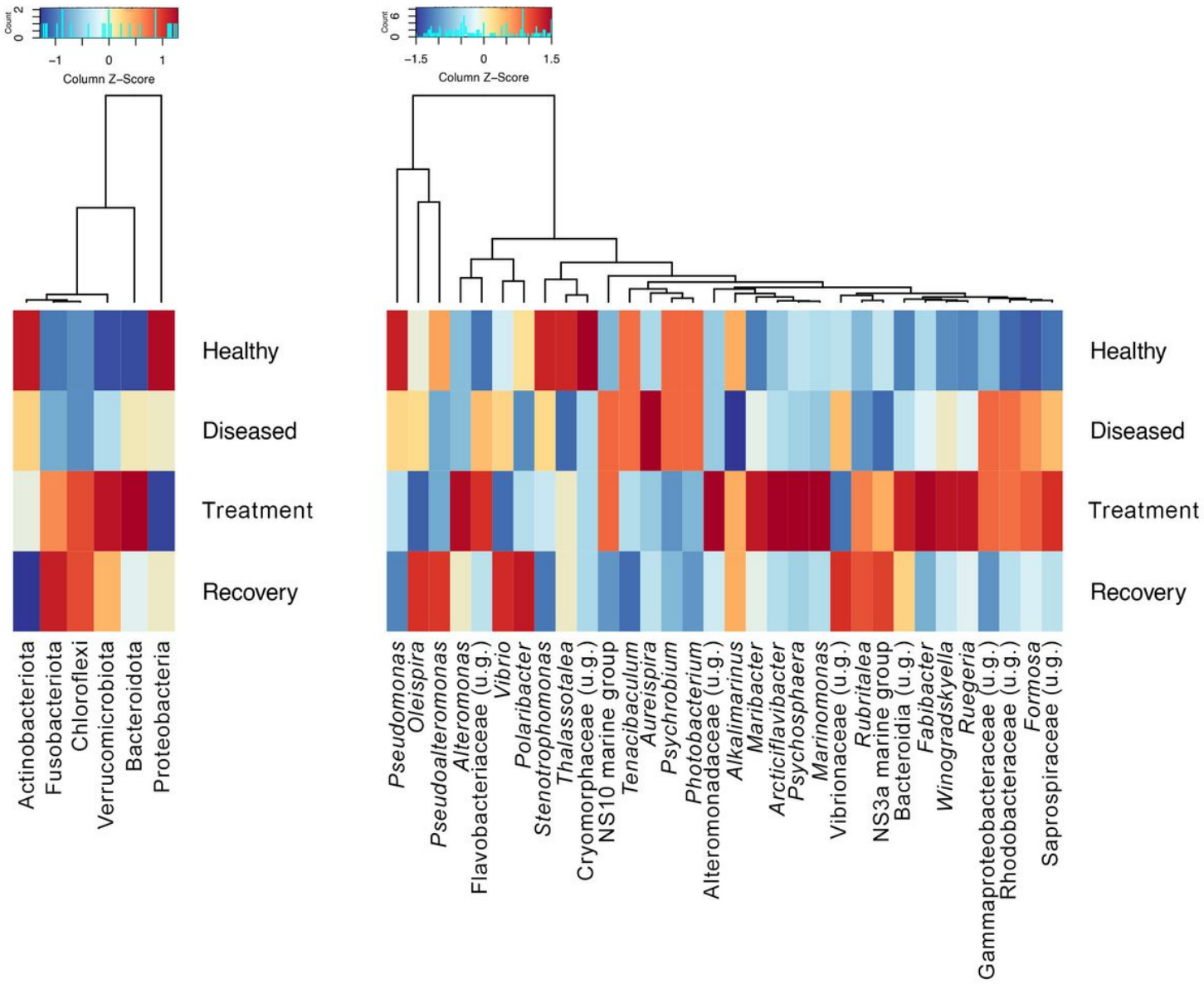

Figure 3

Heat Maps

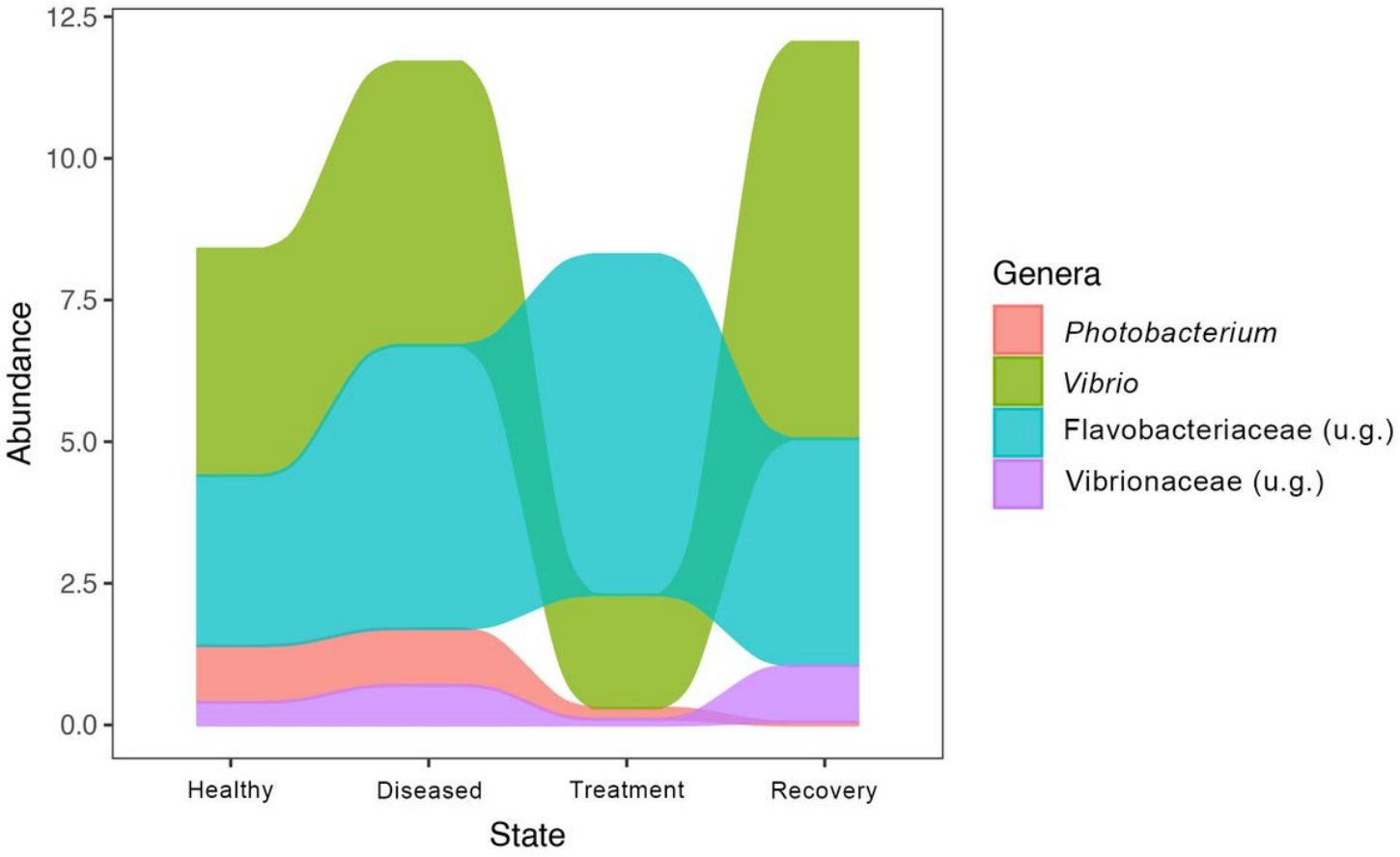

Figure 4

Alluvial Key Taxa

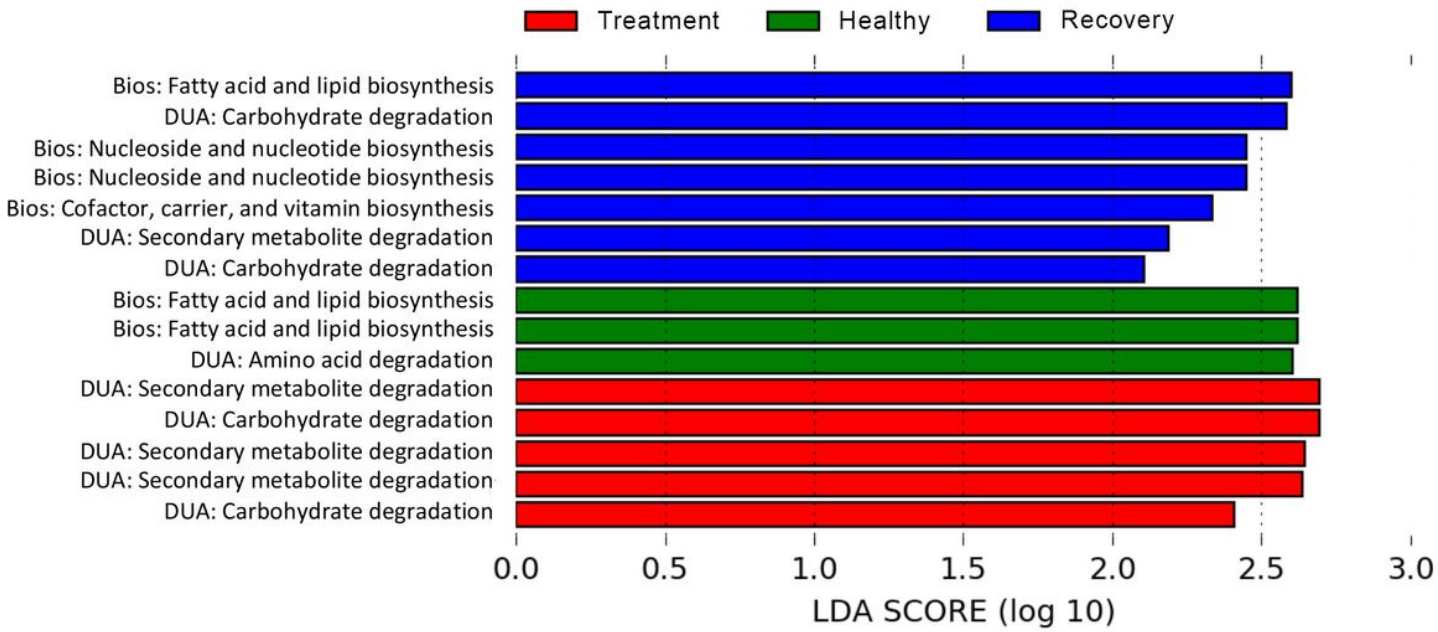

Figure 5

Enriched Pathways

Supplementary Files

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