Peer Community In Microbiology

Getting closer to the host-microbe evolutionary relationship

Konstantinos Kormas *based on peer reviews by* ***Laetitia Wilkins***, ***Marco Basili*** *and 1 anonymous reviewer*

Charlotte Evangelista, Stefaniya Kamenova, Beatriz Diaz Pauli, Joakim Sandkjenn, Leif Asbjørn Vøllestad, Eric Edeline, Pål Trosvik, Eric Jacques de Muinck (2023) Within-species variation in the gut microbiome of medaka (*Oryzias latipes*) is driven by the interaction of light intensity and genetic background. bioRxiv, ver. 2, peer-reviewed and recommended by Peer Community in Microbiology. https://doi.org/10.1101/2023.02.17.528956

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The issue of whether there is a clear and detectable relationship -either deterministic or stochastic- of fish gut microbiota with evolutionary processes is far from being resolved. Studies on fish microbiota are more perplexed as this animal group includes species both from wild and farmed populations (for food production, ornamental fish and animal models), with variable life cycles and ecophysiologies, and all these features expand the type of interactions to be studied. Based on this biological features variability, multiple methodological limitations, especially for the species with wild populations, are perhaps among of the central reasons for this knowledge gap. Therefore, experimental approaches, which can eliminate some of this variability, seem to be the best approach.

The preprint by Evangelista et al. (2023) entitled "Within-species variation in the gut microbiome of medaka (*Oryzias latipes*) is driven by the interaction of light intensity and genetic background" is an example of such a targeted study with a freshwater fish species. Due to the paper's finely detailed experimental design, the interdisciplinary skills of the participating co-authors and exhaustive data analysis, this paper manages to draw solid and reproducible results and conclusions. This renders it not only an insightful contribution towards the more general host-microbe interactions in an evolutionary framework, but also a perfect example on how current and future relevant research should be conducted. I feel confident that this paper will assist other scientits of the field to move forward with their current working hypotheses but also to generate novel ones.

Reference :

Evangelista C, Kamenova S, Diaz Pauli B, Sandkjenn J, Vollestad A, Edeline E, Trosvik P, de Muinck E (2023) Within-species variation in the gut microbiome of medaka (*Oryzias latipes*) is driven by the interaction of light

intensity and genetic background. bioRxiv, 2023.02.17.528956, ver. 2 peer-reviewed and recommended by Peer Community in Microbiology. https://doi.org/10.1101/2023.02.17.528956

Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.02.17.528956
Version of the preprint: 1

Authors' reply, 16 August 2023

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Decision by Konstantinos Kormas, posted 25 May 2023, validated 26 May 2023

A very well performed research on fish microbiomes on a rather understudied subject

This study investigated the gut microbiota of two medaka genetic lines to investigate wehther fishing pressure and/or environmental affect the development of the gut microbiota. Significant differences in the gut microbiota profiles were found between the two lines, and this effect seems to have been mediated by light intensity, but the host's fitness seemed unrelated to these microbiota changes. This part, host fitness in relation to the gut microbiota, could be explored in greater depth by the authors, with more targeted data analysis (no need for extra data or reanalysis of their raw sequencing data). Similar studies are not so common in the field, especially ones that paid extra attention to its experimental design and maticulate data analysis like this one. There are also several comments and suggestions provided by the three reviewers, which I also find accurate and righfully spotted, that could considerable increase the quality and the reading flow of the paper. Overall, I find this paper an important contribution in the field of fish-microbe interactions and co-evolutionary mechanisms which I believe it will be fully appreaciated by the scientists in the microbiome field when published.

Reviewed by anonymous reviewer 1, 11 April 2023

The authors of this study investigated the gut microbiota of two lines of medaka to explore possible fishing pressure and/or environmental effects on the development of the gut microbiota. They detected significant differences in the gut microbiome composition and richness between the two lines, and this effect was mediated by light intensity (represented nutrient availability). However, fitness was not correlated to changes in the gut microbiota.

Understanding the relationship between environmental conditions and fish gut microbiota diversity could reveal important mechanisms influencing the adaptability and resilience of wild fisheries stocks, thus leading to improved management strategies and more sustainable fish stocks.

With that being said, the approach used in this study (e.g., Illumina MiSeq short-amplicon sequencing) is adequate for revealing temporal changes at higher taxonomic levels. However, it lacks the resolution required for species identification, but more importantly, functional services offered by the gut microbiota.

This comment has nothing against the methods used; it just prevents understanding how changes within the community structure impact provided functional services, which could impact fitness in a particular life stage. The authors, however, do point this out in their discussion.

Overall, the paper is well-written and nicely structured. There are only a few discussion points:

Introduction

1) In addition to diet composition and water quality, what about links to deterministic or stochastic differences that might arise between different lines? This has at least been reported in other fish species, such as S. salar.
2) Lines 100-104 – move to methods
3) Lines 105-108 – turn this information into a paragraph, linking it to other studies

Methods

Line 139-142 – starting with the ”On average … ” sentence, move to results

Line 144-148 – move to results

Line 195 – more information about how the tools were sterilized (e.g., 10% was used, followed by a sterile water wash).

Line 227-230 – The final dataset ….. move to results

Results

Line 291…if you change the Additionally, to However, it will be easier for the reader to understand a comparison switch.

Discussion

Line 326: Would this prediction become true given more time or across different life stages?

Line 332-333: Aeromonas is a decent size genus, so it would be nice if you also mentioned beneficial species and expanded on the pathogenic ones.

Line 335-336: LB medaka produced more offspring and grew faster – isn't this correlated to fitness? Fitness is about success at surviving and reproducing, so it appears that LB could have higher fitness.

Line 384: do you mean phenotypic plasticity or genomic plasticity?

Reviewed by Marco Basili, 06 May 2023

The manuscript describes the experimental analysis performed in the gut microbiome of two different lineages of medaka, selected based on the size. The authors test the variation that occurred in microbiomes in different environmental conditions.

The topic analyzed is certainly of great interest, even if the species used is not among the most commonly studied in the bibliography. In terms of expository clarity, the title, abstract, introduction, and final discussion appear sufficient to satisfy the journal parameters.

The authors fail to adequately describe the results obtained: the description of beta diversity is very hasty and lacks the part concerning the different densities of organisms tested. This result, although not significant, should be described in more detail, also associating it with the results obtained from the different exposure to light. The host fitness part should be well explored in term of values and correlation with other factors.

One of the main questions that occur reading the manuscript is in relation to the size of the population, wherein the methods have described the mean and SD of the two different lineages, showing the significative differences. Not having certainty about the actual differences of genetic background, I would suggest to the authors to show more details on the correlations between size and alpha diversity (also in terms of microbial composition of each individual), or even how size is distributed in relation to beta diversity.

line 272: Desulfovibrionaceae were significantly more abundant in the gut of LB than SB medaka, while in the plot is oriented in the SB direction (even if is colored in orange).

Figure 2c: In the barplot, the family labeled as ”Family II” and ”Family XI” should be better characterized with the name of the higher taxa level.

line 700: Figure S4 caption, change the letter ”d” with ”c”

Reviewed by Laetitia Wilkins, 21 May 2023

GENERAL

The manuscript entitled “Within-species variation in the gut microbiome of fish is driven by the interaction of light intensity and genetic background” written by C. Evangelista *et al*. is very well written. It is relatively short, concise, and easy to understand. Applying a very sophisticated mesocosm experiment, the authors tried to quantify the effects of evolution and environment on medaka gut microbiome composition. Said microbial composition was characterized using 16S amplicon sequencing of the V3-V4 region. Evolution was defined by creating two breeding lines where fish were selected for size during 10 generations. Environmental effects were experimentally varied by keeping fish at different abundance (low and high) and then keeping each of those groups at either low or normal light conditions. All in all, a very well-designed set-up that must have taken several months if not years to be developed and carried out.

TITLE/ABSTRACT/INTRODUCTION

The title reflects the content of the article. The word “fish” should be replaced by “medaka” because the results of this current study cannot be generalized for all fishes.

The abstract presents the supported findings of the study. The first sentence of the abstract is too general in my viewpoint and could simply be removed. It ignores the large body of literature investigating the consequences of host genetic background and environmental conditions on gut microbiome composition.

Line 45: is a speculation. Functional importance of the gut microbiome was not investigated in the present study.

Key words: “Medaka” could be added, “mesocosm”, “light”, and “fish density”

Line 84: It would be worth considering reading and including the following literature on human-induced selection on fish sizes in the introduction:

https://doi.org/10.1016/j.tree.2016.04.001

https://www.pnas.org/doi/abs/10.1073/pnas.0809235106

https://doi.org/10.1146/annurev-ecolsys-112414-054339

The introduction is not well connected with the discussion section. I only fully understood the rationale of this manuscript after reading the discussion section.

Lines 109-111: the experimental design is introduced pretty late and does not connect well to the first parts of the introduction.

- Size selected lineages: human impact through fishing is clear
- Why light? Unclear from introduction.
- Why also population density?

Is this microbiome manuscript maybe a side-project of a bigger project where it is laid out more clearly what the motivations behind the experiment were?

Renneville *et al*. 2020 seems to be the main study.

Please build some components into the current introduction where you introduce why you would expect light and density to affect the fish gut microbiome composition.

When reading the methods section, it becomes clear that this study is its own experiment. This was not clear from the introduction.

MATERIALS AND METHODS

The methods and analysis are described in sufficient detail to allow replication by other researchers. I listed a few places where I would need more detail:

Line 125: how many tank replicates?

Line 131: how many individuals were removed and how often?

Line 146: how were families (sib-families == genotypes) kept separate? How were tank effects separated from family effects? Were fish genotyped?

Line 230: standardization of samples for sequencing depth, please give more details.

Line 234: not fully clear how family was defined.

–> Analyses should be run by tank. Within tank replication is most probably pseudo-replication. It is not clear to me whether host families were treated individually in the statistical analyses (= pseudoreplication) or whether tanks were the units of replication.

Line 264: Why were no UniFrac distances calculated? Ideally, UniFrac distances are calculated among groups which take into account the phylogenetic relationships of the bacterial taxa in the microbiome. Weighted UniFrac takes into account the relative abundance of species/taxa shared among groups, whereas unweighted UniFrac only considers presence/absence. The latter counts the fraction of branch lengths unique to either community. Your analysis is most closely related to using weighted UniFrac. It is useful for examining differences in community structure. However, it would also be valuable to know what difference low-abundance features make in a community. Your sophisticated experimental design might have the power to also detect subtle differences in less abundant taxa.

I evaluated the R script.

With regard to the rarefaction analysis in the R script, I wonder whether you sequenced deeply enough to capture most of the microbiome composition (code is in the R script)? Why not reporting this in the main manuscript?

RESULTS

Line 267 and onwards: Relative abundance!! No absolute quantification was performed. Please be very careful in the wording of your text. Features in your dataset can only be compared within the dataset as you did not perform any sort of absolute quantification. Abundance is relative to your dataset.

Table 1: It looks like there is a significant interaction effect between line x light intensity. An interaction effect occurs when the effect of one independent variable on the dependent variable changes across different levels of another independent variable. In other words, the impact of one factor depends on the level of another factor. When there is a significant interaction effect, it indicates that the combined influence of the factors is not adequately captured by the main effects alone. To assess the significance of the individual factors in the presence of an interaction effect, you should conduct follow-up analyses or post-hoc tests. These tests allow you to examine the effects of each factor while controlling for the levels of the other factor. I think the sophisticated experimental design of your study allows you to investigate further and look for pairwise comparisons or simple effects analyses.

Line 274: what about the less abundant bacteria? What bacterial taxa were specific to treatment groups? Please see my comment further up in the methods about using unweighted UniFrac distances.

Negative controls: Did you include any negative controls in your study? Please include them in the manuscript. This could include: sequencing of PCR water, sequencing blank extractions, or sequencing the water of your mesocosm tanks. Typical contaminants from extraction kits can be highly abundant (*e.g.*, Microbacteriaceae). Fish guts are a typical low bacterial biomass niche and this needs to be incorporated in the analysis. Guidelines can be found here: https://journals.asm.org/doi/10.1128/mSystems.00290-19

DISCUSSION

Overall, the discussion is very well written. It answered several questions I had after reading the introduction section. The conclusions are adequately supported by the results. After reading the results section, I came up with several questions and hypotheses. These were all addressed in the discussion section. For example:

Renneville *et al*. 2020 showed that the two lines differ in foraging strategies. Could the difference seen here in microbiome composition represent their preferred diet, which of course is also affected by light conditions? Where is the evidence that differences are caused by host genetics? What mechanisms? Lines 338 – 369 nicely

cover these points.

The discussion section (as well as the introduction) did not take into account a few references from relevant recent and past research performed in the field. I suggest including and discussing the following references:

Sullam KE, Essinger SD, Lozupone CA et al. (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Molecular Ecology, 21, 3363–3378.

Sullam KE, Rubin BER, Dalton CM et al. (2015) Divergence across diet, time and populations rules out parallel evolution in the gut microbiomes of Trinidadian guppies. ISME Journal, 9, 1508–1522.

Sevellec M, Pavey SA, Boutin S et al. (2014) Microbiome investigation in the ecological speciation context of lake whitefish (*Coregonus clupeaformis*) using next-generation sequencing. Journal of Evolutionary Biology, 27, 1029–1046.

Ghanbari M, Kneifel W, Domig KJ (2015) A new view of the fish gut microbiome: advances from next-generation sequencing. Aquaculture, 448, 464–475.

Boutin S, Sauvage C, Bernatchez L, Audet C, Derome N (2014) Inter individual variations of the fish skin microbiota: host genetics basis of mutualism? PLoS ONE, 9, 1–17.

Bolnick DI, Snowberg LK, Caporaso JG et al. (2014b) Major histocompatibility complex class IIb polymorphism influences gut microbiota composition and diversity. Molecular Ecology, 23, 4831–4845.

Wilkins LGE, Fumagalli L, and Wedekind C (2016) Effects of host genetics and environment on egg-associated microbiota in brown trout (Salmo trutta). Molecular Ecology 25(19): 388-394.

Overall, beyond the family level, I am curious which core bacteria and functional pathways could be affected by the genetic lines used in this study and their interaction with light and fish density? Since a lot of work has gone into designing and performing this elaborate experiment, it would be nice to go a bit more into the detail and look at the more rare taxa at greater resolution than the family level of bacteria.

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