Shared environment overcomes host genetic differences in shaping the gut microbiota of distinct fish strains within two species of ictalurid catfish

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Background

Channel catfish (*Ictalurus punctatus*) represent the greatest market share in U.S. aquaculture production, both in terms of total economic value and numbers produced. The blue catfish *I. furcatus* is also of great interest to U.S. aquaculture, because interspecific crosses (*I. punctatus x I. furcatus*) produce offspring with hybrid vigor and desirable phenotypes such as increased disease resistance and fillet yield. Ictalurid catfish have received much breeding attention, and multiple aquaculture strains with unique performance traits exist within both species, yet little is known regarding their gut microbiota.

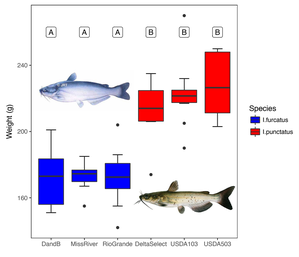
Objectives:

1. Compare the gut microbiota of blue catfish and channel catfish (three distinct strains within each) to determine if gut microbiota may be involved in differences seen in growth performance and disease resistance between the species.
2. Determine if differences in the composition of gut microbiota are correlated with genetic differences among the host fish strains.

Methods

- Nested design:
  - 2 species, 3 strains each
  - *I. punctatus* – USDA103, USDA503, and Delta Select
  - *I. furcatus* – Mississippi River, Rio Grande, and D & B
- $n = 10$ / strain; $n = 30$ / species

Fig 1. Fish weights on the day of sampling.



- Eggs collected from ponds at USDA-WARU, then reared indoors in flow-through system to 193 dph
- Entire intestinal tract removed and homogenized for DNA isolation
- 16S V4 rRNA gene sequencing

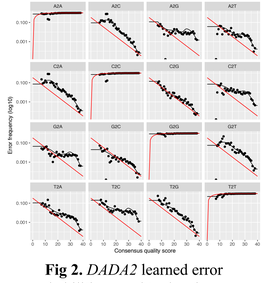


Fig 2. *DADA2* learned error probabilities, used to denoise raw Illumina MiSeq sequencing data.

- *DADA2* used to infer "ribosomal sequence variants" (RSVs)
- *phyloseq* used to filter data and analyze alpha- and beta-diversity
- *DESeq2* used to test for differentially abundant RSVs

- Host genetic data gathered from separate individuals ($n = 20$) in each strain using 22 microsatellite loci [(1)]
- Genepop 4.5.1 used to access allele frequencies and HW-tests
- Analysis of Molecular Variation (AMOVA) and estimates of genetic distances (Fst and Rst) determined with GenAlEx 6.5
- Mantel test used to test for correlation between gut microbiota beta diversity distances and host genetic distances

Results

Differences in fish growth followed expectations, with the blue catfish showing significantly slower growth than channel catfish (ANOVA; Fig. 1). No significant differences (Strain / Species; $p \leq 0.05$) were detected in the gut microbiota alpha- (ANOVA; Obs., Chao1, ACE, and Shannon; Fig. 3) or beta-diversity metrics (perMANOVA; weighted and unweighted UniFrac, and Bray-Curtis dissimilarity; Fig. 6). *Cetobacterium somerae* was the dominant microbe in all strains, with the exception of Delta Select in which *Clostridium* was slightly more abundant (Fig 4). Forty differentially abundant sequence variants were dectected, with *Vibrio* being the only genus enriched in *I. furcatus* while many potential pathogens were enriched in *I. punctatus* (Fig 5). Estimated host genetic distance (Rst) showed clear separation among fish species, and blue catfish showed greater intraspecies distance among strains. A Mantel test of gut microbiota distances and host genetic distances, among catfish strains, showed no significant correlation (Fig. 9).

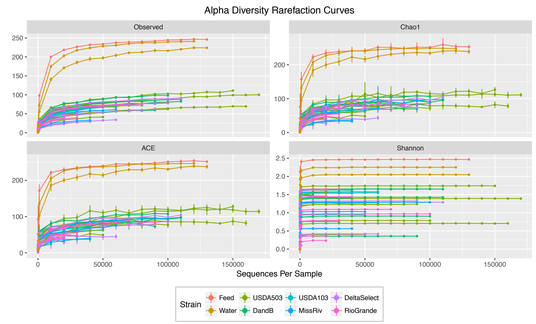


Fig 3. Rarefaction analysis of alpha diversity metrics (richness and diversity) of the gut microbiota of strains of channel and blue catfish, as well as the environmental microbiota (feed and water). No statistical differences were detected in alpha diversity (ANOVA, $p \leq 0.05$; strain nested within species).

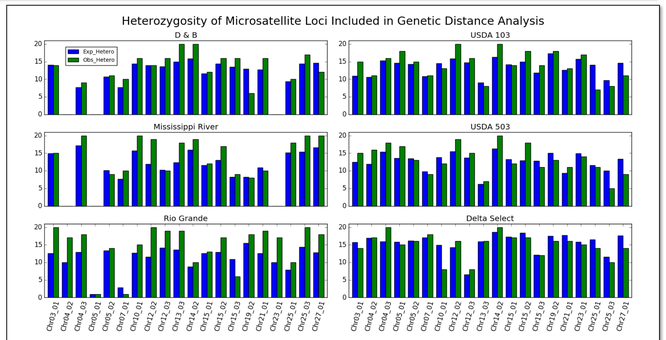


Figure 7. Expected and observed heterozygosity by strain, at the 20 microsatellite loci included in analyses of host genetic distance. Populations of the left are strains of *I. furcatus*, while populations listed on the right are strains of *I. punctatus*.

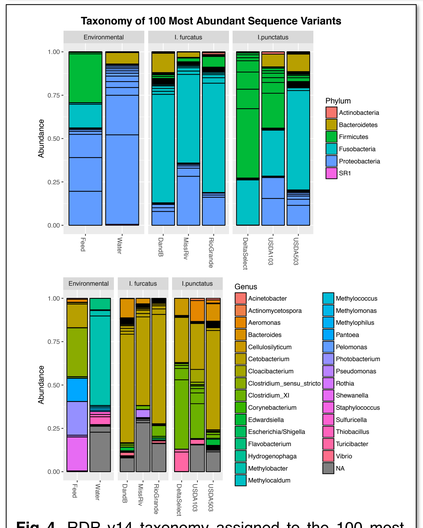


Fig 4. RDP v14 taxonomy assigned to the 100 most abundant 16S rRNA V4 ribosomal sequence variants by fish strain. Cyanobacteria/Chloroplast removed from analyses.

	Delta Select	USDA503	USDA103	D & B	Miss River	Rio Grande
DeltaSelect		0.41	0.37	0.45	0.51	0.41
USDA503	0.51		0.45	0.49	0.53	0.48
USDA103	0.26	0.32		0.49	0.50	0.43
DandB	0.67	0.26	0.51		0.59	0.52
MissRiv	0.50	0.22	0.37	0.42		0.50
RioGrande	0.63	0.34	0.51	0.19	0.45	

unweighted UniFrac

weighted UniFrac

Table 1. Pairwise beta diversity distances of gut microbiota. Top half of matrix shows unweighted UniFrac distances, while the lower half represents weighted UniFrac distances. No significant differences in beta-diversity were detected using either UniFrac index, or Bray-Curtis dissimilarity (perMANOVA (adonis) $p \leq 0.05$; strain nested within species). Data rarefied at 14,895 RSVs.

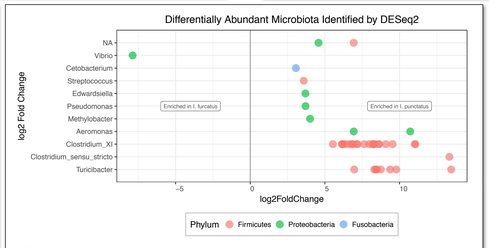


Fig 5. Differentially abundant (DA) sequence variants detected between *I. punctatus* and *I. furcatus* by DESeq2. A parametric-fit was used to estimate dispersion of RSV count data, before testing for DA microbiota using a negative binomial distribution ($q \leq 0.05$). Non-functional taxa and samples with less than 14,000 seqs were removed prior to analysis.

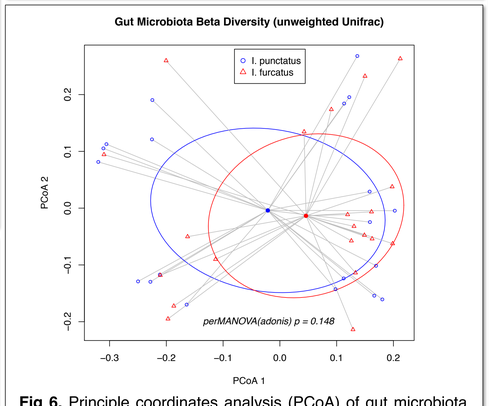


Fig 6. Principle coordinates analysis (PCoA) of gut microbiota beta diversity between the two species of ictaulrid catfish, based on pairwise unweighted UniFrac distances. Individual samples are plotted as hollow points, group centroids are solid filled circles, and ellipses represent 1 standard deviation of dispersion from the centroid. Data rarefied at 14,895 RSV.

	Delta Select	USDA503	USDA103	D & B	Miss River	Rio Grande
DeltaSelect		0.16	0.14	0.31	0.31	0.30
USDA503	0.27		0.00	0.39	0.40	0.39
USDA103	0.25	0.02		0.37	0.38	0.37
DandB	0.65	0.67	0.63		0.18	0.16
MissRiv	0.66	0.71	0.65	0.30		0.23
RioGrande	0.65	0.66	0.63	0.16	0.37	

Fst

Rst

Table 2. Pairwise host genetic distance. Top half of matrix shows estimated Fst distances, while the lower half shows estimated Rst distances.

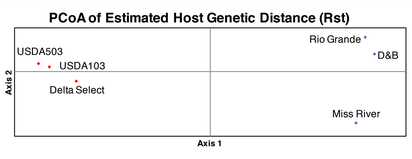


Figure 8. PCoA of estimated pairwise host genetic distances (Rst). Strains within *I. punctatus* (red) are more similar to one another than strains within *I. furcatus* (blue), and distance among species is much greater than distances among strains within species.

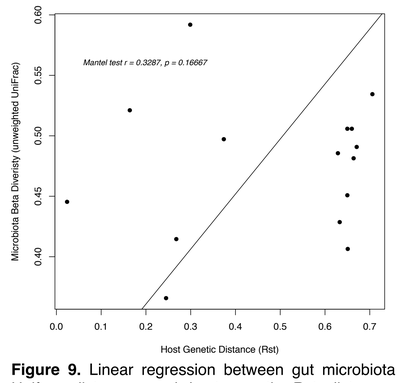


Figure 9. Linear regression between gut microbiota Unifrac distances and host genetic Rst distances among strains of ictalurid catfish. No sig. correlation.

Conclusions

- When reared in a common flow-through system and fed the same diets the gut microbiota among the six strains of ictalurid catfish were relatively similar in alpha and beta, with individual variation being statistically similar to that among experimental groups (strain or species).
- Blue catfish showed an enrichment of *Vibrio*, a genus typical of saltwater fish species. Channel catfish were found to harbor more potentially pathogenic taxa within their gut microbiota (*Edwardsiella, Psuedomonas, Streptococcus,* and *Aeromonas*), although all fish exhibited no signs of disease at the time of sampling. This potentially indicates that channel catfish tolerate more opportunistically pathogenic microbes in their gut microbiota, leading to higher disease susceptibility, under perturbations to homeostasis.
- Analysis of microsatellite data showed rather clear separation in genetic distance among the species or strains.
- We found no correlations between UniFrac distance and host Rst distance.
- Smith et al. (2015)[(2)] found a significant correlation ($p = 0.02$) between host Rst and UniFrac in six allopatric populations of three spine stickleback; however geographic and environmental differences were also present. Together with the results of this study, this suggests that host genetics does effect gut microbiota assemblages, yet diet and environment are more robust drivers.

Cited and Referenced

1. Waldbieser G. - unpublished data. (*In prep.*)
2. Smith C, Snowberg L, Caporaso J, Knight R, Bolnick D (2015). Dietary input of microbes and host genetic variation shape among-population differences in stickleback gut microbiota. *ISME Journal.* 9; 2515-2526.

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