The Effects of Social Experience on Host Gut Microbiome in Male Zebrafish (*Danio rerio*)

Emily Scott, Michael S. Brewer, Ariane L. Peralta*, and Fadi A. Issa†

Department of Biology, East Carolina University, Greenville, North Carolina 27858

Abstract

*Although the gut and the brain vastly differ in physiological function, they have been interlinked in a variety of different neurological and behavioral disorders. The bacteria that comprise the gut microbiome communicate and influence the function of various physiological processes within the body, including nervous system function. However, the effects of social experience in the context of dominance and social stress on gut microbiome remain poorly understood. Here, we examined whether social experience impacts the host zebrafish (*Danio rerio*) gut microbiome. We studied how social dominance during the first 2 weeks of social interactions changed the composition of zebrafish gut microbiome by comparing gut bacterial composition, diversity, and relative abundance between socially dominant, submissive, social isolates and control group–housed communal fish. Using amplicon sequencing of the 16S rRNA gene, we report that social dominance significantly affects host gut bacterial community composition but not bacterial diversity. At the genus level,* Aeromonas *and unclassified Enterobacteriaceae relative abundance decreased in dominant individuals while commensal bacteria (*e.g., Exiguobacterium *and* Cetobacterium*) increased in relative abundance. Conversely, the relative abundance of* Psychrobacter *and* Acinetobacter *was increased in subordinates, isolates, and communal fish compared to dominant fish. The shift in commensal and pathogenic bacteria highlights the impact of social experience and the accompanying stress on gut microbiome, with potentially similar effects in other social organisms.*

Introduction

The gut microbiome plays key roles in biochemical functions in vertebrates and is a diverse ecosystem composed of trillions of microorganisms that support the digestive tract (Cresci and Bawden, 2015; Shreiner *et al.*, 2015; Shanahan *et al.*, 2021). Gut bacterial metabolism has been linked to many systemic diseases in humans and influences numerous immune system pathways (Kinross *et al.*, 2011; Geuking *et al.*, 2014; van der Meulen *et al.*, 2016; Foster *et al.*, 2017; Lazar *et al.*, 2018). Additionally, commensal bacteria within the gut microbiome serve as a line of defense to viral infections through interferon signaling (Stefan *et al.*, 2020). Along with these known functions of the gut biome, it has recently been linked to affecting brain function in the context of neurodevelopmental (Warner, 2019; Tamana *et al.*, 2021), behavioral (Christian *et al.*, 2015; Sharon *et al.*, 2016; Ntranos and Casaccia, 2018), and neurodegenerative (Ghaisas *et al.*, 2016; Chandra *et al.*, 2020; Tan *et al.*, 2021) disorders. For instance, the gut microbiome produces certain biochemicals (*i.e.*, dopamine and oxytocin) that regulate neural nuclei involved in social behavior through vagus nerve and hypothalamus-pituitary-adrenal (HPA) activation (Lynch and Hsiao, 2019; Sgritta *et al.*, 2019; Hamamah *et al.*, 2022). This link is known as the "microbiota-gut-brain axis," and its dysfunction has been associated with many behavioral disorders such as autism spectrum disorder, depression, and anxiety, with long-lasting negative impact on health and well-being (Hughes *et al.*, 2018). Thus, understanding the mechanisms of disease onset and progression related to the gut microbiome is critical. Given that many of these studies highlight the directionality of the gut influencing behavior, it is important to consider

Received 4 August 2023; Accepted 21 December 2023; Published online 20 February 2024.

* Corresponding author; email: peraltaa@ecu.edu.

† Corresponding author; email: issaf14@ecu.edu.

Abbreviations: HPA, hypothalamus-pituitary-adrenal; IP, isolation period; OTU, operational taxonomic unit.

Online enhancement: appendix.

The Biological Bulletin, June 2023, volume 244, number 3: 000–000. https://doi.org/10.1086/729377

that bidirectionality may be observed as well, in that behavior may directly affect gut microbiome composition. For example, the host environment, along with social behaviors, has been shown to impact gut microbiome composition directly in primates, mole rats, and macaques (Fitzpatrick *et al.*, 2022; Johnson *et al.*, 2022). Interestingly, *Streptococcus* was found in higher abundance in less sociable macaques, which emphasizes the hypothesis that social behaviors may drive changes to the gut microbiome (Johnson *et al.*, 2022).

The typical gut microbiome consists of both commensal and pathogenic bacteria (Kamada *et al.*, 2013; Cresci and Bawden, 2015; Jandhyala *et al.*, 2015). While it is known that pathogenic bacteria are more prevalent in gut microbiomes of diseased organisms, evidence from multiple animal models has shown that a lack of commensal bacteria can also contribute to the detrimental progression of various behavioral disorders (Dowlati *et al.*, 2010; Ochoa-Repáraz *et al.*, 2011). In autism spectrum disorder mouse models, the abundance of a commensal bacteria species, *Lactobacillus reuteri*, is significantly reduced in comparison to wild-type mice (Sgritta *et al.*, 2019). Further, *Lactobacillus plantarum* has been used as a probiotic in zebrafish models to reduce stress-related behaviors and prevent stress-induced microbiome dysbiosis (Davis *et al.*, 2016). *Lactobacillus plantarum* was discovered to modulate anxiety-related behavior through the GABAergic and serotonergic pathways (Davis *et al.*, 2016). Additionally, gut bacterial composition influences host behavior through the modulation of brain-derived neurotrophic factor (BDNF) levels and serotonin metabolism (Borrelli *et al.*, 2016). For instance, probiotic treatment with *Lactobacillus rhamnosus* in zebrafish affects shoaling behavior and brain expression levels of *bdnf* and serotoninergic pathways, both of which have been implicated in aggression and multiple psychiatric disorders (Mondelli *et al.*, 2011; Ventriglia *et al.*, 2013). In addition to influencing neurotransmitter levels, bacteria from the genus *Lactobacillus* can activate afferent neurons in the intestine that modulate pain sensation and actions of defensive behavior in response to stress (Chiu *et al.*, 2013). This evidence supports the notion that certain bacteria can influence the activity of neuromodulatory pathways responsible for social behavior.

Zebrafish social behavior has been studied extensively (Oliveira *et al.*, 2011; Teles *et al.*, 2013; Clements *et al.*, 2018; Carver *et al.*, 2021; Orr *et al.*, 2021). When paired, zebrafish interact agonistically with conspecifics and quickly form stable dominance relationships consisting of dominant and submissive fish (Oliveira *et al.*, 2011; Teles and Oliveira, 2016). Once dominance forms, dominants have priority to food, shelter, and mates, while submissive fish experience social stress (Miller *et al.*, 2017; Clements *et al.*, 2023). The well-described social behaviors of male zebrafish make them an ideal model system for such experiments. Although the neurobiological bases of zebrafish social aggression and their long-term impact on social activity have been investigated, the effects of social dominance on gut microbiome are less understood (Sylvia and Demas, 2018). This is of particular importance because the effects of social stress on organisms are detrimental and have been adversely linked to anxiety-like behavior relevant for survival in many social species (Chrousos and Gold, 1992; Blanchard *et al.*, 2001; Bartolomucci *et al.*, 2005; Egan *et al.*, 2009; Mudra Rakshasa and Tong, 2020). Specifically, reduction of glucocorticoid signaling activity (caused by insufficient hormone availability) in social stress models has been associated with stress-related disorders (Raison and Miller, 2003). Glucocorticoid activation plays a critical role in the maintenance of fundamental metabolic processes throughout the body (Kadmiel and Cidlowski, 2013). Glucocorticoids also contribute to the activation of the HPA axis, which the gut microbiota has recently been thought to modulate. These interactions between gut microbes, glucocorticoids, and the HPA axis result in behavioral abnormalities, highlighting the relationship between stress and the gut microbiome (Luo *et al.*, 2018). The stability of zebrafish social dominance and the ease of quantifying their aggressive social activity provide an opportunity to examine the brain-gut microbiome axes and the impact of social stress on the gut biome as dominance relationships are established.

The zebrafish gut microbiome has been examined in embryos and adults. A past study showed that the gut microbiome composition of host zebrafish changes throughout its development (Stagaman *et al.*, 2020). Specifically, as embryonic zebrafish develop, the diversity of gut bacteria decreases significantly in terms of the number of operational taxonomic units (OTUs), clusters of bacteria that exhibit high sequence similarity to the *16S* rRNA gene (Chen *et al.*, 2013; Stephens *et al.*, 2016). However, microbiome composition was similar between stages of adult fish, which indicates that major gut microbial changes occur before and during major developmental changes such as sexual differentiation (Stephens *et al.*, 2016). The adult zebrafish gut microbiome is composed primarily of bacteria within the Proteobacteria, Firmicutes, and Fusobacteria phyla (Roeselers *et al.*, 2011). Pathogenic bacteria within the Proteobacteria phylum are found in significantly lower relative abundance of healthy human gut microbiomes, and higher relative abundance of proteobacterial members can be used as a diagnostic for disease and dysbiosis (Shin *et al.*, 2015). Additionally, in zebrafish, Proteobacteria, including pathogenic genera *Vibrio* and *Plesiomona*, were reduced in probiotic-treated fish as opposed to commensal Firmicutes bacterial members (Borrelli *et al.*, 2016). Thus, the presence of pathogenic bacteria in both zebrafish and humans can indicate microbial dysbiosis and potential health issues. In this study, we examined whether social dominance has opposing effects on gut microbiome in dominant *versus* stressed submissive animals. Submissive zebrafish have higher blood cortisol levels; this supports the hypothesis that submissive animals experience higher levels of stress compared to

dominant counterparts (Filby *et al.*, 2010; Bozi *et al.*, 2021). We tested the hypothesis that the relative abundance, diversity, and overall composition of zebrafish gut microbiome is socially regulated. We also sought to determine whether inherent differences in gut microbiome prior to dominance formation would predict future social rank. We compared gut microbiomes (based on amplicon sequencing of the *16S* rRNA gene) between socially dominant, submissive, socially isolated, and group-housed communal fish. Results from this work improve our understanding of how social status and stress impact host gut microbiome and may help future studies to understand the gut-brain axis and how the onset of behavioral disorders affects gut dysbiosis.

Materials and Methods

Zebrafish husbandry

Zebrafish (*Danio rerio* (Hamilton, 1822)) used in this study were wild-type (WT) fish between the ages of 8 and 12 months old. Male and female zebrafish were housed communally (20 fish per tank) in an automated flow-through tank system (Z-Hab System, Aquatic Habitats, Speonk, NY) in 33 cm × 21 cm × 19 cm holding tanks with room light cycle (light : dark cycle of 14h : 10h). Fish were fed a consistent diet of brine shrimp and pellets twice daily. All methods and protocols were approved by East Carolina University's Institutional Animal Care and Use Committee.

Pairing and behavioral analysis

Male zebrafish siblings ($n = 24$) were isolated in individual tanks for 7 days to minimize the effects of prior social experience (Fig. 1). All fish used in the experiment were taken from a WT communal tank. After initial isolation, 12 fish were randomly paired and placed into new experimental tanks (Fig. 1, group 3); six fish remained in isolation (Fig. 1, control group 2), and the remaining six fish were placed into one new tank to serve as communal controls (Fig. 1, control group 1). All experimental tanks were 26 cm × 14 cm × 10 cm. Over a period of 14 days, the six zebrafish pairs self-established rank, and their behavior was monitored and recorded over a course of 14 days (2 weeks) (Fig. 1, group 3). Selected fish were similar in size, and behavior was observed visually, while written observations were made regarding dominant and subordinate behavior between the paired and communal fish. Although communal fish do not establish structured dominance hierarchies, periodic aggressive activities are observed. Additionally, tank locations of each fish (top *vs.* bottom of tank) were monitored and recorded, as subordinate fish tend to stay lower in the tank and dominant fish claim the top of the tank. Dominant animals were defined as those that swam throughout the tank, while subordinates were determined as those that spent most of their time at the bottom of the tank (Table S4 [appendix is available online]).

Fecal sample collection

Initial fecal samples were collected from each fish on day 3 of the isolation period. This fecal collection before pairing or establishment of behaviors served as a method of comparison for later fecal collections. Fecal samples were also collected on day 0 (day of pairing), day 7 (1 week after pairing), and day 14 (2 weeks after pairing). Collections were made throughout the progression of social hierarchal development to determine whether the gut microbiome composition of host fish evolved as dominance was established.

On days of fecal collection, fish were removed from tanks, and each fish was placed into an individual container

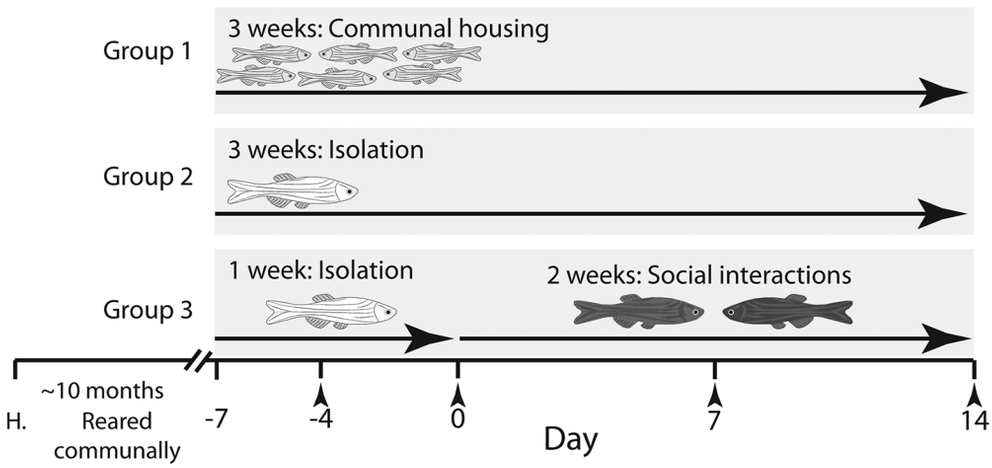

Figure 1. Time line of experimental setup consisting of three groups. All groups were initially reared communally from hatchlings (H) to adulthood prior to experimentation. Group 1 consisted of six communally housed fish for a period of 3 weeks. Group 2 consisted of individually housed fish for a period of 3 weeks. Group 3 consisted of two fish that were initially isolated for a period of 1 week and then paired for 2 weeks, during which their social interactions were monitored. Arrowheads denote fecal sample collection.

to prevent any cross contamination of fecal matter. Before pairing, each fish was carefully observed, and distinct markings, size differences, and color differences were noted to ensure easy recognition of each fish upon isolation and fecal sample collection. Two to four hours after separation and feeding of each fish, fecal samples were collected (Sayah *et al.*, 2005; Dominianni *et al.*, 2014). Fecal samples from each fish were extracted using sterile micropipette tips and were placed into 500 μL of DNAse-free water. Fecal samples were extracted from the container of each individual fish soon after defecation to minimize cross contamination with the water in the container. This method of fecal sample collection has been utilized in previous studies examining gut microbiome composition and was selected as opposed to intestinal tract extraction, which may provide more accurate representation of gut microbiome content (Yan *et al.*, 2012; Tang *et al.*, 2020; Huang *et al.*, 2021; Ahn *et al.*, 2023) in order to analyze microbiome composition over several time periods. Gloves were always worn when handling fecal samples and fish, to minimize any cross contamination. After samples were collected, they were stored at −80 °C until DNA extractions and processing were performed.

Microbiome analysis

To determine differences in microbial composition in zebrafish based on social status, microbial communities in each fecal sample were characterized with Illumina (San Diego, CA) sequencing of the highly conserved *16S* rRNA gene (Caporaso *et al.*, 2012). Following fecal sample collection, DNA extractions were performed on each sample using the PowerLyzer PowerSoil DNA protocol (catalog no. 12855-50, Qiagen, Hilden, Germany). Approximately 0.02 g of feces was collected from each fish for extractions. This extracted genomic DNA was used as the template for polymerase chain reactions (PCRs), where bar-coded primers (515F-806RB) were used to target the V4 region of the *16S* rRNA gene (Caporaso *et al.*, 2012; Apprill *et al.*, 2015). Each fecal sample was run in triplicate PCRs and then combined and purified using the Axygen AxyPrep magnetic bead purification kit (Corning Life Sciences, Durham, NC). After successful cleanup, DNA concentrations (ng mL^{-1}) from each sample were quantified using the Quant-iT dsDNA HS (high-sensitivity) assay (Thermo Scientific, Waltham, MA). Recorded DNA concentrations (ng mL^{-1}) using HS measurements were converted to the final DNA concentration (ng mL^{-1}), and then dilutions using PCR-grade water were performed to ensure that all samples added to the final pooled product were the same mass (ng). The PCR products were pooled in equimolar concentrations and sequenced on an Illumina MiSeq platform, using paired-end reads (Illumina reagent kit ver. 2, 500 reaction kit) at the Center for Genomics and Bioinformatics at Indiana University (Bloomington).

We processed raw *16S* rRNA gene sequences by using a standard mothur pipeline (ver. 1.40.1) (Schloss *et al.*, 2009; Kozich *et al.*, 2013). We assembled contigs from paired-end reads and quality trimmed using a moving average quality score of 35 bp. We aligned sequences to the SILVA rRNA gene database (ver. 128) (Yilmaz *et al.*, 2014), and we removed chimeric sequences using the VSEARCH algorithm (Rognes *et al.*, 2016). We divided sequences based on taxonomic class and binned into OTUs with a 97% sequence similarity level, and we classified OTUs using the SILVA rRNA database (ver. 128). We used an OTU-based 3% distance threshold to avoid splitting the same bacterial genome into distinct clusters (using amplicon sequence variant [ASV] of a single base difference). In addition, the sequencing was conducted using short reads (250 bp) and not assembled genomes, which introduces PCR and sequencing errors resulting in overinflated numbers of ASVs (Schloss, 2021). The broadscale community composition patterns are robust when employing an OTU-based approach (Glassman and Martiny, 2018).

Species diversity and community compositional analysis

We examined the diversity and composition of bacterial communities in each fecal sample. For each microbiome sample, we calculated the Chao 1 OTU richness using the estimateR() function and Shannon diversity (H') using the diversity() function in the vegan package (Oksanen *et al.*, 2017), and we also calculated Simpson's evenness using a custom function after we rarefied the OTU table to 10,181 observations. We calculated the Bray-Curtis dissimilarity matrix and visualized community composition according to social status and day, using principal coordinate analysis. Finally, genus-level compositions with relative abundances greater than 0.05 were plotted after the OTU table was rarefied to 6000 observations.

Statistical analyses

All data analyses were completed using the R statistical environment (R ver. 4.2.0, R Studio ver. 2022.07.1, R Development Core Team 2022). Using the lmer() function in the lmerTest package (Kuznetsova *et al.*, 2017), a linear mixed effects model with social status and day as fixed effects was used to analyze bacterial diversity metrics Chao 1 OTU richness, Shannon diversity, and Simpson's evenness. We ran a permutational multivariate analysis of variance (PERMANOVA) based on the Bray-Curtis dissimilarity of bacterial community composition to determine the extent that social status and day and the interaction explained bacterial community composition. We next identified bacterial species that represented each treatment (social status × day) for bacterial taxa with a relative abundance greater than 0.05 when summed across all samples. We performed the PERMANOVA using the adonis() function in the vegan package (Oksanen *et al.*, 2017) and the indval() function in the indicspecies package (De Caceres and Jansen, 2016).

Data availability

All code and data used in this study are in a public GitHub repository (Scott *et al.*, 2024) and National Center for Biotechnology Information Sequence Read Archive BioProject PRJNA925886 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA925886).

Results

Bacterial species diversity and community composition

We compared gut microbiomes of zebrafish of different social statuses. Bacterial OTU richness (Fig. 2) was similar across social status and day, while the interaction of social status and day but not the main effects influenced Shannon diversity (Fig. 3) and Simpson's evenness increased from day 7 to day 14 (Fig. 4; Table S1). While statistical significance was detected, the linear models explained a relatively low amount of variation (richness: adjusted $R^2 = 0.149$, $P = 0.020$; diversity: adjusted $R^2 = 0.106$, $P = 0.062$; evenness: adjusted $R^2 = 0.215$, $P = 0.003$; Table S1). Communal data points plotted similarly to isolate data points during the isolation period (IP) before any pairing or rank establishment. Additionally, dominant data points trended similarly to those of subordinate animals during the IP. These trends were observed for richness (Fig. 2) and evenness (Fig. 4).

In contrast to bacterial diversity, social status and day influenced bacterial community composition. The interaction of social status and day accounted for 14.4% of the variation in bacterial composition ($F_{9,92} = 1.9214$, $P = 0.001$), while the main effects of day accounted for 12.5% ($F_{3,92} = 5.035$, $P = 0.001$) and social status accounted for 9.1% ($F_{3,92} = 3.671$, $P = 0.001$) of the bacterial community composition (Fig. 5; Table S2). The bacterial gut microbiomes of dominant and isolate fish tended to group together, while the gut microbiomes of communal fish were distinct from all other groups, except for day 14, which grouped by the gut microbiomes of subordinate fish on day 0 (Fig. 5). The IP for subordinate, dominant, and isolated fish resulted in similar gut microbiomes (Fig. 5, diamonds). For subordinate and dominant fish (Fig. 5, blue and red symbols), the gut microbiomes were more variable over time (*i.e.*, symbols were farther apart over ordination space) than were the isolate and communal gut microbiomes (gray and purple symbols).

Taxa-level shifts across social status

We evaluated which bacteria taxa represented gut microbiomes by social status and day, using indicator species analysis. *Paracoccus* spp. and *Achromobacter* spp. represented gut microbiomes of dominant fish during the IP, and unclassified taxa from the families Planctomycetacea, Caldilineaceae, and Comamonadaceae and indicators *Staphylococcus* spp. and *Exiguobacterium* spp. represented gut microbiomes of dominant fish on day 7 (Table S3). *Acinetobacter* spp. and *Psychrobacter* spp. represented gut microbiomes of subordinate fish during day 0 and day 7, respectively (Table S3). Unclassified taxa from the class Betaproteobacteria and family Rhizobiaceae represented gut microbiomes of isolated fish on day 0 (Table S3). For communal fish, an unclassified taxon from the Rhodobacteraceae family, *Pseudomonas* spp., and *Stappia* spp. represented gut microbiomes during the IP,

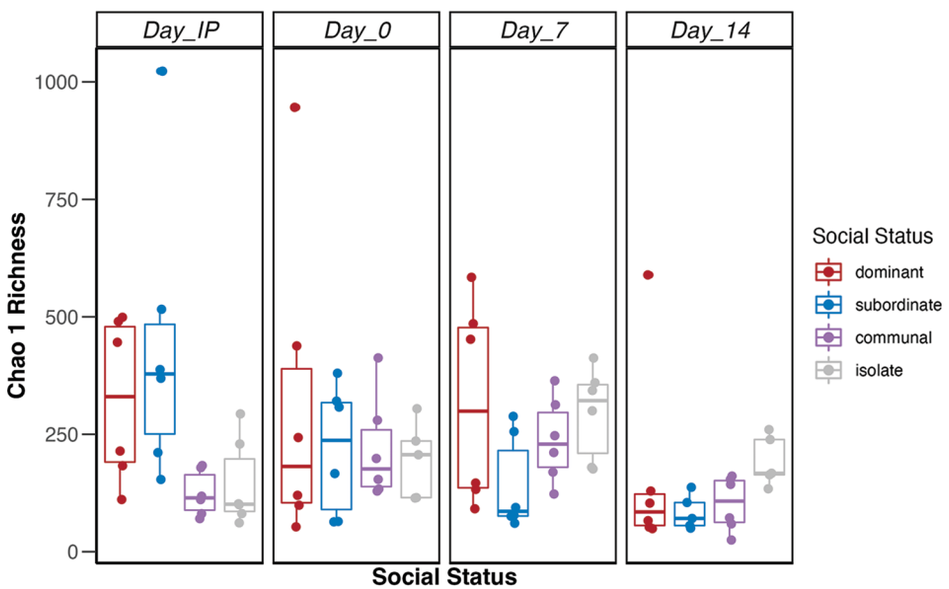

Figure 2. Effect of social status on zebrafish (*Danio rerio*) gut microbiome bacterial richness. Boxplots representing bacterial Chao 1 richness in individuals from dominant (red), subordinate (blue), communal (purple), and isolate (gray) animals throughout the isolation period (day IP), representing collection before pairing and pairing period (day 0, day 7, day 14). The boxplot is a visual representation of five key summary statistics: the median, the 25% and 75% percentiles, and the whiskers, which represent the feasible range of the data as determined by 1.5× the interquartile range. Symbols represent individual raw data points from six replicate samples. See summary of statistical output in Table S1A (available online).

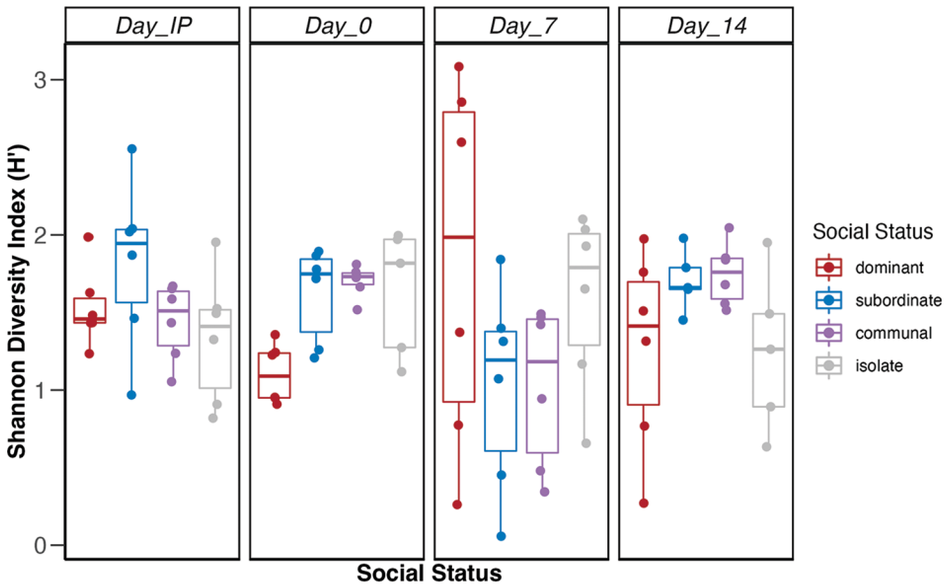

Figure 3. Effect of social status on zebrafish (*Danio rerio*) gut microbiome bacterial diversity. Boxplots representing bacterial Shannon diversity index in individuals from dominant (red), subordinate (blue), communal (purple), and isolate (gray) animals throughout the isolation period (day IP), representing collection before pairing and pairing period (day 0, day 7, day 14). The boxplot is a visual representation of five key summary statistics: the median, the 25% and 75% percentiles, and the whiskers, which represent the feasible range of the data as determined by 1.5× the interquartile range. Symbols represent individual raw data points from six replicate samples. See summary of statistical output in Table S1B (available online).

while *Rhizobiales* spp., *Arthrobacter* spp., and *Acinetobacter* spp. represented day 0; *Chryseobacterium* spp. represented day 7; and *Shewanella* spp. represented day 14 (Table S3). Unclassified taxa from the class Betaproteobacteria and family Rhizobiaceae represented gut microbiomes of isolated fish on day 0 (Table 1).

To further explore trends, we evaluated genus-level composition for genera observed in >5% relative abundance. While community composition varied between different social statuses and day of pairing, we observed changes in dominant individuals on day 7 and day 14 of pairing compared to the same individuals during the IP and on day 0 of

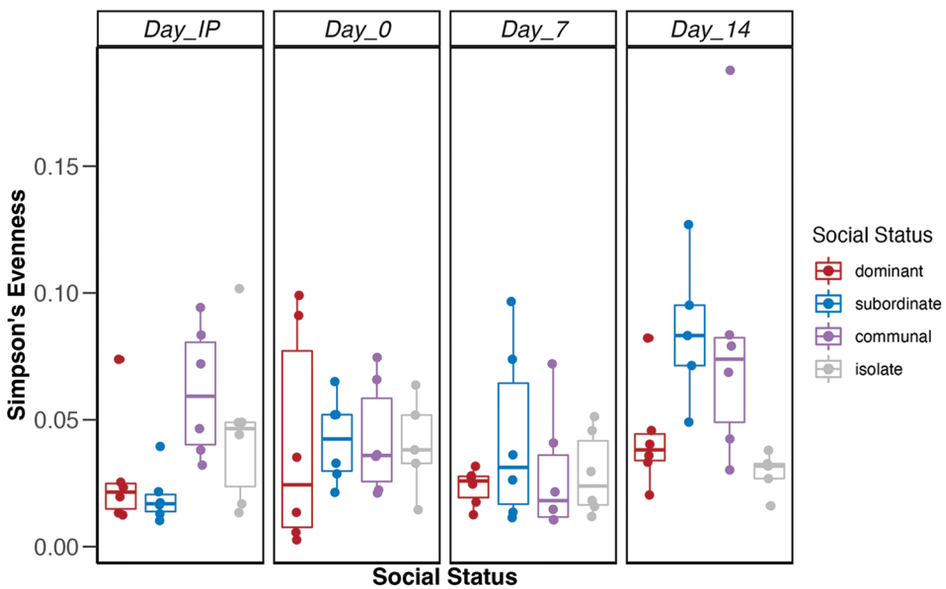

Figure 4. Effect of social status on zebrafish (*Danio rerio*) gut microbiome bacterial Simpson's diversity. Boxplots representing bacterial Simpson's diversity index in individuals from dominant (red), subordinate (blue), communal (purple), and isolate (gray) animals throughout the isolation period (day IP), representing collection before pairing and pairing period (day 0, day 7, day 14). The boxplot is a visual representation of five key summary statistics: the median, the 25% and 75% percentiles, and the whiskers, which represent the feasible range of the data as determined by 1.5× the interquartile range. Symbols represent individual raw data points from six replicate samples. See summary of statistical output in Table S1B (available online).

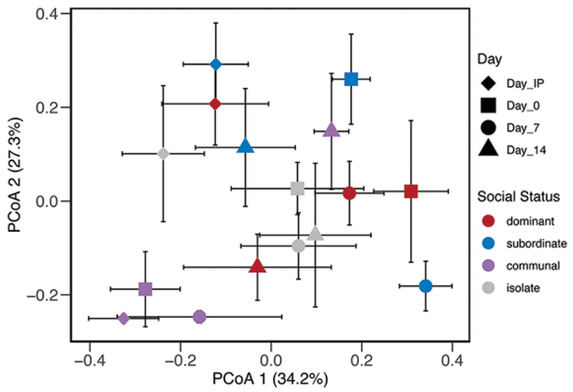

Figure 5. Correlation analysis of social status and gut bacterial composition. Ordination based on a principal coordinates analysis (PCoA) depicting bacterial community composition according to social status and day. Symbols are colored according to social status (red, dominant; blue, subordinate; purple, communal; gray, isolate) and shapes represent day of pairing (square, day 0; circle, day 7; triangle, day 14; diamond, day IP [isolation period]). The centroid and standard error bars (along axes 1 and 2) were calculated for six replicate plots. See summary of statistical output in Table S2 (available online).

pairing (Fig. S1). We observed a reduction in pathogenic genera that were consistently high in relative abundance in nondominant social status groups and throughout the pairing period. Specifically, *Aeromonas* and unclassified Enterobacteriaceae relative abundance was lower in dominant individuals on day 7 and day 14, whereas relative abundance of these genera remained higher in other groups (Fig. S1). We also observed an increased relative abundance of *Chitinobacteria*, *Vibrio*, and *Pseudomonas* spp. in subordinate, isolate, and communal animals, and the presence of these genera was maintained before and during pairing (Fig. S1). In addition, certain commensal genera such as *Exiguobacterium* and *Cetobacterium* increased in relative abundance in dominant animals on day 7 of pairing. These genera were present in dominant animals throughout pairing, but on day 7 the relative abundance increased, especially for the *Cetobacterium* genus (Fig. S1).

Discussion

A growing number of studies highlight the microbial role in the link between the gut and the brain (Flight, 2014; Reardon, 2014; Skonieczna-Żydecka *et al.*, 2018). Our results suggest a link between the host gut microbiome and social experience in zebrafish, building on prior research that gut microbiome may be associated with social dominance (Mondelli *et al.*, 2011; Ventriglia *et al.*, 2013; Flight, 2014; Davis *et al.*, 2016). Here, we studied whether social behaviors related to rank and associated stress due to social subordination influence gut microbiome content in zebrafish. We showed that while there were no significant differences in gut microbiome diversity or richness between different social states, social dominance leads to compositional differences. This result complements prior work in a mouse model that showed a causative relationship between the gut and anxiety/depression phenotypes (Sgritta *et al.*, 2019); however, the directionality in which these interactions occur may differ. These similarities, along with findings in other studies examining how probiotic treatment alters behavioral activity in socially stressed individuals, lend further credence to the strong relationship between the gut-brain axis. Specifically, stressed zebrafish that were treated with probiotics experienced a complete reversal and rescue of their anxiety-like behavior (Davis *et al.*, 2016). Altogether, these ideas support a microbiome-mediated behavior hypothesis in which changes to the gut microbiome affect associated behaviors related to stress and anxiety. However, in those studies, both the composition and diversity of the gut microbiome directly affect behavior, whereas we found that only bacterial composition was affected by social status. While these studies supported the hypothesis that changes to the gut microbiome

Table 1

Condensed Indicator Species Analysis Table Highlighting Unique Taxa Associated with Treatment Type (Social Status × Day)

Cluster	Associated phyla	Associated families	Associated genera
Dominant day IP	Proteobacteria	Rhodobacteraceae, Alcaligenaceae	*Paracoccus, Achromobacter*
Dominant day 7	Planctomycetes, Chloroflexi, Proteobactera, Firmicutes	Planctomycetaceae, Caldilineaceae, Comamonadaceae	*Exiguobacterium, Staphlyococcus, Comamonadaceae_unclassified, Caldilineacea_unclassified, Planctomycetaceae_unclassified*
Subordinate day 0	Proteobacteria	Moraxellaceae	*Acinerobacter*
Subordinate day 7	Proteobacteria	Moraxellaceae	*Psychrobacter*
Communal day IP	Proteobacteria	Pseudomonadaceae, Rhodobacteraceae	*Rhodobacteraceae_unclassified, Pseudomonas, Stappia*
Communal day 0	Proteobacteria, Actinobacteria	Moraxellaceae, Rhizobiales_unclassified, Micrococcaceae	*Rhizobiales_unclassified, Arthrobacter, Acinetobacter*
Communal day 7	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*
Communal day 14	Proteobacteria	Shewanellaceae	*Shewanella*
Isolate day 0	Proteobacteria	Betaproteobacteria_unclassified, Rhizobiaceae	*Betaproteobactera_unclassified, Rhizobiaceae_unclassified*

directly affect behavior, the results of our study support that social rank and associated behavior affect gut microbiome composition. While we observed compositional differences between fish of different social rank, these differences were more pronounced after 7–14 days of social interactions when social dominance solidified. This signifies that stress due to submission or isolation may directly impact bacterial composition. Additionally, it seems that social dominance promotes an increased presence of commensal bacterial genera. Previous research in autism models shows an inverse relationship between the gut and behavior, as individuals born with gut microbiota dysbiosis are predisposed to social deficits and autism-like behaviors (Sharon *et al.*, 2019). This increased commensal bacteria presence in dominant individuals could offer support toward a hypothesis that dominant fish experience lower levels of stress compared to subordinate counterparts.

Given the large amount of research supporting the influence of the gut microbiome on social behaviors, one of the questions we sought to answer was whether inherent differences in gut microbiome prior to social dominance formation would predispose individuals to become either dominant or submissive. Based on our results of fecal samples that were collected prior to pairing during the IP (day IP), this hypothesis is not supported. However, it would be interesting to determine in future studies whether repeated probiotic or antibiotic treatments over extended periods are sufficient to modulate aggression levels and reverse social dominance. Such results would be very exciting and would strongly indicate that the microbiome could directly affect social aggression.

Based on previous research in zebrafish and other animal models, the genus *Lactobacillus*, from phylum Bacteroidetes, is a common protective bacterial species often found in the guts of many animal models (Davis *et al.*, 2016). Interestingly, the only genus of bacteria found from the Bacteroidetes phylum in this study was *Chryseobacterium* spp., and it was primarily found in commensal fish on day 7 (Table S3). While this genus does not come from the same family as *Lactobacillus*, both genera seem to have similar protective qualities in zebrafish gut microbiota. In a study examining bacterial key players in pathogenic infection in zebrafish, *Chryseobacterium massillae* was determined to be important in protecting both larvae and adult zebrafish from pathogenic infection (Stressmann *et al.*, 2021). Interestingly, this bacterial genus was present only in low abundance in both communal and dominant individuals (Table S3; Fig. S1).

Within the Firmicutes phylum, the bacterial genus *Exiguobacterium* was also determined as a component of compositional analysis of the zebrafish gut in our study. This genus of bacteria has been shown to produce cyclic dipeptides, which have many antimicrobial, antifungal, antiviral, and anti-inflammatory properties in humans and other animals (Graz *et al.*, 2000; Jinendiran *et al.*, 2020). Intriguingly, *Exiguobacterium acetylicum* has therapeutic properties in zebrafish colorectal cancer models, which highlights the protective properties of this genus of bacteria in the gut of both zebrafish and humans (Ström *et al.*, 2002). While the bacterial genus *Exiguobacterium* was present in socially submissive and isolated zebrafish, this OTU increased in relative abundance only in dominant animals (Fig. S1). The absence of the bacterial genus *Exiguobacterium* in communal animals should be noted, which may be due to the heterogeneity among midranked members living in a communal setting whereby aggression and dominant behaviors are minimal.

Given the high percentage of Proteobacteria communities in zebrafish gut microbiomes, the presence of pathogenic bacteria is not surprising, particularly since a large portion of the species in these genera are waterborne. Therefore, tank water was analyzed, and microbiome tested grouped in the middle of subordinate, dominant, isolate, and communal fish microbiomes (Fig. S2). However, the varying relative abundance of certain potential pathogenic bacteria like *Aeromonas*, unclassified Enterobacteriaceae, *Staphylococcus*, *Vibrio*, and *Pseudomonas* spp. in the community composition is noteworthy. *Aeromonas* and unclassified Enterobacteriaceae were the main putatively pathogenic genera in all groups and at all time points during pairing, but several putatively pathogenic genera were primarily seen in subordinates, isolates, and even communal individuals (Fig. S1). Interestingly, *Staphylococcus* relative abundance is increased in dominant individuals even though other pathogenic genera are decreased. This could contribute to the increase in Firmicutes relative abundance seen in dominant individuals. This finding is inconsistent with other trends observed, as other potential pathogenic bacterial communities decreased in dominant individuals; however, these observations still provide insight into gut microbiome composition and associated social experience. In addition to the presence of *Staphylococcus*, indicator species analysis also revealed the presence of *Achromobacter* and *Paracoccus* in dominants during the IP (Table S3). *Achromobacter* and *Paracoccus* are genera of bacteria with pathogenic properties in both mammals and fish (Decewicz *et al.*, 2019; Loftie-Eaton *et al.*, 2021; Menetrey *et al.*, 2021). While they are present in dominant fish prior to pairing, they decrease in relative abundance after pairing, supporting the hypothesis that social status may affect gut bacterial composition.

Prior studies did not describe the role for the unclassified genera indicators observed for dominant day 7, which included OTUs in *Planctomycetaceae*, *Caldineaceae*, and *Comamondaceae*. The *Aeromonas* genus, which has a high relative abundance in almost all zebrafish individuals regardless of social status, has been observed in several pathogenic models of zebrafish. Interestingly, specific species within the *Aeromonas* genus have been seen to induce severe infection of zebrafish models, triggering an immune response accompanied by massive inflammatory reaction and

increased mortality rates (Rodríguez *et al.*, 2008). While our results do not specify the species of *Aeromonas* present, higher relative abundances of this genus may result in pathogenesis in stressed animals (*e.g.*, socially submissive or isolates) (Rolig *et al.*, 2015, 2018). We also acknowledge the limitations to *16S* rRNA gene sequencing and discuss the compositional differences, with emphasis on putative pathogenic and commensal genera. While we observe notable shifts in both commensal and potential pathogenic bacteria abundance between fish of different social conditions across time, we acknowledge that the effect on host health depends on the interactions within microbial communities and the interactions between a host and its microbiomes. To further identify how these specific genera may be involved in the development of social status, future studies examining the changes to specific commensal and pathogenic genera and their effects on social status are needed by taking advantage of antibiotic and probiotic treatments, as demonstrated in mice and zebrafish, respectively, in studying depression and anxiety (Borrelli *et al.*, 2016; Davis *et al.*, 2016; Zhang *et al.*, 2020; Foroozan *et al.*, 2021).

Collectively, our results show that gut microbiome composition is socially regulated. This is illustrated by a partial shift in the composition from pathogenic to commensal bacteria in dominant animals, while stressed animals (*e.g.*, submissive and socially isolated) showed an increase in potentially pathogenic bacteria and a decline in commensal bacterial genera. Although our results suggest that the physiological mechanisms underlying the shift in microbial composition is slow acting and requires 1–2 weeks for their biological manifestation, prior reports have shown that acute exposure to stress can impact the gut microbiota community composition by changing the relative proportions of the main microbiota phyla (Galley *et al.*, 2014). Moreover, experimental manipulations of gut microbiota affect stress levels and anxiety-like behavior by influencing the function of the HPA stress axis (Crumeyrolle-Arias *et al.*, 2014). Indeed, social stress–induced increase in blood cortisol levels correlates with changes in the diversity of the mammalian gut microbiome, including the abundance of lactic acid and pathogenic bacteria (Galley *et al.*, 2014; Mudd *et al.*, 2017). In addition, chronic injections of glucocorticoids can have positive and negative impact on the presence of specific microbial taxa, which consequently affects host metabolism (Huang *et al.*, 2015; Petrosus *et al.*, 2018; Wu *et al.*, 2018). Thus, it is likely that stress induced by either prolonged social isolation or subordination will result in increased cortisol levels in isolated and submissive zebrafish with substantial increase in expression of gut pathogenic bacteria given that previous research uses cortisol levels as indicators of stress in submissive individuals (Bozi *et al.*, 2021). It is of importance to note that stress may not be exclusive to social subordinates. More specifically, dominant individuals in many species experience higher stress than subordinates, which raises a question as to whether these individuals exhibit a higher concentration of bacteria with pathogenic properties (Creel, 2001; Gesquiere *et al.*, 2011; Milich *et al.*, 2018).

How these status-dependent changes in gut bacterial composition feed back to influence brain function remains poorly understood. Given that zebrafish experiencing induced stress exhibit changes in gut microbiome composition and brain gene expression (Borrelli *et al.*, 2016), future experiments testing this relationship in zebrafish in the context of social dominance and stress will be highly informative. More specifically, it would be of interest to determine whether transcriptional changes in the brains of dominant and subordinate fish can be linked to changes in bacterial abundance in the host gut. A large body of work has shown that dopaminergic, GABAergic, and serotoninergic pathways implicated in social behavior are highly plastic, but whether the plasticity is mediated by feedback regulation of gut microbiome to influence motivational brain centers involved in social aggression remains an open question (Korzan and Summers, 2007; McDonald *et al.*, 2012; Teles and Oliveira, 2016; Weitekamp *et al.*, 2017; Inoue *et al.*, 2022).

Acknowledgments

We thank Dr. Katie Clements for her assistance in the early design of behavioral experiments. This work was supported by the National Science Foundation (grant 754513 to FAI and grant 1845845 to ALP).

Literature Cited

Ahn, J.-S., E. Lkhagva, S. Jung, H.-J. Kim, H.-J. Chung, and S.-T. Hong. 2023. Fecal microbiome does not represent whole gut microbiome. *Cell. Microbiol.* **2023:** e6868417.

Apprill, A., S. McNally, R. Parsons, and L. Weber. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* **75:** 129–137.

Bartolomucci, A., P. Palanza, P. Sacerdote, A. E. Panerai, A. Sgoifo, R. Dantzer, and S. Parmigiani. 2005. Social factors and individual vulnerability to chronic stress exposure. *Neurosci. Biobehav. Rev.* **29:** 67–81.

Blanchard, R. J., C. R. McKittrick, and D. C. Blanchard. 2001. Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiol. Behav.* **73:** 261–271.

Borrelli, L., S. Aceto, C. Agnisola, S. De Paolo, L. Dipineto, R. M. Stilling, T. G. Dinan, J. F. Cryan, L. F. Menna, and A. Fioretti. 2016. Probiotic modulation of the microbiota-gut-brain axis and behaviour in zebrafish. *Sci. Rep.* **6:** 30046.

Bozi, B., J. Rodrigues, M. Lima-Maximino, D. H. de Siqueira-Silva, M. C. Soares, and C. Maximino. 2021. Social stress increases anxiety-like behavior equally in male and female zebrafish. *Front. Behav. Neurosci.* **15:** 785656.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer *et al.* 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **6:** 1621–1624.

Carver, J. J., S. C. Carrell, M. W. Chilton, J. N. Brown, L. Yong, Y. Zhu, and F. A. Issa. 2021. Nuclear androgen and progestin receptors inversely affect aggression and social dominance in male zebrafish (*Danio rerio*). *Horm. Behav.* **134:** 105012.

Chandra, S., M. T. Alam, J. Dey, B. C. P. Sasidharan, U. Ray, A. K. Srivastava, S. Gandhi, and P. P. Tripathi. 2020. Healthy gut, healthy brain: the gut microbiome in neurodegenerative disorders. *Curr. Top. Med. Chem.* **20:** 1142–1153.

Chen, W., C. K. Zhang, Y. Cheng, S. Zhang, and H. Zhao. 2013. A comparison of methods for clustering 16S rRNA sequences into OTUs. *PLoS One* **8:** e70837.

Chiu, I. M., B. A. Heesters, N. Ghasemlou, C. A. Von Hehn, F. Zhao, J. Tran, B. Wainger, A. Strominger, S. Muralidharan, A. R. Horswill *et al.* 2013. Bacteria activate sensory neurons that modulate pain and inflammation. *Nature* **501:** 52–57.

Christian, L. M., J. D. Galley, E. M. Hade, S. Schoppe-Sullivan, C. Kamp Dush, and M. T. Bailey. 2015. Gut microbiome composition is associated with temperament during early childhood. *Brain. Behav. Immun.* **45:** 118–127.

Chrousos, G. P., and P. W. Gold. 1992. The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. *J. Am. Med. Assoc.* **267:** 1244–1252.

Clements, K. N., S. Ahn, C. Park, F. K. Heagy, T. H. Miller, M. Kassai, and F. A. Issa. 2023. Socially mediated shift in neural circuits activation regulated by synergistic neuromodulatory signaling. *eNeuro* **10:** 1–20.

Clements, K. N., T. H. Miller, J. M. Keever, A. M. Hall, and F. A. Issa. 2018. Social status-related differences in motor activity between wild-type and mutant zebrafish. *Biol. Bull.* **235:** 71–82.

Creel, S. 2001. Social dominance and stress hormones. *Trends Ecol. Evol.* **16:** 491–497.

Cresci, G. A., and E. Bawden. 2015. Gut microbiome: what we do and don't know. *Nutr. Clin. Pract.* **30:** 734–746.

Crumeyrolle-Arias, M., M. Jaglin, A. Bruneau, S. Vancassel, A. Cardona, V. Daugé, L. Naudon, and S. Rabot. 2014. Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology* **42:** 207–217.

Davis, D. J., H. M. Doerr, A. K. Grzelak, S. B. Busi, E. Jasarevic, A. C. Ericsson, and E. C. Bryda. 2016. *Lactobacillus plantarum* attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish. *Sci. Rep.* **6:** 33726.

De Caceres, M., and F. Jansen. 2016. Indispecies: relationship between species and groups of sites. R package version 1.7.6. R Foundation for Statistical Computing, Vienna.

Decewicz, P., L. Dziewit, P. Golec, P. Kozlowska, D. Bartosik, and M. Radlinska. 2019. Characterization of the virome of *Paracoccus* spp. (*Alphaproteobacteria*) by combined *in silico* and *in vivo* approaches. *Sci. Rep.* **9:** 7899.

Dominianni, C., J. Wu, R. B. Hayes, and J. Ahn. 2014. Comparison of methods for fecal microbiome biospecimen collection. *BMC Microbiol.* **14:** 103.

Dowlati, Y., N. Herrmann, W. Swardfager, H. Liu, L. Sham, E. K. Reim, and K. L. Lanctôt. 2010. A meta-analysis of cytokines in major depression. *Biol. Psychiatry* **67:** 446–457.

Egan, R. J., C. L. Bergner, P. C. Hart, J. M. Cachat, P. R. Canavello, M. F. Elegante, S. I. Elkhayat, B. K. Bartels, A. K. Tien, D. H. Tien *et al.* 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behav. Brain Res.* **205:** 38–44.

Filby, A. L., G. C. Paull, T. F. Hickmore, and C. R. Tyler. 2010. Unravelling the neurophysiological basis of aggression in a fish model. *BMC Genomics* **11:** 498.

Fitzpatrick, C. R., I. Toor, and M. M. Holmes. 2022. Colony but not social phenotype or status structures the gut bacteria of a eusocial mammal. *Behav. Ecol. Sociobiol.* **76:** 117.

Flight, M. H. 2014. Neurodevelopmental disorders: the gut-microbiome-brain connection. *Nat. Rev. Neurosci.* **15:** 65.

Foroozan, P., M. K. Jahromi, J. Nemati, H. Sepehri, M. A. Safari, and S. Brand. 2021. Probiotic supplementation and high-intensity interval training modify anxiety-like behaviors and corticosterone in high-fat diet-induced obesity mice. *Nutrients* **13:** 1762.

Foster, J. A., L. Rinaman, and J. F. Cryan. 2017. Stress and the gut-brain axis: regulation by the microbiome. *Neurobiol. Stress* **7:** 124–136.

Galley, J. D., M. C. Nelson, Z. Yu, S. E. Dowd, J. Walter, P. S. Kumar, M. Lyte, and M. T. Bailey. 2014. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiol.* **14:** 189.

Gesquiere, L. R., N. H. Learn, M. C. M. Simao, P. O. Onyango, S. C. Alberts, and J. Altmann. 2011. Life at the top: rank and stress in wild male baboons. *Science* **333:** 357–360.

Geuking, M. B., Y. Köller, S. Rupp, and K. D. McCoy. 2014. The interplay between the gut microbiota and the immune system. *Gut Microbes* **5:** 411–418.

Ghaisas, S., J. Maher, and A. Kanthasamy. 2016. Gut microbiome in health and disease: linking the microbiome-gut-brain axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. *Pharmacol. Ther.* **158:** 52–62.

Glassman, S. I., and J. B. H. Martiny. 2018. Broad-scale ecological patterns are robust to use of exact sequence variants *versus* operational taxonomic units. *mSphere* **3:** e00148-18.

Graz, C. J., G. D. Grant, S. C. Brauns, A. Hunt, H. Jamie, and P. J. Milne. 2000. Cyclic dipeptides in the induction of maturation for cancer therapy. *J. Pharm. Pharmacol.* **52:** 75–82.

Hamamah, S., A. Aghazarian, A. Nazaryan, A. Hajnal, and M. Covasa. 2022. Role of microbiota-gut-brain axis in regulating dopaminergic signaling. *Biomedicines* **10:** 436.

Huang, E. Y., T. Inoue, V. A. Leone, S. Dalal, K. Touw, Y. Wang, M. W. Musch, B. Theriault, K. Higuchi, S. Donovan *et al.* 2015. Using corticosteroids to reshape the gut microbiome: implications for inflammatory bowel diseases. *Inflamm. Bowel Dis.* **21:** 963–972.

Huang, S., S. Jiang, D. Huo, C. Allaband, M. Estaki, V. Cantu, P. Belda-Ferre, Y. Vázquez-Baeza, Q. Zhu, C. Ma *et al.* 2021. Candidate probiotic *Lactiplantibacillus plantarum* HNU082 rapidly and convergently evolves within human, mice, and zebrafish gut but differentially influences the resident microbiome. *Microbiome* **9:** 151.

Hughes, H. K., D. Rose, and P. Ashwood. 2018. The gut microbiota and dysbiosis in autism spectrum disorders. *Curr. Neurol. Neurosci. Rep.* **18:** 81.

Inoue, K., C. L. Ford, K. Horie, and L. J. Young. 2022. Oxytocin receptors are widely distributed in the prairie vole (*Microtus ochrogaster*) brain: relation to social behavior, genetic polymorphisms, and the dopamine system. *J. Comp. Neurol.* **530:** 2881–2900.

Jandhyala, S. M., R. Talukdar, C. Subramanyam, H. Vuyyuru, M. Sasikala, and D. Nageshwar Reddy. 2015. Role of the normal gut microbiota. *World J. Gastroenterol.* **21:** 8787–8803.

Jinendiran, S., W. Teng, H.-U. Dahms, W. Liu, V. K. Ponnusamy, C. C.-C. Chiu, B. S. D. Kumar, and N. Sivakumar. 2020. Induction of mitochondria-mediated apoptosis and suppression of tumor growth in zebrafish xenograft model by cyclic dipeptides identified from *Exiguobacterium acetylicum*. *Sci. Rep.* **10:** 13721.

Johnson, K. V.-A., K. K. Watson, R. I. M. Dunbar, and P. W. J. Burnet. 2022. Sociability in a non-captive macaque population is associated with beneficial gut bacteria. *Front. Microbiol.* **13:** 1032495.

Kadmiel, M., and J. A. Cidlowski. 2013. Glucocorticoid receptor signaling in health and disease. *Trends Pharmacol. Sci.* **34:** 518–530.

Kamada, N., G. Y. Chen, N. Inohara, and G. Núñez. 2013. Control of pathogens and pathobionts by the gut microbiota. *Nat. Immunol.* **14:** 685–690.

Kinross, J. M., A. W. Darzi, and J. K. Nicholson. 2011. Gut microbiome-host interactions in health and disease. *Genome Med.* **3:** 14.

Korzan, W. J., and C. H. Summers. 2007. Behavioral diversity and neurochemical plasticity: selection of stress coping strategies that define social status. *Brain. Behav. Evol.* **70:** 257–266.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 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.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: tests in linear mixed effects models. *J. Stat. Softw.* **82:** 1–26.

Lazar, V., L.-M. Ditu, G. G. Pircalabioru, I. Gheorghe, C. Curutiu, A. M. Holban, A. Picu, L. Petcu, and M. C. Chifiriuc. 2018. Aspects of gut microbiota and immune system interactions in infectious diseases, immunopathology, and cancer. *Front. Immunol.* **9:** 1830.

Loftie-Eaton, W., A. Crabtree, D. Perry, J. Millstein, J. Baytosh, T. Stalder, B. D. Robison, L. J. Forney, and E. M. Top. 2021. Contagious antibiotic resistance: plasmid transfer among bacterial residents of the zebrafish gut. *Appl. Environ. Microbiol.* **87:** e02735-20.

Luo, Y., B. Zeng, L. Zeng, X. Du, B. Li, R. Huo, L. Liu, H. Wang, M. Dong, J. Pan *et al.* 2018. Gut microbiota regulates mouse behaviors through glucocorticoid receptor pathway genes in the hippocampus. *Transl. Psychiatry* **8:** 187.

Lynch, J. B., and E. Y. Hsiao. 2019. Microbiomes as sources of emergent host phenotypes. *Science* **365:** 1405–1409.

McDonald, M. M., C. M. Markham, A. Norvelle, H. E. Albers, and K. L. Huhman. 2012. GABAA receptor activation in the lateral septum reduces the expression of conditioned defeat and increases aggression in Syrian hamsters. *Brain Res.* **1439:** 27–33.

Menetrey, Q., P. Sorlin, E. Jumas-Bilak, R. Chiron, C. Dupont, and H. Marchandin. 2021. *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia*: emerging pathogens well-armed for life in the cystic fibrosis patients' lung. *Genes* **12:** 610.

Milich, K. M., A. V. Georgiev, R. M. Petersen, M. Emery Thompson, and D. Maestripieri. 2018. Alpha male status and availability of conceptive females are associated with high glucocorticoid concentrations in high-ranking male rhesus macaques (*Macaca mulatta*) during the mating season. *Horm. Behav.* **97:** 5–13.

Miller, T. H., K. Clements, S. Ahn, C. Park, E. Hye Ji, and F. A. Issa. 2017. Social status–dependent shift

in neural circuit activation affects decision making. *J. Neurosci.* **37:** 2137–2148.

Mondelli, V., A. Cattaneo, M. B. Murri, M. Di Forti, R. Handley, N. Hepgul, A. Miorelli, S. Navari, A. S. Papadopoulos, K. J. Aitchison *et al.* 2011. Stress and inflammation reduce brain-derived neurotrophic factor expression in first-episode psychosis: a pathway to smaller hippocampal volume. *J. Clin. Psychiatry* **72:** 1677–1684.

Mudd, A. T., K. Berding, M. Wang, S. M. Donovan, and R. N. Dilger. 2017. Serum cortisol mediates the relationship between fecal *Ruminococcus* and brain *N*-acetylaspartate in the young pig. *Gut Microbes* **8:** 589–600.

Mudra Rakshasa, A., and M. T. Tong. 2020. Making "good" choices: Social isolation in mice exacerbates the effects of chronic stress on decision making. *Front. Behav. Neurosci.* **14:** 81.

Ntranos, A., and P. Casaccia. 2018. The microbiome-gut-behavior axis: Crosstalk between the gut microbiome and oligodendrocytes modulates behavioral responses. *Neurother. J. Am. Soc. Exp. Neurother.* **15:** 31–35.

Ochoa-Repáraz, J., D. W. Mielcarz, S. Begum-Haque, and L. H. Kasper. 2011. Gut, bugs, and brain: role of commensal bacteria in the control of central nervous system disease. *Ann. Neurol.* **69:** 240–247.

Oksanen, A. J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos *et al.* 2017. Vegan: community ecology package. R package. [Online]. R Foundation for Statistical Computing, Vienna. Available: https://cran.r-project.org/web/packages/vegan/index.html [2022, January 23].

Oliveira, R. F., J. F. Silva, and J. M. Simoes. 2011. Fighting zebrafish: characterization of aggressive behavior and winner-loser effects. *Zebrafish* **8:** 73–81.

Orr, S. A., S. Ahn, C. Park, T. H. Miller, M. Kassai, and F. A. Issa. 2021. Social experience regulates endocannabinoids modulation of zebrafish motor behaviors. *Front. Behav. Neurosci.* **15:** 668589.

Petrosus, E., E. B. Silva, D. J. Lay, and S. D. Eicher. 2018. Effects of orally administered cortisol and norepinephrine on weanling piglet gut microbial populations and *Salmonella* passage. *J. Anim. Sci.* **96:** 4543–4551.

Raison, C. L., and A. H. Miller. 2003. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am. J. Psychiatry* **160:** 1554–1565.

R Development Core Team. 2022. R: a language and environment for statistical computing. [Online]. R Foundation for Statistical Computing, Vienna. Available: http://www.R-project.org [2022, January 23].

Reardon, S. 2014. Gut-brain link grabs neuroscientists. *Nature* **515:** 175–177.

Rodríguez, I., B. Novoa, and A. Figueras. 2008. Immune response of zebrafish (*Danio rerio*) against a newly isolated bacterial pathogen *Aeromonas hydrophila*. *Fish Shellfish Immunol.* **25:** 239–249.

Roeselers, G., E. K. Mittge, W. Z. Stephens, D. M. Parichy, C. M. Cavanaugh, K. Guillemin, and J. F. Rawls. 2011. Evidence for a core gut microbiota in the zebrafish. *ISME J.* **5:** 1595–1608.

Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4:** e2584.

Rolig, A. S., R. Parthasarathy, A. R. Burns, B. J. M. Bohannan, and K. Guillemin. 2015. Individual members of the microbiota disproportionately modulate host innate immune responses. *Cell Host Microbe* **18:** 613–620.

Rolig, A. S., E. G. Sweeney, L. E. Kaye, M. D. Desantis, A. Perkins, A. V. Banse, M. K. Hamilton, and K. Guillemin. 2018. A bacterial immunomodulatory protein with lipocalin-like domains facilitates host-bacteria mutualism in larval zebrafish. *eLife* **7:** e37172.

Sayah, R. S., J. B. Kaneene, Y. Johnson, and R. A. Miller. 2005. Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. *Appl. Environ. Microbiol.* **71:** 1394–1404.

Schloss, P. D. 2021. Amplicon sequence variants artificially split bacterial genomes into separate clusters. *mSphere* **6:** e00191-21.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson *et al.* 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75:** 7537–7541.

Scott, E., M. S. Brewer, A. L. Peralta, and F. A. Issa. 2024. The effects of social experience on host gut microbiome in male zebrafish (*Danio rerio*). [Online]. GitHub. Available: https://github.com/PeraltaLab/ZebrafishMicrobiomes_SocialStatus [2024, January 23].

Sgritta, M., S. W. Dooling, S. A. Buffington, E. N. Momin, M. B. Francis, R. A. Britton, and M. Costa-Mattioli. 2019. Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. *Neuron* **101:** 246–259.e6.

Shanahan, F., T. S. Ghosh, and P. W. O'Toole. 2021. The healthy microbiome: What is the definition of a healthy gut microbiome? *Gastroenterology* **160:** 483–494.

Sharon, G., N. J. Cruz, D.-W. Kang, M. J. Gandal, B. Wang, Y.-M. Kim, E. M. Zink, C. P. Casey, B. C. Taylor, C. J. Lane *et al.* 2019. Human gut microbiota

from autism spectrum disorder promote behavioral symptoms in mice. *Cell* **177:** 1600–1618.e17.

Sharon, G., T. R. Sampson, D. H. Geschwind, and S. K. Mazmanian. 2016. The central nervous system and the gut microbiome. *Cell* **167:** 915–932.

Shin, N.-R., T. W. Whon, and J.-W. Bae. 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* **33:** 496–503.

Shreiner, A. B., J. Y. Kao, and V. B. Young. 2015. The gut microbiome in health and in disease. *Curr. Opin. Gastroenterol.* **31:** 69–75.

Skonieczna-Żydecka, K., W. Marlicz, A. Misera, A. Koulaouzidis, and I. Łoniewski. 2018. Microbiome—the missing link in the gut-brain axis: focus on its role in gastrointestinal and mental health. *J. Clin. Med.* **7:** 521.

Stagaman, K., T. J. Sharpton, and K. Guillemin. 2020. Zebrafish microbiome studies make waves. *Lab Anim.* **49:** 201–207.

Stefan, K. L., M. V. Kim, A. Iwasaki, and D. L. Kasper. 2020. Commensal microbiota modulation of natural resistance to virus infection. *Cell* **183:** 1312–1324.e10.

Stephens, W. Z., A. R. Burns, K. Stagaman, S. Wong, J. F. Rawls, K. Guillemin, and B. J. M. Bohannan. 2016. The composition of the zebrafish intestinal microbial community varies across development. *ISME J.* **10:** 644–654.

Stressmann, F. A., J. Bernal-Bayard, D. Perez-Pascual, B. Audrain, O. Rendueles, V. Briolat, S. Bruchmann, S. Volant, A. Ghozlane, S. Häussler *et al.* 2021. Mining zebrafish microbiota reveals key community-level resistance against fish pathogen infection. *ISME J.* **15:** 702–719.

Ström, K., J. Sjögren, A. Broberg, and J. Schnürer. 2002. *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid. *Appl. Environ. Microbiol.* **68:** 4322–4327.

Sylvia, K. E., and G. E. Demas. 2018. A gut feeling: microbiome-brain-immune interactions modulate social and affective behaviors. *Horm. Behav.* **99:** 41–49.

Tamana, S. K., H. M. Tun, T. Konya, R. S. Chari, C. J. Field, D. S. Guttman, A. B. Becker, T. J. Moraes, S. E. Turvey, P. Subbarao *et al.* 2021. Bacteroides-dominant gut microbiome of late infancy is associated with enhanced neurodevelopment. *Gut Microbes* **13:** 1–17.

Tan, L. Y., X. Y. Yeo, H.-G. Bae, D. P. S. Lee, R. C. Ho, J. E. Kim, D.-G. Jo, and S. Jung. 2021. Association of gut microbiome dysbiosis with neurodegeneration: Can gut microbe-modifying diet prevent or alleviate the symptoms of neurodegenerative diseases? *Life* **11:** 698.

Tang, Q., G. Jin, G. Wang, T. Liu, X. Liu, B. Wang, and H. Cao. 2020. Current sampling methods for gut microbiota: a call for more precise devices. *Front. Cell. Infect. Microbiol.* **10:** 151.

Teles, M. C., and R. F. Oliveira. 2016. Quantifying aggressive behavior in zebrafish. *Methods Mol. Biol.* **1451:** 293–305.

Teles, M. C., S. J. Dahlbom, S. Winberg, and R. F. Oliveira. 2013. Social modulation of brain monoamine levels in zebrafish. *Behav. Brain Res.* **253:** 17–24.

van der Meulen, T. A., H. Harmsen, H. Bootsma, F. Spijkervet, F. Kroese, and A. Vissink. 2016. The microbiome-systemic diseases connection. *Oral Dis.* **22:** 719–734.

Ventriglia, M., R. Zanardini, C. Bonomini, O. Zanetti, D. Volpe, P. Pasqualetti, M. Gennarelli, and L. Bocchio-Chiavetto. 2013. Serum brain-derived neurotrophic factor levels in different neurological diseases. *BioMed. Res. Int.* **2013:** 901082.

Warner, B. B. 2019. The contribution of the gut microbiome to neurodevelopment and neuropsychiatric disorders. *Pediatr. Res.* **85:** 216–224.

Weitekamp, C. A., J. Nguyen, and H. A. Hofmann. 2017. Neuromolecular regulation of aggression differs by social role during joint territory defense. *Integr. Comp. Biol.* **57:** 631–639.

Wu, T., L. Yang, J. Jiang, Y. Ni, J. Zhu, X. Zheng, Q. Wang, X. Lu, and Z. Fu. 2018. Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alterations in rats. *Life Sci.* **192:** 173–182.

Yan, Q., C. J. van der Gast, and Y. Yu. 2012. Bacterial community assembly and turnover within the intestines of developing zebrafish. *PLoS One* **7:** e30603.

Yilmaz, P., L. W. Parfrey, P. Yarza, J. Gerken, E. Pruesse, C. Quast, T. Schweer, J. Peplies, W. Ludwig, and F. O. Glöckner. 2014. The SILVA and "All-species living tree project (LTP)" taxonomic frameworks. *Nucleic Acids Res.* **42:** D643–D648.

Zhang, J., L. Ma, L. Chang, Y. Pu, Y. Qu, and K. Hashimoto. 2020. A key role of the subdiaphragmatic vagus nerve in the depression-like phenotype and abnormal composition of gut microbiota in mice after lipopolysaccharide administration. *Transl. Psychiatry* **10:** 186.