The effects of social experience on host gut microbiome in male zebrafish (*Danio rerio*)

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

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

*Corresponding authors:

Dr. Fadi A. Issa, East Carolina University, Department of Biology, 101 E 10th St., Greenville, NC, Email: issaf14@ecu.edu

Dr. Ariane Peralta, East Carolina University, Department of Biology, 101 E 10th St., Greenville, NC, Email: peraltaa@ecu.edu

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 chronic 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 two weeks of social interactions changed the composition of zebrafish gut microbiome by comparing gut bacterial composition, diversity and relative abundance among 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 control group-housed 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.

IMPORTANCE

Disruptions in the gut microbiome negatively impact various systems in the body. Recently, the gut microbiome has been associated with neurological deficits in both behavioral and neurodegenerative disorders. Given the increasing prevalence in diagnosis of both neurological disease and behavioral disorders, researching the link between social behaviors and the gut microbiome is critical to better understand how the gut and the brain communicate during healthy and diseased states. Our research findings demonstrate the effects of social dominance and chronic stress on host gut microbiome composition. By identifying variations in bacterial relative abundance based on social experience and associated stress, a broader understanding of *pathogenic* (e.g., Enterobacteriaceae*, Aeromonas*) versus commensal communities (e.g., *Exiguobacterium, Cetobacterium*) and related host physiology can be inferred.

INTRODUCTION

The gut microbiome plays key roles in biochemical functions in vertebrates. Gut bacterial metabolism has been linked to many systemic diseases in humans and influences numerous immune system pathways (1–5). Additionally, commensal bacteria within the gut microbiome serves as a line of defense to viral infections through interferon signaling (6). Along with these known functions of the gut biome, it has recently been linked to affecting brain function in the context of neurodevelopmental (7, 8), behavioral (9–11), and neurodegenerative disorders (12–14). For instance, the gut microbiome produces certain biochemicals (i.e., dopamine and oxytocin) that regulate neural nuclei involved in social behavior (15, 16). 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 wellbeing (17). Thus, understanding the mechanisms of disease onset and progression related to the gut microbiome is critical.

The typical gut microbiome consists of both commensal and pathogenic bacteria (18–20). 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 (21, 22). In Autism Spectrum Disorder mouse models, the abundance of commensal bacteria species, *Lactobacillus reuteri* is significantly reduced in comparison to wild type mice (15). Further, *Lactobacillus plantarum* has been used as a probiotic in zebrafish models to reduce stress-related behaviors and prevent stress-induced microbiome dysbiosis (23). *L. plantarum* was discovered to modulate anxiety related behavior through the GABAergic and serotonergic pathways (23). Additionally, gut bacterial composition influences host behavior through the modulation of Brain-Derived Neurotrophic Factor (BDNF) levels and serotonin metabolism (24). 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 (25, 26). In addition to influencing neurotransmitters levels, bacteria from the genera *Lactobacillus* can activate afferent neurons in the intestine that modulate pain sensation and actions of defensive behavior in response to stress (27). This evidence supports the notion that certain bacteria can influence the activity of neuromodulatory pathways responsible for social behavior. Collectively, these relationships between host gut bacteria and nervous system function support the notion that the gut microbiome regulates social activity by influencing neuromodulatory pathways responsible for social behavior.

Zebrafish social behavior has been studied extensively (28–32). When paired, zebrafish interact agonistically with conspecifics and quickly form stable dominance relationships consisting of dominant and submissive fish (28, 33). Once dominance forms, dominants have priority to food, shelter, and mates while submissive fish experience chronic stress (34). 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 (35). This is of particular importance because the effects of chronic social stress on organisms are detrimental and have been adversely linked to anxiety-like behavior relevant for survival in many social species (2, 36–40). Specifically, reduction of glucocorticoid signaling activity (caused by insufficient hormone availability) in chronic stress models has been associated with stress-related disorders (41). Interestingly, the gut microbiota has recently been thought to modulate downstream glucocorticoid receptors in the hippocampus resulting in behavioral abnormalities highlighting the relationship between stress and the gut microbiome (42). The stability of zebrafish social dominance and ease of quantifying their aggressive social activity provides 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 (43). Specifically, as embryonic zebrafish develop, the diversity of gut bacteria decreases significantly in terms of the number of operational taxonomic units (OTUs), which are clusters of bacteria that exhibit high sequence similarity of the 16S rRNA gene (44, 45). 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 (44). The adult zebrafish gut microbiome is composed primarily of bacteria within the Proteobacteria, Firmicutes, and Fusobacteria phyla (46). 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 (47). Additionally, in zebrafish, Proteobacteria including pathogenic genera *Vibrio* and *Plesiomona* were reduced in probiotic treated fish as opposed to commensal Firmicutes bacterial members (24). 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 chronically stressed submissive animals. We tested the hypothesis that the relative abundance, diversity, and overall composition of zebrafish gut microbiome is socially regulated. We compared gut microbiomes (based on amplicon sequencing of the 16S rRNA gene) among socially dominant, submissive, socially isolated, and group-housed communal fish. Results from this work improve our understanding of how social aggression and stress impact host gut microbiome and may help future studies to understand the gut-brain axis and how the onset of behavioral disorders affect gut dysbiosis.

Experimental Methods

Pairing and behavioral analysis:

Male zebrafish (n=24) were isolated in individual tanks for 7 days to minimize the effects of prior social experience. Then, 12 fish were randomly paired and placed into new tanks while 6 fish remained in isolation, and the remaining 6 fish were placed into one new tank to serve as communal controls. Over a period of 14 days, the 6 zebrafish pairs self-established rank and their behavior was monitored and recorded over a course of 14 days (2 weeks). Behavior was observed visually, and written observations were made regarding dominant and subordinate behavior among the paired and communal fish. Specifically, the number of attacks and retreats by each fish were recorded during a 5-minute observation period 3 times a week. Additionally, tank locations of each fish (top vs. bottom of tank) were monitored and recorded as stressed or subordinate fish tend to stay lower in the tank and more aggressive/dominant fish claim the top of the tank. All animal methods and protocols were approved by East Carolina University's Institutional Animal Care and Use Committee (IACUC) AUP# D320b.

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. After pairing of fish after the 7-day isolation period, fecal samples were collected on day 0 (day of pairing), day 7 (one 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 evolves as dominance was established.

On days of fecal collection, fish were removed from tanks and each fish was placed into individual containers 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. 2-4 hours after separation and feeding each fish, fecal samples were collected. Fecal samples from each fish were extracted using sterile micropipette tips and were placed into 500 microliters of DNAse free water. 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 sequencing of the highly conserved 16S rRNA gene (48). Following fecal sample collection, DNA extractions were performed on each sample using the PowerLyzer PowerSoil DNA protocol. Approximately 0.02g of feces were collected from each fish for extractions. This extracted genomic DNA was used as the template for PCR reactions, where barcoded primers (515F-806RB) were used to target the V4 region of the 16S rRNA gene (48, 49). Each fecal sample was run in triplicate PCR reactions and then combined and purified using the Axygen® AxyPrep magnetic bead purification kit (Corning Life Sciences). After successful cleanup, DNA concentrations (ng/mL) from each sample were quantified using the Quant-iT dsDNA HS (high sensitivity) assay (Thermo Scientific, Waltham, Massachusetts, USA). Recorded DNA concentrations (ng/mL) using HS measurements were converted to the final DNA concentration (ng/µl) and then dilutions using PCR grade water were performed to ensure all samples added to the final pooled product were the same mass (ng). PCR products were pooled in equimolar concentrations and sequenced on an Illumina MiSeq platform using paired-end reads (Illumina Reagent Kit v2, 500 reaction kit) at the Center for Genomics and Bioinformatics at Indiana University.

We processed raw 16S rRNA gene sequences using a standard mothur pipeline (v1.40.1) (50, 51). 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 (v.128) (52), and we removed chimeric sequences using the VSEARCH algorithm (53). We divided sequences based on taxonomic class and binned into operational taxonomic units (OTUs) with a 97% sequence similarity level, and we classified OTUs using the SILVA rRNA database (v128). 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 which can result in over-inflated number of ASVs (54). The broadscale community composition patterns are robust when employing an OTU-based approach (55).

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 Chao1 OTU richness using the estimateR() function and Shannon diversity using the diversity() function in the vegan package (56), and 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 (PCoA). Finally, genus level compositions with relative abundances greater than 0.05 were plotted after OTU table was rarefied to 6,000 observations.

Statistical Analyses:

All data analyses were completed using the R statistical environment (R v4.2.0, R Core Development Team 2022; R Studio v2022.07.1). Using the lmer() function from the lmerTest package (57), a linear mixed effects model with 'Day' and 'Social Status' as fixed effects was used to analyze bacterial diversity metrics Chao1 OTU richness, Shannon diversity (H'), and

209 Simpson's evenness. We ran a permutational multivariate analysis of variance (PERMANOVA)
210 based on the Bray-Curtis dissimilarity of bacterial community composition to determine the
211 extent that 'Social Status' and 'Day' and the interaction explained bacterial community
212 composition. We next identified bacterial species that represented each treatment ('Social
213 Status x 'Day') for bacterial taxa with a relative abundance greater than 0.05 when summed
214 across all samples. We performed the PERMANOVA using the adonis() function from the vegan
215 package (56) and the indval() function in the indicspecies package (58).
216
217 **Data Availability**
218 All code and data used in this study are in a public GitHub repository
219 (https://github.com/PeraltaLab/ZebrafishMicrobiomes_SocialStatus) and NCBI SRA BioProject
220 PRJNA925886.
221
222
223
224
225
226
227
228
229
230
231
232
233
234

RESULTS

Bacterial species diversity and community composition

We compared gut microbiomes of zebrafish of different social statuses. Bacterial OTU richness (Fig. 1) was similar across 'Social Status' and 'Day', while the interaction of 'Social Status' and 'Day' but not the main effects influenced Shannon diversity (Fig. 2) and Simpson's evenness increased from Day 7 to Day 14 (Fig. 3) (Table S1). While statistical significance was detected, the linear model explained a relatively low amount of variation (richness: adjusted R^2= 0.1049, p-value=0.0645; diversity: adjusted R^2=0.1089, p-value=0.0585; evenness: adjusted R^2=0.1547, p-value=0.0171) (Table S1). Subordinate data points plotted similarly to isolate data points during the isolation period (IP) before any pairing or rank establishment. Additionally, dominants data points trended similarly to those of communal animals during the isolation period. These trends were observed for richness (Fig. 1), diversity (Fig. 2), and evenness (Fig. 3).

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. 4, Table S2). The bacterial gut microbiomes of subordinate and communal fish tended to group together while the gut microbiomes of dominant fish were distinct from all other groups, except for Day 14 which grouped by the gut microbiomes of isolated fish on Day 0 (Fig. 4). The isolation period (IP) for subordinate, communal, and isolated fish resulted in similar gut microbiomes (diamond symbols). For subordinate and isolated fish (blue and gray symbols), the gut microbiomes changed over time more than the dominant and communal gut microbiomes (red and purple symbols) (Fig. 4).

Figure 1

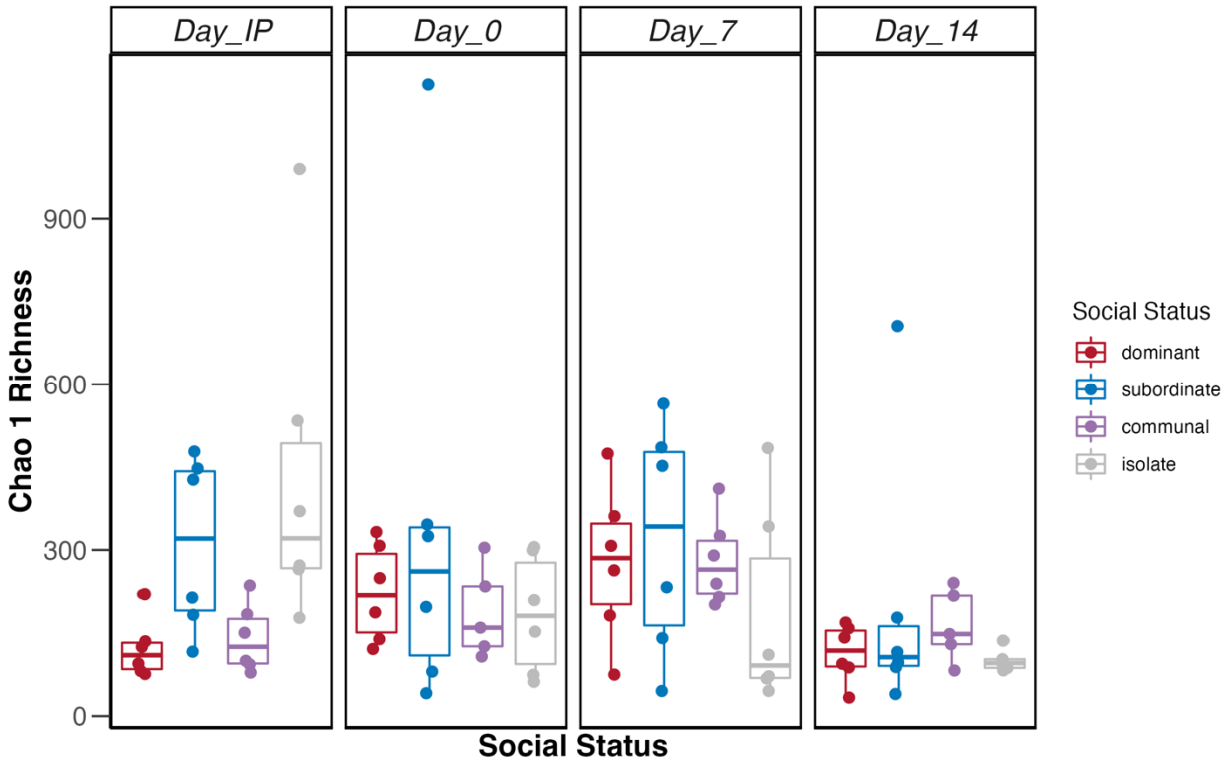
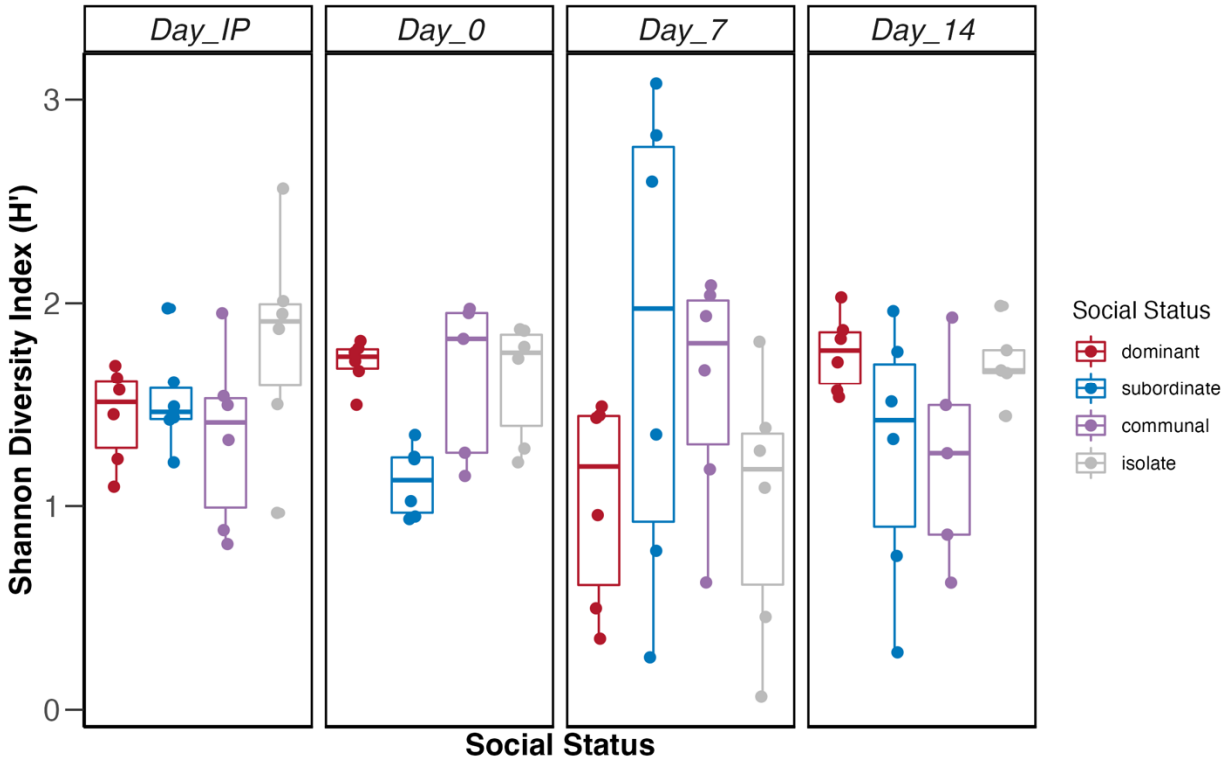


Figure 1. Boxplots representing bacterial Chao 1 richness in individuals from dominant (red), subordinate (blue), communal (purple), and isolate (gray) animals throughout the isolation period (Day IP) representing collection before pairing and pairing period (Day 0, Day 7, Day 14). The boxplot is a visual representation of 5 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. Summary of statistical output in Appendix S1: Table S1A.

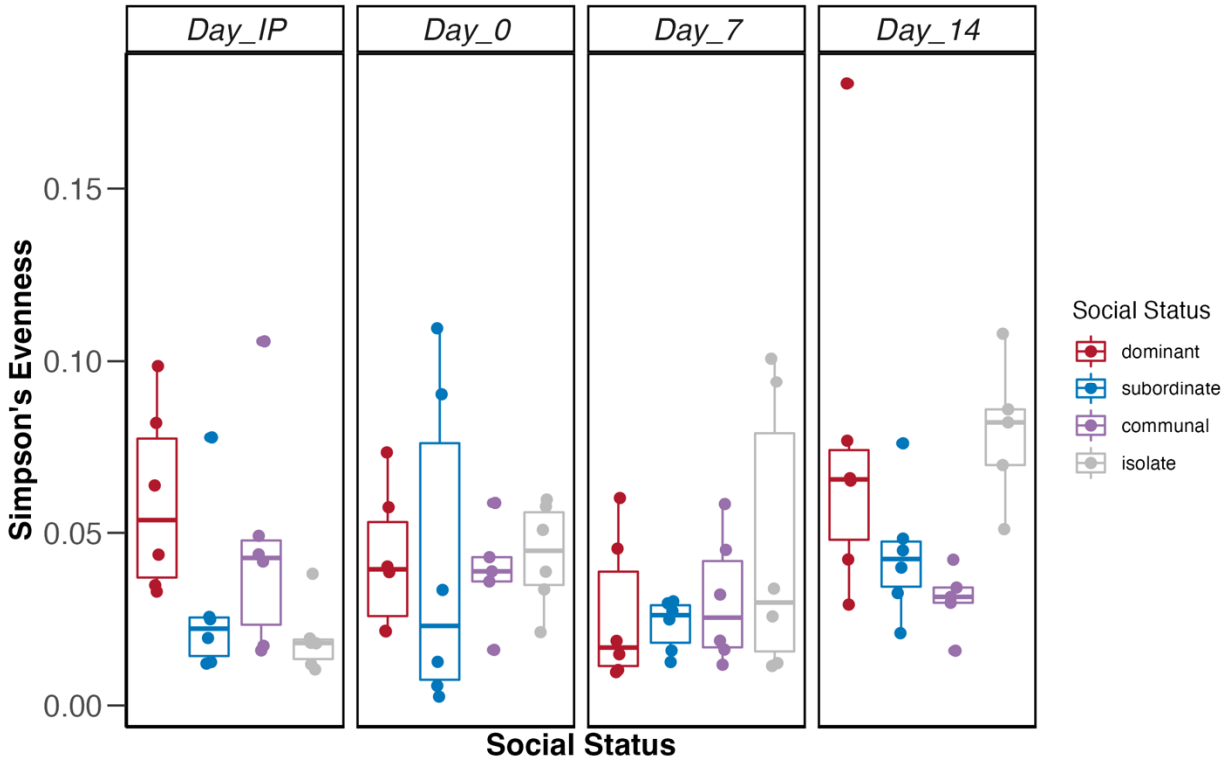
271 Figure 2



272

273 Figure 2. Boxplots representing bacterial Shannon diversity index in individuals from dominant
274 (red), subordinate (blue), communal (purple), and isolate (gray) animals throughout the isolation
275 period (Day IP) representing collection before pairing and pairing period (Day 0, Day 7, Day 14).
276 The boxplot is a visual representation of 5 key summary statistics: the median, the 25% and
277 75% percentiles, and the whiskers which represent the feasible range of the data as determined
278 by 1.5 × the interquartile range. Symbols represent individual raw data points from six replicate
279 samples. Summary of statistical output in Appendix S1: Table S1B.

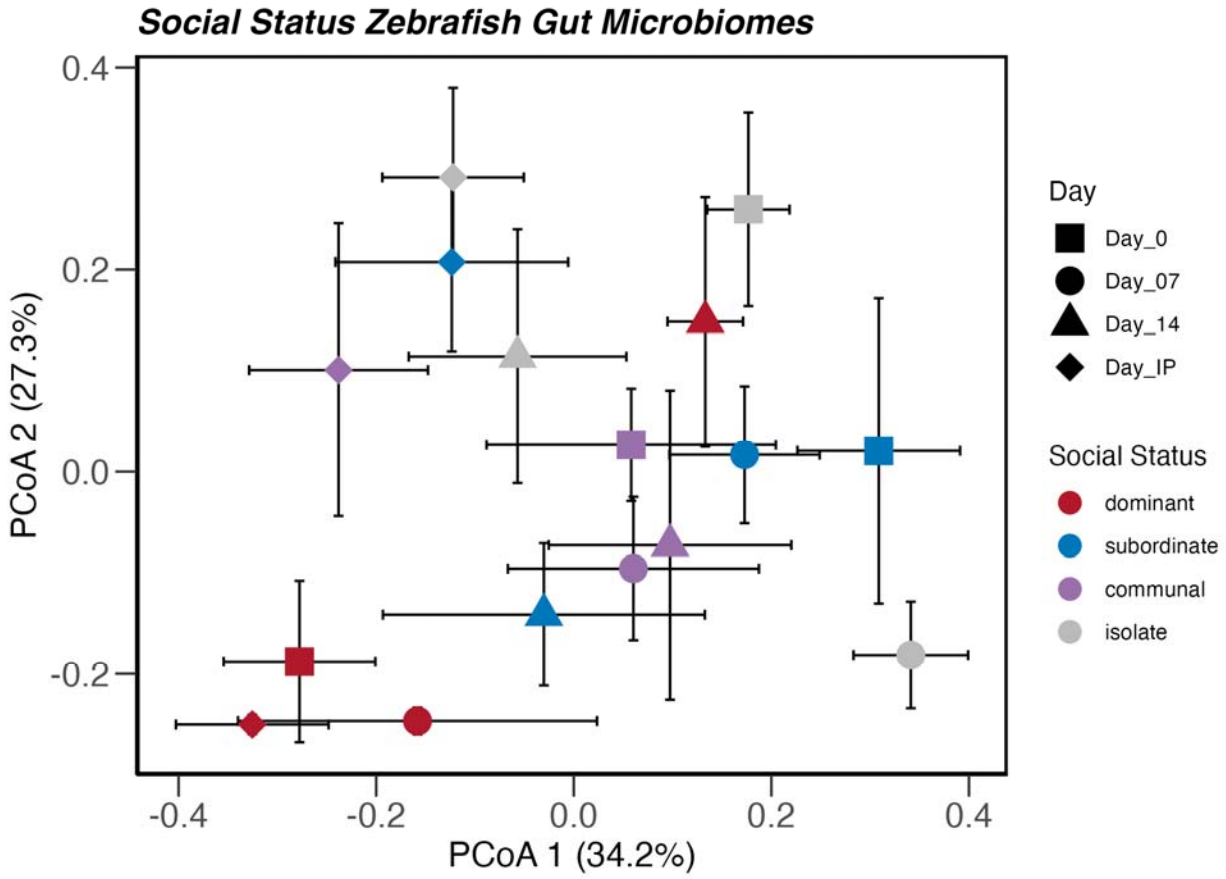
280 Figure 3



281

282 Figure 3. Boxplots representing bacterial Simpson's diversity index in individuals from dominant
283 (red), subordinate (blue), communal (purple), and isolate (gray) animals throughout the isolation
284 period (Day IP) representing collection before pairing and pairing period (Day 0, Day 7, Day 14).
285 The boxplot is a visual representation of 5 key summary statistics: the median, the 25% and
286 75% percentiles, and the whiskers which represent the feasible range of the data as determined
287 by 1.5 × the interquartile range. Symbols represent individual raw data points from six replicate
288 samples. Summary of statistical output in Appendix S1: Table S1B.

289 Figure 4



290

291 Figure 4. Ordination based on a Principal Coordinates Analysis depicting bacterial community
292 composition according to fertilization and hydrology. Symbols are colored according to social
293 status (dominant=red, subordinate=blue, communal=purple, and isolate=gray) and shapes
294 represent day of pairing (Day 0=square, Day 7=circle, Day 14=triangle, Day IP=diamond). The
295 centroid and standard error bars (along axes 1 and 2) were calculated for six replicate plots.
296 Summary of statistical output in Appendix S1: Table S2.

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 isolation period, and unclassified taxa from the families Planctomycetacea, Caldilineaceae, 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 isolation period, 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 S3).

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 14 of pairing (Fig. S1). We observed a reduction in pathogenic genera that were consistently high in relative abundance in non-dominant social status groups and throughout the pairing period. Specifically, *Aeromonas* and unclassified Enterobacteriaceae relative abundance was lower in dominant individuals on day 7 and 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 were 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 (59–61). 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 activity and behavioral disorders. Here, we studied whether social aggression and chronic stress influence gut microbiome content in zebrafish. We showed that while there were no significant differences in gut microbiome diversity or richness among 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 (15). These similarities along with findings in other studies examining how probiotic treatment alters behavioral activity in socially stressed individuals lends further credence of the strong relationship between the gut-brain axis. Specifically, chronically stressed zebrafish that were treated with probiotics, experienced a complete reversal and rescue of their anxiety-like behavior (23). This supports a microbiome mediated behavior hypothesis in which changes to the gut microbiome affects associated behaviors related to stress and anxiety. However, in those studies, both the composition and diversity of the gut microbiome directly affects behavior whereas we found that only bacterial composition was affected by social status.

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 the chronic stress that accompanies social submission or isolation may directly impact bacterial composition. Additionally, it seems that social dominance and aggression promote an increased presence of commensal bacterial genera. Previous research

in autism models show an inverse relationship between the gut and behavior, as individuals born with gut microbiota dysbiosis are predisposed to social deficits and autism-like behaviors (62).

One of the questions we sought to answer in this study 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 isolation period (Day IP), this hypothesis is not supported in this model. 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 (23). Because of these protective qualities, the *Lactobacillus* genus is often used as a probiotic treatment in both zebrafish and mouse models to rescue stressed phenotypes (15, 23). Interestingly, the *Lactobacillus* genus was not present in the taxonomic analysis of this experiment. This is likely due to *Lactobacillus* species being a transient to the gut microbiome, as they primarily colonize the host GI tract from food intake and the oral cavity (63–65). The only genus of bacteria found from the Bacteroidetes phylum was *Chryseobacterium spp. a*nd was primarily found in communal fish on day 7 (Table S3). While this genus doesn't 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 (66). Interestingly, this bacterial genus was only present in low relative 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 (67, 68). 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 (69). While the bacterial genus *Exiguobacterium* was present in socially submissive and isolated zebrafish, this OTU only increased in relative abundance in dominant individuals after a week of pairing (Fig. S1). The absence of the bacterial genus *Exiguobacterium* in communal animals should be noted, which may be due to the heterogeneity among mid-ranked members living in a communal setting whereby individuals may dominate some low-ranking animals while being submissive to high-ranking opponents.

Given the high percent 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 pathogenic genera are waterborne. However, the varying relative abundance of certain pathogenic bacteria like *Aeromonas,* unclassified Enterobacteriaceae*, Staphylococcus, Vibrio,* and *Pseudomonas spp.* in the community composition is noteworthy. *Aeromonas and* unclassified Enterobacteriaceae were the dominant pathogenic genera in all groups and at all time points during pairing, but several pathogenic genera were primarily seen in subordinate, isolate, and even communal individuals (Fig. S1). Many of these genera have been used in zebrafish infection models. A model of *Staphylococcus* infection in zebrafish has been studied, and the interactions between *Staphylococcus aureus* and other bacteria in the gut to create infection (70). This study specifically examined the immune response of the fish in response to this infection, but it is important to note the pathogenesis associated with *Staphylococcus* infection. 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 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* in dominant individuals, indicator species analysis also revealed the presence of *Achromobacter* and *Paracoccus* in dominant individuals during the isolation period (Table S3). *Achromobacter* and *Paracoccus* are genera of bacteria with pathogenic properties in both mammals and fish (71–73). While these genera are present in dominant individuals prior to pairing, they decrease in relative abundance post-pairing which supports 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* genera. 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 by influencing the release of reactive oxygen species (ROS) and nitrogen free radicals triggering an immune response accompanied by massive inflammatory reaction and increased mortality rates (74). While our results do not specify the species of *Aeromonas* present, higher relative abundances of this genus may result in pathogenesis in chronically stressed animals as well (e.g., socially submissive or isolates).

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 chronically stressed animals (e.g., submissive, and socially isolated) showed an increase in pathogenic bacteria and a decline in commensal bacterial genera. The interconnectivity between gut function and cognitive processes are likely to mediated bi-directionally via afferent and efferent neural and neuroendocrine signaling pathways (75).

427 Although our results suggest that the physiological mechanisms underlying the shift in microbial
428 composition is slow acting and require one to two weeks for their biological manifestation, prior
429 reports have shown that acute exposure to stress can impact the gut microbiota community
430 composition by changing the relative proportions of the main microbiota phyla (76). Moreover,
431 experimental manipulations of gut microbiota affect stress levels and anxiety-like behavior by
432 influencing the function of the hypothalamic-pituitary-adrenal stress axis (77). Indeed, social
433 stress induced increase in blood cortisol levels correlates with changes in the diversity of the
434 mammalian gut microbiome, including the abundance of lactic acid and pathogenic bacteria (76,
435 78). In addition, chronic injections of glucocorticoids can have positive and negative impact on
436 the presence of specific microbial taxa, which consequently affect host metabolism (79–81).
437 Thus, it is likely that chronic stress induced by either prolonged social isolation or subordination
438 likely to have increased cortisol levels in isolated and submissive zebrafish with substantial
439 increase in expression of gut pathogenic bacteria.

440 How these status-dependent changes in gut bacterial composition feedback to influence
441 brain function remains poorly understood. Given that zebrafish experiencing induced stress
442 exhibit changes in gut microbiome composition and brain gene expression (24), future
443 experiments testing this relationship in zebrafish in the context of social dominance and stress
444 will be highly informative. More specifically, it would be of interest to determine whether
445 transcriptional changes in the brains of dominant and subordinate fish can be linked to changes
446 in bacterial abundance in the host gut. Indeed, a large body of work has shown that
447 dopaminergic, GABAergic, and serotoninergic pathways implicated in social behavior are highly
448 plastic, but whether the plasticity is mediated by feedback regulation of gut microbiome to
449 influence motivational brain centers involved in social aggression remains an open question (32,
450 82–86).
451

452 **ACKNOWLEDGMENTS**

453 We thank Dr. Katie Clements for her assistance in the early design of behavioral experiments.
454 This work was supported by the National Science Foundation grant (#1754513) to F.A.I. and
455 grant (#1845845) to A.L.P..

456

457 **REFERENCES**

458 1. Kinross JM, Darzi AW, Nicholson JK. 2011. Gut microbiome-host interactions in health and
459 disease. Genome medicine 3:14.

460 2. Foster JA, Rinaman L, Cryan JF. 2017. Stress & the gut-brain axis: Regulation by the
461 microbiome. Neurobiology of stress 7:124–136.

462 3. Lazar V, Ditu L-M, Pircalabioru GG, Gheorghe I, Curutiu C, Holban AM, Picu A, Petcu L,
463 Chifiriuc MC. 2018. Aspects of Gut Microbiota and Immune System Interactions in
464 Infectious Diseases, Immunopathology, and Cancer. Frontiers in immunology 9:1830.

465 4. Geuking MB, Köller Y, Rupp S, McCoy KD. 2014. The interplay between the gut microbiota
466 and the immune system. Gut microbes 5:411–418.

467 5. van der Meulen TA, Harmsen H, Bootsma H, Spijkervet F, Kroese F, Vissink A. 2016. The
468 microbiome-systemic diseases connection. Oral diseases 22:719–734.

469 6. Stefan KL, Kim M V, Iwasaki A, Kasper DL. 2020. Commensal Microbiota Modulation of
470 Natural Resistance to Virus Infection. Cell 183:1312-1324.e10.

471 7. Tamana SK, Tun HM, Konya T, Chari RS, Field CJ, Guttman DS, Becker AB, Moraes TJ,
472 Turvey SE, Subbarao P, Sears MR, Pei J, Scott JA, Mandhane PJ, Kozyrskyj AL. 2021.
473 Bacteroides-dominant gut microbiome of late infancy is associated with enhanced
474 neurodevelopment. Gut microbes 13:1–17.

475 8. Warner BB. 2019. The contribution of the gut microbiome to neurodevelopment and
476 neuropsychiatric disorders. Pediatric research 85:216–224.

477 9. Ntranos A, Casaccia P. 2018. The Microbiome-Gut-Behavior Axis: Crosstalk Between the
478 Gut Microbiome and Oligodendrocytes Modulates Behavioral Responses.
479 Neurotherapeutics : the journal of the American Society for Experimental
480 NeuroTherapeutics 15:31–35.

481 10. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. 2016. The Central Nervous
482 System and the Gut Microbiome. Cell 167:915–932.

483 11. Christian LM, Galley JD, Hade EM, Schoppe-Sullivan S, Kamp Dush C, Bailey MT. 2015.
484 Gut microbiome composition is associated with temperament during early childhood. Brain,
485 behavior, and immunity 45:118–127.

486 12. Tan LY, Yeo XY, Bae H-G, Lee DPS, Ho RC, Kim JE, Jo D-G, Jung S. 2021. Association
487 of Gut Microbiome Dysbiosis with Neurodegeneration: Can Gut Microbe-Modifying Diet
488 Prevent or Alleviate the Symptoms of Neurodegenerative Diseases? Life (Basel,
489 Switzerland) 11.

490 13. Ghaisas S, Maher J, Kanthasamy A. 2016. Gut microbiome in health and disease: Linking
491 the microbiome-gut-brain axis and environmental factors in the pathogenesis of systemic
492 and neurodegenerative diseases. Pharmacology & therapeutics 158:52–62.

493 14. Chandra S, Alam MT, Dey J, Sasidharan BCP, Ray U, Srivastava AK, Gandhi S, Tripathi
494 PP. 2020. Healthy Gut, Healthy Brain: The Gut Microbiome in Neurodegenerative
495 Disorders. Current topics in medicinal chemistry 20:1142–1153.

496 15. Sgritta M, Dooling SW, Buffington SA, Momin EN, Francis MB, Britton RA, Costa-Mattioli
497 M. 2019. Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in
498 Mouse Models of Autism Spectrum Disorder. Neuron 101:246-259.e6.

499 16. Lynch JB, Hsiao EY. 2019. Microbiomes as sources of emergent host phenotypes.
500 Science (New York, NY) 365:1405–1409.

501 17. Hughes HK, Rose D, Ashwood P. 2018. The Gut Microbiota and Dysbiosis in Autism
502 Spectrum Disorders. Current neurology and neuroscience reports 18:81.

503 18. Cresci GA, Bawden E. 2015. Gut Microbiome: What We Do and Don't Know. Nutrition in
504 clinical practice : official publication of the American Society for Parenteral and Enteral
505 Nutrition 30:734–746.

506 19. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy
507 D. 2015. Role of the normal gut microbiota. World journal of gastroenterology 21:8787–
508 8803.

509 20. Kamada N, Chen GY, Inohara N, Núñez G. 2013. Control of pathogens and pathobionts by
510 the gut microbiota. Nature immunology 14:685–690.

511 21. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL. 2010. A
512 meta-analysis of cytokines in major depression. Biological psychiatry 67:446–457.

513 22. Ochoa-Repáraz J, Mielcarz DW, Begum-Haque S, Kasper LH. 2011. Gut, bugs, and brain:
514 role of commensal bacteria in the control of central nervous system disease. Annals of
515 neurology 69:240–247.

516 23. Davis DJ, Doerr HM, Grzelak AK, Busi SB, Jasarevic E, Ericsson AC, Bryda EC. 2016.
517 Lactobacillus plantarum attenuates anxiety-related behavior and protects against stress-
518 induced dysbiosis in adult zebrafish. Scientific reports 6:33726.

519 24. Borrelli L, Aceto S, Agnisola C, De Paolo S, Dipineto L, Stilling RM, Dinan TG, Cryan JF,
520 Menna LF, Fioretti A. 2016. Probiotic modulation of the microbiota-gut-brain axis and
521 behaviour in zebrafish. Scientific reports 6:30046.

522 25. Ventriglia M, Zanardini R, Bonomini C, Zanetti O, Volpe D, Pasqualetti P, Gennarelli M,
523 Bocchio-Chiavetto L. 2013. Serum brain-derived neurotrophic factor levels in different
524 neurological diseases. BioMed research international 2013:901082.

525 26. Mondelli V, Cattaneo A, Murri MB, Di Forti M, Handley R, Hepgul N, Miorelli A, Navari S,
526 Papadopoulos AS, Aitchison KJ, Morgan C, Murray RM, Dazzan P, Pariante CM. 2011.
527 Stress and inflammation reduce brain-derived neurotrophic factor expression in first-
528 episode psychosis: a pathway to smaller hippocampal volume. The Journal of clinical
529 psychiatry 72:1677–1684.

530 27. Chiu IM, Heesters BA, Ghasemlou N, Von Hehn CA, Zhao F, Tran J, Wainger B,
531 Strominger A, Muralidharan S, Horswill AR, Bubeck Wardenburg J, Hwang SW, Carroll
532 MC, Woolf CJ. 2013. Bacteria activate sensory neurons that modulate pain and
533 inflammation. Nature 501:52–57.

534 28. Oliveira RF, Silva JF, Simoes JM. 2011. Fighting zebrafish: characterization of aggressive
535 behavior and winner-loser effects. Zebrafish 8:73–81.

536 29. Clements KN, Miller TH, Keever JM, Hall AM, Issa FA. 2018. Social Status-Related
537 Differences in Motor Activity Between Wild-Type and Mutant Zebrafish. The Biological
538 Bulletin 235:71–82.

539 30. Orr SA, Ahn S, Park C, Miller TH, Kassai M, Issa FA. 2021. Social Experience Regulates
540 Endocannabinoids Modulation of Zebrafish Motor Behaviors. Frontiers in behavioral
541 neuroscience 15:668589.

542 31. Carver JJ, Carrell SC, Chilton MW, Brown JN, Yong L, Zhu Y, Issa FA. 2021. Nuclear
543 androgen and progestin receptors inversely affect aggression and social dominance in
544 male zebrafish (Danio rerio). Hormones and behavior 134:105012.

545 32. Teles MC, Dahlbom SJ, Winberg S, Oliveira RF. 2013. Social modulation of brain
546 monoamine levels in zebrafish. Behavioural brain research 253:17–24.

547 33. Teles MC, Oliveira RF. 2016. Quantifying Aggressive Behavior in Zebrafish. Methods in
548 molecular biology (Clifton, NJ) 1451:293–305.

549 34. Miller TH, Clements K, Ahn S, Park C, Hye Ji E, Issa FA. 2017. Social Status–Dependent
550 Shift in Neural Circuit Activation Affects Decision Making. The Journal of Neuroscience
551 37:2137–2148.

552 35. Sylvia KE, Demas GE. 2018. A gut feeling: Microbiome-brain-immune interactions
553 modulate social and affective behaviors. Hormones and behavior 99:41–49.

554 36. Chrousos GP, Gold PW. 1992. The concepts of stress and stress system disorders.
555 Overview of physical and behavioral homeostasis. JAMA 267:1244–1252.

556 37. Blanchard RJ, McKittrick CR, Blanchard DC. 2001. Animal models of social stress: effects
557 on behavior and brain neurochemical systems. Physiology & behavior 73:261–271.

558 38. Mudra Rakshasa A, Tong MT. 2020. Making “Good” Choices: Social Isolation in Mice
559 Exacerbates the Effects of Chronic Stress on Decision Making. Frontiers in behavioral
560 neuroscience 14:81.

561 39. Bartolomucci A, Palanza P, Sacerdote P, Panerai AE, Sgoifo A, Dantzer R, Parmigiani S.
562 2005. Social factors and individual vulnerability to chronic stress exposure. Neuroscience
563 and biobehavioral reviews 29:67–81.

564 40. Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, Elkhayat SI,
565 Bartels BK, Tien AK, Tien DH, Mohnot S, Beeson E, Glasgow E, Amri H, Zukowska Z,
566 Kalueff A V. 2009. Understanding behavioral and physiological phenotypes of stress and
567 anxiety in zebrafish. Behavioural brain research 205:38–44.

568 41. Raison CL, Miller AH. 2003. When not enough is too much: the role of insufficient
569 glucocorticoid signaling in the pathophysiology of stress-related disorders. The American
570 journal of psychiatry 160:1554–1565.

571 42. Luo Y, Zeng B, Zeng L, Du X, Li B, Huo R, Liu L, Wang H, Dong M, Pan J, Zheng P, Zhou
572 C, Wei H, Xie P. 2018. Gut microbiota regulates mouse behaviors through glucocorticoid
573 receptor pathway genes in the hippocampus. Translational psychiatry 8:187.

574 43. Stagaman K, Sharpton TJ, Guillemin K. 2020. Zebrafish microbiome studies make waves.
575 Lab animal 49:201–207.

576 44. Stephens WZ, Burns AR, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannan BJM.
577 2016. The composition of the zebrafish intestinal microbial community varies across
578 development. The ISME journal 10:644–654.

579 45. Chen W, Zhang CK, Cheng Y, Zhang S, Zhao H. 2013. A comparison of methods for
580 clustering 16S rRNA sequences into OTUs. PloS one 8:e70837.

581 46. Roeselers G, Mittge EK, Stephens WZ, Parichy DM, Cavanaugh CM, Guillemin K, Rawls
582 JF. 2011. Evidence for a core gut microbiota in the zebrafish. The ISME journal 5:1595–
583 1608.

584 47. Shin N-R, Whon TW, Bae J-W. 2015. Proteobacteria: microbial signature of dysbiosis in
585 gut microbiota. Trends in biotechnology 33:496–503.

586 48. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM,
587 Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. 2012. Ultra-high-
588 throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The
589 ISME journal 6:1621–1624.

590 49. Apprill A, McNally S, Parsons R, Weber L. 2015. Minor revision to V4 region SSU rRNA
591 806R gene primer greatly increases detection of SAR11 bacterioplankton . Aquatic
592 Microbial Ecology 75:129–137.

593 50. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a
594 dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence
595 data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79:5112–20.

596 51. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA,
597 Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Horn DJV, Weber
598 CF. 2009. Introducing Mothur: open-source, platform-independent, community-supported
599 software for describing and comparing microbial communities. Appl Environ Microbiol
600 75:7537–7541.

601 52. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO.
602 2013. The SILVA ribosomal RNA gene database project: improved data processing and
603 web-based tools. Nucleic Acids Research 41:D590–D596.

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

606 54. Schloss PD. 2021. Amplicon Sequence Variants Artificially Split Bacterial Genomes into
607 Separate Clusters. mSphere 6:e00191-21.

608 55. Glassman SI, Martiny JBH. 2018. Broadscale Ecological Patterns Are Robust to Use of
609 Exact Sequence Variants versus Operational Taxonomic Units. mSphere 3:e00148-18.

610 56. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR,
611 O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2019. vegan:
612 Community Ecology Package (2.5-5).

613 57. Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. lmerTest Package: Tests in Linear
614 Mixed Effects Models. Journal of Statistical Software 82:1–26.

615 58. De Caceres, M.; Jansen F. 2016. Indispecies: Relationship between species and groups of
616 sites. R package version 1.7.6 R Found. Stat. Comput., Vienna. 1.7.6 R.

617 59. Skonieczna-Żydecka K, Marlicz W, Misera A, Koulaouzidis A, Łoniewski I. 2018.
618 Microbiome-The Missing Link in the Gut-Brain Axis: Focus on Its Role in Gastrointestinal
619 and Mental Health. Journal of clinical medicine 7.

620 60. Reardon S. 2014. Gut-brain link grabs neuroscientists. Nature. England
621 https://doi.org/10.1038/515175a.

622 61. Flight MH. 2014. Neurodevelopmental disorders: the gut-microbiome-brain connection.
623 Nature reviews Neuroscience 15:65.

624 62. Sharon G, Cruz NJ, Kang D-W, Gandal MJ, Wang B, Kim Y-M, Zink EM, Casey CP, Taylor
625 BC, Lane CJ, Bramer LM, Isern NG, Hoyt DW, Noecker C, Sweredoski MJ, Moradian A,
626 Borenstein E, Jansson JK, Knight R, Metz TO, Lois C, Geschwind DH, Krajmalnik-Brown
627 R, Mazmanian SK. 2019. Human Gut Microbiota from Autism Spectrum Disorder Promote
628 Behavioral Symptoms in Mice. Cell 177:1600-1618.e17.

629 63. Walter J. 2008. Ecological role of lactobacilli in the gastrointestinal tract: implications for
630 fundamental and biomedical research. Applied and environmental microbiology 74:4985–
631 4996.

632 64. Dal Bello F, Hertel C. 2006. Oral cavity as natural reservoir for intestinal lactobacilli.
633 Systematic and applied microbiology 29:69–76.

634 65. Reuter G. 2001. The Lactobacillus and Bifidobacterium microflora of the human intestine:
635 composition and succession. Current issues in intestinal microbiology 2:43–53.

636 66. Stressmann FA, Bernal-Bayard J, Perez-Pascual D, Audrain B, Rendueles O, Briolat V,
637 Bruchmann S, Volant S, Ghozlane A, Häussler S, Duchaud E, Levraud J-P, Ghigo J-M.
638 2021. Mining zebrafish microbiota reveals key community-level resistance against fish
639 pathogen infection. The ISME journal 15:702–719.

640 67. Graz CJ, Grant GD, Brauns SC, Hunt A, Jamie H, Milne PJ. 2000. Cyclic dipeptides in the
641 induction of maturation for cancer therapy. The Journal of pharmacy and pharmacology
642 52:75–82.

643 68. Jinendiran S, Teng W, Dahms H-U, Liu W, Ponnusamy VK, Chiu CC-C, Kumar BSD,
644 Sivakumar N. 2020. Induction of mitochondria-mediated apoptosis and suppression of
645 tumor growth in zebrafish xenograft model by cyclic dipeptides identified from
646 Exiguobacterium acetylicum. Scientific reports 10:13721.

647 69. Ström K, Sjögren J, Broberg A, Schnürer J. 2002. Lactobacillus plantarum MiLAB 393
648 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-trans-4-OH-
649 L-Pro) and 3-phenyllactic acid. Applied and environmental microbiology 68:4322–4327.

650 70. Clatworthy AE, Lee JS-W, Leibman M, Kostun Z, Davidson AJ, Hung DT. 2009.
651 Pseudomonas aeruginosa infection of zebrafish involves both host and pathogen
652 determinants. Infection and immunity 77:1293–1303.

653 71. Menetrey Q, Sorlin P, Jumas-Bilak E, Chiron R, Dupont C, Marchandin H. 2021.
654 Achromobacter xylosoxidans and Stenotrophomonas maltophilia: Emerging Pathogens
655 Well-Armed for Life in the Cystic Fibrosis Patients' Lung. 5. Genes 12:610.

656 72. Loftie-Eaton W, Crabtree A, Perry D, Millstein J, Baytosh J, Stalder T, Robison BD, Forney
657 LJ, Top EM. 2021. Contagious Antibiotic Resistance: Plasmid Transfer among Bacterial
658 Residents of the Zebrafish Gut. Applied and Environmental Microbiology 87:e02735-20.

659 73. Decewicz P, Dziewit L, Golec P, Kozlowska P, Bartosik D, Radlinska M. 2019.
660 Characterization of the virome of Paracoccus spp. (Alphaproteobacteria) by combined in
661 silico and in vivo approaches. 1. Sci Rep 9:7899.

662 74. Rodríguez I, Novoa B, Figueras A. 2008. Immune response of zebrafish (Danio rerio)
663 against a newly isolated bacterial pathogen Aeromonas hydrophila. Fish & shellfish
664 immunology 25:239–249.

665 75. Mayer EA. 2011. Gut feelings: the emerging biology of gut-brain communication. Nature
666 reviews Neuroscience 12:453–466.

667 76. Galley JD, Nelson MC, Yu Z, Dowd SE, Walter J, Kumar PS, Lyte M, Bailey MT. 2014.
668 Exposure to a social stressor disrupts the community structure of the colonic mucosa-
669 associated microbiota. BMC microbiology 14:189.

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

673 78. Mudd AT, Berding K, Wang M, Donovan SM, Dilger RN. 2017. Serum cortisol mediates the
674 relationship between fecal Ruminococcus and brain N-acetylaspartate in the young pig.
675 Gut microbes 8:589–600.

676 79. Huang EY, Inoue T, Leone VA, Dalal S, Touw K, Wang Y, Musch MW, Theriault B, Higuchi
677 K, Donovan S, Gilbert J, Chang EB. 2015. Using corticosteroids to reshape the gut
678 microbiome: implications for inflammatory bowel diseases. Inflammatory bowel diseases
679 21:963–972.

680 80. Petrosus E, Silva EB, Lay DJ, Eicher SD. 2018. Effects of orally administered cortisol and
681 norepinephrine on weanling piglet gut microbial populations and Salmonella passage.
682 Journal of animal science 96:4543–4551.

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

686 82. Weitekamp CA, Nguyen J, Hofmann HA. 2017. Neuromolecular Regulation of Aggression
687 Differs by Social Role during Joint Territory Defense. Integrative and comparative biology
688 57:631–639.

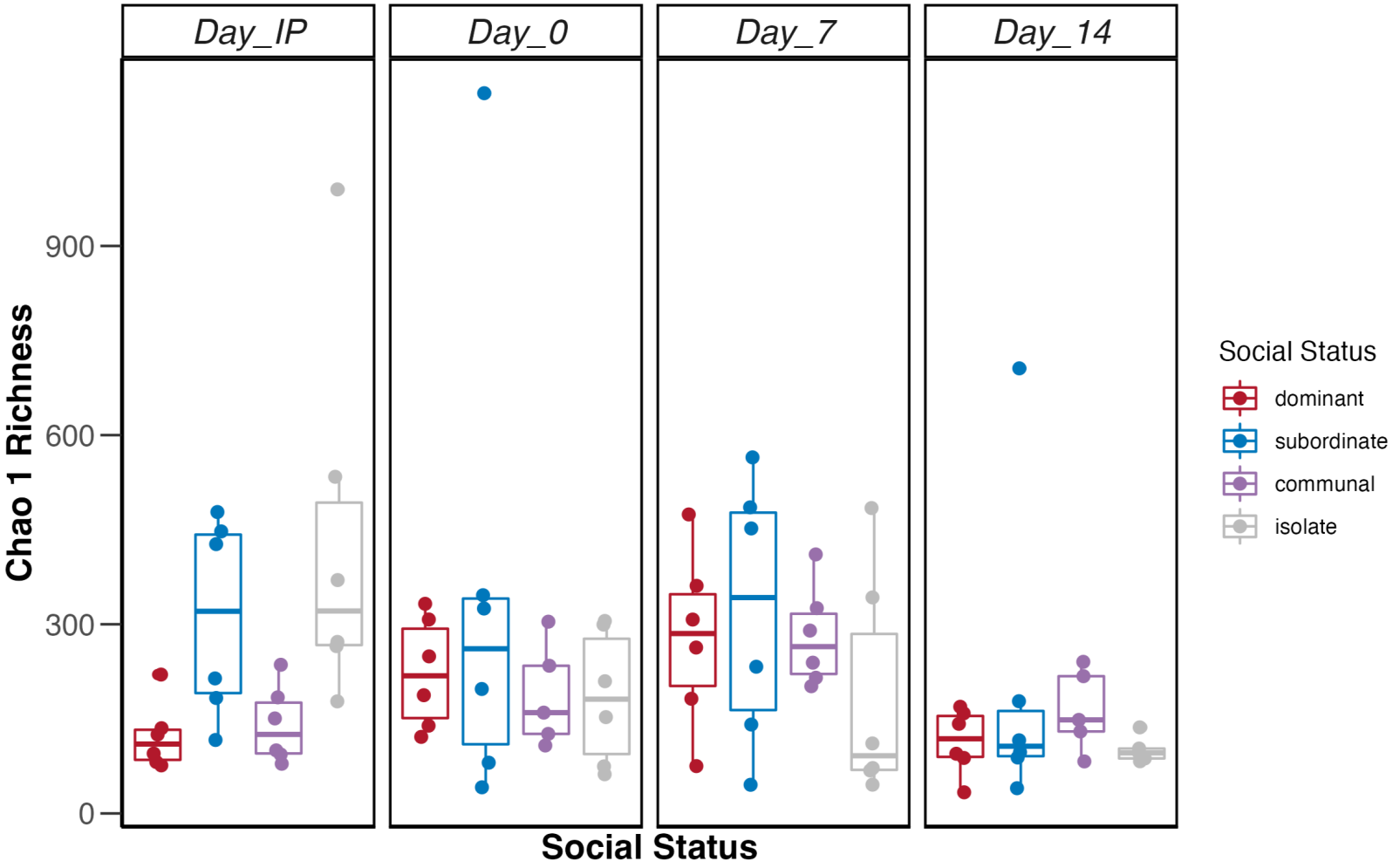
689 83. Inoue K, Ford CL, Horie K, Young LJ. 2022. Oxytocin receptors are widely distributed in
690 the prairie vole (Microtus ochrogaster) brain: Relation to social behavior, genetic
691 polymorphisms, and the dopamine system. The Journal of comparative neurology
692 530:2881–2900.

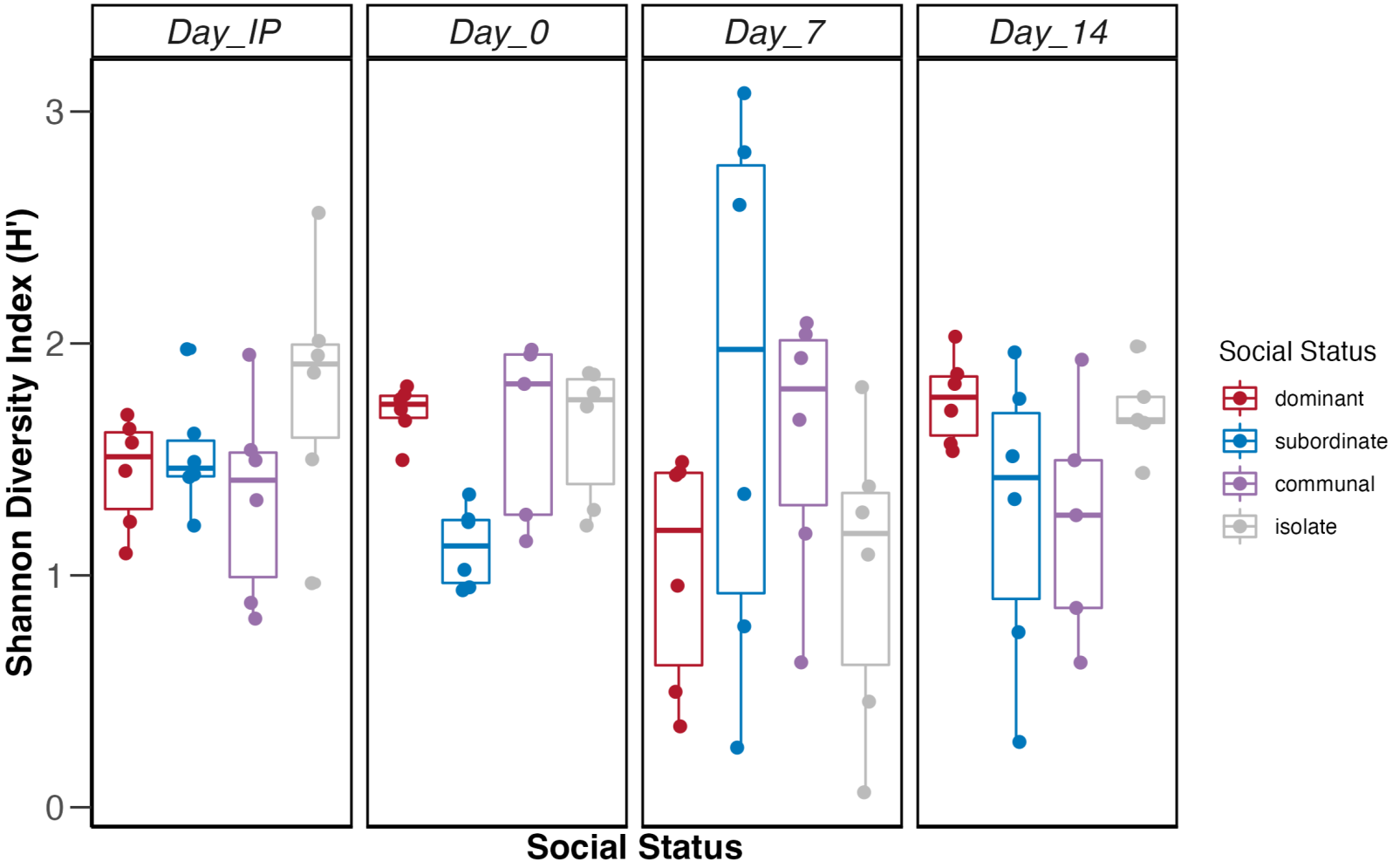
693 84. Korzan WJ, Summers CH. 2007. Behavioral diversity and neurochemical plasticity:
694 selection of stress coping strategies that define social status. Brain, behavior and
695 evolution 70:257–266.

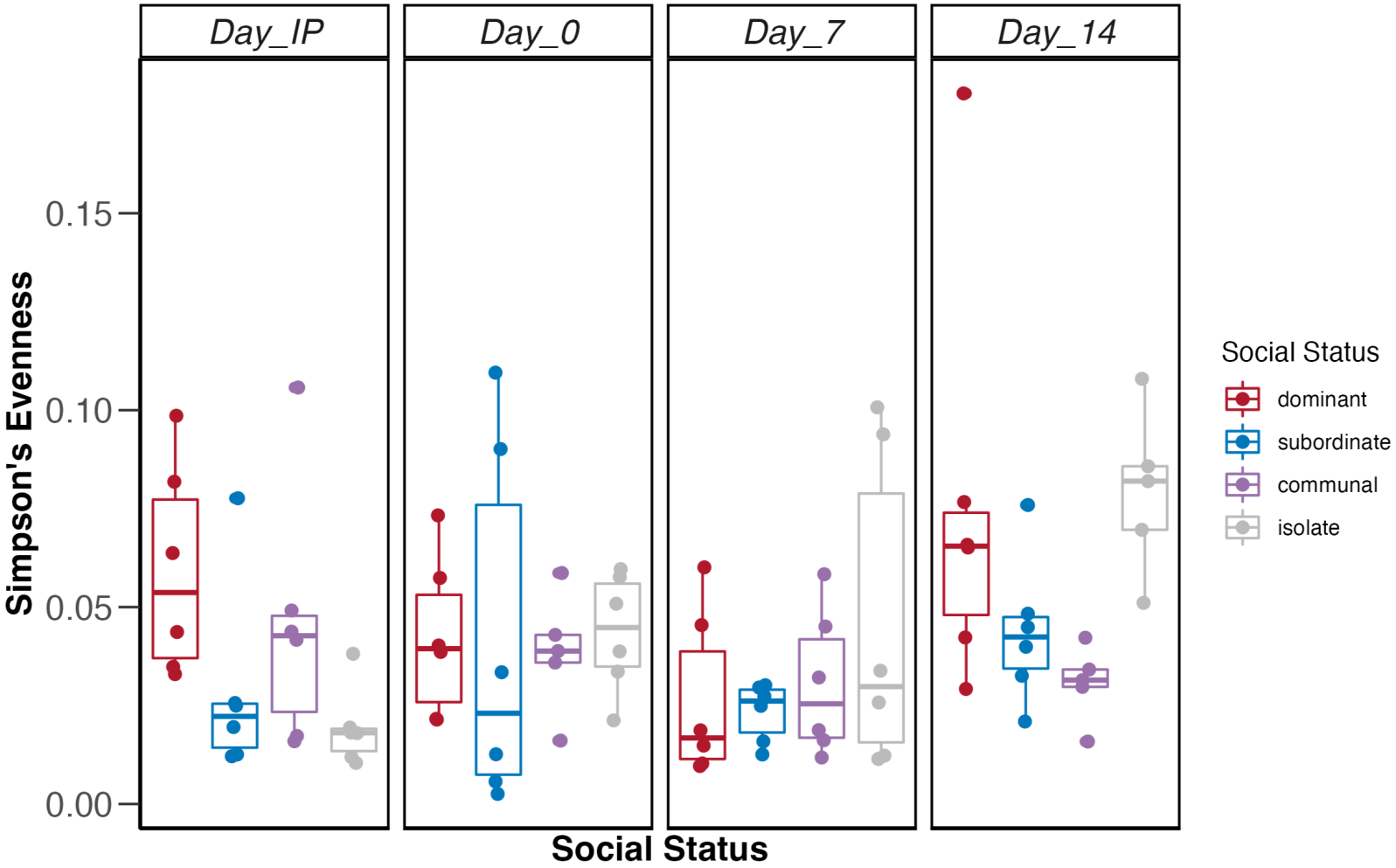
696 85. McDonald MM, Markham CM, Norvelle A, Albers HE, Huhman KL. 2012. GABAA receptor
697 activation in the lateral septum reduces the expression of conditioned defeat and
698 increases aggression in Syrian hamsters. Brain research 1439:27–33.

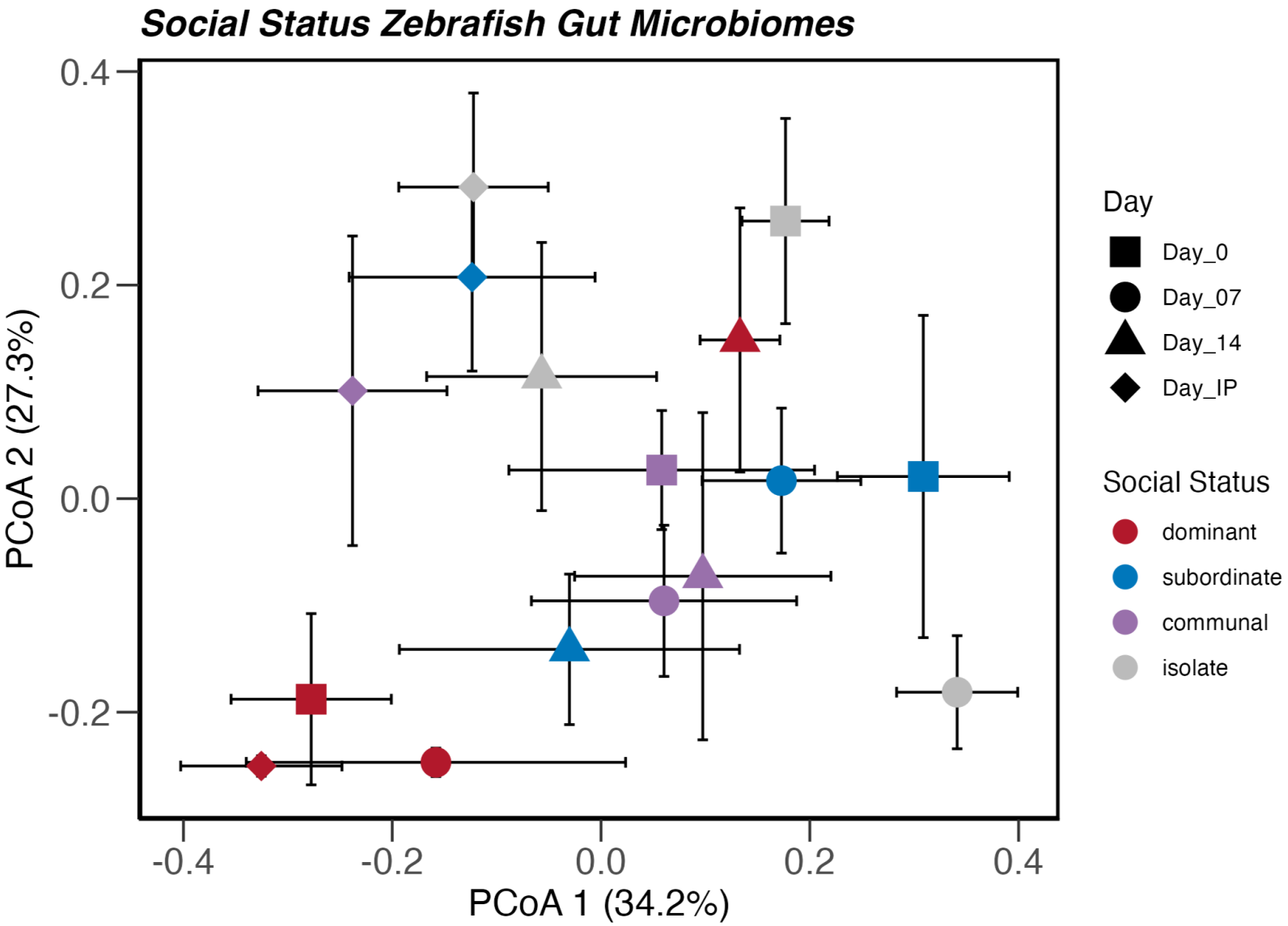
699 86. Weitekamp CA, Nguyen J, Hofmann HA. 2017. Social context affects behavior, preoptic
700 area gene expression, and response to D2 receptor manipulation during territorial defense
701 in a cichlid fish. Genes, brain, and behavior 16:601–611.

702


Day_IP
Day_0
Day_7
Day_14
Chao 1 Richness
900
600
300
0
Social Status
Social Status
dominant
subordinate
communal
isolate


Day_IP
Day_0
Day_7
Day_14
Shannon Diversity Index (H')
0
1
2
3
Social Status
Social Status
dominant
subordinate
communal
isolate


Day_IP
Day_0
Day_7
Day_14
Simpson's Evenness
0.15
0.10
0.05
0.00
Social Status
Social Status
dominant
subordinate
communal
isolate


Social Status Zebrafish Gut Microbiomes
0.4
0.2
0.0
-0.2
PCoA 2 (27.3%)
-0.4
-0.2
0.0
0.2
0.4
PCoA 1 (34.2%)
Day
Day_0
Day_07
Day_14
Day_IP
Social Status
dominant
subordinate
communal
isolate