

**Modeling the zebrafish gut microbiome's resistance and sensitivity to climate change and parasite infection**

Michael J. Sieler Jr.<sup>1</sup>, Colleen E. Al-Samarrie<sup>1</sup>, Kristin D. Kasschau<sup>1</sup>, Mike L. Kent<sup>1,2,3</sup>,  
Thomas J. Sharpton<sup>1,3#</sup>

<sup>1</sup>Department of Microbiology, Oregon State University, Corvallis, OR 97330, USA

<sup>2</sup>Department of Biomedical Sciences, Oregon State University, Corvallis, OR, 97330,  
USA

<sup>3</sup>Zebrafish International Resource Center, University of Oregon, Eugene, OR 97403,  
USA

<sup>4</sup>Department of Statistics, Oregon State University, Corvallis, OR 97330, USA

**#Corresponding Author:** Thomas J. Sharpton, E-mail:

Thomas.Sharpton@oregonstate.edu

Department of Microbiology and Department of Statistics  
Oregon State University, 97330

**Keywords:** Zebrafish; Gut microbiome, Development, Infection, Helminth, Temperature,  
Climate change, *Pseudocapillaria tomentosa*

**Abstract:**

As climate change increases global water temperatures, ecologists expect intestinal helminth infection ranges to expand and increase the health burden on aquatic organisms. However, the gut microbiome can interact with these parasites to influence infection outcomes, raising the possibility that its response to increasing temperatures may help buffer against increased infection burden. To evaluate this hypothesis, we sought to determine if the microbiome is resistant or resilient to the stressors of increased water temperature, helminth exposure, and their combination, and whether this variation linked to infection outcomes. We leveraged the zebrafish (*Danio rerio*) model organism to measure how these variables relate to the temporal dynamics of the gut microbiome. In particular, we exposed adult zebrafish to the parasitic whipworm *Pseudocapillaria tomentosa* across three different water temperatures (28°C, 32°C, 35°C), and analyzed fecal microbiome samples at five time points across 42 days. Our findings show that parasite exposure and water temperature independently alter gut microbiome diversity. Moreover, we find that water temperature moderates the association between parasite infection and the gut microbiome. Consistent with this observation, but at odds with current expectations, we find that increasing water temperature reduces parasitic infection in fish. Overall, our results indicate that water temperature alters the contextual landscape of the gut microbiome to impact its response to an exogenous stressor of an intestinal parasite in zebrafish. Furthermore, our findings represent the first report of the effects of elevated temperature on parasitic nematode development in a fish host. Importantly, our study demonstrates that climate

43 change may have unanticipated and environmentally contingent impacts to vertebrate  
44 gut microbiomes and health outcomes in response to an exogenous stressor.

45

## Introduction

The steady increase in global temperatures due to climate change challenges vertebrate health (1). These threats to vertebrate health take on many forms, including the expected expansion of infectious agents (2,3). Of particular concern are the increased infection burdens faced by aquatic organisms experiencing increasing water temperatures (4). Due in part to the varied coincident effects of climate, the impacts of a warming climate on aquatic organisms are anticipated to be nonuniform in effect (4,5) and vary biogeographically (4,6), which in turn complicates harm mitigation and conservation efforts (7). Consequently, there's an urgent need to better understand climate change's contextual impacts on organisms depending on the unique environmental conditions of the ecosystems they inhabit.

In recent years researchers have considered that climate change may also elicit harm to vertebrates by disrupting the composition of their gut microbiome (8). While prior work has shown that varying temperatures impacts gut microbiome composition across a variety of vertebrate host species (9), less is known about how coincident variables, such as parasite or pathogen exposure, collide with temperature to drive variation in the gut microbiome. These potential interaction effects are important to quantify, because it may be that they elicit even greater effects on the gut microbiome than anticipated by investigations of temperature alone, and could possibly result in increased frequency of dysbiotic disorders. It's important to elucidate these interactions because increasing work points to the gut microbiome as a key determinant of whether vertebrate physiology is able to buffer against stress (9,10), and whether temperature

induced perturbations to the gut microbiome may sensitize individuals to subsequent stressors (11).

To answer these questions, we evaluated the gut microbiome's temporal response to an exogenous stressor across a gradient of environmental conditions. To do so, we levered the zebrafish (*Danio rerio*) model organism to measure how gut microbiomes differ across fish reared to adulthood at one of three water temperature conditions (28°C, 32°C, or 35°C; Fig. 1). Zebrafish are highly thermal tolerant, capable of existing across a wide spectrum of temperature ranges from 4°C to 40°C (12), but living outside their thermal optimum can come at a physiological and microbial cost (12,13). While much is known about the thermal range of zebrafish, the effects of altered water temperature on their gut microbiome structure has not been elucidated. We also sought to determine if water temperature affected how the microbiome and host responds to exposure to and infection by intestinal nematode *Pseudocapillaria tomentosa*, a common source of disease in zebrafish facilities (14). *P. tomentosa* is known to cause high mortality and disrupts the gut microbiome (14,15). Yet, it is not known if or how water temperatures mediates this interaction. Overall, our study sought to clarify the environmentally dependent context of a gut microbiome's resistance and sensitivity to climate change-relevant stressors.

## Results

### Water temperature shapes gut microbiome structure

To determine how zebrafish reared across a gradient of increasing water temperatures impacts the structure of the gut microbiome, we reared 260 zebrafish

91 across one of three water temperatures (28°C, 32°C or 35°C) until 206 days-post  
92 fertilization (dpf; Fig. 1). Additionally, within each temperature cohort, fish were evenly  
93 divided into two additional treatment groups: either unexposed or exposed to the  
94 intestinal helminthic parasite *Pseudocapillaria tomentosa*. Microbiome samples were  
95 collected at five time points between 164 and 206 dpf. In the parasite exposed cohort,  
96 fish were exposed to *P. tomentosa* following microbiome sampling at 164 dpf, or 0 days  
97 post exposure (dpe). Four subsequent microbiome samples were collected at 14 dpe  
98 (178 dpf), 21 dpe (185 dpf), 28 dpe (192 dpf), and 42 dpe (206 dpf). Within the parasite  
99 unexposed fish cohort, we built generalized linear models (GLM) to determine if water  
100 temperature associated with variation in one of four measures of alpha-diversity:  
101 Simpson's Index, Shannon Entropy, richness, and phylogenetic diversity (Table S2A.1).  
102 An ANOVA test of these GLMs revealed that alpha-diversity varied as a function of  
103 temperature for all measures ( $P < 0.05$ ; Fig. 2A; Table S2A.2), except Shannon Entropy  
104 ( $P > 0.05$ ; Table S2A.2). A post hoc Tukey test clarified that alpha-diversity scores did not  
105 significantly differ between 28°C and 32°C water temperature reared fish for each  
106 diversity metric we measured ( $P > 0.05$ ; Table S2A.3). However, we observed significant  
107 differences in diversity between 28°C and 35°C water temperature reared fish across  
108 Simpson's Index, richness and phylogenetic alpha-diversity measures ( $P < 0.05$ ; Table  
109 S2A.2), and between 32°C and 35°C water temperature reared fish as measured by  
110 richness and phylogenetic diversity metrics ( $P < 0.05$ ; Table S2A.2). These results  
111 indicate that water temperature associates with fish gut microbiome diversity, and that  
112 water temperature may differentially impact particular microbial clades of the gut.

To evaluate how temperature associates with microbiome composition in parasite unexposed fish, we quantified dissimilarity amongst all samples and generated distance matrices using the Bray-Curtis, Canberra and half-weighted UniFrac distance metrics. Using permutational multivariate analysis of variance (PERMANOVA), we assessed whether increasing water temperatures explained variance in gut microbial community composition. A PERMANOVA test indicated that microbial communities were significantly stratified by water temperature as measured by all beta-diversity metrics (PERMANOVA,  $P < 0.05$ ; Fig. 2B; Table S2B.1). These results indicate that microbial communities of fish reared at the same water temperature are more consistent in composition to one another than fish reared at different water temperatures. Additionally, we assessed beta-dispersion, a measure of variance, in the gut microbiome community compositions for each water temperature group. We find the beta-dispersion levels did not significantly differ between the water temperature groups ( $P > 0.05$ ; Table S2B.2). These results indicate that fish reared at different water temperatures are consistent in community composition.

Next, we compared our results across five time points between 0- and 42 dpe to determine how water temperature impacts the successional trajectories of gut microbiome diversity and composition. Linear regression revealed gut microbial alpha-diversity was significantly associated with the main effect of time for each alpha-diversity metric we assessed ( $P < 0.05$ ; Fig. 2C; Table S2C.1-2). Moreover, we found a temperature dependent effect on time as measured by richness and phylogenetic diversity metrics ( $P < 0.05$ ; Table S2C.1-2). A post hoc Tukey test clarified that microbiome diversity significantly differed between 0- and 42 dpe fish reared at 28°C as

measured by richness and phylogenetic diversity ( $P < 0.05$ ; Table S2C.3), between 0- and 42 dpe fish reared at 32°C as measured by all alpha-diversity metrics ( $P < 0.05$ ; Table S2C.3), and between 0- and 42 dpe fish reared at 35°C as measured by Shannon Entropy and Simpson's Index ( $P < 0.05$ ; Table S2C.3). These results indicate that gut microbial alpha-diversity increases over time irrespective of water temperature.

A PERMANOVA test detected significant clustering of microbial gut community composition based on the interaction of water temperature and time as measured by all beta-diversity metrics (PERMANOVA,  $P < 0.05$ ; Fig. 2D; Table S2D.1). These results indicate that microbial communities of fish reared at the same water temperature are more consistent in composition to one another across time than fish reared at different water temperatures. Moreover, a pairwise analysis of beta-dispersion found significantly elevated levels of dispersion between fish reared across different temperatures and time as measured by all beta-diversity metrics ( $P < 0.05$ ; Table S2D.2). These results indicate that gut microbial community composition varies inconsistently between water temperature groups in a time-dependent manner. Collectively, these results indicate that zebrafish gut microbiomes communities stratify by temperature, and the trajectory of gut microbiome successional development varies depending on water temperature.

#### **Infection burden is highest in fish reared at lower water temperatures**

Next, we evaluated infection outcomes of zebrafish reared at different water temperatures and exposed to the intestinal helminthic parasite *Pseudocapillaria tomentosa*. To determine whether water temperature affects infection burden, we exposed zebrafish to 50 *P. tomentosa* eggs per liter of tank water at 164 days post-



fertilization (dpf). Infection outcomes were assessed using wet mount and histological evaluation at 0, 14, 21, 28, and 42 days post-exposure (dpe). We built a negative binomial general linear model to compare infection burden (total worm counts) between fish reared at different water temperatures (Table S3B.1). The regression analysis found a statistically significant effect of temperature on infection burden ( $P < 0.05$ ; Fig. 3B; Table S3B. 2). However, we did not find a statistically significant interaction effect between water temperature and time on infection burden ( $P > 0.05$ ; Table S3B.3).

Across time points, fish reared at 28°C exhibited the highest mean infection burden (4.86 worms per fish), followed by those at 32°C (3.6 worms per fish). Notably, at 14 dpe, fish at 32°C had a slightly higher infection burden (3.3 worms per fish) than those at 28°C (2.3 worms per fish). Tukey's post hoc test revealed that infection burden was significantly higher in fish reared at 28°C and 32°C compared to those at 35°C ( $P < 0.05$ ; Fig. 3B; Table S3B.3). Only a single larval worm was detected by wet mount in two fish from the 35°C group, while histological examination revealed a slightly higher prevalence, with larval worms observed in 9 out of 32 fish at this temperature (Fig. 3A; Table S3A.1). These results indicate that infection burden is highest at lower water temperatures. We also examined the development of mature female worms across temperature conditions. At 28°C, mature female worms were first detected at 28 dpe in 7 fish, whereas at 32°C, mature females were only observed in 4 fish. Interestingly, at 14 dpe, a single mature female was identified in a fish reared at 32°C, marking the earliest recorded instance of worm maturation at this temperature.

Additionally, we compared the sensitivity of infection detection between histology and wet mount methods on a subset of fish selected for microbiome analysis ( $n = 120$ ;

Fig. S3C; Table S3C.1). McNemar's test revealed significant differences in detection sensitivity under specific conditions. At 35°C and 21 dpe, histology identified significantly more infections than wet mount ( $\chi^2 = 4.17$ ,  $P < 0.05$ ; Fig. S3C; Table S3C.3), with 6 samples testing positive by histology alone compared to 0 by wet mount alone. No statistically significant differences were observed at other temperature and dpe combinations ( $P > 0.05$ ; Table S3C.3). In cases where all samples were concordant (e.g., 28°C at 28 dpe and 35°C at 28 dpe), McNemar's test could not be computed due to the absence of discordant pairs. These findings suggest that histological methods may be more sensitive than wet mounts, particularly at higher temperatures and intermediate time points. Collectively, these results suggest that higher water temperatures may have a protective effect against infection burden, limiting worm establishment and development in zebrafish.

### **Gut microbiome response to parasite exposure varies across water temperature**

To investigate how parasite exposure affects the gut microbiome under varying water temperatures, we analyzed fecal samples from exposed and control fish at multiple time points. *P. tomentosa* is known to alter the zebrafish gut microbiome (15), but it remains unclear how increasing water temperatures affect this response. We collected fecal samples for microbiome analysis of fish in the parasite exposed cohort at 14-, 21-, 28-, and 42 dpe. Similar to our parasite unexposed fish microbiome analyses, we built generalized linear models (GLM) to determine if temperature, time or their combination associated with variation in measures of microbial diversity and composition of parasite exposed fish (Table S4A.1). An ANOVA test of these GLMs

revealed that alpha-diversity varied as a function of temperature for all measures (P<0.05; Fig. 4A; Table S4A.2). A post hoc Tukey test clarified that gut microbial diversity between 28°C and 32°C water temperature reared fish significantly differed for all alpha-diversity metrics (P<0.05; Table S4A.3), and gut microbial diversity differed between 28°C and 35°C water temperature reared fish as measured by Simpson's Index. However, we did not find significant differences in gut microbial diversity between 32°C and 35°C water temperature reared fish for all alpha-diversity metrics, or between 28°C and 35°C water temperature reared fish as measured by Shannon Entropy, richness and phylogenetic diversity metrics. These results indicate that moderate increases in water temperature promotes gut microbial diversification in parasite exposed fish, but diversification of gut microbes plateaus in parasite exposed fish reared at higher water temperatures.

For each beta-diversity metrics we considered, PERMANOVA tests found that temperature significantly explained the variation in microbiome composition in parasite exposed fish (PERMANOVA, P<0.05; Fig. 4B; Table S4B.1). These results indicate that gut microbiome communities of parasite exposed fish reared at the same water temperature are more similar to each other than fish reared at different water temperatures. Additionally, we found beta-dispersion levels were significantly elevated between water temperature groups (P<0.05; Table S4B.2). A post hoc Tukey test clarified that beta-diversity dispersion levels were highest in fish reared at 28°C, followed by fish reared at 32°C and 35°C water temperatures (P<0.05; Table S4B.3). These results indicate that that gut microbiome communities of parasite exposed fish

reared at lower water temperatures are more inconsistent in composition than parasite exposed fish reared at higher water temperatures.

Next, we compared our results across five time points to evaluate how parasite exposure and water temperature impacted gut microbiome diversity and composition. An ANOVA test did not find significant main effects of time as measured by Shannon Entropy and Simpson's Index ( $P>0.05$ ; Table S4C.2), but found marginally significant main effects of time as measured by richness and phylogenetic diversity ( $P=0.064$  and  $P=0.078$ ; Table S4C.2). Moreover, linear regression did not reveal significant interaction effects between temperature and time across all alpha-diversity metrics ( $P>0.05$ ; Fig. 4C; Table S4C.2). These results indicate increasing water temperatures generally do not consistently impact microbial gut diversification over time in parasite exposed fish, and particular microbial clades appear more sensitive to the effects of time depending on temperature.

PERMANOVA tests found that community composition was best explained by the interaction effects between temperature and time using the Canberra beta-diversity metric (PERMANOVA,  $P<0.05$ ; Fig. 4D; Table S4D.1), but a significant interaction effect was not observed using the Bray-Curtis and half-weighted UniFrac dissimilarity metrics ( $P>0.05$ ; Table S4D.1). Given how these metrics weigh the importance of rarer (e.g., Canberra) versus abundant (e.g., Bray-Curtis) microbial community members, these results indicate that abundant members of the microbiome community are more robust to the effects of temperature across time in parasite exposed fish, while rarer taxa are more sensitive to the effects of time depending on temperature. Moreover, a pairwise analysis of beta-dispersion found significantly elevated levels of dispersion between fish

reared across different temperatures and time as measured by all beta-diversity metrics ( $P < 0.05$ ; Table S4D.2). These results indicate that parasite exposure inconsistently impacts gut microbial community composition across time depending on temperature ( $P < 0.05$ ; Table S4D.2). Collectively, these results indicate that parasite exposure can impact gut microbiome diversity and composition, and these impacts are greatest at lower temperatures.

### **Gut microbiome response has a non-linear relationship with infection burden**

Given the differences we observed in gut microbiome diversity and composition across parasite exposed fish reared at different water temperatures, we further investigated whether gut microbiomes of parasite exposed fish vary depending on presence of infection and infection burden. Linear regression did not find significant main effects of presence of infection or significant interaction effects between presence of infection and temperature on gut microbial alpha-diversity for all metrics we measured ( $P > 0.05$ ; Fig 5A; Table S5A.1-2). These results indicate that gut microbial diversity does not differ as a function of presence of infection. Moreover, a PERMANOVA analysis found microbial community composition was best explained by presence of infection as measured by Canberra (PERMANOVA,  $P < 0.05$ ; Fig. 5B; Table S5B.1), but a significant result was not observed by the other beta-diversity metrics we measured. Additionally, we did not find statistically significant results between the interaction effects of water temperature and presence of infection on gut microbial community composition. These results indicate that rarer members of microbial communities of parasite exposed fish vary by presence or absence of infection, but

abundant microbes do not. However, we did detect elevated levels of beta-dispersion across fish reared at different water temperatures depending on presence of infection ( $P < 0.05$ ; Table S5B.2). These results indicate that gut microbiome composition inconsistently varies between fish depending on presence of infection and water temperature.

Next, we investigated how infection burden (i.e., number of intestinal worms detected) impacted parasite exposed fish gut microbiome diversity and composition. We used GLMs to determine if infection burden associated with variation in gut microbial alpha-diversity (Table S5C.1). An ANOVA test of these GLMs revealed that alpha-diversity varied as a function of infection burden as measured by Shannon Entropy and Simpson's Index ( $P < 0.05$ ; Table S5C.2.2), but the interaction effects between infection burden and water temperature did not significantly explain variation in alpha-diversity across all measures ( $P > 0.05$ ; Table S5C.2.2). These results indicate that gut microbial diversity varies as a function of parasitic worm count. A PERMANOVA analysis found microbial community composition was best explained by infection burden as measured by all beta-diversity metrics (PERMANOVA,  $P < 0.05$ ; Table S5C.2.1), but the interaction effect between infection burden and temperature was not significant ( $P > 0.05$ ; Table S5C.2.1). These results indicate that higher infection burden drives increased inconsistency in gut microbial composition, regardless of water temperature.

Upon closer inspection of our infection burden results, we observed a non-linear relationship between infection burden and alpha-diversity scores, where highest infection burden associated with either highest or lowest alpha-diversity scores. To further explore this non-linear relationship between gut microbial diversity and infection

burden, we grouped parasite exposed fish samples based on their alpha-diversity scores and infection burden. Parasite exposed fish samples with at least one intestinal worm detected were classified as “Low” or “High” if their alpha-diversity score was less than or greater than the median alpha-diversity score, respectively. Fish with zero detected worms were classified as “Other”. When grouping samples either Low or High based on alpha-diversity scores as measured by the Simpson’s index, we find that the samples in the Low group are composed of 67% 28°C and 33% 32°C water temperature reared fish, samples in the High group are composed of 33% 28°C and 67% 32°C water temperature reared fish, and samples in the Other group are composed of 14% 28°C, 27% 32°C, and 59% 35°C water temperature reared fish (Table S5C1.0). These results indicate that group membership trends with water temperature. To test this supposition, we used GLMs to determine if infection burden associated with variation in alpha-diversity score grouping (Table S5C.1.1). An ANOVA test of these GLMs revealed significant main effects of group for all alpha-diversity measures ( $P < 0.05$ ; Fig 5C; Table S5C.2.1), and significant interaction effects between group and alpha-diversity score. Notably, fish in the Low group had a significant negative slope and fish in the High group had a significant positive slope between alpha-diversity and infection burden as measured by Shannon Entropy and Simpson’s Index. These results indicate that parasite exposed fish have diverging gut microbial alpha-diversity responses to high infection burden.

Additionally, we find that these groups of samples – based on high versus low alpha-diversity scores of parasite exposed fish – also formed two distinct clusters in beta-diversity space. A PERMANOVA analysis detected significant clustering between

Low, High, and Other groups across each measure of beta-diversity (PERMANOVA,  $P < 0.05$ ; Fig. 5D; Table S5D.1.1). However, this effect was weakest when considering the Canberra metric. Furthermore, a pairwise analysis of beta-dispersion finds significantly elevated dispersion levels between group membership as measured by Canberra metric, but not the other beta-diversity metrics (Table S5D.2). Given that the Canberra metric gives rarer taxa greater importance in its beta-diversity calculations than the other metrics we evaluated, these results suggest there is more consistency in microbial composition among abundant taxa within samples that share Low or High group membership, but not among more rarer taxa. A post-hoc Tukey test also clarified that beta-dispersion levels are significantly different between fish in the High and Other groups compared to fish in the Low group as measured by the Canberra metric (Table S5D.3). Together, these results indicate that rarer members of the gut microbiome are less consistently represented across fish in the Low cluster group as compared to fish in the High and Other cluster groups. Collectively, these results indicate that the microbiome response of fish with heaviest infection burden diverge into two distinct trajectories, which may be influenced by water temperature.

### **Parasite exposure exacerbates water temperature differences in gut microbiome structure**

Next, we sought to determine whether the gut microbiomes of zebrafish exposed to the parasite *Pseudocapillaria tomentosa* respond differentially compared to parasite unexposed control fish across increasing water temperatures. Prior to the parasite exposure at 164 dpf (or 0 dpe), we collected fecal samples from both cohorts of control



and parasite exposed fish. Following fecal sample collection, fish in the parasite exposure group were exposed to *P. tomentosa*. We collected subsequent fecal samples at 14-, 21-, 28- and 42 dpe. Fecal samples were then measured for gut microbial diversity and composition and compared between parasite unexposed and exposed fish. We built generalized linear models (GLM) to determine if parasite exposure as a function of water temperature associated with microbial diversity and composition measures (Table S6A.1). Within pre-exposed (i.e., 0 dpe) samples, we did not observe any significant associations between the interaction effect of parasite exposure and water temperature across any of the alpha-diversity measures ( $P > 0.05$ ; Fig. 6A; Table S6A.2). These results indicate that at 0 dpe prior to parasite exposure, gut microbial diversity measures of fish reared at the same water temperature are not different from one another. Furthermore, PERMANOVA tests revealed significant differences in microbiome composition between control and pre-exposed fish across all beta diversity metrics. Homogeneity of dispersion tests revealed a significant difference in group variability for Bray-Curtis ( $P < 0.05$ ; Fig. 6B; Table S6B.2), but not for Canberra or Generalized UniFrac. Post hoc Tukey tests indicated no significant pairwise differences in dispersion for any metric (Table S6B.3), suggesting that the observed dispersion effect in Bray-Curtis was not driven by specific group outliers. Given that temperature alone consistently explained the variation in microbial communities followed by treatment effects, these results suggest that prior to parasite exposure microbial communities vary by water temperature and possibly stochasticity of tank effects.

We next compared our results between control and exposed fish across each water temperature to determine how parasite exposure impacts gut microbiome

diversity and composition. Linear regression revealed microbial gut alpha-diversity was significantly associated with the interaction effect between temperature and treatment for any alpha-diversity metric we assessed ( $P < 0.05$ ; Fig. 6C; Table S6C.1-2). A post hoc Tukey test clarified that microbiome diversity was significantly different between exposure groups of fish reared at 28°C water temperature as measured by Simpson's Index ( $P < 0.05$ ; Table S6C.3), at 32°C water temperature as measured by all alpha-diversity metrics ( $P < 0.05$ ; Table S6C.3), and at 35°C as measured by richness and phylogenetic diversity ( $P < 0.05$ ; Table S6C.3). These results indicate that gut microbial diversity differs between unexposed and exposed fish depending on water temperature, and parasite exposure uniquely impacts particular microbial clades, rare and abundant taxa depending on water temperature. Additionally, PERMANOVA tests found that microbiome composition differed between control and exposed fish reared at all water temperatures as measured by all beta-diversity metrics ( $P < 0.05$ ; Table S6D.1). These results suggest that the gut microbiomes compositions between control and parasite exposed differed in microbiome community composition regardless of water temperature. Moreover, a pairwise analysis of beta-dispersion found elevated levels of dispersion across all beta-diversity metrics measured, and dispersion levels were highest among parasite exposed fish reared at lower water temperatures ( $P < 0.05$ ; Table S6D.2). These results suggest that gut microbiome community composition is less consistent between parasite unexposed and exposed fish reared at lower water temperatures. Collectively, these results demonstrate that water temperature dictates how exposure to parasites alters the temporal trajectory of the gut microbiome.

## **Gut microbial relative abundance significantly associates with environmental conditions and stressors**

Finally, to evaluate how gut microbial abundance is influenced by environmental conditions and stressors (e.g., worm infections), we quantified differential abundance using MaAsLin2. Our analysis revealed 277 unique taxa at the Genus taxonomic level with at least one significant associations between taxon abundance and a covariate ( $Q < 0.05$ , Fig. 7; Table S7A.1). We observed several taxa were significantly associated with the effect of water temperature. Fish reared at 35°C water temperature were enriched for 37 taxa, and depleted of 54 taxa relative to fish reared at 28°C water temperature. Fish reared at 32°C were enriched for 42 taxa, and depleted of 47 taxa relative to fish reared at 28°C water temperature (Fig. 7). Notably, *Aeromonas* and *Pseudomonas* bacterial abundance significantly associated negatively and positively with the effects of water temperature, respectively. *Aeromonas* and *Pseudomonas* are common members of the zebrafish gut microbiome (16,17), and these genera's bacterial abundance has previously been observed to associate with the effects of water temperature in zebrafish (13). These results indicate that gut microbes are differentially selected for across varying water temperatures and time. We also measured how taxon abundance change over time regardless of water temperature. Over the course of 42 days, fish were enriched for 73 taxa and depleted of 36 taxa (Fig. 7). Notably, *Bosea* and *Cloacibacterium* bacterial abundance were negatively associated with the effects of time. *Bosea* and *Cloacibacterium* are common members of the zebrafish gut microbiome (16–18), and were also previously identified as having negative associations with the effects of time in zebrafish (15). These results indicate that

particular members of the gut microbiome associate with time irrespective of water temperature.

Next, we sought to determine how parasite exposure impacted gut microbial abundance in fish. Fish exposed to *P. tomentosa* were significantly enriched for 74 taxa, and depleted of 35 taxa relative to parasite unexposed fish (Fig. 7). Notably, we find *Aeromonas*, *Chitinibacter*, and *Flavobacterium* are positively associated with parasite exposure, while *Plesiomonas*, *Phreatobacter* and *Cetobacterium* are negatively associated with parasite exposure. With the exception of *Phreatobacter* and *Cetobacterium*, these data are consistent with our prior work that found *P. tomentosa* exposure associated with altered bacterial abundance of these members of the zebrafish gut microbiome (15). We further investigated the effects of parasite exposure and measured how infection burden or presence of infection impacted gut microbial abundance. Fish with higher infection burden (i.e., number of parasitic worms present) enriched for 49 taxa and were depleted of 13 taxa, while fish that were positively infected enriched for 117 taxa and were depleted of 5 (Fig. 7). Notably, we find abundance of members of *Cetobacterium*, *Shewanella*, *Vibrio* and *Zoogloea* are negatively associated with infection burden. These taxa are identified as common members of the zebrafish gut microbiome (19). These results indicate that exposure to the intestinal parasitic worm *P. tomentosa* impacts common zebrafish gut microbiota, and presence of infection and severity have varying impacts to gut microbial abundance.

To deepen our analysis of parasite exposure on the zebrafish gut microbiome, we investigated how taxon relative abundance associated with gut microbiome diversity

and composition. Previously, we found that parasite exposed fish reared at 28°C and 32°C water temperatures clustered into two distinct groups of community composition, which associated with high infection burden and either high or low alpha diversity scores. This observation led us to investigate which gut microbiota might be driving the clustering of the gut microbiomes of heavily infection burdened fish. We did not find significantly abundant taxa in the High group. We detected 1 taxon that was significantly enriched and 192 taxa that were significantly depleted among fish in the Low group (Fig. 7). Notably, we find *Aeromonas* was enriched, while *Mycobacterium* were depleted in the Low group fish. Some species of *Mycobacterium* are common pathogens in zebrafish facilities (20). These results indicate that the gut microbiome communities of parasite exposed fish experiencing heavy infection burden stratify into two distinct groups represented by the unique depletion of particular members of the gut microbiome. Collectively, these results indicate that environmental conditions associate with altered gut microbial abundance, and the response of specific members of the gut microbiome to environmental stressors varies depending on environmental conditions.

## Discussion

The zebrafish is an important model organism for understanding how environmental stressors impact the microbiome (17,18). Our work capitalized on the experimental control and scale afforded by the zebrafish model system to investigate how temperature and parasite exposure interact to influence infection and microbiome outcomes. While previous research has investigated how water temperature (13) and parasite exposure (15) independently impact the zebrafish gut microbiome, no studies

in any *in vivo* experimental system, to our knowledge, have examined the microbiome's temporal response to the combined effects of increasing water temperature and parasite exposure. Overall, we found that water temperature serves as a key contextual variable that dictates the severity of infection, the developmental state of worms, the composition of the gut microbiome in unexposed fish, and how the gut microbiome responds to parasite exposure and infection. These results underscore that the gut microbiome's response to—and potentially its ability to buffer against—intestinal parasitic infection is influenced by other exogenous factors, in this case, water temperature. Furthermore, our findings challenge current expectations of climate change's anticipated impact on aquatic organismal parasite burden (2,3). Consequently, it is important that we consider going forward how stacking multiple stressors, an experience inherent to life in the Anthropocene, may accelerate the arrival of dysbioses.

We found that parasitic infection burden was highest among zebrafish reared at ambient water temperatures. While field studies have documented arrested development in parasitic nematodes (e.g., *P. tomentosa*) during cold winter months (21), this study represents the first report of the effects of elevated temperature on parasitic nematode development in a fish host. Consistent with our prior work, temporal trends in *P. tomentosa* infection burden were similar for fish at ambient temperatures (15). However, contrary to expectations that elevated temperatures increase infection burden, we observed the opposite outcome: fish reared at the highest temperatures exhibited the lowest infection burden, with only a few larval-stage worms detected. Because parasite eggs were larvated at ambient temperature before being transferred to warmer tanks, we hypothesize that elevated temperatures may have impaired hatching once the

eggs were ingested, reducing overall abundance of worms. Nevertheless, at 35°C, worms that did establish infections persisted but remained in an arrested state out to 28 and 42 days post-exposure (dpe), whereas at 28°C and 32°C, worms completed development and mated within 3 to 4 weeks, consistent with previous observations (14). These findings suggest that worm hatching and development may be temperature-sensitive, adding to prior research showing that many fish pathogens, from viruses to parasites, exhibit well-defined upper thermal boundaries for infectivity and pathogenesis. For example, the development of the ciliate *Ichthyophthirius multifiliis*, a common cause of disease in aquarium fishes, is arrested by elevating water temperatures above 30°C (22). While we are not aware of this phenomenon with nematode parasites of fishes, analogous examples exist in terrestrial nematodes, such as *Wuchereria bancrofti*, where larval development is restricted to temperatures below 31°C in mosquitoes (23). Our findings address a gap in understanding of nematode development at a host's upper thermal limit in an aquatic organism, and offers new insights into how climate change may disrupt aquatic disease ecological dynamics. Future research should investigate whether arrested development in *P. tomentosa* is a direct effect of temperature or mediated by host physiological responses.

Beyond direct effects on parasite development, poikilothermic (i.e., animals with variable body temperature and the inability to regulate it) hosts may gain protections against infection through temperature-dependent immune responses or gene expression changes. While studies on zebrafish immunity under elevated temperatures are limited, prior research in teleosts indicates that immune responses are host- and environment-specific, varying with the direction and duration of temperature shifts

(24,25). For example, Dittmar et al. found that immune activity was highest at thermal limits and inversely related to acute temperature shifts in three-spine sticklebacks (26), whereas Bailey et al. observed suppressed immunity and increased parasite burden in rainbow trout exposed to chronic upper optimal thermal ranges (27). Although these studies differ in exposure regimes to ours, they highlight that colonization resistance may be influenced by temperature-sensitive immune responses and gene expression. Future research integrating immune function, gene expression, and histopathological assessments will be crucial to disentangling the host's role in colonization resistance under chronic parasite exposure and elevated temperatures. Notably, controlled temperature manipulation is already used to mitigate certain aquaculture pathogens, such as *Ichthyophthirius multifiliis* (22), where increasing tank temperature to 30°C can eliminate infections in susceptible fish. Our findings suggest that similar interventions may help mitigate or delay parasite infection in aquaculture settings.

We also found that zebrafish gut microbiome structure stratified depending on the environmental conditions of increasing water temperature. Our results are congruent with previous research that found increased water temperatures altered zebrafish gut microbial diversity and composition (13). Moreover, Wang *et al.* observed that zebrafish reared at different water temperatures manifested distinct liver carbohydrate metabolism profiles and temperature-dependent sensitivity to irradiation. A unique aspect of our study considered how the gut microbiome temporally varies as a function of water temperature. We found that water temperature acts as a filter on initial zebrafish gut microbiome assembly, and these initial differences in assembly between water temperature remained stable across time. Beyond zebrafish, analogous investigations



have investigated how temperature variation shapes gut microbiome composition and function in mammals, fish, and other animal species (28,29). In particular, a recent meta-analysis of aquatic organisms' response to temperature found similar, but inconsistent results to our study, wherein increasing water temperature is associated with both increases and decreases to gut microbial diversity, differences in gut microbiota community composition, and altered gut taxon abundance (29). Inconsistencies between prior work and ours could be driven by differences in magnitude of the stressor (i.e., press vs pulse; (30)), host species (31), facility or habitat effects (29,32,33), or diet (20). Despite these differences, the results of prior studies in conjunction with ours are consistent with the concept of environmental conditions acting as a habitat filter to shape initial gut microbiome assembly (34) and illicit environmentally dependent responses to exogenous stressors.

Finally, we observed a nonlinear relationship between gut microbiome diversity and infection outcomes, with water temperature moderating these dynamics. Consistent with our prior research on zebrafish infected by *Pseudocapillaria tomentosa* (15) exhibiting dysbiotic microbiomes described by the Anna Karenina Principle (AKP) (35). The AKP postulates that dysbiotic microbiome communities display higher rates of dispersion or are more inconsistent between dysbiotic individuals, whereas the microbiome community composition of healthy individuals are less dispersed or are more similar to one another. In particular, we observed elevated microbial community dispersion among heavily parasite infected fish, reflecting unstable microbial states characteristic of the AKP. However, when accounting for water temperature, the AKP effect diminished, and multiple alternative stable states emerged, which may explain

inconsistencies in prior studies that failed to detect the AKP or found contrary results (29). Our findings underscore the importance of accounting for individuals' temporal and spatial contexts to adequately assess microbiome stability in response to stressors (36). However, current definitions of microbiome stability (11,37,38), which rely on a homeostatic framework, may be insufficient to describe these dynamic shifts. A homeorhetic framework (39), conceptualizing stability as a change along a stable trajectory rather than a fixed state, could improve our ability to measure microbiome stability and dysbiosis across dynamic temporal and spatial scales. Shifting to such a model may reconcile discrepancies in AKP detection across studies and provide deeper insights into how microbiomes respond to exogenous stressors in different host systems.

In conclusion, we found that water temperature alters the contextual landscape of the microbiome to impact its response to an exogenous stressor of an intestinal parasite. Our work revealed that differences in environmental conditions of water temperature were sufficient to temporally change the gut microbiome's response to parasitic exposure and impact infection outcomes in zebrafish. While the zebrafish gut microbiome differs taxonomically from other animal-microbiome systems, a considerable amount of functional capacity is shared between animals (40). Thus, zebrafish serve as a powerful model for investigating how environmental changes and stressor exposures influence microbiomes and host health. Our findings have important implications for microbiome research in the context of climate change, demonstrating that rising temperatures may have unexpected effects on gut microbiomes and infection outcomes. Future work should further clarify how gut microbiomes and host responses

572 buffer against combined environmental stressors, ultimately shaping health outcomes in  
573 vertebrates.

574

575

## **Methods**

### **Fish husbandry**

5D strain zebrafish embryos were obtained from the Sinnhuber Aquatic Resource Center at Oregon State University, and reared in our vivarium at Nash Hall (Corvallis, OR, USA). The vivarium is a single pass flow through, using dechlorinated city water. Fish were then randomly divided into twelve 2.8 L tanks. The temperature was recorded daily and the ambient temperature ranged from 27 to 28°C. All other water conditions were monitored weekly, pH was maintained at 7.6, total ammonia was not detected, and conductivity ranged from 102 to 122. Light in the vivarium was provided for 14 hours/day. Fish were fed Gemma Micro 300 (Skretting; Fontaine-les-Vervins, France) at 1.5% body weight twice daily, except on weekends or during exposure to parasitic eggs. One plastic aquatic plant piece, approximately six inches in length, was added to each tank for enrichment. The use of zebrafish in this study was approved by the Institutional Animal Care and Use Committee (IACUC) at Oregon State University (permit number: 5151).

### **Temperature exposure**

Fish at 5 month-old were randomly divided into 12 9.5-L tanks (approximately 25 fish/tank). Each tank was outfitted with a 50W (28 °C treatment only) or 100W HG-802 Hygger titanium aquarium heater (Hygger, Shenzhen Mago Co., Ltd., Shenzhen City, Guangdong Province, China). Four of the twelve tanks were assigned to each of the temperature treatments: 28°C, 32°C, or 35°C. Two tanks for each temperature were held as pathogen negative controls and two tanks were exposed to *Pseudocapillaria*

*tomentosa* as described below. Fish were acclimated to the prescribed temperature treatments by increasing the heater thermostat settings by 1°C every two days until the final prescribed temperature was achieved. Two temperature logging thermometers, one for the six pathogen negative control tanks and one for the six *P. tomentosa* exposed tanks, were rotated through the tanks every two days on weekdays to monitor temperature at each temperature treatment. The average range recorded for the water temperature treatments was +/- 1°C.

### ***Pseudocapillaria tomentosa* exposure**

Eggs were collected from a population of infected zebrafish that we constantly maintain in our laboratory as described by Martins *et al.* 2017 (41). Eggs were allowed to larvate for 6 days at 28°C, and fish were exposed at 25 larvated eggs/fish. Water flow was turned off for 36h to enhance exposures, while an airstone was provided to each tank to maintain adequate oxygen levels. This was a lower exposure dose than many of our previous studies (14). Therefore, we enhanced exposure adding 1 L of water from a stock tank holding infected fish twice a day during the 36 h hour post exposure period. This additional water supplement was created by siphoning water from the bottom of the exposed stock fish tank because the infectious stage is a larvated egg, which sinks in water.

### **Infection assessment**

Exposed and control fish were collected and examined for worm prevalence, abundance and state of development using wet mounts of whole intestines as described

in Schuster *et al.* 2023 (42). After recording observations in wet mounts, the individual intestine was preserved in Dietrich's solution and intestines of 95 fish were processed for histology prepared as described in Gaulke *et al.* 2019 (15). Here we focused on selected samples from fish from the 35°C group as very few worms were detected by wet mounts in this group. Two stepwise sections, 50 um apart, were obtained from each block to enhance the possibility of larval worms

### **Fecal collection**

Five fish from each tank were randomly selected for fecal sampling at 0 dpe (n=60; 5 samples/tank), prior to parasite exposure. Subsequent fecal sampling took place at 14- (n = 54), 21- (n = 48), 28- (n = 47), and 42 (n = 51) dpe to parasites. Fecal material was collected as previously described (20). In brief, fish were transferred to 1.4 L tanks (1 fish/tank) containing ~ 0.4 L of fish water at least 30 min after the last feeding of the day. Fish were left to defecate overnight and all fecal material was collected from each tank the following morning in a 1.5ml microcentrifuge tube. Fecal samples were immediately spun at 10k rpm for 2 min, excess tank water was removed, and samples were snap frozen on dry ice and stored at -80 °C until processing. However, not all fish produced a fecal sample for a variety of reasons. For instance, experiments involving fish have expected mortality, and fish which died prematurely did not produce fecal samples. Additionally, infection conditions may have prevented infected fish from producing a fecal sample. Instances where fish failed to produce a fecal sample are noted in the metadata sheet.

## **Microbial 16S rRNA library preparation and sequencing**

Microbial DNA was extracted from zebrafish fecal samples and 16S rRNA gene sequence libraries were produced and analyzed following previously described methods (43). DNA was isolated from fecal samples using the DNeasy 96 PowerSoil Pro DNA kits (Qiagen, Hilden, Germany), in accordance with the manufacturer's directions. In brief, samples were subjected to bead beating for 10 minutes using the Qiagen TissueLyser II, spun a max speed in the centrifuge, supernatant was process using 96 well columns, and DNA was eluted with 100µl Tris buffer. The V4 region of the 16S rRNA gene was PCR amplified using dual-index 16S primers and protocols (44). PCR was performed using 1 µl of purified DNA, 2µl of a 5µM mix of the forward and reverse dual-index primers, 5µl of Platinum II Hot-Start PCR Master Mix (Thermo Fisher, Carlsbad, CA), and 2µl water with the following conditions, 94°C, 3m; (94°C, 30s; 50°C, 30s; 68°C, 1m)x 35; 68°C 10m. PCR products were visualized on a 1.5% agarose gel and quantified on the BioTek Synergy H1 Hybrid Multi-Mode Plate Reader using the Quand-iT 1X dsDNA HS Assay kit (Thermo Fisher, Carlsbad, CA, USA). A 100ng aliquot of DNA was selected from each of the 300 samples, the pooled DNA was cleaned using the QIAGEN QIAquick PCR purification kit, and quantified using Qubit HS kit (Carlsbad, CA). The quality of the pooled library was verified on the Agilent TapeStation 4200. The prepared library was submitted to the Oregon State University Center for Quantitative Life Sciences (CQLS) for paired end 2x300 bp read sequencing on an Illumina MiSeq System

## **Bioinformatic processing**

All microbiome DNA sequence analyses and visualization were conducted in R (v 4.3.3)(45). Raw reads were filtered for quality, merged, and assigned using the DADA2 R package (v 1.26.0) as previously described (46). In brief, forward and reverse reads were trimmed at 250 and 225 bp, respectively, subsequently merged into contigs, and subject to amplicon sequence variant (ASV) identification. ASVs unannotated at the Phylum level or identified as non-bacterial were removed, which resulted in 674 remaining detected ASVs. Samples containing reads below the minimum required read count (<5000) were dropped from downstream analysis. The final sample number for microbiome analysis was 260. Phylogenetic analysis was conducted using MOTHUR (v 1.46.1)(47) with default parameters as previously described (17). Phylogeny was inferred using FastTree2 (48), an approximately-maximum-likelihood method. Microbiome and sample data were contained in a Phyloseq object using the Phyloseq R package (49), and the tidyverse (v 2.0.0)(50) and microViz (v 0.12.1) R packages were used for downstream data processing, analyzing, and visualization (51). Code for bioinformatic processing are available at [https://github.com/sielerjm/Sieler2025\\_ZF\\_Temperature\\_Parasite/](https://github.com/sielerjm/Sieler2025_ZF_Temperature_Parasite/).

### **Microbiome diversity metrics**

All microbiome analyses were conducted at the genera level unless otherwise noted. We estimated four alpha-diversity metrics for each microbiome fecal sample: Simpson (52), Shannon (53), phylogenetic diversity (Faith's PD (54); ASVs), and richness. We also estimated beta-diversity between each pair of microbiome fecal



690 samples using three metrics. These included Bray-Curtis (55), Canberra (56), and half-  
691 weighted generalized UniFrac (57).

## 693 **Statistical Analyses**

694 All statistical analyses were conducted in R (v 4.3.3)(45) with a significance level  
695 of  $\alpha = 0.05$ , and randomization procedures employed a fixed seed (42) to ensure  
696 reproducibility. Code for statistical analyses are available at  
697 [https://github.com/sielerjm/Sieler2025\\_ZF\\_Temperature\\_Parasite/](https://github.com/sielerjm/Sieler2025_ZF_Temperature_Parasite/).

698 Using methods previously described, we assessed normality of alpha-diversity  
699 scores using Shapiro-Wilk test (45,58,59), transformed non-normal scores using  
700 Tukey's Ladder of Powers (59,60) and normalized from 0 to 1 (20) before incorporation  
701 into linear models. We used generalized linear models (GLM), we assessed the  
702 relationship between alpha-diversity score and experimental parameters. Post hoc  
703 Tukey Tests evaluated pairwise comparisons of models using the multcomp R package  
704 (v 1.4-25)(61). We corrected for multiple tests using Benjamini-Hochberg correction  
705 (62). Two-way ANOVA was used to determine if the expanded models of these GLMs  
706 significantly improved the response variable relative to the null model.

708 Beta-diversity models were generated using methods described previously (20).  
709 In brief, we assessed the relationship between experimental parameters and beta-  
710 diversity by applying a step-wise model selection approach as implemented in the  
711 capscale function (vegan R package v 2.6-4)(63). Optimal models were subsequently  
712 subject to permutation analysis of variance (PERMANOVA) with anova.cca using the

vegan R package to determine if the selected model parameters significantly explained the variation in microbiome composition across samples. Differential abundance was measured using MaAsLin2 (64). We assessed beta-diversity dispersion within groups with betadisper using the vegan R package.

To assess the relationship between parasite infection outcomes and experimental parameters, we used negative binomial generalized linear models (GLM) with the glm.nb function from the MASS R package (v 7.3-60.0.1)(65) and used the negative binomial distribution to account for overdispersion in the count data, a common characteristic of parasite infection data (14). Significance of main effects and interactions was assessed using two-way ANOVA implemented through the Anova function with the Car R package (v 3.1-2)(66). Post-hoc comparisons were conducted using Tukey's HSD tests via the emmeans package, where we estimated marginal means and performed pairwise contrasts with p-value adjustment using the Tukey method (67). Detection method comparisons were analyzed on a subset of samples used for microbiome analysis (120 samples, 20 samples/tank, ~10 samples/time point; Table S3C.1). To compare detection methods between wet mount and histology, we used McNemar's test (68), with discordant pairs (wet only vs histology only) examined at each temperature and DPE combination through 2×2 contingency tables Table S3C.2-3). Using similar methods as described above, we assessed the relationship between infection outcomes and microbiome diversity using GLMs (Table S3B.1).

## **Abbreviations**

dpe = days post exposure

736

## 737 **Acknowledgements**

738       The authors thank the members of the Oregon State University Center for  
739 Quantitative Life Sciences for technical assistance with sequencing and maintenance of  
740 our computational infrastructure, and Dr. Corbin Schuster and Kelan Elliot for sample  
741 collection assistance.

742

## 743 **Data Availability**

744       All code generated during this analysis is available in the GitHub repository at the  
745 following URL: [https://github.com/sielerjm/Sieler2025\\_ZF\\_Temperature\\_Parasite](https://github.com/sielerjm/Sieler2025_ZF_Temperature_Parasite).

746       Supplementary tables and figures can be in the accompanying supplementary files. The  
747 raw sequence files generated during the current study are available at the NCBI  
748 Sequence Read Archive (SRA) project number: [PRJNA1219243](#).

749

## 750 **Author contributions**

751       TJS and MLK conceived and designed the study. CEA and MJS conducted the  
752 experiments. MJS, TJS, and KDK performed the gut microbiome and integrated  
753 analyses. MJS, TJS, MLK, CEA, KDK, contributed to the preparation and editing of the  
754 manuscript. MJS prepared the figures. All authors read and approved the final  
755 manuscript.

756

## 757 **Funding**

758           This work was supported in part by a National Foundation Grant (#2025457) to  
759 TJS, and a fellowship to MJS offered by the Oregon Department of Fish and Wildlife.

760

761   **Declarations**

762           The authors declare no competing interests.

- 764 1. Acevedo-Whitehouse K, Duffus ALJ. Effects of environmental change on wildlife  
765 health. *Phil Trans R Soc B* [Internet]. 2009 Nov 27 [cited 2024 Oct  
766 31];364(1534):3429–38. Available from:  
767 <https://royalsocietypublishing.org/doi/10.1098/rstb.2009.0128>
- 768 2. Mas-Coma S, Valero MA, Bargues MD. Effects of climate change on animal and  
769 zoonotic helminthiases. *Rev Sci Tech*. 2008;27(2):443–57.
- 770 3. El-Sayed A, Kamel M. Climatic changes and their role in emergence and re-  
771 emergence of diseases. *Environ Sci Pollut Res* [Internet]. 2020 Jun [cited 2025 Mar  
772 18];27(18):22336–52. Available from: [https://link.springer.com/10.1007/s11356-020-](https://link.springer.com/10.1007/s11356-020-08896-w)  
773 [08896-w](https://link.springer.com/10.1007/s11356-020-08896-w)
- 774 4. Sydeman WJ, Poloczanska E, Reed TE, Thompson SA. Climate change and marine  
775 vertebrates. *Science* [Internet]. 2015 Nov 13 [cited 2024 Oct 31];350(6262):772–7.  
776 Available from: <https://www.science.org/doi/10.1126/science.aac9874>
- 777 5. Tomanek L. Variation in the heat shock response and its implication for predicting  
778 the effect of global climate change on species' biogeographical distribution ranges  
779 and metabolic costs. *Journal of Experimental Biology* [Internet]. 2010 Mar 15 [cited  
780 2024 Oct 31];213(6):971–9. Available from:  
781 [https://journals.biologists.com/jeb/article/213/6/971/10173/Variation-in-the-heat-](https://journals.biologists.com/jeb/article/213/6/971/10173/Variation-in-the-heat-shock-response-and-its)  
782 [shock-response-and-its](https://journals.biologists.com/jeb/article/213/6/971/10173/Variation-in-the-heat-shock-response-and-its)
- 783 6. Ackerly DD, Loarie SR, Cornwell WK, Weiss SB, Hamilton H, Branciforte R, et al.  
784 The geography of climate change: implications for conservation biogeography.  
785 *Diversity and Distributions* [Internet]. 2010 [cited 2024 Oct 31];16(3):476–87.  
786 Available from: [https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1472-](https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1472-4642.2010.00654.x)  
787 [4642.2010.00654.x](https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1472-4642.2010.00654.x)
- 788 7. Duarte CM, Agusti S, Barbier E, Britten GL, Castilla JC, Gattuso JP, et al. Rebuilding  
789 marine life. *Nature* [Internet]. 2020 Apr [cited 2024 Oct 31];580(7801):39–51.  
790 Available from: <https://www.nature.com/articles/s41586-020-2146-7>
- 791 8. Greenspan SE, Migliorini GH, Lyra ML, Pontes MR, Carvalho T, Ribeiro LP, et al.  
792 Warming drives ecological community changes linked to host-associated  
793 microbiome dysbiosis. *Nat Clim Chang* [Internet]. 2020 Nov [cited 2024 Oct  
794 31];10(11):1057–61. Available from: [https://www.nature.com/articles/s41558-020-](https://www.nature.com/articles/s41558-020-0899-5)  
795 [0899-5](https://www.nature.com/articles/s41558-020-0899-5)
- 796 9. Fontaine SS, Kohl KD. The microbiome buffers tadpole hosts from heat stress: a  
797 hologenomic approach to understand host–microbe interactions under warming.  
798 *Journal of Experimental Biology* [Internet]. 2023 Jan 1 [cited 2023 Aug  
799 25];226(1):jeb245191. Available from:

800 <https://journals.biologists.com/jeb/article/226/1/jeb245191/286604/The-microbiome->  
801 [buffers-tadpole-hosts-from-heat](https://journals.biologists.com/jeb/article/226/1/jeb245191/286604/The-microbiome-)

802 10. Fontaine SS, Mineo PM, Kohl KD. Experimental manipulation of microbiota reduces  
803 host thermal tolerance and fitness under heat stress in a vertebrate ectotherm. *Nat*  
804 *Ecol Evol* [Internet]. 2022 Mar 7 [cited 2025 Feb 11];6(4):405–17. Available from:  
805 <https://www.nature.com/articles/s41559-022-01686-2>

806 11. Fassarella M, Blaak EE, Penders J, Nauta A, Smidt H, Zoetendal EG. Gut  
807 microbiome stability and resilience: elucidating the response to perturbations in  
808 order to modulate gut health. *Gut* [Internet]. 2021 Mar [cited 2023 Jun 7];70(3):595–  
809 605. Available from: <https://gut.bmj.com/lookup/doi/10.1136/gutjnl-2020-321747>

810 12. López-Olmeda JF, Sánchez-Vázquez FJ. Thermal biology of zebrafish (*Danio rerio*).  
811 *Journal of Thermal Biology* [Internet]. 2011 Mar [cited 2024 Oct 31];36(2):91–104.  
812 Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0306456510001440>

813 13. Wang B, Zhang S qin, Dong J li, Li Y, Jin Y xiao, Xiao H wen, et al. Ambient  
814 temperature structures the gut microbiota of zebrafish to impact the response to  
815 radioactive pollution. *Environmental Pollution* [Internet]. 2022 Jan [cited 2024 Jun  
816 19];293:118539. Available from:  
817 <https://linkinghub.elsevier.com/retrieve/pii/S0269749121021217>

818 14. Kent M, Gaulke C, Watral V, Sharpton T. *Pseudocapillaria tomentosa* in laboratory  
819 zebrafish *Danio rerio*: patterns of infection and dose response. *Dis Aquat Org*  
820 [Internet]. 2018 Nov 6 [cited 2024 Oct 31];131(2):121–31. Available from:  
821 <https://www.int-res.com/abstracts/dao/v131/n2/p121-131/>

822 15. Gaulke CA, Martins ML, Watral VG, Humphreys IR, Spagnoli ST, Kent ML, et al. A  
823 longitudinal assessment of host-microbe-parasite interactions resolves the zebrafish  
824 gut microbiome's link to *Pseudocapillaria tomentosa* infection and pathology.  
825 *Microbiome* [Internet]. 2019 Dec [cited 2023 Aug 25];7(1):10. Available from:  
826 <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-019-0622-9>

827 16. Semova I, Carten JD, Stombaugh J, Mackey LC, Knight R, Farber SA, et al.  
828 Microbiota Regulate Intestinal Absorption and Metabolism of Fatty Acids in the  
829 Zebrafish. *Cell Host & Microbe* [Internet]. 2012 Sep 13 [cited 2022 Oct  
830 31];12(3):277–88. Available from:  
831 <https://www.sciencedirect.com/science/article/pii/S1931312812002740>

832 17. Sharpton TJ, Stagaman K, Sieler Jr. MJ, Arnold HK, Davis EW. Phylogenetic  
833 Integration Reveals the Zebrafish Core Microbiome and Its Sensitivity to  
834 Environmental Exposures. *Toxics* [Internet]. 2021 Jan [cited 2022 Feb 16];9(1):10.  
835 Available from: <https://www.mdpi.com/2305-6304/9/1/10>

836 18. Stagaman K, Sharpton TJ, Guillemin K. Zebrafish microbiome studies make waves.  
837 *Lab Anim (NY)* [Internet]. 2020 Jul [cited 2022 Nov 2];49(7):201–7. Available from:  
838 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7755162/>

19. Stephens WZ, Burns AR, Stagaman K, Wong S, Rawls JF, Guillemin K, et al. The composition of the zebrafish intestinal microbial community varies across development. *ISME J* [Internet]. 2016 Mar [cited 2022 Jul 14];10(3):644–54. Available from: <https://www.nature.com/articles/ismej2015140>
20. Sieler MJ, Al-Samarrie CE, Kasschau KD, Varga ZM, Kent ML, Sharpton TJ. Disentangling the link between zebrafish diet, gut microbiome succession, and *Mycobacterium chelonae* infection. *anim microbiome* [Internet]. 2023 Aug 10 [cited 2023 Aug 25];5(1):38. Available from: <https://doi.org/10.1186/s42523-023-00254-8>
21. Moravec F. FIRST RECORD OF THE NEMATODE PSEUDOCAPILLARIA BREVISPICULA. *FOLIA PARASITOLOGICA (PRAHA)* [Internet]. 1984 [cited 2025 Mar 18];31:241–5. Available from: <https://folia.paru.cas.cz/pdfs/fol/1984/03/06.pdf>
22. Dickerson HW. *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (phylum Ciliophora). In: Woo PTK, editor. Fish diseases and disorders Volume 1: protozoan and metazoan infections [Internet]. 2nd ed. UK: CABI; 2006 [cited 2025 Mar 19]. p. 116–53. Available from: <http://www.cabidigitallibrary.org/doi/10.1079/9780851990156.0116>
23. Lardeux F, Cheffort J. Temperature thresholds and statistical modelling of larval *Wuchereria bancrofti* (Filariidea: Onchocercidae) developmental rates. *Parasitology* [Internet]. 1997 Feb [cited 2025 Mar 18];114(2):123–34. Available from: [https://www.cambridge.org/core/product/identifier/S0031182096008359/type/journal\\_article](https://www.cambridge.org/core/product/identifier/S0031182096008359/type/journal_article)
24. Islam MJ, Kunzmann A, Slater MJ. Responses of aquaculture fish to climate change-induced extreme temperatures: A review. *Journal of the World Aquaculture Society* [Internet]. 2022 [cited 2024 Oct 30];53(2):314–66. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/jwas.12853>
25. Makrinos DL, Bowden TJ. Natural environmental impacts on teleost immune function. *Fish & Shellfish Immunology* [Internet]. 2016 Jun [cited 2024 Oct 29];53:50–7. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1050464816300870>
26. Dittmar J, Janssen H, Kuske A, Kurtz J, Scharsack JP. Heat and immunity: an experimental heat wave alters immune functions in three-spined sticklebacks (*asterosteus aculeatus*). *Journal of Animal Ecology* [Internet]. 2014 [cited 2024 Oct 29];83(4):744–57. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/1365-2656.12175>
27. Bailey C, Segner H, Casanova-Nakayama A, Wahli T. Who needs the hotspot? The effect of temperature on the fish host immune response to *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease. *Fish & Shellfish Immunology* [Internet]. 2017 Apr [cited 2024 Oct 30];63:424–37. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1050464817301110>

28. Sepulveda J, Moeller AH. The Effects of Temperature on Animal Gut Microbiomes. *Frontiers in Microbiology* [Internet]. 2020 [cited 2023 Apr 6];11. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.00384>
29. Li P, Zhang J, Liu X, Gan L, Xie Y, Zhang H, et al. The Function and the Affecting Factors of the Zebrafish Gut Microbiota. *Front Microbiol* [Internet]. 2022 Jun 2 [cited 2024 Jun 19];13:903471. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2022.903471/full>
30. Relman DA. The human microbiome: ecosystem resilience and health. *Nutrition Reviews* [Internet]. 2012 Aug [cited 2025 Mar 19];70:S2–9. Available from: <https://academic.oup.com/nutritionreviews/article-lookup/doi/10.1111/j.1753-4887.2012.00489.x>
31. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JL. Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat Rev Microbiol* [Internet]. 2008 Oct [cited 2022 Nov 2];6(10):776–88. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2664199/>
32. Roeselers G, Mittge EK, Stephens WZ, Parichy DM, Cavanaugh CM, Guillemin K, et al. Evidence for a core gut microbiota in the zebrafish. *ISME J* [Internet]. 2011 Oct [cited 2022 Nov 3];5(10):1595–608. Available from: <http://www.nature.com/articles/ismej201138>
33. Breen P, Winters AD, Nag D, Ahmad MM, Theis KR, Withey JH. Internal Versus External Pressures: Effect of Housing Systems on the Zebrafish Microbiome. *Zebrafish* [Internet]. 2019 Aug [cited 2022 Jul 14];16(4):388–400. Available from: <https://www.liebertpub.com/doi/10.1089/zeb.2018.1711>
34. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. The Application of Ecological Theory Toward an Understanding of the Human Microbiome. *Science* [Internet]. 2012 Jun 8 [cited 2024 May 1];336(6086):1255–62. Available from: <https://www.science.org/doi/10.1126/science.1224203>
35. Zaneveld JR, McMinds R, Vega Thurber R. Stress and stability: applying the Anna Karenina principle to animal microbiomes. *Nat Microbiol* [Internet]. 2017 Sep [cited 2022 Jan 30];2(9):17121. Available from: <http://www.nature.com/articles/nmicrobiol2017121>
36. Jeltsch F, Roeleke M, Abdelfattah A, Arlinghaus R, Berg G, Blaum N, et al. The need for an individual-based global change ecology. *IBE* [Internet]. 2025 Mar 27 [cited 2025 Mar 28];1:1–18. Available from: <https://ibe.pensoft.net/article/148200/>
37. Sommer AJ, Peters A, Rommel M, Cyrus J, Grallert H, Haller D, et al. A randomization-based causal inference framework for uncovering environmental exposure effects on human gut microbiota [Internet]. 2021 Mar [cited 2022 Jan 13] p. 2021.02.24.432662. Available from: <https://www.biorxiv.org/content/10.1101/2021.02.24.432662v2>



- 917 38. Schlomann BH, Parthasarathy R. Timescales of gut microbiome dynamics. *Current*  
918 *Opinion in Microbiology* [Internet]. 2019 Aug [cited 2024 Aug 10];50:56–63. Available  
919 from: <https://linkinghub.elsevier.com/retrieve/pii/S1369527419300463>
- 920 39. Waddington CH. Canalization of development and the inheritance of acquired  
921 characters. 1942;
- 922 40. Rawls JF, Samuel BS, Gordon JL. Gnotobiotic zebrafish reveal evolutionarily  
923 conserved responses to the gut microbiota. *Proc Natl Acad Sci USA* [Internet]. 2004  
924 Mar 30 [cited 2024 Oct 31];101(13):4596–601. Available from:  
925 <https://pnas.org/doi/full/10.1073/pnas.0400706101>
- 926 41. Martins ML, Watral V, Rodrigues-Soares JP, Kent ML. A method for collecting eggs  
927 of *Pseudocapillaria tomentosa* (Nematoda: Capillariidae) from zebrafish *Danio rerio*  
928 and efficacy of heat and chlorine for killing the nematode's eggs. *Journal of Fish*  
929 *Diseases* [Internet]. 2017 [cited 2025 Mar 18];40(2):169–82. Available from:  
930 <https://onlinelibrary.wiley.com/doi/abs/10.1111/jfd.12501>
- 931 42. Schuster CJ, Leong C, Kasschau KD, Sharpton TJ, Kent ML. Early detection of  
932 *Pseudocapillaria tomentosa* by qPCR in four lines of zebrafish, *Danio rerio*  
933 (Hamilton 1882). *Journal of Fish Diseases* [Internet]. 2023 Jun [cited 2024 Jan  
934 4];46(6):619–27. Available from: <https://onlinelibrary.wiley.com/doi/10.1111/jfd.13773>
- 935 43. Hammer AJ, Gaulke CA, Garcia-Jaramillo M, Leong C, Morre J, Sieler Jr. MJ, et al.  
936 Gut microbiota metabolically mediate intestinal helminth infection in zebrafish. Rawls  
937 JF, editor. *mSystems* [Internet]. 2024 Aug 27 [cited 2024 Aug 28];e00545-24.  
938 Available from: <https://journals.asm.org/doi/10.1128/msystems.00545-24>
- 939 44. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a  
940 Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon  
941 Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied and*  
942 *Environmental Microbiology* [Internet]. 2013 Sep [cited 2025 Mar 26];79(17):5112–  
943 20. Available from: <https://journals.asm.org/doi/full/10.1128/aem.01043-13>
- 944 45. R Core Team. R: A Language and Environment for Statistical computing. [Internet].  
945 Vienna, Austria: R Foundation for Statistical Computing, Vienna; 2025. Available  
946 from: <https://www.R-project.org/>
- 947 46. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2:  
948 High-resolution sample inference from Illumina amplicon data. *Nat Methods*  
949 [Internet]. 2016 Jul [cited 2025 Feb 11];13(7):581–3. Available from:  
950 <https://www.nature.com/articles/nmeth.3869>
- 951 47. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al.  
952 Introducing mothur: Open-Source, Platform-Independent, Community-Supported  
953 Software for Describing and Comparing Microbial Communities. *Appl Environ*  
954 *Microbiol* [Internet]. 2009 Dec [cited 2025 Feb 11];75(23):7537–41. Available from:  
955 <https://journals.asm.org/doi/10.1128/AEM.01541-09>

- 956 48. Price MN, Dehal PS, Arkin AP. FastTree 2 – Approximately Maximum-Likelihood  
957 Trees for Large Alignments. Poon AFY, editor. PLoS ONE [Internet]. 2010 Mar 10  
958 [cited 2025 Feb 11];5(3):e9490. Available from:  
959 <https://dx.plos.org/10.1371/journal.pone.0009490>
- 960 49. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive  
961 Analysis and Graphics of Microbiome Census Data. Watson M, editor. PLoS ONE  
962 [Internet]. 2013 Apr 22 [cited 2025 Feb 11];8(4):e61217. Available from:  
963 <https://dx.plos.org/10.1371/journal.pone.0061217>
- 964 50. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome  
965 to the Tidyverse. JOSS [Internet]. 2019 Nov 21 [cited 2025 Feb 11];4(43):1686.  
966 Available from: <https://joss.theoj.org/papers/10.21105/joss.01686>
- 967 51. Barnett D, Arts I, Penders J. microViz: an R package for microbiome data  
968 visualization and statistics. JOSS [Internet]. 2021 Jul 10 [cited 2025 Feb  
969 11];6(63):3201. Available from: <https://joss.theoj.org/papers/10.21105/joss.03201>
- 970 52. Simpson EH. Measurement of Diversity. Nature [Internet]. 1949 Apr [cited 2025 Feb  
971 11];163(4148):688–688. Available from: <https://www.nature.com/articles/163688a0>
- 972 53. Shannon C, Weaver W. The Mathematical Theory of Communication.
- 973 54. Faith DP. Conservation evaluation and phylogenetic diversity. Biological  
974 Conservation [Internet]. 1992 [cited 2025 Feb 11];61(1):1–10. Available from:  
975 <https://linkinghub.elsevier.com/retrieve/pii/0006320792912013>
- 976 55. Bray JR, Curtis JT. An Ordination of the Upland Forest Communities of Southern  
977 Wisconsin. Ecological Monographs [Internet]. 1957 Oct [cited 2025 Feb  
978 11];27(4):325–49. Available from:  
979 <https://esajournals.onlinelibrary.wiley.com/doi/10.2307/1942268>
- 980 56. Lance GN, Williams WT. Mixed-data classificatory programs I - agglomerative  
981 systems. Aust Comput J [Internet]. 1967;1:15–20. Available from:  
982 <https://api.semanticscholar.org/CorpusID:43168398>
- 983 57. Chen J, Bittinger K, Charlson ES, Hoffmann C, Lewis J, Wu GD, et al. Associating  
984 microbiome composition with environmental covariates using generalized UniFrac  
985 distances. Bioinformatics [Internet]. 2012 Aug 15 [cited 2025 Feb 11];28(16):2106–  
986 13. Available from:  
987 <https://academic.oup.com/bioinformatics/article/28/16/2106/324465>
- 988 58. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples).  
989 Biometrika [Internet]. 1965 Dec 1 [cited 2025 Feb 11];52(3–4):591–611. Available  
990 from: <https://academic.oup.com/biomet/article-lookup/doi/10.1093/biomet/52.3-4.591>
- 991 59. Kundu P, Torres ERS, Stagaman K, Kasschau K, Okhovat M, Holden S, et al.  
992 Integrated analysis of behavioral, epigenetic, and gut microbiome analyses in

- 993 AppNL-G-F, AppNL-F, and wild type mice. *Sci Rep* [Internet]. 2021 Feb 25 [cited  
994 2022 Dec 6];11(1):4678. Available from: [https://www.nature.com/articles/s41598-](https://www.nature.com/articles/s41598-021-83851-4)  
995 021-83851-4
- 996 60. Exploratory data analysis. In: *The concise encyclopedia of statistics* [Internet]. New  
997 York, NY: Springer New York; 2008. p. 192–4. Available from:  
998 [https://doi.org/10.1007/978-0-387-32833-1\\_136](https://doi.org/10.1007/978-0-387-32833-1_136)
- 999 61. Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S, et al.  
1000 Package ‘multcomp.’ Simultaneous inference in general parametric models Project  
1001 for Statistical Computing, Vienna, Austria. 2016;
- 1002 62. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing  
1003 under dependency. *Ann Statist* [Internet]. 2001 Aug 1 [cited 2023 Jan 29];29(4).  
1004 Available from: [https://projecteuclid.org/journals/annals-of-statistics/volume-](https://projecteuclid.org/journals/annals-of-statistics/volume-29/issue-4/The-control-of-the-false-discovery-rate-in-multiple-testing/10.1214/aos/1013699998.full)  
1005 29/issue-4/The-control-of-the-false-discovery-rate-in-multiple-  
1006 testing/10.1214/aos/1013699998.full
- 1007 63. Oksanen J, Kindt R, Legendre P, Hara B, Simpson G, Solymos P, et al. The vegan  
1008 Package. 2009 Jan;
- 1009 64. Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, et al. Multivariable  
1010 association discovery in population-scale meta-omics studies. Coelho LP, editor.  
1011 *PLoS Comput Biol* [Internet]. 2021 Nov 16 [cited 2025 Feb 11];17(11):e1009442.  
1012 Available from: <https://dx.plos.org/10.1371/journal.pcbi.1009442>
- 1013 65. Ripley B, Venables B. MASS: Support Functions and Datasets for Venables and  
1014 Ripley’s MASS [Internet]. 2009 [cited 2025 Feb 11]. p. 7.3-64. Available from:  
1015 <https://CRAN.R-project.org/package=MASS>
- 1016 66. Fox J, Weisberg S, Price B. car: Companion to Applied Regression [Internet]. 2001  
1017 [cited 2025 Feb 11]. p. 3.1-3. Available from: [https://CRAN.R-](https://CRAN.R-project.org/package=car)  
1018 [project.org/package=car](https://CRAN.R-project.org/package=car)
- 1019 67. Lenth RV. emmeans: Estimated Marginal Means, aka Least-Squares Means  
1020 [Internet]. 2017 [cited 2025 Feb 11]. p. 1.10.7. Available from: [https://CRAN.R-](https://CRAN.R-project.org/package=emmeans)  
1021 [project.org/package=emmeans](https://CRAN.R-project.org/package=emmeans)
- 1022 68. McNemar Q. Note on the Sampling Error of the Difference Between Correlated  
1023 Proportions or Percentages. *Psychometrika* [Internet]. 1947 Jun [cited 2025 Feb  
1024 11];12(2):153–7. Available from:  
1025 [https://www.cambridge.org/core/product/identifier/S0033312300045178/type/journal](https://www.cambridge.org/core/product/identifier/S0033312300045178/type/journal_article)  
1026 [\\_article](https://www.cambridge.org/core/product/identifier/S0033312300045178/type/journal_article)

Figure 1. Experimental design showing treatments and husbandry events during the course of the study. Symbols indicate when an experimental event occurred at each time point. (1) 260 fish were assigned and acclimated to one of three water temperature groups (e.g., 28°C, 32°C, or 35°C) and reared from 0 to 164 days post fertilization (dpf). (2a) At 164 dpf (or 0 days post exposure; DPE), fecal collections were collected from a random selection of five fish per tank (n = 60). Additionally, histological and wet mount assessments were conducted on selected fish to assess presence of infection and infection burden. (1b) Afterwards, a cohort of fish from each water temperature group were exposed to *Pseudocapillaria tomentosa*. (3-6) Subsequent fecal samples were collected and histopathological assessments were conducted at 14 dpe (n = 54), (3) 21 dpe (n = 48), (4) 28 dpe (n = 47), and (5) 42 dpe (n = 51).

Figure 2. Effects of water temperature on zebrafish gut microbiomes. (A) Simpson's Index of diversity shows that gut microbiome diversity significantly differs between fish reared at 28°C and 35°C water temperatures. (B) Capscale ordination based on the Bray-Curtis dissimilarity of gut microbiome composition constrained on the main effect of temperature. The analysis shows that gut microbiome composition significantly differs between fish reared at different water temperatures. (C) Simpson's Index of diversity shows microbial gut diversity increases with time from 0 days post exposure (dpe) to 42 dpe, irrespective of water temperature. (D) Capscale ordination of gut microbiome composition based on the Bray-Curtis dissimilarity constrained on the main effects of water temperature and time (days post exposure, dpe), and their interaction. The analysis shows that shows that gut microbiome composition differs between fish across

time depending on water temperature. Ribbons and ellipses indicate 95% confidence interval. Only statistically significant relationships are shown. A “\*” indicates statistical significance below the “0.05” level. Black arrows indicate direction of greatest change in the indicated by covariates.

Figure 3. Infection outcomes in zebrafish exposed to *Pseudocapillaria tomentosa*. (A) Histological sections stained with H&E stain in zebrafish exposed to *P. tomentosa* examined at 35°C at 21 days post exposure. Arrow = larval worms, sagittal and cross sections. Bar = 50 µm (B) Infection outcome analysis of fish exposed to *P. tomentosa* (n = 89) by temperature. Fish reared at 28°C and 32°C water temperatures had significantly different infection burden to fish reared at 35°C water temperature. Only one fish in our microbiome analysis reared at 35°C was identified as being positively infected by wet mount. Only statistically significant relationships are shown. A “\*” indicates statistical significance below the “0.05” level.

Figure 4. Effects of *Pseudocapillaria tomentosa* exposure on zebrafish gut microbiomes reared at different water temperatures. (A) Simpson’s Index of diversity shows that gut microbiome diversity significantly differs between fish reared at 28°C water temperature to fish reared at 32°C and 35°C water temperatures. (B) Capscale ordination based on the Bray-Curtis dissimilarity of gut microbiome composition constrained on the main effect of temperature. The analysis shows that gut microbiome composition significantly differs between parasite exposed fish reared at different water temperatures. (C) Simpson’s Index of diversity shows microbial gut diversity decreases with time from 0 days post exposure (dpe) to 42 dpe in parasite exposed fish reared at 28°C water

temperature. (D) Capscale ordination of gut microbiome composition based on the Canberra dissimilarity constrained on the main effects of water temperature and time (days post exposure, dpe), and their interaction. The analysis shows that gut microbiome composition differs between parasite exposed fish across time depending on water temperature. Ribbons and ellipses indicate 95% confidence interval. Only statistically significant relationships are shown. A “\*” indicates statistical significance below the “0.05” level. Black arrows indicate direction of greatest change in the indicated covariates.

Figure 5. The impacts of presence of infection and infection burden on the gut microbiomes of *Pseudocapillaria tomentosa* exposed zebrafish. (A) Simpson’s Index for diversity of parasite exposed fish. Gut microbial alpha-diversity does not significantly differ between fish reared at the same water temperature depending on presence of infection. (B) Capscale ordination based on the Canberra dissimilarity of gut microbiome composition of parasite exposed fish constrained on the main effects of temperature and pathology result. The analysis shows that gut microbiome composition significantly differs between positively infected fish reared at different water temperatures. (C) Infection burden (total worm counts ) is positively correlated with lowest or highest alpha diversity scores in positively infected fish. (D) Capscale ordination based on the Bray-Curtis dissimilarity of gut microbiome composition constrained on the main effects of water temperature and infection burden. The analysis shows that gut microbiome composition significantly differs between clusters of Low, High and Other fish. Samples points are colored by water temperature, and filled by “Cluster” grouping. Samples with

at least one detectable worm and an alpha-diversity score less than 0.5 are categorized as Low (orange fill), samples with at least one detectable worm and an alpha-diversity score greater than 0.5 are categorized as High (purple fill), and samples with no observable infection are categorized as Other (white and transparent fill). Ribbons and ellipses indicate 95% confidence interval. Only statistically significant relationships are shown. A “\*” indicates statistical significance below the “0.05” level. Black arrows indicate statistically significant covariates and direction of greatest change in the indicated covariates.

Figure 6. Comparison of the effects of water temperature on the gut microbiome between parasite exposed fish and parasite unexposed fish. (A) Simpson's Index for diversity of parasite unexposed and pre-exposed fish at 0 days post exposure (dpe). Prior to parasite exposure gut microbial alpha-diversity does not significantly differ between fish reared at the same water temperature. (B) Capscale ordinations based on the Bray-Curtis dissimilarity of gut microbiome composition constrained on the main and interaction effects of temperature and parasite exposure (treatment) of pre-exposure samples at 0 dpe. (C) Simpson's Index for diversity of parasite unexposed and exposed fish. Gut microbial alpha-diversity significantly differs between parasite exposed fish reared at 28°C and 32°C water temperature relative to unexposed control fish, but gut microbial alpha-diversity does not differ between parasite unexposed and exposed fish reared at 35°C water temperature. (D) Capscale ordinations based on the Bray-Curtis dissimilarity of gut microbiome composition constrained on the main and interaction effects of temperature and parasite exposure (treatment) of post-exposure samples

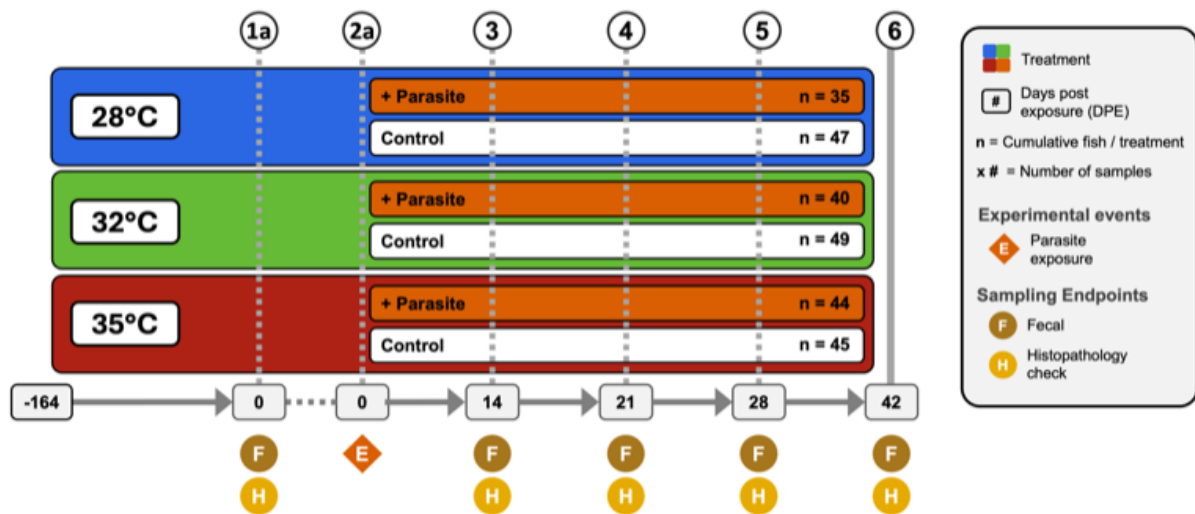
1120 after 0 dpe. The analysis shows gut microbiome composition differs between fish reared  
1121 at different water temperatures prior to parasite exposure, and parasite exposure further  
1122 drives these temperature associated differences in microbiome community composition.  
1123 Ribbons and ellipses indicate 95% confidence interval. Ribbons and ellipses indicate  
1124 95% confidence interval. Only statistically significant relationships are shown. A "\*\*\*  
1125 indicates statistical significance below the "0.05" level. Black arrows indicate statistically  
1126 significant covariates and direction of greatest change in the indicated covariates.

1127

1128 Figure 7. A heatmap of model coefficient values of the top 50 statistically significant  
1129 abundant gut microbial taxa identified by MaAsLin2. The color of each cell represents  
1130 the coefficient value and direction (red is positive, blue is negative). A "+" or "-" indicates  
1131 a statistically significant association was observed between taxon abundance and a  
1132 covariate. Gray colored cells indicate a significant effect was not observed.

1133





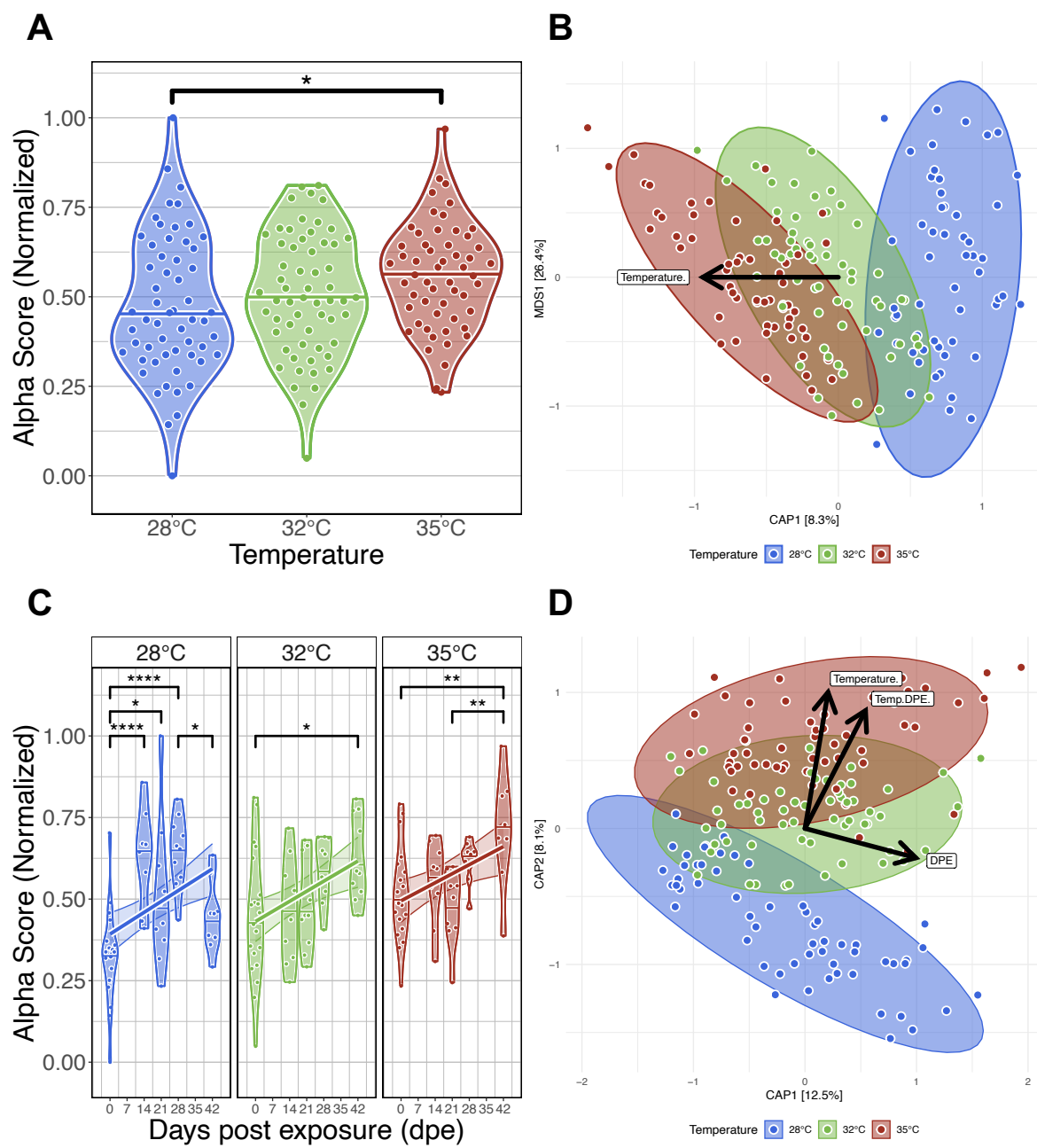
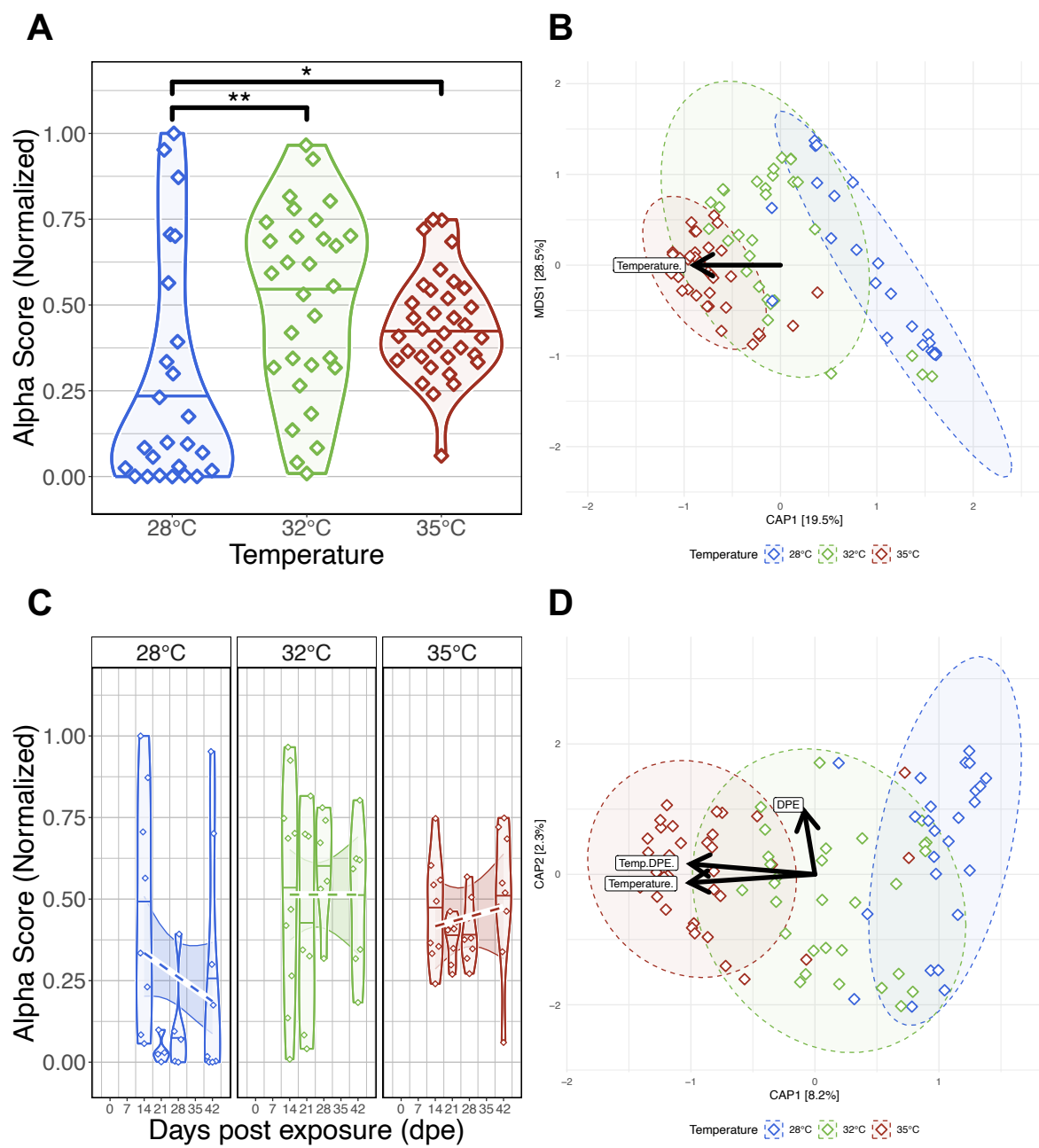
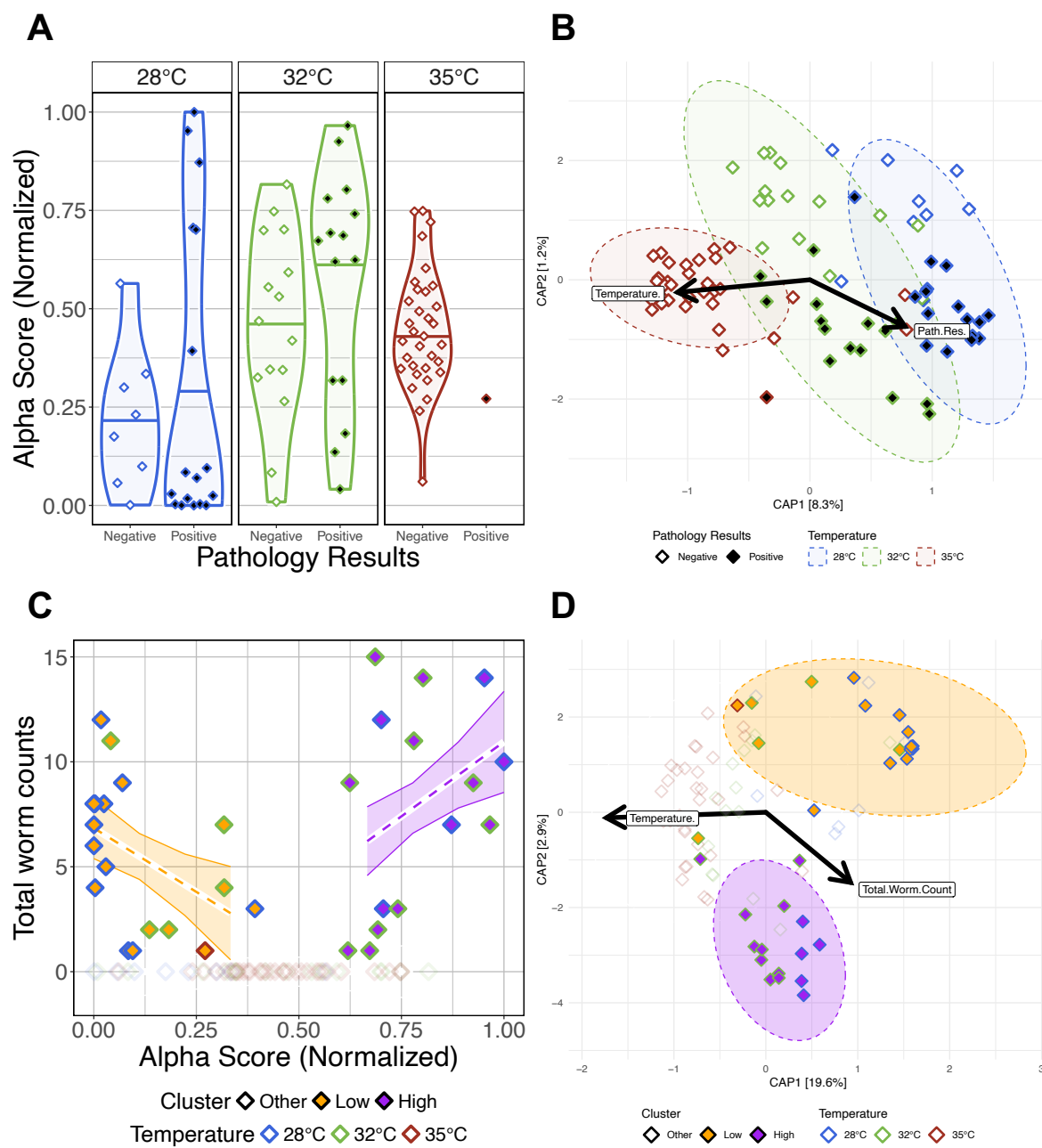
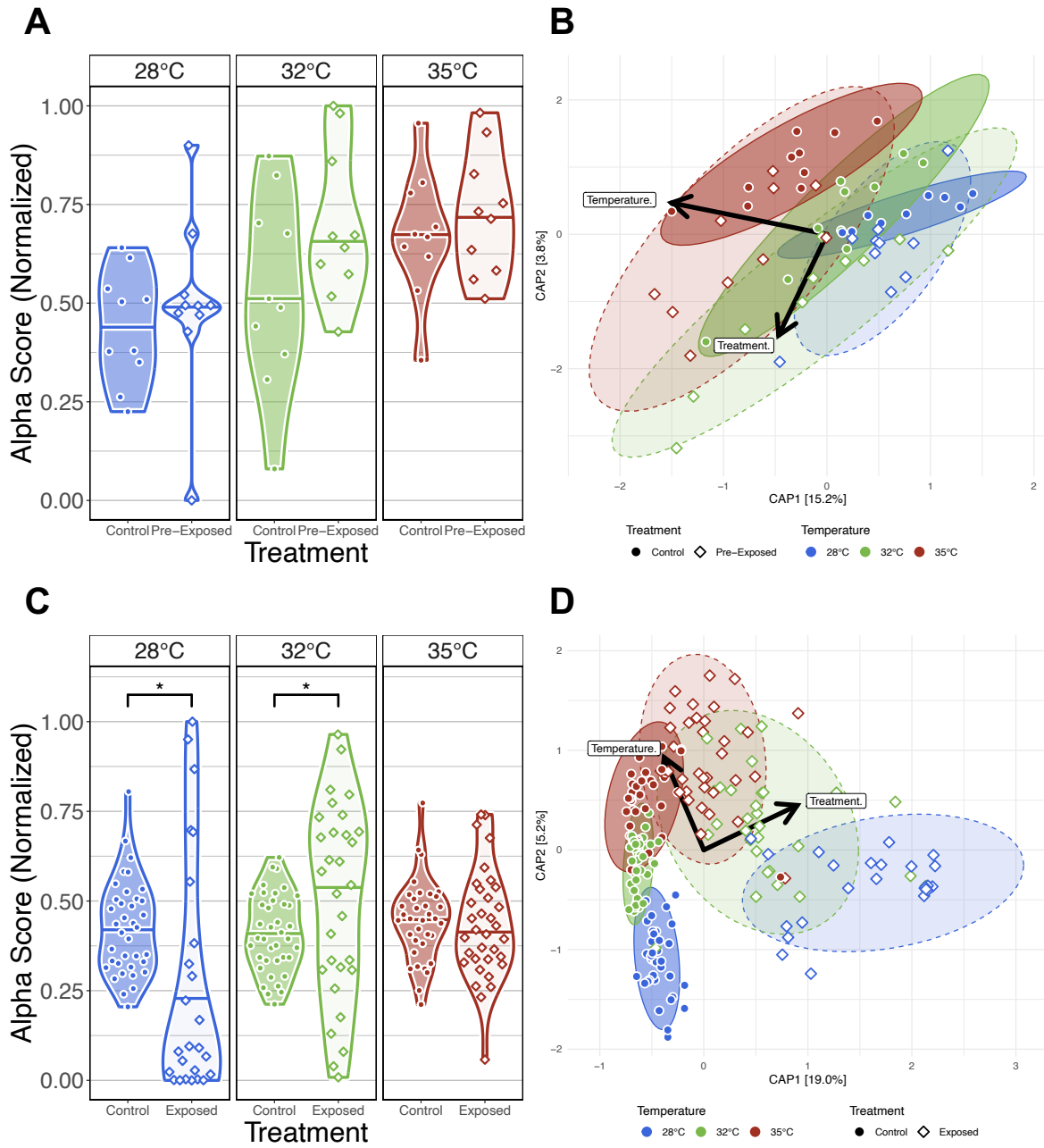


Figure 1 is a density plot showing the distribution of total worm counts for three temperatures: 28°C (blue), 32°C (green), and 35°C (red). The y-axis represents 'Total worm counts' (0 to 16), and the x-axis represents 'Temperature' (28°C to 35°C). The 28°C distribution is broad, peaking around 5.5 worms. The 32°C distribution is narrow, peaking around 3.2 worms. The 35°C distribution is very narrow, peaking around 0.2 worms. Significance brackets (\*\*\*\*) indicate differences between 28°C and 32°C, and between 32°C and 35°C.







Taxon	Temp: 32°C	Temp: 35°C	Time (DPE)	Cluster: Low	Parasite exposed	Infection present	Infection burden
<b>Actinobacteriota</b>							
IMCC26207	+		-	-	-		
Nocardiaceae Genus	+	+	+				
PeM15 Genus	+		-	-	-		
<b>Bacteroidota</b>							
Barnesiellaceae Genus			-		-		
Cloacibacterium		+	-				
Flavobacterium	-	-	-	-	+		
Fluviicola		-	-		-	-	
Microscillaceae Genus	+	+	+	-			
Terrimonas				-		+	+
env OPS 17 Genus		-		-	-		
<b>Chloroflexi</b>							
JG30 KF CM45 Genus			+	-	+	+	
<b>Cyanobacteria</b>							
Candidatus Obscuribacter	-	-	+	-	+	+	
Obscuribacteraceae Genus	+		+	-		+	-
Vampirovibrionaceae Genus			+	-	+	+	
<b>Desulfobacterota</b>							
Desulfobacterota Genus				-	+	+	
<b>Firmicutes</b>							
Clostridium sensu stricto 3			+	-	+	+	
<b>Gemmatimonadota</b>							
Gemmatimonadaceae Genus		-		-	+	+	
<b>Myxococcota</b>							
Sandaracinaceae Genus	+	+	+	-	+	+	
<b>NB1-j</b>							
NB1   Genus		+	+	-	+	+	
<b>Nitrospirota</b>							
Nitrospira		-	+	-	+	+	
<b>Patescibacteria</b>							
Saccharimonadales Genus	+	+	+	-	+		
<b>Planctomycetota</b>							
CL500 3			+	-	-	+	
Gemmata		+	+	-	+	+	
Pirellulaceae Genus	+	-	+	-	-		
Planctomycetales Genus			+	-	+	+	
Planctomycetes Genus			+	-	+	+	
Rhodopirellula				-	+	+	-
SM1A02			+	-	+	+	
Telmatocola			+	-	+	+	
<b>Proteobacteria</b>							
A0839 Genus	+			-	+	+	
Alphaproteobacteria Genus		+	+	-	+	+	
Bauldia	+		+	-	+	+	
Beijerinckiaceae Genus		-	+	-		+	-
Bosea	-	-	-	-			
Candidatus Alysiosphaera	+		+	-	+	+	
Chitinibacter		+	+		+		-
Comamonadaceae Genus			-	-	+	+	
Gammaproteobacteria Genus	-	-	+	-		+	
Luteimonas	-			-		+	+
Methylobacteriaceae Genus	+	+	+	-	+	+	
Nordella		-		-	+	+	
Plesiomonas	+	+		-		-	-
Pseudomonadales Genus	-	-	-	-	-	-	
Rhizobacter				-	+	+	+
Rhizobiales Incertae Sedis Genus				-		+	
Rhodobacteraceae Genus				-	+	+	
Rhodovastum		-		-			
Steroidobacteraceae Genus			+	-	+	+	
Xanthobacteraceae Genus	-	-	+	-		+	
<b>Verrucomicrobiota</b>							
Oikopleura			+	-	+	+	

Coefficient

8



-8