1   **Modeling the zebrafish gut microbiome's resistance and sensitivity to climate**

2   **change and parasite infection**

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Abstract:

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22   As climate change increases global water temperatures, ecologists expect

23   intestinal helminth infection ranges to expand and increase the health burden on aquatic

24   organisms. However, the gut microbiome can interact with these parasites to influence

25   infection outcomes, raising the possibility that its response to increasing temperatures

26   may help buffer against increased infection burden. To evaluate this hypothesis, we

27   sought to determine if the microbiome is resistant or resilient to the stressors of

28   increased water temperature, helminth exposure, and their combination, and whether

29   this variation linked to infection outcomes. We leveraged the zebrafish (Danio rerio)

30   model organism to measure how these variables relate to the temporal dynamics of the

31   gut microbiome. In particular, we exposed adult zebrafish to the parasitic whipworm

32   Pseudocapillaria tomentosa across three different water temperatures (28°C, 32°C,

33   35°C), and analyzed fecal microbiome samples at five time points across 42 days. Our

34   findings show that parasite exposure and water temperature independently alter gut

35   microbiome diversity. Moreover, we find that water temperature moderates the

36   association between parasite infection and the gut microbiome. Consistent with this

37   observation, but at odds with current expectations, we find that increasing water

38   temperature reduces parasitic infection in fish. Overall, our results indicate that water

39   temperature alters the contextual landscape of the gut microbiome to impact its

40   response to an exogenous stressor of an intestinal parasite in zebrafish. Furthermore,

41   our findings represent the first report of the effects of elevated temperature on parasitic

42   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

68   induced perturbations to the gut microbiome may sensitize individuals to subsequent

69   stressors (11).

70   To answer these questions, we evaluated the gut microbiome's temporal

71   response to an exogenous stressor across a gradient of environmental conditions. To

72   do so, we levered the zebrafish (*Danio rerio*) model organism to measure how gut

73   microbiomes differ across fish reared to adulthood at one of three water temperature

74   conditions (28°C, 32°C, or 35°C; Fig. 1). Zebrafish are highly thermal tolerant, capable

75   of existing across a wide spectrum of temperature ranges from 4°C to 40°C (12), but

76   living outside their thermal optimum can come at a physiological and microbial cost

77   (12,13). While much is known about the thermal range of zebrafish, the effects of

78   altered water temperature on their gut microbiome structure has not been elucidated.

79   We also sought to determine if water temperature affected how the microbiome and

80   host responds to exposure to and infection by intestinal nematode *Pseudocapillaria*

81   *tomentosa*, a common source of disease in zebrafish facilities (14). *P. tomentosa* is

82   known to cause high mortality and disrupts the gut microbiome (14,15). Yet, it is not

83   known if or how water temperatures mediates this interaction. Overall, our study sought

84   to clarify the environmentally dependent context of a gut microbiome's resistance and

85   sensitivity to climate change-relevant stressors.

86

87   **Results**

88   **Water temperature shapes gut microbiome structure**

89   To determine how zebrafish reared across a gradient of increasing water

90   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.

113   To evaluate how temperature associates with microbiome composition in parasite

114   unexposed fish, we quantified dissimilarity amongst all samples and generated distance

115   matrices using the Bray-Curtis, Canberra and half-weighted UniFrac distance metrics.

116   Using permutational multivariate analysis of variance (PERMANOVA), we assessed

117   whether increasing water temperatures explained variance in gut microbial community

118   composition. A PERMANOVA test indicated that microbial communities were

119   significantly stratified by water temperature as measured by all beta-diversity metrics

120   (PERMANOVA, $P<0.05$; Fig. 2B; Table S2B.1). These results indicate that microbial

121   communities of fish reared at the same water temperature are more consistent in

122   composition to one another than fish reared at different water temperatures. Additionally,

123   we assessed beta-dispersion, a measure of variance, in the gut microbiome community

124   compositions for each water temperature group. We find the beta-dispersion levels did

125   not significantly differ between the water temperature groups ($P>0.05$; Table S2B.2).

126   These results indicate that fish reared at different water temperatures are consistent in

127   community composition.

128   Next, we compared our results across five time points between 0- and 42 dpe to

129   determine how water temperature impacts the successional trajectories of gut

130   microbiome diversity and composition. Linear regression revealed gut microbial alpha-

131   diversity was significantly associated with the main effect of time for each alpha-diversity

132   metric we assessed ($P<0.05$; Fig. 2C; Table S2C.1-2). Moreover, we found a

133   temperature dependent effect on time as measured by richness and phylogenetic

134   diversity metrics ($P<0.05$; Table S2C.1-2). A post hoc Tukey test clarified that

135   microbiome diversity significantly differed between 0- and 42 dpe fish reared at 28°C as

136   measured by richness and phylogenetic diversity (P<0.05; Table S2C.3), between 0-

137   and 42 dpe fish reared at 32°C as measured by all alpha-diversity metrics (P<0.05;

138   Table S2C.3), and between 0- and 42 dpe fish reared at 35°C as measured by Shannon

139   Entropy and Simpson's Index (P<0.05; Table S2C.3). These results indicate that gut

140   microbial alpha-diversity increases over time irrespective of water temperature.

141   A PERMANOVA test detected significant clustering of microbial gut community

142   composition based on the interaction of water temperature and time as measured by all

143   beta-diversity metrics (PERMANOVA, P<0.05; Fig. 2D; Table S2D.1). These results

144   indicate that microbial communities of fish reared at the same water temperature are

145   more consistent in composition to one another across time than fish reared at different

146   water temperatures. Moreover, a pairwise analysis of beta-dispersion found significantly

147   elevated levels of dispersion between fish reared across different temperatures and

148   time as measured by all beta-diversity metrics (P<0.05; Table S2D.2). These results

149   indicate that gut microbial community composition varies inconsistently between water

150   temperature groups in a time-dependent manner. Collectively, these results indicate that

151   zebrafish gut microbiomes communities stratify by temperature, and the trajectory of gut

152   microbiome successional development varies depending on water temperature.

153

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

155   Next, we evaluated infection outcomes of zebrafish reared at different water

156   temperatures and exposed to the intestinal helminthic parasite *Pseudocapillaria*

157   *tomentosa*. To determine whether water temperature affects infection burden, we

158   exposed zebrafish to 50 *P. tomentosa* eggs per liter of tank water at 164 days post-

159   fertilization (dpf). Infection outcomes were assessed using wet mount and histological

160   evaluation at 0, 14, 21, 28, and 42 days post-exposure (dpe). We built a negative

161   binomial general linear model to compare infection burden (total worm counts) between

162   fish reared at different water temperatures (Table S3B.1). The regression analysis found

163   a statistically significant effect of temperature on infection burden ($P < 0.05$; Fig. 3B;

164   Table S3B. 2). However, we did not find a statistically significant interaction effect

165   between water temperature and time on infection burden ($P > 0.05$; Table S3B.3).

166   Across time points, fish reared at 28°C exhibited the highest mean infection

167   burden (4.86 worms per fish), followed by those at 32°C (3.6 worms per fish). Notably,

168   at 14 dpe, fish at 32°C had a slightly higher infection burden (3.3 worms per fish) than

169   those at 28°C (2.3 worms per fish). Tukey's post hoc test revealed that infection burden

170   was significantly higher in fish reared at 28°C and 32°C compared to those at 35°C ($P <$

171   0.05; Fig. 3B; Table S3B.3). Only a single larval worm was detected by wet mount in

172   two fish from the 35°C group, while histological examination revealed a slightly higher

173   prevalence, with larval worms observed in 9 out of 32 fish at this temperature (Fig. 3A;

174   Table S3A.1). These results indicate that infection burden is highest at lower water

175   temperatures. We also examined the development of mature female worms across

176   temperature conditions. At 28°C, mature female worms were first detected at 28 dpe in

177   7 fish, whereas at 32°C, mature females were only observed in 4 fish. Interestingly, at

178   14 dpe, a single mature female was identified in a fish reared at 32°C, marking the

179   earliest recorded instance of worm maturation at this temperature.

180   Additionally, we compared the sensitivity of infection detection between histology

181   and wet mount methods on a subset of fish selected for microbiome analysis ($n = 120$;

182   Fig. S3C; Table S3C.1). McNemar's test revealed significant differences in detection

183   sensitivity under specific conditions. At 35°C and 21 dpe, histology identified

184   significantly more infections than wet mount ($\chi^2 = 4.17$, $P < 0.05$; Fig. S3C; Table

185   S3C.3), with 6 samples testing positive by histology alone compared to 0 by wet mount

186   alone. No statistically significant differences were observed at other temperature and

187   dpe combinations ($P > 0.05$; Table S3C.3). In cases where all samples were concordant

188   (e.g., 28°C at 28 dpe and 35°C at 28 dpe), McNemar's test could not be computed due

189   to the absence of discordant pairs. These findings suggest that histological methods

190   may be more sensitive than wet mounts, particularly at higher temperatures and

191   intermediate time points. Collectively, these results suggest that higher water

192   temperatures may have a protective effect against infection burden, limiting worm

193   establishment and development in zebrafish.

194

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

196   To investigate how parasite exposure affects the gut microbiome under varying

197   water temperatures, we analyzed fecal samples from exposed and control fish at

198   multiple time points. *P. tomentosa* is known to alter the zebrafish gut microbiome (15),

199   but it remains unclear how increasing water temperatures affect this response. We

200   collected fecal samples for microbiome analysis of fish in the parasite exposed cohort at

201   14-, 21-, 28-, and 42 dpe. Similar to our parasite unexposed fish microbiome analyses,

202   we built generalized linear models (GLM) to determine if temperature, time or their

203   combination associated with variation in measures of microbial diversity and

204   composition of parasite exposed fish (Table S4A.1). An ANOVA test of these GLMs

205   revealed that alpha-diversity varied as a function of temperature for all measures

206   ($P<0.05$; Fig. 4A; Table S4A.2). A post hoc Tukey test clarified that gut microbial

207   diversity between 28°C and 32°C water temperature reared fish significantly differed for

208   all alpha-diversity metrics ($P<0.05$; Table S4A.3), and gut microbial diversity differed

209   between 28°C and 35°C water temperature reared fish as measured by Simpson's

210   Index. However, we did not find significant differences in gut microbial diversity between

211   32°C and 35°C water temperature reared fish for all alpha-diversity metrics, or between

212   28°C and 35°C water temperature reared fish as measured by Shannon Entropy,

213   richness and phylogenetic diversity metrics. These results indicate that moderate

214   increases in water temperature promotes gut microbial diversification in parasite

215   exposed fish, but diversification of gut microbes plateaus in parasite exposed fish

216   reared at higher water temperatures.

217   For each beta-diversity metrics we considered, PERMANOVA tests found that

218   temperature significantly explained the variation in microbiome composition in parasite

219   exposed fish (PERMANOVA, $P<0.05$; Fig. 4B; Table S4B.1). These results indicate that

220   gut microbiome communities of parasite exposed fish reared at the same water

221   temperature are more similar to each other than fish reared at different water

222   temperatures. Additionally, we found beta-dispersion levels were significantly elevated

223   between water temperature groups ($P<0.05$; Table S4B.2). A post hoc Tukey test

224   clarified that beta-diversity dispersion levels were highest in fish reared at 28°C,

225   followed by fish reared at 32°C and 35°C water temperatures ($P<0.05$; Table S4B.3).

226   These results indicate that that gut microbiome communities of parasite exposed fish

227 reared at lower water temperatures are more inconsistent in composition than parasite

228 exposed fish reared at higher water temperatures.

229   Next, we compared our results across five time points to evaluate how parasite

230 exposure and water temperature impacted gut microbiome diversity and composition.

231 An ANOVA test did not find significant main effects of time as measured by Shannon

232 Entropy and Simpson's Index (P>0.05; Table S4C.2), but found marginally significant

233 main effects of time as measured by richness and phylogenetic diversity (P=0.064 and

234 P=0.078; Table S4C.2). Moreover, linear regression did not reveal significant interaction

235 effects between temperature and time across all alpha-diversity metrics (P>0.05; Fig.

236 4C; Table S4C.2). These results indicate increasing water temperatures generally do not

237 consistently impact microbial gut diversification over time in parasite exposed fish, and

238 particular microbial clades appear more sensitive to the effects of time depending on

239 temperature.

240   PERMANOVA tests found that community composition was best explained by the

241 interaction effects between temperature and time using the Canberra beta-diversity

242 metric (PERMANOVA, P<0.05; Fig. 4D; Table S4D.1), but a significant interaction effect

243 was not observed using the Bray-Curtis and half-weighted UniFrac dissimilarity metrics

244 (P>0.05; Table S4D.1). Given how these metrics weigh the importance of rarer (e.g.,

245 Canberra) versus abundant (e.g., Bray-Curtis) microbial community members, these

246 results indicate that abundant members of the microbiome community are more robust

247 to the effects of temperature across time in parasite exposed fish, while rarer taxa are

248 more sensitive to the effects of time depending on temperature. Moreover, a pairwise

249 analysis of beta-dispersion found significantly elevated levels of dispersion between fish

250 reared across different temperatures and time as measured by all beta-diversity metrics

251 (P<0.05; Table S4D.2). These results indicate that parasite exposure inconsistently

252 impacts gut microbial community composition across time depending on temperature

253 (P<0.05; Table S4D.2). Collectively, these results indicate that parasite exposure can

254 impact gut microbiome diversity and composition, and these impacts are greatest at

255 lower temperatures.

256

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

258 Given the differences we observed in gut microbiome diversity and composition

259 across parasite exposed fish reared at different water temperatures, we further

260 investigated whether gut microbiomes of parasite exposed fish vary depending on

261 presence of infection and infection burden. Linear regression did not find significant

262 main effects of presence of infection or significant interaction effects between presence

263 of infection and temperature on gut microbial alpha-diversity for all metrics we

264 measured (P>0.05; Fig 6A; Table S5A.1-2). These results indicate that gut microbial

265 diversity does not differ as a function of presence of infection. Moreover, a

266 PERMANOVA analysis found microbial community composition was best explained by

267 presence of infection as measured by Canberra (PERMANOVA, P<0.05; Fig. 5B; Table

268 S5B.1), but a significant result was not observed by the other beta-diversity metrics we

269 measured. Additionally, we did not find statistically significant results between the

270 interaction effects of water temperature and presence of infection on gut microbial

271 community composition. These results indicate that rarer members of microbial

272 communities of parasite exposed fish vary by presence or absence of infection, but

273   abundant microbes do not. However, we did detect elevated levels of beta-dispersion

274   across fish reared at different water temperatures depending on presence of infection

275   (P<0.05; Table S5B.2). These results indicate that gut microbiome composition

276   inconsistently varies between fish depending on presence of infection and water

277   temperature.

278   Next, we investigated how infection burden (i.e., number of intestinal worms

279   detected) impacted parasite exposed fish gut microbiome diversity and composition. We

280   used GLMs to determine if infection burden associated with variation in gut microbial

281   alpha-diversity (Table S5C.1). An ANOVA test of these GLMs revealed that alpha-

282   diversity varied as a function of infection burden as measured by Shannon Entropy and

283   Simpson's Index (P<0.05; Table S5C.2), but the interaction effects between infection

284   burden and water temperature did not significantly explain variation in alpha-diversity

285   across all measures (P>0.05; Table S5C.2). These results indicate that gut microbial

286   diversity varies as a function of parasitic worm count. A PERMANOVA analysis found

287   microbial community composition was best explained by infection burden as measured

288   by all beta-diversity metrics (PERMANOVA, P<0.05; Table S5C.2), but the interaction

289   effect between infection burden and temperature was not significant (P>0.05; Table

290   S5C.2). These results indicate that higher infection burden drives increased

291   inconsistency in gut microbial composition, regardless of water temperature.

292   Upon closer inspection of our infection burden results, we observed a non-linear

293   relationship between infection burden and alpha-diversity scores, where highest

294   infection burden associated with either highest or lowest alpha-diversity scores. To

295   further explore this non-linear relationship between gut microbial diversity and infection

296   burden, we grouped parasite exposed fish samples based on their alpha-diversity

297   scores and infection burden. Parasite exposed fish samples with at least one intestinal

298   worm detected were classified as "Low" or "High" if their alpha-diversity score was less

299   than or greater than the median alpha-diversity score, respectively. Fish with zero

300   detected worms were classified as "Other". When grouping samples either Low or High

301   based on alpha-diversity scores as measured by the Simpson's index, we find that the

302   samples in the Low group are composed of 67% 28°C and 33% 32°C water

303   temperature reared fish, samples in the High group are composed of 33% 28°C and

304   67% 32°C water temperature reared fish, and samples in the Other group are

305   composed of 14% 28°C, 27% 32°C, and 59% 35°C water temperature reared fish (Table

306   S5C1.1). These results indicate that group membership associates with water

307   temperature. To test this supposition, we used GLMs to determine if infection burden

308   associated with variation in alpha-diversity score grouping (Table S5C.1.2). An ANOVA

309   test of these GLMs revealed significant main effects of group for all alpha-diversity

310   measures ($P<0.05$; Fig 5C; Table S5C2), and significant interaction effects between

311   group and alpha-diversity score. Notably, fish in the Low group had a significant

312   negative slope and fish in the High group had a significant positive slope between

313   alpha-diversity and infection burden as measured by Shannon Entropy and Simpson's

314   Index. These results indicate that parasite exposed fish have diverging gut microbial

315   alpha-diversity responses to high infection burden.

316   Additionally, we find that these groups of samples – based on high versus low

317   alpha-diversity scores of parasite exposed fish – also formed two distinct clusters in

318   beta-diversity space. A PERMANOVA analysis detected significant clustering between

319   Low, High, and Other groups across each measure of beta-diversity (PERMANOVA,

320    $P<0.05$; Fig. 5D; Table S5D.1). However, this effect was weakest when considering the

321   Canberra metric. Furthermore, a pairwise analysis of beta-dispersion finds significantly

322   elevated dispersion levels between group membership as measured by Canberra

323   metric, but not the other beta-diversity metrics (Table S5D.2). Given that the Canberra

324   metric gives rarer taxa greater importance in its beta-diversity calculations than the

325   other metrics we evaluated, these results suggest there is more consistency in microbial

326   composition among abundant taxa within samples that share Low or High group

327   membership, but not among more rarer taxa. A post-hoc Tukey test also clarified that

328   beta-dispersion levels are significantly different between fish in the High and Other

329   groups compared to fish in the Low group as measured by the Canberra metric (Table

330   S5D.3). Together, these results indicate that rarer members of the gut microbiome are

331   less consistently represented across fish in the Low cluster group as compared to fish in

332   the High and Other cluster groups. Collectively, these results indicate that the

333   microbiome response of fish with heaviest infection burden diverge into two distinct

334   trajectories, which may be influenced by water temperature.

335

336   **Parasite exposure exacerbates water temperature differences in gut microbiome**

337   **structure**

338   Next, we sought to determine whether the gut microbiomes of zebrafish exposed

339   to the parasite *Pseudocapillaria tomentosa* respond differentially compared to parasite

340   unexposed control fish across increasing water temperatures. Prior to the parasite

341   exposure at 164 dpf (or 0 dpe), we collected fecal samples from both cohorts of control

342   and parasite exposed fish. Following fecal sample collection, fish in the parasite

343   exposure group were exposed to *P. tomentosa*. We collected subsequent fecal samples

344   at 14-, 21-, 28- and 42 dpe. Fecal samples were then measured for gut microbial

345   diversity and composition and compared between parasite unexposed and exposed

346   fish. We built generalized linear models (GLM) to determine if parasite exposure as a

347   function of water temperature associated with microbial diversity and composition

348   measures (Table S6A.1). Within pre-exposed (i.e., 0 dpe) samples, we did not observe

349   any significant associations between the interaction effect of parasite exposure and

350   water temperature across any of the alpha-diversity measures ($P>0.05$; Fig. 6A; Table

351   S6A.2). These results indicate that at 0 dpe prior to parasite exposure, gut microbial

352   diversity measures of fish reared at the same water temperature are not different from

353   one another. Furthermore, PERMANOVA tests revealed significant differences in

354   microbiome composition between control and pre-exposed fish across all beta diversity

355   metrics. Homogeneity of dispersion tests revealed a significant difference in group

356   variability for Bray-Curtis ($P<0.05$; Fig. 6B; Table S6B.2), but not for Canberra or

357   Generalized UniFrac. Post hoc Tukey tests indicated no significant pairwise differences

358   in dispersion for any metric (Table S6B.3), suggesting that the observed dispersion

359   effect in Bray-Curtis was not driven by specific group outliers. Given that temperature

360   alone consistently explained the variation in microbial communities followed by

361   treatment effects, these results suggest that prior to parasite exposure microbial

362   communities vary by water temperature and possibly stochasticity of tank effects.

363   We next compared our results between control and exposed fish across each

364   water temperature to determine how parasite exposure impacts gut microbiome

365   diversity and composition. Linear regression revealed microbial gut alpha-diversity was

366   significantly associated with the interaction effect between temperature and treatment

367   for any alpha-diversity metric we assessed ($P<0.05$; Fig. 6C; Table S6C.1-2). A post hoc

368   Tukey test clarified that microbiome diversity was significantly different between

369   exposure groups of fish reared at 28°C water temperature as measured by Simpson's

370   Index ($P<0.05$; Table S6C.3), at 32°C water temperature as measured by all alpha-

371   diversity metrics ($P<0.05$; Table S6C.3), and at 35°C as measured by richness and

372   phylogenetic diversity ($P<0.05$; Table S6C.3). These results indicate that gut microbial

373   diversity differs between unexposed and exposed fish depending on water temperature,

374   and parasite exposure uniquely impacts particular microbial clades, rare and abundant

375   taxa depending on water temperature. Additionally, PERMANOVA tests found that

376   microbiome composition differed between control and exposed fish reared at all water

377   temperatures as measured by all beta-diversity metrics ($P<0.05$; Table S6D.1). These

378   results suggest that the gut microbiomes compositions between control and parasite

379   exposed differed in microbiome community composition regardless of water

380   temperature. Moreover, a pairwise analysis of beta-dispersion found elevated levels of

381   dispersion across all beta-diversity metrics measured, and dispersion levels were

382   highest among parasite exposed fish reared at lower water temperatures ($P<0.05$; Table

383   S6D.2). These results suggest that gut microbiome community composition is less

384   consistent between parasite unexposed and exposed fish reared at lower water

385   temperatures. Collectively, these results demonstrate that water temperature dictates

386   how exposure to parasites alters the temporal trajectory of the gut microbiome.

387

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

411   particular members of the gut microbiome associate with time irrespective of water

412   temperature.

413   Next, we sought to determine how parasite exposure impacted gut microbial

414   abundance in fish. Fish exposed to *P. tomentosa* were significantly enriched for 74 taxa,

415   and depleted of 35 taxa relative to parasite unexposed fish (Fig. 7). Notably, we find

416   *Aeromonas*, *Chitinibacter*, and *Flavobacterium* are positively associated with parasite

417   exposure, while *Plesiomonas, Phreatobacter* and *Cetobacterium* are negatively

418   associated with parasite exposure. With the exception of *Phreatobacter* and

419   *Cetobacterium*, these data are consistent with our prior work that found *P. tomentosa*

420   exposure associated with altered bacterial abundance of these members of the

421   zebrafish gut microbiome (15). We further investigated the effects of parasite exposure

422   and measured how infection burden or presence of infection impacted gut microbial

423   abundance. Fish with higher infection burden (i.e., number of parasitic worms present)

424   enriched for 49 taxa and were depleted of 13 taxa, while fish that were positively

425   infected enriched for 117 taxa and were depleted of 5 (Fig. 7). Notably, we find

426   abundance of members of *Cetobacterium, Shewanella, Vibrio* and *Zooglogea* are

427   negatively associated with infection burden. These taxa are identified as common

428   members of the zebrafish gut microbiome (19). These results indicate that exposure to

429   the intestinal parasitic worm *P. tomentosa* impacts common zebrafish gut microbiota,

430   and presence of infection and severity have varying impacts to gut microbial

431   abundance.

432   To deepen our analysis of parasite exposure on the zebrafish gut microbiome, we

433   investigated how taxon relative abundance associated with gut microbiome diversity

434 and composition. Previously, we found that parasite exposed fish reared at 28°C and

435 32°C water temperatures clustered into two distinct groups of community composition,

436 which associated with high infection burden and either high or low alpha diversity

437 scores. This observation led us to investigate which gut microbiota might be driving the

438 clustering of the gut microbiomes of heavily infection burdened fish. We did not find

439 significantly abundant taxa in the High group. We detected 1 taxon that was significantly

440 enriched and 192 taxa that were significantly depleted among fish in the Low group (Fig.

441 7). Notably, we find *Aeromonas* was enriched, while *Mycobacterium* were depleted in

442 the Low group fish. Some species of *Mycobacterium* are common pathogens in

443 zebrafish facilities (20). These results indicate that the gut microbiome communities of

444 parasite exposed fish experiencing heavy infection burden stratify into two distinct

445 groups represented by the unique depletion of particular members of the gut

446 microbiome. Collectively, these results indicate that environmental conditions associate

447 with altered gut microbial abundance, and the response of specific members of the gut

448 microbiome to environmental stressors varies depending on environmental conditions.

449

450 **Discussion**

451   The zebrafish is an important model organism for understanding how

452 environmental stressors impact the microbiome (17,18). Our work capitalized on the

453 experimental control and scale afforded by the zebrafish model system to investigate

454 how temperature and parasite exposure interact to influence infection and microbiome

455 outcomes. While previous research has investigated how water temperature (13) and

456 parasite exposure (15) independently impact the zebrafish gut microbiome, no studies

457 in any *in vivo* experimental system, to our knowledge, have examined the microbiome's

458 temporal response to the combined effects of increasing water temperature and

459 parasite exposure. Overall, we found that water temperature serves as a key contextual

460 variable that dictates the severity of infection, the developmental state of worms, the

461 composition of the gut microbiome in unexposed fish, and how the gut microbiome

462 responds to parasite exposure and infection. These results underscore that the gut

463 microbiome's response to—and potentially its ability to buffer against—intestinal

464 parasitic infection is influenced by other exogenous factors, in this case, water

465 temperature. Furthermore, our findings challenge current expectations of climate

466 change's anticipated impact on aquatic organismal parasite burden (2,3). Consequently,

467 it is important that we consider going forward how stacking multiple stressors, an

468 experience inherent to life in the Anthropocene, may accelerate the arrival of dysbioses.

469   We found that parasitic infection burden was highest among zebrafish reared at

470 ambient water temperatures. While field studies have documented arrested

471 development in parasitic nematodes (e.g., *P. tomentosa*) during cold winter months (21),

472 this study represents the first report of the effects of elevated temperature on parasitic

473 nematode development in a fish host. Consistent with our prior work, temporal trends in

474 *P. tomentosa* infection burden were similar for fish at ambient temperatures (15).

475 However, contrary to expectations that elevated temperatures increase infection burden,

476 we observed the opposite outcome: fish reared at the highest temperatures exhibited

477 the lowest infection burden, with only a few larval-stage worms detected. Because

478 parasite eggs were larvated at ambient temperature before being transferred to warmer

479 tanks, we hypothesize that elevated temperatures may have impaired hatching once the

480   eggs were ingested, reducing overall abundance of worms. Nevertheless, at 35°C,

481   worms that did establish infections persisted but remained in an arrested state out to 28

482   and 42 days post-exposure (dpe), whereas at 28°C and 32°C, worms completed

483   development and mated within 3 to 4 weeks, consistent with previous observations

484   (14). These findings suggest that worm hatching and development may be temperature-

485   sensitive, adding to prior research showing that many fish pathogens, from viruses to

486   parasies, exhibit well-defined upper thermal boundaries for infectivity and pathogenesis.

487   For example, the development is of the ciliate *Ichthyophthirius multifiliis,* a common

488   cause of disease in aquarium fishes, is arrested by elevating water temperatures above

489   30°C (22). While we are not aware of this phenomenon with nematode parasites of

490   fishes, analogous examples exist in terrestrial nematodes, such as *Wuchereria*

491   *bancrofti*, where larval development is restricted to temperatures below 31°C in

492   mosquitoes (23). Our findings address a gap in understanding of nematode

493   development at a host's upper thermal limit in an aquatic organism, and offers new

494   insights into how climate change may disrupt aquatic disease ecological

495   dynamics. Future research should investigate whether arrested development in *P.*

496   *tomentosa* is a direct effect of temperature or mediated by host physiological responses.

497   Beyond direct effects on parasite development, poikilothermic (i.e., animals with

498   variable body temperature and the inability to regulate it) hosts may gain protections

499   against infection through temperature-dependent immune responses or gene

500   expression changes. While studies on zebrafish immunity under elevated temperatures

501   are limited, prior research in teleosts indicates that immune responses are host- and

502   environment-specific, varying with the direction and duration of temperature shifts

503   (24,25). For example, Dittmar et al. found that immune activity was highest at thermal

504   limits and inversely related to acute temperature shifts in three-spine sticklebacks (26),

505   whereas Bailey et al. observed suppressed immunity and increased parasite burden in

506   rainbow trout exposed to chronic upper optimal thermal ranges (27). Although these

507   studies differ in exposure regimes to ours, they highlight that colonization resistance

508   may be influenced by temperature-sensitive immune responses and gene expression.

509   Future research integrating immune function, gene expression, and histopathological

510   assessments will be crucial to disentangling the host's role in colonization resistance

511   under chronic parasite exposure and elevated temperatures. Notably, controlled

512   temperature manipulation is already used to mitigate certain aquaculture pathogens,

513   such as *Ichthyophthirius multifiliis (22)*, where increasing tank temperature to 30°C can

514   eliminate infections in susceptible fish. Our findings suggest that similar interventions

515   may help mitigate or delay parasite infection in aquaculture settings.

516   We also found that zebrafish gut microbiome structure stratified depending on

517   the environmental conditions of increasing water temperature. Our results are congruent

518   with previous research that found increased water temperatures altered zebrafish gut

519   microbial diversity and composition (13). Moreover, Wang *et al.* observed that zebrafish

520   reared at different water temperatures manifested distinct liver carbohydrate metabolism

521   profiles and temperature-dependent sensitivity to irradiation. A unique aspect of our

522   study considered how the gut microbiome temporally varies as a function of water

523   temperature. We found that water temperature acts as a filter on initial zebrafish gut

524   microbiome assembly, and these initial differences in assembly between water

525   temperature remained stable across time. Beyond zebrafish, analogous investigations

526   have investigated how temperature variation shapes gut microbiome composition and

527   function in mammals, fish, and other animal species (28,29). In particular, a recent

528   meta-analysis of aquatic organisms' response to temperature found similar, but

529   inconsistent results to our study, wherein increasing water temperature is associated

530   with both increases and decreases to gut microbial diversity, differences in gut

531   microbiota community composition, and altered gut taxon abundance (29).

532   Inconsistencies between prior work and ours could be driven by differences in

533   magnitude of the stressor (i.e., press vs pulse; (30)), host species (31), facility or habitat

534   effects (29,32,33), or diet (20). Despite these differences, the results of prior studies in

535   conjunction with ours are consistent with the concept of environmental conditions acting

536   as a habitat filter to shape initial gut microbiome assembly (34) and illicit

537   environmentally dependent responses to exogenous stressors.

538   Finally, we observed a nonlinear relationship between gut microbiome diversity

539   and infection outcomes, with water temperature moderating these dynamics. Consistent

540   with our prior research on zebrafish infected by *Pseudocapillaria tomentosa* (15)

541   exhibiting dysbiotic microbiomes described by the Anna Karenina Principle (AKP) (35).

542   The AKP postulates that dysbiotic microbiome communities display higher rates of

543   dispersion or are more inconsistent between dysbiotic individuals, whereas the

544   microbiome community composition of healthy individuals are less dispersed or are

545   more similar to one another. In particular, we observed elevated microbial community

546   dispersion among heavily parasite infected fish, reflecting unstable microbial states

547   characteristic of the AKP. However, when accounting for water temperature, the AKP

548   effect diminished, and multiple alternative stable states emerged, which may explain

549   inconsistencies in prior studies that failed to detect the AKP or found contrary results

550   (29). Our findings underscore the importance of accounting for individuals' temporal and

551   spatial contexts to adequately assess microbiome stability in response to stressors (36).

552   However, current definitions of microbiome stability (11,37,38), which rely on a

553   homeostatic framework, may be insufficient to describe these dynamic shifts. A

554   homeorhetic framework (39), conceptualizing stability as a change along a stable

555   trajectory rather than a fixed state, could improve our ability to measure microbiome

556   stability and dysbiosis across dynamic temporal and spatial scales. Shifting to such a

557   model may reconcile discrepancies in AKP detection across studies and provide deeper

558   insights into how microbiomes respond to exogenous stressors in different host

559   systems.

560   In conclusion, we found that water temperature alters the contextual landscape of

561   the microbiome to impact its response to an exogenous stressor of an intestinal

562   parasite. Our work revealed that differences in environmental conditions of water

563   temperature were sufficient to temporally change the gut microbiome's response to

564   parasitic exposure and impact infection outcomes in zebrafish. While the zebrafish gut

565   microbiome differs taxonomically from other animal-microbiome systems, a

566   considerable amount of functional capacity is shared between animals (40). Thus,

567   zebrafish serve as a powerful model for investigating how environmental changes and

568   stressor exposures influence microbiomes and host health. Our findings have important

569   implications for microbiome research in the context of climate change, demonstrating

570   that rising temperatures may have unexpected effects on gut microbiomes and infection

571   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*

599   *tomentosa* as described below. Fish were acclimated to the prescribed temperature

600   treatments by increasing the heater thermostat settings by 1°C every two days until the

601   final prescribed temperature was achieved. Two temperature logging thermometers,

602   one for the six pathogen negative control tanks and one for the six *P. tomentosa*

603   exposed tanks, were rotated through the tanks every two days on weekdays to monitor

604   temperature at each temperature treatment. The average range recorded for the water

605   temperature treatments was +/- 1°C.

606

607   ***Pseudocapillaria tomentosa* exposure**

608   Eggs were collected from a population of infected zebrafish that we constantly

609   maintain in our laboratory as described by Martins *et al.* 2017 (41). Eggs were allowed

610   to larvate for 6 days at 28°C, and fish were exposed at 25 larvated eggs/fish. Water flow

611   was turned off for 36h to enhance exposures, while an airstone was provided to each

612   tank to maintain adequate oxygen levels. This was a lower exposure dose than many of

613   our previous studies (14). Therefore, we enhanced exposure adding 1 L of water from a

614   stock tank holding infected fish twice a day during the 36 h hour post exposure period.

615   This additional water supplement was created by siphoning water from the bottom of the

616   exposed stock fish tank because the infectious stage is a larvated egg, which sinks in

617   water.

618

619   **Infection assessment**

620   Exposed and control fish were collected and examined for worm prevalence,

621   abundance and state of development using wet mounts of whole intestines as described

622   in Schuster *et al.* 2023 (42). After recording observations in wet mounts, the individual

623   intestine was preserved in Dietrich's solution and intestines of 95 fish were processed

624   for histology prepared as described in Gaulke *et al.* 2019 (15). Here we focused on

625   selected samples from fish from the 35°C group as very few worms were detected by

626   wet mounts in this group. Two stepwise sections, 50 um apart, were obtained from each

627   block to enhance the possibility of larval worms

628

629   **Fecal collection**

630   Five fish from each tank were randomly selected for fecal sampling at 0 dpe

631   (n=60; 5 samples/tank), prior to parasite exposure. Subsequent fecal sampling took

632   place at 14- (n = 54), 21- (n = 48), 28- (n = 47), and 42 (n = 51) dpe to parasites. Fecal

633   material was collected as previously described (20). In brief, fish were transferred to

634   1.4 L tanks (1 fish/tank) containing ~ 0.4 L of fish water at least 30 min after the

635   last feeding of the day. Fish were left to defecate overnight and all fecal material

636   was collected from each tank the following morning in a 1.5ml microcentrifuge

637   tube. Fecal samples were immediately spun at 10k rpm for 2 min, excess tank

638   water was removed, and samples were snap frozen on dry ice and stored at -80

639   °C until processing. However, not all fish produced a fecal sample for a variety of

640   reasons. For instance, experiments involving fish have expected mortality, and fish

641   which died prematurely did not produce fecal samples. Additionally, infection conditions

642   may have prevented infected fish from producing a fecal sample. Instances where fish

643   failed to produce a fecal sample are noted in the metadata sheet.

644

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 Assy 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

668   All microbiome DNA sequence analyses and visualization were conducted in R (v

669   4.3.3)(45). Raw reads were filtered for quality, merged, and assigned using the DADA2

670   R package (v 1.26.0) as previously described (46). In brief, forward and reverse reads

671   were trimmed at 250 and 225 bp, respectively, subsequently merged into contigs, and

672   subject to amplicon sequence variant (ASV) identification. ASVs unannotated at the

673   Phylum level or identified as non-bacterial were removed, which resulted in 674

674   remaining detected ASVs. Samples containing reads below the minimum required read

675   count (<5000) were dropped from downstream analysis. The final sample number for

676   microbiome analysis was 260. Phylogenetic analysis was conducted using MOTHUR (v

677   1.46.1)(47) with default parameters as previously described (17). Phylogeny was

678   inferred using FastTree2 (48), an approximately-maximum-likelihood method.

679   Microbiome and sample data were contained in a Phyloseq object using the Phyloseq R

680   package (49), and the tidyverse (v 2.0.0)(50) and microViz (v 0.12.1) R packages were

681   used for downstream data processing, analyzing, and visualization (51). Code for

682   bioinformatic processing are available at

683   https://github.com/sielerjm/Sieler2025__ZF_Temperature_Parasite/.

684

685   **Microbiome diversity metrics**

686   All microbiome analyses were conducted at the genera level unless otherwise

687   noted. We estimated four alpha-diversity metrics for each microbiome fecal sample:

688   Simpson (52), Shannon (53), phylogenetic diversity (Faith's PD (54); ASVs), and

689   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).

692

693   **Statistical Analyses**

694   All statistical analyses were conducted in R (v 4.3.3)(45) with a significance level

695   of α = 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/.

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.

707

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

713   vegan R package to determine if the selected model parameters significantly explained

714   the variation in microbiome composition across samples. Differential abundance was

715   measured using MaAsLin2 (64). We assessed beta-diversity dispersion within groups

716   with betadisper using the vegan R package.

717   To assess the relationship between parasite infection outcomes and experimental

718   parameters, we used negative binomial generalized linear models (GLM) with the

719   glm.nb function from the MASS R package (v 7.3-60.0.1)(65) and used the negative

720   binomial distribution to account for overdispersion in the count data, a common

721   characteristic of parasite infection data (14). Significance of main effects and

722   interactions was assessed using two-way ANOVA implemented through the Anova

723   function with the Car R package (v 3.1-2)(66). Post-hoc comparisons were conducted

724   using Tukey's HSD tests via the emmeans package, where we estimated marginal

725   means and performed pairwise contrasts with p-value adjustment using the Tukey

726   method (67). Detection method comparisons were analyzed on a subset of samples

727   used for microbiome analysis (120 samples, 20 samples/tank, ~10 samples/time point;

728   Table S3C.1). To compare detection methods between wet mount and histology, we

729   used McNemar's test (68), with discordant pairs (wet only vs histology only) examined

730   at each temperature and DPE combination through 2×2 contingency tables Table

731   S3C.2-3). Using similar methods as described above, we assessed the relationship

732   between infection outcomes and microbiome diversity using GLMs (Table S3B.1).

733

734   **Abbreviations**

735   dpe = days post exposure

736

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Data Availability

All code generated during this analysis is available in the GitHub repository at the

following URL: https://github.com/sielerjm/Sieler2025__ZF_Temperature_Parasite.

Supplementary tables and figures can be in the accompanying supplementary files. The

raw sequence files generated during the current study are available at the NCBI

Sequence Read Archive (SRA) project number: PRJNA1219243.

Author contributions

TJS and MLK conceived and designed the study. CEA and MJS conducted the

experiments. MJS, TJS, and KDK performed the gut microbiome and integrated

analyses. MJS, TJS, MLK, CEA, KDK, contributed to the preparation and editing of the

manuscript. MJS prepared the figures. All authors read and approved the final

manuscript.

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760

761   Declarations

762   The authors declare no competing interests.

763 **References**

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-
773   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-
782   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-
787   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-
795   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:

https://journals.biologists.com/jeb/article/226/1/jeb245191/286604/The-microbiome-buffers-tadpole-hosts-from-heat

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

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

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

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

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

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

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

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

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

839 19. Stephens WZ, Burns AR, Stagaman K, Wong S, Rawls JF, Guillemin K, et al. The
840   composition of the zebrafish intestinal microbial community varies across
841   development. ISME J [Internet]. 2016 Mar [cited 2022 Jul 14];10(3):644–54.
842   Available from: https://www.nature.com/articles/ismej2015140

843 20. Sieler MJ, Al-Samarrie CE, Kasschau KD, Varga ZM, Kent ML, Sharpton TJ.
844   Disentangling the link between zebrafish diet, gut microbiome succession, and
845   Mycobacterium chelonae infection. anim microbiome [Internet]. 2023 Aug 10 [cited
846   2023 Aug 25];5(1):38. Available from: https://doi.org/10.1186/s42523-023-00254-8

847 21. Moravec F. FIRST RECORD OF THE NEMATODE PSEUDOCAPILLARIA
848   BREVISPICULA. FOLIA PARASITOLOGICA (PRAHA) [Internet]. 1984 [cited 2025
849   Mar 18];31:241–5. Available from: https://folia.paru.cas.cz/pdfs/fol/1984/03/06.pdf

850 22. Dickerson HW. *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (phylum
851   Ciliophora). In: Woo PTK, editor. Fish diseases and disorders Volume 1: protozoan
852   and metazoan infections [Internet]. 2nd ed. UK: CABI; 2006 [cited 2025 Mar 19]. p.
853   116–53. Available from:
854   http://www.cabidigitallibrary.org/doi/10.1079/9780851990156.0116

855 23. Lardeux F, Cheffort J. Temperature thresholds and statistical modelling of larval
856   *Wuchereria bancrofti* (Filariidea: Onchocercidae) developmental rates. Parasitology
857   [Internet]. 1997 Feb [cited 2025 Mar 18];114(2):123–34. Available from:
858   https://www.cambridge.org/core/product/identifier/S0031182096008359/type/journal
859   _article

860 24. Islam MJ, Kunzmann A, Slater MJ. Responses of aquaculture fish to climate
861   change-induced extreme temperatures: A review. Journal of the World Aquaculture
862   Society [Internet]. 2022 [cited 2024 Oct 30];53(2):314–66. Available from:
863   https://onlinelibrary.wiley.com/doi/abs/10.1111/jwas.12853

864 25. Makrinos DL, Bowden TJ. Natural environmental impacts on teleost immune
865   function. Fish & Shellfish Immunology [Internet]. 2016 Jun [cited 2024 Oct
866   29];53:50–7. Available from:
867   https://linkinghub.elsevier.com/retrieve/pii/S1050464816300870

868 26. Dittmar J, Janssen H, Kuske A, Kurtz J, Scharsack JP. Heat and immunity: an
869   experimental heat wave alters immune functions in three-spined sticklebacks (
870   asterosteus aculeatus). Journal of Animal Ecology [Internet]. 2014 [cited 2024 Oct
871   29];83(4):744–57. Available from:
872   https://onlinelibrary.wiley.com/doi/abs/10.1111/1365-2656.12175

873 27. Bailey C, Segner H, Casanova-Nakayama A, Wahli T. Who needs the hotspot? The
874   effect of temperature on the fish host immune response to Tetracapsuloides
875   bryosalmonae the causative agent of proliferative kidney disease. Fish & Shellfish
876   Immunology [Internet]. 2017 Apr [cited 2024 Oct 30];63:424–37. Available from:
877   https://linkinghub.elsevier.com/retrieve/pii/S1050464817301110

878   28. Sepulveda J, Moeller AH. The Effects of Temperature on Animal Gut Microbiomes.
879   Frontiers in Microbiology [Internet]. 2020 [cited 2023 Apr 6];11. Available from:
880   https://www.frontiersin.org/articles/10.3389/fmicb.2020.00384

881   29. Li P, Zhang J, Liu X, Gan L, Xie Y, Zhang H, et al. The Function and the Affecting
882   Factors of the Zebrafish Gut Microbiota. Front Microbiol [Internet]. 2022 Jun 2 [cited
883   2024 Jun 19];13:903471. Available from:
884   https://www.frontiersin.org/articles/10.3389/fmicb.2022.903471/full

885   30. Relman DA. The human microbiome: ecosystem resilience and health. Nutrition
886   Reviews [Internet]. 2012 Aug [cited 2025 Mar 19];70:S2–9. Available from:
887   https://academic.oup.com/nutritionreviews/article-lookup/doi/10.1111/j.1753-
888   4887.2012.00489.x

889   31. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds:
890   evolution of the vertebrate gut microbiota. Nat Rev Microbiol [Internet]. 2008 Oct
891   [cited 2022 Nov 2];6(10):776–88. Available from:
892   https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2664199/

893   32. Roeselers G, Mittge EK, Stephens WZ, Parichy DM, Cavanaugh CM, Guillemin K, et
894   al. Evidence for a core gut microbiota in the zebrafish. ISME J [Internet]. 2011 Oct
895   [cited 2022 Nov 3];5(10):1595–608. Available from:
896   http://www.nature.com/articles/ismej201138

897   33. Breen P, Winters AD, Nag D, Ahmad MM, Theis KR, Withey JH. Internal Versus
898   External Pressures: Effect of Housing Systems on the Zebrafish Microbiome.
899   Zebrafish [Internet]. 2019 Aug [cited 2022 Jul 14];16(4):388–400. Available from:
900   https://www.liebertpub.com/doi/10.1089/zeb.2018.1711

901   34. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. The
902   Application of Ecological Theory Toward an Understanding of the Human
903   Microbiome. Science [Internet]. 2012 Jun 8 [cited 2024 May 1];336(6086):1255–62.
904   Available from: https://www.science.org/doi/10.1126/science.1224203

905   35. Zaneveld JR, McMinds R, Vega Thurber R. Stress and stability: applying the Anna
906   Karenina principle to animal microbiomes. Nat Microbiol [Internet]. 2017 Sep [cited
907   2022 Jan 30];2(9):17121. Available from:
908   http://www.nature.com/articles/nmicrobiol2017121

909   36. Jeltsch F, Roeleke M, Abdelfattah A, Arlinghaus R, Berg G, Blaum N, et al. The need
910   for an individual-based global change ecology. IBE [Internet]. 2025 Mar 27 [cited
911   2025 Mar 28];1:1–18. Available from: https://ibe.pensoft.net/article/148200/

912   37. Sommer AJ, Peters A, Rommel M, Cyrys J, Grallert H, Haller D, et al. A
913   randomization-based causal inference framework for uncovering environmental
914   exposure effects on human gut microbiota [Internet]. 2021 Mar [cited 2022 Jan 13]
915   p. 2021.02.24.432662. Available from:
916   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 JI. 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-
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

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-
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-
1018 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-
1021 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
1026 _article

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1028   Figure 1. Experimental design showing treatments and husbandry events during the

1029   course of the study. Symbols indicate when an experimental event occurred at each

1030   time point. (1) 260 fish were assigned and acclimated to one of three water temperature

1031   groups (e.g., 28°C, 32°C, or 35°C) and reared from 0 to 164 days post fertilization (dpf).

1032   (2a) At 164 dpf (or 0 days post exposure; DPE), fecal collections were collected from a

1033   random selection of five fish per tank (n = 60). Additionally, histological and wet mount

1034   assessments were conducted on selected fish to assess presence of infection and

1035   infection burden. (1b) Afterwards, a cohort of fish from each water temperature group

1036   were exposed to *Pseudocapillaria tomentosa*. (3-6) Subsequent fecal samples were

1037   collected and histopathological assessments were conducted at 14 dpe (n = 54), (3) 21

1038   dpe (n = 48), (4) 28 dpe (n = 47), and (5) 42 dpe (n = 51).

1039

1040   Figure 2. Effects of water temperature on zebrafish gut microbiomes. (A) Simpson's

1041   Index of diversity shows that gut microbiome diversity significantly differs between fish

1042   reared at 28°C and 35°C water temperatures. (B) Capscale ordination based on the

1043   Bray-Curtis dissimilarity of gut microbiome composition constrained on the main effect

1044   of temperature. The analysis shows that gut microbiome composition significantly differs

1045   between fish reared at different water temperatures. (C) Simpson's Index of diversity

1046   shows microbial gut diversity increases with time from 0 days post exposure (dpe) to 42

1047   dpe, irrespective of water temperature. (D) Capscale ordination of gut microbiome

1048   composition based on the Bray-Curtis dissimilarity constrained on the main effects of

1049   water temperature and time (days post exposure, dpe), and their interaction. The

1050   analysis shows that shows that gut microbiome composition differs between fish across

1051 time depending on water temperature. Ribbons and ellipses indicate 95% confidence

1052 interval. Only statistically significant relationships are shown. A "*" indicates statistical

1053 significance below the "0.05" level. Black arrows indicate direction of greatest change in

1054 the indicated by covariates.

1055

1056 Figure 3. Infection outcomes in zebrafish exposed to *Pseudocapillaria tomentosa*. (A)

1057 Histological sections stained with H&E stain in zebrafish exposed to *P. tomentosa*

1058 examined at 35°C at 21 days post exposure. Arrow = larval worms, sagittal and cross

1059 sections. Bar = 50 µm (B) Infection outcome analysis of fish exposed to *P. tomentosa* (n

1060 = 89) by temperature. Fish reared at 28°C and 32°C water temperatures had

1061 significantly different infection burden to fish reared at 35°C water temperature. Only

1062 one fish in our microbiome analysis reared at 35°C was identified as being positively

1063 infected by wet mount. Only statistically significant relationships are shown. A "*"

1064 indicates statistical significance below the "0.05" level.

1065 Figure 4. Effects of *Pseudocapillaria tomentosa* exposure on zebrafish gut microbiomes

1066 reared at different water temperatures. (A) Simpson's Index of diversity shows that gut

1067 microbiome diversity significantly differs between fish reared at 28°C water temperature

1068 to fish reared at 32°C and 35°C water temperatures. (B) Capscale ordination based on

1069 the Bray-Curtis dissimilarity of gut microbiome composition constrained on the main

1070 effect of temperature. The analysis shows that gut microbiome composition significantly

1071 differs between parasite exposed fish reared at different water temperatures. (C)

1072 Simpson's Index of diversity shows microbial gut diversity decreases with time from 0

1073 days post exposure (dpe) to 42 dpe in parasite exposed fish reared at 28°C water

1074   temperature. (D) Capscale ordination of gut microbiome composition based on the

1075   Canberra dissimilarity constrained on the main effects of water temperature and time

1076   (days post exposure, dpe), and their interaction. The analysis shows that shows that gut

1077   microbiome composition differs between parasite exposed fish across time depending

1078   on water temperature. Ribbons and ellipses indicate 95% confidence interval. Only

1079   statistically significant relationships are shown. A "*" indicates statistical significance

1080   below the "0.05" level. Black arrows indicate direction of greatest change in the

1081   indicated covariates.

1082

1083   Figure 5. The impacts of presence of infection and infection burden on the gut

1084   microbiomes of *Pseudocapillaria tomentosa* exposed zebrafish. (A) Simpson's Index for

1085   diversity of parasite exposed fish. Gut microbial alpha-diversity does not significantly

1086   differ between fish reared at the same water temperature depending on presence of

1087   infection. (B) Capscale ordination based on the Canberra dissimilarity of gut microbiome

1088   composition of parasite exposed fish constrained on the main effects of temperature

1089   and pathology result. The analysis shows that gut microbiome composition significantly

1090   differs between positively infected fish reared at different water temperatures. (C)

1091   Infection burden (total worm counts) is positively correlated with lowest or highest alpha

1092   diversity scores in positively infected fish. (D) Capscale ordination based on the Bray-

1093   Curtis dissimilarity of gut microbiome composition constrained on the main effects of

1094   water temperature and infection burden. The analysis shows that gut microbiome

1095   composition significantly differs between clusters of Low, High and Other fish. Samples

1096   points are colored by water temperature, and filled by "Cluster" grouping. Samples with

1097   at least one detectable worm and an alpha-diversity score less than 0.5 are categorized

1098   as Low (orange fill), samples with at least one detectable worm and an alpha-diversity

1099   score greater than 0.5 are categorized as High (purple fill), and samples with no

1100   observable infection are categorized as Other (white and transparent fill). Ribbons and

1101   ellipses indicate 95% confidence interval. Only statistically significant relationships are

1102   shown. A "*" indicates statistical significance below the "0.05" level. Black arrows

1103   indicate statistically significant covariates and direction of greatest change in the

1104   indicated covariates.

1105

1106   Figure 6. Comparison of the effects of water temperature on the gut microbiome

1107   between parasite exposed fish and parasite unexposed fish. (A) Simpson's Index for

1108   diversity of parasite unexposed and pre-exposed fish at 0 days post exposure (dpe).

1109   Prior to parasite exposure gut microbial alpha-diversity does not significantly differ

1110   between fish reared at the same water temperature. (B) Capscale ordinations based on

1111   the Bray-Curtis dissimilarity of gut microbiome composition constrained on the main and

1112   interaction effects of temperature and parasite exposure (treatment) of pre-exposure

1113   samples at 0 dpe. (C) Simpson's Index for diversity of parasite unexposed and exposed

1114   fish. Gut microbial alpha-diversity significantly differs between parasite exposed fish

1115   reared at 28°C and 32°C water temperature relative to unexposed control fish, but gut

1116   microbial alpha-diversity does not differ between parasite unexposed and exposed fish

1117   reared at 35°C water temperature. (D) Capscale ordinations based on the Bray-Curtis

1118   dissimilarity of gut microbiome composition constrained on the main and interaction

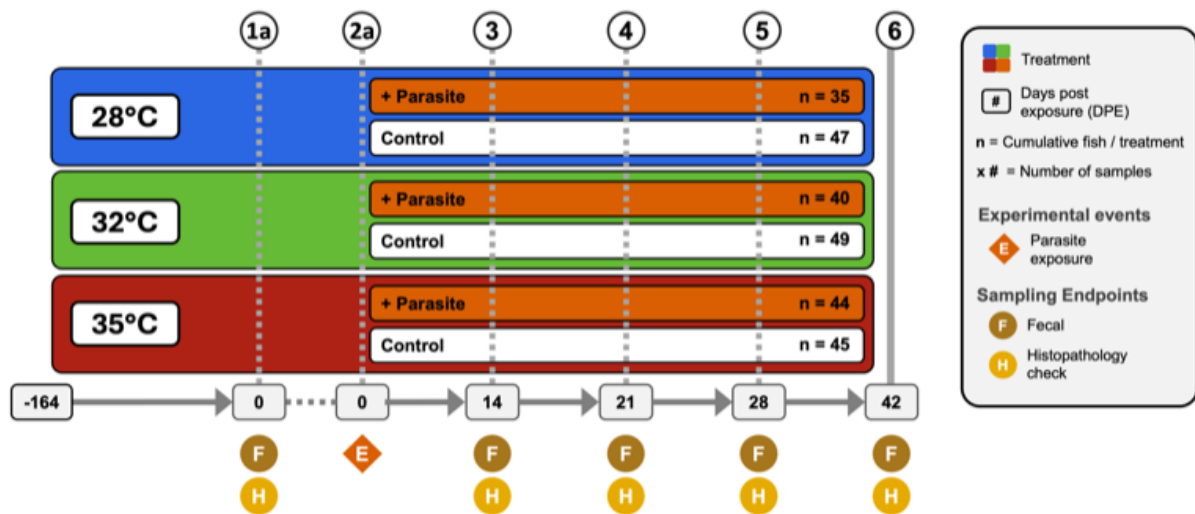1119   effects of temperature and parasite exposure (treatment) of post-exposure samples

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

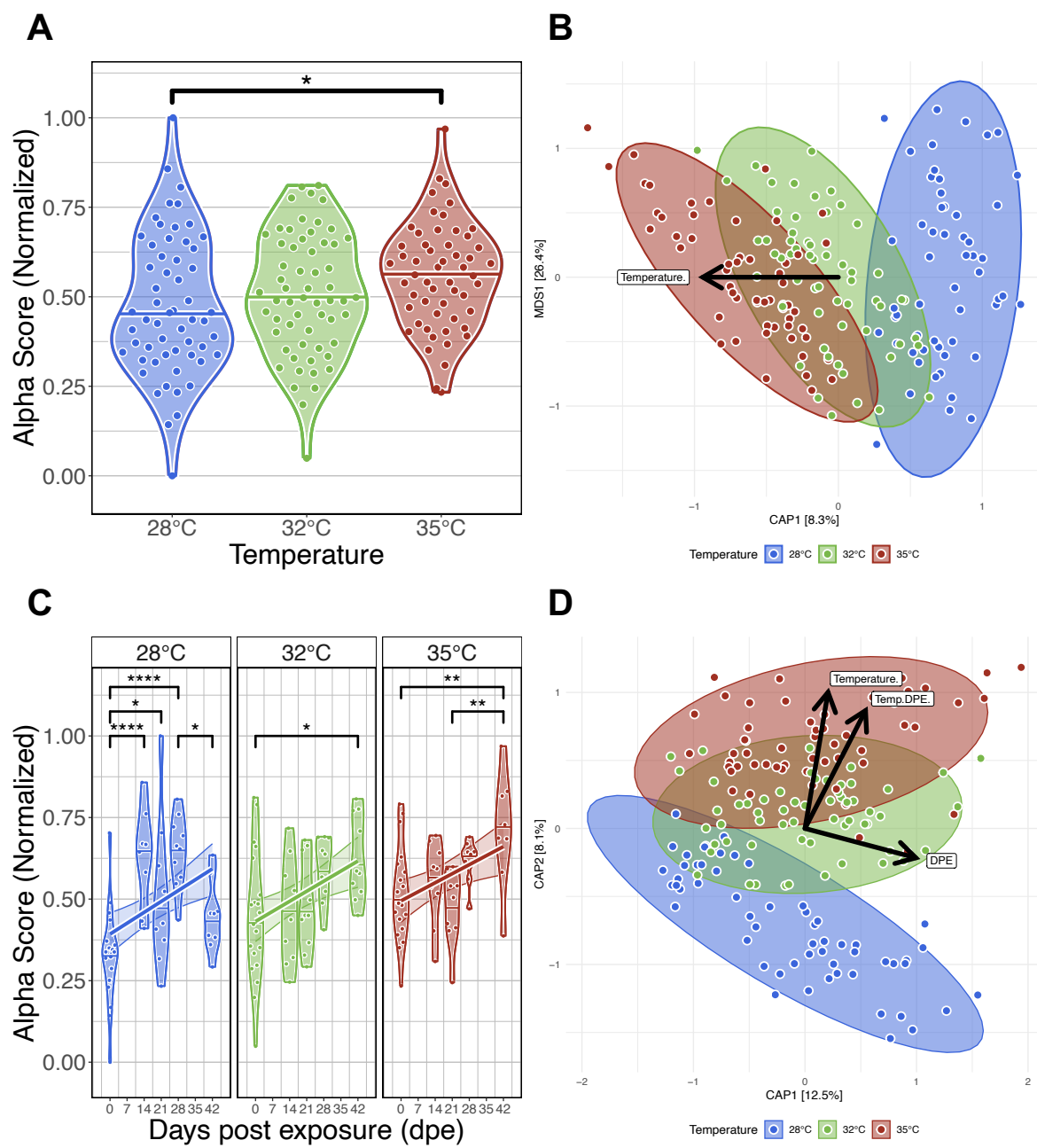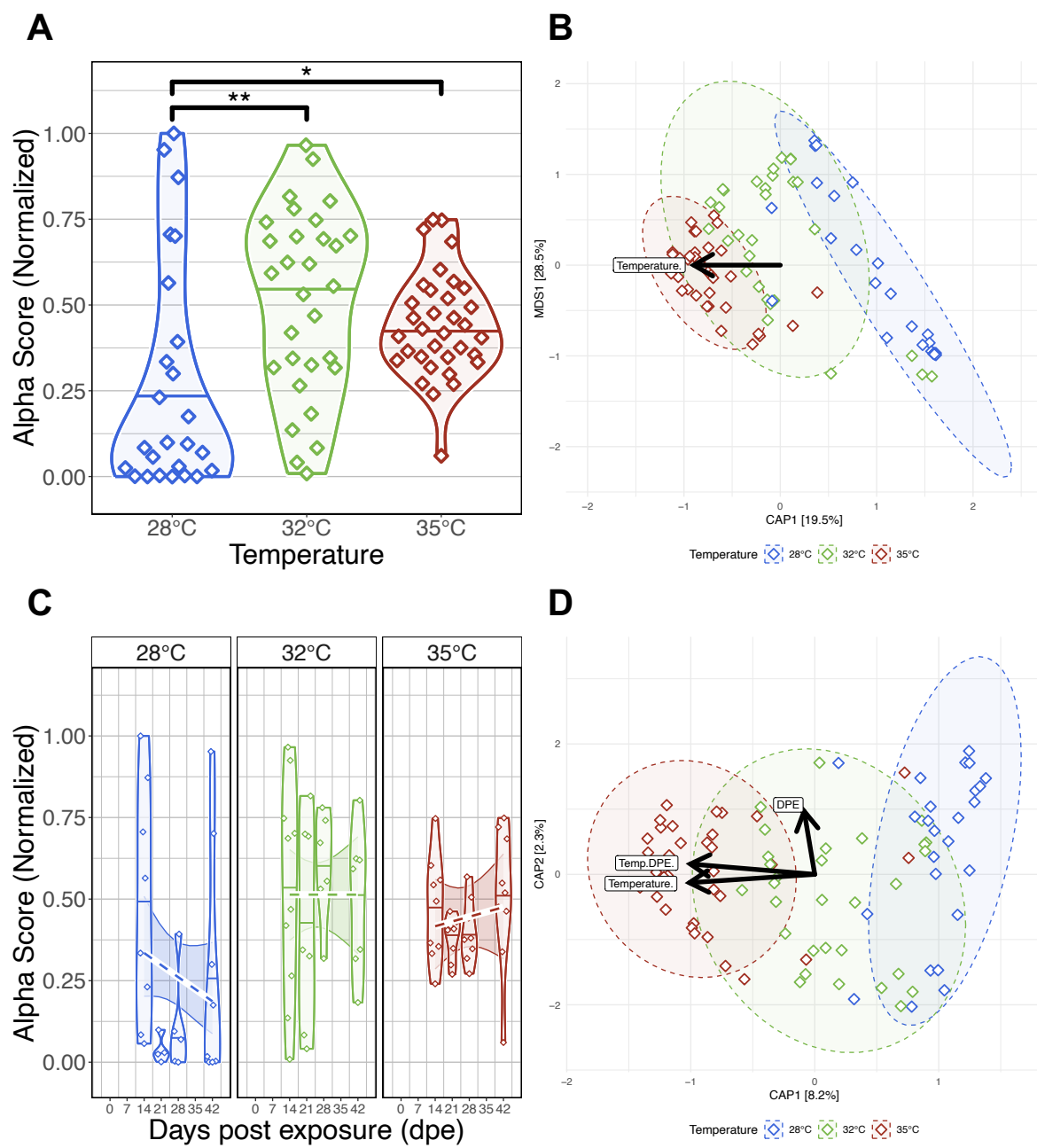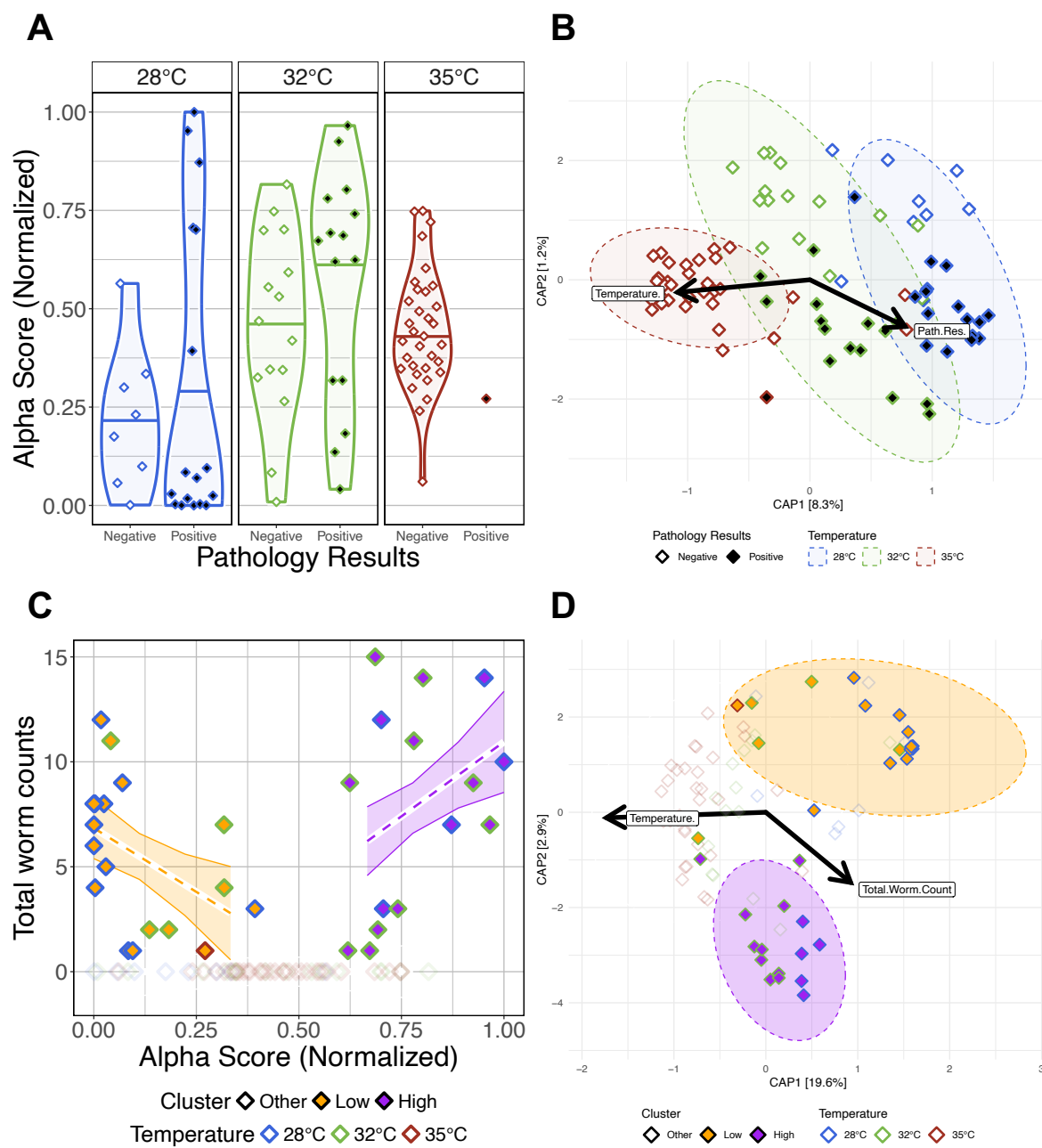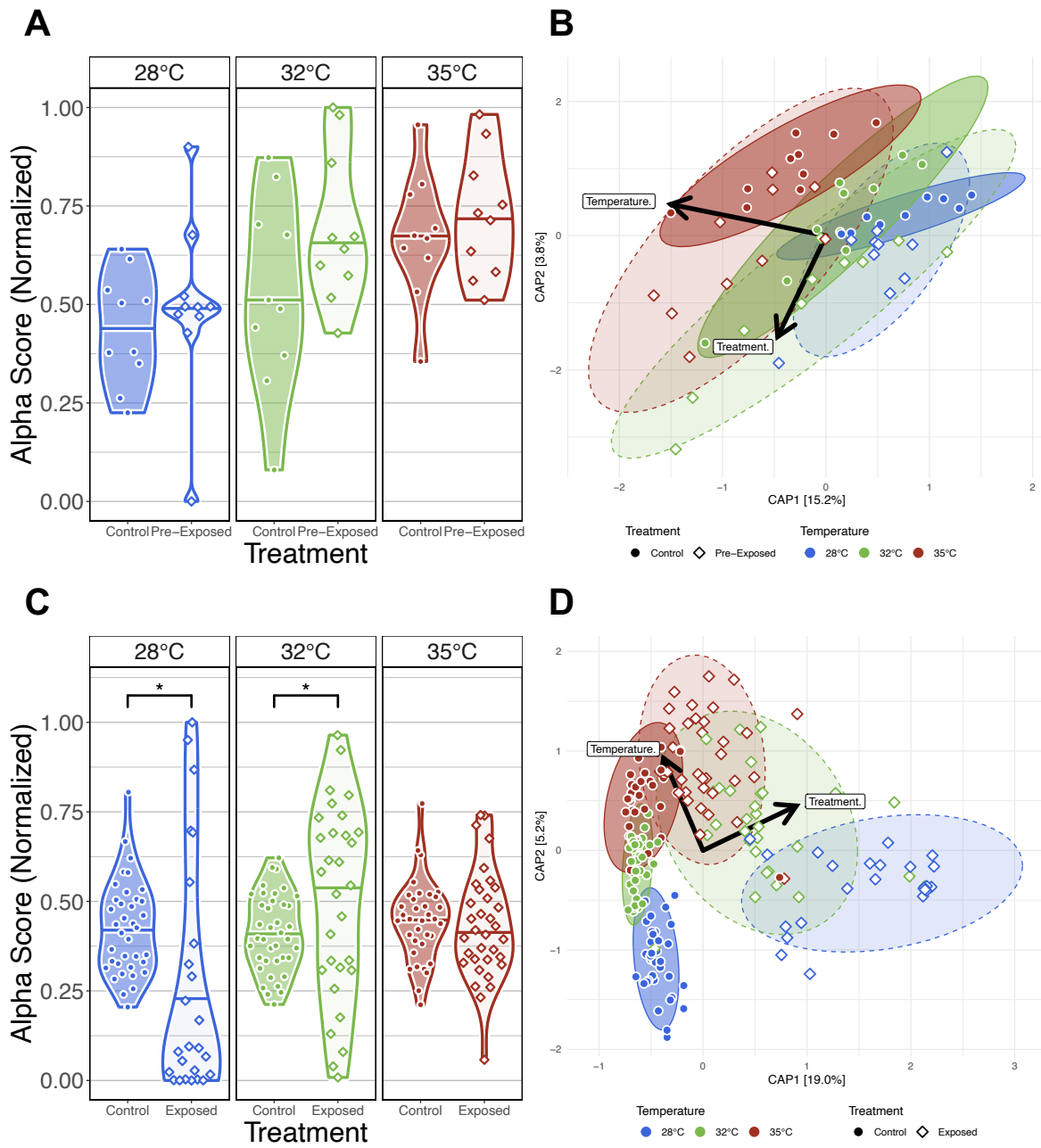1132 covariate. Gray colored cells indicate a significant effect was not observed.

1133

1134

1135

A

B

1136

1138

1139

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

Coefficient

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