Gut Microbiome Composition and Diversity Are Associated with Heat Tolerance Plasticity in a Tropical Lizard

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ABSTRACT

Climate change is detrimentally affecting species worldwide. Against this backdrop, tropical ectotherms, which have evolved under historically stable conditions, are considered especially vulnerable to changing environmental conditions. Phenotypic plasticity may provide a rapid coping mechanism in response to warming. While plasticity is typically attributed to host mechanisms, vertebrates house diverse gut microbial communities that impact host traits and can change rapidly within the host's lifetime. Thus, it is possible that the microbiome may contribute to the capacity of ectotherms to mount plastic responses to environmental warming. We explored this possibility using the Panamanian slender anole (*Anolis apletophallus*), a tropical forest-dwelling lizard experiencing population declines induced by climate change. We tested for associations between the composition of the gut microbiome in slender anoles and the capacity for plasticity in their heat tolerance at three time points after exposure to an acute heat stress event. We found that the magnitude of host plasticity at two of three time points was significantly associated with gut microbiome composition before heat stress. Furthermore, up to 94% of the variation in host plasticity was explained by gut microbiome diversity at one time point. Our work suggests a potentially key role for the microbiome in modulating plastic responses to climate change in ectothermic vertebrates, although future manipulative experiments are required to confirm causality.

Keywords: climate change, host-microbe interactions, warming tolerance, thermal physiology, thermal plasticity, reptile physiology.

Introduction

Climate change is resulting in an unprecedented warming of habitats that animals must cope with to persist (Somero 2010; Jørgensen et al. 2022). One mechanism animals use to mitigate the effects of rapid changes in thermal regimes is phenotypic plasticity, including shifts in traits like heat tolerance that can occur rapidly after exposure to thermal stress (Morley et al. 2019). These changes can increase the probability of survival in the new warmer environment (Walsh et al. 2021). However, the capacity for physiological plasticity in response to warming is considered to be limited in some species, particularly tropical terrestrial ectotherms, as they have evolved under relatively stable climatic conditions (Huey et al. 2009; Sinervo et al. 2010; Hoffmann et al. 2013; Gunderson and Stillman 2015; van Heerwaarden et al. 2016). Understanding the mechanisms that drive the capacity for animal plasticity will be key in determining their potential responses to climate change.

Physiological mechanisms of ectothermic hosts, such as heat shock proteins (HSPs; Fangue et al. 2006; Rosso et al. 2024; Wuthrich et al. 2025), facilitate heat tolerance plasticity; however, functional changes in the microbial communities that live in and on animals might also facilitate rapid shifts in thermal tolerance when hosts experience acute heat stress (Alberdi et al. 2016; Voolstra and Ziegler 2020; Gillingham et al. 2024). Indeed, gut microbial communities of ectotherms impact host thermal tolerance (Hector et al. 2022; Fontaine and Kohl 2023*a*)

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and can change rapidly within an animal's lifetime, suggesting that they could play a key role in modulating host thermal plasticity. Evidence to support this idea includes the observation that gut microbiome composition and function changes with temperature in ectotherms (Fontaine et al. 2018; Moeller et al. 2020; Sepulveda and Moeller 2020; Fontaine and Kohl 2023*b*), including over relatively short timescales (Fontaine and Kohl 2020, 2023*b*). Additionally, experimental colonization of a range of animal host species with symbiotic microbes increases their heat tolerance relative to hosts raised without these microbes, including in sea anemones and corals (Rosado et al. 2019; Baldassarre et al. 2022), roundworms (Nakagawa et al. 2016; Hoang et al. 2019), insects (Montllor et al. 2002; Russell and Moran 2006; Dunbar et al. 2007; Moghadam et al. 2018; Jaramillo and Castañeda 2021), and larval amphibians (Fontaine et al. 2022; Dallas et al. 2024). In lizards, the composition of the gut microbiome is correlated with heat tolerance (Moeller et al. 2020) and host survival under warming (Bestion et al. 2017).

Nonetheless, while microbiome composition can change under heat stress and there is a clear link between baseline heat tolerance and microbial communities, the impact of the microbiome on host variation in heat tolerance plasticity is unclear. Indeed, empirical evidence demonstrating relationships between any form of host plasticity and the microbiome is lacking (Martin-Bideguren et al. 2024). However, there are several mechanisms by which the gut microbiome might influence plasticity in ectotherm heat tolerance. In aphids, the production of a single bacterial HSP after heat stress dramatically impacts host thermal tolerance and fitness (Dunbar et al. 2007). Thus, if important bacterial HSPs are inducible upon exposure to warming and increase host heat tolerance, it is likely that this process would enhance heat tolerance plasticity as well. Furthermore, exposure to heat stress changes the abundance of genes associated with various metabolic pathways in the gut metagenome (Fontaine and Kohl 2023*b*) and impacts the abundance of gut microbe–associated metabolites in the intestine (Fang et al. 2023; Zhou et al. 2023). Microbial metabolites such as amino acids (Fontaine and Kohl 2023*b*) and vitamins (Xie et al. 2013) can be protective for hosts under heat stress and may become more abundant after exposure to warming. In addition to the direct impact of microbially produced molecules on host physiology, these molecules can also stimulate changes in host gene expression. For example, microbial colonization increases the expression of host HSPs in aphids, enabling them to occupy warmer habitats (Porras et al. 2020). Furthermore, particular strains of bacteria can stimulate host genes associated with the antioxidant defense pathway, which can mitigate the effects of oxidative stress under heat shock and allow hosts to withstand higher temperatures (Nakagawa et al. 2016). Despite these possibilities, to our knowledge, there has not been a test of the microbiome's influence on the host's capacity to increase thermal tolerance after a heat stress event.

Here, we tested for associations between heat tolerance plasticity in a tropical lizard, the Panamanian slender anole (*Anolis apletophallus*), and the composition and diversity of their gut microbiomes. Slender anoles are lowland rainforest lizards from central and eastern Panama that have been shown to exhibit plasticity in heat tolerance and gene expression under experimental warming (Rosso et al. 2024; Wuthrich et al. 2025). Although baseline heat tolerance in this species was previously shown to be uncorrelated with microbiome features (Williams et al. 2022), the extent to which plasticity in heat tolerance varies among individuals and depends on microbiome composition has not been investigated. This question is of particular importance because the slender anole is experiencing population declines as a result of climate warming (Stapley et al. 2015). Understanding the links between the gut microbiome and host plasticity is important for the conservation of slender anoles and other vulnerable tropical species because of the potential to augment bacteria to achieve beneficial outcomes (Trevelline et al. 2019; Fontaine and Kohl 2023*a*).

Methods

Animal Collection and Husbandry

We captured adult slender anoles ($N = 15$ males and 15 females; snout-vent length = 38–46 mm) by hand along a central trail (Pipeline Road) that bisects Soberanía National Park, Panama, in June 2023. Animals were individually placed in plastic 100-mL conical tubes with an air hole in the lid and, using a cooler backpack, were transported to laboratory facilities at the Smithsonian Tropical Research Institute (STRI) in Gamboa, Panama. In the laboratory, we housed animals individually in small Tupperware containers with air holes and a wet paper towel to maintain humidity. Containers were kept in a Percival incubator set to 26°C and 65% humidity overnight for acclimation to the laboratory, approximating average field conditions (Logan et al. 2021). Animal collections and experiments were conducted in accordance with STRI's Animal Care and Use Committee (protocol SI-23021) and Panama's Ministerio de Ambiente (scientific collecting permit ARG-098-2023).

Fecal Sample Collection and Microbiome Sequence Processing

The morning after field capture (at ~07:00 a.m.), we collected any lizard feces present using sterile forceps. We placed individual fecal samples into sterile cryovials that were frozen at −20°C until processing. DNA was extracted from fecal samples using a Zymo Research Quick-DNA HMW MagBead Kit with modifications to enhance bacterial lysis. We washed fecal samples twice with TE buffer and then digested samples overnight at 55°C in lysozyme (25 mg/mL) suspended in the same TE buffer. After centrifuging the sample and retaining the supernatant for extraction, we bead beat samples at 40 m/s for three 40-s rounds. The supernatant was then combined with that from the enzymatic lysis and used to isolate DNA following the manufacturer's protocol.

We amplified the V4 region of the bacterial 16S ribosomal RNA (rRNA) gene using a two-step polymerase chain reaction (PCR) protocol following previously described methods (Williams et al. 2022). In the first step, the V4 region was amplified using the

primers 515F and 806R (Caporaso et al. 2011). In the second step, dual-index barcodes were added to amplicon products (i7 SB704-07 and i5 SD501-08; Kozich et al. 2013). The resulting PCR products were purified and normalized using a Charm Biotech PCR normalization and purification plate. We then pooled, purified, and concentrated the libraries using solid-phase reversible immobilization beads. We assessed the final library concentration and quality using a Qubit fluorometer dsDNA high-sensitivity assay and a high-sensitivity DNA assay on an Agilent bioanalyzer, respectively. Amplicons were sequenced at the STRI's Naos Molecular Laboratory on an Illumina MiSeq, generating 2 × 250 base paired-end reads (Caporaso et al. 2012).

To process the raw sequencing data, we used QIIME2 (ver. 2023.9; Bolyen et al. 2019) and the DADA2 pipeline (Callahan et al. 2016) to filter sequences for quality, remove chimeric reads, and merge and assign reads to amplicon sequence variants (ASVs). We built a phylogenetic tree using FastTree (Price et al. 2010) and assigned ASVs to taxonomy using the SILVA database classifier (ver. 138; Quast et al. 2012). Any reads identified as archaea, chloroplast, or mitochondria were removed from analysis. We calculated four metrics of microbiome α diversity (number of observed ASVs, Shannon diversity, Pielou's evenness, and Faith's phylogenetic diversity) and four metrics of microbiome β diversity (Jaccard, Bray-Curtis, unweighted UniFrac, and weighted UniFrac distances), rarefying to a depth of 19,987 reads per sample.

Voluntary Thermal Maximum Plasticity Experiment

The voluntary thermal maximum (VT_{max}) is the body temperature at which an individual begins to engage in escape behavior during a warming challenge (Cowles and Bogert 1944; Camacho and Rusch 2017) and is considered an index of physiological heat tolerance. Using this measure of heat tolerance is advantageous compared to more thermally extreme measurements, such as the critical thermal maximum, because VT_{max} occurs at body temperatures that are more likely to be experienced by wild lizards and are thus more ecologically relevant (Neel et al. 2021).

After fecal sample collection (at ~7:30 a.m.), an initial measurement of VT_{max} was taken for each lizard in a randomized order. To measure VT_{max}, lizard Tupperware containers (with the lizard inside) were removed one at a time from the Percival incubator and placed into a laboratory benchtop incubator (Benchmark MyTemp mini) set to 50°C. Lizard behavior was observed through the clear door of the incubator as their body temperature warmed. Lizards were immediately removed from the incubator once they demonstrated clear signs of escape behavior (e.g., running, jumping, digging; Logan et al. 2021; Neel et al. 2021). To avoid observer bias, all VT_{max} trials were conducted by the same person. After removal, lizard body temperature was immediately recorded as VT_{max} by inserting a thermocouple probe attached to a type K thermometer (Omega HH801B) into the cloaca.

Next, to measure plasticity in VT_{max}, we divided lizards into two groups: a warm treatment and an unmanipulated group. We placed treatment animals into a Percival incubator set to 32°C for 3 h. Environmental temperatures at the field site can reach this temperature at midday but only rarely and for short durations; thus, we consider this a moderate heat shock for the slender anole (Logan et al. 2021). Animals in the unmanipulated group were returned to the 26°C incubator for the same 3 h. After this period, VT_{max} was measured again in all individuals and then, regardless of treatment group, animals were returned to 26°C. VT_{max} was remeasured 13 times over the course of the next 3 d for a separate study focused on the circadian rhythm of heat tolerance. All animals remained at 26°C between measurements. We did not feed animals during this time to avoid confounding effects of specific dynamic action (Wall and Shine 2008) or novel gut microbes that could be introduced via captive diet.

For the purposes of this experiment and to reduce an already large number of comparisons (and thus potential for spurious results), we chose a subset of three time points to assess relationships between microbiome features and host plasticity (hereafter, time points 1–3). First, to assess plasticity in VT_{max}, we calculated each individual's heat tolerance plasticity score at each measurement time point by subtracting the individual's initial VT_{max} value measured before the heat treatment from the VT_{max} value measured at that time point. We chose the following three time points before assessing relationships with microbiome features. Time point 1 is the first measurement of VT_{max} after the heat treatment, which occurred during morning hours. Time point 2 is the second measurement after heat exposure, which was selected for analysis because it was the time when the difference in average plasticity score between treatment and unmanipulated animals was greatest. This time point occurred during midday hours, 4 h after the end of heat exposure. Time point 3 is the sixth measurement after heat exposure, which was selected for analysis because it was the time when the average of both VT_{max} and plasticity score was highest in the heat-exposed animals. This time point occurred 28 h after the end of heat exposure, during midday hours.

Statistical Analyses

Our main objective was to understand how the microbiome may influence the host's plastic response to heat stress. Because unmanipulated animals were not exposed to a heat stressor, we restricted our microbiome analyses to animals in the warm treatment group only. Unmanipulated animals were used solely to verify that a plastic response had occurred in the heat-stressed group. To verify this plastic response after confirming equal variance between groups, we used *t*-tests to compare VT_{max} values between heat-exposed and unmanipulated animals before heat exposure and at the three time points analyzed in this study. We found no difference in VT_{max} between groups before treatment (table A1 [tables A1, A2 are available online]; fig. A1 [figs. A1–A3 are available online]). After heat exposure, VT_{max} was significantly higher in heat-exposed animals relative to unexposed animals at time point 1 (table A1; fig. A1). While average VT_{max} remained higher in heat-exposed animals at each subsequent time point and individual values did not overlap between groups, differences in VT_{max} were nonsignificant at these time points likely because of small sample size. However, based on data from time point 1

and differences in means across all time points, we can conclude that our treatment was successful at inducing VT_{max} plasticity after heat exposure, in accordance with our group's previous work with this species (Rosso et al. 2024).

We collected a total of 11 fecal samples from the heat-stressed group (some animals did not defecate), which was reduced to eight samples ($N = 5$ females, $N = 3$ males) after removing those with low sequence read counts (<300). Despite our reduced sample size, we were able to capture individual-level variation in host heat tolerance plasticity and microbiome features at a similar or greater magnitude than during our previous studies in this species (Williams et al. 2022; Rosso et al. 2024). For each of the previously described time points, we tested correlations of the microbiome features of the fecal sample collected before heat exposure with the value of VT_{max} at that time point and the plasticity score at that time point. All analyses were conducted in R (ver. 3.6.3). We conducted permutational multivariate ANOVAs (PERMANOVAs) with 999 permutations on each of the four β diversity matrices using the adonis2 function in the vegan package (Dixon 2003) to explore the relationship between microbiome composition and host VT_{max} and plasticity score. Additionally, for each β diversity metric, we used linear models (LMs) to test for correlations of principal coordinate analysis (PCoA) axes 1 and 2 with VT_{max} and plasticity score. We tested for correlations between both VT_{max} and plasticity score and each of the four microbiome α diversity metrics using LMs. Last, we used MaAsLin2 (Mallick et al. 2021) to test for relationships between VT_{max} and plasticity score and the relative abundances of bacterial taxa at the phylum, genus, and ASV level, removing taxa from analysis that were present in less than two samples. In MaAsLin2, we used an arcsine square root transformation, and P values were corrected using the false discovery rate method.

Results

Time Point 1

At time point 1 in heat-exposed animals (measurement of VT_{max} immediately after heat exposure), VT_{max} ranged from 29.6°C to 36.3°C, with an average of 32.2°C. Plasticity scores in these animals ranged from −0.9°C to 3.2°C, with an average of 0.93°C. At this time point, we did not uncover significant relationships between host heat tolerance phenotypes and gut microbiome α or β diversity (table 1). There were no significant associations between the relative abundance of any bacterial taxon and VT_{max} or plasticity score after P value correction. Before P value correction, however, the abundances of 73 bacterial taxa were associated with either host VT_{max} or plasticity score (table A2).

Time Point 2

At time point 2 in heat-exposed animals (4 h after heat exposure), VT_{max} ranged from 30.7°C to 34.4°C with an average of 32.65°C. Plasticity scores in these animals ranged from −1.1°C to 3.1°C with an average of 1.35°C. At this time point, we observed the strongest relationships between microbiome features and both VT_{max} and its plasticity. VT_{max} was correlated with one metric of microbiome composition, unweighted UniFrac PCoA axis 2 (LM, $P = 0.04$; table 1; fig. A2). No other microbiome features were associated with VT_{max}. However, all metrics of microbiome β diversity were significantly associated with host plasticity score at this time point (PERMANOVA, $P < 0.05$ for all; table 1; fig. 1*A*–1*D*). Plasticity scores were significantly associated with the following individual PCoA axes: Jaccard PCoA axis 1, weighted UniFrac PCoA axis 1, and unweighted UniFrac PCoA axis 1 (LM, $P < 0.01$ for all; table 1; fig. 1*E*–1*G*). Plasticity scores at this time point were strongly negatively correlated with all metrics of microbial α diversity (LM, $P < 0.05$ for all; table 1; fig. 2). There were no individual bacterial taxa whose abundances correlated with host phenotypes at this time point after P value correction. Before correction, the abundances of 57 bacterial taxa were correlated with host VT_{max} or plasticity score (table A2).

Time Point 3

At time point 3 in heat-exposed animals (28 h after heat exposure), VT_{max} ranged from 31.1°C to 35.0°C, with an average of 32.88°C. Plasticity scores in these animals ranged from −0.6°C to 4.0°C, with an average of 1.56°C. At this time point, there were again no significant associations between VT_{max} values and microbiome features, but host plasticity score was associated with microbiome composition based on weighted UniFrac distance (PERMANOVA, $P = 0.047$; table 1; fig. A3). Plasticity scores were also significantly associated with two individual PCoA axes of microbiome composition: Jaccard PCoA axis 1 and weighted UniFrac PCoA axis 1 (LM, $P < 0.05$ for both; table 1; fig. A3). Plasticity scores at this time point were negatively correlated with two metrics of microbial α diversity: the number of observed ASVs and Shannon diversity (LM, $P < 0.05$ for both; table 1; fig. A3). There were no associations between the abundance of any bacterial taxon and VT_{max} or plasticity score after P value correction. There were 47 associations between bacterial taxa and host VT_{max} or plasticity score before correction (table A2).

Discussion

The composition and diversity of the anole gut microbiome was strongly associated with host plasticity in heat tolerance at multiple time points, including over a full day after heat exposure. While we did not observe significant correlations between the abundances of individual bacterial taxa and host heat tolerance or plasticity after P value correction, we did identify several taxa with potential roles in shaping the thermal physiology of lizards based on uncorrected P values. Our work provides rare evidence that gut microbes might facilitate rapid physiological compensation of hosts during environmental challenges (Alberdi et al. 2016; Martin-Bideguren et al. 2024) and suggests that the role of the microbiome in host plastic capacity is worthy of additional study in the context of conservation strategies for wild vertebrates.

We discovered a temporal component in the relationship between the gut microbiome and host plasticity. There was no relationship between microbiome composition and host plasticity

Table 1: Results from statistical models testing for associations between host voluntary thermal maximum (VT_{max}) and plasticity score with gut microbiome features at three experimental time points

		Time point 1				Time point 2				Time point 3			
		VT_{max}		Plasticity		VT_{max}		Plasticity		VT_{max}		Plasticity	
Microbiome metric	Statistical test	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
		β diversity											
BC	PERMANOVA	.92	.52	.71	.67	.43	.92	**2.44**	**.04**	.70	.70	1.01	.43
BC axis 1	LM	1.31	.30	.01	.94	.06	.81	4.81	.08	.73	.73	.31	.60
BC axis 2	LM	.22	.66	2.35	.18	.43	.54	3.94	.10	.20	.67	1.46	.28
JC	PERMANOVA	.86	.79	1.00	.45	.71	.93	**1.60**	**.01**	1.00	.45	1.30	.10
JC axis 1	LM	.08	.79	1.05	.35	<.01	.98	**23.53**	**<.01**	.37	.37	**8.27**	**.03**
JC axis 2	LM	.02	.90	.07	.80	<.01	.99	.05	.83	.09	.78	.03	.88
WUF	PERMANOVA	.23	.95	.98	.44	.67	.52	**7.51**	**<.01**	1.28	.32	**3.27**	**.04**
WUF axis 1	LM	.92	.92	1.08	.34	.52	.50	**25.38**	**<.01**	.20	.20	**7.39**	**.04**
WUF axis 2	LM	.99	.99	.59	.47	.74	.43	.51	.51	.93	.93	.57	.48
UWUF	PERMANOVA	.84	.67	.94	.51	1.04	.41	**2.23**	**.03**	.83	.71	1.17	.26
UWUF axis 1	LM	.07	.80	.73	.43	.02	.89	**14.42**	**.01**	.48	.48	2.81	.35
UWUF axis 2	LM	.66	.45	1.22	.31	**8.20**	**.04**	.21	.67	.30	.61	1.08	.15
		α diversity											
No. observed ASVs	LM	.54	.49	.44	.53	.01	.94	**26.77**	**<.01**	.39	.56	**6.89**	**<.05**
Shannon diversity	LM	.07	.80	1.21	.31	.29	.61	**83.35**	**<.01**	1.43	.28	**.02**	**.02**
Faith's phylogenetic diversity	LM	.20	.67	.23	.65	.33	.59	**12.70**	**.02**	.39	.56	3.95	.10
Pielou's evenness	LM	.01	.91	1.45	.27	1.01	.36	**8.81**	**.03**	1.61	.23	5.60	.06

Note. Plasticity score was calculated by subtracting the value of an individual's VT_{max} before heat treatment from their VT_{max} at that time point after treatment. We tested four metrics of microbiome β diversity: Bray-Curtis distance (BC), Jaccard distance (JC), weighted UniFrac distance (WUF), and unweighted UniFrac distance (UWUF). For β diversity, results are shown from a permutational multivariate ANOVA (PERMANOVA) associating host phenotypes with overall microbiome composition and from linear models (LMs) testing associations with axes 1 and 2 from principal coordinate analyses. We tested four metrics of microbiome α diversity: number of observed ASVs, Shannon diversity, Faith's phylogenetic diversity, and Pielou's evenness using LMs. *F* statistics and *P* values are displayed for each comparison, with significant values shown in bold. Degrees of freedom (df) for comparisons at each time point are as follows: for time point 1, df = 1, 6; for time point 2, df = 1, 5; for time point 3, df = 1, 5.

immediately after heat exposure (morning), a strong relationship 4 h after exposure (midday), and a weaker relationship 1 d after exposure (midday). These dynamics support previous work conducted in humans that demonstrates a short lag in the gut microbial compositional and functional response to environmental change, followed by a peak change and eventual return to baseline (David et al. 2014). Because the gut microbiome may impact host plasticity up to a day after heat exposure, microbial function may have the potential to buffer hosts from rapid spikes in temperature, which are increasing in intensity and frequency as a result of climate change (Domeisen et al. 2023).

The strongest and most consistent host-microbiome relationships we observed were negative correlations between gut microbial diversity and host heat tolerance plasticity. For example, microbial Shannon diversity explained 94% of variation in host plasticity at time point 2 (fig. 2). While microbial diversity is often assumed to be beneficial for host health and function, recent perspectives challenge the generality of this view (Johnson and Burnet 2016; Williams et al. 2024). A less diverse microbiome may be composed of a smaller group of more specialized taxa that facilitate key host functions. Some extreme cases occur in invertebrates. For example, the Hawaiian bobtail squid relies on a single bacterial species (*Vibrio fischeri*) for bioluminescence (Nyholm and McFall-Ngai 2021), and in pea aphids, essential amino acids are supplied by one obligate endosymbiont (*Buchnera aphidicola*; Hansen and Moran 2011). Even in vertebrates, which house more complex and labile gut microbial communities (Petersen and Osvatic 2018), associations have been observed between low microbiome diversity and reduced incidence of host disease in both humans and wildlife (Ponnusamy et al. 2011; Steelman et al. 2012; Williams et al. 2016). Additionally, in the cow rumen, lower microbial diversity is associated with increased energy extraction from feed (Shabat et al. 2016). It is possible that individual lizards that are more plastic in response to warming house a less diverse community of microbes overall but with a high abundances of taxa that help facilitate this plasticity.

Despite the negative correlations we observed between microbial diversity and host plasticity, we were unable to identify

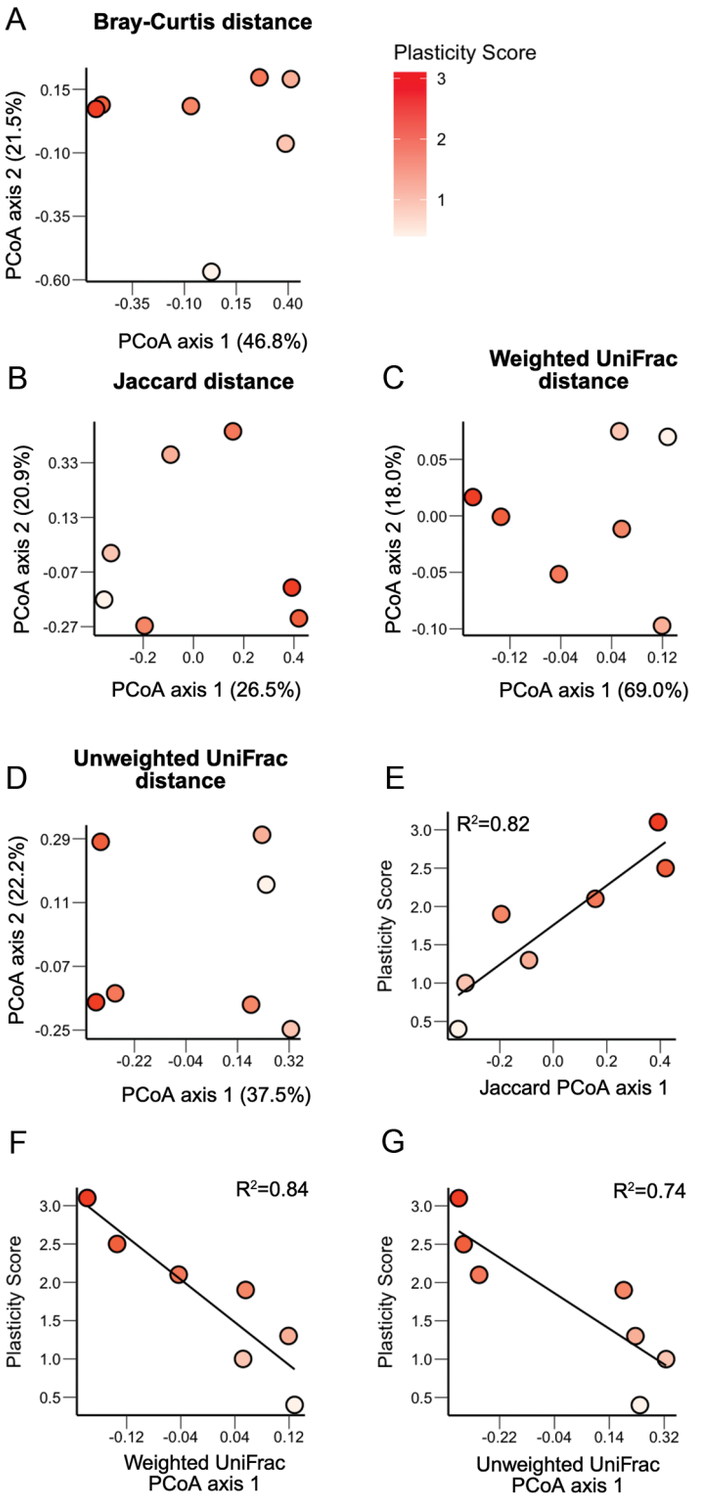

Figure 1. Significant associations between gut microbiome community composition before heat exposure and host heat tolerance plasticity at time point 2. Plasticity score was calculated by subtracting the value of an individual's voluntary thermal maximum before heat treatment from their voluntary thermal maximum at that time point after treatment. Microbial community dissimilarity was calculated and plotted using principal coordinate analysis (PCoA) based on Bray-Curtis distance (*A*), Jaccard distance (*B*), weighted UniFrac distance (*C*), and unweighted UniFrac distance (*D*) between samples colored by plasticity score. Percentages represent the proportion of variation explained by each axis. Associations were observed between host plasticity score and axis 1 of the Jaccard PCoA (*E*), weighted UniFrac PCoA (*F*), and unweighted UniFrac PCoA (*G*).

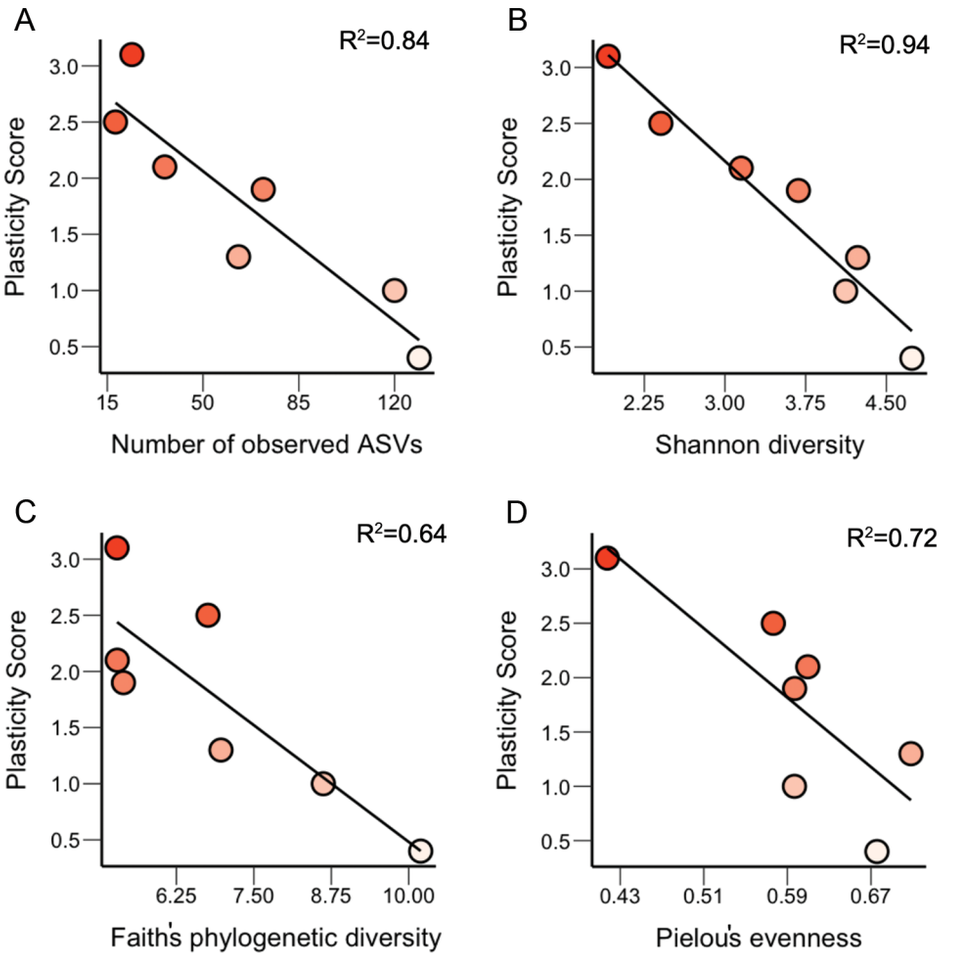

Figure 2. Significant correlations between gut microbiome diversity before heat exposure and host heat tolerance plasticity at time point 2. Plasticity score was calculated by subtracting the value of an individual's voluntary thermal maximum before heat treatment from their voluntary thermal maximum at that time point after treatment. Microbiome diversity was calculated as the number of observed amplicon sequence variants (ASVs) in the community (*A*), Shannon diversity (*B*), Faith's phylogenetic diversity (*C*), and Pielou's evenness (*D*).

individual bacterial taxa that were significantly associated with host phenotypes after *P* value correction. This result is similar to those in a previous study investigating relationships between the lizard gut microbiome and host heat tolerance (Moeller et al. 2020). It can be difficult to detect significant correlations between host phenotypes and bacterial taxa abundance after false discovery rate correction if the relationships are relatively weak (Moeller et al. 2020) or sample sizes are small (Swift et al. 2023). However, before *P* value correction, we identified 73, 57, and 47 bacterial taxa at the phylum, genus, or ASV level, respectively, that were associated with host VT_{max} or plasticity score at each time point.

Some associations between microbial taxa and host traits were consistent across time points, suggesting that these relationships may not only be artifacts of multiple comparisons. For example, the genus *Eisenbergiella* was positively associated with host VT_{max} and negatively associated with VT_{max} plasticity at all time points, and three additional bacterial genera (*Monoglobus*, *Caproiciproducens*, and *Marvinbryantia*) were negatively associated with plasticity at all time points. Some of these bacteria are present in the gut of other lizard species and may have relevance for host physiology under changing environmental conditions. The relative abundance of *Caproiciproducens* increased in the gut of two cold-adapted lizards under experimental warming (Chen et al. 2022). Likewise, the relative abundance of *Eisenbergiella* increased in the gut of desert-dwelling multiocellated racerunner lizards (*Eremias multiocellata*) in response to warming (Yang et al. 2024) and in island-dwelling Italian wall lizards (*Podarcis siculus*) in response to changing food availability (Buglione et al. 2022). The observation that *Eisenbergiella* was always positively associated with VT_{max} but negatively associated with its plasticity suggests the potential for trade-offs for hosts in which housing certain bacteria could be beneficial under static conditions but detrimental under fluctuating conditions, mirroring trade-offs in host physiology for these phenotypes (van Heerwaarden and Kellermann 2020).

Despite strong relationships between the gut microbiome and host plasticity, we observed weak or nonexistent correlations

between microbiome features and baseline values of lizard heat tolerance. This result aligns with previous work by our group that showed no significant relationships between gut microbiome structure and lizard thermal tolerance or preference (Williams et al. 2022). This result could be because different physiological mechanisms underlie basal values of heat tolerance and its plasticity (Comte and Olden 2017; van Heerwaarden and Kellermann 2020), and perhaps the gut microbiome has more influence on the factors that drive plasticity. For example, microbiome composition can impact host gene expression under heat stress (Fontaine and Kohl 2023*b*). Additionally, the gut microbiome itself is functionally responsive to heat (Fontaine and Kohl 2020, 2023*b*), and microbial communities can exhibit "ecological memory" in which community functions are shaped by past environmental exposures (Letourneau et al. 2022). Thus, exposure to warming may stimulate shifts in microbial community structure or function that enhance host plasticity. Regardless, our observation of limited covariation between baseline heat tolerance and the microbiome should be taken with caution, given our low sample sizes.

It is important to note that while the relationships we observed between host heat tolerance plasticity and the microbiome are strong, they are correlational and there are potential lurking variables that could drive these relationships. Although we did not feed animals during the experiment, we cannot control for their diets before capture. Diet quality, quantity, and frequency have important effects on animal gut microbial communities, including reptiles (Costello et al. 2010; Jiang et al. 2017; Xie et al. 2024), and diet can also impact ectotherm thermal physiology (Camacho and Rusch 2017; Hardison and Eliason 2024). Furthermore, there are host genetic variants that impact microbiome composition in wild animals (Suzuki et al. 2019), and plasticity in host thermal tolerance can also have a genetic basis (Mallard et al. 2020). Therefore, our data cannot rule out the possibility of causation in the opposite direction, namely, that host genotypes that confer higher plasticity are also likely to house certain gut microbial communities. A host's previous thermal environment can also impact both their microbiome structure and their thermal physiology (Fontaine et al. 2022). However, the slender anole is a thermoconforming species inhabiting a highly homogenous thermal environment in the dense tropical rainforest understory; therefore, individuals of this species exhibit little variation in body temperature (Logan et al. 2021; Neel et al. 2021). Thus, it is unlikely that thermal exposure in the wild before capture shaped our results.

Although in our view, the most logical explanation for our results is that gut microbes contribute directly to host plasticity, a next important step will be to confirm causality and uncover its mechanism using manipulative studies. For example, future work could focus on conducting similar experiments with a larger number of host individuals to identify the strongest and most consistent relationships between host heat tolerance plasticity and gut microbiome taxa. It would then be important to test causation between individual bacteria and host physiology, through the administration of either microbial cultures or fecal transplants (Yang et al. 2024). Furthermore, these experiments can include a true control group in which animals with unmanipulated microbiomes are also exposed to a heat stressor and plastic responses in microbially manipulated versus unmanipulated animals are compared, which we were unable to assess here. The identification of microbial taxa associated with heat tolerance plasticity may have important conservation implications because there is potential to use probiotic inoculations to increase host resilience and adaptability under environmental change (McKenzie et al. 2018; Rosado et al. 2019; Trevelline et al. 2019; Peixoto et al. 2022; Dallas and Warne 2023; Fontaine and Kohl 2023*a*). In general, when considering how hosts respond and evolve to changing environments, it will be important to consider not only interactions between host genotypes and their environments but also how these relationships are modulated by their associated microbiome (Henry et al. 2021).

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Literature Cited

Alberdi A.O., K.B. Bohmann, M.L. Zepeda-Mendoza, and M.T.P. Gilbert. 2016. Do vertebrate gut metagenomes confer rapid ecological adaptation? Trends Ecol Evol 31: 689–699.

Baldassarre L., H. Ying, A.M. Reitzel, S. Franzenburg, and S. Fraune. 2022. Microbiota mediated plasticity promotes thermal adaptation in the sea anemone *Nematostella vectensis*. Nat Commun 13:3804.

Bestion E., S. Jacob, L. Zinger, L. Di Gesu, M. Richard, J. White, and J. Cote. 2017. Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. Nat Ecol Evol 1:161.

Bolyen E., J.R. Rideout, M.R. Dillon, N.A. Bokulich, C.C. Abnet, G.A. Al-Ghalith, H. Alexander, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857.

Buglione M., E. Ricca, S. Petrelli, L. Baccigalupi, C. Troiano, A. Saggese, E. Rivieccio, and D. Fulgione. 2022. Gut microbiota plasticity in insular lizards under reversed island syndrome. Sci Rep 12:12682.

Callahan B.J., P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, and S.P. Holmes. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581.

Camacho A. and T.W. Rusch. 2017. Methods and pitfalls of measuring thermal preference and tolerance in lizards. J Therm Biol 68:63–72.

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

Caporaso J.G., C.L. Lauber, W.A. Walters, D. Berg-Lyons, C.A. Lozupone, P.J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 108:4516–4522.

Chen J.Q., L.W. Zhang, R.M. Zhao, H.-X. Wu, L.-H. Lin, P. Li, H. Li, Y.-F. Qu, and X. Ji. 2022. Gut microbiota differs between two cold-climate lizards distributed in thermally different regions. BMC Ecol Evol 22:120.

Comte L. and J.D. Olden. 2017. Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. Glob Change Biol 23:728–736.

Costello E.K., J.I. Gordon, S.M. Secor, and R. Knight. 2010. Postprandial remodeling of the gut microbiota in Burmese pythons. ISME J 4:1375.

Cowles R.B. and C.M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. Bull Am Mus Nat Hist 83:5.

Dallas J.W., A. Kazarina, S. Lee, and R.W. Warne. 2024. Cross-species gut microbiota transplantation predictably affects host heat tolerance. J Exp Biol 227:jeb246735.

Dallas J.W. and R.W. Warne. 2023. Captivity and animal microbiomes: potential roles of microbiota for influencing animal conservation. Microb Ecol 85:820–838.

David L.A., C.F. Maurice, R.N. Carmody, D.B. Gootenberg, J.E. Button, B.E. Wolfe, A.V. Ling, et al. 2014. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505:559.

Dixon P. 2003. Vegan, a package of R functions for community ecology. J Veg Sci 14:927–930.

Domeisen D.I.V., E.A.B. Eltahir, E.M. Fischer, R. Knutti, S.E. Perkins-Kirkpatrick, C. Schär, S.I. Seneviratne, A. Weisheimer, and H. Wernli. 2023. Prediction and projection of heatwaves. Nat Rev Earth Environ 4:36–50.

Dunbar H.E., A.C.C. Wilson, N.R. Ferguson, and N.A. Moran. 2007. Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. PLoS Biol 5:e96.

Fang M., Z. Lei, M. Ruilin, W. Jing, and D. Leqiang. 2023. High temperature stress induced oxidative stress, gut inflammation and disordered metabolome and microbiome in Tsinling lenok trout. Ecotoxicol Environ Saf 266:115607.

Fangue N.A., M. Hofmeister, and P.M. Schulte. 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. J Exp Biol 209:2859–2872.

Fontaine S.S. and K.D. Kohl. 2020. The gut microbiota of invasive bullfrog tadpoles responds more rapidly to temperature than a non-invasive congener. Mol Ecol 29:2449–2462.

———. 2023*a*. Ectotherm heat tolerance and the microbiome: current understanding, future directions and potential applications. J Exp Biol 226:jeb245761.

———. 2023*b*. The microbiome buffers tadpole hosts from heat stress: a hologenomic approach to understand host-microbe interactions under warming. J Exp Biol 226:jeb245191.

Fontaine S.S., P.M. Mineo, and K.D. Kohl. 2022. Experimental manipulation of microbiota reduces host thermal tolerance and fitness under heat stress in a vertebrate ectotherm. Nat Ecol Evol 6:405–417.

Fontaine S.S., A.J. Novarro, and K.D. Kohl. 2018. Environmental temperature alters the digestive performance and gut microbiota of a terrestrial amphibian. J Exp Biol 221: jeb187559.

Gillingham M.A.F., H. Prüter, B.K. Montero, and B. Kempenaers. 2024. The costs and benefits of a dynamic host microbiome. Trends Ecol Evol 40:255–272.

Gunderson A.R. and J.H. Stillman. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc R Soc B 282:20150401.

Hansen A.K. and N.A. Moran. 2011. Aphid genome expression reveals host-symbiont cooperation in the production of amino acids. Proc Natl Acad Sci USA 108:2849–2854.

Hardison E.A. and E.J. Eliason. 2024. Diet effects on ectotherm thermal performance. Biol Rev 99:1537–1555.

Hector T.E., K.L. Hoang, J. Li, and K.C. King. 2022. Symbiosis and host responses to heating. Trends Ecol Evol 37:611–624.

Henry L.P., M. Bruijning, S.K.G. Forsberg, and J.F. Ayroles. 2021. The microbiome extends host evolutionary potential. Nat Commun 12:5141.

Hoang K.L., N.M. Gerardo, and L.T. Morran. 2019. The effects of *Bacillus subtilis* on *Caenorhabditis elegans* fitness after heat stress. Ecol Evol 9:3491–3499.

Hoffmann A.A., S.L. Chown, and S. Clusella-Trullas. 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? Funct Ecol 27:934–949.

Huey R.B., C.A. Deutsch, J.J. Tewksbury, L.J. Vitt, P.E. Hertz, H.J. Álvarez Pérez, and T. Garland Jr. 2009. Why tropical forest lizards are vulnerable to climate warming. Proc R Soc B 276: 1939–1948.

Jaramillo A. and L.E. Castañeda. 2021. Gut microbiota of *Drosophila subobscura* contributes to its heat tolerance and is sensitive to transient thermal stress. Front Microbiol 12:886.

Jiang H.Y., J.E. Ma, J. Li, X.-J. Zhang, L.-M. Li, N. He, H.-Y. Liu, et al. 2017. Diets alter the gut microbiome of crocodile lizards. Front Microbiol 8:2073.

Johnson K.V.-A. and P.W.J. Burnet. 2016. Microbiome: should we diversify from diversity? Gut Microbes 7:455–458.

Jørgensen L.B., M. Ørsted, H. Malte, T. Wang, and J. Overgaard. 2022. Extreme escalation of heat failure rates in ectotherms with global warming. Nature 611:93–98.

Kozich J.J., S.L. Westcott, N.T. Baxter, S.K. Highlander, and P.D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79:5112–5120.

Letourneau J., Z.C. Holmes, E.P. Dallow, H.K. Durand, S. Jiang, V.M. Carrion, S.K. Gupta, et al. 2022. Ecological memory of prior nutrient exposure in the human gut microbiome. ISME J 16:2479–2490.

Logan M.L., L.K. Neel, D.J. Nicholson, A.J. Stokes, C.L. Miller, A.K. Chung, J.D. Curlis, et al. 2021. Sex-specific microhabitat use is

associated with sex-biased thermal physiology in *Anolis* lizards. J Exp Biol 224:jeb235697.

Mallard F., V. Nolte, and C. Schlötterer. 2020. The evolution of phenotypic plasticity in response to temperature stress. Genome Biol Evol 12:2429–2440.

Mallick H., A. Rahnavard, L.J. McIver, S. Ma, Y. Zhang, L.H. Nguyen, T.L. Tickle, et al. 2021. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput Biol 17:e1009442.

Martin-Bideguren G., O. Razgour, and A. Alberdi. 2024. Quantitative synthesis of microbe-driven acclimation and adaptation in wild vertebrates. Evol Appl 17:e70025.

McKenzie V.J., J.G. Kueneman, and R.N. Harris. 2018. Probiotics as a tool for disease mitigation in wildlife: insights from food production and medicine. Ann NY Acad Sci 1429:18–30.

Moeller A.H., K. Ivey, M.B. Cornwall, K. Herr, J. Rede, E.N. Taylor, and A.R. Gunderson. 2020. The lizard gut microbiome changes with temperature and is associated with heat tolerance. Appl Environ Microbiol 86:e01181-20.

Moghadam N.N., P.M. Thorshauge, T.N. Kristensen, N. de Jonge, S. Bahrndorff, H. Kjeldal, and J.L. Nielsen. 2018. Strong responses of *Drosophila melanogaster* microbiota to developmental temperature. Fly 12:1–12.

Montllor C.B., A. Maxmen, and A.H. Purcell. 2002. Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. Ecol Entomol 27:189–195.

Morley S.A., L.S. Peck, J.M. Sunday, S. Heiser, and A.E. Bates. 2019. Physiological acclimation and persistence of ectothermic species under extreme heat events. Glob Ecol Biogeogr 28:1018–1037.

Nakagawa H., T. Shiozaki, E. Kobatake, T. Hosoya, T. Moriya, F. Sakai, H. Taru, and T. Miyazaki. 2016. Effects and mechanisms of prolongevity induced by *Lactobacillus gasseri* SBT2055 in *Caenorhabditis elegans*. Aging Cell 15:227–236.

Neel L.K., M.L. Logan, D.J. Nicholson, C. Miller, A.K. Chung, I. Maayan, Z. Degon, et al. 2021. Habitat structure mediates vulnerability to climate change through its effects on thermoregulatory behavior. Biotropica 53:1121–1133.

Nyholm S.V. and M.J. McFall-Ngai. 2021. A lasting symbiosis: how the Hawaiian bobtail squid finds and keeps its bioluminescent bacterial partner. Nat Rev Microbiol 19:666–679.

Peixoto R.S., C.R. Voolstra, M. Sweet, C.M. Duarte, S. Carvalho, H. Villela, J.E. Lunshof, et al. 2022. Harnessing the microbiome to prevent global biodiversity loss. Nat Microbiol 7:1726–1735.

Petersen J.M. and J. Osvatic. 2018. Microbiomes in natura: importance of invertebrates in understanding the natural variety of animal-microbe interactions. mSystems 3:100179-17.

Ponnusamy K., J.N. Choi, J. Kim, S.Y. Lee, and C.H. Lee. 2011. Microbial community and metabolomic comparison of irritable bowel syndrome faeces. J Med Microbiol 60:817–827.

Porras M.F., C.A. Navas, J.H. Marden, M.C. Mescher, C.M. De Moraes, S. Pincebourde, A. Sandoval-Mojica, et al. 2020. Enhanced heat tolerance of viral-infected aphids leads to niche expansion and reduced interspecific competition. Nat Commun 11:1184.

Price M.N., P.S. Dehal, and A.P. Arkin. 2010. FastTree 2: approximately maximum-likelihood trees for large alignments. PLoS ONE 5:e9490.

Quast C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F.O. Glöckner. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596.

Rosado P.M., D.C.A. Leite, G.A.S. Duarte, R.M. Chaloub, G. Jospin, U.N. da Rocha, J.P. Saraiva, et al. 2019. Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. ISME J 13:921–936.

Rosso A.A., B. Casement, A.K. Chung, J.D. Curlis, E. Folfas, M.A. Gallegos, L.K. Neel, et al. 2024. Plasticity of gene expression and thermal tolerance: implications for climate change vulnerability in a tropical forest lizard. Ecol Evol Physiol 97:81–96.

Russell J.A. and N.A. Moran. 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. Proc R Soc B 273:603–610.

Sepulveda J. and A.H. Moeller. 2020. The effects of temperature on animal gut microbiomes. Front Microbiol 11:384.

Shabat S.K.B., G. Sasson, A. Doron-Faigenboim, T. Durman, S. Yaacoby, M.E.B. Miller, B.A. White, N. Shterzer, and I. Mizrahi. 2016. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. ISME J 10:2958–2972.

Sinervo B., F. Mendez-De-La-Cruz, D.B. Miles, B. Heulin, E. Bastiaans, M. Villagrán-Santa Cruz, R. Lara-Resendiz, et al. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328:894–899.

Somero G.N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers." J Exp Biol 213:912–920.

Stapley J., M. Garcia, and R.M. Andrews. 2015. Long-term data reveal a population decline of the tropical lizard *Anolis apletophallus*, and a negative effect of El Niño years on population growth rate. PLoS ONE 10:e0115450.

Steelman S.M., B.P. Chowdhary, S. Dowd, J. Suchodolski, and J.E. Janečka. 2012. Pyrosequencing of 16S rRNA genes in fecal samples reveals high diversity of hindgut microflora in horses and potential links to chronic laminitis. BMC Vet Res 8:231.

Suzuki T.A., M. Phifer-Rixey, K.L. Mack, M.J. Sheehan, D. Lin, K. Bi, and M.W. Nachman. 2019. Host genetic determinants of the gut microbiota of wild mice. Mol Ecol 28:3197–3207.

Swift D., K. Cresswell, R. Johnson, S. Stilianoudakis, and X. Wei. 2023. A review of normalization and differential abundance methods for microbiome counts data. WIREs Comput Stat 15: e1586.

Trevelline B.K., S.S. Fontaine, B.K. Hartup, and K.D. Kohl. 2019. Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. Proc R Soc B 286:20182448.

van Heerwaarden B. and V. Kellermann. 2020. Does plasticity trade off with basal heat tolerance? Trends Ecol Evol 35:874–885.

van Heerwaarden B., V. Kellermann, and C.M. Sgrò. 2016. Limited scope for plasticity to increase upper thermal limits. Funct Ecol 30:1947–1956.

Voolstra C.R. and M. Ziegler. 2020. Adapting with microbial help: microbiome flexibility facilitates rapid responses to environmental change. BioEssays 42:2000004.

Wall M. and R. Shine. 2008. Post-feeding thermophily in lizards (*Lialis burtonis* Gray, Pygopodidae): laboratory studies can provide misleading results. J Therm Biol 33:274–279.

Walsh B.S., S.R. Parratt, N.L.M. Mannion, R.R. Snook, A. Bretman, and T.A.R. Price. 2021. Plastic responses of survival and fertility following heat stress in pupal and adult *Drosophila virilis*. Ecol Evol 11:18238–18247.

Williams C.E., T.J. Hammer, and C.L. Williams. 2024. Diversity alone does not reliably indicate the healthiness of an animal microbiome. ISME J 18:wrae133.

Williams C.E., J.G. Kueneman, D.J. Nicholson, A.A. Rosso, E. Folfas, B. Casement, M.A. Gallegos-Koyner, et al. 2022. Sustained drought, but not short-term warming, alters the gut microbiomes of wild *Anolis* lizards. Appl Environ Microbiol 88:e00530-22.

Williams C.L., K.A. Dill-McFarland, M.W. Vandewege, D.L. Sparks, S.T. Willard, A.J. Kouba, G. Suen, and A.E. Brown. 2016. Dietary shifts may trigger dysbiosis and mucous stools in giant pandas (*Ailuropoda melanoleuca*). Front Microbiol 7:661.

Wuthrich K.L., A.K. Chung, A.A. Rosso, W.O. McMillan, M.L. Logan, and C.L. Cox. 2025. Beating the heat: a lowland tropical lizard expresses heat shock protein networks in response to acute thermal stress. Integr Comp Biol 65:1109-1120.

Xie B., S. Bishop, D. Stessman, D. Wright, M.H. Spalding, and L.J. Halverson. 2013. *Chlamydomonas reinhardtii* thermal tolerance enhancement mediated by a mutualistic interaction with vitamin B_{12}–producing bacteria. ISME J 7:1544–1555.

Xie H., J. Yang, G. Tang, Y. Zeng, S. Luo, B. Sun, Y. Nie, Z. Du, and W.-G. Du. 2024. Consumption of centipedes promotes growth and gut microbiome homeostasis in a lizard. Biol Divers 1:29–39.

Yang J., W. Liu, X. Han, X. Hao, Q. Yao, and W. Du. 2024. Gut microbiota modulation enhances the immune capacity of lizards under climate warming. Microbiome 12:37.

Zhou C., P. Gao, and J. Wang. 2023. Comprehensive analysis of microbiome, metabolome, and transcriptome revealed the mechanisms of intestinal injury in rainbow trout under heat stress. Int J Mol Sci 24:8569.