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RESEARCH ARTICLE

Environmental temperature alters the digestive performance and gut microbiota of a terrestrial amphibian

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ABSTRACT

Environmental temperature and gut microbial communities can both have profound impacts on the digestive performance of ectothermic vertebrates. Additionally, the diversity, composition and function of gut microbial communities themselves are influenced by temperature. It is typically assumed that the temperature-dependent nature of ectotherm digestive performance is due to factors such as host physiological changes and adaptation to local climatic conditions. However, it is also possible that temperature-induced alterations to gut microbiota may influence the relationship between temperature and digestion. To explore the connections between these three factors, we compared digestive performance and gut microbial community diversity and composition in red-backed salamanders housed at three experimental temperatures: 10, 15 and 20°C. We also investigated associations between specific bacterial taxa and temperature or salamander digestive performance. We found that salamander digestive performance was greatest at 15°C, while gut microbial diversity was reduced at 20°C. Further, gut microbial community composition differed among the three temperature treatments. The relative abundance of 25 bacterial genera was dependent on temperature, with high temperatures being associated with reductions in the relative abundance of disease-resistant bacteria and increases in pathogenic taxa. The relative abundance of four bacterial genera was correlated with salamander energy assimilation, two of which are known to digest chitin, a main component of the red-backed salamander diet. These findings suggest that gut microbiota may mediate the relationship between temperature and digestion in ectotherms. We discuss how global climate change may impact ectotherms by altering host–microbe interactions.

KEY WORDS: Salamander, Ectotherm, Energy assimilation, Digestive efficiency, Gut microbiome, Thermal biology

INTRODUCTION

Environmental temperature is a crucial factor impacting the physiology, development and behavior of ectotherms (Gillooly et al., 2002; Huey, 1979). Specifically, multiple aspects of digestive performance in ectothermic vertebrates are temperature dependent, including foraging rates, energy assimilation, digestive efficiency (McConnachie and Alexander, 2004), gut passage time (Waldschmidt et al., 1986) and metabolic response to feeding (Wang et al., 2002). The thermal sensitivity of whole-organism digestive performance traits can be defined using standard thermal performance curves, where performance slowly increases until reaching a thermal optimum and then rapidly decreases until reaching the critical thermal maximum (Huey and Kingsolver, 1989). This relationship has been demonstrated in a number of ectothermic taxa such as fish (Nicieza et al., 1994), tadpoles (Benavides et al., 2005), salamanders (Clay and Gifford, 2017), lizards (Angilletta, 2001) and snakes (Naulleau, 1983). However, other abiotic (seasonality, habitat quality; Ortega et al., 2014) and biotic factors (prey availability, foraging behavior; Adams et al., 1982; Ayers and Shine, 1997) may interact with temperature to impact an organism's digestive performance. Understanding the factors that influence the relationship between temperature and physiological performance in ectotherms is becoming increasingly important because – while already some of the most threatened vertebrate taxa (Gibbons et al., 2000; Stuart et al., 2004) – they are expected to be highly sensitive to the deleterious effects of global climate change (Paaijmans et al., 2013).

Recently, a rapidly growing body of research has demonstrated that microbial communities living in the vertebrate gut have a major impact on many aspects of host physiology, including digestive performance (Kohl and Carey, 2016; McFall-Ngai et al., 2013). Gut microbiota can facilitate enhanced digestion through various functions such as fermentation of plant materials (Mackie, 2002), detoxification of typically unpalatable food (Kohl et al., 2014) or provision of an alternative energy supply during food scarcity (Amato et al., 2015). While most studies have focused on the relationship between microbiota and digestion in mammalian hosts, the gut microbiome is important for digestion in ectothermic vertebrate hosts as well. For example, in both tadpoles and lizards, the gut houses diverse microbial communities with high levels of fermentative activity (Mackie et al., 2004; Pryor and Bjorndal, 2005).

The ability of gut microbiota to provide digestive services may be dependent on temperature. For example, in mammals, exposure to cold leads to characteristic shifts in the community composition of gut microbiota, resulting in marked impacts on overall energy homeostasis (Chevalier et al., 2015). Additionally, in a controlled laboratory study with tadpoles, environmental temperature was determined to be a significant factor shaping community membership and structure of the gut microbiome (Kohl and Yahn, 2016), though the functional consequences of these changes were not studied. Because ectotherm body temperature fluctuates more widely than that of other organisms, impacts on whole-animal performance due to temperature-mediated alterations of gut microbiota may be most pronounced in this group. Indeed, small increases in temperature resulted in decreased diversity and altered community composition of gut microbiota in lizards, which correlated with reduced animal survival (Bestion et al., 2017). However, the mechanisms driving these associations are unclear.

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It is possible that temperature-mediated alterations to gut microbiota composition or function may be an additional factor underlying the relationship between environmental temperature and digestive performance in ectothermic vertebrates. However, studies exploring this possibility are lacking. To address this knowledge gap, we assessed the impacts of environmental temperature on the digestive performance and gut microbiota of a terrestrial amphibian, the eastern red-backed salamander (*Plethodon cinereus* Green 1818). Additionally, we investigated potential connections between digestive performance and gut microbiota that may mediate the relationship between environmental temperature and digestion. We hypothesized that (1) salamander digestive performance – energy intake, energy assimilation and digestive efficiency– would be significantly impacted by environmental temperature; (2) the diversity and community composition of salamander gut microbiota would be temperature dependent; and (3) the relative abundance of specific bacterial taxa would be temperature dependent and correlate with aspects of host digestive performance.

MATERIALS AND METHODS

Animal husbandry

Animals were collected with permission from Virginia Department of Game and Inland Fisheries (permit #056084), interstate transport was permitted under a Federal Fish and Wildlife injurious species permit (permit #MA90136B-0) and vertebrate research was approved by the University of Maryland (protocol FR-15-72).

We collected 19 sexually mature (>32 mm snout–vent length; Sayler, 1966) eastern red-backed salamanders from the Blue Ridge Mountains of Pembroke, VA, USA, in October 2015. To avoid the potentially confounding physiological effects of color polymorphism, we only collected individuals that clearly displayed the striped, rather than unstriped, phenotype (Fisher-Reid et al., 2013; Moreno, 1989). Based on nocturnal summer surveys, body temperature of this population of salamanders ranges from 7.4 to 20.9°C in the wild (Novarro, 2018).

Upon collection from the field, salamanders were transported to the University of Maryland (College Park, MD, USA). Salamanders were housed individually in plastic containers lined with unbleached paper towels and were provided an additional rolled-up paper towel to use as a retreat. Salamanders were acclimated to a constant temperature of 15°C for 4 weeks prior to experiments, and held on a 12 h:12 h light:dark cycle for the duration of the study. Salamanders were fed 15–20 live, adult flightless fruit flies (*Drosophila hydei*) weekly and sprayed with spring water as necessary.

Feeding trials and digestive performance metrics

Following acclimation, each individual salamander underwent three temperature-controlled feeding trials performed in the order 10, 15 and 20°C, following the protocol of Clay and Gifford (2017). At the beginning of each trial, 50 live, adult flightless fruit flies (*D. hydei*) were offered to each salamander. After 24 h, the number of flies remaining was counted and eaten flies were replenished. Counting and replenishing flies continued for five consecutive days. Remaining flies were counted and subsequently removed from enclosures on the sixth day. Feces and shed skin were collected from each individual during trials until the digestive tract was clear (3–5 days without fecal production). Following each trial, salamanders were transferred to the next experimental temperature and allowed to acclimate for 7–10 days prior to beginning the next trial. During this time, they were not fed.

Energy assimilation and digestive efficiency were calculated for each individual during each trial as:

$$\text{Energy assimilation} = \text{EA} - (\text{EF} + \text{ES}), \quad (1)$$

$$\text{Digestive efficiency} = \text{EA} - (\text{EF} + \text{ES})/\text{EA} \times 100, \quad (2)$$

where EA is the total energy acquired through ingestion (kJ), EF is the energy lost as feces (kJ) and ES is the energy lost as shed skin (kJ). As salamanders shed skin more frequently at higher temperatures, we chose to quantify ES to account for variation in energy expenditure among temperatures (Merchant, 1970). All energy measurements were quantified using a Parr 6725 Semimicro Calorimeter (Parr Instrument Company, Moline, IL, USA). Fruit flies were subsampled at different points during the adult life stage and the mean energy content was determined to be 0.064 kJ per fly. This measurement was multiplied by the number of flies ingested during each trial to calculate EA. Fecal and skin samples from individual salamanders were too small to process on their own to calculate energy content and therefore samples from each trial were combined. Combined samples were weighed, dried at 80°C for 24–48 h and pelletized into subsamples, and the energy content was quantified. The mean energy content of fecal and skin subsamples from each trial was multiplied by the mass of each individual's feces and shed skin samples from the same trial to obtain EF and ES for each individual.

Microbiome sample collection

Fecal samples for microbiome analysis were collected from each salamander immediately after each feeding trial ended, before animals were transferred to the next experimental temperature. Samples were kept frozen at −80°C until processing.

DNA extraction

DNA was extracted from fecal samples using a PowerFecal DNA isolation kit (MoBio, Carlsbad, CA, USA) following the manufacturer's protocol. Extracted DNA was sent to Argonne National Laboratory (Argonne, IL, USA). At the laboratory, the V4 region of the 16S rRNA gene was amplified using primers 515F and 806R. PCR amplification was conducted in triplicate, and the resulting products were pooled within a single sample. DNA was cleaned using the UltraClean PCR Clean-Up Kit (MoBio), and amplicons were sequenced on the Illumina MiSeq platform (Caporaso et al., 2012).

Sequence processing

Raw sequence data were processed using the QIIME2 pipeline version 2017.8 (Caporaso et al., 2010). Following demultiplexing, using the DADA2 pipeline within QIIME2, forward sequence reads were filtered, processed and assigned to operational taxonomic units (OTUs) (Callahan et al., 2016). Singleton OTUs were removed, and a phylogenetic tree was built using FASTTREE (Price et al., 2010). Taxonomy was assigned to OTUs using the Greengenes Database (McDonald et al., 2012) and sequences identified as chloroplast or mitochondria were removed from downstream analysis. OTU tables were rarefied to 27,285 reads, excluding one sample with fewer than 10 reads from analysis (from the 10°C trial). To measure bacterial community diversity within each rarefied sample, the number of observed OTUs (OTU richness), Shannon diversity and Faith's phylogenetic diversity were calculated within QIIME2. Shannon diversity is a measure of biodiversity which accounts for OTU richness and evenness (Shannon, 1948). Faith's phylogenetic diversity is a measure of biodiversity which compares phylogenetic relatedness among OTUs in a community by taking the sum of their

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branch lengths (Faith, 1992). To compare bacterial community composition between samples, unweighted and weighted UniFrac distances between samples were calculated in QIIME2 (Lozupone and Knight, 2005). Unweighted UniFrac distance compares samples on the basis of presence and absence of bacterial OTUs, which we call community membership. Weighted UniFrac distance compares samples on the basis of presence, absence and relative abundance of bacterial OTUs, which we call community structure.

Statistical analyses

We used linear mixed effect models (LMMs) with Tukey's *post hoc* HSD in JMP version 12.0 to test for differences across temperature treatments in digestive performance metrics (total energy intake, energy assimilation and digestive efficiency) and microbial community diversity (OTU richness, Shannon diversity and Faith's phylogenetic diversity). We included individual as a random effect in all models, and checked residuals for normality with a Shapiro–Wilk test before proceeding.

To visualize dissimilarity in microbial community composition across temperature treatments, we used principal coordinate analysis (PCoA) with unweighted and weighted UniFrac distances. To test for significant differences in the distance between temperature groups, we used permutational multivariate analysis of variance (PERMANOVA) with 999 permutations and false discovery rate (FDR)-corrected *P*-values, calculated in QIIME2.

To identify specific bacterial genera which had a relative abundance that was significantly associated with temperature, energy assimilation or digestive efficiency, we used multivariate association with linear models (MaAsLin) with default settings. MaAsLin uses boosted, additive general linear models to find associations between the relative abundance of specific bacterial taxa and metadata (Morgan et al., 2012). MaAsLin controlled for individual effects and provided FDR corrected *P*-values. MaAsLin was run in R version 3.4.3 using the package Maaslin. Additionally, we used linear discriminant analysis effect size (LEfSe; Segata et al., 2011), with default settings, to find non-linear associations between the relative abundance of specific bacterial genera and temperature, controlling for individual effects. LEfSe uses a Kruskal–Wallis test to determine differentially abundant taxa between classes, and subsequently ranks them by their linear discriminant analysis score. LEfSe was run on the Galaxy platform (http://huttenhower.sph.harvard.edu/galaxy/).

RESULTS

Digestive performance analysis

Salamander digestive performance was significantly reduced at the highest (20°C) and lowest (10°C) experimental temperatures, relative to that at the intermediate temperature (15°C). Mean total energy intake and energy assimilation were significantly greater at 15°C compared with values at 10 and 20°C (Fig. 1A,B; LMM, energy intake: F=38.6, P<0.001, energy assimilation: F=45.7, P<0.001). Mean digestive efficiency was significantly greater at 15°C than at 10°C (LMM, F=5.7, P<0.01), but digestive efficiency at 20°C was not significantly different from that at 10 or 15°C (Fig. 1C).

Microbial community analysis

After DADA2 processing and removal of chloroplast and mitochondrial sequences, we retained 2,671,629 16S rRNA sequences (mean±s.d. of 47,708±12,374 per sample) representing 2109 unique bacterial OTUs.

Salamander gut microbial diversity was significantly decreased at high temperatures. Bacterial OTU richness decreased by 24.9% at 20°C compared with values at 10 and 15°C (Fig. 2A; LMM, F=23.5, P<0.001). Similarly, Shannon diversity was 8.3% lower at 20°C compared with values at 10 and 15°C (Fig. 2B; LMM, F=7.6, P<0.01) and Faith's phylogenetic diversity was 20.5% lower at 20°C compared with that at 10 and 15°C (Fig. 2C; LMM, F=23.2, P<0.001). Further, salamander gut microbial community composition was distinct at each environmental temperature on the basis of both community membership and structure (Fig. 3A, unweighted UniFrac PERMANOVA, P<0.001; Fig. 3B, weighted UniFrac PERMANOVA, P<0.01).

The relative abundance of 25 bacterial genera was significantly associated with temperature. Specifically, four genera were positively correlated with temperature, 14 genera were negatively correlated with temperature, six genera were enriched at 15°C and one genus decreased in relative abundance at 15°C (Table 1; MaAsLin and/or LEfSe, P<0.05). Significant correlations were detected between host energy assimilation and the relative abundance of four bacterial genera: *Cellvibrio* (Fig. 4A; MaAsLin, coefficient=0.418, P=0.01), *Stenotrophomonas* (Fig. 4B; MaAsLin, coefficient=0.091, P=0.01), *Sphingopyxis* (MaAsLin, coefficient=0.025, P=0.013) and *Roseococcus* (MaAsLin, coefficient=0.011, P=0.012). The relative abundance

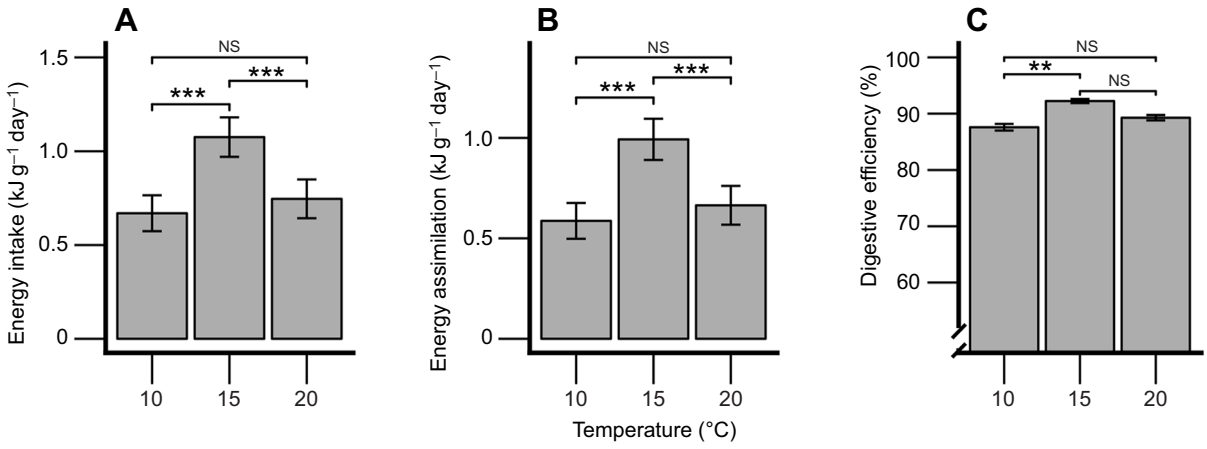

Fig. 1. Digestive performance metrics of salamanders housed at three temperatures. (A) Energy intake per day, (B) energy assimilation and (C) digestive efficiency. Temperature had a significant effect on all measures (LMM, *P<0.05, **P<0.01, ***P<0.001; NS, not significant). Means±s.e.m., n=19 animals per treatment.

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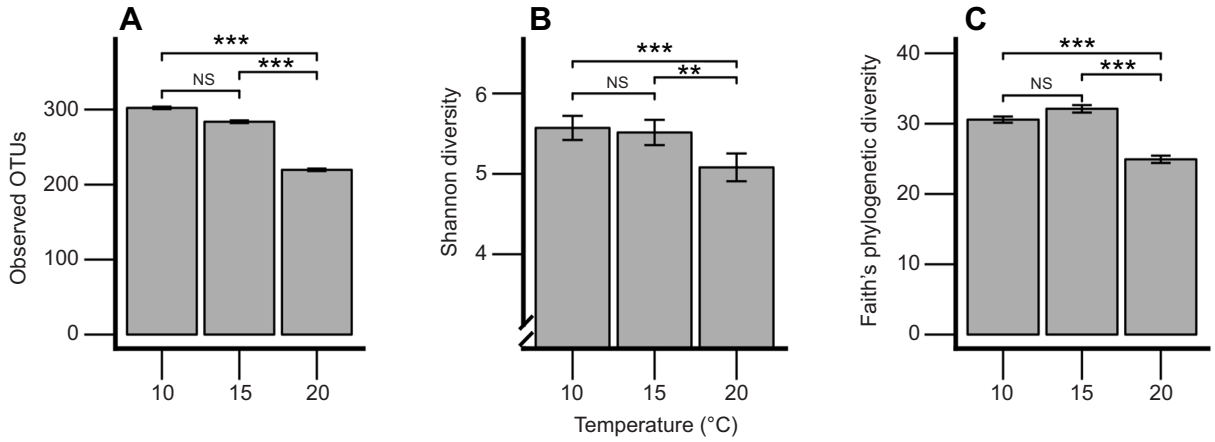

Fig. 2. Diversity metrics of gut microbial communities of salamanders housed at three temperatures. (A) Bacterial operational taxonomic unit (OTU) richness, (B) Shannon diversity and (C) Faith's phylogenetic diversity. Temperature had a significant effect on all metrics (LMM, **P*<0.05, ***P*<0.01, ****P*<0.001; NS, not significant). Means±s.e.m., *n*=19 animals per treatment.

of *Cellvibrio* and *Stenotrophomonas* was significantly enriched at 15°C (Table 1), and although *Sphingopyxis* and *Roseococcus* were not statistically associated with temperature, they were also most abundant at 15°C. No significant associations were observed between the relative abundance of any bacterial genera and salamander digestive efficiency.

DISCUSSION

Our findings demonstrate that environmental temperature significantly impacts digestive performance of salamanders (energy intake, energy assimilation and digestive efficiency), which is consistent with the understanding that temperature has profound impacts on ectotherm physiology (Huey, 1979) and, specifically, digestive performance (McConnachie and Alexander, 2004; Waldschmidt et al., 1986; Wang et al., 2002). Similar to a recent study of energy assimilation in plethodontid salamanders (Clay and Gifford, 2017), we found that the performance of eastern red-backed salamanders is highest at an intermediate temperature (15°C) and is reduced at relatively cool and warm temperatures (10 and 20°C). Although it is possible our results were influenced by the order of temperature trials, which was consistent among individuals, Clay and Gifford (2017) were able to detect species- and population-level differences in thermal optima using a similarly repetitive order of trials. Therefore, we expect that our results are generally reflective of the host's physiological response to its thermal environment rather than other factors. Our results contrast with those of Bobka et al. (1981), who measured energy assimilation of *P. cinereus* fed fruit flies at the same experimental temperatures, and found energy assimilation to be optimal at 10°C, and to decrease significantly with increasing temperature (Bobka et al., 1981). Multiple factors may explain the contradictory findings of these studies, as population-level differences in thermal preference and physiological optima can be due to differences in geographic locality (Clay and Gifford, 2017), habitat (Huey and Bennett, 1987), seasonality (Ortega et al., 2014) and morphological or genetic differentiation between populations (Moreno, 1989). Ectotherm-associated microbiota may exhibit similar local adaptations, as significant variation in community diversity and composition has been observed in spatially separated host populations (Muletz Wolz et al., 2018; Zhang et al., 2018). The degree to which such changes in microbial communities are due to differing host thermal environments or other factors would be interesting to test.

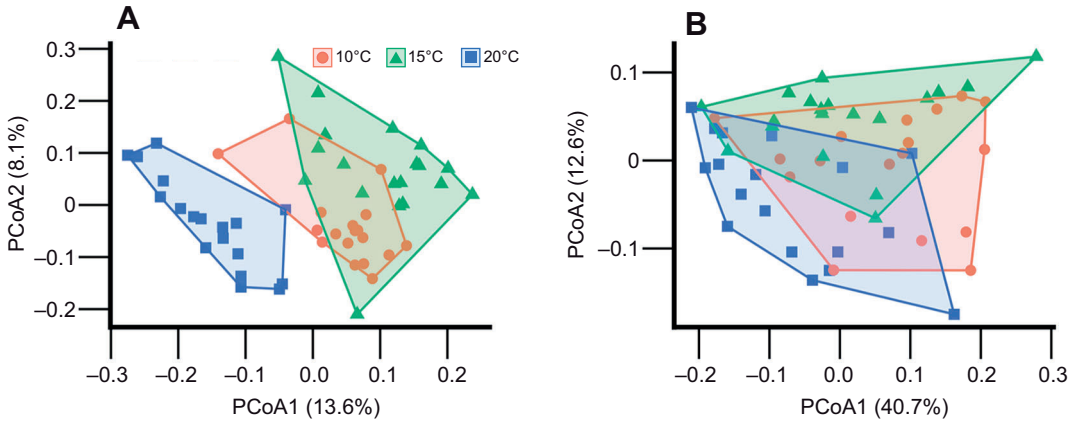

Fig. 3. Principal coordinate analysis (PCoA) of gut microbial communities of salamanders housed at three temperatures. (A) Community membership PCoA was constructed using unweighted UniFrac distance between samples, which considers the presence and absence of bacterial OTUs. (B) Community structure PCoA was constructed using weighted UniFrac distance, which considers both presence and absence of bacterial OTUs and their relative abundance. Temperature had a significant effect on both microbial community membership and community structure (PERMANOVA, community membership *P*<0.001, community structure *P*<0.01). Percentages represent the proportion of total variation explained by each axis. *n*=19 animals per treatment.

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Table 1. Mean (±s.e.m.) relative abundance from MaAsLin and LEfSe outputs for bacterial genera that differed significantly in the gut of salamanders housed at 10, 15 and 20°C

Relationship	Genera	% Abundance 10°C	15°C	20°C	LEfSe *P*	MaAsLin *P*
	*Morganella*	0.04±0.02	0.80±0.20	**1.68±0.44**	<0.001	<0.001
	Delftia	N.O.	0.01±0.01	**0.19±0.06**	<0.001	<0.001
	Agrobacterium	0.06±0.02	0.16±0.03	**0.29±0.06**	N.S.	0.016
	Dysgonomonas	<0.01	0.05±0.04	**0.06±0.20**	<0.001	<0.01
	*Pseudomonas*	**13.67±2.35**	7.34±1.73	1.68±0.55	<0.001	<0.01
	Janthinobacterium	**5.81±1.13**	3.35±0.65	0.14±0.06	<0.001	<0.001
	AF12	**0.43±0.10**	0.19±0.05	0.07±0.03	<0.001	<0.001
	Anaerotruncus	**0.35±0.04**	0.17±0.02	0.13±0.03	<0.001	<0.01
	Paenibacillus	**0.20±0.10**	0.09±0.02	<0.01	NS	<0.01
	Devosia	**0.08±0.02**	0.02±0.01	<0.01	NS	<0.001
	Sedimentibacter	**0.07±0.01**	0.04±0.02	0.02±0.01	NS	0.014
	Wohlfahrtiimonas	**0.03±0.01**	<0.01	<0.01	<0.001	<0.001
	Erysipelothrix	**0.02±0.01**	NO	NO	NS	<0.001
	Rhodobacter	**0.02±0.01**	<0.01	<0.01	<0.01	<0.001
	Rhodococcus	**0.02±0.01**	<0.01	<0.01	NS	0.022
	Bdellovibrio	**0.06±0.01**	0.02±0.01	0.03±0.01	<0.01	NS
	Cytophaga	**0.08±0.05**	<0.01	N.O.	<0.01	NS
	Sphingobacterium	**0.04±0.02**	<0.01	<0.01	<0.001	NS
	*Cellvibrio*	0.80±0.70	**9.81±1.70**	3.47±1.26	<0.001	NS
	Stenotrophomonas	0.01±0.01	**0.58±0.10**	0.10±0.03	<0.001	NS
	Methylotenera	0.09±0.01	**0.12±0.01**	0.04±0.01	<0.001	NS
	Sphingomonas	0.01±0.01	**0.12±0.04**	<0.01	<0.001	NS
	Megamonas	0.06±0.01	**0.07±0.03**	<0.01	NS	<0.001
	Prosthecobacter	0.01±0.01	**0.05±0.01**	<0.01	NS	0.012
	*Citrobacter*	0.22±0.09	0.03±0.02	**0.28±0.27**	NS	<0.01

Genera are organized by the direction of their relationship with temperature. *P*-values are FDR corrected. Highest abundance of each genera is in bold. LEfSe, linear discriminant analysis effect size; MaAsLin, multivariate association with linear models; NO, not observed; NS, not significant.

Additionally, we found that temperature is a significant factor impacting the diversity and composition of salamander gut microbial communities. These results are consistent with the relatively few other studies that have addressed the relationship between temperature and the gut microbiome of ectotherms (Bestion et al., 2017; Kohl and Yahn, 2016). Specifically, we found that increases in environmental temperature are associated with reduced gut microbial diversity and altered bacterial community membership and structure. Underlying these changes is the significant effect of temperature on the relative abundance of 25 bacterial genera. The overall trend was a reduction in relative abundance of these taxa with temperature, with the abundance of 14 genera significantly decreasing as temperature increased.

Notably, we detected a significant decrease in the abundance of the genus *Janthinobacterium* at high temperatures (Table 1). At 10°C, *Janthinobacterium* represented 5.8% of the gut bacterial community (the fifth most abundant bacterial genus), but was diminished to just 0.14% at 20°C. This genus commonly occurs on

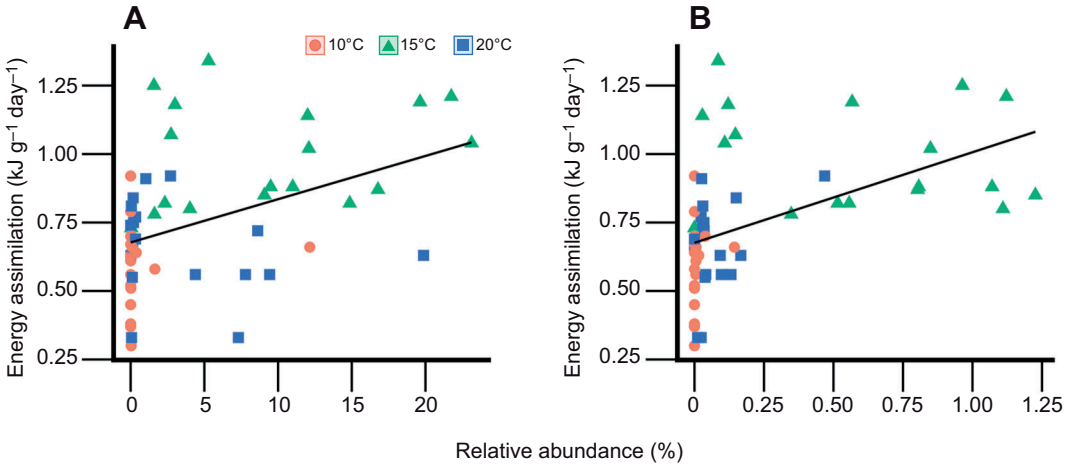

Fig. 4. Correlation between the relative abundance of two bacterial genera in the salamander gut and host energy assimilation at three temperatures. Correlations were calculated in MaAsLin (multivariate association with linear models), which controlled for individual and temperature effects and corrected for multiple comparisons. (A) *Cellvibrio* (coefficient=0.418, *P*=0.01) and (B) *Stenotrophomonas* (coefficient=0.091, *P*=0.01). *n*=19 animals per treatment.

the skin of *P. cinereus* and, when present, has been shown to protect individuals from the globally devastating fungal disease chytridiomycosis, through production of antifungal metabolites (Becker et al., 2009). Additionally, this genus has been successfully used as a probiotic to protect other susceptible species from chytridiomycosis (Harris et al., 2009; Kueneman et al., 2016). Because of its typically high abundance and ability to survive passage through the gastrointestinal tract, it has been suggested that the gut harbors important reservoirs of *Janthinobacterium*, allowing colonization of the skin upon exit from the cloaca (Wiggins et al., 2011). Our results suggest this reservoir may become depleted under warming conditions, potentially hindering the ability of salamanders to resist cutaneous pathogens.

Furthermore, we detected shifts related to temperature in the relative abundance of *Citrobacter* (Table 1), a genus of pathogenic bacteria associated with bacterial dermatosepticemia (red leg syndrome) in amphibians (Densmore and Green, 2007). *Citrobacter* relative abundance exhibited a non-linear relationship with temperature, decreasing dramatically from 10°C to 15°C, and increasing again to its highest relative abundance at 20°C. A previous study detected significant increases in *Citrobacter* abundance in the gut of brown tree frogs when temperature was decreased as a result of induced hibernation in the laboratory (Weng et al., 2016). It is possible that the contrasting effects of temperature on this pathogenic bacterium are a function of a fluctuating host immune response rather than a direct effect of environmental temperature on bacterial growth. Indeed, temperature shifts can depress the amphibian immune system (Raffel et al., 2006). However, the degree to which temperature-induced changes in the microbiome are direct effects, or mediated through the host, remains to be tested.

Lastly, we identified connections between salamander gut microbiota and host digestive performance. Specifically, we found correlations between measurements of energy assimilation and the relative abundance of four bacterial genera: *Cellvibrio*, *Stenotrophomonas*, *Sphingopyxis* and *Roseococcus*. Notably, bacteria within the genus *Cellvibrio* produce numerous carbohydrate-degrading enzymes, including potent chitinases (Forsberg et al., 2016; Monge et al., 2018). Bacteria within *Stenotrophomonas* are also capable of digesting chitin (Ryan et al., 2009). Chitin is the dominant component of arthropod exoskeletons, and the diet of the red-backed salamander consists almost exclusively of arthropods (Maglia, 1996), which suggests a potential explanation for the relationship between the abundance of these bacteria and energy assimilation in these animals. However, explicit testing of this hypothesis through experimental manipulation of gut microbiota is needed.

If *Cellvibrio* and *Stenotrophomonas* do indeed directly facilitate enhanced host energy assimilation, temperature-induced changes in the relative abundance of these genera may have contributed to the relationship observed between temperature and salamander digestive performance. The relative abundance of *Cellvibrio* in the salamander gut was significantly enriched at 15°C, making up almost 10% of the bacterial community (Table 1). *Stenotrophomonas* relative abundance was also significantly increased at 15°C, and although still uncommon (<1% of the community; Table 1), rare microbes serve important functional roles (Jousset et al., 2017). The relationship between the abundance of microbial taxa and salamander digestive performance suggests the possibility that temperature-mediated effects on digestive performance may not only be driven by host physiology but also be influenced by alterations to the gut microbiome. Interestingly, in addition to demonstrating a correlation between altered gut microbial communities and reduced animal survival in lizards, Bestion et al. (2017) detected changes in the functional profile of gut microbiota related to energy metabolism at increased temperatures, providing further support for this hypothesis. Global climate change is already expected to hinder ectotherm digestive performance by decreasing animal foraging rates (Sinervo et al., 2010). We suggest another mechanism by which digestive performance may be reduced under increased temperature regimes: compositional and functional changes to the gut microbiome. More accurate depictions of these impacts may be quantified in the future by incorporating temperature variability into studies, as experiments conducted at constant temperature can actually overestimate measures of digestive performance in ectotherms (Ruppert, 1980).

Overall, our results demonstrate that temperature is a critical factor impacting ectotherm digestive physiology and structuring of gut microbial communities. Fluctuations or increases in environmental temperature, as predicted under current and future global climate change (Parmesan and Yohe, 2003), may negatively impact salamanders as a result of reduced digestive performance, reductions in gut microbial diversity and alterations to microbial community composition, with implications for host health and physiology. Identifying the mechanism by which temperature alters the microbiome, as well as further understanding the direct contribution of microbial alterations to reduced animal performance, will be important directions for future investigation.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.J.N., K.D.K.; Methodology: A.J.N.; Formal analysis: S.S.F., K.D.K.; Investigation: A.J.N.; Resources: A.J.N.; Writing - original draft: S.S.F.; Writing - review & editing: S.S.F., A.J.N., K.D.K.; Visualization: S.S.F.; Supervision: K.D.K.; Funding acquisition: K.D.K.

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Data availability
Sequences are accessible from GenBank (accession number: PRJNA477306).

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