environmental microbiology

Environmental Microbiology (2016) **18**(5), 1561–1565 doi:10.1111/1462-2920.13255

Effects of environmental temperature on the gut microbial communities of tadpoles

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Summary

Numerous studies have investigated the effects of diet, phylogeny and immune status on the gut microbial communities of animals. Most of these studies are conducted on endotherms, especially mammals, which maintain constant body temperature in the face of environmental temperature variability. However, the majority of animals and vertebrates are ectotherms, which often experience fluctuations in body temperature as a result of their environment. While there have been several studies investigating the gut microbial diversity of ectotherms, we lack an understanding of how environmental temperature affects these communities. Here, we used high-throughput sequencing to inventory the gut microbial communities of tadpoles exposed to cool (18°C) or warm (28°C) temperature treatments. We found that temperature significantly impacted the community structure and membership of the tadpole gut. Specifically, tadpoles in the warm treatment exhibited higher abundances of the phylum Planctomycetes and the genus *Mycobacterium*. These results may be due to the direct effects of temperature, or mediated through changes in host physiology. Given that environmental temperatures are expected to increase due to global climate change, understanding the effects of temperature on the diversity and function of gut microbial communities is critical.

Introduction

Recent research has revealed that the communities of microorganisms living within the guts of animals can largely affect the physiology, development and fitness of their animal hosts (McFall-Ngai *et al.*, 2013). Specifically, changes in host physiology can be brought about by differences in the presence or absence of microbial taxa or in their relative abundances (Turnbaugh *et al.*, 2006; Kohl *et al.*, 2014; 2015a). Numerous experimental and comparative studies have investigated how factors such as diet, infection status or host phylogeny may impact gut microbial communities (Ley *et al.*, 2008; David *et al.*, 2014; Moeller *et al.*, 2014; McKenney *et al.*, 2015). However, we still lack an understanding of how basic exogenous, environmental factors may impact the gut community structure of animals.

One crucial factor, environmental temperature, has been understudied in relation to gut microbial community structure. To date, most gut microbiome studies have been conducted on mammals, especially laboratory rodents and humans, which maintain consistent body temperatures in the face of environmental variation. While hibernating mammals exhibit changes in gut microbial community structures, these changes seem to be driven by low nutrient availability for microbes rather than by host body temperature (Carey *et al.*, 2013; Stevenson *et al.*, 2014). Conversely, roughly 99% of animal species and 75% of vertebrate species are considered ectotherms (Hammond, 1992), which experience variations in body temperatures in response to their environments. In addition, temperature is known to influence the community structures of environmental microbiomes such as soil or sediment (Yergeau *et al.*, 2012; Sharp *et al.*, 2014). Further, culture-based studies have demonstrated that environmental temperature does alter the gut community structure of ectotherms such as frogs (Carr *et al.*, 1976; Gossling *et al.*, 1982) and fish (Lésel and Péringer, 1981; Sugita *et al.*, 1989). However, the effect of temperature on gut microbial communities has never been investigated using high-throughput sequencing techniques.

Amphibians represent a diverse class of animals that is experiencing rapid population declines and species extinctions (Beebee and Griffiths, 2005). Given the large effects that microbial communities have on animal performance and fitness (McFall-Ngai *et al.*, 2013), it is crucial to understand host-microbe interactions in this

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group. The gut microbial ecology of amphibians has only recently been studied using high-throughput sequencing methods demonstrating that the microbiota is largely restructured through metamorphosis (Kohl *et al.*, 2013), and that environmental pollutants can cause persistent alteration of the microbiota (Kohl *et al.*, 2015b). Given the threat that climate change represents to amphibian populations (Beebee and Griffiths, 2005), it is important to study the effects of temperature on the amphibian gut microbiota.

Here, we conducted a laboratory experiment to investigate the effects of environmental temperature on the gut microbial communities of tadpoles of the northern leopard frog (*Lithobates pipiens*, also known as *Rana pipiens*). We raised tadpoles under either cool (18°C) or warm (28°C) temperature treatments. All animals were housed and fed identically, details of which can be found in the supporting information. Gut contents were collected from tadpoles at similar developmental time points (Gosner stage = 38.3 $\pm$ 0.31; no difference between treatments: two sample $t(30) = 0.20$; $P = 0.84$). Microbial communities were inventoried by sequencing the 16S rRNA gene from gut contents. Details regarding sequencing and data analysis can be found in the supporting information. We compared measurements of alpha diversity, community membership and structure, and abundances of microbial taxa. We predicted that temperature treatments would significantly influence the gut microbial community composition of tadpoles.

Results and discussion

We obtained an average of 35,557 $\pm$ 1225 sequences per sample. There were no differences in sequencing efforts between treatments or across replicate tanks (Nested ANOVA; temperature: $P = 0.50$; Tanks (nested within temperature): $P = 0.16$). Measurements of alpha diversity of the gut microbiome (Shannon Index, Faith's Phylogenetic Diversity, evenness, or number of observed OTUs) were not significantly different between tadpoles reared at cool and warm temperatures ($P > 0.1$ for all).

Temperature significantly impacted microbial community membership (presence and absence of microbial lineages), and the tank of origin had a near-significant effect (Fig. 1; nonparametric ANOVA: temperature: $F = 3.76$, $P = 0.002$; Tank [nested within temperature]: $F = 1.12$, $P = 0.08$). Similarly, we observed significant effects of both temperature and tank on microbial community structure (which takes relative abundance into account; Fig. 1; temperature: $F = 16.46$, $P = 0.003$; tank [nested within temperature]: $F = 2.22$, $P = 0.007$). The significant effects of tank may be due to distinct microbial communities in the water of each tank, or perhaps due to slight localized variation in temperature, light, oxygenation, or other variables in each tank. The contributions of varying environmental microbial communities (such as in the water) to the gut microbiota of tadpoles demands further research. Interestingly, the tadpoles from warmer temperatures seemed to exhibit higher inter-individual variation, especially in terms of community structure (Fig. 1). These results are consistent with other

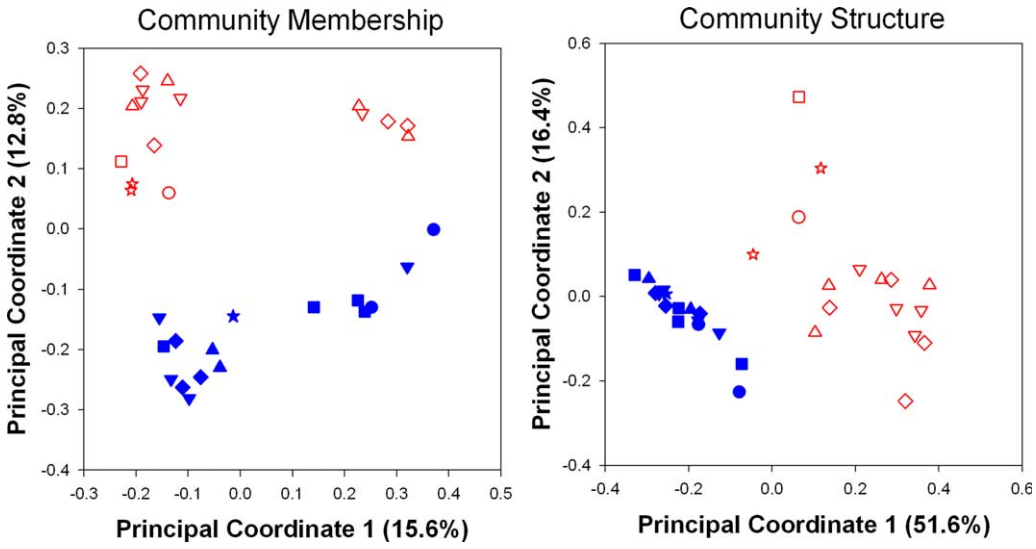

Fig. 1. Principal coordinate analysis of gut communities from tadpoles reared at cool (18°C; closed symbols) or warm (28°C; open symbols) temperatures ($N = 16$ for each temperature treatment). Different shapes of symbols correspond to different replicate tanks. Community membership utilized unweighted Unifrac distances (which investigates the presence and absence of bacterial lineages). Community structure uses weighted Unifrac distances (which takes relative abundances of bacterial lineages into account). See supporting information for details on sequence and data analysis.

studies demonstrating that higher temperatures increase heterogeneity of microbial communities among individuals (Erwin *et al.*, 2012; Lokmer and Wegner, 2015).

Comparisons of relative abundances of microbial taxa revealed a number of phyla and genera that were differentially abundant. We identified four microbial phyla and 20 genera that exhibited differential abundances between the two treatments (Fig. 2 and Table 1). Most notably, the phylum Planctomycetes composed almost 30% of the community of tadpoles reared at the warm temperature, but less than 0.5% of the gut microbiome of tadpoles in the cool treatment. The function if this phylum is poorly understood, and usually composes <3% of the gut microbiota of most animals (Rawls *et al.*, 2006; Ley *et al.*, 2008). However, this phylum is dominant in the guts of soil-feeding termites (Köhler *et al.*, 2008). Our results are consistent with a previous study demonstrating that elevated environmental temperatures increase the abundance of Planctomycetes in oyster gill tissue (Wegner *et al.*, 2013). Further, a study comparing the stomach microbial community composition

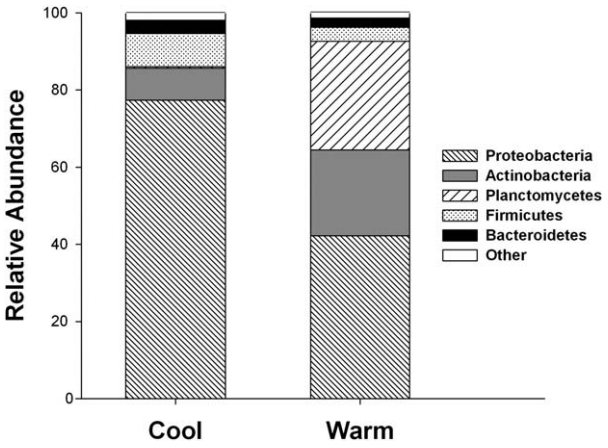

Fig. 2. Relative abundances of bacterial phyla in the guts of tadpoles reared at cool (18°C) or warm (28°C) temperatures ($N = 16$ for each temperature treatment).

Table 1. Relative abundances and statistics for microbial phyla and genera that differed significantly in abundance between tadpoles reared at cool (18°C) or warm (28°C) temperatures. Higher abundances for each taxonomic group are bolded. Asterisks denote a significant effect of tank (nested within treatment) in the model. N.D. = not detected.

	Cool % abundance	Warm % abundance	*F*	FDR-corrected *P*
Phyla				
Firmicutes	**8.66 ± 1.81**	3.55 ± 1.10	9.53	0.05
Proteobacteria	**77.36 ± 3.90**	42.24 ± 4.10	21.55	0.002
Planctomycetes	0.39 ± 0.07	**28.22 ± 2.33**	299.04	< 0.001
TM6	0.08 ± 0.03	**0.19 ± 0.03**	9.71	0.05
Genera (split by class)				
Alphaproteobacteria				
Aminobacter	**0.77 ± 0.19**	0.05 ± 0.02	21.16	0.002
Methylobacterium	**0.05 ± 0.02**	N.D.	9.34	0.04
Devosia	< 0.01	**0.05 ± 0.01**	38.82	< 0.001
Mesorhizobium	< 0.01	**0.02 ± 0.01**	30.60	< 0.001
Pedomicrobium	N.D.	**0.02 ± 0.01**	75.61	< 0.001
Rhodoplanes	0.01 ± 0.01	**0.63 ± 0.07**	164.71	< 0.001
Gammaproteobacteria				
*Aquicella**	N.D.	**0.30 ± 0.16**	213.69	< 0.001
*Rheinheimera**	N.D.	**0.05 ± 0.04**	42.31	< 0.001
Shewanella	N.D.	**0.02 ± 0.01**	49.41	< 0.001
Actinobacteria				
Frigoribacterium	**0.01 ± 0.01**	N.D.	12.73	0.014
Micrococcus	**0.02± 0.01**	0.01 ± 0.01	17.49	0.004
Gordonia	0.01 ± 0.01	**0.87 ± 0.23**	28.47	< 0.001
Mycetocola	<0.01	**0.01 ± 0.01**	16.68	0.005
*Mycobacterium**	0.21 ± 0.11	**20.26 ± 3.50**	99.04	< 0.001
Rathayibacter	N.D.	**<0.01**	11.24	0.02
*Tsukamurella**	N.D.	**0.02 ± 0.01**	28.26	< 0.001
Chlamydiia				
Parachlamydia	N.D.	**0.03 ± 0.01**	43.34	< 0.001
Clostridia				
Dorea	**0.25 ± 0.05**	0.01 ± 0.01	18.83	0.003
Cytophagia				
*Runella**	N.D.	**0.01 ± 0.01**	36.12	< 0.001
Planctomycetia				
Planctomyces	0.07 ± 0.04	**15.34 ± 1.25**	313.48	< 0.001

between populations of oysters found high abundances of Planctomycetes in one population (>20%) and low abundance (~2%) in another (King *et al.*, 2012). Our results raise the question of whether environmental temperature may have contributed to this population difference.

Additionally, tadpoles reared at the warm temperature exhibited a higher abundance of the genus *Mycobacterium* in their guts (Table 1). This genus grows optimally at 31–35°C and is largely considered a gut pathogen, including for ectothermic hosts (Clark and Shepard, 1963; Clayton, 2005). Interestingly, infection and death as a result of *Mycobacterium* is more severe in ectotherms held at warmer temperatures compared to those at cooler temperatures (Clark and Shepard, 1963). Although we cannot confirm that the *Mycobacterium* in our experiment were pathogenic, it would be interesting to further study interactions between environmental temperature and pathogenic gut infections of *Mycobacterium* in tadpoles.

Tadpoles held at the cool temperature were enriched in much fewer taxa compared to those reared at the warm temperature. Tadpoles reared at the cool temperature harbored gut communities with higher abundances of Firmicutes and Proteobacteria. Additionally, the guts of tadpoles reared in the cool temperature had 15× higher abundances of *Aminobacter* and 25× higher abundances of *Dorea* compared to tadpoles reared at the warm temperature.

Overall, temperature had a demonstrable effect on the gut microbial communities of tadpoles. Future studies could elucidate whether these changes are the direct results of contrasting temperatures, or whether they may be mediated through alterations in host physiology. Some of our evidence suggests that temperature may directly be mediating this effect. For example, the warm-adapted genus *Mycobacterium* exhibited higher abundances in tadpoles reared at warm temperatures. Conversely, temperature is known to alter many aspects of host physiology in ectotherms, such as immune function (Maniero and Carey, 1997) or gut transit time (van Marken and Lichtenbelt, 1992), both of which can influence microbial community structure (Hooper *et al.*, 2012; Kashyap *et al.*, 2013). To remove host-induced effects, other researchers have conducted *in vitro* incubations of gut microbial communities exposed to different treatments to look for rapid changes in microbial community structure or function (Maurice *et al.*, 2013). These techniques could be used to disentangle whether temperature and/or host-mediated effects underlie our findings.

Our results are timely given that global temperatures are predicted to increase under models of climate change, which represents a substantial threat to amphibian populations (Beebee and Griffiths, 2005). Gut microbial communities provide a number of functions for their hosts, especially in terms of immunity and nutrition (Hooper *et al.*, 2012; Semova *et al.*, 2012). Understanding the effects of environmental temperatures on the functions provided to ectotherms by their gut microbes may be important for conserving these species.

Acknowledgements

We would like to thank Cherry Tsai-Brown and Logan Laatsch for assistance with animal husbandry and dissections. Animal husbandry and sample collection were sponsored by the University of Wisconsin Sea Grant Institute (grant NA16RG2257, project R/EH-2), the National Oceanic and Atmospheric Administration, and the U.S. Department of Commerce (grant NA10OAR4170070, project R/HCE-14). Microbial inventories were supported by the National Science Foundation (DEB 1210094 and DBI 1400456 to K.D.K.).

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