RESEARCH ARTICLE

Unique and shared responses of the gut microbiota to prolonged fasting: a comparative study across five classes of vertebrate hosts

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Abstract

Many animals face unpredictable food sources and periods of prolonged fasting, which likely present significant challenges to gut microorganisms. While several studies have demonstrated that fasting impacts the gut microbiota, experiments have not been carried out in a comparative context. We used *16S rRNA* gene sequencing to document changes in colonic and cecal microbiomes of animals representing five classes of vertebrates at four time points through prolonged fasting: tilapia, toads, geckos, quail, and mice. We found differences in the starvation-induced changes in the microbiome across host species and across gut regions. Microbial phylogenetic diversity increased as a result of fasting in the colons of fish, toads, and mice, while quail exhibited a decrease in diversity; geckos exhibited no change. Microbial diversity in the cecum decreased in fish and exhibited no change in mice. Alterations in relative abundances of microbial taxa varied across hosts. Fish exhibited the most significant changes due to fasting, while geckos maintained a stable community over 28 days of fasting. We uncovered several shared responses of the microbiota across hosts. For example, all tetrapods exhibited decreases in the abundances of *Coprobacillus* and *Ruminococcus* in response to fasting. We also discuss host-mediated physiological mechanisms that may underlie these community changes.

Introduction

Gut microorganisms provide a number of nutritional functions to their hosts, such as fermenting fiber and synthesizing essential amino acids (Stevens & Hume, 2004). In return, hosts provide symbiotic microorganisms with a stable, protected, and nutrient-rich environment. The importance of these symbiotic relationships to the success of animals is only recently being appreciated by ecologists and evolutionary biologists (McFall-Ngai *et al.*, 2013). However, environmental challenges can alter these relationships and impact host performance or health (Hawrelak & Myers, 2004).

Food limitation, or fasting, is a physiological challenge faced by many animals that may affect their gut microbiota (McCue, 2012). First, fasting represents an 'energy crisis' for microorganisms due to a reduction in the availability of nutrients (McCue, 2012). Second, many animals reduce the size of their intestines in response to fasting (Starck, 2003; Karasov *et al.*, 2004; Zaldúa & Naya, 2014), thereby generating a 'housing crisis' for the microbiota. These changes likely result in alterations of microbial diversity and relative abundances of microbial taxa. For example, early studies using culture-based approaches found that fasting reduced bacterial density in fish (Margolis, 1953) and altered microbial communities in rats (Morishita & Miyaki, 1979); however, such methods are likely to greatly underestimate microbial diversity (Rappe & Giovannoni, 2003). More recently, studies using sequence-based approaches have documented differences in microbial community structure between fed and fasted seabass (Xia *et al.*, 2014), pythons (Costello

et al., 2010), hamsters (Sonoyama *et al.*, 2009) and laboratory mice (Crawford *et al.*, 2009).

There may be universal responses of the gut microbiota to fasting. The absence of free nutrients or physiological responses of hosts to fasting may exert similar selection on resident microorganisms and cause complementary shifts in diversity and abundances of taxa. For example, the microbial genus *Akkermansia* consumes host-produced mucus (Derrien *et al.*, 2004) and increases in relative abundance in response to fasting in hamsters (Sonoyama *et al.*, 2009) and pythons (Costello *et al.*, 2010). To date, there has not been a comprehensive, comparative study investigating the effects of fasting on gut microbial communities of different host taxa.

Understanding the responses of gut microorganisms to fasting is imperative, given that the gut microbiota promote host survival during periods of fasting. For example, mice and rats lacking gut microbiota (germ-free) incur higher mortality rates than conventional hosts when starved, despite similar rates of body mass loss (Einheber & Carter, 1966; Tennant *et al.*, 1968). Likewise, germ-free chickens are less tolerant of starvation than conventional chickens (Hiro-Omi *et al.*, 1992). These outcomes are due to gut microorganisms aiding in the supply of alternative energy sources (e.g. volatile fatty acids and ketone bodies) or nitrogen recycling when hosts are faced with fasting and starvation (Dintzis & Hastings, 1953; Einheber & Carter, 1966; Crawford *et al.*, 2009). Further, maintenance of other functions provided by the gut microbiota, such as immune development and resistance to pathogens (Endt *et al.*, 2010), may be important for hosts to survive periods of fasting.

Here, we compared the effects of fasting on the microbial communities of hosts from five vertebrate classes: Osteichthyes, Amphibia, Reptilia, Aves, and Mammalia. We investigated changes in the microbial communities of two gut chambers: the colon and the cecum. The colon contains a dense microbial communities across vertebrate hosts, with $> 10^9$ bacterial cells per gram of contents (Stevens & Hume, 2004). We also compared microbial communities within cecal chambers. The cecum is a blind pouch of the gut near the beginning of the large intestine (Stevens & Hume, 2004). Not all vertebrate hosts maintain a cecum, but those that do house dense microbial communities of 10^6–10^{10} bacterial cells per gram of contents (Stevens & Hume, 2004). We conducted inventories of microbial communities in the colons and ceca of animals in a nourished state and at various time points during prolonged fasting. We also compared the overall diversity and the relative abundances of microbial taxa across time points to explore the possibility of universal responses of the microbiota to prolonged fasting.

Materials and methods

Animals

All experiments involving vertebrates were conducted at St. Mary's University (StMU) under the auspices of the StMU Institutional Animal Care and Use Committee (StMU #2010-4; 2011-5, 2012-1; 2012-2; 2012-3). Laboratory temperature (28 ± 1 °C) and photoperiod (14L : 10D) remained constant during the experiments.

Nile tilapia, *Oreochromis niloticus* (males and females; fry *c.* 1.5 cm total length; TL), were obtained from a commercial breeder (Tilapia Depot, Saint Augustine, FL) and raised on a standard pelleted tilapia diet for approximately 4 months in a 400-gallon aquarium until reaching *c.* 20 cm TL. Wild-captured adult and subadult southern toads, *Anaxyrus terrestris* (males and females; 11–32 g), were purchased from a commercial distributor (Gulf Hammock Herps, Dade City, FL) and maintained for 60 days on a diet of live crickets (Fluker Farms, Port Allen, LA). Captive-bred, adult leopard geckos, *Eublepharis macularius* (males and females; 50–60 g), were obtained from Leopardgecko.com (Boerne, TX) and maintained in the laboratory for approximately 9 months on a diet of live tenebrionid beetle larvae raised in the laboratory. Geckos were given supplementary heat lamps to permit voluntary basking. Japanese quail, *Coturnix coturnix*, hatchlings (males and females; 2 days old) were obtained from a commercial breeder (Diamond H Ranch, Bandera, TX) and raised in the laboratory for approximately 3 months on a standard quail diet (Nature Wise; Nutrena) until adulthood (*c.* 250 g). Weanling mice, *Mus musculus* (males; *c.* 10–12 g), were obtained from a commercial breeder (Alamo Aquatic Pets, San Antonio, TX) and raised in the laboratory on a standard rodent diet (Teklad, Harlan Laboratories) for approximately 2 months until adulthood (*c.* 25–30 g).

Populations of each host species were housed communally to maximize the homogeneity of the gut microbiota at the start of the fasting period (Ridaura *et al.*, 2013). To ensure that animals were in a postabsorptive condition, food was removed from the toads and geckos 24 h prior to the first sampling time point (e.g. fasting day 0; Table 1). Similarly, food was removed from the tilapia, quail, and mice 6 h prior to the first sampling time point (e.g. fasting day 0). Water was available at all times to fasting animals. Sample sizes are presented in Table 1.

At predetermined time points, animals were euthanized (Table 1) in accordance with standard guidelines for euthanasia (AVMA, 2013). The 'late-fasting' time point was determined using preliminary data with the goal of achieving a 20–30% loss of body mass (Toth & Gardiner,

Table 1. Length of time without food (in days) when samples were collected for various fasting stages

Host	Nourished	Early-fasting	Mid-fasting	Late-fasting
Tilapia	0 (7 – 7)	7 (7 – 6)	14 (4 – 6)	21 (5 – 6)
Toad	0 (6 – NA)	7 (5 – NA)	14 (6 – NA)	21 (6 – NA)
Gecko	0 (7 – NA)	7 (5 – NA)	14 (6 – NA)	28 (7 – NA)
Quail	0 (7 – 7)	2 (7 – 7)	4 (7 – 7)	7 (4 – 6)
Mouse	0 (7 – 7)	1 (6 – 6)	2 (5 – 6)	3 (8 – 8)

Numbers in parentheses represent sample sizes (Number of colonic samples – number of cecal samples). Hosts that do not have ceca show NA (not applicable) for cecal sample size.

2000; Rowland, 2007). The fasting time frame was divided in thirds to determine the 'early-fasting' and 'mid-fasting' time points. Within ten minutes of euthanasia, the gastrointestinal tract from the distal esophagus to the rectum was removed intact. A central section (approximately 1–2 cm in length) of each colon was removed from all host species and promptly frozen. For animals that had ceca (tilapia, quail, mice), the whole cecum was removed and promptly frozen. Necropsy tools were sterilized between samples. Samples were stored for up to 4 weeks at −80 °C.

Microbial inventories

All samples were transported on dry ice to the University of Utah for DNA extraction. The gut chambers of some animals appeared to be completely empty. Therefore, we extracted DNA from whole organ samples (cecum or colon), and not solely gut contents, to inventory the resident microbiota. Samples from all time points were thawed on ice, and sterilized tools were used to cut open the cecum or the colonic cylinder and expose contents and/or the mucosal surface. This strip of tissue was further cut into several pieces to expose deep mucosal layers. We then extracted whole DNA using a QIAamp DNA Stool Mini Kit (Qiagen, Germantown, MD). Extracted DNA was sent to Argonne National Laboratories for sequencing. Bacterial inventories were conducted by amplifying the V4 region of the *16S rRNA* gene using primers 515F and 806R and paired-end sequencing on an Illumina MiSeq platform (Caporaso *et al.*, 2012).

Sequences were analyzed using the QIIME software package (Caporaso *et al.*, 2010). Sequences underwent standard quality control and were split into libraries using default parameters in QIIME. The sequences were grouped into *de novo* operational taxonomic units (OTUs) using UCLUST (Edgar, 2010) with a minimum sequence identity of 97%. The most abundant sequences within each OTU were designated as a 'representative sequence' and then aligned against Greengenes 13_5 (DeSantis *et al.*, 2006) using PYNAST (Caporaso *et al.*, 2009) with default parameters set by QIIME. Chimeric sequences were detected and removed using CHIMERASLAYER (Haas *et al.*, 2011). A PH Lane mask supplied by QIIME was used to remove hypervariable regions from aligned sequences. FASTTREE (Price *et al.*, 2009) was used to create a phylogenetic tree of representative sequences. OTUs were classified using the Ribosomal Database Project classifier with the standard minimum support threshold of 80% (Wang *et al.*, 2007). Singleton OTUs and sequences identified as chloroplasts or mitochondria were removed from analysis.

We calculated Faith's phylogenetic diversity (Faith, 1992), which measures the cumulative branch lengths from randomly sampling OTUs from each sample. For each sample, we calculated the mean of 20 iterations for a subsampling of 1900 sequences. We then compared phylogenetic diversity using ANOVAs within each host species. Relative abundances of microbial phyla and genera were normalized using variance stabilizing transformation of arcsin (abundance$^{0.5}$); (Shchipkova *et al.*, 2010; Kumar *et al.*, 2012). Transformed abundances were compared using ANOVAs within each host species, using the false discovery rate correction.

Shared responses of the microbiota

We conducted a series of statistical tests to identify shared microbial responses to host fasting. We investigated all microbial genera simultaneously using only the 'fed' and 'late-fasting' time points. We used the Response Screening function in the statistical package JMP 11 to conduct numerous ANOVAs simultaneously. The independent variables were host species and fasting time point, as well as the interaction term. The dependent variables were the transformed relative abundances of all detected microbial genera. We ran several models using different combinations of host species. For the colon, we tested for universal responses in vertebrates (all host species), tetrapods (toads, geckos, quail, and mice), and amniotes (geckos, quail, and mice). For the cecum, we tested for responses shared by tilapia, quail, and mice, as well as shared between quail and mice. We corrected *P*-values using the false discovery rate correction. We designated several criteria that a microbial genus had to meet to be considered a universal response: (1) The *P*-values for the 'fasting time point' variable had to be < 0.05 after the false discovery rate correction, (2) the genus had to be detected in at least 2 individuals of each host species, and (3) the direction of change in relative abundance between fed and late-fasting had to be shared by all host species.

Sequence deposition

All sequences were deposited in the Sequence Read Archive of NCBI under accession number PRJNA244306.

Results

Colonic samples

Our sequencing resulted in an average of 35 217 ± 1364 high-quality sequences per sample for the colonic samples. Sequencing was similar across species with the exception of the quail samples, which resulted in roughly a third as many sequences per sample (13 231 ± 1960). There were no differences in number of sequences across time points. It is worth noting that we controlled for number of sequences by rarefying each inventory to an even sequencing depth.

Fasting differentially altered the phylogenetic diversity of microbial communities in the colon across host taxa. Tilapia exhibited a continual increase in phylogenetic diversity, with diversity being 3× higher in late-fasted fish compared with nourished fish (Fig. 1). In toads, microbial diversity was 33% higher in early-fasted individuals and 51% higher in late-fasted individuals compared with nourished toads. There were no differences in phylogenetic diversity among the fasting geckos. Quail exhibited a variable response, with late-fasted individuals exhibiting 37% less phylogenetic diversity compared with nourished individuals (Fig. 1). Last, fasted mice exhibited 15–22% higher phylogenetic diversity compared with nourished mice (Fig. 1). These trends were also reflected in estimated measurements of species richness (Fig. S1, Supporting information).

The changes in diversity were driven by differential changes in abundances of microbial taxa across host species. For example, in tilapia, five bacterial phyla exhibited significant differences depending on feeding state (Fig. 2). The most drastic of the responses was an increase followed by a decrease in the abundance of Fusobacteria. Additionally, the relative abundances of Proteobacteria were 5× higher in late-fasting tilapia compared with nourished tilapia (Fig 2). Late-fasting toads exhibited a 4× higher relative abundance of Bacteroidetes compared with nourished toads (Fig. 2). Geckos and quails did not demonstrate any significant changes in the relative abundances of specific microbial phyla. Mice exhibited an increase in the abundance of Bacteroidetes and a decrease in the abundances of Tenericutes during early to late fasting. A number of microbial genera also exhibited significant changes in abundance due to prolonged fasting, especially in tilapia (Table 2). However, toads and quail did not demonstrate any changes in the relative abundances of identifiable microbial genera. Abundances for all phyla and genera detected can be found in Data S1.

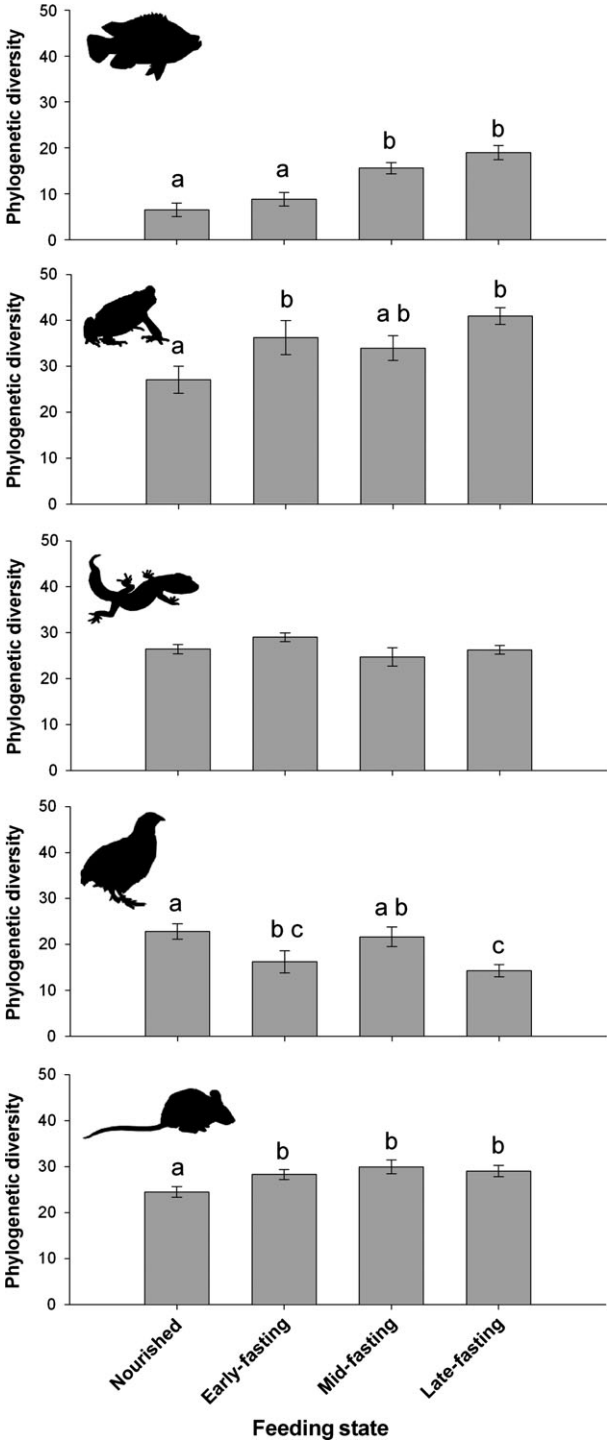

Fig. 1. Phylogenetic diversity of the colonic microbial communities of various hosts at different time points over prolonged fasting. Values were calculated using 20 rarefactions of 1900 sequences per sample. Different letters above bars indicate significant differences. Letters only compare data within a host species. Bars represent means ± 1 SEM.

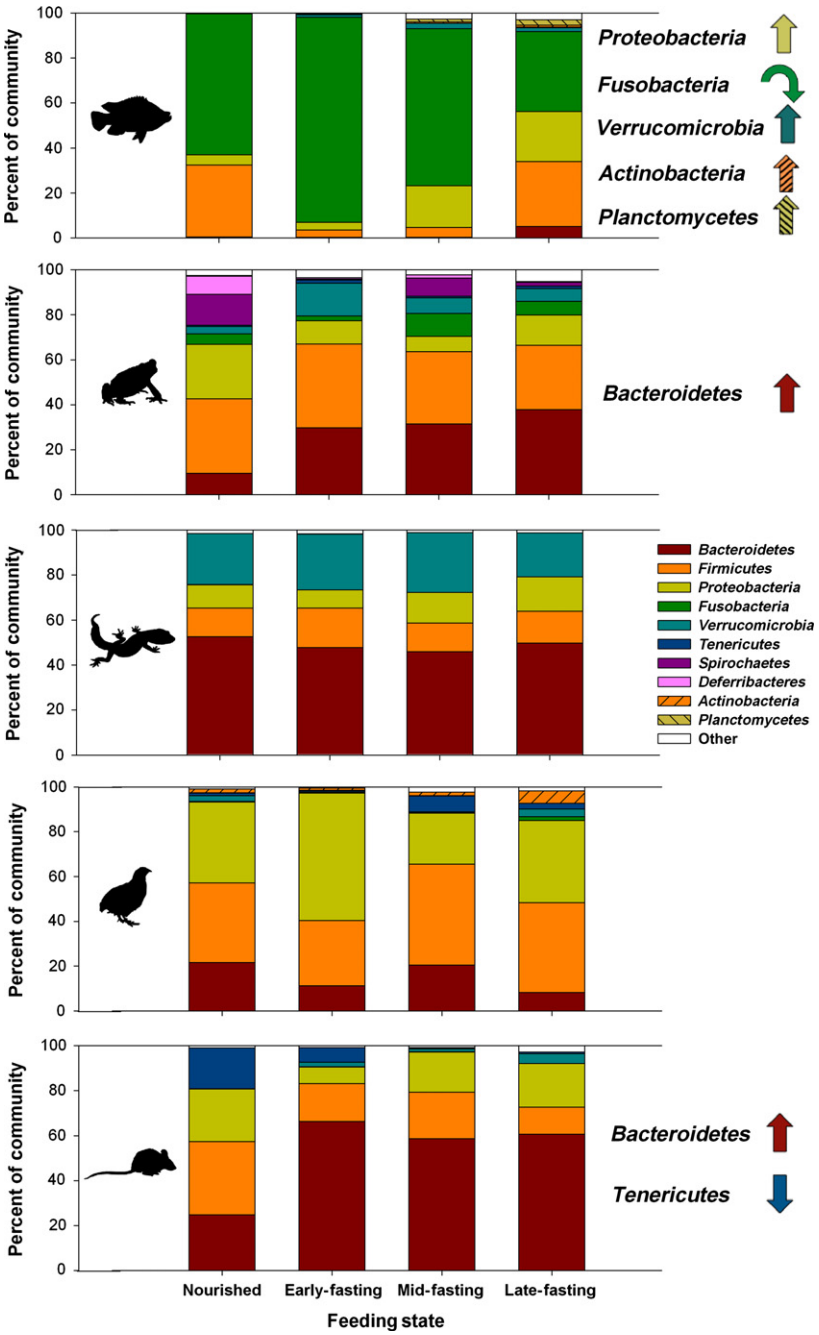

Fig. 2. Relative abundances of microbial phyla in the colonic microbial communities of various hosts at different time points over prolonged fasting. Colored arrows represent significant changes in the abundances of microbial taxa as a result of fasting.

Cecum samples

The number of sequences from cecal samples was similar between quail and mice (56 774 ± 1905 sequences), while sequencing of tilapia samples resulted in a lower number (20 116 ± 2930). There were no differences in number of sequences across time points within a species.

Responses of cecal diversity to fasting varied across host taxa, and in several cases, the changes in diversity did not parallel those observed in the colon. Tilapia exhibited roughly a 40–52% decrease in phylogenetic diversity between fed and fasted states (Fig. 3). Conversely, quail demonstrated a 13% increase in phylogenetic diversity between fed and early-fasting state, but

Table 2. Significant changes in microbial genera of colonic samples

Microbial genera	*P*-value	Direction of change from nourished animals
Tilapia		
Aquicella	0.035	↑
Citrobacter	< 0.0001	↑
Gemmata	0.039	↑
Hyphomicrobium	0.004	↑
Legionella	0.002	↑
Mycobacterium	0.003	↑
Nocardia	0.004	↑
Planctomyces	0.001	↑
Pseudomonas	0.035	↑
Rhodobacter	< 0.0001	↑
Rhodoplanes	0.019	↑
Roseomonas	0.037	↑
Turicibacter	0.035	↓
Toad		
Methanocorpusculum	0.039	↑
Gecko		
Brevundimonas	0.029	↑

P-values have been corrected using the false discovery rate correction. No differences were found in quail or mice.

returned to normal diversity levels in the mid- and late-fasting stages (Fig. 3). No changes in phylogenetic diversity were observed in the ceca of mice (Fig. 3). These trends were also reflected in estimated measurements of species richness (Fig. S2).

The relative abundances of microbial taxa also varied as a result of prolonged fasting. At the level of bacterial phyla, we did not detect any significant changes in tilapia, quail, or mice (Fig. 4). At the genus level, tilapia exhibited significant decreases in the relative abundances of 21 microbial genera (Table S1; Data S1). Quail exhibited decreases in the abundances of *Prevotella* ($P < 0.0001$) and *Faecalibacterium* ($P = 0.028$). Additionally, the abundance of *Methanobrevibacter* was significantly higher in early-fasting individuals ($P = 0.004$), but did not differ across other time points. Mice did not demonstrate any changes in the abundances of bacterial genera. Abundances for all detectable phyla and genera can be found in Data S1.

Shared responses of the microbiota

In the colon, we were unable to identify any microbial genera that met our criteria for shared responses across all five host species. Across tetrapods (toads, geckos, quail, mice), there were shared responses such that these animals exhibited decreases in the abundances of *Coprobacillus* ($P = 0.008$) and *Ruminococcus* ($P = 0.012$). These decreases remained significant when investigating the colonic responses across amniotes (geckos, quail, mice; *Coprococcus:* $P = 0.015$; *Ruminococcus*: $P = 0.036$). We did not find any additional shared responses across amniotes.

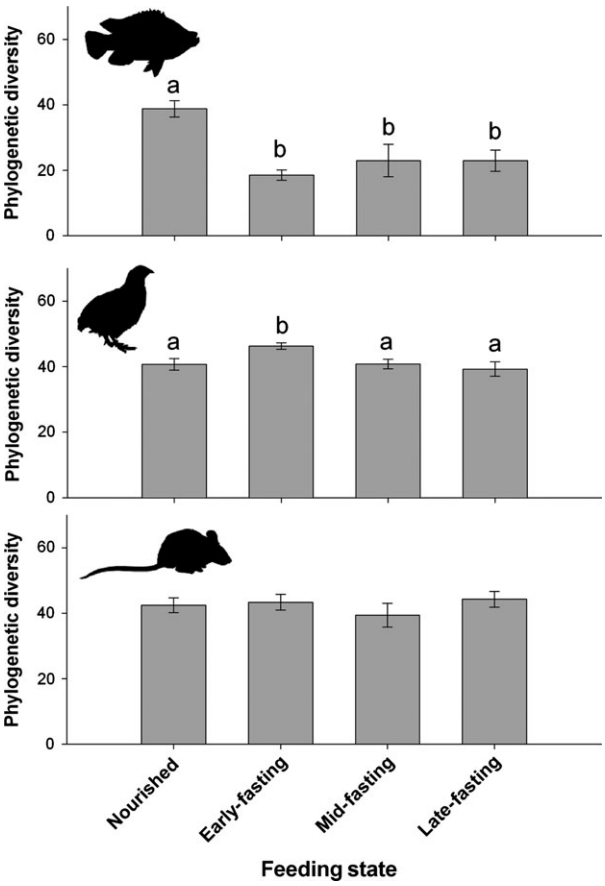

Fig. 3. Phylogenetic diversity of the cecal microbial communities of various hosts at different time points over prolonged fasting. Values were calculated using 20 rarefactions of 1900 sequences per sample. Different letters above bars indicate significant differences. Letters only compare data within a host species. Bars represent means ± 1 SEM.

We uncovered several shared responses in the cecal samples. Tilapia, quail, and mice all exhibited an increase in the abundance of *Oscillospira* and decreases in the abundances of *Prevotella* and *Lactobacillus* after long-term fasting (Table 3). Additionally, we found a number of responses that were shared between the ceca of quail and mice (Table 3).

Discussion

We compared responses of the gut microbiota to fasting across several vertebrate hosts. The responses of the microbiota were largely idiosyncratic across hosts and gut chambers, an outcome that underscores the complex nature of the microbiome. However, we did uncover several shared responses of the microbiota across tetrapod hosts. Below, we discuss the mechanisms and implications that may be associated with these differential responses. We limit our

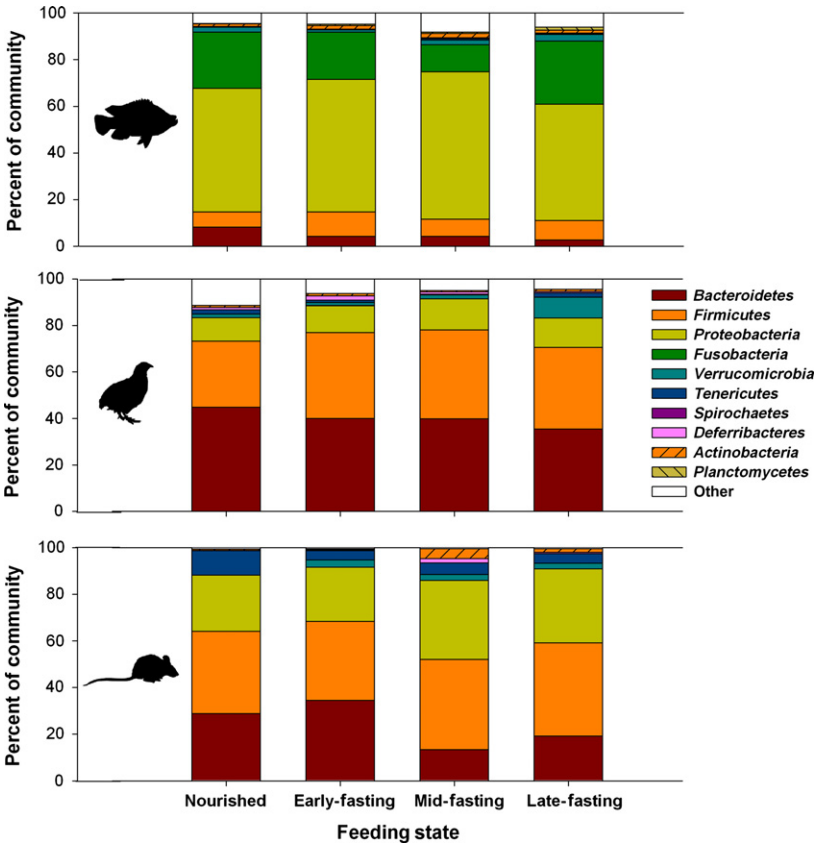

Fig. 4. Relative abundances of microbial phyla in the cecal microbial communities of various hosts at different time points over prolonged fasting. No differences were detected at the phylum level.

Table 3. Shared responses of microbial taxa to prolonged fasting in cecal communities

Shared by tilapia, quail, and mice			Shared by quail and mice		
Genus	Change	*P*-value	Genus	Change	*P*-value
Lactobacillus	↓	< 0.0001	*Dehalobacterium*	↑	0.009
Oscillospira	↑	0.0003	*Lactobacillus*	↓	0.0004
Prevotella	↓	< 0.0001	*Odoribacter*	↑	0.035
			Oscillospira	↑	0.0009
			Prevotella	↓	0.0003

All *P*-values have been corrected using the false discovery rate correction.

discussion to previous studies investigating fasting. While food reduction during hibernation significantly alters gut microbial communities (Carey *et al.*, 2013; Stevenson *et al.*, 2014), the reduction in body temperature acts as a confounding variable that yields unique alterations in the microbiota (Gossling *et al.*, 1982; Sonoyama *et al.*, 2009).

Fasting-induced changes in microbial diversity of the colon varied across host taxa. We observed an increase in the phylogenetic diversity of colonic microbial communities as a result of fasting in tilapia, toads, and mice. Conversely, prolonged fasting decreased phylogenetic diversity of quail microbial communities, while the gecko microbiota exhibited no changes. These equivocal results add to a variety of previous studies that found differing effects of fasting on microbial diversity. Fasting increases microbial community diversity in locusts (Dillon *et al.*, 2010), yet decreases diversity in zebrafish and pythons (Costello *et al.*, 2010; Semova *et al.*, 2012). Thus, prolonged fasting does not seem to have a universal effect on microbial diversity across host taxa.

Changes in microbial diversity also varied within hosts across tissues. For example, tilapia exhibited increased microbial diversity in their colons as a result of fasting, but a decrease in the phylogenetic diversity of their cecal microbiota. We observed no changes in the phylogenetic diversity of the mouse cecum. These findings were consistent with a previous study that also found no changes in the cecal microbial diversity of mice (Crawford *et al.*, 2009). However, we found an increase in the phylogenetic diversity of the mouse colon. Thus, prolonged fasting does not seem to have a universal effect on microbial diversity across gut chambers within a host.

The abundances of various microbial taxa also exhibited differential shifts across host taxa. These differences are unlikely to be driven solely by the loss of transient microorganisms present in the food, as diets usually comprise a small portion of the gut microbiota (Costello *et al.*, 2010; Nelson *et al.*, 2013; Kohl & Dearing, 2014). Further, while feeding drastically alters the microbiota of fasting pythons, it seems that the resident microorganisms flourish to repopulate the gut as opposed to an influx of transient microorganisms from the food (Costello *et al.*, 2010). Thus, it is likely that observed differences represent shifts in the abundances of the resident microbiota rather than loss of transient microorganisms present in food.

Across hosts, tilapia were the most responsive to prolonged fasting with significant changes in the relative abundances of five microbial phyla and 13 microbial genera in the colon. In this case, prolonged fasting resulted in an increase in the abundance of Proteobacteria, which has also been demonstrated in zebrafish (Semova *et al.*, 2012), but not seabass fasted for 12 days (Xia *et al.*, 2014). Proteobacteria are common members of gut communities in fish, although the functionalities of these communities are poorly understood compared with mammals (Sullam *et al.*, 2012; Clements *et al.*, 2014). Interestingly, fasting also resulted in an increase in the relative abundance of Bacteroidetes, but only in toads and mice. These results are consistent with another study that demonstrated a higher relative abundance of Bacteroidetes in fasting pythons (Costello *et al.*, 2010) and seabass (Xia *et al.*, 2014). The relative abundances of Bacteroidetes may increase due to the ability of some members to utilize host-produced mucosal glycans in the absence of dietary nutrients (Macfarlane & Gibson, 1991; Sonnenburg *et al.*, 2005; Martens *et al.*, 2008). However, the isolation of glycan-metabolizing microorganisms from other host species would reveal whether this is a common trait of this microbial phylum.

The community composition of the colons of geckos was the most stable of all species studied. They did not exhibit significant changes in microbial diversity and only exhibited changes in the relative abundance of one microbial genus during prolonged fasting. This consistency is remarkable, given that they were fasted for 28 days, the longest interval in our study. However, some lizard species appear to be starvation-adapted (Wang *et al.*, 2006; McCue, 2008) and are able to survive > 100 days of starvation by relying on lipid stores in their tails (Daniels, 1984). Thus, the stability of the gut microbiota during fasting may be suggestive of a high level of control by the host over the microbial community. The high representation of Bacteroidetes within the geckos could be the result of continual glycan production to maintain this community during unpredictable food periods. Further experiments extending the period of fasting or comparing responses between species pairs that exhibit different abilities to tolerate starvation may be useful to explore this possibility.

Cecal communities were also remarkably stable through prolonged fasting at the level of bacterial phyla. While we detected a number of changes in the abundances of bacterial phyla in the colon, we did not find any changes in cecal communities. This stability may be controlled by the host in times of prolonged fasting and may highlight the importance of the cecal microbial community to host performance. Microbial stability within the human gut has only recently been observed, although the mechanisms underlying this stability remain unclear (Claesson *et al.*, 2011; Lozupone *et al.*, 2012). Further work investigating how animals maintain stability of the cecal microbiota over other chambers may be warranted.

One potential explanation for the variation in responses of gut microbial communities to fasting is the variation in the communities of nourished animals. Fish tend to host microbial communities dominated by *Proteobacteria* and *Fusobacteria* (Rawls *et al.*, 2006; Sullam *et al.*, 2012), while tetrapods tend to host communities rich in *Firmicutes* and *Bacteroidetes* (Ley *et al.*, 2008; Scupham *et al.*, 2008; Costello *et al.*, 2010; Kohl *et al.*, 2013). Variation in resident communities may have limited our abilities to detect 'shared' responses. For example, the microbial phylum *Tenericutes* comprised 18% of the colonic community in nourished mice and decreased in abundance as a result of fasting. However, *Tenericutes* comprised < 1.5% of communities in other nourished hosts, potentially limiting our ability to detect a decrease in the abundances of *Tenericutes* in other hosts. Likewise, *Fusobacteria* were largely abundant in the colons of tilapia, but not other hosts. Controlling this variation across host species would be difficult; when germ-free mice are inoculated with the *Proteobacteria*-rich communities of fish, the few *Firmicutes* present in the fish gut expand to dominate the recipient mouse gut (Rawls *et al.*, 2006). This demonstrates that hosts select the types of bacteria that flourish within the gut.

Therefore, a number of host-driven mechanisms may dictate the idiosyncratic responses that we observed across host species and gut chambers. For example, fasting results in the impairment of host production of antimicrobial proteins and other aspects of mucosal immune function (Fukatsu & Kudsk, 2011; Hodin *et al.*, 2011), which are known to influence microbial community structure (Salzman *et al.*, 2010). Additionally, fasting animals produce less mucus on the gut lining compared with fed animals (Thompson & Applegate, 2006). These alterations may alter microbial diversity given that several gut microorganisms thrive on mucus (Banas *et al.*, 1988; Sonnenburg *et al.*, 2005; Martens *et al.*, 2008), and

differential production of glycans may support the growth of different types of microorganisms (Hooper & Gordon, 2001; Marcobal *et al.*, 2013). Fasted animals also tend to exhibit higher gut pH compared with fed animals (Ward & Coates, 1987), a chemical difference known to alter microbial growth (Palframan *et al.*, 2002). Last, many fasting animals reduce the size of their intestines (Karasov *et al.*, 2004; Thompson & Applegate, 2006), resulting in a 'housing crisis' for microorganisms that could result in increased competition for space. These host-driven mechanisms likely interact to shape the microbial community structure of the guts of fasted animals, and thus, it may be difficult to determine the underlying mechanisms driving these differential communities.

In addition to host-driven mechanisms, it is likely that differential survival of microorganisms underlies the changes observed in this study. The absence of dietary nutrients may cause a severe energy crisis for microorganisms, resulting in widespread microbial death. For example, fasting decreases microbial density by 99.7% in the rumen of reindeer (Aagnes *et al.*, 1995) and by 93.7% in the cecum of hamsters (Sonoyama *et al.*, 2009). Although we did not measure microbial density in our study, it is likely that fasting resulted in substantial decreases in cell density.

Despite the large variability in responses of the microbiota across host taxa and gut chambers, we were able to uncover a number of shared microbial responses to prolonged fasting. We did not detect any shared response across colonic communities of the vertebrate hosts in this study. However, all tetrapod hosts exhibited decreases in the abundances of *Coprobacillus* and *Ruminococcus* in the colon as a result of fasting. The normal function of *Coprobacillus* in the gut is unclear, although it has been implicated in several gastrointestinal diseases (Keren & Gophna, 2011; Pontarelli *et al.*, 2013). *Ruminococcus* is known for its fiber-degrading capabilities (Smith *et al.*, 1973), and thus, lack of dietary substrates during host fasting may cause its decline in relative abundance. All hosts with ceca (tilapia, quail, mice) exhibited increases in the abundance of *Oscillospira* and decreases in *Prevotella* and *Lactobacillus* within this chamber. Both *Oscillospira* and *Prevotella* are regularly found in rumina (Mackie *et al.*, 2003; Stevenson & Weimer, 2007) and are thought to degrade complex carbohydrates. High abundances of *Oscillospira* are associated with feeding on fresh green forage (Mackie *et al.*, 2003), and so, it may play a role in fiber degradation. *Prevotella* and *Lactobacillus*, on the other hand, are noncellulolytic and instead degrade xylans (Miyazaki *et al.*, 1997) or simple sugars (Barrangou *et al.*, 2006). The glycoside hydrolases maintained by saccharolytic bacteria often allow them to forage on host-produced glycans when nutrients are absent (Sonnenburg *et al.*, 2005; Martens *et al.*, 2008). Thus, we hypothesize that *Oscillospira* may have a diverse glycoside hydrolase repertoire that allows it to forage on host-produced glycans in times of nutrient deprivation. Members of *Lactobacillus* are unable to degrade host-produced mucins (Zhou *et al.*, 2001), which may underlie their decrease in relative abundance in the absence of dietary compounds. However, it is unclear why *Prevotella* decreases in abundance during fasting, especially given its ability to degrade host-produced mucins (Wright *et al.*, 2000). Interestingly, we did not detect universal changes in the genus *Akkermansia*. This genus is known to degrade host-produced mucus (Derrien *et al.*, 2004) and increases in relative abundance as dietary substrates become scarcer during fasting in hamsters (Sonoyama *et al.*, 2009) and pythons (Costello *et al.*, 2010). Thus, further studies could investigate the mechanisms and consequences of these shared responses to fasting.

Our study monitored changes in taxonomic diversity and did not investigate functional diversity. Gut microorganisms play a number of functions in host physiology that may be altered by prolonged fasting. Metagenomic analysis to monitor microbial functions over prolonged fasting would reveal these changes. In mammals, the gut microbiota play an important role in aiding the supply of alternative energy sources, such as ketone bodies, when hosts are faced with fasting and starvation (Crawford *et al.*, 2009). It is unknown whether the same types of microorganisms perform similar adaptive functions within other host taxa. While responses at the level of microbial taxa to fasting vary across hosts, there may be certain microbial functions that increase or decrease in abundance in fasted animals (Xia *et al.*, 2014). Metagenomic sequencing would reveal whether these functional responses are consistent across host taxa. Last, most studies investigating host–microorganisms interactions are conducted in mammals. Our study reveals that those results may not be applicable to all vertebrate hosts.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Rarefaction curves of estimated species richness (Chao1) of colonic samples.

Fig. S2. Rarefaction curves of estimated species richness (Chao1) of cecal samples.

Table S1. Significant changes in microbial genera of cecal samples from tilapia.

Data S1. Relative abundances of bacterial phyla and genera in all samples.