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Animal-Microbial Symbioses in Changing Environments

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Abstract

The environments in which animals have evolved and live have profound effects on all aspects of their biology. Predictable rhythmic changes in the physical environment are arguably among the most important forces shaping the evolution of behavior and physiology of animals, and to anticipate and prepare for these predictable changes animals have evolved biological clocks. Unpredictable changes in the physical environment have important impacts on animal biology as well. The ability of animals to cope with and survive unpredictable perturbations depends on phenotypic plasticity and/or microevolution. From the time metazoans first evolved from their protistan ancestors they have lived in close association with a diverse array of microbes that have influenced, in some way, all aspects of the evolution of animal structure, function and behavior. Yet, few studies have addressed whether daily or seasonal rhythms may affect, or be affected by, an animal's microbial symbionts. This survey highlights how biologists interested in the ecological and evolutionary physiology of animals whose lifestyles are influenced by environmental cycles may benefit from considering whether symbiotic microbes have shaped the features they study.

Keywords

Gut microbes; Symbiosis; Microbiota; Hibernation; Biological rhythms; Environmental change

1. Introduction

The environments within which animals have evolved and live have profound effects on all aspects of their biology. Predictable rhythmic changes in the physical environment (daily, seasonal) are arguably among the most important forces shaping the evolution of behavior and physiology of animals. To anticipate and prepare for these predictable changes in the physical environment, life on Earth evolved biological clocks. Zeitgebers (e.g., temperature

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cycles, photoperiod) entrain the biological clock whose outputs manifest as biological rhythms. Unpredictable changes in the physical environment (e.g., catastrophic weather and geologic events, global climate change) also have profound impacts on animal biology. The ability of animals to cope with and survive these unpredictable perturbations depends on phenotypic plasticity and/or microevolution (Reed et al., 2011; Reed et al., 2010; Vander Wal et al., 2013; Wingfield et al., 2011).

From the time metazoans first evolved from their protistan ancestors they have lived in close association with a diverse array of microbes. Microbes are a vital component of the environments in which animals evolved; they have colonized animals inside and out, and thus have likely influenced in some way all aspects of the evolution of animal structure, function and behavior (Gilbert et al., 2012; McFall-Ngai et al., 2013). Research on biological rhythms has focused primarily on the animal and the environmental cues that entrain its rhythms. To date, only a few studies have addressed the possibility that an animal's biological rhythms may influence, or be influenced by, its microbial symbionts (Heath-Heckman et al., 2013; Mukherji et al., 2013; Wier et al., 2010).

Until recently, examinations of a host's microbiota have been limited in part by the techniques available to study the microbial community. Culture-dependent analyses (e.g., BIOLOG[®], culture plating) result in significant underestimations of diversity and population size (Vaughan et al., 2000) because the vast majority of gut microbes are unculturable with current techniques. Culture-independent DNA fingerprinting techniques (e.g., T-RFLP, DGGE) eliminate culture bias, allow for rapid assessment of diversity, and when paired with additional analytical methods (e.g., cloning and sequencing), allow identification of community members; however, these techniques miss less abundant members of the community and are only semi-quantitative. The more recent development of culture-independent high-throughput next-generation (next-gen) sequencing techniques (e.g., Roche 454, Illumina) now allows for an in-depth analysis of microbial community structure (via 16S rRNA genes), physiological potential (metagenomics), and function (community transcriptomics). Although the cost-per run of next-gen sequencing methods can be high (depending on sample number and desired sequencing depth), costs are rapidly declining, 1000's of sequences are generated per sample (compared to 1/sample via traditional Sanger sequencing), and price per base is significantly less than Sanger sequencing (by ~200x) (Liu et al., 2012). In addition to next-gen sequencing techniques, methods to analyze the microbial metabolites that allow communication between animals and their symbionts are being developed (metabolomics). Finally, the development and use of specialized animal models (e.g., germ-free mice) facilitate design of experiments to uncover mechanistic relationships between specific microbes and changes in host biology (Faith et al., 2014).

With the development of new tools to deeply interrogate diverse microbial communities has come a wealth of knowledge on the ways microbial partners affect animal biology and how animal hosts shape the biology of their symbionts (McFall-Ngai et al., 2013). In this article we provide examples of how an animal's biological rhythms may impact the structure and/or function of its microbial community, and how activity of symbiotic microbes may influence seasonal or daily rhythms of the host. We focus primarily on non-laboratory, vertebrate animals and their gut symbionts, although other examples are included in which significant

progress has been made toward understanding the mechanisms by which microbes influence their host's biological rhythms. This survey highlights how consideration of animal-microbe symbioses can enrich studies of the evolutionary and ecological physiology of animals whose lifestyles are influenced by environmental cycles.

2. Gut microbiota

The majority of a vertebrate's microbial symbionts are found in the gut. Microbes that reside in the gastrointestinal tract of animals are members of complex ecosystems in which microbes can number in the trillions and vastly outnumber host cells (Ley et al., 2008; Muegge et al., 2011). The gut microbiota comprises two communities, the "luminal microbiota" that is associated with bulk contents in the lumen, and the "mucosal microbiota" that resides within the mucus layer that overlies the intestinal epithelium. To meet their nutritional needs, gut microbes metabolize dietary components ingested by their hosts that either cannot, or have not been digested in the small intestine, or endogenous substrates such as mucin glycans and nutrients in sloughed epithelial cells. Products of microbial metabolism are utilized by other members of the community, and some, such as short-chain fatty acids (SCFA) and vitamins, can be absorbed and utilized by the host to meet energetic and nutritional needs (Bugaut and Bentejac, 1993). It is becoming increasingly clear that beyond this nutritional input, the resident microbiota protect their hosts from invading pathogens and influence a diverse array of host characteristics including innate and adaptive immunity, cell signaling and proliferation, neural function, behavior and various aspects of organ physiology (Backhed et al., 2004; Crawford et al., 2009; McFall-Ngai et al., 2013; Velagapudi et al., 2010). Gut microbes can detoxify exogenous and endogenous compounds and alter expression of host metabolic genes that affect glucose and lipid metabolism (den Besten et al., 2013). Given its location, the mucosal microbiota is thought to be more strongly affected by host secretions than is the luminal community (Schluter and Foster, 2012), and to exert a greater influence on the host's immune system and other physiological traits (Van den Abbeele et al., 2011; Wang et al., 2010).

The abundance and composition of the microbiota are affected by several factors including host genetics and immune status, colonization history, physical aspects of the gut microenvironment and host diet (Campbell et al., 2012; Deloris Alexander et al., 2006; Friswell et al., 2010; Ley et al., 2006). Diet plays a dominant role among these factors (Scott et al., 2013; Sonnenburg et al., 2005) in large part because different microbial species are better equipped, through the complement of metabolic enzymes encoded in their genomes, to metabolize different substrates in support of growth (Flint et al., 2012). Dietary shifts driven by season or other environmental perturbations (such as drought or habitat fragmentation) can alter the gut microbiota through changes in taxonomic composition, and/or changes in expression of metabolic genes within the same species. For example, some members of the genus *Bacteroides* can alter their expression of carbohydrate-degrading enzymes depending on the availability of complex plant-derived substrates vs. endogenous host substrates such as mucins (McNulty et al., 2013; Salyers et al., 1977; Sonnenburg et al., 2005).

The microbial contribution to host nutritional physiology may be particularly important in fasted animals (Crawford et al., 2009), or in metabolic states that catabolize large amounts of body protein (Singer, 2003). Under these conditions, microbes can assist by “recycling” endogenous compounds lost in the feces (e.g., mucin and epithelial glycans), or what would normally be waste products (e.g., urea), and thus contribute to host energy balance and survival (Fuller and Reeds, 1998; Singer, 2003). This may benefit animals in environments where food availability varies significantly on a seasonal basis leading to substantial reductions in food intake or to complete cessation of feeding, such as species living in high latitudes, migratory species, and hibernators. Some examples of effects of seasonal environmental rhythms on gut microbiota are described below.

3. Circannual Hibernation Rhythms

Seasonal hibernators that rely on endogenous fuels during winter typically exhibit circannual cycles of hibernation, reproduction, growth and fattening (Lyman et al., 1982), as exemplified by many ground-dwelling sciurid rodents such as ground squirrels and marmots. Ground squirrels are homeothermic during most of the active season and become heterothermic during the hibernation season, which is characterized by weeks of torpor when animals profoundly decrease body temperature (T_b) and metabolic rate (MR). Torpor is periodically interrupted by interbout arousals of <24h when animals resume normothermia and high MR. Hibernating squirrels fast for 5–9 months, depending on species and sex, until arousal in spring. Thus, seasonal hibernators shift from reliance on a mixed diet during the active season to a primarily lipid-based metabolism with no dietary intake during hibernation. The physical environment within the gut lumen, and thus the ecosystem in which gut microbes exist, changes during hibernation. Many microbes (e.g., mesophiles) have limited or no growth at the T_b typical of deep torpor (<10°C) whereas others are psychophilic or psychrotolerant and are able to grow at temperatures regularly experienced by torpid animals (–2–10°C) (Morita, 1975). Interbout arousals provide brief periods that return thermal, metabolic, and physiologic conditions to levels similar to the active season, and include conditions in which most gut microbes readily proliferate. Small intestine and, to a lesser extent, hindgut tissues undergo substantial atrophy during hibernation although overall architecture of the mucosa is well maintained (Carey, 1990). Transport of nutrients and electrolytes is depressed during torpor, but increases during interbout arousals to levels similar to or greater than those in active season squirrels (Carey and Sills, 1992). Thus, despite the absence of food intake during the hibernation season, solute transport can still occur in the hibernator gut, allowing absorption and utilization of molecules present in the lumen such as microbially-derived SCFA, vitamins and ammonia. Compared with active season squirrels, gut microbes of hibernators have access only to endogenous, host-derived substrates - primarily glycans and proteins found in mucins and sloughed epithelial cells (Johansson et al., 2011). Although enzyme activity is relatively slow at the low temperatures of deep torpor (<10 °C), host-derived substrates can be rapidly degraded during interbout arousals and result in production of SCFAs and other microbial metabolites (Carey et al., 2013; Duddlestone et al., 2012; Stevenson, 2014).

Previous culture-based studies in thirteen-lined ground squirrels indicated that microbes associated with cecal contents (“luminal microbiota”) were reduced in number and species

composition during hibernation compared to summer active (“summer”) squirrels (Barnes and Burton, 1970), and bacteria could be readily cultured from mucosal tissue of hibernators (Cloud-Hansen et al., 2007). However, as noted previously, culture-based studies are limited because the majority of gut microbes are unculturable with current techniques. In a study that assessed gut microbiota of Syrian hamsters using flow cytometry and quantitative PCR, hibernation had no effect on numbers or taxonomic diversity of cecal microbes relative to non-hibernating hamsters (Sonoyama et al., 2009). This is not surprising because these animals feed during interbout arousals. A study we conducted of the luminal microbiota in thirteen-lined ground squirrel cecum using qPCR with taxa-specific primers indicated that hibernation reduced overall bacterial abundance and relative abundance of the *Firmicutes* phylum (Carey et al., 2012).

Because qPCR analysis of the microbiota may miss changes in taxa that are not identified with targeted probes, 16S rDNA pyrosequencing was used to track relative abundances of luminal microbiota in squirrels born from wild-caught mothers and sampled in summer, early (1 month) and late (4 months) winter, and spring (2 weeks after refeeding). Luminal microbiotas clustered strongly by season, whereas maternal effects, host age and host T_b had minimal impact (Carey et al., 2013). Phylogenetic diversity and numbers of unique phylogenetic groups (as assessed by operational taxonomic units (OTUs)) were lowest in late winter and highest 2 weeks after refeeding in spring. The relative abundances of certain taxa changed with season; hibernation increased relative abundance of *Bacteroidetes* and *Verrucomicrobia*, phyla that contain species capable of surviving on host-derived substrates such as mucins, and reduced relative abundance of several *Firmicutes* taxa which prefer dietary polysaccharides. Some groups were nearly absent during hibernation, such as *Lactobacillus*. Similar patterns were observed in studies with hibernating arctic ground squirrels (Duddlestone et al., 2012; Stevenson, 2014). These results suggest that competition for the limited amount of usable substrates in the hibernator gut may drive the altered microbial communities that emerge by late winter. That is, species less able to utilize endogenous substrates (or intermediate metabolites produced from endogenous substrates) are gradually reduced in number until dietary substrates are available when feeding resumes in the spring. Gut microbes produce a wealth of metabolites that can influence host biology, among these being the SCFAs acetate, propionate and butyrate, and the branched chain fatty acids produced by protein degradation. In hibernating thirteen-lined ground squirrels concentrations of total SCFAs in cecal fluid are about 75% lower relative to the active season when animals are feeding (Carey et al., 2013). Similar results were also observed in arctic ground squirrels hibernating at $T_b < 0^\circ\text{C}$ (Duddlestone et al., 2012; Stevenson, 2014). Interpretation of these static measurements is limited without flux analyses that can estimate relative rates of SCFA production and disappearance (i.e., absorption and excretion). However, the results do confirm that even in hibernators that have not eaten for several months, a baseline level of SCFA is still generated by microbial degradation of host-derived substrates. This presumably occurs during interbout arousals, because total SCFA are greater in hibernators sampled during arousal vs. torpor (Carey et al., 2013; Duddlestone et al., 2012; Stevenson, 2014). Interestingly, the molar proportions of the main SCFAs change with season, with acetate increasing during hibernation, butyrate falling to very low levels, and propionate showing no change (Carey et al., 2013). That similar changes occur in cecal

contents of both thirteen-lined and arctic ground squirrels during hibernation (Carey et al., 2013; Duddlestone et al., 2012; Stevenson, 2014) likely reflects that the amount and type of substrate present in the gut environment, as well as the relative abundance of different microbial taxa, affect the amount and proportions of SCFAs produced (den Besten et al., 2013). SCFAs generated by microbes during winter fasting could provide several benefits to the hibernator host; they can be metabolized to produce ATP, can be converted to ketone bodies and serve as glucose substitutes in tissues like brain and heart, and can be substrates for gluconeogenesis. Because luminal SCFA provide hindgut epithelial cells with as much as 60–70% of energy requirements for maintenance and proliferation, even a relatively low production of SCFA during hibernation may limit the degree of mucosal atrophy that occurs over the course of the hibernation season (Carey, 1990). By binding to cell surface receptors such as GPR43, SCFAs can act as signaling molecules, regulating such processes as lipid and glucose metabolism (den Besten et al., 2013; Ge et al., 2008) and intestinal immune function (Maslowski et al., 2009; Smith et al., 2013). Because SCFA receptors are found on a wide variety of cell types including white and brown adipose tissue, pancreas and intestine (Regard et al., 2008), bacterially-derived SCFA may contribute to metabolic changes during the annual hibernation cycle.

The intestinal immune system is the primary sensor of changes in microbial activity (Artis, 2008), and therefore provides the closest link between dietary changes, their effect on the microbiota, and host responses. Diet composition influences the composition and activity of the microbiota, which in turn directly affects the number, type and function of gut immune cells. These interactions have important implications for animal health and survival, because the absence of food intake in most mammals increases intestinal permeability, thus increasing the risk of bacterial translocation and hyper-stimulation of the immune system. Interestingly, gut permeability is higher in aroused hibernators compared with summer thirteen-lined ground squirrels (Carey, 1992; Carey et al., 2012), and although numbers of certain immune cell populations also increase, there is no evidence of mucosal damage or pathologic inflammation (Kurtz and Carey, 2007). Rather, it appears that the gut's immune system is remodeled, perhaps in response to altered host-microbial communication, in a way that promotes tolerance to the microbiota and suppresses inflammatory pathways in the setting of enhanced permeability.

4. Ruminant-Microbe Symbioses

Ruminant physiologists have long been on the forefront in understanding the importance of microbial symbionts to host nutrition and energetics. Indeed, recent revelations into the link between microbes, host physiology and biological rhythms come from the study of wild ruminants, and we highlight some of those studies here. Soay sheep are highly photosensitive and display strong seasonal rhythms in many aspects of their physiology and behavior. Sheep from a semi-feral flock housed under short day photoperiod (simulating winter conditions) had different rumen microbial diversity and reduced SCFA concentrations compared to those housed in long-day photoperiods (McEwan et al., 2005). Because all sheep were fed the same diet, the change in microbiota and the SCFAs they produced were likely due to the almost 50% reduction in food intake that occurs under short day photoperiods (McEwan et al., 2005). Large mammalian herbivores in the high Arctic

experience drastic reductions in abundance and quality of their plant forage during winter, which leads to reduced substrate availability for their gut symbionts. In reindeer on Svalbard, numbers and taxonomic composition of rumen microbes change seasonally from summer to winter as food resources contract (Orpin et al., 1985). Muskoxen, which also remain on the arctic tundra throughout the year, typically become hypophagic as winter progresses (Peltier et al., 2003), and this affects the abundance and activity of rumen microbes (Barboza et al., 2006; Crater et al., 2007).

The lower latitudes also contain examples of mammals dependent on nutritional symbioses with microbes that are sensitive to seasonal changes. Analysis of fiber-digesting capability of fecal microbes obtained from three ruminants (buffalo, wildebeest, impala) and one hindgut fermenter (zebra) in southern Africa demonstrated that specific activities of four fibrolytic enzyme systems varied between winter (drier) and summer (wetter) seasons (Fon and Nsahlai, 2012). Whether enzymatic function increased, decreased or remained unchanged between seasons varied among the enzymes, and also among the herbivores that housed the microbial communities. This may be due to differing levels of particular fiber types ingested by the animals in the two seasons and the resulting effects on microbiota composition, the functional capacities of individual microbes, and the functional output of the microbial ecosystem as a whole.

While these studies show that symbiotic microbiota diversity and activity in mammals vary with season, the importance of these changes to host biology (i.e. cause, effect, or a reciprocal feedback loop) are largely unexplored. Metagenomics, community transcriptomics and metabolomics analyses could shed light on the nature of the symbiotic relationship by revealing the genetic capacity of the symbiotic population, the changes in microbial gene expression and the varying products of their metabolism. Results of such investigations would be especially revealing when paired with manipulations of symbiotic communities or host diet and subsequent assessment of microbial community dynamics and host biology.

5. Photoperiod and circadian cycles

The biological rhythms of some species are particularly sensitive to changes in photoperiod, and there is evidence that this affects their microbial symbionts. In contrast to other seasonal hibernators, Siberian hamsters lose body and fat mass when maintained under short photoperiods that mimic onset of winter (Wade and Bartness, 1984). Although photoperiod had no effect on food intake nor microbiota species richness or taxonomic diversity, exposure of hamsters to long day lengths increased relative abundance of the phylum *Proteobacteria* compared to hamsters exposed to short days, and this increase was correlated with higher body and fat masses in the long-day exposed animals (Bailey et al., 2010). Six bacterial genera also differed in long- vs short-day hamsters, including a lower abundance of *Citrobacter* spp. in short day animals. Although only a correlation, the results suggest that the intestinal microbiota are responsive to changes in photoperiod, and that changes in certain taxa may contribute to photoperiod-induced changes in body and fat mass.

Direct experimental evidence that microbial symbionts can induce or regulate biological rhythms in animals is scant. However, in a series of elegant studies McFall-Ngai and colleagues showed that the bacterial symbiont *Vibrio fischeri* and its luminescence are critical in the entrainment of cryptochrome proteins in their host, the bobtail squid *Euprymna scolopes* (Heath-Heckman et al., 2013). The squid specifically harbor *V. fischeri* within their light organ (which requires the bacterium for its initial development), and use counterillumination from bacterial luminescence to avoid predators that might otherwise detect their shadows cast by moonlight or starlight. Exposure of the squid to a combination of blue light and two bacterial cell wall metabolites induce daily cycling of cryptochrome genes that are known components of central and peripheral circadian oscillators in other systems (Heath-Heckman et al., 2013). Furthermore, this symbiosis drives a diel rhythm of transcriptional changes in both the squid and bacterium (Wier et al., 2010). Recent work in laboratory mice showed that gut microbes regulate circadian rhythmicity of genes involved in metabolic homeostasis and microbial sensing in intestinal epithelial cells (Mukherji et al., 2013). It is probable that examples such as these are widespread throughout the metazoa. Within mammals, peripheral clocks outside of the brain have been found in multiple cells and tissues, including, among others, the liver, intestines, adrenal gland, lung, thyroid gland, pancreas, thymus, and skeletal muscle (Dibner et al., 2010), providing many target sites for synchronization with microbial metabolites (Wikoff et al., 2009).

6. Animal-microbial symbioses in unpredictable and rapidly changing environments

Biological rhythms are shaped by evolution in response to predictable environmental changes and result in adaptive behavioral and physiological responses that promote health and survival (Reed et al., 2010). Environmental change that is less predictable, however, such as natural catastrophic events, abrupt climatic shifts, or anthropogenic impacts such as herbicide/pesticide use can put animals at risk due to such factors as habitat fragmentation (Amato et al., 2013), changes in the amount and quality of food resources, toxin exposure and changes in numbers of interacting species in an ecosystem (Reed et al., 2010). We suggest that effects on animal-microbe symbioses should not be overlooked when considering the potential impacts of unpredictable environmental change on animal biology (Fig. 1).

A long-term study of the ecophysiology of two populations of arctic ground squirrels in northern Alaska may provide insight as to the potential effects of environmental change on animal-microbe symbioses. Both populations are found in the northern foothills of the Brooks Range; one resides at Atigun, a site that is snow-free earlier in the spring and remains snow-free longer in the autumn compared to Toolik Lake, 20 km to the north where the second population lives. Differences exist in the timing of emergence from hibernation among adults, juveniles, males and females at both locations, and all cohorts of arctic ground squirrels emerge from hibernation earlier, and to less snow-cover and earlier green-up, at Atigun compared to their counterparts at Toolik (Sheriff et al., 2011). We collected fecal samples from the two populations in early May, 2010, examined bacterial diversity using pyrosequencing of 16S rRNA genes, and found that gut microbiotas clustered by

population and not by sex or age (Fig. 2; Duddleston et al., unpublished results). To date no explanation for these differences is evident; more studies are needed to determine whether the difference in microbiotas between the two populations is due to food availability, time spent foraging above ground at the time of collection, genetic differences between the two squirrel populations, or some combination of those factors. Elucidating the factors driving microbiota composition, combined with studies on microbiota function, may help reveal whether the differences in these microbiotas are important to the biology of their hibernator hosts, and suggest how changes in the environment may influence host biology through their gut microbes. After thousands of years of relative stability the Arctic is changing rapidly in response to climate warming (Overpeck et al., 1997). Altered plant diversity and phenology in response to increases in ambient temperature and depth and duration of snowpack can affect food quality and availability for arctic species and impact their biology in numerous ways. Because gut microbiota are influenced by diet and host physiological conditions, the effects of these changes on host-microbe symbioses could be significant.

Changes in an animal's food resources due to unpredictable or rapid environmental perturbations have the potential to affect host-microbe relationships because they affect the substrates available for microbial growth. However, other mechanisms may also be in play. Abrupt or extreme changes in local habitats, including biotic (e.g., predators, social configuration) and abiotic (e.g., weather, food availability) factors can lead to acute activation of neuroendocrine stress responses or enhance an animal's allostatic load, with potential long-term effects on health and survival (McEwen and Wingfield, 2003; Wingfield et al., 2011). It has been known for some time, mainly through studies with laboratory rodents, that a variety of stressors can influence the composition of the gut microbiota (Bailey et al., 2011; Tannock and Savage, 1974), and there is evidence that gut microbiota can influence animal stress responses through activation of the hypothalamic-pituitary-adrenal axis (Dinan and Cryan, 2012) and possibly other mechanisms, such as hyperactivation of the immune system. Gut microbes may have contributed to the evolution of immunity in animals that regularly experience environmental stressors such as extreme cold (Sinclair et al., 2013). For example, pre-emptive activation of immune responses in overwintering insects may have been selected for to reduce the risk of invasion of gut bacteria into the hemocoel after tissue damage induced by cold exposure (MacMillan and Sinclair, 2011). Although the role of gut microbes in shaping stress responses of free-living animals is only beginning to be explored, the possibility that environmental change may affect animal health and survival by direct or indirect effects on host-microbial symbioses is an intriguing possibility that deserves further study.

Adopting an ecological perspective can help to understand whether a particular environmental change has the potential to alter a stable gut microbiota that promotes health into an unstable state that disrupts beneficial host-microbe symbioses (Lozupone et al., 2012) (Fig. 1). The "resiliency" of the community to disruption depends on several factors including the type and severity of the perturbation and its effect on microbial species richness (i.e., the number of species present in the ecosystem) and phylogenetic diversity (how closely species are related to one another). Reduced species richness and diversity ("dysbiosis") have been linked to pathological states such as inflammatory bowel disease and obesity in humans and experimental animals (Lozupone et al., 2012), a situation often

exacerbated by increased gut permeability which can compromise the host's defenses against microbial invasion (Brenchley and Douek, 2012). Interestingly, for hibernating ground squirrels the annual shift from the summer, fed state to the winter, fasted state reduces species richness and diversity of the gut microbiota (Carey et al., 2013; Duddleston et al., 2012; Stevenson, 2014) and increases intestinal permeability (Carey, 1992; Carey et al., 2012). Yet, these changes are well tolerated and may be facilitated by enhanced immune defenses during winter fasting (Kurtz and Carey, 2007). Thus, the evolution of the circannual cycle in hibernators may have favored the maintenance of multiple stable states in their gut microbial communities throughout the year despite significant perturbations that affect microbial growth and community diversity.

7. Perspectives

In this review we provided examples in which gut microbial communities change in concert with seasonal or daily rhythms of their hosts. However, most of these studies are correlative and do not shed light on the degree to which gut microbiota respond to, or contribute to, host physiology. Experiments are needed in which gut microbes are manipulated (e.g., through antibiotics or microbial transplants) and impacts on host physiology assessed. Also unknown is the potential impact of endogenous rhythms of the symbionts on host physiological processes. Bacteria exhibit a variety of important temporal (cell cycle and circadian), and spatial (cell division, DNA segregation, and cell polarity) oscillations (Lenz and Sogaard-Andersen, 2011). Of these, only the circadian oscillator is a true clock (Lenz and Sogaard-Andersen, 2011). While circadian rhythms are well studied in photosynthetic bacteria (Lenz and Sogaard-Andersen, 2011; Min et al., 2005; Van Praag et al., 2000), little is known about circadian rhythms in non-photosynthetic prokaryotes and to date only one non-photosynthetic prokaryote is confirmed to have diurnally entrained rhythms (Whitehead et al., 2009). Future research should explore whether gut microbes express rhythmic oscillations, what host signal(s) microbes entrain to (e.g., food intake, T_b) and how microbial oscillations impact host physiology. As our understanding of the mechanisms that underlie animal-microbe associations grows, and the technological tools to study them become more widely available to all biologists, we will be better equipped to incorporate these remarkable relationships into investigations of biological rhythms and organismal responses to changing environments.

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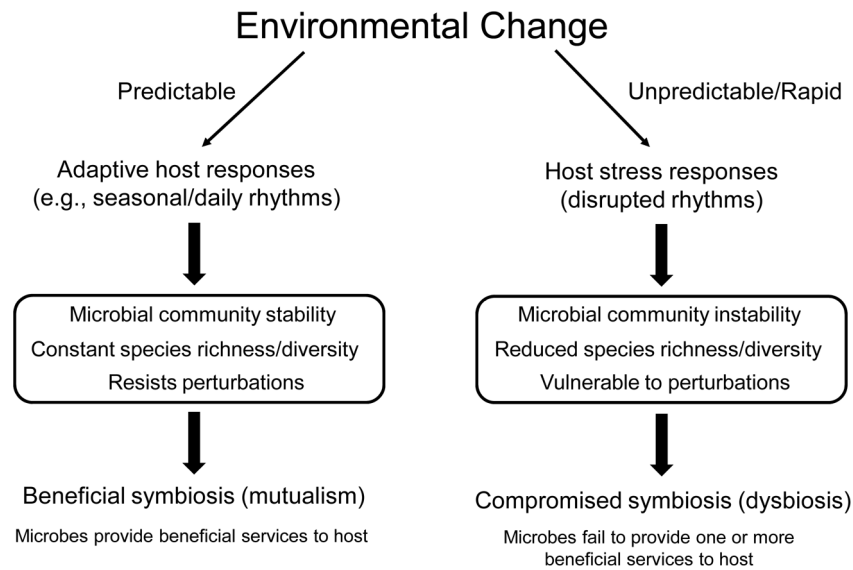
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Highlights

- Biological clocks anticipate and prepare for predictable environmental changes.
- Unpredictable environmental changes also impact animal biology.
- Microbes can influence many aspects of animal structure, function and behavior.
- Microbes may influence or be influenced by host responses to environmental change.

**Figure 1.**

Potential effects of environmental change on host responses and gut microbial communities. Predictable environmental changes such as seasonal (photoperiod, thermal regime, food resources) and circadian (day/night light and feeding cycles) cycles contribute to development and entrainment of animal biological rhythms. Gut microbial communities may respond to, or in some cases drive development and maintenance of host rhythms; although communities may shift rhythmically with their host, they remain in stable configurations with structural properties (e.g., species richness/diversity) that maintain mutualistic relationships and are resilient to perturbations. In these “beneficial symbioses”, microbiotas provide services to the host such as increased energy extraction from diet (degradation of indigestible substrates), vitamin production, detoxification of xenobiotics and endogenous toxins, avoidance of pathobiont colonization, healthy priming of the immune system, etc.). Environmental changes that are unpredictable and/or too rapid for host/microbiota adaptation have the potential to disrupt host responses and induce stress. Gut microbiotas may shift into temporary or long-term unstable states with altered composition and diversity (i.e. dysbiosis). Beneficial symbioses may be compromised such that services to the host are impaired resulting in increased risk of pathobiont invasion, aberrant stimulation of the immune system (inflammation), and/or reduced ability to harvest energy from the diet. (Adapted after Fig. 6 in (Lozupone et al., 2012).

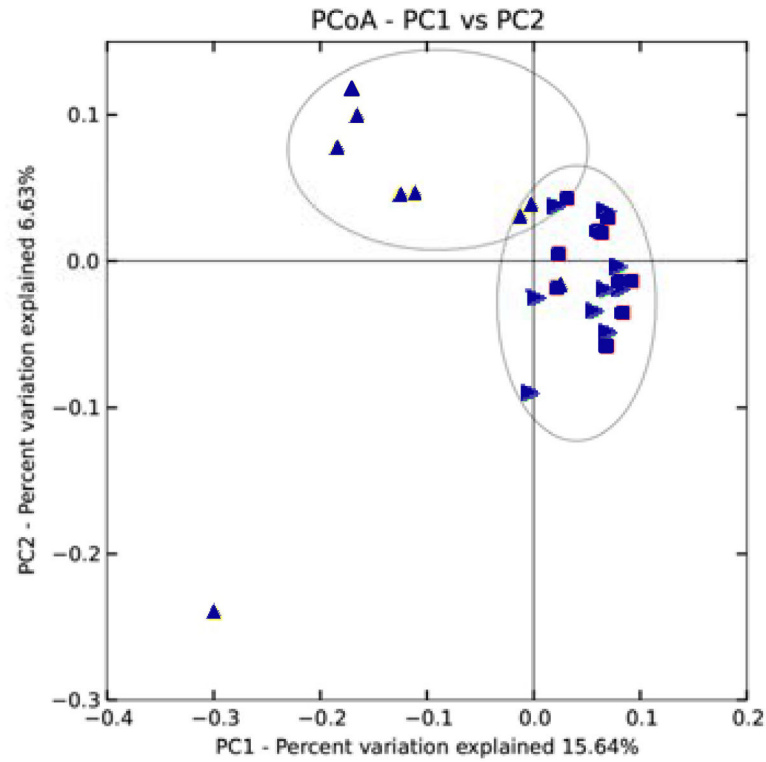


Figure 2.

Principal coordinates analysis of gut microbiotas of arctic ground squirrels from Toolik Lake (▲) and West (■) and East (►) Atigun, Alaska. West and East Atigun refer to areas on the West and East sides of the Dalton Highway, respectively. (From Duddleston et al., unpublished results)