

Leveraging circadian rhythms to study host-gut microbe interactions in wildlife

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Abstract

Daily light-dark cycles shape the activity patterns and physiology of nearly all organisms. Many biological processes undergo circadian rhythms, yet rhythms in immunity and metabolism are particularly important for the maintenance of biological homeostasis. Recent evidence that food intake and the gut bacterial microbiota synchronise system-wide circadian rhythms spanning immunity, metabolism, and behaviour point towards gut microbial oscillations being a crucial component of microbiome function. Findings from model systems suggest that gut microbial oscillations are likely widespread across species and pivotal for shaping immune and metabolic responses, yet their prevalence and functional significance are rarely tested in natural populations. Here we summarize results from experimental studies on how circadian interactions between the gut microbiota and the host act to synchronise rhythms in host metabolism and immunity. We outline how these circadian interactions are likely to mediate diverse biological processes, including host pathogen susceptibility and seasonal switches in metabolism, and discuss how the breakdown of these interactions, for example during senescence and urbanisation, can lead to dysbiosis and declines in health. Lastly, we provide practical guidelines for the measurement of microbial oscillations in wildlife, highlighting that whilst wild animals are rarely available over a 24-hour period, characterising even parts of the cycle can be informative. Light-dark cycles are an almost universal environmental cue that provide a rare opportunity to generalise gut microbial responses across species, yet to fully appreciate their ecological relevance an understanding of how microbial rhythms manifest in wildlife is essential.

Introduction

Circadian rhythms describe the synchronization of multiple biochemical and physiological processes across a 24-hour cycle, allowing organisms to anticipate predictable biotic and abiotic conditions across the day¹. Circadian rhythms are self-sustaining in the absence of environmental cues, yet they are synchronised (‘entrained’) across multiple facets of physiology and behaviour by environmental cues. Light cues entrain the master pacemaker located in the brain, yet peripheral clocks in organs and tissues are largely entrained by non-photic cues such as temperature and feeding schedules^{2–5}. Collectively, these cues interact with clock genes to influence 24-hour rhythms in gene expression⁶. Whilst food intake was previously thought to have localised effects on metabolic rhythms^{7,8}, mounting evidence points towards feeding being fundamental for orchestrating system-wide physiological homeostasis in innate immune function and metabolism across the day^{3,9–14}, even feeding back to influence the master clock¹⁵. The far-reaching effects of food intake on host circadian rhythms are mediated by the gut microbiota, which rhythmically interact with the host to regulate rhythms in both innate immunity and metabolism^{10,11,13}. However, despite a long-standing appreciation for the importance of both circadian rhythms^{1,4,16–19} and the gut microbiota^{20–25} for mediating host biological, ecological, and evolutionary processes, their interaction has largely been neglected in the study of natural populations.

Gut microbial communities are highly responsive to dietary and physiological cues, leading to high temporal variation within and across host individuals over months and years^{26–28}. However, the predictable dynamics of gut microbes over the day in response to food intake and host physiology has recently become a research focus for experimental studies on model systems. These have uncovered strong diurnal oscillations of the gut microbiota^{13,14,29–33} and metabolome^{7,11}, with bacterial numbers estimated to change 10-fold over the course of each day in laboratory mice¹¹. Indeed, gut microbial oscillations have been identified in captive settings from a diverse suite of species, from birds and mammals to fish^{13,26,31,32,34}, and their effects often dominate over that of individual identity^{32 3526,29}. Microbial rhythms are underpinned by a combination of diet and time of feeding^{36–39}, yet are also under host circadian control: mice without functioning clock genes have disrupted gut microbial rhythms⁴⁰. Rhythms in gut microbial communities are therefore likely to be responses diurnal shifts in food intake and host physiology, rather than self-sustaining circadian rhythms.

Gut microbial rhythms are profoundly important for regulating host metabolism and innate immunity across the day^{7,8,41–44}. Their disruption, for example due to jet lag in humans, leads to increased risk of metabolic disease, gut inflammation, and pathogen susceptibility^{12,13,34}. The importance of gut microbial rhythms for mediating both metabolic and immune homeostasis has broad implications for our understanding of gut microbiome function and their adaptive significance in natural populations. Circadian interactions between the gut microbiota and host immunity are of particular relevance for ecologists because pathogens are disproportionately important for mediating host fitness and evolutionary trajectories in natural populations^{45–47}, with pathogen defence potentially being the principal evolutionary advantage of the gut microbiome⁴⁸.

Diurnal oscillations in the gut microbiota are known to be strong, widespread across studied species, and have profound biological functions for the host. As such, research on host-microbe interactions in wildlife must begin to account for these daily dynamics. With an aim of encouraging the incorporation of circadian rhythms into wildlife microbiome research, we review hallmarks of gut microbial rhythms that have been identified across species, describe their molecular mechanisms, and outline how including microbial rhythms can advance our understanding of microbiota-mediated host-pathogen interactions and metabolic regulation in natural populations. Finally, we apply this information to provide recommendations for how to advance our understanding of gut microbial rhythms and their associations with host physiology in wildlife.

Hallmarks of gut microbial rhythms

Gut microbial oscillations are widespread across studied species, having been identified in humans³⁴, meerkats²⁶, mice¹³, cows^{29,33}, fish³¹, and chickens^{30,32}. Even host-associated microbiota of zooplankton undergo diurnal cycles⁴⁹. The proportion of gut members that show oscillating behaviour varies between studies and species, with between ~35% (humans) and ~80% (meerkats) of common taxa being identified as oscillators^{12,26,34,50}. This suggests natural variation in the strength of microbial oscillations across species. In industrialized societies of humans, population-wide gut microbial oscillations identified from cross-sectional studies appear to be weak³⁴, and explain only a modest amount of variation in gut microbiota composition. In other species, circadian rhythms of the gut microbiota are strong and dominate over individual identity effects^{32 35 26,29}.

Across the mammalian species studied thus far, there are some similarities in gut microbial dynamics across the day. In laboratory mice, the absolute abundance of bacteria inhabiting the mucosal epithelial layer peaks in the middle of the active phase^{11,13,40}, with a 10-fold increase in bacterial numbers compared to the rest phase¹¹. This pattern is supported by increased number of bacteria in the gut more generally during the active phase⁴⁰. Similar findings were reported for wild meerkats, where reference standards were used to quantify 16S faecal abundance²⁶, and in humans, where the number of bacterial species in faecal samples peaks at midday³⁴. Importantly, dissections of the mouse intestine show that faecal microbial rhythms reflect real changes to the composition of the intestinal microbiota^{10,13}, and are not simply a product of shedding patterns. Collectively, these findings also suggest that diurnal dynamics of gut microbes may be similar across host species.

Even though oscillating microbial taxa are likely to differ between host species, members of Clostridiales

undergo some of the strongest and most consistent oscillations in mammals ^{7,11,35,39,40} and this may also be true for birds ^{30,32}. There is also growing evidence from mice that different types of gut microbes peak at different times of the day. Some bacteria, termed here *mucosal commensals*, colonise the mucosal gut lining, whilst others, termed here *luminal bacteria*, are mostly found in the gut lumen. Mucosal commensals are hypothesized to have co-evolved with the host and form a protective layer against other bacteria between the gut epithelium and the gut lumen. In mice, mucosal commensals such as segmented filamentous bacteria (SFBs; order Clostridiales) peak at the start of the active phase and then commence to decline over the feeding period^{11,13}. In contrast, many luminal bacteria have low abundances at the start of the active period yet increase after feeding^{11,13} (**Fig. 1**). Because the majority of taxa are luminal bacteria, these contrasting patterns result in increasing bacterial load over the active phase. However, the identification of oscillating taxa is biased by the fact that most studies apply relative rather than absolute abundances ⁴⁰, which can generate misleading results, and by the difficulty of distinguishing between mucosal and luminal bacteria from metagenomic data.

Molecular mechanisms underpinning circadian host-gut microbe interactions

Food intake introduces both nutrients and food-borne pathogens into the gut, therefore the upregulation of both metabolism and components of innate immunity during feeding is crucial for gut function and pathogen defence during this period of acute pathogen exposure⁴². Yet what are the major mechanisms underpinning this process? Whilst this field of research is in its infancy, a number of recent experimental studies on murine models outline some of the mechanisms underpinning circadian host-gut microbe interactions. These mechanisms generally involve cyclical interactions between food intake, components of the immune system including antimicrobial peptides (AMPs) and the antibody secretory Immunoglobulin A (SIgA), and certain mucosal commensals (**Fig. 1**).

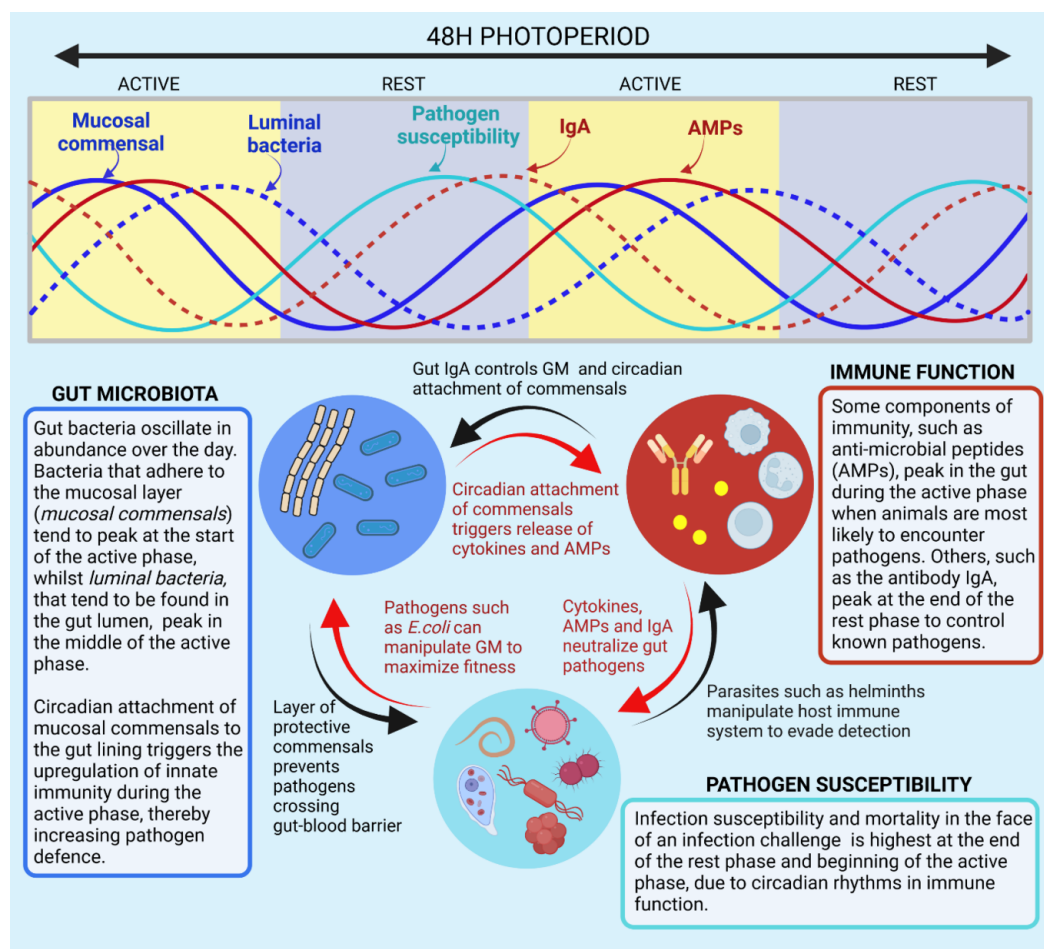


Figure 1) Summary of the circadian crosstalk between gut microbes, components of the host innate immune system, and pathogen susceptibility, based on findings from laboratory mice. GM = Gut microbiota.

During the active phase, when animals are awake and feeding, high densities of diverse gut microbes are tolerated because they generate crucial metabolites, which are absorbed into the bloodstream via a porous gut lining (**Fig. 2a**). Because metabolites are crossing the gut-blood barrier during feeding, the permeable gut lining is vulnerable to opportunistic bacterial attack. To lower infection risk, most non-commensal bacteria are kept away from the mucosal layer by allowing only specific mucosal commensals to adhere to the gut lining^{10,13}. In mice, this function appears to be largely performed by commensal SFBs. SFBs, as well as mucosal commensals *Bacteroidetes fragilis* and *Akkermansia muciniphila*, are suggested to perform this role in humans⁴². The physical interaction between mucosal commensals and host epithelial cells, in particular at the start of the active phase^{11,13}, triggers the mass release of components of innate immunity, including AMPs¹³, that protect the host against a broad range of pathogens during feeding¹³, and feed back to control gut microbial rhythms¹¹. Mucosal commensals also trigger the release of major histocompatibility complex (class II)-mediated cytokines¹⁰, which, whilst part of the adaptive arm of the vertebrate immune system, act to modulate the innate immune response⁵¹. Innate immune protection does not last the entire active phase, but rather begins to drop in the second half of the active phase^{10,13}. The reason for this is unclear, although it may be due to the feeding bouts that typically occur at the start of the active phase in mice⁵².

Maintaining a high level of immune control across a 24-hour period is energetically expensive, and inflammation caused by pro-inflammatory cytokines also damages tissue⁵³. Many aspects of innate immunity are therefore downregulated during the rest phase when the gut lining becomes less permeable, and the host is less likely to encounter pathogens (**Fig. 2b**). This downregulation is preceded by mucosal commensals such as SFB detaching from the mucosal layer, the mechanisms of which remain unclear, thereby reducing the number of cytokines and AMPs secreted into the gut. In the absence of nutrients from food, the gut bacterial population declines, and remaining bacteria migrate to the gut epithelium to feed on the mucosal layer, replacing the protective layer of commensals^{11,13}. Perhaps to protect the integrity of the epithelial layer from feeding bacteria, the intestinal mucosal layer thickens during the rest phase¹¹.

The downregulation of pro-inflammatory components of innate immunity during the rest phase is likely responsible for the well-studied phenomenon whereby animals are more susceptible to infection and mortality when challenged at the end of the rest phase compared with the middle of the active phase⁵⁴. Nevertheless, animals are not altogether undefended during the rest phase. A key gut antibody, sIgA, is upregulated during sleep⁵⁵. Secretory IgA is the most abundant antibody produced by mammals and is present across all mammals and bird species^{56,57}. It acts as bridge between innate and adaptive immunity, being able to distinguish between gut commensals and non-commensals⁵⁸. During the rest phase, upregulated sIgA neutralises non-commensals and their toxins that are otherwise tolerated during the active phase, thereby ensuring that any potential pathogens that were introduced and proliferated during the active phase are killed. Another function of sIgA is to bind to beneficial mucosal commensals and control their adhesion to the mucosal layer^{58,59}, and it is therefore a key agent in triggering the circadian cycles of the gut microbiota at the start to the active phase⁵⁵. A peak in sIgA just prior to the start of the active phase is likely involved in bringing mucosal commensals back to the epithelial layer to begin the circadian cycle anew, although the exact mechanisms are still unknown. Interestingly, sIgA secretion is controlled by food intake rather than the master clock, with food intake repressing sIgA levels⁵⁵ in order to increase tolerance to gut bacteria during the active phase.

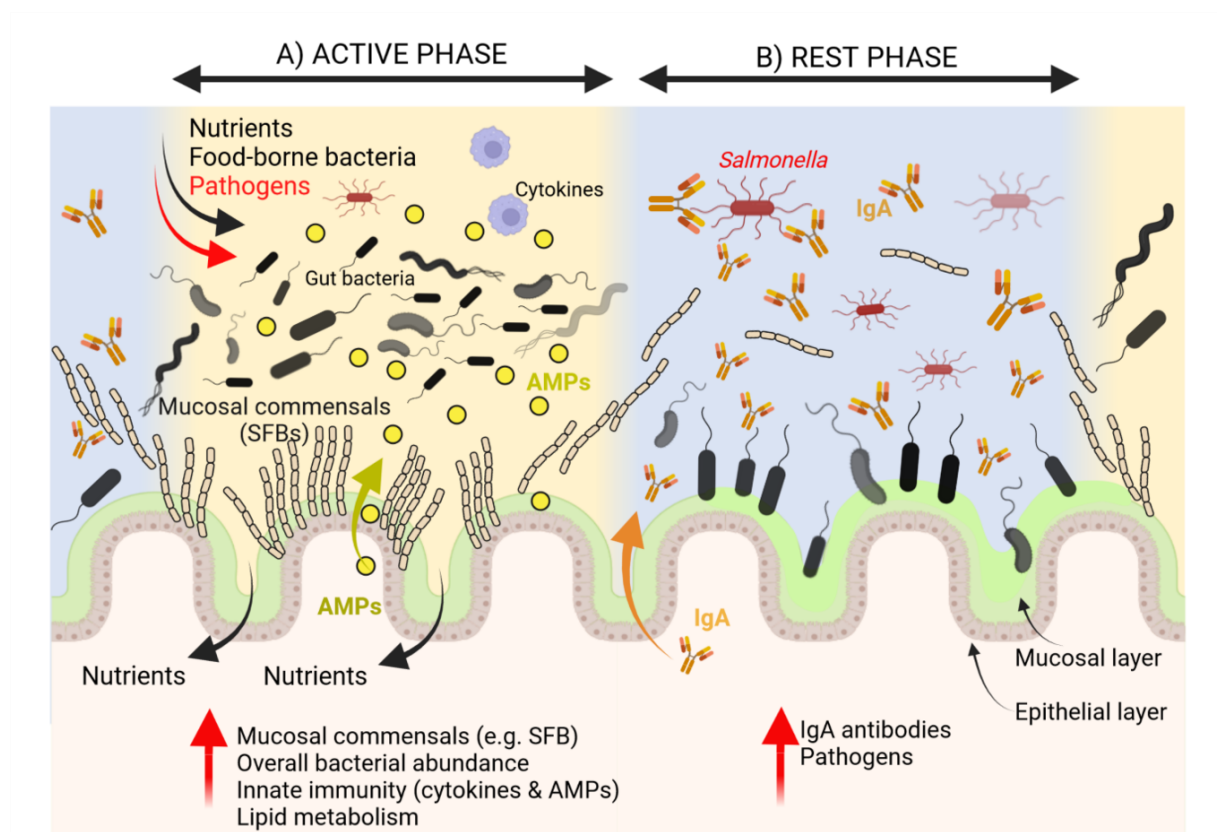


Figure 2) Diurnal rhythms of the gut microbiota, host immunity and pathogen abundance across a) the active phase and b) the rest phase, as characterised in laboratory mice. SFB = Segmented Filamentous Bacteria.

Interactions between food intake, mucosal commensals, and sIgA together regulate gut microbial oscillations over the day. However, an additional mechanism that has received less attention is the role of ecological dynamics in regulating microbial oscillations. An increase in gut microbes post-feeding alter the chemistry of the gut, increasing CO₂ and methane levels and decreasing the pH²⁹. Changes to gut conditions after rapid proliferation of microbes post-feeding may be less favourable for many microbes, potentially contributing to the consequent reduction in the bacterial population late in the active phase despite food still being available and probably ingested. Changes to gut conditions may therefore reinforce microbial rhythms by ensuring that they are only triggered once at first food intake after fasting. This pattern is supported by microbial dynamics in wild meerkats, where bacterial load peaks after dawn foraging, but not in the late afternoon prior to sunset when meerkats forage a second time²⁶.

Whilst we focus here on mechanisms underpinning interactions between gut bacteria and the innate immune system, gut microbial rhythms also trigger molecular cascades that regulate metabolism and hormone production across the day^{7,9,14,41,44,60}. Circadian changes to some bacterial metabolites, such as short-chain fatty acids (SCFAs) and bile acids, are particularly important for upregulating lipid metabolism and absorption during the active phase^{7,11}. The bacterial compounds lipopolysaccharide (LPS) and flagellin, which are found in the cell walls of gram-negative bacteria, have also been implicated in the diurnal dynamics of body weight and corticosterone synthesis in mice⁴⁴. Notably, these pathways are mediated by the host innate immune system, with LPS and flagellin being detected by Toll-like receptors (TLRs)⁴⁴. The gut microbiota

also generate neuro-active metabolites such as tryptophan and serotonin, therefore oscillations of the gut microbiota may cause circadian rhythms in neuro-active compounds that can directly communicate with the nervous system and influence cognitive processes and stress responses⁹. However, the link between microbial oscillations and circadian behaviour remains speculative.

Avenues of future research

A major objective for future investigations on the daily rhythms of the gut microbiome is to quantify their prevalence and strength across natural populations. Currently, our knowledge on gut microbial oscillations largely stems from laboratory mice, whilst our understanding of circadian rhythms of wildlife is largely restricted to behaviour⁶¹. To understand the adaptive significance of circadian rhythms and their entrainment by the gut microbiota, we need to move the study of circadian rhythms to natural populations. This is particularly true given the importance of food intake on system-wide circadian rhythms, because feeding times of captive animals generally do not mirror foraging regimes of wild counterparts. As such, whilst studies on captive animals may help disentangle drivers of circadian rhythms, they may not actually reflect circadian rhythms in nature or capture how the interactions between multiple environmental and social cues act to entrain rhythms. Below we briefly outline how integrating gut microbiome and circadian rhythm research in wildlife can advance several outstanding questions in ecology (**Fig. 3**).

1) *The adaptive significance of gut microbial oscillations*

In which evolutionary contexts do we expect the evolution of gut microbial oscillations, and when would we expect food intake and the gut microbiome to entrain host immunity? Based on data from murine models, one predicts that food intake, metabolic requirement, and pathogen exposure are synchronised to peak at the start of the active phase (i.e., at dusk for mice). Such correlation between feeding, metabolic and immune requirements is expected to be the norm, given that feeding introduces both nutrients and pathogens to the gut. Hence, hosts appear to have co-opted the gut microbiota to mediate both metabolic and innate immune function simultaneously.

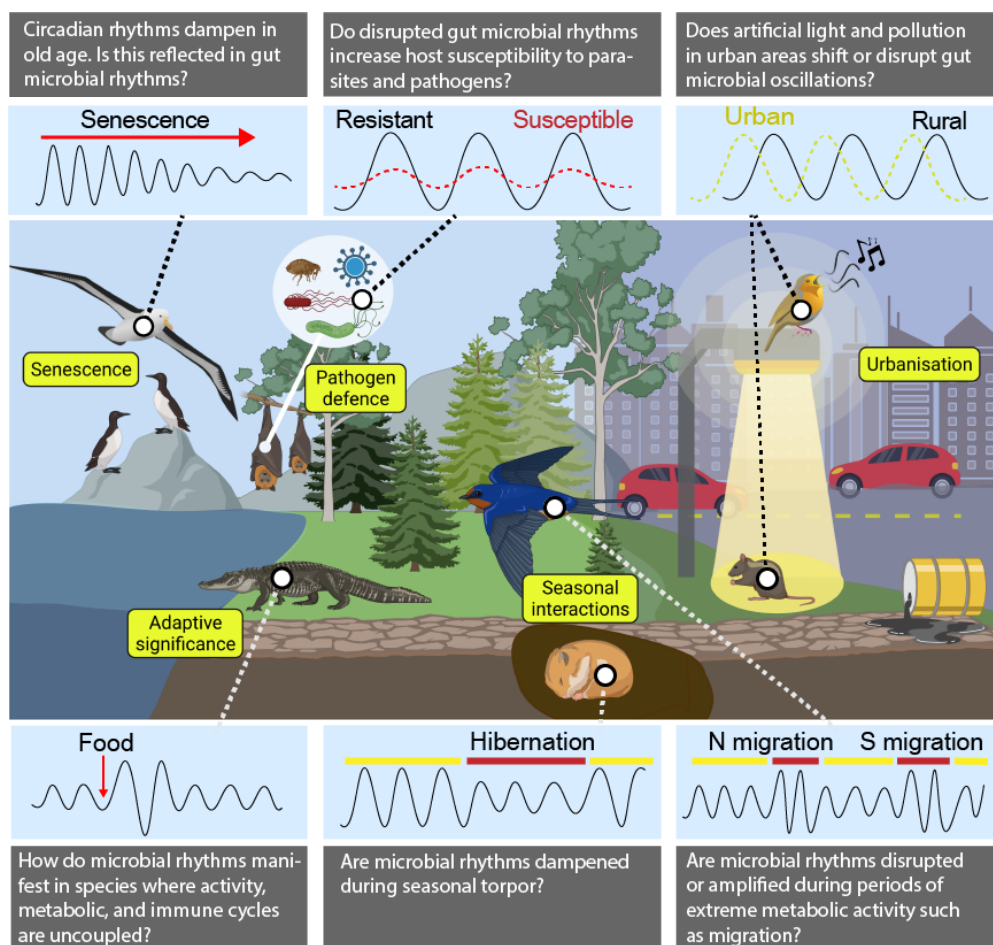


Figure 3) The involvement of food intake and gut microbial oscillations in mediating both metabolism and innate immunity raises several questions regarding their function across a range of ecological contexts. The figure visualizes the expected rhythms of mucosal commensals like under six ecological contexts.

Yet, microbiota-independent mechanisms may be expected in species where feeding, metabolic, and immune requirements are uncoupled. For example, ectotherms exhibit circadian rhythms in body temperature and activity^{62–64}, and have some level of circadian cycles in metabolism⁶⁵ and immunity^{66,67}, but feeding patterns are often not circadian (e.g. for large reptiles such as snakes and crocodiles). In these cases, does the gut microbiota undergo diurnal oscillations, and is the entrainment of innate immunity completely independent of the gut microbiota? Given findings from laboratory mice, one might expect that diurnal rhythms of the gut microbiota to be strongest after feeding (**Fig. 3**). In social or gregarious animals, microbiota are often shared and pathogen exposure increases^{68–70}, providing another example where pathogen exposure may not be closely correlated with food intake. Therefore, peaks in pathogen exposure or activation of immunity may not be limited to mealtimes. This raises the question as to whether social animals have altered circadian rhythms in immune function compared to solitary species, and whether such adaptations are mediated by the gut microbiota.

Considering microbial rhythms in the context of metabolic and immune requirements throughout the day may provide a useful framework to predict the strength and the functional role of gut microbial oscillations that goes beyond light and temperature cycles. Nevertheless, investigating microbial oscillations across latitudes

and in environments with extreme light or temperature conditions (e.g. cave, arctic, or desert animals) will aid our understanding of the circumstances under which microbial rhythms occur. For example, gut microbiome rhythms in meerkats may be particularly strong due to the arid environment they inhabit²⁶, which is characterised by steep temperature differentials between day and night. This extreme fluctuation induces nightly torpor in small desert mammals⁷¹, and whilst it is unclear whether meerkats undergo a similar process, it might be expected that extreme temperatures exert metabolic constraints that both influence and are influenced by the gut microbiota.

2) Gut microbial rhythms and pathogen defence

In mice, gut microbial oscillations increase host resistance to gut pathogens such as *Salmonella* during the active phase by triggering the release of AMPs into the gut¹³. Reducing the abundance of mucosal commensal SFB increases host susceptibility to *Salmonella* infection and also removes circadian rhythms in susceptibility¹³, demonstrating that the rhythmic activity of gut mucosal commensals is a key mechanism governing microbiome-mediated pathogen defence. Testing for associations between the abundance and rhythmicity in mucosal commensals and infection status may therefore be a more effective method of uncovering the link between the gut microbiota and pathogen susceptibility than focusing on overall gut microbial diversity. Arrhythmic gut microbial communities have been linked to disease in humans³⁴, and therefore it might be expected that individuals with disrupted or dampened gut microbiota rhythms are more susceptible to infection (**Fig. 3**).

Circadian rhythms in animal susceptibility and pathogen reproduction and transmission are well documented^{18,72}, with hosts and parasites having coevolved defensive and offensive rhythms, respectively. Yet the role of the gut microbiota in mediating infection outcomes is unclear and many questions remain. Do gut microbial rhythms protect the host against a broad range of pathogens, or are they only effective for specific gut pathogens? Microbial rhythms control the release of AMPs, which are effective against a wide range of pathogens including bacteria, fungi and viruses⁷³. Thus, it is likely that microbial rhythms protect the host against a broad range of pathogenic agents entering the gut. However, the gut is not the only entry point of pathogens and it remains unknown whether microbial rhythms also play a role in pathogen defence more generally.

Even less explored is the connection between gut microbial oscillations and adaptive immunity, which is an essential pillar of resistance against recurring parasitic challenges in jawed vertebrates⁷⁴. Gut microbiome disruption impairs antibody responses in mice⁷⁵, and gut microbial metabolites influence the expression of the mammalian circadian clock gene *Per2*^{7,76}, which is responsible for mounting both innate and adaptive responses to infection⁷⁷. Investigating which components of the gut microbiota affect the expression of clock genes and in which ecological contexts may identify the mechanisms by which the microbiota shape pathogen defence.

3) Interactions between circadian and seasonal rhythms

Seasonal shifts in gut microbiome composition and function have been well described^{78–83}, but emerging evidence suggests that changes to function may be mediated via increasing or decreasing the amplitude of host circadian rhythms⁷⁶. In giant pandas, seasonal switching of diet from bamboo leaves to shoots causes an increase in the bacterial metabolite butyrate in the gut microbiota, and when transferred to mice, this causes the upregulation of clock gene *Per2*, which increases lipid production and fat deposition in spring⁷⁶. This study does not measure gut microbial oscillations directly however, and it is unclear whether microbial rhythms also increase in amplitude during spring. Yet, the findings suggest that seasonal cycling of the gut microbiota functions via interacting with host circadian rhythms.

In addition to seasonal diet switches, seasonal changes to life history stages that involve metabolic restructuring such as migration, hibernation, and even reproduction may also be paired with changes to the amplitude of their gut microbial rhythms (**Fig. 3**). Shifts in the gut microbiota during hibernation adaptively lower metabolism and recycle nitrogen^{84–86}, yet it remains unknown how these functional changes interact with or are mediated by diurnal rhythms. Seasonal switches in strategies may take more unpredictable and fascinat-

ing forms. For instance, the circadian rhythms of some arctic-breeding shorebirds become uncoupled from environmental cues during breeding due to pressures of incubation and predators, with social cues becoming the dominant form of entrainment⁸⁷. How might such changes be reflected in the gut microbiome?

4) *The role of microbial oscillations in animal senescence*

Understanding rates of animal senescence is crucial for predicting demographic processes, and the mechanisms underpinning senescence is an active area of research^{88–90}. Whilst ecological research on animal senescence has focused on changes to immunity⁹¹, telomeres⁹², stress hormones⁹³, and gut microbiota composition²⁴, research on humans and primates have demonstrated that an additional characteristic of ageing is the dampening of circadian rhythms^{93–95}, leading to disrupted sleep-wake cycles and physiology. Changes to gut microbiome rhythmicity with age are implicated in this process^{96–98}.

The involvement of microbial oscillations in senescence suggests that microbial oscillations should decline in old age (**Fig. 3**), yet this has rarely been tested in either captive or wild settings. In wild meerkats, there was little evidence for microbial senescence with old meerkats demonstrating microbial rhythms that were as strong as younger individuals²⁶. However, it is unclear whether meerkats are a good model system for ageing because only dominant individuals reach old age, and these individuals enjoy the benefits of group living and alpha status, potentially slowing senescence⁸⁸. Exploiting systems with high survival rates, such as seabirds, may help clarify this question.

5) *The effect of urbanisation on gut microbial rhythms*

Urbanization is rapidly altering wildlife environments and activity patterns. Medium to large mammals are becoming more nocturnal to escape human disturbance⁹⁹ whilst small mammals that are normally nocturnal are active around the clock in urban areas¹⁰⁰. Artificial light is causing birds and bats to extend and reduce their activity periods, respectively^{101,102}, and is also associated with altered physiology and immune responses^{103–105}. Urban habitats also offer different diets, with many urban animals becoming scavengers or being provisioned by humans¹⁰⁶, and are associated with pollution¹⁰⁷ and higher pathogen diversity¹⁰⁸ than natural habitats. How these shifts in behaviour and exposure to pathogens and pollution are affecting health for both humans and wildlife via circadian mismatching is an outstanding question of urgent need of attention^{109–111}, given ongoing and rapid human encroachment into natural habitats.

How might urbanization affect the gut microbiota, and what are the consequences for wildlife health? Accumulating evidence from across phylogenetically-diverse species suggests that urbanization generates a more ‘humanized’ gut microbiota, with a higher proportion of opportunistic pathogens^{112–117}. Yet, whether urbanisation is altering microbial rhythms is still unclear. In humans, urbanisation is associated with a loss of seasonal rhythms in the gut microbiota^{78,118}, indicating that biological rhythms might be disrupted by urban lifestyles. Wildlife health may be negatively affected by urbanisation and artificial light if changes to activity patterns (e.g., timing of feeding) or altered diet disrupts gut microbial oscillations (**Fig. 3**). Constant light or dark leads to a loss of microbial rhythms in both chickens³² and mice³⁵, and this alteration is at least in part due to sensory signalling from the brain rather than changes to feeding times¹¹⁹. Diets high in fat also dampen microbial rhythms and thereby lead to dysbiosis – an imbalance in the microbiome that has negative health outcomes^{39,120,121}. Together, these indicate that urbanisation may alter microbial rhythms via multiple mechanisms.

Studying gut microbial rhythms in wildlife

Field ecologists face a number of challenges that may have acted to delay the integration of circadian rhythms into field ecology, such as limited availability of study animals across a 24-hour period. However, as long as individuals can be sampled over the morning and preferably also the afternoon (e.g.²⁶) then many questions on microbial oscillations can be tackled. Indeed, the period after the start of the active phase is often when the largest changes occur and therefore reporting just this part of the diurnal cycle is informative. Whilst a longitudinal study design is preferable, the strength of microbial oscillations reported so far suggest that cross-sectional study designs may also have sufficient statistical power to detect predictable microbial

oscillations. For example, in meerkats, sensitivity analyses that restricted analysis to only 20 (cross-sectional) samples per hour during daylight hours (total n [?] 240) still detected the same microbial oscillations reported with the full dataset (total n [?]1100) ²⁶.

A common obstacle in identifying meaningful associations between the gut microbiota and host physiology is the sheer diversity of gut microbial communities and available physiological markers. Future studies on non-model organisms may therefore benefit from focusing on the key taxa and physiological markers identified from experimental studies to date. Findings from mice indicate that mucosal-associated commensals, in particular SFBs which are found across vertebrates¹²², play a fundamental role in mediating physiological homeostasis and immunomodulation by attaching to the intestinal epithelium at the start of the active phase. The identity and oscillations of these specific commensals are therefore likely to be disproportionately important for identifying associations between the gut microbiota and host physiology in natural populations. In addition, gut sIgA and antimicrobial peptides (AMPs) are two facets of immunity that have been strongly implicated in circadian interactions with the gut microbiota, whilst the microbial metabolites butyrate, flagellin, and LPS have been implicated in circadian interactions that regulate metabolic signalling pathways. Applying these physiological markers may therefore be particularly suitable for determining whether mechanisms identified in laboratory systems have broad biological relevance for natural populations.

Conclusions

Microbial diurnal rhythms are likely widespread and pivotal for mediating physiological homeostasis and pathogen defence, yet their study has been neglected in wild populations. Whilst the mechanisms underpinning the circadian crosstalk between the host immune system and the gut microbiota is still an active area of research, key commensal taxa that rhythmically attach to the host intestinal epithelium play a critical role in triggering the upregulation of innate immunity and metabolism at the start of the active phase. A future focus on how gut microbiomes change over the day across diverse host species will advance our understanding of their function and adaptive significance, and may illuminate the processes underpinning the breakdown of gut microbiota function during infection, senescence, or global change.

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