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

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Abstract

Daily light-dark cycles shape the physiology and activity patterns of nearly all organisms. Recent evidence that gut microbial oscillations synchronise circadian rhythms in host immunity and metabolism indicate that diurnal dynamics is a crucial component of microbiome function. However, their prevalence and functional significance are rarely tested in natural populations. Here we summarize the hallmarks of gut microbiota oscillations and the mechanisms by which they synchronise rhythms in host immunity and metabolism. We discuss the consequences for diverse biological processes such as host pathogen susceptibility and seasonal switches in metabolism, and outline how the breakdown of these circadian interactions, for example during senescence and as a consequence of urbanisation, may affect wildlife infection risk and disease. 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 and provide a rare opportunity to generalise gut microbial responses across species. An improved understanding of how microbial rhythms manifest in wildlife is essential to fully comprehend their ecological significance.

Introduction

Circadian rhythms describe the synchronization of multiple biochemical and physiological processes across a 24-hour cycle, allowing organisms to anticipate and respond to predictable biotic and abiotic conditions across the day [1]. 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 expression [6]. 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 clock [15]. The far-reaching effects of food intake on host circadian rhythms are mediated by the gut microbiota, which periodically interact with the host to regulate rhythms in both innate immunity and metabolism [10,11,13], and potentially the gut-brain axis [9]. 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]. Recently, gut microbial dynamics over the course of a day has 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 mice [11]. Gut microbial oscillations have been identified in several species in captivity, from birds and mammals to fish [13,31,32,34], and these oscillations are often strong enough to mask identify effects [29,32,35]. Similar findings were recently demonstrated in a wild population of meerkats [26], which tend to forage in the early morning and again in the evening (Fig. 1a). Peaks in foraging corresponded with shifts in many microbial taxa, with *Clostridium* in particular peaking strongly at dawn and declining in the afternoon (Fig. 1b). These shifts between sunrise and sunset structured the entire ecological community (Fig. 1c). Whereas time of feeding and diet largely govern these microbial rhythms[36–39], genetically-coded immune regulation by the host is a crucial contributing factor: mice lacking clock genes have disrupted gut microbial rhythms [40]. Together, these studies suggest that microbial oscillations may be widespread across host species and that they are likely in response diurnal shifts in food intake and clock-encoded rhythms in host physiology, rather than self-sustaining circadian rhythms.

Seasonal rhythms in the gut microbiota are known to modulate energy metabolism [41–43], and potentially pathogen susceptibility [41]. Consequently, short-term gut microbial oscillations across the day are likely equally important to biological function [7,8,45–48]. The disruption of gut microbial rhythms, for example due to jet lag in humans, leads to increased risk of metabolic disease, gut inflammation, and pathogen susceptibility [12,13,34]. Circadian interactions between the gut microbiota and host immunity are of particular relevance for evolutionary ecologists because pathogens are disproportionally important for mediating host fitness and evolutionary trajectories in natural populations [49–51], with pathogen defence suggested to be the principal evolutionary advantage of the gut microbiome [52]. Understanding the role of gut microbial rhythms in mediating host immune and metabolic homeostasis in natural populations would elucidate the functional importance and adaptive significance of gut microbiome rhythms for individual fitness and, more generally, wildlife health.

In summary, diurnal oscillations in the gut microbiota are known to be strong, widespread across model species, and have profound biological functions for the host. As such, it is important for research on host-microbe interactions in wildlife to account for these daily dynamics. With the aim to encourage 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 wildlife 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 and health in wildlife.

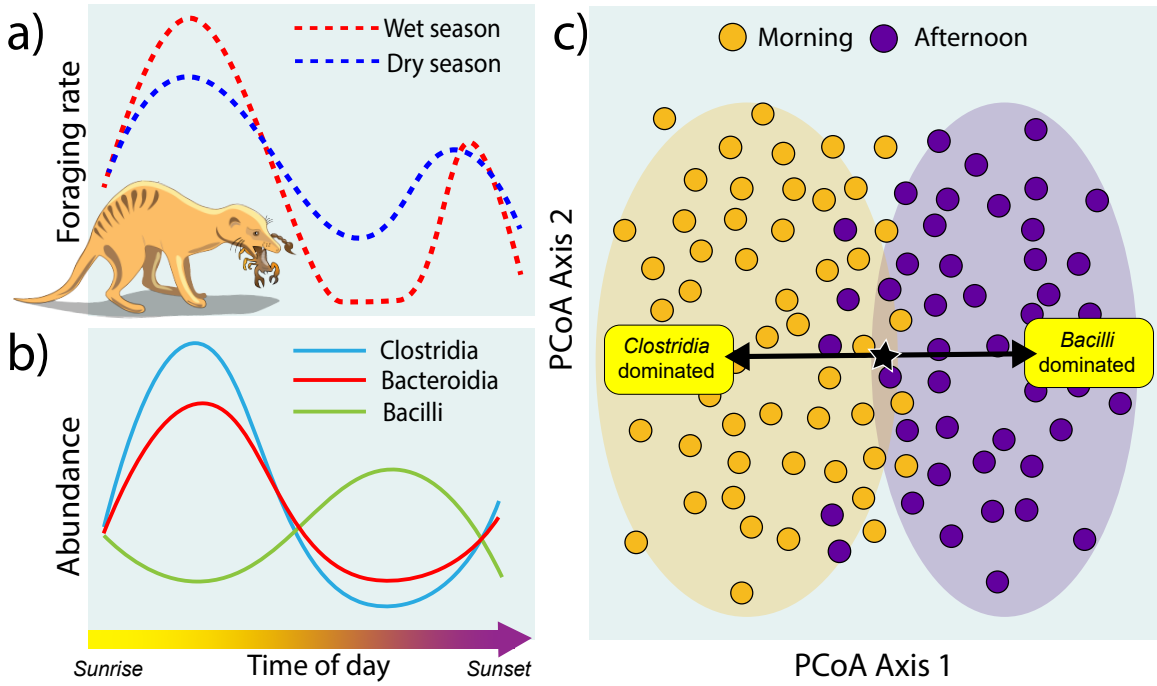

Figure 1: *Conceptual figure representing meerkat foraging schedules and corresponding shifts in the gut microbiota, based on findings from* [26]*. a) Meerkats forage mostly in the morning and again before dusk to avoid the midday heat, although during the cool dry season they can also forage through the entire day; b) Oscillations in selected taxa, highlighting peaks in Clostridia and Bacteroidia in the morning, and Bacilli in the afternoon; c) Diurnal shift in many taxa, especially Clostridia, cause community-wide structuring of the gut microbiota according to time of day, as represented by a PCoA plot.*

Hallmarks of gut microbial rhythms

Gut microbial oscillations have been identified in a wide range of species, including humans [34], meerkats [26], mice [13], cows [29,33], fish [31], and chickens [30,32] - even host-associated microbiota of zooplankton undergo diurnal cycles [53]. The proportion of gut members that show oscillating behaviour varies between studies and species, with the proportion of common taxa being identified as oscillators ranging between ~35% (humans) and ~80% (meerkats) [12,26,34,54]. In industrialized human societies, population-wide gut microbial oscillations identified from cross-sectional studies appear to be weak [34], and explain only a modest amount of variation in gut microbiota composition. In other species, diurnal rhythms of the gut microbiota are strong and dominate over individual identity effects [26,29,32,35], suggesting natural variation in the strength of microbial oscillations across species.

Nevertheless, there are some similarities in diurnal gut microbial dynamics across the mammalian species studied thus far. 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 phase [11]. This pattern is supported by increased number of bacteria in the gut more generally during the active phase [40]. Similar findings were indicated for wild meerkats [26], with bacterial load increasing after dawn, although dynamics in the rest phase were not measured. In humans, the number of bacterial species in faecal samples peaks at midday, potentially indicating a similar pattern [34]. 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 suggest that at least some aspects of diurnal dynamics of gut microbes may be partly conserved across mammal 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, some 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], presumably due to food availability driving population increases. 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

How and why do gut microbiota oscillations mediate host immunity and metabolism, and what are regulatory mechanisms that entrain cycles? 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 exposure [46]. Whilst this field of research is in its infancy, several 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. 2).

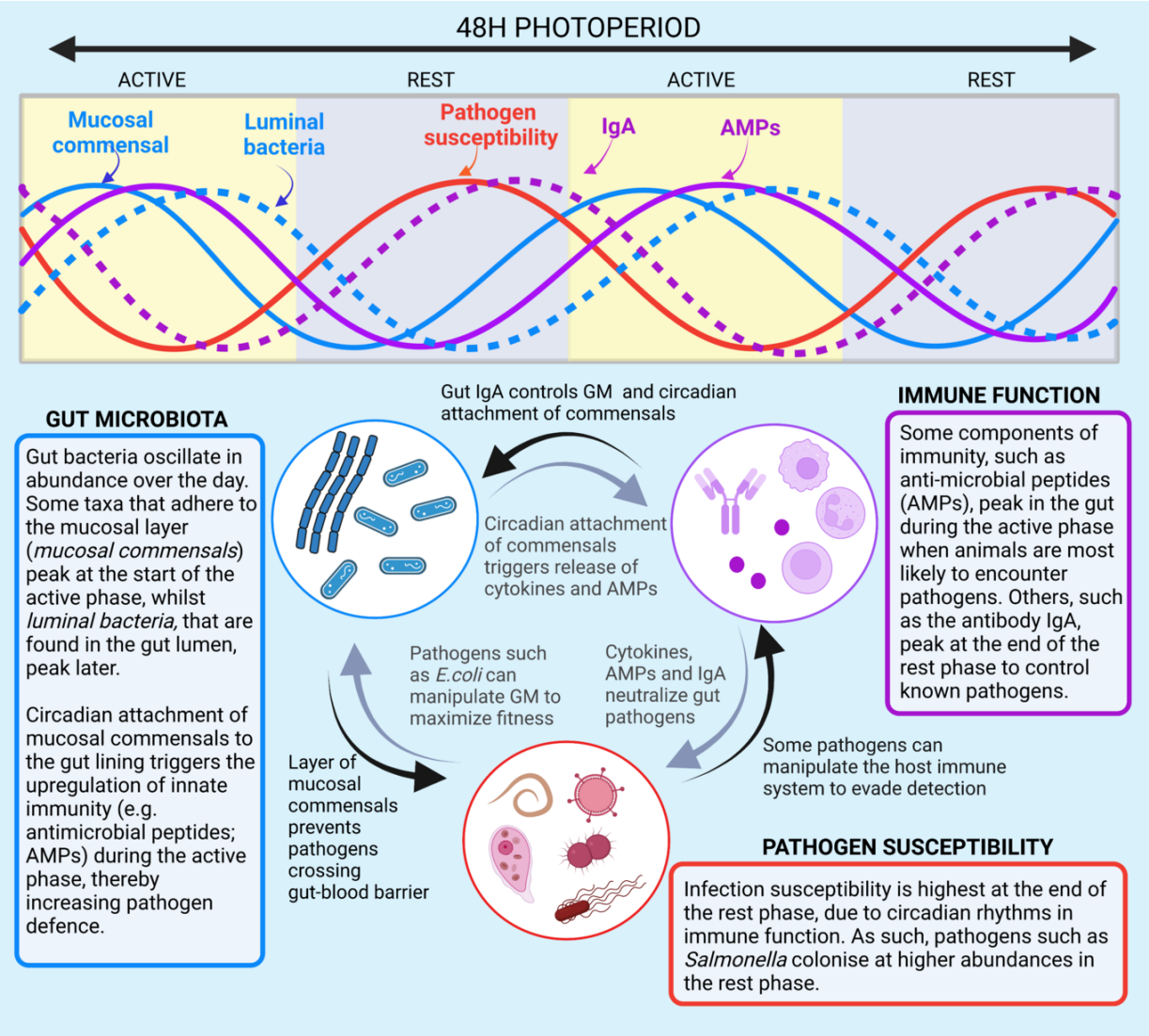

Figure 2: *Summary of the circadian crosstalk between gut microbes and components of the host immune system, and consequences for host pathogen susceptibility, as characterised in laboratory mice* [10,13,55]. *GM = Gut microbiota.*

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 humans [46]. 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 AMPs [13], that protect the host against a broad range of pathogens during feeding [13], and also feed back to control gut microbial rhythms [11]. Mucosal commensals also trigger the release of major histocompatibility complex (class II)-mediated cytokines [10], which, whilst part of the adaptive arm of the vertebrate immune

system, act to modulate the innate immune response [55]. Innate immune protection does not last the entire active phase, but begins to decline 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 mice [56].

Maintaining a high level of immune control across a 24-hour period is energetically expensive, and excessive inflammation causes immunopathology [57]. 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. 3b). This leads to higher host susceptibility to pathogens during the rest phase [58], with pathogens such as Salmonella colonising at higher abundances compared to the active phase [16]. The downregulation of innate immunity in the gut is preceded by the detachment of mucosal commensals from the mucosal layer via mechanisms which remain unclear to date, thereby triggering a reduction in 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] (Fig. 3b). Perhaps to protect the integrity of the epithelial layer from feeding bacteria, the intestinal mucosal layer thickens during the rest phase [11].

Despite higher infection susceptibility during the rest phase, animals are not altogether undefended. A key gut antibody, secretory (s)IgA, is upregulated during sleep [59] (Fig 3b). SIgA is secreted by mucosal membranes and is present across all mammals and bird species [60,61]. It acts as bridge between innate and adaptive immunity, being able to distinguish between gut commensals and non-commensals [62]. During the rest phase, upregulated sIgA neutralises non-commensals and their toxins, which are tolerated during the active phase. Thus, IgA ensures that any potential pathogens introduced and proliferating during the active phase are neutralized. Another function of sIgA is to bind to beneficial mucosal commensals and control their adhesion to the mucosal layer [62,63], and it is therefore a key agent in triggering the circadian cycles of the gut microbiota at the start to the active phase [59]. 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 levels [59] in order to increase tolerance to gut bacteria during the active phase.

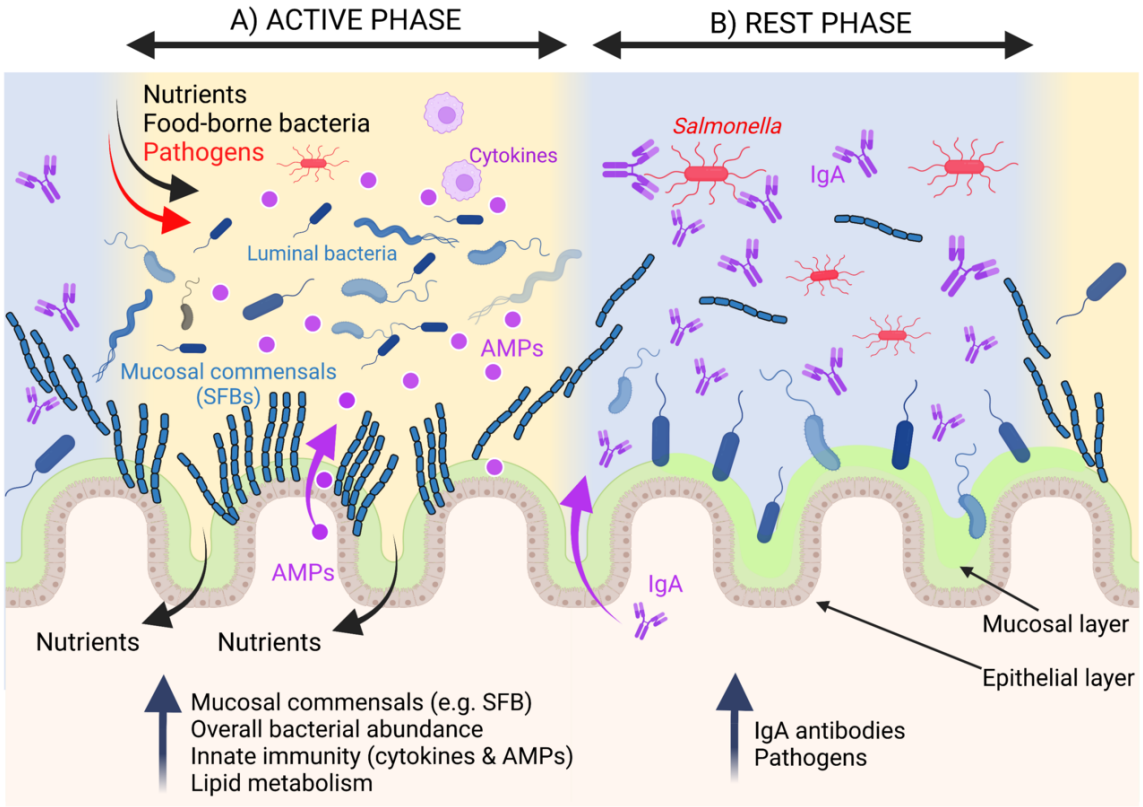

Figure 3: *Diurnal rhythms and gut geography of the microbiota, host immunity and pathogen abundance across a) the active phase and b) the rest phase, as characterised in laboratory mice* [10,13,55]. *SFB = Segmented filamentous bacteria; AMP = Anti-microbial protein.*

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 CO_2 and methane levels and decreasing the pH [29]. 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 time [26].

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 [7,9,14,45,48,64]. 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 mice [48]. Notably, these metabolic pathways are mediated by the host innate immune system, with LPS and flagellin being detected by Toll-like receptors (TLRs) [48,65]. Upon contact with LPS, TLRs initiate the release of α-defensin [66], which increases mucosal defences against

ingested bacteria during feeding [67]. In addition, 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 HPA axis, thereby potentially influencing cognitive processes, stress responses and behaviour [9]. 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 behaviour [68]. 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 [69]. This is because feeding times and diet of captive animals generally do not mirror foraging regimes of wild counterparts, and, together with microbial transmission between captive animals and humans, leads to captive animals having perturbed and 'humanised' gut microbiotas [70,71]. Furthermore, many complex ecological processes are difficult or impossible to replicate in captivity. As such, whilst studies on captive animals can disentangle drivers of circadian rhythms, they may not actually reflect circadian rhythms in nature. A first step is to simply account for time of day samples were collected in analyses. Below we briefly outline how integrating gut microbiome and circadian rhythm in wildlife research can advance several outstanding questions in ecology (Fig. 4).

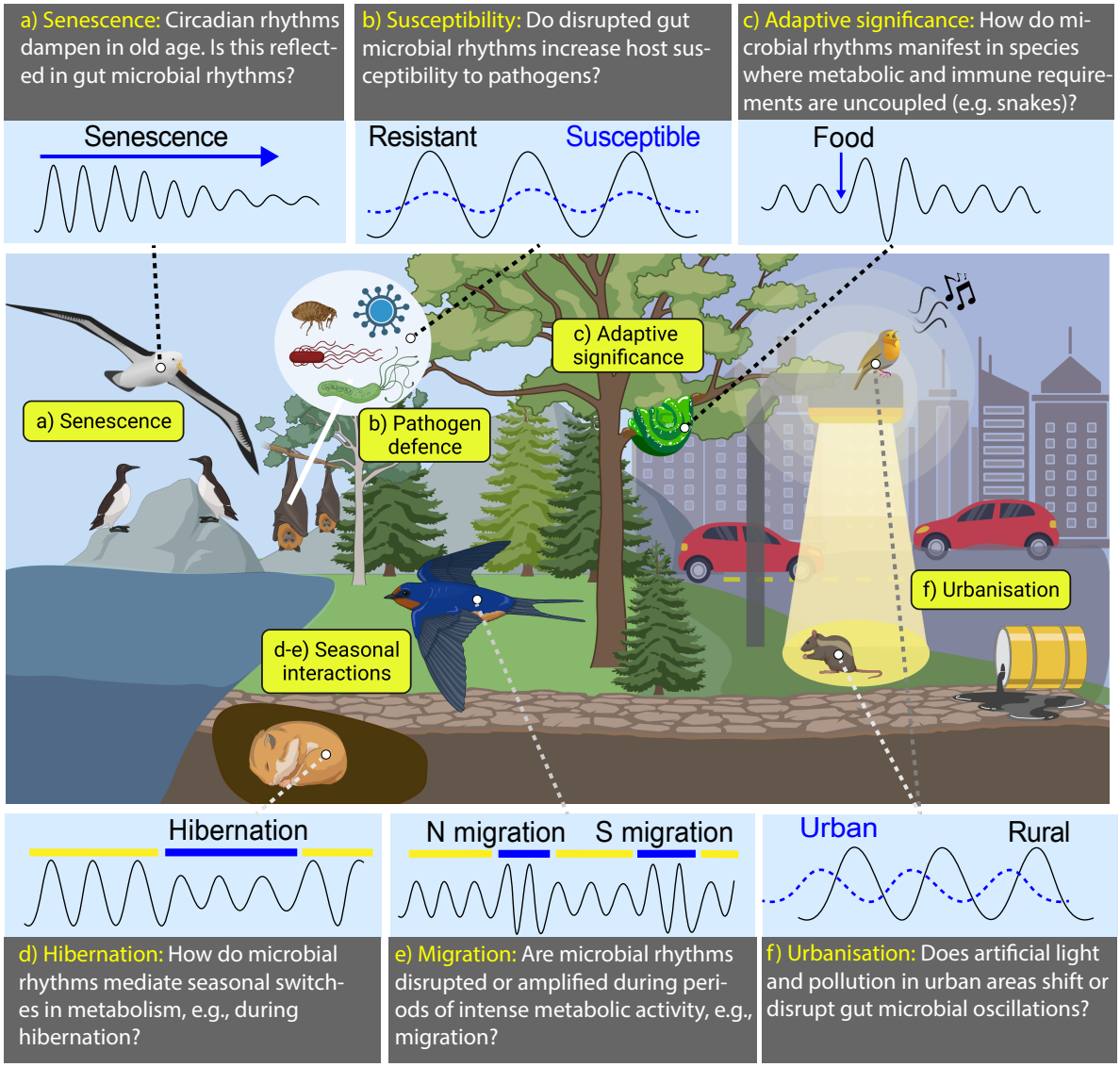

Figure 4: *The involvement of food intake and gut microbial oscillations in mediating both metabolism and innate immunity raises several questions regarding their function across a range of ecological contexts. The figure visualizes the predicted rhythms of mucosal commensals in the context of: a) senescence; b) pathogen defence; c) adaptive significance; d) hibernation; e) migration (N = north, S = south); and e) urbanisation.*

1) 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 [72–74]. Whilst research on animal senescence has focused on changes to immunity [75], telomeres [76], stress hormones [77], and gut microbiota composition [24], research on humans and primates have demonstrated that an additional characteristic of ageing is the dampening of circadian rhythms [77–79], leading to disrupted sleep-wake cycles and physiology. Changes to gut microbiome rhythmicity with age are implicated in this process [80–82].

The involvement of microbial oscillations in senescence suggests that microbial oscillations should decline in old age (Fig. 4a), 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 individuals [26], despite old (and generally dominant) individuals generally losing body condition [83] and having higher rates of telomere loss [84]. However, because only dominant individuals tend to reach old age in this species, physiological senescence may be mitigated by the benefits of group living and alpha status, which decrease mortality risk [72]. Exploiting systems with high survival rates that have commonly been used to model senescence and demography, such as seabirds [85,86], may help clarify this question.

2) Gut microbial rhythms and pathogen defence

In mice, gut microbial oscillations reduce host susceptibility to gut pathogens such as *Salmonella* during the active phase by triggering the release of AMPs into the gut [13]. Reducing the abundance of mucosal commensal SFB increases host susceptibility to *Salmonella* infection and also removes circadian rhythms in susceptibility [13], 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 alone. Arhythmic gut microbial communities have been linked to disease in humans [34], and therefore it might be expected that individuals with disrupted or dampened gut microbiota rhythms are more susceptible to infection (Fig. 4b).

Circadian rhythms in animal susceptibility and pathogen reproduction and transmission are well documented [18,87], with hosts and pathogens having coevolved defensive and offensive rhythms, respectively [18]. Nevertheless, many fundamental questions remain entirely unanswered: 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 viruses [88]. 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 again recurring pathogenic challenges in jawed vertebrates [89]. Ample evidence that adaptive and innate immunity interact to regulate the gut microbiota exist. For example, the co-expression of MHC class II molecules together with LPS-activated TLR4 enhances the production of AMPs [90]. In addition, gut microbial metabolites influence the expression of the mammalian circadian clock gene *Per2* [7,91], which is responsible for mounting both innate and adaptive responses to infection [92]. The maintenance of gut microbial homeostasis, which is crucial for effective pathogen defence, might therefore represent a joint venture of adaptive and innate immunity [93]. Still, information on whether gut microbial oscillations synergise with innate and adaptive immunity are lacking.

3) 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 findings 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 processes and immune activity 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.

Conversely, microbiota-independent mechanisms may be expected in species where metabolic requirements are not circadian, as well as in species where metabolic requirements and pathogen exposure are uncoupled. For example, ectotherms exhibit circadian rhythms in body temperature and activity [94–96], and have some

level of circadian cycles in metabolism [97] and immunity [98,99], but feeding patterns are often not circadian (e.g. for large reptiles such as snakes and crocodiles that are infrequent feeders). 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. 4c). However, evidence from Burmese pythons suggests that shifts in the post-prandial gut microbiota last for many days [100], although this study did not record time of day samples were harvested, therefore it is unknown whether feeding shifted microbial rhythms (if any) as well as composition.

Species where pathogen exposure is not closely correlated with timing of feeding provide an addition example where mechanisms regulating circadian rhythms in immunity are microbiota-independent. In social or gregarious animals, microbiota are often shared [101] and pathogen exposure is high [102–104]. Peaks in pathogen exposure or activation of immunity may therefore 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 particular strong due to the arid environment they inhabit [26], which is characterised by steep temperature differentials between day and night. This extreme fluctuation induces nightly torpor in small desert mammals [105], 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.

4) Interactions between circadian and seasonal rhythms

Many species undergo striking changes in life-history strategies between seasons, with hibernation and long-distance migration representing two of the most extreme life-history responses to seasonal changes in climate. Seasonal shifts in gut microbiome composition and function have been well described [43,106–110], but emerging evidence suggests that changes in function may be mediated via increasing or decreasing the amplitude of host circadian rhythms [91]. 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 spring [91]. 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 hibernation (Fig. 4d), and migration (Fig. 4e), and even reproduction may also be paired with changes to the amplitude of their gut microbial rhythms. Shifts in the gut microbiota during hibernation adaptively lower metabolism and recycle nitrogen [42,44,111], 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 fascinating 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 entrainment [112]. How might such changes be reflected in the gut microbiome?

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 disturbance [113], whereas small mammals that are normally nocturnal are active around the clock in urban areas [114]. Artificial light is causing birds and bats to extend

and reduce their activity periods, respectively [115,116], and is also associated with altered physiology and immune responses [117–119]. Urban habitats also offer different diets, with many urban animals becoming scavengers or being provisioned by humans [120], and are associated with pollution [121] and higher pathogen diversity [122] 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 [69,123,124], given ongoing and rapid human encroachment into natural habitats.

Urbanised environments offer the rare opportunity to experimentally test the impact of changes to abiotic (e.g., light, temperature) and biotic (e.g., diet, pathogen pressure) condition on microbial circadian rhythms compared with wildlife inhabiting natural environments [125]. How might the interacting pressures faced by urban-adapted species 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 [126–131]. 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 [106,132], 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 patters (e.g., timing of feeding) or altered diet disrupts gut microbial oscillations (Fig. 4e). Constant light or dark leads to a loss of microbial rhythms in both chickens [32] and mice [35], and this alteration is at least in part due to sensory signalling from the brain rather than changes to feeding times [133]. Diets high in fat also dampen microbial rhythms and thereby lead to dysbiosis – an imbalance in the microbiome that has negative health outcomes [39,134,135]. 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]), 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 can be 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]. Notably, a huge array of wildlife gut microbial datasets exists, and where the time of collection is known, these can be reanalysed to further our understanding of microbial diurnal rhythms, with comparative studies across species being particularly informative.

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 vertebrates [136], 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 disproportionally important for identifying associations between the gut microbiota and host physiology in natural populations. In addition, gut sIgA and 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 and innate immunity. 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 metabolism and at innate immunity the start of the active phase. A future focus on how gut microbiomes change over the day across host species with diverse biology (e.g., ectotherms, hibernating animals) and ecology (e.g., social animals, urban wildlife) 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, and global change.

References

1. Yerushalmi, S. & Green, R. M. Evidence for the adaptive significance of circadian rhythms. *Ecology letters* **12**, 970–981 (2009).

2. Segers, A. & Depoortere, I. Circadian clocks in the digestive system. *NATURE REVIEWS GASTROENTEROLOGY & HEPATOLOGY* **18**, 239–251 (2021).

3. Lewis, P., Oster, H., Korf, H. W., Foster, R. G. & Erren, T. C. Food as a circadian time cue—evidence from human studies. *Nature Reviews Endocrinology* **16**, 213–223 (2020).

4. Bell-Pedersen, D. *et al.* Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nature Reviews Genetics* **6**, 544–556 (2005).

5. Buhr, E. D., Yoo, S.-H. & Takahashi, J. S. Temperature as a universal resetting cue for mammalian circadian oscillators. *science* **330**, 379–385 (2010).

6. Xiao, Y., Yuan, Y., Jimenez, M., Soni, N. & Yadlapalli, S. Clock proteins regulate spatiotemporal organization of clock genes to control circadian rhythms. *Proceedings of the National Academy of Sciences* **118**, (2021).

7. Leone, V. *et al.* Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell host & microbe* **17**, 681–689 (2015).

8. Frazier, K. & Chang, E. Intersection of the Gut Microbiome and Circadian Rhythms in Metabolism. *TRENDS IN ENDOCRINOLOGY AND METABOLISM* **31**, 25–36 (2020).

9. Teichman, E., O'Riordan, K., Gahan, C., Dinan, T. & Cryan, J. When Rhythms Meet the Blues: Circadian Interactions with the Microbiota-Gut-Brain Axis. *CELL METABOLISM* **31**, 448–471 (2020).

10. Tuganbaev, T. *et al.* Diet diurnally regulates small intestinal microbiome-epithelial-immune homeostasis and enteritis. *Cell* **182**, 1441-1459. e21 (2020).

11. Thaiss, C. A. *et al.* Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* **167**, 1495-1510. e12 (2016).

12. Thaiss, C. A. *et al.* Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* **159**, 514–529 (2014).

13. Brooks, J. *et al.* The microbiota coordinates diurnal rhythms in innate immunity with the circadian clock. *CELL* **184**, 4154-+ (2021).

14. Choi, H., Rao, M. & Chang, E. Gut microbiota as a transducer of dietary cues to regulate host circadian rhythms and metabolism. *NATURE REVIEWS GASTROENTEROLOGY & HEPATOLOGY* **18**, 679–689 (2021).

15. Tognini, P. *et al.* Reshaping circadian metabolism in the suprachiasmatic nucleus and prefrontal cortex by nutritional challenge. *Proceedings of the National Academy of Sciences* **117**, 29904–29913 (2020).

16. Bellet, M. M. *et al.* Circadian clock regulates the host response to Salmonella. *Proceedings of the National Academy of Sciences* **110**, 9897–9902 (2013).

17. Panda, S. Circadian physiology of metabolism. *Science* **354**, 1008–1015 (2016).

18. Westwood, M. L. *et al.* The evolutionary ecology of circadian rhythms in infection. *Nature ecology & evolution* **3**, 552–560 (2019).

19. Spoelstra, K., Wikelski, M., Daan, S., Loudon, A. S. & Hau, M. Natural selection against a circadian clock gene mutation in mice. *Proceedings of the National Academy of Sciences* **113**, 686–691 (2016).

20. Shapira, M. Gut Microbiotas and Host Evolution: Scaling Up Symbiosis. *Trends in Ecology & Evolution* **31**, 539–549 (2016).

21. Rosshart, S. P. *et al.* Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. *Cell* (2017).

22. Gould, A. L. *et al.* Microbiome interactions shape host fitness. *Proceedings of the National Academy of Sciences* **115**, E11951–E11960 (2018).

23. Kolodny, O., Callahan, B. J. & Douglas, A. E. *The role of the microbiome in host evolution. Philosophical Transactions of the Royal Society B* vol. 375 20190588 (The Royal Society, 2020).

24. Wilmanski, T. *et al.* Gut microbiome pattern reflects healthy ageing and predicts survival in humans. *Nature metabolism* **3**, 274–286 (2021).

25. Worsley, S. F. *et al.* Gut microbiome composition, not alpha diversity, is associated with survival in a natural vertebrate population. *Animal microbiome* **3**, 1–18 (2021).

26. Risely, A., Wilhelm, K., Clutton-Brock, T., Manser, M. B. & Sommer, S. Diurnal oscillations in gut bacterial load and composition eclipse seasonal and lifetime dynamics in wild meerkats. *Nature communications* **12**, 1–12 (2021).

27. Ji, B. W., Sheth, R. U., Dixit, P. D., Tchourine, K. & Vitkup, D. Macroecological dynamics of gut microbiota. *Nature Microbiology* **5**, 768–775 (2020).

28. Lloyd-Price, J. *et al.* Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature* **550**, 61 (2017).

29. Shaani, Y., Zehavi, T., Eyal, S., Miron, J. & Mizrahi, I. Microbiome niche modification drives diurnal rumen community assembly, overpowering individual variability and diet effects. *The ISME journal* **12**, 2446–2457 (2018).

30. Hieke, A.-S. C., Hubert, S. M. & Athrey, G. Circadian disruption and divergent microbiota acquisition under extended photoperiod regimens in chicken. *PeerJ* **7**, e6592 (2019).

31. Parris, D. J., Morgan, M. M. & Stewart, F. J. Feeding rapidly alters microbiome composition and gene transcription in the clownfish gut. *Applied and environmental microbiology* **85**, (2019).

32. Zhang, Y. *et al.* Absence of Circadian Rhythm in Fecal Microbiota of Laying Hens under Common Light. *Animals* **11**, 2065 (2021).

33. Ouyang, J. *et al.* Ruminal Microbes Exhibit a Robust Circadian Rhythm and Are Sensitive to Melatonin. *FRONTIERS IN NUTRITION* **8**, (2021).

34. Reitmeier, S. *et al.* Arrhythmic gut microbiome signatures predict risk of type 2 diabetes. *Cell host & microbe* **28**, 258-272. e6 (2020).

35. Wu, G. *et al.* Light exposure influences the diurnal oscillation of gut microbiota in mice. *Biochemical and biophysical research communications* **501**, 16–23 (2018).

36. Zheng, D., Ratiner, K. & Elinav, E. Circadian Influences of Diet on the Microbiome and Immunity. *TRENDS IN IMMUNOLOGY* **41**, 512–530 (2020).

37. Voigt, R. M. *et al.* Circadian disorganization alters intestinal microbiota. *PloS one* **9**, e97500 (2014).

38. Voigt, R. M. *et al.* The circadian clock mutation promotes intestinal dysbiosis. *Alcoholism: Clinical and Experimental Research* **40**, 335–347 (2016).

39. Zarrinpar, A., Chaix, A., Yooseph, S. & Panda, S. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. *Cell metabolism* **20**, 1006–1017 (2014).

40. Liang, X., Bushman, F. D. & FitzGerald, G. A. Rhythmicity of the intestinal microbiota is regulated by gender and the host circadian clock. *Proceedings of the National Academy of Sciences* **112**, 10479–10484 (2015).

41. Sommer, F. *et al.* The gut microbiota modulates energy metabolism in the hibernating brown bear Ursus arctos. *Cell reports* **14**, 1655–1661 (2016).

42. Wu, Q. *et al.* Seasonal variation in nutrient utilization shapes gut microbiome structure and function in wild giant pandas. in vol. 284 20170955 (The Royal Society, 2017).

43. Regan, M. D. *et al.* Nitrogen recycling via gut symbionts increases in ground squirrels over the hibernation season. *Science* **375**, 460–463 (2022).

44. Weng, F. C.-H., Yang, Y.-J. & Wang, D. Functional analysis for gut microbes of the brown tree frog (Polypedates megacephalus) in artificial hibernation. *Bmc Genomics* **17**, 31–42 (2016).

45. Lopez, D. E. G., Lashinger, L. M., Weinstock, G. M. & Bray, M. S. Circadian rhythms and the gut microbiome synchronize the host's metabolic response to diet. *Cell Metabolism* (2021).

46. Nobs, S., Tuganbaev, T. & Elinav, E. Microbiome diurnal rhythmicity and its impact on host physiology and disease risk. *EMBO REPORTS* **20**, (2019).

47. Kuang, Z. *et al.* The intestinal microbiota programs diurnal rhythms in host metabolism through histone deacetylase 3. *SCIENCE* **365**, 1428-+ (2019).

48. Wang, Y. *et al.* The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. *Science* **357**, 912–916 (2017).

49. Fumagalli, M. *et al.* Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. *PLoS genetics* **7**, e1002355 (2011).

50. Shaw, A. K. & Binning, S. A. Migratory recovery from infection as a selective pressure for the evolution of migration. *The American Naturalist* **187**, 491–501 (2016).

51. Weinstein, S. B., Buck, J. C. & Young, H. S. A landscape of disgust. *Science* **359**, 1213–1214 (2018).

52. McLaren, M. R. & Callahan, B. J. Pathogen resistance may be the principal evolutionary advantage provided by the microbiome. *Philosophical Transactions of the Royal Society B* **375**, 20190592 (2020).

53. Pfenning-Butterworth, A., Cooper, R. O. & Cressler, C. E. Daily feeding rhythm linked to microbiome composition in two zooplankton species. *PloS one* **17**, e0263538 (2022).

54. Kaczmarek, J. L., Musaad, S. M. & Holscher, H. D. Time of day and eating behaviors are associated with the composition and function of the human gastrointestinal microbiota. *The American journal of clinical nutrition* **106**, 1220–1231 (2017).

55. Cyktor, J. C. & Turner, J. Interleukin-10 and immunity against prokaryotic and eukaryotic intracellular pathogens. *Infection and immunity* **79**, 2964–2973 (2011).

56. Ahloy-Dallaire, J., Klein, J. D., Davis, J. K. & Garner, J. P. Automated monitoring of mouse feeding and body weight for continuous health assessment. *Laboratory animals* **53**, 342–351 (2019).

57. Labrecque, N. & Cermakian, N. Circadian Clocks in the Immune System. *J Biol Rhythms* **30**, 277–290 (2015).

58. Comas, M. *et al.* A circadian based inflammatory response–implications for respiratory disease and treatment. *Sleep Science and Practice* **1**, 1–19 (2017).

59. Penny, H. A. *et al.* Rhythmicity of Intestinal IgA Responses Confers Oscillatory Commensal Microbiota Mutualism. *bioRxiv* (2021).

60. Macpherson, A. J., Geuking, M. B. & McCoy, K. D. Homeland security: IgA immunity at the frontiers of the body. *Trends in immunology* **33**, 160–167 (2012).

61. Pietrzak, B., Tomela, K., Olejnik-Schmidt, A., Mackiewicz, A. & Schmidt, M. Secretory IgA in intestinal mucosal secretions as an adaptive barrier against microbial cells. *International Journal of Molecular Sciences* **21**, 9254 (2020).

62. Huus, K. E., Petersen, C. & Finlay, B. B. Diversity and dynamism of IgA- microbiota interactions. *Nature Reviews Immunology* **21**, 514–525 (2021).

63. Donaldson, G. P. *et al.* Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science* eaaq0926 (2018).

64. Mukherji, A., Kobiita, A., Ye, T. & Chambon, P. Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. *Cell* **153**, 812–827 (2013).

65. Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **118**, 229–241 (2004).

66. Ayabe, T. *et al.* Secretion of microbicidal α-defensins by intestinal Paneth cells in response to bacteria. *Nature immunology* **1**, 113–118 (2000).

67. Froy, O., Chapnik, N. & Miskin, R. Mouse intestinal cryptdins exhibit circadian oscillation. *The FASEB journal* **19**, 1920–1922 (2005).

68. Dominoni, D. M. The effects of light pollution on biological rhythms of birds: an integrated, mechanistic perspective. *Journal of Ornithology* **156**, 409–418 (2015).

69. Dominoni, D. M., Åkesson, S., Klaassen, R., Spoelstra, K. & Bulla, M. Methods in field chronobiology. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**, 20160247 (2017).

70. Clayton, J. B. *et al.* Captivity humanizes the primate microbiome. *Proceedings of the National Academy of Sciences* **113**, 10376–10381 (2016).

71. Trevelline, B. K. & Moeller, A. H. Robustness of Mammalian Gut Microbiota to Humanization in Captivity. *Frontiers in ecology and evolution* **9**, (2022).

72. Gaillard, J.-M. & Lemaître, J.-F. *An integrative view of senescence in nature. Functional Ecology* vol. 34 4–16 (Wiley Online Library, 2020).

73. Nussey, D. H., Coulson, T., Festa-Bianchet, M. & Gaillard, J.-M. Measuring senescence in wild animal populations: towards a longitudinal approach. *Functional Ecology* 393–406 (2008).

74. Roper, M., Capdevila, P. & Salguero-Gómez, R. Senescence: why and where selection gradients might not decline with age. *Proceedings of the Royal Society B: Biological Sciences* **288**, 20210851 (2021).

75. Peters, A., Delhey, K., Nakagawa, S., Aulsebrook, A. & Verhulst, S. Immunosenescence in wild animals: meta-analysis and outlook. *Ecology Letters* **22**, 1709–1722 (2019).

76. Young, A. J. The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**, 20160452 (2018).

77. Thompson, M. E. *et al.* Wild chimpanzees exhibit humanlike aging of glucocorticoid regulation. *Proceedings of the National Academy of Sciences* **117**, 8424–8430 (2020).

78. Patke, A., Young, M. W. & Axelrod, S. Molecular mechanisms and physiological importance of circadian rhythms. *Nature reviews Molecular cell biology* **21**, 67–84 (2020).

79. Held, N. M. *et al.* Aging selectively dampens oscillation of lipid abundance in white and brown adipose tissue. *Scientific reports* **11**, 1–13 (2021).

80. Paschos, G. K. & FitzGerald, G. A. Circadian clocks and metabolism: implications for microbiome and aging. *Trends in Genetics* **33**, 760–769 (2017).

81. Manoogian, E. N. & Panda, S. Circadian rhythms, time-restricted feeding, and healthy aging. *Ageing research reviews* **39**, 59–67 (2017).

82. Partridge, L., Deelen, J. & Slagboom, P. E. Facing up to the global challenges of ageing. *Nature* **561**, 45–56 (2018).

83. Thorley, J. *et al.* Sex-independent senescence in a cooperatively breeding mammal. *Journal of Animal Ecology* **89**, 1080–1093 (2020).

84. Cram, D. L. *et al.* Rank-related contrasts in longevity arise from extra-group excursions not delayed senescence in a cooperative mammal. *Current Biology* **28**, 2934-2939. e4 (2018).

85. Patrick, S. C. & Weimerskirch, H. Senescence rates and late adulthood reproductive success are strongly influenced by personality in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20141649 (2015).

86. Fay, R., Barbraud, C., Delord, K. & Weimerskirch, H. From early life to senescence: individual heterogeneity in a long-lived seabird. *Ecological monographs* **88**, 60–73 (2018).

87. Martinez-Bakker, M. & Helm, B. The influence of biological rhythms on host–parasite interactions. *Trends in ecology & evolution* **30**, 314–326 (2015).

88. Huan, Y., Kong, Q., Mou, H. & Yi, H. Antimicrobial peptides: classification, design, application and research progress in multiple fields. *Frontiers in microbiology* 2559 (2020).

89. Pearson, J., Wong, F. & Wen, L. Crosstalk between circadian rhythms and the microbiota. *IMMUNOLOGY* **161**, 278–290 (2020).

90. Frei, R. *et al.* MHC class II molecules enhance Toll-like receptor mediated innate immune responses. *PloS one* **5**, e8808 (2010).

91. Huang, G. *et al.* Seasonal shift of the gut microbiome synchronizes host peripheral circadian rhythm for physiological adaptation to a low-fat diet in the giant panda. *CELL REPORTS* **38**, (2022).

92. Silver, A. C., Arjona, A., Walker, W. E. & Fikrig, E. The Circadian Clock Controls Toll-like Receptor 9-Mediated Innate and Adaptive Immunity. *Immunity* **36**, 251–261 (2012).

93. Slack, E. *et al.* Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* **325**, 617–620 (2009).

94. Oishi, T. *et al.* Circadian rhythms in amphibians and reptiles: ecological implications. *Biological Rhythm Research* **35**, 105–120 (2004).

95. Tawa, Y., Jono, T. & Numata, H. Circadian and temperature control of activity in Schlegel's Japanese gecko, Gekko japonicus (Reptilia: Squamata: Gekkonidae). *Current Herpetology* **33**, 121–128 (2014).

96. Bartell, P. A., Miranda-Anaya, M. & Menaker, M. Period and phase control in a multioscillatory circadian system (Iguana iguana). *Journal of biological rhythms* **19**, 47–57 (2004).

97. Roe, J. H., Hopkins, W. A., Snodgrass, J. W. & Congdon, J. D. The influence of circadian rhythms on pre-and post-prandial metabolism in the snake Lamprophis fuliginosus. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **139**, 159–168 (2004).

98. Tripathi, M. K., Singh, R. & Pati, A. K. Daily and seasonal rhythms in immune responses of splenocytes in the freshwater snake, Natrix piscator. *PLoS One* **10**, e0116588 (2015).

99. Singh, A., Singh, R. & Tripathi, M. K. Photoperiodic manipulation modulates the innate and cell mediated immune functions in the fresh water snake, Natrix piscator. *Scientific Reports* **10**, 1–12 (2020).

100. Costello, E. K., Gordon, J. I., Secor, S. M. & Knight, R. Postprandial remodeling of the gut microbiota in Burmese pythons. *The ISME journal* **4**, 1375–1385 (2010).

101. Sarkar, A. *et al.* Microbial transmission in animal social networks and the social microbiome. *Nature ecology & evolution* **4**, 1020–1035 (2020).

102. McCabe, C. M., Reader, S. M. & Nunn, C. L. Infectious disease, behavioural flexibility and the evolution of culture in primates. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20140862 (2015).

103. Kappeler, P. M., Cremer, S. & Nunn, C. L. *Sociality and health: impacts of sociality on disease susceptibility and transmission in animal and human societies. Philosophical Transactions of the Royal Society B: Biological Sciences* vol. 370 20140116 (The Royal Society, 2015).

104. Yarlagadda, K., Razik, I., Malhi, R. S. & Carter, G. G. Social convergence of gut microbiomes in vampire bats. *Biology letters* **17**, 20210389 (2021).

105. Kortner, G. & Geiser, F. The key to winter survival: daily torpor in a small arid-zone marsupial. *Naturwissenschaften* **96**, 525–530 (2009).

106. Smits, S. A. *et al.* Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science* **357**, 802–806 (2017).

107. Baniel, A. *et al.* Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas. *Microbiome* **9**, 1–20 (2021).

108. Carey, H. V., Walters, W. A. & Knight, R. Seasonal restructuring of the ground squirrel gut microbiota over the annual hibernation cycle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **304**, R33–R42 (2013).

109. Ren, T. *et al.* Seasonal, spatial, and maternal effects on gut microbiome in wild red squirrels. *Microbiome* **5**, 163 (2017).

110. Risely, A., Waite, D. W., Ujvari, B., Hoye, B. J. & Klaassen, M. Active migration is associated with specific and consistent changes to gut microbiota in Calidris shorebirds. *Journal of Animal Ecology* **87**, 428–437 (2018).

111. Wiebler, J. M., Kohl, K. D., Lee Jr, R. E. & Costanzo, J. P. Urea hydrolysis by gut bacteria in a hibernating frog: evidence for urea-nitrogen recycling in Amphibia. *Proceedings of the Royal Society B: Biological Sciences* **285**, 20180241 (2018).

112. Bulla, M. *et al.* Unexpected diversity in socially synchronized rhythms of shorebirds. *Nature* **540**, 109–113 (2016).

113. Gaynor, K. M., Hojnowski, C. E., Carter, N. H. & Brashares, J. S. The influence of human disturbance on wildlife nocturnality. *Science* **360**, 1232–1235 (2018).

114. Łopucki, R. & Kiersztyn, A. The city changes the daily activity of urban adapters: Camera-traps study of Apodemus agrarius behaviour and new approaches to data analysis. *Ecological Indicators* **110**, 105957 (2020).

115. Stone, E. L., Harris, S. & Jones, G. Impacts of artificial lighting on bats: a review of challenges and solutions. *Mammalian Biology* **80**, 213–219 (2015).

116. Sanders, D., Frago, E., Kehoe, R., Patterson, C. & Gaston, K. J. A meta-analysis of biological impacts of artificial light at night. *Nature Ecology & Evolution* **5**, 74–81 (2021).

117. Ziegler, A.-K. *et al.* Exposure to artificial light at night alters innate immune response in wild great tit nestlings. *Journal of Experimental Biology* **224**, (2021).

118. Raap, T., Casasole, G., Pinxten, R. & Eens, M. Early life exposure to artificial light at night affects the physiological condition: an experimental study on the ecophysiology of free-living nestling songbirds. *Environmental Pollution* **218**, 909–914 (2016).

119. Walker, W. H. *et al.* Light at night disrupts biological clocks, calendars, and immune function. in *Seminars in immunopathology* 1–9 (Springer, 2021).

120. Cox, D. T. C. & Gaston, K. J. Human–nature interactions and the consequences and drivers of provisioning wildlife. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**, 20170092 (2018).

121. Murray, M. H. *et al.* City sicker? A meta-analysis of wildlife health and urbanization. *Frontiers in Ecology and the Environment* **17**, 575–583 (2019).

122. Albery, G. F. *et al.* Urban-adapted mammal species have more known pathogens. *Nature Ecology & Evolution* 1–8 (2022).

123. Kronfeld-Schor, N., Visser, M. E., Salis, L. & van Gils, J. A. Chronobiology of interspecific interactions in a changing world. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**, 20160248 (2017).

124. Scheer, F. A., Hilton, M. F., Mantzoros, C. S. & Shea, S. A. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proceedings of the National Academy of Sciences* **106**, 4453–4458 (2009).

125. Johnson, M. T. & Munshi-South, J. Evolution of life in urban environments. *Science* **358**, eaam8327 (2017).

126. Murray, M. H. *et al.* Gut microbiome shifts with urbanization and potentially facilitates a zoonotic pathogen in a wading bird. *PLoS One* **15**, e0220926 (2020).

127. Alpízar, P., Risely, A., Tschapka, M. & Sommer, S. Agricultural fast food: bats feeding in banana monocultures are heavier but have less diverse gut microbiota. *Frontiers in Ecology and Evolution* 608 (2021).

128. Berlow, M., Phillips, J. N. & Derryberry, E. P. Effects of urbanization and landscape on gut microbiomes in white-crowned sparrows. *Microbial Ecology* **81**, 253–266 (2021).

129. Fackelmann, G. *et al.* Human encroachment into wildlife gut microbiomes. *Communications Biology* **4**, 1–11 (2021).

130. Dillard, B. A., Chung, A. K., Gunderson, A. R., Campbell-Staton, S. C. & Moeller, A. H. Humanization of wildlife gut microbiota in urban environments. *bioRxiv* (2022).

131. Ruiz-Calderon, J. F. *et al.* Walls talk: microbial biogeography of homes spanning urbanization. *Science advances* **2**, e1501061 (2016).

132. Sonnenburg, E. D. & Sonnenburg, J. L. The ancestral and industrialized gut microbiota and implications for human health. *Nature Reviews Microbiology* **17**, 383 (2019).

133. Lee, C.-C. *et al.* External light dark cycle shapes gut microbiota through intrinsically photosensitive retinal ganglion cells. *EMBO REPORTS* **e52316**, (2022).

134. Martchenko, S. *et al.* Diurnal changes in the murine small intestine are disrupted by obesogenic Western Diet feeding and microbial dysbiosis. *SCIENTIFIC REPORTS* **11**, (2021).

135. Refinetti, R. Western diet affects the murine circadian system possibly through the gastrointestinal microbiota. *Biological Rhythm Research* **48**, 287–296 (2017).

136. Hedblom, G. A., Reiland, H. A., Sylte, M. J., Johnson, T. J. & Baumler, D. J. Segmented filamentous bacteria–metabolism meets immunity. *Frontiers in microbiology* 1991 (2018).