Eurasia Specialized Veterinary Publication

Entomological Research Letters

ISSN: 3108-639X

2024, Volume 4, Issue 2, Page No: 63-72
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Available online at: www.esvpub.com/

Circadian Organization Coordinates Insect Metabolism, Behavior, Immunity, and Reproduction Across Daily Physiological Cycles

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ABSTRACT

Insect physiology is temporally structured, yet circadian studies are commonly organized around individual outputs rather than the relationships among them. This systems perspective argues that daily coordination is better understood as distributed phase organization across molecular clocks, metabolic state, behavior, immune function, and reproduction. Evidence from *Drosophila*, mosquitoes, and *Bombyx* shows that clock networks receive multiple environmental inputs, distribute time through several neural and molecular routes, and generate output-specific rhythms whose expression depends on tissue, diet, microbiome, sleep pressure, light, infection context, sex, and life-history state. The evidence therefore does not support a single invariant schedule or a universally acting master pacemaker. Instead, it supports a distinction between endogenous clock state, environmental timing, subsystem phase, and the organismal phenotype measured at a particular time. We develop this distinction toward a proposed daily physiological organizer in which coordination is relational and polyphasic, while misalignment can arise through altered phase, amplitude, coupling, masking, or output-specific failure. The framework is intended as an evidence-bounded systems interpretation rather than a validated mechanism.

Keywords: Circadian clock, Insect physiology, Temporal coordination, Metabolic rhythms, Immune timing, Reproductive rhythms

Received: 03 September 2024
Revised: 20 October 2024
Accepted: 22 October 2024

How to Cite This Article: Ricci P, De Luca M, Ferraro G, Russo A. Circadian Organization Coordinates Insect Metabolism, Behavior, Immunity, and Reproduction Across Daily Physiological Cycles. Entomol Res Lett. 2024;4(2):63-72.
https://doi.org/10.51847/aZJ4v7tTXe

Introduction

The insect circadian literature has resolved molecular oscillators and daily behavior with unusual mechanistic detail, particularly in *Drosophila*, where transcriptional feedback loops, pacemaker neurons, sleep, and locomotor activity can be experimentally separated [1]. This depth can nevertheless encourage a simplified interpretation in which a central clock is treated as a common driver of every rhythmic phenotype. Current circuit evidence instead favors interacting oscillator populations whose peptidergic coupling is distributed and not adequately represented as a single rigid hierarchy [2]. Mosquito research adds a second caution: 24-h patterns in host seeking, feeding, mating, and locomotion reflect both endogenous timing and immediate environmental signals, with substantial species- and sex-specific variation [3].

The problem addressed here is therefore not whether insects possess circadian clocks, but how temporally distinct physiological systems can remain coordinated without being synchronous, mechanistically identical, or equally clock dependent. We distinguish four layers throughout the article: environmental time cues, oscillator state, subsystem-specific output, and the measured organismal consequence. “Circadian” is reserved for endogenous

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clock-dependent organization where the evidence permits that inference; diel or photoperiodic changes are not automatically relabeled as circadian. The central contribution is a proposed systems interpretation in which metabolism, behavior, immunity, and reproduction occupy different but interacting phases within daily physiological organization. The model is deliberately constrained by taxonomic concentration in Diptera, adult-biased experimental designs, and the scarcity of studies that measure several physiological systems simultaneously.

Molecular clock architecture

Circadian organization begins with entrainment, but environmental information does not enter the insect clock through a single channel. In *Drosophila*, light and temperature are integrated through distinguishable sensory and clock-neuron mechanisms: *nocte* contributes to integration of these inputs, illumination can reconfigure relationships among oscillators, and the TRPA channel PYREXIA participates in temperature synchronization of PERIOD [4–6]. These findings support an input architecture in which the same external day can alter both molecular phase and network configuration. They do not establish that all insects use the same thermosensory route, or that environmental responsiveness itself proves circadian control.

Temperature also reaches the clock through molecular processing within the oscillator. PSI-dependent alternative splicing of *timeless* changes circadian period and behavioral phase under temperature cycles, demonstrating that thermal timing can be encoded through transcript processing rather than solely through acute neuronal sensing [7]. At the output side, clock cells use multiple signaling mechanisms to distribute time-of-day information across the brain [8]. Pacemaker neurons also show rhythmic basal calcium levels and faster calcium fluctuations, indicating that neuronal phase is represented in more than one activity dimension [9].

The architecture is therefore both recurrent and output-diverse. A recent synaptic connectome reveals dense interconnections among clock neurons, providing an anatomical substrate for distributed coupling rather than a simple one-way hierarchy [10]. Structural connectivity, however, does not by itself identify which synapses coordinate metabolism, immunity, behavior, or reproduction. For this article, molecular clock architecture is treated as a timing infrastructure: environmental inputs alter oscillator state; oscillator populations interact; and temporal information is transmitted through multiple outputs. The downstream physiological meaning of that information must be established separately for each system.

Metabolic rhythms

Metabolic timing illustrates why clock output cannot be reduced to one central signal. In *Drosophila*, clock-mediated regulation of *Gart* contributes to feeding rhythms and energy homeostasis, but its effects differ among gut, glial, fat-body, and central contexts [11]. Tissue specificity is therefore part of the temporal mechanism: an organism may share a common external day while different metabolic compartments receive or express clock information differently.

Circulating lipid rhythms are similarly conditional. Hemolymph lipid profiles in male flies show clock-dependent oscillation, yet their temporal pattern changes with photic condition, diet, and feeding regime; under some conditions rhythmicity is attenuated or changes modality rather than simply disappearing [12]. This makes a fixed "metabolic peak" inappropriate as a cross-context descriptor. Phase is a property of a measured metabolite, tissue, and environmental state, not of metabolism as an undifferentiated whole.

Peripheral timing is also shaped by biological context outside the canonical clockwork. The *Drosophila* microbiome can stabilize gut rhythmicity and promote temporal coordination between gut and brain, whereas imposed feeding schedules can strengthen cycling while also changing stress sensitivity [13]. Thus, greater rhythmic amplitude or tighter synchrony should not be equated automatically with improved physiological performance. The metabolic evidence instead supports conditional coordination among oscillator state, feeding time, tissue function, and microbial context.

Behavioral rhythms

Behavior is the most visible circadian output, but it is also where non-circadian processes most clearly intersect with the clock. Sleep deprivation in *Drosophila* produces time-dependent rebound because morning and evening clock neurons differentially program the sleep homeostat [14]. Circadian control and homeostatic pressure are therefore separable processes that interact, rather than alternative labels for the same mechanism.

A quantitative two-process analysis likewise indicates that better descriptions of fly sleep can require interdependence between clock speed and the decay of sleep pressure [15]. This is a modeling result, not evidence for a single molecular pathway, but it reinforces a systems point: the behavioral state observed at a given time depends on more than oscillator phase alone.

Circuit experiments provide an even sharper boundary. A subclass of evening cells promotes the transition from arousal to sleep around dusk, yet removing the intrinsic molecular clock from those cells does not reproduce all effects of manipulating their activity; environmental light and network state remain important [16]. Morning wakefulness is also shaped by dopaminergic input onto identified clock neurons, linking neuromodulatory state to phase-specific behavior [17]. These findings argue against treating every neuron labeled "circadian" as an autonomous clock output. Behavioral phase emerges from clock state interacting with homeostatic, sensory, and neuromodulatory conditions.

Immune rhythms

Evidence for insect circadian immunity is more limited than for locomotion or sleep, and the distinction between immune mechanism and infection outcome is especially important. In *Drosophila*, the clock-controlled neuronal gene *Achilles* regulates immune transcription: neuronal knockdown increases antimicrobial-peptide expression and bacterial resistance while leaving core locomotor rhythmicity largely intact, although constitutive knockdown carries a lifespan cost [18]. This provides a direct cross-tissue route from clock-controlled neural output to immune state, but it does not demonstrate that the entire immune system oscillates with one common phase.

Infection timing adds a second layer because temporal effects can arise from both host and parasite biology. Evolutionary and ecological analyses of infection rhythms emphasize that host clocks, parasite rhythms, exposure timing, and behavior may interact, so a time-of-day effect on infection cannot be assigned automatically to immunity alone [19]. In *Anopheles gambiae*, *Plasmodium falciparum* infection outcome changes with mosquito temporal state; reversing the light–dark cycle and silencing CLOCK alter infection phenotypes, while microbiota and parasite processes remain plausible contributors [20]. The measured endpoint is therefore a host–parasite system outcome rather than a pure immune readout.

For the present systems perspective, immune rhythmicity is best separated into clock-output gating of immune transcription, temporal state at challenge, host–parasite temporal concurrence, and whole-infection outcome. These dimensions generate different predictions and require different tests. The distinctions are condensed below because prose alone can obscure which observation supports which level of inference.

Table 1. Distinguishing immune-clock mechanisms from time-of-challenge and host–parasite concurrence in insect infection

Clock-linked outputs and environmental recoding			
Immune time comparison dimension	**Clock-output gating of immune transcription**	**Environmental recoding of host time**	**Clock perturbation with retained rhythmic outputs**
Biological context and observable basis	Adult *Drosophila*; neuronal-to-immune signaling Rhythmic clock control of a neuronal regulator; antimicrobial-peptide expression after perturbation	Reversed or shifted light–dark conditions Infection phenotype changes after environmental phase manipulation	Genetic clock manipulation Immune or infection changes while some behavioral rhythms persist
Permitted immune-clock reading	Clock-controlled neural output can modulate immune state	Environmental timing can alter the host state presented to the parasite	Subsystems can dissociate under clock perturbation
Inference boundary	One regulator does not define the phase of the whole immune system	Light effects may include masking or non-clock physiology	Pleiotropy prevents assignment of every phenotype to one pathway
Discriminating test and synthesis state	Measure multiple immune effectors across circadian time while perturbing the identified neural route Evidence-supported mechanism	Compare shifted entrainment with free-running and clock-disrupted conditions Evidence-supported distinction; proposed test	Simultaneously phenotype immune, metabolic, and behavioral outputs after the same perturbation Proposed cross-system validation
Challenge timing and host–parasite concurrence			

Immune time comparison dimension	Infection-time phenotype	Host–parasite temporal concurrence
Biological context and observable basis	Insect challenged at different temporal states Time-dependent resistance, prevalence, or infection intensity	Host and parasite both possess temporally structured biology Infection differs with relative timing of host and parasite activity
Permitted immune-clock reading	Host temporal state can contribute to infection outcome	Relative phase may matter more than host phase alone
Inference boundary	The endpoint integrates immunity, exposure, physiology, and pathogen processes	Direct host–parasite phase coupling is rarely measured
Discriminating test and synthesis state	Hold pathogen state constant while varying host circadian phase Evidence-supported inference boundary	Cross-factorial challenge of host phase and parasite phase under constant environment Proposed systems test
	Whole-infection outcome	
Immune time comparison dimension	**Whole-infection outcome**	
Biological context and observable basis	Mosquito–parasite–microbiota system Oocyst prevalence/intensity or resistance after timed infection	
Permitted immune-clock reading	Clock state can shape vector–pathogen outcome	
Inference boundary	Outcome is not equivalent to immune mechanism	
Discriminating test and synthesis state	Add immune-effector, microbiome, feeding, and parasite-stage measurements to the same temporal design Evidence-supported outcome; mechanistic decomposition proposed	

The immune evidence is most faithfully represented as nested routes rather than a single clock-to-immunity arrow. **Figure 1** therefore separates neural clock output, immune state at challenge, parasite temporal state, and the infection phenotype, while retaining the boundary between mechanistic immune evidence and whole-host infection inference.

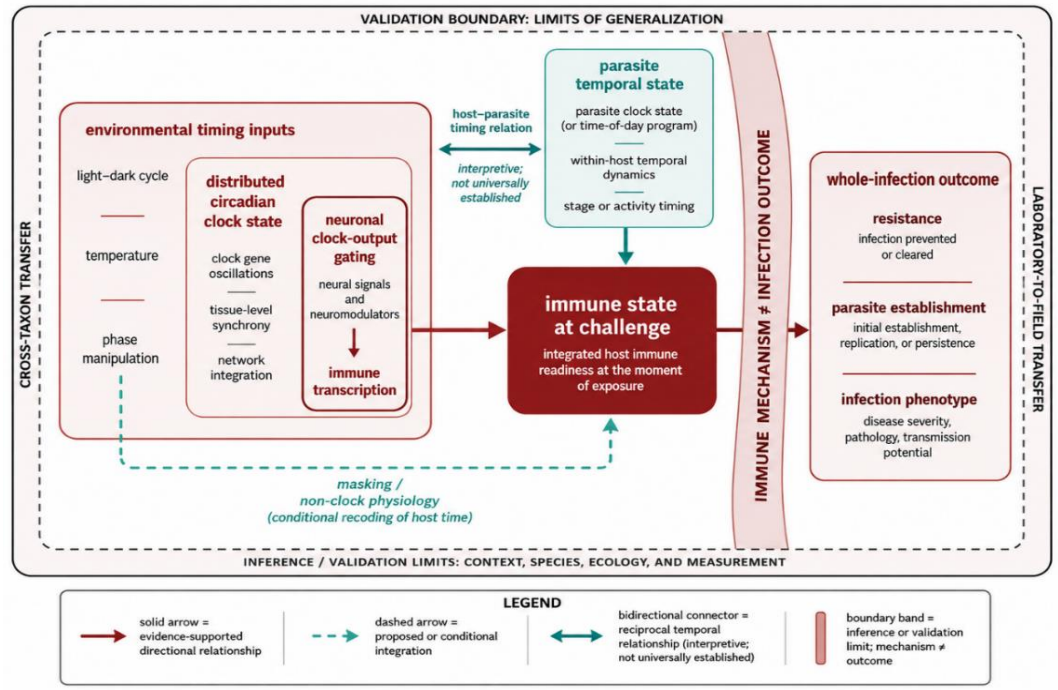


Figure 1. Clock-output gating and host–parasite temporal concurrence create separable routes to time-dependent infection outcomes

Reproductive rhythms

Reproduction shows why daily coordination must accommodate behaviors that depend on both clock state and ecological opportunity. In *Anopheles* mosquitoes, circadian control of flight-tone audibility aligns male acoustic communication with mating-swarm timing, linking a measurable behavioral signal to the temporal structure of reproduction [21]. This is a specific mating mechanism, not evidence that every reproductive process shares the same phase.

Clock-gene manipulations in *Aedes albopictus* and *Culex quinquefasciatus* likewise alter mating-activity rhythms, demonstrating that reproductive behavior can depend on canonical clock machinery across more than one vector species [22]. Yet even within mosquitoes, clock disruption does not move all reproductive and life-history traits in the same direction. In *Aedes aegypti*, disabling *cycle* removes normal activity peaks and reduces short-window mating success, egg hatching, and survival, while increasing blood-feeding propensity and leaving some other endpoints comparatively unchanged [23]. The clock therefore shapes a set of partially dissociable outputs rather than one reproductive syndrome.

A Lepidopteran boundary case further separates daily circadian organization from seasonal photoperiodism. In *Bombyx mori*, *period* contributes to short-day production of diapause eggs, but disruption does not abolish every photoperiodic developmental response [24]. Seasonal timing can thus recruit clock genes without becoming equivalent to a daily circadian rhythm. Across these studies, the defensible conclusion is that reproductive timing is output-specific, taxon-dependent, and embedded in behavioral and environmental context. The next analytical step is consequently to ask how phases among metabolic, behavioral, immune, and reproductive systems relate, rather than assuming that successful organization requires simultaneous peaks.

Phase relationships among systems

The relevant unit of circadian coordination is not simultaneous peak activity but the relationship among subsystem phases. *Drosophila* clock circuits can distribute different phases to distinct downstream rhythmic centers, demonstrating that temporal output is polyphasic even within one neural system [25]. This finding provides a mechanistic basis for rejecting the assumption that coordinated physiology requires metabolic, behavioral, immune, and reproductive maxima to coincide. A phase difference may itself be organizationally meaningful if it preserves ordering among processes, prevents incompatible demands from coinciding, or places an output within an appropriate environmental window; however, those functional interpretations require direct testing rather than inference from rhythmicity alone.

The metabolic evidence reinforces that point. Tissue-specific clock regulation, environmentally conditional lipid oscillations, and microbiome-dependent gut–brain synchrony show that metabolic timing is distributed and state dependent [11–13]. Behavioral data add a separate distinction: sleep pressure, light, and neuromodulatory input can shift the expression of clock-related behavior without implying that the underlying oscillator has moved identically. Phase should therefore be treated as relational: the phase of an output is meaningful only relative to its oscillator, environmental schedule, internal state, and other outputs.

This distinction also prevents inappropriate comparison among measurement classes. Molecular phase may be inferred from transcript or protein oscillation, neural phase from activity dynamics, behavioral phase from event timing, and physiological phase from metabolic or immune endpoints. A phase relationship across these measures is not established merely because each varies over 24 h. Demonstrating coordination requires temporal resolution sufficient to estimate their relative ordering under the same entrainment history and biological state.

We consequently propose that daily physiological organization consists of coordinated phase differences rather than organism-wide synchrony. Some relationships may be stable, whereas others may be conditional on sex, life stage, diet, infection, or ecological setting. The distributed-oscillator evidence supports the plausibility of such an arrangement [2], but no study simultaneously measures all four focal systems in the same insects. The proposed phase-relational interpretation is therefore a synthesis requiring direct multisystem validation.

Consequences of circadian misalignment

Misalignment should not be defined only as arrhythmia. Artificial light at night alters clock-gene expression during mosquito overwintering while many temporal patterns remain detectable, and responses differ between vector species and physiological states [26]. A temporally organized insect can therefore be misaligned because its phase, amplitude, environmental relationship, or coupling among outputs has changed, even when oscillation

persists. This distinction matters experimentally because a retained rhythm can conceal displacement relative to feeding, mating opportunity, pathogen exposure, or another internal subsystem.

Different manipulations also disturb different parts of the temporal system. Diet and photic condition alter the modality and expression of lipid rhythms without providing evidence that the entire clock has failed [12]. Microbiome status and feeding schedule change peripheral rhythmicity and gut–brain synchrony [13]. Reversing the light–dark cycle or disrupting CLOCK changes malaria infection outcomes in *Anopheles*, but those outcomes also integrate parasite and microbiota processes [20]. Core-clock disruption in *Aedes* produces a mixed phenotype in which some behavioral and life-history outputs deteriorate, others are retained, and blood-feeding propensity can increase [23]. These are not interchangeable forms of "circadian disruption."

The consequence also depends on what is being measured. A behavioral phase shift may alter exposure to hosts or mates without demonstrating metabolic impairment; a peripheral metabolic displacement may occur while locomotor timing remains recognizable; and altered infection outcome may reflect the relative temporal states of vector, parasite, and microbiota rather than a generalized collapse of immunity. "Misalignment" therefore needs a specified reference: misaligned with the external cycle, with another tissue, with another physiological system, or with a biologically relevant opportunity.

We therefore propose a taxonomy that distinguishes environmental phase mismatch, oscillator perturbation, peripheral phase displacement, inter-system coupling mismatch, output-specific dysregulation, and masking by acute environmental signals. The categories are analytical, not validated diagnostic states. They are useful because they generate different evidential requirements: a shifted environmental relationship requires phase measurement; oscillator failure requires endogenous rhythmicity assessment; coupling mismatch requires simultaneous subsystem measurements; and masking requires separation of acute environmental effects from endogenous timing.

The resulting decision problem is condensed in **Table 2**, which prevents biologically different perturbations from being interpreted as one generic misalignment phenotype.

Table 2. Circadian misalignment states require different evidence before physiological consequences can be inferred

Misalignment source	Perturbation locus	Observed rhythm	Diagnostic reading with rival explanation	Resolution test and state
External schedule and masking				
Environmental phase mismatch	Light, temperature, feeding, or other zeitgeber schedule altered relative to prior entrainment	Output phase changes relative to external time	External schedule and internal timing may no longer coincide Acute masking can resemble entrainment change	Measure persistence and phase under constant conditions after the shift Proposed classification; evidence-supported phenomenon
Environmental masking	Immediate light or other stimulus changes overt behavior	Behavioral output changes without proof of endogenous phase change	Overt timing may be environmentally driven Masking can be mistaken for circadian resetting	Compare behavior during stimulation with subsequent free-running phase Proposed inference boundary
Oscillator and peripheral timing				
Oscillator perturbation	Genetic or molecular clock manipulation	Loss, weakening, or altered timing of endogenous clock markers	Core timing machinery has been altered in the tested system Pleiotropy can produce non-circadian phenotypes	Pair molecular oscillation with multiple downstream outputs Proposed classification
Peripheral or tissue-phase displacement	Metabolic or organ-level rhythm changes while central timing remains measurable	Tissue-specific phase or rhythmicity differs across conditions	Peripheral timing can change without global clock failure Diet, microbiome, or local physiology may contribute	Measure central and peripheral phase concurrently Proposed classification grounded in metabolic evidence
Inter-system and output-specific mismatch				

Inter-system coupling mismatch	Two or more outputs retain rhythms but change their relative phase	Altered phase relation among simultaneously measured systems	Coordination may fail despite preserved individual rhythms Relative phase has rarely been measured across focal systems	Longitudinal multisystem sampling within the same individuals or cohorts Proposed classification and validation requirement
Output-specific dysregulation	Clock perturbation affects some behaviors or life-history traits but not others	Divergent phenotype responses to the same perturbation	Clock dependence is subsystem specific Direct and indirect effects cannot be separated automatically	Rescue or pathway-specific perturbation for each output Evidence-supported pattern; proposed systems interpretation

Figure 2 separates these perturbation classes from their consequences so that retained rhythmicity, changed phase relations, and output-specific failure remain distinguishable rather than being collapsed into a binary aligned-versus-disrupted state.

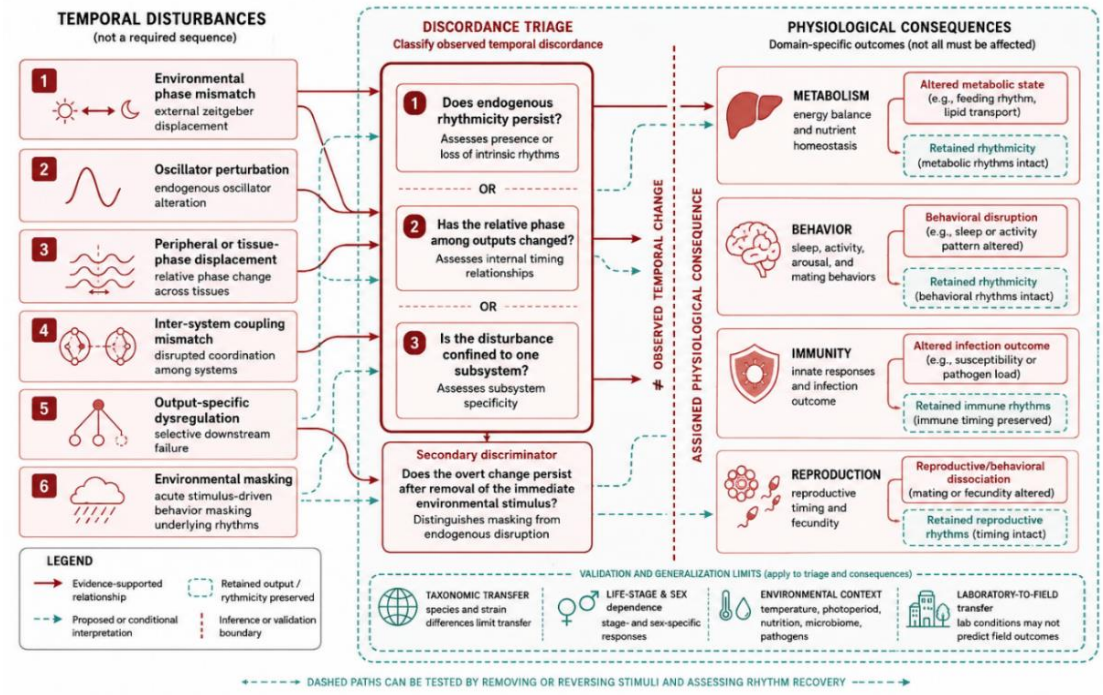


Figure 2. Distinguishing phase mismatch, oscillator disturbance, coupling failure, and masking before assigning physiological consequence

Integrated daily physiological organizer model

The evidence assembled here supports a proposed Daily Physiological Organizer model in which circadian coordination is distributed across four separable functions: environmental alignment, oscillator-network timing, subsystem-specific output, and state-dependent gating. The organizer is not a new anatomical structure. It is a systems-level description of how temporal information can remain coherent while being transformed differently across tissues and behaviors. This formulation deliberately avoids treating the molecular clock, a clock-neuron population, or any single endocrine or metabolic pathway as the organizer itself.

Environmental light and temperature can reconfigure or entrain clock networks; clock cells transmit time through multiple neural mechanisms; metabolic outputs depend on tissue, diet, and microbiome; and behavior depends additionally on homeostatic and neuromodulatory state. Immune and reproductive outcomes then add interaction with pathogens, mates, sex-specific ecology, and life-history condition. The model therefore predicts no single universal physiological peak. Its defining property is constrained phase relationship: subsystem timing may differ, but those differences should remain reproducible within a specified biological and environmental context if they are functionally coordinated.

The proposed architecture contains three forms of coupling. Vertical coupling links environmental input to oscillator state and then to subsystem output. Horizontal coupling describes relationships among outputs that occupy different phases within the same daily cycle. Contextual gating modifies either route according to internal or external state. These coupling classes are conceptual distinctions rather than separately validated mechanisms. Their value is to prevent evidence at one level—for example, a rhythmic neural signal—from being used automatically as proof of coordinated organismal physiology.

The model also incorporates partial failure. An oscillator may remain rhythmic while a peripheral phase shifts; two systems may each remain rhythmic while their relationship changes; or an overt behavior may change through masking without equivalent oscillator displacement. These alternatives convert "circadian disruption" from a single explanatory label into a set of testable temporal failure modes. A decisive test would quantify at least two subsystem phases together with an oscillator marker under controlled phase perturbation and then ask whether their relative timing is restored, preserved, or reorganized.

Results and Discussion

This perspective reframes insect circadian physiology around relations among outputs rather than around isolated rhythms. The strongest mechanistic evidence comes from *Drosophila* neural circuits, metabolism, and sleep, while mosquito work extends the problem into mating, host seeking, infection, and environmental disturbance. That distribution is informative but taxonomically narrow. Mosquito synthesis itself shows species- and sex-specific temporal niches [3], so neither *Drosophila* circuitry nor vector ecology should be treated as an insect-wide template. Evidence from *Bombyx* broadens the taxonomic range but addresses a seasonal photoperiodic problem rather than validating the same daily architecture.

A second implication is that robustness can coexist with disturbance. Evening-cell clock removal can leave some sleep and arousal functions intact, *period* disruption in *Bombyx* does not eliminate every photoperiodic response, and artificial light at night can alter mosquito clock expression without uniformly removing rhythmicity [16, 24, 26]. Such dissociations argue for redundancy, parallel control, environmental compensation, or output-specific dependence, but the available studies do not determine which explanation applies generally. The proposed organizer therefore treats retained function after perturbation as informative evidence about architecture, not as proof that timing is irrelevant.

The proposed organizer also separates physiological timing from adaptive value. Infection timing can involve interacting host and parasite rhythms [19], and stronger or more coherent rhythmicity need not imply improved performance under every stress condition. Similarly, laboratory mating, survival, feeding, or infection phenotypes are not equivalent to population fitness. Claims about adaptation require ecological and evolutionary evidence beyond the temporal mechanisms considered here. The framework is consequently descriptive and mechanistic in ambition, but not a demonstration that any particular phase relationship has been favored by selection.

Methodologically, the principal limitation is fragmentation across experiments. Clock neurons are often measured in one design, metabolites in another, sleep in another, and infection or reproduction in still others. Cross-study synthesis can identify compatible temporal logic but cannot recover individual-level covariance among those systems. Differences in sampling interval, entrainment regime, phase definition, sex, age, and environmental conditions can also create apparent phase differences that are methodological rather than biological.

Direct validation should therefore move beyond one-output chronobiology. The critical experiment is not simply to demonstrate another daily rhythm, but to measure multiple systems in the same taxon across circadian phase while manipulating one timing component at a time. Designs should distinguish endogenous from environmentally driven timing, include both sexes where biology warrants, span relevant life stages, and test whether phase relationships persist under field-realistic temperature, feeding, light, infection, or reproductive conditions. The model would be weakened if cross-system phase relations prove unstable even under controlled states, or if apparent coordination disappears once common environmental forcing is removed.

Conclusion

Insect circadian organization is best treated as distributed temporal coordination rather than simultaneous activation of physiology by a single master signal. Molecular clocks provide environmental alignment and internal timing, but metabolic, behavioral, immune, and reproductive outputs are filtered through tissue identity,

homeostatic state, diet, microbiome, infection, sex, life history, and ecological context. The proposed Daily Physiological Organizer therefore defines coordination through reproducible phase relationships and explicitly allows partial failure, masking, and subsystem dissociation. Its principal value is to convert broad claims of circadian control into testable questions about which oscillator, which output, which phase relationship, and under which conditions. Validation now requires simultaneous multisystem measurements across taxa and realistic environments rather than further accumulation of isolated rhythms.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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