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Decentralized Clocks and Direct Photoreception: The Zebrafish as an Integrative Model for Circadian Biology

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eLife Assessment

This Review Article provides an overview of circadian findings obtained using the zebrafish model and will be of particular interest to researchers working with zebrafish in chronobiology and behavioural neuroscience. The article would benefit from a broader conceptual framework that more clearly positions zebrafish within the wider landscape of animal models used in circadian biology, including comparisons with other extensively studied systems. In addition, several citation inaccuracies and interpretational issues identified during peer review should be carefully addressed to strengthen the accuracy and impact of the review.

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Abstract

Circadian rhythms are fundamental, endogenously generated biological mechanisms that have evolved to synchronize organisms with the diurnal cycles resulting from Earth's rotation. The zebrafish (*Danio rerio*) has emerged as a premier vertebrate model for investigating these rhythms due to its unique combination of features: optical transparency during embryonic and larval stages, rapid external development, genetic tractability, and high evolutionary conservation of core circadian clock genes and neural pathways with humans. A particularly distinctive trait is the intrinsic photosensitivity and autonomous circadian oscillatory capacity found in cells throughout the zebrafish body, including peripheral organs and tissues. This provides an unparalleled system for dissecting the coordinated and independent regulation of central and peripheral clocks. This review systematically synthesizes recent advances in zebrafish circadian research, focusing on the molecular architecture of the core clock, the redundant multi-tissue light-input pathways, and the clock's precise regulation of key physiological processes such as neural function, cardiovascular activity, metabolism, immunity, and reproduction. We further discuss current limitations and challenges, including the mapping of relevant neural circuits, understanding inter-tissue communication, and conducting lifespan-wide investigations. Finally, we outline promising future directions, such as leveraging emerging technologies for circuit analysis, exploring the impact of environmental disruptors, and advancing translational medical applications.

1. Introduction

Circadian rhythms constitute a universal endogenous timekeeping system that enables organisms to align gene expression, cellular physiology, and organ function with predictable daily environmental cycles, thereby maintaining homeostasis and optimizing fitness [1, 2]. Disruption of this intrinsic rhythmicity—whether due to lifestyle factors such as shift work and jet lag, environmental perturbations, or genetic mutations—is strongly associated with a wide spectrum of human disorders, including sleep disturbances, metabolic syndrome, cardiovascular diseases,

neuropsychiatric conditions, and cancer [3]. Therefore, deciphering the molecular basis, regulatory networks, and physiological outputs of circadian rhythms is not only a central question in basic biology but also holds profound implications for translational medicine and public health.

Among the model organisms used in circadian research, zebrafish occupy a crucial evolutionary and experimental niche, bridging the gap between invertebrate models such as *Drosophila melanogaster* and mammalian systems like the mouse [4]. Unlike *Drosophila*, which utilize distinct clock genes and regulatory logic, or mammals, where circadian control is centralized in the hypothalamic suprachiasmatic nucleus (SCN), zebrafish exhibit a high degree of conservation in core clock genes (*Clock*, *Bmal*, *Per*, *Cry*) and pathways with humans. Concurrently, they possess unique biological features that expand the scope of inquiry. These include external fertilization, transparent embryos permitting real-time visualization of rhythmic processes, rapid organogenesis within 24-48 hours post-fertilization, and a robust genetic toolkit for manipulation via CRISPR/Cas9, transgenesis, and morpholino knockdown [5–7].

A defining characteristic that sets zebrafish apart is its decentralized circadian regulatory architecture [8]. In contrast to mammals, where peripheral tissues exhibit dampened autonomy and rely heavily on the SCN for synchronization, virtually all zebrafish cells—from classical photoreceptive tissues such as the retina and pineal gland to peripheral organs such as the liver, heart, and intestine, and even cultured cell lines—harbor intrinsic circadian oscillators and possess autonomous photosensitivity [4, 9, 10]. This allows zebrafish to integrate light signals directly at both central and peripheral levels, offering a powerful model to dissect the independent functions and synchronization mechanisms of distributed clocks [11]. The core circadian oscillator is built around a conserved transcription-translation feedback loop (TTFL). In this loop, CLOCK/BMAL heterodimers activate the transcription of the *per* and *cry* genes, and the resulting PER/CRY proteins subsequently inhibit CLOCK/BMAL activity, establishing an ~24-hour cycle [12, 13]. Teleost-specific genome duplication has further endowed zebrafish with multiple paralogs of clock genes, particularly within the *Cry* family, which have undergone subfunctionalization to mediate specialized roles in rhythm generation, light entrainment, and tissue-specific regulation [14–16]. This molecular oscillator is complemented by a redundant, multi-tiered light-input system encompassing the retina, deep-brain photoreceptors, and peripheral tissue photoreceptors, ensuring robust environmental signal detection and precise phase calibration [17]. As a fundamental form of energy and sensory input, light not only enables image perception through the eyes but also mediates non-image-forming (NIF) functions. The zebrafish genome encodes 42 opsin genes, providing a prototype for understanding NIF functions of light [18].

This sophisticated circadian network governs a wide array of physiological processes in zebrafish, including sleep-wake cycles, learning and memory, cardiovascular function, energy metabolism, immune responses, reproduction, and gut-microbiota interactions, many of which are evolutionarily conserved with humans [19–25]. For example, disruption of zebrafish clock genes recapitulates human disease phenotypes such as sleep fragmentation, metabolic dysregulation, and cardiovascular defects, providing valuable insights into disease mechanisms [26]. Despite significant progress, critical knowledge gaps persist. The intracellular signaling cascades linking light perception by opsins, Cryptochromes, and other photoreceptors to the core clock machinery have not been fully elucidated; the neural circuits mediating communication between the central pacemaker (in the telencephalon) and peripheral tissues remain incompletely mapped; and the dynamics of circadian rhythms across the zebrafish lifespan, from embryogenesis to aging, are not fully characterized [27–29].

This review comprehensively synthesizes recent progress in zebrafish circadian rhythm research. We begin with the molecular mechanisms of the core oscillator, followed by a detailed analysis of multi-tissue light-input pathways. We then summarize the circadian regulation of key physiological systems and address existing limitations and challenges. Finally, we outline future research directions that harness emerging technologies to resolve unanswered questions and promote translational applications. Our goal is to provide a consolidated overview of the zebrafish model in circadian research and to inform novel approaches for exploring the evolution, function, and dysfunction of biological clocks.

2. Core Architecture and Multisystem Regulation of the Zebrafish Circadian Clock

2.1 A Highly Conserved Molecular Oscillator

The core circadian oscillator in zebrafish is constructed around a TTFL that is highly homologous to the mammalian system. CLOCK and BMAL proteins dimerize and bind E-box motifs (CACGTG) in the promoters of *per* and *cry* genes to drive their transcription [15]. Newly synthesized PER and CRY proteins form complexes in the cytoplasm, undergo phosphorylation by kinases such as CK1δ/ε, and translocate to the nucleus, where they directly inhibit CLOCK/BMAL activity, repressing their own transcription [30]. The rhythmic degradation of PER/CRY proteins, primarily via the ubiquitin-proteasome system with a peak at late night, releases this inhibition, allowing the next cycle of activation to begin [31].

This primary loop is reinforced by secondary stabilizing loops. REV-ERBα represses, while RORα activates, *Bmal* transcription through competitive binding to ROR elements (ROREs) in its promoter [32]. Notably, RORaa has been shown to directly regulate *per2* expression via an RORE, adding a layer of cross-regulation [33, 34]. Additionally, D-box elements in the promoters of many clock-controlled genes are bound by PAR bZIP transcription factors (e.g., Tef, Hlf, Dbp). The expression of these factors is dually controlled by the core clock and acute light exposure, providing a direct conduit for photic input to shape circadian outputs [35].

A distinctive genomic feature of zebrafish is the presence of multiple paralogs for nearly all core clock genes, a result of teleost-specific genome duplication. The zebrafish genome contains two *clock* genes (*clock1a, clock1b*), two *bmal* genes (*bmal1*, *bmal2*), three *per* genes, and at least six *cry* genes. Functional analyses have revealed extensive subfunctionalization. For example, *cry1a, cry1b, cry3a*, and *cry3b* retain transcriptional repressor activity and participate in light-dependent phase resetting, with Cry1a acting as a bona fide photoreceptor undergoing light-induced conformational changes [36] (Figure 1). In contrast, Cry2 and Cry4 lack the motifs necessary for CLOCK/BMAL interaction and transcriptional repression [16]; Cry4 is enriched in UV cones and may function in non-circadian light detection, such as magnetoreception [37]. This expanded and diversified genetic repertoire enhances regulatory flexibility and provides a natural system for probing structure-function relationships within the circadian machinery.

2.2 Redundant and Efficient Photoreceptive Input Systems

Light, a fundamental form of energy and sensory input, has profoundly shaped life on Earth [18]. Zebrafish possess a remarkable capacity for light detection, supported by redundant light-input pathways at both the cellular and molecular levels, ensuring reliable environmental signal capture by the circadian system (Figure 2).

Retinal Pathway

The retina of zebrafish serves not only as a sensory organ but also as an intrinsic circadian system that maintains its own rhythmic cycles. Unlike mammals, which depend on the retinal-SCN pathway for centralized clock regulation, the zebrafish retina integrates light signals directly with its biological rhythms via inherent molecular clocks [10]. This integration enables the retina to regulate both visual function and behavioral rhythms independently [38]. The retinal circadian clock system responds to external light cues and, equally importantly, sustains intrinsic rhythms even in the absence of light, through multiple feedback loops [39–42]. These feedback mechanisms are driven by core clock genes, including *clock* and *bmal1*, which regulate the expression of genes crucial for maintaining the daily rhythm of visual function [39]. Specifically, the CLOCK/BMAL1 complex regulates the expression of long-wavelength cone opsin mRNA, exhibiting a circadian rhythm with peak levels in the afternoon and a trough in the early morning [39]. Notably, this rhythmic expression persists even in constant darkness, indicating the retina's autonomous circadian nature [39]. In addition, light exposure directly induces the expression of light-responsive clock genes such as *per2* and *cry1a*, which subsequently influence retinal and systemic

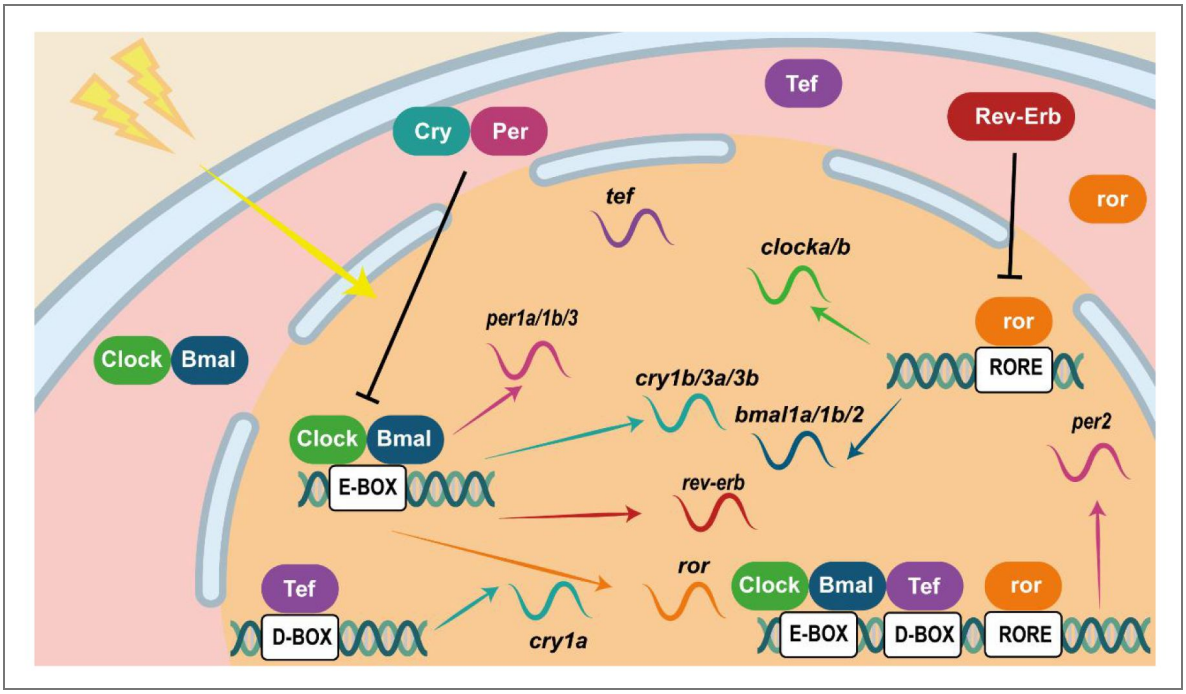

Figure 1. Core Circadian TTFL Molecular Regulatory Diagram in Zebrafish.

This figure illustrates the core transcription-translation feedback loop (TTFL) and auxiliary regulatory network of the zebrafish circadian clock. Clock (clock1a/1b) and Bmal (bmal1/2) form heterodimers, which bind E-box (CACGTG) elements to activate the transcription of the Per (per1/2/3) and Cry (cry1a/1b/3a/3b) genes. Cytoplasmic Per/Cry complexes, which are phosphorylated by CK1δ/ε kinases, translocate into the nucleus to inhibit Clock/Bmal activity, resulting in a 24-hour oscillation. Per/Cry is rhythmically degraded via the ubiquitin-proteasome system to relieve inhibition. Auxiliary loops involve Rev-Erbα/RORα (e.g., roraa) competitively regulating Bmal via RORE, whereas Tef (PAR bZIP family) mediates light-core clock coupling through D-box elements.

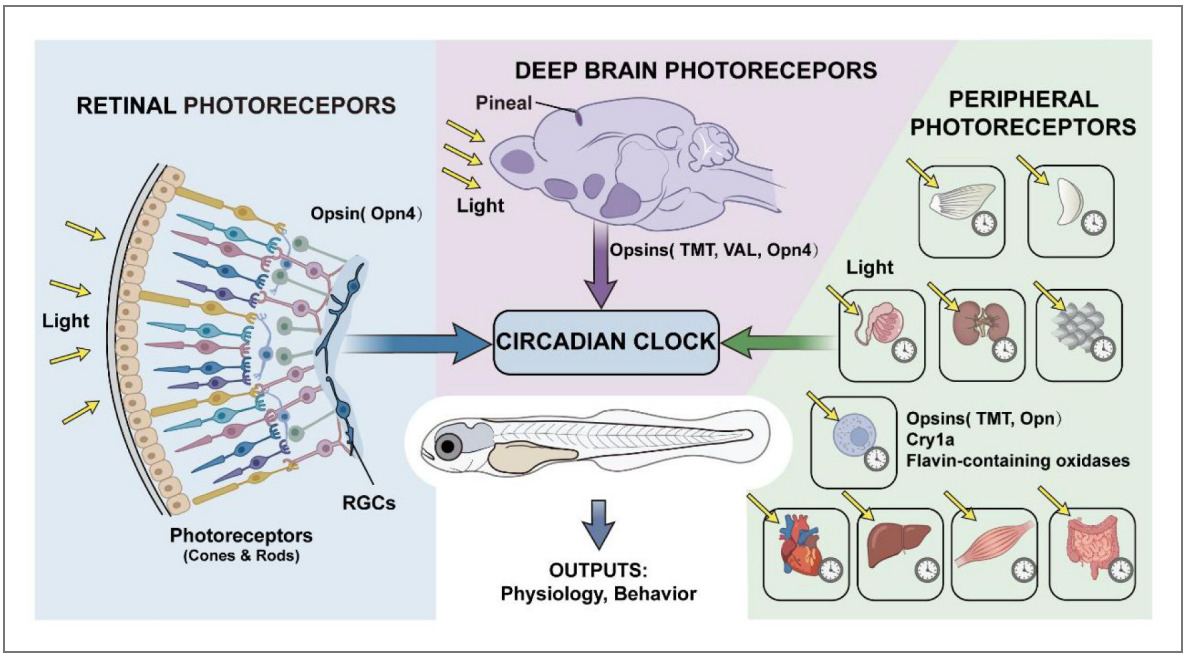

Figure 2. Schematic Diagram of Zebrafish Retinal Photoreceptors, Deep Brain Photoreceptors, and Peripheral Photoreceptors and their Roles in Circadian Photoentrainment.

This figure depicts three core light-sensing systems that mediate zebrafish circadian photoentrainment and light-driven behaviors. The zebrafish retina directly integrates light signals with its biological rhythms via its intrinsic molecular clock, enabling the regulation of behavioral rhythms. Distributed deep-brain photoreceptors form a non-visual light perception system essential for phase entrainment. Zebrafish possess a decentralized circadian system, with peripheral tissues/organs sustaining autonomous oscillations and responding directly to light via photopigments and ROS signaling.

circadian rhythms, facilitating synchronization with environmental light-dark cycles [40–42]. Studies have shown that mutations in *per2* result in phase delays in zebrafish locomotor rhythms, lengthened cycles, and a reduction in both visual sensitivity and contrast sensitivity [42].

Retinal circadian rhythms also impact visual processing through the modulation of neurotransmitter systems, notably dopamine and melatonin [43, 44]. During daylight, dopamine release in the retina promotes cone cell function, enhancing visual sensitivity and color discrimination [43]. Furthermore, dopamine regulates the transmission of visual signals by altering electrical coupling between photoreceptors and bipolar and horizontal cells [43]. At night, increased melatonin secretion shifts retinal function towards rod-dominated, low-light vision [44]. In this manner, the retina adjusts its photoreceptor activity in response to the specific visual demands of day and night. Mutations in *per1b* cause damage to dopaminergic cells in the retina, resulting in dopamine deficiency and decreased contrast sensitivity [45]. These findings emphasize the critical role of retinal clocks and dopamine in maintaining both visual perception and behavioral rhythms [45]. In addition to regulating visual function, retinal clock genes play a role in the development of the visual system [46]. For example, the transcription factor NeuroD is regulated by clock genes after photoreceptor differentiation, contributing to the rhythmic expression of phototransduction genes [46]. Moreover, circadian rhythms regulate synaptogenesis along the retinal-tectal pathway, further underscoring the involvement of retinal clocks in the development of visual neural circuits [47].

The effects of retinal rhythms extend beyond local visual functions, influencing systemic behavioral rhythms as well [38]. Even under constant darkness, zebrafish exhibit clear circadian rhythms in visual sensitivity, with faster recovery of visual responses in the morning, better suited for rapid visual tasks, and slower recovery at night [48]. The retinal clock adjusts the dominance of cone and rod photoreceptors and modulates visual sensitivity, ensuring optimal performance under varying light conditions [43]. In larvae, light regulates the rhythmic phagocytosis of retinal rod and cone outer segment (OS) tips by the retinal pigment epithelium (RPE), thereby preventing the accumulation of harmful compounds in photoreceptors [49, 50]. Furthermore, zebrafish larvae undergo disassembly of cone synaptic ribbons at night, exhibiting “night blindness” as an energy-saving mechanism [48, 51]. Retinal rhythms also synchronize with the central nervous system through neural projections, thus influencing behavioral responses in zebrafish [17]. Pathways from the retina to the optic tectum and hypothalamus regulate circadian changes in behaviors such as visual escape responses, optokinetic responses (OKR), and visual motor responses (VMR) [17]. Although larvae lacking retinal ganglion cells can still maintain basic locomotor rhythms, the retina plays an essential role in adjusting the phase of behavioral rhythms, particularly during subjective night, when behavioral phase advancement occurs [41, 52].

Deep Brain Photoreceptors

The network composed of multiple neural pacemakers and light-responsive regions in the zebrafish brain expresses clock genes in regions including the ventral thalamic nuclei, periventricular gray zone (PGZ), dorsal nucleus of the ventral telencephalic area (Vd), hypothalamus, torus longitudinalis (TL), preglomerular nuclei, and valvula cerebelli. Among these regions, only the ventral thalamic nuclei, PGZ, and Vd receive retinal input in zebrafish, while the hypothalamus, TL, preglomerular nuclei, and valvula cerebelli are directly photosensitive [53, 54]. *In vitro*, clock genes in multiple brain regions of zebrafish, including the telencephalon, diencephalon, mesencephalon, optic tectum, pituitary, and rhombencephalon, can all be photo-induced [54]. Therefore, it appears that retinofugal inputs are not necessary for clock gene expression. The zebrafish brain may contain various types of photosensitive cells, and photosensitive neurons are also widely distributed in non-image-forming brain regions, such as the thalamus, hypothalamus, and preoptic area. Each type of cell may contain the same or different photopigments, including opsins, cryptochrome 4, and flavin-containing oxidases [8]. Among the family of non-visual opsins, TMT-opsin is expressed broadly in neural and non-neural tissues, paralleling clock gene expression [53]. VAL-opsin subtypes in the thalamus are distinctly regulated: *valopa* rhythm is endogenous, whereas *valopb* is light-modulated [55]. Furthermore, in larvae lacking eyes and pineal glands, *opn4m-1* mediates dark phototaxis, a behavior that is

absent in *otpa* mutants but rescued by *opn4m-1* overexpression in the *otp* domain [56]. Melanopsin double mutants exhibit reduced daytime locomotor activity, requiring sustained melatonin inhibition to restore normal activity levels [57].

In the absence of a discrete SCN, zebrafish utilize distributed deep-brain photoreceptive centers [58]. The pineal gland, a photosensitive neuroendocrine organ, generates robust circadian rhythms in melatonin secretion [59, 60]. Rhythmic melatonin is detectable by 2 days post-fertilization and originates from the differentiating pineal before retinal photoreceptors become functional [61]. Even in mutants lacking eyes and forebrains, the pineal maintains rhythmic expression of clock and melatonin synthesis genes, confirming its autonomy as a pacemaker [62, 63]. Larvae without pineal glands exhibit blunted locomotor responses to darkness, underscoring their behavioral role [64]. Collectively, these findings highlight a complex non-visual light perception system essential for phase entrainment and specific light-driven behaviors. However, the complete molecular cascades linking deep-brain photoreceptor activation to the core clock remain to be fully defined.

Peripheral Photoreceptors

Unlike the SCN-centric model in mammals, zebrafish exhibit a decentralized circadian system [5]. Their peripheral organs (e.g., heart, liver), tissues (e.g., skin, muscle, intestine), and embryonic cell lines can sustain endogenous, undamped circadian oscillations *in vitro* for multiple cycles without central input [25, 65–68]. Moreover, these tissues can respond directly to light-dark cycles to adjust their circadian phase. This autonomy stems from the widespread expression of photopigments and associated signaling components in peripheral tissues [69]. The zebrafish genome encodes 42 opsin genes (10 visual, 32 non-visual), providing a molecular basis for tissue-specific light detection [70] (Figure 3). For example, *opn6b*, *opn7*, *opn8*, and *opn9* are highly expressed in the heart, whereas *opn5* is specific to the skin. Among the family of non-visual opsins, the zebrafish-specific TMT-opsin subfamily shares homology with known photopigments; *in vitro* studies have confirmed its activation by blue light and initiation of G protein signaling, supporting its role as a photopigment [71]. Despite the widespread expression of opsins in these non-retinal tissues, the light-sensitive physiological functions of many of these opsins remain to be fully elucidated.

In addition to opsins, cryptochromes and flavin-containing oxidases are also candidate photoreceptive proteins. In zebrafish, Cry proteins possess multiple isoforms. Cry1a functions as an ultraviolet/blue light photoreceptor that can directly communicate with the molecular oscillator [72]. *cry2b* shows high-amplitude rhythmic expression across all tissues, underpinning peripheral light perception [73]. Notably, light can induce hydrogen peroxide (H_2O_2) production, which activates the MAPK pathway [74]. This may involve light-responsive flavoenzymes that absorb near-UV/blue light and promote H_2O_2 generation [75]. Light-responsive D-box enhancers can serve as nuclear targets for reactive oxygen species (ROS), and the antioxidant enzyme catalase can modulate clock gene expression by regulating H_2O_2 levels [76]. Thus, ROS signaling may complement classical pathways in a sophisticated circadian regulatory network.

2.3 Circadian Orchestration of Multi-System Physiology

Neural Rhythmicity

Zebrafish display clear diurnal sleep-wake patterns, with consolidated rest at night and daytime activity [20] (Figure 4). Genetic ablation of *clock* or *bmal1* causes severe sleep fragmentation and arrhythmic locomotion, modeling human circadian sleep disorders. At the cellular level, the clock regulates synaptic homeostasis in the optic tectum, where synapse number and strength increase during wakefulness and are pruned during sleep—a process dependent on intact sleep-wake cycles [47]. Learning and memory also fluctuate diurnally; enhanced avoidance learning at specific phases correlates with peak *per1* expression in the brain. Disruption of rhythms impairs hippocampal-like O-GlcNAcylation rhythms and downregulates nuclear OGT, leading to cognitive deficits [22]. Anxiety-like behaviors show circadian variation, suggesting a model for mood

	Zebrafish												Mouse							
Opsin	Eye	Liver	Gut	Fin	Heart	Skin	Gill	Muscle	Pineal	Pituitary	Testis	Brain	Eye	Liver	Gut	Heart	Skin	Muscle	Testis	Brain
rh1	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	Y	N	N	N
rh2	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
sws1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	Y	N	N	N
sws2	Y	N	N	Y	Y	Y	N	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
lws	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	Y	N	N	N
exorh	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
va	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
parapinopsin	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
parietopisin	N	N	N	Y	Y	N	N	N	Y	N	Y	N	N	N	N	N	N	N	N	N
opn3	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y
tmt	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
opn4m	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	Y	N	N	Y
opn4x	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
opn5	Y	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	N	N	N	Y	Y	Y	Y
rrh	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
rgr	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
opn6	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
opn7	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
opn8	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
opn9	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
Total	20	11	11	20	20	19	18	18	20	18	20	19	6	1	0	0	6	2	3	3

visual opsin *cone-like non-visual opsin* *tmt/opn3*
melanopsin *rgr/rrh/opn5* *opn6-9*

Figure 3. The distribution of photoproteins in various tissues of zebrafish and mice.

This figure shows the distribution of all opsin classes in zebrafish and mouse, two representative vertebrate species. Presence is marked as Y and absence as N. Opsins are classified into six major groups: (1) visual opsins (green), (2) cone-like non-visual opsins (blue), (3) opn3/tmt opsins (purple), (4) rgr/rrh/opn5 opsins (yellow), (5) opn4 opsins (black), and (6) the new opsins (red). *(Figure 3 adapted from Figure 3 from [70])*

disorders. For example, protein kinase Cα (prkcaa), which is linked to neuropsychiatric disorders, modulates the morning expression of immediate early genes; its loss attenuates their normal nocturnal repression [77].

Cardiovascular Homeostasis

The molecular clock plays specific roles within each type of cardiovascular tissue [66, 78] (Figure 4). The occurrence of adverse cardiovascular events also exhibits circadian rhythmicity. Heart rate and cardiac output are under strict circadian control [78]. *bmal2* mutants lose heart rate rhythms and develop bradycardia and reduced stroke volume, directly linking the core clock to cardiovascular function [51]. Vascular development and regeneration also exhibit daily rhythms. Clock genes in endothelial cells rhythmically regulate VEGF and other angiogenic factors. Circadian disruption severely impairs developmental angiogenesis and adult fin regeneration [79]. Notably, *bmal1* knockdown inhibited vessel formation, whereas *per2* knockdown accelerated vessel formation, revealing antagonistic effects. Mechanistically, BMAL1 binds E-boxes in the *vegf* promoter, and its deficiency compromises Notch inhibition-induced sprouting, highlighting crosstalk between circadian and developmental pathways [19]. Hypoxia-inducible factors (HIFs) and their targets, such as EPO and VEGF, are also clock-modulated. Circadian disruption in developing zebrafish impairs hypoxic responses, alters erythropoiesis, disrupts vascular patterning, and increases mortality [80–82].

Hepatic Rhythmicity

The liver exemplifies peripheral clock-metabolism crosstalk. Transcriptomic analyses have revealed circadian oscillations in hundreds of metabolic genes involved in lipogenesis, gluconeogenesis, and detoxification [22] (Figure 4). Mutations in core clock genes induce hepatic steatosis, dyslipidemia, glucose intolerance, and insulin resistance, modeling the metabolic syndrome observed in shift workers [82, 83]. Constant darkness reduces metabolic and immune gene expression and induces fatty liver [84]. Melatonin exerts beneficial metabolic effects by increasing satiety signals in the liver and gut and suppressing Igf-I expression [85]. *roraa*$^{-/-}$ mutants show downregulation of fatty acid oxidation genes (*cpt2*) and transporters (*fabp2*), which are direct RORaa targets and part of the PPAR signaling axis, positioning RORaa as a key integrator of circadian and metabolic regulation [33]. PAR bZIP factors and AhR targets also exhibit light- and clock-dependent rhythms, aligning detoxification capacity with daily environmental exposure [23].

Immune Rhythmicity

Innate immune responses are under circadian regulation (Figure 5) [24]. The migratory efficiency of zebrafish neutrophils to injury sites peaks during the day and is modulated by both the immune cell-intrinsic clock and systemic glucocorticoid signaling. Inflammatory cytokine expression (e.g., *i1-1β*, *tnfα*) also oscillates, indicating preprogramming of the inflammatory pathway by the clock [86, 87]. The core molecular clock component Clock1a regulates the rhythmic recruitment behavior of neutrophils by modulating the antioxidant response through the *nfe212a/duox* pathway [88]. *per1b* and *per2* mutations have distinct effects: Per2 is required for neutrophil bactericidal activity, driving ROS production and enhancing infection-induced *hmgb1a* expression to clear bacteria [89]. The Cry-binding domain of Per2 is essential for this regulation. Conversely, Cryla-deficient neutrophils show increased bactericidal activity and *hmgb1a* expression [90]. Conserved BMAL1 and NF-κB motifs in the *hmgbla* promoter constrain its induction to the light phase. Mutating the BMAL1 motif impairs the light-dependent priming of bactericidal activity, indicating that light optimizes neutrophil function via the circadian timer. In contrast, *per1b* mutation downregulates ERK and subsequent p65 phosphorylation, reducing NF-κB activation and proinflammatory gene expression [91].

Reproductive Rhythmicity

Circadian rhythmicity is an inherent feature of the reproductive system. Circadian clock-controlled retinoic acid (RA) signaling plays a significant role in spermatogonial differentiation and fertilization [92] (Figure 4). In Sertoli cells, the circadian clock regulates RA synthesis and

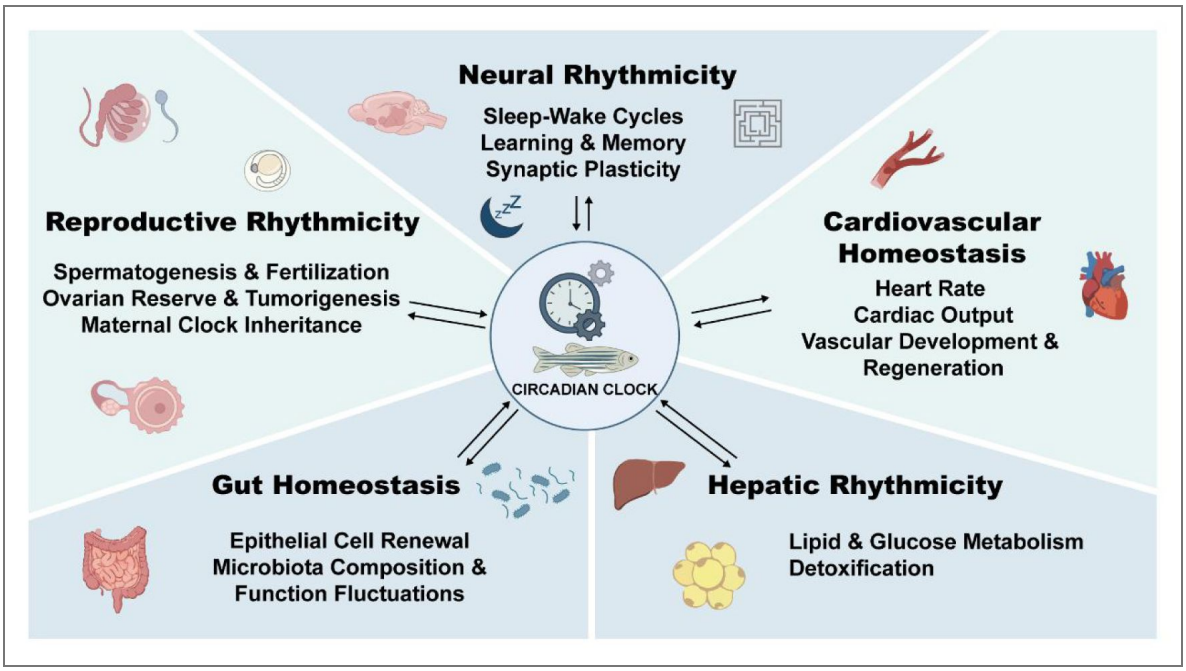

Figure 4. Circadian Clock's Regulatory Network for Zebrafish Multi-System Physiology.

This figure centers on zebrafish to illustrate the regulation of multi-system physiological functions by the circadian clock. The functional modules involved include: (1) the nervous system, encompassing sleep-wake cycles, learning and memory, and synaptic plasticity; (2) the cardiovascular system, including heart rate, cardiac output, and vascular development and regeneration; (3) the metabolic system, covering lipid and glucose metabolism as well as detoxification; (4) the reproductive system, involving spermatogonial differentiation and fertilization, ovarian reserve and tumorigenesis, and maternal clock inheritance; (5) the intestinal system, comprising epithelial cell renewal and gut microbiota homeostasis. The associations between these functional modules and zebrafish reflect the coordinated control of the circadian clock over multiple physiological systems.

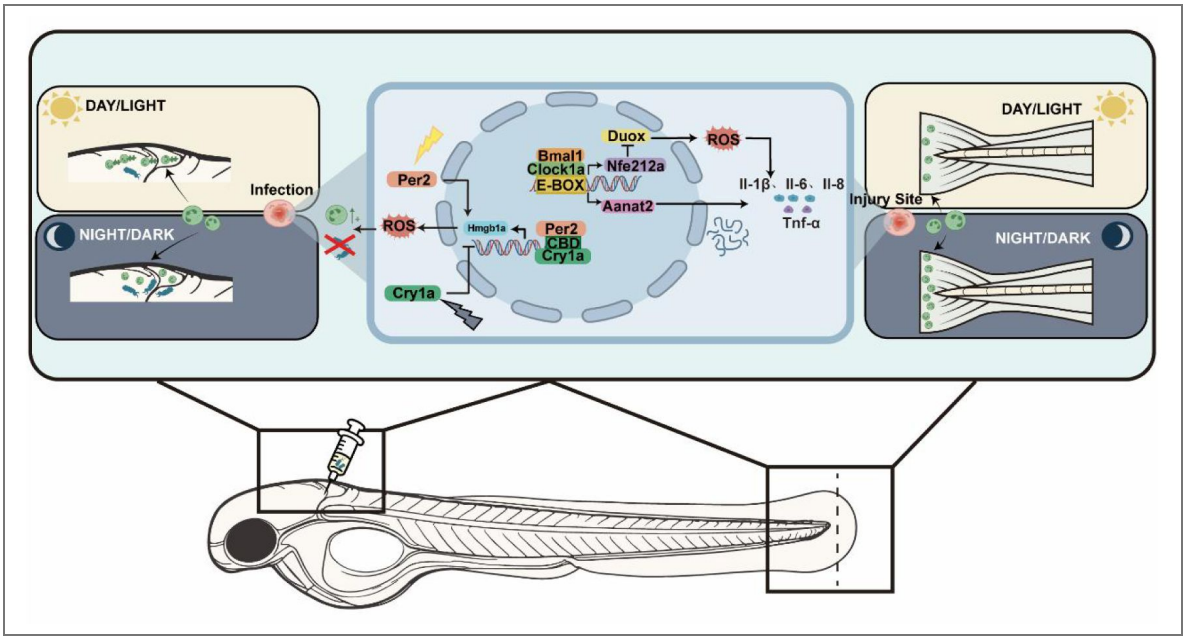

Figure 5. The immune system is regulated by the circadian rhythm.

This figure illustrates the diurnal variation of neutrophils in the model of bacterial infection and tail fin injury in zebrafish larvae. *clock1a* gene regulates neutrophil migration by coordinating the rhythmic expression of *nfe212a* and *duox* genes to control the reactive oxygen species (ROS) level. Light-regulated Per2 increases reactive oxygen species (ROS) production and bacterial killing in zebrafish neutrophils by controlling Hmgb1 expression.

receptor expression by binding to the E-box elements of the *aldh1a2* and *rarga* genes [92]. After RA diffuses to spermatogonia, it inhibits the transcriptional repressor Zbtb16a to promote spermatogonial differentiation. Meanwhile, RA signaling upregulates the expression of the sperm surface fusion factor Izumo1, enhancing sperm fertilization capacity without affecting sperm count [92]. Circadian disruption, either global or Sertoli cell-specific disruption of *clock1a*/*bmal1*, or temporal perturbation or desynchronization of *clock1a* expression, all result in arrested spermatogonial differentiation and reduced fertilization [92]. Clock genes also regulate ovarian function [93]. Loss-of-function mutations in *Per1*/*Per2* lead to premature depletion of the ovarian reserve, resulting in declined reproductive capacity. In addition, circadian rhythm disruption disturbs reproductive hormone levels and ultimately induces ovarian tumorigenesis in zebrafish [94, 95]. Embryos inherit maternal clock gene products through oogenesis and initiate their own rhythms [96]. Furthermore, preconceptional circadian rhythm disruption impairs the ovarian function of female offspring, specifically manifested as compromised follicular development, reduced oocyte quality, and decreased embryonic developmental potential, an impairment closely associated with abnormal lipid metabolism and disruption of the ovarian immune microenvironment in the offspring [96, 97].

Gut Homeostasis

The zebrafish intestine functions as a peripheral clock tissue with autonomous regulatory capacity. Its intrinsic pacemaker can be directly photoentrained for precise daily mitosis, regulating cell cycle genes such as *cdc2*, *wee1*, and *p21*, with modest effects on cyclins [25] (Figure 4). The proliferation and apoptosis of intestinal epithelial cells and the expression of nutrient absorption genes are systemically clock-regulated [98]. Notably, the composition and function of the commensal gut microbiota exhibit diurnal fluctuations synchronized with the host clock. Disruption of clock genes or constant light alters the microbial composition, increasing opportunistic bacteria and reducing probiotics, leading to dysbiosis [99]. This makes zebrafish an ideal model for studying the “circadian clock-gut-brain axis.” For example, constant light in larvae elevates cortisol, inhibits intestinal peristalsis, and creates a stress-induced constipation model characterized by inflammation, impaired neural activity, and dysregulated aquaporin/VIP expression [99]. Probiotic supplementation, particularly with *Bifidobacterium longum*, alleviates these phenotypes by reducing cortisol, modulating inflammation, and restoring gut motility and neural activity [99, 100], demonstrating functional crosstalk within the circadian-gut-microbiota network.

3. Future Perspectives

Compared with classical circadian models, zebrafish are distinguished by their unique combination of vertebrate-level physiological complexity, optical accessibility, and distributed photoreceptive capacity. The presence of decentralized photoreceptors further enhances the theoretical value of this model. In mammals, peripheral clocks are typically regarded as subordinate oscillators indirectly regulated by a central pacemaker. In zebrafish, however, peripheral tissues function as primary light-responsive units capable of autonomous entrainment. This distributed sensory architecture suggests that the relationship between central and peripheral clocks may not strictly follow a hierarchical structure, but instead be dynamically modulated across tissues depending on environmental conditions, developmental stage, or metabolic state. Such a framework redefines a central question in chronobiology. Whereas previous research has focused on how the central clock imposes order on passive peripheral oscillators, future investigations may shift toward understanding how multiple semi-autonomous oscillators achieve coordinated coherence. Does the brain integrate temporal signals from peripheral tissues, or do peripheral clocks collectively constrain central timing? By enabling direct experimental manipulation of both central and peripheral photoreceptive units within the same organism, zebrafish provide a powerful platform to address these questions. Future studies integrating tissue-specific optogenetic control, longitudinal whole-body imaging, and network modeling may reveal that vertebrate circadian systems operate as adaptive, multi-node temporal

networks rather than rigid hierarchical structures. Such insights could reshape our understanding of how central and peripheral clocks coordinate under physiological conditions and how their uncoupling contributes to disease.

Exploring clock genes holds profound significance for disease treatment, as their value extends beyond circadian rhythm regulation to encompass complex organismal interactions and multi-dimensional functional roles. Increasing evidence supports the existence of bidirectional communication between biological clocks and physiological systems, as well as tight coupling among clocks across organs. Intestinal clocks influence the sleep-wake cycle by temporally maintaining the homeostasis of glutamatergic neurons in hypothalamic nuclei, and can also participate in regulating brain cognitive processes, whereas defects in gut biological clocks exert a significant negative impact on cognitive performance [101]. In parallel, the synergistic interaction between the gut microbiota and liver biological clocks has also attracted considerable attention [102]. To meet the host's glucose demand, the gut microbiota can activate hepatic gluconeogenesis through extracellular vesicles, and the production of these extracellular vesicles exhibits circadian rhythmicity related to the host's nutritional status, which further confirms the synergistic regulatory network of biological clocks among systems [103].

Notably, in addition to their core function in circadian rhythm regulation, clock genes also possess significant "non-canonical functions" and can participate in numerous pathways outside traditional circadian control. The CLOCK gene can reshape neuronal connectivity networks, and its abnormal expression is one of the important contributing factors to neuropsychiatric disorders such as autism and schizophrenia [104]. BMAL1 exhibits even more diverse functions: its deletion can cause pluripotent stem cells to partially reverse to a totipotent-like state, a process independent of CLOCK involvement but achieved by synergistically inhibiting the activation of *MERVL* and *2C* genes with TRIM28 [105]. In the field of vascular diseases, the regulatory role of BMAL1 has also been re-evaluated; relevant studies have revealed its pathogenic role in vascular calcification and suggested that targeting dysregulated circadian rhythm factors may serve as a novel therapeutic strategy for preventing diabetic vascular calcification [105]. The discovery of these non-canonical functions has opened new avenues for understanding the pathogenesis of complex diseases such as cognitive disorders and developing targeted therapeutic regimens. Furthermore, the research and development of small-molecule drugs targeting clock gene regulatory pathways have achieved phased progress. Some compounds capable of regulating the activity of the BMAL1/CLOCK complex have entered the preclinical research phase and are expected to be used in combination with chronochemotherapy, enabling dual precision therapy of "targeted + temporal" [106, 107].

Although chronotherapy mediated by clock genes has achieved numerous breakthroughs in clinical applications, it still faces many challenges in translating from basic research to clinical practice. First, the regulatory network of clock genes is extremely complex: there are significant differences in the expression rhythms and regulatory mechanisms of clock genes across different tissues and disease states, and the clock gene regulatory mechanisms of most diseases have not been fully elucidated, limiting the development of targeted chronotherapeutic regimens. In the future, with the development of multi-omics technologies such as genomics, transcriptomics, and metabolomics, as well as the popularization of wearable devices (which can monitor rhythmic indicators and physiological parameters in real-time), clock gene research and chronotherapy will move towards a more precise, personalized, and systematically integrated direction. As an excellent model for circadian rhythm biology research, zebrafish has unique advantages in mechanism elucidation, drug screening, and regimen validation. By leveraging the zebrafish model to deeply analyze the inter-system regulatory mechanisms and non-canonical functions of clock genes, and to identify key regulatory targets for different diseases and individuals, it can provide more precise theoretical support for the development of chronotherapeutic regimens. Meanwhile, using the zebrafish model to rapidly verify the effectiveness of clock gene-targeted drugs and the rationality of chronotherapeutic administration schedules can strongly support improving therapeutic efficacy, reducing adverse reactions, and enhancing patients' quality of life, thus ushering in a new era of "temporal precision + systematic synergy" in disease treatment.

4. Conclusion

Zebrafish circadian research has established a comprehensive framework encompassing molecular mechanisms, multi-tiered photic inputs, and systemic physiological regulation. While conserving the canonical TTFL architecture of vertebrates, zebrafish uniquely combine genomic expansion (e.g., subfunctionalized *cry* paralogs), a tripartite redundant photoreceptive system, and autonomous peripheral photoreception—collectively illuminating the evolutionary adaptability and functional sophistication of biological clocks. This model has yielded profound insights into how circadian networks orchestrate neural plasticity, cardiovascular dynamics, metabolic flux, immune vigilance, reproductive function, and host-microbiome symbiosis, providing an indispensable *in vivo* platform to investigate "clock-organ-environment" interactions and model human diseases rooted in circadian disruption.

Despite these advances, fundamental questions remain—most critically, the intracellular signaling cascades coupling photoreception to the oscillator and the molecular identity of synchronizing cues between central and peripheral clocks. Addressing these gaps through interdisciplinary approaches will not only deepen our understanding of circadian biology but also catalyze the development of novel diagnostics, therapeutics, and preventive strategies for a wide spectrum of human disorders. Owing to its unparalleled combination of genetic tractability, optical accessibility, physiological relevance, and evolutionary position, the zebrafish is poised to remain a cornerstone model in advancing both foundational chronobiology and the emerging era of precision time medicine.

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Peer reviews

Reviewer #1 (Public review):

Summary:

Wang Liao and colleagues aim to provide a comprehensive synthesis of zebrafish circadian research, with particular emphasis on the decentralized photoreceptive architecture that distinguishes teleosts from mammals, and to outline future research directions leveraging emerging technologies for translational applications. The authors frame zebrafish as occupying a "crucial evolutionary and experimental niche" and argue that the model system is uniquely suited to address open questions in chronobiology.

Strengths:

The review is broad in scope and up to date in its citation of recent primary literature. The coverage of physiological outputs - spanning cardiovascular rhythmicity, hepatic metabolism, immune function, reproduction, and gut homeostasis - is more comprehensive than many existing reviews in this area, and researchers seeking an entry point into any of these subfields will find a useful orientation. The figures are well-designed and effectively summarise complex regulatory relationships. The section on immune rhythmicity is a

particular strength, providing mechanistic detail on how specific clock components (Clock1a, Per1b, Per2, Cry1a) differentially regulate neutrophil behaviour, bacterial killing, and cytokine expression; this level of molecular specificity distinguishes it from comparable sections in the review. The brief discussion of non-canonical clock gene functions (CLOCK in neuronal connectivity, BMAL1 in stem cell state, vascular calcification) raises genuinely interesting points that are underexplored in the field and might deserve more prominence.

The future perspectives section makes a conceptually interesting move in suggesting that the zebrafish decentralized architecture could reframe a central question in chronobiology - from how a master clock imposes order on passive peripheral oscillators, to how semi-autonomous oscillators achieve coherence. This is the most original conceptual contribution in the manuscript, and it would benefit from much further development.

Weaknesses:

The core limitation of this review is that it functions primarily as an annotated bibliography rather than a critical synthesis. Section after section follows the same pattern: a physiological system is introduced, several findings from recent papers are described in sequence, and the section ends. Missing throughout is an evaluative voice - where does the field agree, where does it disagree, which findings have been replicated versus remain preliminary, and which conceptual questions are genuinely unresolved versus merely unstudied? Readers with expertise in the field will find little that reframes their understanding; readers new to the field will receive information but not the interpretive scaffolding needed to assess its significance.

The framing of zebrafish as occupying a "crucial evolutionary and experimental niche" is asserted but not substantiated. The experimental advantages of zebrafish - optical transparency, external development, genetic tractability - are real, but they apply primarily to larval stages, typically the first two weeks of development. The review does not adequately address whether the key features it highlights, particularly peripheral photosensitivity and autonomous peripheral oscillators, have been demonstrated in adult animals, where optical transparency is lost. Many of the physiological findings described (sleep-wake cycles, cardiovascular function, reproduction, and immune function) are most relevant in adult or juvenile fish, yet the mechanistic underpinnings often come from larval studies. Whether the mechanisms generalise across developmental stages is not discussed, and this is an important gap that the review could acknowledge explicitly.

The claim that zebrafish bridge invertebrate and mammalian models is a conventional framing that appears in most zebrafish review articles; its repetition here adds little. More interesting - and underexplored - is the comparative question of how the decentralised clock architecture of teleosts compares with that of other non-mammalian vertebrates, or indeed with invertebrate systems such as Drosophila, where peripheral tissue clocks and non-visual photoreception have also been studied. The review does not engage with this comparative dimension, which would be the natural intellectual context for the claims being made.

The future perspectives section identifies several promising directions - optogenetic circuit mapping, whole-body longitudinal imaging, inter-organ communication, network modeling - but these are described at a high level of generality. Most are not specific to the questions raised by the zebrafish decentralized clock architecture; they would appear in any forward-looking review of circadian biology. The one conceptually distinctive idea - that zebrafish could be used to ask how distributed oscillators achieve coordinated coherence without hierarchical control - is identified but not developed into concrete experimental questions or testable predictions. The discussion of non-canonical clock gene functions in the Future Perspectives section would benefit from being more directly connected to what zebrafish specifically can offer: given that teleost genome duplication has produced additional paralogues of clock genes, there is a concrete opportunity to dissect canonical from non-

canonical functions through comparative analysis of paralogues with diverged expression patterns. This point is hinted at but not made explicitly.

Appraisal of conclusions:

The conclusions are broadly consistent with the evidence cited, and the authors are appropriately cautious in noting that many signalling cascades and inter-tissue communication mechanisms remain incompletely characterised. The conclusion that zebrafish represents a valuable and underexploited model for circadian-disease translational research is well-supported. However, the review would be significantly strengthened if the authors distinguished more clearly between what is firmly established, what is supported by preliminary or single-study evidence, and what remains genuinely speculative.

Likely impact and utility:

This review will be useful as an orientation document for researchers new to zebrafish circadian biology, and the comprehensive treatment of physiological outputs across organ systems is a genuine service to the field. Its impact as an intellectual contribution is limited by the descriptive approach and the absence of original synthesis or conceptual reframing. The most interesting ideas in the manuscript - the reframing of the central/peripheral clock hierarchy question, and the potential of clock gene paralogues for probing non-canonical functions - could be further developed and, if pursued, could form the basis of a more distinctive and impactful contribution.

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Reviewer #2 (Public review):

Summary:

This review is valuable in principle because circadian rhythms in zebrafish are unexplored and therefore this degree is valuable in principle. There are a number of significant weaknesses that should be addressed for it to have an impact. First, while the review covers a broad range of topics in chronobiology, it does not put them in context. Placing zebrafish work in the context of other model organisms that are better understood and other fish species would broaden the appeal. The review could also expand to a discussion of sleep, where the understanding in zebrafish is much more advanced. Critically, providing a novel framework, identifying new areas of opportunity and limitations of the system would expand the interest to non-zebrafish research groups. In addition, there are a number of misstatements/mis-citations that are critical to correct. Therefore, I find this review potentially impactful, but its current form is likely to limit its impact.

Strengths:

Focusing on decentralized photo sensing is a strength because it is relatively unique to zebrafish.

The breadth of discussion in zebrafish is a strength.

Weaknesses:

It might be helpful to reorganize the review with an introduction on what is known in other better studied systems to be highly conserved, then to focus in on the components of zebrafish that are discussed here.

A weakness is the lack of integration with other model organisms and other fish systems. Therefore, the narrow focus on zebrafish is unlikely to appeal to broader audiences.

It's surprising that there is not more discussion of sleep, which has been studied in detail, and its relationship to the clock.

Discussions of limitations of the model, including adult vs larval analysis and challenges performing long-term behavioral analysis in fish, would be valuable.

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Reviewer #3 (Public review):

Summary:

Over the past 3 or 4 decades, our understanding of the molecular mechanism underlying the circadian clock has increased substantially. This is in large part due to successful forward and reverse genetics approaches applied to a broad range of genetic model systems, notably Drosophila, Neurospora, mouse, Arabidopsis and cyanobacteria. Although the clock components in these species are diverse, the basic operating principles are highly conserved, allowing us to build a general view of clock mechanisms. Looking forward, there are still many unanswered questions regarding how clocks are organized at the systems level and, in turn, how they are coupled to key aspects of physiology. Each model species has its own set of advantages and disadvantages for tackling particular questions. As this timely review aims to illustrate, the zebrafish has become a particularly valuable model for exploring circadian clock biology. This is in part due to its technical advantages, accessibility of early developmental stages and its directly light-entrainable peripheral clocks. This provides unparalleled opportunities for studying the circadian clock hierarchy and its links with physiology.

Strengths:

This review does a good job of integrating the many lines of circadian clock research where the zebrafish has been used as a model and provides an overview of many future challenges it is well-suited to tackle.

Weaknesses:

There are citation errors, as well as inaccurate and misleading statements that must be remedied in a revised version.

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Author response:

We sincerely thank the reviewers and editors for the thorough, constructive, and insightful comments, which have greatly helped us improve the accuracy, clarity, and rigor of the manuscript. We acknowledge that the current version has several limitations, including insufficient contextualization with other model systems and lack of critical synthesis. These important weaknesses will be comprehensively addressed in a future revised version of the review.

For the present revision, we have focused exclusively on correcting objective errors, factual inaccuracies, and citation mistakes as pointed out by the reviewers. All specific factual and reference issues raised by Reviewer 2 and Reviewer 3 have been carefully corrected in the revised manuscript, including inaccurate statements, incorrect citations, missing references, and inconsistent descriptions of zebrafish clock genes, photoreception, and physiological functions.

We appreciate the reviewers' thoughtful suggestions regarding the conceptual depth, comparative context, critical synthesis, and expanded discussion of sleep and model limitations. While we fully agree that these aspects would significantly strengthen the review, we plan to systematically incorporate these broader conceptual improvements in a future, more substantial revision.

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