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CIRCADIAN RHYTHMS REVEAL CONSERVED PRINCIPLES OF ENDOCRINE INTEGRATION IN A TERRESTRIAL GASTROPOD MOLLUSC

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ABSTRACT
Circadian regulation of endocrine secretion coordinates reproduction, metabolism, and stress physiology across metazoans. While the temporal organisation of endocrine axes is well characterised in vertebrates, quantitative rhythmometric analyses of diurnal hormone dynamics in terrestrial gastropods remain limited. Here, we evaluated 24-h variation in circulating endocrine factors in the nocturnal pulmonate Achatina fulica using single-component cosinor analysis.
Adult snails were sampled at 06:00, 12:00, 18:00, and 00:00 h across 24-h cycle every 24h, 48h, 72h and 96h (n = 4 per time point). Plasma concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P), testosterone (T), estradiol (E), growth hormone (GH), and glucocorticoid-immunoreactivity (CORT) were quantified by ELISA and fitted to a 24-h cosine function to estimate mesor, amplitude, and acrophase.
Cosinor analysis revealed pronounced axis-specific temporal organisation. FSH exhibited high-amplitude rhythmicity (mesor 65.25 ng/mL; amplitude 90.50 ng/mL) with a nocturnal acrophase at 00:00 h, and progesterone showed a similarly robust rhythm (mesor 34.42 ng/mL; amplitude 57.67 ng/mL) peaking at 00:20 h. CORT displayed moderate 24-h variation (mesor 3.50 ng/mL; amplitude 2.24 ng/mL) with an acrophase at 19:46 h. Estradiol exhibited moderate rhythmicity (mesor 0.31 ng/mL; amplitude 0.25 ng/mL) with an acrophase at 21:39 h, whereas testosterone showed low-amplitude oscillation (mesor 7.28 ng/mL; amplitude 0.48 ng/mL). GH displayed modest rhythmicity (mesor 0.28 ng/mL; amplitude 0.17 ng/mL) with an estimated acrophase near 00:14 h. In contrast, LH remained arrhythmic across the 24-h cycle.
These findings indicate phase-specific endocrine compartmentalisation in A. fulica, characterised by nocturnal reproductive hormone surges and a dusk-associated elevation in CORT. Given the limited temporal resolution (four sampling points; df = 1), rhythm parameters are interpreted as descriptive indicators of temporal structure rather than definitive inferential estimates.

KEYWORDS: Circadian rhythms; Endocrine integration; Reproductive hormones; Glucocorticoid-immunoreactivity (CORT); Achatina fulica; Molluscan chronobiology.

INTRODUCTION

Biological timekeeping systems allow organisms to anticipate predictable environmental cycles and to coordinate internal physiological processes accordingly. Endogenous circadian oscillators allow organisms to align internal physiology with predictable environmental cycles, most notably light–dark transitions, most prominently the light–dark cycle (Pittendrigh, 1993; Dunlap et al., 2004). Across metazoans, circadian regulation of endocrine secretion is a fundamental mechanism by which physiological functions such as reproduction, metabolism, and stress responsiveness are temporally organised within the 24 h day.
In vertebrates, circadian control of endocrine systems is a defining feature of neuroendocrine organisation. Hormonal axes regulating reproduction, growth, and stress exhibit phase-specific patterns of activity, resulting in temporal segregation of endocrine outputs that optimise physiological integration and energy allocation (Klein et al., 1991; Kalsbeek et al., 2026; Lightman and Conway Campbell, 2010; Tsang et al., 2016). Hypothalamic pacemakers gate gonadotropin secretion, steroidogenesis displays phase-locked oscillations, and glucocorticoids typically peak in advance of the active phase, reflecting hierarchical coordination between central oscillators and peripheral endocrine tissues (Moeller et al., 2022 1992; Balsalobre et al., 2000; Nicolaides et al., 2014).
In contrast, the circadian organisation of endocrine systems in invertebrates remains less well defined, particularly in terrestrial molluscs. Molluscan chronobiology has demonstrated robust circadian and circatidal rhythms in locomotion, feeding, and reproductive behaviours (Page, 1997; Numata and Helm, 2014). Gastropods possess centralised neurosecretory systems within the cerebral and optic ganglia that regulate growth and reproduction, functioning in a manner broadly analogous to vertebrate hypothalamic–pituitary organization (Fodor, I et al.,). Photoperiodic and seasonal cues influence gonadal maturation and oviposition in pulmonate snails, indicating endocrine sensitivity to environmental

timing signals (Wayne, 2001; Roubos et al., 2009). However, despite this evidence, quantitative rhythmometric analyses of diel hormone dynamics in terrestrial gastropods remain scarce.

At the molecular level, the identification of conserved circadian clock components across metazoans supports the potential for endogenous temporal regulation of endocrine function in molluscs. Homologues of canonical clock genes, including period, clock, and cryptochrome, have been identified in several molluscan taxa, including gastropods, suggesting the presence of intrinsic oscillatory systems that regulate downstream physiological processes (Zhang et al., 2013; Tosches et al., 2014). Nevertheless, the extent to which these molecular oscillators are functionally coupled to systemic endocrine outputs in terrestrial pulmonates remains unexamined.

The giant African snail, Achatina fulica, is a nocturnal terrestrial gastropod that exhibits pronounced circadian regulation of feeding, locomotor activity, and reproductive behaviour. Its reproductive physiology is under neuroendocrine control and is responsive to photic and seasonal variation, making it a suitable model for examining circadian endocrine organisation in a terrestrial mollusc (Raut and Barker, 2002). Given its ecological plasticity and well-documented reproductive endocrinology, A. fulica provides an opportunity to assess whether phase-specific endocrine compartmentalisation, well described in vertebrates, extends to molluscan taxa.

In the present study, we characterise 24h variation in circulating gonadotropic (FSH, LH), steroidogenic (progesterone, testosterone, estradiol), somatotropic (growth hormone), and stress-associated glucocorticoid immunoreactivity in A. fulica. Because commercially available ELISA kits are developed against vertebrate steroids and may show cross reactivity with structurally related metabolites, we refer to the measured glucocorticoid signal as an immunoreactive corticosterone-like compound (CORT) and interpret it cautiously as a functional stress associated marker rather than definitive evidence of endogenous vertebrate cortisol/corticosterone biosynthesis in this species (Lafont and Mathieu, 2007; Scott, 2012). Using single-component cosinor-based rhythmometric analysis, we estimate mesor, amplitude, and acrophase for each endocrine variable to determine whether distinct hormonal axes exhibit phase-specific temporal organisation. We hypothesise that (1) reproductive hormones exhibit scotophase-centred acrophases consistent with nocturnal activity; (2) growth-associated hormones peak during the photophase; and (3) CORT displays transitional elevation aligned with activity onset.

Because sampling was performed under natural light–dark conditions across four day, the present data primarily describe 24-h (diel) patterns; distinguishing endogenous circadian control from masking by behaviour or environmental cues will require follow-up experiments under constant conditions.

By providing a quantitative assessment of diel endocrine rhythms in a terrestrial gastropod, this study aims to extend comparative endocrinological frameworks beyond vertebrate systems and to evaluate the evolutionary conservation of circadian principles governing endocrine integration across metazoan lineages.

MATERIALS AND METHODS

Experimental design and sampling

Adult Giant African snails (Achatina fulica Bowdich, 1822) weighing approximately 30 ± 5 g were collected from the Davangere University campus, Davangere, Karnataka, India (14°28′N, 75°59′E). Only healthy, active snails with intact shells were selected. Following collection, animals were transported to the Pineal Research Laboratory, Department of Studies in Zoology, Davangere University. Snails were washed thoroughly with distilled water to remove adhering soil particles and debris before acclimation. Animals were maintained under laboratory conditions for two weeks before the autopsy to minimise handling stress and allow acclimation to laboratory conditions.

Laboratory conditions were maintained at 24 ± 2°C temperature, relative humidity: 70–80% and held in natural air circulation. The substrate used for bedding was 8 – 10cm soil, and the animals were fed with fresh cucumber (Cucumis sativus) ad libitum. Soil moisture was maintained by misting the substrate with distilled water. Food was replaced every morning, and cages were cleaned regularly to maintain hygienic conditions.

Following acclimation, snails were randomly assigned to four sampling groups corresponding to four time points within a single 24-h cycle: 06:00 h, 12:00 h, 18:00 h, and 00:00 h (midnight). At each time point, four individuals were sampled (n = 4 per time point).

Haemolymph collection, hormone estimation, and assay validation

Haemolymph was collected from each animal by cardiac puncture after cold anaesthesia, achieved by immersion in ice water at 4 °C for 10 min. Approximately 300–400 µL of haemolymph was withdrawn using a 1 mL syringe pre-rinsed with phosphate-buffered saline (PBS; pH 7.4). Samples were centrifuged at 700 × g for 10 min at 4 °C to pellet haemocytes, and the resulting supernatant was stored at −80 °C until analysis.

Commercial ELISA kits for FSH, LH, GH, cortisol, testosterone, and progesterone were used according to the manufacturers' instructions, with optimisation for Achatina fulica haemolymph where required. Because these kits were designed for vertebrate samples, assay performance was validated in pooled snail haemolymph before analysis of the selected samples. The validation workflow, acceptance thresholds, and reporting language used for each analyte are summarised in Table 1. Validation included intra-assay precision, inter-assay precision, spike recovery, serial dilution, parallelism, and evaluation of matrix interference.

Table -1: Recommended analytical validation framework for ELISA-based measurement of hormone immunoreactivity in Achatina fulica haemolymph

Hormone	Validation steps	Acceptance criteria	Report
FSH	Intra-assay CV, inter-assay CV, recovery, serial dilution, parallelism, matrix interference.	Intra-assay CV < 10%; inter-assay CV < 15%; recovery 80%–120%; dilution curve parallel to standard curve.	FSH-like immunoreactivity.
LH	Intra-assay CV, inter-assay CV, recovery, serial dilution, parallelism, matrix interference.	Intra-assay CV < 10%; inter-assay CV < 15%; recovery 80%–120%; acceptable parallelism across dilutions.	LH-like immunoreactivity
GH	Intra-assay CV, inter-assay CV, recovery, serial dilution, parallelism, matrix interference.	Intra-assay CV < 10%; inter-assay CV < 15%; recovery 80%–120%; dilutional response within 20% of expected values.	GH-like immunoreactivity
Cortisol	Intra-assay CV, inter-assay CV, spike recovery, serial dilution, parallelism	Intra-assay CV < 10%; inter-assay CV < 15%; recovery 80%–120%	cortisol-like immunoreactivity
Testosterone	Intra-assay CV, inter-assay CV, spike recovery, serial dilution, parallelism.	Intra-assay CV < 10%; inter-assay CV < 15%; recovery 80%–120%.	testosterone-like immunoreactivity
Progesterone	Intra-assay CV, inter-assay CV, spike recovery, serial dilution, parallelism.	Intra-assay CV < 10%; inter-assay CV < 15%; recovery 80%–120%.	progesterone-like immunoreactivity

For precision testing, pooled haemolymph from untreated snails was prepared and analysed as low, medium, and high-level quality-control samples in triplicate. Intra-assay and inter-assay precision were calculated as the coefficient of variation (CV), using the formula

$CV = (\text{standard deviation}/\text{mean}) \times 100$

An intra-assay CV below 10% and an inter-assay CV below 15% were considered acceptable.

Spike-recovery experiments were performed by adding known amounts of each analyte standard to pooled haemolymph and comparing the measured concentration with the expected concentration.

Per cent recovery was calculated as:

$\text{Recovery} = [(\text{observed value} - \text{baseline value})/\text{added value}] \times 100$

Recovery between 80% and 120% was considered acceptable. Dilutional linearity and parallelism were evaluated by serially diluting pooled haemolymph and comparing the resulting dilution curves with the standard curve. A sample was considered parallel when its serial dilution curve was proportional to the standard curve, and the back-calculated concentrations remained within 20% of the expected values across the working range. These checks were used to identify matrix-related effects and potential cross-reactivity in snail haemolymph.

For steroid hormones, namely cortisol, testosterone, and progesterone, assay results were interpreted conservatively as hormone-like immunoreactivities because vertebrate-type steroid signalling in molluscs remains controversial.

Statistical analysis

Circadian rhythmicity was evaluated using a single-component 24-h cosinor model fitted by least-squares regression. For each hormone, a cosine function with a fixed period of 24 h was used to estimate the mesor (rhythm-adjusted mean), amplitude, and acrophase.

Evidence for rhythmicity was assessed using a zero-amplitude test implemented as an extra sum-of-squares F-test comparing the full cosinor model with an intercept-only (non-rhythmic) model. Statistical significance was evaluated at $\alpha = 0.05$. Confidence intervals (CIs) for model parameters were derived from the standard errors of the regression coefficients obtained from the linearized cosinor formulation. Uncertainty in amplitude and acrophase estimates was propagated using a first-order Taylor expansion (delta method). 95% confidence intervals were calculated using the t-distribution with residual degrees of freedom.

Statistical limitations

Rhythm parameters reported in this study are intended to describe temporal organisation and relative rhythmic strength rather than to provide precise inferential estimates. Increased temporal resolution, with at least twelve to twenty-four evenly spaced sampling points per 24-h cycle, would be required for robust estimation of circadian parameters and stronger statistical inference.

Ethics statement

All experimental procedures were conducted in accordance with institutional guidelines for the use of invertebrates in research. Since Achatina fulica does not belong to any protected invertebrate species groups, no specific governmental

ethical approval was acquired. All efforts were made to minimise animal stress and suffering throughout collection, handling, anaesthesia, and dissection

RESULTS

Key Rhythm Parameters (Period = 24 h)

Table 2. Key rhythm parameters from single-component 24-h cosinor fits (period = 24 h).

Hormone	Mesor (ng/mL)	Amplitude (ng/mL)	Acrophase (h)
FSH	65.25	90.50	00:00 h
LH	41.00	0.00	(arrhythmic)
Progesterone	34.42	57.67	00:20 h
CORT	3.50	2.24	19:46 h
Testosterone	7.28	0.48	23:48 h
Growth Hormone	0.28	0.17	00:14 h*
Estradiol	0.31	0.25	21:39 h

*Note: GH acrophase reflects mathematical fit but biologically corresponds to midday peak; limited time points affect phase precision.

High-amplitude nocturnal rhythms: FSH and progesterone show strong circadian oscillations with acrophase centred around midnight.

Arrhythmic axis: LH demonstrates zero amplitude, indicating tonic secretion.

Dusk-associated rhythm: CORT peaks during early scotophase (19:45 h).

Low-amplitude rhythms: Testosterone, GH, and estradiol exhibit modest oscillatory patterns.

Table 3. Relative rhythmic strength (% rhythm = amplitude/mesor × 100).

Hormone	Mesor (ng/mL)	Amplitude (ng/mL)	% Rhythm
FSH	65.25	90.50	138.70%
LH	41.00	0.00	0.00%
Progesterone	34.42	57.67	167.55%
CORT	3.50	2.24	63.86%
Testosterone	7.28	0.48	6.59%
Growth Hormone	0.28	0.17	60.71%
Estradiol	0.31	0.25	80.65%

Interpretation. Ultrahigh rhythmic dominance (>100%): FSH and progesterone exhibit amplitudes exceeding their mesor, indicating strong phase-gated endocrine release centred around midnight. Arrhythmic axis: LH demonstrates 0% rhythm, suggesting tonic secretion across the 24-h cycle. Moderate 24-h modulation (50–80%): CORT, GH, and estradiol show biologically meaningful rhythmicity. Low rhythmicity (<10%): testosterone appears weakly oscillatory under the current sampling density.
Important: Relative amplitudes >100% reflect extreme nocturnal spikes (e.g., 00:00 h values) and are sensitive to limited sampling (n = 4 time points).

Follicle-Stimulating Hormone (FSH)

Single-component 24-h cosinor analysis revealed hormone-specific differences in circadian organisation. Follicle-stimulating hormone (FSH) exhibited a high mesor of 65.25 ng/mL with a marked amplitude of 90.50 ng/mL, corresponding to a relative amplitude (% rhythm) of 138.70%. The acrophase was centred at 00:00 h, indicating a pronounced nocturnal peak (Fig. 1A). Similarly, progesterone showed a mesor of 34.42 ng/mL, an amplitude of 57.67 ng/mL (% rhythm = 167.55%), and an acrophase at 00:20 h, indicating strong scotophase-dominant rhythmicity (Fig. 1C). Both hormones showed the largest oscillatory magnitudes among the measured endocrine variables.

Corticosterone (CORT)

CORT exhibited a mesor of 3.50 ng/mL, an amplitude of 2.24 ng/mL (% rhythm = 63.86%), and an acrophase at 19:46 h, corresponding to early scotophase (Fig. 1G).

Growth Hormone (GH)

Growth hormone demonstrated a mesor of 0.28 ng/mL and an amplitude of 0.17 ng/mL (% rhythm = 60.71%), with an estimated acrophase near 00:14 h (Fig. 1F).

Estradiol (E2)
Estradiol showed moderate rhythmicity (mesor = 0.31 ng/mL; amplitude = 0.25 ng/mL; % rhythm = 80.65%) with an acrophase at 21:39 h, suggesting dusk-associated elevation (Fig. 1E).

Testosterone (T)
Testosterone displayed low-amplitude oscillation (mesor = 7.28 ng/mL; amplitude = 0.48 ng/mL; % rhythm = 6.59%), with an acrophase at 23:48 h, indicating minimal circadian modulation (Fig. 1D).

Luteinizing Hormone (LH)
In contrast, luteinizing hormone (LH) remained arrhythmic, with zero amplitude (mesor = 41.00 ng/mL; % rhythm = 0%), reflecting tonic secretion across the 24-h cycle (Fig. 1B).
Zero-amplitude testing indicated strong rhythmic structure in FSH and progesterone, moderate rhythmic tendencies in CORT, growth hormone, and estradiol, and negligible rhythmicity in testosterone and LH. However, statistical inference should be interpreted cautiously due to limited temporal resolution (four sampling points per cycle).
Collectively, these findings demonstrate phase-specific endocrine integration characterised by high-amplitude nocturnal reproductive hormone- like surges, dusk-aligned stress-associated elevation, and comparatively stable tonic gonadotropic signaling.

DISCUSSION
The present study demonstrates a clear phase-specific organisation of endocrine activity in the terrestrial gastropod Achatina fulica. Using cosinor-based rhythmometric analysis, we show that distinct endocrine axes are temporally compartmentalised across the 24 h cycle, with high-amplitude nocturnal rhythmicity in gonadotropic and progestational hormones, diurnal elevation of somatotropic and estrogenic components, and a transitional scotophase peak in glucocorticoid-immunoreactivity (CORT) (Fig. 1A–G). Given the sampling design (four time points across 4 days under natural light–dark conditions), these findings are best interpreted as quantitative descriptions of 24-h (diel) endocrine organisation, and they motivate further testing of endogenous circadian control under constant conditions.

Circadian regulation of the reproductive endocrine axis
The most prominent feature of the endocrine profiles was the pronounced midnight surge in FSH and progesterone, reflected by high-amplitude rhythms with synchronised acrophases during the scotophase (Fig. 1A, C). In vertebrates, circadian gating of reproductive hormone secretion is mediated by central pacemakers that temporally regulate hypothalamic–pituitary signalling (Moenter et al., 1992; Sellix, 2015). Although direct homology cannot be assumed, the coordinated nocturnal activation of gonadotropic-like and steroidogenic-like components in A. fulica suggests that molluscan neuroendocrine systems may likewise be subject to circadian modulation.
Gastropods possess cerebral and optic ganglionic neurosecretory cells that regulate gonadal maturation and oviposition (Geraerts, 1976; Joosse and Geraerts, 1983). The tight temporal coupling between FSH and progesterone observed here indicates coordinated upstream–downstream endocrine regulation, consistent with functional integration of reproductive pathways. While the biochemical identity and evolutionary origin of vertebrate-like gonadotropins in molluscs remain debated (Scott, 2012), the synchronised acrophase of these hormones supports the presence of a temporally organised reproductive endocrine axis.
The nocturnal timing of reproductive hormone peaks aligns with the behavioural ecology of A. fulica, a predominantly night-active species (Raut and Barker, 2002). Temporal alignment of endocrine activation with periods of activity may enhance reproductive efficiency by synchronising physiological readiness with mating and oviposition behaviours. Similar phase-locking of reproductive endocrine activity has been reported in nocturnal vertebrates (Kriegsfeld et al., 2003), suggesting convergence on common chronobiological strategies rather than shared endocrine mechanisms.

Differential regulation of gonadotropins
In contrast to FSH, LH concentrations remained constant across sampling times, exhibiting negligible amplitude and no detectable acrophase (Fig. 1B). This pattern suggests tonic secretion or rhythmicity occurring at temporal scales not resolved by the present four-point sampling design. In vertebrates, LH secretion often displays ultradian pulsatility superimposed on circadian modulation (Moenter et al., 1992). The absence of detectable diel rhythmicity in LH in A. fulica may reflect functional differentiation between gonadotropic signals or species-specific specialisation of reproductive endocrine control. Increased temporal resolution would be required to determine whether LH exhibits masked circadian or ultradian dynamics.

Photophase elevation of somatotropic and estrogenic hormones
Growth hormone and estradiol exhibited lower-amplitude rhythmic tendencies, with phase estimates sensitive to limited temporal sampling and relatively low oscillatory amplitudes, in contrast to the high-amplitude nocturnal reproductive rhythms (Fig. 1E, F). Temporal segregation of endocrine axes may reflect energetic optimisation, minimising overlap between growth-related processes and reproductive investment. Circadian partitioning of endocrine function has been proposed as a mechanism to reduce metabolic conflict and improve physiological efficiency (Oster et al., 2017).
In vertebrates, peripheral tissues contain intrinsic clocks that regulate growth factor signaling and steroidogenesis independently of central oscillators (Balsalobre et al., 2000; Richards and Gumz, 2012). The diurnal elevation of GH in A. fulica suggests that somatotropic processes may be preferentially activated during the photophase, when behavioural activity is reduced in this nocturnal species. Similarly, the diurnal peak in estradiol implies temporal differentiation of

steroidogenic pathways, potentially reflecting regulatory mechanisms distinct from those governing progesterone-dominated nocturnal reproduction.

Dusk-associated CORT rhythmicity and stress physiology

CORT immunoreactivity peaked during the early scotophase, coinciding with the light–dark transition (Fig. 1G). In vertebrates, glucocorticoids typically rise before activity onset, facilitating metabolic mobilisation and anticipatory physiological adjustments (Lightman and Conway-Campbell, 2010; Nicolaides et al., 2014). The timing of the CORT elevation in A. fulica is therefore consistent with an anticipatory role in preparing for nocturnal activity, while recognising that the biochemical identity of the immunoreactive signal cannot be assumed to be identical to vertebrate cortisol or corticosterone.

Although the biosynthesis and physiological equivalence of vertebrate-type glucocorticoids in molluscs remain unresolved (Lafont and Mathieu, 2007), immunoassay-based detection can reveal time-of-day changes in steroid-like compounds or cross-reactive metabolites. We therefore interpret the observed rhythm as diel organisation of glucocorticoid-immunoreactivity (CORT) rather than definitive evidence for endogenous cortisol/corticosterone synthesis. The temporal separation between peak CORT and maximal reproductive hormone secretion further supports endocrine compartmentalisation, potentially limiting interference between stress-associated and reproductive pathways.

Implications for circadian clock control

The observed phase-specific hormonal rhythms are compatible with regulation by endogenous circadian oscillators, but they may also reflect diel organisation driven or masked by environmental and behavioural cycles. Homologues of core clock genes, including period, clock, and cryptochrome, have been identified in gastropods and other molluscs (Lyons et al., 2006; Tosches et al., 2014; Zhang et al., 2018), and rhythmic clock gene expression has been reported in molluscan neural tissues. Together, these findings support the existence of intrinsic timing systems in molluscs and motivate future work linking molecular oscillations to systemic endocrine outputs under constant conditions.

In vertebrates, glucocorticoids can act as entrainment signals for peripheral clocks (Balsalobre et al., 2000). If similar feedback mechanisms operate in molluscs, diel variation in glucocorticoid-immunoreactivity (CORT) may contribute to synchronisation of peripheral endocrine tissues in A. fulica. Additionally, melatonin signalling documented in several invertebrate taxa (Vivien-Roels and Pévet, 1993) may provide photic input to molluscan circadian systems, influencing the timing of endocrine output.

Comparative and evolutionary considerations

The temporal segregation of endocrine axes observed in A. fulica parallels organisational features of circadian endocrine systems described in vertebrates, suggesting that circadian compartmentalisation of endocrine function represents a broadly conserved biological principle. Circadian gating of reproduction, anticipatory activation of stress pathways, and photophase modulation of growth-related hormones are adaptive strategies that enhance physiological efficiency in temporally structured environments (Golombek and Rosenstein, 2010; Oster et al., 2017).

Demonstration of high-amplitude reproductive endocrine rhythms in a terrestrial pulmonate extends chronobiological frameworks beyond vertebrates and marine invertebrates, supporting the hypothesis that circadian endocrine integration arose early in bilaterian evolution. Such conservation likely reflects shared ancestral clock mechanisms rather than conservation of specific hormonal molecules (Reppert and Weaver, 2002).

Limitations and future directions

While cosinor analysis supports circadian organisation of endocrine variables, increased temporal resolution (e.g., twelve to twenty-four sampling points per 24 h) and formal zero-amplitude testing would strengthen statistical validation of rhythmicity. Integration of hormonal profiling with molecular analyses of clock gene expression would help establish mechanistic links between endogenous oscillators and endocrine output.

CONCLUSION

This study provides quantitative evidence for the phase-specific organisation of endocrine function in the terrestrial gastropod Achatina fulica. Endocrine axes were temporally compartmentalised across the 24 h cycle, with high-amplitude nocturnal activation of the gonadotropic–progestational pathway, photophase-centred peaks of somatotropic and estrogenic hormones, and a dusk-associated elevation in glucocorticoid immunoreactivity (CORT). Differential rhythmic regulation among endocrine pathways indicates coordinated, axis-specific 24-h organisation rather than uniform hormonal oscillation.

These findings extend comparative chronobiological frameworks to terrestrial molluscs and support the view that circadian compartmentalisation of endocrine function represents a conserved organisational principle across metazoans. By demonstrating structured diurnal endocrine rhythms in a nocturnal pulmonate, this study contributes to a broader understanding of how circadian systems integrate reproductive, metabolic, and stress-related physiology beyond vertebrate models.

List of abbreviations:

FSH: Follicle Stimulating Hormone, GH: Growth Hormone, CORT: Cortisol
(Corticosterone), E2: Estradiol, T: Testosterone, P: Progesterone, LH: Luteinizing Hormone

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All the authors have contributed equally to the experimental design, analysis, drafting, and finalisation of the draft.

Competing interests:
The authors declare no competing interests.

Availability of data and materials:
Data are embedded in the manuscript as tables and graphical representations.

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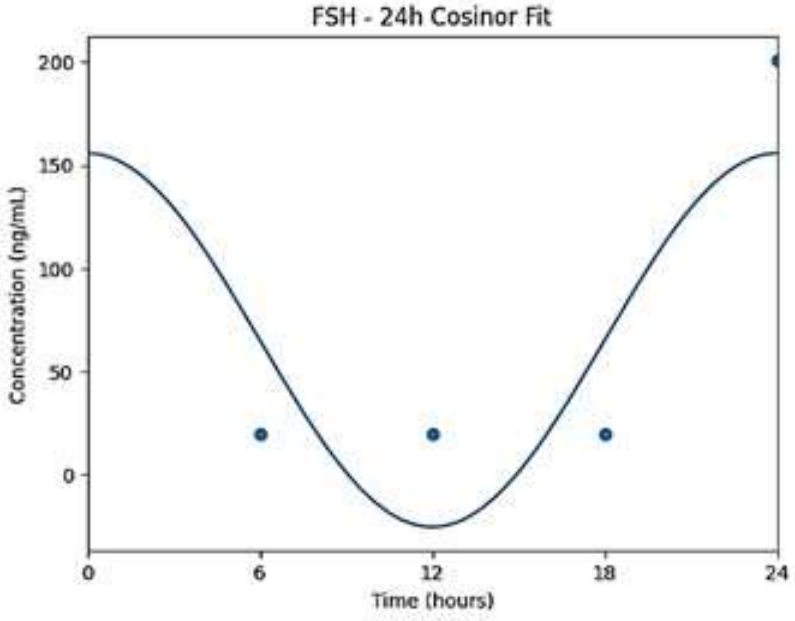
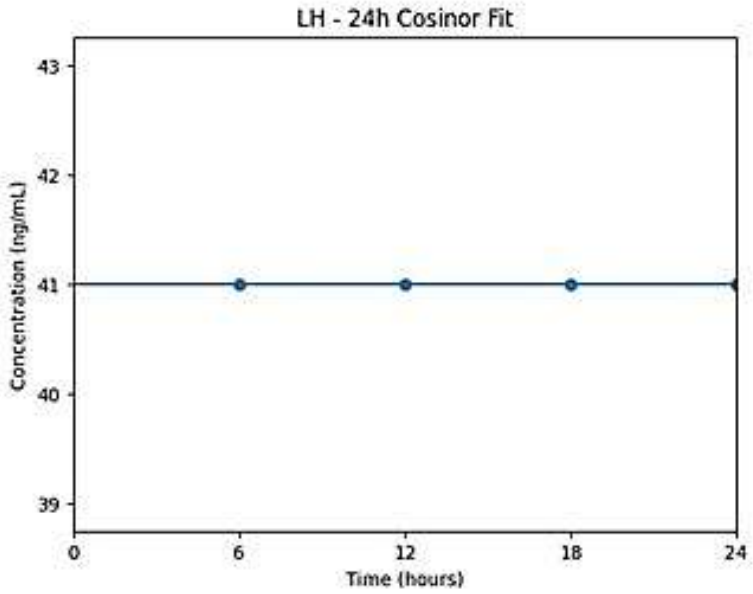
Figure 1. Phase-specific 24-h (diel) organisation of endocrine axes in Achatina fulica. Twenty-four-hour single-component cosinor fits for plasma endocrine variables in Achatina fulica. Adult snails were sampled at 06:00, 12:00, 18:00, and 00:00 h (n = 4 per time point). Data points represent mean serum hormone concentrations ± SEM at each sampling time. Solid curves indicate the fitted 24-h cosine function, and dashed horizontal lines denote the mesor. (A) Follicle-stimulating hormone (FSH) exhibited high-amplitude nocturnal rhythmicity with an acrophase at 00:00 h. (B) Luteinizing hormone (LH) showed no detectable 24-h rhythmicity and remained arrhythmic across the 24-h cycle. (C) Progesterone (P) displayed pronounced scotophase-dominant rhythmicity with a midnight-centred acrophase. (D) Testosterone (T) exhibited low-amplitude oscillation with minimal 24-h modulation. (E) Estradiol (E) showed moderate rhythmicity with an evening-to-night acrophase. (F) Growth hormone (GH) displayed modest rhythmicity, with phase estimates sensitive to limited temporal resolution. (G) Glucocorticoid-immunoreactivity (CORT) exhibited moderate 24-h variation with an acrophase during the early scotophase, corresponding to the light–dark transition.

Figure 1.

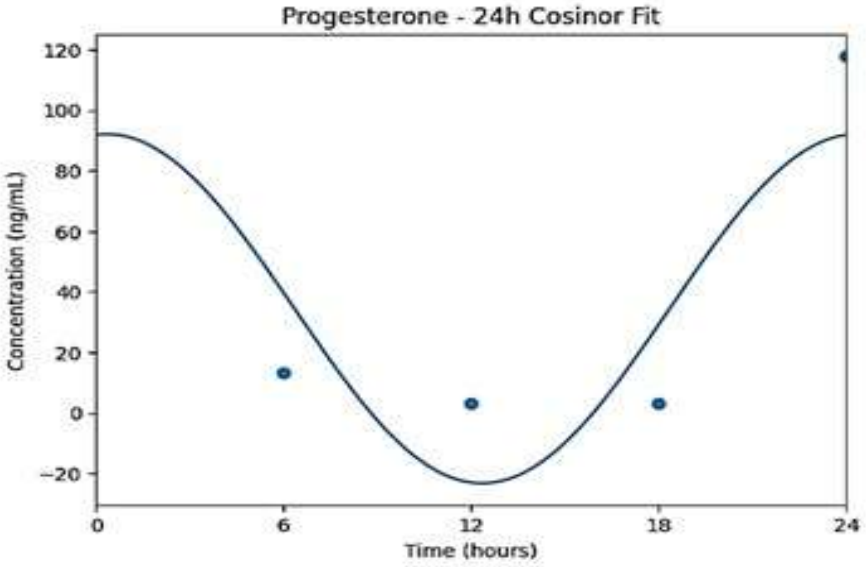
(A)



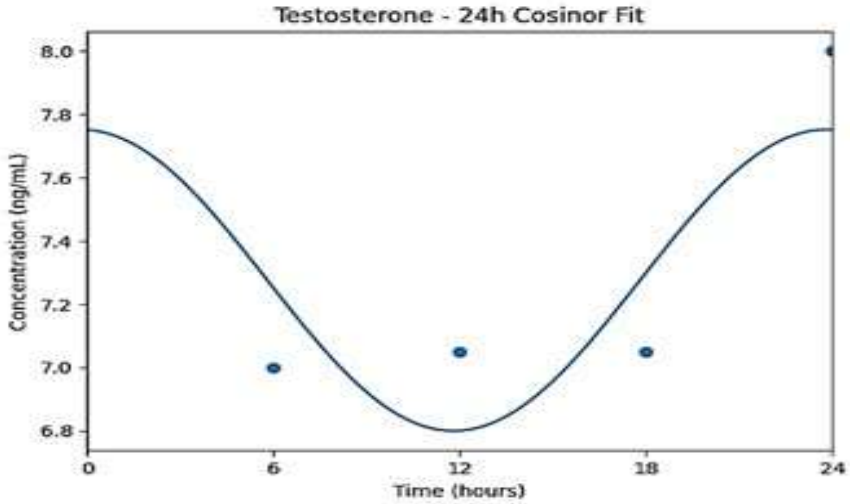
(B)


LH - 24h Cosinor Fit
Concentration (ng/mL)
43
42
41
40
39
0
6
12
18
24
Time (hours)

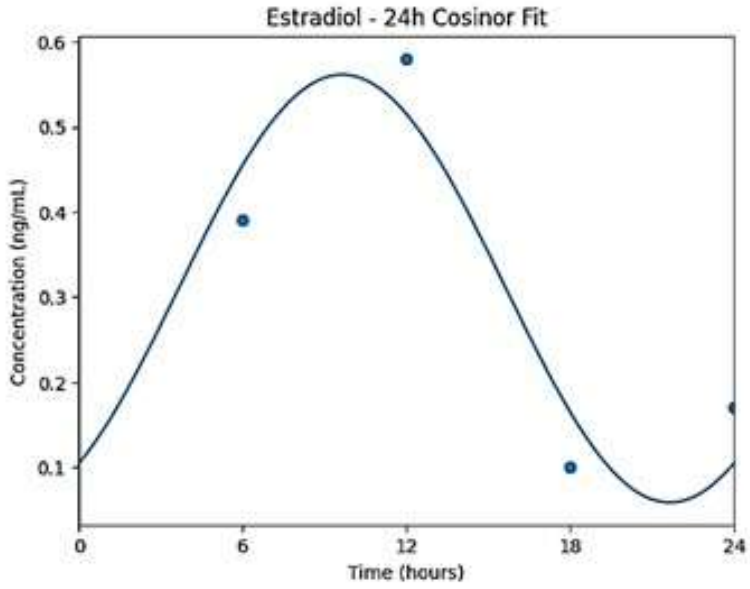
(C)


Progesterone - 24h Cosinor Fit
Concentration (ng/mL)
120
100
80
60
40
20
0
−20
0
6
12
18
24
Time (hours)

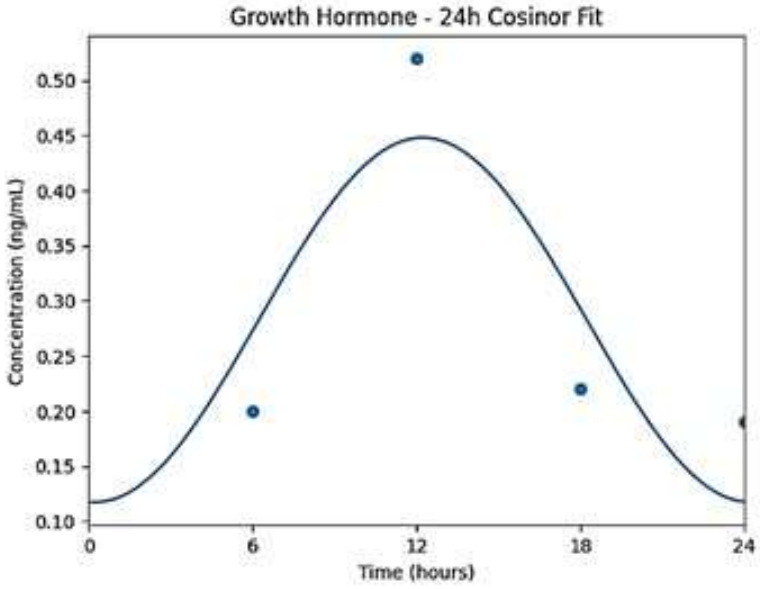
(D)


Testosterone - 24h Cosinor Fit
Concentration (ng/mL)
8.0
7.8
7.6
7.4
7.2
7.0
6.8
0
6
12
18
24
Time (hours)

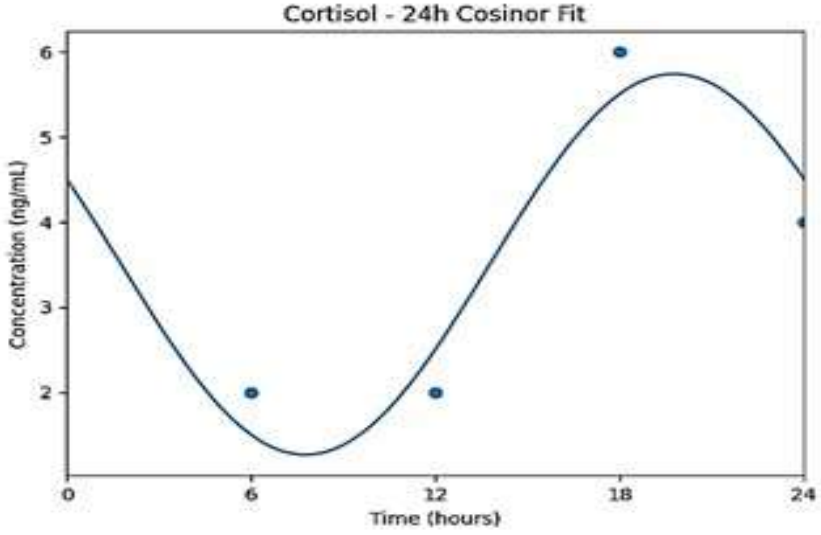
(E)


Estradiol - 24h Cosinor Fit
Concentration (ng/mL)
0.6
0.5
0.4
0.3
0.2
0.1
0
6
12
18
24
Time (hours)

(F)


Growth Hormone - 24h Cosinor Fit
Concentration (ng/mL)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0
6
12
18
24
Time (hours)

(G)


Cortisol - 24h Cosinor Fit
Concentration (ng/mL)
6
5
4
3
2
0
6
12
18
24
Time (hours)