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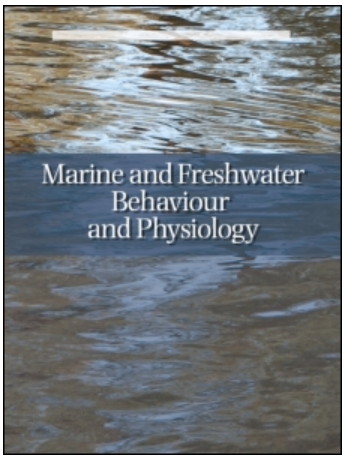


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# A novel running wheel apparatus to monitor locomotor rhythms in land crabs

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Biological rhythms have been identified and analyzed in many organisms, from single-celled alga to vertebrates. Apparatus and methods used for detection and analysis of these rhythms depend on the type of rhythm under investigation, the species, and the investigator, with new methods constantly being developed. For locomotor rhythms, one of the most overt biological rhythms, data are recorded in an actograph. Various types of actographs are commonly used, including tipping carousels (Palmer 1971), photosensor-based detectors (Brown and Unwin 1961), and running wheels (Roberts 1960), and the results of experiments may depend on the type of actograph used (Brady 1967; Honma et al. 1991).

Once data are obtained, several methods are available for analysis of rhythmicity and period, each with its own inherent advantages and disadvantages. A common method is periodogram analysis (Enright 1965). While widely used, periodogram analysis is fairly low resolution and deals poorly with noisy data (Dowse and Ringo 1989). Some investigators choose to fit regression lines to some phase point in actograms, using the regression slope to estimate period length (Palmer 1971). Although this method provides an estimate of period length, it is somewhat subjective, as regression lines are sometimes fit visually, requiring interpretation of the actograms by multiple people (Palmer 1971). Substantially different results may be obtained if a different phase point is used, and the choice is arbitrary. Additionally, this technique often fails to detect interesting or relevant details that may be present in the data (Dowse and Ringo 1989). More robust and accurate techniques are now commonly used, including autocorrelation (Chatfield 1980) and maximum entropy spectral analysis (MESA) (Dowse et al. 1987; Dowse and Ringo 1989). These techniques allow for detection of rhythmicity and accurate estimation of period length in noisy or shorter time series and overcome many of the problems faced when using older methods such as periodogram or actogram analysis.

A novel running wheel apparatus was designed and constructed for use with terrestrial crabs. This apparatus, combined with modern statistical techniques, was used to analyze endogenous locomotor rhythms in the tropical land crab *Gecarcinus lateralis* (Fréminville). This species lives its adult life entirely on land, returning to the sea only briefly to release larvae which develop in the plankton before emerging onto land as megalopae or juveniles

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(Bliss et al. 1978). This crab's abundance and hardiness in captivity make it an attractive model system for the study of biological rhythms. Using these methods, high levels of activity were observed in the running wheels, lasting for the duration of the experiment. A circadian rhythm with a bimodal, crepuscular component was identified.

Adult *G. lateralis* (31–40 mm carapace width) were collected from two locations in Bocas del Toro, Panama. Seven individuals were collected from Isla Colon (9°21.08′ N, 82°15.23′ W) and three individuals were collected from Bastimentos Island (9°21.22′ N, 82°11.99′ W). All crabs were collected from lightly forested or vegetated areas located ~10–50 m inland of a sandy beach. Crabs were transported to the Smithsonian Tropical Research Institute in Bocas del Toro and each crab was placed individually into a running wheel where they were monitored for 5–6 days under constant darkness at room temperature (~25°C). To prevent desiccation, 1 mL of seawater was added to each of the wheels every 2 days during the hours of darkness in the field, starting on day 2. The room was dark during water additions with the only light coming from a small red LED headlamp. Based on their visual pigments (Lall and Cronin 1987), *G. lateralis* should be insensitive to red light.

Running wheels were constructed from 20.5 cm diameter, 5.3 cm deep, opaque plastic containers (Figure 1). The inside of each wheel was lined with marine-grade nonslip tape to provide traction. The wheel was attached to a bearing and the whole assembly rotated around a 9.5 mm stainless steel axle supported by a PVC stand. A magnetic reed switch (PR-20445WH, Ademco/Honeywell) mounted on one of the horizontal PVC supports and two 12.7 × 6.35 × 6.35 mm neodymium magnets mounted 180° apart on the outside of the wheel were used to detect wheel rotation. With this arrangement, a switch closure occurred every half-revolution of the wheel. Each switch was connected to a USB data acquisition device (USB-6501, National Instruments) driven by a laptop computer. Switch closures were monitored using a custom monitoring program created in LabView 8.0 (National Instruments) that checked the status (open or closed) of each of the switches every 10 ms and counted the number of open-to-closed transitions. It then summed the data for each wheel into number of switch closures per 30 min bin.

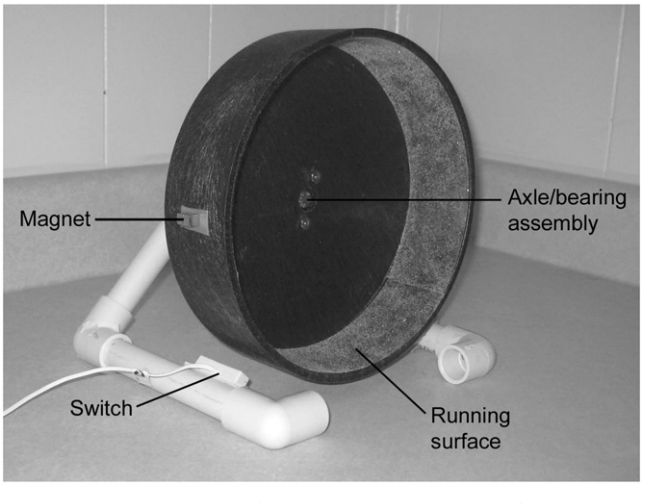


Figure 1. Running wheel apparatus used to monitor activity of *G. lateralis* under constant conditions. Cover is not shown.

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Time series of activity data were analyzed for periodicity using a combination of autocorrelation and MESA (Levine et al. 2002). Rhythmicity was assessed by autocorrelation, which plots the autocorrelation coefficients as a function of lag at 0.5 h intervals. Peaks with autocorrelation coefficients exceeding $\pm 2/\sqrt{N}$, where $N$ is number of 0.5 h intervals, indicated statistical significance at $p < 0.05$ (Dowse and Ringo 1989). Period estimates were obtained using MESA, which fits an autoregressive model to the data and uses Fourier analysis to construct a power spectrum, from which period estimates can be obtained (Levine et al. 2002). Because MESA does not indicate the statistical significance of period estimates, peaks in the spectrum were validated by comparison with peaks in the correllogram. As several crabs showed a high level of activity immediately upon placement in the running wheels, data from day 1 were excluded from all analyses.

Circular statistics were used to assess synchrony between activity and time of day for each crab. Data from day 2 only were used for these analyses in order to reduce the effect of a free-running period that shifted the time of activity over the course of the monitoring period. The time of each switch closure, to the nearest 0.5 h, was treated as an observation and converted to a corresponding angular value, with 0° representing 00:00 (midnight) and 180° representing 12:00 (noon). Rao's spacing test was used to determine whether timing of activity differed significantly from a uniform distribution. Mean angles of activity were calculated using one of two methods. For crabs exhibiting unimodal activity on day 2, mean angles were calculated using standard circular statistical methods (Zar 1996). For crabs exhibiting bimodal activity on day 2, a mean angle for each mode was calculated using the broken axis approach, which allows mean angles to be calculated for each mode for asymmetrical, bimodal data (Holmquist and Sandberg 1991). Mean angles of activity were then converted back into time format and indicated the mean time of activity for each crab.

The running wheel apparatus effectively produced results comparable to previous studies of circadian locomotor rhythms in *G. lateralis*. Bliss and Sprague (1958) monitored locomotor activity in the field and found a robust nocturnal activity pattern. Upon laboratory tests under constant conditions, the rhythm weakened and disappeared only to reappear after the first week. Palmer (1971) monitored locomotor behavior in nocturnally active *G. lateralis* from South Bimini, Bahamas. Under constant conditions in tipping carousel actographs, a circadian locomotor rhythm was identified with activity limited primarily to the hours of darkness in the field.

Of the 10 crabs tested in our running wheels, 8 displayed circadian locomotor rhythms under constant conditions (Figures 2–3). Autocorrelation detected significant ($p < 0.05$) rhythmicity in 8 of the 10 crabs, but not for crabs 6 and 9 (Table 1). Period estimates from the MESA spectra ranged from 19.9–26.1 h for the rhythmic crabs, with a mean period length of $23.3 \pm 0.75$ h (mean ± SEM), comparable to the mean period length of 23.2 h obtained by Palmer (1971). No significant differences in period length were detected between crabs from the two collection sites or between the two sexes.

Times of activity were not random but were associated with the diel cycle. Significant deviations from a uniform distribution were detected for all crabs (Rao's spacing test, $p < 0.01$).

The circadian locomotor rhythm was expressed as one of two forms: (1) a unimodal circadian rhythm of nocturnal activity and (2) a bimodal circadian rhythm. For crabs exhibiting unimodal activity, mean time of activity ranged from 21:05 to 00:19 (Table 1). For crabs exhibiting bimodal activity, mean time of activity for the earlier mode ranged from 2:10 to 7:43 and mean time of activity for the later mode ranged from 17:52 to 20:11

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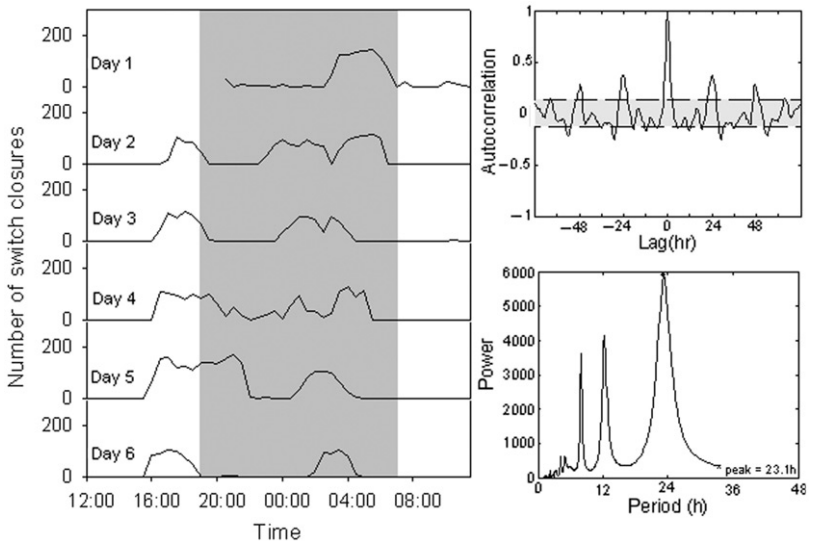


Figure 2. Actogram of number of switch closures per 0.5 h interval under constant conditions for a representative *G. lateralis* (crab 5) that displayed bimodal activity. Each day is plotted beneath the preceding day. The gray bar indicates the hours of darkness in the field. Corresponding correllogram (upper right) and MESA spectrum (lower right) are included.

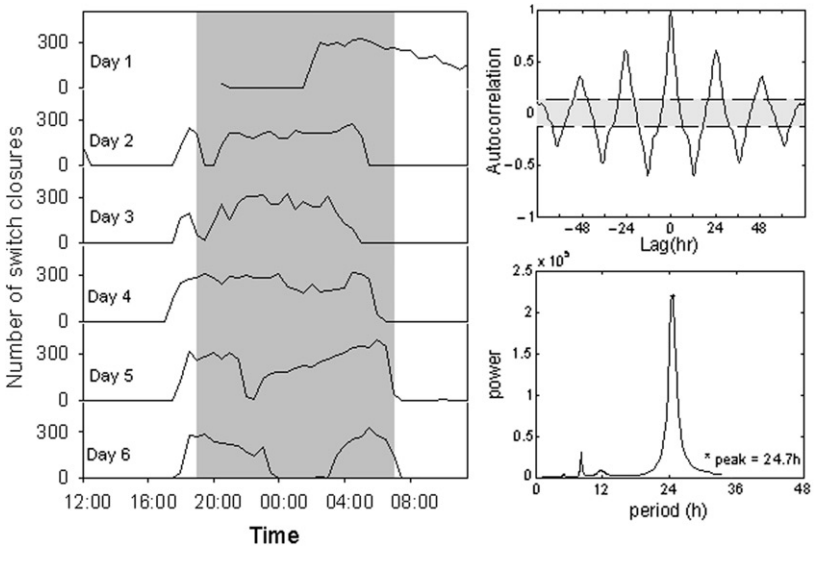


Figure 3. Actogram of number of switch closures per 0.5 h interval under constant conditions for a representative *G. lateralis* (crab 8) that appeared to switch from nocturnal to crepuscular activity. Each day is plotted beneath the preceding day. The gray bar indicates the hours of darkness in the field. Corresponding correllogram (upper right) and MESA spectrum (lower right) are included.

(Table 1). These results suggest crepuscularity, as two modes fall around the times of sunrise and sunset.

Several of the crabs appeared to switch between the two forms. Four of the crabs exhibited a bimodal activity pattern throughout the study period (Figure 2). Two crabs initially exhibited nocturnal activity but appeared to become bimodal after several days in

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Table 1. Summary of results.

| Crab | Carapace width (mm) | Sex | Period estimate (h) | Mean time(s) of activity (h) |
|---|---|---|---|---|
| 1 | 40 | M | 25.1 | 21:05 |
| 2 | 35 | F | 26.1 | 6:53, 18:47 |
| 3 | 40 | M | 23.9 | 7:43, 17:52 |
| 4 | 36 | F | 20.8 | 2:10, 20:11 |
| 5 | 31 | M | 23.1 | 3:16, 18:41 |
| 6 | 35 | M | arr. | – |
| 7 | 35 | F | 19.9 | 5:42, 19:47 |
| 8 | 36 | M | 24.7 | 00:15 |
| 9 | 37 | F | arr. | – |
| 10 | 39 | F | 22.6 | 00:19 |
| | | Mean | 23.3 | |

Notes: Period estimates derived from MESA spectra are presented, along with mean times of activity derived from circular statistics. Carapace width and sex of each crab are shown for reference.

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constant conditions (Figure 3). One crab exhibited a unimodal, nocturnal activity pattern throughout the study period and one individual started out exhibiting a bimodal activity pattern but appeared to exhibit unimodal, nocturnal activity starting on day 3 of the experiment. Longer activity records are needed to determine if this switch persists, though these observations are consistent with the observations of Palmer (1971), who reported that the activity rhythm of at least one individual became bimodal after several days in constant darkness, with bimodality lasting for several weeks.

Results of this study were consistent with those obtained by Palmer (1971), indicating that the running wheel apparatus and methods used here are viable methods for the analysis of locomotor rhythms in terrestrial crabs. The running wheels are inexpensive to construct and provide high-resolution activity data. The robust activity levels detected in the running wheels enabled autocorrelation analysis to detect significant rhythmicity for 80% of the crabs tested using only 5–6 days of data, rather than the several weeks of data needed for other methods. MESA, used here to provide period estimates, is an objective method that does not rely on subjective analysis of times of onset or offset of activity or arbitrary decisions about which activity transition should be used for analyses. These methodological advances can be used to study other aspects of biological rhythms in *G. lateralis* and can be applied to additional terrestrial crab species.

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## References

Bliss DE, Sprague PC. 1958. Diurnal locomotor activity in *Gecarcinus lateralis*. Anat Rec. 132:416–417.

Bliss DE, van Montfrans J, van Montfrans M, Boyer JR. 1978. Behavior and growth of the land crab *Gecarcinus lateralis* (Fréminville) in southern Florida. Bull Am Mus Nat Hist. 160:113–151.

Brady J. 1967. Control of circadian rhythm of activity in the cockroach: I. Role of corpora cardiaca, brain and stress. J Exp Biol. 47:153–163.

Brown RHJ, Unwin DM. 1961. An activity recording system using infra-red detection. J Insect Physiol. 7:203–209.

Chatfield C. 1980. The analysis of time series: an introduction. London, New York: Chapman and Hall.

Dowse HB, Hall JC, Ringo JM. 1987. Circadian and ultradian rhythms in period mutants of *Drosophila melanogaster*. Behav Genet. 17:19–35.

Dowse HB, Ringo JM. 1989. The search for hidden periodicities in biological time-series revisited. J Theor Biol. 139:487–515.

Enright JT. 1965. Search for rhythmicity in biological time-series. J Theor Biol. 8:426–468.

Holmquist B, Sandberg R. 1991. The broken axis approach – a new way to analyze bidirectional circular data. Experientia. 47:845–851.

Honma S, Honma KI, Hiroshige T. 1991. Methamphetamine effects on rat circadian clock depend on actograph. Physiol Behav. 49:787–795.

Lall AB, Cronin TW. 1987. Spectral sensitivity of the compound eyes in the purple land crab *Gecarcinus lateralis* (Freminville). Biol Bull. 173:398–406.

Levine JD, Funes P, Dowse HB, Hall JC. 2002. Signal analysis of behavioral and molecular cycles. BMC Neurosci. 3:1–25.

Palmer JD. 1971. Comparative studies of circadian locomotory rhythms in 4 species of terrestrial crabs. Am Midl Nat. 85:97–107.

Roberts SKD. 1960. Circadian activity rhythms in cockroaches: 1. The free-running rhythm in steady-state. J Cell Comp Physiol. 55:99–110.

Zar JH. 1996. Biostatistical analysis. Upper Saddle River, N.J.: Prentice Hall.

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