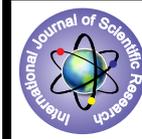


Circadian Locomotor Activity in The Freshwater Crab, *Barytelphusa Cunicularis* (Westwood, 1836): Under Artificial Illumination



Science

KEYWORDS : *Barytelphusa cunicularis*, locomotor activity, freshwater crab, circadian activity, artificial illumination.

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ABSTRACT

In the present study, it was examined if the locomotor activity of both male and female Indian freshwater crab, Barytelphusa cunicularis is rhythmic under LD 12:12 photo cycles and if this rhythm free runs following their transfer from LD to DD or LL. Further, it was investigated if B. cunicularis exhibits bimodal pattern of locomotor activity under LD, DD and LL schedules. The specimens were placed for recording the activity at each light regime in between 10-12 days for male crabs and 15 days for female crabs. The locomotor activity was recorded at three different light regimes; DD, LD (12:12h) and LL at 150 lux. Data were analyzed for documenting a circadian rhythm in locomotor-activity ($\tau = 24h$) with the help of Cosinor rhythmometry. The locomotor activity rhythm in B. cunicularis, when exposed to LD the crab shows the bimodal and multimodal patterns of activity anticipating lights-on and lights-off condition. The free running rhythm under constant light and dark regimen are observed irrespective of gender. The 24-h average mean is more in male as compare to female. Amplitude is higher in DD and lower in LL conditions. It may be possible that, the crab possesses a functional oscillator, which regulates its overt circadian rhythm in locomotor activity.

INTRODUCTION

Among the crustaceans, the freshwater and marine decapods have been used as experimental models to study circadian organization in invertebrates (Warner, 1977; DeCoursey, 1983; Brown, 1983; Arechiga et al., 1993; Fuentes-Pardo and Hernandez-Falcon, 1993). Notably among those are: (1) American horseshoe crab, *Limulus polyphemus* (Chabot et al., 2007, 2004); (2) American freshwater crab, *Pseudothelphusa americana* (Vania et al., 2005); (3) Eastern Pacific fiddler crab, *Uca princeps* Smith (Stillman and Barnwell, 2004); (4) the fiddler crab, *Uca subcylindrica* Stimpson (Thurman and Broghammer, 2001; Thurman, 1998); (5) shore crab, *Carcinus maenas* (Naylor, 1996; Reid and Naylor, 1990); (6) the East African fiddler crabs, *Uca urvillei* and *Uca annulipes* (Lehmann et al., 1974). They prominently exhibit bimodal activity pattern under LD schedules characterized by occurrence of peaks in activity corresponding to light-on and light-off timings. The locomotor activity rhythm has also been reported to free-run under DD and LL in freshwater crab, *Pseudothelphusa americana* (Miranda-Anaya et al., 2003) and fiddler crab, *Uca subcylindrica* (Thurman and Broghammer, 2001; Thurman, 1998). Interestingly, the bimodality in the pattern of locomotor activity has been found to remain unaltered upon transfer from LD to DD, whereas following transfer to LL unimodal pattern in locomotor activity has been conspicuous (Miranda-Anaya et al., 2003).

MATERIALS AND METHODS

Procurement and recording of locomotor activity

Live specimens of *B. cunicularis* were collected from local wetlands and freshwater ponds during rainy season and transferred to animal house. Male and female crabs were segregated and kept in separate stock aquaria containing tap water. The crabs were fed with boiled egg white on alternate day and fresh tap water was replenished a day after each feeding. They were acclimated to the laboratory conditions for 15 days. During the period of acclimation the crabs were exposed to LD 12:12 (Light onset: 06:00) photoperiod and relatively constant room temperature that varied between 22°C and 24°C. The light fraction of the LD had an intensity of 150 lux. Crabs were kept individually in a specially design transparent rectangular aquaria measuring 40 x 10 x 15 cm. The bottom of each aquarium consisted of two parts, such as aquatic zone and terrestrial zone. The former was filled with tap water up to 5 cm height. Each crab was provided with the option to select either of the zones. The rhythmic locomotor activity of the crabs were monitored and recorded by using IR sensors placed in the middle of the aquatic zone. IR beam interruptions caused by the movement of crabs were fed to a 20-channel Angus event recorder.

Experimental protocol

Experiment 1: Five LD-acclimated male crabs (average body weight = 58.59 g) were randomly selected and were exposed to DD for 10 days. Thereafter, they were exposed under LD 12:12 (Light onset: 06:00), DD, LD 12:12 (Light onset: 06:00), and LL

in a sequential manner. In each schedule they had an experience of 10-12 days. Animal husbandry and maintenance activities were carried out under red dim light during the dark fraction of LD schedules and DD.

Experiment 2: The experimental protocol was identical with that of the experiment 1, but the specimens were chosen female crabs (average body weight: 57.81 g) and that they were exposed under DD, LD, DD, LD, and LL sequentially for 15 days each.

Construction of actogram and digitization of data

Actogram was constructed day wise by placing locomotor activity record of each day, one below the other. Double plotting of activity was executed for the better visualization of rhythmic patterns. The qualitative data on the actogram were digitized, using the technique adopted by Thurman and Broghammer (2001). Each hour on the actogram was divided into six 10-minute bins and scored for activity. To represent the sum of the activities for the entire hour integers between 1 and 7 were used. The former digit indicated that the crab was dormant during the entire hour and the latter indicated that the crab was active at least once during all six 10-minute bins. The process of digitization was completed for each day and each time series.

Statistical Analysis of Data

Data were analyzed for documenting a circadian rhythm in locomotor activity ($t = 24$ h) with the help of Cosinor rhythmometry (Nelson et al., 1979). A rhythm was characterized by estimating three parameters, such as the Mesor (M, rhythm-adjusted mean), the amplitude (A, half of the difference between the highest and the lowest value of the rhythmic function) and the acrophase (\emptyset , timing of the highest value of the rhythmic function). A power spectrum method was also employed for detecting prominent period (t) in individual time series for locomotor activity (De Prins et al., 1986).

RESULTS

Both male and female crabs were more active during the onset of dark phase. The daily records of locomotor activity of one male and one female, chosen as representatives, are depicted (Fig. 1 & Fig. 2), respectively. A bout of increased activity was also noticeable corresponding to the timings of light onset, while crabs were under LD 12:12. This bimodality in locomotor activity of crabs disappeared, when they were subjected to DD or LL, irrespective of gender (Fig. 1 & Fig. 2). The observed bimodality was more distinct and precise in case of female crab (Fig. 2). However, in general the rhythm detection ratio was of lower order in the group of female crabs (Table 1) cosinor summary of the characteristics of locomotor activity rhythm in freshwater crab, *B. cunicularis*.

Rhythm-adjusted mean, Mesor

The male crabs were always more active as compared with the females, irrespective of the photo regimens. This was gauged

from the values of circadian Mesor (Table 1). However, the mesor in the group females was not statistically significant from that of the males when they were exposed under LL and LD 12:12 following DD. In males mesor under DD was always higher as compared with that when they were exposed under LL. This difference was not marked in case of female crabs.

Amplitude

The amplitude of locomotor activity remained invariant irrespective of gender and photo regimens. Although differences among values of amplitudes were not statistically significant in respect of gender, it was always lower in females irrespective of photo regimens, namely LD 12:12, DD and LL.

Period

Prominent periods gauged from spectral analysis are shown (Fig. 3 & Fig. 4). They depict periodograms of two representative male and female crabs, respectively. In general under LD 12:12, a bimodal pattern in locomotor activity was evident in both male and female crabs. This distinction was more prominent in case of female crabs as evident from the actogram (Fig. 5). The locomotor activity free-ran under constant conditions, such as DD and LL. The period length was shorter under LL as compared with that under DD, irrespective of gender, although the difference was not statistically significant (Fig. 5).

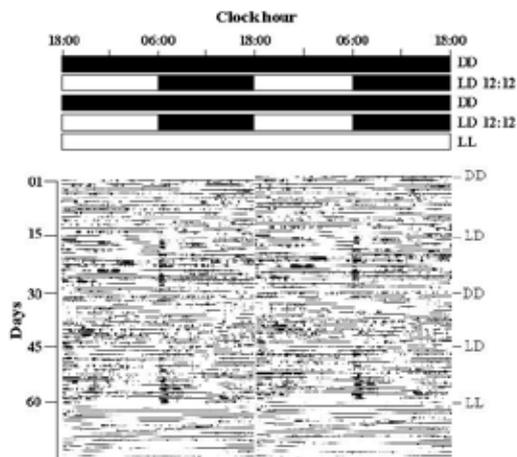


Figure 1 Double-plotted actogram of a representative male *Barytelphusa cucicularis* (BcM # 05) under DD following LD 12:12, DD, LD 12:12 and LL.

Each line represents one 48-h period and successive days are shown from top to bottom.

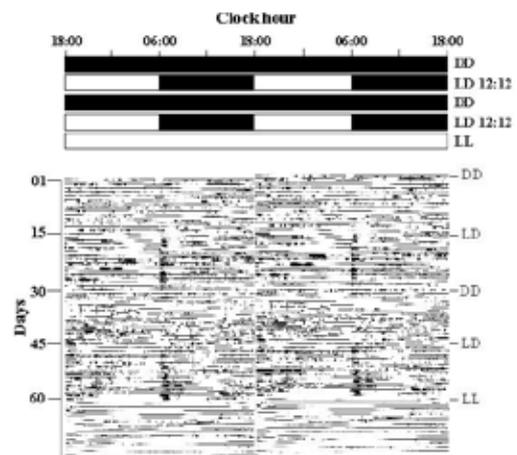


Figure 2 Double-plotted actogram of a representative female *Barytelphusa cucicularis* (BcF # 05) under DD following LD 12:12, DD, LD 12:12 and LL.

Each line represents one 48-h period and successive days are shown from top to bottom.

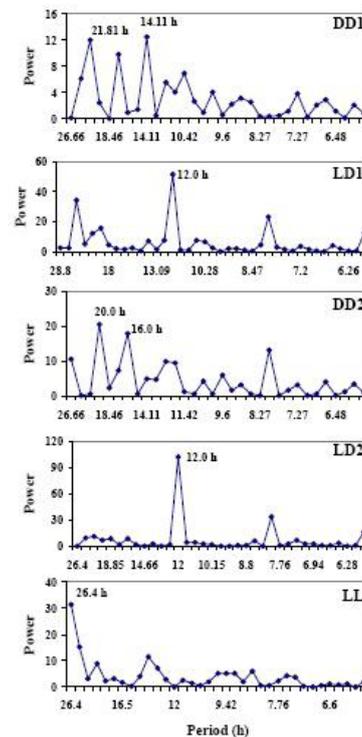


Figure 3 Periodogram of the locomotor activity rhythm of a representative male *Barytelphusa cucicularis* (BcM # 05) under DD following LD 12:12, DD, LD 12:12 and LL.

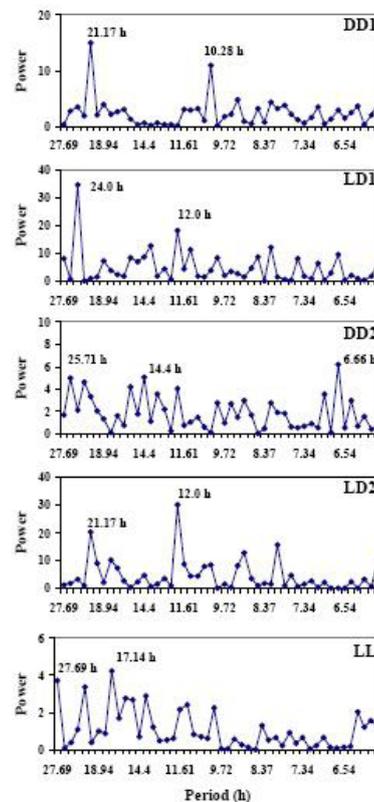


Figure 4 Periodogram of the locomotor activity rhythm of a representative female *Barytelphusa cucicularis* (BcF # 05) under DD following LD 12:12, DD, LD 12:12 and LL.

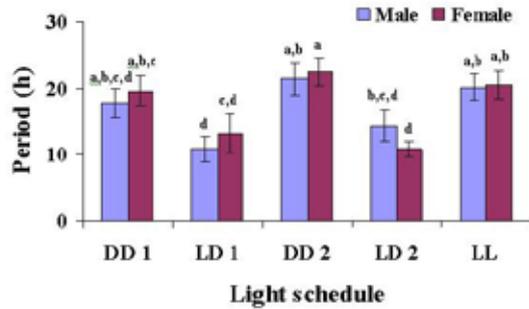


Figure 5 Average prominent periods of locomotor activity rhythm in the groups of male and female crabs exposed to different light schedules.

Table 1 Cosinor summary of the characteristics of locomotor activity rhythm in freshwater crab, *Barytelphusa cunicularis*

Light schedule	Bins/ animal		Rhythm detection ratio		M ± 1 SE		A ± 1 SE		Æ ± 1 SE	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
DD1	240	360	0.6	0.4	2.81 ± 0.14a,b	2.25 ± 0.11c,d	0.45 ± 0.10a	0.23 ± 0.02a	19.92 ± 0.92a,b	18.71 ± 1.05a,b,c
LD1	288	360	0.4	0.4	2.80 ± 0.14a,b	2.22 ± 0.09c,d	0.33 ± 0.13a	0.27 ± 0.1a	5.80 ± 1.89d	10.94 ± 3.9c,d
DD2	240	360	0.6	0.6	2.85 ± 0.12a	2.08 ± 0.08c,d	0.44 ± 0.13a	0.32 ± 0.07a	13.98 ± 1.47a,b,c,d	21.25 ± 0.9a
LD2	264	360	0.8	0.4	2.43 ± 0.17b,c	2.32 ± 0.13c,d	0.45 ± 0.10a	0.25 ± 0.05a	10.53 ± 2.86c,d	13.21 ± 3.3a,b,c,d
LL	264	360	0.4	0.4	2.18 ± 0.12c,d	1.92 ± 0.17d	0.33 ± 0.08a	0.19 ± 0.06a	12.23 ± 4.52b,c,d	15.95 ± 2.13a,b,c

M, Rhythm-adjusted average of the best-fitting cosine function + 1 standard error; A, half the difference between the maximum and the minimum of the best-fitting cosine function ± 1 SE; Æ, time in hour of the maximum in the best-fitting cosine function ± 1 SE

DISCUSSION

In the present study, a conspicuous bimodality in locomotor activity rhythm of *Barytelphusa cunicularis*, irrespective of gender, is observed under LD 12:12. This phenomenon seems to be of common occurrence among crabs, both freshwater and marine species (Chabot et al., 2007; Vania et al., 2005; Miranda-Anaya, 2004; Miranda-Anaya et al., 2003; Reid and Naylor, 1990). In *B. cunicularis* activity pattern entrains to LD with two distinct bouts; one coincides with the onset of darkness and the other one with the onset of light. Present results support earlier findings for *Uca subcylindrica* (Thurman and Broghammer, 2001).

The peak in locomotor activity rhythm always advances when the crabs are exposed to LD 12:12 schedules as compared to their exposure under either DD or LL. This could be imputed to free-running nature of the locomotor activity rhythm in both male and female crabs. The period of the locomotor activity rhythm in crabs, both males and females, shortens, when they are exposed to LD 12:12 light schedule. Under LD, there is no significant difference between males and females as regards lengths of period are concerned. This further consolidates bimodality as a prominent feature in *B. cunicularis* under conditions of LD entrainment and corroborates earlier findings on bimodality in locomotor activity rhythm in different species of

crabs (Chabot et al., 2007; Vania et al., 2005; Miranda-Anaya, 2004; Miranda-Anaya et al., 2003; Reid and Naylor, 1990). The rhythm of locomotor activity turns unimodal and free runs under constant conditions, such as DD and LL. It is also noteworthy to mention here that free runs of locomotor activity rhythm in *B. cunicularis* are not very much conspicuous. In *P. americana* unimodal free-running rhythm of locomotor activity has been reported only under LL, but not DD (Miranda-Anaya et al., 2003). It is concluded that the locomotor activity rhythm of both male and female *B. cunicularis* has the ability to entrain to LD 12:12 cycles and that the rhythm free runs under DD or LL. However, in general the bimodality in locomotor activity rhythm under LD 12:12 disappears, when they are exposed under either DD or LL. This phenomenon appears to be independent of gender. It seems that circadian component in locomotor activity in *B. cunicularis* is strong, irrespective of gender.

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