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Preliminary data on locomotor activity rhythms on epigean and cave snails, genus *Potamolithus* (Hydrobiidae), from southeastern Brazil

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ABSTRACT

The locomotory activities of two freshwater hydrobiid snails, genus *Potamolithus*, one epigean and other troglobite (*Potamolithus* sp., an undescribed species and *P. troglobius*, respectively), were compared in order to detect possible differences associated with the colonization and isolation in the subterranean environment. Individuals were hand-collected in streams from the Upper Ribeira Valley, southeastern Brazil, and maintained collectively in laboratory. The free-running activity (under light conditions of 21-28 lux intensities) was observed consecutively for 48h, when 12 individuals of each species had their position in three liters aquaria recorded every 20 minutes. Both species showed significant ultradian rhythms in three individuals (inds. 2, 10 and 12 of *Potamolithus* sp. and inds. 1, 2 and 12 of *P. troglobius*). However, individual differences were found: the troglobite species, *P. troglobius*, showed higher values for the rhythmicity components (> 6h) and smaller variations in the cycles comparing with the epigean one, *Potamolithus* sp. (peaks between 3 and 14h). When analyzed in groups, the graphs showed that individuals of *P. troglobius* had peaks every 5, 6 and 7h and *Potamolithus* sp. every 2 and 4h. Besides, individuals of *P. troglobius* had a dislodgement significantly smaller than *Potamolithus* sp. (Z=-5.577; p=0.000). Other cave environment characteristics than the permanent darkness, such as the smaller predation pressure and lower temperature fluctuations could be regulating the rhythmicity in the troglobite species, mainly observed in the dislodgement and individual variation of the rhythmicity.

Key words: locomotor activity, freshwater snails, *Potamolithus*, southeastern Brazil.

INTRODUCTION

Biological rhythms are defined as the occurrence, on regular intervals, of biochemical, physiological and behavioural events (Aschoff 1981). In laboratory constant conditions, without environmental time cues, diverse biological rhythms persist for several days, months or years, these are called free-running rhythms, representing the expression of endogenous timing systems (Marques et al 1997).

In the subterranean environment, important "zeitgebers" (external synchronizers), such as light-dark and temperature cycles are normaly damped if not altogether absent. Consequently, circadian components can regress evolutively in the troglobitic species (organisms restricted to subterranean habitats- sensu Holsinger and Culver 1988) (Parzefall 1993; Trajano and Menna-Barreto 1995, 1996, 2000). Barr (1968) stated that if the circadian rhythms are endogenous, genetically controlled, we can suppose evolutive changes in these in the same way we interpret the regression of the eyes and melanic pigmentation observed in cave organisms.

A regression of the free-running circadian locomotory rhythmicity was veryfied in part of troglobitic catfish populations from southeastern and northeastern Brazil (Trajano and Menna-Barreto 1995, 1996, 2000). This character was also observed for tetra-characins blind fishes, genus *Astyanax*, from Mexico (Lamprecht and Weber 1992) and troglobitic beetles and crustaceans (Culver 1982; Lamprecht and Weber 1982; Kane and Richardson 1985).

Biological rhythms are present in many molluscs (Ali 1992). However, most works focused circatidal rhythms of marine gastropods (e.g. Moulton 1962; Newell 1962; Zann 1973; Barnes 1986) and few focusing on freshwater or terrestrial species (e.g. Bailey 1975; Beeston and Morgan 1977, 1979; Blanc et al 1989; Blanc 1993).

Most subterranean gastropods belong to Hydrobiidae family (Bole and Velkovrh 1986), common in many karst regions of the world, including Europe, North América, Africa, Japan, Australia and New Zealand (Hershler and Longley 1986). For South America, there are records of *Andesipyrgus sketi* Hershler and Velkovrh, 1993 in caves from Ecuador and Colombia, and *Potamolithus troglobius* Simone and Moracchioli, 1994 and *P. karsticus* Simone and Moracchioli, 1994 in caves from Upper Ribeira Valley, southeastern Brazil. In addition, a faunistic survey in this karst area, resulted in 14 different morphological species of *Potamolithus* (Bichuette 1998).

Works focusing Neotropical hydrobiid snails are related with species descriptions and taxonomy and papers focusing behaviour and physiology are scarce. The first results focusing behavioural and ecological studies of *Potamolithus* species from southeastern Brazil are presented elsewhere (Bichuette and Trajano 1999; 2003). This paper represents the third part of this study and the first one where free-running locomotor activity rhythms of *Potamolithus* species are studied (one epigean, *Potamolithus* sp., cited as *Potamolithus* sp. 1 in Bichuette and Trajano 1999, 2003, and other troglobite, *P. troglobius*). The aim of the present work is to investigate if there are differences in biological rhythmicity in these species possibly related to the evolution in subterranean environment.

MATERIALS AND METHODS

Collecting, transport and maintenance of snails

Two *Potamolithus* species were studied in relation to locomotory activity, one epigean (*Potamolithus* sp., from Betari River and under description, showing an elliptical brown shell) and one troglobite (*P. troglobius*, from Aguas Quentes Cave, showing a globose transparent shell and no visible eyes). These species are very diminute, with up to 2.0-3.0mm shell length. Both localities are located in the Upper Ribeira karst area, situated in southeastern Brazil.

Specimens were collected by hand after visual inspection of lower surface of boulders and other submerged substrates where these animals concentrate and transported to São Paulo city (430km distant from the localities of snails) in two liters bottles conditioned in thermic boxes, under constant aeration and with temperature kept by addition of recycled hypergel. At São Paulo, the gastropods were maintained in groups of 30-40 individuals, in three liters aquaria installed in a room without illumination, except during maintainance activities (aquaria cleaning and feeding), at a temperature of 24.0 ± 1°C. The aquaria had continuous aeration and pH maintained between 7.0 and 8.0 through introduction of limestone blocks brought from the locality of snails.

Locomotor activity

For locomotor activity recordings, squared paper ($5mm^2$ each square) was attached under the bottom and on three lateral walls of the aquaria. Then the position of the snails was visually recorded every 20 minutes for 48 consecutive hours (allowing for the detection of ultradian rhythms). In order to enter in free-running conditions, specimens were maintained, before recordings, for three days under constant illumination of intensities varying from 21 to 28lux (both species showed no reaction to these light intensities - Bichuette and Trajano 1999) and constant aeration. The water temperature was measured every hour (between 21.7 and 21.9°C) simultaneously to the aquaria aeration. The snails were not fed during the observations.

In total, twelve individuals of each *Potamolithus* species were tested. To individualize them, each one was painted with oil ink of different colors, with a small spot in the dorsal region of their shells.

For the detection of periodical components in the individual time series (data sequences in relation to the time), the Fourier Fast Transform spectral analysis was used, where the graphical representation is the periodogram (Menna-Barreto et al 1993; Benedito-Silva 1997). In order to detect dominat frequencies, the components were submitted to the Sokolove-Bushell statistical test using the software El Temps (version 1, http//www.ub.es/dpfisiv/soft/ElTemps/principal.html). In order to verify another differences in the locomotor activity of *Potamolithus* spp., the dislodgement for pooled data was compared by Wilcoxon non-parametric test (Z) and the daily activity was showed in a graphic.

RESULTS

Fig. 1 and 2 show the daily activity phase for the pooled data of *Potamolithus* species (12 individuals grouped), where the dislodgement means are showed for every hour. In this preliminary analysis, we can note that the epigean species (*Potamolithus* sp.) showed variable activity peaks, every 4-7h (Fig. 1) when compared to the troglobite species (*P. troglobius*), that showed activity peaks mainly every 12h (Fig. 2). In relation to the dislodgement per hour, troglobite species showed significantly smaller values (0.433 cm in average) comparing to the epigean species (3.888 cm in average) ($Z=-5.577$; $p=0.000$).

Individually, the activity rhythms for *Potamolithus* species obtained through the spectral analysis for all time series with the significant ultradian components (more than one cycle per 24h) are presented in Table 1. For the epigean species, *Potamolithus* sp., significant ultradian rhythms of 7h (ind. 12), 11h (ind. 2) and 13h (ind. 10) were registered (Table 1). Fig. 3 shows the periodogram referent to the locomotory activity of the individual 10, representing significant activity rhythms of 13h observed for this individual.

For the troglobite species, *P. troglobius*, the activity ultradian rhythms components showed smaller variation compared to *Potamolithus* sp.. Significant ultradian rhythms of 8h (ind. 11), 10h (ind. 2) and 14h (ind. 1) were registered (Table 1). Fig. 4 shows the periodogram referent to the locomotory activity of the individual 11, representing significant activity rhythms of 8h observed for this individual.

It is interesting to note that from 12 individuals tested in each species, *Potamolithus* sp. showed more than one activity peak in seven of them. Otherwise, only four individuals of *P. troglobius* showed more than one activity peak (Table 1). These results confirm the higher individual variability in the rhythmicity of the epigean species.

DISCUSSION

The two-day period of the experiments allowed ultradian activity components to be detected for the *Pota-*

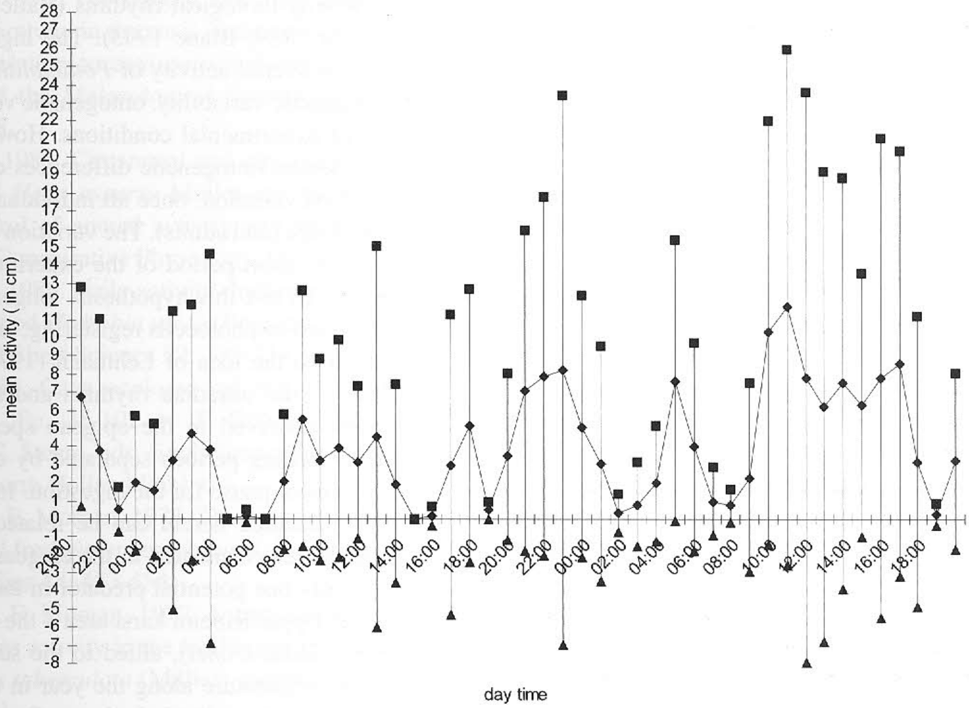

Fig. 1- Mean activity observed in 12 individuals of *Potamolithus* sp. along daytime. *x* axis shows the daily time, for 48h of experiment; y axis shows the mean dislodgement; vertical bars represent standard deviation.

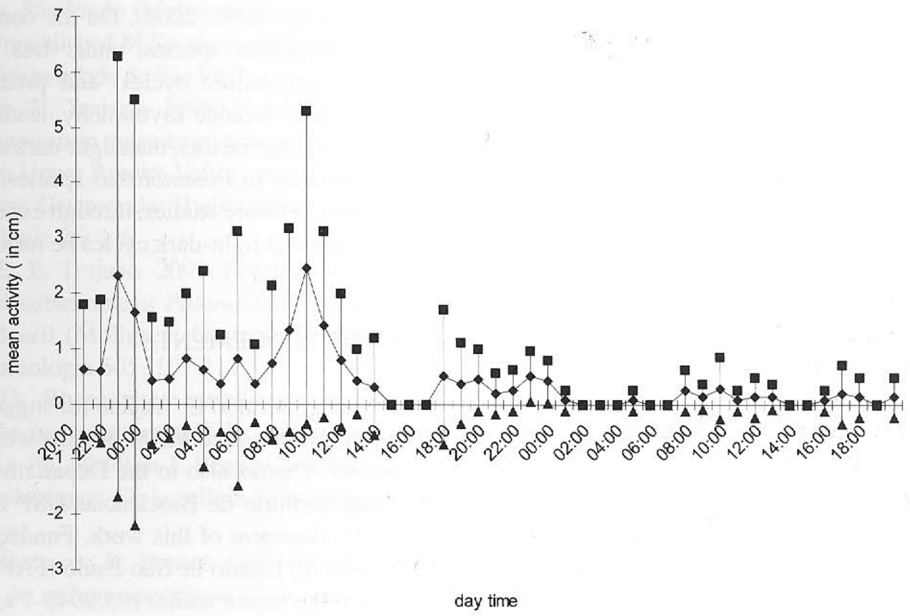

Fig. 2- Mean activity observed in 12 individuals of *P. troglobius* along daytime. *x* axis shows the daily time, for 48h of experiment; *y* axis shows the mean dislodgement; vertical bars represent standard deviation.

Table 1- Activity rhythms componets registered for the *Potamolithus* species from Upper Ribeira Valley, southeastern Brazil, resulted from the Fourier spectral analysis.* significant peaks in the periodogram; e, epigean species; tb, troglobite species.

Individuals	*Potamolithus* sp. (e)	*P. troglobius* (tb)
1	5h and 9h	14h*
2	11h* and 12h	10h*and 14h
3	3h	14h
4	10h and 13h	13h
5	6h and 14h	10h
6	6h and 8h	12h
7	10h	8h
8	11h	10h
9	7h and 10h	10h and 13h
10	6h and 13h*	10h
11	10h and 14h	8h* and 10h
12	7h*	6h and 8h

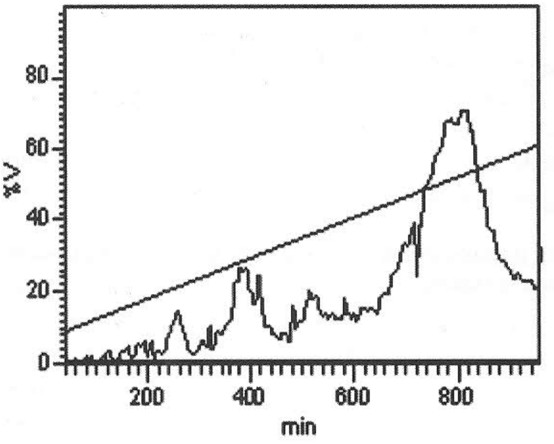

Fig. 3- Periodogram referent to the locomotor activity of *Potamolithus* sp. (ind. 10). The *x* axis represents the activity period data set and the *y* axis, the spectral contributions.

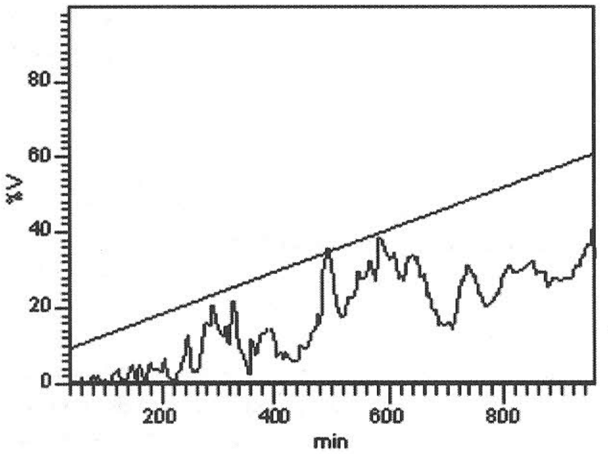

Fig. 4- Periodogram referent to the locomotor activity of *P. troglobius* (ind. 11). The *x* axis represents the activity period data set and the *y* axis, the spectral contributions.

molithus species. The epigean species, *Potamolithus* sp., showed higher variation in the ultradian cycles, in individual level, when compared to *P. troglobius*. Activity ultradian rhythms of 4 and 12h and individual variation in the activity components (ultradian and free-running circadian rhythms) were also observed for the terrestrial pulmonate *Helix aspersa*, one of the best studied molluscs showing biological rhythms (Bailey 1975, 1981; Blanc et al 1989; Blanc 1993). The higher individual variation in overall activity of *Potamolithus* sp. may be result of genetic variability, ontogenetic variation, maintenance or experimental conditions. However, for both studied species, ontogenetic differences cannot explain the observed variation, once all individuals were of the same shell size (and adults). The variation could be probably due the short period of the experiment or genetic variability. To test this hypothesis, longer experiments, through video or photocells registering, are necessary.

Similar to the idea of Lehmann (1976), the higher variation on the ultradian rhythms and the larger dislodgement observed in the epigean species could be related to feeding periods separated by changes in the feeding sites or pause for the digestion. In addition, differences presented herein can be related to predation pressure, more accentuated in the epigean environment (there is only one potential predator in the subterranean streams of Upper Ribeira karst area – the troglobite catfish *Pimelodella kronei)*, allied to the scarce food and the stable temperature along the year in the cave environment (2°C of amplitude in Aguas Quentes Cave and 4°C in Betari River - Bichuette and Trajano 2003).

From the results presented herein, it is not possible to state that the troglobite species, *P. troglobius*, has lost any components of rhythmicity, which could represent a regression in this character-state, like observed for circadian rhythmicity in other cave organisms (Trajano and Menna-Barreto 1995, 2000). On the contrary, it seems that the troglobite species, under less environmental changes (temperature cycles) and predation pressure showed a less variable rhythmicity, leading to the conclusion that other factors, than light-dark cycles, regulate the rhythmicity in *Potamolithus* species, an hypothesis to be tested in future studies, through experiments where temperature and light-dark cycles be introduced.

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