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Circadian locomotor activity in the juvenile red eared turtle *Trachemys scripta elegans*: free running and entrainment

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Long-term circadian locomotor activity under different light conditions and its corresponding free running periods were studied in the juvenile red-eared turtle *Trachemys scripta elegans*. Diurnal activity and a long free running period in darkness was observed in LD 12:12, skeleton photoperiod, and long and short days (LD 16:08, LD 08:16, respectively). Anticipation of activity to lights-on and length of activity phase depends on the photoperiod used. After-effects were observed in free running conditions after long day photoperiods. Constant light effect produces mainly the lack of circadian rhythm after a few days of period lengthening and the lack of rhythm may persist for a few days after re-entering constant darkness conditions. The results of the present report indicate that in *T. scripta elegans*, it is suitable to perform studies on circadian biology in juvenile turtles and its interaction with changes of photoperiod at constant temperature.

Keywords: *Trachemys scripta elegans*; photoperiod; locomotor activity; circadian rhythm

Introduction

Biological rhythms allow organisms to fit their physiology and behavior to the cyclic environment produced by the periodic movements of our planet. A biological clock is therefore a characteristic that may be found in a diverse range of living beings, ranging from bacteria to mammals. The way in which the biological clock confers an advantage depends on multiple interactions between the organism and its environment. A particular temporal niche defines the phase of different interactions between the organism and its ecosystem, such that major benefit can be obtained (Daan 1981; DeCoursey 2004).

In order to understand the mechanisms of circadian regulation, several organisms have been intensively used. In vertebrates, mammals have received special attention, however in order to understand the evolution of circadian biology as well as the adaptive meaning, it is fundamental to study several species of animals.

In reptiles, circadian organization and its mechanisms have been studied mainly in lacertilians and iguanids. The pineal gland, homologous to the suprachiasmatic

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nucleus (SCN) and the retina, are major circadian components (reviewed in Tosini et al. 2001; Underwood 2001). However studies on circadian biology on turtles are scarce. Behavioral studies in turtles indicate seasonal changes in activity patterns, basically because of their temperature preferences (Cloudsley-Thompson 1982; Jarling et al. 1989; Smith and Iverson 2003; Plummer 2004) but there are no clear data related to the effect of day length itself upon locomotor activity. A recent finding indicates that the red-eared turtle *Trachemys scripta elegans* is an interesting model for circadian physiology studies (Reyes and Milsom 2009).

The red-eared turtle *Trachemys scripta elegans* is a freshwater species widely distributed because of its preference to shallows with aquatic vegetation in lakes, channels and rivers of slow stream (Ernst and Barbour 1972; Gibbons et al. 1979). It is mainly diurnal and its preference to bask is noted from the juvenile period (Cloudsley-Thompson 1982). Thermal preferences are between 25 and 30°C and heat tolerance reaches 42°C (Hutchinson et al. 1966). Its distribution ranges from the Mississippi area in Illinois to the southern Gulf of Mexico (Hutchinson 1992). This species is common in pet markets and aquaria, mainly as juveniles.

The present work aims to study the basic circadian behavior of locomotor activity in the juvenile *T. scripta elegans* when exposed to different light conditions. Entraining to different photoperiods, both parametric and non-parametric, and its correspondent after-effects were analyzed in order to establish an antecedent for this species and its value with respect to the study of circadian biology in reptiles.

Methods

Animals

Juvenile turtles *Trachemys scripta elegans* ($n = 13$) of about 3–4 g average weight were obtained with no sex distinction from a local pet shop in Mexico City. Animals were fed pellets (Premium turtle bites, Wardley, USA) twice a week. A tap water layer 3 cm deep was replaced once a week in all aquaria used.

Recording of locomotor activity

Locomotor activity was recorded from individually housed animals in small glass aquaria (30 × 10 × 10 cm) equipped with infrared light crossings along the bottom, as previously reported (Miranda-Anaya et al. 2003). Briefly, locomotor activity was considered as the number of infrared beams crossing every 10 minutes. Data were collected by using a PC by means of a data acquisition board and stored until final analysis. Recordings were maintained at 23.5 ± 2°C in an isolated room. Light was provided by using a lamp (Phillips, 20 W, 150 lux) and controlled by means of a domestic timer.

Data analysis

Data were analyzed using a double plotted actogram and period values were calculated by means of a chi-square periodogram (Sokolove and Wayne 1978) provided by the software DISPAC (IFC, UNAM; Aguilar-Roblero et al. 1997). Period values of at least 10 days in steady-state rhythms were compared by a

Student's t-test using the software Statistica (Jandel Scientific, CA, USA). The significance value considered for differences was $p < 0.05\%$.

Experimental protocol

Animals were exposed to each illumination protocol, which was used for at least 15 consecutive days. After each photoperiod used, animals were exposed to constant darkness (DD) in order to observe the free running period rhythm (FRP, τ) of locomotor activity and evaluate the correspondent after-effects (Pittendrigh and Daan 1976a). Illumination protocols consisted of Light–Darkness (LD) 12:12 (parametric entrainment), Skeleton Photoperiod (SP) L:D, 10:1, 12:1 (non-parametric entrainment), constant light (LL), long days (LD 16:08), and short days (LD 08:16).

Results

Parametric and non-parametric entrainment

Figures 1A and B present two representative double plotted actograms of different animals exposed to LD 12:12 and then constant darkness (indicated by the arrowhead). The actograms show typical diurnal bouts of activity spread over the

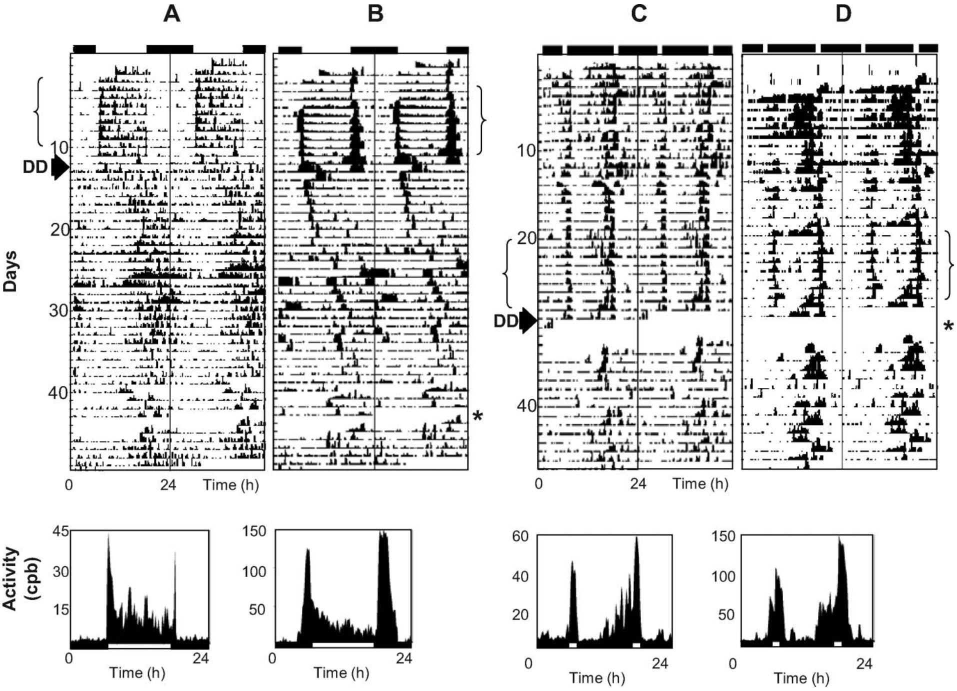

Figure 1. Actograms showing parametric (A, B) and non-parametric entrainment (C, D) of locomotor activity of juvenile turtles *T. scripta elegans*. LD 12:12 induces main activity with the lights-on peak, while during the parametric photoperiod, the main bout of activity is related to the evening peak, also shown in each waveform below actograms. Black bars indicate darkness. DD in the actograms is indicated with an arrowhead. Days used for the average waveform are indicated for each actogram (}).

light period; stronger bouts are present after lights-on as seen in the average waveform below each actogram. When released in DD, the rhythm shows phase control to the previous photic condition and a long free running period is shown during the first 10 days. In Figure 1B, a more marked activity on light transitions during the LD cycle is shown. After lights-on and lights-off, activity bouts are noticeable. When released in DD, free running rhythms are related to the morning peak, maintaining the FRP for about 30 days. The bout concerning the lights-off response does not free run during the first 15 days in DD, but is then observed after. Average waveforms below each actogram indicate the activity profile corresponding LD (fitted to 24 h).

Figure 1C and 1D show two representative actograms during non-parametric entrainment. In both examples activity is displayed during the light pulses, indicating a positive masking effect, then activity is associated with the evening pulse, which free runs in DD for at least 15 more days in DD. Average waveforms below each actogram indicate bimodal activity around each pulse and the endogenous circadian activity is mainly unimodal in DD. Lack of data in both recordings was due to an equipment failure (indicated with a star next to each actogram). All the organisms tested displayed quite similar responses.

Constant light

After DD from SP, constant light was used in the recording of locomotor activity. Two main behaviors were observed, one group of animals ($n = 5$) presented short free running rhythm ($\tau = 23.89 \pm 0.2$ h) and then a progressive lack of circadian activity. A second group displayed lack of rhythm after the first day under this condition. Circadian activity was tested by means of the chi-square periodogram. When in DD again, most of the animals displayed clear circadian activity after three days in this condition and only one remained arrhythmic during the recording after LL (data not shown).

Effect of short and long days (LD 8:16, 16:8)

Figure 2 shows actograms corresponding to short (A, B) and long-day protocols (C, D). In both conditions, the turtle's activity was detected mainly during the light period. In Figure A, activity is seen spread over the light period, but when released in DD, weak circadian activity is observed.

In Figure B, activity is mainly present around light transitions, a robust rhythm of activity free runs with a short period. Animals that display this kind of activity usually anticipate light transitions, and a strong bout of activity is observed after lights-off as denoted in the average waveform. Long-day exposure (2C, 2D) is maintained as spread all over the light phase as the main group of activity, which also free runs and is associated with the phase corresponding to lights-on in the LD cycle.

Phase relationship: onset and length of activity and FRP after-effects

Figure 3A presents the phase relationship regarding lights-on transition (zero value in the graph) and the average ($\pm$SE) onset of activity. Negative values indicate anticipation of lights-on. In three different photoperiods used, anticipation was

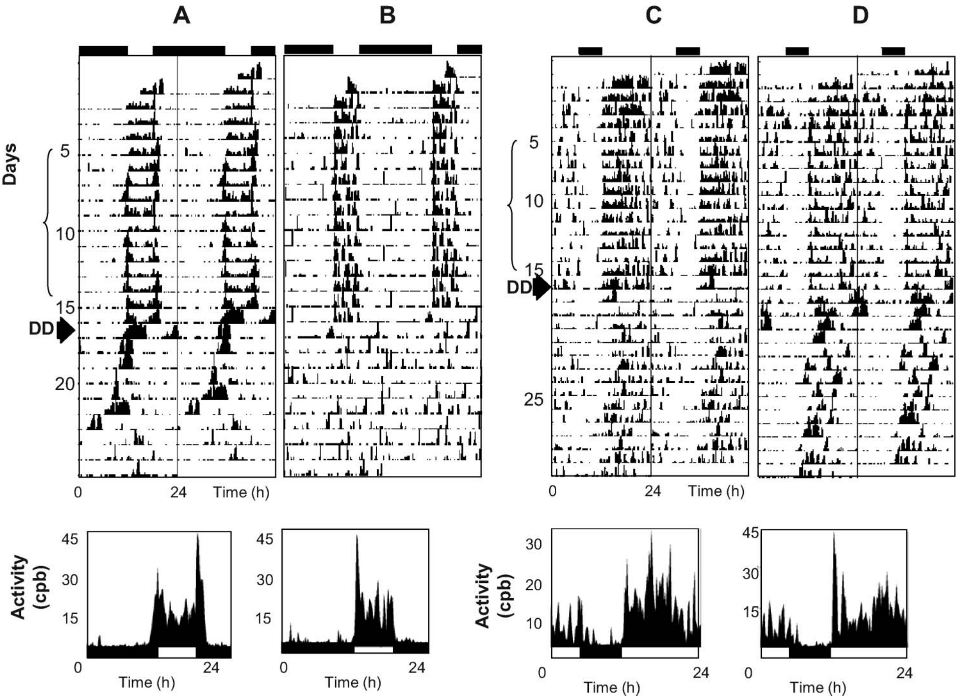

Figure 2. Distribution of circadian activity and free running rhythms of juvenile turtles exposed to short (A, B) and long (C, D) photoperiods. Activity in both protocols is confined to the diurnal phase and circadian activity is locked to the lights-on shift, also shown in each waveform below actograms. Black bars indicate darkness.

observed except on the long days protocols during which most of the animals showed onset immediate or right after the lights transition. The onset of activity is not constant among animals exposed to skeleton photoperiod. Anticipatory activity regarding the lights transition can also be observed in average waveforms, as shown below the actograms in Figures 1 and 2.

Figure 3B shows the average ($\pm$SE) of locomotor activity length during at least 10 consecutive days. The only difference was found in short-day protocols during which activity is constricted to the light phase (stars denote statistical differences, $p < 0.05$). FRP after-effects were observed only between constant darkness after SP ($\tau = 24.21 \pm 0.21$) and long days ($\tau = 23.9 \pm 0.12$ h; $p < 0.05$), since they were the largest and the shortest respectively. The short-day protocol produced the largest dispersion of data ($\tau = 23.79 \pm 0.33$ h).

Discussion

The interaction between organisms and their ecosystem as well as the underlying biological mechanisms need to be pointed to different levels of biological organization. Particularly the physiological and behavioral adaptations are of interest for understanding the complexity of such interactions. Studies on circadian rhythms in different species give us another research tool that allows study of the organism's relationships with its ecosystem. Since important physicochemical factors of the environment change according to the time of day, organisms must find the time when

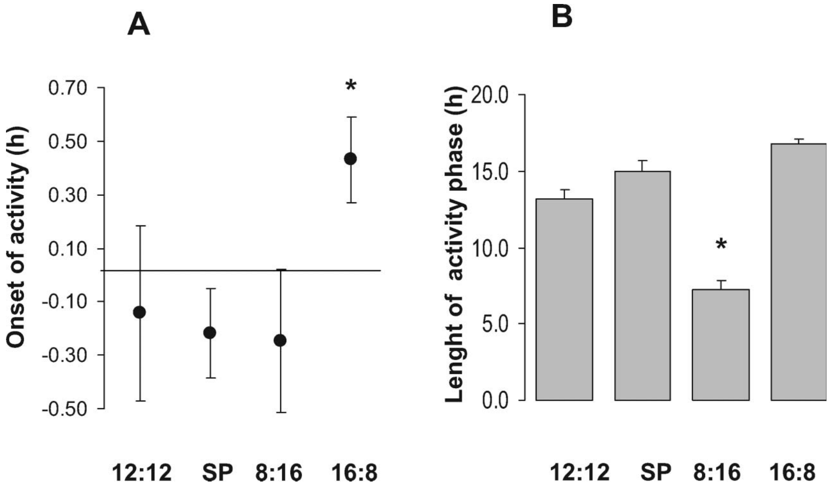

Figure 3. Phase relationship of the onset of activity related to lights-on transitions (A) and length of activity period in each light protocol. Long-day protocols produce a delay in the onset after lights-on.

conditions are more suitable. Therefore, studying circadian activity in different organisms allows us to project fine temporal and spatial relationships between species sharing the same environment (Horton 2001).

The juvenile red-eared turtle *Trachemys scripta elegans* in laboratory conditions has shown clear circadian activity as well as an ability to entrain to both parametric and non-parametric photoperiod protocols. Its locomotor activity is mainly diurnal, however, according to the photoperiod, anticipatory activity to lights-on can be observed in some animals mainly when exposed to non-parametric entrainment. Anticipation of any environmental clue indicates fine tuning of the biological clock with the cyclic environment, establishing a constant phase relationship among them (Pittendrigh and Daan 1976b; Rapp 1980).

Field observation of adults indicates that the animals may change activity phase in the growing season that ranges from May to September (reviewed in Gourley 1979; Graham and Hutchison 1979). Also endogenous circadian and circannual rhythms have been observed in this species, regarding metabolism, ventilation, and breathing patterns (Reyes and Milsom 2009). The way in which activity is compressed or extended according to daylight indicates that this species may have some physiological correlation with the season.

The turtles studied in this work had clear responses to light conditions. The free running conditions studied after all the light protocols indicate that a secondary peak of activity often associated with lights-off is often lost under constant conditions. A particular observation is the manifestation of intense activity when light pulses are given during the skeleton photoperiod. This behavior indicates a positive masking and this species may be of particular interest for study such interesting responses (Mrosovsky 1996).

The free running period after each light protocol was too variable, therefore clear after-effects of each photoperiod were not clearly distinguished. After-effects of photoperiods are the result of previous exposure of particular light–dark conditions (Pittendrigh and Daan 1976a). In *T. scripta*, statistical differences were observed in

FRP between SP and long-day protocols, however there was larger variability after short-days, indicating that behavioral responses observed during different seasons may not only be produced by the thermal preferences but also as a photoperiodic response.

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