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Circadian Locomotor Activity in Freshwater Decapods: An Ecological Approach

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Abstract

Circadian rhythms of locomotor activity shown by freshwater decapods display different patterns among crayfish, *Procambarus*, and crabs, when exposed to artificial light-dark cycles. Crayfish are mainly nocturnal while a crepuscular activity is observed in crabs of the genus *Pseudothelphusa*. In constant darkness, free running rhythms are displayed in unimodal or bimodal patterns by crayfish *Procambarus*; however, *Pseudothelphusa* continues to show bimodal rhythms. The many studies using locomotor activity indicate that the rhythm in freshwater crabs is circadian in nature, but that a multioscillatory system may be controlling the overt rhythm. In the present study, the implications of different locomotor activity patterns are analyzed in selected freshwater decapods with regard to the interactions between light and the organisms. Crabs and crayfish are commonly found in similar habitats, often sharing the same environment; however, different patterns of locomotor activity as well as different sensitivities of the bouts of activity with regard to entrainment by light, indicate that distinct temporal niches may exist that result in temporal exclusion or low competition.

Keywords: Freshwater crab, circadian rhythm, locomotor activity.

Introduction

Ecological studies on biological timing in organisms allows an understanding of the temporal relationship of behavior and geophysical changes; therefore it is possible to study the mechanisms underlying the interactions of organisms and their environment by studying their clock output. Decapods are a diverse group found in a wide diversity of habitats, from deep sea to fresh water and land. Their ability to adapt to diverse

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conditions generates many questions about physiological adaptations that allow this group to be successful. Many studies on circadian organization in crustaceans have used locomotor activity as a measure of the output of the clock, and most of the observations have been in performed in laboratory, presumably because of difficulties in long-term observation in natural aquatic habitats (DeCoursey, 1983).

Decapods have, for several years, been a biological model for studying circadian organization in Crustacea (Warner, 1977; DeCoursey, 1983; Brown, 1983; Aréchiga et al., 1993; Fuentes-Pardo & Hernández-Falcón, 1993). The crayfish, *Procambarus,* has been extensively studied, since it displays circadian rhythms in physiological features; locomotor activity, in particular, has been widely studied (Page & Larimer, 1972, 1975; Fanjul-Moles, 1998; Fuentes-Pardo et al., 1996).

Studies on circadian rhythms of activity in freshwater decapods have shown that activity patterns may be different among crayfishes and crabs sharing similar habitats. The freshwater crab, *Pseudothelphusa,* is often found in habitats where other crayfish species coexist (Álvarez & Villalobos, 1997; López-Hernández, 1997). Studying differences in their circadian activity under laboratory conditions may be useful in understanding the temporal relationship between each species regarding the physical and biological rhythmic environment where they are found. In this paper, we present a summary of the circadian characteristics of locomotor activity displayed by species of *Procambarus* and the freshwater crab, *Pseudothelphusa*, as well as an interpretation of the ecological significance of these locomotor activity patterns.

I. Freshwater decapods and the environment

Freshwater decapods are found in habitats such as standing water (for example, lakes and ponds — lentic) and flowing water (for example, streams and rivers — lotic). Each of these habitats presents a varied set of environmental characteristics. The most obvious factor in freshwater environments is the low salinity and the complex interaction of this with other factors such as temperature. Other differences associated with the temporary and isolated nature of freshwater ponds and streams may limit the distribution of crustaceans (Vernberg & Vernberg, 1983). Freshwater decapods are represented by crayfish of the family Cambaridae, Astacidae and Parastacidae (Hobbs et al., 1989) and by crabs of the family Pseudothelphusidae and Trichodactilidae (Álvarez & Villalobos, 1997). There is little information about the biology of freshwater Brachyurans (true crabs); however, extensive information about of crayfish biology has been documented elsewhere (Hobbs, 1988; Holdich, 2002).

Crayfish and crabs are frequent in slow streams of clean water and near the river shore, living in places where they can hide, like in flooded roots or under stones. Crayfish dig tunnels in soft soil and can remain inside for long periods of time. Field observations of locomotor activity of crayfish indicate that adults are mainly nocturnal, but activity may begin in the late afternoon.

Most of the species of freshwater decapods present small populations, high morphologic differentiation within the population, and reduced distribution areas (Álvarez & Villalobos, 1997). It is common to find both crayfish and crabs in lakes and rivers from Southern Mexico to tropical South America as a component of the aquatic fauna (López-Hernández, 1997). Both crayfish and crabs are omnivorous and usually feed on plants and small animals.

II. Laboratory studies on locomotor activity in freshwater decapods

Freely moving locomotor activity rhythms in freshwater decapods have often been analyzed by means of tilt cages (Page & Larimer, 1972) or of aquaria equipped with infrared light-crossings (Fanjul-Moles et al., 1996; Miranda-Anaya et al., 2003a). Locomotor activity has been also recorded from restrained animals, with a thread tied to the ambulatory legs and movement recorded by a force transducer (Fuentes-Pardo & Inclán-Rubio, 1981; Viccon-Pale & Fuentes-Pardo, 1994). Locomotor activity in decapods is often mathematically 'noisy'; however, some statistical tools have been used to analyze significant circadian behavior (DeCoursey, 1983). Methods used include plotting data in conventional actograms, and calculating circadian periods by means of Enright's and the X^2 periodograms (Enright, 1965; Sokolove & Bushell, 1978). In species like *Procambarus clarkii*, circadian rhythms of locomotor activity manifest themselves gradually after the first week post hatching, and this has allowed study of the ontogenetic expression of locomotor activity rhythms (Fanjul-Moles et al., 1996; Miranda-Anaya & Fanjul-Moles, 1997). Studies of circadian locomotor activity rhythms in crustaceans have been performed not only in *Procambarus clarkii* but also in other crayfish species like *P bouvierii* (Fuentes-Pardo & Inclán-Rubio, 1981) and *P. diguetti* (Fanjul-Moles et al., 1998b).

Both freely moving and restrained animals kept in LD cycles display mainly two peaks of activity, an induced short burst right after lights on and an endogenous long burst after lights off, typical for a nocturnal animal (Page & Larimer, 1972). When placed in constant darkness (DD), their locomotor activity displays mainly a unimodal pattern (Page & Larimer, 1972; Fanjul-Moles, 1998); however, bimodal patterns have been observed in some studies (Fuentes-Pardo et al., 1996).

Figure 1 represents a typical actogram obtained from an adult crayfish in freely moving conditions using infrared light crossings. In constant darkness (DD) locomotor activity is usually noisy during the first days of recording; however, circadian rhythms are seen after about four days in DD. Activity becomes bimodal with multiple short bouts. The circadian rhythm of locomotion in crayfish has been considered as unimodal (Page & Larimer, 1972; Fanjul-Moles et al., 1996) but sometimes it is possible to observe bimodal activity with alternate dominance of each bout (Fuentes-Pardo & Inclán-Rubio, 1981; Gherardhi, 2002; and Fig. 1).

During LD, a short bout of activity is observed at lights on, and a larger bout of activity which starts before lights off and continues until well after lights off. The corresponding waveform (upper right) shows the average activity during DD, there being a clear indication of its bimodal nature. Entraining locomotor activity of the crayfish to an LD cycle denotes typical nocturnal behavior, again with bimodal rhythmicity (lower right). The waveform derived from 9 consecutive days shows that activity peaks are coincident with lights on and lights off respectively (arrow). The duration of the two peaks indicates that the lights-off bout represents the main activity episode, and

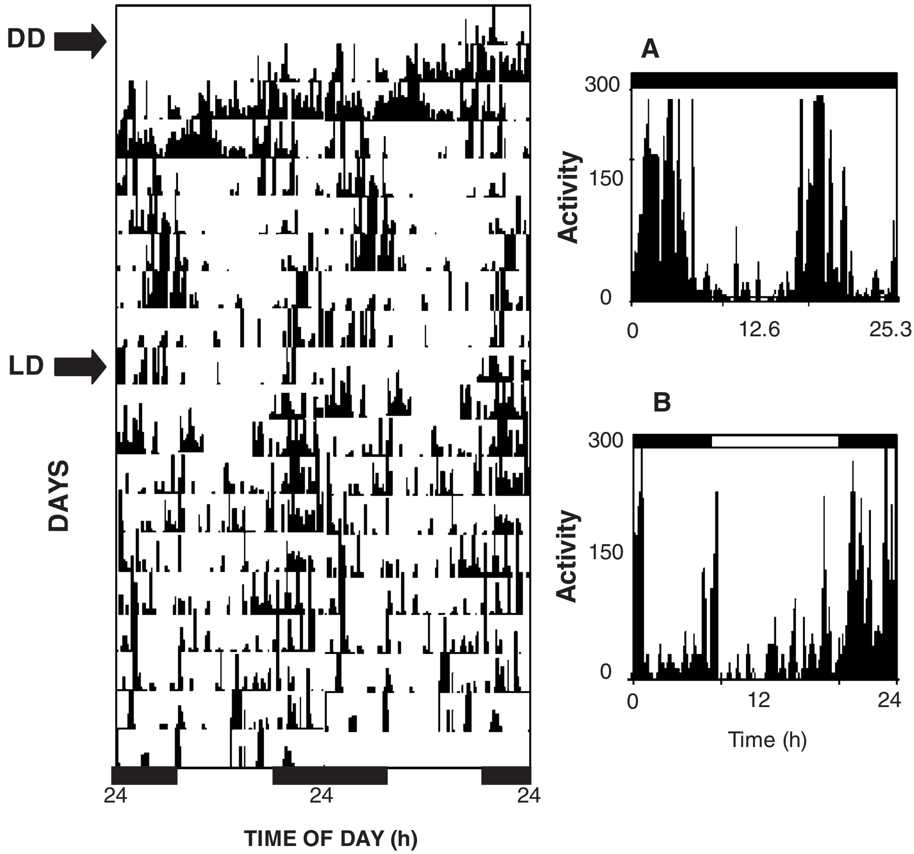

Figure 1. Double-plot of locomotor activity in a freshwater crayfish *Procambarus clarkii*. In DD (upper arrow), a circadian rhythm is observed with two bouts of activity, represented in the waveform (A) fitted to the dominant period. In LD (second arrow) an activity peak is coincident with lights on, also observed in the waveform (B).

the lights-on peak has been described as masking (Page & Larimer, 1975; Miranda-Anaya & Fanjul-Moles, 1997).

The freshwater crab *Pseudothelphusa* presents a circadian rhythm of locomotor activity with a typical bimodal pattern. One bout begins near lights-on and lasts about 4 h, and a second, longer interval is observed a few hours before lights-off. In DD, the circadian rhythm of activity maintains its bimodal pattern. Moreover, each bout of activity often displays a different free-running circadian period (Miranda-Anaya et al., 2003a, b). Figure 2 shows a typical record of locomotor activity in a crab *Pseudothelphusa americana*, where the bouts of activity are restricted to daytime; however, activity is clearly crepuscular.

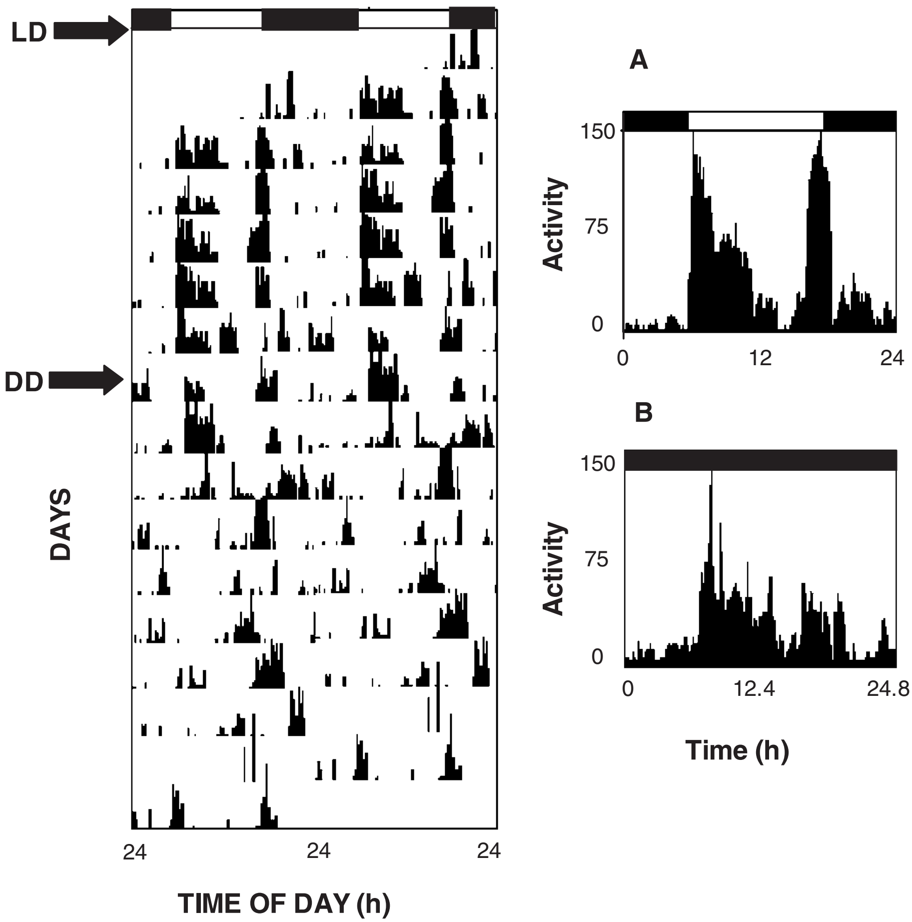

Figure 2. Double-plot of circadian locomotor activity of a freshwater crab *Pseudothelphusa americana*. During the first 7 days in LD, a clear bimodal (crepuscular) rhythm is observed; when exposed to DD, a bimodal circadian rhythm is still present. Waveforms for LD (A) and DD (B) are shown on the right. In DD, the waveform is fitted to the dominant period.

Differences between crayfish and crabs in the daily timing of activity patterns might represent a strategy to inhabit the same environment, with low competition between these species. Differences in daily timing are also the means by which the locomotor pattern changes during the course of the year. Whether a population is diurnal, nocturnal or crepuscular, possibly resulting from the evolutionary history of the species, its physiology indicates its tolerance of daily environmental stresses and adjustment to the presence of significant predators, the habits of prey, the location of food, and social interactions related to resource sharing and reproduction (Powers & Bliss, 1983).

III. Relationship of locomotor activity patterns and environmental factors

Day length is an important environmental factor that modulates circadian behavior in animals. Freshwater decapods have evolved adaptations that may be related to reproductive fitness in seasonal environments; moulting in crayfish also responds to daylight changes (Bittner & Kopanda, 1973); ovarian maturation responds to photoperiodic induction experiments (Fanjul-Moles et al., 2001); and entrainment takes place with skeleton photoperiods of different length (Fanjul-Moles et al., 1998a). Studies of the patterns of locomotor activity in freshwater crabs *Pseudothelphusa* under different photoperiods indicate that the ability to entrain to skeleton photoperiods is also present (Ramírez-Lomelí et al., 2002). By contrast, locomotor activity patterns in *Pseudothelphusa* reflect the photophase length of short and long days, with a different phase relationship of each peak according to the length of the photoperiod. These results indicate that *Pseudothelphusa* might also have physiological responses influenced by the season, during the course of the year (Miranda-Anaya et al., 2003b). However, further studies on this issue are needed.

IV. Entrainment of the clock by light

Entrainment of the circadian system to light cycles is achieved by resetting the phase of the pacemaker every day. The sensitivity of the clock to the entraining agent may change during the course of the inner (biological) day, in such way that there are times when light does not produce significant changes and times where notable changes in phase are produced. The effect of resetting the phase of the internal clock by light pulses is represented by the phase response curve (PRC). The magnitude of the phase shifts observed for a particular species depends on the experimental protocols used, such as the light pulse duration, intensity and wavelength. It is a useful tool for understanding the circadian sensitivity of the organism to light and the entraining ability of light (Daan & Aschoff, 2001).

The PRC to light pulses of the circadian locomotor activity in crayfish presents high variability. However there is a well-defined peak of advances at circadian time 5 (considering CT0 the beginning of the activity), the time when the main bout of activity of the circadian cycle is displayed; at other circadian times, light produces mainly short delays (Viccon-Pale & Fuentes-Pardo, 1994). Even though there are no other reports on the PRC of locomotor activity in freshwater crabs, preliminary results indicate that, in *Pseudothelphusa americana*, the sensitivity to light differs between the bouts of activity at light-on and light-off (Carmona-Alcocer et al., 2003).

It has been hypothesized that, in the fresh water crab *Pseudothelphusa americana*, two independent oscillators are driving the bimodal activity and that the light, through retinal photoreceptors, is important for coupling them (Ramírez-Lomelí et al., 2002; Miranda-Anaya et al., 2003b). The way in which day length may influence the locomotor patterns may depend on the light sensitivity of the oscillator controlling each bout of activity. Conclusions as to the meaning of different PRC's between crayfish and crabs would currently be premature; however, a detailed study on differences between species in their PRC's might elucidate the adaptive responses to photoperiod during the course of the year (Pittendrigh, 1981).

Terrestrial and semi-terrestrial crustaceans that live far from the seashore exhibit predominantly an activity pattern based on light-dark intervals, rather than changes in water level. Many species are nocturnal or crepuscular, taking advantage of lower temperature and increased ground moisture in the evening, at night or at dawn (Powers & Bliss, 1983). Locomotor activity in the freshwater decapods examined to date displays circadian rather than circatidal (circalunidian) rhythmicity. However, the bimodal structure of activity and the different free-running periods of activity bouts in DD indicate that at least two oscillators regulate the circadian activity in freshwater decapods (Fuentes-Pardo et al., 1996; Viccon-Pale & Fuentes-Pardo, 1994; Miranda-Anaya et al., 2003b). Locomotor activity rhythms observed in marine crabs have been interpreted in two different ways: a circadian clock coupled to a circatidal oscillator (Naylor, 1996); or two circalunidian clocks with different free-running periods (Palmer, 2000). There is, however, agreement that the locomotor activity rhythms are driven by multiple oscillators.

V. Habitat influence and locomotor activity patterns

Most species of crayfish are found in permanent sources of freshwater, but some species are capable of living through prolonged intervals of drought, provided that a small amount of water remains in the living chamber of their burrows. True crabs (Brachyura) have adapted to land via diverse ecological routes, resulting in unique combinations of physiological and behavioral features. Pseudothelphusids are among those crabs that can be found inland for several hours (Powers & Bliss, 1983). Freshwater decapods are exposed to a wide variety of habitats, each of which presents a different set of environmental conditions. Crayfish living in streams characterized by rapidly flowing water generally have higher metabolic rates than those species inhabiting slow-moving waters, where interactions with other species are usually sporadic and infrequent. Mutual avoidance is the most common result of potential encounters; therefore, a different pattern of locomotor activity benefits different species sharing the same habitat. Freshwater crabs and crayfish remain in their specific habitat throughout their lives, even though the former are able to live in terrestrial habitats for a longer period of time than can crayfish.

The environment occupied by freshwater decapods is shared with other animals; therefore, inter-specific competition is expected, mainly with a variety of insects, gastropods and other crustaceans (López-Hernández, 1997). The major factors limiting density and distribution appear to be physical — such as substrate, the amount and quality of water present, the availabilities of standing water or refuges of high humidity, and the presence of suitable vegetation for shade or food (or both). Studies of the timing of different species interacting in the same environment will be useful for an understanding of the differences in the activity patterns observed among freshwater decapods. Circadian rhythms and the evolution of the temporal organization of behavior are valuable means of studying the fundamental concept of the co-evolution of physiology, morphology and behavior (Horton, 2001).

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