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Ecological physiology of the circadian pigmentation rhythm in the fiddler crab *Uca panacea*

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ABSTRACT

Color change can serve a number of functions, and can be a singular aperiodic event or a rhythmic process driven by responses to environmental cues or endogenous rhythms. Endogenous pigmentation rhythms have been identified in a number of taxa, with fiddler crabs being the subject of much of this research. Despite a long history of experimental studies of chromatophore-level rhythmicity in fiddler crabs, few studies have considered the entrainment cues for these rhythms or extended findings to whole-organism coloration, information important for understanding the biological properties of circadian systems and understanding the functional significance of these rhythms. This study examined the circadian pigmentation rhythm in the subtropical fiddler crab *Uca panacea* at both the cellular (melanophore) and organismal levels, including expression in artificial light/dark cycles and constant darkness, entrainment by light/dark and temperature cycles, and relationships between melanophore rhythms and the spectral reflectance of the carapace. On the melanophore level, crabs exhibited a circadian rhythm in pigment dispersion, with maximum dispersion occurring during the day and maximum concentration occurring during the night. This rhythm persisted under ambient or reversed light/dark cycles, with maximum pigment dispersion occurring during the light phase, or under constant darkness. Both light/dark and temperature cycles entrained the rhythm, although light/dark cycles resulted in greater phase shift. The circadian rhythm in melanin dispersion within melanophores is associated with a circadian rhythm in organismal coloration, with carapace reflectance low during the day and high at night. Because of the high absorption of UV radiation by melanin, the functional significance of this rhythm may be as a mechanism of UV-protection during the day when crabs are exposed to high levels of UV radiation while foraging on open sand flats of the intertidal zone.

1. Introduction

The ability to alter body pigmentation (color) is widespread in the animal kingdom, especially in ectotherms including crustaceans (Bowman, 1942; Thurman, 1988), cephalopods (Cooper et al., 1990; Hanlon et al., 1990; Masthay, 1997), insects (Hinton and Jarman, 1972), amphibians (King et al., 1994; Novales and Davis, 1969), fishes (Kodric-Brown, 1998; Osborn, 1939; Sugimoto, 1993), and reptiles (Stuart-Fox et al., 2006; Taylor and Hadley, 1970). Such color changes can have a number of functions, including (1) inter- or intra-specific communication (e.g., Andersson, 1994; Cummings et al., 2008; Detto et al., 2006), (2) camouflage (e.g., Hemmi et al., 2006; Stevens and Merilaita, 2009), (3) thermoregulation (e.g., Norris, 1967; Silbiger and Munguia, 2008; Wilkens and Fingerman, 1965), and (4) protection from the deleterious effects of ultraviolet radiation (Coohill et al., 1970; Garcia et al., 2004; Masthay, 1997). In invertebrates and poikilothermic vertebrates, color changes are effected by the chromatophores, stellate cells located in the hypodermis containing black (melanophores), red (erythrophores), yellow (xanthophores), or white (leucophores) pigment granules (Fingerman, 1965). Physiological color change results from migration of pigment granules into and out of dendritic cell processes and takes place over time scales of seconds to hours. Morphological color change, which consists of changes in the amount of pigment or number of chromatophores, takes place over longer time scales of days to weeks.

Physiological and morphological color changes can occur as singular responses to aperiodic environmental or ontogenetic events or may be rhythmic, recurring at regular intervals. Aperiodic color changes include those occurring with age (Booth, 1990; Detto et al., 2008; Hawlena et al., 2006; Wilson et al., 2007), color changes associated with social displays during breeding seasons (Heath and Frederick, 2006; Kodric-Brown, 1998; Ries et al., 2008), and responses to a number of environmental stimuli including temperature (e.g., Garcia et al., 2003; King et al., 1994), illumination (e.g., Gras and Weber, 1977; Shiraki et al., 2010), background coloration (e.g., Kats and Dragt, 1986; King et al., 1994; Sumner, 1911), or predation risk (e.g., Garcia and Sih, 2003; Hemmi et

Abbreviations: LD cycle, light/dark cycle; DD, constant darkness; LL, constant light; TC cycle, thermophase/cryophase (temperature) cycle; τ, free-running period; Φ, acrophase (time of maximum pigment dispersion); $\Delta\Phi$, phase shift (shift in the time of maximum pigment dispersion after entrainment).

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al., 2006). Rhythmic color change is also widespread, have been observed in both invertebrates (e.g., Granato et al., 2004; Hendler, 1984; Thurman, 1988) and vertebrates (e.g., Caswell, 1950; Filadelfi et al., 2005; Kavaliers et al., 1980). Rhythmic pigmentation changes can be under exogenous (environmental) or endogenous control; an exogenous rhythm results from responses to rhythmic environmental stimuli (e.g., diel temperature or light cycles, seasonal cycles in temperature or photoperiod), whereas an endogenous rhythm relies on an internal, self-sustaining oscillator or "biological clock" (Pittendrigh, 1960) and persists in the absence of environmental stimuli. Endogenous rhythms allow an organism to anticipate a rhythmic environmental event and adjust physiology or behavior as necessary to optimize fitness, and can reduce costs associated with delays that can occur when relying on responses to environmental cues. In crustaceans, these rhythms are likely driven by one or more oscillators in the eyestalks (Arechiga et al., 1993), resulting in rhythmic synthesis or release of pigment-dispersing hormone, as well oscillator(s) intrinsic to the chromatophores, resulting in an endogenous rhythm of responsiveness to pigment-dispersing hormone (Granato et al., 2004).

Despite the prevalence of endogenous pigmentation rhythms in crustaceans, few studies have examined the basic ecophysiology of these rhythms, including entrainment cues (zeitgebers) for these rhythms and variation in the period and phase of the rhythm among individuals (Stephens, 1957, 1962). Even fewer studies have examined these rhythms on multiple levels, integrating information at both the cellular and organismal level to better understand the function and functional significance of these rhythms. Although the functional significance of aperiodic (ontogenetic or responsive) color change is clear, the functional significance of circadian pigmentation rhythms is less clear due to the lack of information on (1) expression of the rhythm under various environmental conditions, (2) environmental cues entraining these rhythms, and (3) relationships between the dispersion of pigment within melanophores and coloration on the whole-organism level.

The fiddler crab (genus *Uca*) pigmentation system has been the subject of much research over the past century because of (1) high inter- and intra-specific variation in coloration (Crane, 1975; Detto et al., 2008; Thurman, 1990), (2) capability for color vision (Detto, 2007; Rajkumar et al., 2010) and use of color as a social signal (Detto, 2007; Detto and Backwell, 2009; Detto et al., 2006), and (3) clear circadian and circatidal pigmentation rhythms exhibited by many species (reviewed by Palmer, 1991). Fiddler crabs exhibit endogenous circadian and circatidal rhythms in pigment dispersion within chromatophores (e.g., Brown et al., 1953). As in some other crustaceans, pigment is maximally dispersed within chromatophores during the day and maximally concentrated at night (Abramowitz, 1937). The circatidal rhythm identified in several species results in exceptionally high dispersion of pigment occurring during daytime low tides (Brown et al., 1953). Although *Uca pugnax* (Abramowitz, 1937; Brown and Hines, 1952; Brown and Stephens, 1951; Brown et al., 1953; Hines, 1954; Kelly, 1975; Webb and Brown, 1965) and *U. pugilator* (Abramowitz, 1937; Fingerman, 1956; Fingerman and Yamamoto, 1967; Fingerman et al., 1958) have been the subject of most of this work, endogenous chromatophore rhythms have been identified in at least 12 other fiddler crab species (Barnwell, 1963, 1968a; Fingerman, 1956; Fingerman et al., 1958; Thurman, 1990). In addition to endogenous rhythms controlling pigmentation, fiddler crab chromatophores are also under exogenous control, responding to temperature (Barnwell, 1968a; Brown and Sandeen, 1948; Silbiger and Munguia, 2008; Thurman, 1990), background (Barnwell, 1968a; Brown and Sandeen, 1948; Thurman, 1990), illumination (e.g., Brown and Sandeen, 1948; Coohill and Milton, 1975; Coohill et al., 1970; Thurman, 1990), handling stress (Zeil and Hofmann, 2001), and perceived predation risk (Hemmi et al., 2006). This diversity of responses reflects the multiple, interacting functions of the chromatophore system.

The purpose of this study was to investigate the ecological physiology of the circadian pigmentation rhythm in the subtropical fiddler crab *Uca panacea* (Novak and Salmon, 1974). This species inhabits sandy shorelines from the Florida panhandle to Tabasco, Mexico (Barnwell and Thurman, 1984), often lives in mixed-species assemblages with other *Uca* species, primarily *U. longisignalis* and *U. rapax* (Thurman, 1984), and is a sister-species to the more commonly studied *Uca pugilator* (Levinton et al., 1996). *U. panacea* is active on the surface during both day and night during the peak activity and breeding season when temperatures are above 20 °C (Powers and Cole, 1976). Thurman (1990) previously examined rhythmic changes in melanin dispersion in *U. panacea* under constant light and found that, as in many other *Uca*, dispersion of melanin peaks during the daytime hours, although this rhythm was suppressed in crabs adapted to a black background. Here, I examined circadian pigmentation rhythms at both the cellular and organismal level, specifically: (1) proximate control of melanophore rhythms by artificial light/dark cycles (2) the circadian rhythm in melanin dispersion under constant conditions, (3) light/dark and temperature cycles as potential entrainment cues for this rhythm and (4) the relationship between melanophore rhythms and spectral reflectance of the carapace. Individual crabs were monitored, and records were analyzed using modern statistical methods to estimate period and phase of the circadian rhythm.

2. Methods

2.1. Proximate control of melanophore rhythms by artificial light/dark cycles

Adult *U. panacea* were collected from shoreline habitats near Port Aransas, Texas during July–September, 2010 and June–August, 2011. Crabs were transported to the University of Texas Marine Science Institute and placed into experimental conditions within 24 h. During experiments, crabs were held individually in clear plastic cups containing 12 mL of ambient seawater (~7 mm depth), which were placed in environmental chambers under either an ambient (lights on: 06:30 h, lights off: 20:30 h) or reversed (lights on: 20:30 h, lights off: 06:30 h) light/dark (LD) cycle and constant temperature of 25 °C. During the light phase, lighting was provided by fluorescent lights (4.8×10^{19} photons m^{-2} s^{-1}). Monitoring began immediately after placement in the environmental chamber for crabs exposed to an ambient LD cycle ($n = 15$). For crabs exposed to a reversed LD cycle, monitoring began either immediately ($n = 15$) or after 3 d of exposure ($n = 14$) and all crabs were monitored for 3 d.

During the monitoring period, the dispersion of melanin within melanophores was assessed every 3 h using the pigment dispersion index of Hogben and Slome (1931). The melanophores of the posterior surface of the 3rd walking leg on the right side of each crab were examined under a dissecting microscope and the pigment dispersion index was determined as the average melanophore stage of the central 1/3 of the merus. Melanophore stages ranged from 1 to 5, with 1 indicating full concentration of pigment and 5 indicating full dispersion of pigment (Fig. 1). Intermediate values were assigned if melanophores in the focal area were in multiple stages and represented the approximate mean value. Stages were assigned based on qualitative assessment of the degree of dispersion of pigment within the melanophores. Observations were performed in darkness using only dim red light. Based on their visual pigments, fiddler crabs are generally insensitive to red light (Jordao et al., 2007), so this method approximated constant darkness. Crabs were monitored for 3 d each, with water changes occurring every 18–48 h at haphazard times.

Time series of pigment dispersion indices were analyzed for periodicity using autocorrelation and maximum entropy spectral analysis (MESA) for each individual crab. Data from crabs that escaped or died during the experiments were excluded from all analyses. Rhythmicity (i.e., the presence or absence of a rhythm) was assessed using autocorrelation, which plots autocorrelation coefficients as a function of lag at 3-h intervals. Peaks in the correlogram with autocorrelation