Effect of light intensity and wavelength on diurnal activity of the banded coral shrimp *Stenopus hispidus* (Decapoda, Stenopodidae): a possible adaptation for a cleaner shrimp in reef environments

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**Abstract**

Most decapod crustaceans are nocturnal. However, since cleaner shrimp at cleaning stations act in concert with their hosts, they clean debris and parasites off the body surfaces of diurnal hosts during daytime. It is not known how cleaner shrimp physiologically accommodate diurnal environments and perform cleaning behaviors. We examined the effect of light cues on the daily rhythmicity of the banded coral shrimp *Stenopus hispidus* (Decapoda, Stenopodidae), a cleaner shrimp in coral reefs. Shrimp were individually kept in aquaria under conditions of 12-h light and 12-h darkness (LD) and constant darkness (DD). Double-plotted actogram analyses revealed that shrimp under LD were active during scotophase and inactive during photophase. Locomotor rhythms were observed in most shrimp under DD. Periodogram analyses showed a weak circadian rhythm in shrimp under DD. Our results show that this species is nocturnal and that its locomotor activity is controlled primarily by the LD cycle. Day–night differences in locomotor activity were reduced in shrimp under LD with weak irradiance, and they remained active during photophase. Shrimp under LD using blue or green light-emitting diode (LED) light, but not red LED light, showed a day-inactive and night-active rhythmicity. These results indicate that this species can be active under environments with low green- and blue-spectra - e.g., during twilight hours, at depth, or on cloudy days - even during daytime, and this weakness of the circadian clock may be advantageous in their role as a cleaner shrimp. It is concluded that in addition to the presence of visiting hosts, light conditions at cleaning stations are likely to influence cleaning activity.

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Introduction

Many shallow water animals exhibit behavioral rhythms repeated at an interval of 24 h (Takemura et al. 2010). Each animal acts synchronously with other members of its species to increase reproductive and feeding success, although the active and inactive phases of diurnal animals are opposite to those of nocturnal ones. Additionally, predator avoidance and other selective pressures alter and may even reverse the daily activity patterns of prey species. Overall, these day–night behavioral patterns appear to be controlled by a biological clock system entrained to environmental factors, especially light (Aréchiga & Atkinson 1975, Aréchiga et al. 1993, Aréchiga & Rodriguez-Sosa 1997, Jury et al. 2005).

Many decapod crustaceans are nocturnal, and therefore active only during dim or twilight hours (Strauss & Dircksen 2010). Such nocturnal activity likely reduces the risk of predation by diurnal species (Lima & Dill 1990). Field and laboratory studies have shown that the environmental light–dark cycle strongly predicts the locomotor activity of lobster (Williams & Dean 1989, Jury et al. 2005), crayfish (Page & Larimer 1972), crab (Rebach 1985, 1987, Chatterton & Williams 1994, Forward et al. 1997), and shrimp (Moller & Jones 1975, Pontes et al. 2006). Involvement of biological clocks in the persistence of locomotor activity has also been seen in some decapod crustaceans under constant artificial conditions (Rebach 1985, Fanjul-Moles & Prieto-Sagredo 2003, Miranda-Anaya et al. 2003).

Some decapod crustaceans form symbiotic associations with diverse aquatic species (Baker et al. 2001, Tominaga et al. 2004, Henkel & Pawlik 2005). Thus, the daily locomotor activity of symbionts should be influenced by the periodical activities of their hosts. However, how decapod crustaceans behave as symbionts and entrain their daily locomotor activity to host animals is not clear.
The banded coral shrimp *Stenopus hispidus* (Decapoda, Stenopodidae) is widely distributed in shallow waters throughout the tropics and subtropics (Limbaugh et al. 1961), and is commonly found in crevices in coral reefs and rocks. It is known as a cleaner shrimp and helps to remove mucous, diseased tissues, parasites, and fungus from the body surface of a large variety of reef fishes—particularly morays, tangs, grunts, and groupers (Limbaugh et al. 1961)—and one turtle species (Sazima et al. 2004). Thus, cleaner shrimp such as the banded coral shrimp likely play an important role in sustaining the coral reef ecosystem (Sazima et al. 2004). Most potential host species are diurnal, and consequently, most banded coral shrimp cleaning activity occurs during photophase, although this species may also be a nocturnal species. It is hypothesized that physiological adaptation of and/or environmental conditions around the banded coral shrimp allow for behavior in accordance with diurnal hosts. Few experimental studies have been carried out on involvement of light cues in locomotor activity in cleaner shrimp, although field observation revealed day-night differences in their locomotor activity (Limbaugh et al. 1961, Johnson 1977).

Here, we examine the effects of light environments on the diurnal locomotor activity of the banded coral shrimp under laboratory conditions to clarify the determinants of diurnal cleaning activity by this otherwise nocturnal species. We kept shrimp in aquaria under several fluorescent and light-emitting diode (LED) light regimes and monitored their locomotor activity using an infrared sensor system.

**Materials and methods**

**Study species and experiments**

Mature banded coral shrimp were collected from coral reefs around Sesoko Island (26°38′N, 127°51′E), Okinawa, Japan, by scuba diving. They were transferred to Sesoko Station, Tropical
Biosphere Research Center, University of the Ryukyus, Okinawa, Japan, and individually accommodated under a natural photoperiod (sunrise and sunset times were at 05:40 and 19:27 in the summer solstice and 07:14 and 17:46 in the winter solstice, respectively) and natural water temperature in 30-L aquaria with running seawater and polyvinyl chloride pipes or coral bones as a nest. Shrimp were fed at 12:00 each day with commercial pellets (Kyorin, Himeji, Japan), and an infrared sensor system (E3Z-T61; Omron, Tokyo, Japan) connected to a personal computer was placed externally 5 cm above the substrate to record their locomotor activity. Feeding under constant dark conditions (DD) was done at circadian time (CT) 6.

First, we examined the daily and circadian activities of shrimp (n = 8). We used 20 W fluorescent bulbs as the light source, resulting in an illuminance level at the water surface of 2.32 W/m². After shrimp acclimated to the aquaria under natural light conditions for at least 2 weeks, we changed the light conditions to light–dark (LD 12:12, lights on at 07:00 and off at 19:00). We recorded shrimp locomotor activity under LD 12:12 for 14 days, then under DD for 16 days, and finally under LD for a further 7 days. To examine effect of alternation of the light-dark cycle, we additionally examined shrimp locomotor activity (n = 8) under a schedule of LD 12:12 for 14 days, LD 6:6 (light on at 06:00 and 18:00, and off at 12:00 and 24:00) for 14 days, and finally LD 12:12 for 4 days.

We also evaluated the effect of light intensity on shrimp locomotor activity. Following acclimation under LD 12:12 (lights on at 07:00 and off at 19:00) with a 20 W fluorescent bulb, shrimp were maintained under LD 12:12 conditions at light intensities of 2.135 ± 0.134 W/m² (mean ± S.D.; n = 6), 0.142 ± 0.001 W/m² (n = 6), and 0.020 ± 0.008 W/m² (n = 6), which were approximately 100%, 10%, and 1%, respectively, of the first conditions. One-tenth the illuminance of the first conditions was approximately equivalent to the illuminance around natural nests at the sampling site (3.9 m in depth) 1 h before sunset. We manipulated the light
intensity by covering aquaria with black mesh sheets as required and monitored shrimp locomotor activity for 14 days.

Finally, we examined the effect of light wavelength on shrimp locomotor activity using 2.5 W LEDs (OPTILED; Optiled Lighting International Ltd., Kwun Tong, Hong Kong) that emit blue (peak at 455 nm), green (530 nm), and red light (627 nm). Following acclimation under LD 12:12 using a florescent bulb (20 W), shrimp were exposed to LD 12:12 of blue, green, or red LED light (n = 6 each). We set LED lights at 22-32 cm from water surface to adjusted irradiance of each LED to approximately 0.9 W/m$^2$. Shrimp locomotor activity was monitored under LD 12:12 with a florescent bulb for 14 days, and then under LEDs for a further 14 days.

**Data analyses**

We recorded the number of times the infrared beam was interrupted at 6-min intervals on a personal computer and tested mean activity for all individuals by comparing day vs. night activity (paired U-test). All summary statistics are presented as the mean ± S.E. Significance was assessed at $P < 0.05$, while rhythmicity was statistically determined via chi-square periodogram analysis (Sokolove & Bushell 1978). A one-way analysis of variance (ANOVA) was used to test for statistically significant differences in the average locomotor activities among different conditions of illuminance.

**Results**

Shrimp were first maintained under LD for 14 days. Under these conditions, all shrimp showed a clear cycle of day-inactivity and night-activity (Figs. 1A and B). Locomotor activity during scotophase was significantly (U-test, $P < 0.05$) higher than during photophase (Fig. 2). Following LD, shrimp were exposed under DD for 16 days. One day after this change, a similar
behavior pattern to that under LD remained in all shrimp. Later (two days after this change), six of the eight shrimp remained active even during the subject day. Significant rhythms of these shrimp disappeared (Figs. 1A and C). On the other hand, the remainder exhibited a weak circadian activity pattern (\( \tau = 24.1 \pm 0.14 \), Table 1). When light conditions were returned from DD to LD, all shrimp once again synchronized their locomotor activity to the light–dark cycle.

When light cycles were changed from LD 12:12 to LD 6:6, shrimp became active during scotophase and inactive during photophase (Fig. 3A). Periodogram analyses revealed significant locomotor activities on a 24-h cycle under LD 12:12 (Fig. 3B) and a 12-h cycle under LD 6:6 (Fig. 3C).

Figure 4 shows the effect of light intensity on locomotor activity. In this study, we changed the light intensity during photophase from 100% (Figs. 4A and B) to 100% (i.e., no change), 10%, or 1%, and shrimp were maintained under the new conditions for 14 days (Figs. 4C-H). We found no change in the locomotor activity of shrimp maintained under constant 100% conditions (Figs. 4C and D). When the light intensity decreased to 10% of the first conditions, most shrimp (four of six individuals) still retained a 24-h rhythm in their locomotor activity (Figs. 4E and F, Table 1), while the remaining two shrimp did not (data not shown). On the other hand, reduction of light intensity to 1% of the first conditions resulted in decrement of daily rhythm and active locomotion for 24 hours (Figs. 4G and H). Overall, shrimp locomotor activity was significantly (ANOVA, \( P < 0.05 \)) higher at 1% illuminance than at 10% (Fig. 5).

Shrimp were maintained under LD with a fluorescent bulb for 14 days and then under LD with LED lights (blue, green, and red) for 14 days (Fig. 6). Under LD with a fluorescent bulb,
these shrimp showed day–night cycles in their locomotor activity (Figs. 6A and B). When the light source was changed from fluorescent to LED lights, five of six under the blue LED (Figs. 6C and D), and all shrimp under the green LED (Figs. 6E and F), were inactive during photophase. Under the red LED, four of six shrimp showed no day–night difference in their locomotor activity (Figs. 6G and H). The other two shrimp under the red LED light maintained a very weak diel activity pattern but were intermittently active during both photophase and scotophase (Table 1). There was no statistically significant difference in locomotor activity among the groups (data not shown).

<<Figure 6 near here>>

**Discussion**

Shrimp exhibited a day-inactive and night-active cycle in their locomotory behavior under our experimental light–dark treatments. This behavior was kept in aquaria over the monitored period. This result confirms that the banded coral shrimp is nocturnal, as are many decapod crustaceans (Aréchiga *et al.* 1993, Strauss & Dircksen 2010). Field surveys of this species have shown that mated pairs remain relatively sedentary and within antenna-touching range of each other during daytime, but (especially males) separate and wander to scavenge off the substratum at night (Johnson 1977). Behavioral patterns observed under laboratory conditions are likely to support those from the field. If banded coral shrimp cleaning behavior occurs even in the daytime (Limbaugh *et al.* 1961, Johnson 1966, 1969), they are active during the day and cannot be fully nocturnal species. Thus, the species must be physiologically and/or behaviorally adapted to daylight to function as a diurnal cleaner shrimp.

The present study revealed that locomotor activity of the banded coral shrimp resulted in ambiguity under constant conditions; small number of the banded coral shrimp (25%; 2 out of 8
individuals), but not the others (75%; 6 out of 8 individuals), persisted a free-running rhythmicity. This result is comparable to lobsters; for example, the analyses of locomotor activity using a custom-designed running wheel revealed that the American lobster, *Homarus americanus*, shows a clear daily rhythm at 24.0 ± 0.1 h under LD and a free-running circadian rhythm at 24.2 ± 0.3 h under DD (Jury *et al.* 2005). Video analysis also showed that a weak diurnal burrow-emergence rhythm persisted for at least 9 days in some Norway lobster *Nephrops norvegicus* under DD (Aguzzi *et al.* 2009). These findings suggest that lobsters have an endogenous circadian system related to oscillation in their locomotor activity (Jury *et al.* 2005). Our results do not preclude the possibility that the banded coral shrimp possesses an endogenous circadian clock, as circadian systems are reflected in sensory systems (e.g., electroretinogram amplitude and caudal photoreceptor amplitude for various decapods, and distal/proximal retinal pigment position for crayfish) and neuronal parameters (e.g., spike rate of X-organ neurons and spontaneous multiunit activity of brain neurons for crayfish; Strauss & Dircksen 2010). However, we speculate that an endogenous circadian clock, if present, contributes little to the species’ locomotor activity. Alternatively, there may be individual variation in responses to environmental cues. Furthermore, 10% of the American lobsters did not express clear free-running rhythms even under constant experimental conditions (Jury *et al.* 2005), and their rhythmicity was less than that of the Norway lobster under LD (Aguzzi *et al.* 2009). These observations imply individual variation in responses to environmental cues.

A day-inactive and night-active rhythm persisted at 10% of the original illuminance, whereas at 1% the original illuminance, we observed a marked decrease in sedentary time during daylight hours. Our results suggest that the banded coral shrimp possesses a threshold for light perception and that its locomotor activity can persist even when daylight is weak, perhaps because most crustaceans become active during twilight hours (Aréchiga *et al.* 1993). Since the antennae of
banded coral shrimp can be seen in the mouths of natural nests from the twilight hours onward (Esaka et al. unpublished data), the species apparently emerges from its nest when sunlight is decreasing, which may also occur during daylight hours on cloudy or rainy days.

We used LED lights to clarify the effects of spectrum sensitivity on shrimp locomotor activity. As a result, behavior patterns under LD with blue and green LEDs were similar to those under LD with fluorescent bulbs. In contrast, maintenance of shrimp under LD with red LEDs resulted in less sedentary time and more locomotory behavior even during photophase. These results suggest that the banded coral shrimp is sensitive to blue and green wavelengths, resulting in the oscillation of locomotor activity. Johnson et al. (2002) determined the spectral sensitivity of five marine decapod crustaceans using electroretinograms and found that all species were sensitive to blue–green wavelengths, but that the degree of sensitivity depended on adapted depth: pelagic species (Crangon allmani, Pandalus montagui, and N. norvegicus) were most sensitive to light wavelengths of 510–525 nm, while deep-water species [Paromola cuvieri and Chaceon (Geryon) affinis] had spectral sensitivity maxima below 500 nm. Aguzzi et al. (2009) also reported that a robust diurnal burrow-emergence rhythm in the Norway lobster persisted under a monochromatic blue LD cycle of 0.1 lux, which simulates the photic conditions of 200–300 m depth. These findings indicate that some decapod crustaceans, including the banded coral shrimp, are adapted to benthic environments in which the blue–green spectrum is common.

The relative lack of blue-green wavelengths allows for increased activity in the banded coral shrimp. In habitats in which these wavelengths are weak, the species can be active even in daytime. The lack of a robust free-running circadian system may be adaptive to the banded coral shrimp’s role as a diurnal cleaner. It is concluded that in addition to the presence of visiting hosts at cleaning stations, physiological adaptation to light environments is needed for successful cleaning activity of this cleaner shrimp. In the present study, we did not evaluate effect of the
presence of visiting hosts on the level of diurnal activity in the banded coral shrimp under our experimental conditions. It is likely that cleaning behavior of this species during daytime may be exerted by an interlocked effect of an external attraction (the presence of visiting hosts) and a physiological adaptation (the perception of photic conditions). Further studies are needed to clarify how these two factors collaboratively act for locomotor rhythmicity in *Stenopus hispidus*.

**Acknowledgements**

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**Literature Cited**


Table 1. Rhythmicity of the shrimp maintained under several light regimes.

<table>
<thead>
<tr>
<th>Regime</th>
<th>Source</th>
<th>Intensity</th>
<th>Total Number of Individuals</th>
<th>Number of Significant Rhythm</th>
<th>Period (Mean ± S.D.)</th>
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<tr>
<td>LD 12:12</td>
<td>Fluorescent bulb</td>
<td>100%</td>
<td>8</td>
<td>8</td>
<td>24.0 ± 0.04</td>
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<tr>
<td>DD*</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>2</td>
<td>24.1 ± 0.14</td>
</tr>
<tr>
<td>LD 12:12</td>
<td>Fluorescent bulb</td>
<td>100%</td>
<td>6</td>
<td>6</td>
<td>24.2 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>Fluorescent bulb</td>
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<td>6</td>
<td>4</td>
<td>24.0 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Fluorescent bulb</td>
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<td>6</td>
<td>1</td>
<td>24.0</td>
</tr>
<tr>
<td>LD 12:12</td>
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<td>23.9 ± 0.06</td>
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<tr>
<td></td>
<td>Green LED</td>
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<td>6</td>
<td>6</td>
<td>24.0 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Red LED</td>
<td></td>
<td>6</td>
<td>2</td>
<td>23.9 ± 0.14</td>
</tr>
</tbody>
</table>

*DD was subsequently carried out after LD 12:12.
Figure 1. Representative double-plotted actogram of the banded coral shrimp *Stenopus hispidus* maintained under LD 12:12 for 14 days, DD for 16 days and LD 12:12 for 7 days (A), and chi-square periodograms under LD 12:12 (B) and DD (C). Open and dark bars on actogram indicate photophase and scotophase under LD 12:12, respectively, while dark bar shows constant conditions of DD. A number in periodogram (B) indicates significant rhythm in locomotor activity with $\tau$ of 24.0 under LD 12:12.
Figure 2. Comparison of locomotor activity of the banded coral shrimp *Stenopus hispidus* between scotophase (dark column) and photophase (open column). Data represent the mean ± standard error of the mean. Asterisk shows significant difference at \( P < 0.05 \).
Figure 3. Representative double-plotted actogram of the banded coral shrimp *Stenopus hispidus* maintained under LD 12:12 for 14 days, LD 6:6 for 14 days and LD 12:12 for 4 days (A), and chi-square periodograms under LD 12:12 (B) and LD 6:6 (C). Open and dark bars on actogram indicate photophase and scotophase under LD 12:12 and LD 6:6. Numbers in periodograms (B and C) indicate significant rhythms in locomotor activity with $\tau$ of 24.0 under for LD 12:12 and of 12.0 and 24.0 for LD 6:6.
Figure 4. Effect of light intensity on locomotor activity of the banded coral shrimp *Stenopus hispidus* maintained under LD 12:12. Shrimp were maintained using the original conditions (100%) for 14 days (A and B) and then placed under conditions of 100% to the original conditions for 14 days (C and D), of 10% to the original conditions for 14 days (E and F), and of 1% to the original conditions for 14 days (G and H). Open and dark bar on each actogram (A, C, E, and G) indicates photophase and scotophase under LD 12:12, respectively. Numbers in periodograms (B, D, and F) indicate significant rhythms in locomotor activity with $\tau$ of 24.0 for each condition.
Figure 5. Comparison of locomotor activity of the banded coral shirmp *Stenopus hispidus* among different illuminance. Average locomotor activities (n = 6 in each group) were monitored under 100%, 10%, and 1% of the original conditions. Data represent the mean ± standard error of the mean. Different letters in the figure show significant difference at \( P < 0.05 \).
Figure 6. Effect of light wavelength on locomotor activity of the banded coral shrimp *Stenopus hispidus*. Shrimp were maintained using a fluorescent bulb for 14 days (A and B) and then placed under LD 12:12 of a blue LED for 14 days (C and D), of a green LED for 14 days (E and F), and of a red LED for 14 days (G and H). Open and dark bar on each actogram (A, C, E, and G) indicates photophase and scotophase under LD 12:12, respectively. Numbers in periodograms (B, D, and F) indicate significant rhythms in locomotor activity with \( \tau \) of 24.0 for a fluorescent bulb and blue LED and of 23.9 for a green LED.