Entrainment of the Circatidal Swimming Activity Rhythm in the Cumacean Dimorphostylis asiatica (Crustacea) to 12.5-hour Hydrostatic Pressure Cycles

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Entrainment of the Circatidal Swimming Activity Rhythm in the Cumacean *Dimorphostylis asiatica* (Crustacea) to 12.5-hour Hydrostatic Pressure Cycles

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ABSTRACT—The cumacean *Dimorphostylis asiatica* (Crustacea) exhibits a circatidal swimming activity rhythm. The animals were exposed to a 12.5 hr sinusoidal change of hydrostatic pressure of 0.3 atm amplitude in the laboratory. Under constant dark conditions, most of the specimens were entrained to a daily bimodal swimming activity rhythm by the hydrostatic pressure cycle. A small number of individuals exhibited a unimodal daily rhythm, with no apparent entraining from the administered cycles. A marked feature was a flexible phase relationship between the entrained daily bimodal rhythm and the hydrostatic pressure cycles: the swimming activity of most of the specimens occurred around the pressure-decreasing phase, but for a small number of individuals it coincided with the pressure-increasing phase. Such flexibility suggests a weak entraining effect of hydrostatic pressure on the circatidal rhythm of this species. When exposed to 24 hr light-dark cycles and a hydrostatic pressure cycle simultaneously, the specimens exhibited a rhythmic activity entrained by the hydrostatic pressure cycle during the dark period, which closely resembles the temporal activity pattern of this species in the field. The light cycles entrained the swimming activity via direct inhibition and induction of activity (i.e., masking). Under light-dark conditions, the specimens exhibited activity on the pressure-increasing phase more frequently compared with specimens kept in constant darkness.

Key words: Crustacea, circatidal rhythm, entrainment, hydrostatic pressure cycle, light-dark cycle

INTRODUCTION

Endogenous rhythms synchronized with environmental tides (*i.e.*, circatidal rhythms), have been reported in many marine organisms (Neumann, 1981; Naylor 1988; Morgan, 1991; Palmer, 1995). In contrast to the circadian rhythms of terrestrial organisms, which are predominantly make use of sunlight to entrain their circadian rhythms (Binkley, 1990), circatidal rhythms are entrained by various environmental time cues such as mechanical turbulence, temperature, or salinity (Morgan, 1991). Such variety of sensory inputs in marine organisms is expected to provide important insight into the mechanisms of biological clocks in marine organisms.

Periodical change of hydrostatic pressure caused by tidal fluctuation of water level has been regarded as one of the dominant tidal time cues (Morgan, 1991). However, while many marine organisms respond to sudden change of hydrostatic pressure (Enright, 1961; Digby, 1972; Morgan,

1978), the entraining effect of hydrostatic pressure cycles on circatidal rhythms have been reported in few species, including the amphipod *Colophium* (Morgan, 1965), crab larvae (Naylor and Atkinson, 1972), and shore fishes (Gibson, 1971; Northcott, 1991; Northcott *et al.*, 1991a). Although the entraining effect on the fish was apparent, further entraining experiments in invertebrates using animals which express distinct, persistent circatidal rhythms have not been performed previously.

For animals living in a calm, shallow bay where tidal amplitude is much larger than wave amplitude, it is expected that hydrostatic pressure would be the most reliable tidal time cue compared with other stimuli caused by tides. The cumacean *Dimorphostylis asiatica* is a small crustacean living on the sea bed at the surface of muddy sand. In the central region of the Seto Inland Sea, it lives just below the intertidal zone (1–3 m below LWMS), under the influence of semidiurnal unequal tides of 2–3 m amplitude. The amplitude of wave action is within 0.5 m except during storms. Under constant dark conditions in the laboratory, *D. asiatica* has been shown to sustain circatidal swimming activity rhythms (Akiyama, 1995). When the animals were exposed

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to a 4 hr stimulus pulse of hydrostatic pressure equivalent to a 3m amplitude rise in water level, distinct phase responsiveness was observed (Akiyama, 1997). However, the phase response curve (PRC) in *D. asiatica* was somewhat different from the clear daily bimodal form of PRCs of tidal rhythms reported previously (Enright, 1976; Naylor and Williams, 1984; Northcott *et al.*, 1991b). One plausible reason is that the entraining effect of a single pulse is somewhat weak. In the present study, individual *D. asiatica* were exposed to 12.5 hr cycles of sinusoidal change of hydrostatic pressure to further investigate pressure effects on entrainment.

Also investigated in the present study is the entraining effect of light-dark cycles on circatidal rhythms (cf. Barnwell, 1966; Saigusa, 1992). In *D. asiatica*, a field investigation of the emergence rhythm established their nocturnal activity, which is probably entrained by the environmental light-dark cycle (Akiyama and Yoshida, 1990). In the present study, *D. asiatica* was exposed to 24 hr cycles of artificial light in the laboratory.

MATERIALS AND METHODS

Collection and recording aquaria

Adult male *Dimophostylis asiatica* were collected at Ushimado (Okayama Prefecture), in the central region of the Seto Inland Sea. The animals were collected at night at high tide using an electric lamp (10V, 200W) and a small hand net of 10 cm diameter with nylon mesh of 1mm opening. The detailed procedure for collection is described elsewhere (Akiyama and Yoshida, 1990). Within 30–60 min of collection, the specimens were carried to the laboratory and transferred individually to recording aquaria, comprising a small glass tube (35 mm in diameter, 75 mm high) containing 30 ml of sea water and mud substratum sterilized by boiling. All experiments were performed from December to May, during which the ambient tides are more distinct than in other seasons .

Recording of activity

The recording aquaria were housed in two light-tight incubators (Sanyo, MIR 150). Swimming activity of the specimens was detected using a photoelectrical switch (OMRON, E3S) receiving a thin infrared light beam about 3 mm under the water surface. The number of interruptions of the light beam by the swimming specimen was counted and stored in 30-min bins by two personal computers, each storing the data from eight specimens. The activity data were then converted to double-plotted actograms.

Administration of hydrostatic pressure cycles

The hydrostatic pressure stimulus was produced using an electric air pump (100V, 100W, IWAKI Co.) connected to the recording aquaria with plastic tubing of 8 mm diameter. A circuit diagram of the apparatus is shown in Fig. 1. Each aquarium set on the recording apparatus was covered with plastic plates, to which the air tubing was connected. The upper ridge of each aquarium was sealed by a rubber seat and four iron bolts to prevent air leak. A hand made plastic valve was placed in series with the air tubing to regulate the amplitude of the hydrostatic pressure stimulus by varying the air leakage. To eliminate the high frequency air vibration caused by pumping, two plastic cylinders (15 cm long, 5cm in diameter) choked with cotton wool were placed at the inlet and outlet of the air valve.

The valve was operated using thin rubber strings of about 50 cm in length. One end of the strings was connected to a part of the air valve, and the other was connected to the edge of a plastic disc of 7 cm diameter, mounted on a stepping motor rotating with once every 12.5 hr. The apparatus was calibrated to produce a sinusoidal change in hydrostatic pressure of 0.3 atm amplitude (1.2 atm at minimum and 1.5 atm at maximum), equivalent to the hydrostatic pressure caused by a 3m amplitude change in water level, by regulating tension of the rubber strings. Hydrostatic pressure was monitored using a pressure gauge constructed from a section of glass pipette with one end sealed with glue. The gauge was submerged in a water chamber connected in series with the air tubing system. During periods when the specimens were not exposed to a hydrostatic pressure cycle, the air pump was switched off, so the animals were kept at a hydrostatic pressure equivalent to that at normal air pressure .

Administration of light-dark cycles

In the experiments to examine the entraining effect of the hydrostatic pressure cycles, the specimens were kept in complete darkness. In other experiments, 24 hr light-dark cycles were admin-
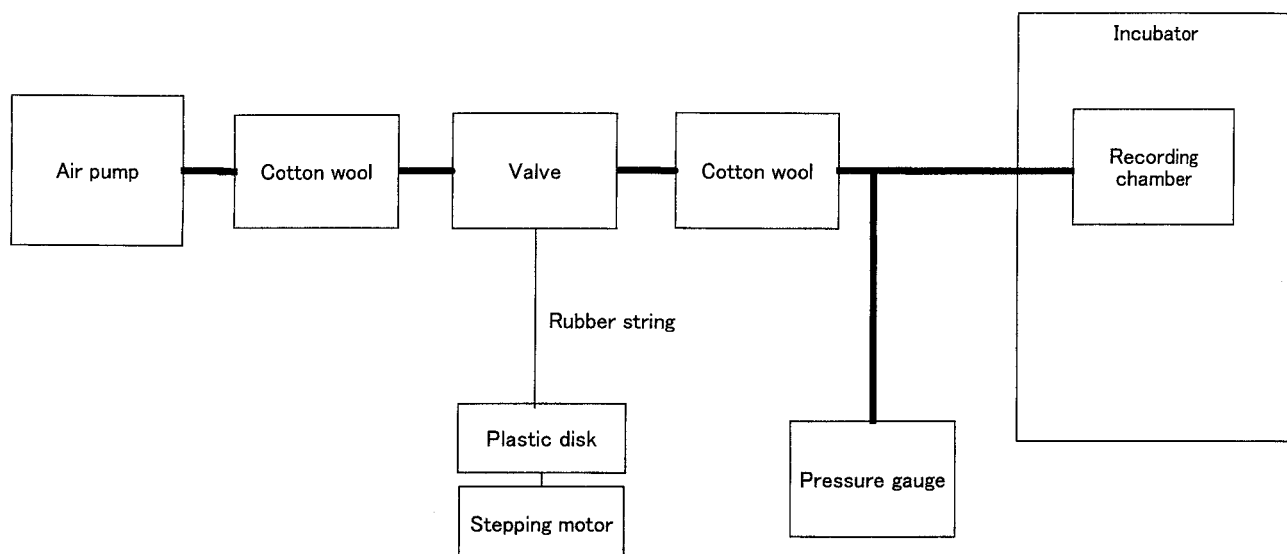

Fig. 1. The circuit diagram of the apparatus used for administration of the 12.5 hr hydrostatic pressure cycles. Bold lines indicate air tubings.
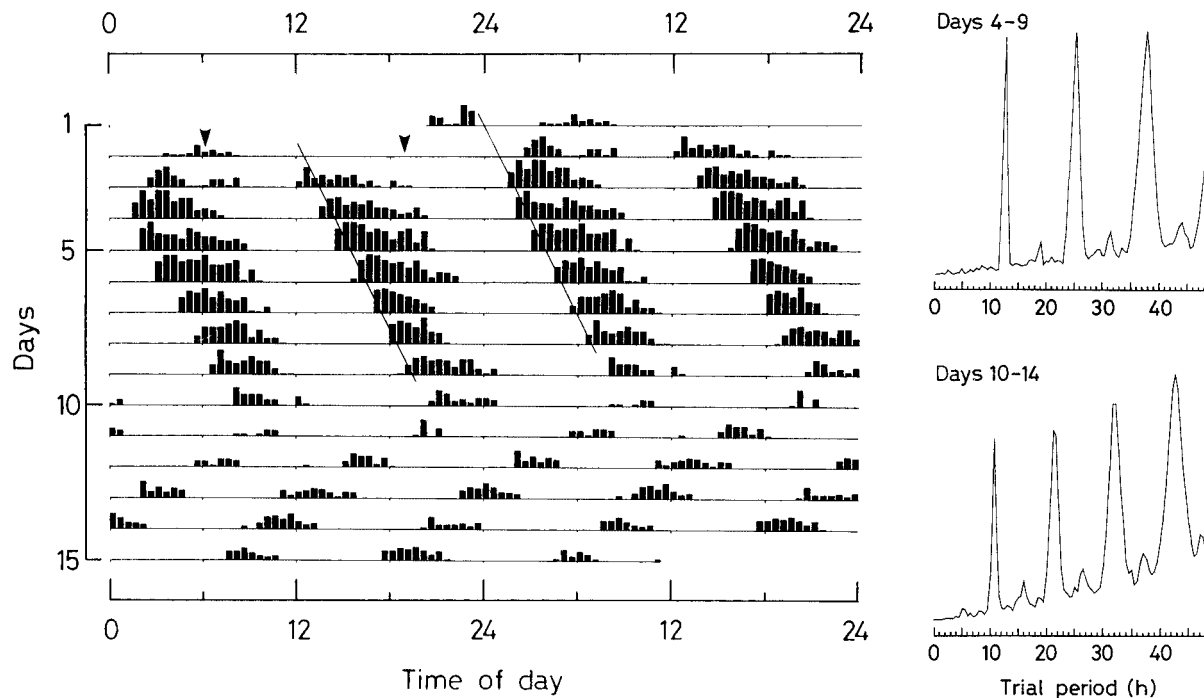
Fig. 2. Activity record of an adult male of *Dimorphostylis asiatica* under constant darkness. A 12.5 hr hydrostatic pressure cycle of 0.3 atm amplitude (1.2–1.5 atm) was administered from 21:40 on day 1 to 01:40 on day 10. Two oblique lines indicate the times of maximum hydrostatic pressure. Arrows indicate the time of high water in the field on day 1. Sea water temperature maintained at 10°C. Right panels; periodogram analysis based on the data for days 4–9 and days 10–14.

istered to the specimens. Two fluorescent tubes (100 V, 20 W) were set in each incubator. 24 hr light-dark cycles with different duration of photo phase (LD 12:12, LD 9:15, LD 6:18) were controlled using electric timers (Matsushita Electric Co.). The light intensity was about 1000 lx at the floor where the recording aquaria were placed. In all experiments, the temperature of the sea water was kept at 10±1°C in the incubators.

Activity period and phase relationship

Activity periods of the specimens were calculated using generic statistics software (e.g., Lotus 123) as circa 24 hr periods for comparison between the daily bimodal and unimodal activity rhythms. In each activity record, linear regression lines of the onsets for 5–10 days of successive daytime and nighttime activities were calculated to estimate the activity period. For daily bimodal rhythms, the activity period of each specimen was estimated as the mean value of the day-time and night-time activity periods (for the daily unimodal rhythms, single successive daytime or nighttime activities were used). The activity period of the specimens shown in Fig. 2 was also calculated by periodogram analysis based on a computer software developed by Dr. N. Matsumoto. The time of high tide in the field was taken from tide tables published by the Japanese Meteorological Agency.

The phase relationship between swimming activity rhythms and administered hydrostatic pressure cycles was estimated by calculating the mean time of activity onset and cessation to relative to hydrostatic pressure maxima and minima. For each specimen, this was based on 4–10 days of successive activity cycles.

RESULTS

Entraining effect of 12.5 hr hydrostatic pressure cycles in constant darkness

Fig. 2 shows the activity record of one animal exposed

to hydrostatic pressure cycles of 0.3 atm amplitude under constant darkness during the first nine days of recording. On day 2, swimming activity occurred from 3:00 to 8:30, which was coincided approximately with ambient high tide (arrowheads). After days 2–4, swimming activity occurred earlier day by day, with the onsets of swimming activity delayed in close association with the time of maximum hydrostatic pressure. Thus activity occurred during the time of hydrostatic pressure decrease. Periodogram analysis of days 4–9 (upper right panel of Fig. 2) shows very sharp peaks at 12.5 hr and 25.0 hr, coinciding with the peaks of the administered hydrostatic pressure cycle. Activity period assessed as double tidal period during the same days based on the linear regression lines of activity onsets was 25.09 hr, which was only about 0.1 hr longer than the period of the administered hydrostatic pressure cycle. There was no indication of any masking effects, such as direct inhibition or the induction of swimming activity, caused by the hydrostatic pressure cycle. After the end of treatment on day 9, the specimen sustained a free-running daily bimodal swimming rhythm. The periodogram analysis of days 10–14 (lower right panel of Fig. 2) shows sharp peaks of free-running activity at 10.5 hr and 21–21.5 hr, which is much shorter than the activity period on days 4–9.

D. asiatica kept under constant conditions in the laboratory is known to spontaneously change its temporal activity pattern from daily bimodal to daily unimodal (Akiyama, 1995). The specimen shown in Fig. 3 was kept for 14 days under constant darkness before exposure to a hydrostatic
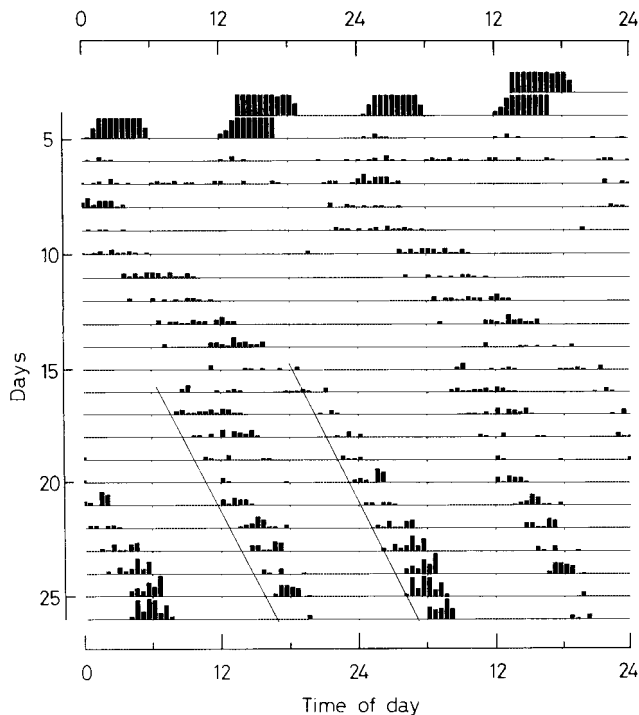
Fig. 3. Activity record of an individual *Dimoprphostylis asiatica* under constant darkness. A 12.5 hr hydrostatic pressure cycle was administered from 12:00 on Day 15. Oblique lines indicate the times of maximum administered hydrostatic pressure.

Fig. 4. Histogram of activity periods of 41 individual *Dimorphostylis asiatica* exposed to 12.5 hr hydrostatic pressure cycles. The activity period of each specimen was calculated as circadian period, based on the linear regression lines of activity onsets over 5–10 days.

pressure cycle. On day 6, the specimen changed its temporal activity pattern from bimodal to unimodal. The activity period of the unimodal rhythm (days 8–15) was 26.20 hr. Thereafter, treatment with a bimodal pressure cycle from day 15 reverted the activity pattern to daily bimodal, and induced a phase advance of 4.2 hr. Each activity bout occurred during the pressure-decreasing phase. On days 17–25, the activity period was 25.0 hr.

Most of the specimens sustained a daily bimodal swimming rhythm during exposure to 12.5 hr hydrostatic pressure cycles under constant darkness. Fig. 4 shows the frequency histogram of the activity periods of the daily bimodal rhythms of 41 specimens (a few of the specimens were excluded because their activity period could not be calculated due to obscure periodicity or no activity). The modal activity period was 24.9 ± 0.6 hr, which is almost exactly the same as a double period of the administered hydrostatic pressure cycle, and significantly different from the period of the free-running daily bimodal rhythm at 10°C ($P < 0.01$; the free-running period is 23.1 ± 1.6 hr; see Akiyama, 1995). Thus the entraining effect of the hydrostatic pressure cycle on the daily bimodal rhythm of this species was clearly demonstrated. In two specimens, the activity period was two hr or more shorter than the period of the administered cycles, suggesting that these specimens sustained the free-running rhythms and were not entrained by pressure cycles.

A small portion of the specimens under constant darkness exhibited a daily unimodal activity rhythm during the
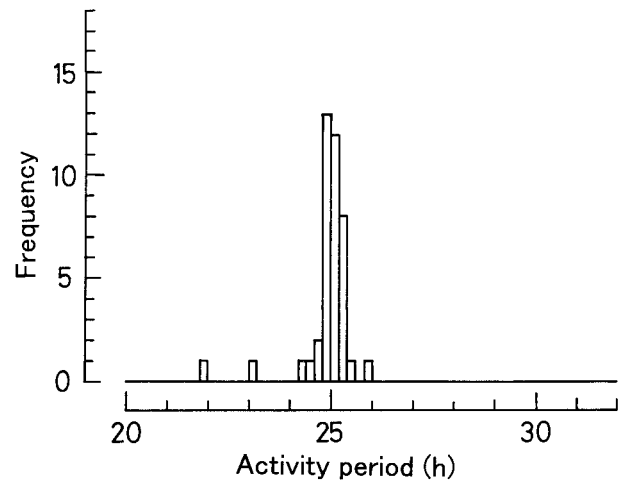
treatment with the hydrostatic pressure cycle. One such case is shown in Fig. 5A. The specimen was exposed to hydrostatic pressure cycles on days 15–25. The activity period before the treatment (days 8–14) was 26.63 hr. During the treatment (on days 8–14), the activity period was 26.30 hr, slightly shorter than before the pressure treatment. The phase shift following commencement of the pressure cycle was calculated as 0.4 hr. It is interesting to note the enhancement of activity near the time the hydrostatic pressure reached a maximum (around day 20). Fig. 5B shows a specimen that changed its activity pattern from daily bimodal to daily unimodal during the experiment (on days 7–9). Although the activity period of the daily bimodal rhythm was not calculated because of its short duration, each activity occurred around the time of maximum hydrostatic pressure, suggesting an entraining effect of the pressure cycle stimulus. The activity period of the daily unimodal activity rhythm (30.45 hr) was more than 5 hr longer than the double period of the administered cycle, suggesting that the endogenous rhythm of the specimen free-ran.

Eleven specimens exhibited daily unimodal rhythms in which the activity period could be estimated during pressure cycles under constant darkness. Fig. 6 shows a frequency histogram of the activity period. The mean activity period was 25.9 ± 1.6 hr, which was close to the free-running period of the daily unimodal rhythm of this species kept under constant darkness at 10°C (25.5 ± 1.8 hr, Akiyama, 1995). Although the activity period of some specimens was near 25 hr, there was no evidence that the hydrostatic pressure cycles entrained the daily unimodal activity rhythm of *D. asiatica*.

Fig. 7 shows the phase relationship between the daily bimodal rhythm of 25 individual *D. asiatica* (whose activity period ranged from 24.8 hr to 25.2 hr) and maximum hydrostatic pressure. In most specimens, swimming activity occurred during the pressure-decreasing phase; the onset
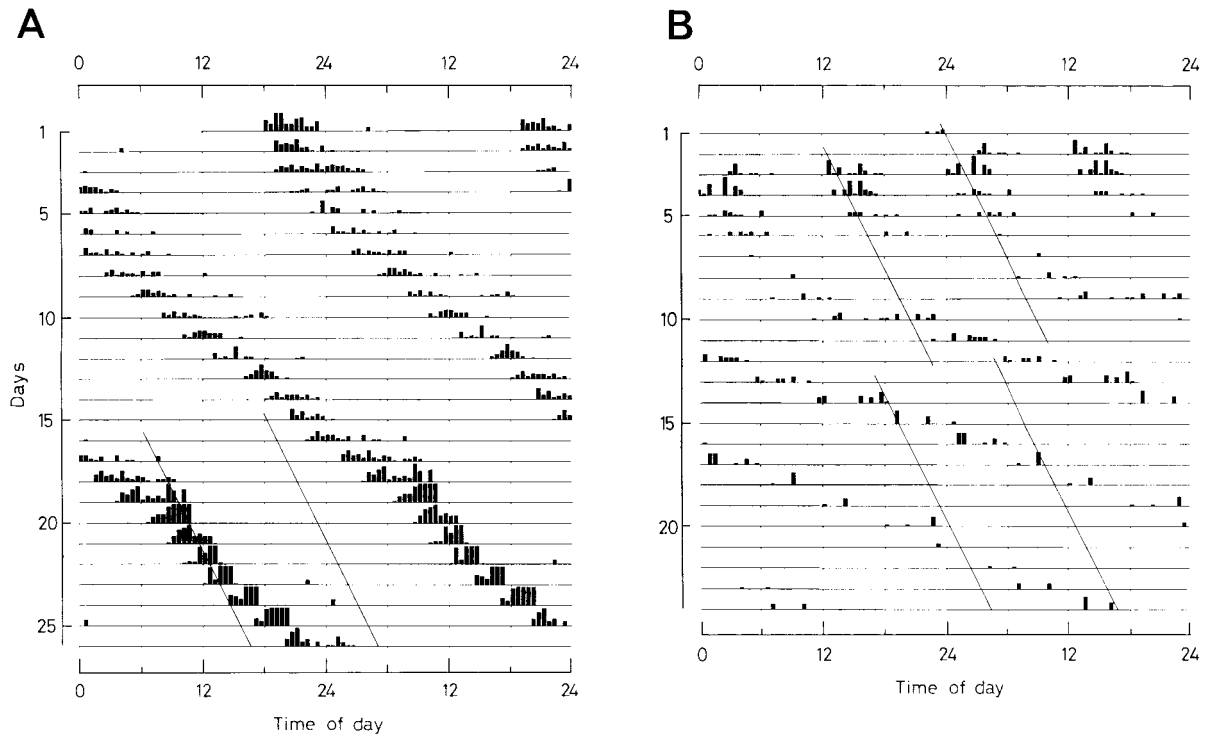
Fig. 5. Activity records of individual *Dimorphostylis asiatica* under continuous darkness. (A) An individual exhibiting a unimodal activity pattern, subjected to the hydrostatic pressure cycles from 12:00 on day 15 to the end of the record. (B) An individual treated with hydrostatic pressure cycles throughout the record: 6.25 hr initially shifted at 22:30 on Day 12. Oblique lines indicate the times of maximum administered hydrostatic pressure.
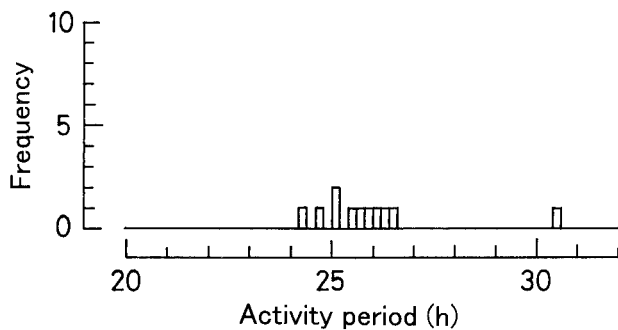

Fig. 6. Histogram of the activity periods of 11 individuals exhibiting a unimodal daily swimming rhythm during treatment with 12.5 hr hydrostatic pressure cycles.

of activities coincided with the time of pressure maximum and cessation of activity coincided with about one hour before the time of minimum hydrostatic pressure. It is noted that four specimens exhibited quite a different phase relationship, showing activity during the pressure-increasing phase. In these specimens, the activity started around the time of minimum hydrostatic pressure and ceased about two hour before the time of maximum hydrostatic pressure.

Fig. 8A shows an example of an activity record where the specimen exhibited activity during the pressure-increasing phase. The period of the daily unimodal activity before exposing it to pressure cycles was 22.90 hr, based on the four successive activity cycles on days 12–15, shifting to a
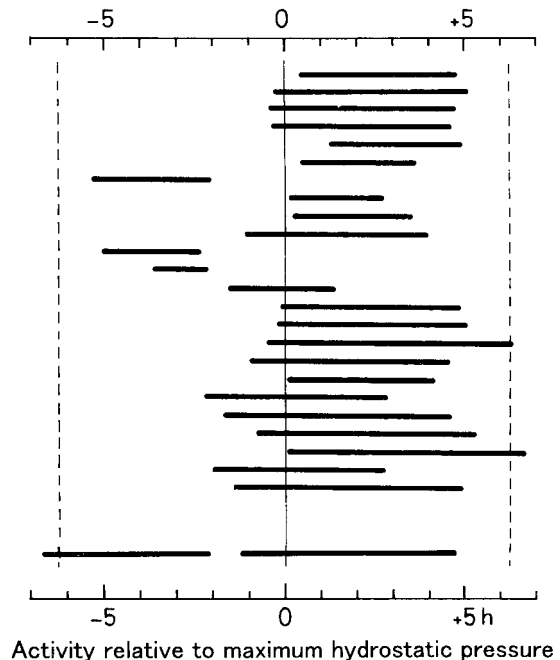

Activity relative to maximum hydrostatic pressure

Fig. 7. Phase relationship of the entrained daily bimodal swimming rhythms of 25 specimens exposed to 12.5 hr hydrostatic pressure cycles. Horizontal bars indicate active time of each specimen. Solid vertical line (time 0 of the abscissa) indicates the time of maximum hydrostatic pressure; broken lines minimum. On one specimen (at bottom of chart)), active times before and after phase shift are shown (activity record shown in Fig. 8B).

daily bimodal activity period (during pressure cycling) of 24.81 hr: a phase delay of 5.6 hr. In another activity record (Fig. 8B), the specimen exhibited the activity during the pressure decreasing phase on days 1–6, then on days 7–9 the specimen underwent a spontaneous phase shift, changing its activity pattern to swimming during the pressure-
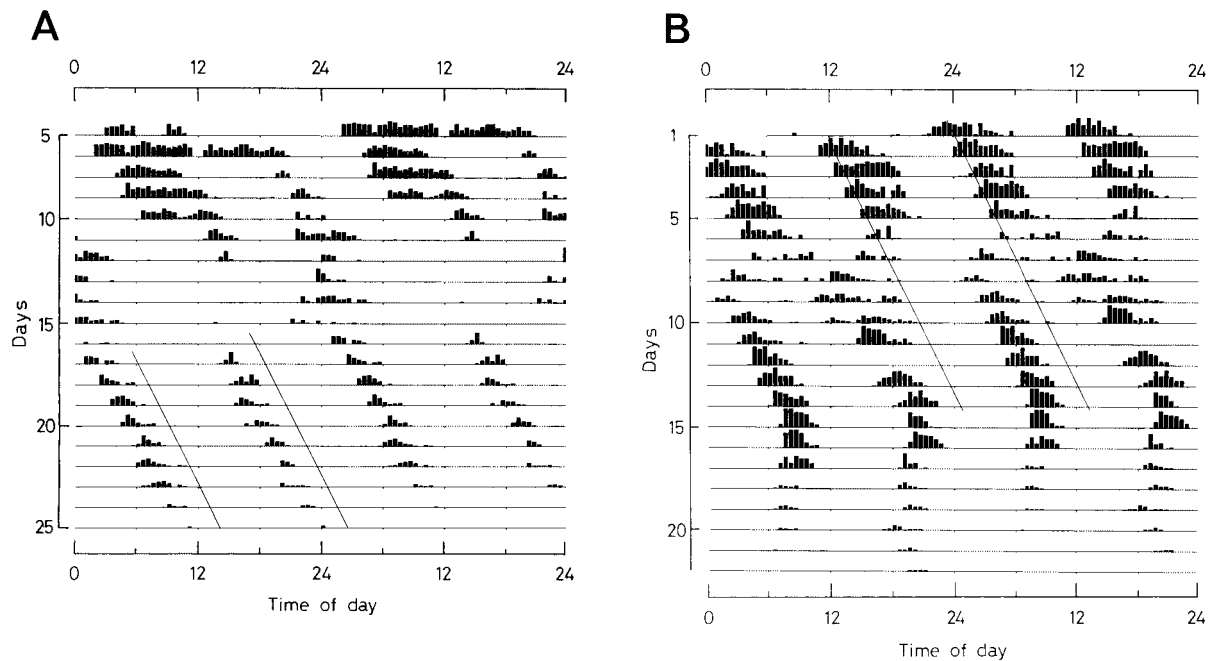

Fig. 8. Activity records of individuals subjected to the hydrostatic pressure cycles (A) administered from 12:00 on day 16 to the end of the record, and (B) administered from 18:00 on day 1 to 20:00 on Day 15. Oblique lines indicate times of maximum hydrostatic pressure.
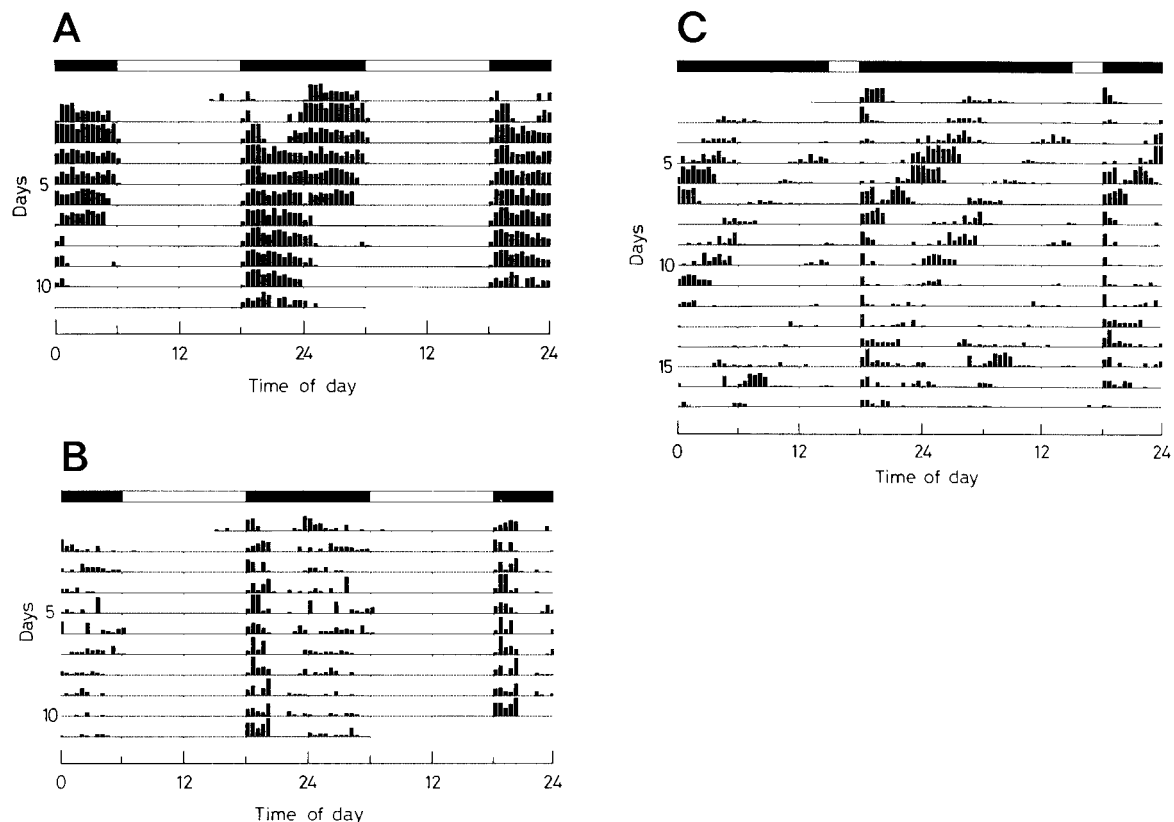

Fig. 9. Activity records of individuals under 24 hr light-dark cycles of LD 12:12 (A, B) or LD 3:21 (C).
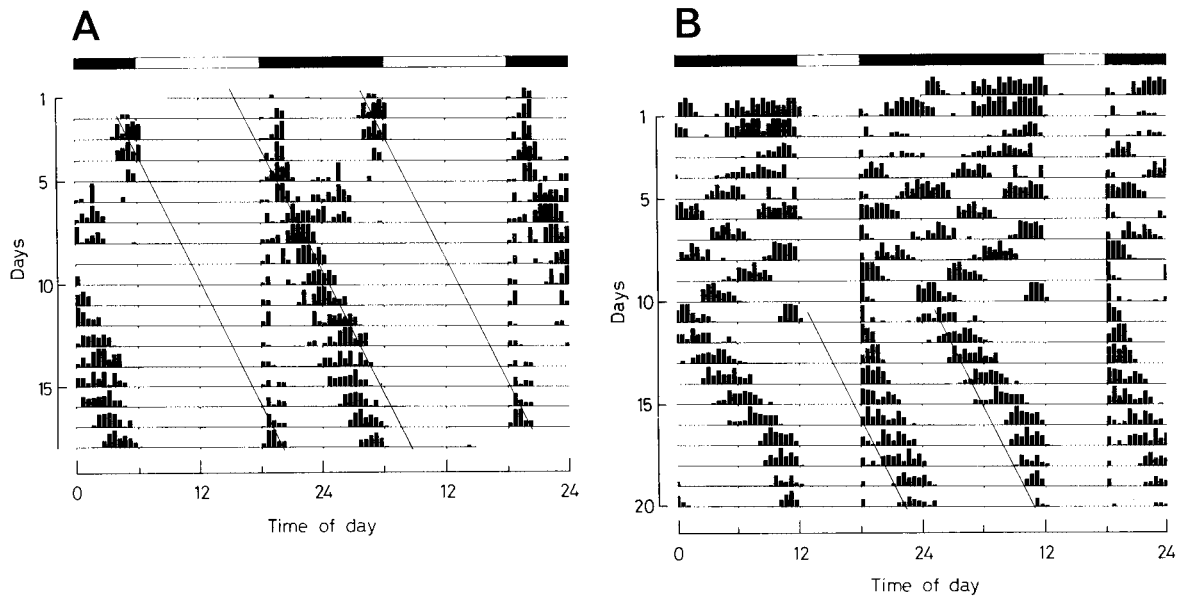
Fig. 10. Activity records of individuals (A) subjected to a 24 hr light-dark cycle (LD 12:12) and a 12.5 hr hydrostatic pressure cycle throughout the recording, and (B) under a 24 hr light-dark cycle (LD 6:18) with a 12.5 hr hydrostatic pressure cycle from 12:25 on Day 11 to the end of recording. Oblique lines indicate times of maximum hydrostatic pressure.

increasing phase. Activity periods before and after the phase shift were 25.25 and 25.04 hr, respectively (the mean of these was 25.15 hr). After cessation of pressure cycling on day 15, the specimen sustained an endogenous daily bimodal rhythm with a free-running period of 23.75 hr.

Entraining effect of 24 hr light dark cycles

Fig. 9A and 9B show two activity records of *D. asiatica* exposed to 24 hr light-dark cycle (LD 12:12). The specimens both show a clear 'nocturnal' activity pattern with no activity observed during the photo-phase. In these records, the entraining effect of light-dark cycles on the endogenous rhythm was not apparent. Fig. 9C shows the activity record where the specimen was exposed to a light-dark cycle with a short photo-phase (LD 3:21). The daily bimodal activity rhythm in which activity onsets become earlier day by day suggests that the specimen exhibits a free-running rhythm under these conditions. Two kinds of masking effect of the light stimulus were observed as direct inhibition of swimming activity during the photo-phase throughout the recording and a small burst of activity shortly after lights off (18:00) on days 10–13.

Fig. 10A shows the activity record of a specimen simultaneously exposed to a 12.5 hr hydrostatic pressure cycle and 24 hr light-dark cycle (LD 12:12). The specimen shows a swimming rhythm entrained to the hydrostatic pressure cycle, with activity bursts occurring on the pressure-increasing phase during the scoto-phase. This rhythmic pattern closely resembles the temporal activity pattern in the field, including the small burst of activity shortly after lights off (Akiyama and Yoshida, 1990). In the latter study, it could not be determined whether the specimen exhibited an endogenous daily bimodal activity or daily unimodal activity under

these light conditions, because of direct inhibition of activity by light.

Fig. 10B shows the activity record in which a specimen was exposed to a light cycle with a shorter photo-phase (LD 6:18). During the period when the specimen was exposed to the light cycle (on days 1–10), it exhibited a daily bimodal free-running rhythm. With a hydrostatic pressure cycle superimposed on the light cycle, the specimen apparently sustained a daily bimodal swimming rhythm entrained by the hydrostatic pressure cycle.

Fig. 11 shows the phase relationship between the hydrostatic pressure cycle maximum and entrained activity
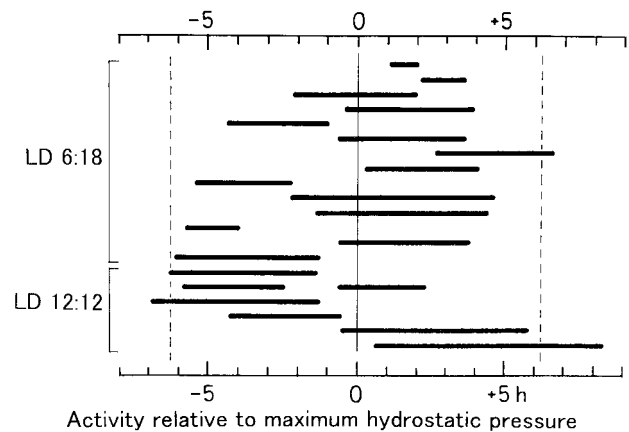

Fig. 11. Phase relationships of the entrained swimming rhythm of 20 individual *Dimorphostylis asiatica* with hydrostatic pressure cycles administered under two different light-dark cycles. Solid vertical line (time 0 of abscissa) indicates hydrostatic pressure maximum; vertical broken lines minimum. Horizontal bars indicate active time of each specimen.

under two different light regimes. Under LD 6:18, 10 out of 14 specimens showed activity during the pressure-decreasing phase or around the time of pressure maximum. Under LD 12:12, four of seven specimens exhibited swimming activity during the pressure-increasing phase. Although the number of specimens tested was not sufficient, the results suggests that light cycles affect the relationship between the swimming rhythm of *D. asiatica* and the administered hydrostatic pressure cycle, in comparison with the specimens kept under constant darkness (Fig. 7).

DISCUSSION

Entraining effect of hydrostatic pressure cycles

The results clearly show that the circatidal swimming rhythm of *Dimorphostylis asiatica* is entrained by hydrostatic pressure cycles of 0.3 atm amplitude. The onset of entrained activity occurred in most specimens shortly before the time of maximum hydrostatic pressure. Onset of the first bout of activity in the free-running rhythm under constant darkness was 0.5 ± 2.1 hr before the expected time of high tide (N=197; data based on the specimens collected in all seasons, water temperature was kept at 10°C or 15°C), suggesting that the animals utilize hydrostatic pressure as the dominant environmental time cue in their natural habitat. This distinct, persistent rhythmicity, with no masking effects (such as inhibition and induction of activity) during treatment with a hydrostatic pressure stimulus in this species suggests that this experimental system is well suited to further detailed investigation of circatidal rhythms.

A noteworthy feature of entrainment in this species is the stability of the phase relationship between entrained activity and the administered hydrostatic pressure cycle. It is also interesting to note that while most individuals were active during the pressure-decreasing phase, a small number swam on the pressure-increasing phase. Such a difference appears not to be simply inter-individual variation because of the change of phase relationship noted one individual (Fig. 8B).

The flexible phase relationship between entrained endogenous rhythms and the administered cycles of entraining stimulus have been reported in diurnal-nocturnal conversion of the circadian activity rhythm in fishes (Sánchez-Vázquez *et al.*, 1995; Iigo and Tabata, 1996). Iigo and Tabata concluded that the flexible phase relationship of fishes is a result of the weak entraining effect of light on animals living in an aqueous environment, which might be applicable also to the circatidal rhythm of *D. asiatica.*

Such an unstable phase relationship could pause a problem for further studies on the mechanism of circatidal pacemakers. Previous studies on the detailed characteristics of circadian rhythms have depended on a specific, stable phase relationship between rhythmicity and light-dark cycles or light pulses. Fine studies on the punctual swimming rhythm of *Excirolana* (Crustacea, Isopoda), which exhibits a complex temporal pattern and regular phase

response curve comparable with circadian rhythms, gave researchers the impression that the phase relationship of circatidal rhythms and environmental cycles is rather stable (Enright, 1972, 1976). The distinct daily bimodal pattern of the PRC of circatidal rhythms studied previously (Enright, 1976; Naylor and Williams, 1984; Northcott *et al.*, 1991b) supports this idea. However, administration of a 4 hr pulse of hydrostatic pressure stimulus in *D. asiatica* did not produce the expected PRC pattern (Akiyama, 1997). Studies on the phase relationship between tidal activity rhythms and administered cycles of physical stimuli have been rather few, partly because of the noisy, less persistent nature of tidal rhythms of the species studied so far. The tidal entraining experiments on *D. asiatica* reported here suggest that this is a suitable model for further detailed study of tidal rhythms.

Another characteristic of the entraining effect of the hydrostatic pressure in *D. asiatica* concerns the temporal activity pattern. While the entrained activity was daily bimodal (circa 12 hr) activity rhythm, there was no evidence that the daily unimodal activity rhythm (circa 24 hr rhythm) could be entrained by hydrostatic pressure cycles. Previous studies focusing on changes in the temporal activity pattern of marine organisms between daily bimodal and daily unimodal rhythms have hypothesized that the change in rhythmicity is related to a change in the temporal pattern of environmental tides (Enright, 1972; Saigusa and Kawagoye, 1997). However, a previous study (Akiyama, 1997) demonstrated that the daily unimodal rhythm of *D. asiatica,* which was not entrained by a single 4hr hydrostatic pressure pulse, was the circadian rhythm. Although the response of the daily unimodal rhythm to light was not tested in the present study, the results support the contention that the two rhythmic patterns observed in this species are governed by pacemaker(s) of quite different characteristics.

In the present study, the specimens were exposed to hydrostatic pressure cycles but not cycles of other physical stimuli. Because of the very wide geographical distribution of *D. asiatica,* from Southeast Asia (Vietnam) to Hokkaido (Gamô, 1968), the environmental cycles affecting the endogenous rhythm of this species probably differ among different local populations. Previous studies have reported that circatidal rhythms are entrained by more than two kinds of tidal time cues in accordance with habitat (Morgan, 1991). Investigation of the entraining effects of other physical time cues, such as mechanical turbulence and temperature is therefore necessary to further investigate circatidal rhythms in *D. asiatica*.

Entraining effect of light cycles

The temporal activity pattern of *D. asiatica* exposed to light-dark cycles and hydrostatic pressure cycles simultaneously (Fig. 10) is closely similar to the temporal emergence pattern of this species in the field (Akiyama and Yoshida, 1990), suggesting that the light stimulus also plays an important role in entraining the activity rhythm in the nat-

ural habitat. In contrast to the entraining effect of the hydrostatic pressure stimulus, which strongly entrained the endogenous tidal pacemaker, entrainment of the activity rhythm by the light stimulus was attained by direct inhibition of swimming activity during the photo-phase, with the induction of a brief burst of activity shortly after lights off; that is, a masking effect.

The masking effect of light on circadian rhythms has been well documented (*e.g.*, Page, 1989). Similar nocturnal activity was reported in the diel vertical migration of certain planktonic species (Enright and Hamner, 1967). The day-night change of photic environment in shallow water would provide regular, reliable time cues compared with complex, noisy stimuli associated with the tides. So for animals living in such an environment, there would be no need to develop endogenous light-phased pacemakers.

Previous studies of circatidal rhythms have paid little attention to the masking effect of light, but it is an important aspects of any further studies on the underlying pacemakers of tidal rhythms, which generally express complex temporal activity patterns. For example, the masking effect will be a problem in studies of *D. asiatica*, whose swimming rhythm changes from circatidal to circadian in the laboratory (Akiyama, 1997). Exposure of these animals to long-term light-dark cycles would lead to obscure results. To discriminate the circadian rhythm from the circatidal rhythm in *D. asiatica*, the specimens were exposed to single light pulses to avoid obscuring the results caused by masking effect of light.

Studies on the circatidal rhythms of crabs have reported the entraining effect of light-dark cycles on cicatidal rhythm (Palmer and Round, 1967; Saigusa, 1992; Saigusa and Kawagoye, 1997). Although the results in the present study suggest the light stimulus also affects the circatidal rhythm in *D. asiatica* (Fig. 11), the structure of the pacemaker and sensory pathways serving it would be quite different from those of the crabs, which were phased by light and tidal stimuli simultaneously. Further comparative studies of the entraining effect of light on circatidal rhythms of various species should reveal more information about the adaptive aspects of the endogenous rhythms of marine organisms.

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