クルマエビにおける摂餌リズムの人為的調節

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Artificial Controls of Feeding Rhythm of the Prawn *Penaeus japonicus*

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Feeding experiments were undertaken to investigate the acclimation ability of the prawn *Penaeus japonicus* to different photoperiods. Activity patterns were observed under 24 h photoperiods as 12L12D (12 h, light; 12 h, dark), 12D12L, 24dim, 24D and 12dim12D. For the individuals of 12D12L and 24D, histological samplings of the brain and optic ganglion were conducted to examine activities of the intercerebral and medulla terminalis cells. The cellular activity was estimated by measuring the relative size of the nucleus (nucleo-cytoplasm ratio). The natural feeding rhythm was disturbed or changed according to the feeding time under respective photoperiods. Achievements of the artificial control of the daytime feeding were shown in the cases of 12D12L, 24dim and 12dim12D, provided that feedings were conducted during morning hours. The cellular activity on the nucleo-cytoplasm ratio indicated that the medulla terminalis cell would continue to show higher values during the daytime than during the night-time regardless of reversal and continuous dark conditions. For the activity of the intercerebral cell, its proper rhythm was disturbed under 24D condition. These results seemed to support the existence of the biological clock in the medulla terminalis.

The behaviours of crustaceans as well as of other organisms are influenced in various ways by environmental factors, and activity is altered at least in part by such factors. However, some behaviours seem to be independent of these environmental parameters when animals are kept under constant conditions. For the prawn *Penaeus japonicus*, environmental effects have been observed especially regarding burrowing behaviour under non-photoperiod conditions.[1] Under these constant conditions, burrowing occurred only during the real daytime, maintaining the original rhythm observed under natural photoperiod. This rhythm seemed to be controlled endogenously, in other words, by a biological clock.

In prawn culture, feeding is usually conducted after sunset, to coincide with the prawn's natural night-time activity. Though it is convenient for practical purposes to shift the feeding time to morning, it is difficult under natural photoperiod. In order to improve the feeding conditions and further, to support the theory of the existence of the biological clock, the present study was performed by observing the ability of the prawns to acclimate to different photoperiods and by investigating cellular activities of their brain and optic ganglion.

Materials and Methods

Behaviour Observations

Postlarvae and juveniles of the prawn *Penaeus japonicus*, reared under natural photoperiod, were obtained from the culture facilities. Sizes and numbers are shown in Table 1. Feeding experiments were conducted under 5 differing photoperiods on a 24 h cycle basis: 12L12D, 12D12L, 24dim, 24D and 12dim12D. Each number represents the number of hours of each of the following treatments: L (light), D (dark) or dim. For all experiments, wooden boxes of 90 cm (W)×90 cm (H)×60 cm (D) were prepared. Each contained a 60 *l* tank provided with a bottom sand layer and a filter pump. To the ceiling was attached a fluorescent lamp, and to a side wall a red lamp for the darkroom was set up. The former lamp was regulated with a timer to control the respective light phases as described above. Only for the dim condition, the lamp was covered with semitransparent vinyl sheet to reduce the light intensity. The red lamp was kept on throughout the experimental periods, in order to observe the prawn's behaviour without the influence of its on-off stimuli. The red lamp was not stimulative in such a condition as

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Table 1. Schedules of acclimation experiments under different photoperiod and feeding time

Photoperiod	Exp. No.	Light phase Feeding time	Specimen number Body weight (g)	Experimental period Water temperature (°C)
12L12D	1.1	08:00–20:00 20:00	200 (postlarvae) 0.03– 0.12	Jun. 1–Jul. 2, 1987 26.8–29.3
	1.2	09:00–21:00 21:00	15 6.05– 6.65	Jul. 10–Jul. 24, 1988 26.8–29.3
12D12L	2.1	21:00–09:00 09:00	200 (postlarvae) 0.03– 0.13	Jun. 1–Jul. 2, 1987 26.7–28.7
	2.2	21:00–09:00 09:00	30 1.67– 3.99	Sept. 9–Oct. 10, 1987 28.4–30.7
	2.3	20:00–08:00 08:00	15 6.21– 9.21	Aug. 8–Sept. 7, 1988 28.4–30.5
	2.4	20:00–08:00 08:00	11 11.04–12.25	Sept. 1–Sept. 28, 1988 27.4–28.8
24dim	3.1	24 h 08:00	40 1.10– 3.78	Jul. 4–Aug. 6, 1988 28.3–31.2
	3.2	24 h 21:00	40 1.76– 3.78	Jul. 4–Aug. 6, 1988 28.0–31.1
24D	4.1	0 h 09:00	24 2.93– 3.89	Aug. 18–Sept. 7, 1988 27.8–29.5
	4.2	0 h 21:00	24 2.88– 4.14	Aug. 18–Sept. 7, 1988 27.8–29.5
12dim12D	5.1	08:00–20:00 08:00	50 0.30– 0.90	Aug. 11–Sept. 8, 1987 29.3–31.3
	5.2	08:00–20:00 08:00	30 1.77– 4.76	Sept. 3–Oct. 4, 1987 28.5–30.5

confirmed by a preparatory experiment. The light intensities for the middle level of the tank water were 500 lx during the L period, 50 lx during the D period, and 60 lx during the dim period. Water temperature was recorded continuously with an electric thermometer. On the hotter days of the summer, an electric fan was used in the box to curb an increase in water temperature. Daily feeding was conducted at a fixed time each day. Concerning this feeding time, however, feeding on the light phase was not conducted during photoperiods 12L12D and 12D12L because of light suppression of the prawn's feeding behaviour or irrelevance to the present purpose at the experiment. After 12 h following each feeding, uneaten pellets, feces and exuviae were removed daily. 50% of total water volume was exchanged weekly. The schedules of respective experiments are presented in Table 1.

Observations on the behaviour of prawns were conducted weekly at fixed times each day. The time interval was 1–4 h, and at occasion, the number of non-burrowing individuals was determined. Activity was expressed as the percentage of non-burrowing prawns in the total population.

Histological Investigations

Samplings of the brain and optic ganglion were conducted for histological observation on prawns reared under 12D12L and 24D. For experiment 2.4 (12D12L), 5 and 6 individuals were sacrificed at 11:00 and 22:00, respectively. For experiments 4.1 and 4.2 (both 24D), 10 individuals were sampled at 08:00–14:00 and 20:00–02:00. In order to investigate the cellular activities of the intercerebral and medulla terminalis cells in the above tissues, diameters of both cells and their nuclei were measured using histological techniques.[1)] Next, the ratio of the nucleus to its cytoplasm (nucleo-cytoplasm ratio) was calculated in order to compare values between daytime and night-time specimens, belonging to the same photoperiod. For the intercerebral cells, the total number of the values was 30 per respective group. For the medulla terminalis cells, that was 100, respectively.

Results

Activity Rhythms under Different Photoperiods

Temperature ranges in tank water during the experimental period are indicated in Table 1.

Daily changes in temperature were within 0.9°C, a value which was recorded only in experiment (exp.) 2.1. For the most part, changes were within 0.5°C, exhibiting in general a gradual and convex curve of which the maximum was located at 20:00–24:00.

Activity patterns obtained from exps. 1.1 and 1.2 both under 12L12D are shown in Fig. 1. Postlarvae of exp. 1.1 exhibited night-time activity; however, the percentage of which was not exact due to difficulties in counting large numbers of swimming prawns. About 20% of the prawns did not burrow during the daytime, throughout the experimental period. On the other hand, young prawns of exp. 1.2 showed nocturnal behaviour only. This would indicate that circadian rhythms are apparently starting from such a juvenile stage of development.

For the reversal photoperiod of 12D12L, postlarvae of exp. 2.1 showed high activities during real daytime under dark conditions (Fig. 2). In this experiment, as well as in the exp. 1.1, 10–20% of the postlarvae did not burrow at the light phase. The young prawns of the exp. 2.2, however, did show clear corresponding rhythms of feeding activity to the dark phase during real daytime (Fig. 2). The above indicates that there is the potential of artificially controlling the feeding rhythm of the prawn. The same can be concluded from the results

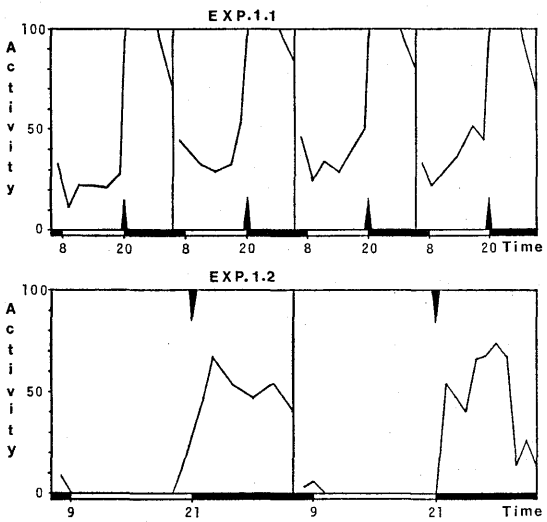

Fig. 1. Activity rhythms obtained from weekly 24h observations of the exp. 1.1 and exp. 1.2. The lower bar means a photoperiod of 12L12D. Arrows indicate the feeding time. For the exp. 1.1, the percentage of night-time activity is not exact due to an error in counting.

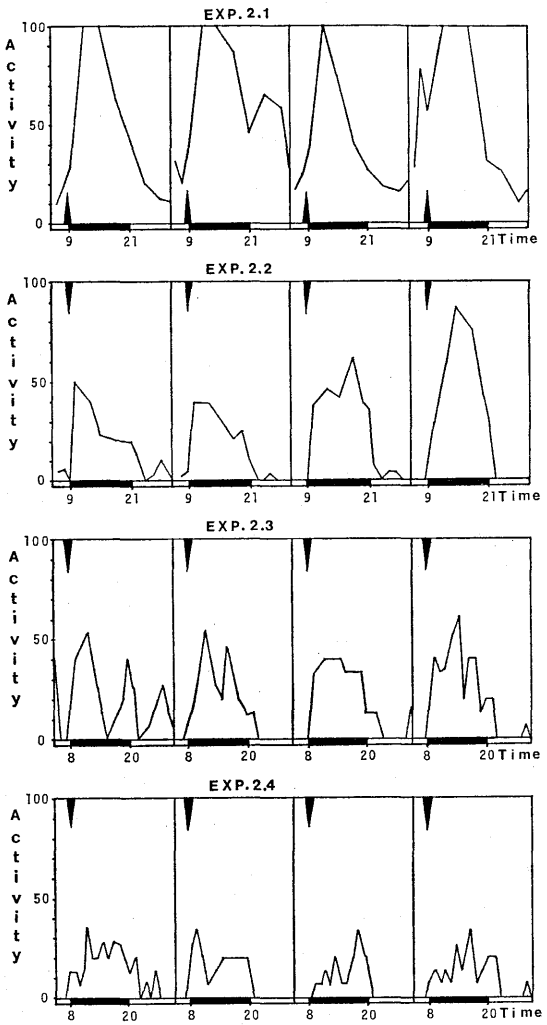

Fig. 2. Activity rhythms obtained from weekly 24h observations of the exps. 2.1, 2.2, 2.3 and 2.4. The lower bar means a photoperiod of 12D12L. Arrows indicate the feeding time. For the exp. 2.1, the percentage of the dark phase is not exact due to an error in counting.

of exps. 2.3 and 2.4, even though activity was low at 20–30% during the dark phase (Fig. 2).

Under 24 dim condition of exp. 3.1, the activity peak seems to occur just after feeding with a lapse of time (Fig. 3), suggesting that the activity rhythm is influenced by feeding time. In the case of exp. 3.2, such a relationship between activity and feeding is also suggested.

Activity patterns of exps. 4.1 and 4.2 under a photoperiod of 24D are shown in Fig. 4. For exp. 4.1, the weekly patterns indicate an

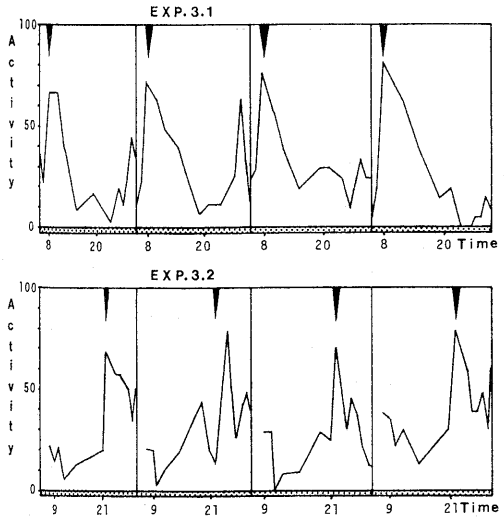

Fig. 3. Activity rhythms obtained from weekly 24h observations of the exp. 3.1 and exp. 3.2. The lower bar means a photoperiod of 24dim. Arrows indicate the feeding time.

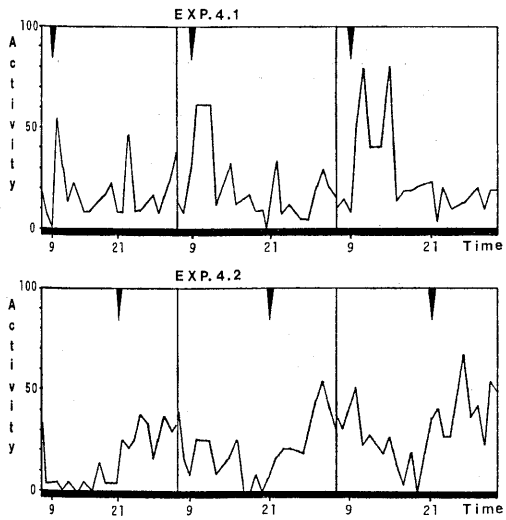

Fig. 4. Activity rhythms obtained from weekly 24h observations of the exp. 4.1 and exp. 4.2. The lower bar means a photoperiod of 24D. Arrows indicate the feeding time.

increasing dependence of activity on feeding time. However, evidence of any clear relationship between the activity pattern and the feeding time was not found in exp. 4.2, although prawns of the same size as those of exp. 4.1 (Table 1) were used.

Activity patterns of exps. 5.1 and 5.2 under 12dim12D condition are presented in Fig. 5. In the results of the exp. 5.1, high activity and

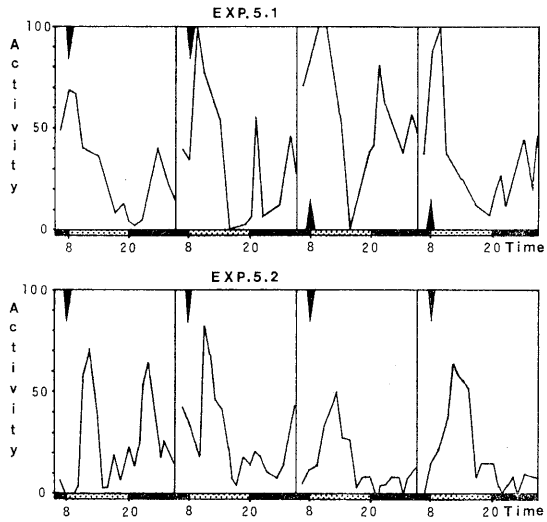

Fig. 5. Activity rhythms obtained from weekly 24h observations of the exp. 5.1 and exp. 5.2. The lower bar means a photoperiod of 12dim12D. Arrows indicate the feeding time.

feeding time occurring in real morning time seem correlated. At the same time, relatively high activity was seen during real night-time. On the other hand, the night-time activity of the exp. 5.2 decreased with the lapse of time. Finally, high activity was observed only during real daytime, regardless of light phase. Considering the prawn size of the exp. 5.2 was 1.8–4.8 g (Table 1), it can be concluded that daytime feeding can be induced under dim and dark photoperiod.

Activities of the Intercerebral and Medulla Terminalis Cells

For the 12D12L, the mean values of the nucleo-cytoplasm ratio of the intercerebral cell are 42.6±2.3 (SD) and 44.9±3.2 at 11:00 and 22:00, respectively. Corresponding values of the medulla terminalis cell are 46.4±3.2 and 44.6±2.8. Both cells show a significant difference between the mean values of the daytime and night-time (*t*-test, $p<0.005$). For the 24D, mean values for the intercerebral cell at 08:00–14:00 and 20:00–02:00 are 44.1±2.8 and 45.2±1.9, respectively. Such values for the medulla terminalis cell are 51.5±3.8 and 49.8 ±3.5. The significant difference between the mean values of the daytime and night-time were only obtained from the medulla terminalis cell ($p<0.005$). These results indicate higher activity of the medulla terminalis cell at day-

time than at night-time, even under different photoperiodic conditions.

Discussion

It was revealed in the present study that the activity rhythm due to feeding changed according to the photoperiod and feeding time. Under photoperiods such as 12D12L, 24dim and 12-dim12D, it was possible to control activity rhythms, if feeding were carried out in morning. However, for practical applications, further investigation must be conducted on how growth and feed efficiency change with feeding time.

As it has been shown for the prawns *P. setiferus* and *P. monodon*,[2] the feeding time can be considered as one of the exogenous factors for *P. japonicus*, though light is probably the most effective factor. For the internal factors, the endogenous rhythm related to prawn behaviour has been examined ethologically for some species under 24L or 24D conditions. In the case of 24L conditions, Fuss and Ogren[3] and Bishop and Herrnkind[4] confirmed the existence of an endogenous rhythm in *P. duorarum*. However, in *P. aztecus*, this was refuted by Lakshimi et al.[5] Under 24D condition, suppositive evidence was obtained for *P. duorarum*, *P. plebejus* and *Metapenaeus mastersii* by Wickham,[6] Racek[7] and Dall.[8] On the contrary, results were negative in *P. aztecus*[5] and *P. duorarum*.[3,4] For *P. japonicus*, one of the authors obtained previously evidence for this rhythm under 24D and 24dim conditions.[1] However, in the present study, especially under 24D conditions, the activity rhythm was disturbed. The above inconsistencies in experimental results may be due to differing experimental conditions employed by the various research groups to such as in length of rearing period and timing of feeding.

The histological results for *P. japonicus* suggest the existence of an endogenous rhythm in terms of cellular activity in the optic ganglion setting aside the issue of behaviour. This cellular rhythm shows the same pattern as that of previously obtained results.[1] The prawn's natural cellular rhythm seems to persist regardless of external condition of light. This presumption correlates with the previous supposition, in which the medulla terminalis was determined to be the biological clock in *P. japonicus*. Furthermore, based on the results of behaviour patterns and the cellular rhythm, the following deduction is possible: the prawn's biological clock does not function as the intrinsic controller of feeding activity.

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