

Periodic Nocturnal Activities in the Catfish *Silurus asotus* in Captivity

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Abstract Nocturnal activities in the catfish *Silurus asotus* were investigated experimentally with an actograph. Most of their nocturnal action commenced nearly at or soon after sunset when ambient light intensities were close to 0.001 lux at various water temperatures (10°~25°C) under natural light conditions. Their nocturnal action, however, terminated a little or more before sunrise depending on the difference of water temperature, when the ambient light intensities were 0.0001 lux or less. The nocturnal activities of this catfish were controlled not only by the daily changes in the ambient light but also by an endogenous circadian rhythm which was detected in light: dark pulses repeated with a short period, and in constant darkness. Their daily activities in nature were regarded as a result of synchronous cooperation of the endogenous rhythmical system and daily light: dark stimuli.

The present author previously reported on nocturnal activities of the catfish *Pseudobagrus aurantiacus* (Temminck et Schlegel) that were subject exclusively to the changes of ambient light intensity (Mashiko, 1979). For instance, *P. aurantiacus* commenced most of its nocturnal action when the environmental light intensity dropped to 0.01 lux at a water temperature of 25°C. Its activity continued for a whole day in experimental constant darkness. The catfish *Silurus asotus* Linnaeus also behaves nocturnally in nature with nearly the same life mode as *P. aurantiacus*, being distributed in stagnant water in the middle and lower reaches of rivers, in lakes and in swamps in Japan (Miyadi et al., 1976). On the nocturnal activities of *S. asotus*, however, few analytical investigations have been made except some fragmentary observations in the field. In this study the nocturnal activity of *S. asotus* was investigated experimentally with special references to its relationship to ambient light intensities from a view point of comparison between these two nocturnal species.

Materials and methods

Silurus asotus about 19 to 23 cm in body length (two years old estimated from the body length, and nearly adult) were collected from the River Kuji in Fukushima Prefecture in the summer of 1979, and kept in aquaria in our labora-

tory until used for experiments. The experimental fish were individually introduced into a glass aquarium (30×60×30 cm), in which a meshed black vinyl pipe (5 cm in diameter and 25 cm in length) was fixed on the bottom. The fish utilized this pipe for their nest-shelter, and concealed themselves in it in the daytime. Daily movements of the fish into and out of the nest-shelter were automatically recorded with an actograph as electric spikes. The number of electric spikes was counted hourly with an automatic counter. Simultaneously with the recording of fish movements, the ambient light intensity in the glass aquarium was continuously measured with a highly sensitive microscopic exposure meter (Olympus Co. Ltd., EMM-II), and also recorded automatically. The measurable minimum light intensity by this exposure meter is 0.0001 lux. The experimental apparatus adopted here was nearly the same as what was previously described in detail (Mashiko, 1979). The whole experimental apparatus except the recorders was enclosed by sheets of thick black cloth in order to shut out unnecessary external light. Then, two kinds of light conditions were adopted for the experiments, i. e. light: dark changes in natural light (nLD), and artificial light: dark cycles (LD). The nLD condition was produced by introducing only external indirect natural light into the experimental

aquarium through a window facing north and an opening in the enclosing black sheets. The artificial LD cycle was made with two 20W-fluorescent lamps hung about 50 cm above the water surface of the experimental aquarium. The light intensity beside the experimental aquarium dropped lower than 0.0001 lux at night in nLD and at dark periods in LD, and the maximum in nLD was about 200 lux throughout the experiments. Water in the aquarium was circulated and filtered through grains of sand and active carbon, and glass wool packed together in a filter box. Water temperature was controlled constant within a fluctuation of $\pm 1^\circ\text{C}$. The fish were fed frozen shrimps from markets, after thawing. Foods were introduced into the aquarium with a small amount of water through

a polyvinyl-chloride pipe which was extended from the upside of the aquarium to the exterior of the black sheets enclosing the apparatus. Thus intrusions of external light were avoided.

Results

Nocturnal fish movements in nLD (natural light: dark changes) and LD (artificial light: dark cycles) were recorded as series of frequently repeated electric spikes which appeared throughout a night or a dark period. While sporadic electric spikes sometimes appear in the daytime or in the light period, most of those were ascertained to be produced by the fish's action against the sensors, staying inside the nest-shelter and not by actual movements in the aquarium. Serial frequent electric spikes, occurring at night or in

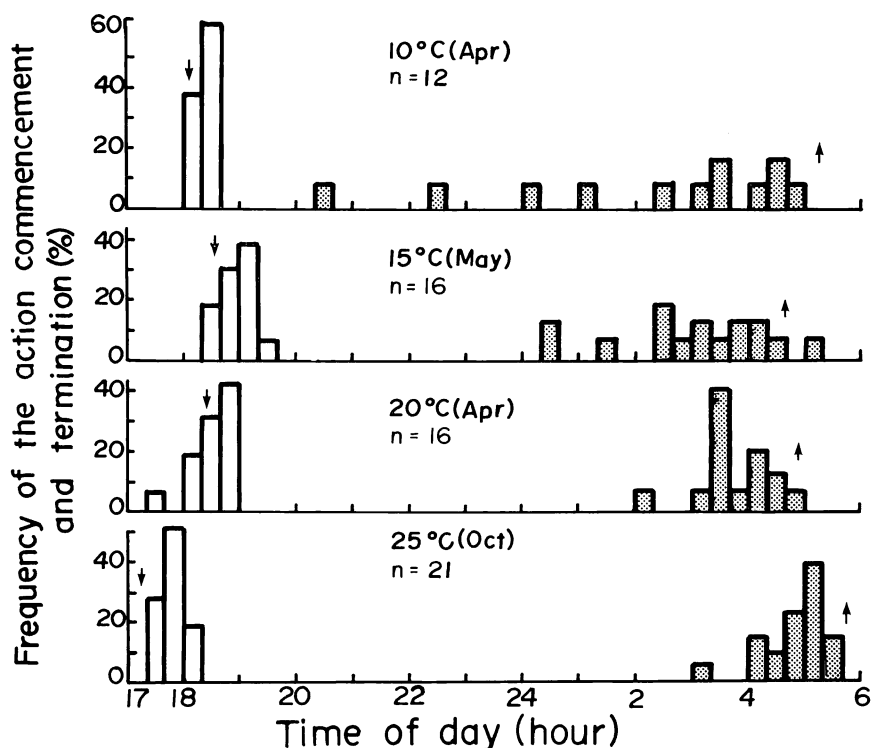


Fig. 1. Frequency distributions of the commencement (left bars) and termination (right shaded bars) of nocturnal action by time of day in one individual (male) at various water temperatures in nLD. The time of day was divided into 20 minute intervals. Frequencies are expressed by the percentages of number of days when the nocturnal action commenced (terminated) within each 20 minute division against the total number of days examined, which are indicated by the symbols *n* in the figures. The arrows \uparrow and \downarrow show the mean time of sunset and sunrise respectively in the experimental month which is indicated in parentheses.

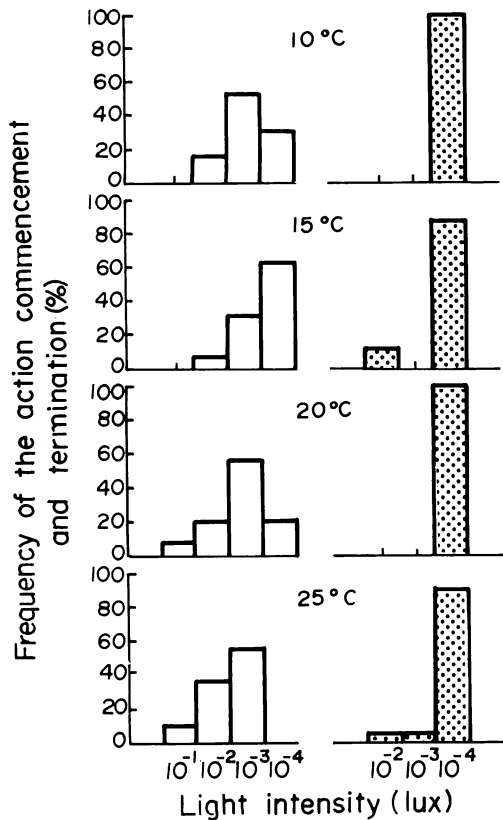


Fig. 2. The relationship between ambient light intensities in nLD and frequencies (%) of action commencement (left bars) and termination (right shaded bars) at the various water temperatures. Light intensities are expressed only by orders of an exponent of 10 in lux.

the dark period, were easily distinguishable from the sporadic spikes, as demonstrated previously in the study on *Pseudobagrus aurantiacus* (Mashiko, 1979). In the present study, the commencement and the termination of nocturnal action were defined respectively by the first appearance of frequent electric spikes and their disappearance on the recorder, as in the previous study.

Fig. 1 shows frequency distributions of the time of day when the commencement and termination of nocturnal action were observed in one individual in nLD at various water temperatures (10°, 15°, 20° and 25°C). The time of day is divided into 20 minute intervals, and the

frequency of action commencement and termination are expressed by percentages of number of days when each item was observed within the respective 20 minute division against the total number of days of the study. The experimental fish was acclimatized in advance to each water temperature for five days at least, then the examinations were made successively. Most nocturnal action commenced nearly at or soon after sunset at all degrees of water temperature examined. However, the termination of nocturnal action covered a wide range of times of day. The unsettled time in the action termination is remarkable at lower water temperatures, and some nocturnal actions terminated even at midnight at 10°C.

Fig. 2 shows the relationship of frequencies of action commencement and termination to the ambient light intensity in the above mentioned experiment. The light intensities are expressed only by orders of exponents of 10 in lux; for instance, 0.01 lux in the figure contains values from 0.01 lux to 0.09 lux. However, the frequency at the lowest 0.0001 lux contains values under this level, because no discrimination of such minute light intensity was possible with the measuring instrument adopted here. The frequencies of the action commencement are clearly correlated with ambient light intensities, dominating in 0.001 lux in all degrees of water temperature except at 15°C. On the other hand, much nocturnal action terminated in 0.0001 lux or less.

The relationships between ambient light intensity and action commencement and termination in two other individuals are shown in Fig. 3. The action commencements of both fish dominated in 0.001 lux, as in the foregoing individual. They terminated most of their nocturnal action in 0.0001 lux or less. These light intensities are also equal to those in the previous experiment. The results obtained hitherto in the several individuals in nLD indicate that *Silurus asotus* commences its nocturnal action depending on the fall of ambient light intensity nearly to 0.001 lux. However, the concentration of the termination of nocturnal action at the fixed range of light intensity (0.0001 lux) shown in Figs. 2 and 3 are only apparent. This minimum range includes also cases occurring at lower intensities

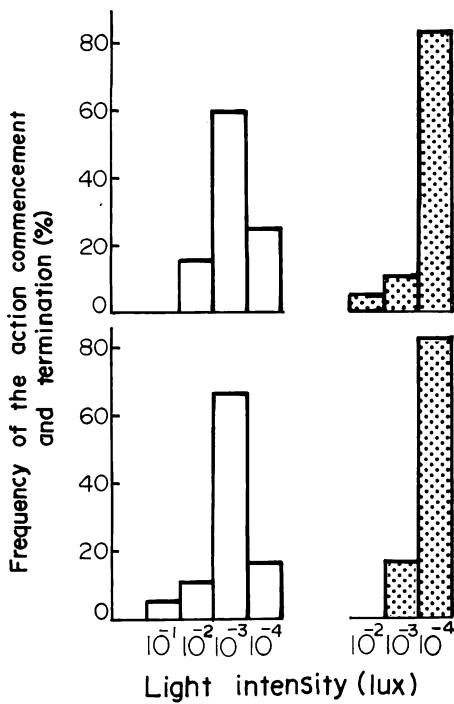


Fig. 3. The relationship between ambient light intensities (expressed only by orders of an exponent of 10 in lux) and frequencies (%) of action commencement (left bars) and termination (right shaded bars) in two other individuals (males) at a water temperature of 20°C. Total numbers of days examined are 20 in the upper and 18 in the lower respectively.

than 0.0001 lux. Therefore, these concentrations do not necessarily mean the dependence of the action termination on changes in the ambient light intensity, but only indicate the maximum level of light intensity before which the nocturnal action terminates in most cases. Moreover, the fact of the termination of action at midnight or considerably long before sunrise shown in Fig. 1 cannot be accounted for by increment of the ambient light intensity, because the light intensity at these times was kept extremely low. Other motivating factors than a change in light intensity may be necessary for a reasonable interpretation of these early terminations.

Fig. 4 shows diel changes in electric spike frequency per hour in one individual at various water temperatures in nLD. Diel activi-

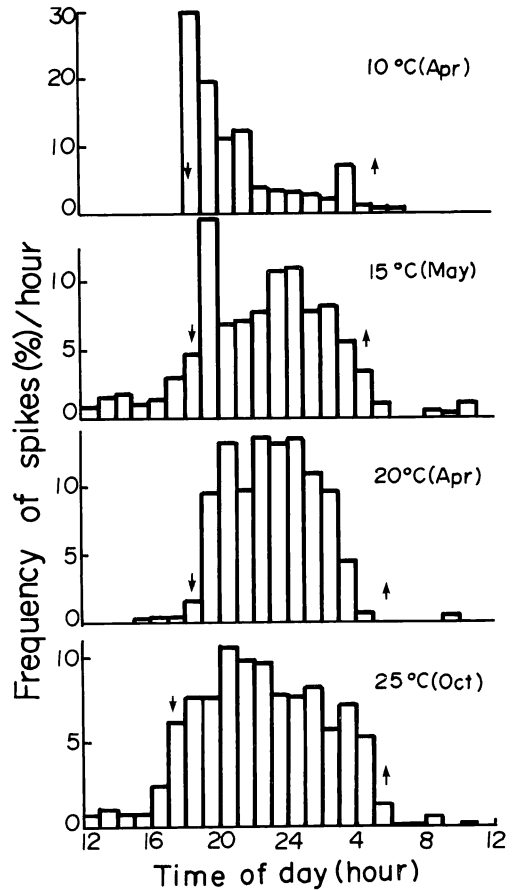


Fig. 4. Diel changes in the electric spike frequency per hour in an individual (male) at the various water temperatures in nLD. Spike frequencies are indicated by the mean values in ten day experiments. Arrows \uparrow and \downarrow indicate the mean time at sunset and sunrise in the experimental month, indicated in the parentheses.

ties were kept nearly uniform throughout the night from sunset until a little before sunrise in higher water temperatures. In lower water temperatures, however, the activities decreased remarkably late at night, and most of the activity was limited to the period before midnight at 10°C. These noticeable decreases in activity late at night at lower water temperatures reasonably correspond to the early terminations of nocturnal action shown previously.

Whether *Silurus asotus* has a free-running rhythm was investigated in the artificial light:

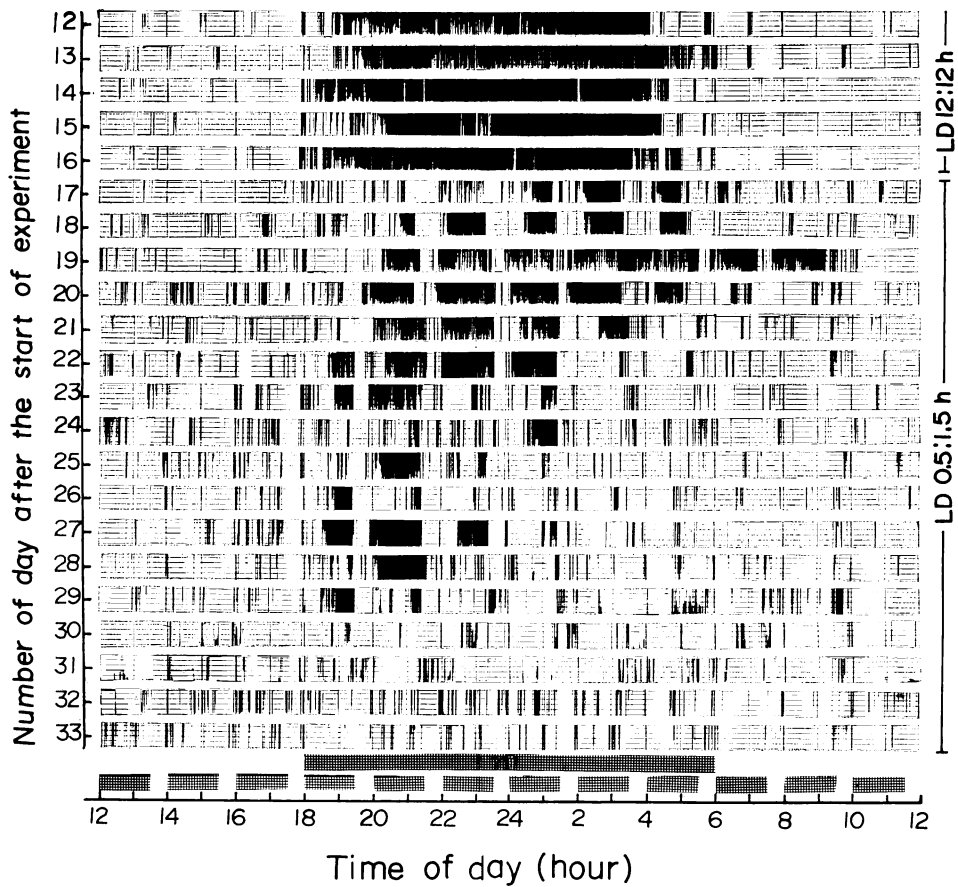


Fig. 5. An original recording of daily activities of an individual (male) when the light condition was changed on the 17th day from the cycles of L (200 lux) D 12: 12 h to repeated pulses of L (0.1 lux) D 0.5: 1.5 h for free-running. The periods of these light conditions are shown at the right side. The activities before the 12th day in the first LD cycles are omitted. The shaded horizontal bars on abscissa indicate the dark periods in LD 12: 12 h (the upper) and LD 0.5: 1.5 h (the lower).

dark (LD) regime. The experimental fish was previously entrained in the cycles of L(200 lux)D 12: 12 h for 16 days, then exposed to repeated pulses of 0.5: 1.5 h with a dim light (0.1 lux in the light period at the water surface) which was produced by a 5W-electric bulb instead of the fluorescent lamps used for the LD cycles. Water temperature was controlled at 25°C. Sufficient food was given once every four days about noon. Fig. 5 shows the original recording of electric spikes during this experiment. The fish's activity (a duration of frequently repeated spikes) in the first LD 12: 12 h are almost completely confined within the dark period. The periodicity in spike frequency per day continues for about two

weeks even after the change of light condition from LD 12: 12 h cycles to LD 0.5: 1.5 h pulses. But the duration time of frequent spikes per day shortened, and the center of the duration time seems to advance only a little day by day. In the LD 0.5: 1.5 h pulses, vigorous activities are nearly completely concentrated within dark periods every 1.5 h. Spotting by the intensity of 0.1 lux may suppress the appearance of activities by free-running rhythm.

Fig. 6 shows correlogram analysis of the spike frequency during free-running, from the 17th to the 29th day in Fig. 5. Serial correlation coefficients (r_c) were calculated according to Itô and Murai (1977). This analysis detected

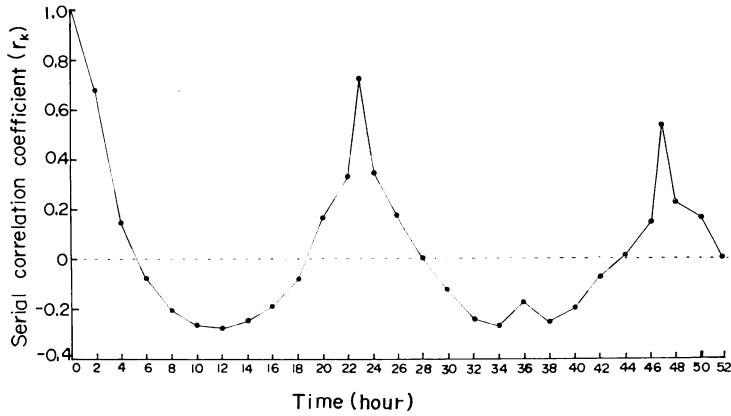


Fig. 6. Analysis of serial correlation coefficients (r_k) in the spike frequencies in a free-running rhythm, from the 17th to 29th days shown in Fig. 5.

a clear periodicity during free-running with a period of about 23 hours with the r_k value 0.73 which is statistically significant, according to Suzuki (1968). The period of about 23 hours in this free-running rhythm shows its independence of external changes of the diel 24 hour period, i. e. that it is endogenous according to its own period. Such endogenous rhythmical activities in free-running conditions were detected also in other individuals in repeated LD pulses.

Free-running rhythm was investigated in constant darkness (DD) after entrainments by LD cycles, and further in repetitious alternations of LD and DD, in order to know the character of its rhythmical systems. Fig. 7 shows changes in daily activities when a fish was entrained by a cycle of L (200 lux) D 12: 12 h for eight days, then exposed to DD and the previous LD alter-

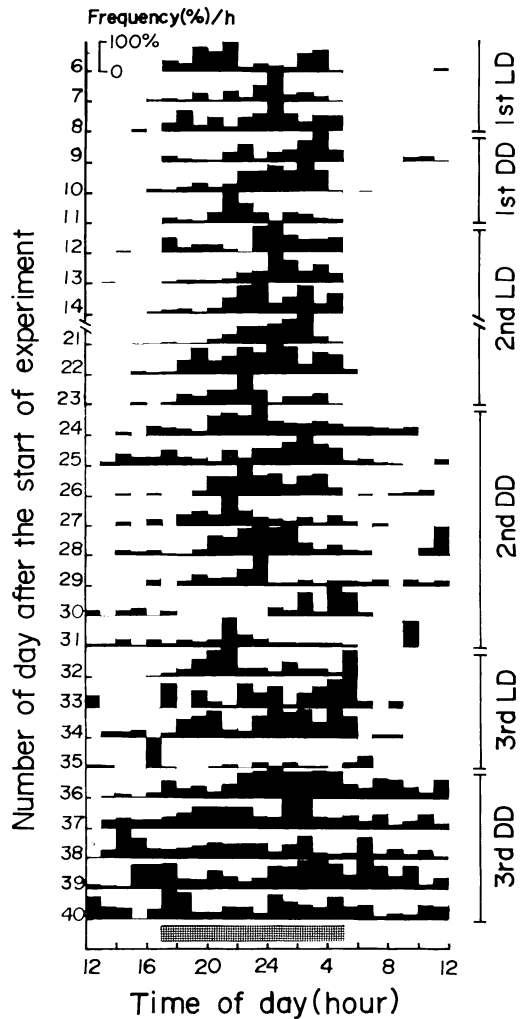


Fig. 7. Daily changes in the activities when an individual (male) was exposed to repetitious alternations between cycles of L (200 lux) D 12: 12 h and constant darkness (DD). The periods of LD and DD are shown at the right side. The spike frequencies are shown as the percentages of number of spikes per hour against the maximum number of spikes recorded at an hour in the day concerned. Activities for the initial five days, and from the 15th to 20th days in LD are omitted.

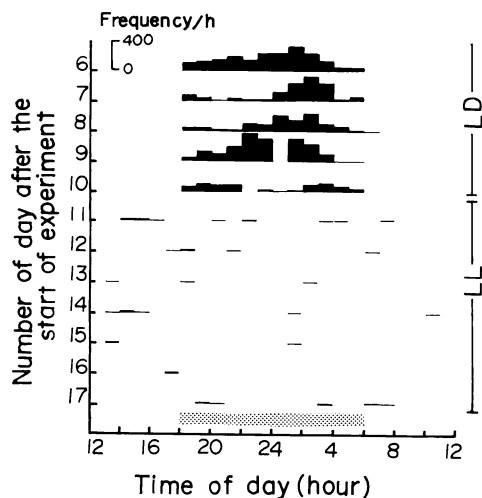


Fig. 8. Daily changes in the activities of an individual (male) when the light condition was changed from cycles of L(200 Lux)D 12: 12 h for ten days to LL(200 lux). The periods of LD and LL are shown at the right side. Spike frequencies are expressed by the actual number recorded every hour. Each daily compartment in the figure corresponds to 400 spikes per hour. The activities for the initial five days in LD are omitted. The horizontal bar on abscissa indicates the dark period in LD.

nately. The schedule of alternation after the first entraining LD was the first DD from the 9th to 11th day, the second LD from the 12th to 23rd day, the second DD from the 24th to 31st day, the third LD from the 32nd to 35th day and the third DD from the 36th to 40th day after the start of the experiment. These light conditions were changed at 5: 00 h from DD to LD, and at 17: 00 h from LD to DD. Water temperature was controlled at 25°C. Food was given ad lib. in the daytime. In the first DD, almost all spikes are restricted in the time corresponding to the dark period of LD, keeping a free-running rhythm throughout the experimental three days. While rhythmical activities seem to appear slightly in the second DD, some amount of spikes also appear at the time corresponding to the light period of LD. Then, only irregular activities appeared by the third DD. Thus the endogenous rhythmical system in *Silurus asotus* seems to be of little stability, and very hard in re-entrain-

ing when the fish was introduced into free-running conditions.

Fig. 8 shows changes in daily activities of a fish when the light condition was changed to continuous illumination (LL) after ten day entrainments by the cycles of L(200 lux) D 12: 12 h. Water temperature was controlled at 20°C. Food was given every day at about noon. LL activities of the fish were inhibited thoroughly for a whole day.

Discussion

Endogenous rhythmical systems are known as "physiological" or "biological" clocks in various vital activities of many organisms (Bünning, 1973; Chiba, 1975). In fishes, endogenous rhythms are known in some behavioral and physiological activities; diel movements of retinal pigment in the catfish *Ameiurus nebulosus* (Welsh and Osborn, 1937), diel locomotor activities of the white sucker *Catostomus commersoni* (Kavaliers, 1980), changes in body color of the killifish *Fundulus heteroclitus* (Kavaliers and Abbott, 1977; Kavaliers et al., 1980), growth activities in scales of the roach *Rutilus rutilus* (Ottaway, 1978), etc.

Silurus asotus, subjected to the pulses of LD 0.5: 1.5 h, exhibited periodical activities in a circadian manner with a period of about 23 hours according to previous entrainment. Such endogenous periodical activities were also found in constant darkness. Eriksson and his coworker detected an endogenous circadian rhythm with a period of about 23 hours under repeated pulses of LD 0.75: 0.25 h in the brown bullhead *Ictalurus nebulosus* (= *Ameiurus nebulosus*) (Eriksson, 1978). In detecting endogenous rhythm, these LD pulses seem to be more adequate conditions than constant darkness or constant light which have been generally adopted hitherto (Chiba, 1975), because they failed to detect a free-running rhythm in constant light conditions in *I. nebulosus*.

The free-running circadian rhythm in daily movements of *Silurus asotus* in LD pulses and in constant darkness may be induced by an inner timekeeping system(s), i. e. biological clocks, independent of diel changes of external stimuli. In nLD, however, *S. asotus* does respond to the fall of ambient light intensity lowered to near

0.001 lux, which works as "Zeitgeber" or a synchronizer. Actual nocturnal action of *S. asotus* in nature may be regulated by both endogenous rhythmical system and natural light:dark changes. In such a situation the inner timekeeping system is expected to contribute to anticipate the time for nocturnal action before exact darkening.

The endogenous rhythmical system of *Silurus asotus* is not so stable, and difficult in re-entrainment. In Fig. 7, the free-running activity rhythm which was exhibited in the first DD lost its clarity at the second DD in spite of twelve day entrainments which were longer than the period of the first entrainment. This rhythm did not reappear by the third DD after four days of entrainments. These arrhythmical trends may be triggered by the first introduction of the fish into free-running conditions for three days, when the endogenous rhythmical system is freed from interaction with external light:dark changes. Re-entrainment longer than in this experiment may be needed for reappearances of distinct free-running rhythm at the second DD and the third one.

Contrary to *Silurus asotus*, no endogenous rhythm was detected in nocturnal activities in *Pseudobagrus aurantiacus*, and they commenced their nocturnal action exclusively depending the change of ambient light intensities (Mashiko, 1979). The lack of endogenous rhythm is also reported in swimming activities in the American shad *Alosa sapidissima* (Katz, 1978). The difference in the inner mechanism of daily movements between *S. asotus* and *P. aurantiacus* may be concerned with each phylogenetic progressive grade in nocturnalism, i. e. *S. asotus* seems to be a more specialized species than *P. aurantiacus*.

Some differences between *Silurus asotus* and *Pseudobagrus aurantiacus* were observed also in the pattern of diel movements in nLD. Almost all nocturnal action of *P. aurantiacus* terminated after sunrise when ambient light intensities are in the order of 0.001 lux or higher (Mashiko, 1979). On the other hand, *S. asotus* terminated most of its nocturnal action far earlier than sunrise when ambient light intensities fell under 0.0001 lux, which is significantly lower than in *P. aurantiacus*.

Silurus asotus, kept at lower water tempera-

tures, often terminated its nocturnal action even at midnight, and remarkably decreased its movements late at night (Fig. 4). *Pseudobagrus aurantiacus*, however, did not show such changes in the pattern of diel movemental activities. *P. aurantiacus* continued its movements throughout the night nearly uniformly despite differences in water temperature which ranged from 7.5° to 30°C, though its absolute frequency of action decreased according to drops in water temperature (Mashiko, unpublished data). This difference in their diel activity pattern in movements at lower water temperatures may be related to a different inner mechanism.

The ambient light intensities at which *Silurus asotus* and *Pseudobagrus aurantiacus* commence most of their nocturnal action are 0.001 lux and 0.01~0.001 lux (Mashiko, 1979) respectively. These approximately equal light intensities are reasonable values for a nocturnal fish's action commencements, because these values are only a little lower than 1~0.01 lux at which diurnal fishes decline their visual feeding activities (Blaxter, 1970, cited in Winslade, 1974).

Acknowledgments

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実験条件下におけるナマズの活動の日周変動

益子計夫

ナマズ *Silurus asotus* の夜間活動について、特に光の昼夜変化との対応に関連して室内実験を行った。ナマズは日没直後に活動を開始し、その時の環境照度は水温 10~25°C の範囲内でほぼ 0.001 ルックスのレベルに保持され、環境照度との強い相関関係が認められた。一方、夜間活動の終了は夜半から日の出前にかけて観察され、またそれは低温環境におけるほどより早い時刻に移行する傾向が認められたので、環境照度への直接的依存性は少ないものと考えられる。ナマズは人工的な短い明暗周期下および恒暗条件下で、その活動に概日的自由継続リズムを示した。従って、自然条件下における本種の日周活動は、自然の昼夜変化と自己の活動リズムの両方の働きで制御されているものと考えられる。これらの結果について、すでに著者により報告されたギバチの夜間活動との比較検討を行った。

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