STUDIES ON THE DAILY RHYTHMIC ACTIVITY OF THE STARFISH, *ASTROPECTEN POLYACANTHUS* MÜLLER ET TROSCHEL, AND THE ACCOMPANIED PHYSIOLOGICAL RHYTHMS[1,2]

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With 9 Text-figures

I. INTRODUCTION

A considerable number of works have hitherto been published on the periodic activity of animals (refer to MORI, 1948; KLEITMAN, 1950). These knowledges show that the rhythmic phenomenon is one of the important basic phenomena prevailing in the world of living organisms, and it is this periodic phenomenon by which the complex ecosystem is maintained in harmonized condition. It is obvious, in this field, that thorough researches are desirable which give some consistent explanations to various knowledges concerning the external manifestations of behaviors, internal physiological conditions and environmental states. Nevertheless, we have now only little knowledges about these unifying researches. The senior author have been elaborated several works on this line since 1935 using some freshwater snails (MORI, 1946, 1948), a sea-pen (MORI, 1950) and a loach (MORI, 1951). This report is one of the works on this line, dealing with the starfish, *Astropecten polyacanthus* MÜLLER et TROSCHEL, as the material. This animal inhabits on the shallow sandy bottom near the Seto Marine Biological Laboratory, and we studied their daily rhythmic behaviors during 1947 to 1949 at this Laboratory.

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II. NATURAL FEATURES OF THE RHYTHMIC ACTIVITY

Materials and Methods

Procedure of one of the observations performed will be described below. Seventy nine starfishes were collected on July 13, 1947 and placed in a large aquarium (1.0×0.8×0.4 m) with sandy bottom. They buried themselves in the sand during midday and midnight, but came out of the sand at down and dusk and moved about in search of food. At 8 o'clock of July 15, a plenty of fishmeat was given as food, and they were left without supplying any food material to the end of our observation. Observations of the activity were started at 11.30^{h} of July 15, and the numbers of animals moving on the sand were counted generally at every 3 hours, but at dusk or dawn, when the animals moved most actively, more frequent counts were made. The observation was ended at 7 o'clock of July 19.

Results

Results obtained are shown in Figure 1. It is clear from this figure that; 1. The starfishes move about actively twice a day, namely, at dusk and at dawn,

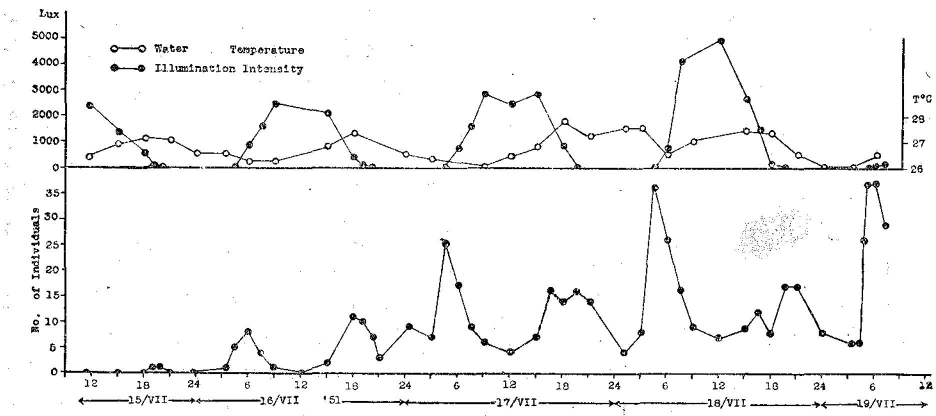

Fig. 1. Graphic representation of daily rhythmic activity of the starfish. Upper column shows changes of environmental factors and lower column shows changes of numbers of animals moving on the sand.

and the intensity of activity is stronger at dawn. On the other hand, during midday and midnight, they generally remain moveless beneath the sand.

2. Activities become more and more vigorous as they become hungry, but the vigor seems to attain its maximum at the 3rd day and thereafter remains constant.

III. RELATIONS BETWEEN ACTIVITY AND ENVIRONMENTAL FACTORS

Major factors seem to be light and food. Thermic factor seems to play no significant rôle so long as the daily cycle is concerned.

1. Light

This is the most important factor by which the periodicity is controlled. They never move about when the intensity of light is above 2000 Lux, and move most actively below 100 Lux. Also they do rarely move during night under complete darkness.

a) Activities under constant darkness

They maintain their rhythmic activities 2 or 3 days under constant darkness. During this period the the rhythmicity become gradually irregular and after 3 days it becomes obscure. The experiments were repeated twice, during July 14 to 19 of 1947 and July 15 to 22 of 1949. The results obtained at the latter case are shown in Figure 2. It must be noted in this case that the

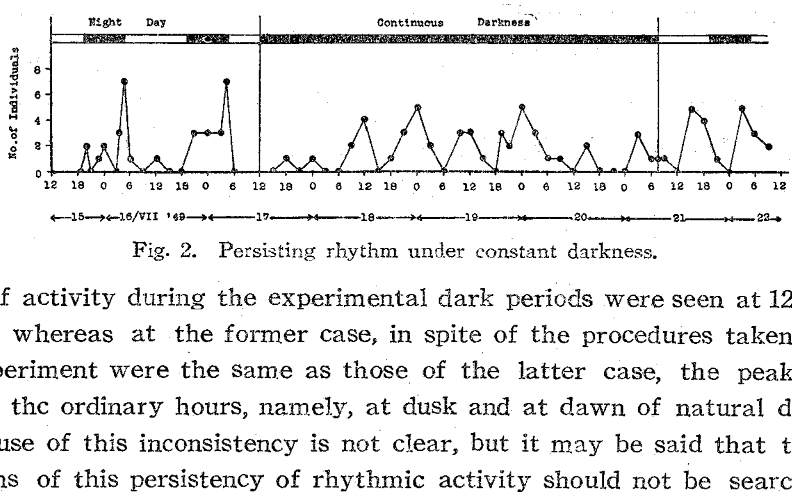

Fig. 2. Persisting rhythm under constant darkness.

peaks of activity during the experimental dark periods were seen at 12 and 24 o'clock, whereas at the former case, in spite of the procedures taken during the experiment were the same as those of the latter case, the peaks were seen at thc ordinary hours, namely, at dusk and at dawn of natural daytime. The cause of this inconsistency is not clear, but it may be said that the mechanisms of this persistency of rhythmic activity should not be searched for in the exogenous affairs but in the endogenous one.

b) Diurnal changes in photic reactions

During our observation of the activity at night by using the illumination of pocket-lamp, we noticed that the reaction of animals to light was considerably different through the course of night, i.e., the tendency to come out from beneath the sand, stimulated by sudden illumination, grew stronger as the time of sunrise approached. It seemed to be clear that the sensibility to light was changed periodically, which was responsible, at least partly, for the occurrence of daily rhythmic activity of the starfish. To test this phenomenon more precisely, we attempted the experiment as below. Forty individuals collected on July 13, 1949, were placed in an aquarium under diffused daylight and the ordinary activity was previously observed. From July 16, the room was darkened once a day for an hour at different times from day to day. The numbers of animals moving about on the sand were counted at every 1.5-3 hours during the whole course of the experiment, and especially duriug darkened periods countings were made at every 15 minutes. The procedures and the results of the experiment are shown in Table 1.

Considering about the photic reaction indices and the χ^2 values, it can be said that the sensibility of the starfish to sudden decrease of illumination intensity during daytime is most raised at dawn, and then falls markedly, and

Table 1. Numbers of animals moving on the sand before and after darkening the environment.

Date		16/VII, '49	17	19	20	18
Time when the environment was darkened		6	9	12	15	18
No. of individuals moving on the sand	Just before darkening (A)	2	2	2	6	0
	15 minutes after darkening (B)	18	8	8	18	6
"Photic reaction index" B/A		9	4	4	3	6+
χ^2 values,* showing the significance of the difference between A and B		15.000	2.857	2.857	7.202	4.504

* χ^2 value at some time was calculated, using 2 by 2 table, as follows:

	Number of individuals		Total
	Remaining still beneath the sand	Moving on the sand	
Just before darkening	a_1	a_2	Ta
15 minutes after darkening	b_1	b_2	Tb
Total	T_1	T_2	T

$$\chi^2=\frac{(a_1b_2-a_2b_1\pm T/2)^2\times T}{T_a\times T_b\times T_1\times T_2}$$

again in the afternoon towards dusk it rises to a fair degree. This change in photic sensibility may undoubtedly be playing some important rôle in the performance of the daily rhythmic activity of the starfish. The experiment with the change of sensibility to light during nighttime has not been performed, but it is now under consideration.

2. Food

As is shown in Figure 1, food as an environmental factor exerts great influence upon the activity. But its rôle is anyway restricted within the range of the modifier to "intensity" or "quantity" of activity, and is, therefore, different from the light which is the real controlling factor of the rhythm. In order to see clearly the changes of activity during hunger, the next experiment using actogram was attempted. The disc of the starfish was bound by

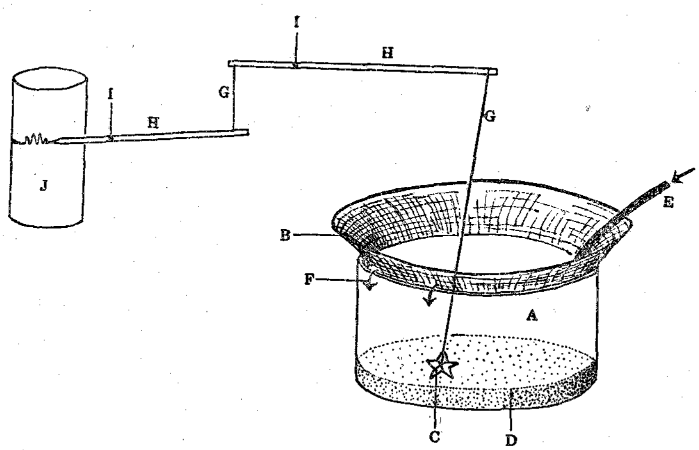

Fig. 3. Arrangement to record automatically the activity of the starfish. A: glass vessel in which the animal is reared (diameter. 30 cm, height 15 cm). B: wire gauze. C: starfish. D: sand. E: tube for inlet of water. F: overflow of water. G: thread. H: lever. I: fulcrum. J: recording drum rotating once a day.

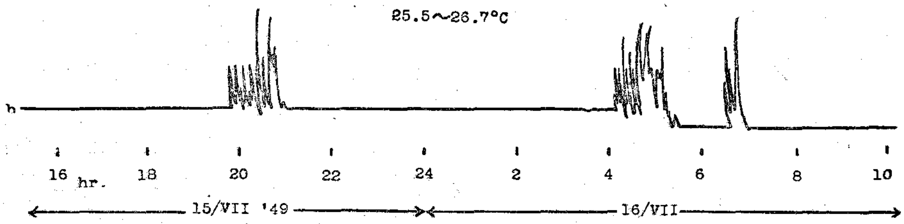

Fig. 4. An instance of the actographic representation of activity.
b: basal line.

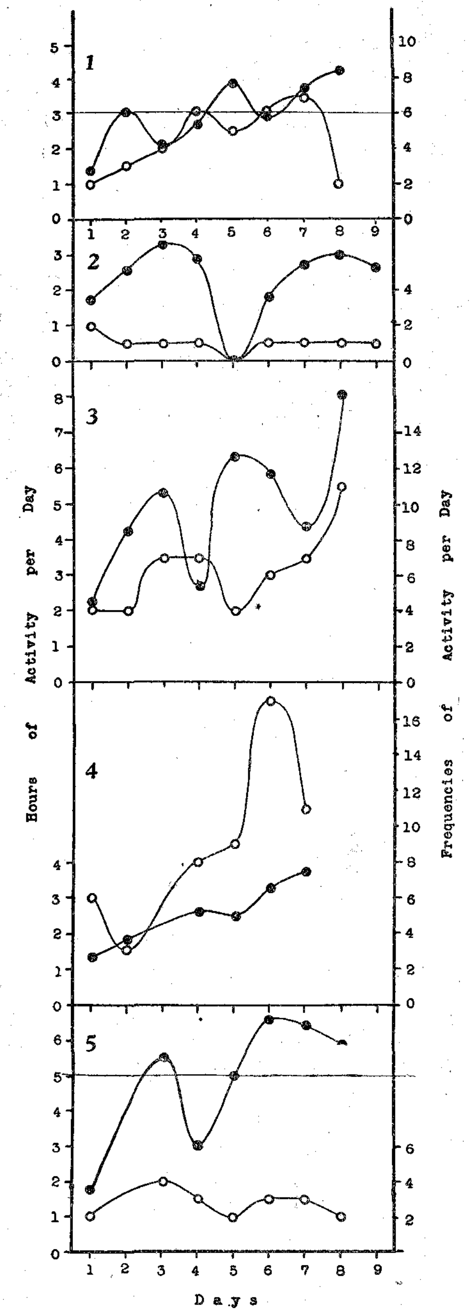

Fig. 5. Changes in hours and frequencies of activity per day during hunger. 1 to 5 show the results of 5 individuals respectively.

a fine wire, tightly but carefully not to injur the animal body, and this wire was connected with the describing lever by a thread. Figure 3 shows general arrangements.

The starfishes were sufficiently fed at the first day of the experiment and thereafter were kept without supplying any foods. Records of 5 individuals during consecutive 7 to 9 days (from July 13 to 22, 1949) were obtained. An instance of the record is shown in Figure 4.

Generally speaking, both hours and frequencies of activity per day increase as hungry condition proceeds. These trends could be clearly represented by performing adequate operations to the records. Namely, when the inclination of the activity curve to the basal line was above 45° and its amplitude exceeded 5 mm in height within an hour, it was regarded as the animal was in the active state. The hours of activity per day were obtained by summing up the periods of this condition through a day and the frequencies of activity per day were obtained by counting the numbers of the continuous phase of activity. The results obtained are shown in Figure 5.

It is concluded from this experiment that the hours of activity of 4 individuals out of 5 increase gradually, in a broad way, with the lapse of time and after a week the quautities become 3 to 4 times of the initial condition. It is conspicuous that there are some periodic fluctuations, the cycle of which is about 3 days. The increases in the frequencies of activity per day are rather less prominent when compared with the increases of the hours of activity, but at 3 individuals out of 5 the general trend of increment may be recognizable, and in this case also some fluctuations of large periods can be seen.

3. Temperature and other Factors

The temperature and other environmental factors, so long as their daily periodicities concern, seem to have no effect upon the rhythmic activity.

IV. SOME PHYSIOLOGICAL ANALYSES OF THE RHYTHMIC ACTIVITY

1. Photoreceptors

Whether the eye spots are photoreceptors regulating the rhythmic activity or not? To ascertain this point, all of the eye spots of 10 individuals were severed and the activities of these individuals were examined. But we could not find out any influences, and they exhibited routine rhythmic activities.

2. Rhythmic activities of severed arms and amputated individuals

How are the autonomic movements of each arm and the coordinations of the body as a whole in the exhibition of the rhythmic activity? Two

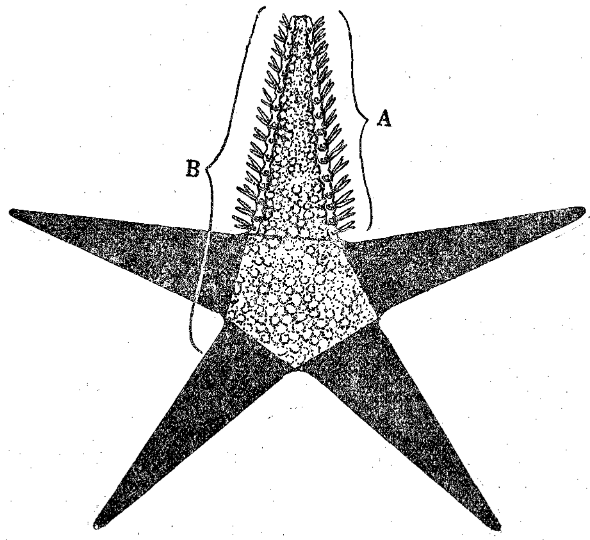

Fig. 6. Severed arm (A) and amputated individual (an arm+disc, B). Eye spot of the arm is severed.

series of experiments were performed in order to clarify these questions, the one by using 40 arms without eye spots severed from the discs as is shown in Figure 6 A, and the other by using 10 individuals, each with only one arm without eye spot as is shown in Figure 6 B.

i. Results with amputated individuals.

Although the rhythmic activity was not vigorous, tendency to move about at dawn could be clearly recognized. They rest quietly at dusk. Except righting and burrowing movements, which were slightly disharmonized, general features of movement were the same as seen in normal individuals.

ii. Results with severed arms.

Results obtained are shown in Table 2.

Table 2. Rhythmic activities of severed arms without eye spots. Operations were performed for 40 arms at 9.30 on July 15, '49.

	Date	15/VII	16								17								18			
	Time (o'clock)	21	0	3	6	9	12	15	18	21	0	3	6	9	12	15	18	21	0	3	6	9
No. of individuals	Buried in the sand	32	36	36	26	29	27	25	26	32	32	32	29	30	30	32	32	32	32	30	27	29
	Resting on the sand	7	4	4	9	11	12	12	14	8	8	8	9	9	10	8	8	8	10	10	12	11
	Moving on the sand	1	0	0	5	0	1	3	0	0	0	0	2	1	0	0	0	0	0	0	1	0

Severed arms repeat gentle bending motion and seldom go further than 1–2 cm in one of the successive movements. They burrow into the sand from the top of the arm. General modes of the rhythmic activity resemble to those of amputated individuals, and move about, although sluggishly, only at dawn. This trend coincides with the results of observation in normal individuals in which the maximum activity occurred at dawn.

3. Daily changes of oxygen consumption

Changes of oxygen consumption may be one of the best indicators of the general physiological conditions of animal body. From this standpoint we attempted the experiment to show whether there were any daily changes in the quantities of oxygen consumed per unit time or not, and if any, what kinds of correlations existed between these changes in oxygen consumption and the external manifestations of activity. General arrangements for experiment are shown in Figure 7.

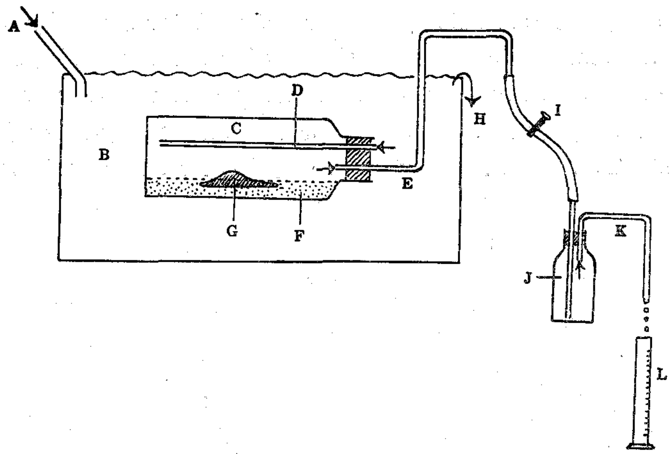

Fig. 7. Arrangements to measure the quantities of oxygen consumed. A: inflow of sea water. B: large vessel for reservoir of water. C: experimental vessel. D: glass tube for inflow of water to C. E: glass tube for outflow of water from C to J. F: sand. G. starfish. H: overflow of water. I: pinch cock. J: bottle for sampling the water (about 100 cc.) K: glass tube for outflow of water from J. L: messcylinder.

One or three starfishes were placed in the experimental vessel (C), the pinch cock of which (I) were opened sufficiently so that the water in the experimental vessel was thoroughly circulated. The rate of oxygen consumption of the starfishes was measured at every 3 hours throughout the day. Just before the time of the measurement, the pinch cock was so screwed that the quantity of water flowing through the experimental vessel containing one animal was made to be about 10 cc per minute. The rate of the flow was determined by the messcylinder L. After an hour the sampling bottle was

Table 3. Data for the experiment of oxygen consumption

No. of the experimental vessel	No. of individuals contained in the vessel	Weight (g)	Volume of water in the experimental vessel (cc)	Volume of sand in the experimental vessel (cc)
1	3	10.6 10.7 15.1	1460	690
2	1	9.1	320	175
3	1	7.9	320	180

taken off and the pinch cock was loosened so as to let the water run as before. The oxygen contents of water were determined by the Winkler's method. The numbers and the weights of animals used in the experiment, and the amounts of water and sand in the experimental vessels are shown in Table 3. The experiments were performed during July 15 to 16 and July 17 to 18 of 1949. The results obtained at the former case are shown in Figure 8.

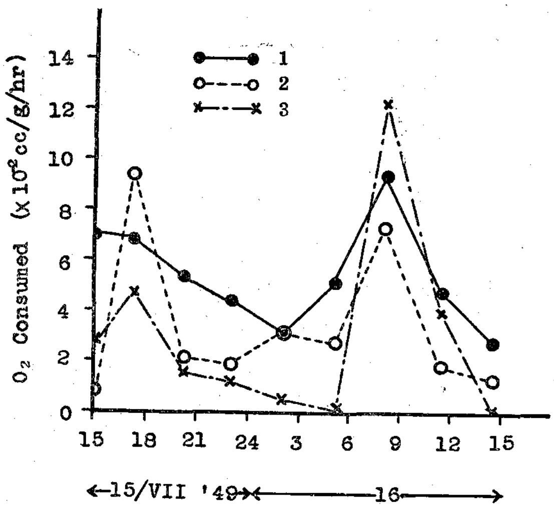

Fig. 8. Daily changes in the rate of oxygen consumption of the starfishes. 1, 2, and 3 indicate the numbers of the experimental vessels. Values shown in the curve 1 are those obtained by treating 3 individuals as a group.

It is clear from this figure that two peaks can be recognized, of which the peak at dawn is higher. These facts coincide exactly with the features of ordinary rhythmic activity.

4. Daily changes of hydrogen ion concentration in body fluids

As well as the rate of oxygen consumption, this factor may also be one of the general indices of the physiological states. The methods employed were as follows: Taking out the animal from the aquarium in which it was reared for several days before the experiment, the water attached on the surface of the body was absorbed by using blothing paper. Then, cutting off the end of one arm, the body fluids flowing out from there were collected in a test tube and the hydrogen ion concentration was determined by using the indicater method. Through the course of treatments, we took care to treat the animal quietly and quickly as possible. The experiments were executed at every 3 hours throughout the consecutive 2 days, during July 19 to 21, 1949, using 5 individuals each time. Figure 9 shows the results obtained.

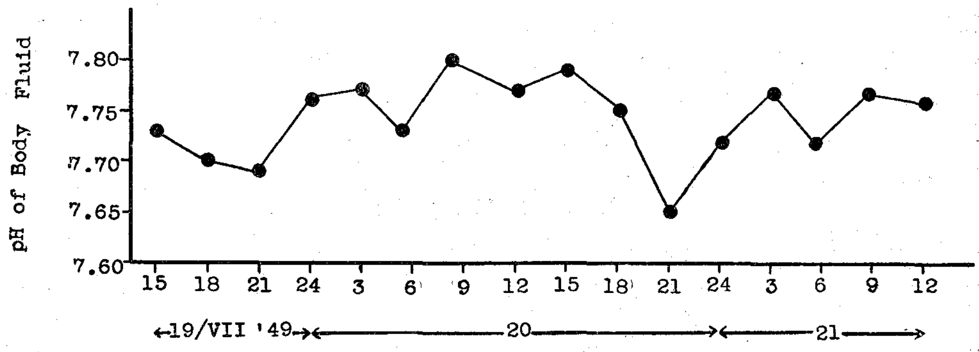

Fig. 9. Daily changes of the hydrogen ion concentrations of body fluids. Mean values of 5 individuals at every time are represented.

It may be concluded from this figure that the body fluid becomes more acidic twice a day, at dawn and at dusk, namely, when the starfishes move most actively. There is a tendency to exhibit higher pH values during daytime than during night, and the reason for this phenomenon is not clear.

V. CONSIDERATIONS

1. The senior author has long been emphasizing that the nature of the rhythmic activity can not be clarified ultimately unless the thorough knowledges about the actions of the environmental factors and the internal physiological conditions of animal body are obtained (MORI, 1947, 1948a, 1948b, 1950). To his opinion, the normal rhythmic activity is maintained under the dual controls, the one by the periodic changes of enviromental factors and the other by the rhythmic changes of internal physiological conditions which have more or less intrinsic, hereditary characters. The rhythm of activity can be modified or put into disorder by disturbing balances of the forces from environments and from internal conditions. The degrees of influences from these two sources on the exhibition of the rhythmic activity are different in different animals. Considerable amounts of works have hitherto been done concerning the effects of the changes of environmental factors. The modes of changes of environmental factors tried by many investigators may be sorted into two main categories, namely, maintaining the factors in constant conditions or changing the cyclic phases of factors (reversing the alternative phases, or lengthening or shortening the periods). By these experiments it has been found that some animals are affected seriously by the changes of environmental factors and

others are scarcely affected having conspicuous tendencies to persist the original rhythmic activities. In this way, it seemed to be the best way to pay efforts in finding the correlations between the external manifestations of rhythmic behaviors and internal physiological rhythms. Nevertheless, we can scarcely find this sort of research at present, and especially there is no conclusive report except with the sea-pen, *Cavernularia obesa*, whose daily rhythmic behavior was forced to modify by altering artificially the internal physiological conditions (MORI, 1945, 1950).

The present report is the work along this line, and at the first step general features of the daily rhythmic activity of the starfish are observed, and at the second step degrees of persistency of the rhythm, in other word, degrees of independency of the rhythm to the environments, are investigated, and at the third step some physiological changes that are considered as having intimate relations with the behaviors are researched.

2. The rhythmic activity of the starfish is principally controlled by the changes of light, as in most other animals studied. The temperatnre and other physico chemical factors of environments seemed to have no important influences on the activity so long as their daily marches concerned. This is also the ordinary circumstances at many animals living in water.

3. The establishment of the intrinsic physiological rhythms is relatively weak, so that the rhythmic behavior is persisted only during 2 or 3 days after the animal had been placed in constant dark conditions.

4. The states of hunger affect considerably the intensities of activity, i.e., they move more and more vigorously as they become hungry. However, it must be noted here that the times of a day when they search for foods are generally restricted to dawn or dusk, so that the rôle of this factor is quite a modifying one.

5. After all, it may be concluded that the crepuscular activity of the starfish is regulated and controlled by the change of light, the liveliness of which is greatly influenced by the states of hunger. Although the physiological conditions show rhythmic alterations with the changes of activity, the establishment of the internal physiological rhythms is rather incomplete, and the animal can only persist its daily rhythmic behavior during 2 or 3 days under constant darkness.

VI. SUMMARY

1. The observations of the daily rhythmic activity of the starfish, *Astropecten polyacanthus*, which is the common inhabitant on the shallow sandy bottom and the physiological analyses of the behavior were performed at the

Seto Marine Biological Laboratory, Wakayama Prefecture, during 1947 to 1949.

2. They showed conspicuous daily rhythmic behavior, moving on the sand most actively at dawn and then less actively at dusk, and generally stayed quietly beneath the sand during midday and midnight.

3. The controlling environmental factor was the light, and other physico-chemical factors seemed to have scarcely been concerned.

4. The food as an environmental factor played the important rôle on the vigorousness of the activity. As more and more they are hungered, so the activity become livelier. But this factor was the modifier against the intensity of rhythmic activity, and not the real controller that alters the phases of rhythm.

5. Eye spot was not concerned with the rhythmic activity.

6. Not only the amputated individual with only one arm, but also the severed single arm, showed tendency to move daily rhythmically. Generally speaking, active movements in these cases were observed at dawn.

7. The amounts of oxygen consumed per unit time showed clear daily periodic changes. Two peaks were observed, the one at dawn and the other at dusk, the former was the greater.

8. The hydrogen ion concentrations of body fluids also revealed the daily periodic changes. Two peaks were observed at dawn and at dusk, the latter was the larger (contrary to the case of the oxygen consumption).

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