ANNAL. BIOL. TIHANY	33	135—142	HUNGARIA 1966

DAILY ACTIVITY RHYTHM OF TWO MEDITERRANEAN LAMELLIBRANCHIA *(PECTEN JACOBAEUS* AND *LITHOPHAGA LITHOPHAGA)* REGULATED BY LIGHT-DARK PERIOD

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Received: December 29th 1965

In the course of research on biological rhythms the greatest attention has been devoted to investigations and hypotheses concerning the regulatory mechanisms of endogenous *circadian* rhythms both in plants and animals (BROWN 1959, BÜNNING 1963, HARKER 1964, SOLLBERGER 1965). Most of the work performed on invertebrates tried to prove that rhythms are less dependent on environmental factors and are regulated by a so-called "clock" mechanism. With the animals placed under "constant conditions", observations have been made on the length of time, that previously observed rhythms have continued, without normal environmental stimulation. In spite of numerous investigations the essence and functioning of the "clock" mechanism is not known in detail, therefore the question, how does the "clock" regulate the rhythm of the living processes hardly arises.

Investigations of the basic mechanisms of the nervous system in animals — on the other hand — have given more and more informations on the problem of its integrative and correlative functions. Correlation takes place, without doubt, first of all between environmental factors and the organism. In most of cases it is obvious, that the daily rhythm helps the organism to adapt to major environmental factors and that this is one of the modes of regulating equilibrium with the environment. Therefore, in spite of the fact that most of the "circadian rhythms" continue under constant conditions, thus suggesting the presence of an "internal clock", in normal life these rhythms run parallel with changes of very effective stimuli from the environment, such as light, t°, humidity and others.

For this very reason, these factors affecting the sense organs must play a continuously important role. This is proven by the fact, that in many cases the rhythms which do not change under constant conditions, will change by altering the rhythm of the controlling factor (light-dark conditions, etc.).

To understand the central regulatory mechanisms responsible for animal behaviour, including rhythmic behaviour, it seems to be more convenient to investigate the mode of action of factors maintaining the rhythm and at the

* The study was conducted at the Stazione Zoologica Napoli, by the scholarship of the Italian Foreign Ministry, within the scope of the cultural agreement between the Hungarian and Italian Governments.

same time directly affecting animal behaviour. In other words, it seems very important to investigate entirely and in detail the mechanisms affecting rhythm including long term environmental factors. Such an investigation could produce data and conclusions which on the one hand lead to an understanding of the nervous control of behaviour, on the other hand to the discovering of the basic mechanism of the "internal clock".

Concerning the papers dealing with the Lamellibranchia, some of them insist on the independence of their rhythms from usual environmental factors (BARNES 1955, RAO 1959) others have proved that they are in close correlation with the changes of the outside world (LOOSANOFF 1958, BROWN 1956, KOSHTOYANTS and SALÁNKI 1958, SALÁNKI 1965). The effects investigated in these experiments were the tide, the pressure, the t°, the O_2-level and the influence of some chemicals. There are a few allusions that illumination has also some effect on the behaviour of mussels (DODGSON 1928, SALÁNKI 1964). Nevertheless, owing to the fact that these animals usually have no eyes, this problem was not investigated in detail, although photosensitive structures are known in some Lamellibranchia species (HECHT 1919, 1920, KENNEDY 1960, 1964).

The aim of the present work was to obtain data on whether illumination and its changes play a role in the regulation of the rhythmic activity of Lamellibranchia, and if they do, is there any daily periodicity in the activity, corresponding to diurnal changes. It was also investigated, whether these factors have a direct role, or are coincident with an independent, persistent, internal daily rhythm.

Investigations were carried out on two different species. The one has well developed mantle eyes *(Pecten Jacobaeus)*, the other has no eyes *(Lithophaga lithophaga)*.

Material and methods

Marine Lamellibranchia, *Pecten Jacobaeus* and *Lithophaga lithophaga* were used. The animals were collected in the Gulf of Naples and kept for several days in tanks supplied with running, aerated water.

During the experiments the rhythmic movements of the shells were recorded. These are corresponding to the rhythmic contractions and relaxations of the closer muscles. For recording the activity, one of the shells was fixed to the bottom while the other was connected by a thread to a lever. The records were made on a kymograph which could run at very slow speeds, thus assuring continuous recording for six days, but at the same time making it possibile to distinguish between separate contractions. During this period the animals were placed in separate vessels with 5—6 litres of see water, without additional aeration. The room temperature was kept constant (22 ± 0.5°C).

The experiments were performed in ordinary daylight. When required, the room was darkened by covering the window with a black blind (which yet did not result in total darkness in the room); at night, light periods were obtined by using a 60 Watt bulb situated at one meter from the animal. The experiments were carried out in May and June 1965.

Results

During the investigations both *Pecten* and *Lithophaga* showed quite regular rhytmic activity. There was a rhythmicity with a frequency 1—5/hour, which continued for several days without any interruption by long adductions of the valves as noted elsewhere (SALÁNKI 1966).

Two types of daily fluctuations occurred in the rhythm:

a) the frequency of the fast contractions and relaxations were different during the night and the day, the difference being about 100%. The higher frequency occurred with *Pecten* in the night while in *Lithophaga* this peak was observed during daytime.

b) The amplitude of the shell movements also showed marked differences, which were manifest for both species in about 30—50% greater amplitude at night compared with day-time records.

This augmentation of the amplitude was caused by the increased opening of the valves, which was about twice as much at night as in the day. At night the valves usually failed to close completely, in contrast to daytime, when maximum adduction could be observed. With *Lithophaga*, at night, regular and more powerful contractions were also observable *(Fig. 1)*.

It must be noted, that the changes in the amplitude of the rhythmic movements strictly correspond with the changes of room-illumination, getting light between 6—7 o' clock in the morning and beginning the nightfall at about 5—6 p. m. (This was a shorter period of daylight, than normal, and was caused by trees overshadowing the window of the laboratory).

Since illumination was the most noticeable diurnal factor to change, this was investigated further. It was planned to examine whether changes in the light-dark period produce a shift or complete change in the rhythm of activity or not. For this purpose, in one case, the daylight period was lengthened by illumination of the animal during the night, in another experiment the dark period was extended to the next day by darkening the room.

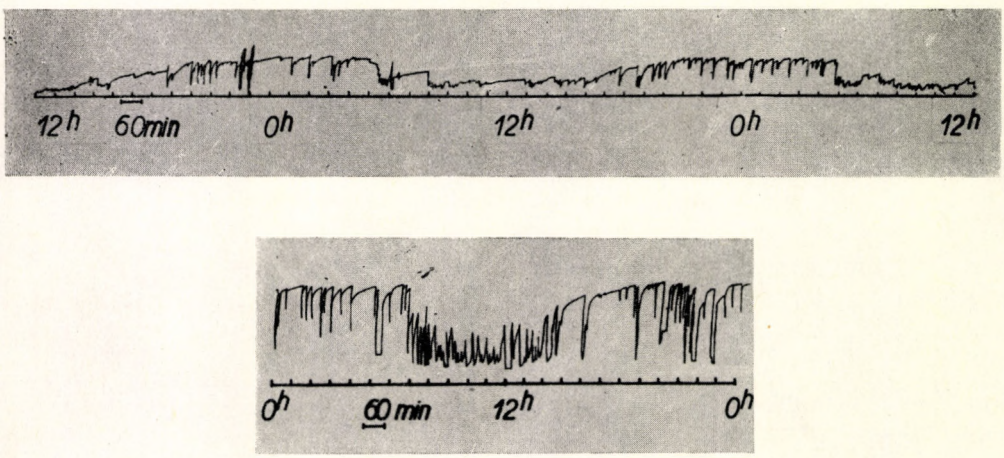

Fig. 1. Diurnal rhythm of activity: *a) Pecten Jacobaeus*; b) *Lithophaga lithophaga*
1. ábra Az aktivitás napi ritmusa

If the dark period continued for 36 hours, in both *Pecten* and *Lithophaga*, the frequency and amplitude of the rhythmic valve-movements assumed nearly completely the night time characteristics *(Fig. 2)*.

T is means that the change to the typical daytime pattern of activity was prevented merely by the lengthening of the dark period.

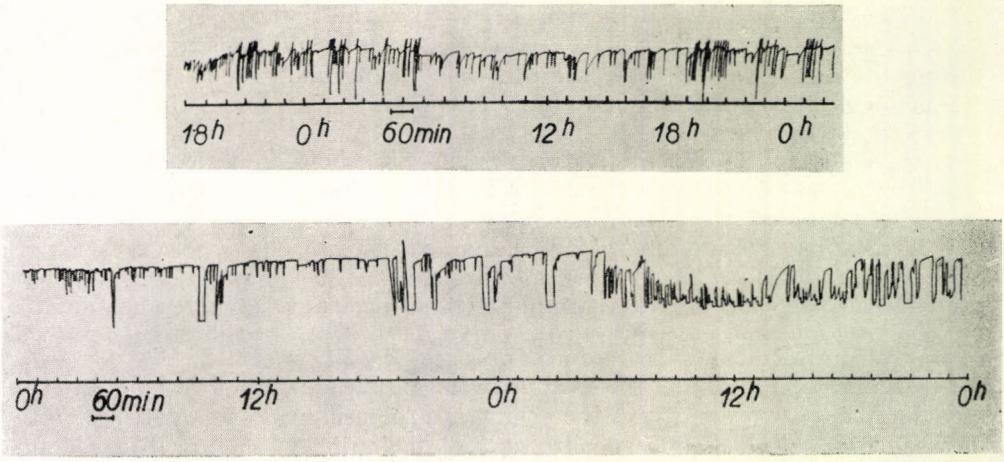

Fig. 2. Effect of 24 hours darkness on the activity of a) *Pecten Jacobaeus*; b) *Lithophaga lithophaga* (on the second day returning to the normal light)
2. ábra 24 órás sötét hatása a napi ritmusra A — *Pecten Jacobaeus* B — *Lithophaga lithophaga* (második nap visszatérés a normál megvilágítási ritmusra)

On the following days, when natural light-dark conditions were allowed to operate, the daily periodicity usually returned. However, in some cases *Pecten* did not return exactly to the pattern shown before the long dark period, but as can be seen in *Fig. 3*, during the subsequent light interval there was only a slight decrease in amplitude, and about a 50% decrease in frequency of the rhythmic contractions.

Fig. 4. shows the effect of a long light period. Continuous light caused in general the lengthening in the period of the partially open position of the valves. A lengthening of the "daily" frequency of the rhythmic contractions was also observed. In the case of *Lithophaga* some decrease in the frequency

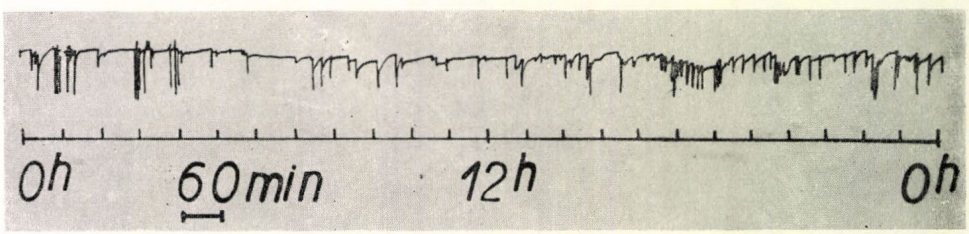

Fig. 3. Rhythmic activity of *Pecten* in normal light after 24 hours continuous dark period
3. ábra Pecten ritmikus aktivitása 24 órás sötét periódus után normál megvilágításban

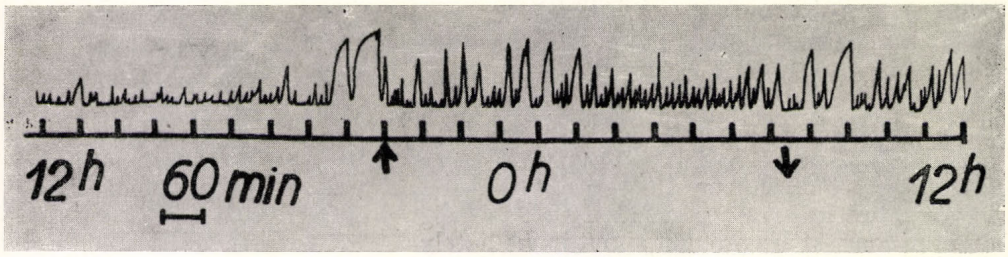

Fig. 4. Effect of light (↑ — ↓) on the activity of *Lithophaga lithophaga*
4. ábra Fény hatása (↑ — ↓) *Lithophaga* aktivitására

of the rhythmicity occurred but it is very different from that which could be seen in darkness. This was probably caused by the inadequate illumination, using bulb-light instead of natural light, being more diffuse and of a broader spectrum.

Discussion

The photosensitivity of Lamellibranchia is a well-known phenomenon (MILNE 1959), though except in the case of *Pecten* it has not been investigated in detail. HARTLINE'S classical work (1938) on the visual reception of *Pecten* gave evidence that two photosensitive layers exist in the eyes, one giving discharges in darkness and the other stimulated by light. There is also good evidence that in *Mya* the syphons respond by retracting when stimulated by light (HECHT 1919, 1920). In the pallial nerve of *Spisula solidissima* also photosensitive nerve elements were found (KENNEDY 1960). It seems probable that the non specialized "general" or "dermal" photosensitivity is widespread among Lamellibranchiates and that photoreception is located in specially pigmented areas of the mantle (KENNEDY 1964).

The general significance of the light-dark periodicity as the signalizing system of the daily rhythm is well established, but there are only a few references to this problem in the case of Lamellibranchia (DODGSON 1928, SALÁNKI 1964).

The present investigation shows that on *Pecten* and on *Lithophaga* the illumination level really play a significant role in the regulation of the rhythmic activity. This rhythm depends directly on the effect of illumination. Both in the case of *Pecten* which has well developed photoreceptors — the eyes — and *Lithophaga*, having no eyes at all, it is probable that long-term photoreception takes place in the receptor structures which, by way of nervous impulses, influence the centres controlling the rhythm. It is noteworthy, that the effect of illumination is the same in both species, pointing to the similar function of the primary photoreceptors in this respect. It may be suggested therefore that the well-developed eyes of *Pecten* might be capable not only of perception of light differences, but also of more complex functions.

The question arises whether the activity of the rhythmic centres is affected by light or by darkness. Maybe both light and dark have their own regula-

tory influence. The results of HARTLINE (1938) and KENNEDY (1960) showing that both light and dark cause changes in the activity of the photoreceptor elements support the latter possibility.

The changes in the daily rhythm of activity caused by lengthening the light or dark periods show the close relationship between illumination and activity, and prove that the activity rhythm depends to a large extent upon daily changes of light and darkness. In the case of the two Lamellibranchia investigated it can be concluded that the regulation of rhythm is external, and the changes are brought about immediately after changing the light conditions. The fact, that in some cases after long dark periods the former periodicity did not return in an analogous form shows that disturbances in the external rhythm can produce a prolonged effect on the regulatory mechanism. It is difficult to decide, whether this effect is due to some alterations of the primary photoreceptor or to that of the nervous control. However, starting from the fact that under normal conditions changes in illumination cause changes both in frequency and in amplitude of the rhythmic contractions, it may be suggested that if only one of these is disturbed, there must be probably a central mechanism involved.

Two questions remain to be answered:

(1) what is the mechanism for the regulation of the rhythm and how can it be altered by changes in illumination;

(2) what is the ecological significance of this reaction? Neither of these questions can be answered at the moment. By investigating the effect of continous light and darkness on the photoreceptive elements and especially on the integrative and coordinative centre of the rhythmic activity i.e. the central nervous system it should be possible to get data concerning the mechanism of this regulation not only in Lamellibranchia but in other light sensitive rhythm too.

Summary

The rhythmic activity of the closer muscles of *Pecten* and *Lithophaga* was recorded and a daily rhythm in the amplitude and frequency of the contractions as well as a difference in the degree of opening of the valves was established. The daily rhythm depends on the illumination changes and can be shifted by lengthening the light or dark periods.

It is concluded that basic functions of the well developed eyes of *Pecten* and the primitive dermal photoreceptors of *Lithophaga* do not differ from each other from the viewpoint of regulation by light-dark periodicity. This means that primitive photoreceptors are also capable of perception of long-term light and dark changes.

It seems probable that the effect of illumination realizes, through the receptors, on the tonus- and rhythm-regulating centre of the nervous system, which latter in the absence of external periodicity do not have a diurnal "clock" mechanism.

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I wish to express my gratitude to Dr. P. DOHRN, Director of the Stazione Zoologica Napoli and his staff for their interest and help, that made possible to carry out these experiments.

REFERENCES

BARNES, G. E. (1955): The behaviour of *Anodonta cygnea L.*, and its neurophysiological basis. — *J. exp. Biol.* **32,** 158–174.

BROWN, F. A. JR., M. F. BENNETT, H. M. WEBB, C. L. RALPH (1956): Persistent daily, monthly, and 27-day cycles of activity in the oyster and quahog. — *J. exp. Zool.* **131,** 235–262.

BROWN, F. A. JR. (1959): The rhythmic nature of animals and plants. — *American Scientist* **47,** 147–168.

BÜNNING, E. (1963): Die physiologische Uhr. — *Springer, Berlin.* 1958.

DODGSON, R. W. (1928): Report on mussel purification. — *Fish. Invest., Ser.* **10,** 1–498.

HARKER, J. E. (1964): The physiology of diurnal rhythms. — *Cambridge* 1964. *University Press* 114. p.

HARTLINE, A. K. (1938): The discharge of impulses in the optic nerve of *Pecten* in response to illumination of the eye. — *J. Cell. Comp. Physiol.* **11,** 465–478.

HECHT, S. (1919): Sensory equilibrium and dark adaptation in *Mya arenaria.* — *J. Gen. Physiol.* **1,** 545–558.

HECHT, S. (1920): The photochemical nature of the photosensory process. — *J. Gen. Physiol.* **2,** 229–246.

KENNEDY, D. (1960): Neural photoreception in a Lamellibranch Mollusc. — *J. Gen. Physiol.* **44,** 277–299.

KENNEDY, D. (1964): The photoreceptor process in lower animals. in: *Photophysiology,* **2,** 79–121. (Ed. A. C. Giese).

KOSHTOYANTS, CH. S., J. SALÁNKI (1958): On the physiological principles underlying the periodical activity of *Anodonta.* — *Acta Biol. Hung.* **8,** 361–366.

LOOSANOFF, V. L. (1958): Some aspects of behaviour of oysters at different temperatures. — *Biol. Bull.* **114,** 57–70.

MILNE, L. J., M. MILNE (1959): Photosensitivity in invertebrates. In: *Handbuch of Physiology.* Sec. 1. *Neurophysiology* **1,** 621–645.

RAO, K. P. (1959): Tidal rhythmicity of rate of water propulsion in *Mytilus,* and its modifiability by transplantation. — *Biol. Bull.* **106,** 353–359.

SALÁNKI, J. (1964): Contributions to the problem of daily rhythm in the activity of the fresh-water mussel *Anodonta cygnea L.* — *Annal. Biol. Tihany* **31,** 109–116.

SALÁNKI, J. (1965): Oxygen level as a specific regulator in the rhythmic activity of fresh-water mussel *(Anodonta cygnea L.).* — *Acta Biol. Hung.* **15,** 3. 299–310.

SALÁNKI, J. (1966): Comparative studies on the regulation of the periodic activity in marine Lamellibranchs. — *Comp. Biochem. Physiol.* **18.** 829–843.

SOLLBERGER, A. (1965): Biological Rhythm Research. — *Elsevier* pp. 461.

KÉT MEDITERRÁN KAGYLÓ *(PECTEN JACOBAEUS* ÉS *LITHOPHAGA LITHOPHAGA)* MEGVILÁGÍTÁSFÜGGŐ NAPI AKTIVITÁSI RITMUSA

Salánki János

Összefoglalás

Szerző vizsgálta két tengeri kagylófaj *(Pecten Jacobaeus* és *Lithophaga lithophaga)* ritimikus aktivitását. A kísérletek során a héjmozgásokat (záróizomműködést) napokon át folyamatosan regisztrálta. Napi ritmust állapított meg a ritmikus aktivitás frekvenciájának és az egyes kontrakciók amplitúdójának változásában. Ugyancsak napi ritmust észlelt a maximális héjnyílás (izomrelaxáció) mértékében is. A ritmus megvilágításfüggő, minthogy a világos vagy sötét periódus megnyújtásával befolyásolható.

A vizsgálatok azt mutatják, hogy a *Pecten* kifejlett szeme és a Lithophaga fényérzékeny köpenyterülete a megvilágításfüggő napi aktivitás szabályozása szempontjából nem különbözik egymástól. Ez azt is jelenti, hogy a primitív fotoreceptor képes tartós megvilágítás ill. sötét érzékelésére.

Valószínű, hogy a megvilágítás a receptorokon keresztül az idegrendszer ritmus- és tónusszabályozó központját befolyásolja, mely utóbbi környezeti periodicitás hiányában nem rendelkezik napi ritmust fenntartó független belső mechanizmussal.

РИТМ СУТОЧНОЙ АКТИВНОСТИ У ДВУХ СРЕДИЗЕМНОМОРСКИХ ДВУСТВОРЧАТЫХ МОЛЛЮСКОВ *(PECTEN JACOBAEUS* И *LITHOPHAGA LITHOPHAGA)* РЕГУЛИРУЕМЫЙ ПЕРИОДИЧНОСТЬЮ СВЕТА И ТЕМНОТЫ

Янош Шаланки

При регистрации ритмической активности запирательных мышц у *Pecten Jacobaeus* и *Lithophaga* была установлена суточная периодичность в амплитуде и частоте сокращений, а также различия в степени раскрывания створок. Суточная периодичность зависит от смены света и темноты и может быть сдвинута продлением периодов света или темноты.

Делается вывод, что основная функция хорошо развитых глаз *Pecten* и примитивных дермальных фоторецепторов *Lithophaga* не различается в отношении регуляции этой периодичности. Это значит, что примитивные фоторецепторы также способны к восприятию длительных изменений света и темноты.

Возможно, что освещение действует, через посредство рецепторов, на центры нервной системы, регулирующие тонус и ритм, которые в отсутствие периодичности во внешней среде не обладают внутренним «часовым» механизмом.