

**CONTRIBUTIONS TO THE PROBLEM OF DAILY RHYTHM
IN THE ACTIVITY OF THE FRESH WATER MUSSEL
ANODONTA CYGNEA L.**

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The daily rhythm in the movements of the living organisms and also in the living processes and activities of plants and animals are widely investigated and in many aspects well known (BÜNNING 1958, WEBB and BROWN 1959, LOBASHOV and SAVVATEYEV 1959). In the majority of the investigated organisms the daily rhythm can be connected — even if not always individually but in general philogenetically — with the daily fluctuations of the physical environment (light, temperature, moisture, etc.) or with the food supply depending on these physical factors. In marine organisms the phenomenon of ebb and tide is acting as important physical but ultimately as biological factor, deviating somewhat from the 24 hours solar periodicity.

It was demonstrated in several organisms — amongst them also in marine Lamellibranchiates — that when the environmental factors are constant, they maintain under laboratory conditions their former life rhythm (RAO 1954).

As consequence of its living conditions the fresh water mussel is only scarcely in connection with the daily fluctuation of the water temperature as well as the O_2 and pH.

So far no data are known whether under natural conditions the *Anodonta cygnea* L. has any daily rhythm or not. Whereas we know that the quick closure and the relatively slow opening of the valves ("rhythmic activity") take place in the animals in the course of the active function. Further the active phase lasting for hours changes with the rest periods characterised by the complete closure of the valves (the "periodic activity") (BARNES 1955).

When investigating the regulation of the periodic activity in mussels — complementing earlier experiments — we wanted to learn more in detail about the correlation between the periodic activity of *Anodonta* and the daily cycle under laboratory conditions. Our present paper summarises the results of these observations.

Methods

Exemplars of *Anodonta cygnea* L. were used kept in aquaria in running fresh water for 1—2 weeks. These conditions remained the same during the observations.

The 10 weeks long continuous registration of the periodic activity was carried out by the method of KOSHTOYANTS and SALÁNKI (1958) with a mussel

actograph (SALÁNKI and BALLA 1964). We took each trace on the actograms as actions of equivalent values.

We registered simultaneously the activity of 10 animals. To check the daily cycle we divided the 24 hours solar cycle into 4 daily periods: from 0—6, 6—12, 12—18 and from 18—24 hours. From the point of view of the rhythmic activity we investigated how many quick contractions took place within a day and how this muscle activity was distributed in the chosen periods. According to each daily period the activity of 10 animals was calculated and given in mean values in the figures.

On the other hand we considered the complete closure of the valves, resp., their opening after a lasting rest period as periodic activity. We investigated in the 10 animals the distribution of the numbers of transitions from activity to rest and from rest to activity. For 1 animal the results were for the four daily periods similarly evaluated as above.

The investigations were carried out from 22 October till 31 December, during 10 weeks. In this period the temperature of the running water decreased from 12 °C to 6 °C, but its daily fluctuation never surpassed 1 °C. This fluctuation followed the room temperature showing a maximum once a day, before noon.

The experiments took place in laboratory illumination approximatively corresponding to the natural conditions. The daybreak fell on the 2nd, the darkening on the 3rd period.

The animals were not isolated from the "daily noises" of the working place and as the actographs were not placed vibration-free — even if the sense organs of the mussels are incomplete — these "noises" could influence to a certain degree the animals. To learn about the possible effect of the "laboratory noises" we evaluated the results of the rest days, when the laboratory used to be completely quiet.

Results

a) Daily fluctuation of the rhythmic activity.

We found that rhythmic activity showed significant deviations not only in the different animals but also in the same individuum at identical periods of the day. The number of the adductor activity in six hours (a quarter of a day) can result in highly different values from 0 — to 23—30.

The highest rates of activity values for 6 hours were: from 0 — 6 hours 25, from 6—12 hours 31, from 12—18 hours 22 and from 18—24 hours 23. Among these numbers 31 is outstanding as it surpasses with 35% the mean values of the other three. *Fig. 1/a* demonstrates the mean values for 1 animal, calculated from the results of the 10 weeks long examinations of the 4 daily periods.

It can be seen that during the different periods the rhythmic activity does not show excessive differences but an increase of some degree can be observed in the second quarter of the day. This phenomenon can be also stated when the rhythmic activity was calculated for shorter (monthly) periods. This is demonstrated in *Fig. 1/b.*, where the data of October—November—December are separately given.

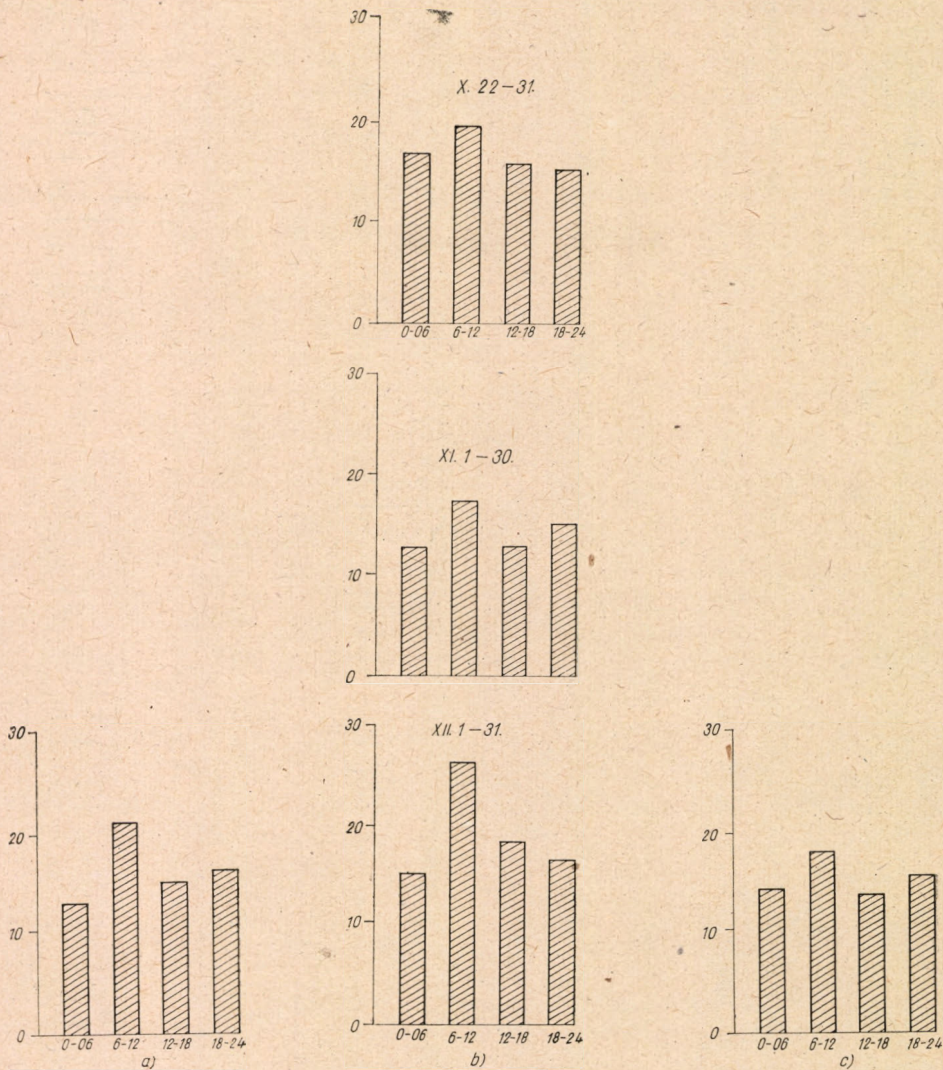


Fig. 1. Daily fluctuation of the rhythmic activity

a — mean values of the activity of 10 animals in 10 weeks; *b* — mean values of the activity of 10 animals divided for the periods from 22—31 October, from 1—30 November and from 1—31 December; *c* — mean values of the activity of 10 animals on rest days from 22 October till 31 December

Abscisse: periods of the day; ordinate: number of contractions of 1 animal for the given period of the day

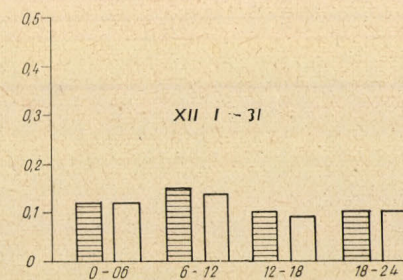
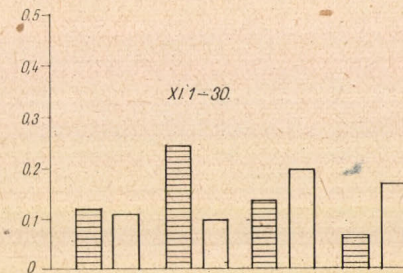
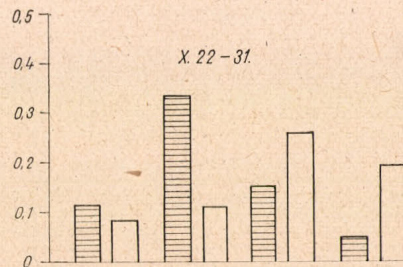
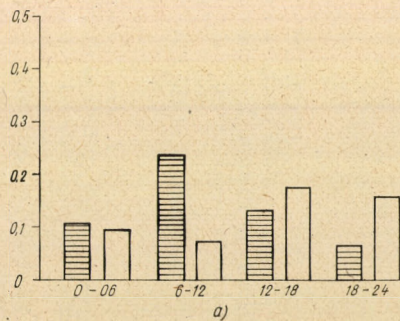
1. ábra. A ritmikus aktivitás napszakos ingadozása

a — 10 állat 10 heti aktivitásának átlaga; *b* — 10 állat aktivitásának átlaga október 22—31-ig, november 1—30-ig és december 1—31-ig tartó időszakra felbontva; *c* — 10 állat aktivitásának átlaga munkaszüneti napokon, október 22—december 31-ig

Abszcissza — napszakok; ordináta — a napszakra eső, egy állatra vonatkoztatott kontrakciós szám

Fig. 2. The transitions rest-activity and activity-rest according to the daily periods (mean values of 10 animals) \equiv = closure; \square = opening

a — mean values of observations in 10 weeks; *b* — in periods from 22–31 October, 1–30 November and 1–31 December; *c* — in rest days from 22 October till 31 December. Ordinate: number of closures and openings related to 1 animal



2. ábra. A nyugalmi, valamint az aktív állapotba való átmenet alakulása napszakonként (10 állat adatainak átlaga). \equiv — zárás; \square — nyitás

a — 10 heti vizsgálat átlaga; *b* — október 22–31, november 1–30 és december 1–31-ig tartó időkből; *c* — munkaszüneti napokon október 22–december 31-ig. Ordínáta: 1 állatra vonatkoztatott zárás-, ill. nyitásszám



The mean values of the rest days we summarised in *Fig. 1/c*. It can be observed that a certain daily fluctuation of the rhythmic activity took place even on rest days. The number of the contractions is in this case also the highest in the period from 6—12 hours, surpassing with about 20% the mean values of the other three periods' rhythmic activity, differing only slightly from each-other.

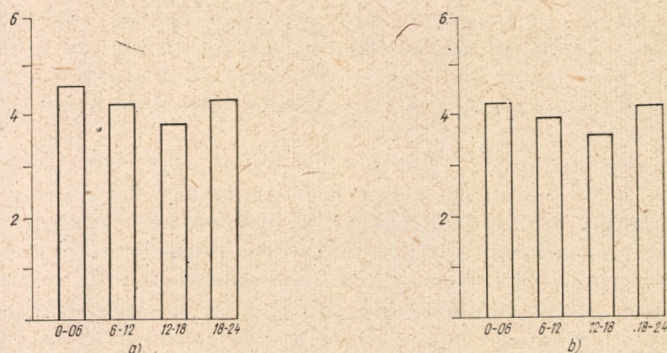


Fig. 3. Daily distribution of the duration of activity (mean values of 10 animals)
a — mean values of observations in 10 weeks; *b* — on rest days
 Ordinate: duration of activity (hours) related to 1 animal

3. ábra. Az aktivitási állapot időtartamának napszakos eloszlása (10 állat adatainak átlaga)
a — 10 heti vizsgálat átlaga; *b* — munkaszüneti napokon október 22—december 31-ig.
 Ordinátá: 1 állatra vonatkoztatott aktivitási idő (óra)

b) Daily fluctuation of the periodic activity

We separately evaluated the results of the transitions from the open state into the closed-one and from rest into activity. The summarised data (and also grouped according to months) are given in *Figs 2/a* and *2/b*. They demonstrate that the mean value of the numbers of closures referred to 1 animal reaches a peak from 6—12 hours and that the mean value of the numbers of openings is from 12—18 hours higher than in the other three periods.

What to the mean values obtained on rest days (*Fig. 2/c*) they lead to similar conclusions as those of the summarised data, that is to say the number of the closures shows for the 2nd period of the day an increase of about 100% compared to the other periods and also the number of the openings is the highest between 12—18 hours.

The distribution of the duration of the active state is rather homogeneous between the different periods, it seemed only to be smaller with some degrees between 12—18 hours (*Fig. 3/a*). The same was found in the rest days.

Discussion

On the hand of our investigations it can be stated that as in the rhythmic activity also in the periodic activity a daily fluctuation of a certain degree takes place. Between 6—12 hours the rhythmic movement of the adductor muscles clearly surpasses the results of the other three daily periods. This

phenomenon is in close contact with the fact that the highest transitory values from activity to rest also appear in the mentioned period, as in general the highest frequency of the rhythmic activity takes place immediately before the closure of the valves too.

As the increase of the number of the rhythmic contractions of the adductor muscles and also the transition of the valves into the closed state took place in the rest days between 6—12 hours too, it may be suggested that this phenomenon is not connected with the "daily noises" of the laboratory. The same is true for the increase of the beginning of the new active phase in the 3rd period. This latter is of course closely related to the increased closure observed in the former period, as the lasting rest period did not use to be longer than 3—6 hours.

In the other periods of the day we found approximatively similar or — expressed for the months — not homogeneously distributed values not only in the rhythmic activity but also in the closures and openings. We suppose that the daily fluctuation in the function of the mussels in *Anodonta* observed during our experimental conditions can be connected with 2 facts: with the daily fluctuation of the illumination and that of the temperature. The daily fluctuation of illumination might play a role because in the investigated period (22 October till 31 December) the daybreak begun just in the second period and because the mantel around the siphos is richly pigmented, therefore its high sensitivity towards light must be taken in consideration. It could happen that the increasing light causes through these receptors or through some other indirect ways an increasing effect of activity, resp., it provokes the tonic contraction of the adductors manifested finally in the rest period. The rise of the temperature can be taken also as a factor increasing the activity. It is known that the oxygen consumption of the tissues in mussels increases with the rising temperature (LUKACSOVICS and SALÁNKI 1964) and this can be registered in the increase of the activity even at temperatures rising with some tenth °C. Further investigations taking place under constant illumination and temperature may clear this problem.

The data showing noticeable differences between the daily periods should be estimated in any case as a sign of daily rhythm, as the results contain the mean values of a rather large material (10 animals per 70 days). At the same time it must be mentioned that even for 1 animal and for the same period extremely large deviations were observed. This is valid not only for the rhythmic activity but also even more for the periodic activity. In single cases we observed a long lasting activity of 2—3 days, but the periods of activity lasted in the very same animal sometimes only for 2—3 hours. This assures that the pattern of the activity, what to its origin, is not essentially depending on the daily periodicity. The basic element in the regulation of the periodic activity — in our interpretation — must be hidden in the factors determining the aerob metabolism of the animal, influenced only to some degree by the daily (solar) periodicity. This hypothesis is well supported by former investigations on the regulatory role of metabolism (KOSHTOYANTS and SALÁNKI 1958, SALÁNKI 1960, 1964), showing that by adding KCl, SH-inhibitors and also in the case of oxygen insufficiency — instead of the daily 1 closure and 1 opening even 4—5 closures and openings in 24 hours were counted. This rate is the manifold of the fluctuation observed even in the extreme cases of the daily deviations in periodicity.

For this reason we suppose that the daily cycle does not influence basically the total of the activity, it is only in connection with the distribution of the activity determined by other factors within 24 hours.

Summary

We investigated in *Anodonta cygnea* L. the daily fluctuations of the rhythmic activity (quick contractions of the adductor muscle) and of the periodic activity (transition to rest from activity and reversely) under laboratory conditions.

We found the rhythmic activity compared to the other three periods to increase in the 2nd period of the day (from 6—12 hours). Simultaneously the values of the transitions from activity to rest are also the highest between 6—12 hours.

The beginning of the new active period is the most frequent in the third period of the day. The results were the same on rest days, when the laboratory was practically "noiseless". The daily fluctuations are supposed to origin in the fluctuations of the illuminations and in the water temperature. The observed daily rhythm does not play determining role in the rhythmic and periodic activity of the fresh water mussel, but influences to a certain degree the daily distribution of them.

REFERENCES

- BARNES, G. E. (1955): The behaviour of *Anodonta cygnea* L., and its neurophysiological basis. — *J. exp. biol.*, **32**, 158—174.
- BÜNNING, E. (1958): Die physiologische Uhr. — Springer Verlag, Berlin-Göttingen—Heidelberg.
- KOSHTOYANTS, CH. S., J. SALÁNKI (1958): On the physiological principles underlying the periodical activity of *Anodonta*. — *Acta Biologica* **8**, 361—366.
- LOBASHOV, M. E., V. B. SAVVATEYEV (1959): Лобашев, М. Е., Савватеев, В. Б.: Физиология суточного ритма животных. Изд. Акад. Наук СССР, Москва.
- LUKASOVICS F., SALÁNKI J. (1964): Effect of substances influencing tissue respiration and of the temperature on the O₂ consumption of the gill tissue in *Unio tumidus*. — *Annal. Biol. Tihany* **31**, 55—63.
- 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. (1960): Шалянка, Я.: О зависимости медленного ритма периодической активности беззубок (*Anodonta cygnea*) от состояния сульфгидрильных групп белковых тел. Ж. общ. биол., **21**, 229—232.
- SALÁNKI J. (1964): Oxygen level as a specific regulator in the rhythmic activity of fresh-water mussel (*Anodonta cygnea* L.). *Acta Biol. Hung.*, in press.
- SALÁNKI J., BALLA L. (1964): Ink-lever equipment for continuous recording of activity in mussels. (mussel-actograph) — *Annal. Biol. Tihany* **31**, 117—121
- WEBB, H. M., F. A. BROWN (1959): Timing long-cycle physiological rhythms. — *Physiological Reviews* **39**, 1, 127—161.

VAN-E NAPSZAKOS INGADOZÁS A TAVI KAGYLÓ (*ANODONTA CYGNEA*)
RITMIKUS ÉS PERIODIKUS AKTIVITÁSÁBAN LABORATÓRIUMI
KÖRÜLMÉNYEK KÖZÖTT?

Salánki János

Összefoglalás

Vizgáltuk tavi kagyló (*Anodonta cygnea* L.) ritmikusz héjmozgásának (záróizomkontrakcióinak) és periodikus aktivitásának (tartós nyugalomba, valamint aktív állapotba való átmenet) napszakos ingadozását, laboratóriumi viszonyok között.

Megállapítást nyert, hogy a ritmikusz héjmozgás a második napszakban (6–12 óra) a másik három napszakhoz képest fokozott.

Ugyancsak 6–12 órák között leggyakoribb a tartós aktivitásból nyugalomba való átmenet is, míg az új aktív állapot kezdete a harmadik napszakban mutat legnagyobb gyakoriságot.

Ezek az adatok munkaszüneti napokon is hasonlóan alakultak, amikor pedig a laboratóriumokban teljes csend uralkodott. A napszakos ritmus feltehetően a fényviszonyok, valamint a víz kistokú hőmérsékletingadozásával van okozati összefüggésben.

A leírt napszakos ritmus a tavi kagyló periodikus aktivitásának meghatározásában vezetőszerepet nem játszik, annak napszakos eloszlására azonban befolyást gyakorol.

СУТОЧНЫЕ КОЛЕБАНИЯ РИТМИЧЕСКОЙ И ПЕРИОДИЧЕСКОЙ АКТИВНОСТИ
БЕЗЗУБОК В ЛАБОРАТОРНЫХ УСЛОВИЯХ

Я. Шаланки

Было установлено, что ритмическая активность створок (т. е. быстрые сокращения запирающих мышц) увеличена во второй четверти дня (с 6 до 12 часов) по сравнению с остальными 6-часовыми периодами дня.

Переход с длительной активности в покойное состояние дает самую большую частоту также с 6 до 12 часов; а начало нового активного состояния можно наблюдать чаще всего в третьей четверти дня (от 12 до 18 часов).

Совершенно совпадающие результаты были получены в свободные дни, когда в лабораториях была полная тишина. Суточные колебания активности находятся в причинной взаимосвязи с небольшими колебаниями температуры воды и также освещенности.

Описанный здесь суточный ритм по мнению автора не играет решающую роль в определении периодической активности беззубок, влияет лишь на ее суточное распределение.