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BEHAVIOURAL STUDIES ON MUSSELS UNDER CHANGING ENVIRONMENTAL CONDITIONS

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Abstract

The pumping behaviour of the fresh water mussel (*Anodonta cygnea* L.) was investigated under laboratory conditions with reference to artificial environmental factors, namely the presence of various pollutants.

The continuous recording of shell activity and evaluation of changes in activity proved to be good indicators for testing the effect of chemicals.

Mussels are rather sensitive to sulfhydryl blocking agents (Hg, Cd), which cause a marked shortening in their active periods. Some insecticides and herbicides shortening the mussels' activity also result in the reduction of water cleaning performed by mussels.

The visible consequences of using hazardous agents in the environment are in some cases most dramatic, like massive fishkills in rivers and lakes, severe disease or death of birds and other animals in the mainland. In water ecosystems, as a rule, massive chemical pollution originating from industry or agriculture proved to be the cause of such events. Coincidence of a number of unfavourable conditions in the environment and within the animal, e.g. low O_2 pressure, accumulation of toxic agents, sudden change of temperature, etc. may cause similarly grave damage to water life.

Although mortality very clearly shows the toxicity of various substances, in such cases only the irreversible result of environmental deterioration and the level of toxicity of various factors for a given species can be registered. However, environmental pollutants do not only cause acute mortality, but may also produce chronic diseases, influencing the physiological processes and survival of animals, and can thus result in a reduction in the number of population. For this reason, besides determining the toxic level of recently used chemicals under various environmental conditions, also investigation of the effect of sublethal doses is extremely important. This must be especially emphasized in water ecosystems because animals living there are permanently exposed to substances dissolved in the water, and also due to the possible accumulation of these chemicals in the organisms both directly from the water and via the food chain. The development of appropriate methods for demonstrating chronic, sublethal effects of hazardous agents in different species seems to be a very important task also in the biological monitoring of the occurrence of dangerous substances and situations in water ecosystems.

Among the animals inhabiting both marine and fresh waters, mussels living on the shore or at the bottom are most widespread. Mussels do not only feed at their location, but when obtaining food and oxygen they also filter the water thus taking part in the cleaning of water. The filter feeding behaviour results in the accumulation in their body of various substances present at low concentrations in the water.

Investigations on the fresh water mussel have been conducted primarily not for determining the lethal level of various agents coming from the environment, but for elucidating their influence on the behaviour of these animals.

MATERIAL AND METHODS

Experiments were made all the year round on *Anodonta cygnea* L. collected from fish-ponds and kept for several months in Lake Balaton. The wet weight of animals without shells varied between 100–120 g. The animals under investigation were kept in tanks containing water from Lake Balaton, which was exchanged regularly. Depending on the season, the temperature varied between 15 °C and 25 °C. It was not cooler in winter either, due to the fact that water was not pumped directly from the lake into the tanks, but through a water reservoir situated on top of the Institute's building.

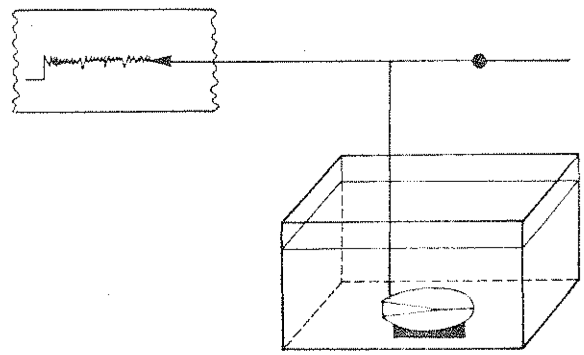

Fig. 1. Scheme of recording the activity of mussels

For monitoring the behaviour of mussels the motor activity of adductor muscles and, consequently, the pumping activity were recorded by using mussel actograph (Salánki and Balla 1964). According to this method, one of the shells is fixed, while the other is connected to a lever. The closed or open position of the shells and the contraction or relaxation of adductors can thus be recorded (Fig. 1) on a slowly rotating paper. Another method used for recording shell movements does not require the fixation of animals (Véró and Salánki 1969). This system includes electronic devices fitted to the shells and movements of the latter cause current changes fed into a recording apparatus. By using this method, the pumping activity of freely moving animals can be recorded under natural conditions. The records obtained from mussels kept under natural and under laboratory conditions were very similar, indicating that fixation of animals on one of their sides does not influence their pumping activity. Recording of activity could be continued with both methods for several weeks or months without interruption. Evaluation of data can follow afterwards.

All the experiments in which the effect of different chemicals was tested, were conducted on fixed animals under laboratory conditions. Mussels were kept separately in vessels containing 3 l water, and control activity was recorded for several days. Water was changed once daily, at a given hour. Substances previously dissolved were directly added to the vessel. After a testing period of more than 24 hours, also the test solution was changed daily.

The effect of various types of substances was investigated. Among them, heavy metals and plant protecting chemicals deserve special attention.

RESULTS

By recording the activity of *Anodonta* either in natural environment or under laboratory conditions, characteristic patterns can be obtained. In accordance with the finding of Marceau (1909) and Barnes (1955), the rhythmic pumping movement of the shells occurs as a result of fast contractions and relaxations of the adductors during the period when shells are generally in an open state. This is the active filtering period of the animal when uptake of food and oxygen occurs. From time to time this active state is interrupted by rest periods when, as a result of tonic contraction of the adductors, the shells are tightly closed for hours, communication of the animal with the surrounding stops, and food and oxygen uptake are blocked. This pattern of the filtering behaviour is called periodic activity. The duration of both the active and rest periods varies widely. It can be different even in animals living under the same conditions. Nevertheless, activity usually lasts from 10 to 20 hours, but sometimes it can be several days long,

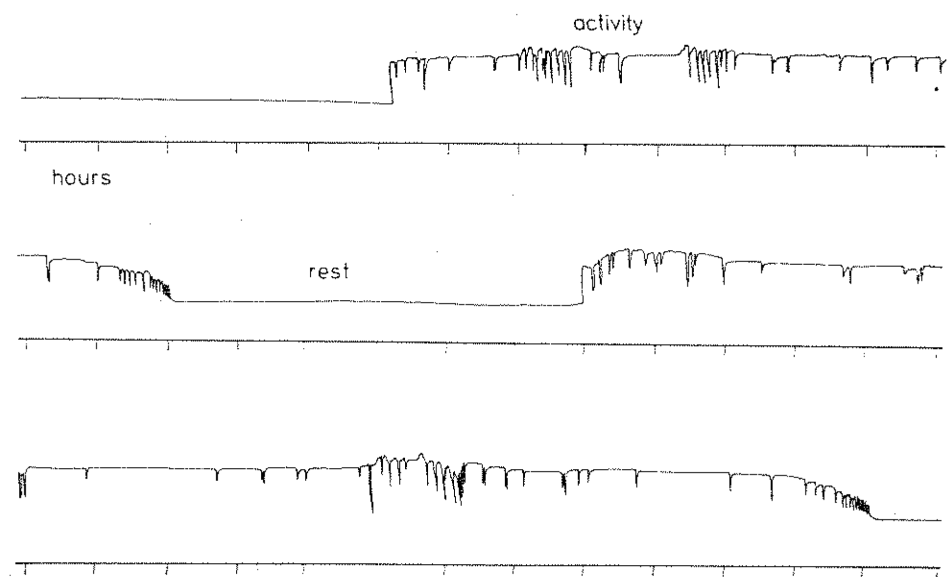

Fig. 2. Pattern of the filtering behaviour of *Anodonta*. Alternation of activity and rest is called periodic activity, while during the active period a rhythmic activity is observable

while duration of rest is normally 5–10 hours. This, however, can also be variable (Salánki et al. 1970). The frequency of rhythmic movements during the active period is variable, too. The range usually is 5–20 contractions per hour (Fig. 2).

Owing to the great variability of time and frequency values, experiments were carried out with self-control. Drugs were applied after a certain time of recording the control activity, and the effect of substances was estimated as a deviation from the pattern observed in the control period. As an indicator of the change of filtering behaviour, the mean duration of the active periods was considered.

Effect of Heavy Metals on Filtering Behaviour

Figure 3 shows a typical example of the effect of para-chloromercuribenzoate on periodic activity. With the substance added to the water, there was an obvious change in the absolute and relative length of active and rest periods. Particularly the duration of the activity shortened, and so rest periods occurred more frequently. The proportion of activity within a given time decreased to less than 50 per cent as compared to the control.

Fig. 3. Effect of *p*-chloromercuribenzoate on the filtering activity of *Anodonta*. Arrow indicates adding of pCMB (10^{-8} g per l). Time: hours

The effect produced by salts of heavy metals depended considerably on concentration, and elimination by washing out usually required several days. If concentration was too high (over 10^{-6} g per l), the animals did not survive but died as a result of intoxication.

Testing the effect of $CdCl_2$, $CuSO_4$ and $PbNO_3$, it was found that both Cd and Cu produce similar effect as Hg, but Pb proved to be ineffective on filtering behaviour. The effect caused by $CuSO_4$ — shortening of the mean duration of active periods — plotted against the concentration is

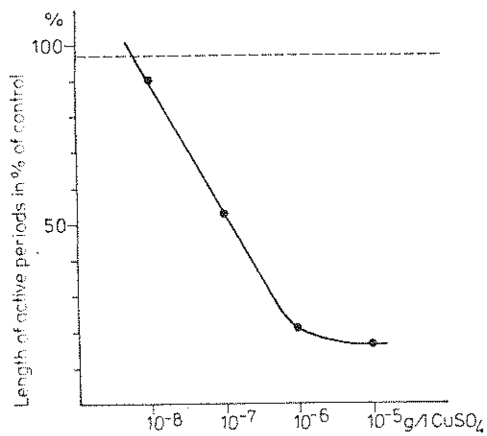

Fig. 4. Effect of $CuSO_4$ on the mean duration of active periods of *Anodonta*. Both control and treatment periods lasted for one week

presented in Fig. 4. It is remarkable that the range of $CuSO_4$ concentrations causing at least 20 per cent decrease in activity and leading to the death of animals was rather narrow, between 10^{-8} and 10^{-6} g per l. In case of Hg and Cd, this range was about the same, varying between 10^{-9} and 10^{-7} g per l. Nevertheless, these concentrations can be considered high even as compared with the levels found in polluted waters, and mussels seem to be rather insensitive to these substances in acute experiments (Salánki and Varanka 1976).

Effects of Chemicals Used in Plant Protection

Mercury and copper are not only industrial waste materials, but are also used in agriculture against fungi, being components of various inorganic and organic compounds. Besides metal-containing substances, also other pesticides and herbicides are washed into rivers and lakes greatly influencing the life of mussels. The effect of some insecticides, containing lindane, phosphamidon and phorate, has been described in another paper (Salánki and Varanka 1978). Recently, the effect of a herbicide called Gramoxon has been demonstrated. Its active compound is paraquat dichloride. Mussels did not show high sensitivity to Gramoxon, however, the range between the threshold and lethal concentrations was very narrow. Although 10^{-3} ml per l did not noticeably cut the duration of the active periods, 10^{-2} ml per l resulted already in an 85 per cent shortening of active periods (Fig. 5). Increasing the concentration up to 10^{-1} ml per l, the mussels died within 72 hours.

It is interesting to note that the death of the animals did not usually occur during the rest period but at the end of a comparatively long activity. The mussel can be considered to be dead when its adductors are totally

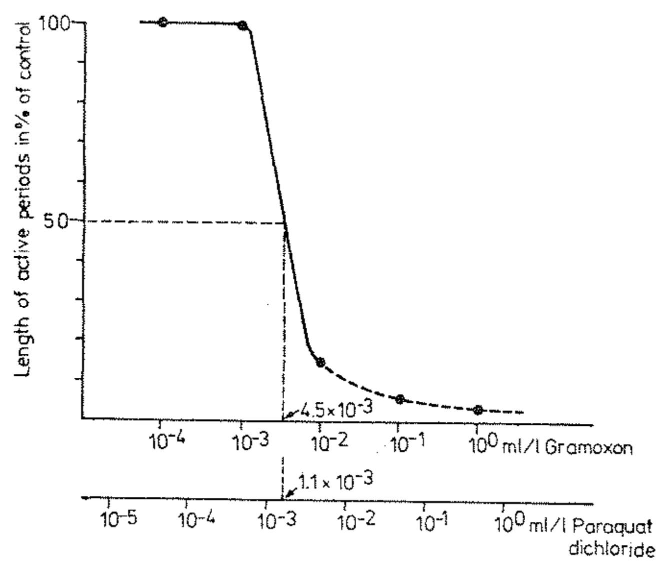

Fig. 5. Effect of Gramoxon and its active ingredient on the duration of active periods of *Anodonta*

relaxed without any rhythmic movements. Prior to this stage, as a sign of deterioration, the adductors become incapable of tonic contractions (to keep "catch"). However, for some time — sometimes even for 10 or 20 hours — they show rhythmic activity of a lower amplitude.

DISCUSSION

The functioning of adductors is necessary for all the vital processes of bivalvian molluscs. This can serve as a basis for investigating rhythmic and periodic activity being also indicative of the behaviour of the animal. Although filtration and uptake of food are mainly connected with the ciliary activity being a continuous process on the gill surface and at other parts of the mantle cavity, pumping of water with the aid of shell movements is a prerequisite for the effective work of the cilia. In previous investigations it was found that both water cleaning and oxygen uptake are concurrent with the activity of mussels (Salánki and Lukacsovics, 1967), and both of these physiological processes are depressed during the rest period. It was also shown that heart rate is lower during rest than during activity (Pécsi and Salánki 1964, Taylor 1976), and also digestion shows the same periodicity as activity and rest (Morton 1969). There is also evidence suggesting that ciliary activity itself has a different rate depending on the animal's behaviour. Of course, it remains to be seen whether pumping as a functional process influences the heart rate, digestion and ciliary activity, or whether there is a central regulation of these processes including the rhythmic and periodic activity of the adductors. As to the heart rate, it seems that the same neural mechanism which regulates the movement of the adductors is simultaneously involved in the increase or decrease of heartbeats (Pécsi and Salánki 1964). In securing the periodicity of digestion, food uptake seems to have a regulatory role (Morton 1969) and, according to Taylor (1976), heart rate is also influenced by the O_2 pressure in the mantle cavity. Food and oxygen uptake as well as excretion depend on the flow of water through the animal, which, in turn, is regulated by the pumping activity.

The substances used in our experiments influenced the pumping behaviour of *Anodonta* significantly in most of the cases. The considerable shortening of the active periods resulted in a decrease in the amount of water filtered by the animal, and consequently, oxygen and food uptake came to be depressed. Thus the rate of metabolism in the animal was probably lowered, and growth must also have been retarded. Decrease in the rate of growth (Butler et al. 1960, Frazier 1976) and also the death of various bivalves kept in waters of increased salinity or containing pollutants have been reported (Imlay 1973, Okazaki 1976).

Heavy metals are known to block the SH groups of various enzymes. In our case it is supposed that respiratory enzymes should in the first place be considered in this respect (Salánki 1960). Our earlier experiments had revealed that oxygen deficiency may cause a heavy depression in the activity of mussels (Salánki 1965), and heavy metals, especially Hg and Cd, can exert their effect via the respiratory system as well. The effect of Cu should probably be interpreted differently, and also that of plant protecting chemicals might be connected with intracellular metabolic processes.

Nevertheless, the fact that different substances, e.g. the herbicide paraquat and also insecticides like lindane, phosphamidon, phorate (Salánki and Varanka 1978) decrease the activity of mussels, shows that this effect can be regarded as a generalized reaction to the change of the chemism of the water.

In our short-term experiments, mussels proved to be rather insensitive to low concentrations of the substances used. Nevertheless, it can still be supposed that during a longer period, when accumulation of the drugs occurs, mussels would be the most suitable organisms for the biological monitoring of the quality of rivers and lakes.

Considering the various ways in which the substances used may act, probably none of them influences the physiological properties and functioning of the adductors directly. This means that all the described effects were mediated by the nervous system which can be influenced directly, but the activity and regulatory function of the ganglia can be modified also by afferent pathways arriving from peripheral and visceral receptors or by metabolites which are produced in the different organs. In the central regulation of the periodic activity of mussels and also at neuromuscular level, serotoninergic and catecholaminergic mechanisms play the key role (Hiripi and Salánki 1973). It is highly probable that all the factors changing the behaviour of *Anodonta* act through metabolic pathways or by releasing monoamines in the ganglia and in the adductors at nerve terminals. Both catecholamines and serotonin strongly influence the duration of active periods (Salánki et al. 1974), and also drugs influencing the synthesis and breakdown of monoamines change the periodicity of activity and rest effectively (Hiripi 1973).

Further investigations are required to explain both the mode of action of various substances and also the way they modify the central neural mechanism. Additional research is also needed to clarify the effect of the various types of chemicals occurring in lakes and rivers as a result of environmental pollution on the behaviour of mussels. Thereby valuable information may be obtained as to the degree to which different substances are dangerous for mussels, a species playing an important role in water purification.

REFERENCES

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

BUTLER, P. A., WILSON, A. J., JR. and RICK, A. J. (1960): Effect of pesticides on oysters. *Proc. Nat. Shellfish Ass.* **51**, 23–32.

FRAZIER, J. M. (1976): The dynamics of metals in the American Oyster, *Crassostrea virginica*. II. Environmental effects. *Chesapeake Science* **17**, 188–197.

HIRIPI, L. (1973): Pharmacological investigations on the regulation mechanisms of the periodic activity of the fresh water mussel (*Anodonta cygnea* L.). *Annal. Biol. Tihany* **40**, 27–53.

HIRIPI, L. and SALÁNKI, J. (1973): Role of monoamines in the central regulation of periodic activity in *Anodonta cygnea* L. (Pelecypoda). In *Neurobiology of Invertebrates*. Ed. by SALÁNKI, J. Akadémiai Kiadó, Budapest, pp. 391–401.

IMLAY, M. J. (1973): Effects of potassium on survival and distribution of freshwater mussels. *Malacologia* **12**, 97–113.

MARCEAU, F. (1909): Recherches sur la morphologie, l'histologie et la physiologie comparées des muscles adductors des Mollusques acéphales. *Arch. Zool. Exp. Gen.* **2**, 295–469.

Morton, B. (1969): Studies on the biology of *Dreissena polymorpha*. II. Correlation of the rhythms of adductor activity, feeding, digestion and excretion. *Proc. malac. Soc. London* **38**, 401–414.
Okazaki, R. K. (1976): Copper toxicity in the Pacific Oyster *Crassostrea gigas*. *Bull. Environm. Contam. Toxicol.* **16**, 658–664.
Pécsi, T. and Salánki, J. (1964): The role of pressure in the periodical changes of cardiac action in the fresh water mussel (*Anodonta cygnea* L.). *Annal. Biol. Tihany* **31**, 65–76.
(Salánki, J.) Шаланки, Я. (1960): О зависимости медленного ритма периодической активности беззубок (*Anodonta cygnea*) от состояния сульфгидридальных групп белковых тел (On the dependence of the slow rhythm of periodical activity of *Anodonta cygnea* on the condition of sulfhydryl groups in protein bodies). *Ж. общ. биол.* **21**, 229–232.
Salánki, J. (1965): Oxygen level as a specific regulator in the rhythmic activity of fresh-water mussel (*Anodonta cygnea* L.). *Acta biol. Acad. Sci. hung.* **15**, 299–310.
Salánki, J. and Balla, L. (1964): Ink-lever equipment for continuous recording of activity in mussels (mussel-actograph). *Annal. Biol. Tihany* **31**, 117–121.
Salánki, J., Glaizner, B. and Lábos, E. (1970): On the temporal organization of periodic and rhythmic activity in fresh-water mussel (*Anodonta cygnea* L.). *J. interdiscipl. Cycle Res.* **1**, 123–134.
Salánki, J., Hiripi, L. and Nemcsók, J. (1974): Regulation of periodicity by monoamines in the mussel *Anodonta cygnea* L. *J. interdiscipl. Cycle Res.* **5**, 277–285.
Salánki, J. and Lukacsovics, F. (1967): Filtration and O_2 consumption related to the periodic activity of freshwater mussel (*Anodonta cygnea* L.). *Annal. Biol. Tihany* **34**, 85–98.
Salánki, J. and Varanka, I. (1976): Effect of copper and lead compounds on the activity of the fresh-water mussel. *Annal. Biol. Tihany* **43**, 21–27.
Salánki, J. and Varanka, I. (1978): Effect of some insecticides on the periodic activity of the fresh water mussel (*Anodonta cygnea* L.). *Acta biol. Acad. Sci hung.* **29**, 173–180.
Taylor, A. C. (1976): The cardiac responses to shell opening and closure in the bivalve *Arctica islandica* (L.). *J. exp. Biol.* **64**, 751–759.
Véró, M. and Salánki, J. (1969): Inductive attenuator for continuous registration of rhythmic and periodic activity of mussels in their natural environment. *Med. biol. Engng.* **7**, 235–237.