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CHANGES IN ACTIVITY IN A FRESHWATER CLAM IN RESPONSE TO OXYGEN CONCENTRATION

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Abstract—1. Shell movements of *Pleurobema coccineum* were recorded in the presence and absence of oxygen.

2. A constant, continuous level of activity was present when aerated.

3. In the absence of oxygen, a striking activity shift occurred, consisting of prolonged shell closure punctuated by periods of intense valve activity.

4. The critical level of oxygen separating the two types of activity at 20°C was 0.4 mg/l.

INTRODUCTION

A FEW physiological workers have recently examined freshwater bivalve activity and the factors controlling it. Barnes (1955) recorded the activity of *Anodonta cygnea* and investigated the neuromuscular basis of rhythmic contractions exhibited by these animals. He found, as did some early workers (Marceau, 1906; Koch & Hers, 1943), a pattern of alternate rapid adductions and slow abductions of the valves. This pattern, in *A. cygnea*, was part of a larger, intrinsically controlled, rhythmical pattern, which recurred with frequencies ranging from 3 to 30 per week.

Salánki & Balla (1964) devised a continuous recording method, which was later used to study the effect of oxygen on *A. cygnea* activity (Salánki, 1964). In contrast to the report of Barnes, Salánki found only a weak degree of diurnal rhythmicity in aerated animals. Further, he found that extrinsic factors, such as a reduction in oxygen concentration below a certain level, resulted in a change in the normal pattern of activity.

The freshwater bivalve *Pleurobema coccineum* has been found to be relatively unaffected by hypoxic conditions (Badman & Chin, 1973). That is, only a weak Pasteur effect is present, longevity in the absence of oxygen is great, and the presence of 3–10% oxygen at 20°C (0.6–1.8 mg/l.) seems to be adequate for normal oxygen demands. In the course of preparation of those animals for the above experiments, it was observed that when placed in oxygen-free water, they spent a large amount of time with closed valves. It was thus decided to examine the activity response of *P. coccineum* to changes in oxygen concentration and to attempt to determine the critical oxygen concentration.

MATERIALS AND METHODS

P. coccineum was collected from the Kalamazoo River, near Galesburg, Michigan, and allowed to dry about 20 min. The left valve of each clam was then fastened to a small (6 × 8 cm) glass plate with Dow Corning silicone rubber aquarium sealer. A small glass ring was fastened in the center of the right valve. The clam was allowed to remain dry for 24 hr to cure the sealer, and then placed in 20°C spring water for at least 48 hr to allow acclimation and final curing of the sealer.

Figure 1 shows a clam placed in position for recording. The glass plate to which the clam was fixed was inserted vertically into a Plexiglas "throne" which previously had been fastened to the bottom by a slot in the throne and at the top with a clothespin. The vertical position

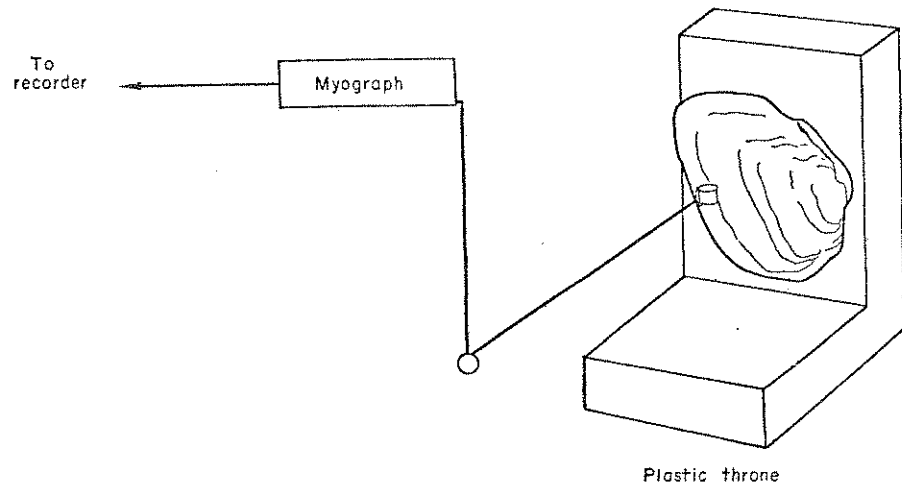


FIG. 1. Orientation of clam on plastic throne used for activity recording.

was used to approximate the natural orientation of the animal. A thread was attached at one end to the glass ring in the right valve of the clam, passed through a similar ring attached to the side of the aquarium, and connected to a Narco Bio-systems Myograph. Thus, valve movements could be transmitted via the myograph to a Physiograph recorder.

The aquarium was fitted with a plastic lid sealed on with a Vaseline-paraffin mixture. Through the lid were inserted a rubber tube for bubbling the water with gas, a Yellow Springs oxygen probe for oxygen monitoring and threads running to the myograph. With the oxygen probe, the water could be monitored constantly with a Yellow Springs Biological Oxygen Monitor (Model 53) and a Heath Servo-recorder.

A small aquarium pump was used to aerate the water for normal activity recording, while nitrogen gas was bubbled into the water to remove oxygen. In order to regulate the oxygen levels between 100 and 0% saturation, a small amount of a mixture of 95% O₂-5% CO₂ was added.

The aquarium was placed in a bath regulated at 20°C, in a room chosen for its minimal exposure to vibration, and maintained in constant darkness, except for a yellow dark-room lamp for making observations.

RESULTS

Specimens in aerated water (Fig. 2) spend nearly 100 per cent of the time with open valves. Periodically, rapid adductions occur, followed by slow abductions.

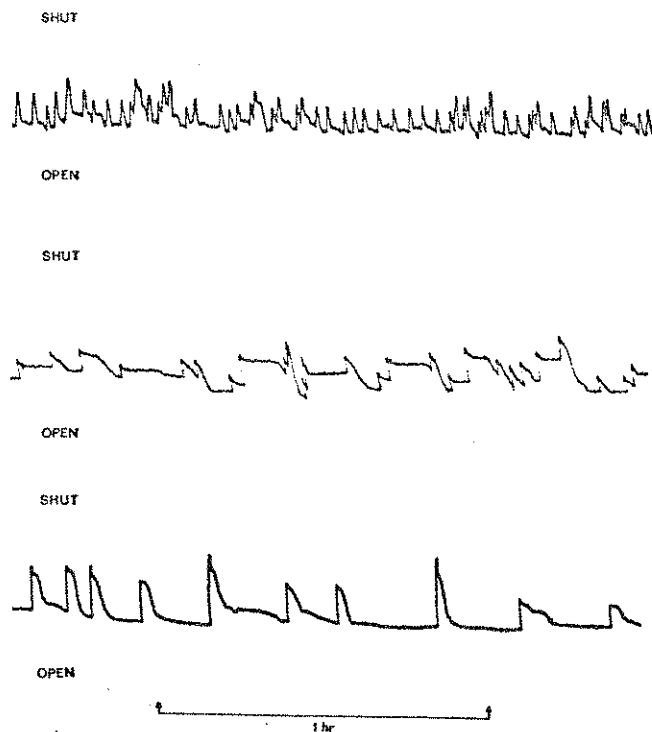


FIG. 2. Examples of activity recordings of three different *P. corbiculus* individuals in aerated spring water at 20°C. In all recordings, contractions (adductions) are upwards.

Usually, such adductions are incomplete. The particular activity pattern of an individual seems to be unique to that individual, no two clams showing exactly the same pattern. Further, no diurnal or other rhythm is apparent.

The specimens in 20°C water devoid of oxygen remain active only 7.6–13.1 per cent of the time. Moreover, they exhibit an activity pattern (Fig. 3) which is characteristic for the absence of oxygen, consisting of an abrupt gaping, followed by nearly complete adduction. This is repeated several times, each time the valves opening further and closing less completely, until the animal is fully active. There follows a fairly regular pattern of abrupt partial adductions and slow abductions, each of similar magnitude. After a time the valves close in a characteristic stepwise fashion, similar to the opening, except that the successive partial abductions are not as extensive, and the clam again becomes inactive.

The activity of three individuals was recorded for approximately 200 hr at 20°C in the absence of oxygen. Results are summarized in Table 1. For each individual, the average duration of each inactive period is not significantly different from those of the other individuals. There is, however, a significant difference ($P < 0.05$) in mean duration of active periods.

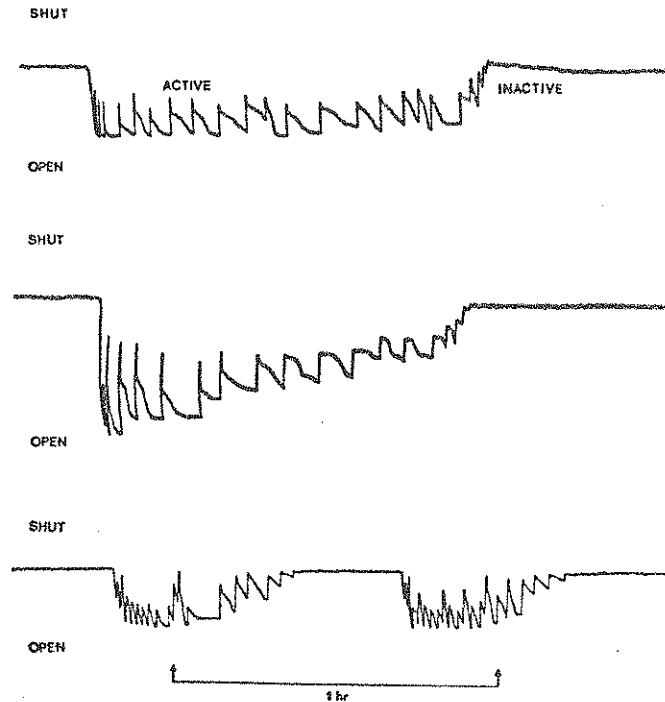


FIG. 3. Examples of active periods of three different *P. coccineum* individuals in oxygen-free spring water at 20°C.

TABLE 1—SUMMARY OF *P. coccineum* ACTIVITY IN OXYGEN-FREE SPRING WATER AT VARIOUS TEMPERATURES

Clam No.	Active period: mean duration ± S.E.M. (min)	Inactive period: mean duration ± S.E.M. (hr)	Total time of recording (hr)
20°C			
1	85.42 ± 5.83	8.67 ± 0.71	216
2	51.79 ± 2.05	10.50 ± 0.72	210
3	59.53 ± 3.25	10.05 ± 0.26	196
25°C			
1	144.54 ± 12.73	9.67 ± 1.00	126
2	66.73 ± 4.22	11.46 ± 0.80	134
3	83.09 ± 4.66	10.80 ± 0.90	123
30°C			
2	58.00 ± 7.34	3.39 ± 0.44	44
4	31.37 ± 3.16	1.70 ± 0.13	41

Table 1 also summarizes activity at 25 and 30°C. The duration of the inactive period increases slightly in each, but the increase is not significant. As at 20°C, the difference in duration of inactive periods at 25°C between individuals is not

significant. The mean active period duration shows a significant increase ($P < 0.01$) over that at 20°C. At 30°C, two of the individuals died and were replaced by a fresh individual. At this temperature the duration of both inactive and active periods drops significantly.

The data were analysed in an attempt to determine whether there was any correlation between the duration of an inactive period and the duration of the following active period, and vice versa. In only three instances was such a correlation found: 25°C—clams Nos. 2 and 3 ($P < 0.05$) and 30°C—clam No. 1 ($P < 0.01$).

It was found that by simultaneously bubbling the water with pure nitrogen gas from one tank and a mixture of 95% O₂–5% CO₂ from another tank and using some care in regulating their respective proportions, the oxygen concentration of the water could be maintained at a fairly constant level. Experiments were then performed to determine the oxygen concentration which could elicit the characteristic anaerobic activity. At 20°C, this critical oxygen level was found to be about 0.4 mg/l. At 30°C, the critical level rises to 1.1 mg/l.

DISCUSSION

In the earlier work referred to above, clams were recorded while resting on one valve, an abnormal position. It was hoped that the upright position used in the present work would offer a more natural orientation for the animals, leading to uninhibited activity. While this orientation possibly is not so critical for activity recording itself, it may be important in obtaining a true analysis of the correlation between the observed valve activity and filtration.

The normal *P. coccineum* in aerated spring water appears to be in constant activity. The valves are open most of the time and the siphons are visible. The sporadic partial adductions in the recordings are similar to those reported by Barnes (1954) in *A. cygnea*, and were found by him to be controlled by ganglia associated with the striated portions of the adductor muscles, under apparently intrinsic stimuli. It is not possible to say, for the moment, whether the open valves mean that the animals are filtering water. It is apparent that when the valves are open, the siphons usually are also. Previous investigations by Salánki & Lukacsovics (1967) showed that during the active periods of *A. cygnea*, the amount of suspended neutral red decreases rapidly, while during inactive periods the filtration stops. Certainly *P. coccineum* is not completely isolated from the surrounding water when the valves are closed, since a rise in oxygen concentration of the water from 0.4 mg/l. to 0.6 mg/l. at 20°C can be detected almost immediately. Such an oxygen rise results in an abrupt increase in valve activity (Fig. 4).

The shift in activity pattern which occurs at 20°C and 0.4 mg O₂/l. is characteristic and striking. It is apparently a result of the animals' direct sensing of the oxygen concentration, since they can be induced to alternate between the aerated behavior and hypoxic activity by raising or lowering the oxygen concentration slightly when at the critical oxygen level.

As pointed out above, under hypoxic conditions *P. coccineum* is active 7.6–13.1 per cent of the time. The function, if any, of this active period is not known. It is

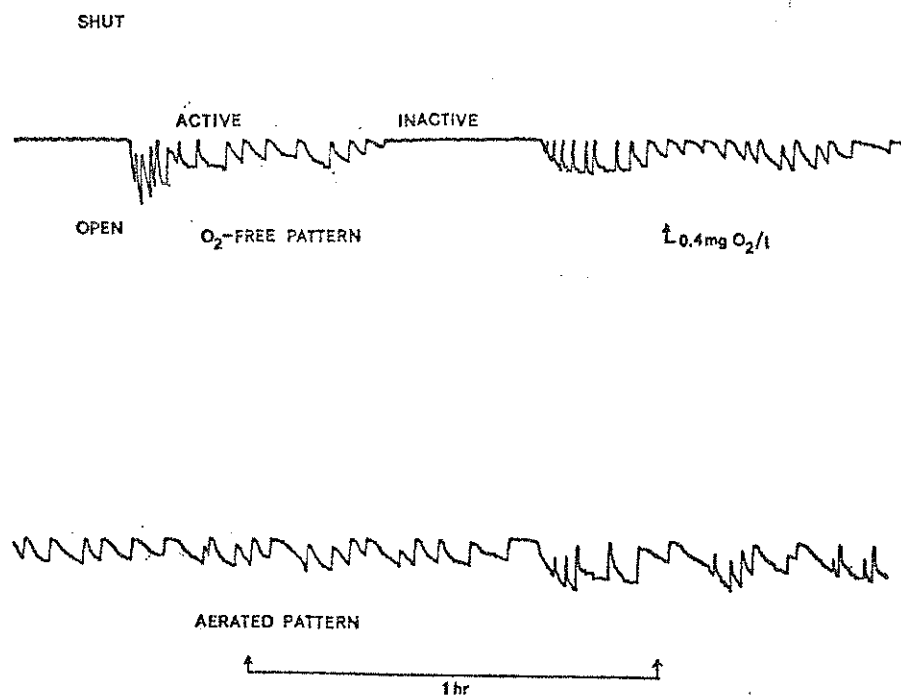


FIG. 4. Example of the shift in activity pattern in *P. coccineum* in response to an increase in oxygen concentration from 0 to 0.4 mg/l. at 20°C. (The lower recording is a continuation of the upper.)

possible to speculate that it may be used to flush out accumulated waste products. Alternatively, the active period might be a time of active filtration of water, in an effort to gain oxygen. If it were caused primarily by an internal physiological condition, such as waste product accumulation, it would be expected that duration of open period would be correlated with body size. There is, however, no such discernible correlation. Instead, each individual appears to have its own characteristic pattern.

Further, if an accumulation of anaerobic waste products produces the characteristic activity pattern, it might be possible to induce such activity with an electron transport poison. Accordingly, one individual was recorded in 10^{-3} M, 4×10^{-3} M and 10^{-2} M sodium azide. The first two concentrations had no apparent effect on activity. The third caused prolonged adduction, followed by death after about 12 hr exposure. This result was probably due to the increased ionic strength of the medium, rather than the inhibitory effect of the azide, since a similar concentration of NaCl also results in prolonged closure. From this isolated experiment, little can be assumed, since other inhibitors and more individuals should be tried. Also, molluscs may have limited cell permeability (Martin, 1961) and the azide, in lower

quantities, simply may not have penetrated the tissues in quantities sufficient for inhibition.

It has become apparent from numerous reports (Stokes & Awapara, 1968; De Zwaan & Zandee, 1972) that many bivalves are facultative anaerobes with the ability to exist in the absence of oxygen by a shift in metabolism. Under such conditions, it would be of advantage to reduce energy expenditure, and reduce contact with the oxygen-free environment. Thus it is not surprising to find prolonged shell closure, resulting in the observed activity shift in *P. coccineum*. The periodic short active periods could perform the necessary function of allowing the animal to rid itself of accumulated waste products and test the water for oxygen availability.

The characteristic shift in activity below a critical oxygen concentration is interesting from another point of view. By its very nature of appearing abruptly as a result of deteriorating environmental conditions, this activity shift may possibly be used in future investigations as an indicator of substandard water quality.

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Key Word Index—Activity; anaerobiosis; Mollusca; fresh-water bivalve; *Pleurobema coccineum*.