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EXPERIMENTS FOR DETERMINING THE OXYGEN CONSUMPTION OF NYMPS OF PALIGENIA LONGICAUDA (EPHEMEROPTERA)

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Abstract

On a closed-system apparatus suitable for measuring the oxygen consumption of burrowing Ephemeroptera, supplied with respiratory water in continuous circulation. The change in the oxygen concentration is measured in electrochemical way.

Introduction

The distribution of benthos organisms is influenced considerably by the quality of the substrate and the dissolved oxygen content of water. Several works are known that are regarding the effects of these two factors as independent of each other. But the establishments that prove a close connection between these two parameters become more and more conspicuous (ERIKSEN 1963, 1964, 1968, LINDUSKA 1942, LYMAN 1965, OLSON *et al.* 1968). This refers particularly to the burrowing water organisms and nymphs, respectively.

The presence of species is influenced by the oxygen concentration. The quantity of the dissolved oxygen is namely reduced by pollution in several cases that changes the fauna-composition in the area. Some species — bio-indicators — respond to this change, sensitively, therefore it is of no small interest to recognize their oxygen requirement.

At the bio-energetic researches, practised of late years, it is indispensable, as well, to know the oxygen requirement characteristic of the organisms investigated. It became necessary to elaborate some methods for investigating the respiration, in case of which a primary point of view is to adapt themselves to the natural circumstances as well as possible (KAMLER 1969, KLEKOWSKY *et al.* 1968, NAGELL 1963). Our experiments carried out with nymphs of *Palingenia longicauda* — and taking into consideration the above principle — to construct an apparatus for measuring oxygen consumption, its essence being: (1) to keep the respiratory water in continuous flowing; (2) to contain a substrate that is ideal both to the animal and in respect of measuring, as well.

Description of the measuring system

The most important part of our closed-system apparatus is control electrode Argox-M-$ZnDO_2$ whose functioning is based upon measuring the dissolved oxygen in electrochemical way. A signal current proportionate to the quantity of the dissolved oxygen is supplied by the voltametric sensing-

divices and, after calibrating satisfactorily, the changes in the oxygen content of the water dissolved can be concluded from the changes in the intensity of the signal current (electrode description by the Central Research Laboratory).

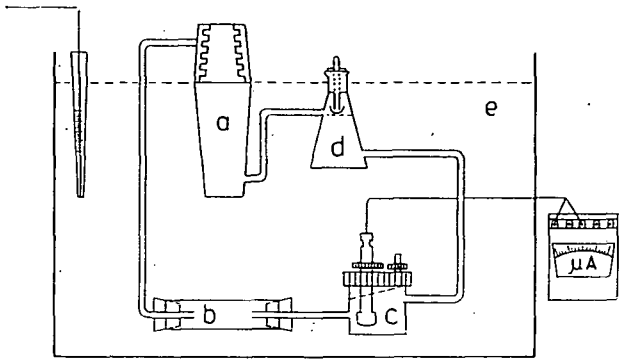

Fig. 1. Schematical representation of the apparatus measuring the flowing-water oxygen consumption. (a: water-circulator, b: respiartory chamber, c: measuring chamber, d: carbon dioxide adsorber, e: water bath).

Measuring Technique.

The most important parts of the apparatus are (Fig. 1):

1. WATER-CIRCULATOR AND TANK (I/a): Motorized plexiglass-tank capable of piping 4.000 ml/min water. It is fundamentally necessary to maintain the continuous waterflow for both the control electrode and animals, as well.
2. RESPIRATORY CHAMBER (1/b): 20 cm×5 cm glass pipe connected to the other parts of the system with connecting pipes. On the occasion of measuring, the experimental animal is placed there.
3. MEASURING CELL (1/c): A plexiglass-tank equipped with a detachable deck, with an incorporated control electrode. The cathode and anode of the electrode are silver, the anode is covered with a zink case, both of them taking place in a gel tank of special formation.
4. CARBON DIOXIDE ABSORBER(1/d): Glass vessel in which, in a small bowl stretching into the air-space over the circulating water, a filter-paper was placed, impregnated with 0.2 ml 20 per cent KOH, for binding the carbon dioxide- released in the course of respiration, and diffusible from the water.
5. WATER BATH (1/e): The system described so far is immersed in a water bath the water of which can be kept, by means of an ultra-thermostat mixing motor and a fine thermo-regulator at a temperature given, with an accuracy of 0.1—0.2 °C.

The system fit together of the enumerated parts was filled in with 3,000 ml river water. We have chosen the quantity and oxygen content of the water so that plenty of oxygen remained even at the end of measuring and we could eliminate the inhibiting effect of its shortage.

In order to destroy the living micro-organisms, we have added chloramphenicol and streptomycin to the water, in 20 mg/l concentration. In this way, we could exclusively measure the oxygen quantity consumed by the experimental animals. On the basis of our investigations this antibiotic quantity is not pernicious to the larvae.

In order to get a reference also to the part played by the substrate in the respiration, we have carried out two series of measurings: in the first series, we have measured the oxygen consumption of nymphs without substrate, in the second one in the presence of a substrate. The intervering effect of light was eliminated by the respiratory chamber being made dark. Further on, we have set the water bath, together with the complete respiratory system and animals, for the temperature wished and then, about 30 minutes later, we began our measuring. We regarded this time as satisfying bacause the animals had already been kept at the experimental temperature for 24 hours. We have performed measuring for two hours and recorded the current drop every fifteen minutes. The values measured can be reckoned over, by means of a calibrating curve, into μg/l oxygen concentration. The calibration curve was drawn at every temperature on the basis of current intensity values measured in 0 per cent (5 p. c. Na_2SO_3) and 100 per cent oxigen-saturated solutions after ventilating, mixing, and bubbling air through them for two hours.

Results and their discussion

We could compare the results of the two measuring series to the data obtained by means of the traditional and mostly-used Warburg-method (CSOKNYA 1973). As it is known, the essence of Warburg's method is that the vessels of the manometer are to be vibrated with a definite speed. This vibration and the fact that the animals take place in a "closed-bottle" water of small quantity, are in our opinion factors considerably different from the natural conditions of larvae. The effect of these facts can be demonstrated in the form of a larger oxygen consumption.

Have we measured without any substrate but in flowing water then the larvae performed, contrary to the natural circumstances, an intensive movement changing their places in full length of the respiratory chamber, accompanied with the lively movement of branchiae. The respiratory values measured by us in this way — in case of animals of identical weight-class that means the same stage of development, as well — were higher as compared to the data measured with Warburg's apparatus (Fig. 2). We attribute the increase in respiratory values to taking place of a stronger excitement than before.

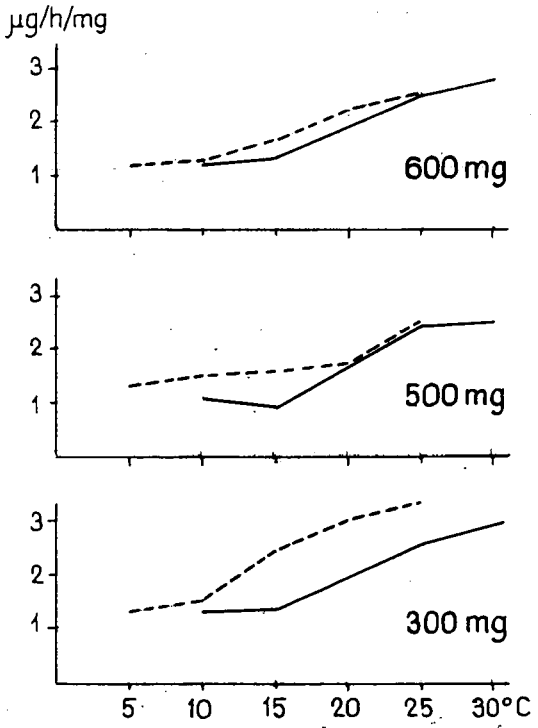

Fig. 2. Comparison of the values of oxygen consumption of the mayfly nymphs (of 300, 500, and 600 mg weight) by means of various methods)———Warburg's method, — — — flowing-water procedure).

In order to decrease this excitement, we put the artificial substrate in the respiratory chamber. A natural substrate is not suitable for that purpose because of its own consumption. And after being burned out or treated in another way (*e. g.*, with

chemical agents), the animals perished for in the course of the procedure the substrate had lost its proper concentration, consistence. After trying repeatedly, we found most suitable for this purpose the common plasticine because its composition (clay powder, kaolin, zink oxide) and particularly its consistence are similar to those of the silt which the animals are living in, but which has no own oxygen consumption. We made from the placticine some ducts similar to those of mayfly nymphs but being straight. They often took place in these voluntarily and got in that way into the measuring cell.

The values of oxygen consumption obtained by us were generally lower than the results of either of both former methods. For instance:

body weight	Oxygen consumption at 25° C µg/h/mg	
mg	without substrate	with substrate
200	4.51	2.95
500	2.45	0.91

We find the explanation of the lower values nearly unambiguously in that the larvae were in the „ducts" of the substrate under similar conditions to those in natural circumstances, therefore the irritated movement that looked for place ceased to be continued. The quieter state, characterized by the more regular gill-movement, as well, manifested itself in lower values of oxygen consumption.

The water-circulating oxygen-measuring apparatus, in which the mayfly nymphs can be placed in a substrate, is most ideal for them showing the respiration under natural conditions perhaps in the most real way.

Later on, we want to complete the data obtained by means of the latter method by investigating the effects of the development of nymphs and other environmental factors.

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