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Effect of Temperature on Respiration Metabolism of Two Starved Species of Millipedes (*Diplopoda*)

Wpływ temperatury na metabolizm oddechowy u dwu głodzonych gatunków krocionogów (*Diplopoda*)

Влияние температуры на дыхательный метаболизм у двух видов голодающих многоножек (*Diplopoda*)

Ambient temperature is undoubtedly a factor clearly defining oxygen consumption level in invertebrates. The connection between the temperature of the habitat in which these animals live and their body temperature, and consequently intensity of respiratory metabolism, is on the one hand due to the evolutionary adaptation of this species (2), and on the other constitutes a response to the currently prevailing temperature conditions (5, 8, 10, 12, 14, 15, 16, 19, 41, 43).

Respirometric measurements of oxygen consumption revealed the ranges of ambient temperature in which it increases rapidly, and also those in which respiratory metabolism exhibits a certain degree of independence of increased temperature (2, 10, 13, 19, 24, 26, 33, 42).

More detailed studies showed that the range of temperature characterized by balanced oxygen requirements in these animals depends not only on the thermic history of the species and the current ambient temperature, but also on the degree of the animals satiation with food (4, 11, 20, 27, 28, 36).

On account of the lack of studies on this problem in relation to millipedes, an attempt has been made in the present study to define the effect of starvation on the oxygen consumption curve at different ambient

temperatures, in two species of *Diplopoda: Orthomorpha gracilis* C. L. K. and *Cylindroiulus frisius* (V e r h o e f f).

MATERIAL AND METHODS

The experimental material consisted of males and females of the above two species of *Diplopoda*. The animals were supplied to the laboratory from the hothouses of the Town Gardens Department, Lublin, and kept at a constant temperature of 22°C, in terraria containing soil, decayed leaves and wood (group I) or peat (group II). The millipedes were starved by keeping on well-washed sand (group III). The body weight of *Orthomorpha gracilis* varied from 29.4 mg to 86.8 mg, and of *Cylindroiulus frisius* from 7.1 mg to 19.0 mg. Before starting measurements of oxygen consumption the animals were adapted to the current experimental temperature for 24 hours. Oxygen consumption was determined in Drastich volumetric respirometers, as modified by K l e k o w s k i (23). Animals and apparatus were adapted to the experimental conditions for 20 minutes. At the end of this time the manometer taps were closed and measurements of oxygen consumption started, lasting for 3 hours. The experiments were carried out at 8 temperature levels for *Orthomorpha gracilis* and at 9 for *Cylindroiulus frisius*, from 10°C to 34°C, mainly at intervals of 3°C. The millipedes were weighed before beginning measurements of respiratory metabolism.

Results obtained for oxygen requirements were converted to a temperature of 0°C and pressure of 760 mm Hg (23).

RESULTS AND DISCUSSION

Results of oxygen consumption measurements for *Orthomorpha gracilis* are set out in Table 1, and for *Cylindroiulus frisius* in Table 2; and in diagram form for both species in Figure 1. Values for the thermic coefficient are given in Figure 2.

The data obtained show that oxygen requirements for millipedes kept on decayed parts of plants distinctly increase with increase in temperature, although differences between species in this respect are fairly considerable. The respiratory metabolism of *Cylindroiulus frisius* exhibits decidedly greater dependence on ambient temperature than that of *Orthomorpha gracilis*.

This may be the expression of the poorer adaptation of the former species to average high ambient temperatures prevailing in hothouses. The relatively balanced and faintly expressed increase in respiratory metabolism in *Orthomorpha gracilis* from 10° to 22°C as compared with the preceding species may be attributed to the reaction to the non-typical ambient temperature. This explanation would appear to be the right one, as *Orthomorpha gracilis* lives in a natural habitat only in tropical countries, and in hothouses in the temperature zone lives in places where

the temperature is suitably high and maintained between 21° and 26°C. This millipede does not persist in cooler hothouses. *Cylindroiulus frisius*, on the other hand, commonly occurs in hothouses, garden frames, gardens and parks (38), and in addition occurs in the natural habitat of Western Poland (22, 29).

Table 1. The effect of different temperatures and starvation on oxygen consumption of *Orthomorpha gracilis* C.L.K.

Food	Temperature in °C	10	13	16	19	22	25	28	31	34
Mouldy part of plants	No of animals	18	18	18	18	18	24	24	23	22
	Mean body weight /in mg/	12.0	13.1	11.8	13.9	12.3	12.1	11.7	11.3	11.3
	$mm^3/O_2/g/hr$	92.8* ±17.8	127.0 ±36.5	145.4 ±48.7	215.5 ±49.4	265.2 ±68.5	269.1 ±94.3	349.0 ±84.9	426.1 ±110.9	467.5 ±114.7
Peat	No of animals	30	38	42	47	40	40	38	38	45
	Mean body weight /in mg/	12.5	13.8	13.2	13.0	12.8	13.8	12.9	13.2	12.6
	$mm^3/O_2/g/hr$	80.0 ±23.1	94.4 ±27.3	116.0 ±29.5	141.6 ±38.9	173.6 ±41.7	174.4 ±35.0	197.5 ±53.9	258.4 ±70.5	335.5 ±58.6
Starved for 14 days	No of animals	18	18	17	17	18	17	16	18	17
	Mean body weight /in mg/	11.7	10.9	11.2	12.9	11.6	11.7	11.9	10.8	10.7
	$mm^3/O_2/g/hr$	81.0 ±13.1	89.2 ±20.0	92.6 ±20.6	112.2 ±31.3	123.7 ±21.0	130.2 ±17.4	130.2 ±31.8	201.8 ±37.6	288.5 ±53.6

* Means ±SD.

Table 2. The effect of different temperatures and starvation on oxygen consumption of *Cylindroiulus frisius* (V e r h o e f f)

Food	Temperature in °C	10	14.5	19	22	25	28	31	34
Mouldy part of plants	No of animals	34	36	36	35	36	34	32	34
	Mean body weight /in mg/	57.3	62.5	60.7	59.9	58.7	59.4	57.6	61.2
	$mm^3/O_2/g/hr$	46.4* ±5.7	71.0 ±11.2	107.1 ±17.2	140.3 ±24.1	146.8 ±22.9	179.8 ±24.7	228.6 ±35.5	221.7 ±38.3
Peat	No of animals	18	22	24	24	22	23	18	22
	Mean body weight /in mg/	42.3	43.9	44.2	43.7	41.9	41.3	43.0	42.7
	$mm^3/O_2/g/hr$	47.8 ±9.2	64.1 ±10.3	70.8 ±9.9	75.4 ±8.4	81.0 ±6.9	91.9 ±10.1	116.3 ±11.0	147.9 ±19.3
Starved for 14 days	No of animals	20	23	18	22	24	24	23	23
	Mean body weight /in mg/	56.1	55.9	53.7	53.0	53.2	52.5	52.2	51.8
	$mm^3/O_2/g/hr$	43.7 ±6.9	48.8 ±7.3	64.3 ±8.1	69.9 ±8.8	76.2 ±9.4	79.9 ±10.1	110.4 ±15.4	140.7 ±13.3

* Means ±SD.

It may be concluded from the data given in Tables 1 and 2 and Figures 1 and 2 that peat is less suitable food for both species of millipede than decayed leaves and pieces of wood, this applying particularly to *Orthomorpha gracilis*. Oxygen consumption by individuals kept in peat or without food is very similar in this millipede, curves illustrating oxygen requirements taking almost parallel courses over the whole study range of temperatures.

Millipedes kept in peat taken from places in which these animals occur are characterized not only by a lower level of respiratory metabolism, but also, within the range from 10° to 28°C, by a lesser degree of dependence of oxygen consumption on the experimental temperature. The group of individuals kept for 14 days without food are characterized by even smaller increases in oxygen consumption in this range of temperatures, particularly in *Cylindroiulus frisius*.

A similarly lesser dependence of respiratory metabolism on ambient temperature to that in the study of millipedes kept on peat or starved, was found in starving *Balanus balanoides* (4), in *Patella vulgata* (11), *Carcinus maenas* (25) and *Nadabius coloradensis* (34).

Newell and Pye (27), when determining respiratory metabolism in homogenates of *Littorina* and using concentrations of pyroracemic

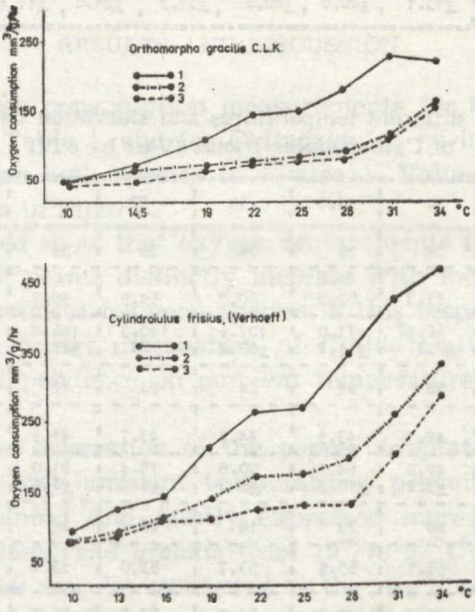

Fig. 1. The effect of different temperatures and starvation on the temperature coefficient (Q_{10}) of millipedes; 1 — from mouldy part of plants, 2 — from peat, 3 — starved for 14 days

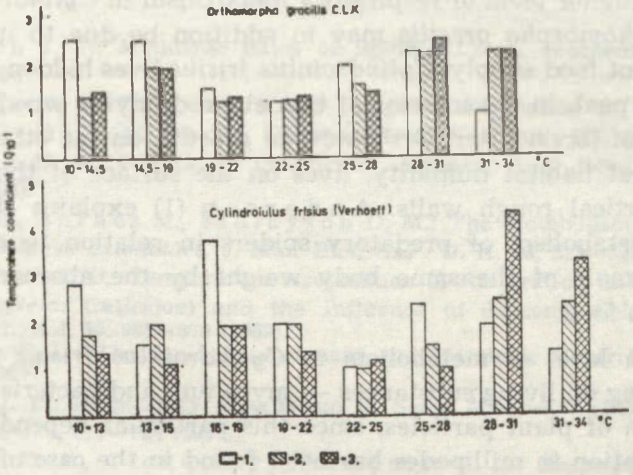

Fig. 2. The effect of different temperatures and starvation on oxygen consumption of millipedes; 1 — from part of plants, 2 — from peat, 3 — starved for 14 days

acid as a substrate, found that with low concentrations of this acid such as 0.05—0.10 mM, "oxygen consumption — temperature" curves (MT) of homogenates correspond to the curves for oxygen requirements in starving individuals examined *in vivo*. With higher concentrations of pyroracemic acid of 0.30—2.00 mM, the "metabolism—temperature" curve is similar to that for respiratory metabolism in non-fasting animals. Thus the slow increase in respiratory metabolism in millipedes from peat, or those deprived of food, is most likely connected, over a wider range of temperatures than for individuals cultured on decayed parts of plants, with the reduced concentration of energetic substances in the body cells, and in consequence in the hemolymph, and possibly also in the fat body. On the other hand, as can be concluded from studies by numerous authors (3, 6, 21, 36, 37) the affinity of respiratory enzymes to the substrate decreases in millipedes taken from peat or starved, over a wider range of temperatures.

The greater respiratory metabolism of the somewhat inactive *Cylindroiulus frisius* in relation to the more active *Orthomorpha gracilis* at all experimental temperatures is, in the authors' opinion, conditioned at least to some degree by its smaller body dimensions. Greater oxygen requirements were found in other species of *Diplopoda* with smaller body weight by Byzova (7) and Gromysz-Kałkowska (17). A similar phenomenon was also found in crabs (42) and other species of *Arthropoda* (30, 31) and in marine polychaetes (35).

The far higher level of respiratory metabolism in *Cylindroiulus frisius* than in *Orthomorpha gracilis* may in addition be due to its potentially more constant food supply. *Cylindroiulus frisius* lives hidden in its food — in lumps of peat, in the interior of tunnels in decaying wood and in compact layers of forest litter. *Orthomorpha gracilis*, on the other hand, with suitably great habitat humidity, lives on the surface of the ground and even on vertical rough walls. Anderson (1) explains the lower respiratory metabolism of predatory spiders in relation to other heterothermic animals of the same body weight by the absence of constant contact with food.

The high level of metabolism in *Cylindroiulus frisius* may also be due to feeding on living substances — mycelium and bacteria which cause fermentation of plant particles, since this particular dependence of oxygen consumption in millipedes has been found in the case of *Polydesmus complanatus* (17) and in *Proteroiulus fuscus* (18). In both these species oxygen consumption was found to decrease as the mycelium on litter was used up by feeding millipedes. The data given by other authors confirm this assumption, for instance Prokopiewa (30) found higher respiratory metabolism in terrestrial insect larvae, and Reichle and Crossley (32) more rapid ^{134}Cs circulation in animals feeding on fresh parts of plants in comparison with those feeding on the decayed parts.

The high oxygen consumption in individuals of *Cylindroiulus frisius* living on decaying parts of plants may be conditioned by their easier access to simple sugars formed as the result of cellulose fermentation. The studies made by Tracz (40) on food preferences in the millipede *Proteroiulus fuscus* lead to this conclusion. The above author found distinctly larger numbers of animals in the part of the apparatus with mono- and disaccharides than with polysaccharides.

The authors' own observations, and data in literature (9, 38, 39) lead to the conclusion that *Diplopoda* may be encountered on plant organs damaged by fungi, protozoa or viruses. The damaged surfaces of plants are rich in simple sugars and attract millipedes for which they form excellent food. This interpretation is borne out by large groups of these animals in places in the terraria where table sugar had been placed. On the other hand, the diseased parts of plants adequately supply the millipedes requirements for humidity, which they may lack at dry periods of the year, especially during dry summers. In the light of the foregoing Niezgodziński's observations (29) of large numbers of *Cylindroiulus frisius* on maize and sunflower crops, particularly in years of mass occurrence, this fact would become fully understandable.

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STRESZCZENIE

Zużycie tlenu u *Orthomorpha gracilis* C. L. K. i *Cylindroiulus frisius* (Verhoeff) przebadano w różnej temperaturze: od 10 do 34°C i w różnym stopniu nasycenia zwierząt pokarmem.

Ustalono, że krocionogi przetrzymywane na torfie wykazują niższy metabolizm oddechowy aniżeli pobrane ze ściółki i zbutwiałych części drewna. Większe różnice w zapotrzebowaniu na tlen między tymi grupami krocionogów u dwu gatunków zaznaczają się w wyższej temperaturze, co zwiększa zakres ciepłoty, w której krzywa zużycia tlenu przebiega pod małym kątem nachylenia. Jeszcze większą niezależność przemiany oddechowej od temperatury wykazują krocionogi głodzone.

Znacznie wyższy metabolizm oddechowy *Cylindroiulus frisius* w porównaniu z *Orthomorpha gracilis* wynikać może z mniejszych rozmiarów ciała, ciągłego kontaktu z pokarmem, z preferowania w większym stopniu jako pokarmu drobnoustrojów dokonujących fermentacji części roślinnych i ewentualnie cukrów prostych powstających przy rozkładzie celulozy przez mikroorganizmy.

РЕЗЮМЕ

Потребление кислорода *Orthomorpha gracilis* C. L. K. и *Cylindroiulus frisius* (Verhoeff) исследовали при разной степени насыщения многоножек кормом и при различной температуре — в пределах от 10 до 34°C. Констатировали, что многоножки, которых держали на торфе, характеризовались более низким дыхательным метаболизмом, чем животные, собранные с лесной подстилки и прелой древесины. Более четкие различия в потреблении кислорода между этими группами многоножек у обоих видов наблюдали при более высокой температуре, что повышает диапазон температуры, при которой кривая потребления кислорода проходит под небольшим углом наклона. Большую независимость дыхательного обмена от температуры проявляют голодающие многоножки.

Значительно высший дыхательный метаболизм *Cylindroiulus frisius* по сравнению с *Orthomorpha gracilis* вытекает, как нам кажется, из меньших размеров тела, постоянного контакта с кормом, из преобладания в корме микроорганизмов, а также благодаря потреблению простейших сахаров, образующихся в результате разложения клетчатки этими микроорганизмами.