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# Respiratory Responses in a Brazilian Millipede, *Pseudonannolene tricolor*, to Declining Oxygen Pressures

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**Abstract**
*Oxygen uptake and the influence of declining oxygen pressures ($Po_2$'s) were examined in a Brazilian spirostreptid millipede,* Pseudonannolene tricolor. *The data were obtained in a Warburg respirometer at 25° C from both male and female animals, sexually inactive, in the intermolt stage, and fasting for 24 h. In a sudden exposure to a decreased $Po_2$ the millipedes regulated respiration down to at least 71 mmHg $O_2$. From a $Po_2$ of 35 mmHg $O_2$ downward the animals started to show oxyconformity. When the millipedes were exposed to a stepwise declining $Po_2$ the results indicated only conformation. After exposure to hypoxia,* P. tricolor *showed a pattern of "underrepayment" on return to normoxia, but larger millipedes accumulated more $O_2$ debt than smaller ones.*

## Introduction

Respiratory responses of terrestrial arthropods to variations in the environment remains a relatively poorly explored field of investigation. This holds mainly for the responses to variations in certain parameters such as the available $O_2$, temperature, and humidity, especially in the noninsect arthropods inhabiting tropical regions. Millipedes are among the arthropods in which the study of respiratory metabolism in relation to environmental $O_2$ still requires more attention.

When respiration (or metabolism) is used as a "rate function" to study the influence of declining ambient $O_2$ pressures ($Po_2$'s), animals behave more or less as conformers or regulators. The nature of the response depends on the species' phylogenetic position, temperature, habitat, molting cycle,

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size, acclimation period, activity, rhythms, and other factors. Respiratory regulation frequently occurs in tracheates, being reported as a characteristic of insects (Chauvin 1956; Wigglesworth 1972), but it has also been attributed to another group of terrestrial tracheates, the millipedes (Daly and Siegel 1964; Byzova 1967; Penteado and Mendes 1978; Penteado 1987). Stewart and Woodring (1973), however, claim that conformation may occur in millipedes, so respiratory regulation may not be universal in Diplopoda.

Terrestrial arthropods also differ in tolerance to hypoxic or anoxic conditions. Some species acquire $O_2$ debts that are paid off on return to normoxia; others do not acquire debts. Cryptozoic and burrowing forms, like millipedes, may be exposed to hypoxic conditions in nature. One typical occasion is during and after heavy rains, when the soil becomes temporarily flooded with water, forcing the animals to move to safe places or tolerate a period of submersion. In this case, an $O_2$ debt may accumulate, as reported for millipedes (Dwarakanath and Job 1974; Dwarakanath, Gabriel, and Joseph 1977; Penteado and Mendes 1978). Similar responses may occur when millipedes are exposed to experimental hypoxia or anoxia, and in this case debt repayments have been referred to as incomplete or partial (Penteado 1987).

The present work aims to determine the responses of the millipede *Pseudonannolene tricolor* exposed to acute or stepwise lowering of the environmental $P_{O_2}$. It is a continuation of a comparative study of respiratory regulation in Brazilian species of this interesting group of arthropods. The repayment of $O_2$ debt after exposure to hypoxia is also investigated.

## Material and Methods

*Pseudonannolene tricolor* is a millipede widely spread in São Paulo State, Brazil, and easily found on the campus of the Universidade Estadual Paulista (UNESP) in Rio Claro (22°24′36″S, 47°33′36″W).

The $O_2$ consumption ($\dot{V}_{O_2}$) expressed as $\mu L\ O_2 \cdot g^{-1} \cdot h^{-1}$ (mass-related $O_2$ consumption or $O_2$ consumption rate) was determined in a Warburg apparatus at 25°C, with flasks of 60-mL capacity. Males and females, sexually inactive, in the intermolt stage and 24 h after the last ingestion of food, were used. During the fasting periods the animals were kept in petri dishes lined with moistened cotton in a climatic chamber at 25°C. In order to maintain the animals in a quiescent state, and since there was no fluid phase in the respirometric flasks, these were not shaken during the experiments. In each experiment, after 1 h run in normoxia the Warburg flasks (each containing one animal) were thoroughly flushed for 3 min with air or mixtures of $N_2$ and $O_2$ adequately prepared by Oxigenio do Brasil, containing 20.93%, 15.00%, 10.00%, 5.00%, 2.65%, and 1.00% $O_2$. As the mean local barometric pressure was 708 mmHg (altitude 612 m), the $Po_2$'s were 148.18, 106.20, 70.80, 35.40, 18.76, and 7.08 mmHg, respectively. Before the initial readings were taken, 30 min was allowed for the adjustment of the animal at the experimental $Po_2$. During the flushing period the flux rates were not measured, but the flask ventilation for 3 min was sufficient to assure a complete replacement of the gases by the perfusing mixture.

Two types of experiments were performed. In the first, the $\dot{V}o_2$ of the animals (weight range 1.073–2.655 g) was measured in air, soon afterwards at a lowered $Po_2$, and finally again in air. In the second type, after 1 h in normoxia, millipedes (0.410–2.802 g) were subjected to a stepwise declining $Po_2$, down to 7.08 mmHg, and then returned to air for 1 h. In both cases, the $\dot{V}o_2$ upon return to normoxia was used to check whether the millipedes acquired $O_2$ debts during the exposure to hypoxia. In the second procedure the purpose was also to determine whether the eventual debts bore any relation to the size (body mass) of the animals. The possibility of interference due to daily respiratory rhythms was also checked.

## Results

*Oxygen Consumption Rates on Sudden (Acute) Exposure to Lowered $Po_2$*

As mentioned, the $\dot{V}o_2$ of *Pseudonannolene tricolor* in this case was measured in three periods: first in air, then in air or in a lowered $Po_2$, and finally in air. The data are in table 1.

A control experiment running for 3 h in constant normoxia (series 1; probabilities $P_1$, $P_2$, and $P_3$) assured that during this time no significant differences in the $\dot{V}o_2$ of the animals occurred.

In declining $Po_2$'s significant differences in the $\dot{V}o_2$ were recorded only below 70.80 mmHg $O_2$, indicating that *P. tricolor* is capable of regulation down to a relatively low $Po_2$ (first hour × second hour probability, $P_1$). No significant differences in $\dot{V}o_2$ were found between the values of the second and third hours, down to 35.40 mmHg $O_2$ (series 4; $P_2$ and $P_3$).

On return to normoxia (third hour) the $\dot{V}o_2$ was higher than in the first hour in air, after the exposures to 18.76 and 7.08 mmHg $O_2$ (series 5 and 6; $P_2$ and $P_3$), indicating that an $O_2$ debt had been acquired at these two low pressures and was at least partially paid off on return to normoxia. The critical $Po_2$ (Pc) was not determined, but it was assumed to lie somewhere between 70.80 and 35.40 mmHg $O_2$, since from this range downward the millipedes showed oxyconformity.

TABLE 1

*Oxygen consumption rates (µL $O_2 \cdot g^{-1} \cdot h^{-1}$) of* Pseudonannolene tricolor *in declining $Po_2$'s, at 25° C*

| Experiment Series | Mean Body Mass (g) | $Po_2$ (mmHg) First Hour | Second Hour | Third Hour | Mean $\dot{V}O_2$ in Different $Po_2$ First Hour | Second Hour | Third Hour | Probability[a] $P_1$ (first × second) | $P_2$ (second × third) | $P_3$ (first × third) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 ........ | 1.607 (.364) | 148.18 | 148.18 | 148.18 | 61.86 (26.76) | 72.40 (19.59) | 80.33 (20.81) | >.05 | >.05 | >.05 |
| 2 ........ | 1.597 (.171) | 148.18 | 106.20 | 148.18 | 98.33 (14.32) | 88.43 (25.52) | 99.68 (15.33) | >.05 | >.05 | >.05 |
| 3 ........ | 1.526 (.255) | 148.18 | 70.80 | 148.18 | 75.28 (32.49) | 73.52 (21.85) | 81.90 (25.73) | >.05 | >.05 | >.05 |
| 4 ........ | 1.747 (.405) | 148.18 | 35.40 | 148.18 | 80.95 (26.60) | 68.65 (19.35) | 77.82 (17.45) | <.05 | >.05 | >.05 |
| 5 ........ | 1.489 (.232) | 148.18 | 18.76 | 148.18 | 73.26 (27.44) | 41.06 (11.08) | 103.32 (53.26) | <.01 | <.01 | <.05 |
| 6 ........ | 1.530 (.172) | 148.18 | 7.08 | 148.18 | 61.53 (26.08) | 36.81 (7.44) | 95.82 (13.49) | <.01 | <.01 | <.01 |

Note. SDs are given in parentheses below values. Ten animals were used for each series. Mean local atmospheric pressure = 708 mmHg. $Po_2$ in normal air (normoxia) = 148.18 mmHg.

[a] Measured by *t*-tests for paired comparisons (mean $\dot{V}O_2$ values of first, second, and third hours).

In figure 1 the change in $\dot{V}O_2$ in each series due to lowered $PO_2$ is expressed in percentages of the second-hour values in relation to the first hour in normoxia to minimize the influence of individual variation.

*Oxygen Consumption Rates in a Stepwise Declining $PO_2$*

The results shown in figure 2 indicate that *P. tricolor* does not control $\dot{V}O_2$ when exposed to a stepwise declining $PO_2$. The decrease in $\dot{V}O_2$ observed showed conformation, and this was checked by a *t*-test for paired comparisons (each case in relation to the first hour in normoxia). In a control experiment in which six animals were kept in constant normoxia for the same time (6 h), it became clear that an eventual circadian respiratory rhythm did not interfere with the results. In fact, the $\dot{V}O_2$ did not significantly differ, as confirmed by a similar test for paired comparisons. Figure 3 compares the data from control and experimental tests in stepwise declining $PO_2$.

In figure 4, the data from the first and sixth hours of figure 2 were used in a ratio of $\dot{V}O_2$ after to $\dot{V}O_2$ before hypoxia to calculate the "oxygen debt index" of *P. tricolor* in relation to size, according to the method of Bayne and Livingstone (1977). The results demonstrated that larger millipedes accumulated more $O_2$ debt than smaller ones.

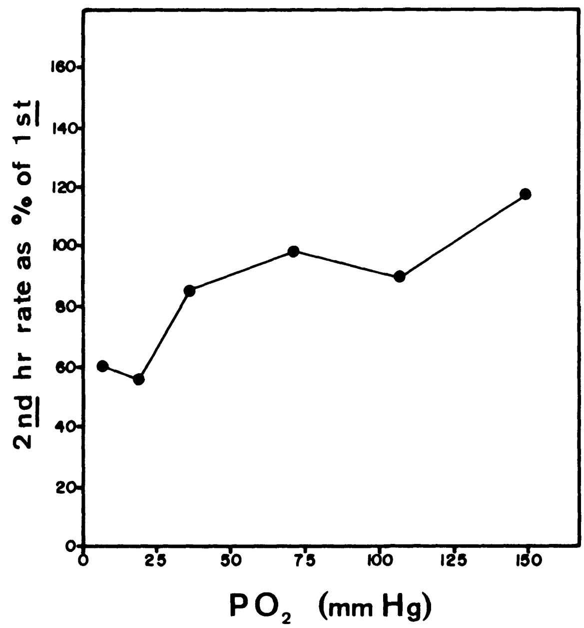


*Fig. 1. Second-hour $\dot{V}O_2$'s in normal air or lowered $PO_2$'s (sudden exposure) for* Pseudonannolene tricolor *expressed as percentages of the first-hour rates in normoxia (calculated from table 1).*

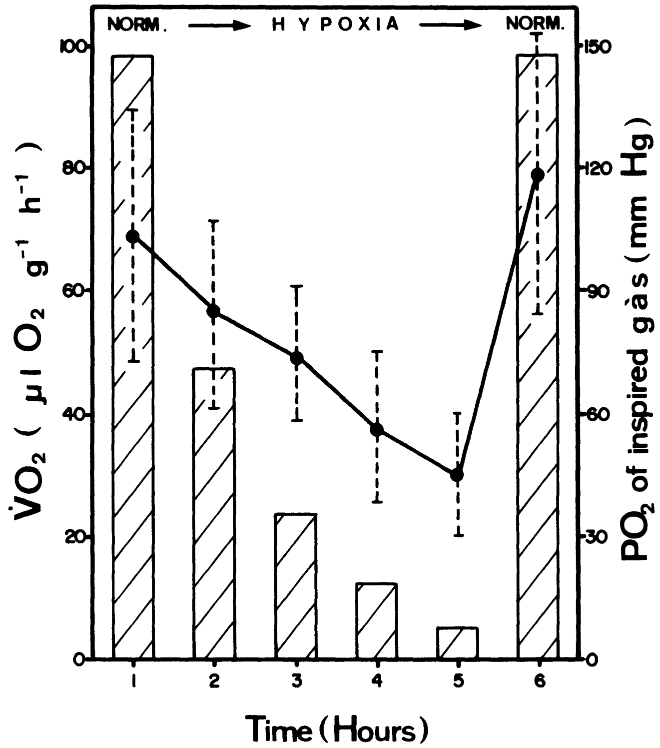


*Fig. 2. Oxygen consumption rates of* Pseudonannolene tricolor *exposed to a stepwise decline of* $Po_2$ *(1 h in each pressure).* N = *13; weight range 0.410–2.802 g. Columns are the* $Po_2$ *values, and the points the mean* $\dot{V}o_2$*. Dashed vertical lines represent SDs.*

## Discussion

The spirostreptid millipede *Pseudonannolene tricolor* was able to regulate $\dot{V}o_2$ down to at least 70.8 mmHg $O_2$ (10% $O_2$). A Pc was assumed to lie between 70.8 and 35.4 mmHg $O_2$, since from this range downward the animals started to show oxyconformity. When the animals were exposed to a stepwise declining $Po_2$ down to 7.08 mmHg $O_2$, results indicated conformation. Millipedes are typical soil invertebrates, subject to hypoxic or even anoxic conditions in nature. This may occur during soil flooding or by the obstruction of their galleries underground. Millipedes are also slow-moving land arthropods, thence expected to be independent of environmental $Po_2$. Besides, as tracheates, they respond as insects, which, as a rule, are regulators. The explanation is that tracheae offer a quick way of conveying $O_2$ to the tissues, in many respects even better than transportation through the circulation of blood containing an $O_2$ carrier (Penteado and Mendes 1978).

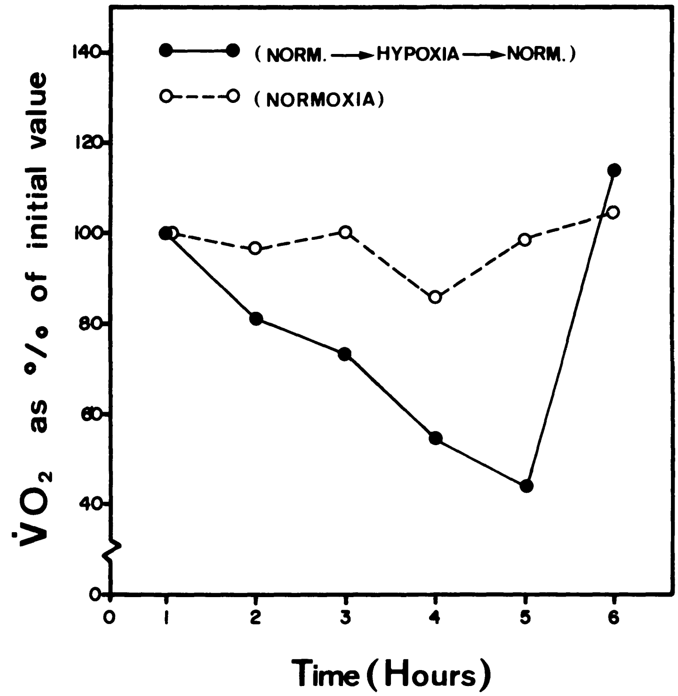


*Fig. 3. Oxygen consumption rate as a percentage of the initial* $\dot{V}O_2$ *in normoxia, as measured in* Pseudonannolene tricolor *exposed to a stepwise declining* $Po_2$*. The dashed line represents a control in constant normoxia.*

Thus tracheates contrast with protracheates (onychophores), in which cutaneous respiration still coexists with tracheal respiration and which are claimed to be conformers (Mendes and Sawaya 1958).

Regulation of respiration was found in an American spirobolid millipede, *Spirobolus* sp. (Daly and Siegel 1964), and in a julid millipede from Russia, *Chromatoiulus rossicus* (Byzova 1967). In both species regulation was detected down to 5% $O_2$. Similar respiratory regulations were found in *Rhinocricus padbergi,* a Brazilian spirobolid millipede (Penteado and Mendes 1978) and in *Plusioporus setiger,* a Brazilian spirostreptid millipede (Penteado 1987). Regulation down to as low as 2.5% $O_2$ was also recorded for *P. setiger,* according to Penteado (1987), during experiments using a stepwise decline in $Po_2$.

Millipedes, however, were also reported to be unable to regulate respiration in declining $Po_2$. According to Stewart and Woodring (1973), two American species, *Pachydesmus crassicutis* (a polydesmid) and *Orthoporus texicolens* (a spirostreptid) behave as oxyconformers from 15% $O_2$ (ca. 106

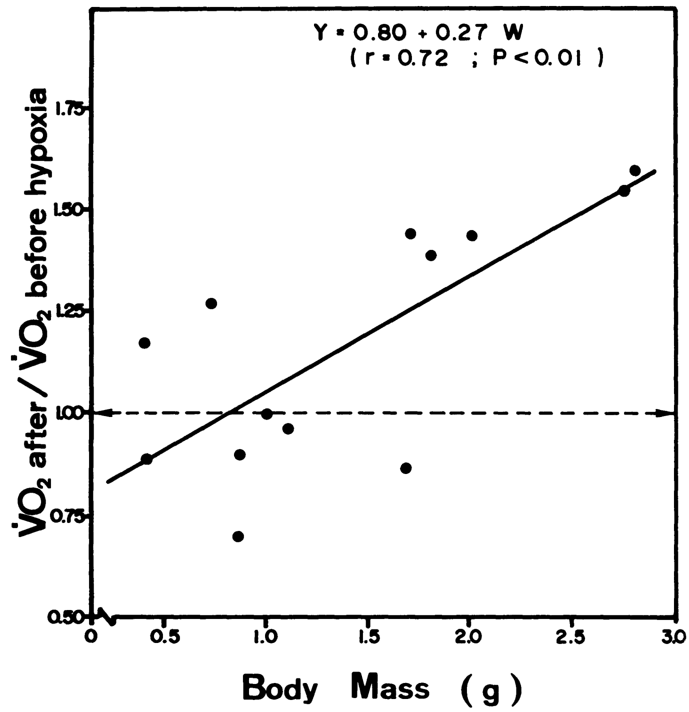


*Fig. 4. Oxygen debt index in relation to size (mass) in* Pseudonannolene tricolor. *Indexes higher than 1.00 mean that the oxygen debt is repaid.*

mmHg) downward. Stewart and Woodring claimed that conformity, not regulation, would be expected, since in both millipedes the respiratory gases simply diffuse in and out through the spiracles, although possibly aided by body movements. This functional morphology was also reported by Penteado and Mendes (1978) for *R. padbergi* and for *P. setiger* (Penteado 1987) and possibly occurs in *P. tricolor* and other millipedes. It is hard, however, to explain why millipedes differ in their responses to declining ambient $Po_2$. Differences in experimental procedures used, or interference with the animal mobility inside the respirometric flasks, might account for the divergences, as mentioned by Penteado and Mendes (1978), but ecological, genetic, and physiological factors should not be disregarded.

When *P. tricolor* was suddenly exposed to 18.76 or 7.08 mmHg $O_2$, the millipedes, as shown also by *P. setiger* (Penteado 1987), accumulated an $O_2$ debt that was paid off in 1 h on return to air. When exposed to a stepwise declining $Po_2$ over 5 h, $O_2$ debts acquired were also paid off in a sixth hour run in air. This is indicated by the ratios of $\dot{V}o_2$ after to $\dot{V}o_2$ before hypoxia (the oxygen debt indexes). In both experimental series, however, as claimed for *P. setiger* by Penteado (1987), the millipedes showed in a subsequent hour in normoxia a pattern of partial repayment (an "underrepayment," according to von Brand [1952], or a "sub-normal oxygen debt," as referred to by Herreid [1980]). This was assumed since, after the millipedes returned to air from hypoxia, the $\dot{V}o_2$ stayed between 11% and 160% above the hypoxic rates. But, in contrast with *P. setiger,* in which the effects of size were not significant (Penteado 1987), the $O_2$ debts paid off by *P. tricolor* bore a relation to size (larger animals showing higher indexes than smaller ones).

Underrepayment and overrepayment (respiratory rebound), may occur in different animals, but according to T. von Brand, in a personal communication quoted by Dwarakanath and Job (1974), "quite frequently much lower values are encountered, and there is really no good ground to assume that 100% repayment is the ideal situation, because there would be very little connection between the amount of oxygen repaid and that lost. All would depend on the nature of the anaerobiotic processes, on the question of whether or not anaerobiotic metabolites are stored in tissues, and the nature of the recovery processes."

Oxygen debt repayments in diplopods were also reported for the Brazilian spirobolid millipede *R. padbergi* (Penteado and Mendes 1978) and for the Indian spirostreptid millipede *Spirostreptus asthenes* (Dwarakanath and Job 1974). Dwarakanath and Job, however, dealt only with the $O_2$ debt acquired during submersion in water, and they reported that, when submersion was prolonged (up to 6 h), there was a decrease in the $O_2$ debt repaid. Similar decreases were also obtained by Dwarakanath et al. (1977), when dealing with the same species of millipedes, but they showed that the decreases were compensated by an increase of lactic acid in the hemolymph of the animals.

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