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Influence of body mass and environmental oxygen tension on the oxygen consumption rates of an enteropneust, *Glossobalanus crozieri*

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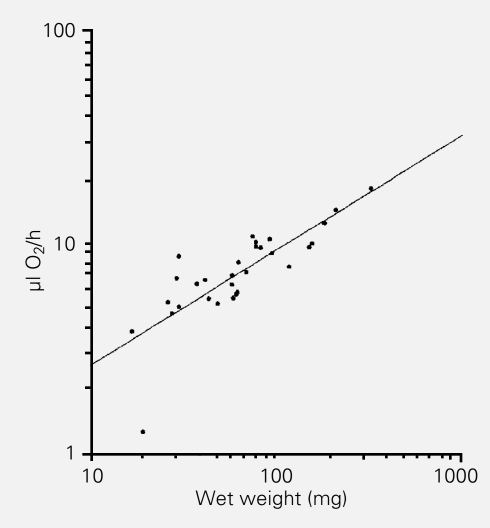
Abstract

The present study deals with a species of enteropneust, *Glossobalanus crozieri*, focusing on two aspects of its respiration: a) oxygen consumption and body mass, and b) the influence of environmental oxygen tension on the respiratory rate. Preliminarily, the body water content was shown to be 85% of the whole body weight. The regression coefficient of the oxygen consumption on the wet body mass (0.578) seems to agree with the view that in enteropneusts respiration is mainly cutaneous. The respiratory rate was significantly reduced at O_2 tensions from 76 mmHg downwards, suggesting conformity rather than regulation.

Key words
- Enteropneusta
- *Glossobalanus crozieri*
- Respiration
- Body mass
- Oxygen tension

Tongue or acorn worms are hemichordates of the class Enteropneusta, in which a short anterior structure is identified as notochorda. They are marine animals inhabiting mostly shallow waters and digging galleries in the sandy or muddy substrate. Different aspects of their biology have been studied (1,2), as well as their body chemical constituents (3-5), but their physiology has received much less attention, because of “their sluggish responses and tendency to break into pieces on handling” (2). Two of the existing papers refer to respiration. The ecology and respiration of *Ptychodera flava* from India were studied at both high and low tides (6). The respiratory rate (weight-specific oxygen consumption) determined at 25.5ºC was 0.762 µl O_2 for the smallest animal (89 mg), about 1.5 times that of the largest (375 mg). Individual variations in O_2 uptake were large. The authors calculated a linear regression of the observed data (Y on x) and obtained the following regression equation: $Y = -251x + 469.46$, where Y = weight and x = the rate of oxygen consumption (*sic*). The results also indicated a respiratory rhythm associated with the tidal cycle. In an accurate study of 31 invertebrates from 10 phyla, the oxygen consumption of *Sacchoglossus kowalevski* in the presence of declining oxygen (expressed as the term B_2, when the data were analyzed to fit the quadratic equation) showed regulation rather than conformity (7). We now describe a species found in Brazil, *Glossobalanus crozieri* van der Horst 1924, focusing on the influence of body

Figure 1 - Regression line of oxygen consumption *vs* log body mass in *Glossobalanus crozieri.*



mass and environmental oxygen tension on the oxygen consumption rate.

The worms were obtained from a small population of *Glossobalanus crozieri* van der Horst 1924 located in the intertidal zone of the Baleeiro isthmus near the Centro de Biologia Marinha of the University of São Paulo, at São Sebastião, SP. The animals were caught during low tide by carefully shovelling the sand with the fingers to obtain intact animals. In the laboratory, the animals were kept in small vessels, on a 2-cm thick layer of sand covered by running sea water. Before the experiments, the worms remained 24 h in filtered sea water to clear their guts. Due to the scarcity of females, only adult, sexually quiescent males were used. Cleaned of their mucous coat, the worms were placed individually in 15-17-ml flasks of a Warburg apparatus to measure their oxygen consumption at 25ºC. To avoid disturbing the animals, the flasks were not shaken, but as the animals were placed in a shallow amount of sea water (3 ml, 0.5 cm high), it was assumed that an effective gas exchange took place between the liquid and gas phases. The P_{O_2} of the sea water (34 S) at the collecting site, as measured at 25ºC with an oxygen electrode connected to a Beckman physiological gas analyzer (model 160), was 146 mmHg. The results are reported as $\dot{V}_{O_2}$ = μl O_2/h and $\dot{V}_{O_2}$/Mb = μl O_2 mg wet weight^{-1} h^{-1}. After the experiment, the animals were quickly washed in distilled water, blotted on filter paper to determine their wet weight and then placed in an oven (at 85ºC) until their dry weight reached a constant value.

The mean dry body weight of 30 worms (wet weight range: 17.5-324.1 mg), reported as percent of wet weight, was 15.13 (SD, 4.34). The regression equation of % dry weight on wet weight was $\%Wt_{dry} = 18.88\ Wt_{wet}^{0.02}$. Thus, the percentage of dry weight does not significantly change with increasing body weight. On the basis of the dry weight value, the body water content of *G. crozieri* amounts to 85% of the wet body weight.

When the hourly oxygen consumption of the same 30 worms was related to wet body mass, the regression equation was $\dot{V}_{O_2} = 0.663\ Wt^{0.578}$, $r^2 = 0.662$. This coefficient of determination was within the confidence limits and found to be significant (ANOVA). O_2 consumption (in μl/h) was 3.79 for the smallest worm and 19.470 for the largest. A log-log plot of the relation between body mass and oxygen consumption is shown in Figure 1.

The influence of environmental oxygen tension on respiratory rate was assessed using 4 groups of worms. The animals of each group were kept in sea water in equilibrium with atmospheric air (P_{O_2} = 146 mmHg) during the first hour. In the following hour, after gassing the flasks with N_2/O_2 mixtures of a desired O_2 percentage, the animals were exposed to reduced oxygen tensions (69.5, 34.8, 17.36 and 6.9 mmHg). The results are shown in Table 1 for groups of 6 worms. The respiratory rates in normoxia and at a lower oxygen tension and the hypoxic rates are also reported as percent of normoxic rates. One-way repeated measures analysis of variance applied to the data showed no significant differences among normoxic classes (P = 0.11). Medians were calculated for the results as percentage. When the Wilcoxon

Table 1 - Respiratory rates (µl O_2 mg wet weight^{-1} h^{-1}) and oxygen tension in an enteropneust, *Glossobalanus crozieri*.

P_{O_2} (normoxia): 146 mmHg. Sea water: 34 S. Temperature: 25ºC. N (per series): 6. *P<0.05 compared to normoxia (paired sample *t*-test); **Wilcoxon matched-pair test. S = Significant at P≤0.05; NS = nonsignificant.

P_{O_2} (mmHg)	Weight range (mg)	Mean respiratory rates (SD)		Hypoxic rates (% of normoxic)	Percent (medians)	P**			
		normoxic	hypoxic			(1)	(2)	(3)	(4)
69.5 (10%)	70.6-27.1	0.133 (0.024)	0.088 (0.018)*	66.9 (12.0)	69.0 (1)	-	NS	S	S
34.8 (5%)	97.1-42.6	0.097 (0.031)	0.060 (0.019)*	62.6 (6.1)	58.9 (2)	NS	-	S	S
17.36 (2.5%)	324.1-17.5	0.102 (0.072)	0.045 (0.022)*	44.7 (10.7)	55.4 (3)	S	S	-	S
6.9 (1%)	158.2-20.6	0.078 (0.026)	0.023 (0.011)*	30.0 (10.3)	27.3 (4)	S	S	S	-

matched-pair test was applied to detect acceptance or rejection of H_o between the percentage medians in the various groups, acceptance was only found when the 10% O_2 group was compared with the 5% O_2 group.

The only previous report on the body water content of enteropneusts seems to be that of De Jorge and Petersen (5), who studied the water distribution in different regions of the body in two species (*Balanoglossus clavigerus* and *Willeya loya*) from Brazil. The mean values found for *B. clavigerus* and *W. loya* were 83.2% (3.9) and 83.3% (2.3) of the whole body wet weight, respectively, as compared with 85% determined in *G. crozieri*. The sacs or branchial pouches of enteropneusts are small cavities between the pharyngeal clefts and the external dorsal pores which may participate in filtration or osmoionic processes. The well-developed blood irrigation of this region suggests (8) a site for respiratory exchange, but more probably respiration in acorn worms is mainly cutaneous (9). Assuming that a regression coefficient of the oxygen consumption on weight of about 0.6 means a rise in O_2 uptake with body surface rather than with body mass, the value determined in *G. crozieri* (0.578) is in accordance with a respiratory gas exchange preponderantly across the skin. Yet, the meaning of this coefficient in terms of surface or weight is open to discussion (10). Azariah et al. (6) studied the relation between O_2 consumption and body mass in *P. flava* using breeding worms collected during low and high tides and non-breeding worms during high tide only. However, they commented the results only in terms of the regression of weight on respiratory rate (weight-specific oxygen consumption) and provided no table listing the individual rates and respective weights. These parameters, however, can be obtained approximately from the points of a plot relating rate and weight, and from the results of individual oxygen consumption. When this was done for non-breeding worms collected during high tide, the individual O_2 uptake ranged from 59.6 to 96.5 µl, a rather small increase, considering the weight range (593-2173 mg, wet weight). The regression equation of uptake on body mass was $\dot{V}_{O_2} = 4.899\ M_b^{0.400}$, for $r^2 = 0.851$. The lack of statistical significance of the determination coefficient corroborated the limited variation of the O_2 consumption with increasing body mass. The tide factor was not considered in the case of *G. crozieri*, but in the laboratory the worms stayed in a sort of continuous "high tide", since they were maintained completely submerged in running sea water throughout the experiment. The animals were also in the non-breeding stage and O_2 uptake measurements were performed at about the same temperature as in the case of *P. flava*. Yet, as expected, in *G. crozieri* the oxygen consumption increased rather regularly with increasing weight (ca. 25 times in the weight range 7.5-340 mg *vs* 1.6 times in the weight range 59.6-96.5 mg, in *P. flava*).

Marine invertebrates living temporarily or permanently in burrows or galleries, periodically exposed to low oxygen, tend to be oxyconformers. Examples are the polychete *Nereis*, the sipunculid *Sipunculus* and the echiurid *Urechis.* Oxygen dependence or metabolic conformity to low environmental oxygen is mostly found in less active animals, such as intertidal or bottom dwelling invertebrates (11). No reference was found in the literature about the respiratory behavior of enteropneusts in declining oxygen tensions. In the present paper, water oxygen tensions are reported as P_{O_2} or O_2 percentage in the gas phase of the flasks, according to Schmidt-Nielsen (12). The results obtained for *G. crozieri* point to a significant decrease of the respiratory rate as the oxygen tension of the liquid phase declined. This agrees with the fact that this worm is a rather sluggish animal living in galleries dug in the sand of the intertidal zone, in which stagnant water may accumulate during ebb tide. In this case, oxyconformity would help the animals to face lesser oxygen availability. The reduction in O_2 uptake in this situation may also reflect a depression in the ventilation (water pumping) of the galleries to save energy in an adverse condition. The resort to anaerobiosis, however, cannot be ruled out.

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