THE RELATION OF ANIMAL SIZE TO OXYGEN CONSUMPTION IN SOME FRESH-WATER TURBELLARIAN WORMS

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(With Two Text-figures)

INTRODUCTION

In the course of work started four years ago on the metabolism of fresh-water turbellarians from different habitats it became necessary to investigate the effect which body size had on the respiratory rate of the worms. The work had to be discontinued at the outbreak of war, but at that time the data concerning the effect of size on oxygen consumption were fairly complete and the chief results, which are given in this paper, may be of interest in the general subject of the effect of animal size on metabolism.

The size factor in the metabolism of animals is now recognized as one which must be taken into account in all metabolic studies. A considerable amount of work has been done in the past from the point of view of the size factor itself, and an extensive literature on the subject now exists. It would be out of place to attempt here to review the work which has been done; the homoiotherms which have largely figured in past investigations present different problems from those of the poikilothermal animals such as the turbellarian worms on which my work was done, and a summary of previous work on the poikilotherms has recently been given by Wingfield (1939). I will therefore confine myself to the Turbellaria. The work of Allen (1918), on *Planaria agilis* and *P. maculata*, and that of Hyman (1919), on *P. dorotocephala*, *P. velata* and *P. maculata*, demonstrated the higher respiration per unit weight of smaller individuals; the analysis of the relation between size and respiration was not taken further.

MATERIAL AND METHODS

In the work described below the oxygen consumption of four fresh-water species of Turbellaria was investigated.

*Crenobia* (*Planaria*) *alpina* (Dana) is particularly characteristic of cold springs. According to Beauchamp & Ullyott (1932) it is typical of rheocrene springs—that is, the type of spring in which the water begins to flow immediately from the source. This was the type of habitat in which I found the animals used in my experiments. I investigated the respiration of animals from two sources: during the summer of 1937 at the Freshwater Biological Station, Wray Castle (Westmorland), where the animals were collected from a spring at a temperature of 12–13° C.; during the summer of 1938 at the Biologisches Station at Lunz, where the prevailing temperature of the spring from which the animals were collected was 7–8° C. At Lunz the animals reached a much larger size than those at Wray, individual worms often exceeding a wet weight of 17 mg., whereas
The largest worms at Wray rarely exceeded 6 mg. This is probably another case of the effect of environmental temperature on maximum size (Fox, 1939).

*Polycelis cornuta* (Johnson) is typical of cold brooks, tending also to replace *Crenobia alpina*, according to Beauchamp & Ulyott (1932), in springs of the limnocrene type where a stagnant pool is formed at the source. The animals used in these investigations were sent from Wray during the summer of 1939.

*Polycelis nigra* (Ehrenb.) is usually found in weedy ponds, but is also found to some extent on stones towards the edges of brooks. The animals used in my work were collected from a pond at Alvechurch, Worcestershire, in the spring and summer of 1939, during which time the diurnal temperature of the pond water fluctuated from 11.7 to 14.2°C.

*Planaria polychroa* (O. Schm.). I have observed this species to be present in weedy ponds only, but Whitehead (1921–2) summarizes its habitats as 'slowly running or stagnant water'. The animals used in my work came from a pond in Newdigate, Surrey,* and were collected during the spring and early summer of 1939.

Careful attention was paid to the treatment of the animals before their use in respiration experiments. All four species could be easily maintained in the laboratory, food being provided by pieces of *Gammarus pulex*. The animals were transferred immediately after collection to water artificially buffered with sodium bicarbonate so that it had the same alkali reserve as the water in which the animals were found in the field. This was considered necessary in view of the work of Anderson (1927) on the effect of alkali reserve on the oxygen consumption of *Planaria dorotocephala*. The respective alkali reserves were: *Crenobia alpina* 0.0004 N at Wray, 0.0030 at Lunz; *Polycelis cornuta* 0.0010 N; *P. nigra* 0.0025 N; *Planaria polychroa* 0.0030 N.

For the majority of experiments the oxygen consumption was determined at 14.5°C, and the animals were kept in the laboratory at this temperature. The animals were starved for 48–72 hr. before the experiments and were kept in dim light both before and during the experiments. The methods used in determining oxygen uptake were essentially those of Fox & Wingfield (1937, 1938). The animals to be tested were placed in glass stoppered respiration bottles which were completely filled with water artificially buffered to the appropriate alkali reserve. The oxygen content of the water in the bottles was determined at the beginning and end of a definite period of time by the method of Fox & Wingfield (1938). The respiration bottles were of 15 c.c. capacity for the smaller animals and of 75 c.c. capacity for the larger specimens, the number of animals introduced into each bottle varying with the size of the individual animal. A combination of size of bottle, size of individual animal and number of animals was chosen so that there should be a reduction of oxygen content of the water of 10–15% in 2 hr. The average number of animals used per experiment for each size group is given in Tables 1 and 2. The initial oxygen content of the water in the bottles was always approximately that of air saturation at 14.5°C, namely, 7.10 c.c. of oxygen per litre.

The experiments on *Crenobia alpina* at 7.0°C were performed in a similar manner, except that the animals were kept at 7.0°C before and during the experiments. The initial oxygen content of the water in the bottles in these experiments was approximately 8.0 c.c. of oxygen per litre.

* The animals were supplied by Mr L. Haig, to whom thanks are due for despatching them to Birmingham.
The oxygen consumption was calculated per unit wet weight of animals. A standard method of weighing was used and its reliability tested by repeated weighing of the same batch of animals. The animals survived the weighing procedure if returned to water, but in the experiments given in this paper a particular batch of animals was used only once.

RESULTS

The results of the experiments at 14.5° C. are summarized in Table 1 and Fig. 1. Table 1 gives the number of experiments done on each size group in each species and the average number of animals used per experiment for each size group. The size range given refers to the limits of the average weights of the animals used in the experiments of each group.

Table 1. Oxygen consumption of the different size groups of each species at 14.5° C.

<table>
<thead>
<tr>
<th>Species and locality</th>
<th>Size range mg. wet wt.</th>
<th>Average size mg. wet wt.</th>
<th>No. of exps.</th>
<th>Av. no. animals per exp.</th>
<th>Oxygen consumption cu.mm./g./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crenobia alpina (Wray Castle)</td>
<td>1'7- 3'0</td>
<td>2'3</td>
<td>19</td>
<td>75</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>3'2- 4'1</td>
<td>3'6</td>
<td>16</td>
<td>47</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>4'6- 5'9</td>
<td>5'4</td>
<td>11</td>
<td>42</td>
<td>148</td>
</tr>
<tr>
<td>Crenobia alpina (Lunz)</td>
<td>1'5- 3'9</td>
<td>2'8</td>
<td>6</td>
<td>17</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>4'1- 7'1</td>
<td>5'3</td>
<td>14</td>
<td>22</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>8'0-11'9</td>
<td>9'8</td>
<td>13</td>
<td>19</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>12'6-17'0</td>
<td>14'0</td>
<td>17</td>
<td>16</td>
<td>119</td>
</tr>
<tr>
<td>Polycelis cornuta</td>
<td>1'6- 2'8</td>
<td>2'3</td>
<td>10</td>
<td>22</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>3'1- 5'9</td>
<td>4'5</td>
<td>11</td>
<td>14</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td>6'3- 9'8</td>
<td>8'3</td>
<td>7</td>
<td>7</td>
<td>159</td>
</tr>
<tr>
<td>Polycelis nigra</td>
<td>1'5- 2'8</td>
<td>2'2</td>
<td>7</td>
<td>16</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>3'1- 4'8</td>
<td>4'0</td>
<td>11</td>
<td>14</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>5'0- 6'8</td>
<td>5'9</td>
<td>11</td>
<td>11</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>7'2-10'8</td>
<td>8'7</td>
<td>9</td>
<td>6</td>
<td>176</td>
</tr>
<tr>
<td>Planaria polychroa</td>
<td>1'7- 4'7</td>
<td>3'5</td>
<td>11</td>
<td>15</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>5'0- 6'3</td>
<td>5'8</td>
<td>9</td>
<td>11</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>7'6- 9'7</td>
<td>8'2</td>
<td>9</td>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>10'0-12'3</td>
<td>10'8</td>
<td>9</td>
<td>8</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>13'1-14'7</td>
<td>14'2</td>
<td>7</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>15'1-16'6</td>
<td>16'9</td>
<td>7</td>
<td>5</td>
<td>97</td>
</tr>
</tbody>
</table>

The mean oxygen consumption for the experiments on each size group is given in cu.mm./g./hr. The standard errors of the means are not given in the table, but they never exceeded 6.6% of the mean oxygen consumption value. This indicates, I think, that the turbellarians used in these experiments were quite uniform from the metabolic viewpoint, since larger standard errors are more general in metabolic studies of this nature.

It will be seen that, with the exception of Polycelis nigra, the small animals of each species have a higher oxygen uptake per unit weight than the large ones. The graph relating size and respiration per unit weight for these three species is a curve of a hyperbolic or exponential form.

In the case of P. nigra, however, small animals have a lower respiratory rate than large ones. Since the standard errors of the means were in this case rather large compared with the differences between the means I thought it necessary to apply further statistical analysis to test the correlation between size and respiration. The data were considered as a case of curvilinear regression and the correlation ratio \( r \) worked out according to the procedure given by Peters & Van Voorhis (1935). The correlation ratio for respiration per unit weight \( W \) is 0.560 with a probable error of 0.075. Since
the correlation ratio is more than seven times its probable error a definite correlation between \( r \) and \( W \) can be said to hold. The reason why \( P. \) nigra is different from the other three species is quite obscure and the matter requires further investigation.

Returning to a consideration of the other three species the question arises as to whether the size factor obeys the surface law of Rubner (1883). This well-known law, relating metabolism to size, postulates a constancy of the metabolic rate per unit body surface and would require in the present instance that the respiration per unit weight (\( r \)) should be related to the individual body weight (\( W \)) according to the formula \( r = kW^{-\frac{1}{4}} \), \( k \) being a constant for a particular species under a particular set of conditions. Since Voit (1901) originally proposed that the surface law should hold for cold-blooded invertebrates as well as for the warm-blooded vertebrates with which Rubner was largely concerned, several investigators have demonstrated that it does in fact hold for many different animal phyla. The most important work of this nature on invertebrates which has come to my notice is that of Cohnheim (1901) on Holothuria; Hesse (1910) on Helix; Weinland (1918) on Anodonta; Hino (1929) on the earthworm Pheretima; Delpech & Terroine (1931) on Helix; Krüger (1940) on Ascaris. So far as the cold-blooded aquatic vertebrates are concerned there is only the work of Cronheim (1911) which shows the application of the
surface law to the respiration of certain fishes, although I have found that the law can be demonstrated to hold also for the data given by Wells (1935) for the oxygen consumption of the fish Fundulus. Several other investigators have demonstrated the existence of a size factor in the metabolism of invertebrates but have not analysed their results from the point of view of the surface law. Most of these references are given in Wingfield (1939), but the work of Scherbakov (1935) on planktonic Crustacea is not mentioned.

In the present work it became apparent that the turbellarian worms are particularly suitable for the analysis of the size factor of oxygen consumption in terms of the surface law. Most of the previous work has depended on the application of the formula of Meeh (1879) for obtaining the surface area of an animal from its weight. Meeh stated that the surface area of an animal was proportional to the $\frac{2}{3}$ power of its weight, and the truth of this has several times been demonstrated, particularly for warm-blooded animals. The computation of the surface area of an invertebrate is often, however, a difficult matter owing to the irregularity of the surface and, very often too, to the existence of respiratory surfaces wholly or partly on the outside. This difficulty has led in the past to the attribution of different meanings to the 'surface' as found in Meeh's formula (Voit, 1901; Weinland, 1918; Pfaunder, 1921). Since, however, Turbellaria have a simple body surface and the proportions of the animals are largely maintained for different sizes of animals, Meeh's formula must hold with a considerable degree of accuracy.

Nevertheless, quite apart from the anomalous case of Polycelis nigra, it is obvious from Fig. 1 that the surface law does not always hold even for the other three species. Planaria polychroa has only a small size factor and the surface law is certainly not obeyed. The adherence to the law which is shown by Polycelis cornuta and Crenobia alpina at 14.5°C can be seen from Fig. 2 (the upper two graphs) in which the logarithm of $r$ (respiration per unit weight) has been plotted against the logarithm of $W$ (individual weight). If the surface law holds, such a graph should be a straight line with a slope of $-\frac{2}{3}$. It will be seen from Fig. 2 that the graphs in the two cases under discussion do in fact approximate to straight lines with slopes of $-\frac{2}{3}$, particularly if the points for the largest $C. alpina$ are disregarded. The slope for $C. alpina$ works out at $-0.34$, which is a good approximation to the theoretical value of $-0.33$, so that it may be assumed that the surface law does hold in this case. For Polycelis cornuta, however, the slope is $-0.18$, which is a considerable divergence from the theoretical requirement and it must be concluded that the respiration per unit surface area is not constant in $P. cornuta$ but becomes progressively lower in smaller animals.

It is possible that there are other factors affecting metabolism which are determined by size of animal. In this case we may expect a divergence from the surface law which depends very largely on the existence of a direct relationship between size and metabolism, although no explanation of this relationship has been given which can be verified by further experiment. This latter problem has recently been discussed by Krüger (1940), but the whole subject seems to me to be very controversial and not profitable for discussion until more work, particularly that of a biochemical nature, has been done on the immediate reasons for the increased metabolism of small animals. However, it is of great interest to investigate other factors which may bring about the size relationship indirectly, particularly age and sexual maturity. I intended to investigate these in the Turbellaria, particularly as it seemed likely that the larger worms would be more sexually mature.
However, I have as yet no conclusive data on this point. Seasonal variations of metabolism, such as those noticed by Wells (1935) in Fundulus and by Morgan & Wilder (1936) in the mayfly nymph Hexagenia, could not have vitiated the results given above, since the work on each species was completed within a few weeks and an attempt was made also to include the whole size range in each series of experiments.

Comparatively little work has been done on the effect of other environmental conditions on the size factor in metabolism. The state of nutrition is almost certainly of importance and Allen (1919) has shown for Planaria maculata and P. agilis that the respiration per unit weight decreases on starvation after feeding until it reaches a constant level, although during the whole of this period the weight of the individual animal is gradually decreasing. In my investigations I aimed at achieving a normal and standard state of nutrition for the experimental animals.

Hotovy (1938), using the branchiopod crustacean Triops (Apus) cancriformis, has demonstrated the dependence of the size factor on temperature; it was diminished at lower temperatures. I have investigated the effect of temperature in the case of Crenobia
alpina. The experiments were done at Lunz at 7° C. at about the same time as the 14.5° C. experiments, so that the two sets of results can be compared for the effect of temperature on the size factor. The results for different sizes at 7° C. are given in Table 2 and illustrated in Fig. 2 (lower graph). It is clear that, in the case of C. alpina, a decrease of temperature of 7.5° C. has only a slight diminishing effect on the size factor. The slope of the logarithm graph as obtained from Fig. 2 is −0.31, which is only slightly different from the 14.5° C. value of −0.34 and is still a good approximation to the −0.33 theoretically required by the surface law.

Table 2. Oxygen consumption of Crenobia alpina at 7.0° C. Experiments done at Lunz

<table>
<thead>
<tr>
<th>Size range mg. wet wt.</th>
<th>Average size mg. wet wt.</th>
<th>No. of exps.</th>
<th>Av. no. animals per exp.</th>
<th>Oxygen consumption cu.mm./g./hr.</th>
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<tr>
<td>o.9—2.3</td>
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<td>7</td>
<td>28</td>
<td>141</td>
</tr>
<tr>
<td>3.1—5.1</td>
<td>4.1</td>
<td>7</td>
<td>31</td>
<td>101</td>
</tr>
<tr>
<td>6.5—11.1</td>
<td>8.5</td>
<td>7</td>
<td>22</td>
<td>82</td>
</tr>
<tr>
<td>12.4—17.4</td>
<td>14.3</td>
<td>8</td>
<td>16</td>
<td>75</td>
</tr>
</tbody>
</table>

The place of origin of the experimental animals has no apparent effect on the nature of the size factor. This is well brought out in a comparison of the results obtained for C. alpina at Wray and Lunz. Fig. 1 shows that there is good agreement between the two sets of results, and this is despite the fact that the prevailing environmental temperatures were different at the two places (12—13° C. at Wray, 7—8° C. at Lunz), and that the size ranges at the two places were 1.7—5.9 mg. per animal at Wray and 1.5—17.0 mg. per animal at Lunz.

With regard to the magnitude of metabolic rate for the different species it will be noticed that for the species which show a normal size factor (i.e. small animals having a higher metabolic rate than large ones) the stream species have a higher respiratory rate than the pond species. This is in agreement with the findings of Fox et al. (1933, 1935, 1937) for other fresh-water poikilotherms.

SUMMARY

1. A size factor is demonstrated for the oxygen consumption at 14.5° C. of four species of fresh-water Turbellaria obtained from different habitats.

2. For three of the species studied (Polycelis cornuta, Crenobia alpina and Planaria polychroa) small animals were found to have a higher oxygen consumption per unit weight than large ones. In the case of Polycelis nigra small animals were shown to have a lower oxygen consumption per unit weight than large ones.

3. The adherence of the size factor to the surface law of Rubner is discussed. Only Crenobia alpina satisfies the theoretical requirements of the law, and in this species it is shown that the size factor is practically unchanged when the oxygen consumptions are determined at 7.0° C. instead of 14.5° C.

I am very grateful to Prof. H. Munro Fox, F.R.S., of Bedford College, London, for the constant encouragement he gave me during the course of the work and for the valuable help given during its publication.
REFERENCES