

THE OXYGEN CONSUMPTION OF AN ECHIUROID, *BONELLIA VIRIDIS* ROLANDO

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The Echiuroidea comprise an interesting group of animals which have, nevertheless, received scant attention as far as their physiology is concerned. All are marine, living in rock crevices or burrowing in the sand. The oxygen consumption of *Urechis caupo*, a sand burrower, has been investigated by Hall (1931), but no other reliable reports have appeared concerning respiration in the group. The present work is an investigation of the oxygen consumption of the crevice-dwelling *Bonellia viridis*. This animal has in the past been the subject of two lines of inquiry. First, the long series of publications concerning the method of sex determination, starting with the work of Baltzer during the beginning of the century and culminating in the recent conclusive demonstration by Wilczynski (1960) that sex is indeed determined genetically, and not by environmental influences as had been thought previously. The second line of inquiry has concerned the pigment bonellin, which gives the animal its characteristic dark green colour, and which is known to be toxic to a wide variety of invertebrates and indeed even to *Bonellia* itself. Lallier (1955) showed that the cell disorganization induced in young gastrulae of *Paracentrotus lividus* by an extract of bonellin occurs much faster in the light than in the dark. A gradual degeneration of the tissues occurs in a specimen of *Bonellia* exposed to the light. Lallier, therefore, suggested that the presence of the pigment in *Bonellia* could be held responsible for 'son comportement lucifuge et ses habitudes nocturnes'. The animal certainly seems to prefer darkness, both in its natural environment (J. Theodor, personal communication) and in a laboratory aquarium. The animal seeks out a rock crevice and conceals its body some centimetres within, only the long and mobile proboscis being projected outside to fulfil its ciliary feeding function. It is not known whether *Bonellia* possesses photoreceptors or not. Although their presence is very likely it is just possible that movement into the dark from the light is a direct result of the irritant pigment promoting muscular activity. The present work, in the light of these remarks and the general lack of information about the physiology of *Bonellia*, was designed with three ends in view. First, to measure oxygen consumption by a reliable method in order to compare it with that typical of related groups such as the annelids, secondly, to investigate the effects of light and darkness on the oxygen consumption and, thirdly, to compare the respiratory rate of an isolated proboscis with that of the body region alone, both in light and in darkness.

The apparatus used was essentially similar to the continuous-flow respirometer used by Brafield and Chapman (1967). A specimen of *Bonellia* was usually placed in a glass tube approximately 25 cm. long and 4 cm. in diameter, but in the case of small specimens a tube about 15 cm. long and 3.5 cm. in diameter was used. A constant

and continuous flow of water was maintained through this respiratory chamber in the way described by Brafield & Chapman (1967). Generally the flow rate was maintained at 7 ml. per minute but occasionally, with particularly large specimens, a slightly faster rate was found to be more suitable.

The oxygen concentration of the water which had just left the respiratory chamber was measured polarographically. The signal from a Beckman 39065 oxygen sensor was fed through a 96260 oxygen adaptor box to a Beckman expandomatic pH meter (model 76). During an experiment the oxygen concentration of the water was recorded from the dial reading at 5 min. intervals. When desired for calibration and checking purposes the aerated water from the stock water aspirator could be passed across the sensor by means of a tube by-passing the respiratory chamber. Water immediately beneath the sensor was agitated by a magnetic flea driven by a small electric motor. The respiratory chamber and the stock water aspirator were immersed in a constant-temperature water bath, at $22 \pm 1^\circ \text{C}$. This temperature is realistic as that of the sea in the locality from which the specimens were collected was about 23°C . at the surface and about 20°C . at 10 m. depth during the period in which the experiments were conducted. The water used had a chlorinity of 21‰.

For experiments concerning oxygen consumption in the dark the respiratory chamber was closely wrapped in two layers of silver foil. When measuring oxygen consumption in the light, the light intensity immediately beside the respiratory chamber was measured at intervals by means of a suitably calibrated waterproof light meter. The light intensity measured on such occasions varied, but always lay between 1000 and 4000 lux. The animals normally experience light intensities in this range, for at a depth of 12 m. in the area from which the specimens were collected the light intensity in the afternoon is normally within the range 2000 to 5000 lux during the summer (Brafield, Chapman & Theodor, 1965).

Bonellia is a difficult experimental animal for three main reasons. First, the proboscis is very long and thin in comparison with the body, creating difficulties in arriving at an optimal size and shape for a respiratory chamber. Secondly, the proboscis is extremely mobile and the animal tends to insinuate it into the minutest channel. Consequently, the effluent tube from the respiratory chamber, although of capillary tubing, became blocked from time to time by the animal introducing its proboscis into it. No reliable method was devised to avoid this, with the result that many experiments had to be discontinued before it was desired to do so. Thirdly, *Bonellia* is not a common animal, nor is it easy to collect.

Knowing the flow rate through the respiratory chamber, the oxygen concentration of the water entering it, and the dry weight of the animal, the oxygen consumption was calculated for each 5 min. record of the oxygen concentration of the water which had just left the chamber. These values for oxygen consumption were then averaged by consecutive groups of three, each then yielding an average oxygen consumption for the quarter of an hour in question. No experimental period less than an hour has been considered in the treatment of the results, and most represent consecutive recordings of six hours or more. The mean oxygen consumption of all records for each of the seven entire specimens used are shown in Table 1. Each value for oxygen consumption is the mean of results for experimental periods of between 2 and 10 hr. The values for consumption in the dark are graphed against the dry weight of the

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specimen in Fig. 1. The customary reduction in consumption with increasing body weight is apparent. Hall (1931) found the average value for the oxygen consumption of *Urechis caupo* to be 0.00021 c.c./g./min. This is apparently in terms of the wet weight of the animal, but as it has been shown (Brafield & Chapman, 1965) that expressing consumptions in terms of dry weight is more satisfactory in several respects,

Table 1. *The mean oxygen consumption of seven specimens of Bonellia*

Oxygen consumption (mg./g. dry wt./hr.)		Percentage increase in consumption in the light	Dry weight of the specimen (g.)
In darkness	In light		
0.198	—	—	2.233
0.273	—	—	1.185
0.314	0.415	32.2	1.182
0.335	0.403	20.3	0.660
0.379	—	—	0.663
0.464	—	—	0.369
0.511	0.728	42.5	0.331

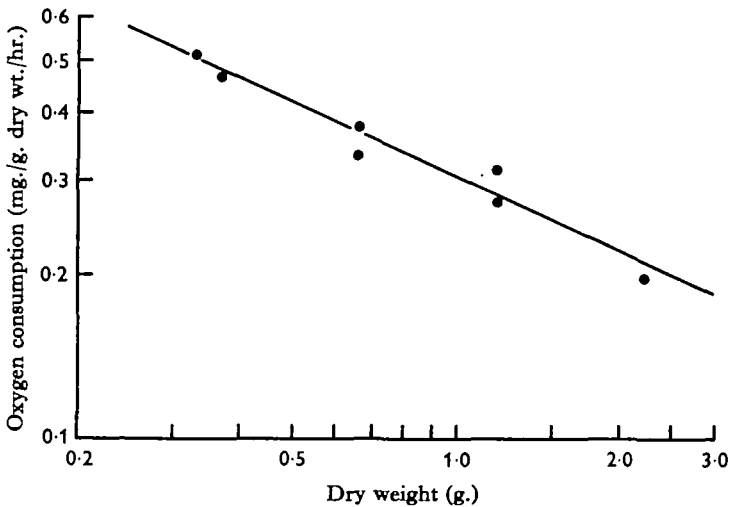


Fig. 1. The relation of mean oxygen consumption in the dark to dry weight for the seven entire specimens studied. Logarithmic scales.

dry weights have been used in the present work. With *Bonellia*, the average value for dry weight as a percentage of wet weight was 10.3%. Assuming a value of 10% for *Urechis* (the two animals are broadly similar in form), Hall's average value can be written as 0.18 mg./g. dry wt./hr. This is less than the average for *Bonellia* of about 0.3 mg./g. dry wt./hr. (Table 1). The oxygen consumption of polychaetes is generally about 0.2 mg./g. dry wt./hr. (Brafield & Chapman, 1965), a broadly similar rate of oxygen consumption to that found for *Bonellia*.

The increase in oxygen consumption when in the light is striking. Table 1 shows the average percentage increase in consumption in the light of three specimens. It appears that in general *Bonellia* increases its oxygen consumption by about 30% when in the light. Fig. 2 shows the changes in oxygen consumption during a typical

experiment involving exposure to light for a period, in this case one of the experiments with the specimen of 0.331 g. dry weight which together provided the average values for consumption in the light and dark shown in the bottom line of Table 1. It can be seen from Fig. 2 that the increase in consumption is almost immediate when light is introduced, and remains at a high level until darkness is restored, whereupon the consumption falls. The fall in consumption on return to darkness was often almost immediate, but occasionally, as in Fig. 2, there was some delay before the consumption rate typical of dark conditions was resumed. (For this reason values for oxygen consumption in the dark after a period of light were not used in calculating the figures for average dark consumption of Table 1.) In the experiment shown in Fig. 2 the

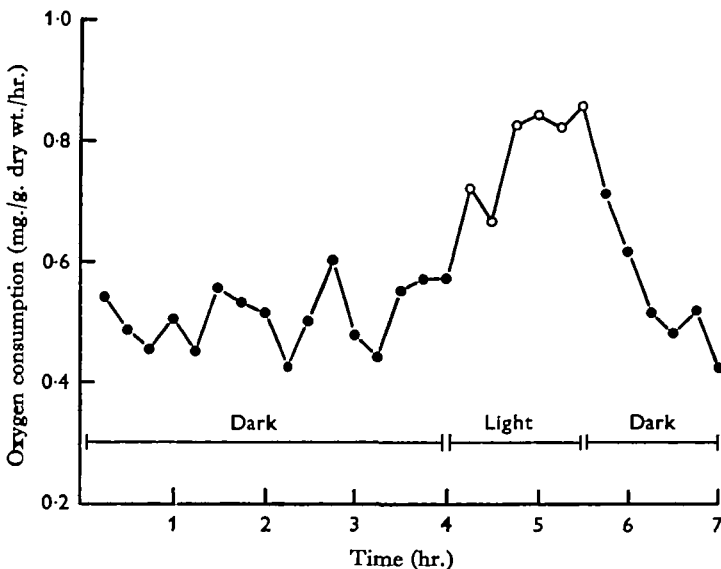


Fig. 2. The variation in oxygen consumption during a typical experiment involving exposure to light. Each point is the mean of three consecutive values for oxygen consumption calculated from records of the effluent oxygen concentration taken at 5 min. intervals.

animal was exposed to the light for $1\frac{1}{2}$ hr., and this was the general practice. In no experiment was an animal exposed to the light for more than 3 hr., in order to minimize the damage resulting from the phototoxic effects of the bonellin.

The higher oxygen consumption in the light is probably due at least in part to increased general activity of the animal. In darkness the proboscis is active but the body generally immobile. In the light, however, the body is soon thrown into the regular sequence of movements shown in Fig. 3. This cycle of contraction and relaxation continues steadily throughout a period of exposure to light, and may represent an attempt to move into a darkened area. The sequence of movements seems to be similar in nature to the respiratory peristaltic waves occurring in sand-dwelling echiuroids, such as those of *Echiurus* (Gislén, 1940) and *Urechis* (Redfield & Florkin, 1931).

In order to determine the proportions of the total oxygen consumption due to the proboscis and to the body region, and to investigate the effect of light on the oxygen

consumption of these two regions, series of experiments were conducted using firstly an isolated proboscis and then the body from which the proboscis had been removed. The mean oxygen consumption derived from such experiments with two specimens are shown in Table 2. The shortage of animals and the general difficulties of experimenting with *Bonellia* prevented complete cycles of experiments (with the entire

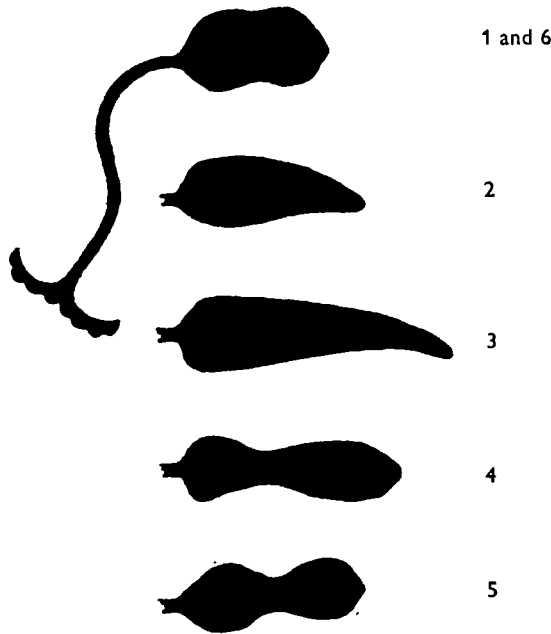


Fig. 3. The sequence of movements of the body of *Bonellia* when exposed to light. The proboscis is drawn in one case only.

Table 2. *The oxygen consumption of an isolated proboscis and of the remaining body region compared with the consumption of the entire specimen, for two animals. The specimens were in darkness throughout the experiments from which these means were calculated*

Oxygen consumption (mg./specimen/hr.) (Dry weights (g.) in brackets)				Oxygen consumption (mg./g. dry wt./hr.)		
Proboscis only	Body region only	Sum of first two columns	Entire animal	Proboscis only	Body region only	Entire animal
0.084 (0.111)	0.200 (0.552)	0.284 (0.663)	0.251 (0.663)	0.757	0.362	0.379
0.119 (0.164)	0.095 (0.496)	0.214 (0.660)	0.221 (0.660)	0.725	0.191	0.335

specimen, with the proboscis alone, and with the body region alone) being completed with more than two animals, but the results from these two correspond fairly closely.

It can be seen from Table 2 that the figures in the third and fourth columns are very similar. This implies that an entire specimen respire at approximately the same rate as the sum of the parts when isolated. Apparently, an isolated proboscis consumes oxygen at about the same rate as when it is part of the entire animal, and similarly for

the body region. Thus it would seem that no physiological stress results from the proboscis breaking away from the body. Certainly such an event must be fairly common, for the proboscis, protruding from a rock crevice, may well be accidentally torn from the body, or taken by a predator, while the body region remains concealed. (It is impossible to withdraw *Bonellia* from its crevice by pulling the proboscis; the latter merely breaks away.) Animals which have lost the proboscis are not uncommon, and they can readily regenerate one (Wilczynski, personal communication). An isolated proboscis is relatively short-lived but can survive for several days, although it cannot regenerate a body.

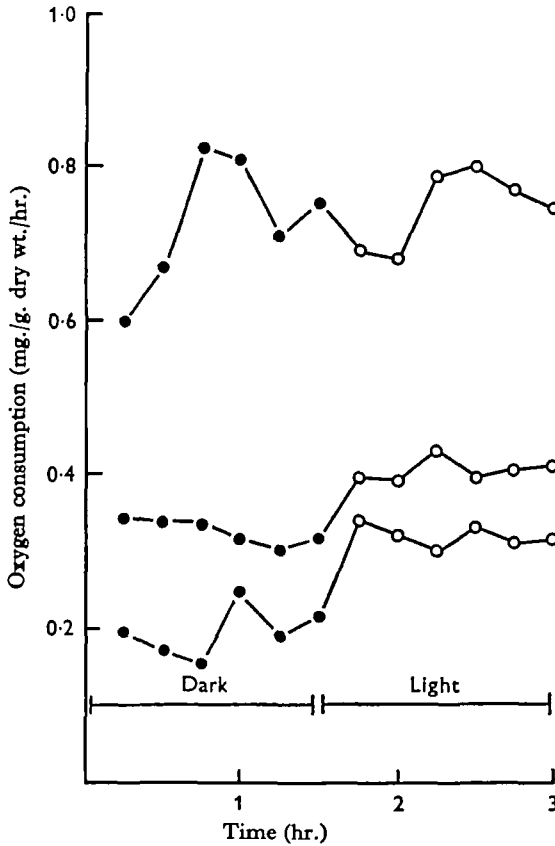


Fig. 4. The variation in oxygen consumption during a typical experiment involving exposure to light of an entire specimen (centre line), its isolated proboscis (top line) and its body region alone (bottom line). The points are derived as in Fig. 2.

Table 2 also shows that the proboscis of *Bonellia* represents only about a sixth to a quarter of the dry weight of the entire animal. Nevertheless, the proboscis has a much higher rate of oxygen consumption per unit dry weight than the body region. This probably reflects the activity of the very numerous cilia on the proboscis, leading to a high respiratory rate as compared with the relatively inactive body region. In one case the respiratory rate per unit weight of the proboscis is more than twice that of the body; in the other specimen almost four times as great.

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Further experiments indicate that whereas the consumption by an isolated body region is much greater when in the light than when in darkness, an isolated proboscis respire at about the same rate in the light as in the dark. This is seen in Fig. 4, which shows the changes in oxygen consumption during a typical experiment with the second specimen of Table 2. All results for this animal yield an average percentage change in oxygen consumption in the light in terms of consumption in the dark of +2.6% for the proboscis alone and +64.4% for the body region alone. Altogether, experiments involving exposure to light were carried out with three isolated proboscides and with two isolated body regions. The results are summarized in Table 3. The mean percentage change in consumption in the light for the proboscides is -6.3% and for the body regions +46.6%. Clearly it is the increased oxygen consumption by the body region when in the light which is responsible for the rise in consumption in the

Table 3. *Summary of experiments with parts of Bonellia in the dark and in the light*

	Oxygen consumption (mg./g. dry wt./hr.)		Percentage change in consumption in the light	Dry weight (g.)
	In dark	In light		
Proboscis	0.725	0.744	+2.6	0.164
only	0.725	0.612	-15.6	0.232
	0.557	0.524	-5.9	0.309
Body region	0.362	0.466	+28.7	0.552
only	0.191	0.314	+64.4	0.496

light of entire animals. This implies that, if photoreceptors are responsible for initiating the increased activity, the body region must possess receptors, for a response to light occurs even when the proboscis is absent. Although no rise in consumption in the light occurs in the case of an isolated proboscis, it cannot be assumed that the latter contains no receptors, for it may be that sensory tracts extend from proboscis receptors to a nerve centre in the body region, from which motor tracts return to the proboscis. Severing the proboscis in such a situation would make increased muscular activity of the proboscis in the light impossible, in spite of the presence of proboscis receptors.

Increased oxygen consumption by the body region of *Bonellia* in the light is probably almost entirely due to the muscular activity already described. These movements occur in an animal from which the proboscis has been removed in exactly the same way as in the entire specimen. The increased metabolic reactions which one may assume to occur in the light to combat the toxic effects of the pigment could account to some extent for the increased oxygen consumption. This is unlikely, however, because the proboscis has bonellin and yet no increase in consumption in the light is discernible. It is very doubtful, therefore, whether the pigment has any significant effect on respiratory rate. It is more probable that photoreceptors are present, and that these initiate the increased muscular activity of a specimen of *Bonellia* in the light.

SUMMARY

1. The oxygen consumption of the echiuroid *Bonellia viridis* has been investigated by means of a continuous-flow polarographic respirometer.
2. The general rate of oxygen consumption per unit dry weight is similar to that characteristic of polychaetes, and declines exponentially with increasing body size.
3. The rate of oxygen consumption rises in the light and falls again if darkness is restored.
4. The oxygen consumption of the isolated proboscis plus that of the isolated body region corresponds closely to that of the entire animal.
5. The oxygen consumption per unit dry weight of the proboscis is considerably higher than that of the body region.
6. The oxygen consumption of an isolated body region increases in the presence of light, but that of an isolated proboscis does not.
7. These findings are discussed in relation to the biology of the animal, observed muscular activity, and the occurrence of the pigment bonellin.

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