

THE RESPIRATORY PHYSIOLOGY
OF THE MARINE NEMATODES *ENOPLUS BREVIS*
(BASTIAN) AND *E. COMMUNIS* (BASTIAN)

I. THE INFLUENCE OF OXYGEN TENSION AND BODY SIZE

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INTRODUCTION

Rogers (1962) has stressed that the major factors influencing the nematode's ability to obtain oxygen by diffusion are the environmental oxygen tension and its body size – especially its body radius. Both he and von Brand (1966, 1968) suggest that the smaller intestinal parasites, such as *Nippostrongylus brasiliensis* (= *muris*), maintain an aerobic metabolism, but that larger nematodes like *Ascaris lumbricoides* lead predominantly anaerobic lives. Saz (1969) considers that stages of certain nematodes, including adult *A. lumbricoides* have no oxygen requirement for normal production of energy. In contrast, Smith (1969*a, b*) raises some fundamental objections to the concept of a unique parasite metabolism. He considers that *A. lumbricoides* may rely on oxygen derived from intermittent contact with the mucosa to raise the level of aerobic metabolism possible within the gut lumen.

Less is known of the oxygen requirements of plant-parasitic or free-living nematodes. Some marine forms have been reported to inhabit muds apparently devoid of oxygen (Moore, 1931; Wieser & Kanwisher, 1961), but the concentration of this gas in the environment does influence the life processes of most plant-parasitic and free-living forms investigated. Low-oxygen concentrations reduce the rate of emergence of larvae from the cysts of *Heterodera schachtii* (Wallace, 1956) and from the eggs of *Meloidogyne* species (Collis-George & Wallace, 1968; Wallace, 1968). The mobility of *M. incognita* larvae is also dependent upon oxygen concentration (Baxter & Blake, 1969). Cooper, van Gundy & Stolzy (1970) have shown that an occasional reduction in the ambient oxygen concentration lowered the rate of reproduction of *Aphelenchus avenae* and *Caenorhabditis* spp. in cultures, and *Hemicycliophora* on tomato plants; the aeration of soils is not constant and may, at times, be greatly reduced (Wallace, 1964). Bair (1955) demonstrated that the onset of a fall in respiration with decreasing oxygen tension occurred at higher oxygen tensions for the soil nematode *Rhabditis elegans* than for the facultative skin parasite *R. strongyloides* from a lower environmental oxygen regime.

This work considers the influence of oxygen tension on oxygen consumption of two species of marine nematodes from different habitats. *Enoplus brevis* occurs in an estuarine mud within the depth of oxygen penetration (Atkinson, 1972); *E. communis*

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is found in *Mytilus* beds, and is exposed to a more constant and a greater level of oxygen concentration. They are the only free-living nematodes shown to possess haemoglobin. In *E. brevis*, the pharynx of both sexes and the tail musculature of males contain considerable quantities of haemoglobin; *E. communis* contains little of this pigment (Ellenby & Smith, 1966). The study of their respiratory physiology has proved a useful basis for further work on the functional significance of the haemoglobin of *E. brevis* (Atkinson, 1973, in preparation).

The measurement of the oxygen consumption of single animals has been preferred to methods using many individuals. In this way, the effect of varying the oxygen tension could be more accurately investigated, for the experimental conditions could be more easily defined. Furthermore, the metabolic capacity of individuals with a weight-dependent metabolism cannot be accurately estimated from the rate measured for a group for the latter is altered by the distribution of sizes within the sample (Ellenby, 1953).

MATERIALS AND METHODS

The oxygen consumption of individuals of both species was examined at four oxygen tensions, 135, 75, 35, and 12 Torr. The highest approaches that of the sea; 75 Torr is approximately half that of the oxygen in air; 35 Torr is the upper limit of those oxygen concentrations termed microaerobic by Cooper *et al.* (1970), and 12 Torr is the lowest value which could be conveniently studied. All experiments were at 15 ± 0.1 °C. In each case individuals were stored at the temperature and oxygen tension of measurement for at least 12 h. In order to avoid possible differences due to sex only adult males were studied, but it should not be concluded that results for females would necessarily differ from those presented in this series of papers.

Collection and storage

E. brevis was collected from the salt marsh at Budle Bay, Northumberland (NU 147347). A sample of the upper 2 cm of the mud was carefully sieved (40-mesh/inch), and *E. brevis* was collected from the matter retained. *E. communis* occurs at Bamburgh in *Mytilus* beds on rocks exposed at low tide (NU. 202341). The matt bound by the *Mytilus* byssus threads was teased apart and *E. communis* was recovered from the debris. Both species were removed from these substrates within 24 h of collection, usually within 6 h.

Each individual was carefully cleaned of the adhering matter with a camel-hair brush, and one or two were placed in each of a number of 10 ml Polythene pots containing filtered sea water. All individuals were stored at 15 ± 1 °C, and at the oxygen tension required for a series of experiments. Providing the water is changed frequently and accumulating debris removed, the nematodes will survive for a period of weeks. However, they were discarded as a routine after 9 days.

Oxygen consumption

For the series of experiments at 135 Torr the pots containing individuals were left exposed to the atmosphere. After at least 12 h the oxygen consumption of the individuals was measured as described in detail previously (Atkinson & Smith, 1973). Essentially, a Radiometer oxygen electrode and ancillary equipment were used for

continuous monitoring of the oxygen tension within a constant-temperature chamber of 250 mm³. After the electrode had been calibrated, the nematode was placed under a net envelope within the cell, which was then filled with freshly filtered sea water and sealed. A slowly rotating micro-stirrer ensured that the oxygen consumed by the nematode caused a steady fall in oxygen tension, and this was recorded potentiometrically for 1 h. Fresh sea water was allowed to flow through valves into the cell before continuing for a second, similar period. The mean of these two rates, usually in reasonable agreement, was used to calculate the oxygen consumption of the individual.

The procedure was similar at oxygen tensions less than that of air but, in these cases, the freshly collected animals and the respirometer were exposed to the reduced oxygen tension. When not in use the respirometer was always perfused with distilled water to which antibiotics had been added. This distilled water, and the sea water used in the respirometer during experimentation, were adjusted to the required oxygen tension with a gas manometer system. The lid of each storage pot was connected by Silicon tubing (Watson-Marlow) to a gas cylinder filled with a mixture of oxygen and nitrogen appropriate to the oxygen tension studied. The supply of gas was controlled by the reduction valve of the cylinder, and a series of screw-valves ensured the flow to each storage pot was sufficient to stir the sea water without disturbing the animal.

The number of animals examined at each oxygen tension was as follows: 18 *E. brevis* males and 19 *E. communis* males at 135 Torr; 15 of each at 75 Torr; 15 of each at 35 Torr; and 20 *E. brevis* males and 18 *E. communis* males at 12 Torr.

Dry weight

The rate of respiration of individual small nematodes has usually been expressed on a unit weight basis, using values for wet weight derived from estimates of volume based on body dimensions (Nielsen, 1949; Wieser & Kanwisher, 1960; Teal & Wieser 1966). It seemed desirable to obtain more direct estimates of the body weight of each enoplid, using dry weight in preference to wet weight because nematodes readily lose water in air.

Nematodes were weighed on a quartz-fibre balance, based on that described by Lowry (1944). The fibre was housed in a Perspex box lined internally with earthed, perforated zinc, and the displacement of its tip by loads was measured with a vernier microscope. Hooked tare weights were pulled from hot Nylon thread; these were easier to handle than weights made of glass. They were weighed on analytical and torsion balances weighing to 1 and 2 µg respectively, and the fibre was calibrated for the narrow weight range required. As small, dried nematodes are difficult to manipulate, each individual was allowed to dry while attached to a hook on the fibre. Activated silica gel was placed in the sealed balance box as a drying agent, and was replaced frequently. The animal was narcotized in a 0.5% solution of propylene phenoxetol in sea water (Ellenby & Smith, 1964), then dipped into distilled water to remove external salts before touching it against the stem of a Nylon hook. The nematode adhered to the Nylon and the hook was transferred to the quartz fibre through an access port in the balance box. At least 5 h drying was allowed, but constant weight was obtained in a shorter period. Repeated observations over periods up to 24 h showed that no further loss of weight occurred. Weighing was by difference, and a virtually constant value

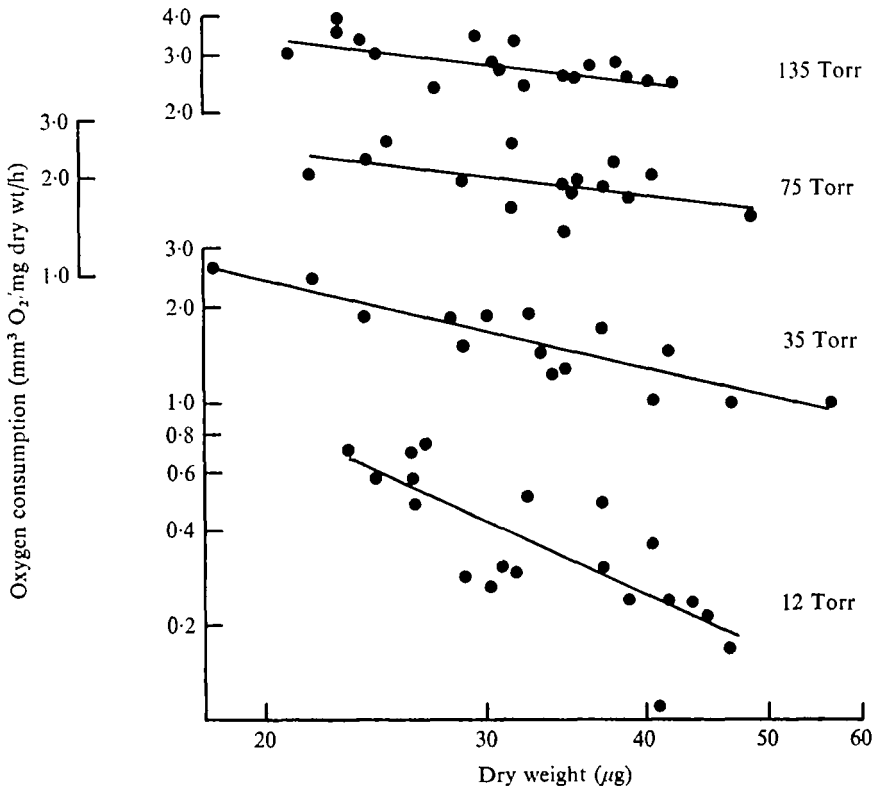


Fig. 1. Respiration of *E. brevis* at four oxygen tensions. All data have been transformed into logarithmic values; the regression lines are fitted by the method of least squares. Ordinate repeated to separate data points from differing oxygen tensions.

was obtained for the hook after the individual had been separated from it in distilled water. The dry weight of each enoplid could be measured to approximately $0.1 \mu\text{g}$, with a reproducibility of about 5%. Dry weight determined by this method gave a very good estimate of body size, free of large, random errors (Atkinson, 1972).

RESULTS

The rate of oxygen consumption/mg dry weight/h of individuals of each species are presented in Figs. 1 and 2 for *E. brevis* and *E. communis* respectively, for the four oxygen tensions studied, using a logarithmic transformation. Regression analysis, assuming a linear relationship, has been carried out on the transformed data by the method of least squares. The lines fitted to these figures are based on these analyses.

The influence of the imposed oxygen tension on the oxygen consumption of E. communis and E. brevis

Co-variance analysis (Snedecor & Cochran, 1968) showed that the slopes of all the lines are similar ($P > 0.05$) except for *E. brevis* at 12 Torr; the latter will be considered in detail later. Clearly, the rate of oxygen consumption of both species is

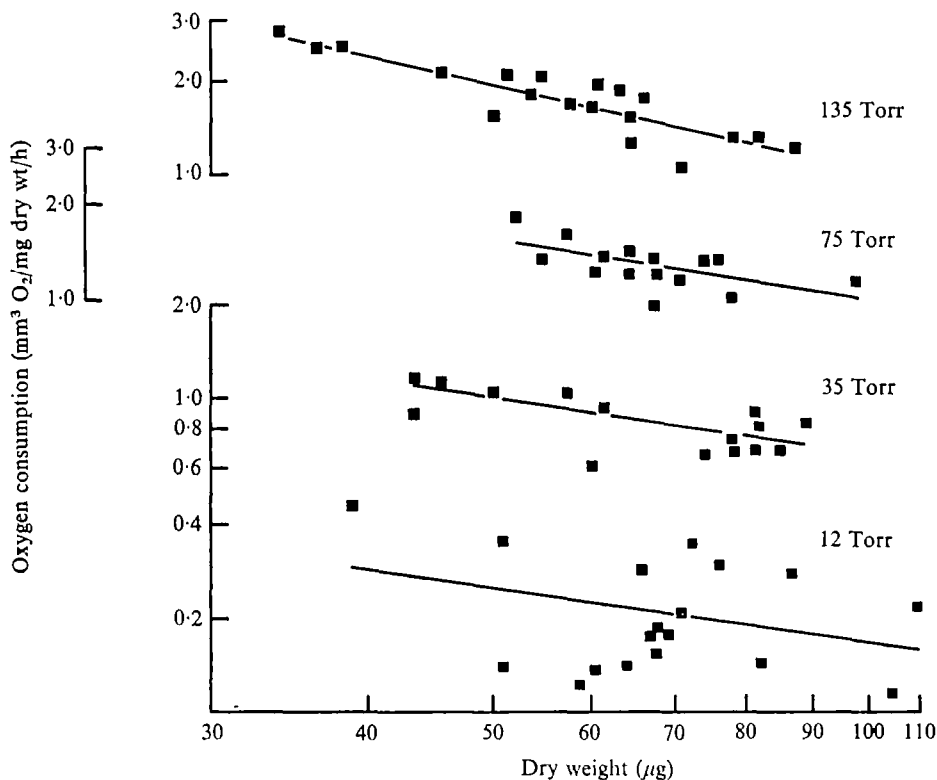


Fig. 2. Respiration of *E. communis* at four oxygen tensions. See legend to Fig. 1.

influenced by the oxygen tension. For example, the smallest recorded difference was between the adjusted mean for *E. brevis* at 75 Torr and the 20% lower value for this species at 35 Torr. Using co-variance analysis, this difference, and all those of other comparable data, was found to be very highly significant ($P < 0.005$). The mean oxygen consumption for *E. brevis* at 35 and 12 Torr, 1.6 and 0.35 mm³ O₂/mg dry wt./h respectively, could not be compared in this way because the two slopes are not similar, but the difference is too great to be accounted for by the dissimilar slopes of the small size range studied.

To facilitate comparison the data for the rates of oxygen uptake of the two species have been presented together in Fig. 3. The mean for each tension has been 'adjusted' along its regression line to the grand mean dry weight for each species, log 32.4 µg and log 64.1 µg for *E. brevis* and *E. communis* respectively. Oxygen consumption shows a progressively greater decline with increasing tension. The decline is greater for *E. brevis* than for *E. communis* between 135 and 75 Torr, but both show the greatest relative difference between 35 and 12 Torr. Clearly, the rate of oxygen consumption of both species is strongly influenced by the environmental oxygen tension.

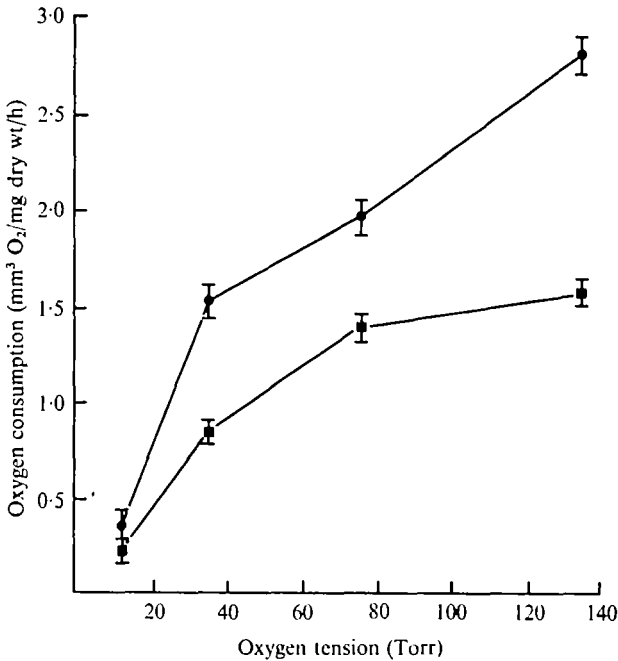


Fig. 3. Respiration of *E. brevis* (●) and *E. communis* (■) at four oxygen tensions. Comparison based on mean oxygen consumption adjusted to the intraspecific grand mean dry weight, 32.4 μg for *E. brevis*, 64.1 μg for *E. communis*. Limits of standard error of adjusted means, \pm .

*The rate of oxygen consumption of E. communis and E. brevis
at four oxygen tensions*

Direct comparison on the basis of the mean dry weight for each species would indicate that the oxygen consumption per unit weight of the smaller *E. brevis* was higher than that of the larger *E. communis* at each oxygen tension. However, the results clearly show that the rate of oxygen consumption varies with the size of the animal, so direct comparison is rather meaningless.

Comparison of the rate of oxygen consumption has again been made by co-variance analysis. In effect, the values for oxygen consumption of the two species at each oxygen tension are compared at the grand mean dry weight for the data of both species, log 45.5 μg . At no oxygen tension was the residual variance after regression greater in one species than the other ($P > 0.05$). The regression coefficients for the two species are similar at 75 Torr ($P > 0.25$) and 35 Torr ($P \approx 0.1$), so the adjusted mean rates of oxygen consumption can be compared at these tensions. There is no significant difference between the two enoplids at either of these tensions ($P > 0.25$ in both cases). Direct comparison at 135 Torr is not possible, since the slopes of their regression lines at this tension differ ($P = 0.025$). As can be seen in Figs. 1 and 2 the weight range of the two species overlap at about 35–45 μg . In this range the rate of oxygen uptake of *E. brevis* is somewhat higher than that of *E. communis*, but the difference is only of the order of 10%, and little importance can therefore be attached to it. The same kind of comparison can also be made for the data at 12 Torr, for here the slope for *E. brevis* is much steeper than that for *E. communis* ($P \approx 0.025$). Com-

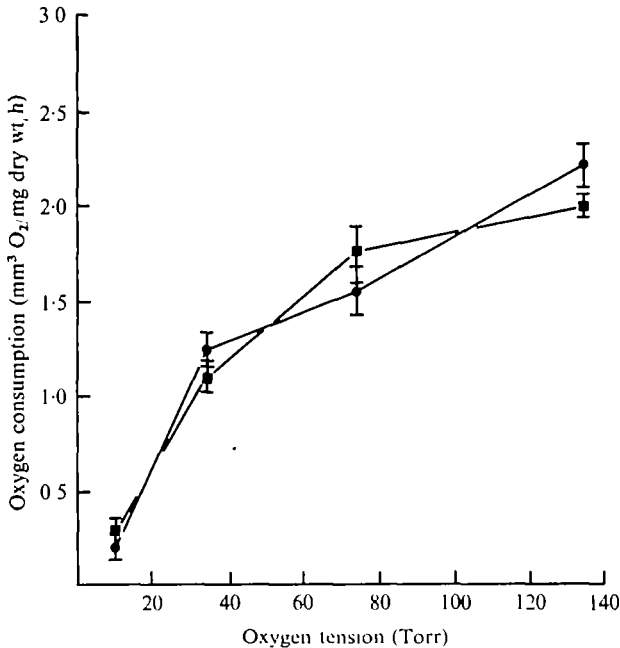


Fig. 4. Respiration of *E. brevis* (●) and *E. communis* (■) at four oxygen tensions. Comparison based on estimates for mean oxygen consumption adjusted to the interspecific grand mean dry weight, 45.5 μ g. Limits of standard error of adjusted means, \pm .

pared at the same body size, there is little difference between the low level of oxygen consumption of the two species at this tension.

In Fig. 4 the mean oxygen consumptions of each species at the four oxygen tensions have been adjusted to the grand, interspecific, mean dry weight, using the common regression coefficient -0.68 . The aberrant data for *E. brevis* at 12 Torr was treated separately, using its much steeper slope of -1.82 ; the adjusted mean values for oxygen consumption of the two enoplids at 12 Torr are, therefore, not strictly comparable. Fig. 4 shows that there is a remarkable similarity in the rate of oxygen consumption of the two species; evidently most of the difference between them in oxygen uptake (Fig. 3) may be correlated with the difference in their body size. There is some evidence that there is a greater change in the rate of oxygen consumption of *E. brevis* between 135 and 75 Torr than there is for *E. communis*. Unfortunately, the statistical difference cannot be established, because the two slopes at 135 Torr differ. At the remaining three oxygen tensions the adjusted mean rates of oxygen consumption of the two species are similar. The difference in the level of their oxygen consumption per unit weight is mainly due to a difference in body size. However, although the size ranges overlap, some extrapolation is involved in these comparisons. They have, therefore, to be interpreted with caution.

DISCUSSION

The results show the following:

(1) The level of oxygen consumption of both *E. brevis* and *E. communis* is reduced by each lowering of the imposed oxygen tension; at 12 Torr the oxygen uptake of both is about 15% of that at atmospheric tensions.

(2) The oxygen uptake of both species decreases with increasing body size.

(3) After allowance for the difference in body size, the rates of respiration of the two species are more or less similar at all oxygen tensions.

Nematodes lack specialized respiratory or circulatory organs, and rely almost entirely on thermal diffusion to supply oxygen to their tissues. Rogers (1962) applied to nematodes the formulae derived by Hill (1929) to characterize the diffusion of oxygen into cylinders of muscle. Rogers lists some, but by no means all, of the limitations of applying a model based on this formula to whole, active animals, and concludes that it fits closely the relationship between oxygen consumption and oxygen tension in *Haemonchus contortus*, *Nematodirus* spp. and *Nippostrongylus brasiliensis* (= *muris*) (Rogers, 1949). Although *E. brevis* is approximately of the same body radius as *Nematodirus* spp. and is smaller than *H. contortus*, it does not follow the model. According to the model, both *E. brevis* and *E. communis* should maintain the oxygen uptake at 135 Torr down to a tension of 10–15 Torr; but at these tensions, the actual consumption is only 15–25% of that at atmospheric tensions. The rate of respiration of the two enoplids is therefore affected at higher tensions than that of the three animal parasites, and in this respect they resemble *Rhabditis elegans* and *R. strongyloides* (Bair, 1955). However, it is possible that the difference between the enoplids and the three intestinal parasites is due to dissimilar experimental conditions; Rogers (1949) measured the oxygen consumption of large numbers of nematodes using a Warburg manometer.

Even if the enoplids do show a greater dependency of oxygen consumption on oxygen tension than the three intestinal parasites, it cannot be assumed that the value for oxygen permeability applied by Rogers (1962) to these three nematodes is greater than that for either *E. brevis* or *E. communis*. The enoplids are inactive at low oxygen tensions and may therefore be affected by a diffusion gradient extending beyond the animal, as a result of their inactivity. Furthermore, diffusion equations which may possibly be applicable to cells or tissues may not be applicable to active animals. Krogh (1919*a, b*) first showed that geometric factors influence the availability of oxygen to cells within tissues; a much greater change in oxygen tension occurs through the tissues than within the mitochondria (Forster, 1964).

In mammalian cells an intracellular oxygen concentration at the mitochondria as low as 1 Torr may be adequate for oxidative phosphorylation (Chance, Schoener & Schindler, 1964). If this is also true of the cells of enoplids, tissue oxygenation should be sufficient for this process at environmental oxygen tensions of 18–20 Torr. At these tensions the tissue haemoglobin of the pharynx of *E. brevis* is saturated with oxygen, indicating that the tissue tension of all this resting musculature is above zero (Atkinson, 1973, in preparation). A greater environmental oxygen tension may be required for adequate oxygen supply during periods of muscular work. Clearly, the relationship between oxygen consumption and oxygen tension is more easily

analysed for cells and tissues than for an active animal. The total oxygen consumed by such an individual is the resultant of a complex interaction of those life processes which utilize oxygen; their relative contribution may alter in a complex way with oxygen tension. For example, at some oxygen tensions the rate of respiration of the enoplids may be less than the maximum dictated by the rate of diffusion at that tension. Adjustments of this sort may have considerable adaptive significance.

Even if a nematode possesses haemoglobin, the amount of oxygen which can be held in store within the tissues will be small. The tissue oxygen tension of all but the largest nematodes will fluctuate rapidly with changes in the environmental oxygen tension. Variation in the oxygen available to *E. brevis* at low oxygen tensions will occur, particularly when the animal moves away from the mud surface (Atkinson, 1972). If the initial rate of oxygen consumption is less than the maximum imposed by the diffusion rate, the enforced change in uptake at the lower tensions would be far less than the theoretical change for a nematode following the model described by Rogers (1962). Moreover, in their natural habitat, temperature may be an important factor; exposure of the mud at Budle Bay by the ebbing tide may be followed by a temperature change of up to 10 °C (Atkinson, 1972). The increase in oxygen consumption following a change of temperature may well be relatively greater than the change in rate of supply due to the increased diffusivity of oxygen. Possibly, the levels of oxygen uptake recorded for the two species represent an attempt by animals lacking the flexibility of specialized respiratory or circulatory organs, to ensure that sufficient oxygen reaches their central tissues when the temperature rises or the ambient oxygen tension falls. If this is so, the animal parasitic forms investigated by Rogers may not exhibit this phenomenon because, living adjacent to the intestinal mucosa of mammals, they experience a constant temperature and, perhaps, more stable oxygen concentrations. However, this interpretation can only be tentative, in view of the differences in experimental technique referred to earlier.

Only the rate of respiration determined within the normal environmental oxygen range of the enoplids can be considered to be a reliable indication of their rates of metabolism; it cannot be assumed, therefore, that the oxygen uptake recorded for *E. brevis* at 135 Torr represents an optimum for this species. The relatively greater change in oxygen consumption recorded for *E. brevis* than *E. communis* between 135 and 75 Torr may even represent a response to an abnormal situation, perhaps inducing a higher level of activity. Both species are certainly capable of maintaining an appreciable level of oxygen consumption down to at least 35 Torr. Neither species is active in the absence of oxygen; presumably they conserve energy reserves by lapsing into a quiescent state. *E. communis*, for example, can survive exposure to anoxia for 24 h in this state (Wieser & Kanwisher, 1959).

Using co-variance analysis, the eight regression coefficients for the present work (Fig. 5), and the four values present elsewhere (Atkinson, 1973) may be compared. Ten slopes do not differ significantly ($P > 0.05$); their pooled value, based on nine of homogeneous residual variance, is -0.68 , with a standard deviation of ± 0.07 . The two dissimilar values are, for *E. brevis* at 12 Torr, -1.82 , and for this species after anoxia, -1.13 (Atkinson, 1973). Their exclusion from the pooled slope is justified because they would seem to represent a response by this species to low oxygen regimes; this is discussed later. -0.68 is statistically different from -1.0 ($P < 0.001$);

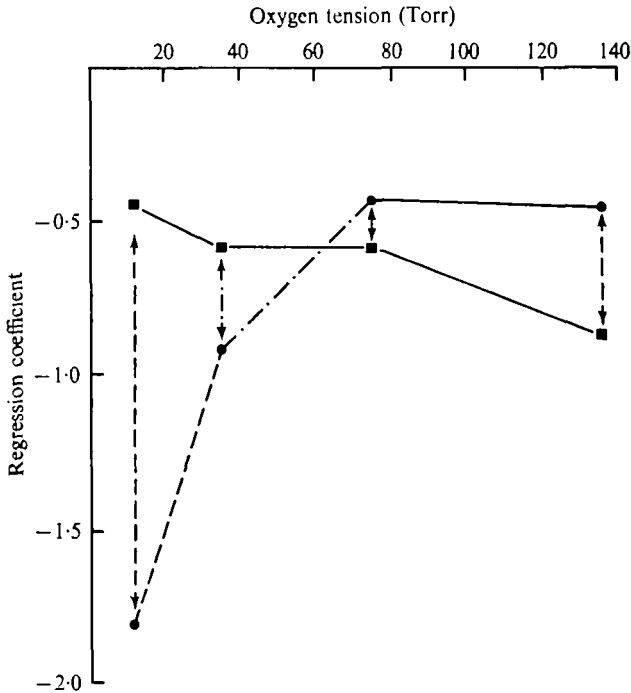


Fig. 5. Regression coefficient for each treatment of *E. brevis* (●) and *E. communis* (■) at four oxygen tensions. Regression coefficients connected by: ———, if significantly different at 5% ($P < 0.05$); - · - · -, if significantly different at 10% ($P < 0.1$); ———, if not significantly different at 10% ($P > 0.1$).

the oxygen consumption per unit weight therefore decreases with increasing body size. The validity of this pooled slope is emphasized by the similarity of the two species, after adjustment has been made for the greater body size of *E. communis* by means of this coefficient (Fig. 4). Clearly, Wieser & Kanwisher (1961), and Teal & Wieser (1966) were not justified in assuming, without investigation, that differences in body size were irrelevant to comparisons of the oxygen consumptions of nematodes. Eventually, nematodes may be shown to be a part of the array of poikilotherms, calculated by Hemmingsen (1960) to have oxygen consumption related to body weight by the exponent $b = 0.73$, and not to have a weight-independent metabolism as suggested by Zeuthen (1953). Hemmingsen (1960) considered it quite likely that $b = 0.73$ is modified for limited, intraspecific size ranges of some poikilotherms. The overall exponent for nematodes is unlikely to range from 0.19 to 0.45, in which case the regression coefficient -0.68 ($b = 0.32$) would be an example of a significant slope modification ($P < 0.001$) characteristic of male enoplids. Numerous exogenous or endogenous factors may be involved, but it is interesting that 0.32 is similar to the 0.33 power relating radius to volume ($P > 0.5$), and to the actual estimate of 0.25 ($P \approx 0.2$) relating mean radius and dry weight of 58 enoplids. This does not necessarily imply a causal relationship between the oxygen consumed by these individuals and their radius; however, there could be a correlation of adaptive significance, for body radius apparently estimates the length of the diffusion pathway in nematodes.

Previous workers have suggested that both endogenous and exogenous factors can influence the power factor ($b-1$): the genotype of the prepupae of *Dryosiphila melano*

gaster (Ellenby & Evans, 1956); temperature on fiddler crabs, *Uca* species (Vernberg & Vernberg, 1966), and the millipede *Spirostreptus astheres* (Dawarakanath, 1971), and salinity on the prawn *Metapenaeus monoceros* (Rao, 1958). The slope for *E. brevis* at 12 Torr was -1.82 , and differed significantly from -1.0 , indicating, in this case alone, a significantly greater total oxygen consumption by smaller than larger individuals ($P < 0.001$). Furthermore, the dissimilar slopes at 12 Torr represent a real difference between the species, for on each day of experiments, individuals of both species were used in an identical manner. Fig. 5, in which slopes for the *E. brevis* and *E. communis* data have been plotted against oxygen tension, clearly shows that a steeper slope occurs for *E. brevis* at lower oxygen tensions. Apparently, under these conditions, the oxygen consumption of smaller individuals of this species is less easily reduced than that of the larger *E. brevis*. Perhaps diffusion is a limiting factor at oxygen tensions less than 12 Torr; smaller animals receiving relatively more oxygen, and those of greater body size, lapsing more easily into a quiescent state. The close agreement of other aspects of the results emphasizes this real difference between the species in the slope of regression at 12 Torr. There are considerable quantities of haemoglobin in the pharynx of both sexes of *E. brevis*, and in the tail musculature of males, but *E. communis* contains little of this pigment (Ellenby & Smith, 1966). The functional significance of the haemoglobin of *E. brevis* is under investigation.

SUMMARY

1. The rate of oxygen consumption of individual males of *Enoplus brevis* and *E. communis* was measured at 15 °C and at each of four oxygen tensions, 135, 75, 35, and 12 Torr, after at least 12 h experience of these conditions.
2. It was clearly demonstrated that the level of oxygen consumption of both species was reduced by each lowering of the imposed oxygen tension.
3. In all cases the oxygen consumption of each species fell with increasing body size. On a unit dry-weight basis the oxygen consumption of *E. brevis* is greater than that of the larger *E. communis*, but after allowing for the difference of body size the two species have more or less similar oxygen uptakes at all oxygen tensions.
4. In *E. brevis* oxygen tension influenced the relationship of body size and metabolism, the slope relating oxygen consumption and body weight becomes steeper with decreasing oxygen tension. This effect was not shown by *E. communis*.
5. Some general factors influencing the availability of oxygen to nematodes are considered.

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