

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

II. THE EFFECTS OF CHANGES IN THE IMPOSED
OXYGEN REGIME

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INTRODUCTION

The influence of a range of stable oxygen tensions on the respiratory physiology of the marine nematodes *Enoplus brevis* and *E. communis* has been reported elsewhere (Atkinson, 1973). Although *E. communis* is found in a habitat where oxygen is usually freely available, *E. brevis* may be subject from time to time to changes in the environmental oxygen concentration. Therefore, an investigation of the effects of altering the imposed oxygen regime is relevant to the study of the influence of oxygen tension on their respiratory physiology.

Little is known of the effects of fluctuating oxygen regimes on the life processes of nematodes, although such changes are a feature of many habitats, including some agricultural soils (Wallace, 1964, 1971). Cooper, Van Gundy & Stolzy (1970) demonstrated that the rate at which populations of *Caenorhabditis* sp. and *Aphelenchus avenae* built up were reduced when they were exposed to 12 h without oxygen every 3 days for 30 days. They considered that fluctuations in oxygen concentrations caused a shunt between oxidative and fermentative metabolism. This, they thought, might prevent rapid catabolism, interfere with lipid metabolism, egg production, and hatch. Many species can withstand periods of anoxia, but vary in their viability. *Caenorhabditis* spp. can survive in a quiescent state for 24-48 h (Nicholas & Jantunen, 1964; Cooper & Van Gundy, 1970). Cooper & Van Gundy (1971) distinguish this from the ability of *Aphelenchus avenae* to recover after 60 days without oxygen. Prolonged anoxia apparently induces cryptobiosis in this species; it utilizes neither glycogen nor lipid stores at these times (Cooper & Van Gundy, 1970). *E. communis* is quiescent in anaerobic conditions, and can survive for at least 24 h without oxygen (Wieser & Kanwisher, 1959).

This paper examines the influence of rapid changes in the imposed oxygen tension on the oxygen consumption of two species of *Enoplus*.

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MATERIALS AND METHODS

Individuals were collected and stored in precisely the same manner as already reported (Atkinson, 1973). The oxygen electrode respirometer was again used to measure the oxygen uptake of individual adult males (Atkinson & Smith, 1973), and dry weight was obtained with a quartz-fibre balance (Atkinson, 1973).

In the first series of experiments individuals were transferred directly from sea water exposed to the atmosphere to the respirometer at 35 Torr, and oxygen consumption was determined. In the second series animals were transferred directly from sea water at zero oxygen tension to 135 Torr in the respirometer. For these experiments each individual was treated as follows. The nematode was transferred from the pot of sea water at atmospheric tension to a glass ladle of approximately 1 ml capacity. This was lowered into a Drechsel bottle of sea water at 15 °C, through which pure nitrogen (British Oxygen Co. 'white spot grade') had been continuously flushed for 1 h. Although it could be seen that the water in the ladle was rapidly exchanged, its small size prevented the turbulence caused by the gas flow from physically disturbing the nematode. The oxygen tension within this water was certainly less than 1 Torr, for the gas could not be detected with the Radiometer oxygen electrode. After an (unfortunately) obligatory 15 min delay to allow the animal to settle, and the apparatus to equilibrate (Atkinson, 1973), the respiration of 15 individual males of each species was measured in the usual way for two 1 h periods.

Throughout this work it is assumed that these animals experienced anoxia for 2 h. This may not be strictly accurate, for oxygen tensions below 1 Torr would not have been detected by the oxygen electrode. If oxygen is consumed by either species at less than 1 Torr, it must be at an extremely low rate, for it has already been shown that both *E. brevis* and *E. communis* have a very low level of oxygen consumption at 12 Torr (Atkinson, 1973).

RESULTS

The rate of respiration of individuals transferred from 135 to 35 Torr

In Fig. 1 the values for oxygen consumption of individuals of both species, after transfer from 135 to 35 Torr, are plotted against their dry weights on double logarithmic grids. Regression lines, assuming a linear relationship, have been fitted by the method of least squares. For comparison, the regression lines obtained for each species, when maintained continuously at 35 Torr ('Acclimated' Prosser, 1964), are included as broken lines.

The mean oxygen consumption of *E. brevis* is 1.04 mm³ O₂/mg dry wt/h when transferred to 35 Torr ('non-acclimated'), and 1.55 mm³ O₂/mg dry wt/h when maintained at this tension (acclimated); for *E. communis*, the rates were 0.56 and 0.86 mm³ O₂/mg dry wt/h respectively. The species, therefore, are influenced in a similar manner: both have a lower oxygen consumption when transferred to 35 Torr than when acclimated to this tension. Co-variance analysis (Snedecor & Cochran, 1968) has been used as before (Atkinson, 1973) to make possible a precise comparison of the data for acclimated and non-acclimated enoplids. In neither case was heterogeneous variance evident ($P > 0.05$). The slopes of the regression lines for animals maintained at 35 Torr did not differ from those obtained for animals transferred to this tension

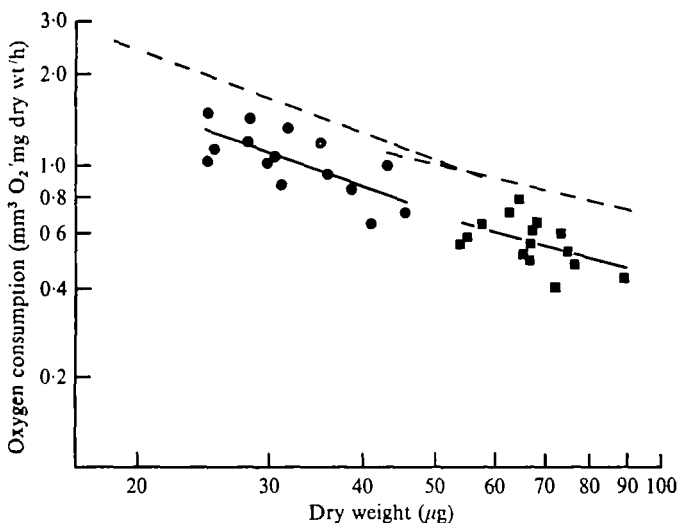


Fig. 1. Respiration of *E. brevis* (●) and *E. communis* (■) at an oxygen tension of 35 Torr after transfer from atmospheric tensions. ---, Regression lines after prolonged exposure to 35 Torr. All data have been transformed into logarithmic values; the regression lines are fitted by the method of least squares.

($P > 0.25$). For *E. brevis*, the respective coefficients were -0.92 and -0.84 and for *E. communis* -0.45 and -0.69 . As seemed likely from Fig. 1, the oxygen consumption of *E. brevis* is greater when the animal is acclimated to 35 Torr than when it is transferred to this tension from atmospheric oxygen concentrations ($P < 0.005$). This is also true for *E. communis* ($P < 0.005$). Furthermore, the size of the effect is similar, if the means of the two different treatments are adjusted to an intraspecific, mean dry weight for each species; the oxygen consumption of *E. brevis*, when transferred to 35 Torr, is only 66% of that of individuals maintained at this tension; the corresponding figure for *E. communis* is 67%.

Clearly, as far as their rates of oxygen consumption are concerned, the two species respond in a similar way to this fall in oxygen tension. This can be confirmed by comparing the non-acclimated data for each species at 35 Torr. Neither the residual variance nor the slopes differ significantly ($P > 0.05$, and $P > 0.25$ respectively). The adjusted mean values for oxygen consumption can therefore be compared at the interspecific, mean dry weight of $45.5 \mu\text{g}$. The values, $0.77 \text{ mm}^3 \text{ O}_2/\text{mg dry wt/h}$ for *E. brevis*, and $0.72 \text{ mm}^3 \text{ O}_2/\text{mg dry wt/h}$ for *E. communis*, are very similar indeed, and do not differ significantly ($P > 0.25$). However, because of the degree of extrapolation involved in this comparison, these results must be treated with caution.

The rate of respiration of individuals transferred from oxygen-free water to 135 Torr

The results of this series of experiments are presented in Fig. 2. The regression lines have been fitted as before, and for comparison those for each species when kept at 135 Torr (Atkinson, 1973) are included as broken lines. The slope of the line for *E. brevis* is apparently steeper for animals after exposure to anaerobic conditions than for animals kept at 135 Torr. The slope for *E. communis* after anoxia appears to be

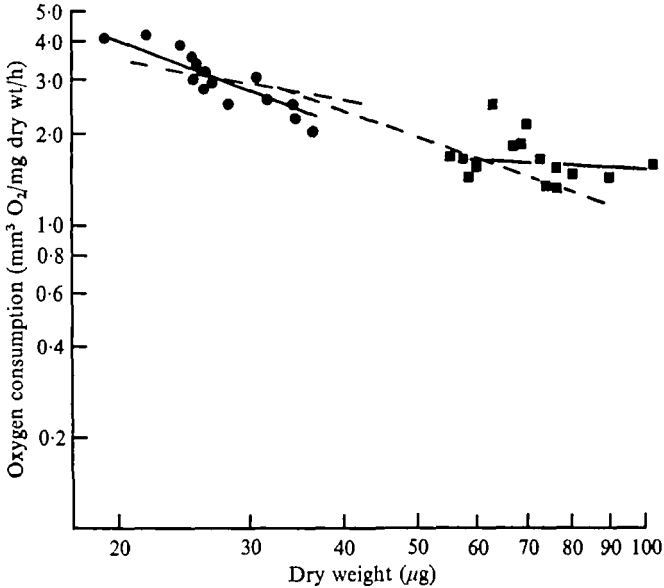


Fig. 2. Respiration of *E. brevis* (●) and *E. communis* (■) at an oxygen tension of 135 Torr after exposure to oxygen-free sea water. ---, Regression lines after prolonged exposure to an atmospheric oxygen tension.

less steep than that after maintenance at 135 Torr. The mean values for oxygen consumption for both species are little different from those for animals kept at 135 Torr: 2.98 and 2.91 mm³ O₂/mg dry wt/h for *E. brevis* and 1.62 and 1.72 mm³ O₂/mg dry wt/h for *E. communis* respectively.

The results for each species were compared with those obtained for individuals maintained at 135 Torr, using co-variance analysis. The residual variance in both cases was not significantly heterogeneous ($P > 0.05$). However, the difference between the slopes for the *E. communis* data, when kept at 135 Torr, -0.87 , and after anoxia, -0.30 , is statistically significant ($P < 0.05$). It is interesting that there is also a significant change in slope for *E. brevis*, but in this case the regression coefficient for individuals maintained at 135 Torr, -0.47 , is less than that after anoxia, -1.13 ($P < 0.01$). It has been shown (Atkinson, 1973) that a greater fall in the oxygen consumption of *E. brevis* with increasing body size occurred at 12 Torr than at other oxygen tensions. It would seem that steeper regression slopes not only occur for this species at low oxygen tensions, but also after exposure to anaerobic conditions. This point is discussed below.

The effect of oxygen lack on the subsequent oxygen uptake at 135 Torr cannot be precisely evaluated for either species because of the difference in the slopes for the two treatments. It would seem that the levels of their oxygen consumptions are not appreciably altered after exposure to anaerobic conditions. Fig. 2 shows that the rates for *E. brevis* at the intraspecific, grand mean dry weight of 32.4 µg are similar. This is also true for the same comparison of the data for *E. communis* at its intraspecific, grand mean dry weight of 64.1 µg. In a simple comparison, the oxygen uptake, after anoxia, at these mean dry weights, expressed as a percentage of the rate at 135 Torr, is 85–100% for *E. brevis* and 100–115% for *E. communis*.

The adjusted mean values for oxygen consumption of the two species at 135 Torr after anoxia are not strictly comparable at an interspecific, grand mean dry weight, because the slopes differ. Furthermore, there is no overlap in the size range of the individuals of the two species, so that comparison at a similar dry weight is not possible without extrapolation. However, Fig. 2 shows that the oxygen consumption of *E. brevis* at the upper weight extreme of 37 μg is approximately 2.2 mm³ O₂/mg dry wt/h, while that of *E. communis* at its lower extreme of 55 μg is 1.7 mm³ O₂/mg dry wt/h. There is, therefore, no large difference between the rates of oxygen consumption of the two species after this treatment.

Since the residual variance after regression is significantly greater for *E. communis* than for *E. brevis* ($P \approx 0.05$), its regression coefficient is less closely defined by the data. Although the slope for *E. brevis* is one of two for this species which are steeper than -1.0, it is extremely well-defined (Fig. 2), and has the smallest residual variance recorded for the twelve regression lines of this and the previous work. The correlation coefficient is 0.91, which suggests that 83 % of the total variance is attributable to the regression on dry weight.

DISCUSSION

Apparently, the males of both *E. brevis* and *E. communis* show similar adaptation to prolonged maintenance at an oxygen tension of 35 Torr. For both species the oxygen consumption after transfer from 135 Torr to this tension was only two-thirds of that of individuals maintained at 35 Torr for at least 12 h. The second series of experiments showed that the oxygen uptake of the two species at 135 Torr was unaltered after exposure to virtually oxygen-free sea water for 2 h.

Prosser (1964) defines 'acclimation' as the compensatory changes which occur in an animal during maintenance under laboratory conditions which have been altered for one stressful parameter. He restricts the term acclimatization to changes under natural conditions, where multiple parameters vary. In both species, the oxygen consumption of nematodes, when exposed directly to an oxygen tension of 35 Torr, was approximately 67 % of that of individuals after acclimation. *Chironomus plumosus* larvae in tubes at low oxygen tensions have, initially, a lower oxygen consumption than they show after prolonged exposure to this oxygen regime (Walshe-Maetz, 1953). Usually, respiratory or circulatory organs play a major role in modifying the rate of oxygen supply to invertebrates, but as nematodes rely mainly on diffusion alone they are not capable of this flexibility. It is, therefore, interesting that the oxygen consumption of enoplids transferred to 35 Torr is not the maximum possible at this tension - after prolonged exposure to this tension, the level of oxygen consumption is appreciably higher. However, it certainly cannot be assumed that this represents an increased affinity for oxygen at the intracellular level, for other factors may be involved. For instance, the animal's initial response to the change in oxygen tension may include a transient suppression of certain activities. Whatever the true nature of the changes which occur, it is noteworthy that the oxygen uptake before acclimation is near the line of a strictly dependent relationship between oxygen tension and oxygen consumption below the level of uptake recorded at 135 Torr (Atkinson, 1973). Mann (1956) recorded a dependent curve for the leech *Erpobdella testacea* when first exposed to reduced oxygen tensions; with time, the oxygen uptake increased and a regulatory

curve was established. If the curvature of the oxygen consumption: oxygen tension relationship for the enoplids (Atkinson, 1973) is due to compensatory mechanisms in the metabolism, it would appear that these are most marked in the region of 30–40 Torr. This range of oxygen tensions may be nearly the lowest at which the acclimated enoplids are able to maintain a full range of metabolic activity.

After exposure to oxygen-free sea water the rates of respiration of *E. brevis* and *E. communis* at 135 Torr were similar to those of individuals maintained continuously at this tension. *Ascaris lumbricoides* (Laser, 1944) does show a higher level of oxygen consumption at atmospheric tensions after anoxia, but this subsequent oxygen uptake can give no clear indication of the rate of metabolism during the period without oxygen. The oxygen consumed by an animal is an integration of separate, oxygen-utilizing processes, and it cannot be assumed that only one is stimulated by the increase in oxygen concentration. Large changes in oxygen tension may even represent an abnormal situation for some animals, perhaps resulting in a high level of locomotor activity. Furthermore, there is no clear correlation between the presence or magnitude of an oxygen debt, and either the ability to survive anoxia or the extent of oxygen lack during anaerobiosis. According to Rogers (1962) and von Brand (1966, 1968), species of nematodes differ in their metabolic responses to the absence of oxygen and the extent of excretion of anaerobic by-products. These workers consider *A. lumbricoides* occurs in an environment which rarely varies from a low oxygen concentration, but do not emphasize that the demonstration of an oxygen debt, repaid at atmospheric oxygen tensions, is more likely to be of adaptive significance to an animal which experiences a fluctuation of oxygen regime. If demonstrated at tensions higher than those of its environment, such measurements of oxygen consumption can only have limited relevance to an understanding of an animal's metabolic economy.

E. brevis undoubtedly experiences a fluctuating oxygen regime. Usually, there will be a low oxygen tension, but on occasions it may encounter an atmospheric oxygen concentration at the mud's surface. However, neither *E. brevis* or *E. communis* showed a postanaerobic increase in oxygen consumption. Both species are immobile in the absence of oxygen, as are many other species of nematodes (*Nippostrongylus brasiliensis* (= muris), Roberts & Fairbairn, 1965; *Meloidoigne javanica* larvae, Baxter & Blake, 1969; and *Caenorhabditis briggsae*, Nicholas & Jantunen, 1964). Although inactive, many are not readily killed by these conditions: *Aphelenchus avenae* can survive without oxygen, apparently in a cryptobiotic state, for 60 days (Cooper & Van Gundy, 1970), *E. communis* can recover from quiescence after 24 h anoxia (Wieser & Kanwisher, 1959). This mechanism probably enables enoplids to survive for some time without oxygen but, as locomotory activity is inhibited, it is probable that the overall metabolic rate is low, as in *Caenorhabditis* sp. (Cooper & Van Gundy, 1970). When oxygen is again available, the rate of respiration is similar to that prior to anoxia, and normal activity of the enoplids is restored. Quiescence enables nematodes, dependent on oxygen for mobility, to live in environments liable to periods of anoxia, but has no survival value for those which fail to avoid permanently anaerobic conditions. Probably a major factor restricting the vertical distribution of *E. brevis* to the upper 2 cm of mud is the presence of an anaerobic substrate below this level (Atkinson, 1972).

It is interesting that, for both species, the slopes after anoxia differ from those of individuals maintained at 135 Torr. However, this may not be entirely due to the

Imposed experimental conditions. The two regression lines for *E. communis* are the only two of the twelve presented in this paper and the previous paper in this series which are not based on similar size ranges (Fig. 2). There is some evidence that the slopes may change over the size range of males studied, as suggested for *Pontanema vulgare* by Nielsen (1949) and for *E. communis* by Wieser & Kanwisher (1960). Unlike the regression coefficient for *E. communis* after anoxia, that for *E. brevis* is much steeper than the value recorded for individuals kept at 135 Torr. In this case the two mean dry weights are similar, and the data after anoxia have the smallest variance of any of the 12 regression lines. There seems little doubt that in this case the slope after anoxia represents a response to the imposed experimental conditions, as shown by this species when acclimated to 12 Torr (Atkinson, 1973).

SUMMARY

1. The rate of oxygen consumption of individual male *Enoplus brevis* and *E. communis* was measured at 15 °C, after altering the oxygen regime experienced since the animals were collected.
2. When both *E. brevis* and *E. communis* were transferred to 35 Torr from atmospheric oxygen tensions, their oxygen consumption was only two-thirds of that of individuals maintained at this lower oxygen tension.
3. The rate of oxygen consumption of the two species at 135 Torr was unaltered by exposure for 2 h to oxygen-free sea water.
4. The results are discussed in relation to the overall influence of fluctuating oxygen regimes on the oxygen requirements of nematodes.

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