ASPECTS OF THE RESPIRATORY PHYSIOLOGY AND OXYGEN PREFERENCES OF FOUR AQUATIC OLIGOCHAETES (ANNELIDA)

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ABSTRACT

Certain aquatic Oligochaeta live successfully in organic-polluted waters while others cannot tolerate such conditions. *Tubifex templetoni* Southern and *Limnodrilus hoffmeisteri* (Clap.), two non-gilled species, are compared to *Branchiura sowerbyi* (Beddard) and *Dero nivea* (Aiyer), two gilled species, concerning their haemoglobin concentration, the influence of low oxygen availability on the respiration rate and their reactions in an oxygen gradient. In contrast with *L. hoffmeisteri* and *T. templetoni* where a very narrow range of respiration dependency (pO_2 0–12 mm Hg) is found, *B. sowerbyi* and *D. nivea* have a level of respiratory dependence from pO_2 0–131 mm Hg. Haemoglobin concentrations are 6,238 – and 6,452 g/100 cm^3 blood in *L. hoffmeisteri* and *T. templetoni* and 3,011 and 1,611 g/100 cm^3 in *B. sowerbyi* and *D. nivea* respectively. In the oxygen gradient all four species have a preference for water with a high oxygen content.

INTRODUCTION

After disturbance of a natural aquatic habitat by organic pollution, certain oligochaete species increase in numbers because competitive and predator species die in, or escape from, the polluted environment. The polluting organic material is used as food while the lowering of the oxygen content of the water does not affect all oligochaete species negatively. The principle on which a biotic index of water quality is based is therefore the typical change occurring in the aquatic community due to pollution.

Aquatic oligochaetes which commonly occur in organically polluted waters are species of *Tubifex* and *Limnodrilus* and to a lesser extent *Branchiura sowerbyi* (Pretorius 1970). *Dero nivea*, however, seems incapable of existing in water with a low oxygen content and is one of the first oligochaetes to disappear from the community after the onset of organic pollution.

Probably the most important single factor regulating the composition of an aquatic fauna, is the oxygen content of the water. Although use of the aquatic Oligochaeta in the biotic index is well known, very little research has been done on their respiratory physiology (Brinkhurst 1965).

Dausend (1931) in the first study on the respiration of an oligochaete, *Tubifex tubifex* (Müller), found that the respiration rate of this species was dependent on the oxygen content of the water. From an oxygen saturation level in water at 19°C of between 15,0–22,3 %, the respiration rate decreased rapidly with a lowering of the oxygen content. Berg *et al* (1962), however,

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found that the rate of respiration of *T. tubifex* was much less dependent on the oxygen content of the water and that the critical oxygen content point below which the respiration rate decreases rapidly was at only 13% saturation. Palmer (1966) again found that oxygen content of water above 7% saturation has no influence on the respiration rate of *T. tubifex*.

From the work quoted above on *T. tubifex* it seems that there is an oxygen content below which, with further lowering, the respiration rate decreases in parallel fashion. The seemingly contradictory results of Dausend (1931), Berg *et al* (1962) and Palmer (1966) can be attributed to the differences in their methods. Dausend used large groups of individuals and tested the oxygen content of the water using the Winkler method, while Berg *et al* worked with much smaller groups and employed a polarographic method for oxygen determinations. The latter doubted whether they were working with a pure *T. tubifex* population. Palmer used only single worms in her study and did the oxygen determinations on the Warburg principle. Berg *et al* and Palmer based the curves from which they derived the critical points on seven and eight respiration rate determinations respectively, which probably were less exact than the great number of determinations made by Dausend.

With few exceptions, oligochaetes have red blood, probably due to the presence of the pigment haemoglobin (Fox & Vevers 1960). This pigment is also responsible for the reddish colour of certain river beds in Europe containing vast numbers of Tubificidae (Fox & Vevers 1960). However, no information is available on the haemoglobin concentration in the blood of different oligochaete species. Also, only Kawaguti (1934), so far, has suggested that the respiratory pigment in the blood of *B. sowerbyi* differs from haemoglobin.

The study reported on below concerned the determination of the influence of oxygen content on the respiration of the four aquatic oligochaetes *Limnodrilus hoffmeisteri* (Clap.), *Tubifex templetoni* Southern, *Dero nivea* (Aiyer) and *Branchiura sowerbyi* (Beddard) as well as the nature and concentration of their respiratory pigments. With the results obtained in mind, the preference of the four oligochaete species for oxygen in an oxygen gradient was investigated.

The reactions of aquatic animals to an oxygen gradient have already been investigated with fish and to a lesser extent with other aquatic animals (Gamble 1971). The general conclusions of these studies were that most fish and Crustacea prefer oxygen-rich water.

The methods used thus far to create an oxygen gradient in water can be divided into two groups, viz. with the gradient parallel to the direction of flow, and with the gradient perpendicular to the flow of the water. The most important disadvantage attached to the first method is that a change in the direction of flow or speed of flow would also lead to a change in the gradient. The second method, known as the fluviarium method, was described and applied for the first time by Höglund (1951) in a study of fish. A qualified description of this method was published by Höglund (1961), while Gamble (1971) adapted the technique to study the preferential oxygen condition of marine Amphipoda.

MATERIALS AND METHODS

Acclimation, collection and mass determination of the oligochaetes were done as described by

Van Hoven (1974). Except for the gradient chamber, all the experiments were done at 25°C. The worms were placed in a respiration chamber of 100 cm^3 filled with water saturated with air having a pO_2 of 131 mm Hg at the prevailing barometric pressure. The pO_2 determinations were done on the polarometric principle using a Clark-type oxygen electrode (Radiometer, Denmark). The respiration rates were measured per hour and are expressed in terms of $\mu l\ mg^{-1}\ hour^{-1}$. When the respiration of a group of worms proceeded very slowly, time intervals of from 4 to 10 hours between measurements were allowed to permit the pO_2 to decrease appreciably. Under other circumstances it was found necessary to use overlapping hour units during measurements. Because of the size of the respiration chamber the possibility of any oxygen gradient forming within it had to be kept in mind, especially when working with smaller groups of worms where respiratory activity may not be sufficient for complete water circulation within the chamber. Following Palmer (1966) the chamber was rotated somewhat before each pO_2 determination to ensure that no oxygen gradient prevailed during measurements.

The transparent respiration chamber was placed in a tin container within a water bath at 25°C and sealed off with a lid, thus preventing any light entering. With each pO_2 determination the lid was removed and the respiration chamber rotated. Whereas Dausend (1931), Berg *et al* (1962) and Palmer (1966) bubbled the water each time with a gas mixture of known oxygen content in order to bring the water to a desired pO_2, the water in the present study was only air-saturated at the onset of the experiments. As the worms consumed oxygen in the respiration chamber, the respiration rate was continuously measured at decreasing pO_2 values.

In determining the haemoglobin content of the different oligochaete species, a number of individuals of a certain species were first homogenized. Of the homogenate 1 cm^3 was diluted with 1 cm^3 of distilled water followed by a 20 minute centrifugation at 4 000 r.p.m. A further diluted sample of the supernatant was used to determine the positions of the absorption bands using a spectrophotometer. The haemoglobin settled too quickly from the solution to do accurate optical density studies. It was also difficult to collect enough specimens for this method which required relatively large volumes of blood.

Following Fox & Vevers (1960) a Hartridge reversion spectroscope was then used. Seven blood samples from each of *L. hoffmeisteri* and *T. templetoni* were treated and three samples each of *B. sowerbyi* and *D. nivea*.

A colorimetric method used by Bakker *et al* (1966) was used to determine the haemoglobin concentration in the blood. This method was also successfully employed by Gibson (1954) in determining the haemoglobin concentration of the blood of two earthworm species. The size of the species dictated the numbers of specimens homogenized before centrifuging the undiluted homogenate for 30 minutes at 4 000 r.p.m. Of the supernatant 0,02 cm^3 was drawn off and added to 5 cm^3 Drabkins' solution which was then shaken well and left for 10 minutes to allow all the haemoglobin to react with the ferricyanide so forming cyanomethaemoglobin. Pure Drabkins' solution was used in the reference column of the colorimeter. Cyanomethaemoglobin with a 56,8 mg/100 cm^3 concentration was used as standard and its optical density was read after the light was first led through a green Ilford no. 625 filter. The haemoglobin concentration determinations were repeated five times for each species and a mean was then calculated.

The gradient apparatus used in this investigation functions on the fluviarium principle as described by Höglund (1961) and Gamble (1971) and was applied with some minor alterations

in the construction. The mixing chamber in the apparatus, containing the glass balls, was reduced in length from 30,5 cm to about 12,25 cm to accommodate the bent edges of the experimental chamber (Figure 10). It has been proved experimentally that this size of mixing chamber produced the most homogeneous oxygen gradient. The length, width and height of the gradient apparatus were doubled, because a bigger experimental chamber is essential for observations on oligochaetes. The pore sizes in the distribution chamber (VK), mixing chamber and experimental chambers were also doubled. The water was kept constant at 10 $\pm$ 1°C throughout all experiments. Water, which had been previously bubbled through with air (pO_2 132 mm Hg) was let in at inlet A. At inlet B of the distribution chamber (VK), water with a pO_2 of 8 mm Hg was let in. This water had previously been bubbled with nitrogen by means of a cross flow system. A 1,5 m long glass tube, filled with porcelain pieces, was used for this purpose. Borehole water, the chemical composition of which was indicated in Van Hoven (1974), was used in the experiments. Water, with a pO_2 of 132 and 8 mm Hg, was let in at A and B respectively, at a rate of 100 cm^3/min. A gradient between pO_2 132 and 8 mm Hg was created in the water. Nine divisions were marked off in the experimental chamber.

The oligochaetes which were used in the gradient experiments had previously been acclimatized in the laboratory for about one week at 10 $\pm$ 1°C after collection in the field. A one-centimetre deep layer of the sediment from the localities where the oligochaetes had been collected, was put in the experimental chamber after drying it well in the sun to ensure that no foreign species were present. The sediment had also been mixed well to ensure that grains and organic material were distributed evenly, so that this could have no influence on the settling of the oligochaetes at a specific oxygen value. Varying numbers of individuals were evenly distributed over the length of the experimental chamber. During the experiment, the light was kept constant by means of a single 150W globe, placed 0,5 m above and in the middle of the gradient apparatus, so that symmetrical lighting was provided. After 24 hours small dividing plates were placed in the sediment opposite the nine markings in the experimental chamber. The sediment in each such compartment was removed and the number of individuals determined after extraction. The individuals which had been damaged by the small plates, were not taken into consideration. The experiment was repeated three times with each species.

RESULTS

The positions of the a- and b- band of the blood of the four species are tabulated in Table 1. The mean of the seven readings is regarded as the position of the absorption bands. Table 2 presents the haemoglobin concentration results.

The relations between respiration rate and the pO_2 of the water are illustrated in Figures 1 to 4.

The results obtained after varying numbers of *L. hoffmeisteri*, *T. templetoni*, *B. sowerbyi* and *D. nivea* were exposed to the gradient, with three repetitions per species are given in Table 3. The distribution of the different species within the gradient after an exposure of 24 hours are illustrated in Figures 5 to 8.

TABLE 1

The mean, standard deviation and standard error of seven measurements of the wavelength positions of the haemoglobin absorption bands of the four species. All values are in nanometers.

	$\bar{X}$	S	*SE*
L. hoffmeisteri			
a-band	574,6	0,081	0,031
b-band	538,2	0,135	0,051
T. templetoni			
a-band	574,3	0,081	0,031
b-band	538,2	0,196	0,074
B. sowerbyi			
a-band	573,7	0,081	0,031
b-band	537,8	0,115	0,044
D. nivea			
a-band	572,3	0,081	0,031
b-band	537,3	0,294	0,111

TABLE 2

The mean, standard deviation and standard error of the haemoglobin concentration as found in five samples of blood from each of the four species. Values are expressed in g/100 cm^3 blood.

	$\bar{X}$	*S*	*SE*
L. hoffmeisteri	6,238	0,085	0,038
T. templetoni	6,452	0,116	0,052
B. sowerbyi	3,011	0,312	0,139
D. nivea	1,611	0,063	0,028

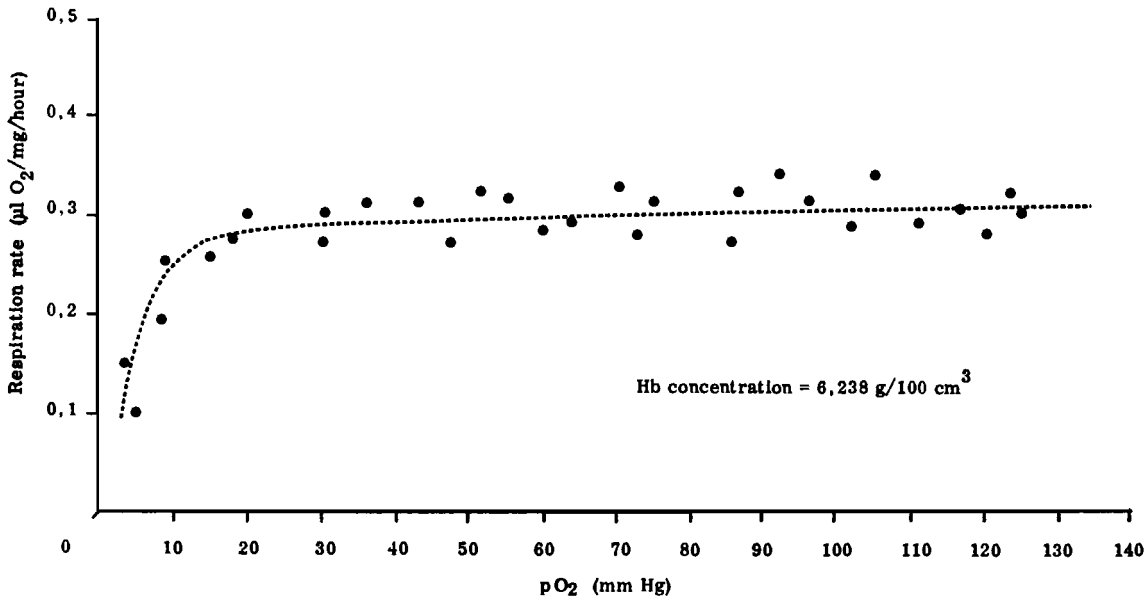


FIGURE 1
The respiration rate of *L. hoffmeisteri* as affected by the pO_2 of the water at 25°C.

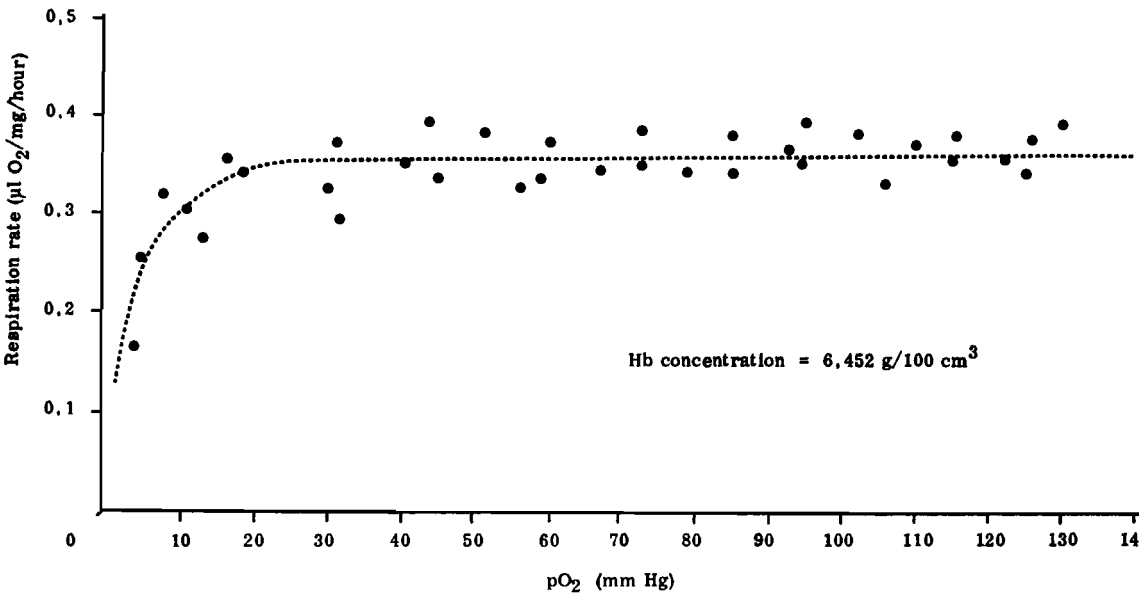


FIGURE 2
The respiration rate of *T. templetoni* as affected by the pO_2 of the water at 25°C.

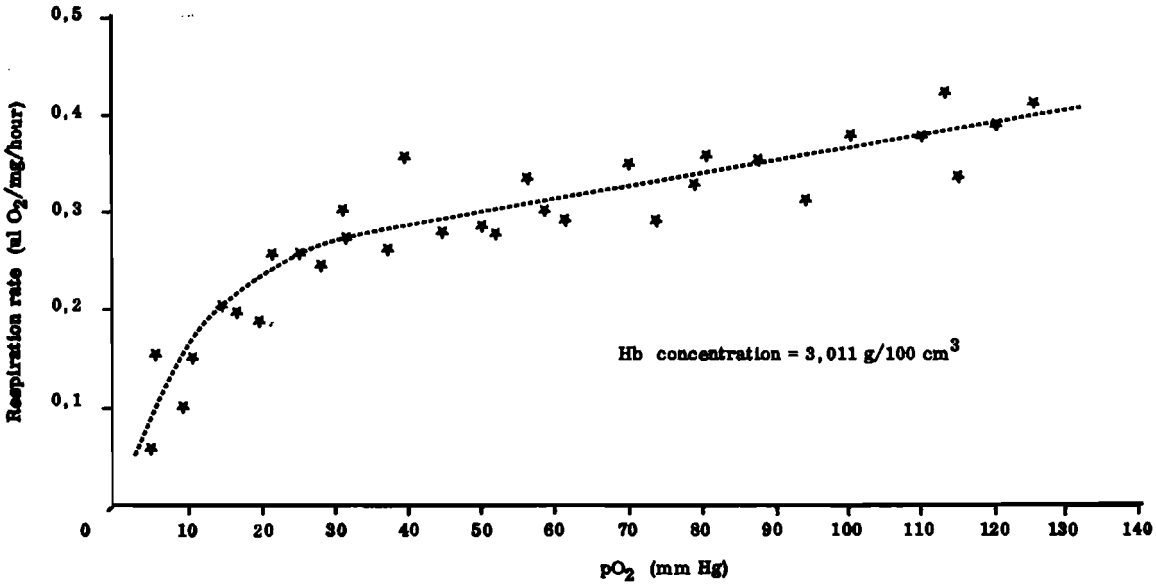


FIGURE 3
The respiration rate of *B. sowerbyi* as affected by the pO_2 of the water at 25°C.

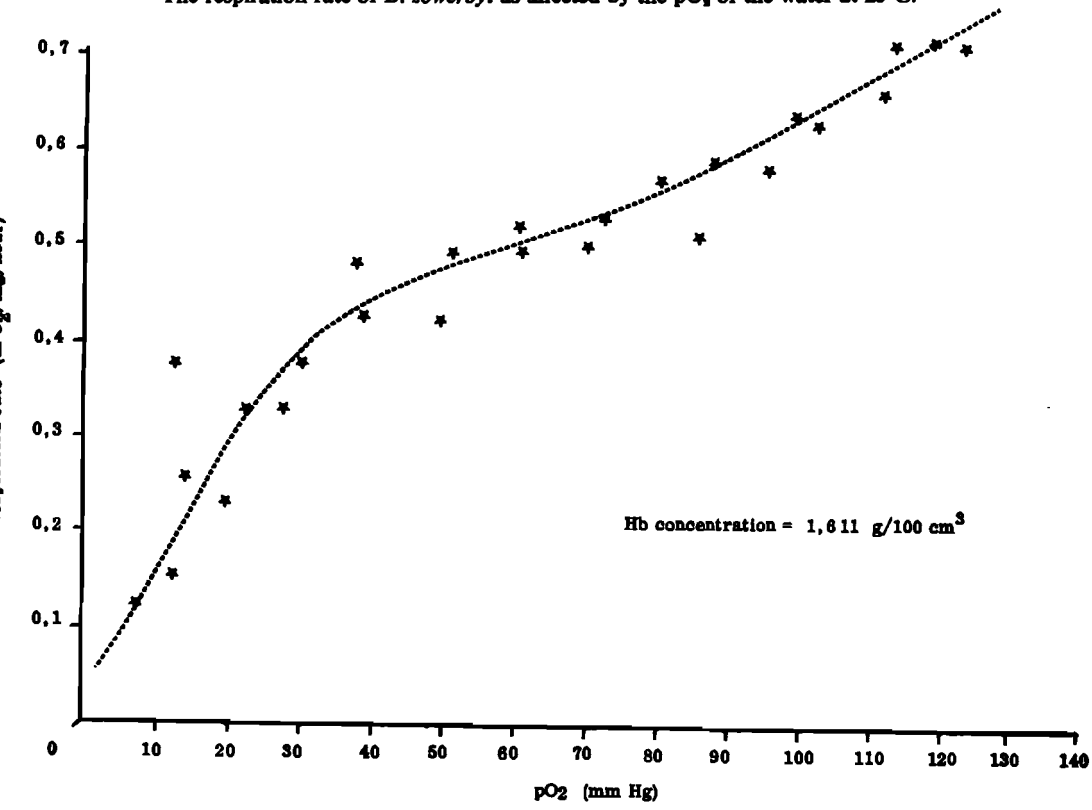
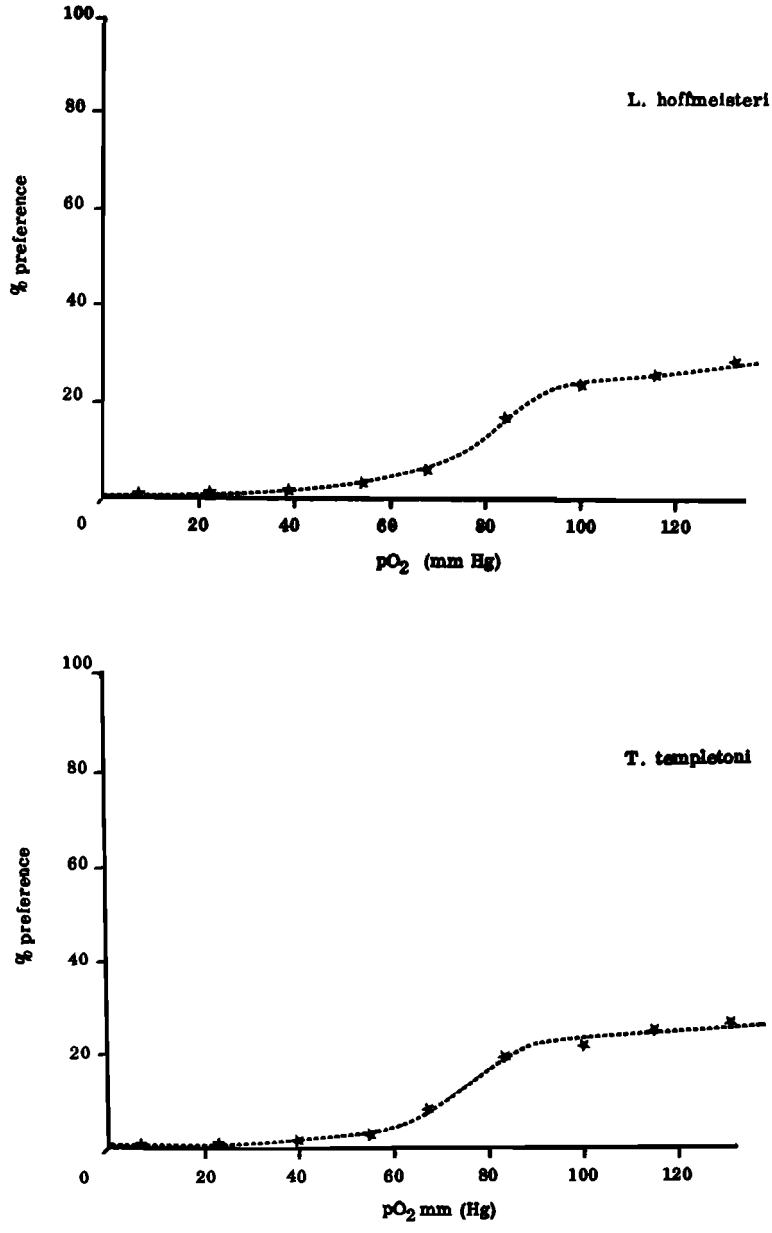


FIGURE 4
The respiration rate of *D. nivea* as affected by the pO_2 of the water at 25°C.

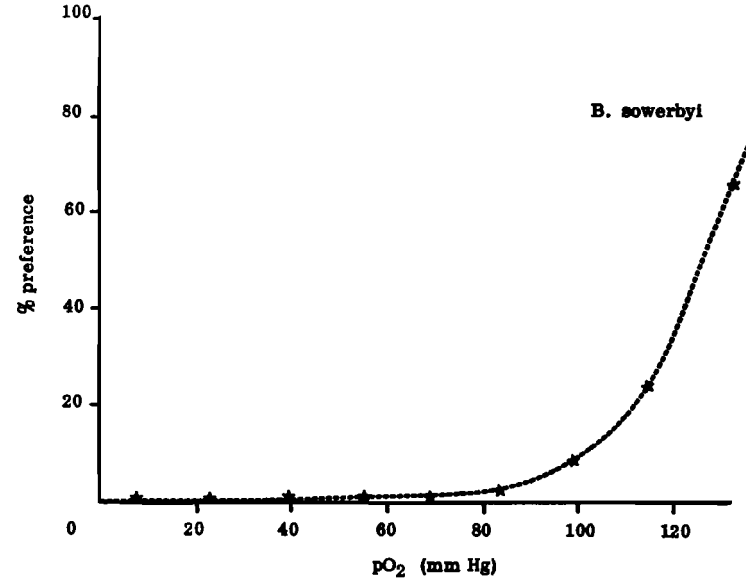
TABLE 3

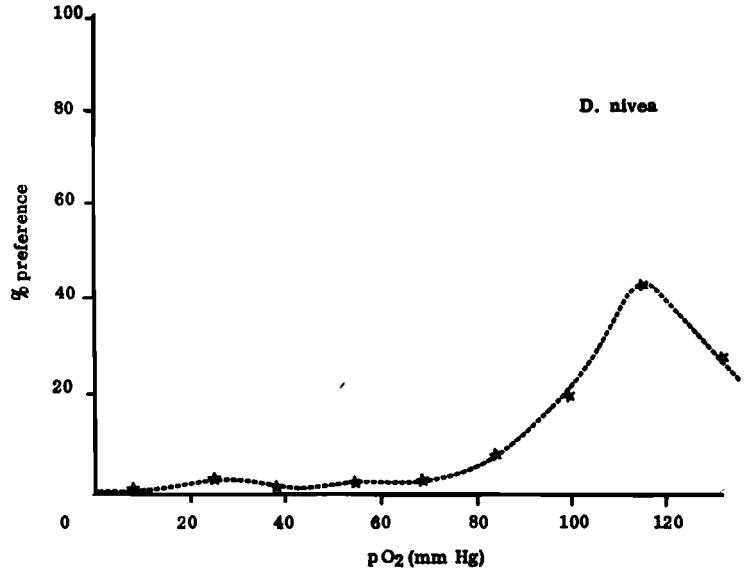
The preference of oligochaetes for different oxygen concentrations in an oxygen gradient from pO_2 132 to 8 mm Hg. Groups of each species were exposed to the gradient three times.

Divisions	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
pO_2	132	116	100	84	69	55	39	23	8
Average number of *L. hoffmeisteri* per division	46,67	42,67	39,00	26,33	8,67	3,00	0,33	0	0
Preference in %	28,00	25,60	23,40	15,80	5,20	1,80	0,20	0	0
Average number of *T. templetoni* per division	118,00	111,00	99,00	82,00	33,67	8,67	1,33	0	0
Preference in %	26,01	24,46	21,82	18,07	7,42	1,91	0,29	0	0
Average number of *B. sowerbyi* per division	27,67	10,00	3,67	0,67	0	0	0	0	0
Preference in %	65,87	23,81	8,73	1,59	0	0	0	0	0
Average number of *D. nivea* per division	27,33	41,66	19,00	6,33	1,00	0,33	0,67	0,67	0
Preference in %	28,17	42,95	19,58	6,52	1,03	0,34	0,68	0,68	0



FIGURES 5 AND 6
The preference of *L. hoffmeisteri* and *T. templetoni* for different oxygen values.





FIGURES 7 AND 8
The preference of *B. sowerbyi* and *D. nivea* for different oxygen values.

DISCUSSION

Figure 9 explains the theoretical relation between the oxygen consumption of an animal and the amount of oxygen available in its environment. The oxygen requirements of an animal are lowest at a minimum level of metabolism (basal metabolism) and referred to as the incipient lethal point (Hoar 1966).

An environment with an oxygen availability which just conforms to the basal metabolism requirements would lead to anoxia with a lowering of the oxygen supply. The upper horizontal line in Figure 9 represents the incipient limiting level of oxygen uptake, that is the minimum oxygen demand level for active metabolism. Most animals would maintain a constant respiratory rate or would be able to regulate it at will depending on the metabolic rate if the oxygen avail-

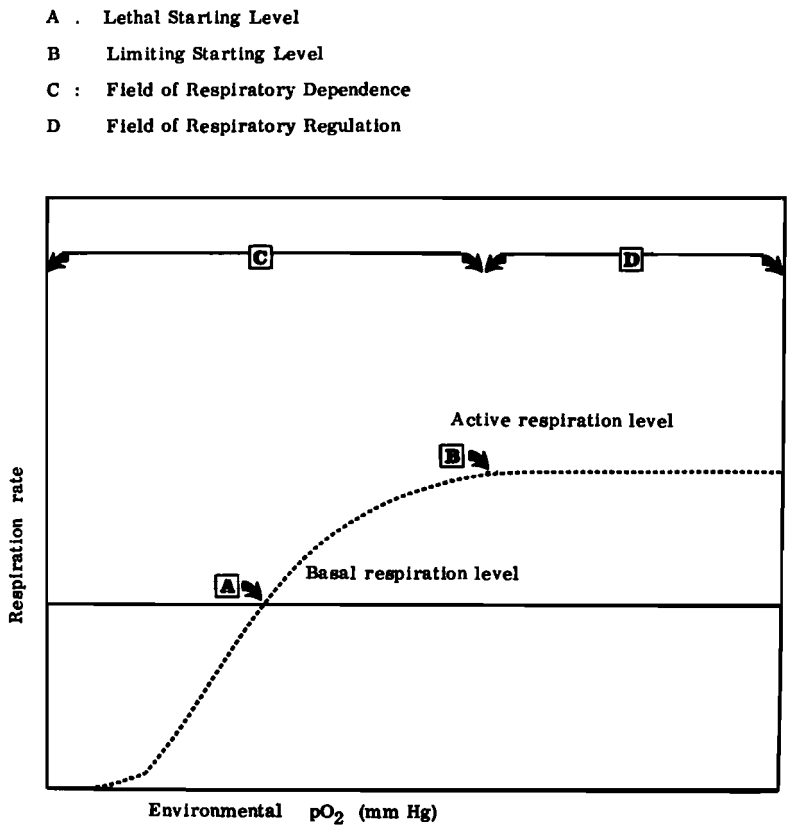


FIGURE 9
The theoretical relation between basal and active respiration at different pO_2 levels in the environment (from Hoar 1966).

ability is higher than the incipient limiting level. (This is the field of respiratory regulation.) Respiration is usually dependent on the available oxygen between the incipient limiting level and the incipient lethal level, this being the field of respiratory dependence. The exact positions of the incipient lethal level and incipient limiting level of animals are specific for different species. Both these incipient levels are dependent on the respiratory organs, the respiratory pigments and the host of enzymes involved in their functioning.

On comparing Figures 1 and 2 with Figure 9, it becomes clear that both *L. hoffmeisteri* and *T. templetoni* have a very narrow range of respiratory dependency stretching from pO_2 0–12 mm Hg. If these oligochaete respiration experiments are assumed to have been done under basal metabolism conditions, nothing can be said about the incipient limiting level for oxygen uptake. The so-called incipient lethal level would then be at a pO_2 of 12 mm Hg and a further decrease in the pO_2 of the water would lead to anoxia for both *L. hoffmeisteri* and *T. templetoni*. However, the oligochaetes have a continuous respiratory activity moving their hind part to and fro in a rapid fashion and when in groups in the respiration chamber they always tend to bundle together, continuously moving. This means that basal metabolism is difficult to define throughout the experiments so that the turning point at pO_2 12 mm Hg in Figures 1 and 2 should rather be regarded as the incipient limiting level.

It is apparent from Figure 3 that no level of respiratory independence exists with *B. sowerbyi*. The range between pO_2 0 and 131 mm Hg should in this case rather be seen as a level of respiratory dependence although it is not as clear-cut as is the case with *D. nivea* (Figure 4). The incipient limiting level in the respiration curve of *B. sowerbyi* can also be regarded as beginning at approximately 20 mm Hg (Figure 3). The level of respiratory dependence for *D. nivea* stretches over the entire range of oxygen values, although an incipient lethal level seems to occur at about pO_2 20 mm Hg (Figure 4).

In order to comply with the increasing demand for oxygen, animal size and complexity evolved parallel with the respiratory mechanisms. The basic problem of a limited gas exchange between environment and animal tissue was overcome by the development of an oxygen transport system which eliminated the long diffusion pathway between body surface and the deeper-lying tissue, and secondly by the development of specialized areas for gas exchange. The primary function of respiratory organs is to bring the oxygen transport system in close contact with the external medium for diffusion. Both *B. sowerbyi* and *D. nivea* have gills while *L. hoffmeisteri* and *T. templetoni* lack them. From the results of this investigation it becomes clear that there is no great difference between the sizes of *L. hoffmeisteri* and *T. templetoni*, while *B. sowerbyi* is on average about four times larger, which justifies in a sense the development of gills. The full-grown worms used in the experiments were in the case of *L. hoffmeisteri* on average 20–35 mm long with a wet mass of 8 mg and *T. templetoni* 15–18 mm long with a wet mass of 5 mg. *Branchiura sowerbyi* was considerably larger and its length varied between 75–100 mm with a wet mass on average of 34 mg per individual in contrast to the 2–6 mm long *D. nivea* with a wet mass of 0,1 mg. In *D. nivea* it seems that a low haemoglobin concentration and a difference in the nature of its haemoglobin could be related to its small size and possession of gills.

In spite of small differences in haemoglobin amongst the four species, all utilize haemoglobin in the blood as a respiratory pigment. This study does not support Kawaguti's (1934) findings that the respiratory pigment of *B. sowerbyi* differs from haemoglobin.

The differences in the nature of the haemoglobin of the four oligochaete species here discussed can be of both taxonomical and physiological importance. Fox (1945) investigated the absorption bands of the haemoglobin of three *Daphnia* species using a Hartridge reversion spectroscope and regarded the a-band difference of between 576,1 nm and 576,6 nm in *Daphnia obtusa* Kurz, *D. pulex* (De Geer) and *D. magna* Straus as of taxonomical importance. Fox & Vevers (1960) are also of opinion that different types of haemoglobin can differ in position of the absorption bands, molecular mass, solubility, crystal shape, alkalinity, acid and heat resistance, isoelectric point as well as oxygen affinity. Against the background of the present study the latter quality becomes of major importance. No information is available, however, on how the oxygen affinity differs with different types of haemoglobin.

It seems from the respiration curves of *L. hoffmeisteri* and *T. templetoni* that the pO_2 of the water does not act as a limiting factor on the metabolism of these two animals within the oxygen range where the respiration curves run horizontal. The pO_2 of the water does appear to be a determining factor of the respiration rate as soon as the available oxygen in the environment of the animals decreases below the critical value of 12 mm Hg.

In contrast to the two species discussed above, the respiration curves of *B. sowerbyi* and *D. nivea* are more diagonal which points to a more direct relation between their respiration rates and the pO_2 of the water. According to Jones (1964) the oxygen content of blood without haemoglobin is a function of the absorption coefficient and the pO_2 in the blood. The absorption coefficient of the blood is dependent on the pO_2 of the water, if this is not higher than the pO_2 in the blood. The low haemoglobin concentration in the blood of *D. nivea* must also be seen against the background of its relatively small size (<0,5 mm diameter). Because of its small size, gases diffuse rapidly into the different parts of the body, making a high haemoglobin concentration unnecessary. Haemoglobin can also be regarded from a physico-chemical point of view as an oxygen buffer, keeping the saturation of haemoglobin constant regardless of the pO_2 of the water down to a specific minimum level. Jones (1964) determined this minimum environmental pO_2 for the saturation of haemoglobin of *Planorbis corneus* through cutaneous respiration, to be 30 mm Hg. Since each haemoglobin molecule only reacts with a specific amount of oxygen, the maximum oxygen capacity of the haemoglobin becomes a function of the pigment concentration in the blood if pCO_2, pH and temperature of the water medium is kept constant.

The so-called loading tension of haemoglobin is in general at a relatively low pO_2, *viz.* 85 mm Hg in man and 5 mm Hg in *Arenicola*. The importance of haemoglobin as a respiratory pigment results from the threshold value for oxygen uptake being in most cases at a low pO_2, and apart from a wide variability of the environmental pO_2 above a certain threshold value, respiration can still proceed freely. The haemoglobin concentration in the blood of *B. sowerbyi* is only about half of that found in *L. hoffmeisteri* and *T. templetoni*. In a way this causes the functioning of the haemoglobin to be overshadowed by the plasma which dissolves oxygen in accordance with the pO_2 of the environment.

According to Yamaguchi (1953) there exist significant indications that the environment has a determining influence on the number of gills of *B. sowerbyi*. In this species it has further been found (Aston 1966) that 90 % of the oxygen uptake takes place via the gills while in fact the gills and tail of this species merely account for 18 % of its body surface.

In view of what has been said above, the respiratory function of haemoglobin in *D. nivea*

would be minimal, since here it has the lowest concentration of the four species investigated. Oxygen would thus be transported in *D. nivea* mainly by the plasma, which also becomes quite evident by looking at the dependency of oxygen uptake of this species on the environmental pO_2. The presence of gills could also in this case be regarded as an adaptation to the low haemoglobin concentration in the blood.

Seeing that amongst others Fox (1954), Gilchrist (1954), Fox & Vevers (1960) and Jones (1964) are of opinion that the Annelida as a group are not capable of synthesizing haemoglobin in the blood under unfavourable oxygen conditions, the haemoglobin concentration in the blood of oligochaetes must be regarded as genetically determined.

From the results obtained in the oxygen gradient experiment it is evident that all four species have a preference for water with a high oxygen content.

According to the values obtained from the three repeat experiments with *L. hoffmeisteri* (Table 3 and Figure 5), it can be concluded that this species does not have a definite preference between oxygen values below pO_2 100. Out of the total number of *L. hoffmeisteri* individuals used, 77% had settled at a pO_2 of 100 mm Hg and above, after 24 hours. Of *T. templetoni* most individuals per division were found in the area with a pO_2 of 132 mm Hg (Figure 6), while 72,29% of the total number of individuals of this species preferred a pO_2 of 100 mm Hg and above. Thus, there was little difference in the distribution pattern of *L. hoffmeisteri* and *T. templetoni* within the gradient chamber.

The distribution of *B. sowerbyi* in the gradient chamber (Figure 7) differs from that of *L. hoffmeisteri* and *T. templetoni*; 98,41% of the individuals preferred a pO_2 of 100 mm Hg and higher, and 1,59% occurred in the area of 84 mm Hg. No individuals were found at lower oxygen conditions. It is evident that the preference for water with a high oxygen content is much greater with *B. sowerbyi* than with *L. hoffmeiseri* and *T. templetoni*.

In comparison with the three species mentioned above, *D. nivea* is an exception (Figure 8). While *L. hoffmeisteri*, *T. templetoni* and *B. sowerbyi* were found in progressively larger numbers from a low pO_2 to 132 mm Hg, the numerical representation of *D. nivea* forms a peak at 116 mm Hg, whereafter a decrease in numbers was found at higher pO_2 values. It is obvious that with *D. nivea* there is a measure of avoidance for the saturation point of air in the water. In general it can be said that a definite preference was shown for the higher oxygen values in the water, if it is considered that 90,7% of all individuals (all four species) were located in the area of pO_2 100 mm Hg and higher. The other 9,3% were found sporadically distributed in the rest of the gradient chamber. A possible explanation for the latter phenomenon is the mobility of *D. nivea*. This species, in contrast with the other three, has the ability to move out of the sediment and into the water by means of active spiralling movements of the body. This swimming activity takes place with certain individuals in a group for a short time. It was noted during the experiments with *D. nivea* that some individuals suddenly swam some way through the water, rested, and then took another course. This apparently aimless sporadic activity is also the reason why single individuals were found at low oxygen contents during the sorting of the worms at the end of the experimental period.

CONCLUSION

The typical changes which take place in the species composition and representation of mainly faunal communities after organic pollution of a water mass forms the basis on which biotic indexes of water quality are formulated. The importance of aquatic oligochaetes in the biotic index is so significant that some species are referred to as "indicator species", and these animals are often associated with organic pollution of water. But these oligochaetes, *viz.* species of *Limnodrilus* and *Tubifex*, can also survive in water of a normal quality, although their population densities do not become high. Competition for the more limited food stock in the non-polluted habitat, and also the curbing of the population numbers by predation, seem to be the most important reasons for this, the most important predators being fish, leeches and crabs.

In water of good quality where the oxygen content fluctuated within normal limits, other ecological factors in the population density control emerge. In such habitats the so-called "indicator species" of water pollution, *viz. L. hoffmeisteri* and *T. templetoni*, live together with *B. sowerbyi*, which can live less successfully in organically polluted water, and *D. nivea*, which is not found in organically polluted water at all.

From this study it is evident that definite physiological mechanisms are responsible for the successful survival of certain species of aquatic oligochaetes in organically polluted water, while others can only live in clean water with a high oxygen content.

This study is also a reaction to a need formulated by Brinkhurst & Jamieson (1971:140) in the first major monograph on aquatic oligochaetes: "It is apparent that nothing has been done on the respiratory tolerance of species known to occur only in unpolluted and unproductive habitats, nor have any physiological mechanisms been described which would account for the ability of a few species like *L. hoffmeisteri* and *B. sowerbyi* to withstand a wider range of environmental conditions than all other fresh-water tubificids".

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