# THE FUNCTION OF HAEMOGLOBIN IN *TANYTARSUS* (CHIRONOMIDAE)

By BARBARA M. WALSHE
*Zoology Department, Bedford College, University of London*

(*Received* 13 *May* 1947)

(With One Text-figure)

Eighty years ago Lankester (1867) identified spectroscopically the red blood pigment of the larvae of *Chironomus plumosus* as haemoglobin. Subsequently (1873) he correlated its presence with the fact that they inhabit stagnant ponds and putrescent mud where the amount of accessible oxygen must often be small. Since then it has been generally assumed that their haemoglobin enables the larvae to live in surroundings deficient in oxygen, and experimental evidence for this has lately been supplied by Harnisch (1936) and by Ewer (1942). These workers compared the metabolism of normal larvae with that of larvae whose haemoglobin had been made functionless by conversion to carboxyhaemoglobin. They found that the pigment was only used as an oxygen carrier at low pressures of oxygen; it is used to the greatest extent in water which is 22% saturated with air at 17° C. Ewer also made a series of measurements of the oxygen content of the pond from which her larvae were obtained and found oxygen concentrations corresponding to 32% air saturation or less for periods of at least 16 consecutive hours. Thus it may be assumed that in nature the haemoglobin of the species with which Ewer worked is of functional value.

The larvae of several chironomid genera other than *Chironomus*, however, contain haemoglobin, and it has frequently been pointed out (Malloch, 1915; Harnisch, 1930) that the possession of haemoglobin by a larva is no proof that it can live in situations poor in oxygen. Thus, although *C. plumosus* is known to be very euroxybiotic the almost identical *C. bathophilus* is less so, while *Tanytarsus* species are markedly sensitive to low oxygen concentrations and only occur in well-aerated waters (Thienemann, 1923). The wealth of limnological data on the distribution of chironomids provides many other instances of stenoxybiosis in larvae with haemoglobin. In such forms the haemoglobin can scarcely function in the same manner as it does in the *Chironomus* species studied by Harnisch and by Ewer, otherwise they would be capable of maintaining a normal aerobic metabolic rate at low oxygen concentrations. The functional significance of the haemoglobin of such oxygen-needy chironomids has never been satisfactorily studied. Harnisch (1930, 1933, 1937) made a comparative study of *Chironomus*, *Prodiamesa* and *Tanytarsus*, three chironomid genera with different capacities of withstanding low oxygen concentrations in nature, but he was mainly concerned with their respiration after periods of anaerobiosis and did not attempt to assess the mode of functioning of the haemoglobin in the stenoxybiotic species.

I therefore chose *Tanytarsus* larvae for an experimental study of the function of the haemoglobin. These small larvae, which contain sufficient haemoglobin to make them red in colour, are very sensitive to oxygen lack and occur in nature in oligotrophic lakes, the oxygen content of which never falls below about 50% air saturation and in running water. The principle and techniques employed were those of Ewer (1942): the rates of oxygen uptake of normal larvae at different oxygen concentrations were compared with those of larvae whose haemoglobin had been converted into carboxyhaemoglobin and was thus incapable of transporting oxygen.

Final instar larvae (7–8 mm. in length) of *Tanytarsus brunnipes* (Zett.) were collected from a small stream in Cambridge. The species was determined from adults which emerged in the laboratory, the identification being checked by comparison with British Museum specimens. Larvae were collected a day or two before experiments and were kept in running aerated water in shallow dishes with a thin layer of mud.

The method used to determine the metabolic rate was that described by Ewer (1942), with minor modifications. Larvae were enclosed in 10 ml. glass syringes in distilled water buffered with sodium bicarbonate (normality 0·004). A small glass bead was put into each syringe to ensure adequate mixing of the water. Four syringes were used at a time, each containing between fifteen and twenty *Tanytarsus* larvae. They were clipped on to a large wheel rotating in a thermostatic water bath at 17° C. At intervals of approximately 1 hr. a sample of water was withdrawn from each syringe and its oxygen content determined by the syringe-pipette micro-Winkler method described by Fox & Wingfield (1938). Each experiment lasted about 3 hr.; during this time the pH of the water altered less than 0·2 unit, and the oxygen concentration fell from air saturation (6·75 ml./l.) to about 2 ml./l., or, in experiments at a lower range of oxygen contents, from 4 ml./l. to about 0·7 ml./l. At the end of the experiment the animals were removed from the syringes, dried on filter-paper and weighed. The oxygen consumption for each hour of the experiment was calculated in cubic millimetres of oxygen per gram wet weight per hour. The average of the oxygen contents of the water at the beginning and end of each hour's interval gave the oxygen content to which the oxygen consumption rate was referred.

The method of treatment with carbon monoxide was as follows. The animals were kept in a 170 ml. bottle in aerated water to which sufficient carbon monoxide-saturated water had been added to make the pressure of carbon monoxide one-sixth of that of the dissolved oxygen. They were then left in the dark until their haemoglobin was entirely converted into carboxyhaemoglobin. The conversion was tested spectroscopically and was found to require 1 hr. It was calculated that the removal of oxygen by the larvae during this time did not increase the relative pressure of carbon monoxide to more than one-fifth. Experiments using animals with carboxyhaemoglobin were made in water containing 0·2 ml./l. carbon monoxide to prevent dissociation of the carboxy-compound. At the end of these experiments the blood was tested spectroscopically to ascertain that the haemoglobin was still in the form of carboxyhaemoglobin; this was judged by the failure of the absorption bands to fade on the addition of sodium hydrosulphite. Experiments with carbon monoxide-

treated animals were alternated with those using normal animals. This ensured that larvae in the same physiological state were compared, since work on *Chironomus* (Walshe, 1947) had shown that seasonal differences in metabolic rate occur.

The utilization of haemoglobin at very low oxygen pressures was also tested in another way: the times of death of larvae with and without haemoglobin in water of low oxygen content were compared. Larvae were enclosed in 1 l. bottles containing water of known low oxygen content (ranging from 9 to 23 % air saturation) and kept in the dark at room temperature, the numbers of dead being noted at intervals. The experimental bottles were set up in pairs: one bottle with normal untreated larvae and the other with larvae previously treated with carbon monoxide, as already described. In all experiments except one (Exp. 3, Table 3) ten larvae were used in each bottle; in Exp. 3, twenty larvae were used. At the end of each experiment the oxygen content of the water was estimated.

The metabolic rate of *Tanytarsus* at 17° C. at various concentrations of dissolved oxygen is given in Table 1 and Fig. 1. The mean rate of oxygen consumption in fully

Table 1. *Oxygen consumption of* Tanytarsus brunnipes *larvae at* 17° C. *at various concentrations of dissolved oxygen*

| Oxygen concentration (ml./l.) | Oxygen consumption (cu.mm./g. (wet weight)/hr.) | |
|---|---|---|
| | Separate values | Mean and S.E. |
| 6·00–5·01 | 630, 507, 590, 494, 532, 528, 424, 522, 541, 544, 469, 552, 489, 553, 454 | 525 ± 13 |
| 5·00–4·01 | 343, 440, 442, 435, 117, 322, 305, 231, 299, 424, 358, 450, 415 | 352 ± 27 |
| 4·00–3·01 | 505, 435, 424, 421, 415, 376, 435, 73, 51, 101, 326, 288, 344, 318, 367, 352 | 327 ± 34 |
| 3·00–2·01 | 309, 362, 316, 248, 223, 357, 298, 240, 354 | 301 ± 18 |
| 2·00–1·61 | 229, 246, 319, 330, 334, 379, 316, 219, 295, 228, 183 | 279 ± 18 |
| 1·60–0·90 | 224, 194, 224, 262, 273, 357, 299, 271, 298 | 267 ± 16 |

aerated water is 525 ± 13 cu.mm./g. (wet weight)/hr., but falls considerably at lower oxygen values. It is seen from the curve that the metabolism is dependent on the oxygen pressure in the water at all pressures below that corresponding to air saturation. This is contrary to Harnisch's statement that the oxygen consumption of *Tanytarsus* is constant over a wide range of oxygen pressures and only begins to decline at the low pressure of 1–2 % of oxygen (equivalent to 7 % air saturation) (Harnisch, 1929). The data from which he draws this conclusion, however, are so badly presented in his paper that it is impossible to estimate their validity. Using a Warburg manometer he plots the decline in pressure in the apparatus caused by the oxygen consumption of the larvae in a series of graphs lacking both ordinates and abscissae, and he judges the effects on the larvae of various gas mixtures by changes in the rate of decrease of the gas pressure. He selects certain experiments as examples for discussion and gives no idea of the actual numbers of experiments made, or of the range of gas mixtures used. The temperature for one experiment was 'etwa 20° C.', that of the others is not given. In comparing the effect of low oxygen

pressures on *Eutanytarsus inermipes* (= *Tanytarsus brunnipes*) with *Chironomus* he states: 'Ich habe leider keinen Versuch, der die Stelle klar trifft, an der *Eutanytarsus* vor *Chironomus* knickt. Sie wird sich aber noch finden lassen, da die Abknickung der Atmungskurve von *Eutanytarsus* stets deutlich stärker ist als die von *Chironomus thummi*.' This conclusion he subsequently quotes (1930) as an established fact.

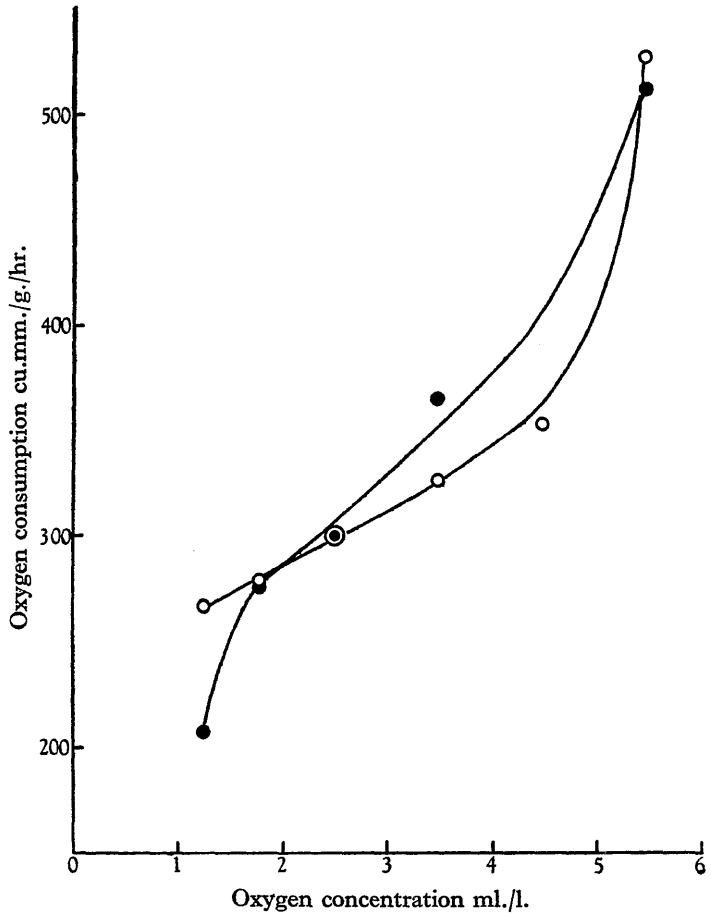


Fig. 1. Rates of oxygen consumption at 17° C. of *Tanytarsus* at various concentrations of dissolved oxygen. ○, normal animals; ●, animals with carboxyhaemoglobin. Data from Tables 1 and 2.

The general significance of dependence or independence of the oxygen consumption of animals on oxygen pressure has been very intensively discussed (Henze, 1910; Amberson, Mayerson & Scott, 1924; Rashevsky, 1933; Maloeuf, 1937*a*, *b*) and will not be elaborated here. Harnisch's theory (1937), however, that a dependent type of curve is an indication of a repayment of oxygen debt by the animals cannot be the explanation in the case of my larvae. In order to avoid the possibility of the accumulation of an oxygen debt before experiments, Harnisch kept his experimental animals in running water in wide glass tubes with bolting silk ends. I repeated this technique in a number of my experiments, but the subsequent

metabolism of larvae so treated was still just as dependent on the oxygen content of the water as without this treatment.

The oxygen consumption at various concentrations of dissolved oxygen of *Tanytarsus* larvae with carboxyhaemoglobin is given in Table 2 and Fig. 1. As in the case of the untreated animals, the oxygen consumption is high in fully aerated water and falls with declining oxygen pressures. A statistical comparison of the metabolic rates of treated and untreated larvae shows that between 6·0 and 1·6 ml./l. oxygen (90%–24% air saturation), the slight differences recorded are not significant. Below 1·6 ml./l. the oxygen consumption of carbon monoxide-treated larvae drops to 78% that of normal larvae: this decrease is statistically significant. It indicates that below this oxygen value the haemoglobin is functional in the normal animal in picking up oxygen.

Table 2. *Oxygen consumption of* Tanytarsus brunnipes *larvae with carboxyhaemoglobin at* 17° C. *at various concentrations of dissolved oxygen*

| Oxygen concentration (ml./l.) | Oxygen consumption (cu.mm./g. (wet weight)/hr.) | | |
|---|---|---|---|
| | Separate values | Mean and S.E. | % normal rate |
| 6·00–5·01 | 513, 524, 424, 622, 394, 547, 547, 455, 689, 531, 471, 478, 630, 663, 412, 283, 639, 411, 369 | 505±25 | 96 |
| 5·00–4·01 | 564, 509, 451, 504, 412, 356, 345, 254, 336, 442, 265, 370, 299, 419, 273 | 387±25 | 109 |
| 4·00–3·01 | 322, 564, 370, 286, 498, 408, 421, 605, 477, 167, 360, 127, 169, 376, 448, 235 | 365±25 | 112 |
| 3·00–2·01 | 427, 494, 246, 291, 313, 358, 277, 249, 307, 271, 212, 168 | 301±27 | 100 |
| 2·00–1·61 | 287, 285, 222, 268, 271, 228, 370, 244, 368, 216 | 276±17 | 99 |
| 1·60–0·90 | 178, 206, 238, 204, 273, 163, 241, 205, 191, 330, 163, 190, 204, 126 | 208±14 | 78 |

The function of the haemoglobin at very low oxygen pressures was confirmed and extended by experiments of another type. The reason for the different technique was as follows. The experiments in syringes were unsuitable for detecting the slight differences in metabolic rate between untreated and treated larvae at very low oxygen pressures for the following reason: unless the differences were considerable they might easily be masked at oxygen concentrations of less than about 1 ml./l. by the random error of the Winkler method. This error, being largely determined by the titration end-point and by the introduction of oxygen in the reagents, is independent of oxygen concentration and is therefore relatively greater at low concentrations. For this reason another method was adopted and the times of death of larvae with and without carboxyhaemoglobin in waters of low oxygen contents were studied. A more rapid death of larvae with carboxyhaemoglobin was taken to mean that the haemoglobin of normal larvae functions at that oxygen pressure. The data from a number of such experiments are summarized in Table 3. Exps. 1 and 6 were made in winter at a lower temperature than the others and the absolute values for survival are thus

not comparable throughout the series, but since experiments with and without haemoglobin were always made simultaneously the assessment of the function of the haemoglobin remains valid. Larvae without functional haemoglobin kept in water with an initial oxygen content of less than 15% air saturation (approximately 1 ml./l.) died more rapidly than normal larvae (Exps. 1–3). It follows that at this low oxygen concentration the haemoglobin is of survival value to the larvae.

A more rapid rate of death of carbon monoxide-treated larvae also occurred when the initial oxygen content of the water was greater than 15% saturation (Exps. 4–6), but the increased rate of dying was not apparent in these larvae until many hours after the start of the experiment. Although the larvae were enclosed in a relatively

Table 3. *Rates of death of* Tanytarsus brunnipes *larvae at low oxygen concentrations*

| Exp. no. | Temp. (° C.) | Larvae with haemoglobin | | | | Larvae with carboxyhaemoglobin | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Oxygen, % air saturation | | Hr. after enclosure | % alive | Oxygen, % air saturation | | Hr. after enclosure | % alive |
| | | Initial | Final | | | Initial | Final | | |
| 1 | 13–14 | 9 | 7 | 44 | 90 | 9 | 8 | 44 | 80 |
| | | | | 52 | 90 | | | 52 | 70 |
| | | | | 68 | 80 | | | 68 | 40 |
| | | | | 75 | 80 | | | 75 | 20 |
| 2 | 18–20 | 12 | 6 | 21 | 100 | 12 | 6 | 21 | 100 |
| | | | | 28 | 100 | | | 28 | 70 |
| | | | | 50 | 100 | | | 50 | 0 |
| 3 | 18–20 | 14 | 5 | 20 | 100 | 14 | 8 | 20 | 100 |
| | | | | 45 | 95 | | | 45 | 0 |
| 4 | 18–20 | 15 | 11 | 20 | 100 | 15 | 12 | 20 | 100 |
| | | | | 27 | 100 | | | 27 | 100 |
| | | | | 43 | 90 | | | 43 | 90 |
| | | | | 49 | 90 | | | 49 | 60 |
| 5 | 18–20 | 20 | 15 | 21 | 90 | 22 | 15 | 21 | 100 |
| | | | | 28 | 90 | | | 28 | 100 |
| | | | | 50 | 90 | | | 50 | 60 |
| 6 | 13–14 | 23 | 15 | 44 | 100 | 25 | 15 | 44 | 100 |
| | | | | 52 | 100 | | | 52 | 100 |
| | | | | 68 | 90 | | | 68 | 80 |
| | | | | 75 | 90 | | | 75 | 70 |
| | | | | 97 | 80 | | | 97 | 40 |

large volume of water their metabolism appreciably reduced its oxygen content during the course of the experiment (as is shown in Table 3) and it is therefore not possible from these experiments to determine precisely the highest oxygen concentration at which the haemoglobin is functional. Since, however, the bottles were opened and their oxygen contents determined as soon as an increased rate of dying was apparent, the critical oxygen value at which the haemoglobin becomes of significance for survival would seem to be about 15% air saturation (1·1 ml./l. at 14° C., 0·9 ml./l. at 19° C.). Above 15% saturation it is possible that the haemoglobin is also of value in oxygen transport and therefore in ultimately increasing the length of survival, but that the fatal effects of a slightly inadequate oxygen supply take some hours to become apparent.

The lower limit of the range of oxygen pressures at which the haemoglobin functions can more easily be determined. To do so larvae were enclosed in water of low oxygen content in small specimen tubes and the state of their haemoglobin was observed with a spectroscope. When the haemoglobin became fully deoxygenated the oxygen concentration of the water was determined. The average oxygen concentration at which this occurred was 5% air saturation. At and below this concentration, therefore, their haemoglobin can have no functional value.

The range of oxygen pressures over which the larvae of *Tanytarsus* use their haemoglobin at 17° C. is thus from 5 to less than 25% air saturation, while that of *Chironomus* is from 9 to 37% air saturation (Ewer, 1942). The haemoglobin therefore functions at lower oxygen pressures in *Tanytarsus* than in *Chironomus*. In normal *Chironomus* larvae the oxygen uptake at 17° C. is constant and independent of the oxygen concentration of the water down to about 15% air saturation. Above 37% air saturation of the water the animal's oxygen requirements are met by oxygen in physical solution in the blood, but between 37 and 15% air saturation of the water the animal's oxygen uptake owes its independence of external oxygen pressure to a functional haemoglobin. The haemoglobin is thus of functional value to the animal in making available enough oxygen for normal metabolism at oxygen pressures too low to allow of this in the absence of haemoglobin.

In *Tanytarsus*, however, it is meaningless to talk of the normal oxygen requirements of the larvae, since their oxygen uptake varies with the external oxygen pressure and one cannot therefore say what is normal. Even when the haemoglobin is functioning at low oxygen pressures it only raises the metabolic rate a little compared with larvae lacking haemoglobin. The haemoglobin still leaves the oxygen uptake considerably below that of animals in higher oxygen pressures. If a dependence of oxygen uptake on oxygen pressure indicates inadequacy of oxygen transport to the tissues (Henze, 1910), then at all oxygen concentrations below air saturation* *Tanytarsus* suffers partial oxygen lack and the haemoglobin does nothing to alleviate this condition except at very low oxygen pressures, and even then the extra oxygen picked up by the haemoglobin only slightly increases the metabolic rate. The tissues of *Tanytarsus*, in fact, have such a high oxygen demand that they suffer oxygen lack at oxygen pressures of the blood far higher than those which will cause dissociation of a haemoglobin with a high oxygen affinity, such as that of *Tanytarsus*; the oxygen linked with the haemoglobin cannot be of use to the oxygen-greedy tissues.

Is it, however, legitimate to assume that at all oxygen pressures at which the oxygen uptake is dependent the animal is necessarily suffering real oxygen shortage? It may be that animals with this type of metabolism have a surfeit of the intracellular oxidation-reduction systems, enabling them to show a high metabolic rate when plentifully supplied with oxygen, but not necessarily being adversely affected at lower oxygen pressures by an incapacity to maintain this rate. This hypothesis could only be tested by determining the lowest oxygen pressure at which such an animal could live indefinitely and normally. This, to my knowledge, has never been

* And possibly above it. My data, not extending above air saturation, do not settle this point.

done. It is known that *Tanytarsus* is very sensitive to poorly aerated water and Thienemann (1928) says that it is not found in nature in water below 50% air saturation. This is well above the upper limit at which the haemoglobin is used. In fact, the haemoglobin of *Tanytarsus* functions by transporting to the tissues an inadequate amount of oxygen, at an external oxygen pressure which in any case is probably ultimately lethal. The most it can do is to delay death a little. From this it is tempting to conclude that the haemoglobin plays no significant part as an oxygen carrier in the life of *Tanytarsus* in nature, in which case its presence may perhaps be due to some quality other than its oxygen transporting ability.

It should, however, be remembered that the animal lives in conditions very different from those presented to it in these experiments. *Tanytarsus* lives in mud tubes in well-aerated water, but nothing is known of the range of oxygen concentrations it actually encounters in these tubes, nor of its metabolic rate or response to declining oxygen pressures under these conditions.*

Evidence of this nature must come before an evaluation can be made of the normal significance of the haemoglobin of *Tanytarsus*.

## SUMMARY

1. The metabolic rate of *Tanytarsus* larvae is higher in air-saturated water than at lower oxygen concentrations; the oxygen consumption is thus dependent on the external oxygen pressure.

2. The haemoglobin in the blood of the larvae does not function in oxygen transport when the larvae are in water which is between 25 and 100% saturated with air at 17° C. Below 25% air saturation of the water the metabolic rate of larvae without functional haemoglobin (i.e. treated with carbon monoxide) is lower than that of normal larvae. When kept in water below this oxygen concentration larvae with carboxyhaemoglobin also die quicker than normal larvae.

3. The external oxygen concentration at which the haemoglobin in the blood of the larvae becomes deoxygenated is 5% air saturation.

4. The range of oxygen concentrations over which the larvae of *Tanytarsus* use their haemoglobin at 17° C. is thus from 5 to 25% of air saturation.

5. The doubtful significance of the haemoglobin in the life of *Tanytarsus* in nature is discussed.

This investigation was made in the Department of Prof. H. Munro Fox.

* Hyman (1932) records that *Nereis* in tubes has a lower metabolism, more independent of declining oxygen concentrations, than free animals.

## REFERENCES


AMBERSON, W. R., MAYERSON, H. S. & SCOTT, W. J. (1924). The influence of oxygen tension upon metabolic rate in Invertebrates. *J. Gen. Physiol.* **7**, 171.

EWER, R. F. (1942). On the function of haemoglobin in *Chironomus*. *J. Exp. Biol.* **18**, 197.

FOX, H. M. & WINGFIELD, C. A. (1938). A portable apparatus for the determination of oxygen dissolved in a small volume of water. *J. Exp. Biol.* **15**, 437.

HARNISCH, O. (1929). Verbreitung und ökologische Bedeutung des Hämoglobins bei den Chironomidenlarven. *Verh. internat. Zoologenkongr. X. Budapest*, p. 345.

HARNISCH, O. (1930). Daten zur Respirationsphysiologie Hämoglobin-führender Chironomidenlarven. *Z. vergl. Physiol.* **11**, 285.

HARNISCH, O. (1933). Respirationsphysiologische Grundlagen der Ökologie der Chironomidenlarven. *Verh. dtsch. zool. Ges.* **35**, 209.

HARNISCH, O. (1936). Primäre und sekundäre Oxybiose der Larve von *Chironomus thummi*. *Z. vergl. Physiol.* **23**, 391.

HARNISCH, O. (1937). *Chironomus* und *Tanytarsus*. *Biol. Zbl.* **57**, 628.

HENZE, M. (1910). Über den Einfluss des Sauerstoffdruckes auf den Gaswechsel einiger Meerestiere. *Biochem. Z.* **26**, 255.

HYMAN, L. H. (1932). Relation of oxygen tension to oxygen consumption in *Nereis virens*. *J. Exp. Zool.* **61**, 209.

LANKESTER, E. R. (1867). Preliminary notice of some observations with the spectroscope on animal substances. *J. Anat., Lond.*, **2**, 114.

LANKESTER, E. R. (1873). A contribution to the knowledge of haemoglobin. *Proc. Roy. Soc.* **140**, 70.

MALLOCH, J. R. (1915). The Chironomidae, or midges, of Illinois, with particular reference to the species occurring in the Illinois River. *Bull. Ill. Lab. Nat. Hist.* **10**, 275.

MALOEUF, N. S. R. (1937*a*). Studies on the respiration (and osmoregulation) of animals. I. Aquatic animals without an oxygen transporter in their internal medium. *Z. vergl. Physiol.* **25**, 1.

MALOEUF, N. S. R. (1937*b*). Studies on the respiration of animals. II. Aquatic animals with an oxygen transporter in their internal medium. *Z. vergl. Physiol.* **25**, 29.

RASHEVSKY, N. (1933). Note on the mathematical theory of oxygen consumption at low oxygen pressures. *Protoplasma*, **20**, 125.

THIENEMANN, A. (1923). Die beiden Chironomusarten der Tiefenfauna der Norddeutschen Seen. *Ver. int. Ver. Limnol. Kiel. Stuttgart*, **1**, 108.

THIENEMANN, A. (1928). *Die Binnengewässer*. Stuttgart.

WALSHE, B. M. (1947). On the function of haemoglobin in *Chironomus* after oxygen lack. *J. Exp. Biol.* **24**, 329.