

THE RELATION OF OXYGEN CONSUMPTION TO BODY
SIZE AND TO TEMPERATURE IN THE LARVAE OF
CHIRONOMUS RIPARIUS MEIGEN

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INTRODUCTION

The experiments described in the present paper were undertaken so that an assessment could be made of the importance of these larvae in the oxygen balance of polluted streams (Phelps, 1944), where under certain conditions they are very abundant (Edwards, 1957). The effect of the oxygen concentration of the water upon the oxygen consumption of fully grown *Chironomus* larvae has been studied by Ewer (1942) and Walshe-Maetz (1953). The influence of two other factors of primary importance in determining the rate of oxygen consumption, namely, body size and environmental temperature, have been investigated in the present study; work has been confined to 3rd- and 4th-instar larvae and to only two environmental temperatures.

The investigation may be of interest, however, from a more general viewpoint for it is possible with this species, as it is to some extent with the majority of the aquatic arthropods, to distinguish experimentally between an increase in size, indicated by total weight, volume and surface area measurements, and growth expressed in terms of the weight of body constituents other than water (dry weight). The former expression of growth is generally discontinuous whilst the latter is continuous (Teissier, 1931). In consequence of this type of growth pattern, the relationship of oxygen consumption to surface area can be separated from the relationship of oxygen consumption to an exponential function of dry weight. Generally such a separation may be made, by a statistical treatment of the data, only when the exponential function relating oxygen consumption to weight is sufficiently different from that relating surface area to weight. The variability of biological material and the limited size range of most experimental animals are such that a satisfactorily conclusive separation is rarely possible (see Kleiber, 1947).

MATERIALS AND METHODS

Specimens were obtained from the Maple Lodge Effluent Channel, Rickmansworth, Hertfordshire, and placed in aerated water containing effluent mud.

Oxygen consumption

Larvae were conditioned at the test temperature $\pm 2^\circ$ C. for at least 24 hr. before testing, mud being present in the conditioning tanks. Oxygen consumption determinations were made principally in October and November 1956, when the effluent temperature was approximately 15° C. The investigation was confined to 3rd- and 4th-instar larvae, and two test temperatures, 10 and 20° C., were used.

Measurements were made with a respirometer of the Warburg constant-volume type. The respirometer flasks described an arc of 1.43 cm. at a rate of 86 complete swings per minute. After a steady state had been reached, the rate of oxygen uptake over 5 hr. was recorded. Groups of from seven to thirty-three larvae were placed in each flask, the number depending upon the body size of each group, and the groups being selected by eye for size uniformity. The respirometer was fitted with a hood which excluded most of the light.

Larvae were subsequently placed on filter-paper to remove surface moisture and weighed (wet weight). They were then dried overnight at 105° C. and reweighed (dry weight). The average weight for each group was determined.

Specific gravity

The specific gravity of larvae was measured by immersing them in solutions of sodium chloride and of sucrose. In Fig. 7 which shows the relationship between specific gravity and wet weight, the close agreement between sodium chloride and sucrose determinations suggests that salt absorption does not change the specific gravity of the larvae appreciably during the period under test. Larvae were divided into groups of similar size, the average body weight of each group being determined. The specific gravity of each size group was measured by immersing several larvae in each of a series of solutions ranging in specific gravity from 1.01 to 1.06. The number of larvae at the surface, suspended in the solution, or resting on the bottom of the vessel, was recorded 1 min. after immersion. This method, though perhaps not as accurate as the displacement method of density determination described by Lowndes (1942) for marine animals, is simpler and quicker.

Linear dimensions

The lengths of larvae were measured by means of a micrometer eyepiece, the larvae being first immersed in 0.5% chloral hydrate to reduce body movements. Larvae were prevented from bending by placing them on a cavity slide, the width of the cavity being approximately that of the larvae. The average length and average wet weight of groups of ten larvae, selected by eye for size uniformity, were recorded. The measured length included the head but not the posterior pseudopods.

RESULTS

Oxygen consumption

An equation of the form $R = aW^b$ (where R is the oxygen consumption of an organism of weight W and a and b are constants) is often given as an expression of the relationship between oxygen consumption and body weight. A plot of the logarithmic transformation, $\log R = \log a + b \log W$, gives a straight line, the slope of which denotes b , relating oxygen consumption to weight. The equation is sometimes expressed in the form $r = aW^{(b-1)}$, where $r = R/W$, the oxygen consumption per unit weight.

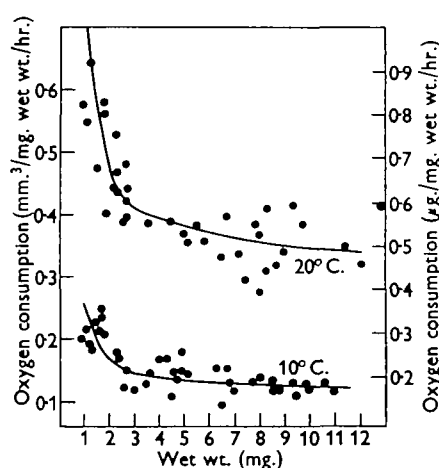


Fig. 1

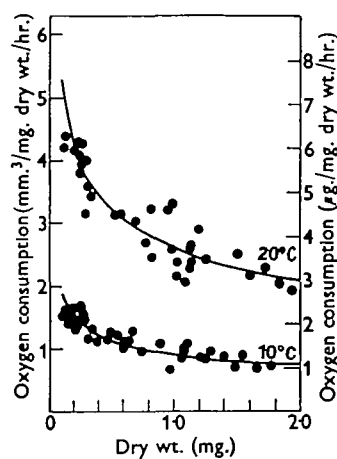


Fig. 2

Fig. 1. Oxygen consumption per unit wet weight plotted against wet weight.

Fig. 2. Oxygen consumption per unit dry weight plotted against dry weight.

Fig. 1 shows the values for oxygen consumption per unit wet weight plotted against wet weight at 10 and 20° C. The wet weight varies between 0.89 and 12.82 mg., and over this size range the oxygen consumption per unit weight of the smallest larvae is about twice that of the largest. Fig. 2 shows the values for oxygen consumption per unit dry weight plotted against dry weight. It is clear from an inspection of the data that there is a progressive decrease in oxygen consumption per unit weight as the size of the animal increases.

Logarithmic transformations of the data shown in Fig. 1 are drawn in Fig. 3. These are clearly not straight lines, that is, b has no constant value and the relationship $R = aW^b$ does not hold when wet weight is used as a measure of body size. On the other hand, logarithmic transformations based on the dry-weight data, shown in Fig. 4, suggest that b has a fixed value. Regression analyses gave coefficients of $-0.29 (\pm 0.023)$ at 10° C. and $-0.30 (\pm 0.025)$ at 20° C. The oxygen

consumption per unit weight is proportional to these powers of the dry weight, and the total oxygen consumption is proportional to the 0.71 power of the dry weight at 10° C. and to the 0.70 power at 20° C.

The larvae consume oxygen 2.6 times as fast at 20° C. as at 10° C. over the size-range studied. The results suggest that the Q_{10} value is not dependent upon size, the difference in the regression coefficients being insignificant and the results of a 't' test giving $P=0.3$.

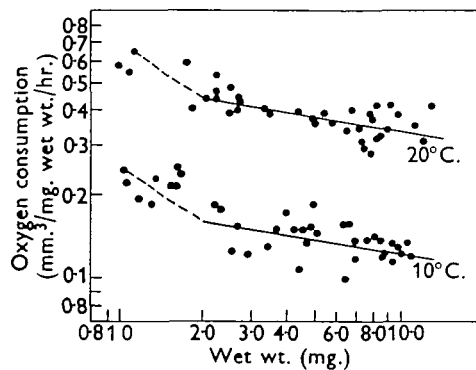


Fig. 3. Log oxygen consumption per unit wet weight plotted against log wet weight. Continuous lines, representing the oxygen consumption of 4th-instar larvae, are drawn from calculations based on regression lines in Figs. 4 and 6.

The oxygen consumptions of several groups of larvae were determined at twice the normal amplitude of shaking of the respirometer flasks, to ascertain the effect of shaking upon the rate of oxygen consumption (Table 1). A regression analysis using the method of concomitant observations (Mather, 1943) applied to the data suggests that the difference in amplitude of shaking did not influence the oxygen consumption of the larvae, the results of a 't' test giving $P=0.6$.

The experiments concerning the effect of shaking on the rate of oxygen consumption were carried out in August 1957, whereas the remainder of the oxygen-consumption determinations were made in October and November 1956. The results of an analysis of covariance suggest that the oxygen consumption per unit dry weight of the 'summer' larvae is significantly higher than that of the 'autumn' larvae ($P < 0.001$), whereas the slopes of the regression lines for 'summer' and

Table 1. *Effect of amplitude of shaking on oxygen consumption*

	Oxygen consumption at 20° C. (mm.³/g. wet wt./hr.)								
	Normal shaking amplitude	487	457	436	332	390	380	410	419
Normal shaking amplitude × 2	528	466	391	390	481	399	366	409	418
Average wet wt. (mg.)	6.23	7.96	10.13	7.23	7.16	11.71	9.23	10.24	9.41

Average wet weights were converted to dry weights for comparing seasonal oxygen consumption rates (Fig. 4) using the relationship between wet and dry weights shown in Fig. 6.

'autumn' larvae (see Fig. 4) are not different ($P=0.3$). The data for both amplitudes of shaking were grouped in these comparisons of seasonal rates of oxygen consumption.

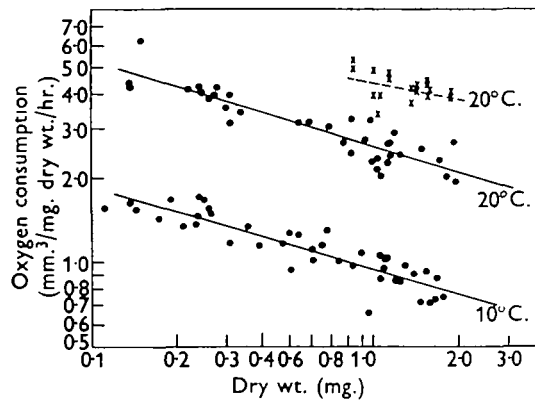


Fig. 4. Log oxygen consumption per unit dry weight plotted against log dry weight. x, August determinations; ●, October–November determinations.

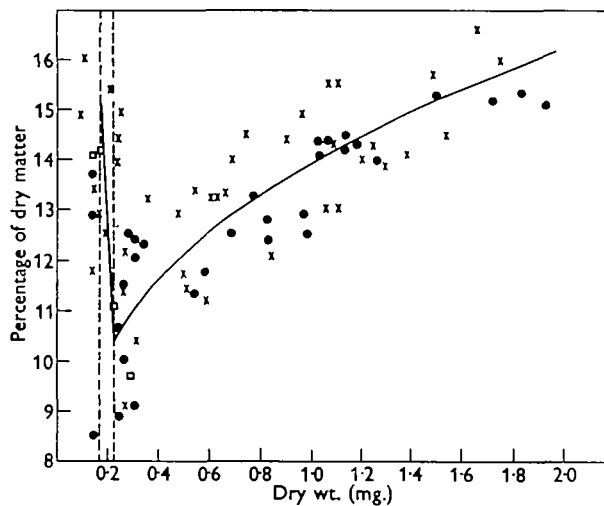


Fig. 5. Change in percentage of dry matter with growth. x, larvae from oxygen consumption determinations at 10° C.; ●, larvae from oxygen consumption determinations at 20° C.; □, data from Table 2.

Percentage of dry matter

The change in the percentage of dry matter with size, indicated by the disparity between the oxygen-consumption relationships based on wet and dry weights, is shown in Fig. 5. The percentage of dry matter decreases suddenly when larvae reach a weight of approximately 0.2 mg. dry weight. Subsequent growth is associated with a gradual increase in the percentage of dry matter. Geng (1925) found that larvae of *Chironomus thummi* K (= *riparius* Meigen) weighing 0.67 mg. dry

weight contained 12.82% dry matter, whilst Karsinkin (1935) found a value of 17.94% for larvae weighing 1.35 mg. dry weight. Geng's value lies close to the curve drawn in Fig. 5.

Larvae weighing between 0.1 and 0.3 mg. dry weight were separated into their respective instars using head size (Tanaka, 1939), grouped, and the percentage of dry matter was determined. Table 2, summarizing these data, indicates that moulting occurs when larvae weigh between 0.17 and 0.23 mg. dry weight. The initial increase in body weight following the moult is principally the result of water intake, this intake being reflected by the change in percentage of dry matter.

The relation between dry weight and wet weight throughout growth in the 4th instar is plotted logarithmically in Fig. 6. The dry weight varies as the 1.186 power (± 0.022) of the wet weight.

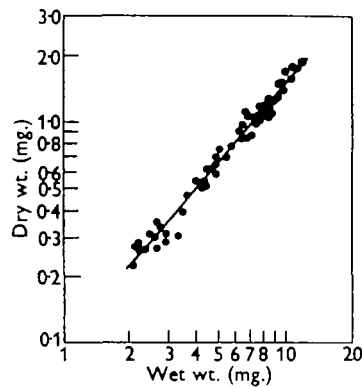


Fig. 6. Log dry weight plotted against log wet weight for 4th-instar larvae.

Table 2. Change in percentage of dry matter associated with the third larval moult

Instar	Average wet wt. (mg.)	Average dry wt. (mg.)	Percentage dry matter
3	1.22	0.1735	14.2
3	1.02	0.1433	14.1
4	2.096	0.223	11.1
4	2.955	0.286	9.7

Although the relationship $R = aW^b$ does not hold for larvae of *C. riparius* over the whole of the size range studied when body size is expressed as wet weight (see Fig. 3), a constant value of b may be found for the 4th instar since it has been shown that (a) the oxygen consumption varies as the 0.7 power of the dry weight, and (b) the dry weight varies as the 1.186 power of the wet weight.

Thus, within the 4th instar oxygen consumption varies as the 0.83 power (1.186×0.70) of the wet weight. Though the value of b appears to be constant within an instar, moulting brings about a change in the value of the constant a , so

that, when log oxygen consumption is plotted against log wet weight over a size range associated with two or more instars, the relationship cannot be represented by a single straight line (Fig. 3).

Specific gravity

An increase of specific gravity from about 1.026 to 1.043 occurs during growth from 3 to 11 mg. wet weight (see Fig. 7). This increase reflects the increase in percentage of dry matter which takes place during growth within the 4th larval instar.

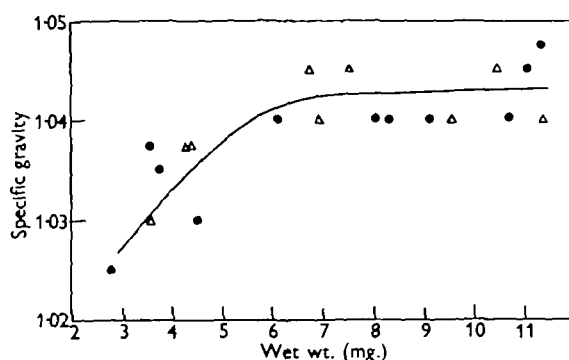


Fig. 7. Change in specific gravity of 4th-instar larvae with growth. Δ , sucrose determinations; \bullet , sodium chloride determinations.

Table 3. *Volume, wet weight, specific gravity and dry weight of early 4th-instar larvae as compared with late 4th-instar larvae*

	Dry wt. (mg.)	Wet wt. (mg.)	S.G.	Volume (mm. ³)
Early	0.3	2.74	1.026	2.67
Late	1.8	11.32	1.043	10.86
Ratio $\frac{\text{late}}{\text{early}}$	6	4.13	—	4.06

$$\frac{\text{Ratio (wet wt.)} - \text{ratio (volume)}}{\text{Ratio (wet wt.)}} \times 100 = 1.7.$$

$$\frac{\text{Ratio (dry wt.)} - \text{ratio (wet wt.)}}{\text{Ratio (wet wt.)}} \times 100 = 45.3.$$

Table 3 shows how closely volume is proportional to wet weight, the discrepancy amounting to no more than 1.7%, whereas dry weight is not proportional to wet weight, the discrepancy being 45.3%.

Surface area estimates

If one assumes that the shape of a *C. riparius* larva approximates to a cylinder, the surface area of such larvae may be calculated from length and volume measurements, the latter being calculated from weight and specific gravity determinations.

One of the more important errors in this approximation of surface area is probably that resulting from the omission of the ventral gills, whose surface area is larger compared with their weight. For the present study, however, where the surface area is being considered primarily in its relationship to oxygen consumption, the surface area of the ventral gills is best omitted in view of Fox's observation that they do not absorb oxygen as does the rest of the body surface (Fox, 1921; Thorpe, 1933). The area through which oxygen enters, being less than the total surface area, is probably determined more accurately by the cylinder approximation than by actual measurement.

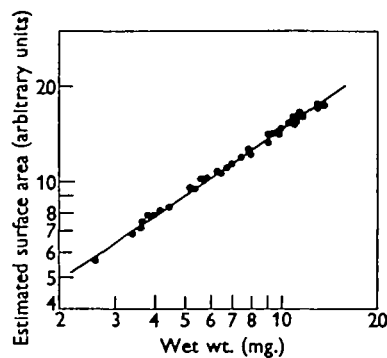


Fig. 8. Log estimated surface area plotted against log wet weight for 4th-instar larvae.

Fig. 8 shows the logarithmic transformation of estimated surface area plotted against wet weight for 4th-instar larvae. The surface area is proportional to the 0.70 power of wet weight. The standard error has not been calculated because the variables are not independent, the surface area estimates being calculated partly from weight.

In Fig. 9 where oxygen consumption at 20° C. is plotted against estimated surface area it is evident that the relationship is not one of simple proportionality. The oxygen consumption per unit area increases with increase in size (throughout the 4th instar). Values for the total oxygen consumption of 3rd-instar larvae have not been included in Fig. 8 as the specific gravity and the relationship between length and weight have only been investigated for the 4th-larval instar.

DISCUSSION

The relationship between size and respiration rate has been extensively reviewed in recent years (Weymouth *et al.* 1944; Kleiber, 1947; Ellenby, 1951; Zeuthen, 1953, 1955; Bertalanffy, 1957). It has been widely observed that the respiration rate falls with increasing size and these observations have some justification on thermodynamic grounds (Prigogine & Wiame, 1946). Generally, b , the exponent relating respiration and size, has a fixed value for wide size ranges of many organisms, though Zeuthen (1955) suggests that it is best considered as a tangent to

a more complicated curve. Kleiber (1947), discussing the theoretical validity of the Surface Law (Rubner, 1883), concludes that there is no satisfactory basis for the acceptance of a causal relationship between surface area and respiration, especially with poikilothermic animals. Zeuthen (1953) 'considers that the surface concept has dominated our thinking in this field far too much', though as Needham (1942)

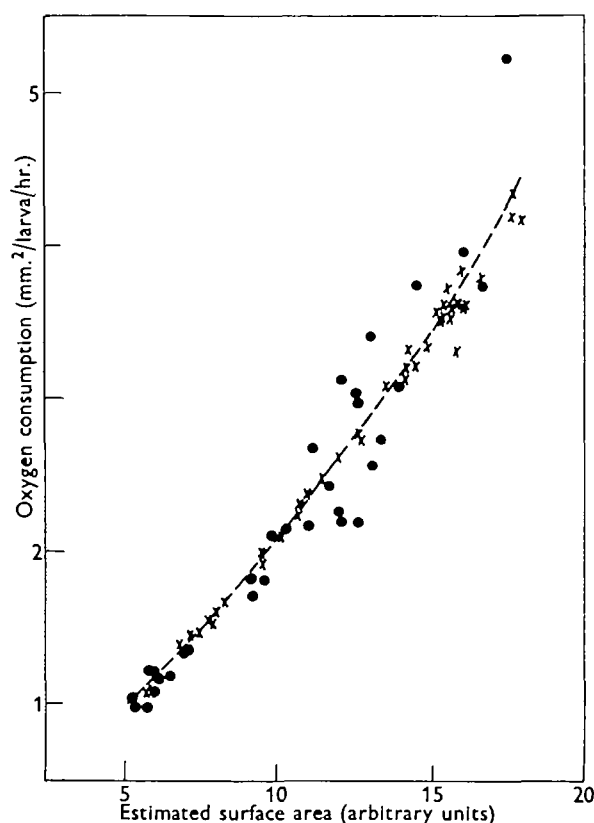


Fig. 9. Oxygen consumption plotted against estimated surface area for 4th-instar larvae. ●, values calculated from measurements of oxygen consumption at 20° C. recorded in Fig. 3, the surface area estimates being assessed from the relationship between weight and surface area shown in Fig. 8; ×, values calculated from surface area estimates recorded in Fig. 8, the oxygen consumption being calculated from the relationship between weight and oxygen consumption at 20° C. shown in Fig. 3.

points out, the 'surface factor' must not be excluded. The 'surface factor' may be of greater relevance if it is not restricted to the external body surface. Rensch (1948) describes how certain changes in tissue structure and cell shape are associated with changes in body size, and such histomorphological changes may be of importance in our more complete understanding of the relationship between body size and rate of respiration.

Ellenby (1945, 1951) has pointed out that there is no *a priori* reason why the surface areas of a series of bodies of differing size should be proportional to the two-thirds power of their weights. There is no justification for the assumption that the Surface Law applies when it has been shown that b has a value not significantly different from two-thirds (Gilchrist, 1956), unless it has been demonstrated that there are no changes in shape and specific gravity. It is also true, however, that the rejection of the Surface Law on the grounds that b differs from two-thirds is equally faulty. This association between surface area and the two-thirds power of the weight has led to much of the disorder now prevailing in this field.

If one were looking for a poikilothermic animal to demonstrate the relationship between surface area and oxygen consumption one might well select the larva of *C. riparius* which has an extremely rudimentary tracheal system (Miall & Hammond, 1900) and which absorbs oxygen through the general body surface (Fox, 1921). The results of the present investigation suggest, however, that the oxygen consumption is not proportional to the estimated surface area, but varies as the 0.7 power of the dry weight of the body.

It seems best to consider the relationship between size and respiration as a further demonstration of the heterauxesis theory (Needham, 1942). This states that when the magnitude of a part of an organism, either chemical or anatomical (y) is compared with its totality (x), a relationship of the form $y = cx^d$, where c and d are constants, is frequently observed. Needham suggests that the fall in metabolic rate is best regarded as a change in the relative proportion of respiring protoplasm and inert material in the cells. Several workers have found falling metabolic rates in tissues taken from animals of increasing size (Weymouth *et al.* 1944; Krebs, 1950), and Rosenthal & Drabkin (1943) observed that cytochrome c concentrations of mammalian epithelium decreased with increasing body weight.

Bertalanffy (1951) has carried the analysis of the size/respiration relationship somewhat further and has divided animals into three metabolic groups according to the values of b . Bertalanffy (1951) and Bertalanffy & Krywienczyk (1953) have suggested that some taxonomic groups are homogeneous in respect of the value of b . It is also suggested that the metabolic groups have characteristic forms of growth. Some evidence does not accord with Bertalanffy's views (see Zeuthen, 1955). Mann (1956) records values of b varying from 0.695 to 1.06 for five species of freshwater leeches; two closely related species *Erpobdella octoculata* and *E. testacea* having values of 1.06 and 0.81. It has been shown, however, that three of the species studied have a similar type of growth (Mann, 1953, 1957). According to Bertalanffy (1951) the metabolic rate of insect larvae is proportional to body weight (see also Edwards in Roeder, 1953); this is at variance with the conclusions of the present investigation.

Ewer (1942) measured the oxygen consumption of fully grown *Chironomus* larvae of the *plumosus* group (subsequently identified as *C. cingulatus* Mg and *C. riparius*

Mg) at 17° C., using the syringe and micro-Winkler method described by Fox & Wingfield (1938). Although the weights of the larvae and the season of the year are not given, a comparison of the results is of some interest. For fully grown winter larvae (10 mg. wet weight), assuming a linear relationship between temperature and oxygen consumption between 10 and 20° C., the calculated oxygen consumption at 17° C. would be about 280 mm.³/g. wet wt./hr. Ewer found an oxygen consumption of about 190 mm.³/g. wet wt./hr. The discrepancy may be attributed to differences of method or material, for the findings of Tanaka (1939) on the temperature/growth relationship of *C. dorsalis* Mg, suggest that the temperature relation is approximately linear over this temperature range ($Q_{10}=1.53$). Walshe-Maetz (1953) found discernible differences between the oxygen consumption rates of two subspecies of *C. plumosus* L. It is unlikely, however, that differences between closely allied species could satisfactorily account for a discrepancy of such magnitude. It seems possible that the difference in methods employed in the oxygen consumption determination may be largely responsible for the apparent difference between the oxygen consumption rates. Both methods are extensively used in respiration studies of aquatic animals, and comparative studies similar to that made by Wilder (1937) with salamanders, would seem to be worthwhile.

Estimates of the total oxygen consumption of natural populations based on laboratory determinations must be treated with caution. Walshe-Maetz (1953) has clearly shown how the degree of artificiality in test conditions influences the oxygen consumption rate. Estimates made from oxygen consumption values presented in this paper agree well, however, with observed changes in the oxygen uptake of stream muds when known numbers and weights of larvae are added to them (Edwards, unpublished).

Seasonal adjustments in oxygen consumption rates similar to those described by Edwards & Irving (1943) for *Emerita talpoida*, where rates in winter are higher than those in summer at comparable temperatures, have not been found with *Chironomus riparius*. It seems that the oxygen consumption rate at 20° C. is higher in summer than in the winter. The depression of metabolism in the winter may be regarded as evidence of an arrested development of the overwintering generation (Lees, 1955) which remains at the larval stage from October until March. There are about seven generations between March and October.*

With *C. riparius* larvae, the relation between oxygen consumption and size is not influenced by temperature. Whitney (1942) recorded a similar independence for *Crenobia (Planaria) alpina*. Hotovy (1938), working with *Triops cancriformis*, found that the size factor diminished with decreasing temperatures. The usual relationship, $R=aW^b$, does not fit Hotovy's data, however, especially at the lower temperatures. Edwards (1946), discussing the influence of temperature upon the oxygen consumption of *Talorchestia* and *Melanotus*, concluded that small animals show a greater response to temperature changes than large ones. This conclusion

* See *Freshwater Biological Association Annual Report*, 23, 1955, p. 38.

was not based on relative values, however, and an inspection of the graphs drawn from the data (Prosser, 1950) suggests that the proportionate response of small animals to a given temperature change was no greater than for large ones.

SUMMARY

1. The oxygen consumption rates of 3rd- and 4th-instar larvae of *Chironomus riparius* have been measured at 10 and 20° C. using a constant-volume respirometer.
2. The oxygen consumption is approximately proportional to the 0.7 power of the dry weight: it is not proportional to the estimated surface area.
3. This relationship between oxygen consumption and dry weight is the same at 10 and at 20° C.
4. The rate of oxygen consumption at 20° C. is greater than at 10° C. by a factor of 2.6.
5. During growth the percentage of dry matter of 4th-instar larvae increases from 10 to 16 and the specific gravity from 1.030 to 1.043.
6. The change in the dry weight/wet weight ratio during the 4th larval instar supports the theory of heterauxesis.
7. At 20° C., 'summer' larvae respire faster than 'winter' larvae.

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