

THE OXYGEN REQUIREMENTS AND THERMAL RESISTANCE OF CHIRONOMID LARVAE FROM FLOWING AND FROM STILL WATERS

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

(Received 25 July 1947)

(With Three Text-figures)

Extensive surveys of the distribution of chironomid larvae have established the fact that the majority of species are strikingly restricted in their habitat: the causes of this specialization, however, remain largely undetermined. For a few species comprehensive ecological work has made it possible to deduce the factors important in determining distribution (Thienemann, 1923, 1928; Lenz, 1925), but an experimental attack on the physiology and behaviour of larval and adult chironomids in relation to their choice of habitat remains almost a virgin field.

In other groups of aquatic invertebrates experimental work has indicated that related forms from different habitats may have well-marked physiological differences. Various species of ephemeropterid nymphs and caddis larvae from streams, for instance, have higher metabolic rates than related species from stagnant water, and the former are more sensitive to oxygen lack than the latter (Fox & Simmonds, 1933; Fox, Simmonds & Washbourn, 1935). May-fly nymphs from swift streams are less resistant to high temperatures than those from slow-moving waters (Whitney, 1939), and the heat tolerance of other aquatic organisms has been shown to be adapted to the temperature conditions of their environment (Plateau, 1872; Mason, 1939).

In order to find the extent to which the physiological requirements of chironomid larvae are related to their special habitats I have made measurements of certain physiological processes in the larvae of a number of species from different types of environment. The rate of oxygen consumption, capacity for anaerobiosis and resistance to high temperatures were measured. As far as possible closely related species with different habitats were selected for comparison.

(1) OXYGEN CONSUMPTION

The oxygen requirements of chironomid larvae of four species were studied. The larvae fell into two comparable pairs, the members of each pair being closely related and of about the same size. One member of each pair occurs in a stream and the other in a still-water environment. The following species were used: *Anatopynia varia* (Fabr.) (Tanypodinae) from a stagnant ditch; *A. nebulosa* (Mg.) from a stream. *Chironomus longistylus* Goet. (Chironominae) from a stagnant ditch; *Tanytarsus brunnipes* (Zett.) (Chironominae) from a stream. Final instar larvae of all species were used, except *Chironomus longistylus* in which case young larvae,

6-7 mm. in length, were selected in order to be more nearly comparable with the 7 mm. larvae of *Tanytarsus*. The three last-mentioned species have sufficient haemoglobin in the blood to make the larvae appear distinctly red; *Anatopynia varia*, however, contains much less, the blood being only pale pink, and in some individuals almost colourless. The larvae were usually collected on the day before the experiments and were kept overnight in running water in shallow dishes with a thin layer of mud. Larvae which had been more than 2 days in the laboratory were never used.

The oxygen consumption of the larvae was determined by the method described by Ewer (1942). Between twelve and twenty *Chironomus* or *Tanytarsus* larvae, or six *Anatopynia*, were used each time.

The results of these experiments are given in Tables 1-4 and Fig. 1. The ditch and stream species differ strikingly in their oxygen requirements. Both *Tanytarsus brunnipes** and *Anatopynia nebulosa*, from streams, have a higher metabolic rate at

Table 1. *Oxygen consumption of young Chironomus longistylus* Goet. larvae at 17° C.

Oxygen concentration (ml./l.)	Oxygen consumption (cu.mm./g. (wet wt.)/hr.)	
	Separate values	Means
6.01-7.00	444, 331, 417, 200, 342, 219, 140, 337, 452	320 ± 37
5.01-6.00	366, 335, 311, 280, 308, 366, 360, 394, 296, 396, 342, 323, 543, 375, 304, 202, 107	330 ± 21
4.01-5.00	292, 266, 247, 312, 259, 379, 244, 284, 381	296 ± 9
3.01-4.00	309, 252, 313, 310, 258, 244, 505, 328, 390, 316	313 ± 22
2.01-3.00	268, 236, 297, 240, 245, 356, 294, 359, 315, 287, 324, 196, 314, 225, 440, 464	304 ± 11
1.01-2.00	350, 219, 379, 215, 279, 330, 315, 267, 291, 277, 213, 377, 266, 264, 184, 332, 283, 295	285 ± 13
0.51-1.00	168, 220, 247, 245, 297, 205, 228, 213, 159	220 ± 13
0.00-0.50	45, 62, 205, 68, 185	117

Table 2. *Oxygen consumption of Tanytarsus brunnipes* (Zett.) larvae at 17° C.

Oxygen concentration (ml./l.)	Oxygen consumption (cu.mm./g. (wet wt.)/hr.)	
	Separate values	Means
6.01-7.00	364, 484, 360, 270, 434, 356, 394, 590, 666, 531, 700, 482	469 ± 38
5.01-6.00	500, 451, 486, 304, 233, 336, 313, 344, 565, 621, 466, 440, 635, 448, 301	430 ± 31
4.01-5.00	305, 418, 361, 354, 363, 210, 371, 440, 435, 467, 238, 399, 315, 320, 404, 296, 467, 436	367 ± 9
3.01-4.00	318, 286, 287, 341, 331, 219, 232, 315, 378	301 ± 17
2.01-3.00	297, 366, 280, 327, 218, 310, 422, 390, 381, 368, 303, 268, 307, 294, 350, 443, 426	342 ± 8
1.01-2.00	250, 119, 329, 248, 251, 315, 237, 263, 226, 224	246 ± 16
0.50-1.00	47, 146, 128, 176, 70, 198, 201, 162	141 ± 20

* Other data of the oxygen consumption at various oxygen pressures of *T. brunnipes* have already been published (Walshe, 1947). The experiments giving these data were made on larvae from a different habitat and at a different season: these factors must account for the slight differences between the two sets of data.

Table 3. Oxygen consumption of *Anatopynia varia* (Fabr.) larvae at 17° C.

Oxygen concentration (ml./l.)	Oxygen consumption (cu.mm./g. (wet wt.)/hr.)	
	Separate values	Means
6.01-7.00	348, 156, 300, 428, 503, 503, 428, 287, 214, 207	337 ± 40
5.01-6.00	402, 304, 263, 324, 326, 430, 511, 271, 413	360 ± 28
4.01-5.00	96, 357, 115, 256, 307, 353, 273, 356, 340, 276, 198, 224, 275, 242	262 ± 22
3.01-4.00	218, 194, 231, 394, 247, 199, 167, 426, 386, 296, 413	288 ± 30
2.01-3.00	340, 357, 350, 414, 195, 247, 200, 381, 429, 441	335 ± 29
1.01-2.00	67, 216, 132, 251, 339, 244, 314, 167, 134, 255, 268, 210, 286, 221, 182	220 ± 20
0.00-1.00	75, 1, 38, 120, 195, 179, 92, 33, 46, 225, 88, 335, 352, 457	160 ± 36

Table 4. Oxygen consumption of *Anatopynia nebulosa* (Mg.) larvae at 17° C.

Oxygen concentration (ml./l.)	Oxygen consumption (cu.mm./g. (wet wt.)/hr.)	
	Separate values	Means
6.01-7.00	519, 253, 542, 371, 423, 346, 440, 370, 324, 454, 466, 612, 668, 223	429 ± 34
5.01-6.00	510, 183, 474, 264, 395, 209, 231, 206, 258, 277, 337, 474, 388	324 ± 33
4.01-5.00	194, 228, 232, 268, 306, 305, 412, 235, 288, 324, 197, 213, 279, 301, 221, 224, 479, 300, 315, 170	275 ± 16
3.01-4.00	134, 267, 299, 174, 157, 232, 293, 261, 215, 290, 232, 129, 197, 250, 173, 118, 453, 611, 131	243 ± 27
2.01-3.00	179, 169, 67, 228, 362, 208, 247, 275, 134, 196, 140, 162, 208, 189	197 ± 19
1.01-2.00	85, 67, 176, 20, 125, 24, 138, 152, 104	99 ± 18
0.00-1.00	86, 39, 132, 98, 106	92

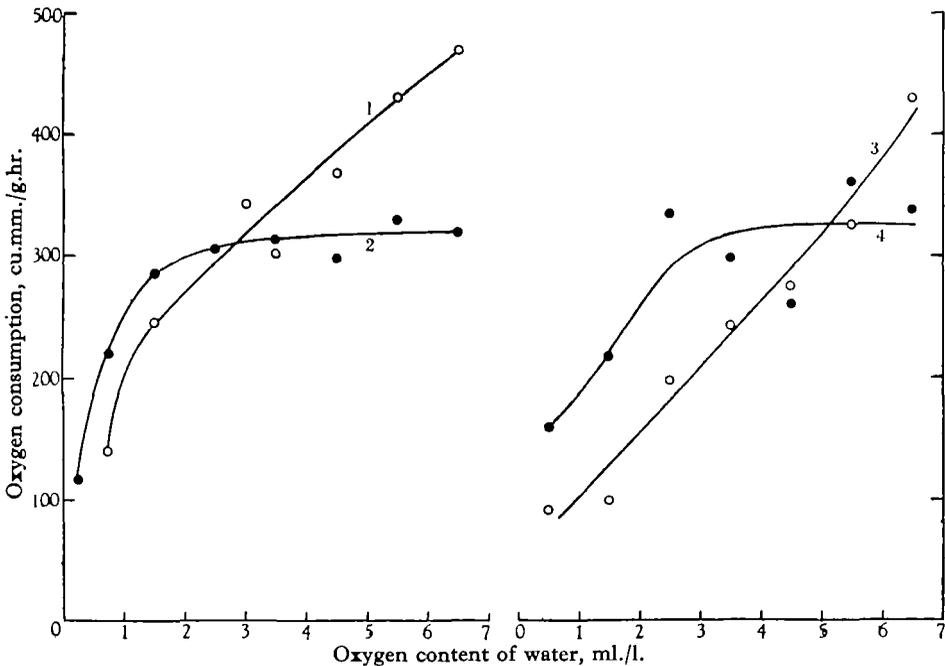


Fig. 1. Rates of oxygen consumption at 17° C. of chironomid larvae at various concentrations of dissolved oxygen. (1) *Tanytarsus brunnipes* (stream); (2) *Chironomus longistylus* (ditch); (3) *Anatopynia nebulosa* (stream); (4) *A. varia* (ditch). Data from Tables 1-4.

air saturation of the water than the corresponding ditch species; but at lower oxygen contents the oxygen consumption of the stream species falls rapidly, the relation between oxygen consumption and oxygen content of the water being roughly linear. The rate of oxygen consumption of the two stagnant water species, however, is almost independent of the oxygen content until this falls to the value of 3 ml./l. (44% air saturation).

The rates of heart beat, in fully aerated water at 17° C., of the four species were found to be 32.4 ± 1.1 beats per min. in *Anatopynia varia* as compared with 32.4 ± 1.0 in *A. nebulosa*, and 72.9 ± 5.1 in *Chironomus longistylus* compared with 64.4 ± 2.1 in *Tanytarsus brunnipes*. There is thus no significant difference in the rate of blood circulation in the comparable ditch and stream species in spite of the differences in oxygen consumption. This is unlike the case of the ephemeropterid nymphs *Cloeon* and *Baetis* (Fox & Simmonds, 1933).

(2) SURVIVAL UNDER ANAEROBIC CONDITIONS

A comparative study was made of the capacity of larvae from different habitats to live under anaerobic conditions. A list of the species used and the habitats from which they were obtained is given in Table 5. In all cases final instar larvae were

Table 5. *Survival times of various chironomid larvae under anaerobic conditions*

No. on Fig. 2	Species	Subfamily	Habitat	50% alive (hr.)
1	<i>Tanytarsus brunnipes</i> (Zett.)	Chironominae	Stream	8
2	<i>Procladius olivacea</i> (Mg.)	Diamesinae	Stream	10
3	<i>Procladius choreus</i> (Mg.)	Tanypodinae	Stream	12
4	<i>Anatopynia nebulosa</i> (Mg.)	Tanypodinae	Stream	14
5	<i>Chironomus albimanus</i> Mg.	Chironominae	Stream	16
6	<i>C. rubeculosus</i> Mg.	Chironominae	River	20
7	<i>Anatopynia varia</i> (Fabr.)	Tanypodinae	Ditch	46
8	<i>Tanytus punctipennis</i> Mg.	Tanypodinae	Stagnant backwater	50
9	<i>Chironomus longistylus</i> Goet.	Chironominae	Ditch	68
10	<i>C. paganus</i> Mg.	Chironominae	Concrete trough	101

used. The length of survival without oxygen was measured by enclosing the larvae in bottles of nitrogen-saturated water, which were then kept in the dark at room temperature and the numbers of dead noted at intervals. In all cases larvae were enclosed in the proportion of 0.2 g. to 400 ml. water: the number of animals used varied from 5 to 25 per bottle according to the size of the larvae. Five bottles of each species were used.

The results of these experiments are given in Fig. 2, which shows clearly that the species fall into two well-defined groups: one in which the capacity to live anaerobically is small, 50% of the larvae being dead after 8–20 hr., and the other in which the animals remain alive much longer, the 50% death points ranging from 46 to 101 hr. The distinction between the two groups is unrelated to any systematic or morphological differences in the larvae, for the various species of *Anatopynia* and *Chironomus* are seen to have very different susceptibilities. On the other hand, the

distinction between the two groups can clearly be correlated with differences in habitat. The group with a slight capacity for anaerobiosis is composed of species taken from running waters, while the larvae in which the capacity is greater are all from stagnant water environments. This is comparable with the case of the ephememerid nymphs and caddis larvae of Fox *et al.* (1933, 1935).

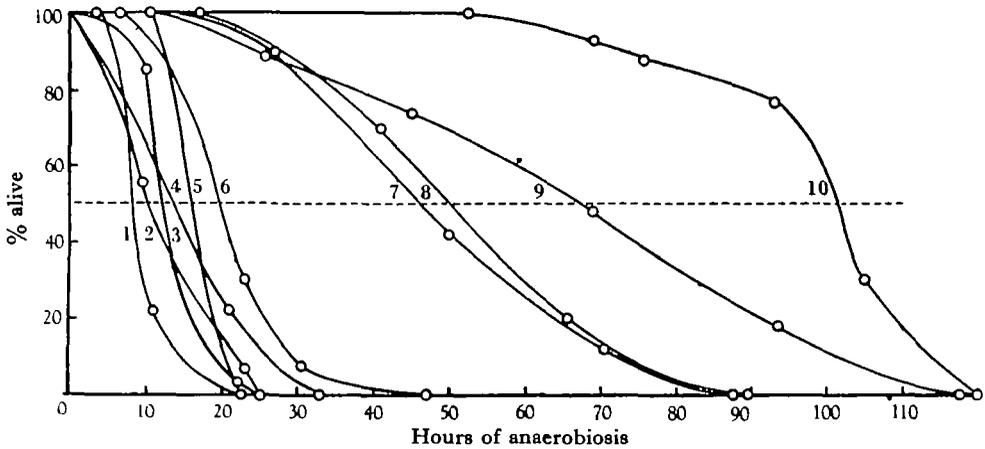


Fig. 2. Duration of life under anaerobic conditions of various chironomid larvae. Species used (nos. 1-10) given in Table 5.

(3) THERMAL RESISTANCE

Experiments were made to determine the heat tolerance of larvae from different habitats. Table 6 gives a list of the seven species with their habitats; fully grown larvae were used. The thermal resistance was determined by placing twenty

Table 6. Heat resistance of various chironomid larvae

No. on Fig. 3	Species	Subfamily	Habitat	Thermal index (° C.)
1	<i>Tanytarsus brunnipes</i> (Zett.)	Chironominae	Stream	29
2	<i>Prodiamesa olivacea</i> (Mg.)	Diamesinae	Stream	30
3	<i>Anatopynia nebulosa</i> (Mg.)	Tanypodinae	Stream	30.5
4	<i>Chironomus riparius</i> Mg.	Chironominae	Concrete trough	34.5
5	<i>C. albimanus</i> Mg.	Chironominae	Stream	35
6	<i>C. longistylus</i> Goet.	Chironominae	Ditch	35.5
7	<i>Anatopynia varia</i> (Fabr.)	Tanypodinae	Ditch	38.8

larvae in an open glass vessel containing 100 ml. of air-saturated water, and keeping them in a thermostat at an appropriate temperature for 22 hr. The number which died during that time was then counted. Temperatures between 23 and 39° C. were used. The water was not aerated during the experiments; consequently less oxygen was available at the higher temperatures. Even at the highest temperatures, however, the diminution of dissolved oxygen could not alone have been responsible for the death of the larvae, but it possibly modified their capacity to resist high

temperatures.* In nature, however, high temperatures would almost always be accompanied by diminished oxygen concentrations: the larvae would thus be influenced by a combination of the two factors.

Fig. 3 shows the decline in numbers of larvae remaining alive after 22 hr. at various temperatures. The capacity to withstand high temperatures varies: taking as a thermal index the temperature at which 50% of the larvae are dead in 22 hr., the values range from 29 to 39° C. The species from flowing water had thermal indices of 29, 30, 30.5 and 35° C., those from stagnant water had indices of 34.5, 35.5 and 38.8° C. Of special interest is the remarkable power of thermal resistance of *Anatopynia varia*.

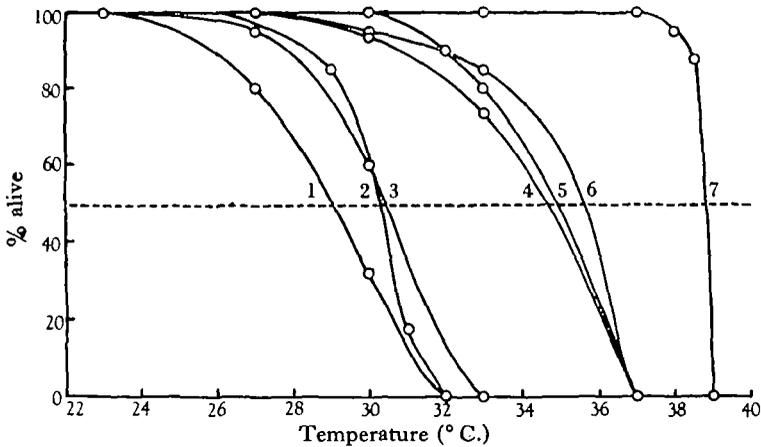


Fig. 3. Thermal resistance of various chironomid larvae. Species used (nos. 1-7) given in Table 6.

(4) DISCUSSION

Of the chironomid larvae whose oxygen requirements have here been studied, the two species from a stream have higher metabolic rates in aerated water than the corresponding species from still water. The similarity in size, structure and activity of the members of the two pairs of larvae eliminates the possibility that the metabolic differences are due to these factors. The fact that relatively high metabolic rates have also been found in stream species of other aquatic arthropods (Fox *et al.* 1933, 1935) suggests that this property may be a general characteristic of animals living in streams. The evidence of Washbourn (1936) that the oxygen consumption of trout fry increases with the current velocity in which they are reared shows that the metabolic difference between fast- and slow-water animals can be acquired during individual life. Although in the case of the trout fry the direct effect of current may well have been the causal factor influencing the metabolic rate, with chironomid larvae, which in their mud tubes are protected from the current, this cannot be so

* Whitney (1939) tested this possibility when working with the nymphs of Ephemeroptera. He found the extra survival in oxygenated water as compared with aerated water never greater than 14%, which was not sufficient to obscure the specific differences in thermal resistance.

and the significant cause seems more likely to be a high oxygen pressure, if indeed the high metabolism is individually acquired. On the other hand, it may be innate.

The high metabolic rate of the stream chironomid larvae is only maintained in fully aerated water, the relation between oxygen concentration and oxygen consumption being approximately a linear one. This contrasts with the non-linear relationship between the two variables in the larvae from stagnant waters. Such differences in animals' responses to low oxygen concentrations have been detected in a wide variety of organisms (Hyman, 1932; Tang, 1933) and have given rise to much speculation as to the general significance of the dependence or independence of oxygen consumption on oxygen pressure. It should be pointed out that the difference between the dependent type of curve shown by the stream chironomids and the independent type of the other chironomids is only a relative one, related to differences in the critical oxygen pressure below which the normal metabolic rate cannot be maintained. This critical oxygen concentration lies between 15 and 44% air saturation of the water in the stagnant water chironomids, and at, or possibly even above, 100% air saturation in the stream ones. That it should differ so markedly in such closely related animals makes the assumption reasonable that it has some special ecological or physiological significance. In the chironomid larvae the differences are related to the habitat of the animals, but Fox *et al.* (1937), measuring the oxygen consumption of various ephemeropterid nymphs, found that although the nymph with the most dependent type of metabolism (*Baetis* sp.) came from a stream and that with the most independent type (*Cloeon dipterum*) from a pond, the correlation between degree of dependence and habitat did not hold for the other species studied.

The main interest of such comparative studies of metabolism lies in the fact that very closely related and structurally similar animals do show these extensive differences in types of metabolic response to oxygen. In these animals different degrees of dependence cannot be the result of differences in the efficiency of oxygen transport to the tissues, since in the chironomid larvae, at any rate, the size and the speed of blood circulation are the same in both members of the contrasted pairs. The larvae which show dependence are, however, those with a high metabolic rate in aerated water, suggesting a difference in cellular respiratory systems.

The differences in type of metabolism shown by these closely related larvae raises the question of the possible function in respiration of the haemoglobin they contain. Ewer (1942) showed that the haemoglobin of the larvae of *Chironomus riparius* and *C. cingulatus* (two very similar species), while functionless as a transporter of oxygen in air-saturated water, enables them to maintain a normal metabolic rate at lower oxygen concentrations: in other words, by lowering the critical oxygen pressure the haemoglobin was to a large extent responsible for their independent type of metabolism curve. One is probably justified in assuming a similar function of the haemoglobin in the closely allied *C. longistylus*, but the independent curve of *Anatopynia varia* can scarcely be the result of the possession of a functional

haemoglobin since the blood of this species contains very little of the pigment. Conversely, the strikingly dependent *Tanytarsus* and *Anatopynia nebulosa* contain as much haemoglobin as *Chironomus longistylus*. In *Tanytarsus* the haemoglobin is only used in oxygen transport at oxygen concentrations below 25% air saturation (Walshe, 1947), at which concentration the larvae, even with the help of their haemoglobin, are only capable of maintaining a metabolic rate less than half that which they have in fully aerated water. To what extent the haemoglobin is really of use to a chironomid with a dependent type of metabolism curve is therefore doubtful: it certainly does not equip them to continue a normal aerobic metabolism at low oxygen pressures.

From an ecological point of view the differences are more easily interpreted. A high metabolic rate and a need for complete air saturation of the water to maintain this rate imply that the stream larvae are only fitted for life in situations where water movement ensures adequate aeration. The oxygen content of the water just above the mud in the brook from which the two stream chironomids were collected was measured at intervals throughout the year and never fell below 78% air-saturation, whereas the oxygen concentration in the ditch containing the other two species was always much lower than this. Oxygen samples were taken in this ditch at intervals during one day in summer and even at its highest point the oxygen immediately above the animals' tubes only rose to 58% saturation and throughout the hours of darkness was at the very low value of 2% saturation. The still-water species therefore have to endure very low pressures of oxygen, at any rate in summer, and their ability to maintain a normal metabolic rate at low oxygen pressures fits them for their life in such an environment. It would appear that in such species adaptation to environment has proceeded to enable the larvae to live in habitats deficient in oxygen whereas the stream chironomids are unmodified in this respect, no selection for such an adapted metabolism having been necessary.

With one exception, the larvae of stream chironomids are much less resistant to high temperatures than still-water forms. Whitney (1939) also found that ephemeropterid nymphs from slow-flowing or still waters had a greater heat tolerance than those from swift streams, and he correlated these differences with greater temperature fluctuations in the former environments. A similar correlation can be made for the chironomids: temperatures in the stream never rose above 15° C. during the summer in which these experiments were made, whereas a water temperature of 20° C. was recorded in the still-water habitats. In general, the thermal resistance of animals is closely related to the temperature conditions of their environment, the maximum temperature for normal life being little above the highest temperature to which the organism is normally subjected, although the maxima can be artificially raised by acclimatization. To what extent the thermal resistance of an animal in its natural state represents an individual acclimatized condition, and to what extent it is the genetical result of adaptation by selection of physiological processes which have temperature optima most fitted to the temperature ranges experienced, is as yet unknown. In this connexion it was interesting to find *Chironomus albimanus* (the stream chironomid with the highest thermal resistance) also living in a stagnant water

environment in association with *Chironomus plumosus* and *Anatopynia varia*. Its choice of habitat therefore seems wide and its thermal resistance correspondingly greater than the more typical stream species. This suggests a genetically determined condition.

The maintenance of a normal metabolic rate at low oxygen pressures, and the capacity to withstand both oxygen lack and high temperatures, are features in the physiology of stagnant-water chironomids which enable them to live in such habitats. It is clear that these physiological characters are adaptive, and it is reasonable to suppose that they have been acquired by such chironomids as necessary conditions for life in difficult habitats. On the other hand, because the stream species have a high metabolic rate and are much more susceptible to adverse conditions one may assume that constantly favourable oxygen pressures and temperatures in the stream habitat have made it unnecessary for them to evolve adaptations to meet unfavourable conditions. This does not necessarily imply, as one might be tempted to conclude, that such an environment was the original type to be colonized by chironomids, but rather that stream and pond larvae have evolved along two independent adaptive lines. Adaptation for life in streams demanded modifications mainly in morphology and behaviour as opposed to the profound respiratory specializations evolved by larvae colonizing stagnant habitats.

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

SUMMARY

1. The larvae of two chironomid species, *Tanytarsus brunnipes* and *Anatopynia nebulosa*, living in streams consume more oxygen than the closely related *Chironomus longistylus* and *Anatopynia varia* from still water.
2. The oxygen consumption of the two stream species falls as the oxygen content of the water diminishes, whereas that of the two still-water species remains approximately constant until the oxygen content has fallen to a low value.
3. Of the larvae of ten chironomid species, those from streams are much less resistant to anaerobic conditions than those from still water.
4. Stream chironomids have a lower thermal resistance than still-water forms.

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