The effects of temperature on certain life stages of Simuliidae (Diptera)

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The effects of temperature on certain life stages of Simuliidae (Diptera)

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Introduction

The Simuliidae have a world-wide distribution, being of great economic importance in many countries where certain species are serious pests of livestock and man due to their biting habits and their role as vectors for several parasites. The death of farm livestock on a large scale has occurred from time to time following the mass emergence of black-flies from large river breeding sites, notably in Saskatchewan (Rempel & Arnason, 1947).

In Great Britain few of the indigenous species are present in large enough numbers to become serious pests although they are known to bite a variety of birds and mammals including man (Davies et al, 1962). Simulium ornatum Meig. is known to transmit the filaroid nematode, Onchocerca gutturosa to cattle in the British Isles (Steward, 1937) and it is probable that black-flies act as vectors in the transmission of various Leucocytozoon spp. (Protozoa, Sporozoa) which are known to occur in birds in Britain (Baker, 1958).

The immature stages of black-flies are entirely aquatic and are common and often abundant in most relatively permanent water courses throughout the British Isles, provided they are not grossly polluted. Both the larvae and pupae have essentially sedentary habits and show an exclusive preference for moving water in their selection of attachment sites. The

reason for the selection of such sites by the larvae has been the subject of much speculation; some authorities claim that in this position they can best fulfil their oxygen requirements (Smart, 1934; Rubzov, 1940), while others suggest that the advantage lies in its suitability as a feeding site (Wu, 1931; Zahar, 1951). Several workers have noted that different assemblages of species are associated with water courses of different velocities and volumes of flow (Zahar, 1951; Maitland & Penny, 1967) , and Phillipson (1957) in his study of the effect of current speed on the distribution of two species of black-fly larvae suggests that velocity alone could explain the larval distribution within a water course. Edington (1968), however, in his study of the influence of water velocity on the habitat preferences of net-spinning Caddis larvae, showed that although different species had definite water velocity preferences, these velocity preferences could not be related to the sequential replacement of the different species along the length of the water course. (This must be at least in part dependent on some other environmental factor, most probably water temperature, while the selection of attachment sites within their range is dependent on water velocity.

The effect of water temperature on the development of both black-fly larvae and pupae has been recorded on several occasions, both in the laboratory and under normal environmental

conditions. The minimum water temperatures seldom prove lethal and it is unlikely that larval development ever ceases in certain species, even at stream temperatures approaching 0°C (Davies, 1961). Moulting of black-fly larvae has been seen to occur between 1°C - 3°C in both the laboratory and the stream (Zahar, 1951) although general development was retarded at these temperatures. Rising temperatures have also been shown to accelerate the development of the larvae and pupae which consequently will determine the dates on which pupation and emergence of the adult flies will occur (Zahar, 1951). Temperature not only appears to be a major factor in determining the rates at which larval and pupal development proceed, but also determines the extent of the development. It has been noted (Smart, 1934; Zahar, 1951) that pupae resulting from the overwintering larvae, developing more slowly in generally lower water temperatures, achieve a greater size than those resulting from the more quickly developing Summer generation larvae. This difference in the sizes of the pupae is reflected in the sizes of the adult flies (Edwards et al, 1939; Davies, 1957) which may in turn have some effect on the reproductive capacity of the adult flies.

The stream temperature seldom becomes high enough to kill quickly and there are no records of high stream temperatures causing mortality of black-fly larvae. The major factors responsible for decimating the larval populations seem

to be spates and to a lesser extent predation by fish and other water creatures (Maitland & Penny, 1967).

Temperature therefore acts mainly through its effect on the rate and extent of growth and its consequent effect on the time of emergence of the adult flies and to some extent on the time of oviposition. It must therefore be a major factor in determining whether or not a particular species of black-fly can complete its life-cycle or cycles at a time suitable for the emergence of the adult flies and for their subsequent oviposition. It must be noted that since in general the temperatures are suitable for development of the larvae, it is not the level of temperature achieved, but the thermal sum to which the larvae are exposed which is important in determining the time of completion of larval development. The thermal sum declines with increase in altitude so the thermal sum at different altitudes in a water course will be suitable for the completion of the larval development of different black-fly species. This could provide a basis for the sequential replacement of black-fly larvae at different altitudes in a water course.

It must be pointed out here that it is not the altitude per se but the physical characteristics of the environment which are dependent on the altitude above sea level, such as temperature or the dissolved oxygen concentration of the water. These physical characteristics, although closely

related to the altitude per se, will be subject to local variation such as the effect of the aspect of the stream on the water temperature. The altitude at which the thermal sum is optimal for the larvae of one black-fly species will vary from year to year with the yearly changes in water temperature, but a general altitudinal range within which development could be satisfactorily completed could be established over a period of years. In species which have more than one generation per year this range would be extended, fewer generations occurring at the higher altitudes (Zahar, 1951).

The previous studies concerning the development of black-fly larvae and its relationship to temperature have been based on general observations of temperature or the temperature levels during the period of study. The present study considers, on a systematic basis, the relationship between the development and altitudinal distribution of Prosimulium hirtipes Fries. larvae and the thermal sums to which the larvae are exposed during their developmental period. It will be shown that development of these larvae is very closely related to the thermal sum and that the completion of development occurs at a later date at the higher altitudes. The altitudinal distribution of P.hirtipes larvae in the stream is compared with those of the larvae of other black-fly species present in the stream during the period of study and an attempt is made to give an explanation for their different altitudinal

distributions. An attempt has also been made to explain the absence of *P.hirtipes* from the stream during the period of higher water temperatures during the Summer months, while other species of larvae are present at the same altitudes, and are able to complete Summer generations.

The study has, for convenience, been divided into two sections, Section 1 dealing with the field studies, and with the larval temperature-mortality experiments, and Section 2 describes laboratory experiments investigating the relationship between air temperature and the activity of the adult flies of *Simulium ornatum* Meig.

This study was carried out from Summer 1956 to Summer 1957, but Dr. L. Davies, Department of Zoology, Durham University, very kindly put at my disposal materials and data obtained from the study area during the previous years 1954-56.

Section 1

Growth of Prosimulium hirtipes Fries. larvae in Hill Streams of Northern England

1.A. General biology of P.hirtipes and associated black-fly species.

The species here described is the North European form of P.hirtipes Fries. as re-defined by Edwards (1915) and Puri (1925). A careful examination of the larvae was not carried out during the 1954-57 period of study to establish the separation of these larvae from Prosimulium arvernense Grenier (Davies, 1966).

All Prosimulium pupae obtained from the study area streams during this period possessed 16 respiratory filaments and were therefore those of P.hirtipes while no P.arvernense pupae, possessing 24-27 respiratory filaments, were obtained. The larvae of P.arvernense are also known to occur in small rapid stony streams, smaller than those inhabited by P.hirtipes, in the Eastern and Midland regions of England and Wales. The identity of all the Prosimulium larvae in Crowdundle and Swindale Becks, other than those of Prosimulium inflatum Davies, is therefore assumed to be Prosimulium hirtipes Fries. This was confirmed in 1969 when some 40 Prosimulium larvae from Swindale Beck were carefully examined and all proved to be P.hirtipes Fries.

In Britain the larvae of P.hirtipes are widely spread throughout the hill streams of the Scottish Highlands and in the Pennines from the Tyne-Solway gap to as far south as Swaledale. They are also known to occur in the Lake District. The larvae inhabit the large rapid mountain streams including the cascading parts of the larger streams on the valley bottoms, so they occur from low altitudes up to about 500m. The life cycles of closely related Prosimulium spp. are well known and have been described by several workers, including O'Kane, Twinn, Davies, D.M. and Rubzov. Oviposition occurs in May or June, the eggs remaining on the bed of the stream until they hatch in the Autumn. The larvae grow semi-continuously during the Winter and early Spring, pupation and emergence occurring in late April or May. The delay in the hatching of the eggs may be due to some form of diapause which prevents the larvae of this species being present in the stream during the period of higher Summer water temperatures. Since its development is restricted to the colder months and to the relatively colder hill streams it is generally regarded as a cold water stenotherm. There is some evidence that the females are autogenous for their first ovarian cycle (Davies, L., unpublished observations), and perhaps because of this little is known about the biting activity of the adult female flies.

Three other black-fly species, Prosimulium inflatum Davies, Simulium monticola Fried., and Simulium variegatum Meig.

occur along with P.hirtipes in both Swindale and Crowdundle Becks.

In both Swindale and Crowdundle Becks the larvae of P.inflatum collected during 1954-57 were all obtained from altitudes of 660m or higher, except for 1 larva obtained at 630m on 22 November 1956. Collections from other streams in the North Pennines, Lake District and Scottish Highlands (Davies, 1957a) also show a similar altitudinal range. The larvae of P.inflatum are therefore confined to the headwaters of rapid mountain streams, at altitudes of 660m or greater, where the waters are permanently cold. The larval development period is much longer than that of P.hirtipes which is completed in 4-5 months, that of P.inflatum taking approximately 8 months. The size of the P.inflatum larvae collected in November 1955 and 1956 suggest that the larvae appear in the stream in early Autumn and result from hatching some time in September or October. Pupation does not occur until the following Summer since pupae were collected from Swindale and Crowdundle Becks on 16 July 1953, 5 August 1954, 31 July 1955, and in the Cairngorms from 30 July - 2 August 1955. The development of P.inflatum larvae is therefore slower than that of P.hirtipes due probably to the lower water temperatures. The slower rate of development of P.inflatum larvae is also illustrated by the fact that the larvae of both P.hirtipes and P.inflatum collected in March were of approximately the same dimensions, but the larvae of P.hirtipes pupated 1 - 1½

months later while those of P.inflatum did not pupate for a further 4 months despite the mature larvae of both species having similar dimensions (Davies, 1966). The emergence of the adult flies of P.inflatum occurred from late July to August. It is therefore unlikely that the eggs of P.inflatum undergo any form of diapause or dormancy as do the eggs of P.hirtipes, since the time between the emergence of the adults and hatching can be accounted for by 2 - 4 weeks from emergence to oviposition by the female, and one month from oviposition to the hatching of the egg, which is not an unusually long period of incubation considering the low water temperatures. Only one generation occurs during the year and therefore like P.hirtipes it is univoltine.

The remaining two species, S.monticola and S.variegatum, are also sometimes regarded as cold water stenotherms since their larvae occur in the relatively cold waters of rapid hill streams. Both species are however bivoltine, the Summer generation larvae being present in the relatively warmer water temperatures at this time of the year. The overwintering larvae of both species produce adults which emerge from early May to June, while emergence of the Summer generation adults occurs from July to September. S.variegatum has a similar altitudinal range to that of P.hirtipes while S.monticola extends to much higher altitudes. At altitudes below 400m therefore these two species often form mixed populations of larvae along with P.hirtipes.

1.B. Site of Fieldwork

The fieldwork was carried out from November 1956 to May 1957 in Crowdundle and Swindale Becks (Nat.grid refs.35/6832, 35/7029). These streams drain part of the western slope of the North Pennine Ridge to the south of Cross Fell (Fig.1) and eventually flow into the River Eden. Crowdundle Beck rises at 2700ft (823m) on the southern slope of Cross Fell and with its tributaries it drains the western slope of the North Pennine Ridge from Cross Fell to Great Dun Fell, while Swindale Beck rises at 2330ft (680m) on the western slope of Knock Fell.

The stream profiles (Fig.2) are typical of those of mountain streams, although the gradients in the extreme upper reaches of both streams are less steep. The steepest gradients encountered in both streams were 1 in 1.7m where the streams flow over the escarpment formed by the faults of the Outer Pennine Fault System, thereafter the gradients steadily decrease until at 180m they are 1 in 40.7m for Crowdundle Beck and 1 in 42.4m for Swindale Beck.

The extreme upper reaches and the steeper escarpment sections of the streams above 400m flow over the rock strata of the Lower Carboniferous Limestone Series with outcrops of the Whin Sill (Dolerite), Sandstone and Melmerby Scar Limestone occurring in the escarpment sections. From 400m to 200m altitude, the streams flow over the rock strata of the Cross

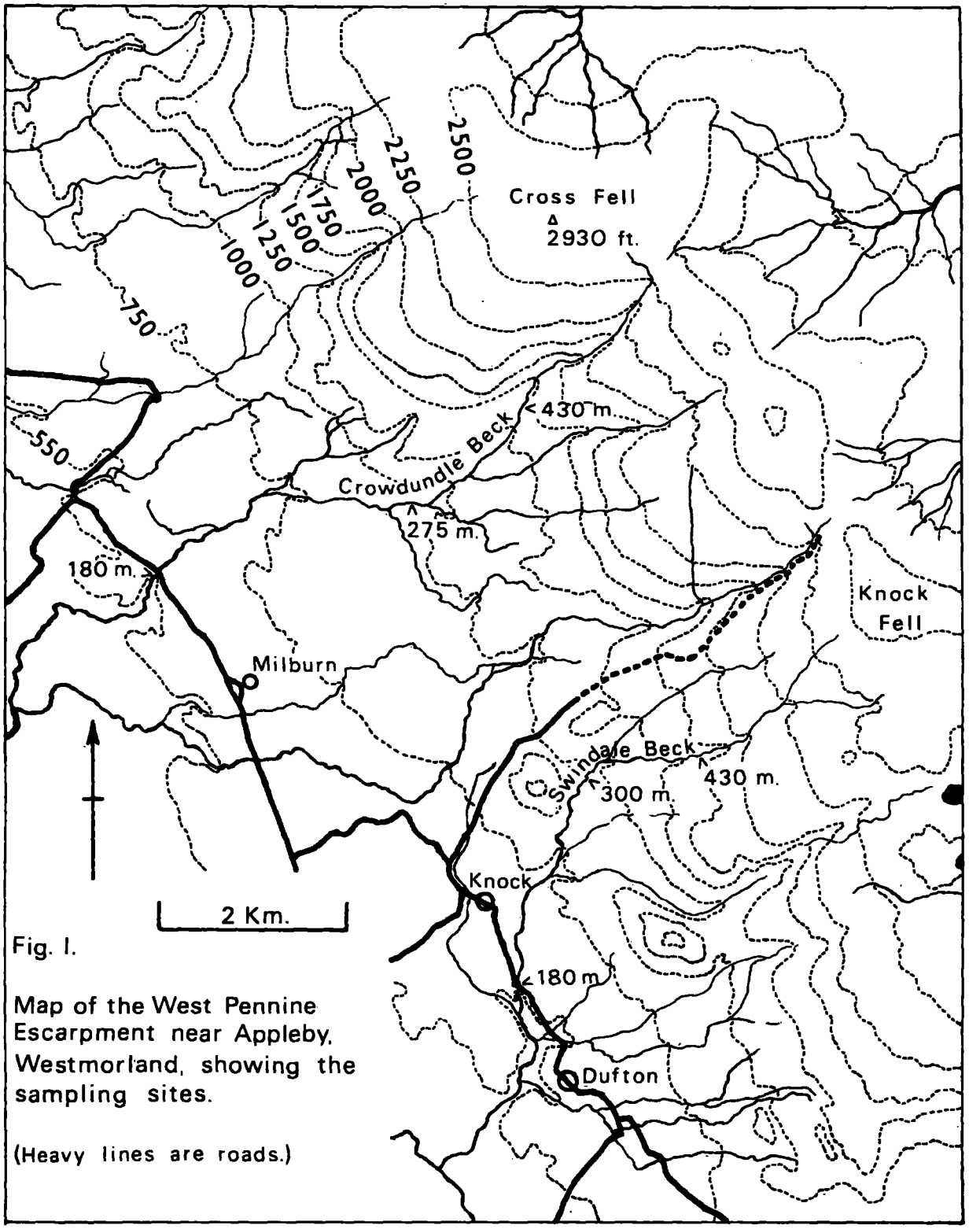
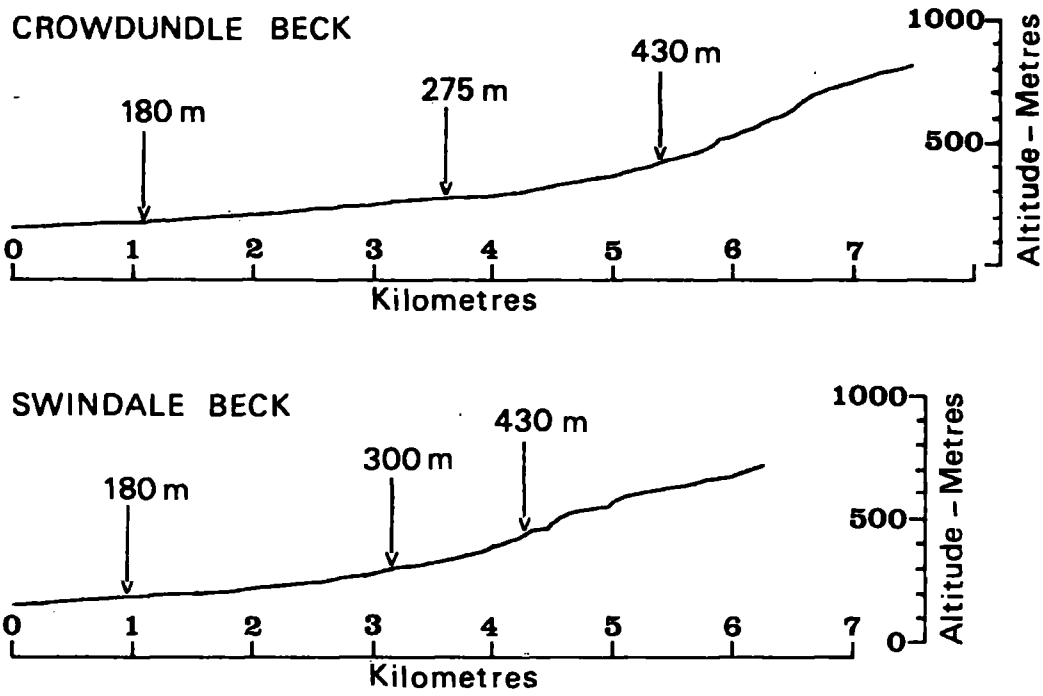


Fig. I.

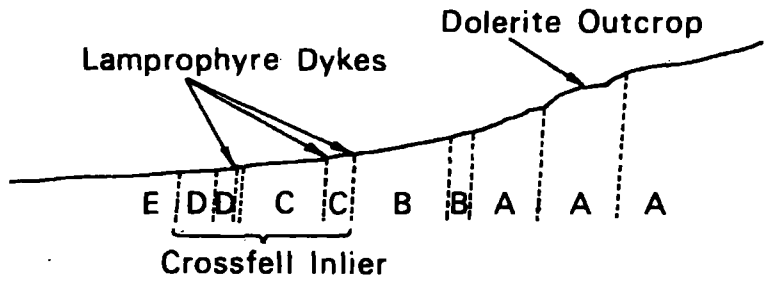
Map of the West Pennine Escarpment near Appleby, Westmorland, showing the sampling sites.

(Heavy lines are roads.)

Fig. 2. Stream profiles of Swindale and Crowdundle Becks.



Swindale Beck – Rock Strata



A. Lower Carboniferous Limestone Series
B. Contorted Strata
C. Ordivician rocks
D. Silurian rocks
E. Permo-Triassic rocks --------- Faults

Fell Inlier consisting of rocks of the Silurian and Ordivician period and several intrusive Lampophyre dykes. Below 200m the streams flow over St. Bees Sandstone of the Triassic era.

The volume of flow in both streams is considerable, even at 700m, and neither stream showed any signs of drying up during the period 1954-57. The annual precipitation in the fells which form the catchment area of these streams is considerable, 102 inches (259mm) being recorded in 1954 (Coulson, 1962) which is sufficient to sustain the water flow even in the drier years.

The widths of the streams vary from 0.5m, up to 1Km from the source, to 3 to 5m in the lower reaches approximately 6 or 7Km from the source. The beds of the streams at all the sampling points, except those above 650m, consisted of loose stones between 5-50cms in length. No rooted vegetation occurred in the streams due to the scouring action of spates which occur during the Autumn and Winter and very little moss growth was noted. Both streams rise on open moorland which forms part of the Moorhouse National Nature Reserve with a vegetation complex of Calluna, Eriophorum and Sphagnum species (Cragg, 1961). The steep western escarpment has only a thin peat covering and one of the dominant plants is Juncus squarrosus, together with Deschampsia flexuosa, Festuca ovina, Gallium spp. and Polytricum sp. in the drier parts and Eriophorum sp. and

Sphagnum sp. in the damper parts. The lower reaches of both streams are overhung by trees, Swindale Beck flowing through a shallow tree lined gorge for 3Km from 280m to 180m while Crowdundle Beck is wooded for approximately 1Km from 210m to 180m.

1.C. Sampling Method

Three sampling sites were selected at 180m, 300m and 430m in Swindale Beck and four sites at 180m, 275m, 400m and 430m in Crowdundle Beck. These sites were selected on the basis of results from sampling along the lengths of both streams during the previous years 1954-56 (Table 1). At the lower stations P.hirtipes larvae were invariably present while they were only obtained from stations above 450m during April 1954. This irregular occurrence of P.hirtipes larvae above 450m and the longer time required to obtain samples due to the generally lower numbers of all black-fly larvae at the higher altitudes led to the selection of sites between 180m and 450m. The sites selected were typical sites for hill stream black-fly larvae where the current is rapid, cascading over and between stones. Both sites at 180m had a slower current due to the lower gradient but they occur in steeper sections of the stream with a pronounced rippling current.

TABLE 1. Percentage of Prosimulium hirtipes larvae at different altitudes, 1954-56

(Sample sizes in parentheses)

	ALTITUDE (m)						
	180	275	400	430	600	630	c 800
CROWDUNDLE BECK							
16 Apr. 54	17.3 (133)	50.0 (60)	9.0 (170)	4.8 (248)	-	1.0 (115)	0.0 (28)
14 Apr. 55	22.3 (614)	31.9 (238)	6.3 (142)	5.8 (69)	-	0.0 (28)	0.0 (7)
SWINDALE BECK		300m					
15 Apr. 54	82.0 (106)	58.0 (143)	37.0 (84)	65.6 (96)	18.3 (71)	4.5 (178)	0.0 (377)
23 Apr. 55	60.0 (437)	72.2 (220)	27.1* (410)	8.4 (93)	0.0 (69)	0.0 (64)	-
23 Mar. 56	36.9(1195)	43.9(1282)	8.1 (161)	3.6 (83)	-	-	-

* 360m

Quantitative methods of sampling were not used since the object was to obtain samples of larvae which represent the size distribution of the P.hirtipes larvae present and not the relative abundance of larvae between the sampling dates. Periodic collections of larvae were made at the sampling sites by selecting and lifting stones from the rapid sections. Five to 30 stones usually yielded sufficient larvae to determine the proportion of P.hirtipes in the black-fly larval population and also their size distribution. All the larvae were removed from the selected stones with the side of the forefinger and transferred into a tube containing 70% alcohol. This sampling technique provided a good cross section of the P.hirtipes larval population, all instar stages, except the first instar, being represented in the samples. There is some evidence however that the 2nd and 3rd instar stages are less efficiently obtained by this method of sampling, especially in the early part of the season (November and December), when the percentage of P.hirtipes in the larval population is at its lowest. In November 1968 a large sample of black-fly larvae were collected while still attached to the petioles of dead leaves (Acer sp.) which were trapped on the stones at the 180m sampling site in Swindale Beck. Of the 15,000 black-fly larvae collected, only 0.9% proved to be P.hirtipes of the 1st to 4th instars, and only 2 first instar larvae were found. It is not surprising therefore that no first instar larvae of

P.hirtipes were obtained in the necessarily smaller samples obtained from 1954-57. The low numbers of 1st instar larvae of P.hirtipes in the samples is due partly to the short duration of this stage; approximately 2 days (Davies, 1960), part of which will be taken up by the dispersal of the larvae from their oviposition sites. It is probable also that the relatively shorter durations of the 2nd and 3rd instars will be a factor contributing to the lower numbers of these larvae in the samples.

Samples of the larval black-fly population were collected from the three sites in Swindale Beck (180m, 300m, 430m) at regular intervals from November 1956 to May 1957, and samples were also collected from four sites in Crowdundle Beck (180m, 275m, 400m, 430m) on 19 December 1956, 8 January 1957, 15 January 1957 and 23 January 1957. Sampling in Crowdundle Beck was discontinued after 23 January 1957 because the percentage of P.hirtipes larvae was too small to provide a reliable estimate of the size distribution of the larvae present.

1.D. Altitudinal distribution of P.hirtipes larvae and their proportion in the black-fly larval population at different times during the year

The percentage of P.hirtipes in all the larval samples was readily determined as the larvae were quite distinct from

those of the other species present at all stages in their development. The larvae of S.monticola and S.variegatum are not easily distinguished, especially in the early instars, so no separation of these larvae was attempted. The percentages of P.hirtipes in the larval samples obtained from Swindale and Crowdundle Becks from November 1956 to May 1957 are given in Table 2. The percentages of P.hirtipes in the larval samples from previous years are given in Table 1.

P.hirtipes larvae form a progressively larger proportion of the total black-fly larval population as the season progresses (Fig.3) until the onset of pupation in April or early May, and this increase is more marked as the altitude decreases. Prior to the hatching of the first P.hirtipes larvae in the Autumn, the black-fly larval population consisted entirely of S.monticola and S.variegatum larvae which have hatched from the eggs of the Summer generation adults. These larvae showed a large size distribution due to an extended period of hatching which was seen to continue through the Winter months. Hatching of P.hirtipes larvae begins in late October or early November and results in the steady increase in the proportion of P.hirtipes larvae in the population. Between 30 January and 19 February there was a marked rise in the proportion of P.hirtipes larvae at 180m which did not occur at the higher altitudes, and corresponded with a large number of the smaller larvae in the sample on 19 February (Fig.4)

TABLE 2. Percentage of *Prosimulium hirtipes* larvae at different altitudes, 1956-57

(Sample sizes in parentheses)

	ALTITUDE (m)						
	180	300	400	430	600	630	c 800
CROWDUNDLE BECK							
19 Dec. 56	0.0 (732)	*0.3 (1391)	0.3 (588)	0.0 (182)	-	-	-
8 Jan. 57	0.1 (900)	-	-	-	-	-	-
15 Jan. 57	0.0 (1101)	*0.6 (1634)	0.0 (167)	0.5 (394)	-	-	-
23 Jan. 57	0.1 (1999)	*0.6 (4249)	0.3 (382)	0.1 (728)	-	-	-
SWINDALE BECK							
22 Nov. 56	1.5 (1033)	1.2 (1692)	-	0.0 (591)	0.0 (182)	0.0 (739)	0.0 (33)
19 Dec. 56	2.1 (3009)	3.0 (3328)	-	0.2 (441)	-	-	-
8 Jan. 57	4.7 (813)	5.5 (943)	-	0.8 (258)	-	-	-
15 Jan. 57	4.6 (2113)	5.3 (2253)	-	0.8 (737)	-	-	-
23 Jan. 57	-	-	-	0.7 (605)	-	-	-
30 Jan. 57	5.7 (3989)	7.8 (5268)	-	0.7 (1966)	-	-	-
19 Feb. 57	13.7 (3320)	7.6 (6209)	-	0.7 (1787)	-	-	-
8 Mar. 57	16.3 (1660)	6.7 (2791)	-	0.7 (1513)	-	-	-
23 Mar. 57	18.9 (1610)	8.3 (3449)	-	0.8 (3196)	-	-	-
5 Apr. 57	18.0 (1546)	9.1 (1300)	-	1.0 (2889)	-	-	-
15 Apr. 57	13.1 (1471)	9.6 (3065)	-	1.2 (2141)	-	-	-
25 Apr. 57	15.5 (1221)	9.0 (2602)	-	1.9 (2178)	-	-	-
4 May 57	16.0 (810)	5.0 (1252)	-	1.1 (985)	-	-	-
15 May 57	-	4.4 (1503)	-	-	-	-	-

*275m

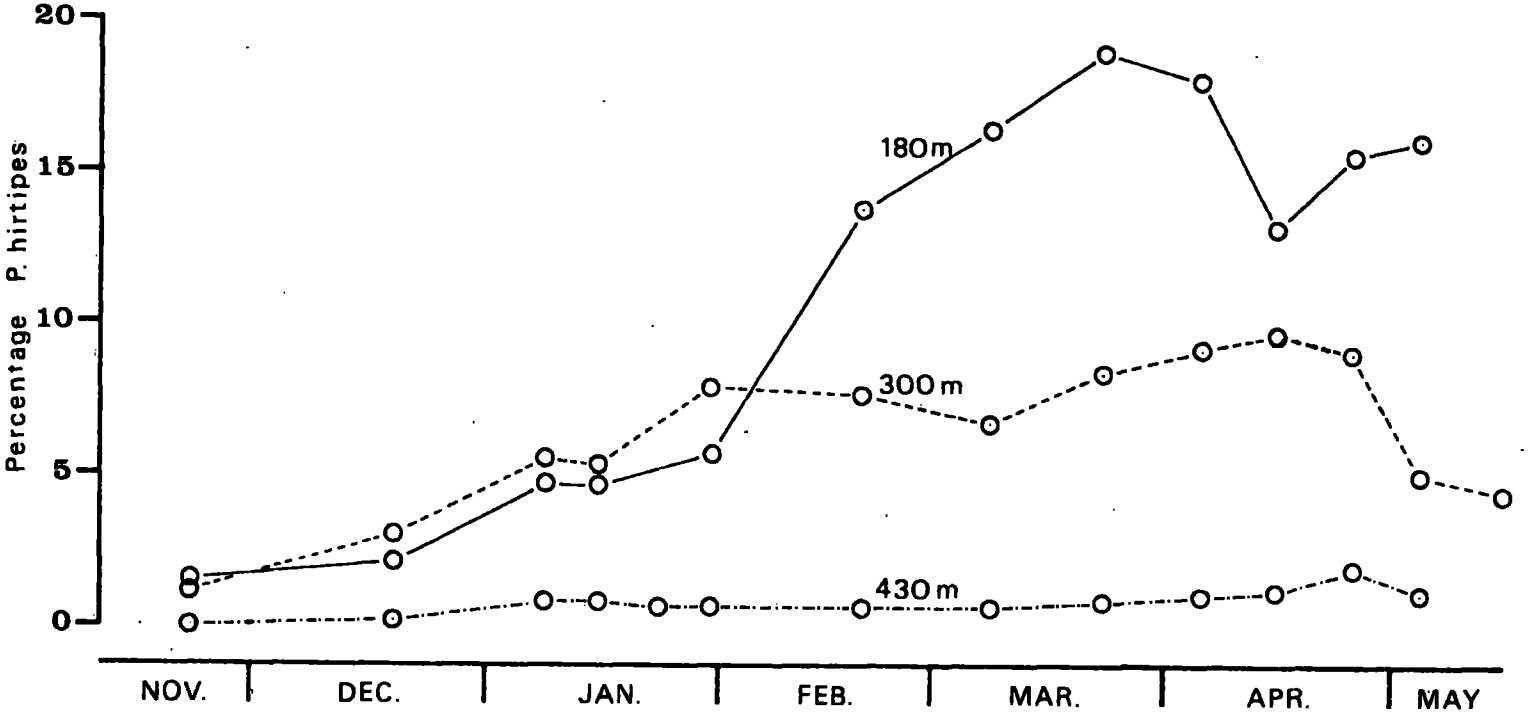


FIG. 3. THE PERCENTAGE OF P. hirtipes AT DIFFERENT ALTITUDES IN SWINDALE BECK, 56-57.

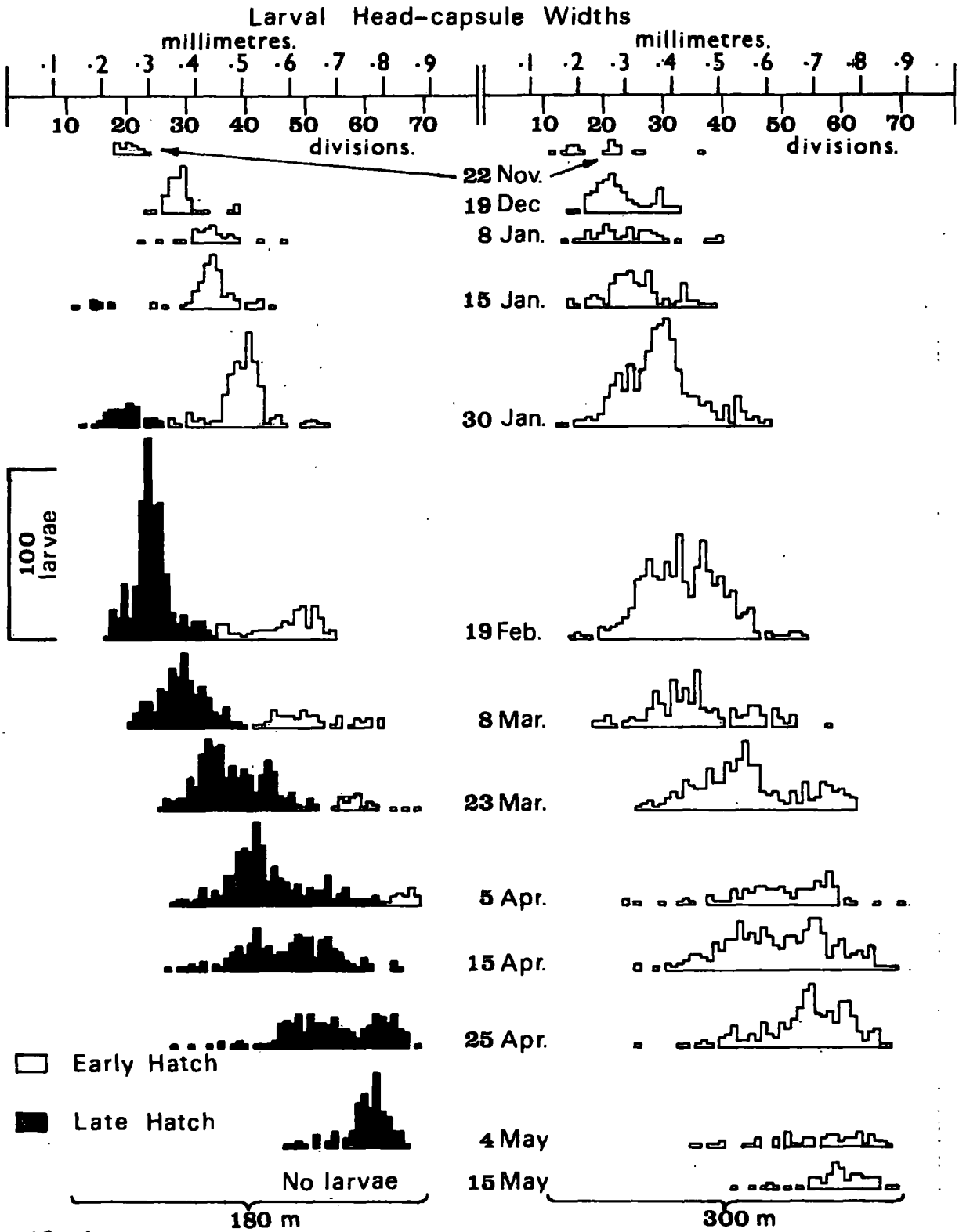


FIG. 4. The larval head-capsule width distributions of P. hirtipes in all samples from 180 m and 300 m in Swindale Beck, 56–57.

which resulted from a large hatch in late January. Very little hatching occurred after 19 February as very small larvae were absent from the samples at all altitudes after this date. The proportion of P.hirtipes larvae in the population however continued to increase, which must have been due to the loss of S.monticola and S.variegatum larvae from the larval population. This continued rise was probably due to limited pupation of the larger S.monticola and S.variegatum larvae with the arrival of the warmer water temperatures in mid March. The fall in the proportion of P.hirtipes larvae on 5 April at 180m, 25 April at 300m and 4 May at 430m coincides with the occurrence of the pupae of P.hirtipes at these altitudes. The fact that the proportion of P.hirtipes larvae falls with the onset of pupation of this species shows that pupation of P.hirtipes larvae is spread over a much shorter period than pupation of the other black-fly larvae present in the population. The rise in the proportion of P.hirtipes in the larval population after 15 April at 180m is due to later pupation of larvae resulting from the large later hatch in January.

The decrease in the proportion of P.hirtipes larvae in the black-fly larval population with increase in altitude between 180m and 430m, and the very low percentage of this species at 430m, indicates that 430m is near to the upper limit of the altitudinal range of P.hirtipes larvae in

Swindale Beck during the 1956-57 season. A similar altitudinal distribution of P.hirtipes larvae is seen when the percentages of P.hirtipes larvae in the samples taken from both Crowdundle and Swindale Becks, 1954-56, are examined. P.hirtipes larvae were invariably present in the samples obtained below 450m where they formed 20-50% of the total larval population, while in samples obtained above 450m they are absent or form a very small proportion (Table 3). The upper limit of the distribution of P.hirtipes larvae in both Swindale and Crowdundle Becks appears, in most years, to occur at approximately 450m. A similar altitudinal distribution of P.hirtipes was also found in other streams (Table 4) draining the western slope of the North Pennine Ridge and in three streams in the Upper Spey Valley, Inverness-shire.

P.hirtipes larvae formed the highest proportion of the black-fly larval population at 275m in Crowdundle Beck on all occasions, and at 300m in Swindale Beck in April 1955 and March 1956. In the remaining two years when samples were taken the proportion was greatest at 180m in Swindale Beck. If we assume that P.hirtipes larvae will form the greatest percentage of the larval population at the centre of its altitudinal range, the central point of its altitudinal range would lie between 180m and 300m in Swindale and Crowdundle Becks. The year 1954 seems to have been an exceptional year for P.hirtipes larvae in Swindale Beck for not only did they form

TABLE 3. The occurrence of Prosimulium hirtipes larvae above and below 450m

	180 - 450m		450 - 800m	
	No. of larvae	% hirtipes	No. of larvae	% hirtipes
CROWDUNDLE BECK				
16 Apr. 54	611	13.2	284	0.7
14 Apr. 55	1063	21.3	35	0.0
SWINDALE BECK				
15 Apr. 54	429	61.5	626	3.3
23 Apr. 55	1160	46.5	148	0.0
23 Mar. 56	2721	37.5	269	0.0
22 Nov. 56	3316	1.1	924	0.0

TABLE 4. The occurrence of *Prosimulium hirtipes* larvae above and below 450m in other Pennine and Inverness-shire Streams

		Below 450m			Above 450m		
West slope of Pennine Ridge	High Cup Gill	4 samples :			3 samples :		
	Ardale Beck	3 contained			none contained		
	Rundale Beck	*P.hirtipes* larvae			*P.hirtipes* larvae		
		Alt.	No. of larvae	% hirt.	Alt.	No. of larvae	% hirt.
Upper Spey Valley, Inverness-shire	River Feshie (at confluence with Spey at Kincraig)	255m	462	48		-	
	Another stream	270m	210	16		-	
	Allt Fhearnagan	400m	253	83	800-1000m	82	0

a very high proportion of the total black-fly larval population but their altitudinal distribution was greatly extended forming 4.5% of the sample at 630m which is only 100m below the source. The proportion of P.hirtipes larvae was also higher during this year in the samples obtained from Crowdundle Beck and there is some evidence of an extended altitudinal distribution in that one larva was present in a sample obtained at 630m. The reason for this extension of the altitudinal range during this year is not clear but it does serve to illustrate that suitable sites are available for the development of P.hirtipes larvae above 450m.

1.E. Seasonal Development of Prosimulium hirtipes larvae at different altitudes

In order to assess the development of the larvae it is necessary to obtain a measure of their growth in size and if possible to achieve their separation into instars so that the development can be followed from instar to instar. Since the morphology of the different instars was not known and only the final and first instar (unknown for this species at the time of study) stages could be identified with any degree of certainty, it was decided to obtain growth measurements and to use these to follow the development of the larvae and also if possible to use them as a basis for the separation of the larvae into their respective instars.

The more highly sclerotized parts of the larval cuticle were selected for measurement since these will show the least variation in dimensions during an instar stage, thus giving a better separation of the instars. Terterjan (1957) showed that the head-capsule width provided a more reliable means of separating the instars of Simulium (Wilhelmia) paraequina Puri. than measurements of the softer parts of the larva's body. Grenier (1960) also showed that measurement of the length of the highly sclerotised mandible provided a reliable basis for the separation of some of the instars of Simulium damnosum Theobald. Measurements of the head-capsule widths and the widths of the hypostomial teeth were made on a trial series of larvae and the former were adopted since they appeared to provide a better separation of the instars.

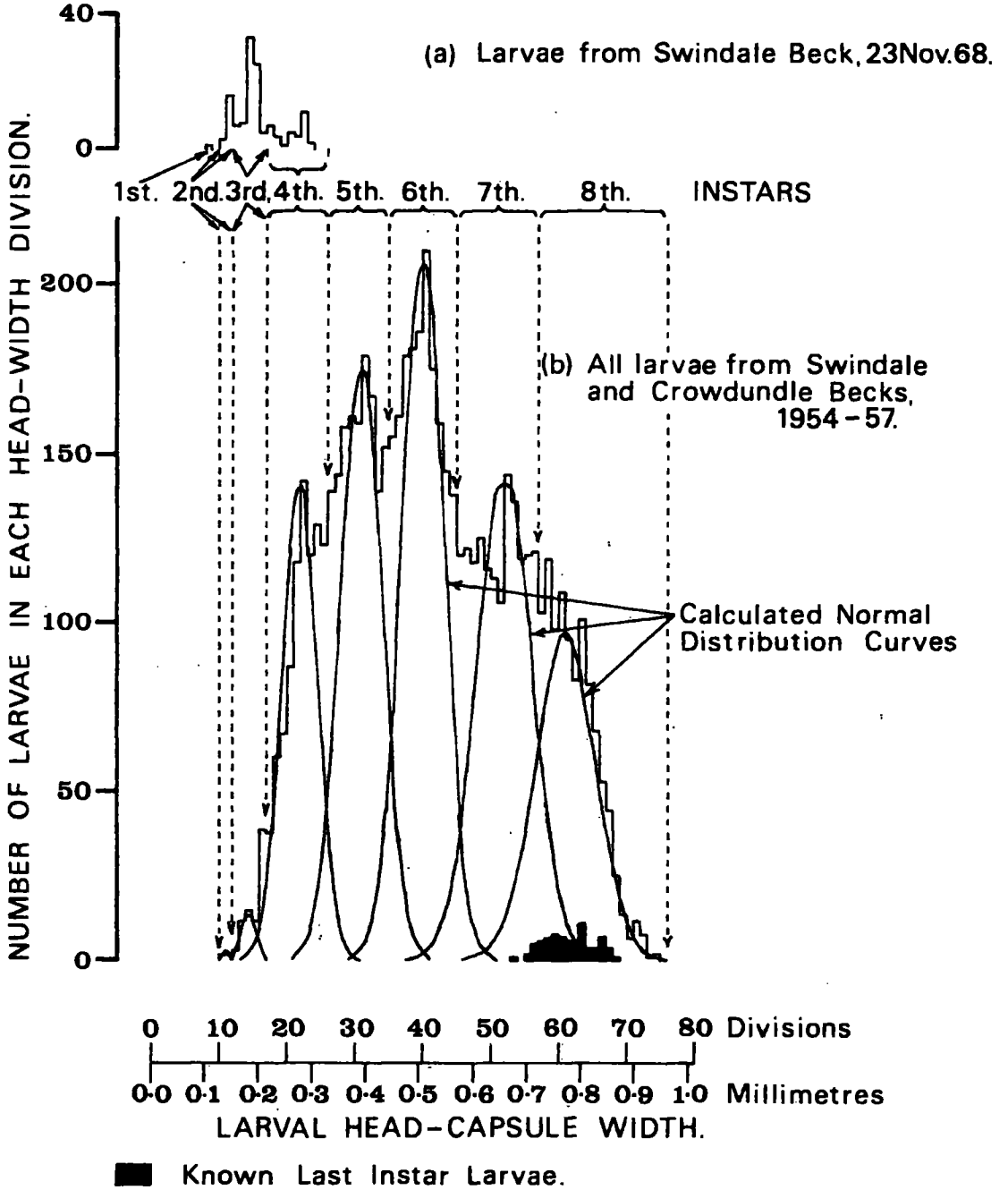
1.E.(i) Sampling and measurement of larval head-capsule width

Samples of larvae were obtained at 2 to 4 week intervals from 180m, 300m and 430m in Swindale Beck, throughout the development period from 22 November 1956 to 15 May 1957. The three sites cover the altitudinal range of P.hirtipes previously described. The samples were collected as described earlier and after separation of the P.hirtipes larvae and determination of their percentage in the sample, the larvae were carefully decapitated in 90% alcohol. The decapitated

heads were then carefully arranged on a microscope slide in a drop of Euparal which facilitated their correct orientation. Each head capsule was arranged with the dorsal surface uppermost and the width of the head-capsule was measured between the eye-spots using a microscope fitted with a micrometer eye piece. The optical system of the microscope gave a magnification of 100x. The micrometer scale in the eye piece had 100 divisions and it was found that 79 divisions of this scale were equivalent to 1mm. The head-capsule widths throughout this account will be measured in "divisions" where 1 division = 1/79th mm. Where possible in the figures the measurements in millimetres are shown along with the measurements in "divisions". Any head capsules which were damaged during sampling were discarded. These amounted to 4.5% of the total number of larvae which is relatively small considering the method of sampling.

In all, some 6632 larvae were measured, these being obtained from different altitudes in two streams over a period of 5 years and for one of these years, being obtained at regular intervals throughout the developmental period. The results of these measurements are shown as a polymodal head-capsule width distribution (Fig.5b) and as head-capsule width distributions for the separate samples from 180m and 300m in Swindale Beck, 1956-57 (Fig.4). The head-capsule width distribution for the larvae obtained from Swindale Beck on 23 November 1968 is also included in Fig.5a.

Fig. 5. Polymodal Head-capsule Width distributions of P. hirtipes larvae and their separation into Instar distributions.



1.E.(ii) Measurement of growth by use of the mean head-capsule width

A measurement of the growth attained by the P.hirtipes larvae in each sample can be obtained by calculation of the mean head-capsule width of the larvae in each sample, those for the samples obtained from Swindale Beck, 1956-57, are given in Table 5.

As expected, the larvae in samples from all altitudes show an increase in their mean head-capsule width from the beginning to the end of the season, but the pattern of this increase is far from uniform. The increase in the mean head-capsule widths of the samples from 180m and 300m remains low during the early period of development until the end of February. This is due partly to the continued addition of small larvae to the sample resulting from the prolonged period of hatching at both altitudes, small 2nd instar larvae being present in the samples taken at both altitudes on 30 January. The occurrence of the very large number of small larvae in the sample from 180m on 19 February 1957, resulting from the large late hatch at this altitude, caused a reduction in the mean head-capsule width or "negative" growth. Although a similar change did not occur at 300m it does emphasise the need for caution when interpreting mean head-capsule width data in terms of growth, while hatching is still occurring. It resulted in an apparent reversal of the growth of the larvae at 180m and 300m. Up to

TABLE 5. The mean head capsule widths ("divisions") of samples from 3 altitudes in Swindale Beck, 1956-57

	180m		300m		430m	
	Mean head width	No. of Larvae	Mean head width	No. of Larvae	Mean head width	No. of Larvae
22 Nov. 56	20.0	16	19.3	21	-	0
19 Dec. 56	28.7	60	22.4	93	27.0	1
8 Jan. 57	33.6	36	24.0	49	2 crushed	2
15 Jan. 57	32.8	95	25.1	111	37.6	6
23 Jan. 57	-	-	-	-	33.2	4
30 Jan. 57	34.5	222	29.1	381	28.4	14
19 Feb. 57	28.5	424	32.8	448	40.3	12
8 Mar. 57	34.0	250	34.9	179	42.0	9
23 Mar. 57	39.2	285	43.4	271	44.1	25
5 Apr. 57	44.7	270	48.9	115	54.6	27
15 Apr. 57	46.2	187	48.8	293	55.4	26
25 Apr. 57	53.9	184	53.3	223	58.9	41
4 May 57	59.3	128	54.6	61	no record	11
15 May 57	-	0	58.2	66	-	-

30 January the larvae at 180m had a mean head-capsule width greater than that of the larvae at 300m, but because of the pattern of hatching, the larvae at 300m had a mean head-capsule width greater than that at 180m from 19 February until 25 April when the former position was restored, due to the more rapid growth of the larvae at 180m during this period. The pattern of development between the earlier sampling dates at 430m was similar to that at 180m, but since there was no evidence of a similar pattern of hatching at this altitude, it must be due to errors in the calculation of the mean head-capsule width resulting from the extremely small numbers of larvae in the samples at this time.

The larvae at 180m reach their maximum growth earlier than those at 300m, the mean head-capsule width of 59.3 divs. being very close to the mean head-capsule width of the known last instar larvae (62.1 divs.), indicating that the majority of the larvae have reached the last instar (81.2%). The maximum mean head-capsule width of 58.2 divs. achieved by the larvae at 300m does not represent their maximum growth, since only 62.5% of the larvae in this sample had reached the last instar and sampling was discontinued after this date (15 May 1957). The larvae at 430m had a mean head-capsule width of 58.9 divs. on 25 April 1957 which was greater than that achieved by the larvae at 180m on the same date, and was approximately the same as the

maximum mean head-capsule width achieved by the larvae at 180m. Since the larvae in the 430m sample consisted of 68.3% final instars, 24.4% 7th instars, 4.9% 6th instars and 2.4% 5th instars, it might be expected that the larvae developing at 430m will achieve a larger mean head-capsule width than those developing at 180m. The early attainment of this large mean head-capsule width by the larvae at 430m may be the result of a short, concentrated hatch in the early part of the season and the absence of, or greatly reduced later hatching which had the effect of reducing the mean head-capsule widths at the lower altitudes.

Last instar larvae, as distinguished by the presence of black pupal respiratory filament histoblasts occurred earlier in the samples obtained from 180m in Swindale Beck, 1956-57 (Table 6) than at the higher altitudes. It will be seen later however that last instar larvae were present in the stream prior to these dates, on 8 March at 180m and 430m, and 23 March at 300m (Fig.11), the early appearance of the last instar larvae at 430m perhaps indicating an earlier starting of Autumn hatching at this altitude.

Pupation of P.hirtipes larvae began between 23 March and 5 April at 180m, between 15 April and 25 April at 300m, and between 25 April and 5 May at 430m, pupae of P.hirtipes being observed in the stream at each of these altitudes on the latter dates stated. Observations of the

TABLE 6. The proportion of larvae with black pupal histoblasts, Swindale Beck, 1957

	180m		300m		430m	
	Sample size	% with black histoblasts	Sample size	% with black histoblasts	Sample size	% with black histoblasts
23 Mar. 57	285	0.0	271	0.0	25	0.0
5 Apr. 57	270	3.6	115	0.0	27	0.0
15 Apr. 57	187	0.5	293	0.0	26	0.0
25 Apr. 57	184	7.9	223	0.0	41	0.0
4 May 57	128	25.4	61	6.5	11	9.1
15 May 57	-	-	66	19.8	-	-

occurrence of the last instar larvae about to pupate, i.e. those with black pupal respiratory filament histoblasts, and the time of pupation of the larvae at the different altitudes, confirm the previous observations that the development of the larvae is quicker at 180m than at 300m and 430m. The later pupation at 430m also suggests that development of the larvae is slowest at the highest altitude. It is also interesting to note that the time from the first appearance of the last instar larvae in the samples to the beginning of pupation increased with altitude, being 28 days at 180m, 33 days at 300m and 58 days at 430m, again suggesting slower development with increase in altitude.

Data from both Swindale and Crowdundle Becks in the previous years, 1954-56 (Table 7), when samples were obtained from different altitudes on the same date, also shows a decrease in the mean head-capsule width as the altitude increases. On 19 May 1955 in Swindale Beck pupation and emergence were completed at 180m while at 300m numerous pupae were present, some empty, but no larvae showing that pupation was complete but emergence of the adult flies was still in process. At 400m however larvae vastly outnumbered the pupae and no empty pupal skins were found, showing that pupation was in process but emergence of the adult flies had not begun at this altitude. Thus pupation of the larvae in Swindale Beck during 1955 was at least 2 to 3 weeks later at

TABLE 7. The mean head capsule widths ("divisions") of the samples from Swindale and Crowdundle Becks, 1954-56

CROWDUNDLE BECK	180m		300m		430m	
	Mean Head width	No. of larvae	Mean Head width	No. of larvae	Mean Head width	No. of larvae
1 Feb. 55	36.7	80	-		-	
1 Mar. 55	43.6	184	-		-	
14 Apr. 55	66.7	120	*59.3	73	-	
13 Mar. 56	34.5	27	-		-	
28 Mar. 56	50.1	66	-		-	
23 Mar. 56	61.4	42	-		-	
SWINDALE BECK						
15 Apr. 54	59.8	87	53.6	80	47.6	75
30 Dec. 55	23.3	22	-		-	
7 Feb. 56	31.6	50	-		-	
13 Mar. 56	36.5	130	38.0	73	-	
28 Mar. 56	46.4	150	44.7	335	-	
23 Apr. 56	60.3	141	56.8	127	-	

* 275m

430m than at 180m, which is similar to what happened in 1956-57 when pupation was 3 to 4 weeks later at 430m than at 180m.

From the evidence of the mean head-capsule widths of the larval samples and the occurrence of the last instar larvae and pupation, there seems little doubt that development of the larvae proceeds more quickly at the lower altitudes, and the differences in the rates of development would have been more marked had it not been obscured by the appearance of a second large hatch at 180m in Swindale Beck in 1957.

The mean head-capsule widths of the larval samples, although providing a general basis on which the development of the larvae could be studied at the different altitudes, was not sufficiently precise to enable a more detailed analysis of the larval development, especially during the early period when hatching was occurring. It was necessary therefore to separate the larval samples into their respective instars and this necessitated the recognition of the larval instars on the basis of their head widths, and later by other criteria.

1.E.(iii) Determination of the number of larval instars for P.hirtipes

(a) Examination of the head-capsule width distributions

An analysis of the polymodal head-capsule width distribution for P.hirtipes (Fig.5b), including measurements

from some 6500 larvae obtained from both Swindale and Crowdundle Becks, 1954-57, was made. Since there is no statistical method available for the analysis of polymodal distributions, certain basic assumptions were made to facilitate this analysis.

Of the several instar head-capsule width distributions which make up the polymodal distribution, only that of the last instar can be separated with any degree of certainty using data relating to the known last instar larvae with black pupal respiratory filaments. These larvae were found to have a mean head-capsule width of 62.1 divs. In the remaining distribution distinct peaks occurred at 23 divs., 32 divs., and 41 divs., and from 41 divs. to 75 divs. only one peak was distinct at 53 divs. Since the mean head-capsule width of the last instar larvae is 62.1 divs. and the range of head-capsule widths from 41 divs. to 75 divs. is greater than the range covered by the rest of the distribution which included three distinct peaks, it can be assumed that this part of the distribution represents two distributions which show a greater degree of mergence. It was also assumed that another peak occurred at the lower end of the distribution at approximately 18 divs. which was obscured by the larger numbers of larvae in the higher distributions. This may be justified since the first instar, which must be less than 11 divs. would have to more than double its size to reach the first distinct

distribution with a peak at 23 divs. It was assumed therefore that 6 peaks were present in the distribution representing 6 larval instar distributions. Since the mean head-capsule widths of all the instars, except the last, cannot be calculated, it was assumed that Dyar's Growth Rule was applicable, and it was found that a Growth Index of 1.28 provided the best fit to the observed peaks of the distribution and the calculated mean head-capsule width of the last instar larvae. The true mean head-capsule widths of the 6 instar distributions were taken as 62.1 divs. for the last instar distribution and the 5 mean head-capsule widths of the remaining 5 instar distributions were calculated from this, using a Growth Index of 1.28.

It was next assumed that :

a. the head-capsule widths in each larval instar distribution show a normal distribution about the mean.

b. the maximum deviation from the mean is equal to the difference between the true mean of the instar head-capsule width distribution and the true mean of the instar head-capsule width distribution above. For the last instar distribution the maximum deviation was taken as being the upper end of the distribution.

c. the maxima of the polymodal distribution are the maximum values at the true means of each instar head-capsule width distribution (= y).

The standard deviation (σ) for each instar head-capsule width distribution was then calculated

$$\sigma = \frac{\text{True mean of 3rd instar distribution - True mean of 2nd I.D.}}{3.2}$$

The number of larval head-capsule widths in each instar distribution can now be calculated from the general equation for a normal curve

$$y = \frac{N}{\sigma\sqrt{2\pi}} e^{-\frac{x^2}{2\sigma^2}} \qquad \text{or} \qquad N = y\sigma\sqrt{2\pi}\, e^{-\frac{x^2}{2\sigma^2}}$$

Since the value of y is taken at the true mean $x = 0$

It follows then that $e^{-\frac{x^2}{2\sigma^2}} = 1$

Therefore $N = y\sigma\sqrt{2\pi}$

Using this formula the numbers of larval head-capsule widths (N) in each instar distribution were calculated and compared with the known total number of larval measurements (6500). The numbers of larval head-capsule width measurements were then proportionately adjusted so that they totalled 6500 and the standard deviations for each distribution were adjusted accordingly.

Example

	y (max.)	True mean	σ	N	N (adj.)	σ (Adj.)
2nd Instar	2	12.0	0.938	5	5	1.026
3rd	15	15.5	1.094	41	45	1.197
4th	142	23.0	2.344	835	913	2.564
5th	179	32.0	2.813	1263	1381	3.077
6th	210	41.0	2.813	1481	1620	3.077
7th	144	53.0	3.750	1354	1481	4.102
8th	95	62.1	4.044	963	1051	4.424
			Total	5942	6499	

Since there are 6500 larval head-capsule width measurements in the polymodal distribution, the calculated numbers of larval measurements (N) in each head-capsule width distribution is increased by $\times\frac{6500}{5942}$, so that the total number of larval head-capsule width measurements is as nearly as possible equal to 6500. The standard deviation is then adjusted to accommodate the increased numbers of measurements in each distribution.

On this basis normal distribution curves were constructed for each instar distribution and a composite polymodal distribution was produced for comparison with the observed polymodal distribution. When this was done the calculated polymodal distribution gave a much greater separation

of the penultimate and final instar distributions than occurred in the original polymodal distribution so we must assume that Dyar's Growth Rule, i.e. a constant growth increase factor, does not hold true for growth between the penultimate and final instars. The remaining instar distributions showed separations of the peaks similar to those in the original polymodal distribution but slightly displaced to the left.

A second polymodal distribution was next calculated taking these observations into account. It was assumed in this case that the peak at 53 divs. represented the true mean head-capsule width of the penultinate instar distribution and that Dyar's Growth Rule only held true for the first 5 instar distributions of the polymodal distribution. Using a Growth Index of 1.31 which produced a good fit with the peaks of the first 5 instar distributions, the true means of these distributions were calculated. The true mean of the final instar distribution remained at 62.1 divs. The same procedure was used as previously, except for the calculation of the standard deviation of the penultimate instar distribution. In this case it was calculated from the difference between the true mean of the penultimate instar distribution and the true mean of the instar distribution below, instead of the true mean of the instar distribution above. This was necessary as the penultimate and final instar distributions have a greater

degree of overlapping, so that calculation of the standard deviation from the difference between their true means would produce a false impression of the deviation of the head-capsule widths in the penultimate instar distribution.

The second calculated polymodal distribution fitted the original polymodal distribution very well and provided a basis on which further work could be done to more clearly separate the larval head-capsule width distributions of the separate instars.

On the basis of this analysis it seemed that there were 7 larval instars during the development of P.hirtipes larvae, the 1st instar which had not been found in the samples and 6 others which were represented in the polymodal distribution.

The earliest instar distribution which was obscured by the larger numbers of measurements in the later instar distributions was next investigated to try to establish its presence with greater certainty. The lower end of the polymodal distribution (11-23 divs.) was plotted on probability paper and an inflexion at 17 divs. in the expected straight line indicated that the distribution was bimodal over this range of larval head-capsule widths. The number of head-capsule width groups was however small so that no accurate separation of the two distributions could be made using the graphical method for the separation of bimodal distributions (Lewis & Taylor, 1968). The calculations made indicated that

the mean head-capsule width of the earliest instar distribution lay between 14.5 - 17.5 divs.

The distributions of the larval head-capsule widths of the penultimate and final instars indicate that the amount of growth occurring between these instars is proportionately less than between the earlier instars which approximately follow Dyar's Growth Rule. This is to be expected since the pupal and imaginal histoblasts show their greatest development at this time and therefore a larger proportion of the resources of the organism will be used for the development of these structures and therefore less to the increase in the size of the larval structures.

1.E.(iii) (b) The morphological study of P.hirtipes larvae

It was decided to obtain a large sample of P.hirtipes larvae at the beginning of the season, during the initial period of hatching, to try to establish the existence of the earliest instar with a mean-head-capsule width of between 14.5 and 17.5 divs. and to make a study of the morphology of the larvae to confirm or reject the different instar distributions as postulated in the analysis of the polymodal distribution of the larval head-capsule widths.

A large sample of black-fly larvae, approximately 15,000, was obtained from Swindale Beck at 180m on 23 November 1968. Since large numbers of dead leaves were trapped on the rocks of the sampling site, the opportunity was taken to employ

a different sampling technique which would ensure the collection of small larvae. The petioles to which many larvae were clinging were detatched and transferred, with the larvae still attached, to tubes containing 70% alcohol. The percentage of P.hirtipes larvae was very low and only 133 P.hirtipes larvae were obtained. The head-capsule widths of the larvae were measured, as previously, using the same scale (79 divs. = 1mm.). The larvae were not however decapitated so that the width of the head-capsule could later be related to the morphology of the entire larva. The distribution of the head-capsule widths of these larvae is shown in Fig.5a. The larvae included 2 first instar larvae and the remaining larvae varied in head-capsule width between 11 - 24 divs. The smallest larvae, excluding the first instar larvae, had head-capsule widths equal to the head-capsule widths of the smallest larvae obtained during sampling in the years 1954-57. The distribution is polymodal showing three distinct peaks at 12 divs., 15-16 divs., and 23 divs., the latter corresponding to the first distinct peak of the polymodal distribution for 1954-57. This indicated that 3 instar distributions were present in addition to the 1st instar. The peak at 15-16 divisions confirms the presence of the suspected instar distribution with a mean head-capsule width between 14.5 - 17.5 divs. in the 1954-57 polymodal distribution, while the peak at 12 divs., which had not been suspected, indicated the presence of an additional instar distribution. The data from all head-capsule width distributions

thus indicates that eight instars are present during the larval development of P.hirtipes. The mean head-capsule widths of these instars are shown in Table 8 along with the respective Growth Indices.

The number of instars during the development of black-fly larvae has been the subject of much research and a historical survey is well set out by Grenier (1960). The concensus of opinion is that there are usually 6 instars during the development of black-fly larvae. Puri (1925), on the basis of morphological characters alone, was able to distinguish 6 instars during the development of both Simulium aureum Fries. and Simulium erythrocephalum Degeer. Most other workers have relied, at least in part, on biometric studies for the separation of the instars. That of Terterjan (1957), who established 6 instars for Simulium (Wilhelmia) paraequina Puri., is particularly notable for the biometrical study of many of the larval structures from which he concluded that, of the measurements, the width of the head-capsule and the comparative dimensions of the antennal segments, give the best separation of the larval instars. Grenier (1960) however relied more on morphological characters for the establishment of 7 instars in the development of Simulium damnosum Theobald larvae, but he relied on measurements of the length of the mandible for the separation of the 5th and 6th instar larvae. The use of good morphological characters in the separation of the instars is to be desired since they are less subject to variation due

TABLE 8. The mean head-capsule widths of the instars of *P.hirtipes* larvae and Growth Indices

INSTAR	1	2	3	4	5	6	7	8
Mean head-capsule width (divs.)	10	12	15.5	23	32	41	53	62.1
Growth Index		1.20	1.29	1.48	1.39	1.28	1.29	1.17

to changing environmental conditions. It is seldom in biometric studies that the instars can be completely separated and most workers have shown considerable overlapping between the instars (Smart, 1934; Terterjan, 1957; Grenier, 1960; Harrod, 1964). The use of preliminary biometric studies is however very useful in indicating the number of instars and the mean head-capsule widths of their instar distributions. This would be very useful as this would enable groups of larvae to be obtained for morphological study which will be almost entirely composed of larvae of the same instar stage.

In this study the reverse is true since the only data available for the separation of all the larval instars are the widths of the head-capsules, these having been discarded after measurement. The purpose of the morphological study in this case is to confirm the previous conclusions drawn from the analysis of the polymodal distribution of the larval head-capsule width measurements. The morphological terms used in this account of the morphology of the larvae are mainly those proposed by Crosskey (1960).

The morphology of 60 P.hirtipes larvae was studied, the smaller larvae being obtained from Swindale Beck at 180m on 23 November 1968 and 4 January 1969, while the larger larvae were collected from the river Belah, Westmorland, on 11 May 1963 and were kindly provided by Dr. L. Davies. In the case of the smaller larvae it was possible to examine a number of larvae with the same head-capsule widths which corresponded to the

peaks of the polymodal distributions, but the smaller number of larger larvae available necessitated the examination of larvae over a range of head-capsule widths lying within the later instar distributions (Table 9).

The larval head widths had been previously determined, so the larvae were decapitated and the head capsules carefully dissected. The large cephalic fans; the mandibles and maxillae; the hypostomium and the antennae were then mounted in Euparal under separate coverslips. The thoracic and abdominal sections of the larvae were next carefully hydrated before the posterior attachment organ and anal sclerite were dissected out and mounted flat in a water mounting medium. This medium caused no hardening of the tissues and this facilitated the more effective flattening of the posterior attachment organ during mounting. The particular features examined were the number of rays in the large cephalic fans; the number of antennal joints and their proportions (Fig.6H); the proportions of the mandibles (Fig.6J) together with the numbers of preapical spines and the number of teeth on the inner preapical ridge (Fig.6K); the proportions of the sclerotised region of the maxillary palp (Fig.7); the number and arrangement of bristles on the hypostomium together with the proportions of the hypostomial teeth (Figs. 7 and 8); the number of radial rows in the posterior attachment organ and the number of hooks per row.

TABLE 9. The place of origin and head-capsule widths of the P.hirtipes larvae used for morphological study

Instar	Mean of head-capsule width distribution (divs.)	No. of larvae examined	Place of origin		
			Swindale Beck 23 Nov. 68	Swindale Beck 4 Jan. 69	River Belah 11 May 63
1	9	1	1 (9)		
2	12	19	19 (11-12)		
3	15.5	5	5 (15)		
4	23	5	5 (23)		
5	32	11	6 (28-37)	4 (30-31)	1 (40)
6	41	2			2 (40-41)
7	53	8			8 (43-54)
8	62.1	6			6 (60-65)

( ) head-capsule widths in "divisions"

Using a Baker microprojector and the mirror of a Camera Lucida the larval structures were projected onto a sheet of drawing paper mounted on an inclined board to avoid distortion. All features which were to be measured were first drawn and after projection of a millimeter scale (0.01mm divisions) onto the same sheet, measurements were made as shown in the figures. All the smaller features, especially with the smaller larvae, were carefully checked with the use of an oil immersion lens (x 800). A full record of all the measurements and observations made is included in Tables 10 (a) - (f), which also include some measurements of the next instar structures which were visible in some of the larvae about to moult into the next instar. The nomenclature of the antennae is the same as that used by Grenier (1960). The method of measurement of the antennae is shown in Fig.6H. Since the exact positions of the antennal joints between the sensilla and joint 3, and between joint 3 and joint 2, were not clear and therefore the measurements of joint 3 refer to the length of the darkly sclerotised region which was more readily discernible. The distal end of joint 2 was also taken as the mid-point of a line drawn between the bases of the more conspicuous spines between joints 3 and 2. The methods of measuring the mandibles, maxillae and hypostomial teeth are illustrated in Figs. 6J and 7. The relationships of the lengths of the central and lateral teeth of the

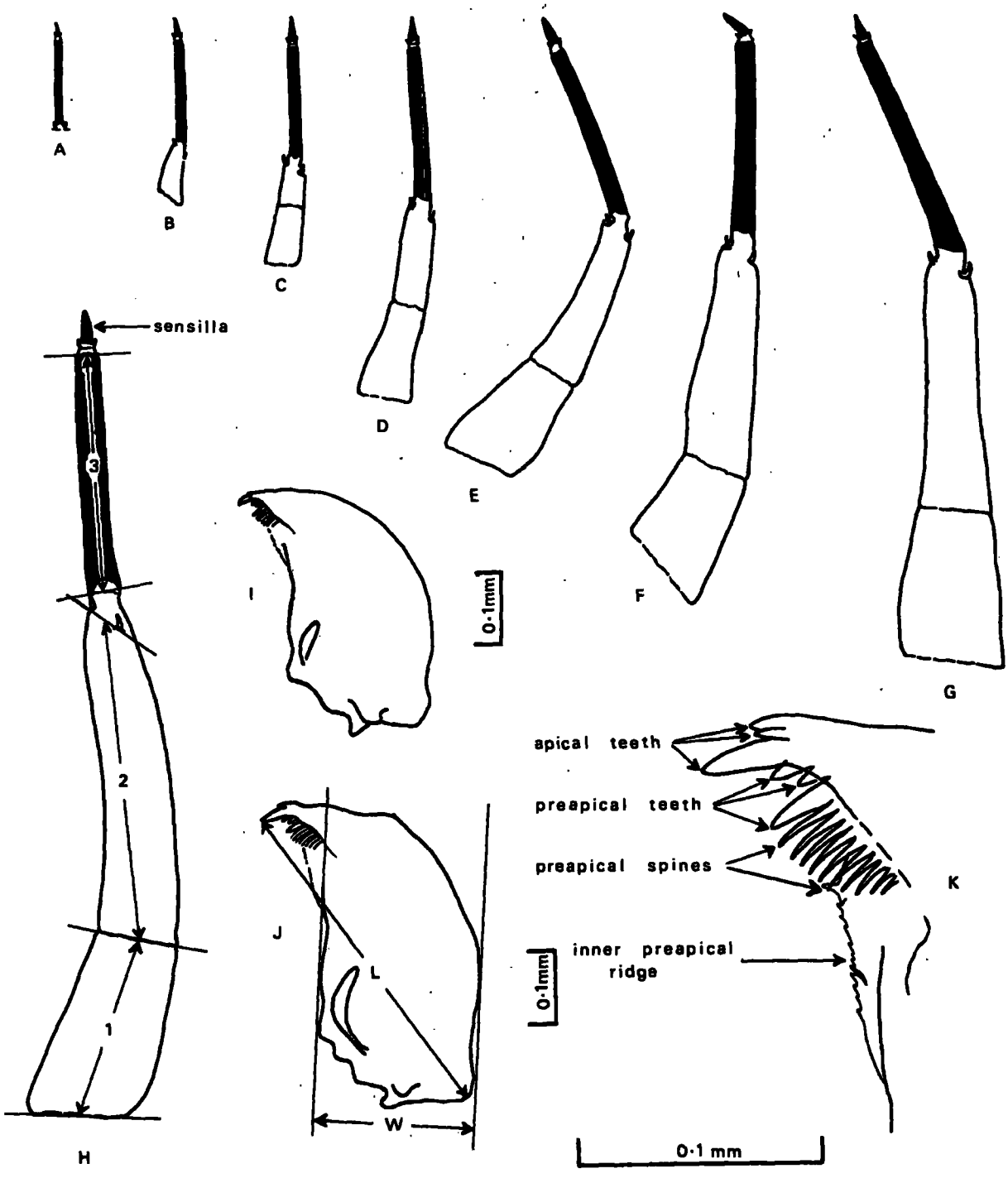
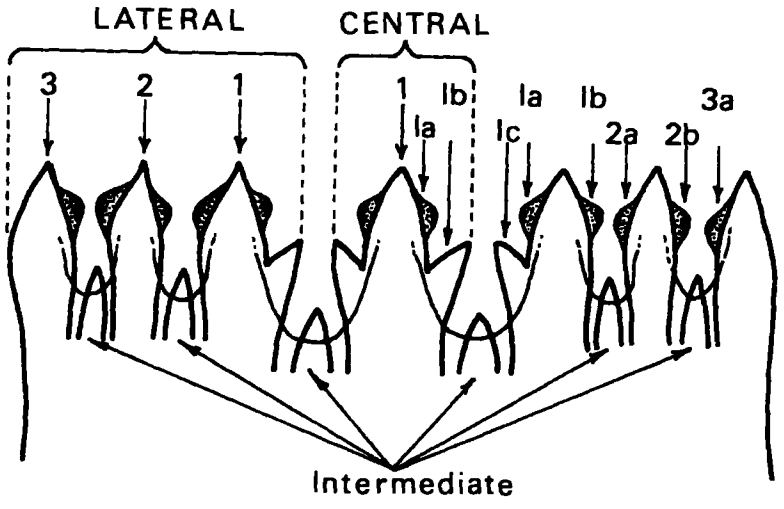
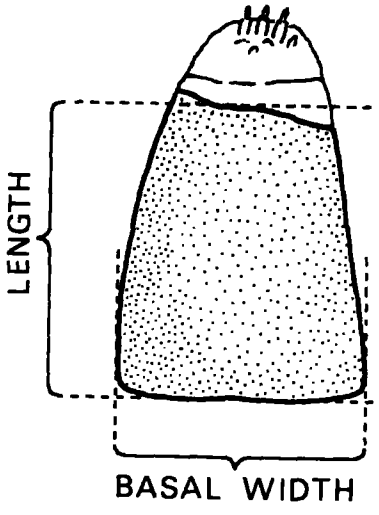


FIG. 6. ANTENNAE and MANDIBLES of Prosimulium hirtipes larvae:

A–H: The growth of the Antennae from 1st. to 8th. Instars
I: Mandible of 7th. Instar larva.
J: Mandible of 8th. Instar larva.
K: Mandible tip of 8th. Instar larva giving the nomenclature of the teeth and spines.

HYPOSTOMIAL TEETH - Nomenclature





MAXILLARY PALP

Fig. 7.

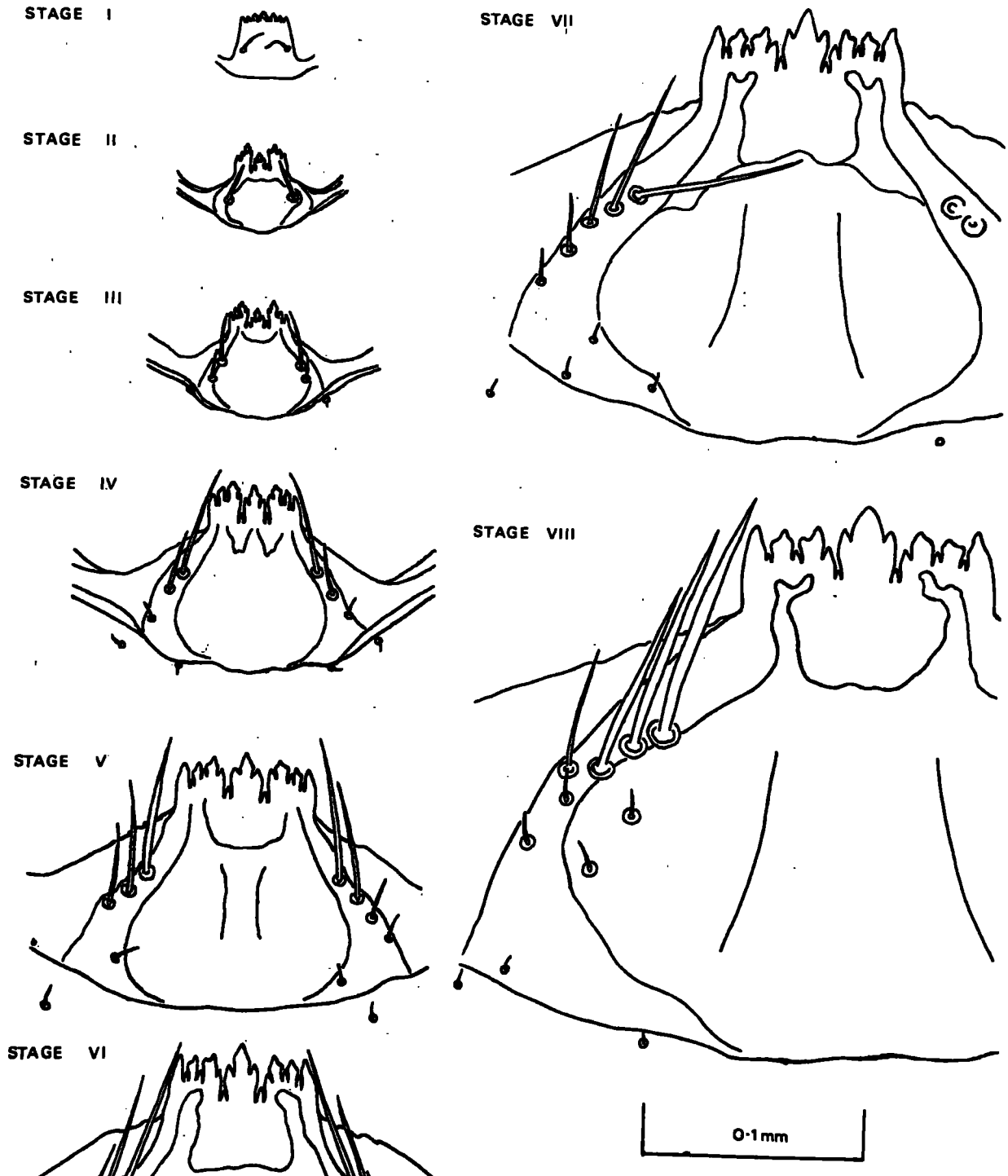


FIG. 8. HYPOSTOMIA OF THE 8 LARVAL INSTARS OF Prosimulium hirtipes.

TABLE 10.

Morphological Data for *Prosimulium hirtipes* larvae

10(a) 2nd Instar larvae

10(b) 3rd and 4th Instar larvae

10(c) 5th Instar larvae

10(d) 6th and 7th Instar larvae

10(e) 8th Instar larvae

10(f) Morphological data for following instars from larvae about to moult

All measurements, excluding the head-capsule width, are given in microns.

Measurements have been made on only one of each pair of paired structures, that having the best orientation being selected.

Damaged or badly orientated structures are indicated by an asterisk.

The proportions of the hypostomial teeth are summarized below each table; the nomenclature used follows that given in Fig.7.

L = Length W = Width

TABLE 10(a) Second Instar larvae

Head Width (Div.)	Posterior disc: No. fan rays		Rows	Hooks/row	Mandible: Pre-apical spines		Ridge teeth		Maxillary palp: L	W	L/W	(Length of Antenna): No. Joints	3	2	1	3/2+1	Hypostomium: 2/1	Bristles in main row	Other Bristles	W
11	8*	10*	59	4	2	-	5	-	65	29	2.25	16	15	1.07	2	36	20	-	1.80	-	1-1	-	22
11	8*	11*	54	4	2	-	5	-	68	33	2.06	17	16	1.06	2	32	21	-	1.52	-	1-1	-	21
11	8	11	33*	4	2	2	5	5	74	37	2.00	18	16	1.13	2	42	20	-	2.10	-	1-1	-	20
12	11	11	-	4	1	2	5	5	69	32	2.16	-	-	-	2	36	19	-	1.90	-	1-1	-	19
12	9	10	-	-	2	2	4	4	71	33	2.15	15	17	0.88	2	24	22*	-	1.09*	-	1-1	-	21*
12	7*	10	-	4	2	2	5	6	75	37	2.03	-	-	-	2	39	18	-	2.17	-	1-1	-	21*
12	9	10	-	4	2	-	6	-	72	34	2.12	-	-	-	2	39	21	-	1.86	-	1-1	-	20
12	8*	11	-	-	2	2	5	5	67	34	1.97	16	15	1.07	2	37	24	-	1.54	-	-	-	-
12	10	11	47	4	2	2	5	5	73	35	2.09	18	17	1.06	2	38	27	-	1.41	-	1-1	-	23
12	8*	8*	57	4	-	-	-	-	-	-	-	-	-	-	2	39	24	-	1.63	-	1-1	-	22
12	7*	10	48	4	2	2	6	5	69	33	2.09	18	17	1.06	2	38	26	-	1.46	-	1-1	-	22
12	10	-	46	4	26	2	6	6	76	36	2.11	15	16	0.94	2	36	22	-	1.64	-	1-1	-	21
12	6*	11*	-	-	2	2	6	6	67	32	2.10	16	17	0.94	2	37	22	-	1.68	-	1-1	-	22
12	7*	11	48	4	2	2	6	5	68	34	2.00	-	-	-	2	31	26	-	1.19	-	1-1	-	22
12	10	10	46	4	2	2	5	5	72	37	1.95	17	16	1.06	-	-	-	-	-	-	1-1	-	21
12	10*	10	54	4	2	2	6	6	71	35	2.03	17	17	1.00	2	39	24	-	1.63	-	1-1	-	23
12	10*	10	53	4	2	2	5	5	73	36	2.03	16	17	0.94	2	37	19	-	1.95	-	1-1	-	23
12	10	8*	56	4	2	2	6	4	72	35	2.06	17	18	0.94	2	38	22	-	1.73	-	1-1	-	22
12	9*	9*	-	4	2	2	5	5	71	33	2.15	19	18	1.06	-	-	-	-	-	-	1-1	-	22

Hypostomial teeth 2nd Instar larvae (head-capsule width 11-12 divs.)

11 larvae Cl < Llc; 6 larvae Cl = Llc; 1 larva Cl > Llc and Clb < Llc; all larvae with Lla and Cla prominent

TABLE 10(b) Third and Fourth Instar larvae

Head Width (Div.)	Posterior disc: No. fan rays		Posterior disc: Rows	Posterior disc: Hooks/row	Mandible: Pre-apical spines		Mandible: Ridge teeth		Maxillary palp: L	W	L/W	(Length of Antenna): L	W	L/W	No. joints	3	2	1	3/2+1	2/1	Hypostomium: Bristles in main row	Other Bristles	W
15	12	11*	59	5	3	3	5	7	99	44	2.25	27	23	1.17	3	46	16	25	1.12	0.64	2-2	-	26
15	12	12	61	5	3	3	6	6	97	47	2.07	24	22	1.09	3	44	13	26	1.13	0.50	2-2	-	26
15	11	11	-	5	3	-	6	-	101	47	2.15	26	25	1.04	3	45	15	23	1.19	0.55	2-2	-	28
15	11	-	58	5	3	-	6	-	96	46	2.09	28	23	1.21	3	49	13	25	1.29	0.52	2-2	-	-
15	12*	12*	58	5	3	3	6	5	98	44	2.23	25	25	1.00	3	48	17	23	1.20	0.74	2-2*	-	30*
23	18	18*	62	7	4	5	8	7	132	62	2.13	40	29	1.38	3	51	29	37	0.77	0.78	3-3*	-	38
23	19	19	63	7	5	5	7	7	144	70	2.06	42	32	1.31	3	64	34	37	0.77	0.92	3-3	-	-
23	19	16*	65	7	6	5	7	6	152	69	2.20	46	29	1.59	3	62	39	39	0.80	1.00	3-3	-	38
23	18*	17*	69	7-8	4	5	-	7	133	63	2.11	42	31	1.35	3	61	37	40	0.79	0.93	3-3	-	38
23	16*	15*	66	6-8	5	5	8	9	149	68	2.16	42	33	1.27	3	61	32	42	0.85	0.76	3-3	2	49

Hypostomial teeth 3rd Instar Larvae (head-capsule width 13-16 divs.)

4 larvae C1 = L1c; 1 larva C1 > L1c and L1c and C1b < L1c; all larvae with L1a and C1a prominent.

4th Instar Larvae (head-capsule width 17-26 divs.)

3 larvae C1 < L1, C1 > L1c and C1b < L1c; 1 larva C1b < L1c; 1 larva C1b = L1c and C1 < L1; all larvae with L1a and C1a prominent.

TABLE 10(c) Fifth Instar larvae

Head Width (Div.)	Posterior disc: No. fan rays	Posterior disc: Rows	Posterior disc: Hooks/row	Mandible: Pre-apical spines	Mandible: Ridge teeth	Maxillary palp: L	Maxillary palp: W	Maxillary palp: L/W	Maxillary palp: L	Maxillary palp: W	Maxillary palp: L/W	(Length of Antenna): No. joints	(Length of Antenna): 3	(Length of Antenna): 2	(Length of Antenna): 1	(Length of Antenna): 3/2+1	(Length of Antenna): 2/1	Hypostomium: Bristles in main row	Hypostomium: Other bristles	Hypostomium: W
28	25*	25*	69	8-9	6 -	9 -	198	96	2.06	54	39	1.39	3	74	56	39	0.78	1.44	3-3	2	49
30	24	25	62	8-11	7 7	8 9	214	105	2.04	62	43	1.44	3	76	55	57	0.68	0.97	4-4	2	52
31	25	25	78	9-10	6 8	10 9	236	118	2.00	62	48	1.29	3	78	67	53	0.65	1.26	4-4	1	58
31	26	26	67	9-11	6 8	10 12	231	114	2.03	62	53	1.17	3	79	68	57	0.63	1.19	4-4	2	57
31	26	26	70	9-11	8 7	11 12	222	109	2.04	60	43	1.40	3	71	63	51	0.62	1.24	4-4	1	52
31	25	25	74	10-11	7 7	11 11	218	109	2.00	65	42	1.55	3	86	69	75	0.60	0.92	4-4	3	53
34	25	24	76	9	7 7	11 10	228	112	2.03	68	47	1.42	3	74	71	45	0.64	1.58	4-3	2	56
35	28	28	72	9-11	7 7	10 12	241	119	2.02	68	55	1.24	3	78	76	58	0.58	1.31	4-4	3	60
36	29	23*	74	9-11	7 7	9 8	228	114	2.00	70	52	1.24	3	77	72	54	0.61	1.33	4-4*	1	57
37	27	20*	74	9-11	8 7	11 10	233	113	2.06	63	46	1.37	3	71	71	47	0.65	1.51	4-3	1	57
40	24	25*	74	8-10	7 7	9 10	244	126	1.94	73	60	1.22	3	82	(112)		0.73	-	4-3*	-	60

Hypostomial teeth 5th Instar Larvae (head-capsule width 27-35 divs.)

8 larvae C1>L1 and C1b = L1c; 1 larva C1b = L1c and C1 = L1; 1 larva C1 = L1 and C1b<L1c;

1 larva* C1< L1, C1>L1c and C1b< L1c (teeth distorted); 9 larvae with L1a and C1a prominent and 2 larvae with only L1a promi

TABLE 10(d) Sixth and Seventh Instar larvae

Head Width (Divs.)	Posterior disc: No. fan rays		Rows	Hooks/row	Pre-apical spines		Mandible: Ridge teeth		L	W	L/W	Maxillary palp: L	W	L/W	No. joints	(Length of Antenna): 3	2	1	3/2+1	2/1	Hypostomium: Bristles in main row	Other bristles	W
40	31	31	75	9-11	9	9	11	10	288	140	2.06	84	57	1.47	3	80	88	56	0.55	1.57	4-4	2	61
41	27	27*	79	10-12	7	8	9	9	286	147	1.94	76	70	1.09	3	78	89	48	0.57	1.86	3-4	2	62
43	33	34	81	10-12	9	8	13	13	319	160	1.99	85	56	1.52	3	88	92	69	0.55	1.34	4-5	3	70
45	33	33	79	10-13	9	7	11	11	343	168	2.04	98	63	1.56	3	87	106	47	0.57	2.26	5-5	2	80
47	36	35	77	11-12	9	9	14	13	331	164	2.02	95	68	1.40	3	88	110	59	0.52	1.87	4-5	2	74
49	36	35	78	10-11	8	8	10	9	323	169	1.91	93	68	1.37	3	90	103	64	0.54	1.61	4-4	1	69
49	35*	35*	75	10-12	8	9	15	12	328	159	2.06	95	63	1.51	3	92	107	78	0.50	1.37	4-4	4	71
50	36	36	79	11-12	8	9	11	11	328	171	1.92	101	72	1.40	3	82	104	58	0.51	1.79	4-5	6	71
51	36	35	81	12	9	9	9	10	388	200	1.94	98	80	1.23	3	84	114	61	0.48	1.87	5-5	3	80
54	36	31*	85*	11-13	9	8	14	13	356	182	1.96	94	80	1.18	3	91	103	67	0.54	1.54	5-5	5	79

Hypostomial teeth 6th Instar larvae (head-capsule width 36-45 divs.)

2 larvae C1 > L1, C1b < L1 and C1b > L1c; 2 larvae L1a and C1a not prominent.

7th Instar larvae (head-capsule width 46-57 divs.)

6 larvae C1 > L1, C1b < L1 and C1b > L1c; 2 larvae C1 > L1 and C1b = L1c.

TABLE 10(e) Eighth Instar larvae

Head Width (Divs.)	Posterior disc: No. fan rays		Rows	Hooks/ row	Pre-apical spines	Mandible: Ridge teeth		L	W	L/W	Maxillary palp: L	W	L/W	(Length of Antenna): No. Joints	3	2	1	3/2+1	2/1	Hypostomium: Bristles in main row	Other Bristles	W
60	42	39*	92	13-15	10	9	10	14	445	206	2.17	128	86	1.49	3	110	136	79	0.51	1.72	5-6	4	101
60	43	42	94	14-15	12	11	14	13	448	210	2.14	120	94	1.28	3	98	144	87	0.46	1.66	6-6	11	99
60	41	42	83*	13-16	11	11	13	13	432	198	2.18	115	84	1.37	3	112	124	88	0.53	1.41	4-6	4	101
61	43	44	95	14-16	11	12	11	11	478	218	2.20	125	84	1.49	3	117	147	81	0.51	1.82	6-6	8	99
63	40	41	89	13-15	11	10	9*	11*	435	190	2.29	120	79	1.52	3	102	135	78	0.48	1.73	5-5	6	102
65	39*	-	77*	14-16	12	9	11	12	430	200	2.15	125	74	1.69	3	92	(172)		0.54	-	6-6	5	90

Hypostomial teeth 8th Instar larvae (head-capsule width 58-76 divs.)

4 larvae C1>L1 and C1b = L1; 1 larva C1>L1 and C1b>L1; 1 larva C1>L1, C1b<L1 and C1b>L1c.

TABLE 10(f) Morphological data for following instars from larvae about to moult

Head Width (Div.)	Posterior disc		Mandible				Hypostomium			
	Rows	Hooks/ rows	Pre-apical spines		Ridge teeth		Bristles in main row	Width	Proportions of hypostomial teeth	
12			3	3	-	-				
12	61	5	3	-	-	-				
12	50	5	3	-	5*	-				3rd Instar structures in 2nd Instar larvae about to moult
12	61	5	3	-	-	-	-	27	C1=L1c	
12	59	-	3	-	-	-				
12	-	5	3	-	-	-				
12	-	5	3	-	-	-	2-2	27	C1<L1c	
23	62	8-9	6	-	6*	-				5th Instar structures in 2nd Instar larvae about to moult
30	71	-	8	8	11*	9*	4-4	59	C1>L1, C1b<L1, C1b>L1c	6th Instar structures in 5th Instar larvae about to moult
31	-	-	9	9	7*	-	-			
40	-	-	8	7	-	-	-	66	C1>L1, C1b<L1, C1b>L1c	
47	-	-	10	-	9*	-				8th Instar structures in 7th Instar larvae about to moult
49	-	-	9	-	-	-		84	C1>L1, C1b<L1, C1b>L1c	
50	-	-	10	-	-	-		87	C1>L1, C1b<L1, C1b>L1c	

hypostomium differ from instar to instar and for ease of description the teeth have been numbered as in Fig.7.

The morphological study of the larvae showed that the first, second, third, fourth and eighth instars could be separated on the basis of morphological characteristics in addition to their distinction on the basis of head-capsule width, as follows :-

Instar 1. Egg bursting tooth present
One antennal joint in addition to the sensilla
No cephalic fans present
One pair of bristles present on the hypostomium

Instar 2. Egg bursting tooth absent
Two antennal joints in addition to the sensilla
Cephalic fans are present
One pair of bristles present on the hypostomium

Instar 3. Three antennal joints in addition to the sensilla, joint 3 being longer than joint 2 + joint 1
Two pairs of bristles present on the hypostomium

Instar 4. Three antennal joints in addition to the sensilla, joint 3 being shorter than joint 2 + joint 1 and joint 2 is longer than joint 1
Three pairs of bristles present on the hypostomium

Instar 8. Pupal respiratory filaments are black when fully developed

Mandible is elongated, the length being 2.14 - 2.29 x the width

Large cephalic fan has 40-44 rays

Radial rows of hooks in the posterior attachment organ have up to 15 or 16 hooks

The above features represent only the principal morphological differences, but differences in the numbers of rays in the large cephalic fan, the number of preapical spines on the mandibles, and the proportions of the hypostomial teeth provide additional evidence for the separation of the instars. A number of second instar larvae which were about to moult showed developing skeletal structures, particularly the number of hooks in the radial rows of hooks in the posterior attachment organ, the number of preapical spines on the mandible, and the number of bristles on the hypostomium which correspond with the features of the third instar larva. The changes in the structure of the antennae described by Puri (1925) and also noted by Terterjan (1957) and Grenier (1960) also hold true for the first four instars of P.hirtipes. The numbers of pairs of bristles on the hypostomium of P.hirtipes larvae however differs from that described by Grenier for Simulium damnosum but agrees with Terterjan's observations of the larval instars of S.(W.) paraequina for the second to fourth

instars, differing only in the presence of one pair of bristles on the hypostomium of the first instar larva. The recognition of the final instar by the separation of the cervical sclerites from the post-occiput or collar, as occurs in several species of black-fly larvae and used by Grenier as one of the principal features by which he recognised the final (7th) instar of S.damnosum, does not occur in the final (8th) instar of P.hirtipes.

Examination of the morphology of the fifth to seventh instars did not provide as clear evidence for their separation as was obtained for the other instars. The sixth to eighth instars do however show the progressive development of the pupal and imaginal histoblasts, no development of these histoblasts being noted in the fifth instar larvae examined. The stage of development of these histoblasts was not used for the separation of the sixth and seventh instars as there seemed to be no distinct change between the instars, but rather progressive development during the instars. If however a number of morphological characters are used, the fifth and seventh instars can be separated with a good degree of certainty.

Instar 5. No evidence of imaginal or pupal histoblasts;
24 to 29 rays in the large cephalic fan;
6 - 8 preapical spines on the mandible (usually 7);
Length of the mandible .198mm to .244 mm;

3 antennal joints in addition to the sensilla.
Joint 3 is shorter than joint 2 + joint 1,
joint 2 + joint 1 being 1.5 x to 2 x joint 3.
Joint 2 is equal to or less than joint 3;
3 - 4 pairs of bristles on the hypostomium;
Width of the hypostomial teeth .049mm to .060mm;
The central tooth of the hypostomium exceeds the
lateral teeth and central tooth 1b is equal in
length to lateral teeth 1b; The posterior
attachment organ has 62 to 78 radial rows of
hooks having 8 to 11 hooks per row;

Instar 7. The imaginal and pupal histoblasts are well
developed; 33-36 rays in the large cephalic fan;
7 - 9 preapical spines on the mandible (usually 9);
Length of the mandible .319mm to .388mm;
3 antennal joints in addition to the sensilla.
Joint 2 + joint 1 is approximately 2 x joint 3.
Joint 2 is longer than joint 3;
4 - 5 pairs of bristles on the hypostomium;
Width of the hypostomial teeth .070mm to .080mm;
The central tooth of the hypostomium exceeds the
lateral teeth and central tooth 1b is greater than
lateral teeth 1b but shorter than lateral teeth 1.
Protuberances 1a are not present on either the
central tooth or the lateral teeth 1;
The posterior attachment organ has 75-81 radial rows
of hooks having 10 to 13 hooks per row.

Only two 6th instar larvae were critically examined and they showed affinities to both the 5th and 7th instar larvae.

Instar 6. The imaginal histoblasts are small but clearly visible;

27-31 rays in the large cephalic fan;

7 - 9 preapical spines on the mandible;

Length of the mandible .286mm to .288mm;

3 antennal joints in addition to the sensilla.

Joint 3 is shorter than joint 2 + joint 1.

Joint 2 + joint 1 is approximately 2 x joint 3.

Joint 2 is longer than joint 3;

3 to 4 pairs of bristles on the hypostomium;

Width of hypostomial teeth .061mm to .062mm;

The central tooth of the hypostomium exceeds the lateral teeth and central tooth 1b is longer than lateral teeth 1b but shorter than lateral teeth 1. Protruberances 1a are not present on either the central tooth or lateral teeth 1;

The posterior attachment organ has 75-79 radial rows of hooks having 9 to 12 hooks per row.

Owing to the small number of larvae examined, the existence of the 6th instar cannot be established with certainty on the basis of morphological characters. For the separation of this instar we must rely on the evidence from the measurements of the head-capsule widths of a large number of larvae.

On the basis of morphological characters, seven instars (1st - 5th and 7th - 8th) can be distinguished, thus confirming the presence of at least seven instars. The presence of the remaining instar, the 6th instar, is established mainly on evidence from the polymodal head-capsule width distribution of the larvae. The mean head-capsule width of the 5th instar corresponds with the peak at 31 divs. in the polymodal distribution and the mean head-capsule width of the 8th instar is that of the known final instar larva, namely 62.1 divs. It seems unlikely that only one instar should lie between 31 divs. and 62.1 divs. since this represents a doubling in the width of the head-capsule and also two peaks, at 41 divs. and 53 divs., occur in the polymodal distribution during this interval. Of these two peaks, the peak at 41 divs. is the more distinct and therefore must represent the mean head-capsule width of a 6th instar.

It must be noted that the larvae used for the morphological examination were obtained from two different localities and developed under very different environmental conditions. The smaller larvae, up to 37 divs. head-capsule width, were obtained from Swindale Beck during the Winter (Nov. 23 & Jan. 4) while the larger larvae with head-capsule widths of 40 divs. and greater were all obtained from the river Belah in mid-May when many larvae had already pupated. These large larvae were thus biased towards being the last or

tail-end of the larvae reaching full growth in Spring and thus are likely to have grown relatively rapidly. There is some evidence that the larvae which develop quickly under conditions of high water temperature fail to achieve head-capsule widths as large as those of the larvae developing more slowly under conditions of low water temperature. The use of larvae developing at such temperature extremes was unfortunate because the point of overlap was in the 6th instar. The position must be clarified at a later date by the use of later instar larvae from Swindale Beck obtained before pupation commences.

The nature of the material used in the morphological study of the instars was therefore likely to show some telescoping of dimensions of the later instars, and, allowing for this, the existence of 8 instars, all-told, seems most likely.

1.E.(iv) Morphology of the first instar larva of *Prosimulium hirtipes* Fries.

The opportunity is taken at this point of giving a brief account of the first instar larva of *P.hirtipes*, since there are no records of the first instar larva of this species having been found previously in the British Isles. The larvae closely resemble the first instar larva of a species of *Prosimulium*, probably *P.fuscum*

Syme & Davies, obtained by Davies (1960) from three streams to the north of Ottawa, Canada, in October 1958, and I am indebted to Dr. L. Davies for the identification of the British specimens. Two first instar larvae were obtained from 180m in Swindale Beck on 23 November 1968, one of which was subsequently lost during mounting. Since the only Prosimulium species known to occur in this stream, at this altitude, is P.hirtipes, it can be assumed that these first instar larvae are P.hirtipes larvae.

The similarity, both in dimensions and morphology, to the Canadian larvae is very striking and only minor differences can be seen.

The general form of the larva resembles that of the later instars (Fig.9A) and has a length, including the head, of 0.9 to 1.0mm. The width of the head-capsule at its broadest point near the posterior margin is 0.14mm and in length it measures 0.18mm. The body pigmentation was light brown while the head capsule was only lightly sclerotised and was for the most part light yellow in colour. The posterior occipital regions and the post-occiput were darkly pigmented producing a black 'collar' while the oval region surrounding the egg-bursting tooth was also darkly pigmented. The 'T' shaped mandibular phragma produced a darker area extending to the antennal socket.

Of the less heavily sclerotised parts of the body, little was discernable without the use of a phase contrast

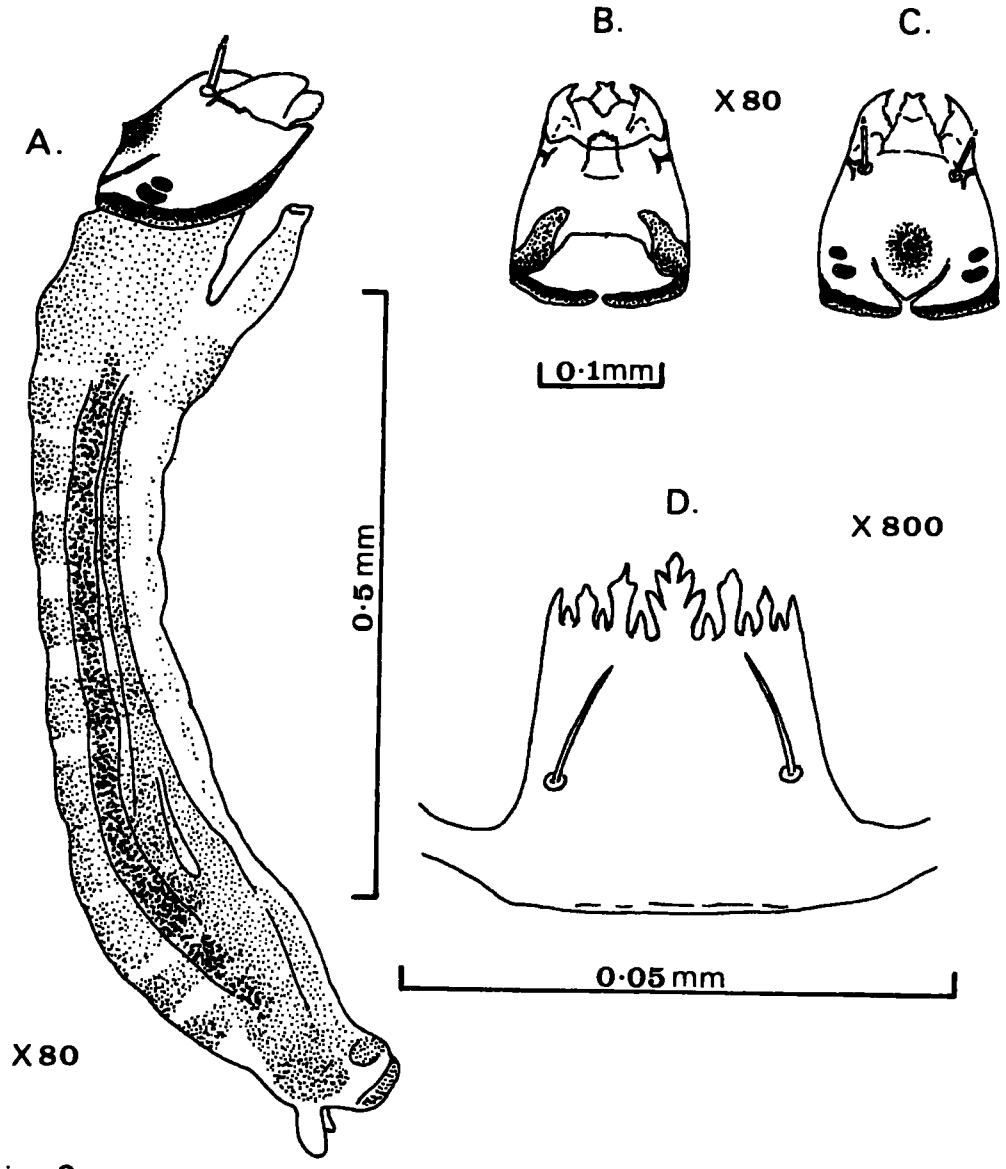


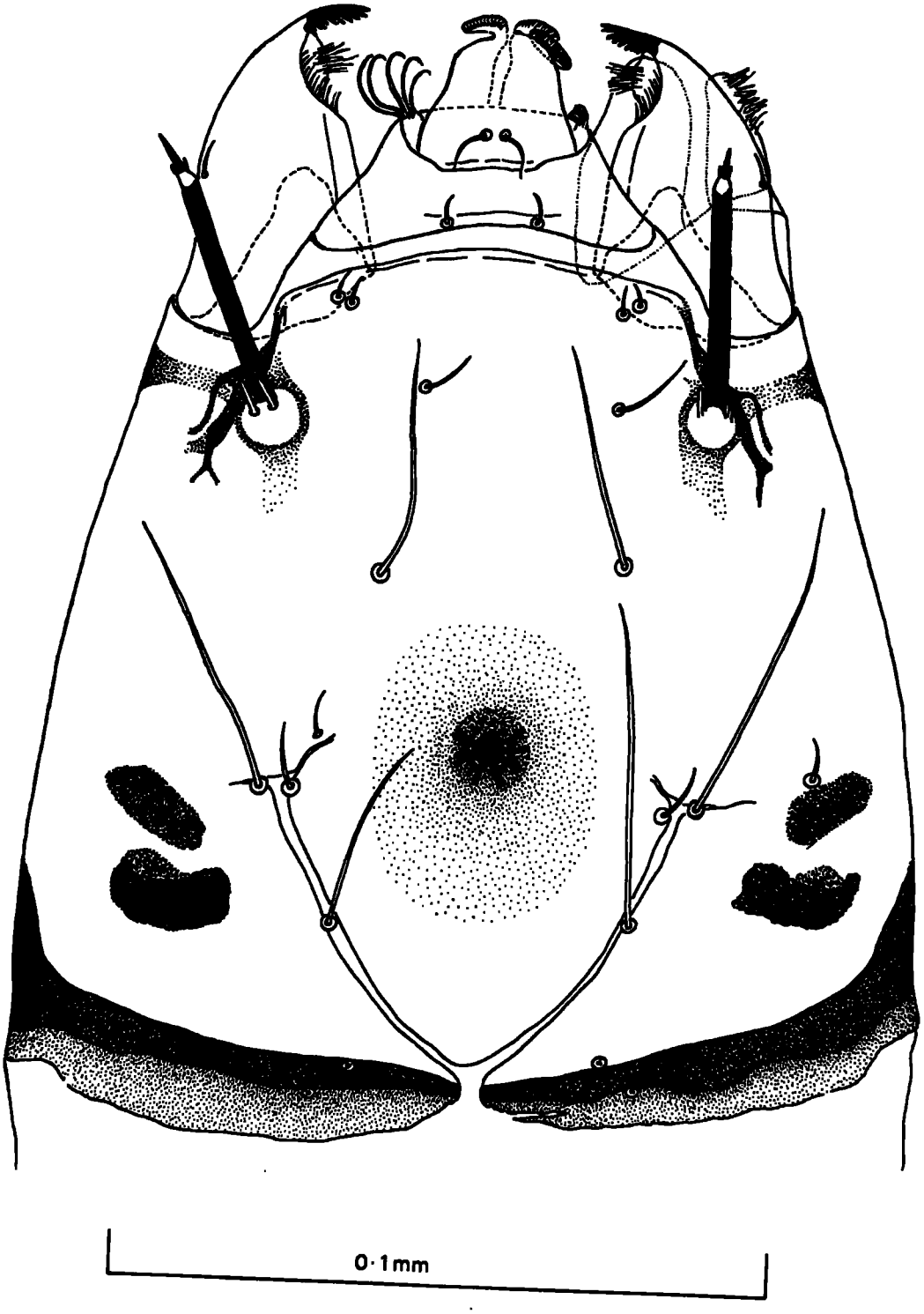
Fig. 9. FIRST INSTAR LARVA of Prosimulium hirtipes Fries. A. Lateral view of Entire larva. B. Ventral and C. Dorsal views of head-capsule. D. Hypostomium.

microscope, the anal gills were however simple and three branched as in the later instars and the posterior attachment organ had 3 hooks in each radial row but the number of rows could not be determined.

The most conspicuous feature of the head capsule was the complete absence of the cephalic fans. The post-occiput and occipital regions are extended, as in the first instar larvae of other Simuliidae so that they almost meet in the midline leaving a narrow occipital cleft from which the sutures bounding the posterior region of the cephalic apotome extent anterior-laterally forming a 'Y' shape. Ventrally (Fig.9B) the postgenal cleft is shallow and rectangular as in the later instars and the dark pigmentation of the collar does not extend onto the postgenal bridge. The eye spots lie near to the posterior margin of the head capsule. The antennae have one joint which is more lightly sclerotised than in the later instars with a conical sensilla at the apex. The antennal socket is surrounded by a lightly sclerotised extension of the mandibular phragma and it contains two sensilla lying near the base of the antenna.

The mandible is darkly sclerotised at the tip (Fig.10) where a curve series of seven teeth are quite distinct followed by two or three more slender spines. The maxillae were less easily distinguished but their general shape resembled that in the later instars. The two prominent spines in the maxillary comb of the later instars were not visible.

FIG. 10. DORSAL VIEW OF THE HEAD-CAPSULE OF THE FIRST INSTAR LARVA OF Prosimulium hirtipes Fries.



The labrum was well developed (Fig.10) as noted by Davies (1960) although in the specimen it appeared to be slightly retracted near the apex. On this section of the labrum Davies noted three pairs of bristles, the hindmost of which had conspicuous sockets. In the first instar larva of P.hirtipes only the hindmost of these three pairs of bristles is present. The uppermost two-branched median plate at the extreme front end of the labrum, described by Davies, is also clearly visible (Fig.10), with a diatom attached. The remaining details of the labrum and hypopharynx were not sufficiently clear to allow accurate description.

The hypostomium has seven principal teeth as in the later instars, one central and three lateral pairs. The central tooth however exceeds the lateral teeth "1", thus differing from the arrangement of the teeth in the second instar larva where the central tooth is equal to or less than lateral teeth "1c". The general shape of the hypostomium appears to be more rectangular than in the later instars and like the second instar it carries a single pair of bristles.

The larger number of instars in the development of P.hirtipes larvae (than is usually found during the development of the larvae of other genera) may be associated with the more primitive position of this genus (Davies, 1960) as exemplified by the structure of the first instar larva.

1.E.(v) Establishment of the instar head-capsule width ranges for the separation of the data into instars

Having established the presence of eight instars it is now possible to effect the separation of the instars on the basis of their head-capsule width. The mean head-capsule widths of each instar distribution can be obtained from the polymodal distribution, then knowing the maximum number of larvae with the mean head-capsule width and assuming a deviation from the mean, equal to the number of divisions between the mean head-capsule width of the instar and the mean head-capsule width of the instar above, normal distribution curves for the larval head-capsule widths of each instar can be constructed (Fig.5). The points at which these distribution curves overlap provide the limits of the head-capsule width range for each larval instar. It is recognised that a small proportion of the larvae will be attributed to the wrong instar, however, when several instars are present these errors will be self compensating (i.e. just as many larvae from the lower instar will be wrongly attributed to the instar above, as larvae from the higher instar will be wrongly attributed to the instar below).

The larval instars of P.hirtipes were thus separated as shown in Table 11.

The calculation of the Growth Index (Table 12) between the different instars reveals that there is no constant relationship between the different instars. This might be expected as these larvae did not develop under controlled experimental conditions, the environmental conditions experienced by the early instar larvae which mostly develop during the colder months being very different from the warm environmental conditions experienced by the later instar larvae during their development in the Spring. In calculation of the Growth Index, the Growth Indices between the earlier instar larvae will be subject to a higher degree of error since the unit for measuring the head-capsule width (1 division = 1/19mm) is equal to 1/3rd of the difference between the mean head-capsule widths of the 1st and 2nd instar larvae, while it is equal to only 1/12th of the difference between the mean head-capsule widths of the 6th and 7th instar larvae.

It can however be seen that the growth occurring between the earlier instars (1st to 5th) is on average greater than that occurring between the later instars (5th to 8th) and that the smallest growth index was obtained for growth between the penultimate and final instar larvae. Similar patterns of development are shown in the data of both Terterjan (1957) and Grenier (1960) for which the

TABLE 11. The larval head-capsule width ranges (divisions) for the separation of the data into instars

Instar	Mean Head-capsule Width	Maximum Head-capsule Width	Minimum Head-capsule Width
1	10.0 (assumed)	10.0	not known
2	12.0	12.0	11.0
3	15.5	16.0	13.0
4	23.0	26.0	17.0
5	32.0	35.0	27.0
6	41.0	45.0	36.0
7	53.0	57.0	46.0
8	62.1	76.0	58.0

TABLE 12. Calculated Growth Indices for *Simulium (Wilhelmia) paraequina*, *Simulium damnosum* and *Prosimulium hirtipes*

Instar	*S.(Wilhelmia) paraequina* Puri (after Terterjan, A.E. 1957)		*S.damnosum* Theo. (after Grenier P. & Feraud, 1960)	*P.hirtipes* Fries.
	Head width	Head width immediately after moulting	Mandible length	Head-capsule width
1 to 2	1.76	-	1.56	1.20
2 to 3	1.47	1.60	1.37	1.29
3 to 4	1.50	1.78	1.40	1.48
4 to 5	1.30	1.28	1.33	1.39
5 to 6	1.32	1.19	1.38	1.28
6 to 7			1.23	1.29
7 to 8				1.17
	(6 instars)	(6 instars)	(7 instars)	(8 instars)

Growth indices have been calculated (Table 12). The increase in the length of the mandible of S.damnosum shows a very similar pattern of growth, while that of the increase in the head-capsule width of S.(W.)paraequina is also similar to the growth of P.hirtipes larvae, except that the proportionate growth between the penultimate and final instars is slightly greater than between the 4th and penultinate instar which is not to be expected. The Growth Indices calculated from measurements of the head-capsule widths of S.(W.)paraequina immediately after moulting however show a decrease in the proportionate growth between the penultimate and final instars.

1.E.(vi) Measurement of larval development using the Percentage Change Index

Using the previously determined head-capsule width ranges for the different instars of P.hirtipes larvae, the numbers of each instar larvae in the samples obtained from Swindale Beck, 1956-57, were determined. They were then expressed as a percentage of the total larvae in each sample to enable the comparison of the instar composition of the samples obtained on different dates and at different altitudes during the season. The instar composition of all the samples obtained throughout the season, 1956-57, at three altitudes (180m, 300m and 430m) in Swindale Beck

is shown in Fig.11. The samples usually contained larvae of several different instars, 70% of the samples having 4 or more instars. These histograms show clearly the instar composition of each sample and how this changes as the season progresses, the smaller instar larvae being more numerous at the beginning of the season and only the larger instar larvae being present at the end of the season. At 300m, where the hatching was more uniform, the changes from the smaller to the larger instar larvae are most regular. The changes at 180m follow a less regular pattern almost forming two separate groups of instars, the parallel development of which can be followed throughout the season. This was due, as has been previously mentioned, to the occurrence of distinct early and late periods of hatching. The samples from 430m show still less regular changes in their instar composition due mainly to errors arising from the small sizes of the samples early in the season. The pattern of later development at this altitude is similar to that occurring at 180m and 300m.

Knowing the instar composition of each sample it is now possible to obtain a measure of the development between the successive sampling dates by calculating the Percentage Change Index. This is done by first expressing the number of larvae in each instar of a sample as a percentage of the whole sample and then taking two successive samples and determining the percentage of the larvae which

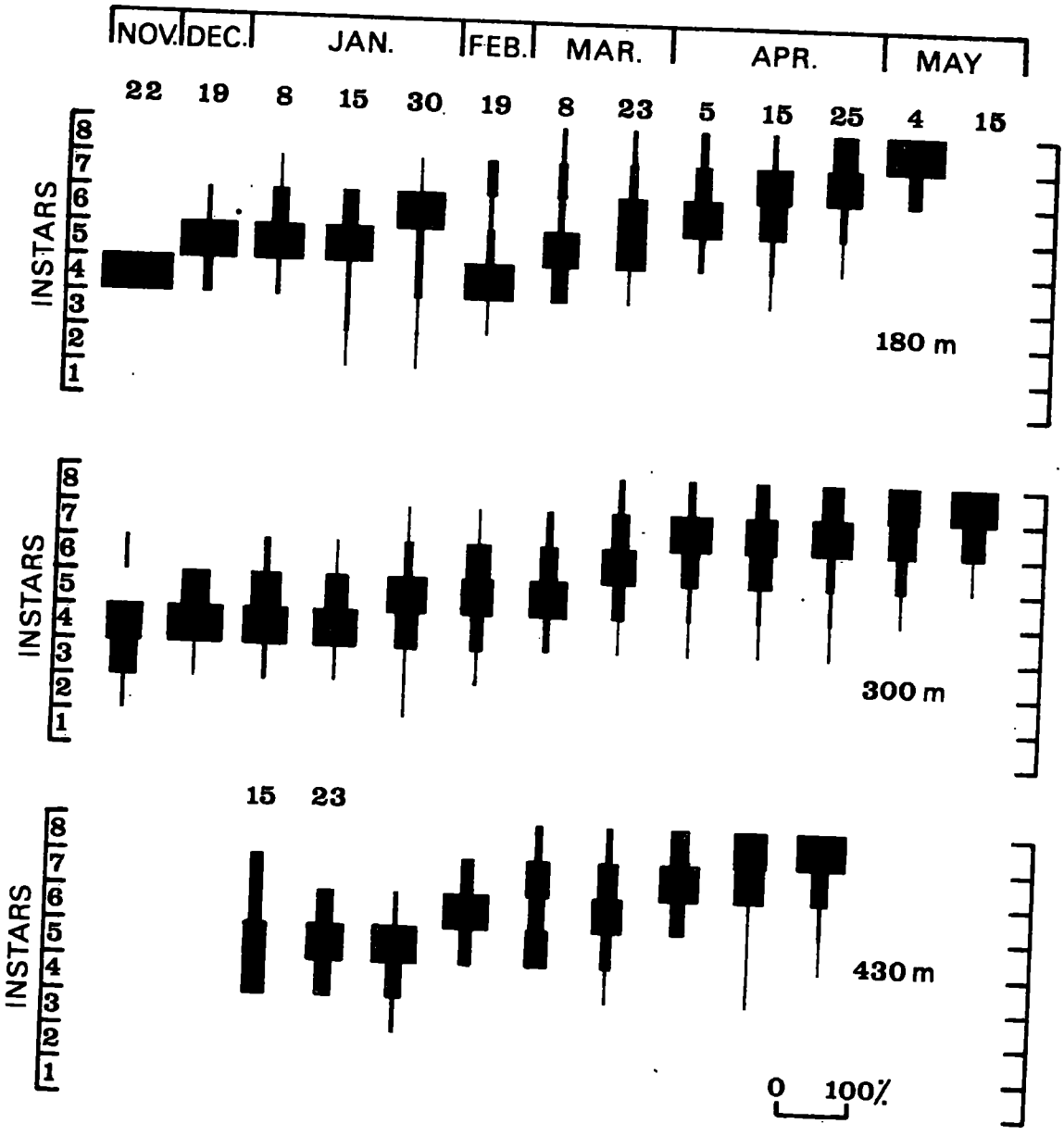


FIG. 11. THE INSTAR COMPOSITION (Percentages) of P. hirtipes LARVAL SAMPLES FROM THREE ALTITUDES IN SWINDALE BECK, 56-57.

have apparently moved from lower to higher instars. If all the larvae move to the next instar above then 100% change has occurred and since there are seven moults (eight instars) the maximum change which could occur during the season would be 700%. In this study the maximum change will be 600% as no first instar larvae were obtained in the 1956-57 samples. An example of the calculation is given below.

In-star	Percentage of sample in each instar				
	Sample A	Sample B (taken 1 week later)			
8	0	0	± 0		
7	0	10	+ 10	∴ 10+10+10-10-10±0-10 larvae moved up	= 0%
6	10	10	± 0	∴ 10+10+10-10-10±0 larvae moved up	= 10%
5	10	20	+ 10	∴ 10+10+10-10-10 larvae moved up	= 10%
4	20	30	+ 10	∴ 10+10+10-10 larvae moved up	= 20%
3	30	20	- 10	∴ 10+10+10 larvae moved up	= 30%
2	20	10	- 10	∴ 10+10 larvae moved up	= 20%
1	10	0	- 10	∴ 10 larvae moved up	= 10%
					100%

∴ The total % change = 100% in 7 days (i.e. all larvae have moved up 1 instar)

∴ The Daily % change = 14.3% per day

The Daily % change and the Cumulative % change between the successive samples from the three altitudes (180m, 300m and 430m) in Swindale Beck were calculated and are given in Table 13. All the calculations did not follow

TABLE 13(a) Percentage change between instars at 180m, Swindale Beck, 1956-57

1956-57	Whole sample (uncorrected)			Early Hatch			Late Hatch			Whole sample (corrected)		
	Daily %	Unacc. %	Cumul. %	Daily %	Unacc. %	Cumul. %	Daily %	Unacc. %	Cumul. %	Daily %	Unacc. %	Cumul. %
22Nov-19Dec	3.3	(0)	90	3.3	(0)	90		-		3.3	(0)	90
19Dec- 8Jan	1.4	(0)	118	1.4	(0)	118		-		1.4	(0)	118
8Jan-15Jan	1.1	(9)	126	0.4	(6)	121		-		0.8	(6)	124
15Jan-30Jan	4.9	(15)	200	4.8	(1)	193	4.5	(1)	67	4.7	(2)	195
30Jan-19Feb	3.3	(106)	265	3.4	(2)	261	1.7	(0)	100	2.4	(2)	243
19Feb- 8Mar	4.5	(3)	341	2.3	(1)	300	3.8	(0)	164	3.5	(1)	303
8Mar-23Mar	3.3	(4)	390	3.5	(0)	352	5.5	(1)	246	5.2	(1)	381
23Mar- 5Apr	4.7	(1)	451	3.7	(0)	400	4.8	(1)	309	4.7	(1)	442
5Apr-15Apr	2.4	(11)	475		-		3.2	(1)	341	2.8	(1)	470
15Apr-25Apr	7.1	(1)	546		-		7.1	(1)	412	7.1	(1)	541
25Apr- 4May	6.6	(0)	605		-		6.6	(0)	471	6.6	(0)	600

Daily % - Daily Percentage Change
Unacc. % - Percentage Change Unaccounted for
Cumul. % - Cumulative Percentage Change

TABLE 13(b) Percentage change between Instars and Thermal Sums, Swindale Beck, 1956-57

	180m				300m				430m			
	Whole sample (corrected)				Whole sample				Whole sample			
1956-57	Daily %	Unacc. %	Cumul. %	Therm.S.	Daily %	Unacc. %	Cumul. %	Therm.S.	Daily %	Unacc. %	Cumul. %	Therm.S.
22Nov-19Dec	3.3	(0)	90	-	2.3	(9)	62	-	-	-	-	-
19Dec- 8Jan	1.4	(0)	118	-	1.3	(7)	87	-	-	-	-	-
8Jan-15Jan	0.8	(6)	124	75.6	0.8	(10)	93	67.3	-	-	-	-
15Jan-23Jan	4.7	(2)	195	92.5	3.4	(1)	144	74.6	1.0	(34)	8	54.4
23Jan-30Jan									0.6	(35)	12	59.1
30Jan-19Feb	2.4	(2)	243	98.3	6.5	(3)	274	87.0	6.3	(2)	137	75.8
19Feb- 8Mar	3.5	(1)	303	89.8	1.3	(1)	296	70.2	4.4	(34)	211	50.6
8Mar-23Mar	5.2	(1)	381	191.9	5.5	(0)	378	171.9	1.5	(12)	233	151.9
23Mar- 5Apr	4.7	(1)	442	171.2	4.4	(4)	435	154.9	6.6	(1)	319	138.8
5Apr-15Apr	2.8	(1)	470	139.5	1.5	(13)	450	122.0	4.4	(17)	363	104.4
15Apr-25Apr	7.1	(1)	541	178.0	4.8	(1)	498	162.6	3.8	(1)	401	147.2
25Apr- 4May	6.6	(0)	600	210.7	2.0	(10)	516	197.7	-	-	-	-
4May-15May	-	-	-	-	3.5	(0)	555	173.1	-	-	-	-

Daily % - Daily Percentage Change
Unacc. % - Percentage Change Unaccounted for
Cumul. % - Cumulative Percentage Change
Therm.S. - Average Daily Thermal Sum (Degree hours above $0^{o}C$)

the above pattern but, due to errors arising from the addition to the sample of newly hatched larvae or the removal of larvae from the sample due to pupation, a certain percentage change could not be accounted for on the basis of the growth of larvae to the instar above. The total percentage change which cannot be accounted for in each calculation are given in Tables 13(a) and (b). These inaccuracies occurred principally in the calculations for the 180m samples during the later period of hatching and for the samples at 430m where only small samples were available.

The daily percentage change shows large fluctuations throughout the sampling period varying from a minimum of 0.6% per day to a maximum of 7.1% per day. It appears then that at no time during the sampling period were the environmental conditions preventing development of the larvae, but they caused great variations in the extent of the development. Examination of the histograms of the daily percentage change (Fig.12) shows that the pattern of development at the different altitudes is similar in many respects. This suggests that the fluctuations in the daily percentage change are not random fluctuations but are the result of one or more environmental factors which have similar effects at all three altitudes. The greatest similarity occurs between the first five samples at 180m and 300m. The similarities between the daily percentage change after 30 January 1957 at these altitudes is

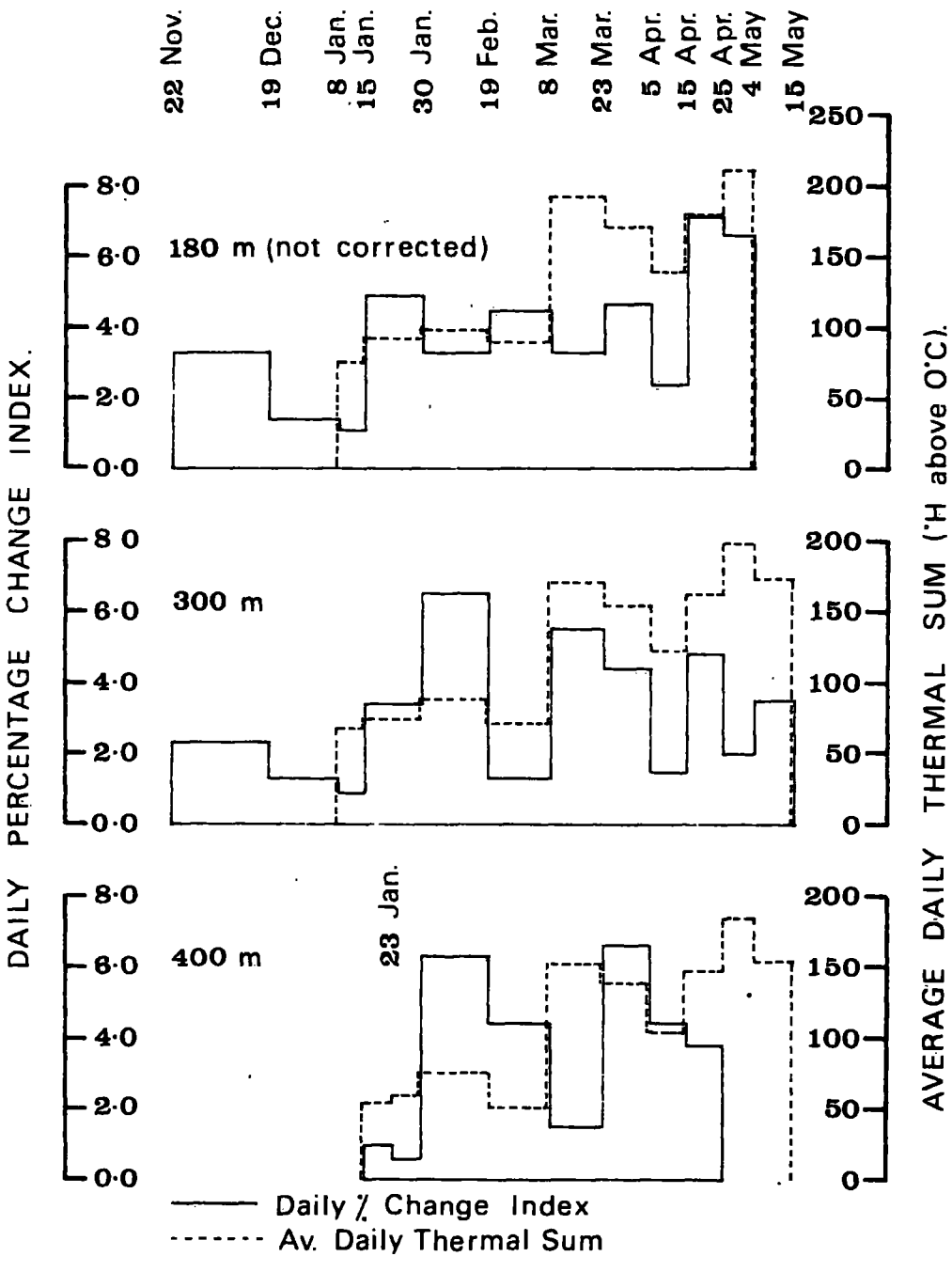


FIG. 12. The Daily Percentage Change Index for P. hirtipes larvae at three altitudes in Swindale Beck, 1956–57, and the Average Daily Thermal Sum at these altitudes.

less marked and coincides with the occurrence of a large number of smaller larvae in the 19 February 1957 sample at 180m resulting from the large late hatch at this altitude. This also accounts for the large percentage change which cannot be accounted for in the calculation of the daily percentage change between the 30 January 1957 and 19 February 1957 samples at 180m. At 300m where the hatching occurred more evenly, the effect on the daily percentage change was less marked.

In order to obtain a truer picture of the daily percentage change at 180m, it is necessary to apply a correction which will reduce the inaccuracies resulting from the late hatch. Examination of the head-capsule width distributions of the individual samples at 180m (Fig.4) shows that two almost separate distributions are present, one derived from the early hatch and the second derived from the late hatch. The two distributions were separated as accurately as possible and for clarity the second distribution has been shaded. The larvae derived from the second hatch appear as second and third instars in the 15 January 1957 sample. The larvae derived from the first Autumn hatch reach the last instar by 8 March 1957. The proportion of last instar larvae falls in the 15 April 1957 sample, rising again in the sample taken on 25 April 1957 with the appearance in the last instar of larvae derived from the second hatch. This separation in the numbers of last instar larvae provides

additional evidence that the two hatches remain reasonably separate throughout their development. Corrections were made to all daily percentage changes between the samples at 180m which contained larvae from both hatches (15 January 1957 to 5 April 1957 samples). The corrections to the daily percentage changes between these samples were made in the following way :-

	EARLY HATCH		LATE HATCH	
	Number of larvae	Daily % Change	Number of larvae	Daily % Change
30 Jan. 57	172		50	
		3.5		3.1
19 Feb. 57	93		331	
	265		381	

Difference between the Daily percentage changes = 0.4%

$\therefore$ Average of Daily percentage changes = $3.5 - (0.4 \times \frac{381}{646})$ %

= 3.3%

It is basically the average of the daily percentage changes for the early and late hatches which is adjusted in proportion to the numbers of larvae present in these hatches. The daily percentage changes for the separate hatches and the corrected daily percentage change at 180m are also given in Table 13(a) and are shown as histograms in Fig.13.

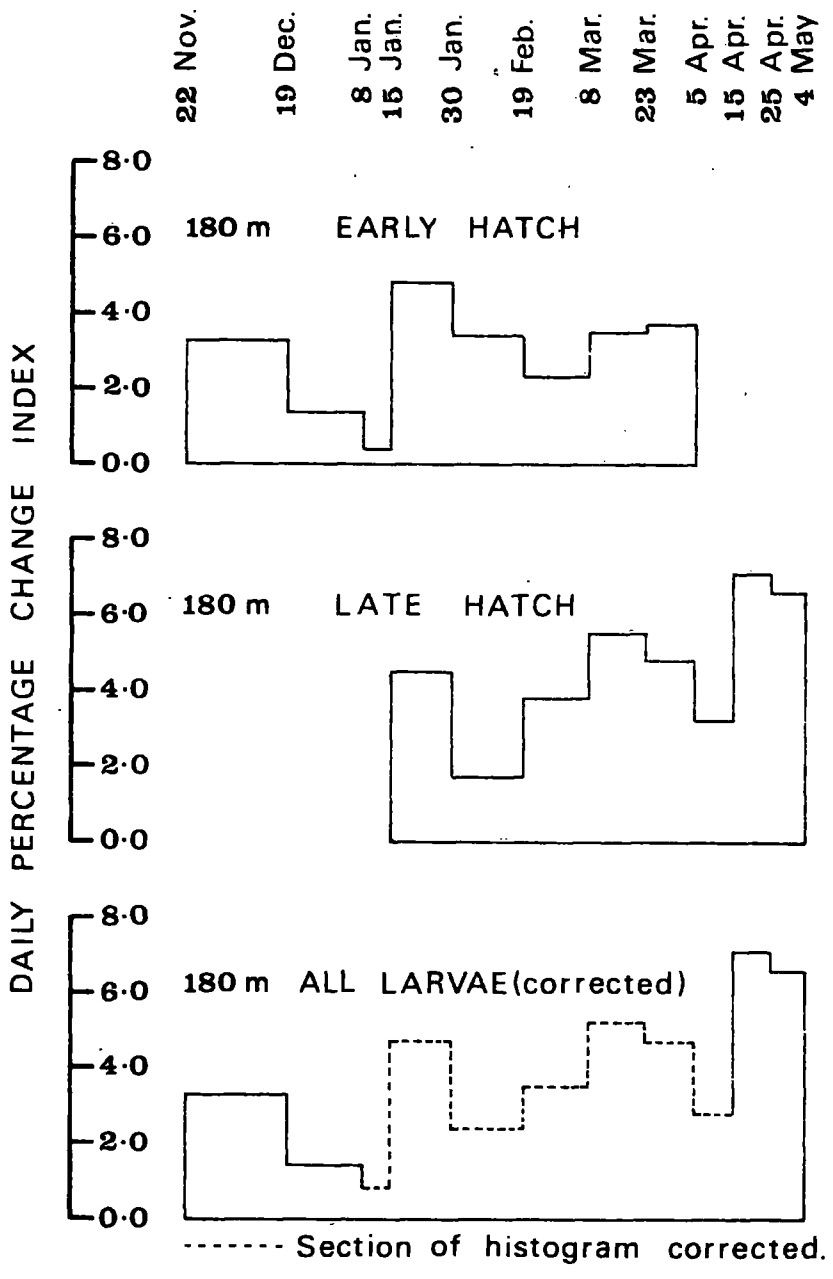


FIG. 13. The Daily Percentage Change Index for the two hatches of P. hirtipes larvae at 180m in Swindale Beck, 1956–57.

Since the hatching at 300m was continuous, the histograms of the head-capsule widths of the separate samples at this altitude (Fig.4) show no separation into two hatches and therefore a similar correction is unnecessary. The effect of the hatching will therefore slightly reduce the values for the daily percentage change. The corrected values for the daily percentage change at 180m show a much improved correlation with the values obtained for 300m.

1.F. Measurement of Stream Temperatures

The water temperature was the only environmental factor studied. During the period of study the water temperature was recorded when each sample was obtained, using a mercury in glass thermometer graduated to 0.1°C. At the time of each reading the thermometer bulb was held in the rapid water and shaded from the sun. The readings for Swindale Beck, 1956-57, are given in Table 14. All the readings were made between 11.00 - 15.00hrs in the same sequence, the higher altitude temperatures being taken first. A temperature gradient which decreases with increasing altitude was evident for most of the sampling period except in the early Spring when the temperature at 300m sometimes exceeded that at 180m.

In order to obtain more detailed and reliable stream water temperature data, two Cambridge mercury-in-steel

TABLE 14 Stream temperatures (^{o}C) in Swindale Beck, 1956-57

	180m	300m	430m
22 Nov. 56	2.5	2.0	0.9
19 Dec. 56	5.2	4.7	4.2
8 Jan. 57	7.8	7.2	6.9
15 Jan. 57	1.6	0.4	0.1
30 Jan. 57	4.8	4.1	3.4
19 Feb. 57	2.1	0.6	0.2
8 Mar. 57	7.0	6.5	5.5
23 Mar. 57	8.2	8.7	6.5
5 Apr. 57	11.8	-	8.5
15 Apr. 57	8.3	7.4	5.8
25 Apr. 57	9.2	11.5	7.4
4 May 57	11.4	13.2	11.0
15 May 57	9.7	-	9.1

thermographs were set up on 8 January 1957 at two stations on Swindale Beck. One was situated at 150m while the other was adjacent to the stream at 400m. The 6 inch thermograph bulbs were placed in the shade in rapid deep water and they were never exposed to the air during their period of operation. The thermographs were checked at 1 to 2 week intervals against a mercury thermometer and gave consistent readings differing from the thermometer by $\pm 0.3^{\circ}C$. The hourly thermograph readings were corrected to correspond with the single thermometer used for checking both instruments. Due to a mechanical defect the clock of the thermograph at 400m occasionally stopped before the chart was due to be changed and, in all, it was out of operation for 18% of the time (23 days out of 127 days). The thermograph at 150m functioned continuously throughout the period.

The thermal sum above $0^{\circ}C$ was obtained from the thermograph readings and the thermal sum for the short periods when the 400m thermograph was out of operation was estimated on the assumption that the hourly water temperatures at 400m bore the same relationship to those at 150m as during the previous seven to twelve days when both instruments were working. There is no reason to believe that the estimation of these thermal sums at 400m introduced sufficient error to invalidate the conclusions drawn from the results. The thermal sums for the periods between samples and the cumulative thermal sums are given in Table 15. The temperature data obtained from the thermographs at 150m and 400m will approximate

TABLE 15 The thermal sums (degree hours above $0^{o}C$) between the sampling dates and the cumulative thermal sum, Swindale Beck, 1956-57

	150m		400m	
1957	Therm. Sum between Samples	Cumulative Therm. Sum	Therm. Sum between Samples	Cumulative Therm. Sum
8-15 Jan	529	529	412	412
15-30 Jan	1388	1918	850	1262
30Jan-19Feb	1965	3883	1515	2777
19Feb- 8Mar	1526	5408	860	3637
8-23 Mar	2878	8286	2279	5915
23Mar-5Apr	2225	10511	1804	7719
5-15 Apr	1395	11906	1044	8763
15-25Apr	1780	13686	1472	10236
25Apr-4May	1896	15582	1662	11898
4May-15May	2112	18525	1696	13594

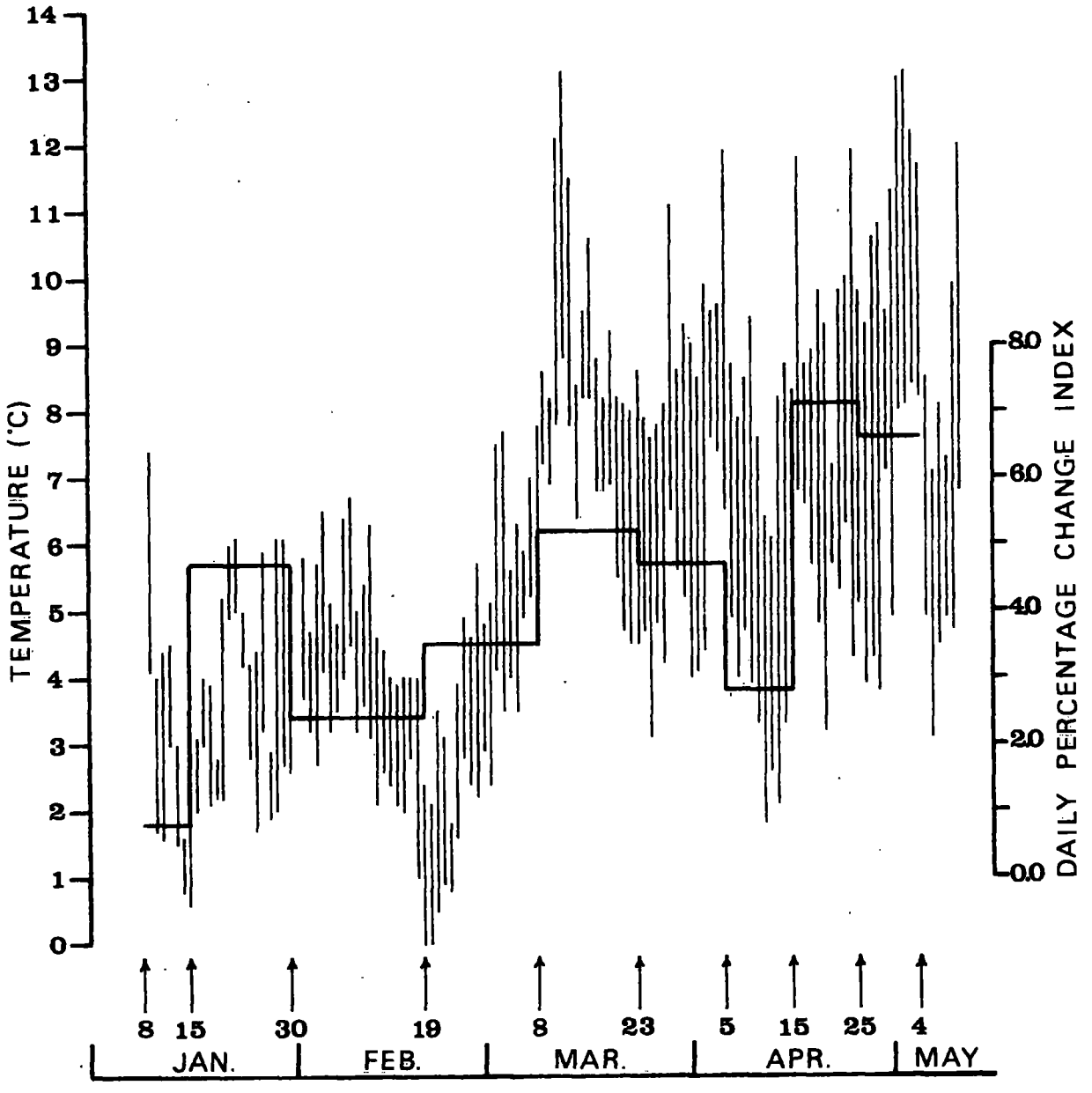
very closely to the water temperature regimes at the 180m and 430m sampling sites respectively. The water temperature regime at 300m is taken as being halfway between that at 150m and 400m, an assumption which is approximately true in view of the temperature readings given in Table 14.

The thermograph water temperature readings at 400m were usually 0.2 - 1.0°C lower than at 150m, only on four occasions, in Spring, were the daily maximum temperatures higher at 400m than at 150m. The daily thermal sum was however always greater at 150m, the cumulative thermal sum for the period from 8 January 1957 to 15 May 1957 being 17,693.4°H above 0°C, while at 400m for the same period it was only 13,593.6°H above 0°C. An estimate of the cumulative thermal sum at 300m will therefore be 15,643°H above 0°C., i.e. :-

$$13{,}593 + \frac{17693.4 - 13593.6}{2} \quad {}^{\circ}H \text{ above } 0^{\circ}C.$$

The general pattern of stream temperatures corresponds well with that described by Macan (1958). The 'winter period' during January and February was a time when the water temperatures were at their lowest with little general tendency to rise (Fig.14) while the 'second-period' which Macan delimits as from just before the Equinox (Mar.20) to just after the Solstice (June 21) is a period of increasing temperatures, the fine weather producing large temperature fluctuations. The winter of 1956-57 was the mildest of the

Fig. 14. Histogram showing the Daily % Change Index (corrected) for P. hirtipes larvae at 180 m in Swindale Beck, 1957, and the Daily Max. and Min. stream temperature ranges.



years 1954-57 (Macan) and the water temperatures showed an earlier rise at the end of February. The water temperature first reached 10°C at 150m and 400m in Swindale Beck on 11 March 1957 which was one day earlier than noted by Macan during the same year for Outgate Beck, a small stony stream near Ambleside, Westmorland. Two periods of particularly low water temperatures occurred during the 'winter-period' on 14 and 15 January 1957 and from 18 to 23 February 1957. During the latter period the minimum temperature reached 0°C on two successive days at 180m. The water temperatures rose steadily from 24 February 1957 at 180m, reaching maximum temperatures of 12.1°C, 13.1°C and 11.5°C on the 11, 12 and 13 March 1957 respectively. For the remainder of the period the temperatures remained high, the maximum water temperature exceeding 8.0°C on 52 of the 62 days. The stream water temperatures at 400m followed a similar pattern of fluctuation but were relatively colder.

1.G. Discussion of the relationship between development (as measured by the percentage change) of the larvae and the water temperature

A comparison of the development of the larvae, as represented by the Cumulative Percentage Change, and the overall water temperature, as represented by the Cumulative Thermal Sum (degree hours above 0°C), can be seen

in Fig.15 for both 180m and 300m in Swindale Beck, 1956-57. The Cumulative Percentage Change between the samples at 180m has been corrected to compensate for the two hatches and the curve produced shows a quite remarkable fit to the curve for the Cumulative Thermal Sum at this altitude. This shows that the development of the larvae, as measured by the changes between the instars, is very closely associated with the overall environmental temperature. The curve obtained for the Cumulative Percentage Change at 300m does not show such a good fit but this might be expected since this curve could not be corrected to compensate for the continued hatching. These errors are therefore particularly associated with the early part of the curve. In the latter part of the curves the larvae developing in the higher water temperatures at 180m show a greater percentage change than those developing in the lower water temperatures at 300m and achieve their maximum development at an earlier date.

The use of cumulative curves, although providing a means of comparing the overall development of the larvae with the overall water temperatures experienced by the larvae at the different altitudes, does not allow any precise comparisons to be made. A more precise comparison can be made if the daily percentage change between the sampling dates is compared directly with the thermal sum above $0^{\circ}C$ for the same period (Figs. 12 & 13). When examined in this way it can be seen that in general, during periods of low

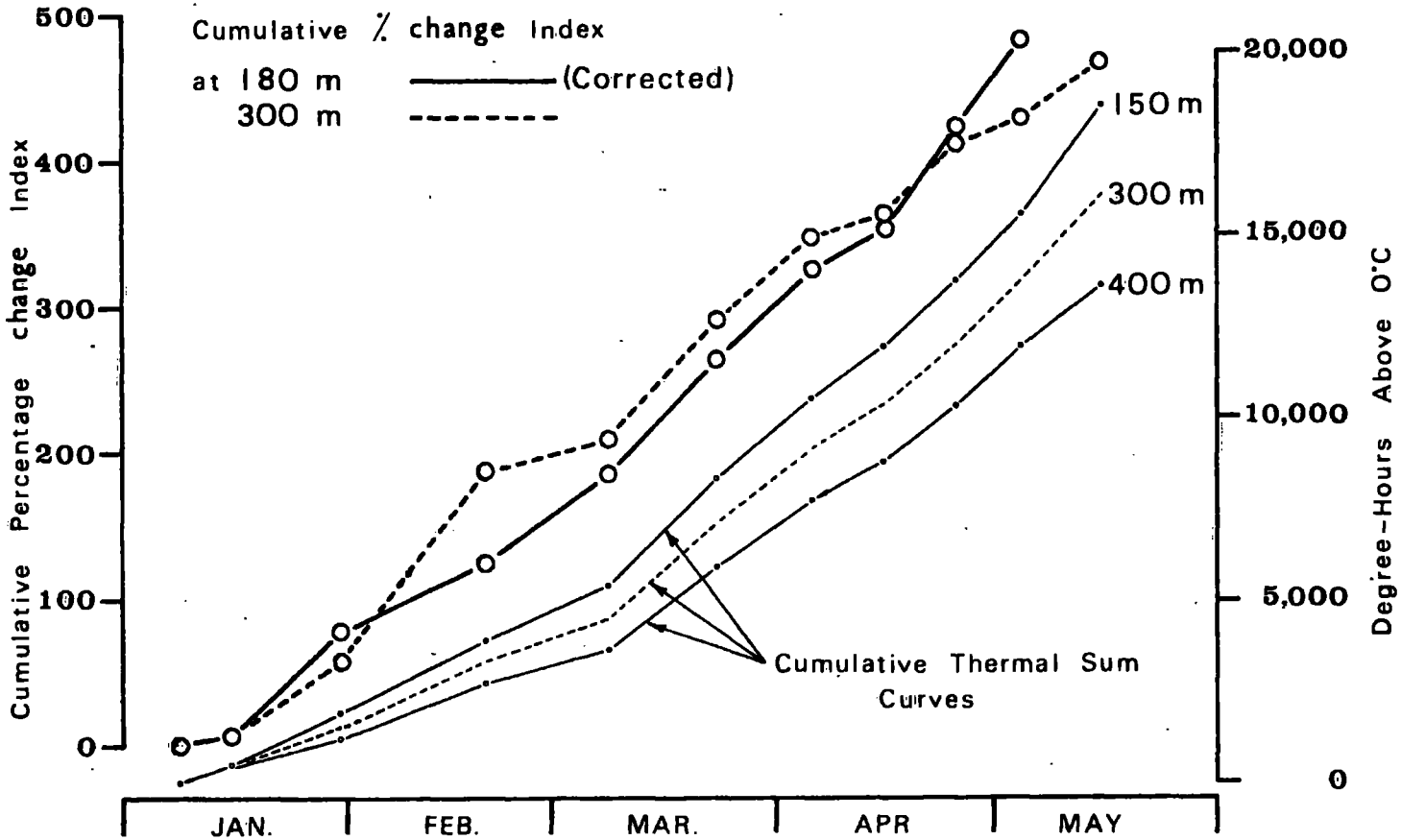


Fig. 15. CUMULATIVE % CHANGE INDEX AND CUMULATIVE THERMAL SUM CURVES FOR SWINDALE BECK, 1957. (C.T.SUM for 300 m interpolated).

water temperatures, less development occurs, while in periods of high water temperatures the development is accelerated. This is particularly well demonstrated in the period between 8 January 1957 - 15 January 1957 when the lowest average daily thermal sum, for the period when thermograph temperatures were taken, was recorded at 150m. This corresponded with the lowest daily percentage change for the larvae at both 180m and 300m. A comparison with the same period at 430m is not possible since the larval samples were too small until 15 January 1957 at this altitude. Although the periods with higher average daily thermal sums in general corresponded with those with high daily percentage changes, the amount by which the daily percentage change increased was not always proportionately related to the amount by which the average daily thermal sum increased and in some instances the average daily percentage change declined while the average daily thermal sum increased. The relationship was however tested for the periods when temperature data was available by calculating the Correlation Coefficients between the total percentage change for each period between sampling and the thermal sum for the same periods, at three altitudes (180m, 300m and 430m). At 180m the Correlation Coefficient = 0.755, which with 7 degrees of freedom is significant at the .02 level of significance. At 300m the Correlation Coefficient = 0.532, which with 8 degrees of freedom is not significant at the .10 level

of significance. (The .10 level of significance for 8 degrees of freedom = .549.) At 430m the Correlation Coefficient = 0.377 which with 6 degrees of freedom is not significant at the .10 level of significance. (The .10 level of significance for 6 degrees of freedom = .622.) A significant correlation therefore exists between the development of P.hirtipes larvae and the overall water temperature to which the larvae are exposed for the greater part of their development period at 180m in Swindale Beck. At the higher altitudes (300m and 430m) the correlation is not significant which will be due in part to the uncorrected errors due to hatching at 300m and the smaller numbers of larvae in the samples at 400m. However, the absence of a precise correlation, especially at the higher altitudes, is not unexpected since the development of the larvae is not uniform but occurs in seven steps or moults. Let us assume that a certain thermal sum, x degree hours, is required for a larva to complete its development during an instar. If the larva receives a thermal sum of x degree hours or x + 1 degree hours before the next sampling period, it will have moulted during the sampling period and will be recorded in the percentage change of the sample. If, however, it only receives x - 1 degree hours it will not moult until the following or a later sampling period and therefore will not be included in the percentage change of the sample. Therefore when dealing with the development of insect larvae in a

population the amount of development they show is not only dependent on the environmental conditions between the sampling dates but also in part on the previous environmental conditions, which do not become effective until the next sampling period. Thus the fact that the percentage change may be lower while the thermal sum has increased, or vice-versa, does not necessarily invalidate a relationship between the two factors for that period. If the corrected histogram for the daily percentage change at 180m (Fig.13) is compared with those for 300m and 430m (Fig.12) for the period between 15 January 1957 and 23 March 57 when the water temperatures declined to low values in mid-February and rose steadily to quite high values in early March (Fig.14), the development of the larvae, as indicated by the daily percentage change, appears to be progressively displaced to the right with increasing altitude. This indicates that development which occurred during one sampling period at the lower altitude could not be completed during the same period at the higher altitudes because of the lower overall temperatures, and was delayed until the following sampling period. Thus at 430m from 19 February - 8 March 1957 the daily percentage change was relatively high despite the lower temperatures, but in the following period, 8 March - 23 March 1957, when the water temperatures rose, many of the larvae which had just completed a moult in the previous sampling period were unable to make sufficient growth to moult during this period

and had to wait until the following period to complete their next moult. This type of development, especially where water temperatures are low, will tend to mask a close correlation which does exist, as is shown by the larvae developing at 180m, between the overall temperature and the development of the larvae.

The relationship between the water temperature and the development of the larvae at 180m in Swindale Beck, 1957, is perhaps best illustrated in Fig.14. At the beginning of the period (8 - 15 January 1957) growth is checked by the generally low temperatures and very few larvae moult while the relatively warmer temperatures of the following period (15 - 30 January 1957) produce a marked rise in the percentage change. The period from 30 January - 19 February 1957 is slightly warmer, having an average daily thermal sum of 98.3°hrs as compared with 92.5° Hours, but the percentage change falls sharply showing that many of the larvae have not made sufficient growth from the moult during the previous period in order to moult during this period. During the following period, when the average daily thermal sum is slightly less (89.8°Hours) and includes a particularly cold period, the percentage change rises due at least in part to the growth made during the previous period. The following two periods, 8 March - 23 March 1957 and 23 March - 5 April 1957, show percentage changes which correspond with the temperature regimes experienced by the

larvae, however a fall in temperature during the period from 5 April - 15 April 1957 causes a marked fall in the percentage change. The subsequent rise in water temperatures from 15 April - 25 April 1957 caused a marked rise in the percentage change which declined slightly from 25 April - 4 May 1957 despite the higher temperatures, due partly to the loss of the larger larvae which were pupating.

Another factor which must be borne in mind, but is difficult to make allowance for, is the different amounts of growth which the different instars undergo. If a given thermal sum produces a given amount of larval growth, the same thermal sum may not cause the moulting of the penultimate instar larva into the final instar larva, while it might enable the moulting of the first instar larva into the second instar larva.

The relationship between the overall temperature and the development of the larvae was also examined by calculating the percentage transfer of larvae which occurred between pairs of instars at different times and altitudes throughout the sampling period (Figs. 16, 17, 18 & 19). Where the hatching of the larvae is prolonged and uneven, errors, similar to those encounted in the calculation of the percentage change between whole samples, occur. This is particularly evident in the uncorrected percentage transfer of larvae between the pairs of instars for 180m in Swindale Beck, 1957, where the moults associated with the main larval

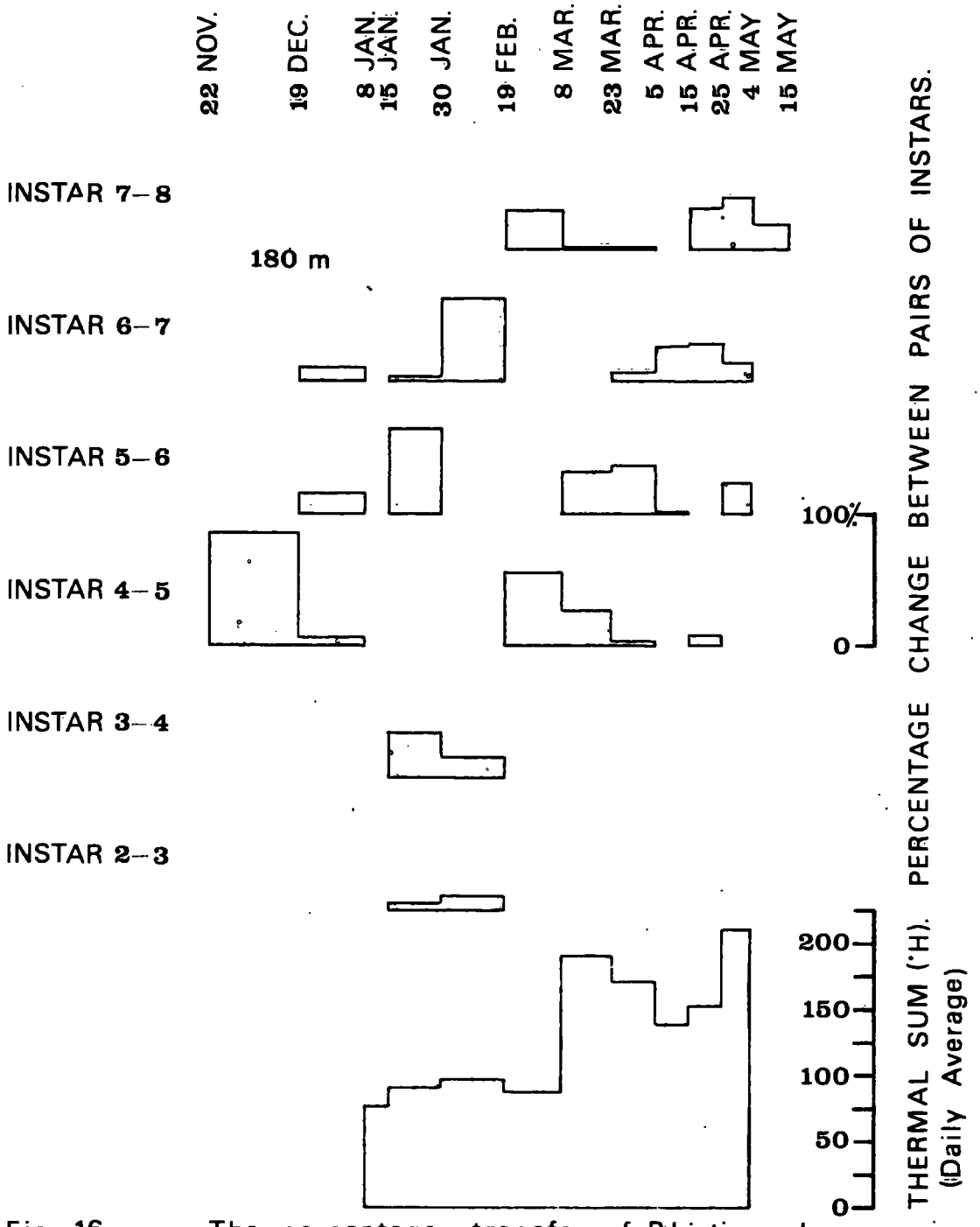


Fig. 16. The percentage transfer of P. hirtipes larvae between pairs of instars. 180 m Swindale Beck, 1956–57.

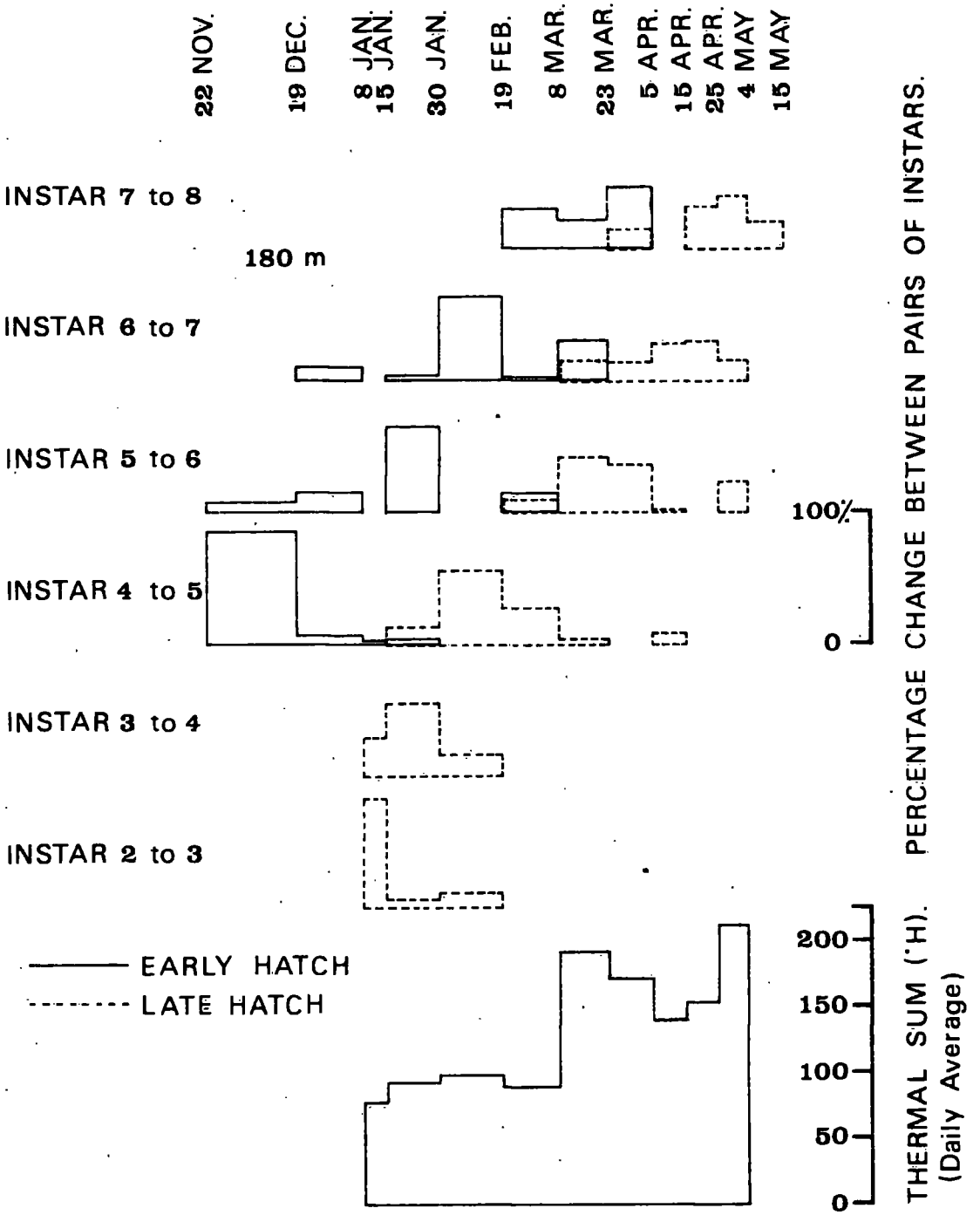


FIG. 17. The percentage transfer of P. hirtipes larvae between pairs of instars. 180m Swindale Beck, 56–57. (The two hatches are treated separately.)

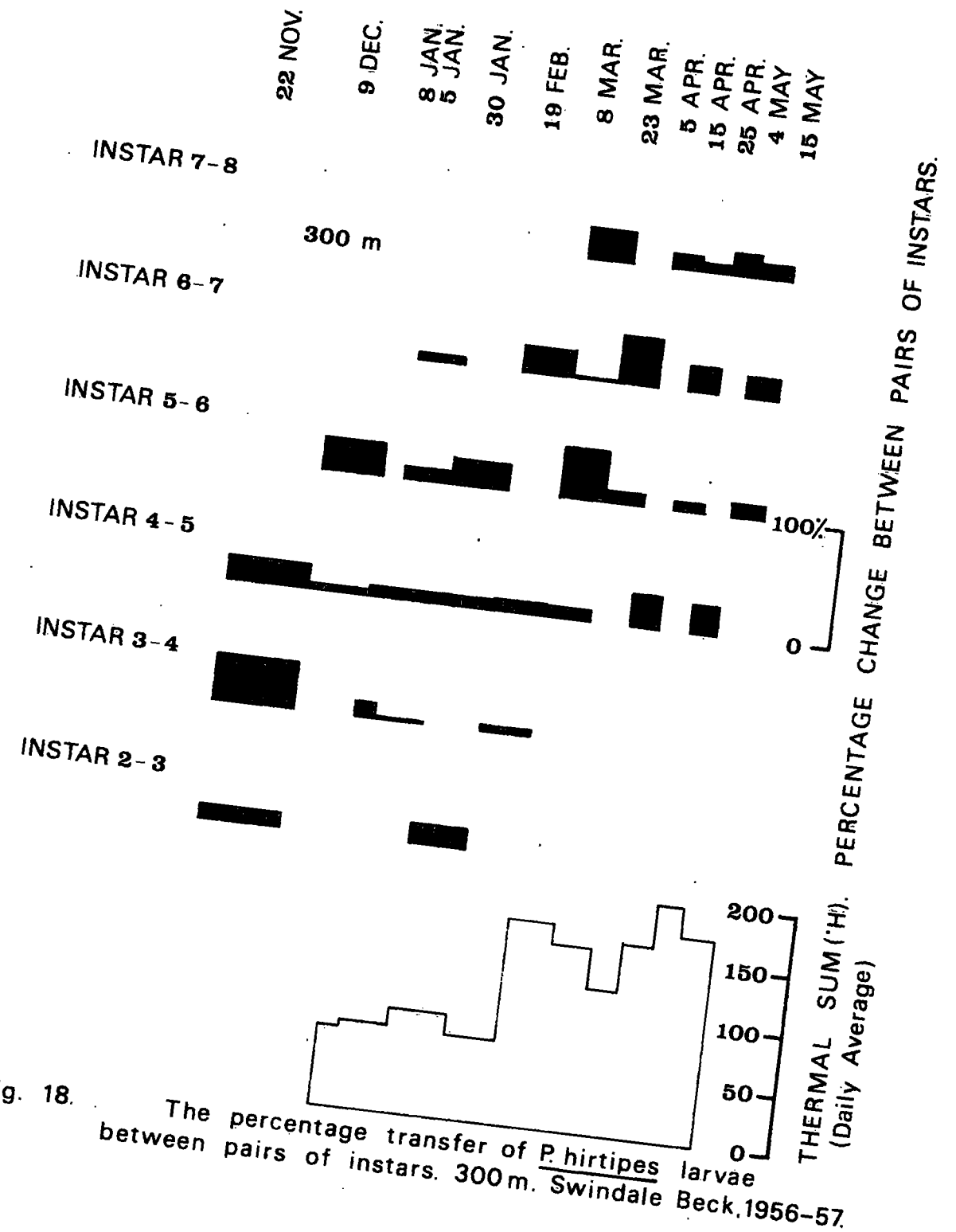


Fig. 18. The percentage transfer of P. hirtipes larvae between pairs of instars. 300 m. Swindale Beck, 1956–57.

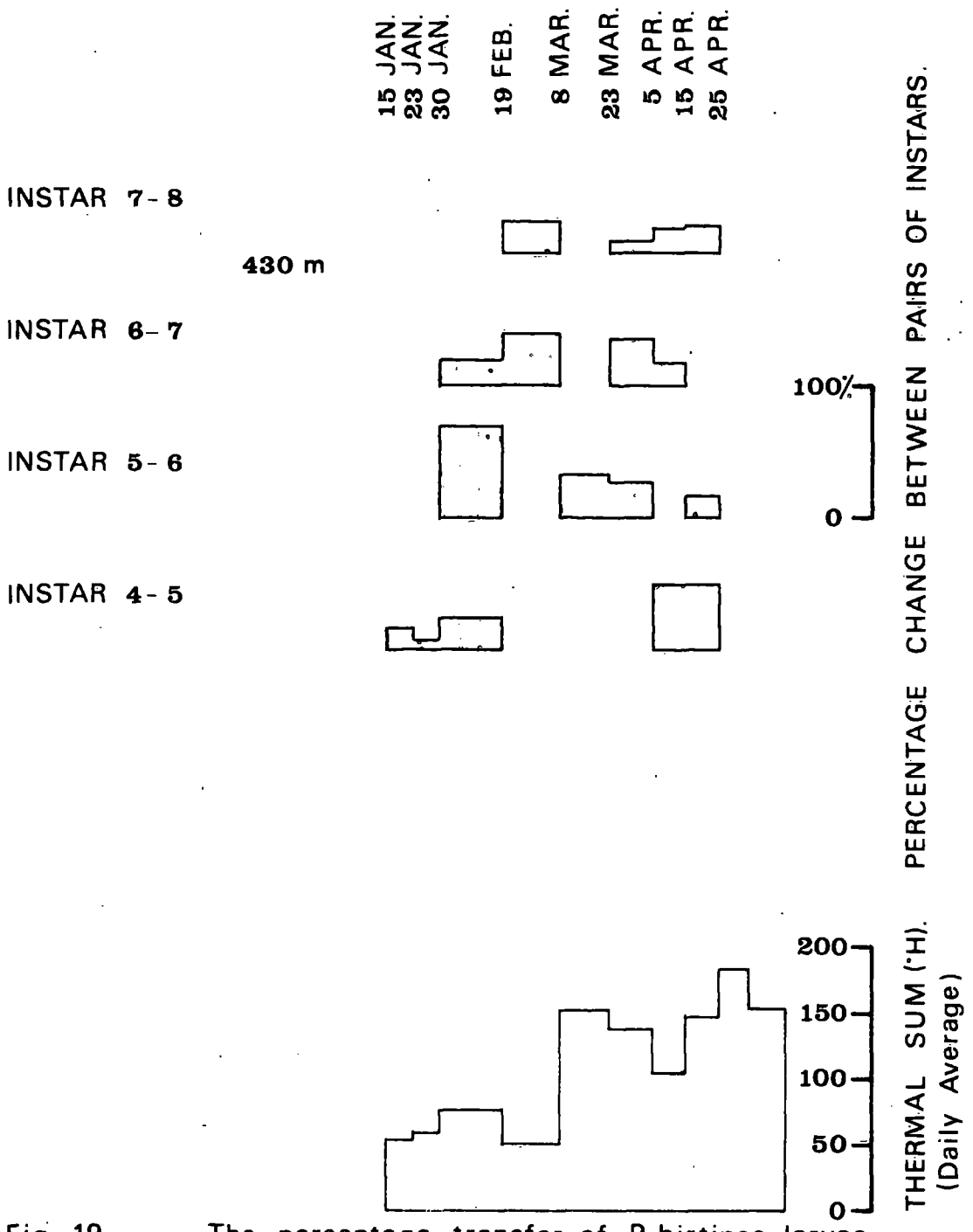


Fig. 19. The percentage transfer of P. hirtipes larvae between pairs of instars. 430m. Swindale Beck, 1956-57.

hatches appear to be completely separated. However when the larvae of the two hatches at this altitude are considered separately (Fig.17) this separation is less distinct and a more correct picture of the actual events can be seen.

At 180m 86% of the first hatch larvae moulted into the fifth instar between 22 November 1956 - 19 December 1956 in which stage the majority of them remained until 15 January 1957 - 30 January 1957 when 65% moulted into the sixth instar. This delay coincided with a period of colder water temperatures. The majority of these sixth instar larvae (64%) moulted into the seventh instar during the following period of higher water temperatures but the moulting of the seventh instar larvae into the last instar were again delayed, 48% of the first hatch larvae not moulting into the last instar until 23 March - 5 April 1957. This delay in moulting again coincided with a period of lower water temperatures from 19 February - 18 March 1957. The larvae of the second hatch at this altitude appeared as second and third instar larvae in the sample taken on 8 January 1957. These larvae showed a high degree of moulting during the cold period from 8 January - 15 January 1957, 83% moulting from the second to third instars and 29% of the third instars moulting into fourth instars; thus although the lower water temperatures produce lower percentage changes between the whole samples, the transfer

of larvae between different pairs of instars is dependent more on the stage of development reached by these larvae.

Of the 80% second instar larvae which became third instars by the 15 January 1957, 56% had reached the fifth instar by 19 February 1957 which is approximately 25 days to complete two moults. Of the 83% fourth instar larvae which became fifth instars by 19 December 1956, 64% had reached the seventh instar by 19 February 1957, approximately 62 days to complete two moults. The temperatures during these periods are roughly comparable so it would appear that the overall temperature required to produce a given number of moults between the early instars is less than that required to produce the same number of moults between the later instars. If this is true, then under constant temperature conditions the time between moults would gradually increase with the later instars. Examination of the histograms in Fig.17 however shows a general convergence of the time interval during which the moults between particular pairs of instars take place. The larvae developing later in the season, when the water temperatures are generally higher, pass through their moults more quickly than those developing early in the season. The effect of the rising water temperatures during the Spring is thus to compress the long period during which small larvae resulting from an extended period of hatching occurred in the stream into a relatively shorter period when mature larvae are present. This may be further shortened by similar temperature effects on the

development of the pupae so that emergence will occur over a shorter period than hatching. It is probably a similar effect to this which re-establishes the definite Spring generation of the bivoltine and trivoltine species, the generations of which show an increasing spread as the Summer progresses.

It is possible, from the data relating to the transfer of larvae between the pairs of instars, to determine the average thermal sum experienced by the larvae between each moult and thus obtain an estimate of the thermal sum required by the larvae to complete their development. Such data might prove useful in the control of black-flies.

There is some evidence that associated with the quicker development of P.hirtipes larvae during the periods of higher water temperatures there is a reduction in the size attained by the larvae, as indicated by a reduction in their head-capsule widths. This is most evident when the mean head-capsule widths of the known last instar larvae (those with black pupal respiratory filaments) are examined (Table 16). There is a decrease in the mean head-capsule width of these larvae from 5 April 1957 to 4 May 1957 at 180m in Swindale Beck. The last instar larvae on 5 April 1957 are derived from the early larval hatch which have developed over a long period of generally low water temperatures, while the last instar larvae present from 25 April 1957 to 4 May 1957 are derived from larvae of the late hatch and have therefore developed over a shorter period of

TABLE 16 The mean head-capsule widths of the known last instar larvae of P.Kirtipes in Swindale Beck, 1957, at 180m, 300m & 430m

	5 Apr	15 Apr	25 Apr	4 May	15 May
180m	66.5 (10)	65.0 (1)	63.7 (15)	59.2 (33)	no larvae
300m	- (0)	- (0)	- (0)	64.5 (4)	62.5 (13)
430m	- (0)	- (0)	69.0 (1)	no sample	no sample

( ) Number of known last instar larvae

generally higher water temperatures. A similar trend is also evident between 4 May 1957 and 15 May 1957 at 300m in Swindale Beck. Since the thermal sums also decrease with increased altitude it might be expected that the mean head-capsule widths of the last instar larvae will increase with increase in the altitude at which the larvae develop. Only two comparable sets of data are available to show this, on 25 April 1957 at 180m and 430m, and on 4 May 1957 at 180m and 300m. However in both cases the number of larvae are too small to be reliable, but in both cases the larvae at the higher altitudes have the greater mean head-capsule widths. The comparison of the mean head-capsule widths of all the last instar larvae, that is those with a head-capsule width of 58 divisions or greater, is less reliable since it will include a number of seventh instar larvae due to the overlapping of the head-capsule width distributions which is especially marked at times just prior to the main final moulting period. At this time there is an overlap of the seventh instar distribution into the eighthinstar range but no compensating overlap of the eighth instar distribution into the seventh instar range. It would be expected therefore that when the mean head-capsule widths of all last instar larvae are examined, the mean head-capsule width would increase up to the time of the main final moult and thereafter follow the pattern described for the known last instar larvae. The mean head-capsule widths of the last instar larvae at three altitudes in Swindale Beck, 1957,

and at two altitudes in both Crowdundle and Swindale Beck during the years 1954-56, are given in Table 17. The head-capsule width of the last instar larvae again show a tendency to decrease after the main period of moulting into the final instar and to increase with increase in altitude. These differences in the mean head-capsule widths of the larvae developing under different conditions of water temperature correspond with the observations of Smart, 1934; Edwards et al, 1939; Zahar, 1951; and Davies 1957; on the different sizes of the Spring and Summer generation pupae and imagines which were perhaps more marked due to the larger water temperature differences at these times of the year. The variation in the maximum head-capsule widths achieved by the larvae developing under different water temperature regimes does however show an additional source of error when attempting the separation of the larval instars on the basis of the mean head-capsule width.

1.H. Effects of temperature on the Mortality of P.hirtipes larvae and the larvae of two associated black-fly species in laboratory experiments

The restriction of P.inflatum larvae to the head waters of the streams where the water temperatures are permanently cold, and the occurrence of P.hirtipes larvae at lower altitudes only during the Winter and early Spring when

TABLE 17 The mean head-capsule widths of the last instar larvae (above 58 divs.) in Swindale and Crowdundle Becks, 1954-57

Swindale Beck 1957

	8 Mar	23 Mar	5 Apr	15 Apr	25 Apr	4 May	15 May
180m	60.1 (14)	60.5 (15)	63.9 (30)	61.0 (11)	62.3 (68)	60.8 (104)	no larvae
300m	- (0)	59.3 (22)	60.2 (11)	61.2 (49)	60.9 (68)	61.9 (27)	61.1 (43)
430m	59.0 (1)	59.5 (2)	62.7 (7)	63.6 (12)	65.8 (28)	no sample	no sample

Swindale and Crowdundle Becks, 1954-56

	Swindale Beck		Crowdundle Beck	
	15 Apr 54	23 Apr 56	14 Apr 55	23 Apr 56
180m	64.9 (62)	63.9 (104)	67.8 (114)	62.6 (38)
300m	63.5 (15)	64.2 (71)	63.8*(40)	

* 275m ( ) Number of last instar larvae

the water temperatures are again cold, suggests that perhaps the higher water temperatures which occur during the Summer months (Macan, 1958) at the lower altitudes may in some way be detrimental to the development of, or even lethal to, the larvae of both Prosimulium species present in the stream. The larvae of both S.monticola and S.variegatum also form part of the black-fly larval population at the lower altitudes in these streams during the Winter and Spring when they develop at approximately the same rate as the P.hirtipes larvae. Both S.monticola and S.variegatum are however bivoltine, producing Summer generations of larvae which can thrive in these streams during the period of high Summer water temperatures. It was decided therefore to examine the water temperature conditions which normally prevail at the lower altitudes in these streams during the Summer months and to perform Temperature - Mortality experiments on larvae of the black-fly population at these altitudes to try to account for the absence of P.hirtipes larvae from the streams during the Summer months.

1.H.(i) Temperature - Mortality experiments

1.H.(i)(a) Apparatus and experimental procedure

All larvae used in the experiments were obtained from Swindale Beck at 300m where large numbers of larvae were readily available and therefore the time taken to collect the large number of larvae required for the experiments was

minimal. The larvae, after collection, were transported in large thermos jars to prevent any excessive rise in temperature during their transfer to the laboratory. In the laboratory the larvae were transferred to large glass jars of approximately 3 litres capacity which contained aerated pond water. The apparatus was essentially the same as that described by Smart (1934), the good aeration and rapid water current, simulating the natural environmental conditions of the larvae, being provided by a continuous stream of air bubbles travelling up a glass tube (2cms diameter) completely immersed in the water. The jars, after setting up, were left in a constant temperature cold room overnight which maintained a water temperature of 4 - 5°C. The larvae were found to re-attach themselves inside the glass tube and at points near the entrances to the tube where the water current was most rapid. The following morning all dead larvae, which had died as the result of collection or their transport to the laboratory, were removed with a pipette and discarded as transport mortality. The variation of the water temperature in the experimental jars required the removal of some of the jars from the C.T. coldroom to other situations with the consequent interruption of the compressed air supply. Such transfers were completed quickly (10 to 15 seconds) to prevent the detachment of the larvae and each time such a transfer was made the compressed air supply to the appropriate control jar was interrupted for a similar period.

The larvae survived well under the experimental conditions. The larvae in the control jars at 4 - 5°C showed cumulative mortalities of 13.4% and 14.0% after nine days in Experiment 1, and 4.1% and 11.9% after seven days in Experiment 2. The greater part of this mortality however occurred in the first two or three days, probably therefore being a delayed transport mortality, and fell to 0% to 2% per day for most of the period. To test whether the high mortality, which occurred in some experimental jars, was caused by poisoning due to the accumulation of the larval excretory material, the water in Jar 2 (Experiment 2) was replaced after $1\frac{1}{2}$ days by slowly running in 5-jar volumes (15 litres) of clean aerated pond water at the correct temperature. The excess water was syphoned off at the same rate from the bottom of the jar where the faecal material had accumulated. It can be seen from Table 19 that the mortality in Jar 2 was comparable with that in Jar 1 where the water was not changed. It was concluded therefore that the poisoning of the water by faecal material was of minor importance as a mortality factor during the course of these experiments.

The lighting conditions were controlled to simulate as nearly as possible the natural diurnal light cycle. Jars 1 and 2 (Experiment 1), Jars 3 and 4 (Experiment 1) and Jars 1 and 2 (Experiment 2) which were in artificial lighting conditions only received light during the day, while the other jars received

a natural diurnal light cycle. Since the control larvae received only artificial lighting and suffered only a low mortality, the lighting conditions cannot be considered to be of great importance as a mortality factor in these experiments.

Temperature readings were taken in all jars at hourly intervals throughout the day using a mercury thermometer. The dead larvae were removed with a pipette each morning, sorted and counted.

Two experiments were performed, each using 3 pairs of jars, one pair being maintained at 4 - 5°C as a control in each experiment. The remaining 2 pairs of jars in each experiment were subjected to different temperature conditions as indicated in Table 18.

The larvae used in Experiment 1 were obtained on 8 March 1957 and contained fourth to seventh instar larvae of P.hirtipes of which 53% were fifth instar larvae, while the larvae used in Experiment 2 were obtained on 23 March 1957 and contained fourth to final instar larvae of P.hirtipes of which 51% were sixth instar larvae. No data concerning the instar composition of the S.monticola and S.variegatum larvae is available, but it can be assumed that the difference between the development of the larvae on the two dates is approximately the same as that for the P.hirtipes larvae. Each jar contained between 1833 and 2331 larvae, of which 4.8 - 11.9% in Experiment 1 and 9.3 to 11.5% in Experiment 2, were P.hirtipes larvae, all the remaining larvae being either

TABLE 18 The water temperatures of the experimental jars in Experiments 1 and 2

Experiment 1	JAR 1	4 - 5°C Control	Experiment 2	JAR 1	4 - 5°C Control
	JAR 2			JAR 2	
	JAR 3	21-22.5°C in C.T.room		JAR 3	4 -19°C Fluctuating temperature
	JAR 4			JAR 4	
	JAR 5	16-18°C in Laboratory		JAR 5	16-18°C
	JAR 6			JAR 6	

S.monticola or S.variegatum larvae. The dead larvae on removal from the jars were separated into P.hirtipes larvae and Non-hirtipes larvae for recording. Since no distinction was made between the two Simulium species present, for ease of description they will be referred to as the Non-hirtipes larvae during the remainder of this account. When the experiments were terminated all the larvae were removed, and in this case the living larvae were also separated and their numbers recorded. All the data relating to the mortality in both experiments is shown in Tables 19(a) and (b) and Tables (a) and (b) of Appendix 1.

1.H.(i)(b) Results

If the daily percentage mortality of P.hirtipes larvae for each member of a pair of jars kept under identical temperature conditions is compared, they are found to be very similar, and the same was true for the mortality of the Non-hirtipes larvae. The larger variations which did occur were at the beginning of the experiments and were also seen in the control jars where they extended over the first three days. They were probably again due to the residual effects of transferring the larvae to the laboratory. This is to some extent confirmed as these differences were less marked in Experiment 2, when the larval experiments were begun 42 hours after the collection of the larvae, while in Experiment 1, which commenced only 18 hours after the collection of the larvae, a smaller proportion of the larvae suffering transport

TABLE 19(a). Mortalities of Black-fly larvae in laboratory experiments. EXPERIMENT 1.
(Pairs of Jars)

JARS 1 & 2	DATE	9 Mar.	10 Mar.	11 Mar.	12 Mar.	13 Mar.	14 Mar.	18 Mar.
4 - 5°C	TIME	9.10am	9.15am	1.15pm	2.15pm	5.15pm	9.30am	5.15pm
P.hirtipes	D%M	0.0	3.9	4.9	3.6		1.0	0.0
184 larvae	C%M	0.0	3.9	8.8	12.4		13.4	13.4
(7.9%)	%RM	0.0	3.9	5.1	4.0		1.1	0.0
Non-hirtipes	D%M	0.0	2.2	3.2	5.8		0.9	1.9
2147 larvae	C%M	0.0	2.2	5.4	11.2		12.1	14.0
(92.1%)	%RM	0.0	2.2	3.2	6.2		1.5	2.4
Cumulative THERMAL SUM	(degree hrs above 0°C)	0	96	215	321		492	924
JARS 3 & 4								
22 - 22.5°C								
P.hirtipes	D%M	0.0	34.6	51.8	13.6			
280 larvae	C%M	0.0	34.6	86.4	100.0			
(6.5%)	%RM	0.0	34.6	79.2	100.0			
Non-hirtipes	D%M	0.0	37.2	25.0	34.3			
4000 larvae	C%M	0.0	37.2	62.2	96.5			
(93.5%)	%RM	0.0	37.2	39.8	90.8			
Cumulative THERMAL SUM	(degree hrs above 0°C)	0	417	1042	1601			

D%M Daily percentage mortality
C%M Cumulative percentage mortality
%RM Percentage mortality of larvae at risk

TABLE 19(a) (Contd.) Mortalities of Black-fly larvae in laboratory experiments. EXPERIMENT 1.
(Pairs of Jars)

	DATE	9 Mar.	10 Mar.	11 Mar.	12 Mar.	13 Mar.	14 Mar.	18 Mar.
	TIME	9.10am	9.15am	1.15pm	2.15pm	5.15pm	9.30am	5.15pm
JARS 5 & 6								
16 - 18°C								
P.hirtipes	D%M	0.0	11.5	5.9	34.1	46.3		
391 larvae	C%M	0.0	11.5	17.4	51.5	97.8		
(8.5%)	%RM	0.0	11.5	6.6	41.2	90.1		
Non-hirtipes	D%M	0.0	12.6	6.3	30.9	37.8		
4198 larvae	C%M	0.0	12.6	18.9	49.8	87.6		
(91.5%)	%RM	0.0	12.6	7.2	38.0	75.2		
Cumulative THERMAL SUM	(degree hrs above 0°C	0	330	848	1341	1884		

D%M Daily percentage mortality
C%M Cumulative percentage mortality
%RM Percentage mortality of larvae at risk

TABLE 19(b). Mortalities of Black-fly larvae in laboratory experiments. EXPERIMENT 2.

(Pairs of Jars)

	DATE	25 Mar.	26 Mar.	27 Mar.	28 Mar.	29 Mar.	30 Mar.	1 Apr.	2 Apr.
	TIME	10.00am	9.00am	8.55am	9.10am	9.10am	9.10am	9.15am	11.30am
JARS 1 & 2									
4 - 5°C									
P.hirtipes	D%M	0.0	0.9	0.7	1.1	0.7	0.7	0.0	0.0
427 larvae	C%M	0.0	0.9	1.6	2.7	3.4	4.1	4.1	4.1
(10.7%)	%RM	0.0	0.9	0.7	1.2	0.7	0.7	0.0	0.0
Non-hirtipes		0.0	3.2	1.7	2.3	1.5	0.5	1.6	1.1
4084 larvae	C%M	0.0	3.2	4.9	7.2	8.7	9.2	10.8	11.9
(89.3%)	%RM	0.0	3.2	1.8	2.4	1.6	0.5	1.7	1.3
Cumulative (degree hrs THERMAL SUM above 0°C)		0	94	188	287	389	490	695	803
JARS 3 & 4									10.00am
4 - 19°C									
P.hirtipes	D%M	0.0	3.4	1.4	0.2	4.1	19.0	29.0	8.7
415 larvae	C%M	0.0	3.4	4.8	5.0	9.1	28.1	57.1	65.8
(10.0%)	%RM	0.0	3.4	1.5	0.3	4.3	20.1	40.3	20.2
Non-hirtipes	D%M	0.0	5.1	2.2	1.2	6.6	21.0	25.2	8.4
4142 larvae	C%M	0.0	5.1	7.3	8.5	15.1	36.1	61.3	69.7
(90.0%)	%RM	0.0	5.1	2.3	1.3	7.3	24.8	39.5	21.7
Cumulative (degree hrs THERMAL SUM above 0°C)		0	195	405	662	918	1162	1385	1498

D%M Daily percentage mortality
C%M Cumulative percentage mortality
%RM Percentage mortality of larvae at risk

TABLE 19(b) (Contd.) Mortalities of Black-fly larvae in laboratory experiments. EXPERIMENT 2.

(Pairs of Jars)

	DATE	25 Mar.	26 Mar.	27 Mar.	28 Mar.	29 Mar.
JARS 5 & 6	TIME	10.00am	9.00am	8.55am	11.00am	
16 - 18°C						
P.hirtipes	D%M	0.0	5.7	29.1	46.2	17.9
440 larvae	C%M	0.0	5.7	34.8	81.0	98.9
(10.7%)	%RM	0.0	5.7	30.8	70.7	94.0
Non-hirtipes	D%M	0.0	7.6	21.6	44.7	19.1
4112 larvae	C%M	0.0	7.6	29.2	73.9	93.0
(89.3%)	%RM	0.0	7.6	23.2	62.9	72.9
Cumulative (degree hrs THERMAL SUM above 0°C)		0	362	786	1188	1648

D%M Daily percentage mortality
C%M Cumulative percentage mortality
%RM Percentage mortality of larvae at risk

mortality will have been removed. The differences in the percentage mortalities of the larvae in the pairs of jars also tended to be higher in those jars where high mortality rates occurred. Since the differences in the mortalities of the larvae in each member of a pair of jars is relatively small, the results for pairs of jars have been combined and are given in Tables 19(a) and 19(b). All the relevant data for the individual jars is given in Tables (a) and (b) of Appendix 1.

The cumulative thermal sums for each jar were calculated to the nearest degree hour above $0^{\circ}C$ for each jar but since the variation between the members of the respective pairs of jars was very small, they are given in Tables 19(a) and (b) as the average cumulate thermal sum for each pair of jars. Where the larvae were not maintained in constant temperature rooms the calculation of the thermal sum required the interpolation of the night temperatures. In jars 5 & 6 of both Experiments 1 and 2 the overall temperature variation was only $2^{\circ}C$. So any errors in the calculation will be quite small. The determination of the thermal sums for jars 3 and 4 of Experiment 2 was more difficult since part of the cooling during each temperature fluctuation occurred at night. These calculations were therefore done graphically and by calculating the cooling curves from the initial rates of cooling and interpolating the results, and the thermal sum was determined from the area under the curves produced.

The delayed transport mortality which occurred especially in the control jars 1 and 2 of Experiment 1, and was also apparent in the other experimental jars of Experiment 1 and to a lesser extent in Experiment 2, will be of the same proportions in all the jars of each experiment, and therefore its presence will not invalidate any conclusions which may be drawn from comparisons of the percentage larval mortality, between the different pairs of jars within each experiment. Care must however be taken when comparing the results of Experiment 1 with those of Experiment 2, not only because of the difference in the delayed transport mortality, but also because of the different instar compositions of the larval populations used.

It is apparent from these experiments that continuous temperatures of both 16 - 18°C and 21 - 22.5°C will cause 100% mortality of both the P.hirtipes and Non-hirtipes larvae within 3 - 5 days, but both temperatures lie beneath the thermal death point for the larvae. The continuous temperature of 4 - 5°C in the controls of both experiments showed a very low mortality in the later periods, showing that this temperature was suitable for the completion of larval development. The critical temperature, that is the temperature above which 100% mortality of the larvae will occur before development is complete, must lie between 5 - 16°C. Above this temperature the mortality rate of the larvae will increase with increase in temperature,

therefore as expected the mortality rate was higher at 21 - 22.5°C than at 16 - 18°C, the mortality rate at the higher temperature being approximately 2 x that at 16 - 18°C as is shown by the times taken to reach 50% larval mortality (Table 20). An accurate determination of the critical temperatures cannot be made on the basis of the present experiments since the only constant temperatures at which the larvae were maintained were above the critical temperature.

The relationships between the mortality of the larvae and the water temperatures to which they were exposed is shown in Figs. 20 and 21. The mortalities are shown as a percentage mortality of the larvae at risk, which compensates for the apparent fall in the percentage mortality during each period especially as 100% mortality is approached. At 21 - 22.5°C the mortality of P.hirtipes larvae occurs at a constant rate throughout the experiment, falling only slightly during the last period, probably due to the achievement of 100% mortality before the dead larvae were removed. At 16 - 18°C the initial mortality of P.hirtipes larvae is low during the first day in both experiments but rises during the third and fourth days in Experiment 1, to a rate of mortality similar to that seen at 21 - 22.5°C. In Experiment 2 this increased mortality begins on the second day. This delay in the mortality at 16 - 18°C may in part be due to the time taken for the water temperature to rise from 4 - 5°C at the beginning of the experiment to 17°C which took approximately 8 hours in both experiments, but the

TABLE 20 Time (Hours) taken to achieve 50% larval mortality in Experiment 1

	Temperature	P.hirtipes larvae	Non-hirtipes larvae
JARS 5 & 6	16-18°C	76hrs (963)	77hrs (1075)
JARS 3 & 4	21-22.5°C	32hrs (603)	38hrs (831)

( ) Cumulative thermal sum in degree hrs above 0°C

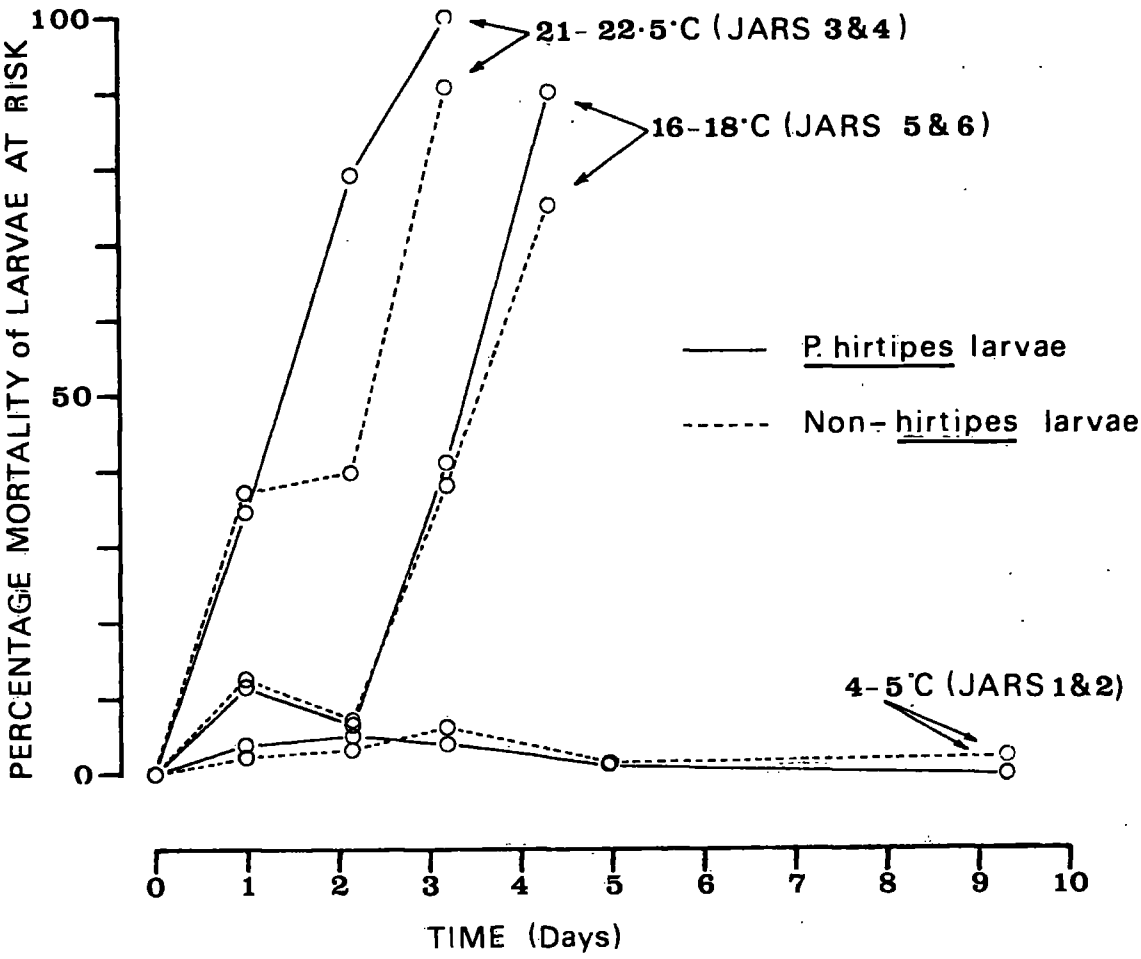


FIG. 20. LARVAL MORTALITY CURVES OF EXPERIMENT 1. (RESULTS for PAIRS of JARS are Combined.)

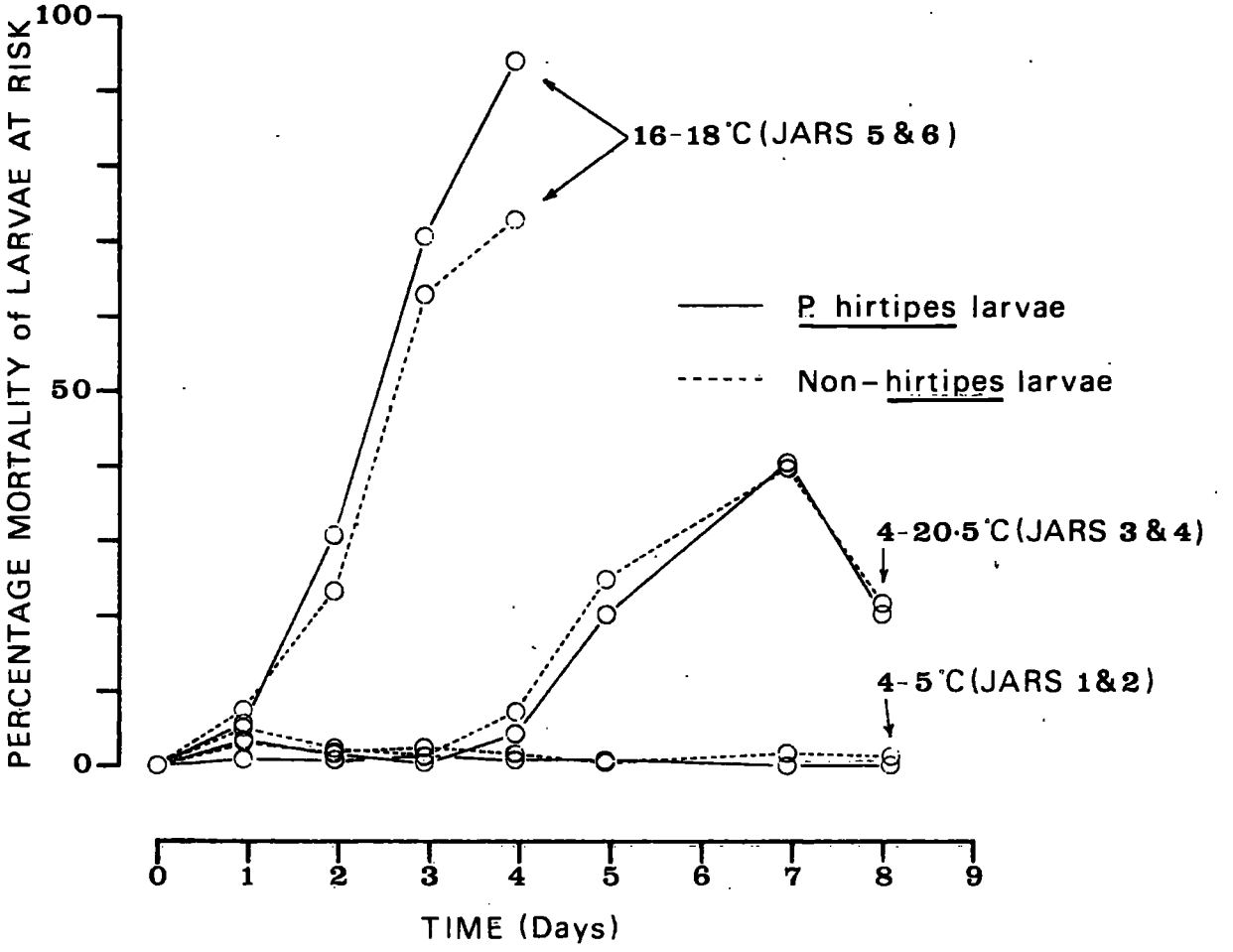


FIG. 21. LARVAL MORTALITY CURVES OF EXPERIMENT 2. (RESULTS for PAIRS of JARS are Combined.)

delay of the mortality on the second day in Experiment 1 at this temperature cannot be due, in any way, to the time taken for the water temperature to rise. At this temperature therefore there is evidence of a delay of one to two days before the temperature has a marked effect on the mortality of the larvae. Bearing in mind the delayed transport mortality of the larvae in the control jars, the early mortality of the larvae could well have been lower. The presence of a delayed transport mortality in the larvae maintained at 16 - 18°C is indicated in Experiment 1 where the percentage mortality of the larvae at risk fell during the second day.

When the larvae were subjected to a water temperature fluctuating between 4 - 20.5°C, having maximum temperatures of 18.5°C, 19.0°C, 20.0°C, 20.5°C and 19.9°C during the first five days before being kept at 4 - 5°C for the remainder of the experiment, the mortality of the larvae remained at a very low level during the first three days, only showing a marked increase in mortality after the fifth fluctuation. This shows clearly that the effects of harmful temperatures may not be immediate but are accumulative as is shown by the continued higher rate of mortality during the last three days of the experiment when the water temperature was at 4 - 5°C, a level which would normally produce only a very low mortality.

At temperatures below 21 - 22.5°C an accumulative effect of temperature can be seen. This can be demonstrated more clearly when the data is plotted graphically (Fig.22),

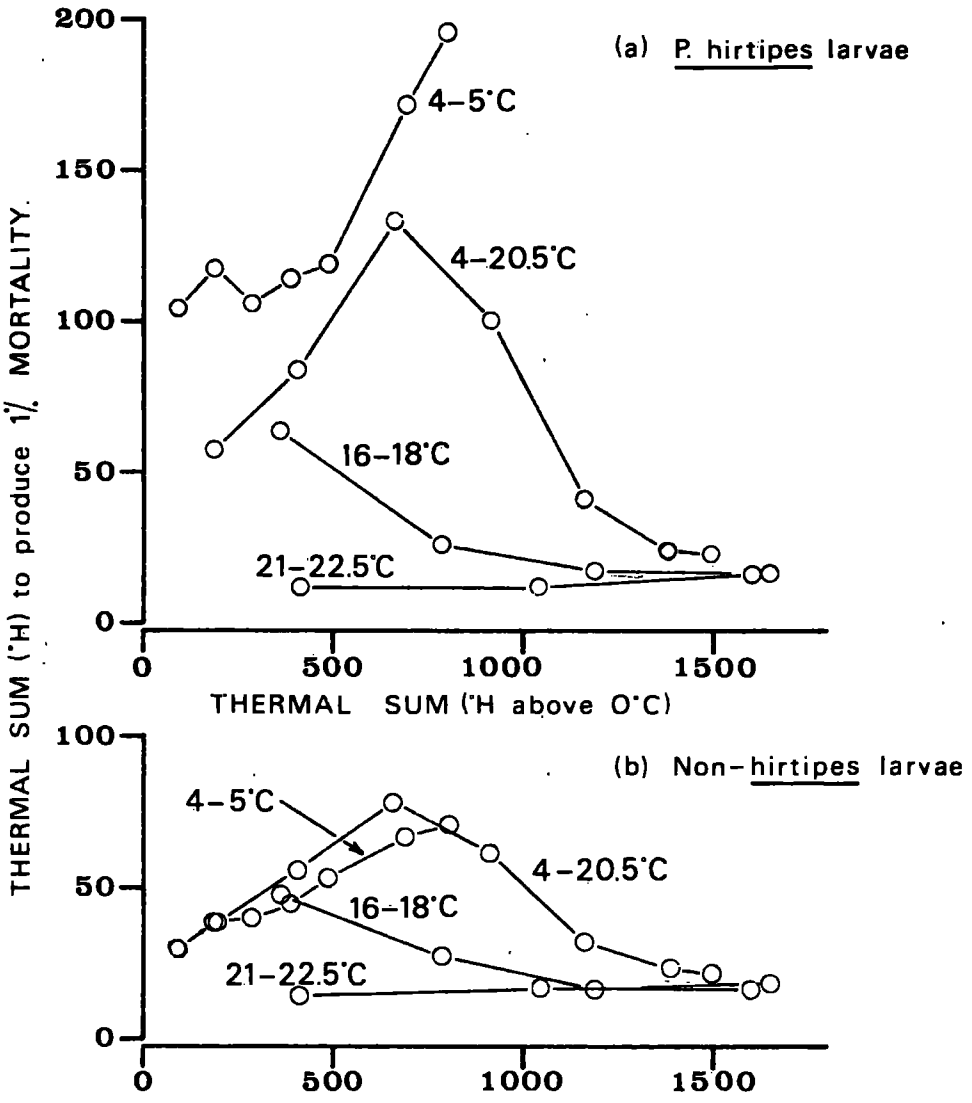


FIG. 22. CURVES RELATING LARVAL MORTALITY TO THE THERMAL SUM, WHEN SUBJECT TO DIFFERENT WATER TEMPERATURES.

the thermal sum to produce 1% Mortality of the larvae being plotted on the X axis and the cumulative thermal sum being plotted on the Y axis. If the temperature has an accumulative effect there will be a decrease in the thermal sum required to produce 1% mortality as the cumulative thermal sum increases, that is, the graph will show a downward slope from left to right. When the graph is parallel to the Y axis the mortality is proportional to the thermal sum and no accumulative effect is evident. At 21 - 22.5°C the mortality of the larvae is proportional to the thermal sum and no accumulative effect is evident, while at 16 - 18°C the temperature becomes more effective in producing mortality of the larvae until by the third day it is almost proportional to the thermal sum, indicating a cumulative effect during the first two days. At 4 - 5°C, after a low mortality during the first three days, the mortality decreases despite the increase in the thermal sum and no accumulative effect occurs. Where the temperature was allowed to fluctuate (4 - 20.5°C) above and below the critical temperature, the graph is similar to that at 4 - 5°C for the first three days. However between the third and fourth days the mortality increases and an accumulative effect can be seen, and even after return to the cooler water temperatures the mortality remained proportional to the thermal sum although at a slightly lower level than at the higher constant temperatures. Although differences do occur in the relationship between the larval mortality and

and the thermal sum for the P.hirtipes and Non-hirtipes larvae, especially in the control jars at 4 - 5°C, where the difference was mainly due to the difference in the initial larval mortalities, the general relationships between the larval mortality and the thermal sum is the same for both P. hirtipes and Non-hirtipes larvae.

We can conclude, from the previous data, that temperatures above 16 - 18°C are too high, if applied continuously to allow any of the larvae to complete their development, and temperatures of 16 - 20.5°C, even when applied for short periods, 2.25hrs, 3hrs, 5.25hrs, 5.5hrs and 5hrs, as they were in Experiment 2, have cumulative effects which cause a marked mortality, perhaps being sufficient to prevent completion of development by the larvae. The critical temperature must therefore lie below 16°C but above the average maximum temperature which they normally experience during the completion of their development during the Spring.

Since both S.monticola and S.variegatum are both bivoltine, their larvae of the Summer generation will experience much higher stream temperatures than those of the Winter generation. The maximum temperatures in several north Pennine streams frequently exceed 15°C during late June, July and August (Table 22). The larvae of P.hirtipes however only occur during the Autumn and Winter when the water temperatures are considerably lower, the maximum temperature at 150m in Swindale Beck only exceeding 13.0°C on three occasions towards

the end of their development period. Since the Summer generation larvae of S.monticola and S.variegatum must be adapted to the higher Summer stream temperatures, we might expect to be able to detect some difference in the effects of high water temperatures on the mortality of P.hirtipes larvae as compared with the Non-hirtipes larvae, which would then provide an explanation for the absence of a Summer generation in P.hirtipes.

The daily percentage mortality for both P.hirtipes and Non-hirtipes larvae correspond well at the lower temperatures of the control jars; however at the higher temperatures differences can be seen. In all the experimental jars maintained at 16 - 18°C and 21 - 22.5°C the cumulative percentage mortality was greater for the P.hirtipes larvae, that is, the effects of the high water temperatures tended to produce 100% mortality of P.hirtipes larvae sooner than for the Non-hirtipes larvae. In jars 3 and 4 of Experiment 1 where the temperature range was 21 - 22.5°C there was also a marked difference between the mortalities of the P.hirtipes and Non-hirtipes larvae on the second day as can be seen by examination of the percentage mortality of the larvae at risk for these jars (Table 19(a). This again shows that the higher temperatures are less rapidly effective in causing the mortality of Non-hirtipes larvae and it may be that at this temperature there is a delayed temperature effect, as at the lower temperatures, on the first day, which is obscured by a high

transport mortality. This may also be true for the P.hirtipes larvae, but the delay does not extend into the second day.

The differences in the mortalities of the P.hirtipes and Non-hirtipes larvae was less clearly defined in the jars maintained at 16 - 18°C, but at this temperature in both Experiments 1 and 2 the percentage mortality during the second and third days was consistently lower for the Non-hirtipes larvae (Table 21). This again indicates that the higher temperatures take longer to become effective on the Non-hirtipes larvae. This effect may have been more marked were it not for the generally higher mortalities of the Non-hirtipes larvae under the experimental conditions as is shown by the mortalities of these larvae in the control jars.

In jars 3 and 4 of Experiment 2, where the larvae were subjected to fluctuating temperatures, it might be expected that, because of the apparent delay in the effectiveness of the high temperatures on the Non-hirtipes larvae, the accumulative effect of the temperature would occur later or cause a lower percentage mortality. This was not the case; the cumulative effect of temperature occurred sooner for the Non-hirtipes larvae and caused a slightly higher percentage mortality.

We may conclude from these experiments that constant water temperatures of 16 - 18°C will cause a high mortality of

TABLE 21 Sum of Percentage Mortalities on Days 2 and 3

		P.hirtipes larvae	Non-*hirtipes* larvae
EXPT. 1	JAR 5	35.0	31.1
(16-18°C)	JAR 6	45.3	43.2
EXPT.2	JAR 5	78.1	63.0
(16-18°C)	JAR 6	73.1	68.6

both P.hirtipes and Non-hirtipes larvae, the rate of mortality increasing with the time of exposure to the temperature. This water temperature range is however slightly less effective in producing mortality of the Non-hirtipes larvae and its effects are more delayed. At constant water temperatures of 21 - 22.5°C the effects of temperature are immediate and do not increase with the time of exposure to the temperature although here again there is some evidence that it is less effective and its effects may be more delayed in the case of the Non-hirtipes larvae. If temperatures in excess of 16°C, even if given for short periods, they will increase the percentage mortality of the larvae and their effect is cumulative. There is no evidence however to suggest that P.hirtipes larvae are any more susceptible to these fluctuations of high temperatures than the Non-hirtipes larvae. The critical temperature above which mortality shows a marked increase is not precisely known but it probably lies below 16°C for both P.hirtipes and Non-hirtipes larvae, and the evidence for these two types of larvae having different critical temperatures is contradictory, although the greater weight of evidence suggests that the critical temperature may be slightly higher for the Non-hirtipes larvae.

1.I. Summer Stream Temperatures in North Pennine Streams

It would seem from the evidence of the larval temperature-mortality experiments that a water temperature of 15°C would be sufficient to prevent the completion of the larval development, and it is of interest therefore to examine the water temperature data which is available for Swindale Beck and other streams in the locality during the months from May to September when P.hirtipes larvae are not present in the stream.

The thermographs at 150m and 400m in Swindale Beck continued to operate until 5 June 1957. At 150m, in the period after 5 May, when the last larvae occurred in the stream until 5 June, the water temperatures remained high, water temperatures in excess of 15°C occurring on 1, 2 and 3 June being 15.9°, 15.0° and 16.4°C respectively. Similar high maximum temperatures occurred at 400m on 28 May, 31 May, 1 June and 3 June being 15.0°, 15.3°, 15.8° and 16.8°C respectively. It is interesting to note that the maximum water temperature was higher at 400m than at 150m. This occurred on a sunny day following a period of consistently high water temperatures. Swindale Beck is exposed to the south-west and is therefore warmed by the sun for most of the day, especially at 400m where no trees are present to provide shade. For 3Km, from 280m - 180m, the stream passes through a shallow tree-lined gorge, the trees being

in leaf from mid-May until Autumn, thus providing shade during these months. This shading reduced the heating effects of the sun sufficiently to prevent the maximum temperature at 180m attaining a value as high as that at 400m. A similar effect has been described by Macan (1958) for a similar type of stream rising in open moorland and then flowing down a wooded slope. He noted a fall in temperature from 21.6°C before the stream entered the wooded section to a minimum of 14.0°C in the part shaded by the woodland. He pointed out however that the cooling effect of shading only occurs in warm sunny weather conditions while in periods of high water levels after rainfall such drops in temperature are greatly reduced. Since June, July and August are periods of sustained high temperatures, these inversions of the temperature maxima between the open higher altitudes and the shaded lower section of the stream are probably not uncommon during periods of fine weather. The thermal sums however remained higher at 150m (330.4° Hours) than at 400m (312.4° Hours) on 3 June. Since these high maximum temperatures were achieved before the period of high Summer temperatures, recognised by Macan (1958) to occur during July and August in most years, there seems little doubt that during most periods of fine weather during these months temperatures of 15°C and above will occur frequently over the altitudinal range of P.hirtipes larvae in Swindale Beck. It may also be assumed that the thermal sums at the higher

unshaded altitudes will show less divergence from those at the lower shaded altitudes during these periods and may on occasions even be greater.

Thermograph data is also available for Cross Gill at 380m in which P.hirtipes larvae are known to occur and for Moss Burn at 605m, a smaller stream from which P.hirtipes larvae are absent. This data relates to the Summer water temperatures from 28 June - 5 September 1951 for Cross Gill and from 15 May - 30 September 1953 for Moss Burn. Both streams drain the eastern slope of the Alston Block, having gradients of 1 in 15-30. These lesser gradients may enable these streams to warm more quickly than Swindale and Crowdundle Becks where the gradients reach 1 in 1.7. However their north-easterly exposure may to some extent compensate for this. During these periods of recording, maximum temperatures of 15°C or greater occurred on 38 of the 90 days of complete record in Cross Gill and on 30 of the 155 days of complete record in Moss Burn.

Mercury thermometer readings are also available for Cross Gill, Moss Burn and Trout Beck, a much larger stream 3-6m in width (Table 22).

Water temperatures of 15°C and above are not uncommon in the North Pennine Streams and in the smaller streams, even at relatively high altitudes, they may reach levels at which rapid mortality of all three species of black-fly larvae would occur. The maximum temperature in

TABLE 22 Summer thermometer readings in North Pennine Streams

	Altitude(m)		No. of Readings	Readings exceeding 14.9°C
Moss Burn	605	14May-23Sept 1952	75	21
Cross Gill	300	22Jun-11July 1955	19	16
Cross Gill	300	4-18July 1956	14	2
Trout Beck	600	9-15July 1956	5	5

Moss Burn on 12 successive days from 23 June to 4 July 1952 ranged from 20.3°C to 25.2°C and maximum temperatures of 20°C were again achieved on 7 and 8 August in the same year. There is also evidence that these temperatures occur regularly from year to year.

There is thus evidence that the Summer stream temperatures are unsuitable for the development of P.hirtipes larvae. However from the results of the larval temperature experiments it would appear that these temperatures are also detrimental to the development of S.monticola and S.variegatum larvae. If any difference does exist between the critical temperatures for P.hirtipes and Non-hirtipes larvae, it is small, allowing the larvae of S.monticola and S.variegatum to develop in only slightly warmer water temperatures which may explain the restriction of these species to the larger hill streams at low altitudes. The Summer generation larvae of S.monticola and S.variegatum may well have higher temperature tolerances than the Winter larvae of the same species, due perhaps to acclimatization effects, i.e. under natural conditions the slower rises in water temperatures than those used in the laboratory experiments, might give the larvae scope for acclimatization, so that instead of 18°C causing considerable mortality, 22°C might be needed to have the same effect.

1.J. Discussion

Evidence has been given that in both Swindale and Crowdundle Becks during the period from 1954-57 the larval development period of P.hirtipes is progressively extended as the altitude increases and is longest near the normal upper limit of the altitudinal range for this species at 430m. Pupation at this altitude was between 2-4 weeks later than at 180m which was the lowest altitude studied so that development near the upper limit of the altitudinal range cannot be completed before mid-May, while at the lower altitudes it is completed in early May. The time of hatching is less well demarcated since first instar larvae were not found in the samples from 1954-57. Most of the hatching at 300m however occurred earlier than at 180m in Swindale Beck, 1956-57, since the mean head-capsule widths of the larval samples were for a period during the Winter, larger at 300m than at 180m. The larval samples at 430m were very small in the early season from 22 November 1956 - 8 January 1957 so there is little evidence to indicate the time of hatching at this altitude. However in the samples obtained on 15 January 1957 one seventh instar larva was obtained at 430m while the largest larvae in the samples obtained from 180m and 300m were 6th instar larvae, also the first last-instar larvae obtained in the samples were collected on 8 March 1957 at both 180m and 430m despite the observed slower rate of development

at the higher altitude. This would seem to indicate that hatching of the larvae occurred on average earlier at the higher altitudes, thus compensating to some extent for the slower rate of development.

The thermal sum for January to May 1957 was substantially greater at 150m than at 400m and this difference was consistent throughout the period. From the evidence of other temperature readings along the length of the streams it may be assumed that a decrease of thermal sum with increasing altitude was the norm, at least during the Winter and early Spring. It is suggested that it is the thermal sum or overall temperature experienced by the larvae which is responsible for the increasing time taken to complete larval development as the altitude increases.

The evidence from the percentage change between the instar composition of the successive larval samples obtained from Swindale Beck, 1956-57, and its relation to the thermal sum experienced by the larvae between the sampling dates, shows a very close relationship between the overall temperature experienced by the larvae and their rate of development. This is especially true at 180m where corrections could be made to offset the anomalies created by the continued addition of small larvae to the samples, as a result of an extended hatching period. The existence of a significant correlation between the rate of development of the larvae and the thermal sum they

experienced indicates that the rate of development is, at least in part, directly related to the thermal sum, although it may also be effective indirectly through other factors such as the dissolved oxygen concentration or the availability of food organisms. It is possible that the changes in the instar composition of the samples collected from Swindale Beck, 1956-57, at 180m, 300m and 430m, which gave the appearance of a higher rate of development at the lower altitudes, could have been caused by the steady washing down of the larger larvae from the higher to the lower altitudes. Allowing for the changes which occurred at the times of hatching and pupation, the instar composition of the samples were consistent, with the steady movement to the higher instars and no marked reduction of the larger instar larvae in the samples at the higher altitudes was noted except during times of pupation.

The increase in the length of the development period of P.hirtipes with increasing altitude, due to the lower thermal sum at the higher altitudes, is supported by the observations on the development of P.inflatum larvae for which the development period is about eight months in the much colder temperature regime of the stream at altitudes above 660m.

The annual cycle of P.hirtipes breeding in the lower parts of the North Pennine streams appears to be adapted so that the larvae only occur in the streams from October to May when the water temperatures are low. Over their altitudinal range development of the larvae is completed by mid-May before the

occurrence of the high maximum water temperatures. It is suggested, on the evidence from the larval temperature - mortality experiments, that water temperatures of 15°C or above will cause high larval mortality and that the effect of these high water temperatures is accumulative. Evidence has been presented that maximum water temperatures of 15°C or above occurred in late May and early June at 150m and 400m in Swindale Beck, 1957, and that maximum water temperatures of a similar order will frequently be achieved in periods of fine weather from July to August at these altitudes. These high maximum water temperatures during the late Spring and Summer would therefore be detrimental to the development of any P.hirtipes larvae present at these times. The long egg stage from June to October may therefore be a method of surviving this period of unfavourably high water temperatures. The upper limit of the altitudinal range of P.hirtipes larvae is that altitude at which they can complete their larval development before the onset of the high maximum water temperatures.

There is no evidence however that the thermal sum and the occurrence of high maximum water temperatures in late Spring directly control the altitudinal distribution of P.hirtipes larvae in the streams, the initial distribution will be largely determined by the spatial distribution of the eggs, the adult female flies presumably tending to confine

their oviposition to altitudes below 450m where the water temperatures are generally favourable for the development of the larvae.

It is suggested that any P.hirtipes larvae present in the streams above 450m will not be able to complete their development before the advent of the high maximum water temperatures in late Spring due to the slower rate of development possible in the lower water temperatures. This would then result in high larval mortalities and failure to complete their annual cycle. At still higher altitudes a point will be reached where the maximum Summer water temperatures seldom reach critical values of 15°C or above, and their accumulative effect is insufficient to prevent the completion of larval development. The water temperatures at these altitudes are therefore suitable for the development of P.hirtipes larvae but the lengthening of the larval development period, due to the lower water temperatures, coupled with the long egg stage of this species, would not enable the completion of the annual cycle at these altitudes. It is likely, but not proven, that the larvae of P.inflatum are sensitive to the relatively high water temperatures as has been shown for P.hirtipes larvae. It is therefore suggested that the larvae of P.inflatum occupy this temperature regime in the head-waters of the stream above 660m, the completion of their annual cycle being facilitated by a short egg stage. The absence of a long egg stage in P.inflatum would conversely restrict the lower altitudinal distribution of

the larvae of this species to those altitudes above which the water temperature does not become critical during the Summer months. Although no evidence has been presented for the Summer temperatures in the streams at the higher altitudes, it is unlikely that the water temperatures often exceed 15°C during the Summer months as most of the water at these times is derived from the colder ground waters and less from surface drainage which was shown by Macan (1958) to be responsible for the high maximum temperatures when it occurred during periods of fine weather.

From this evidence it would appear that the overall water temperature conditions prevailing during the development periods and the occurrence of high maximum water temperatures during the late Spring and Summer at the lower altitudes plays an important part in the sequential displacement of P.inflatum by P.hirtipes as the altitude decreases in several North Pennine streams. A similar sequential displacement of Prosimulium species has been recorded for streams in northern Norway (Davies, 1951, 54) where P.ursinum Edwards occupied a position at the higher altitudes in the streams, similar to that of P.inflatum, being displaced at the lower altitudes by P.hirtipes.

Since the larvae of the other black-fly species present in the streams were not the subject of a detailed study little can be said concerning their altitudinal distribution and seasonal development. However the larval temperature - mortality experiments indicated that the water temperature

becomes critical for the Winter generation larvae of both S.monticola and S.variegatum at approximately the same values as for P.hirtipes larvae, although there were some indications that the Non-hirtipes larvae could withstand the effects of high water temperatures for slightly longer periods. There is insufficient evidence however to say that these differences are large enough to explain the presence of both S.monticola and S.variegatum larvae in the stream during the period of apparently harmful water temperatures during the Summer months. It may be that the larvae of the Summer generations of these species are physiologically adapted to the higher water temperatures occurring during the Summer, possibly by acclimatization. If this is so, perhaps the Prosimulium species are univoltine because they have genetically been unable to produce larvae able to acclimatize to the Summer temperatures.

Section 2

The effects of temperature on the activity of the imagines of Simulium ornatum Meigen.

2.A. Biology of S.ornatum

S.ornatum Meig. is the commonest and most widespread of the British Simuliidae, the larvae and pupae being found in almost all lowland water courses with a moderate or slow water current, especially where there is trailing vegetation. The larvae are present throughout the year, the overwintering larvae producing adults from February to April. Further generations of adult flies emerge throughout the Summer and Autumn, the generations overlapping to a large extent. Pupae are present until late October in most years. The adult female fly bites cattle and to a lesser extent horses, chiefly biting the belly and inner side of the thigh. They are also known to bite other domestic mammals and man. The object of the present study, made during the Summers of 1956 and 1957, was to investigate the relationship between the air temperature and the activity of the adult flies under controlled laboratory conditions, and so to determine the optimal temperature range for activity.

2.B. The Collection and Storage of the imagines of S.ornatum

Pupae of S.ornatum were collected from a small stream at Sherburn, 5Km to the east of Durham City, Co. Durham. The

pupae were removed from the stream while still attached to the trailing vegetation on which they had pupated. In the laboratory the pupae were placed in a shaded glass trough at approximately 17°C until the adult flies emerged. The adult flies were then transferred to 2 inch x 1 inch glass longevity tubes similar to those described by Davies, D.M. (1953) and kept in a shaded moist trough. The entrances of the longevity tubes were covered with a narrow nylon mesh and secured by a rubber band. Each tube contained a sugar cube on which the adult flies could feed and a strip of filter paper on which the flies could rest. No more than five flies were placed in each tube and the sexes were kept separate. The day of emergence for all flies was noted so that the ages of the flies could be determined when required. The mortality of the adult flies in these tubes was very low during the first eight days which was the longest period of storage of the flies prior to their use in the temperature experiments.

2.C. The temperature - activity experiments

2.C.(i) Method of measuring activity

The experimental procedure used was similar to that used by Nicholson A.J. (1934) to investigate the influence of temperature on the activity of Sheep Blowflies. The activity of the flies was based on direct observations of the flies under

different temperature conditions. Five easily recognisable types of activity were recorded as follows :

1. COMA : The flies usually lying on their backs or hanging from the paper or cork by one or more legs.
2. REST : There is no movement of the legs or body but the fly is in a normal resting position.
3. "MOVEMENT": This is confined to movements other than locomotory movements, such as cleaning, feeding and small movements of the legs and body.
4. CRAWLING : Self-explanatory.
5. FLIGHT : Self-explanatory.

Any or all of these different types of activity may occur at the same time in tubes containing several flies and they can change quite rapidly from one form of activity to another. The recording of each type of activity must therefore be made at a glance. Since the activity is always in a state of flux and there may be large differences in the activities of individual flies, large numbers of observations are required to give a reliable estimate of the activity. Estimates of flight activity being more difficult to estimate were made at different times from the other observations, by counting the number of individual flights made by the flies in each tube separately during one-minute observation periods.

Observations were made at different times on tubes containing one, six and ten flies per tube. With ten flies per tube, bursts of activity were particularly noticeable, while with only one fly per tube the activity was greatly reduced and the differences between the individual flies were more marked. A record was also kept of the numbers of flies resting on the corks of the experimental containers since Nicholson had shown that their proportion provided a good indication of the suitability of the temperature in the case of Sheep-blowflies.

The effect of temperature was tested, firstly by using a series of constant temperatures at which separate sets of observations were made, and secondly by using a steadily rising temperature and making observations at regular intervals as the temperature rises.

2.C.(ii) Constant Temperature Experiments

The groups of flies on which observations were to be made were placed in 3 inch x 1 inch glass tubes which contained a 2 inch x 1 inch strip of filter paper on which the flies could rest. The tubes were closed with corks which were pierced centrally by a 4mm bore glass tube approximately 5cm in length which was sealed at the upper end and blocked at the lower end which just projected beyond the cork, by cotton wool. These tubes contained a dilute glucose solution and acted as a source of nutriment for the adult flies.

The cork was also pierced eccentrically by a hole of similar dimensions, blocked with dry cotton wool to facilitate a limited exchange of gases with the external atmosphere.

Constant temperatures of 17°, 20°, 25°, 30° and 35°C were maintained using a constant temperature water bath. This consisted of a rectangular glass trough, 20 inches x 11 inches x 11 inches deep, filled with water. The heating was provided by a 2 Kilowatt electric kettle element controlled thermostatically which maintained the water temperature to within ± 0.1°C at 20°C. The water in the bath was circulated by an electric stirrer running at low speed to prevent agitation of the tubes which would stimulate activity of the flies. Two thermometers were immersed in the water at either end of the water bath, adjacent to the tubes of flies, to check the water temperature. The constant temperature of 17°C was maintained by the judicious use of ice during the observation period, the heating element having been disconnected at this temperature as it was very close to the laboratory temperature. The experimental tubes containing the flies were held in position by a 1/3 inch thick perspex strip clamped across the top of the water bath. The glass feeding tubes were inserted into holes in the perspex strip and held in position by the upthrust of the water. The level of water in the water bath just exceeded the lower surfaces of the corks in the experimental tubes. The apparatus was set up and run for one day at 20°C, some of

the glass feeding tubes being replaced by thermometers and the remaining tubes containing Potassium thiocyanate papers (Solomon, M.E., 1945). The air temperature in the experimental tubes remained within $\pm$ 0.1°C of the water temperature and the relative humidity between 95 - 100%. The high humidity is maintained partly by the dilute glucose solution in the feeding tube and partly by the close proximity of a large volume of water. It seems fair to assume that this high level of humidity will be maintained at all experimental temperatures. The water bath was situated on a bench in the laboratory, the illumination coming from a north facing window, so the flies obtained a normal diurnal light cycle and were not exposed to direct sunlight. The constant temperature of 10°C was maintained using a constant temperature cold room in which the illumination was provided by an electric light which was left on from 9.00hrs to 18.30hrs to simulate the natural diurnal light cycle. The constant temperature cold room was not however designed to operate efficiently at this rather high temperature so temperature readings were made of the air temperature near to the experimental tubes at the beginning and end of each set of observations, the average of the two readings then being taken as the temperature at which the observations were made. The variation recorded was from 8.8°C to 12.0°C.

In the experiments where the experimental tubes contained 6 or 10 flies, observations were made at 15 minute

intervals between 9.30hrs and 17.30hrs except for a break of one hour between 12.30hrs and 13.30hrs. Observations were made on sets of ten tubes, so in the case of 6 flies per tube 1800 observations on individual flies were made at each temperature and 3000 observations on individual flies at the same temperatures using 10 flies per tube. Where only 1 fly per tube was used, the observations were made at 10-minute intervals so only 420 observations were made on individual flies during one day. To compensate for this the experiments were repeated, making totals of 840 observations on individual flies. Each set of experimental tubes consisted of equal numbers of tubes containing either male or female flies, but occasionally one or more tubes contained mixed male and female flies depending on the availability of the different sexes. However since there was no marked difference in the activities of the male and female flies the observations for the sexes have been grouped together. Different sets of flies were used for each set of observations at one temperature and only flies from two to six days old were used, except in the experiment using one fly per tube when the ages ranged from 1 to 8 days. The sets of flies were placed in the water bath on the evening before observations were to be made so that all flies had a period of 15hrs in which to acclimatize to the temperature. This however caused a high mortality at 35°C.

Only one constant-temperature water bath was used and in consequence the experiments were spread over a long period from September to October in 1956 and from July to August in 1957. The illumination and the flies themselves will therefore be subject to some seasonal variation.

When the results were analysed the flies in a coma were discounted. The number of flies affected in this way at the lower temperatures from 10°C to 25°C was very small, but at both 30°C and 35°C the mortality rate was high. When two flies in a tube containing 6 flies, or 3 flies in a tube containing 10 flies were in a coma or died during the course of observations the results for this tube were discounted since it was shown that the number of flies in each tube has a marked effect on the level of activity (Fig.23). The numbers of observations on which the results for 30°C and 35°C are based is consequently reduced, especially at 35°C for 6 flies per tube, and no results are available at this temperature for 10 flies per tube. The observations made under the fluctuating temperature (8.8°C to 12°C) have been grouped in 1°C intervals so that here again the number of observations on which the results are based has again been reduced, 50% of the observations being at 11°C and 25% at 10°C.

The experiment using only one fly per tube was designed to test the effect of ageing on the activity of black-flies. Only female flies were used and separate sets of flies from 1 day to 8 days old were maintained at 20°C for observations.

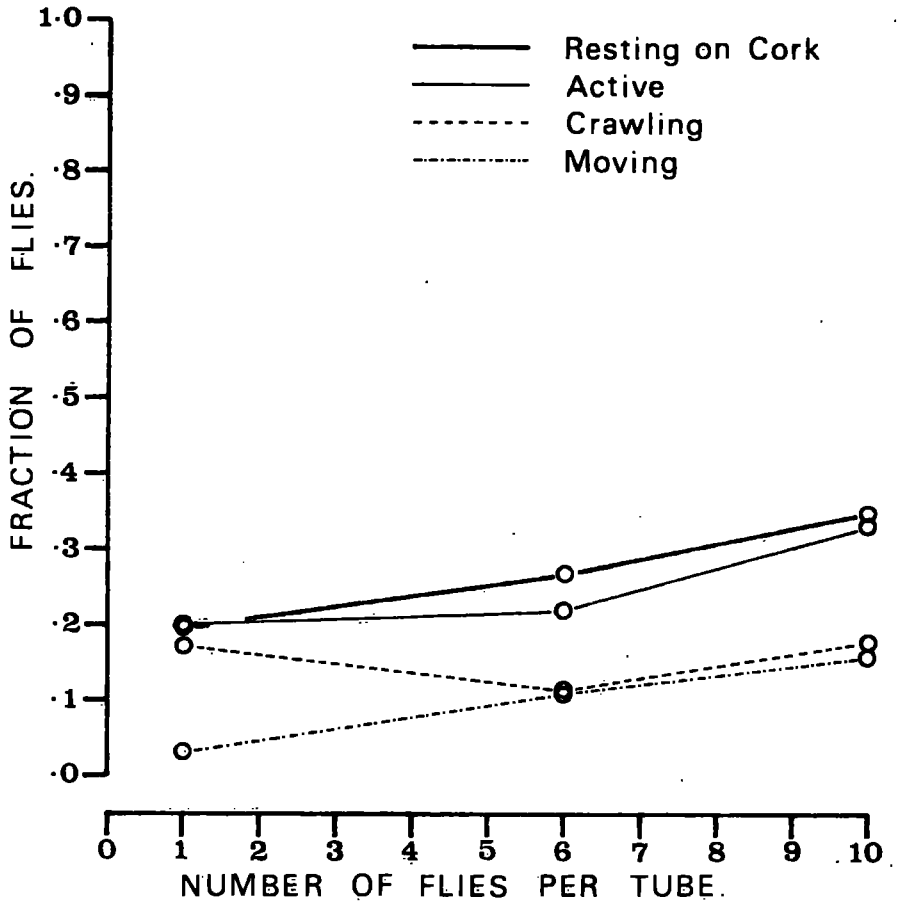


Fig. 23. The effect of the Number of Flies per Tube on the Activity of S. ornatum adults at 20°C.

The results are shown in Table 23. It can be seen that the activity of the flies increases between the first and second days, then remaining fairly constant for the next 6 days, the overall variation being 6.2%. On the eighth day the activity showed a considerable increase. The low activity on the first day may have been due to the lack of time in which the flies could acclimatise since some of these flies were placed in the experimental conditions only 2 - 3hrs after emerging. Since each fly represents 5% of the observations in each age group, the variation between the second and seventh days is due at least in part to the large differences in activity which can occur between the individual flies. It seems fair to assume therefore that the activity of the flies does not vary greatly between the ages of 2 - 6 days. In all other experiments only flies within this age range have been used.

The results for the experiments using 6 and 10 flies per tube are plotted as curves in Fig.24 as the fractions resting, "moving", and crawling. A comparison of the curves shows that the activity is greater for 10 flies per tube in all respects. The shape of the activity curves are not symmetrical but show a rapid increase between 9 - 12°C and a much slower decline as the temperature approaches the thermal death point. The initial steep increase in activity may be due to the stimulating effect of fluctuating temperatures or, as will be seen later, to it coinciding with temperature range in which co-ordinated activity begins. The slower decline in activity

TABLE 23 The activity of Simulium ornatum imagines and ageing at 20°C and 95-100% Relative Humidity

Age (days)	Fractions				
	Resting	"Moving"	Crawling	Active	On Cork
0 - 1	.875	.021	.105	.125	.200
1 - 2	.798	.031	.171	.202	.380
2 - 3	.768	.023	.209	.232	.117
3 - 4	.995	.025	.180	.205	.173
4 - 5	.802	.043	.156	.198	.110
5 - 6	.750	.055	.195	.250	.129
6 - 7	.812	.042	.146	.188	.153
7 - 8	.646	.066	.288	.354	.205

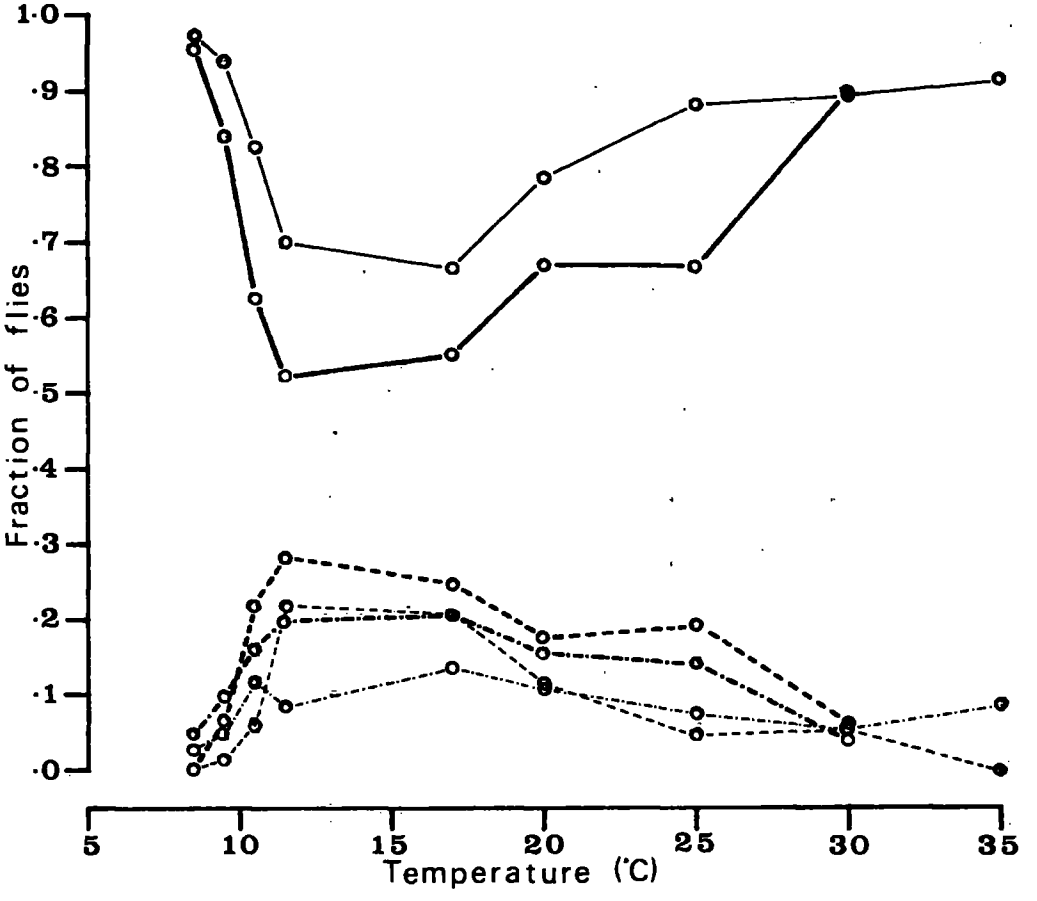


Fig. 24. Activity curves of S. ornatum adults when subject to constant temperatures.

(6 flies/tube { ——— Moving ·—·—· / - - - - - - - Crawling - - - - - - - / ——— Resting } 10 flies/tube)

at higher temperatures is to be expected since the temperatures remain high enough to allow co-ordinated activity to occur but the decline may be less marked as the result of discounting tubes in which mortality occurred. The maximum activity for 10 flies per tube occurs at 12°C and that for 6 flies per tube at 17°C. It is probable however that neither of these two temperatures represents the true optimum temperatures for activity. Examination of the shapes of the curves would suggest that the maximum activity in both cases would lie between these temperatures.

Co-ordinated locomotory movements begin at 9.5°C for both 6 and 10 flies per tube but they are preceded at 8.5°C by "movement" described as "fidgetiness" by Nicholson (1934). The temperature at which these non-locomotory movements occur cannot be determined from these experiments except to say that they probably begin below 8.5°C. Fidgetiness or movement, although forming a high proportion of the general activity below 9.5°C, does not continue to increase once the temperature level is sufficiently high to allow crawling, but remains relatively low, only forming a greater proportion of the activity at the higher temperatures which have a depressing effect on the co-ordinated locomotory movements. The fraction of flies resting on the cork was shown by Nicholson to indicate the unsuitability of the temperature, higher numbers of flies resting on the cork indicating the unsuitable nature of the

temperature. The temperature-preference curve or fraction of flies resting on the cork is shown in Fig.25 for 10 flies per tube only and it shows a similar pattern to that described by Nicholson (1934) and indicating a preferred temperature of 10.5°C - 17°C which corresponds with the range indicated for the optimum temperature for activity.

The observations of flight activity were incomplete, no observations being made at 30°C and 35°C and at 17°C and 20°C for the tubes with six flies. The remaining observations are plotted in Fig.26 as the average number of flights per minute per fly. The results represent observations of a single fly for a period 2.2 to 5.0hrs. No flights occurred at 8.5°C but the flight activity increased rapidly from 9.5° - 11.5°C reaching a higher value for 10 flies per tube at 11.5°C than for 6 flies per tube at the same temperature. The optimum temperatures for flight activity probably again lie between 11.5° - 17.0°C. Although the observations are not complete, the curves for flight activity appear to correspond with the curves for other co-ordinated movement, beginning at the same temperature.

2.C.(iii) Rising Temperature Experiments

The same constant temperature water bath was used but the heater was replaced with one of a lower wattage and

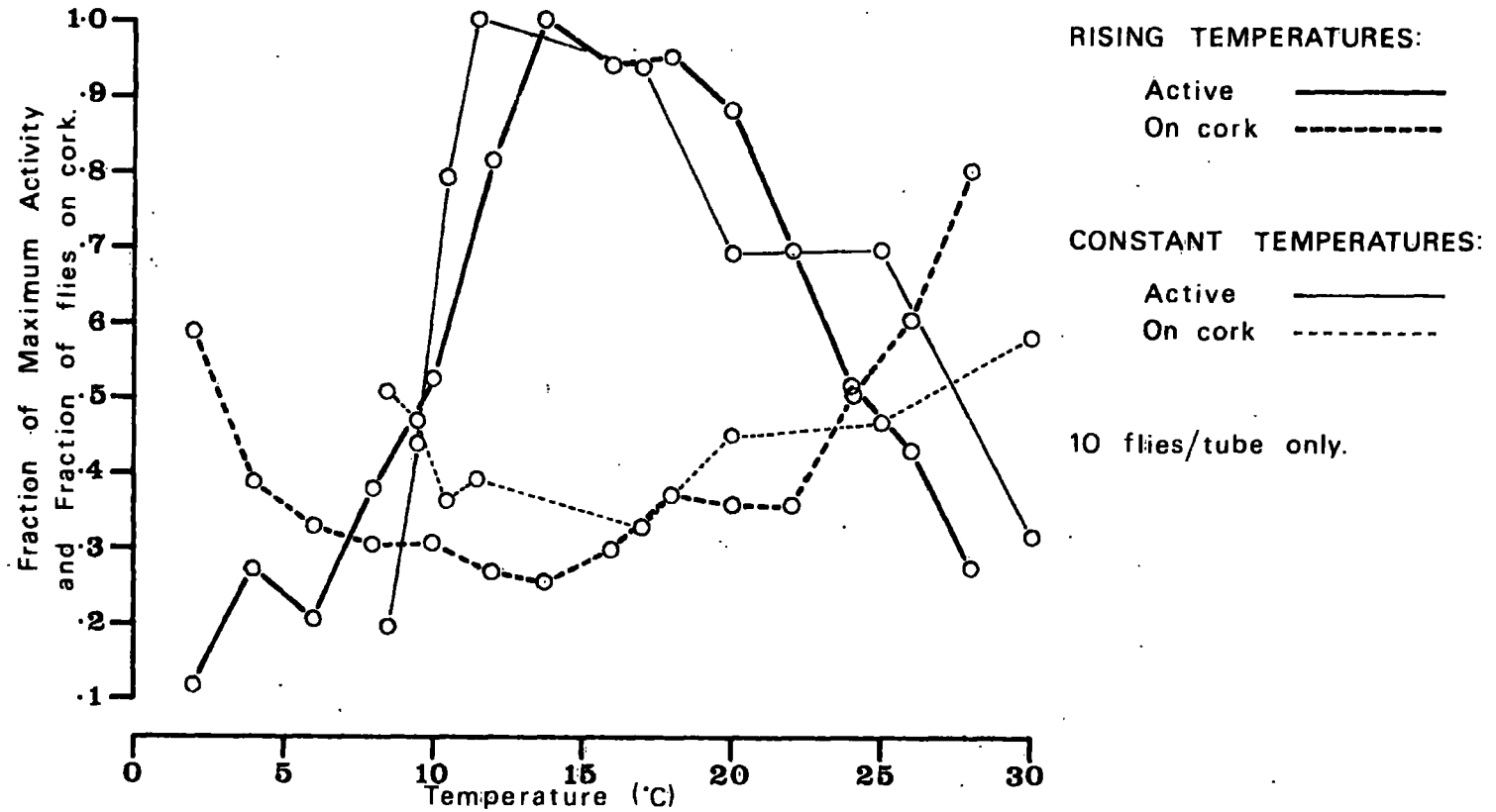


Fig. 25. General activity (combining "movement" and crawling) and temperature-preference curves of S. ornatum adults.

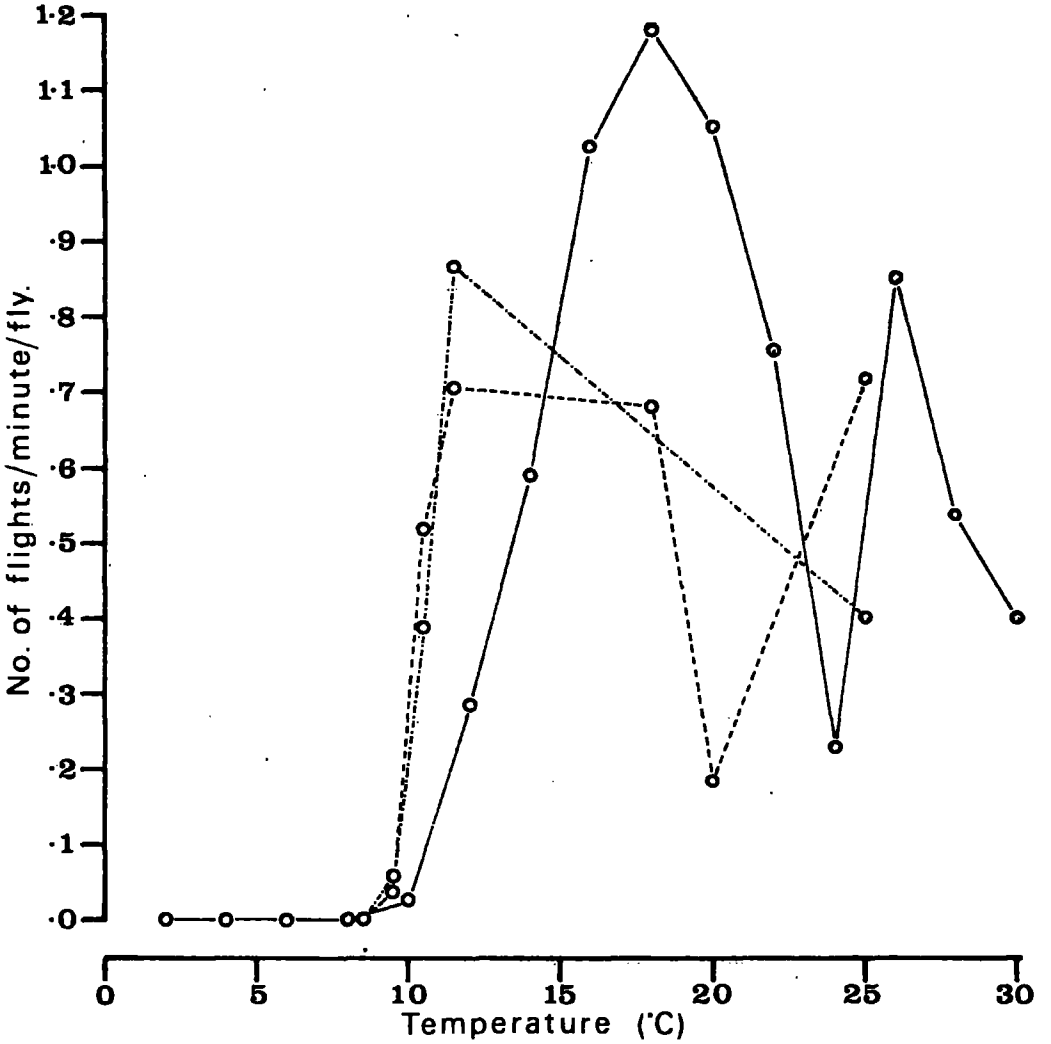


Fig. 26. Flight Activity curves of S. ornatum adults when subject to constant (10 flies/tube ——— & 6 flies/tube - - - - - - -) and progressively rising (10 flies/tube ———) temperatures.

the thermostat was disconnected. When switched on, this apparatus produced a slowly rising temperature, rising on average 1°C in 13.5 minutes. Since the heater was not adjusted during the period of the experiment, the rate at which the temperature rose was slightly quicker at the lower temperatures than at the higher temperatures. In all, three experiments over temperature ranges from 1.5°C - 22.5°C, 5.0°C - 24.2°C, and 11.8°C - 29.0°C were performed and since the water heating curves were the same in all cases, the results of the three experiments were combined, the results being collected in 2°C intervals to produce smoother curves.

The same experimental tubes were used as previously, each tube containing 10 flies of mixed sexes. The sets of 10 tubes were kept in the constant temperature coldroom at 8.8° - 12.0°C overnight before being transferred to the water bath the following morning. The water in the water bath was reduced by the addition of ice, the experimental tubes being placed in the water bath immediately after the ice had melted. The flies were given 5 minutes to settle and then the heater was switched on. Observations of activity were made every 15 minutes throughout the period, the temperature being noted at the beginning and end of each set of observations, the average of the two temperature readings being taken as the temperatures at which the observations were made. Flight observations were made every 30 minutes and since the time

to make the flight observations was much longer, note of the temperature at the beginning and end of the flight observation on each experimental tube was made.

The activity of the flies under conditions of progressively rising temperatures is shown as curves in Fig.27. The general character of the curves is very similar to those of the constant temperature experiments. General co-ordinated activity, such as crawling and flight, increases rapidly above 10°C although occasional flies were observed crawling at lower temperatures, the lowest temperature at which crawling was observed was 3.4°C. The lowest temperature at which flight was noted was not in a tube being directly observed and is not therefore included in the results. This flight occurred at 8.7°C. The next flight observed occurred at 9.4°C, after which flights became more frequent. The lowest temperature at which any form of activity was observed was 1.6°C shortly after the experiment commenced. At temperatures below 10°C the principal form of activity exhibited by the flies is non-locomotory, and above 10°C these non-locomotory activities remain constant up to 20°C before declining at the higher temperatures. The co-ordinated locomotory movements increase rapidly above 10°C reaching maximum values at approximately 15°C for crawling and at 17°C for flight activity, after which they decline, the fraction crawling being less than the fraction of flies showing "movement" above 24°C. The flight activity is

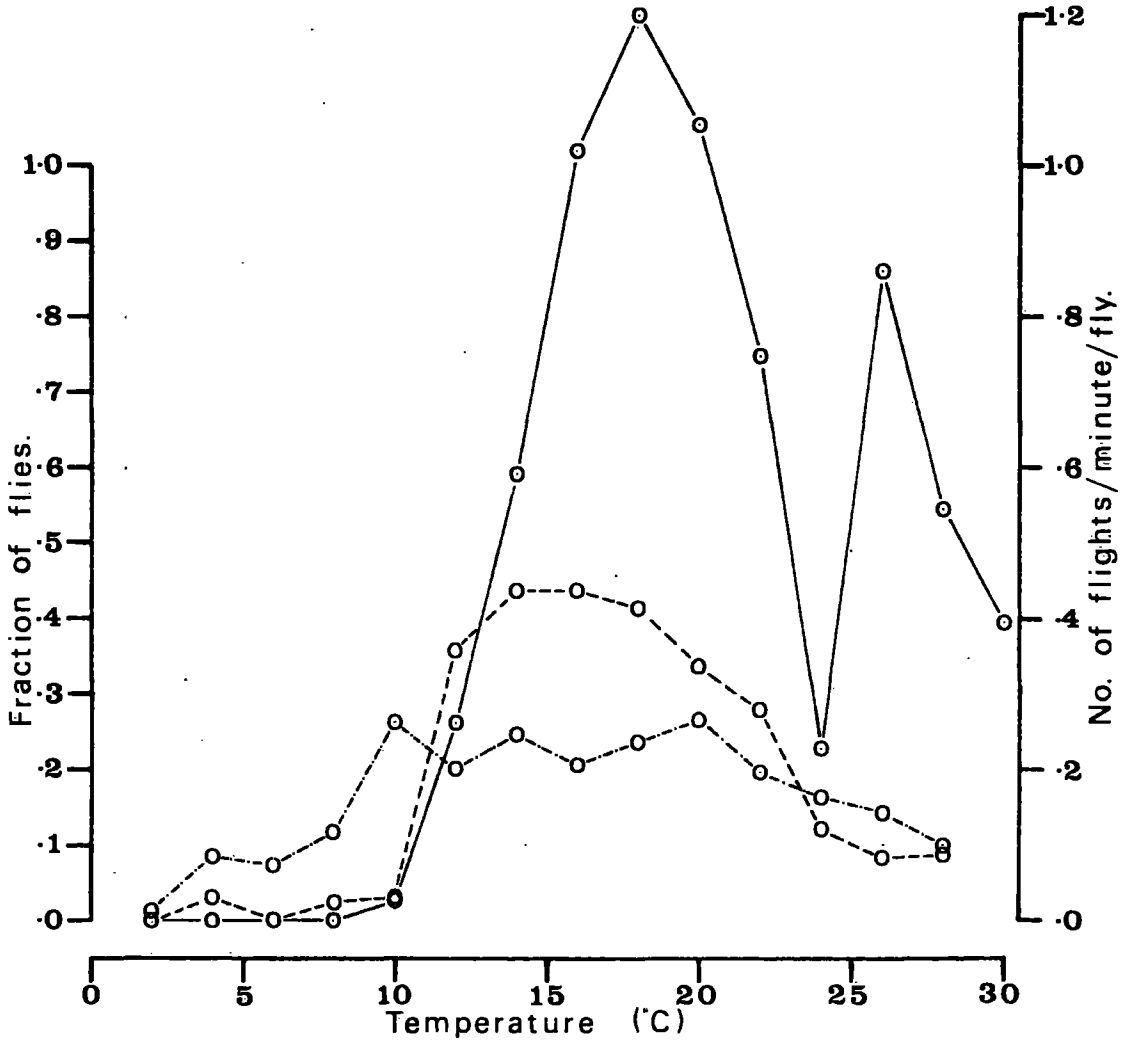


Fig. 27. Activity curves of S. ornatum adults under conditions of progressively rising temperature.

(-·-·-·- Moving: - - - - - Crawling: ——— Flying.)

rather erratic at the higher temperatures, perhaps indicating the distressing effect of these high temperatures although no mortality occurred during these experiments. The temperature - preference curve (Fig.25) also follows a similar pattern to that observed in the constant temperature experiments. This figure gives the comparative activity curves for rising and constant temperatures, the activity (moving + crawling) being expressed as a fraction of the maximum activity observed in each experiment. These activity curves indicated that the optimum activity is achieved at lower temperatures under constant temperatures, which is at variance with the findings of Nicholson who suggested that rising temperatures tend to stimulate activity, so that activity under these conditions would be optimal at a lower temperature than would be expected on the basis of constant temperature experiments. Examination of the temperature-preference curve however shows agreement with Nicholson's findings, indicating that the preferred temperature is higher in the constant temperature experiments. The curve for activity may also be distorted due to the stimulating effect of the fluctuating temperatures experienced by the flies over the temperature range from $8.8^{\circ}C$ - $12.0^{\circ}C$. It is probable therefore that under more rigidly controlled experimental conditions the curve for the activity under conditions of constant temperature would be displaced to the right.

2.D. Discussion

It is clear from these experiments that temperature has a marked effect on the activity of imagines of S.ornatum under conditions of high relative humidity and that this effect is very similar whether the temperatures are maintained at a constant level or progressively rising. It is probable that activity is stimulated under conditions of rising temperature so that maximum activity will be achieved at lower temperatures and it is these values which will be of greatest importance when comparing these experimental results with those observed in the field. Temperatures below 10°C inhibit locomotory movements although the occasional flies are capable of crawling at much lower temperatures and even flight at approximately 9.0°C. The range of temperature from 10°C to 23°C is quite suitable for activity of the flies, the maximum flight activity occurring at 17°C. Of the different forms of activity observed, the flight activity is of greatest importance since it will enable the dispersal of the adult flies from the streams after emergence, mating, feeding, which, in the case of the female fly, involves the seeking out of a suitable host from which to obtain a blood meal, and oviposition. Both the oviposition and biting activity of S.ornatum have been studied by Davies (1957) under field conditions. He found that there was no consistent tendency for the landing activity on the cow to increase with the temperature or to be directly related to the temperature in any way. The

range of temperatures over which he observed the flies landing on the cow was from 10°C - 22°C, which is identical with the values obtained from laboratory experiments. He showed that wind speed was a major factor determining the landing activity and oviposition activity in the field. It will be seldom in the field that temperature will be the only variable environmental factor since it is inter-related with humidity and light intensity. It would not be expected then that activity could be closely related to the flight activity of the fly under normal environmental conditions but it is only when the temperature becomes the factor limiting the flight activity that the association between flight activity and temperature will be seen. Since the environmental conditions in the British Isles will seldom reach values high enough to severely depress the flight activity, the relationship will best be seen at the temperature below which flights are not observed. It would appear then that the results of the field observations confirm that flight activity of S.ornatum adults begins at 10°C.

Acknowledgments

I wish to express my thanks to Dr. L. Davies under whose direction this study was made and with whom I collaborated in carrying out the field studies. I am also indebted to Dr. L. Davies for his comments on the thesis before presentation and his encouragement which ensured its completion. My thanks are also due to Professor J.B. Cragg who was Professor of Zoology at Durham University during the period of this study for his help; to Mr. T. Dargue for access to Coney Garth farm, Dufton, on which a thermograph was installed; to Mr. V.M. Brown for placing certain stream temperature measurements at my disposal; to the Nature Conservancy for allowing work to be done on the Moor House Nature Reserve; to the Agricultural Research Council and Durham Colleges Research Fund for financial assistance in carrying out the field work and to Durham County Education Committee from whom I was in receipt of a County Major Scholarship while engaged in this work. I also wish to acknowledge the help and encouragement of my late father, who read some of the script before its completion.

Summary

1. In two north Pennine streams larvae of Prosimulium hirtipes normally occurred at altitudes below 450m and those of P.inflatum above 660m. Simulium monticola and Simulium variegatum formed a mixed population along with P.hirtipes below 450m and S.monticola probably had an extended range occurring together with P.inflatum at altitudes above 660m.
2. P.hirtipes forms an increasing proportion of the black-fly larval population from November to May due to the addition of larvae resulting from an extended hatching period.
3. The thermal sum from January to May 1957 was greater at 150m than at 400m and is probably the normal case except perhaps during periods of fine summer weather.
4. There are eight larval instars during the development of P.hirtipes and the larval head-capsule width provides a reliable means of separating the larval samples into instar groups.
5. The first instar larva of P.hirtipes is recorded for the first time in the British Isles and its morphology closely resembles that of the first instar larva of a Canadian Prosimulium species, probably P.fuscum.
6. The development of P.hirtipes larvae takes place more quickly at 180m and progressively slower at 300m and 430m, and the development is at least in part directly related

to the thermal sum. There is a significant correlation between the larval development and thermal sum at 180m.

7. The dimensions of P.hirtipes larvae developing over a longer period of lower water temperatures are greater than those of larvae developing more quickly during periods of higher water temperatures.

8. In laboratory experiments temperatures in excess of 16°C - 18°C are harmful to P.hirtipes larvae and the effect of these temperatures is accumulative. The larvae of the winter generations of S.monticola and S.variegatum are only slightly less affected by these temperatures.

9. The restriction of P.hirtipes larvae to altitudes below 450m may be due to the thermal sum being insufficient to allow larval development to be completed by mid-May after which the thermal sum to at least 400m may reach harmful levels.

10. The larvae of P.inflatum develop in constantly cold water temperatures and the larval development period lasts 8 months compared to 4 - 5 months for P.hirtipes. The presence of a long egg stage in P.hirtipes and its absence in P.inflatum and the temperature regimes at the different altitudes provide a basis for the sequential displacement of P.inflatum by P.hirtipes larvae at lower altitudes in the streams.

11. In laboratory experiments the activity of Simulium ornatum adults is related to the air temperature under constant humidity and lighting conditions.

12. Locomotory movements of S.ornatum adults commence at 10°C and the optimum temperature for flight is 17°C when the relative humidity is 95 - 100%.

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APPENDIX 1(a) Mortalities of Black-fly larvae in laboratory experiments. EXPERIMENT 1.

(Individual Jars)

	DATE	9 Mar.	10 Mar.	11 Mar.	12 Mar.	13 Mar.	14 Mar.	18 Mar.
	TIME	9.10am	9.15am	1.15pm	2.15pm	5.15pm	9.30am	5.15pm
JAR 1								
4 - 5°C								
P.hirtipes	D%M	0.0	0.5	4.3	4.4		1.6	0.0
184 larvae	C%M	0.0	0.5	4.8	9.2		10.8	10.8
Non-hirtipes	D%M	0.0	1.1	3.2	7.9		1.3	1.4
2147 larvae	C%M	0.0	1.1	4.3	12.2		13.5	14.9
JAR 2								
4 - 5°C								
P.hirtipes	D%M	0.0	6.6	5.3	3.1		0.4	0.0
228 larvae	C%M	0.0	6.6	11.9	15.0		15.4	15.4
Non-hirtipes	D%M	0.0	3.6	3.1	3.3		1.1	2.0
1863 larvae	C%M	0.0	3.6	6.7	10.0		11.1	13.1
JAR 3								
21-22.5°C								
P.hirtipes	D%M	0.0	26.5	56.0	17.5			
98 larvae	C%M	0.0	26.5	82.5	100.0			
Non-hirtipes	D%M	0.0	31.0	28.6	36.6			
1960 larvae	C%M	0.0	31.0	59.6	96.2			

APPENDIX 1(a) (Contd.)

	DATE	9 Mar.	10 Mar.	11 Mar.	12 Mar.	13 Mar.	14 Mar.	18 Mar.
	TIME	9.10am	9.15am	1.15pm	2.15pm	5.15pm	9.30am	5.15pm
JAR 4								
21-22.5°C								
P.hirtipes	D%M	0.0	39.0	49.5	11.5			
182 larvae	C%M	0.0	39.0	88.5	100.0			
Non-hirtipes	D%M	0.0	43.2	21.5	32.1			
2040 larvae	C%M	0.0	43.2	64.7	96.8			
JAR 5								
P.hirtipes	D%M	0.0	15.0	3.5	31.5	47.0		
199 larvae	C%M	0.0	15.0	18.5	50.0	97.0		
Non-hirtipes	D%M	0.0	14.7	6.1	25.0	37.0		
2132 larvae	C%M	0.0	14.7	20.8	45.8	82.8		
JAR 6								
16-18°C								
P.hirtipes	D%M	0.0	7.8	8.3	37.0	45.0		
192 larvae	C%M	0.0	7.8	16.1	53.1	98.1		
Non-hirtipes	D%M	0.0	10.4	6.5	36.7	38.4		
2066 larvae	C%M	0.0	10.4	16.9	53.6	92.0		

D%M Daily percentage mortality
C%M Cumulative percentage mortality

APPENDIX 1(b) Mortalities of Black-fly larvae in laboratory experiments. EXPERIMENT 2.

(Individual Jars)

	DATE	25 Mar.	26 Mar.	27 Mar.	28 Mar.	29 Mar.	30 Mar.	1 Apr.	2 Apr.
	TIME	10.00am	9.00am	8.55am	9.10am	9.10am	9.10am	9.15am	11.30am
JAR 1									
4 - 5°C									
P.hirtipes	D%M	0.0	1.4	0.5	0.5	1.4	0.0	0.0	0.0
218 larvae	C%M	0.0	1.4	1.9	2.4	3.8	5.2	5.2	5.2
Non-hirtipes	D%M	0.0	3.8	2.1	1.2	2.5	0.4	0.8	1.0
1889 larvae	C%M	0.0	3.8	5.9	7.1	9.6	10.0	10.8	11.8
JAR 2									
4 - 5°C									
P.hirtipes	D%M	0.0	0.5	0.9	1.8	0.0	0.0	0.0	0.0
219 larvae	C%M	0.0	0.5	1.4	3.2	3.2	3.2	3.2	3.2
Non-hirtipes	D%M	0.0	2.6	1.4	3.5	0.3	0.5	2.4	2.7
1758 larvae	C%M	0.0	2.6	4.0	7.5	7.8	8.3	10.7	12.0
JAR 3									
4 - 19°C									10.00am
P.hirtipes	D%M	0.0	3.9	1.0	0.6	4.9	17.6	26.0	10.3
204 larvae	C%M	0.0	3.9	4.9	5.4	10.3	27.9	53.9	64.2
Non-hirtipes	D%M	0.0	6.3	1.9	1.5	6.7	20.5	25.1	6.7
1978 larvae	C%M	0.0	6.3	8.2	9.7	16.4	36.9	62.0	68.7

APPENDIX 1(b) (Contd.)

	DATE	25 Mar.	26 Mar.	27 Mar.	28 Mar.	29 Mar.	30 Mar.	1 Apr.	2 Apr.
	TIME	10.00am	9.00am	8.55am	9.10am	9.10am	9.10am	9.15am	11.30am
JAR 4									
4 - 19°C									
P.hirtipes	D%M	0.0	2.8	1.9	0.0	3.3	20.4	31.7	7.1
211 larvae	C%M	0.0	2.8	4.7	4.7	8.0	28.4	60.1	67.2
Non-hirtipes	D%M	0.0	3.7	2.6	1.0	6.6	21.5	25.2	10.3
1749 larvae	C%M	0.0	3.7	6.3	7.3	13.9	35.4	60.6	70.9
JAR 5									
16-18°C						11.00am			
P.hirtipes	D%M	0.0	3.9	27.4	49.7	18.4			
179 larvae	C%M	0.0	3.9	31.3	81.0	99.4			
Non-hirtipes	D%M	0.0	7.2	17.6	45.4	21.7			
1654 larvae	C%M	0.0	7.2	24.8	70.2	91.9			
JAR 6									
16-18°C									
P.hirtipes	D%M	0.0	6.9	30.2	42.9	18.4			
261 larvae	C%M	0.0	6.9	37.1	80.0	98.4			
Non-hirtipes	D%M	0.0	8.0	24.6	44.0	17.2			
2018 larvae	C%M	0.0	8.0	32.6	76.6	93.8			

D%M Daily percentage mortality
C%M Cumulative percentage mortality