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OBSERVATIONS ON *CULICOIDES CIRCUMSCRIPTUS* KIEFFER

(DIPTERA, CERATOPOGONIDAE = HELEIDAE)

by

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C O N T E N T S

I. ACKNOWLEDGEMENTS

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fluorescent bacteria found in the larvae, Dr D. Patton, of the Botany Department, who identified some of the flora found at Dumbuck, and Dr G.O. Evans, of the British Museum, who examined and identified the mites parasitising the adult midges. Dr R.A. Robb, gave me his valuable assistance and advice with the statistical analysis of the sampling data. To them and to all the members of the teaching and technical staff of the Zoology Department who helped me in numerous ways I wish to express my gratitude.

It was while assisting in the tedious task of extracting larvae from mud samples that my wife, Irene M.C. Becker, R.G.N., first noticed those containing fluorescent bacteria. I should also like to thank her for the valuable assistance she gave me in the preparation of this manuscript.

II. INTRODUCTION

The biting midges of the genus Culicoides Latreille have long been regarded as among the most annoying and troublesome insects in Britain, particularly in Scotland where their activities in some areas often make work in the fields in the evening quite impossible. Midges are also a serious source of annoyance to holiday-makers and tourists whose appreciation of the beauty of Scotland is so often spoiled by the bites of myriads of these insects with the result that the development of the tourist industry in this country has been seriously hampered.

It is only since the end of the 1939-45 war, however, that attention has been given in Britain to the study of the bionomics of the genus. Prior to this date the only comprehensive work on Culicoides was by Edwards (1939). This is primarily of a taxonomic nature, giving descriptions of the British species with keys for their identification. It has since been supplemented by descriptions of new species by Downes & Kettle (1952) and Kettle & Lawson (1955).

In 1945, under the auspices of the Scientific Advisory Committee of the Secretary of State for Scotland, a team of experts started an investigation of the Scottish midge fauna and their/

their habits accompanied by trials of various midge repellents (Cameron, E., 1946; Cameron, A.E., et al., 1946 and Cameron, A.E., et al., 1948). At the same time, working independently, Hill carried out an investigation into the life-cycle and habits of several species found near Liverpool. In her paper (Hill, 1947) she has also given excellent reviews of the previous literature on the subject which it is not proposed to repeat here.

Following these investigations a considerable amount of work has been carried out on different aspects of the midge problem. Hill & Roberts (1947), Cameron (1948) and Kettle (1949) have studied the possibilities of the use of insecticides as a control measure. Research on the life history and habits of different species has been performed by Cameron, A.E., (1946, 1947 and 1948), Parker (1949), Downes (1950 and 1955) and Kettle (1950 and 1951). Parker (1950) has investigated the conditions of egg incubation and Lawson (1951) made a thorough study of the anatomy and morphology of the larva and pupa of C. nubeculosus.

Descriptions of the larvae and pupae of most of the British species of Culicoides, together with keys for their identification, have been given by Kettle & Lawson (1952 and 1955). Roberts (1950) and Megahed (1956a) have given accounts of the methods of maintaining laboratory cultures of C. nubeculosus. Also, the anatomy and histology of the alimentary canal of this species/

species has been studied by Megahed (1956b).

The present work is the result of the study of the habits of a single species C. circumscriptus Kieffer, which is found on many of the coastal marshes of Britain. It is described by Edwards (1939) who regards the following as synonyms:

C. circumscriptus Kieffer, 1918, Ann.hist.-nat.Mus.hung., 16, p.49.

C. nadayanus Kieffer, 1918, Ann.hist.-nat.Mus.hung., 16, p.95.

? C. algarum Kieffer, 1924, Bull.Soc.Hist.nat.Moselle, 30, p.18.

? C. salicola Kieffer, 1924, Arch.Inst.Pasteur Algér., 2, p.405.

C. salicola var. pictidorsum Kieffer, 1924, Arch.Inst. Pasteur Algér., 2, p.406.

C. edwardsi Goetghebuer, 1921, Mem.Mus.Hist.nat.Belg., 8, p.177.

? C. polymaculatus Vimmer, 1932, Sborn.ent.Odd.nár.Mus. Praze, 8, p.144.

? C. albonotatus Vimmer, 1932, Sborn.ent.Odd.nár.Mus.Praze, 8, p.144.

C. pulcher (pulscher) Zilahi, 1934, Mitt.Bulg.ent.Ges., 8, p.155.

The adult was first recorded in Scotland by Cameron et al. (1946). Detailed descriptions of the larva and pupa are given by Tokunaga (1937) but to distinguish them from those of other species the descriptions and keys given by Kettle & Lawson (1952) are more useful.

Apart/

Apart from some information about the breeding places of C. circumscriptus given by the last-named authors virtually nothing was previously known about the bionomics of the species. The present work goes some way towards repairing this deficiency and adding to our knowledge of the biology of the genus Culicoi-:des in general. Although C. circumscriptus is a somewhat specialized species, being a salt-marsh breeder, and not known to suck blood, it is probable that many of the observations made are equally applicable to other species with, perhaps, some minor variations.

If adequate control of the blood-sucking species is to be achieved it is necessary that the habits and life histories of these species, with their specific variations, should be fully understood. Much of the previous work has been confined to the adult stage, and the study of the behaviour of larvae and pupae, which is most important from the point of view of control, has been largely neglected. The present work has shown that there are specific variations in larval behaviour sufficient to make it important that this be studied thoroughly in the economically more important species.

III. LOCATION OF FIELD WORK

Most of the collections and observations concerned in the present work were made at the Dumbuck salt-marsh which extends for about two miles along the north shore of the Clyde estuary between Dumbarton and Bowling. This marsh consists of bare mud flats on the side nearest the river while further inland is an area of saltings thickly covered with vegetation. The saltings are traversed by muddy natural drainage channels and small pools of up to 2 feet in depth are scattered here and there. The area is frequently flooded at high tide.

After the area was sampled extensively for both adults and larvae of Culicoides circumscriptus the main bulk of the work was confined to the vicinity of one of the pools where large concentrations of larvae of that species were to be found (Fig. 1). This pool, which measured 60 feet in length and 11 feet at its broadest point, is situated near the landward edge of the marsh which, at this point, is separated from neighbouring fields by a railway embankment and an artificial drainage ditch. During the course of a year this part of the marsh is covered by 80% - 85% of high tides which cover it normally for periods of up to 5½ hours (Fig. 2).

On the north (i.e. landward) side of the pool there is a narrow/

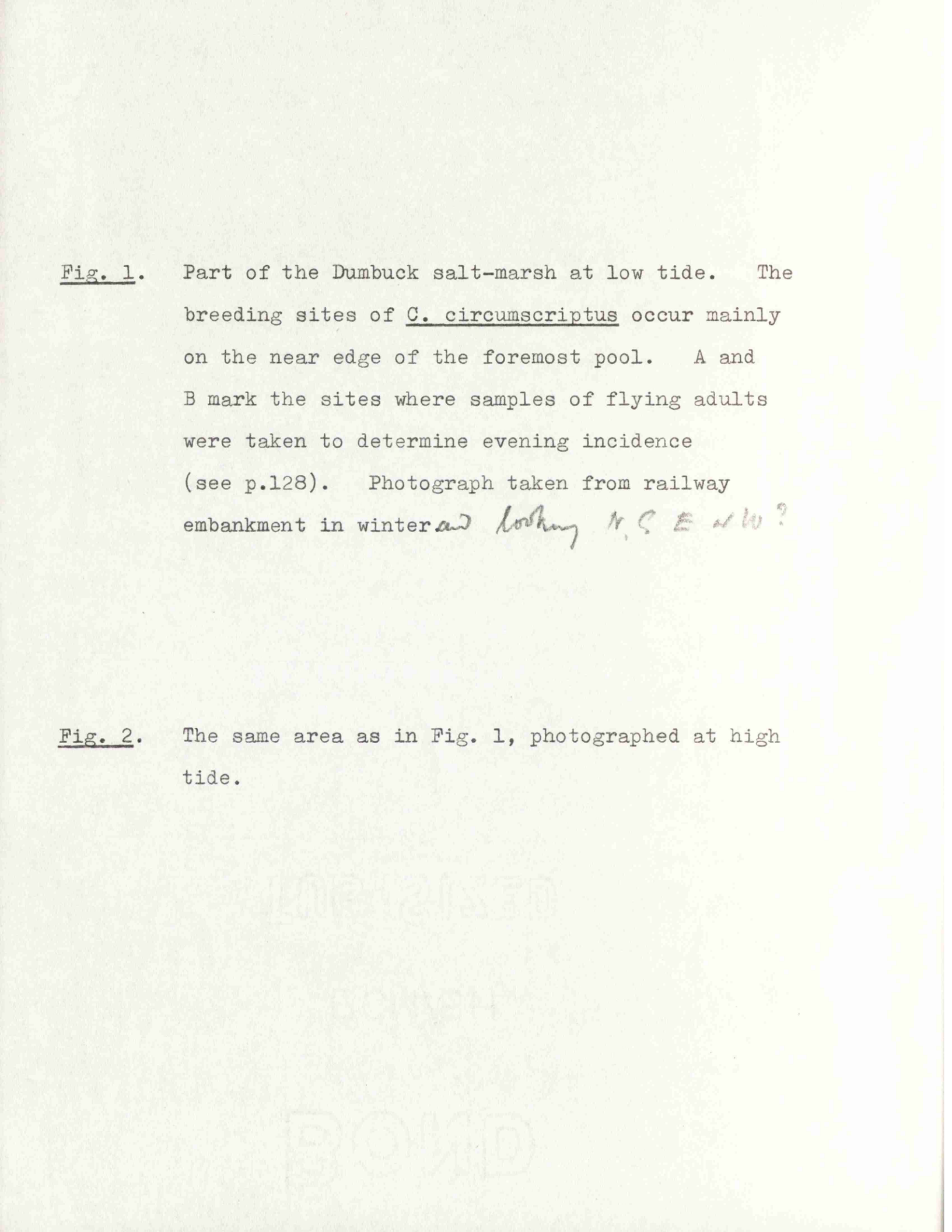

Fig. 1. Part of the Dumbuck salt-marsh at low tide. The breeding sites of C. circumscriptus occur mainly on the near edge of the foremost pool. A and B mark the sites where samples of flying adults were taken to determine evening incidence (see p.128). Photograph taken from railway embankment in winter and looking N. S E or W?

Fig. 2. The same area as in Fig. 1, photographed at high tide.

Fig. 1

Fig. 2

Fig. 3. A salt-marsh of the 'muddy' type, with soft mud and luxuriant vegetation. (Firth of Tay)

Fig. 4. Detail of the Firth of Tay marsh, showing height of reeds.

Fig. 3

Fig. 4

narrow margin of soft mud interrupted by tussocks of grass and small clumps of reeds (Phragmites communis Trin.) and rushes (Scirpus maritimus L.). This mud, on which most of the sampling work was done, is mostly bare of growing vegetation except for a surface layer of green algae which appears during the summer months, but it contains a large amount of rotting plant matter from the adjacent areas. Beyond this is a rough area of grassy tussocks stretching to the railway embankment. To the east and south is a large area densely covered with reeds growing to the very edge of the pool which, on the south side, is for the most part, sharply defined and without the mud margin found on the north side. The area to the west of the pool is covered with reflexed poa (Glyceria maritima Huds.) over which, at various times throughout the summer, is scattered scurvy grass (Cochlearia officinalis L.), sea aster (Aster tripolium L.), sea purslane (Halimione portulacoides L.) and sea milkwort (Glaux maritima L.). (Other plants most commonly found in the vicinity are listed below in Table I.)

While the main part of the investigations were carried out at Dumbuck, many other salt-marshes in Scotland have been visited. The topography of these marshes varies quite con-:siderably. In some places e.g. the Firth of Tay, there may be a dense, luxuriant growth of reeds, up to 10 feet in height, covering most of the area (Figs 3 and 4), while in other marshes these are either patchy in distribution or absent.

TABLE I

List of the flowering plants commonly found in the vicinity of the breeding sites of *C. circumscriptus* at Dumbuck, and from which the adults might obtain nectar. (Classification according to Clapham, Tutin and Warburg, 1952.)

M - Growing on marsh.

E - Growing on railway embankment.

CRUCIFERAE

Cochlearia officinalis L. (Scurvy grass) M

CARYOPHYLLACEAE

Silene maritima With. (Sea campion) E

CHENOPODIACEAE

Halimione portulacoides L. (Sea purslane) M

PAPILIONACEAE

Trifolium dubium Sibth. (Lesser yellow trefoil) E

Vicia cracca L. (Tufted vetch) E

ROSACEAE

Rubus fruticosus L. (Bramble) E

Potentilla anserina L. (Silverweed) E

Rosa sp. L. (Wild rose) E

Crataegus monogyna Jacq. (Hawthorn) E

CRASSULACEAE

Sedum acre L./

Sedum acre L. (Wall pepper)	E
UMBELLIFERAE	
Torilus japonica Houtt. (Upright hedge-parsley)	E
Angelica sylvestris L. (Wild angelica)	E
CAPRIFOLIACEAE	
Sambucus nigra L. (Elder)	E
PRIMULACEAE	
Glaux maritima L. (Sea milkwort)	M
CONVOLVULACEAE	
Calystegia sepium L. (Larger bindweed)	E
SOLANACEAE	
Solanum dulcimara L. (Woody nightshade)	E
COMPOSITAE	
Senecio jacobaea L. (Ragwort)	E
Senecio viscosus L. (Stinking groundsel)	E
Aster tripolium L. (Sea aster)	M
Achillea millefolium L. (Yarrow)	E
Matricaria maritima L. (Scentless mayweed)	E
Cirsium arvense L. (Field thistle)	E
Centaurea nigra L. (Hardhead)	E
Lapsana communis L. (Nipplewort)	E
Sonchus arvensis L. (Field milk-thistle)	E
Sonchus oleraceus L. (Sow thistle)	E
Hieracium sp. L. (Hawkweed)	E

Rushes are present in nearly all, but usually only in scattered clumps. The substrate may be firm, dry and sandy or may consist of mud of varying degrees of softness. Where sandy soil occurs the vegetation is very short and the surface is broken by sharply defined channels and pools (Figs 5 and 6). In muddy marshes such as at Dumbuck the soil is waterlogged to a greater or lesser degree and vegetation is more luxuriant. Drainage channels are fewer and are often marked only by the absence of vegetation and by much softer mud. Deeply cut ditches do occur, however, and represent a considerable hazard for the collector.

The species of adult Culicoides found in the Dumbuck area were C. circumscriptus, C. halophilus, C. maritimus, C. riethi, C. pulicaris, C. punctatus, C. impunctatus, C. chiopterus and C. pallidicornis (?). The first four species formed the great majority of the collected material and are recorded as true salt marsh types. The remainder, it is assumed, were strays from adjoining inland areas. The relative numbers of the species varied considerably, corresponding, no doubt, to the seasonal breeding cycle of each. Almost always, however, C. halophilus was found to be the most abundant. The other three salt marsh species were much less abundant, and their numbers relative to one another varied with the time of year.

From the mud samples taken over the same area the Culicoides/

Fig. 5. Salt-marsh at Inver Bay, Ross and Cromarty.

Fig. 6. Salt-marsh at Tynninghame, East Lothian.

Both are salt-marshes of the sandy type, with short vegetation and sharply defined pools and channels.

Fig. 5

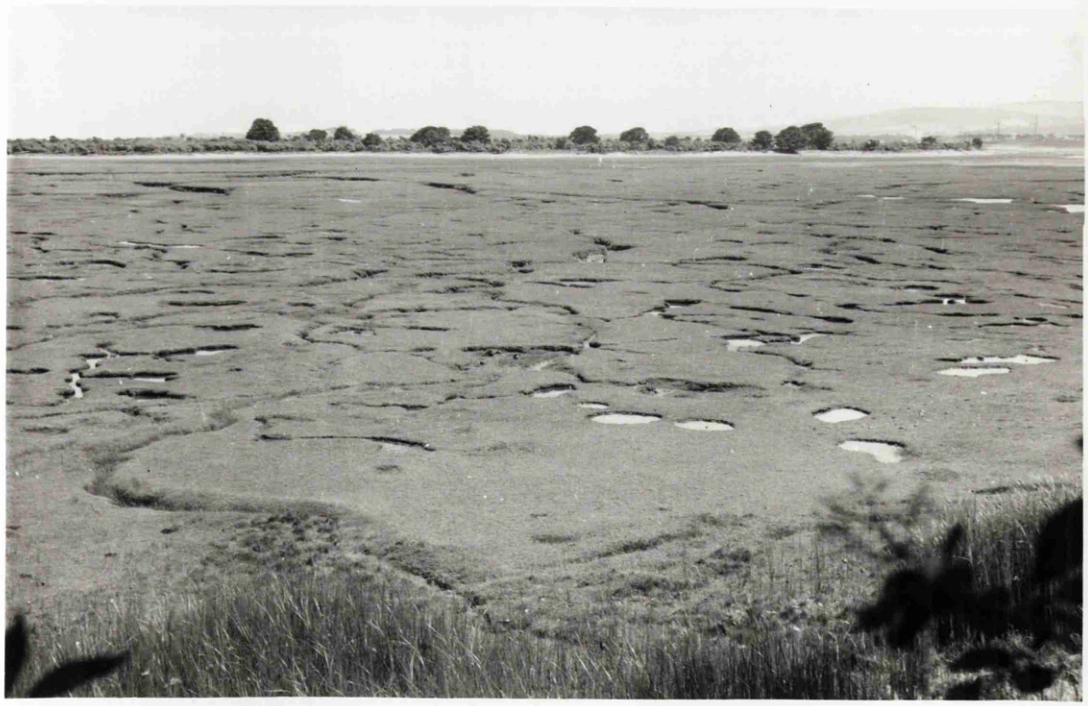

Fig. 6

Culicoides larvae obtained presented a surprisingly different picture. Although C. halophilus was the most common adult midge in the area it was only rarely that a larva of this species was obtained from samples taken either from the vicinity of the pool or from the wide area of the marsh which was examined. C. circumscriptus, C. maritimus and C. riethi, on the other hand, were the species generally found. Around the north side and west end of the pool C. circumscriptus occurred in very large numbers. C. maritimus rarely accounted for more than 20% of the total number in a sample, usually much less, while as a rule only one or two larvae of C. riethi were found in a sample. On the reed-covered south side and away from the immediate vici-:nity of the pool the numbers of C. circumscriptus larvae were much lower and very often C. maritimus was the dominant species, especially in areas covered with reeds, where the mud was tightly interlaced with roots.

Other fauna usually found along with Culicoides larvae in the mud included Nereis diversicolor, Tubifex sp., Gammarus sp. and Asellus sp.. Of the other insects found the larvae of Dolichopodidae, Ephydridae, Limnobiidae, Chironomidae, Psychodidae and Syrphidae were the most common, but their combined numbers per sample were usually considerably below those of Culicoides larvae.

IV. TECHNIQUE

A. Collection of Immature Stages

For more than two years samples of mud have been taken from six breeding sites situated round the large pool in Dumbuck salt-marsh. These were taken as far as possible at fortnightly intervals and the larvae and pupae extracted, identified and counted. To ensure accurate results it was important that the samples taken were as uniform as possible. Dove et al. (1932), Hill (1947) and other workers obtained their samples by filling containers of uniform capacity with mud taken from not more than 1 inch in depth. Hill claims that 90% of the larvae in a given area are thus recovered. Kettle & Lawson (1952), however, have demonstrated in a number of species that a large proportion of the larvae are found below this depth. They have also shown that, while most of the younger stages may be found in the top inch, a large percentage of the late instars occur below that depth. It is not improbable, moreover, that some vertical migration may take place on occasion under certain conditions, for example, in cold weather, drought, or, in the case of salt-marsh species, when the breeding site is flooded.

Owing to the liquid nature of the mud it was not possible to take samples at different depths in the breeding sites under investigation. Rough estimates made, however, have shown that about/

about 78.5% of the larvae occur in the top inch of mud. Taking the above observations into consideration it was decided that if an accurate representation of all instars was to be given, the samples should be taken more than 1 inch in depth.

Accordingly, a tool was devised (Fig. 7) consisting of a shallow metal cylinder mounted on a handle. The cylinder measured 4 inches in diamter and 2¼ inches in depth. (Total capacity approximately 28.3 cubic inches.) The bottom edge was sharpened and the top covered with fine-mesh wire gauze.

When taking a sample the tool was slowly pushed into the mud using a rotary motion to cut through roots etc.. A flat bricklayer's trowel was then pushed underneath and the sample was lifted out, and transferred to a convenient receptacle.

In this way a sample of uniform area and depth was obtained. Tests made on the mud below the depth reached by the tool showed that virtually all the larvae were removed.

B. Separation of Immature Stages from Mud

To recover larvae from samples of mud Dove et al. (1932) and Williams (1951) among others, placed the mud in a dish and put it into a larger dish which was filled with water to cover the sample. In the course of several days larvae migrated out of the mud and were picked out of the moat of water between the two dishes. Williams reports that the method is both time-

and/

Fig. 7. Equipment used for taking mud samples, consisting of a sampling tool, trowel and tin to contain the sample.

Fig. 8. The sampling equipment in use.

Fig. 7

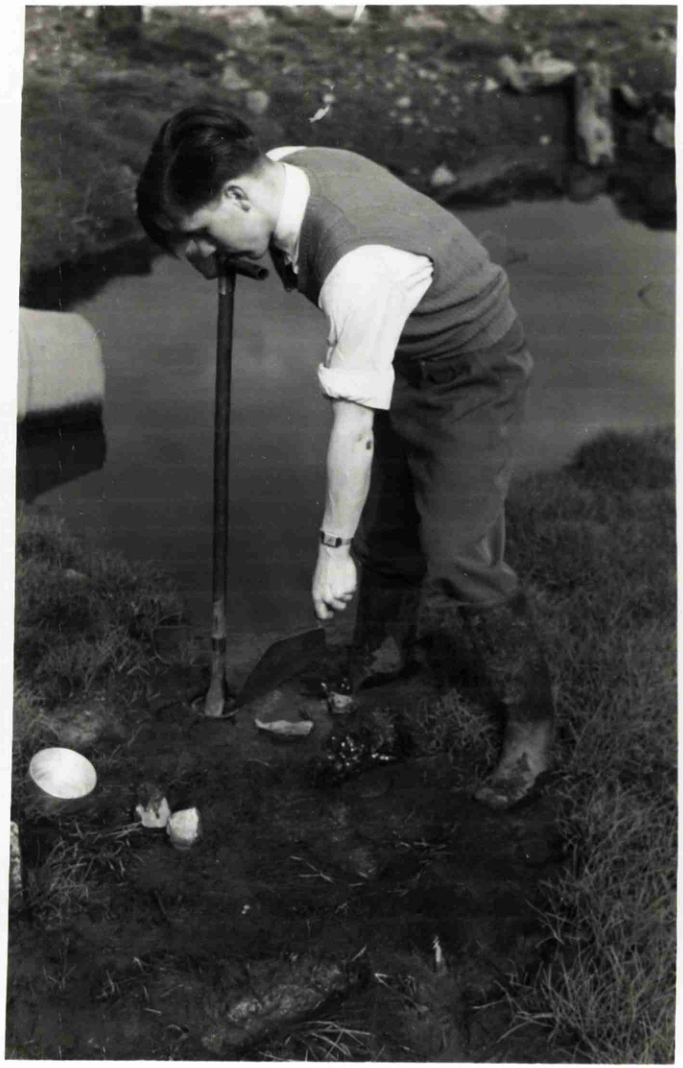

Fig. 8

and space-consuming and is by no means sure to recover all the larvae. Neither does it recover pupae.

Hill (1947) washed her samples through meshes of increasing fineness, recovering the larvae by picking them off bolting silk on which they were retained. This method, to be quantitatively accurate to the degree she estimates, must be extremely laborious.

Kettle & Lawson (1952) used the flotation technique of Ladell (1936) and this method, with some improvements was used in the present work.

The samples were broken up and washed through a 10-mesh sieve with a strong jet of water. The filtrate was then poured through 20-mesh and 100-mesh sieves, and the material retained in them was mixed with a saturated solution of magnesium sulphate, the specific gravity of which allows all inorganic material and much of the dead organic material to sink, while all living and some dead organic material will float on the surface.

When, in a number of test samples, the residues of all the sieves were treated in this way it was found that an average of 45% of the total larvae in the sample may be retained in the 20 mesh-sieve, and an average of 2.9% in the coarse sieve, a large proportion of these being of the larger instars. An increase/

increase in the strength of the water jet reduced this percen-:tage appreciably but a large number of larvae were killed if the force of the water was too great. Thus, to obtain an accurate estimate of the numbers of larvae and of the relative proportions of the different instars, it was considered neces-:sary to treat the residues of both the 20-and 100-mesh sieves.

After mixing the residue with magnesium sulphate and allowing the heavier debris to settle, the larvae and pupae could be picked off the surface with a hooked pin. The larger larvae could be seen easily with the naked eye but a long-arm binocular or other low power magnifier had to be used to recover first and second instar larvae. This, however, can be a very tedious task when a sample yields several hundred larvae, and the following apparatus renders it somewhat easier.

This apparatus (Fig. 9) is a modified version of that used by Fenwick (1940) for the recovery of eelworm cysts from soil. A 1-litre conical flask is fitted with a collar or collecting channel moulded out of "Vinagel 118" plastic (Vinatex Ltd.) and hardened by baking in an oven at 160°C. A funnel and a small glass dish are also required.

The residue is washed into the flask with magnesium sulphate and enough solution poured in to reach the top of the flask. The sediment is allowed to settle and the larvae etc. float to the/

Fig. 9. The apparatus used for the recovery of larvae and pupae from mud samples.

Brief Laboratory.

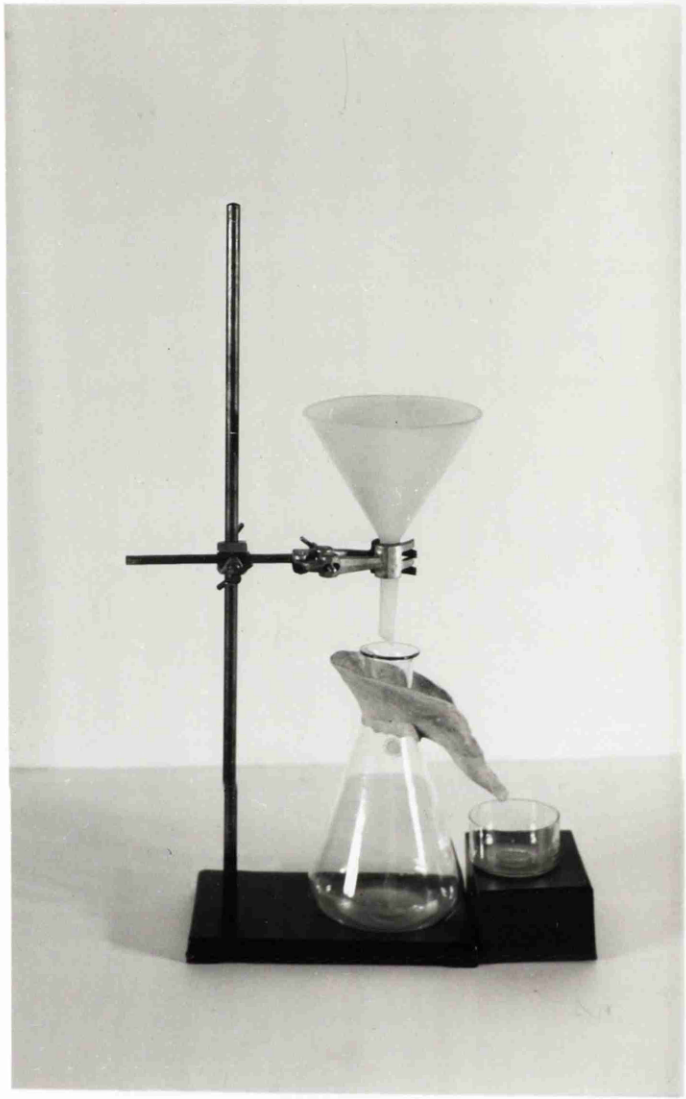

Fig. 9

the top. More solution is then poured in through the funnel causing the floating debris and larvae to be washed over into the collar and thence into the glass dish. The sediment in the flask should then be stirred with a long rod and the pro-:cedure repeated once or twice to ensure that all the larvae and pupae are obtained. Using this method it is possible to con-:centrate large numbers of larvae into a small surface area and to scoop them out more quickly. The task of finding the smaller instars is also made much easier.

C. Maintenance and Rearing Immature Stages in the Laboratory

1) Eggs

All eggs used in the present work have been obtained from gravid females caught in the field. In early experiments the insects were put into a 3 inch by 1 inch tube containing dampened plaster of paris on which the eggs were laid. Later it was found more convenient to follow a variation of the method used by Megahed (1956a). The tubes used for egg-laying con-:tained a small wad of cotton wool wrapped in filter paper and covered by a disc of filter paper cut to fit the inside of the tube. This method not only facilitated removal of the eggs, but enough water was retained in the cotton wool to keep the filter paper moist until the eggs are laid, whereas plaster of paris had to be moistened regularly at the risk of the insects becoming stuck to the plaster or the side of the tube before the/

the eggs are laid. A small piece of moistened raisin was pinned to the cork to provide nourishment for the midge.

Using the above method most of the midges laid their eggs on the disc of filter paper within a few days' time. If, after several days, an obviously gravid female had not laid they could be forced to do so by cutting off their heads. The decapitated fly then wandered over the surface of the filter paper and shortly began to lay. With this method, however, the egg yield was on the average lower than normal and the percentage hatching was much lower, many of the eggs being apparently infertile.

After laying was completed the disc of filter paper was transferred to a solid watch-glass containing a few drops of water. To prevent evaporation a glass cover was sealed on the watch-glass with a thin strip of plasticine. This was found less troublesome than smearing it with vaseline as recommended by Megahed as there was less danger of the larvae becoming trapped in scraps which had been overlooked.

2) Larvae

In the early stages of this work larvae obtained from field samples or by rearing from eggs were transferred to earthenware pots containing mud which had been taken from the natural habitat. This had previously been dried to kill off any macroscopic fauna, powdered, then mixed with tap water. This method/

method, however, was not successful and yielded very few adults.

Since then much more success has been obtained using the technique devised by Megahed (1956a).

Mud, rich in organic matter, was obtained near a byre and spread out on thick paper to dry. It was then powdered in a mortar and sieved through a 10-mesh sieve to remove stones etc..

The pots used were of the type known to gardeners as "saucers", about 6 inches in diameter and $1\frac{1}{4}$ inches deep, without a hole in the bottom.

Two teaspoonfuls of dried, autolysed yeast and one teaspoonful of powdered animal charcoal were added to a quantity of dried mud, sufficient to cover the bottom of the pot to a depth of about 1/4 inch (about 120 c.c.). These were mixed thoroughly and transferred to the pot. Enough water was then added to leave about a 1/4 inch layer of free water on top after absorption by the dried mud.

The pots were transferred to a glass covered tray out of doors and were left for about three weeks to allow growth of algae, bacteria etc.. An ample covering of water over the mud was maintained and the mud stirred occasionally to prevent the formation of brown scum which was caused by the yeast. The covered tray was well aired but also insect-proof. Other insects,/

insects, in particular Psychodidae, will breed in the mud if allowed to do so and this has an adverse effect on cultures of Culicoides.

At the end of three weeks the pots usually contained a rich growth of green algae. This growth was taken as a sign that the pot was ready for use, but it is pointed out by Megahed that it is not essential for successful cultures. The larvae were transferred to the pots which were placed in rearing cages. These were large glass battery jars, or were made of "Perspex" (I.C.I). The open end of the cage was covered with muslin and the cage was placed so that the closed end faced a window or some other source of light. The adult Culicoides are positively phototactic and on emergence most of them congregated at the end nearest the light, making collection easier and lessening the chances of escape while the cage was open.

The temperature of the room in which the cultures were kept was maintained as far as possible around 20°C. Under these conditions the time which elapsed before emergence began was about four weeks, but during hot weather when the temperature was above 20°C. the time dropped to three weeks. During the first two weeks the mud was kept covered by an ample layer of supernatant water but after this it was allowed to evaporate until only a surface film was left. The moisture was maintained/

maintained at this level until the emergence was completed.

It has been suggested that the addition of charcoal to the rearing medium is not necessary. To test this, several pots were made up without this ingredient. The proportion of larvae which emerged from these pots as adults was 44.2% compared with 49.8% for the pots containing normal rearing medium. This difference is just significant ($\chi^2 = 4.239$), therefore the addition of charcoal would appear to be justified.

Pieces of blow-fly larvae were also added regularly to some of the pots in the hope that this additional food might cause a larger percentage of larvae to complete development. The average proportion which did so, however, was 43.2%. This is significantly different from the results obtained from the normal rearing medium ($\chi^2 = 9.581$). The difference may be attributed to the accumulation of the toxic products of putre-:faction which certainly has an adverse effect on larvae when given this type of food in water cultures (see below).

For observation or experimental purposes larvae were kept in water. A ramp or small island of plaster of paris was also placed in the dish to allow the prepupal phase to climb out of the water and to pupate successfully. The larvae fed on green algae obtained from a hay infusion and also on pieces of other insect larvae. (The maggots of blow-fly have been used/

used successfully, though probably any large larvae will do just as well.) If a culture of this type was kept for a long time a bacterial scum often formed on the surface and the larvae became moribund and died. This was probably due to lack of oxygen as the scum could be removed and heavy mortality avoided by aerating the water. If maggots or any other food of animal origin was given the water also had to be changed regularly otherwise the products of decay prove toxic to the larvae.

This method will keep a large proportion of larvae alive but development is much slower than in the culture pots and during winter months pupation hardly ever takes place. This is probably due to the fact that the diet provided is inadequate or unsuitable. The exact food requirements of the larvae are not known. It has, however, proved to be the easiest method of studying the larvae and of obtaining the prepupal phase and pupae.

3) Pupae

Pupae obtained from cultures kept in water or from mud samples were kept in dishes containing soft mud or damp filter paper and covered with gauze to prevent the escape of the emerged adults.

D. Collection, Identification and Maintenance of Adult Midges

In the present work adults were caught for the following reasons:-/

reasons:-

(a) To obtain records of the species present in an area.

(b) To obtain specimens for experimental purposes and to supply eggs for rearing.

(c) To gain data on the diurnal and seasonal activity of midges present in salt marsh areas.

Several methods have been used by other workers to catch adult midges. Some of these are well-known. The use of human-beings and animals to attract the females is a standard procedure employed by investigators on biting flies. Similarly light traps and hand-nets are used frequently to catch many types of insects. Parker (1949) and later Nicholas (1953) have used a hand-net to obtain samples for quantitative data by carefully standardising their netting procedure.

Hill (1947) has observed that the female midge is attracted by dark coloured surfaces and her observations on diurnal and seasonal incidence is based on catches made as the flies alighted on a black cloth screen.

Kettle (1949 *et seq.*) has used sticky traps mounted at varying heights on poles.

Dove *et al.* (1932), Cameron (1947) and others have obtained newly emerged adults by placing box traps over the breeding sites.

Adult *Culicoides* have been found at heights of over 5,000 feet/

feet by Glick (1939) by attaching traps to the wings of aero-:planes.

A neat labour-saving method of catching females of C. austeni has been devised by Hopkins (1952). This species, when unfed, can pass through the apertures of sand-fly netting, but is unable to do so when gorged with blood. This enables the collector to sleep while the midges come to feed and the trapped flies are collected from the inside of the net at intervals throughout the night.

The species of midge which is the main subject of this paper, C. circumscriptus, is not recorded as a biter of humans or of animals. On many occasions throughout this work the author has taken samples of midges biting himself and other people. Samples have also been taken of midges flying around cattle and horses. None of these has yielded any of the species in question. As a result no trapping method could be used which depended on the attraction of the female to a blood meal.

Attempts have been made to estimate the diurnal activity of C. circumscriptus by standardised sweeping with a hand-net and by using a black cloth screen. Since Culicoides will only fly in very calm weather, a series of calm evenings is necessary to carry out this work properly. Because of the unsheltered nature/

nature of the Dumbuck salt-marsh as well as most other salt-marshes it has only once been possible to obtain such conditions for a length of time sufficient to provide data from which any information could be gained. Sweeping with a net of fine gauze was, nevertheless, found to be the best method of obtaining adults for other purposes. Whenever possible aerial sweeps were made as the resulting catch usually contained a large percentage of Culicoides. But when large numbers were required or when it was too windy to allow the midges to fly, the vegetation was swept. As a result of this latter procedure a large number of other insects were caught, but with practice it was quite easy to recognise members of the genus Culicoides and pick them out with an aspirator, even in twilight when they were silhouetted against the light of a torch.

A light-trap has also been employed. Owing to the lack of electricity supply anywhere near the areas of work, a trap was devised which was lit by an acetylene flame. This combined the advantages of brilliance of illumination, long duration of operation without attention, and portability. The trap (Fig. 10) had a wooden base and top, each with a round hole in the centre to accommodate a burner and a lamp-glass respectively. The sides of the trap were constructed of eight pieces of "Perspex" joined at the corners of the trap and sloping inwards towards the middle of each side where the perspex sheets were separated by a narrow opening of about 1/8 inch wide. This allowed/

Fig. 10. Light trap with acetylene generator.

Fig. 11. The light trap being emptied. The base-plate, with the burner attached, has been removed and is lying on the ground. The lamp-glass thus drops to close the hole in the floor and leaves open the gap in the roof through which the insects are removed with a long-nozzled aspirator.

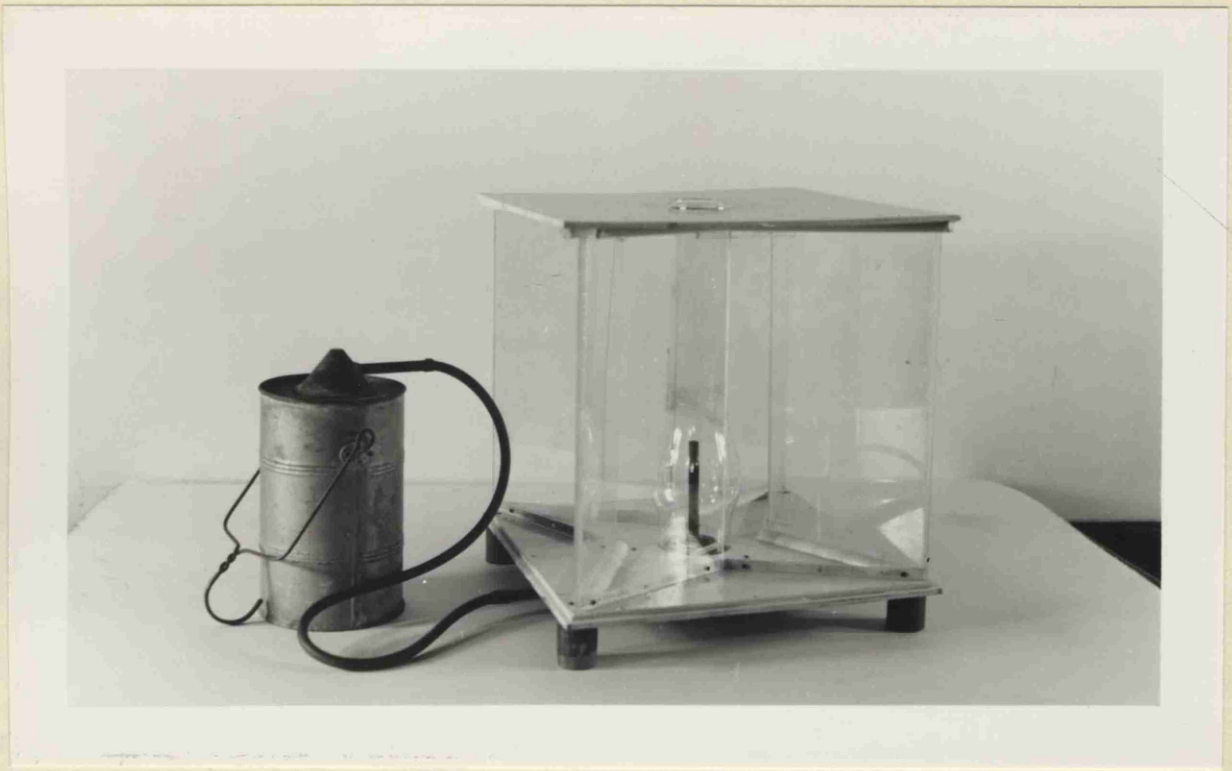

Fig. 10

Fig. 11

allowed midges and other small insects to enter the trap but excluded larger insects also attracted by the light.

The burner was attached to a short length of copper pipe which passed through a brass base-plate covering the hole in the floor of the trap and was connected by a rubber tube to an acetylene generator. The base-plate was perforated to allow ventilation and clipped to the under-side of the floor of the trap so that it could be easily removed. Insects entering the trap were prevented from coming near the flame by a lamp-glass which surrounded the burner. The bottom of this glass fitted into the hole in the floor of the trap and was supported by the base-plate of the burner, while the top protruded through the opening in the roof.

To empty the trap the base-plate was unclipped and the burner removed allowing the lamp-glass to slip down as far as the widened portion, thus closing the opening in the floor. The top of the glass was then below the opening in the roof and the insects were removed through this, using an aspirator with a nozzle long enough to reach all parts of the trap (Fig. 11).

The acetylene generator was of a standard type supplied commercially and working on the same principle as a Kipp's apparatus.

When/

When in use, the trap was placed on a small folding table which raised it about 2 feet from the ground. It was found extremely useful for catching midges when one was engaged on other types of work, and as it could remain alight for up to eight hours it could be left burning all night if required. It has, however, not proved itself to be very efficient in the form described and improvements are necessary. Not all the insects attracted by the light managed to find their way into the trap and some of those which did enter were observed to escape again through the slits in the sides. The method of recovery of the trapped insects proved to be awkward and clumsy and could also be improved.

In spite of its disadvantages, however, the use of the light-trap has been an easy way of obtaining samples of midges present in an area.

With a view to developing a trap which would be effective in catching Culicoides by day as well as night, experiments were made in order to find a substance attractive to the olfactory senses. Pieces of gauze were dampened with solutions in alcohol of some odoriferous ingredients of essential plant oils. Anisaldehyde, bromelia, cidrene, cinnamyl cinnamate, iso-eugenol and yara-yara were used since they happened to be already at hand. The pieces of gauze were stretched on wire frames which were/

were stuck in the mud at different heights on the marsh at Dumbuck and watched carefully for alighting midges. An opportunity has not arisen to put this method to a fair test. On each occasion that the wire frames were placed out on the marsh the weather has been such that few flying midges were about and no conclusion could be reached regarding the efficiency of the substances as attractants. On one occasion pieces of gauze were used dampened with a solution of honey in water and, while conditions for the experiment were again far from ideal, a number of insects were seen to alight and feed on the cloth. These included Ceratopogon spp., a female Culicoides circumscriptus, a female C. halophilus and a male C. riethi. From this it may be reckoned that, given better weather conditions or in a sheltered area, experiments in the use of honey-water, and possibly also of other substances such as those mentioned above, as attractants for Culicoides would have a good chance of success.

After capture the midges were either killed with chloroform or if required alive were anaesthetised with carbon dioxide for identification. Midges recovered from this anaesthesia very quickly however, and frequently they escaped while a batch was being sorted out under a binocular microscope. To avoid this an anaesthetising chamber was made which at the same time permitted examination of the insects under a binocular microscope./

Fig. 12. The apparatus used for anaesthetising and identifying adults. The insects are sucked in and the carbon dioxide introduced through the right-hand tube. The glass slide can be slid back to remove the insects.

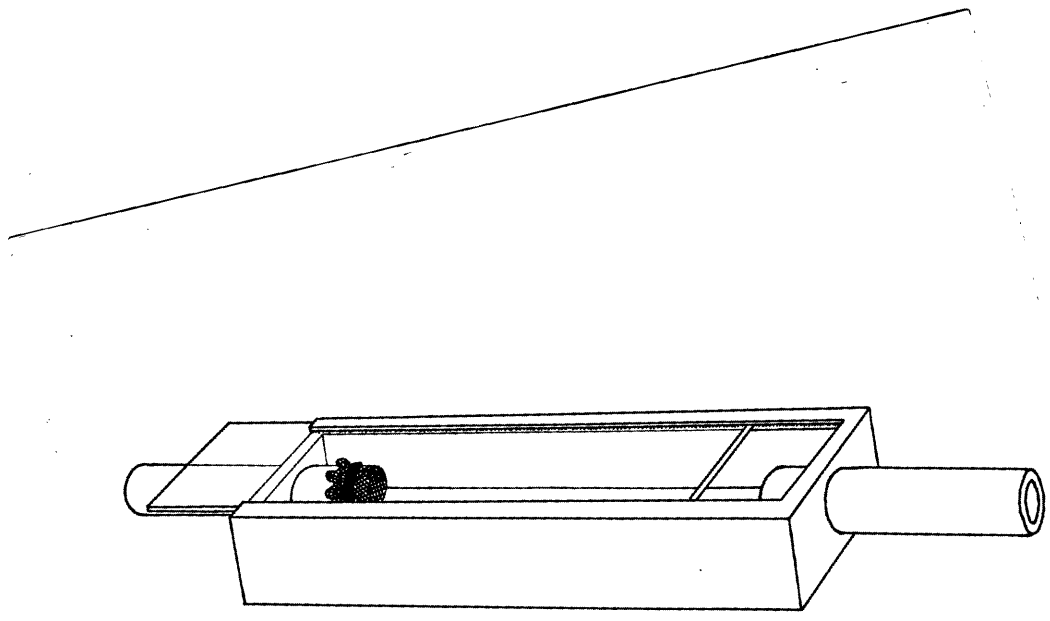

Fig. 12

microscope.

This apparatus (Fig. 12) consisted of a small "perspex" box measuring 3 inches by 1 inch by ½ inch, with grooves on the top edge to allow a 3 inch by 1 inch glass slide to be slipped in to close it tightly. Glass tubes were inserted through holes at either end of the box and the inner end of one was covered with a small piece of gauze while the other end connected to a short piece of rubber tubing.

The insects to be examined were sucked into the chamber through the open tube which was then connected to the carbon dioxide supply. The gas was passed through the chamber gently for about a minute, which was usually sufficient to anaesthetise the midges long enough to allow identification or superficial examination. To empty the chamber the glass top was slide back and the insects taken out with an aspirator. Ribbands (1950) found that anaesthesia of honeybees by carbon dioxide led to abnormalities in their behaviour. Weir (1955) has shown also that carbon dioxide anaesthesia increases the mortality and decreases the egg-yield of worker ants. Adult C. circumscriptus recovered very quickly in fresh air and appeared almost unaffected by the anaesthetic. The only abnormality noted was a twisting of the head in some specimens, which appeared to be the result of a muscular spasm and lasted sometimes for several days. The egg-yield seemed unaffected and compared favourably/

favourably with that of other species (see Table XIII).

Adult midges were kept alive for a few weeks in a "Perspex" cage, well ventilated and fitted with a gauze sleeve. They were supplied with broken raisins dampened with water, and dishes containing wet cotton wool covered with filter paper were also placed in the cage to maintain a high humidity.

E. Dissection of Adults and Larvae

To examine the contents of the alimentary canal the chloroformed adult midge was placed in a drop of water on a slide and the head cut off to free the anterior end of the canal. A small tear was then made with a fine needle on either side of the abdomen between the sixth and seventh segments. Gripping the posterior abdominal segments with one pair of fine forceps and the wings with another, the body was gently pulled away leaving the alimentary canal on the slide attached to the posterior segments. Care had to be taken to ensure that the oesophageal diverticulum was not broken off in the process, particularly when it was full.

A similar procedure was used in dissecting the larvae except that after decapitation the cut edge of the cuticle was held to draw the body away.

F. Analysis of Adult Gut Contents by Paper Chromatography

The technique of paper chromatography is a convenient and easy/

easy method of separation and identification of sugars. It is of particular advantage in the analysis of small quantities of carbohydrates such as are present in the gut contents of adult midges. The methods and materials used in this analysis were based on those described by Block et al. (1955).

The alimentary tracts of adult midges caught in the field were dissected out and placed, a dozen at a time, on spots along a line near the edge of a sheet of Whatman no.1 filter paper. Adult midges which had recently emerged in the laboratory and which were unfed, were similarly dissected and their guts placed on the filter paper as controls. The constituents of nectar, i.e. fructose, glucose and sucrose, were used as standards. These were dissolved to give solutions of 1% wt/vol. and about 3µl. of each solution was applied to the filter paper in line with the other samples.

The chromatograms were run for 12 hours in a Shandon chromatography cabinet using as a solvent a n-butanol/acetic acid/water mixture in the proportions 80:25:100. This solvent mixture was found to give more compact spots on the chromato-:grams than the corresponding proportions 40:10:50 suggested by Block et al.. The filter papers were dried in an oven and developed with aniline hydrogen phthalate as used by Partridge (1949).

After/

After spraying, the paper was dried in front of an electric fire. This treatment showed up the sugars present as coloured spots (Fig. 56) and enabled comparison of the substances contained in the alimentary tracts with the standard sugar solutions.

G. Labelling with Radiophosphorus

No attempt has previously been made to label Culicoides sp. with radioisotopes. A substantial amount of work has been done, however, in labelling mosquitoes with radiophosphorus (P-32). For the purposes of the present paper particular attention has been paid to the experiments of Hasset & Jenkins (1951) and Stich & Grell (1955).

Phosphorus-32 was considered the most suitable isotope with which to label the larvae. It is taken up readily into the body tissues. The short half-life of 14.3 days together with the fact that it only emits β-rays means that it is a relatively safe material with which to work and elaborate precautions are not necessary.

Before experiments could be undertaken in the field it was necessary to make some preliminary tests in the laboratory to determine how long the larvae should be exposed to the radioisotope, and also the most suitable concentration of the radioisotope to use for efficient labelling.

The/

The radiophosphorus was obtained in the form of orthophos-:phate in dilute hydrochloric acid. This was diluted and added to six dishes, each containing 100 ml. of water and 100 fourth instar larvae of C. circumscriptus, so that two dishes contained solutions with acitivity of 0.1µc/ml., two of 0.4µc/ml. and two of 0.6µc/ml..

In order to obtain a measure of the mortality rate due to the radioisotope, four control dishes each containing 100 larvae in 100 ml. of fresh water, were kept for the duration of the experiment. Of the dishes containing the radioactive solutions, one set of three, each with a different activity, was left for 40 hours and the remainder for 65 hours. At the end of this time the larvae were taken out and washed thoroughly to remove all traces of radiophosphorus from their external surfaces. They were then transferred to fresh water.

The radioactivity of the larvae was measured with a scaler shortly after irradiation, at the end of one half-life, and after two half-lives of the isotope (i.e. 14 and 28 days respectively). On the last two occasions they were also tested with a monitor which gave an audible response. For these purposes the larvae were placed on a circle of damp filter paper in a metal planchette which was then placed at a distance of 1 cm. from an end-window Geiger-Muller counter.

At/

At the end of the experiment the number of surviving irradiated larvae was compared with the number surviving in the control dishes. Of those which had been irradiated for 40 hours in different concentrations of the isotopes an average of 64% survived, while 57.5% of those irradiated for 60 hours were still alive. Of the controls 59% remained alive. It should be noted that the larvae had no food over the four weeks duration of the experiment other than their own companions. It could, therefore, be assumed that mortality due to the isotope, if any, was negligible.

The average radioactivity of each set of larvae is shown below (Table II).

Using the monitor at the end of one half-life good audible responses were obtained from those larvae kept for 40 hours in a concentration of 0.6μc/ml. and those kept for 60 hours in concentrations of 0.4μc/ml. and 0.6μc/ml.. At the end of 2 half-lives only those which had been kept for 60 hours in concen-:trations of 0.4μc/ml. and 0.6μc/ml. could be depended on to give a response easily distinguishable from the background.

From the above observations it was decided that for field experiments larvae should be exposed for 65 hours in a concen-:tration of the isotope of 0.5μc/ml. thus ensuring that they would be detectable for at least four weeks after irradiation with

TABLE II

The average radioactivity of larvae of C. circumscriptus after irradiation for 40 and 65 hours in different concentrations of P-32 given in net counts per minute, i.e. background count deducted.

Time of count	40 Hours Irradiation			65 Hours Irradiation		
	0.1µc/ml.	0.4µc/ml.	0.6µc/ml.	0.1µc/ml.	0.4µc/ml.	0.6µc/ml.
Shortly after irradiation	39	296	1090	86	388	1695
1 Half-life (14 days)	38	282	367	48	446	1433
2 Half-lives (28 days)	*	55	77	*	149	409

*Count not above background

a portable monitor fitted with ear-phones and an end-window counter sensitive to β-rays.

H. Vital Staining of Blow-fly Larvae

In the experiments on phototaxis in Culicoides larvae it was necessary to provide the larvae with a food which would not exclude the light and which would be recognisable in their alimentary canals.

It was found that the larvae fed on the larvae of blow-flies which were cut up and dropped into the dish. In order that this food would show through the transparent body walls of the midge larvae three vital stains, Trypan Blue, Trypan Red, and Waxolin Red were used in attempts to stain the blow-fly tissues before killing. Trypan Blue and Trypan Red were dis-:solved in insect Ringer's solution and injected into the anaesthetised blow-fly larvae which were left for two to three days. It was found, however, that when these were cut up and placed in water the fat body was unstained and the other tissues only lightly coloured. In order to stain blow-fly larvae with Waxolin Red, a fat-soluble stain, the powder was sprinkled on pieces of liver to which young blow-fly larvae were transferred. The dish, containing the liver and larvae was kept in a tempera-:ture of about 25°C. to stimulate rapid growth and the fully grown larvae eventually became bright pink. When opened the fat body was seen to be brightly stained but the other tissues were/

were only slightly coloured. However, since a large proportion of the body tissues consists of fat body and since this stains intensely this method was chosen for the experiments.

V. LIFE CYCLE

A. Laboratory Cultures (Table III)

Although the temperature of the laboratory was controlled thermostatically, daily mean temperatures ranging from 14.5°C. to 27.8°C. were experienced while cultures were developing. This naturally had its effect on the rate of development of the different stages. The average period of incubation of the eggs of C. circumscriptus was 4½ days. A number of batches incubated at average temperatures of 23.8°C. - 24.7°C. hatched in 3 days while on one occasion a batch took 8 days to hatch at a mean temperature of 17.2°C.. These times are roughly in accord with Megahed's (1956a) observations on the eggs of C. nubeculosus reared under similar conditions, but differ radically from those of Hill (op. cit.) who reports an average incubation period of two weeks at 16°C. - 19°C. in the eggs of C. impunctatus. Lawson (op. cit.) found that the eggs of C. nubeculosus, kept at room temperature, hatched on the second day after oviposition, but such a short incubation period has only been noted in C. circumscriptus when kept at temperatures of 28°C. and 33°C..

In one or two batches hatching either did not take place or only a few eggs hatched. Apart from these isolated cases the proportion of a batch of eggs which hatched ranged from

TABLE III

Data relevant to the life cycle of C. circumscriptus in laboratory cultures.

Culture Number	No. of Eggs	Incubn. Period (Days)	% Hatch	Larval + Pupal Period (Days)	No. of Adults obtained			Period of Emergence (Days)	% of Larvae Emerged
					M	F	Tot.		
53/1	352	6	92.9	21-45	28	41	69	24	21.1
53/2	354	5	92.9	-	-	-	-	-	-
53/3	323	5	96.9	-	-	-	-	-	-
53/4	346	5	87.0	-	-	-	-	-	-
53/5	221	5	86.9	-	-	-	-	-	-
53/6	77	6	96.1	-	-	-	-	-	-
53/7	45	6	51.1	-	-	-	-	-	-
53/8	201	6	85.6	-	-	-	-	-	-
53/9	271	6	79.7	-	-	-	-	-	-
54/1	178	7-8	92.1	28-45	38	25	63	18	38.4
54/2	292	6-7	79.8	28-45	98	94	192	18	82.4
54/3	313	6	97.4	28-48	69	102	171	21	56.1
54/4	269	5	75.1	27-43	75	79	154	17	76.2
54/5	347	7	24.2	28-42	18	17	35	15	41.7
54/6	120	7	95.8	37-47	0	4	4	11	3.5
54/7	367	4	87.2	30-41	120	150	270	14	84.4
54/8	71	6	90.1	28-50	13	18	31	22	48.4
54/9	54	6	25.9	-	-	-	-	-	-
54/10	328	5	68.3	27-54	10	15	25	27	12.1
54/11	256	6	83.2	26-48	66	80	146	23	67.1
54/12	204	5	99.5	26-41	71	78	149	16	72.4
55/1	287	4	94.8	26-48	15	37	52	13	19.1
55/2	113	4	98.2	24-46	60	31	91	13	82.0
55/3	304	4	95.7	20-26	3	78	81	6	27.8
55/4	401	4	97.3	23-38	106	96	202	15	51.8
55/5	320	4	96.9	20-29	53	41	94	10	30.3
55/6	182	4	98.9	20-28	45	57	102	8	56.7
55/7	194	4	100.0	21-32	28	14	42	11	21.6
55/8	195	4	98.5	20-23	47	44	91	4	47.4
55/9	274	3-5	99.6	25-45	65	81	146	21	53.5
55/10	266	4	98.1	23-33	85	98	183	11	70.1
55/11	275	5	95.3	21-25	7	1	8	5	3.1
55/12	251	4-6	12.7	-	-	-	-	-	-
55/13	307	3-4	98.7	21-35	118	37	155	15	51.2
55/14	309	3	99.7	24-46	100	104	204	23	66.2
55/15	324	3	97.5	25-43	65	49	114	19	36.1
55/16	212	4	83.5	-	-	-	-	-	-
55/17	158	4	91.1	-	-	-	-	-	-
55/18	386	3-4	95.6	24-46	102	102	204	23	55.3
55/19	296	3	98.6	-	-	-	-	-	-
55/20	260	3-4	98.5	30-51	61	80	141	22	55.1
55/21	192	4	96.4	30-45	39	39	78	16	42.2
55/22	333	4	98.5	26-37	0	185	185	12	56.4
55/23	287	3-4	99.0	23-45	100	92	192	23	67.6
55/24	200	4	99.5	-	-	-	-	-	-
55/25	229	4	90.8	-	-	-	-	-	-
55/26	195	※2	90.8	-	-	-	-	-	-
55/27	353	※2	94.1	+16-35	37	32	69	20	20.8
55/28	325	※2	97.8	-	-	-	-	-	-
55/29	351	※2	98.9	‡28-35	6	8	14	8	4.0
55/30	213	3	99.5	-	-	-	-	-	-
55/31	246	3	87.0	+14-33	45	50	95	20	44.4
55/32	197	4	97.0	-	-	-	-	-	-
55/33	271	3	95.6	-	-	-	-	-	-
55/34	157	3	98.1	-	-	-	-	-	-

※Eggs incubated at 28°C and at 33°C.
+Larvae reared at 33°C.
‡Larvae reared at 28°C.

12.7% to 100%. In most of the batches, however, 90% - 100% of the eggs hatched so that the overall average for 55 batches was 88.7%. Parker (1950) has shown that exposure of the eggs of C. pulicaris and C. punctatus to high temperatures in the early stages tends to lower the survival rate, but the viability of the 4 batches of C. circumscriptus eggs which were kept throughout the incubation period at 28°C. and 33°C. was not affected.

The time which elapsed before the first adult midge emerged from the culture pots in the laboratory was from 20 - 37 days and is partly dependent on the temperature during the time of development. (For pots kept at 33°C. the time was 14 - 16 days.) The last adults emerged 23 - 54 days after the start of the culture. Emergences took place from pots over a period of 4 - 27 days, these times being quite independent either of the number of larvae in the pot or of the temperature.

The time when pupation began in the culture pots could not be easily observed, but separate investigations showed that the pupal stage lasts 3 - 9 days with an average of 5 days. Subtracting this from the time spent in the culture pots, the time spent in the larval stage may be estimated to be 15 - 49 days. The complete pre-adult period lasted anything from 24 - 59 days.

Out of 31 cultures reared from egg to adult the number of adults which emerged varied from 3.1% to 82.4% of the larvae introduced/

introduced into the pots, with an average of 48.3%. As Megahed found in his cultures, the percentage of flies which emerged had no relation to the number of larvae in the pot, which varied from 64 - 390.

There are considerable differences among the cultures in the sex ratios of the emerged adults but in the overall totals the ratio is nearly normal, 53.5% being females and 46.5% males. The emergences from two pots consisted entirely of females. The first (Table III, 54/6) may be ignored since the number of flies which emerged was too small to lend any significance to the absence of males. In the second pot (55/22) the females which emerged represented 56.7% of the larvae introduced into the pot, a figure far enough above the average emergence for both sexes to make it unlikely to be the result of a freak chance. The possibility that parthenogenesis had taken place must be considered. Among the Chironomidae cases of partheno-:genesis have been reported in species of Corynoneura by Goetghebuer (1913) and Edwards (1919), in Chironomus clavaticrus by Edwards (1919) and in species of Tanytarsus by von Grimm (1870) and Johannsen (1937). As in the case of the culture in question the offspring of these flies were all females. Since they were unfortunately lost shortly after the culture was ex-:hausted it has not been possible to examine the female parent or the offspring for morphological similarities which might support/

support the suggestion of parthenogenesis. No such case has been previously reported in the Ceratopogonidae and the matter must rest until another case is observed.

In general, the development of male flies was slightly in advance of that of the females. In most of the cultures the earliest emerging adults were preponderantly males. On the average, at any particular fraction of the period over which emergences took place, the percentage of the final number of males which had emerged was greater than that of the females (Fig. 13). Hill found with C. impunctatus and C. obsoletus that the first flies to emerge were all males. Megahed reports that the first adult of C. nubeculosus to emerge might be either male or female, but he gives no indication of the subsequent pattern of emergence.

Two cultures (55/27 and 55/31) were raised in an incubating oven at 33°C.. This reduced the period of development by about a week but the percentage of larvae which reached adulthood was below average. Hill found that cultures of C. impunctatus and C. obsoletus kept at 23°C. - 24°C. were completely unsuccessful. Dove et al. (1932) were also unsuccessful when they attempted to rear C. canithorax, C. melleus and C. dovei at 70°F. (21.1°C.).

It has not been possible to maintain adults of C. circumscriptus/

Fig. 13. The rates of emergence of males and females of C. circumscriptus from laboratory cultures.

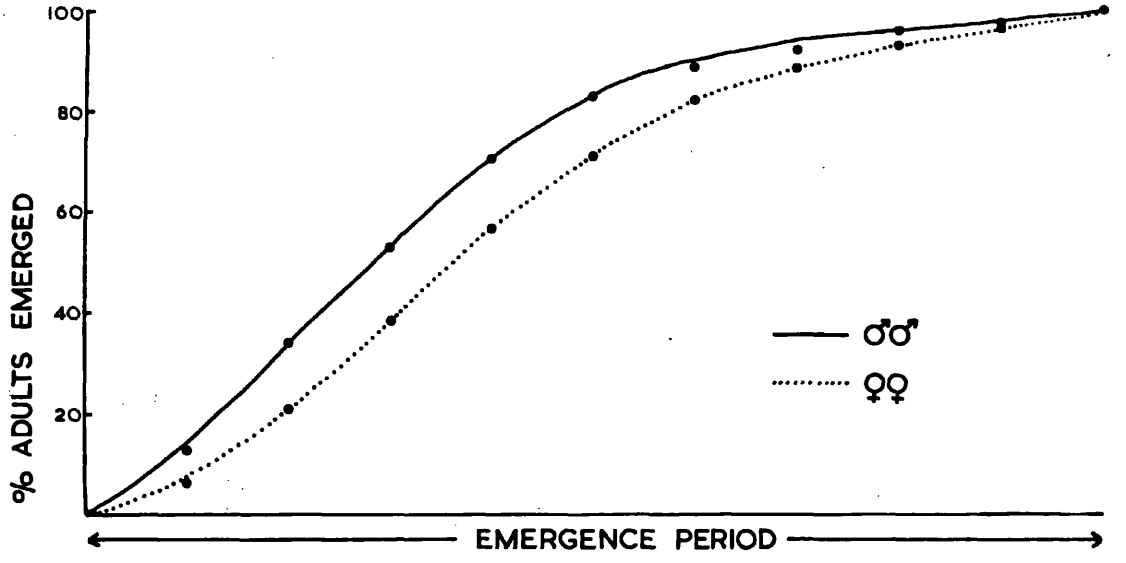

Fig. 13

C. circumscriptus in the laboratory for more than about two and a half weeks, and, as reported elsewhere, no mating or laying of eggs has been observed in laboratory-reared flies.

B. In the Field

1) Method and results

Uniform samples of mud were taken, every fortnight as far as possible, from six sites in the larval breeding area at Dumbuck during the period 2nd May, 1954 to 10th October, 1956. The midge larvae and pupae were recovered from the mud using the methods described earlier, and, after counting and identification, those which were not required for experimental purposes were returned to the areas from which they were obtained.

The results of this sampling are given in Appendix A and shown graphically in Fig. 14. This shows that the numbers of first instar larvae and pupae which were recovered were relatively small. A number of reasons may be given to account for this. In the case of the first instar larvae it is undoubtedly partly due to their small size and transparency which made them extremely difficult to find unless they were moving, and they were not so active as were the later instars. Also, it may be that a larger number of these were killed by the strong jet of water used to wash the samples. The small numbers of pupae recovered may be ascribed to the fact that they are constantly/

Fig. 14. The seasonal distribution of the immature stages of C. circumscriptus, shown by the average numbers re-:covered from 6 sites at Dumbuck during the period between 2nd May, 1954, and 10th October, 1956.

P.T.O.

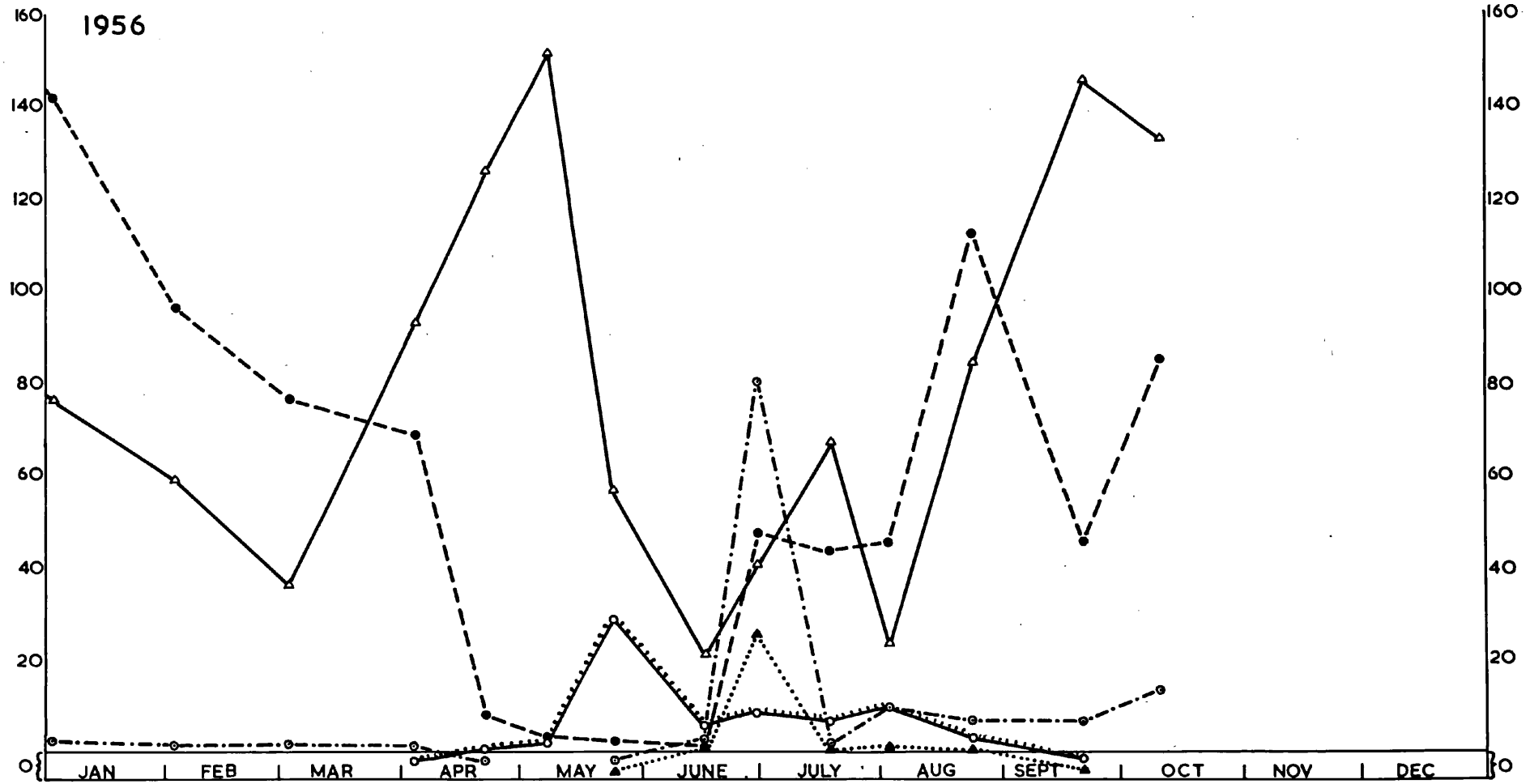
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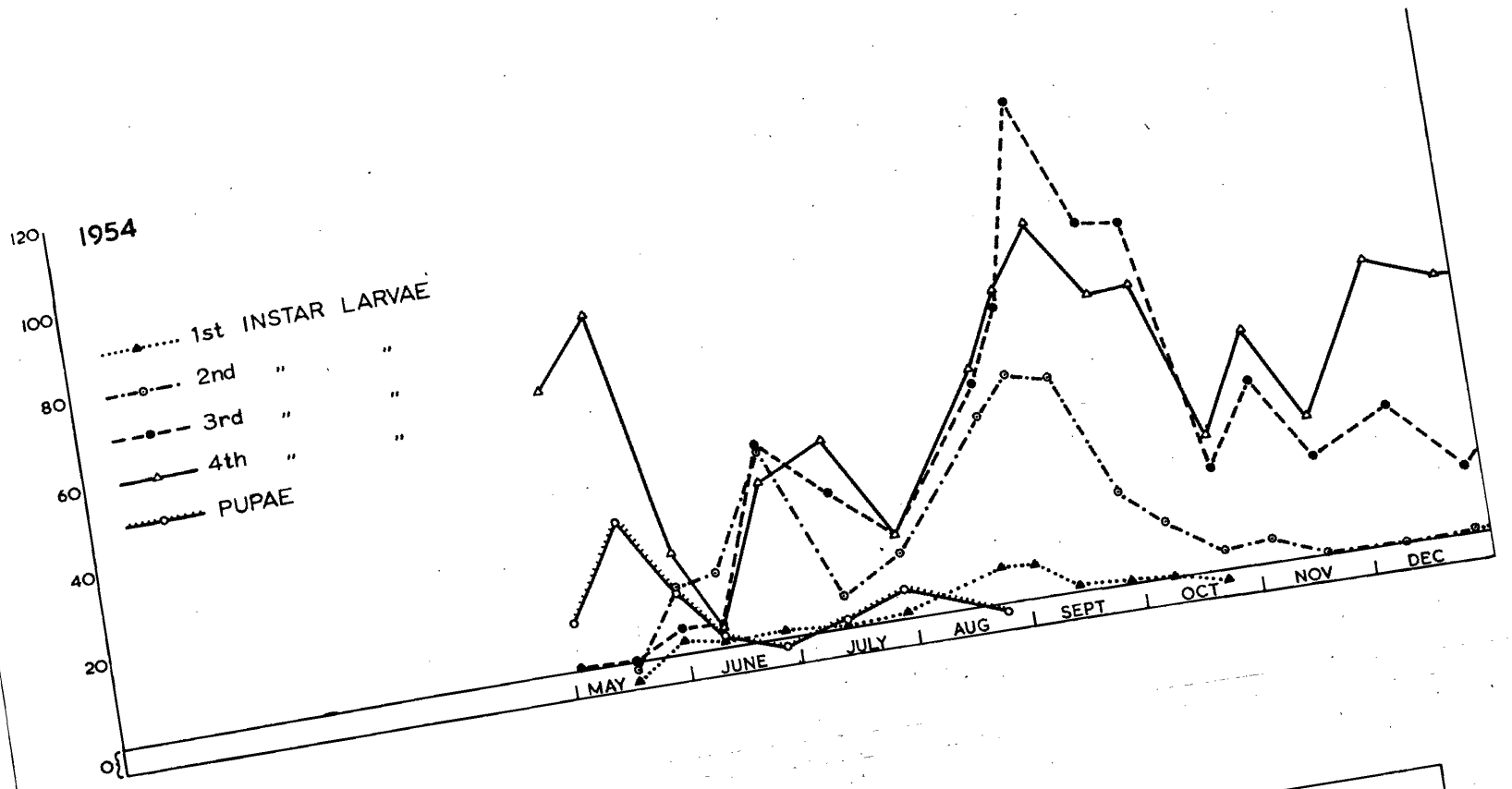
Fig. 14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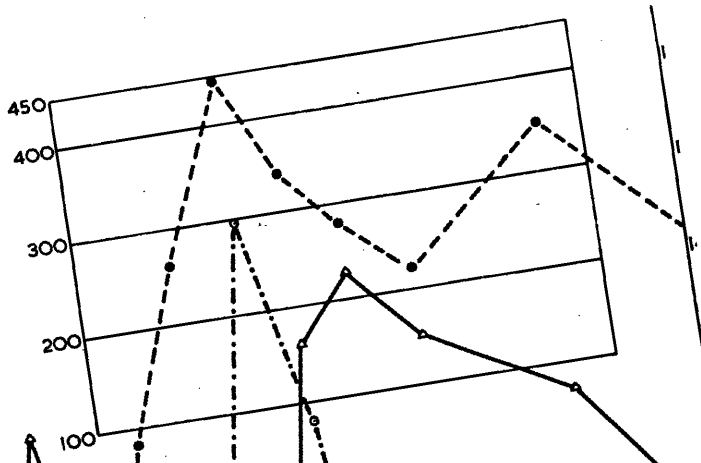
450
400
300
200
100

1955
180
160
140
120
100
80
60
40
20
0
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC

constantly being dispersed by the high tides and winds over an area considerably larger than that occupied by the larvae. Apart from these reasons, the short duration of both stages means that many could pass from the previous stages to the sub-:sequent stages between the times of sampling, thus giving a lower figure than would have been obtained if the sampling times had been more frequent.

It was not possible to distinguish with accuracy the first and second instars of C. circumscriptus from those of C. maritimus and therefore the numbers of these instars embrace both species. However, the third and fourth instars of C. maritimus comprise only 8.5% of the total number of larvae of these instars, so it is unlikely that the error in the numbers of the earlier stages would be much greater than this figure.

The graphs for 1954 and 1956 show a general similarity to one another, but the graph for 1955 is strikingly different. The exceptionally hot summer of that year may account for the differences. The summers of 1954 and 1956 were in the main cool and wet, and may be considered as nearer the normal for this part of the country.

The graph for 1954 shows a seasonal distribution of second, third and fourth instars, each with two clearly defined peaks, occurring in the earlier part of the summer and in the autumn. This/

This is repeated in 1955 and 1956 in the fourth instar only. In order to determine whether these peaks had any statistical significance an analysis of variance was made for the third and fourth instars over the whole sampling period (Appendix A, 2). The logarithms of the numbers to base 10 have been used in the analysis. The table shows that there are significant differences between the sampling times and also between sites.

As seen from the analysis of variance tables the standard error of the logarithm of a single observation is:

$\sqrt{0.2189} = 0.468$ for the third instar, and

$\sqrt{0.1723} = 0.415$ for the fourth instar.

Thus we find that the standard error of the difference between the logarithms of the numbers for any two dates (over the 6 sites) is, for the third instar:

$$\sqrt{\frac{0.2189}{6} + \frac{0.2189}{6}} = 0.270$$

and, for the fourth instar:

$$\sqrt{\frac{0.1723}{6} + \frac{0.1723}{6}} = 0.240$$

Making use of these standard errors of difference between means we find that, while the rise in numbers of third instar larvae occurring between 10th and 26th June, 1954, is statisti-:cally significant, the fall occurring between 26th June and 29th July/

29th July is not. Similarly the fall in numbers of fourth instar larvae occurring between 13th and 29th July, 1954, is not statistically significant. However, the falls in numbers of fourth instar larvae which occur between 19th July and 28th August, 1955, and between 18th July and 2nd August, 1956, are both significant.

2) Conclusions and discussion

It will be noted that the seasonal distribution of the immature stages of C. circumscriptus differs considerably from that of C. impunctatus as given by Hill (op. cit.) as well as differing in itself from one year to another. Hill found that C. impunctatus overwintered only in the fourth instar, but C. circumscriptus occurs during the winter mainly in the third and fourth instars, though a few second instar larvae are also found. Fourth instar and, as a rule, third instar larvae occur in greater or lesser numbers at all times of the year, and in general the pattern of the life cycle is less clearly defined than in C. impunctatus.

The earliest date on which an adult of C. circumscriptus has been obtained at Dumbuck was on 5th May. That this is probably very close to the time when the first emergences take place is indicated by the fact that the first pupae were obtained from mud samples on the 21st and 26th April (1956 and 1955 respectively). At outdoor mean temperatures of 10.5°C. - 13.7°C./

13.7°C. the period of pupation ranges from 9 to 23 days, so that in mild weather the first emergences would take place about the beginning of May.

The period of development of the eggs in the body of the female C. circumscriptus is not known. Megahed (1956a) states that in C. nubeculosus it is commonly 3 - 4 days under labora-:tory conditions and Hill (1947) states that 2 laboratory-reared females of C. impunctatus laid eggs 14 days after their first blood-meal, while in a single specimen of C. obsoletus the time taken was 17 days. Eggs of C. circumscriptus kept out of doors at a daily mean temperature of 13.4°C. hatched in 7 - 9 days.

The earliest date on which first instar larvae were ob-:tained in the field was May 30th in 1954. However, larger numbers of second instar larvae were also obtained in samples taken on that date. In 1955 and 1956 too, when first instar larvae were first observed, larger numbers of second instar larvae were also found. This indicates that the first instar is of short duration, so that a large number of those which had hatched out before the sampling times in question had already progressed to the second instar. Lawson (op. cit.) reports that the first instar of C. nubeculosus lasts for about three weeks, but for C. circumscriptus it seems probable that it only lasts for a few days at the most.

In/

In 1956 first instar larvae were not found until mid-July, when their appearance was also accompanied by rises in the numbers of the other instars. The reason for the late appearance of the new generation of larvae is difficult to find. Pupation had started almost three months previously, and, although the temperatures during May were low, the following month was no colder than the period preceding the appearance of the first instars the previous year. The sudden rise in the numbers of the other instars, coincident with the first appea-:rance of the first instar larvae, may be ascribed to the extremely hot weather of the previous three weeks. Due to the author's absence, samples were not taken during that period and it must be supposed that the unduly hot weather accelerated the development to such an extent that, by the time samples were taken on July 19th, many of the newly hatched larvae had developed to the fourth instar and, in some cases, had pupated. The heat-wave of 1955 was also without doubt responsible for the abnormally high numbers of larvae encountered during the subsequent months.

It had been thought that the apparently bimodal curves of the distribution of the second, third and fourth instar larvae during the summer of 1954 and of the fourth instar in 1955 and 1956 may be the result of the occurrence of either a double generation or, as suggested by Kettle (1950) for C. impunctatus, of/

of two biological races. The analysis of variance showed, however, that the troughs between the double peaks of third and fourth instars in 1954 were not significant, although there was significance in those occurring for the fourth instar in the years 1955 and 1956.

While the statistical analysis does not encourage the idea of there being two generations or two biological races, it would nevertheless appear that some factor is influencing the develop-:ment of the larvae so that their numbers show rises and falls at unexpected times. For, if according to the analysis of variance, the troughs shown between July and August, 1954, are in fact artifacts, this does not explain away the double peaks of the fourth instar in 1955 and 1956. It is also an un-:deniable fact that pupae were found all through the summer of each year in sufficiently high numbers to make it highly unlikely that they were those of the overwintering larvae. It therefore appears that the breeding of C. circumscriptus may be continuous, and that, given the right conditions, any part in the chain of events involved in the life cycle may take place at any time of the year. Temperature plays an important role in the development of all stages of the cycle and it is suggested that, while the majority of the new generation of larvae may not reach the fourth instar until the autumn or the following spring, as would appear to be the case in 1954, a substantial number, given favourable/

favourable temperature conditions, do, in fact, complete their life cycle and emerge as adults in the later part of the summer. These are likely to be the larvae which hatched earliest in the year. This suggestion is further strengthened by the fact that gravid females have been caught at Dumbuck as late as September 14th.

Varying temperature conditions may also largely account for the differences in larval distribution from year to year. While they may not be sufficient to explain fully the double peaks of fourth instar larvae in 1955 and 1956, or to explain the absence of parallel phenomena for the other instars, it is suggested nevertheless, that they constitute an important controlling factor which requires careful consideration in future investigations.

VI. THE EGG

A. Description (Fig. 15)

When laid, the eggs of C. circumscriptus are greyish-white in colour but within an hour of being laid this changes to a dark golden-brown. The average length is 459μ. They are banana-shaped, being often more or less curved, and taper gradually towards one end which is slightly more sharply pointed than the other. At the broadest part, about one third of the length from the blunt end, the average breadth is 65μ.

The surface of the chorion is covered with extremely small sucker-like structures, about 2μ in length. These are arranged more or less in longitudinal rows which sometimes coalesce with one another. They are more numerous than on the eggs of C. vexans and C. impunctatus, described by Jobling (1953) and Hill (1947) respectively, conforming more to Hill's description of the eggs of C. odibilis in this respect. They are not, however, arranged in regular lines, but instead are scattered irregularly along the rows (Fig. 16). Patel (1921) has suggested that these structures might act as floats but this view is not supported by experiment. If a piece of filter paper on which eggs have been laid is carefully covered with water the eggs, as a rule, remain adhering to the surface of the paper. They/

Fig. 15. The eggs of C. circumscriptus.

A. Unhatched eggs.

B. The chorion after hatching, showing the manner of splitting.

Fig. 16. Part of the surface of the chorion of the egg, showing the arrangement of the ansulae. (x 492)

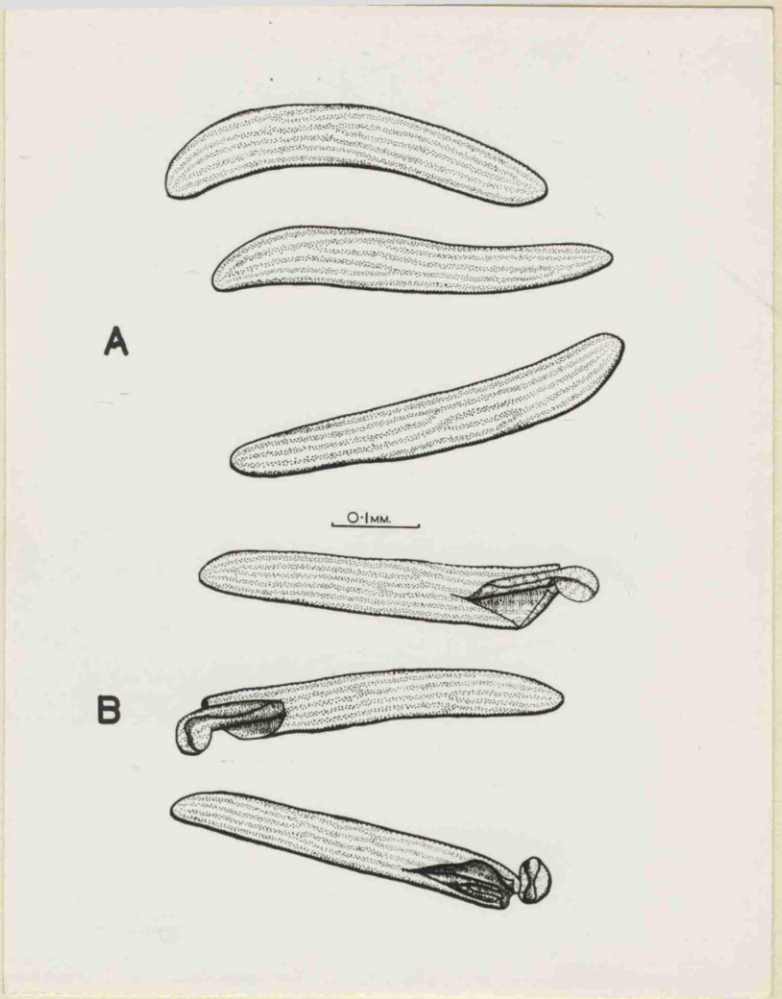

Fig. 15

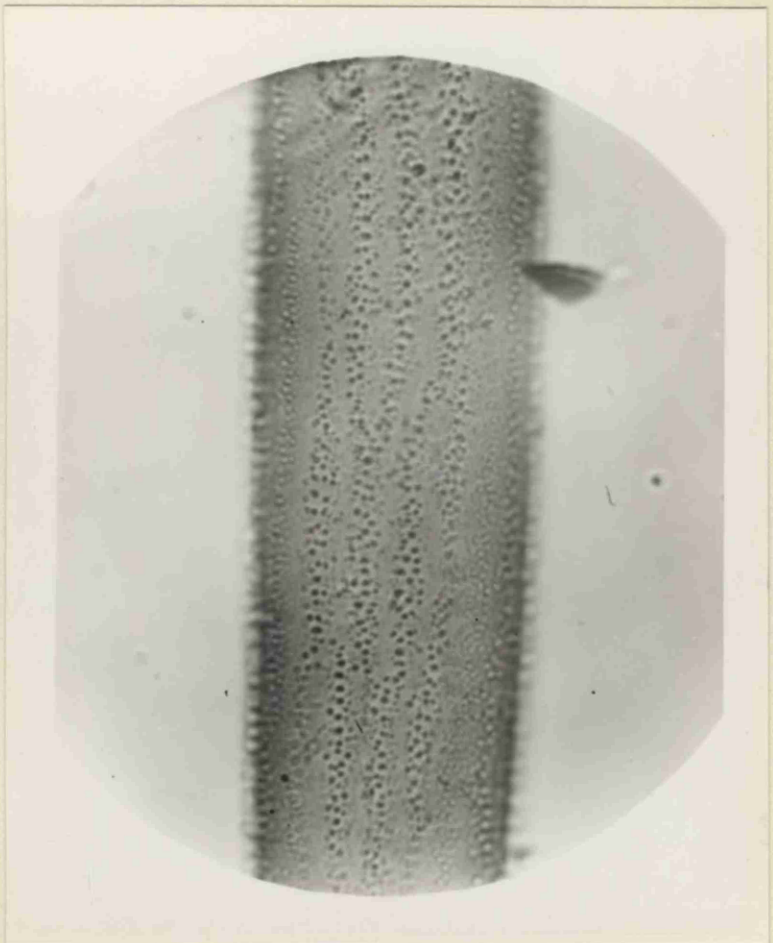

Fig. 16

Fig. 17. An egg of C. circumscriptus which was laid on mud. Particles of stone and debris may be seen adhering to the ansulae. (x 220)

Fig. 18. Histogram showing the distribution of the sizes of the egg batches of C. circumscriptus.

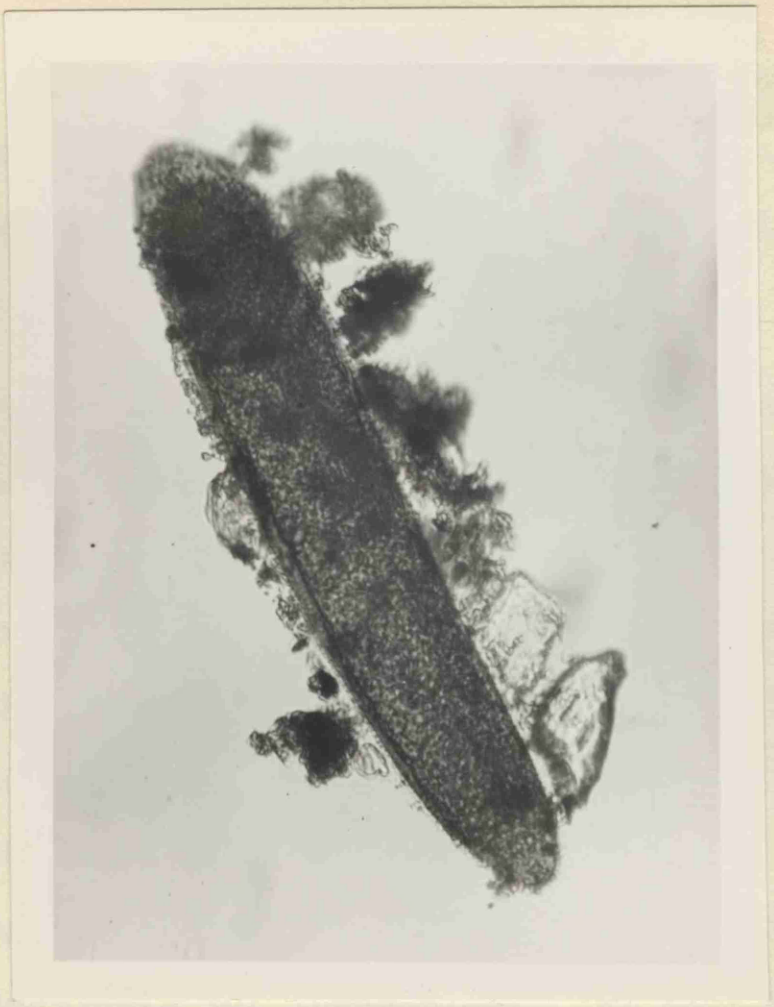

Fig. 17

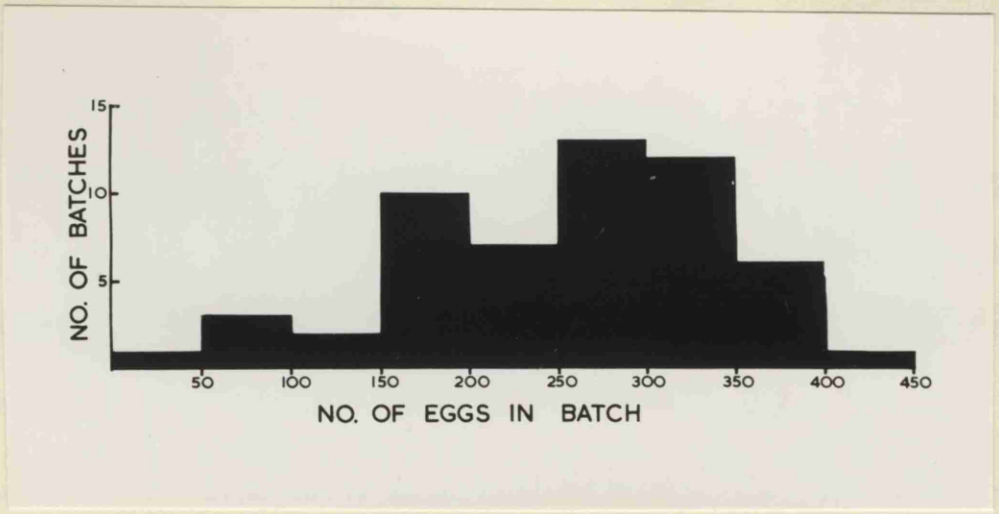
NO. OF BATCHES
15
10
5
50
100
150
200
250
300
350
400
450
NO. OF EGGS IN BATCH

Fig. 18

They will not float even when disturbed with a camel-hair brush. If disturbed before flooding the eggs float on the surface film and, if pushed under the surface with a brush, will sink immediately.

If the filter paper is carefully dissected away from around an undisturbed egg it may be observed that fibres of the paper remain adhering to the chorion. Also, when the eggs are laid on mud, small particles of soil, stones and debris are found attached to the chorion (Fig. 17). There can be no doubt, therefore, that the structures in question provide a means of adhering to the substrate, or particles thereof, thus preventing the eggs being washed away by rain or tides and deposited in sur-:roundings unsuitable for incubation. In view of this function it is proposed to call the structures ansulae (singular; ansula).*

It has been suggested that adhesion to the substrate may be effected simply by the entanglement of the ansulae with the fibres of the filter paper or, under natural conditions, with debris. In my view they are too short and set too closely to-:gether for this to be feasible, in the eggs of C. circumscriptus at any rate. Neither is it likely that they are suckers as their shape suggests. It is more probable that the distal surfaces/

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*I am indebted to Professor C.J. Fordyce, M.A., for his helpful advice in the coining of this name.

surfaces of the ansulae, when the egg is laid, are coated with some sticky material which is the means of securing the egg to the substrate. This material appears to be effective only if the substrate is not too wet. In one case, where it had been noted that the filter paper in the egg-laying tube was much more saturated than usual, the batch of eggs all floated when they were flooded. It may therefore be assumed that the gravid fe-:male, when selecting an oviposition site, must avoid places that are too wet as well as too dry. Further, the adhesive material is not effective a second time. If the eggs are dis-:turbed or transferred to another piece of filter paper they remain loose and will float if they are flooded.

Flooding, even lasting throughout the period of incubation does not affect the development of the embryos. Several batches of eggs have been covered with water soon after laying and hatched under the surface in the normal time without any ill-effects on the larvae, which were reared eventually to adulthood.

B. Oviposition

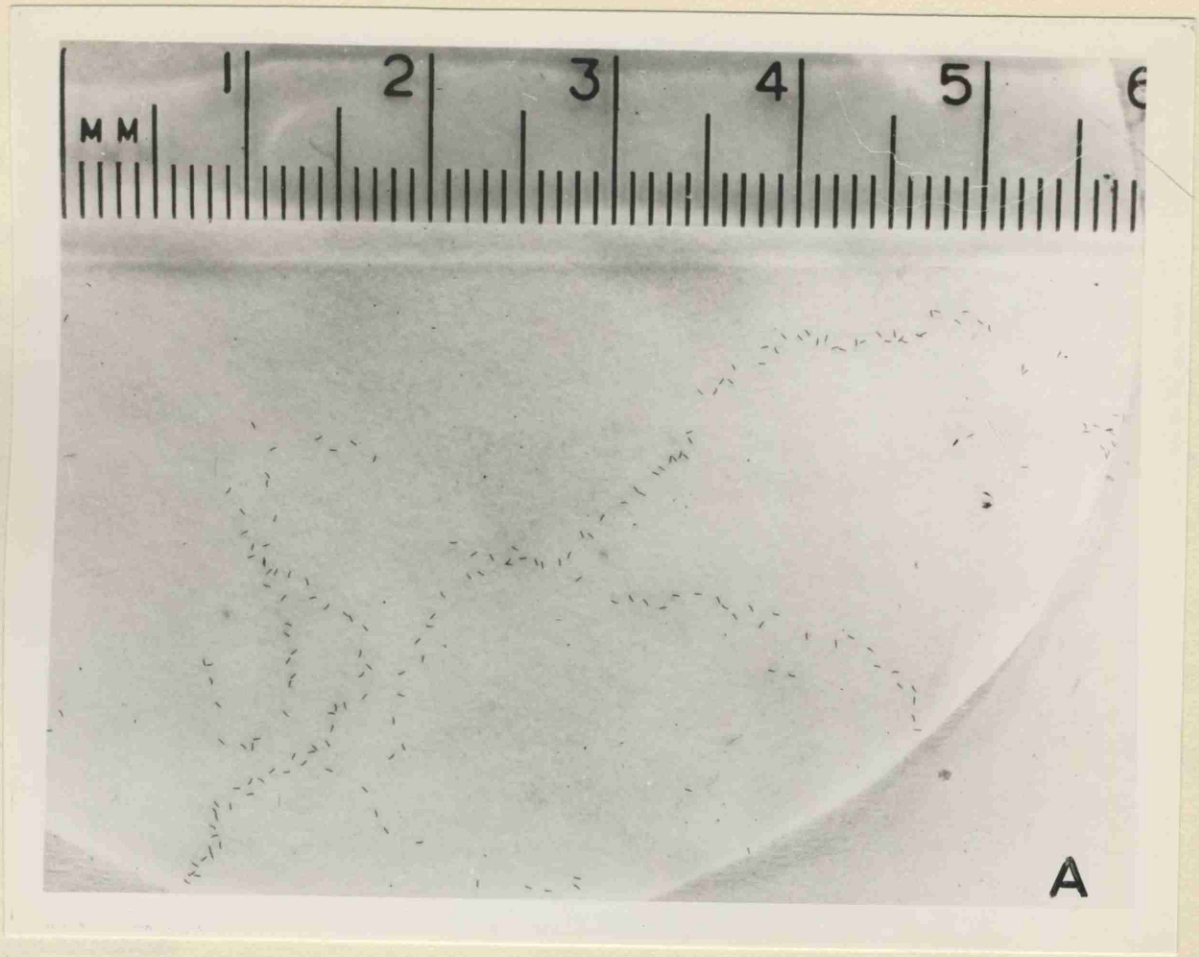
Megahed (1956a) has reported that female midges will not lay eggs on a dry surface. This has been confirmed in the present investigation on C. circumscriptus. Eggs have not been observed in the field but it may be assumed that they are laid on the same damp patches of mud in which the larvae are to be found./

found. These sites are more often than not exposed to direct rays of the sun, but the experiments carried out by Parker (1950) show that, as long as the eggs are kept in moist conditions they will survive any temperature normally experienced in this country.

In the laboratory eggs were laid by wild gravid female midges on damp filter paper. The eggs were not laid in any pattern as was sometimes observed by Hill in the case of C. impunctatus. The female midge sometimes walked slowly over the paper depositing her eggs here and there in her tracks. Sometimes she stood still and laid the eggs in a cluster (Fig. 19). The number of eggs laid by C. circumscriptus varied from 45 to 401, but commonly 150 - 350 eggs are laid (Fig. 18). Oviposition usually took place overnight and was completed by the next morning, but occasionally the eggs were laid in two lots with an interval of 1 - 2 days between them.

Hill found that the females of C. impunctatus usually died immediately after oviposition and that with C. obsoletus this was always the case. Parker (1950) reports that, among the species studied by him, the females died a few days after laying with the exception of C. obsoletus group, the females of which survived for five and a half weeks. (This was probably a different species from that studied by Hill.) With C. nubeculosus, Downes (1950) was able to obtain 5 successive batches/

Fig. 19. A. and B. The eggs of C. circumscriptus laid on damp filter paper. They are laid haphazardly in the tracks of the female as she walks over the paper or occasionally, when she stands still, in small clumps (B).



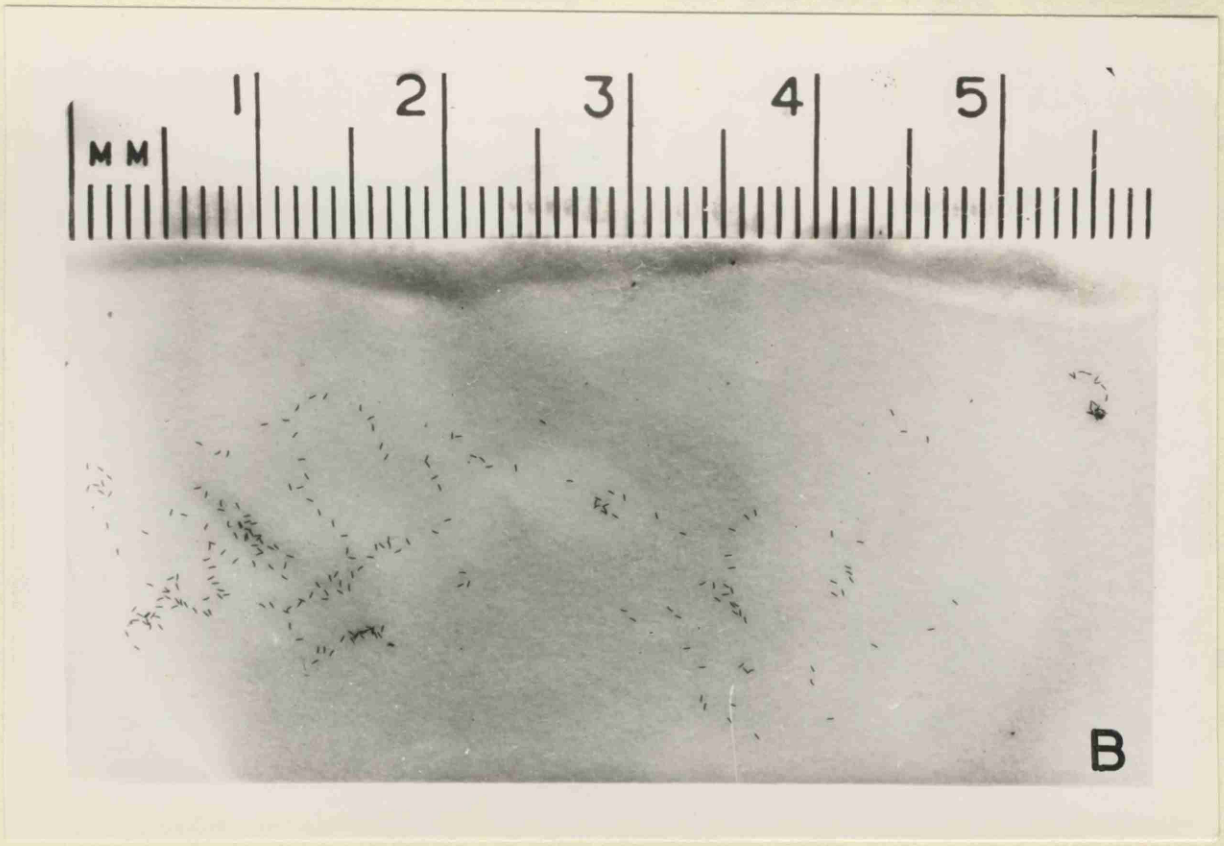


Fig. 19

batches of eggs by giving a blood meal after each batch had been laid. In the case of C. circumscriptus the females appeared to be in a weakened state after laying their eggs and, when replaced in oviposition tubes, often became stuck to the filter paper and died soon afterwards. Those flies which managed to avoid this survived for 3 - 10 days but never laid any more eggs. It is possible that they would have laid other batches if the correct food necessary for the development of the eggs had been known for this species and given to the females after they had laid. However, in view of the large number of eggs laid initially this is perhaps unlikely.

C. Hatching

Of the 55 batches of eggs laid in the laboratory an average of 88.7% hatched successfully. In the majority of cases hatching occurred at night and most of the eggs had hatched by the following morning. In a few cases only did eggs begin to hatch during the day and the hatching of the whole batch extended sometimes over 24 hours. In batches of eggs which were incubated out of doors at a mean temperature of about 13°C. hatching was extended over 2 - 3 days. There is no doubt that the temperature affects the time taken for a batch of eggs to hatch. Jobling (op. cit.), working on C. vexans, showed that at about 6°C. hatching was incomplete after 4 days and ceased altogether at 4°C.. When taken into the laboratory at 16°C. hatching/

hatching was completed in about 3 hours.

The process of hatching has not been observed but eggs examined after hatching (Fig. 15B) show that, in this also, C. circumscriptus closely resembles C. odibilis (see Hill, 1947). A split, partially encircling the blunt end of the egg, almost breaks off a small cap, and from this another split, longitudinal and slightly spiral, extends about one third of the way along the length. The cap is often missing altogether, probably, as Hill reports, having been torn away by the emerging larva.

VII. THE LARVA

A. Description (Figs 20 - 23)

A detailed description of the larva will not be given here as Tokunaga (1937) and Kettle & Lawson (1952) have already described the fourth instar. It is only necessary, therefore, to make a few remarks to supplement their descriptions.

The mean head measurements for each instar are given in Table IV. It will be noted that the sizes for the fourth instar are smaller than those given by Kettle & Lawson, as is also the head ratio. The larvae measured were taken from Dumbuck salt-marsh, while those of Kettle & Lawson were from Dorset and Devon. It is not improbable, therefore, that there is some variation in head sizes from one locality to another. No systematic measurements were made on larvae from other salt-marshes, but larvae taken from the marsh at Kincardine Bridge, Stirlingshire, gave rise to typical C. circumscriptus adults although their heads were smaller than the sizes given by Kettle & Lawson, being about the size given for C. salinarius.

The first instar larva has no pigmentation and therefore, if caught in the field in the presence of other species, is impossible to identify with certainty. On the ventral side of the prothorax is a retractable proleg, used for locomotion.

TABLE IV

Mean head sizes of the larvae of C. circumscriptus with standard deviation from mean. (Measured in microns.)

	1st Instar	2nd Instar	3rd Instar	4th Instar
No. of larvae	18	45	50	50
Head length	70 ± 2.5	103 ± 4	153 ± 6	212 ± 6
Head breadth	51 ± 1.5	73 ± 2	109 ± 4	160 ± 7
Oral ring breadth	37 ± 1.5	53 ± 2	75 ± 3	107 ± 5
Head ratio	1.37 ± 0.05	1.42 ± 0.06	1.41 ± 0.08	1.33 ± 0.06

The larvae of C. circumscriptus. (x 50)

Fig. 20. Newly hatched first instar.

Fig. 21. Second instar.

Fig. 22. Third instar.

Fig. 23. Fourth instar.

Fig. 20

Fig. 21

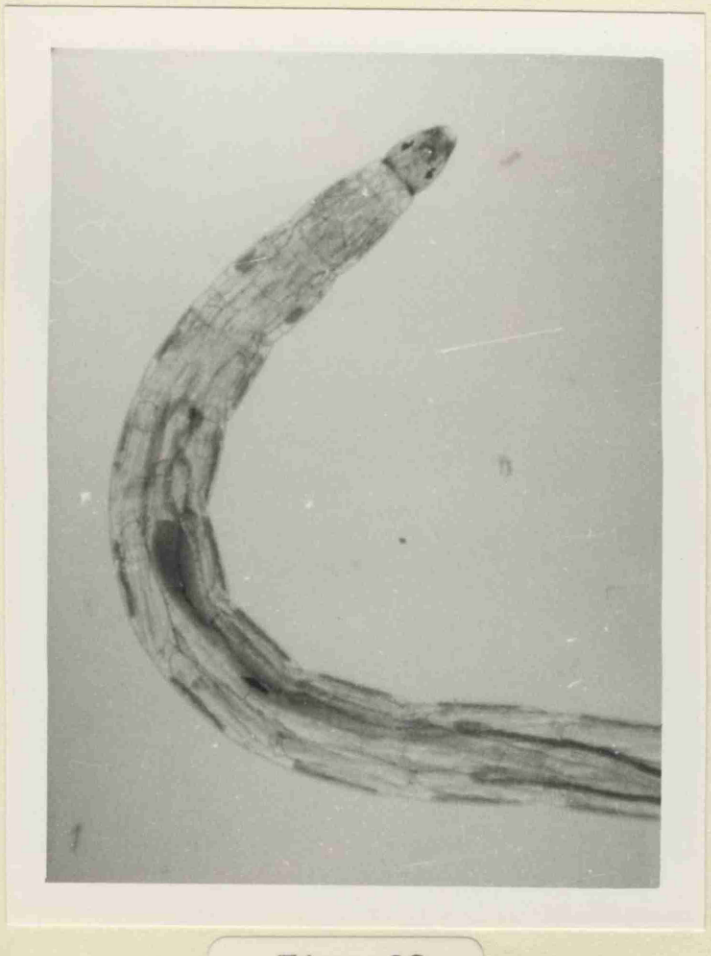


Fig. 22

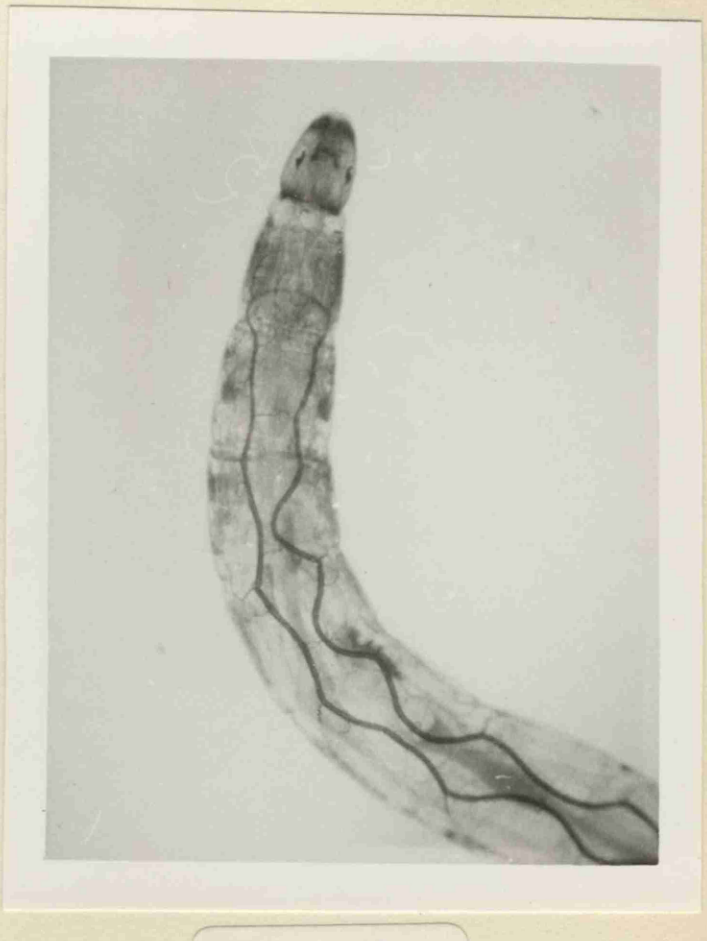


Fig. 23

This organ is further dealt with below.

In the second instar larvae the beginnings of thoracic pigmentation may be seen. If present, this is, as a rule, very faint and cannot be used for purposes of identification.

In the third instar larvae pigmentation is usually clear enough to make a positive identification. In both third and young fourth instars, however, the degree of pigmentation varies considerably. In some specimens it may be so faint as to make it difficult to distinguish the larvae from other species. In older fourth instar larvae the pigment is more concentrated and the colouring more pronounced. The diamond-shaped patch of pigment on the meso-thorax described by Kettle & Lawson, although present, cannot always be discerned, especially in third instar larvae. Sometimes in the third instars and young fourth instars the pigment is distributed over the thorax in discrete patches and spots, but this does not persist to the later stages of the fourth instar where it is distributed more or less uniformly.

Recently a number of specimens in the third and fourth in-:star have been observed, in which the pigmentation of the prothorax was completely lacking. The colour pattern of the remainder of the thorax was typical of C. circumscriptus. These abnormal larvae were not numerous, two or three occurring in a collection of more than 1,200 larvae, and it is likely that they/

they are merely freak mutations. None have so far been reared, so it is not known if the adults show any abnormal features.

B. The Larval Habitat

The larvae of C. circumscriptus are, as a rule, found in the mud of salt-marshes but specimens have also been obtained from farm-yard mud at Chideock, Dorset, which is about a mile from the coast (Kettle & Lawson, 1952). The Dumbuck salt-marsh and the particular part of it where large numbers of larvae were found has already been described. The larval distribution is not uniform. On occasion more than 1,600 larvae of this species alone have been obtained from a single sample taken from the edge of the pool but elsewhere, even in sites which were to all appearances identical, the population was very sparse, 12 - 15 larvae or less being recovered from a sample.

The factors which favour the breeding of large concentra-:tions of larvae are not fully understood but a rough guide may be given to the type of site which is most likely to yield appreciable numbers of C. circumscriptus larvae, though it must be emphasised that larvae will not necessarily be found in every site which fits the description. The sites at Dumbuck (Fig. 24) which were most productive were patches of soft wet mud, which were bare of vegetation except, perhaps, for algae, and which contained plenty of rotting vegetation with the usual characteristic smell. The mud on the surface is dark brown in colour/

Fig. 24. Part of the sampling area at Dumbuck. The breeding sites of the larvae of C. circumscriptus are seen on the near edge of the pool.

Fig. 25. An atypical breeding site of C. circumscriptus on the Montrose Basin. The soil is sandy and firm.

Fig. 24

Fig. 25

colour but underneath it is black, due to the lack of oxygen. In spite of this, however, as has been pointed out earlier, an average of more than 20% of the larvae may be found more than 1 inch below the surface. One may wonder how the larvae live under such conditions. It is possible either that they may build up an oxygen debt while below the surface, or that they may be able to carry a reserve of oxygen in their tracheae. In either case they would obtain fresh supplies when they came to feed at the surface.

While the larvae are quite able to swim when placed in water they have seldom been seen to do so in the field. They are found only in small numbers in mud permanently covered with water, and then only if large populations are to be found at the edge of the pool. This is shown by the results of sampling under water at intervals of 2 feet from the edge and on the edge itself (Table V). Areas covered with dense vegetation where the mud was tightly interlaced with roots also yielded, as a rule, few larvae of C. circumscriptus. They are not found on the bare mud flats on the river side of the marsh, or in the mud of the natural drainage channels and ditches where the water runs quite strongly with the tides. It is reported by Hull et al. (1934), however, that the larvae of C. dovei occur in these last-named situations.

These criteria were followed with some success when investigations/

investigations were made at other Scottish salt-marshes but on a number of occasions large numbers of larvae were found in sites which were quite different. This was particularly the case when the 'typical' sites were not present in the area. Thus at Tynninghame the only site found to contain C. circumscriptus larvae was in a thick clump of reeds where, because of the high proportion of sand it contained, the mud was quite firm. At Montrose highly productive sites were found consisting almost wholly of sand which was very firm underfoot (Fig. 25). The larvae were also found near Dingwall in sites with a fair covering of grass.

TABLE V

The number of larvae of C. circumscriptus recovered from samples taken at the edge of the pool and under water 2 feet and 4 feet away from the edge.

Edge	2 ft from Edge	4 ft from Edge
111	31	1
147	29	2
89	22	0
46	4	0
12	0	0
121	24	3
114	36	3
22	2	0
31	2	0

Samples of mud were taken from salt-marshes in different parts of Scotland and sent for analysis to try to determine what chemical factors, if any, influenced the distribution of the larvae, but from the mass of data obtained no correlation was found between the size of the larval population and chemical characteristics of the samples.

The salinity of the breeding sites in the Dumbuck area usually lies between 19.0°/oo and 23.0°/oo (cf. sea-water 35.02°/oo). After heavy rain the salinity has been found to drop to 14.47°/oo. In warm weather salinities up to 24.21°/oo have been recorded and this is probably increased further during fierce heat. It is unlikely, however, that the larvae have to endure extremes of dilution or concentration since the area is frequently covered by high tides whose salinity varies between 16.82°/oo and 23.67°/oo.

C. Osmo-regulation

The larvae of Culicoides have exceptional powers of osmo-regulation. Thorpe (1927) found a larva of C. nubeculosus, a species normally occurring in mud contaminated with sewage, in a pool of sea-water, and reared it to adulthood in fresh water. In the salt-marshes C. circumscriptus larvae live in conditions where the salinity varies constantly. They have been maintained and reared successfully in the laboratory in fresh water and will also live in sea-water without any ill-effects. When placed/

Fig. 26. Lateral view of the tail of a larva of C. circumscriptus, showing the anal papillae ex-:truded in a dorso-ventral fan. Some debris is clinging to the tips of the papillae. (x 98).

Fig. 26

Fig. 27. Lateral view of first instar larva with proleg extruded. The head is slightly twisted. (x 270).

Fig. 28. Lateral view of the first instar larva with proleg invaginated. (x 540).

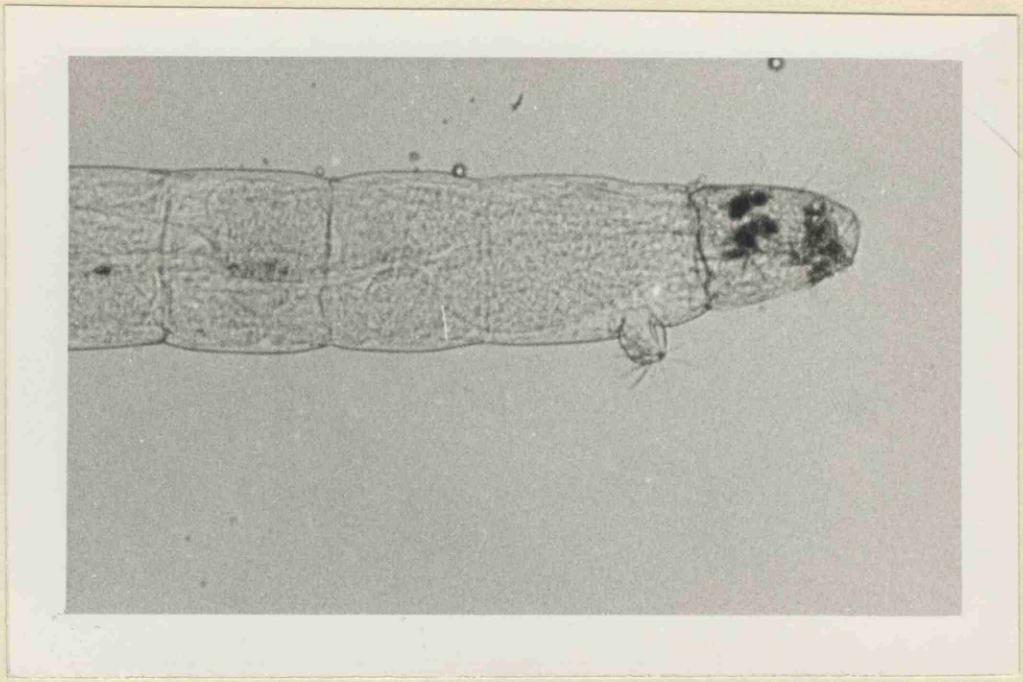


Fig. 27

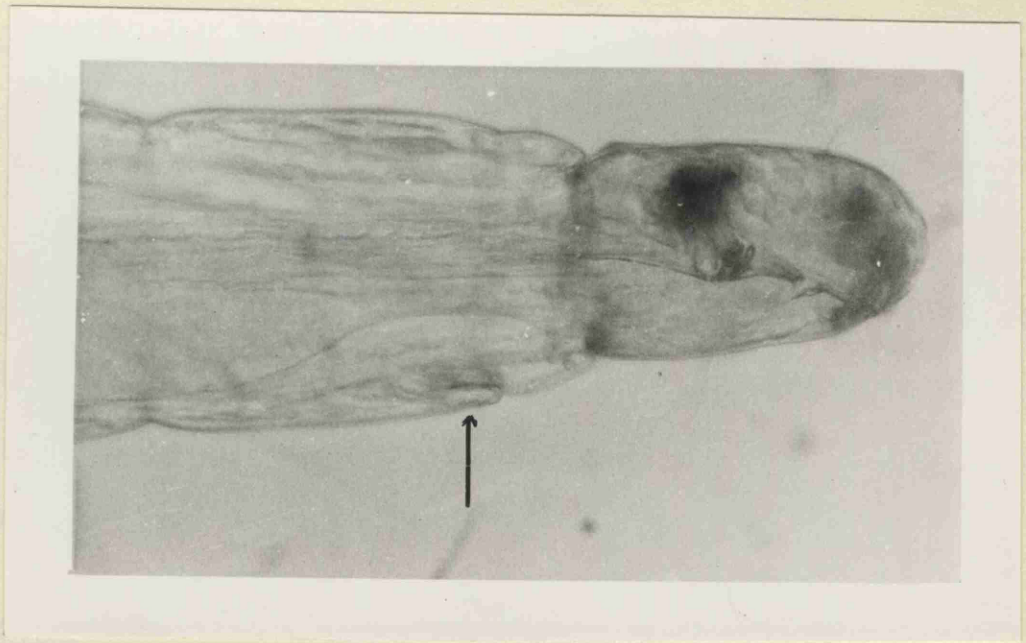


Fig. 28

placed in water whose salinity was 1½ times that of sea-water they did not display their usual activity but nevertheless remained in this medium for 8 days without any deaths having occurred. Dove et al. (1932) have reported that C. dovei is found in areas which on occasion are twice as saline as sea-water, but when C. circumscriptus were kept in such concentra-:tions they died within 12 hours.

The function of osmo-regulation is now thought to be perfor-:med by the anal papillae (Fig. 26). Thorpe (1933) and Wigglesworth (1933) have shown that similar organs in aquatic larvae are not respiratory in function as had been previously supposed. Lawson (op. cit.) has reported that the anal papillae of C. nubeculosus are selectively stained by silver nitrate. This has also been shown by Koch (1938) to occur with the anal papillae in Culicidae, which both he and Wigglesworth (1938) have proved to be osmo-regulatory in function.

In Culicoides larvae the anal papillae are four transparent bifurcate structures which may be extruded fan-wise from a dorso-ventral slit in the anal region. If the larva is dis-:turbed they are immediately withdrawn into the anus.

D. Locomotion

The second, third and fourth instar larvae are all very active in their movements. When on the surface of the mud they move/

move over it with a snake-like motion. When placed in water they can swim rapidly, bending the body into a bow first to one side then the other. Using a stroboscope, it was found that in the fully grown fourth instar larvae these movements took place at a rate of about 550 per minute but were more rapid in smaller larvae. First instar larvae, on the other hand, are much more sluggish. They can swim but seldom do so. On a solid substrate they do not slither over it as do the older instars, but haul themselves along, gripping the substrate by means of the proleg which is situated on the ventral side of the neck region and is armed with stout, slightly curved setae (Fig. 27). This organ, which is lost in later instars, was first described in C. nubeculosus by Lawson (op. cit.) who states that in the newly hatched larva it is invaginated slightly into the neck. Larvae have, however, been observed to retract it fully into the neck (Fig. 28) and later evert it again. The larvae were well-grown and it would appear, therefore, that this is a voluntary action which may take place at any time.

Accounts of climbing of aquatic plants by Culicoides larvae have been given by Patton & Evans (1929) and Taylor (1944) who say this is achieved with the aid of hooked spines on the ninth segment. Lawson & Kettle (1952) report signs of climbing the vertical walls of glass tubes by C. salinarius, C. pulicaris and C. punctatus,/

C. punctatus, but not by other species. This has not been observed to occur voluntarily in the case of C. circumscriptus but if a larva is placed on a wet vertical glass surface it is able to move over it in any direction. The spines mentioned by the first-named authors are not present and it is doubtful, in fact, if they occur in any species of Culicoides.

E. Larval Migration

1) Methods and results

During the periodic sampling of sites in the larval habitat it was found that there was a considerable fluctuation in the number of larvae recovered from samples taken from the same site, which would not be accounted for by seasonal variation. From the figures obtained it appeared that changes in the distribu-:tion of the larvae are constantly taking place.

In order to discover whether the larvae migrated actively through the mud, sheets of aluminium were pushed into the mud to a depth of 3 - 4 inches to 'fence off' an area about 4 feet in length in the shape of an elongated lozenge (Fig. 29). At either end this opened into the gap in a sheet of aluminium bent almost to form a cylinder, which was likewise pushed into the mud. On two occasions two of these traps were left for a fortnight in the larval habitat orientated at right angles to one another. On the second occasion the length of the trap was reduced to 2 feet. If active migration did in fact take place/

Fig. 29. Plan of the trap used to test for larval migration. Samples were taken from the spots lettered.

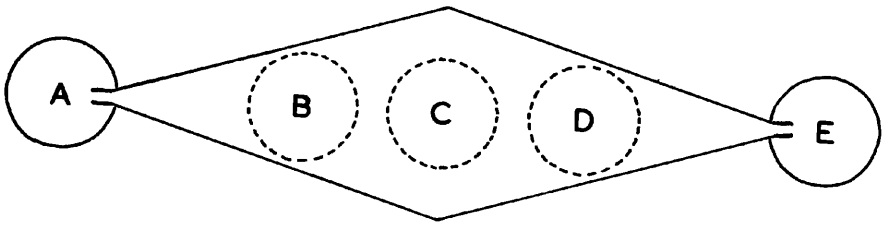


Fig. 29

place it was hoped that the migrating larvae would be guided by the walls of the trap to be eventually concentrated in one or other of the cylinders. At the end of the fortnight the mud contained within the cylinders was taken and the numbers of larvae found in them were compared with the numbers found in samples of similar size taken from inside the area of the traps (Table VI). The data obtained from the traps 4 feet in length did not show any signs of concentrations of larvae in the cylinders. The second experiment, using traps 2 feet in length, showed some comcentration of larvae at one or the other end of the traps.

TABLE VI

The numbers of larvae of C. circumscriptus recovered from the trap shown in Fig. 29.

	A	B	C	D	E
4 ft. Traps	16	19	11	9	17
	37	45	26	37	58
2 ft. Traps	3	92	*	90	112
	123	27	*	57	65

*In the smaller traps it was only possible to take two samples from the area of the lozenge.

Also investigated was the possibility that the high tides may affect the larval distribution by disturbing them and dispersing them, passively or otherwise to other areas. Three sites were selected and on the date of a high spring tide, four adjacent samples of mud were taken from a square marked off on each site. Two of these were taken before high tide and the other two after the water had receded. It was found that at one site the mean number of larvae found in the samples taken before high tide was very close to that found after ebb-tide (96.5 and 100 respectively). At the second site the mean number showed an increase from 81 to 120, and at the third site it decreased from 132.5 to 66. When the water was at a depth of about 2 feet over the area, sweeps were made with a plankton net but no swimming larvae were obtained.

In a further experiment to trace the movements of larvae in the field, a number of fourth instar larvae were made radioactive by keeping them for 65 hours in water containing Phosphorus-32 at a concentration of 0.5µc/ml.. On the marsh at Dumbuck the top inch of mud was removed from an area covered by the sampling tool and 300 radioactive larvae were placed on the surface. This number was used since it is the maximum one might normally expect to find within the area of a single sample, and abnormal effects due to overcrowding might thus be avoided.

A/

A few minutes after depositing the larvae in the mud, the site was scanned with a Geiger counter and it was found that they had spread into a circle of about 6 inches in diameter at the centre of which the reading on the ratemeter was about 18,000 counts/minute.

One hour afterwards six samples of mud were taken in a circle 12 inches from the centre of the site and the larvae obtained from them were tested with the Geiger counter. No radioactive specimens were found. The larvae were returned to the spots from which they had been obtained. 9 hours after the larvae had been deposited, after the area had been covered by a high tide, samples were again taken at the same distance from the central point. No radioactive specimens were recovered. The area of radioactivity had not increased and the activity at the centre was the same as previously recorded.

Samples were subsequently taken from a circle of the same radius round the centre of the area of deposition site at intervals of 1, 6, 11, 16 and 20 days from the beginning of the experiment. From the 6th day onwards samples were also taken from a circle of 18 inches round the site. In all cases the non-radioactive larvae were returned to the spot from which they were taken after testing with the counter. By the 20th day, the activity at the centre of the site had fallen to 1,000 counts/minute but the area of activity had not increased. By this time it/

it was becoming difficult to distinguish radioactive larvae and therefore the experiment was ended at this point. The data obtained from the samples are shown in Table VII.

2) Conclusions and Discussion

The results of the experiments involving the use of traps and of radioactive larvae indicate that some movement of the larvae from place to place does occur, but that this is very slow and does not cover great distances. Only in the smaller traps did there appear any signs of migration. Similarly, of the 13 radioactive larvae recovered in the course of about three weeks, only two were recovered 18 inches from the centre of the deposition site, the rest having been found only about 12 inches away. It would appear that the main bulk of the radioactive larvae remained within the original area in which they had first been deposited. This is indicated by the fact that the area of radioactivity round the central point of deposition did not show any sign of increasing in size during the time of the experiment.

Observation of the behaviour of larvae on the surface of culture pots also shows that the movements of larvae tend to be limited. As a rule the larvae either remain close to their burrows or keep the end of the abdomen inserted in the hole, and feed in the area immediately round about. Occasionally a larva may move an inch or so to a new feeding area but this happens relatively seldom.

TABLE VII

The numbers of fourth instar larvae recovered from samples taken at distances of 12 inches and 18 inches from the site where 300 radioactive larvae were deposited on 26/4/56. The number of radioactive larvae recovered are shown in brackets.

Date	12 Inches from site						18 Inches from site					
	1	2	3	4	5	6	I	II	III	IV	V	VI
27/4/56	74	106	91	26	43(1)	102(1)						
2/5/56	96	84	85	49(1)	61(2)	69(1)	34	64	81	71	47	98
7/5/56	123	127	104	90(1)	71	63	29	66	109	62	80	130
12/5/56	82(2)	132	79	64(1)	60	71	45	74(1)	134	87	68	106
16/5/56	34(1)	79	61	96	49	92	49	89	73(1)	77	22	121

This conclusion would seem to be contradicted by the rapid fall-off in activity measured at the centre of the site. This was more rapid than could be expected as a result of natural decay of the isotope. At the same time however, the audible response obtained from the radioactive larvae at the end of the three weeks was much less than had been obtained at the end of four weeks in the preliminary laboratory experiments. It can only be supposed, therefore, that in their natural environment greater feeding activity may have resulted in a more rapid excretion of the radioactive phosphorus than had occurred in the laboratory experiments. This would be dispersed by the high tides.

It will also be noticed from the sampling data in Table VII that the numbers of larvae obtained from the sampling sites over a period of three weeks show fluctuations similar to those earlier described. In the absence of any evidence of large-scale movements, the writer is at a loss to account for these. Sampling error may partly explain some of the fluctuations but the differences are so great that there can be no doubt that some other unrecognised factor is involved.

The tides themselves do not appear to alter the distribution of larvae. The results of taking adjacent samples at the time of the high tides do not apparently support this assertion but these results are rendered inconclusive by the fact that the differences/

differences in mean numbers of larvae taken before and after high tide are no greater than those found in adjacent samples taken at the same time. When the tides cover the area they do so with no noticeable disturbance of the mud and it is unlikely that any passive dispersion of the larvae could occur in this way unless the water was whipped up by an extremely strong wind. That the larvae do not take advantage of the flooding to swim to other areas has been shown by the negative results achieved with the plankton net and also by the fact that no signs of dispersal were noted among the radioactive larvae when, soon after they were deposited in the mud, they were covered by a high tide.

Hull et al. (1934) report a seasonal migration of the larvae of C. dovei, which seek the shade of trees during the hot season and return to the marshes in winter. They are also of the opinion that the larvae are dispersed by high tides and, in seepage marshes, by being carried along under the soil surface by the tidal water.

F. Behaviour Towards Light Stimuli

1) Experiments and results

Painter (1926), referring to C. furens and C. phlebotomus, and Kettle & Lawson (1952), in their work on the immature stages of British midges, state that the larvae are negatively phototactic. Hull et al. (1934) report similar behaviour in the larvae/

larvae of C. dovei. Carter et al. (1920) state that the larvae of C. acraensis are positively 'phototropic' when kept in jars in the laboratory.

In the present work, when the larvae of C. circumscriptus were removed from mud and placed in a dish of water, it was observed that the great majority exhibited a negative phototaxis while a few were either positively phototactic or did not seem to be attracted one way or the other. When the dish was turned through 180° the larvae immediately reorientated themselves, those of the photonegative group swimming away from the light source and the photopositive larvae towards it. If they were kept in the dish for a few days it was seen that the number of photopositive larvae had increased while that of the other group had correspondingly decreased indicating a change in taxis on the part of some larvae from photonegative to photopositive. Again, when the dish was turned through 180°, the larvae of the two groups exchanged their positions.

To examine this phenomenon experimentally, a "Perspex" trough, measuring 24 inches by 4 inches by 2½ inches and marked off lengthwise into quarters, was placed in a darkened room with no source of light other than a 60 watt bench lamp placed a short distance from one end. The end of the trough farthest from the light was painted on the inner side with flat black paint to prevent the reflection of the light by the "Perspex". The/

The trough was filled to a depth of about 1 inch with water, and 182 larve, which had been taken out of their mud several days before, were introduced into the centre and left for 6 hours. At the end of that time it was found that 109 larvae (59.9%) had migrated to the illuminated quarter of the trough, 21 larvae (11.5%) had migrated to the quarter farthest from the light, and 52 (28.6%) remained in the middle half.

This procedure was repeated with 58 larvae which had been kept in a completely dark room for 3 days beforehand. In this case a still larger proportion (86.2%) showed a photopositive response.

Following the results of the previous two experiments, a series of investigations were made to show how the change of response to light took place over a period of time and to endeavour to discover the cause of the changes. Larvae, which had been recovered from mud 24 hours or less beforehand, were left in the trough for 1 hour. This was found to be sufficient time for the majority of them to move to one or the other end of the trough. At the end of this time the larvae from the end quarters of the trough were transferred into the middle of separate troughs and left for another hour. This gave the larvae a second opportunity to orientate themselves and made it possible to discount any which moved in the opposite direction to which they had done before. The great majority of larvae

moved/

moved to one or other end of the trough and only these have been considered in the experiment. Those which remained in the centre of the trough at the end of each test were ignored. Of these, only a few seemed to be normally active larvae, the rest being dead, moribund, hampered by adhering debris, or, in a few cases, had pupated.

Tests of this type were made periodically over a number of days which varied with each experiment. After each test all dead or pupated larvae were removed and the remainder, unless otherwise stated, transferred to a dish of fresh water. The results of each experiment, where they do not appear with the text, are shown in graphic form in the Appendix.

In experiments 1 - 4, mixed batches of third and fourth instar larvae of C. circumscriptus were used. In experiment 3, the dishes containing the larvae were kept in complete dark-:ness between tests. It was noted that in these and subsequent experiments there was a steep rise in the proportion of positively phototactic larvae over a period of about a week (Figs 30 and 31) by which time the curves began to level off in the case of fourth instar between 70% and 90%. The curve of positively phototactic third instar larvae reached 100% in experiments 3 and 4 (Fig. 31 and Appendix B, 1), but since large numbers of these larvae were not available at the time, the results shown towards the end of the experiments may be somewhat distorted./

Fig. 30. The typical reaction to light of a group of unfed fourth instar larvae of C. circumscriptus over a period of time after extraction from mud, showing the change which occurs on feeding with blow-fly larvae (F). (Derived from the results of experiments 1 - 10 as shown in Appendix B.)

Fig. 31. The typical reaction to light of a group of unfed third instar larvae of C. circumscriptus over a period of time after extraction from mud. (Derived from the results of experiments 1 - 4 as shown in Appendix B.)

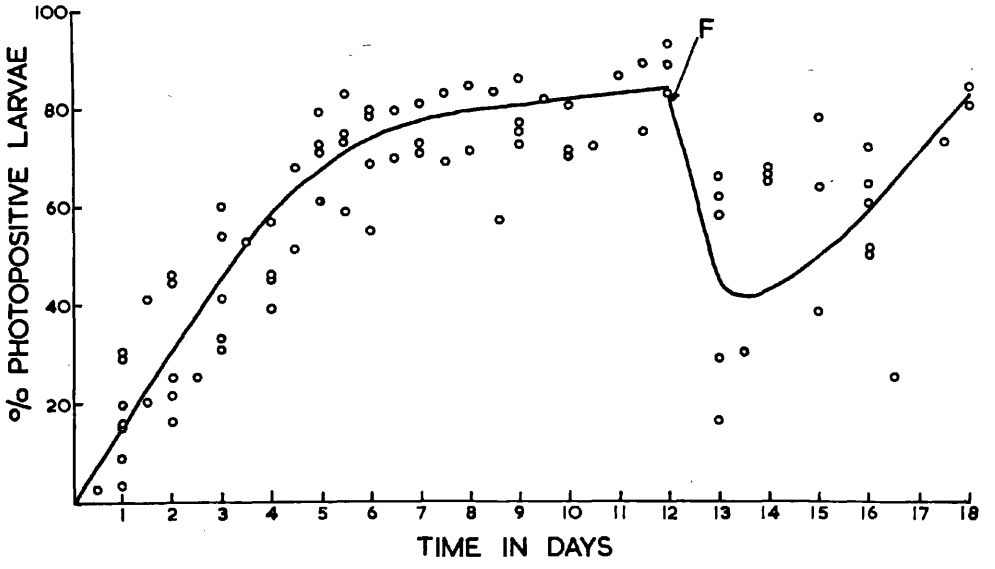

% PHOTOPOSITIVE LARVAE
100
80
60
40
20
F
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
TIME IN DAYS

Fig. 30

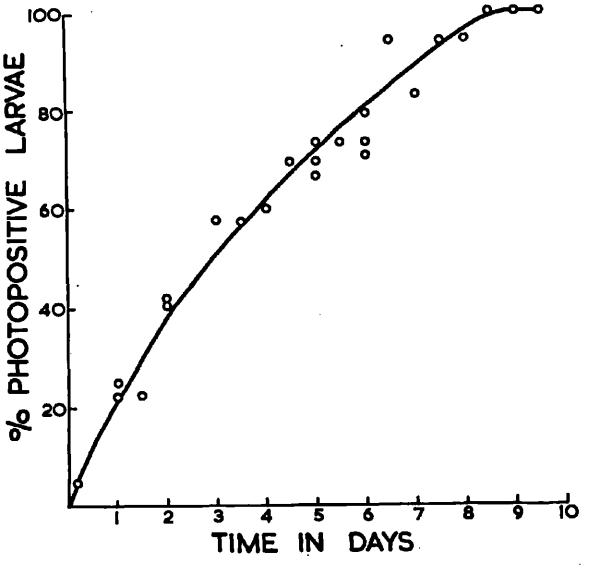

% PHOTOPOSITIVE LARVAE
100
80
60
40
20
1 2 3 4 5 6 7 8 9 10
TIME IN DAYS

Fig. 31

distorted.

In experiments 2 and 4, when the number of photopositive larvae had reached a high level, food consisting of pieces of blow-fly larvae (unstained) was dropped into the dishes and the larvae allowed to feed for 6 days, after which a further light test was made. Using this type of food the larvae were able to feed while still being exposed to light. Thus the change of environment was limited to one factor only. It was found advisable to aerate the water in which they were kept between tests, otherwise many of them died. Also when tests were made subsequent to feeding it was found that many of the larvae were hampered in their movements by adhering pieces of blow-fly tissue. This difficulty was partly overcome by putting a thin layer of clean sand in the dishes, which had the effect of rub-:bing off some of the material. Before tests were made the larvae were carefully extracted from the food with hooked pins and any large adhering shreds of food removed.

When tests were made after a 6 day feeding period it was found that in most cases there was a marked drop in the propor-:tion of photopositive larvae.

In experiments 5 - 10, tests were made with unfed larvae for longer periods, after which they were fed with blow-fly larvae vitally stained with Waxolin Red, which were renewed daily/

daily along with the water in the dishes. After 1 day's feeding, the proportion of photopositive larvae dropped by 23 - 60% after which, in spite of fresh supplies of food, sooner or later it began to rise again (Fig. 30). From all 6 experiments the average of 70.7% of photonegative larvae and 64.0% of photopositive larvae had visible food in their alimentary canals. Since without doubt some of the larvae fed on unstained tissues these figures may represent a slightly low estimate of the actual number of fed larvae.

In a further set of experiments (Nos 11 - 15) larvae were kept in dishes of water for about a week after which time they were tested. Those which were photopositive were separated from the rest, and in experiments 11 and 12 were put into dishes containing larval rearing medium. In experiments 13 and 14, they were put into mud which had previously been powdered and incinerated. All organic matter was thus destroyed but the pH remained the same. Since the incinerated mud did not have the same flocculence as the rearing medium, in experiment 15, it was mixed with enough "Dyox" powder (probably methyl cellulose) to give an approximately similar texture. Some Trypan Red vital stain was added and the larvae introduced into the mixture.

The larvae were recovered at intervals and tested for phototaxis after which they were put back into fresh medium. The results (Fig. 32) showed that when the larvae were kept in rearing/

rearing medium, the proportion of photopositive larvae dropped to between 20% and 30% where it remained as long as the experiment lasted. The proportion of photopositive larvae with material in the alimentary canal was 38.4% while that of the photonegative larvae was 74.6%. In the experiments in which the larvae were kept in incinerated mud, the proportion of photopositive larvae remained at or very near 100%. This was also the case where the incinerated mud was mixed with "Dyox". Of the larvae kept in plain incinerated mud very few had any material in their alimentary canals. Of those kept in the mud/"Dyox" mixture, all had material in their guts a few days after the beginning of the experiment. When a number of these were dissected this material proved to be almost exclusively "Dyox".

When larvae were kept in media of this type there was often a considerable loss of specimens during the course of the experiment. This was not generally due to mortality but mainly to the method employed to recover the larvae before tests. Since they were required alive and in good condition, a water spray was not used. The medium containing the larvae was simply mixed with magnesium sulphate. As a result some lumps of medium were not broken up and the larvae remained buried in them. Although attempts were made to recover these larvae many were obviously overlooked.

Tests carried out on late fourth instar larva immediately after/

Fig. 32. The reactions to light of three groups of previously starved fourth instar larvae of C. circumscriptus after placing in different media. The numbers of larvae at the beginning and end of each experiment are given.

Fig. 33. The reaction to light of a group of unfed late fourth instar larvae of C. circumscriptus over a period of time after extraction from mud (Experiment 17). The numbers of larvae at the beginning and end of the experiment are given.

Fig. 34. The reaction to light of a group of unfed fourth instar larvae of C. maritimus over a period of time after extraction from mud (Experiment 18). The numbers of larvae at the beginning and end of the experiment are given.

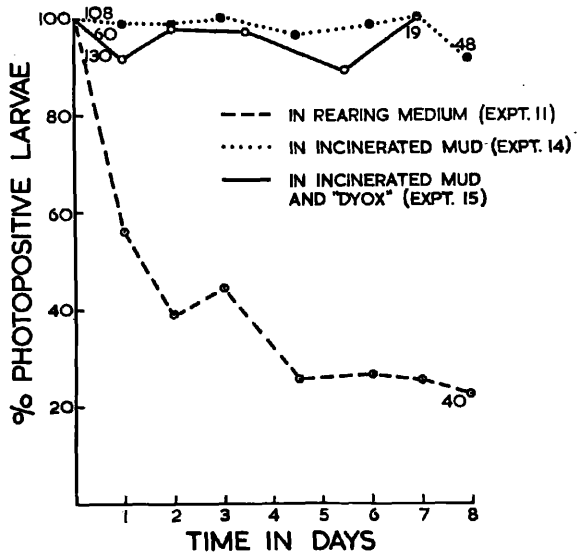


Fig. 32

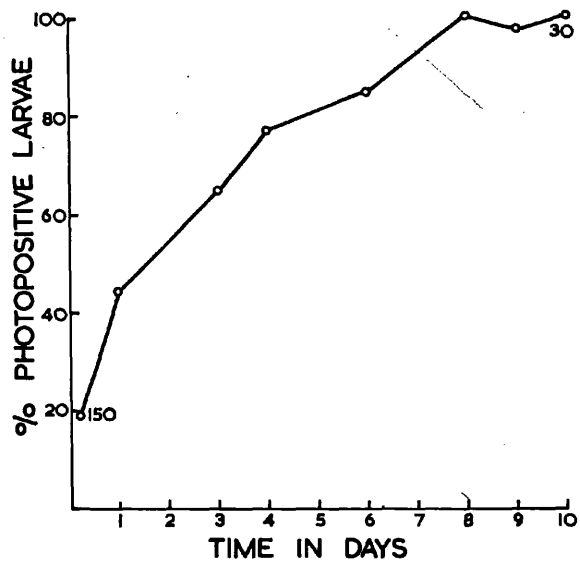


Fig. 33

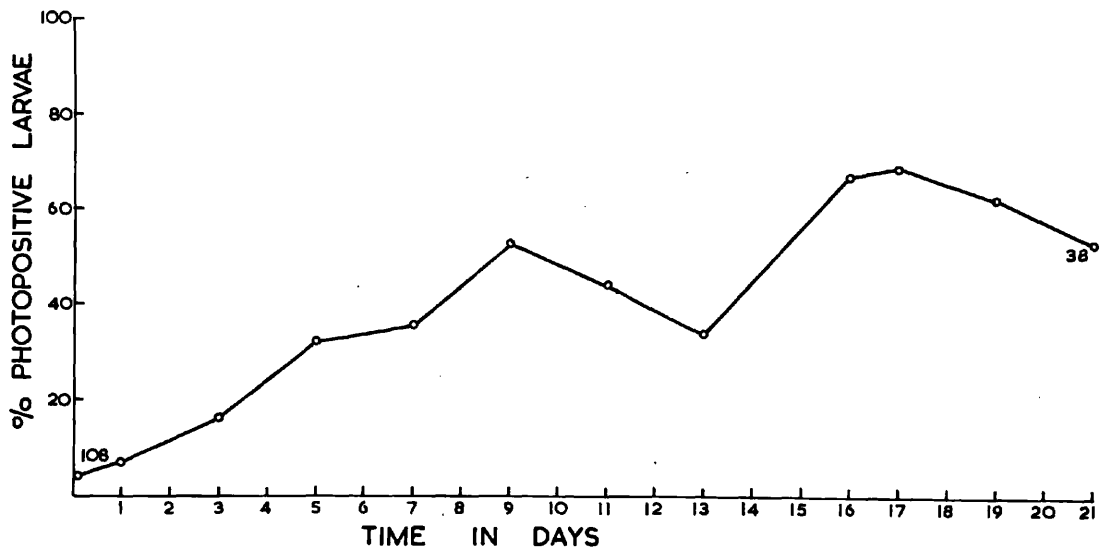


Fig. 34

after recovery from mud (Experiments 16 and 17) show that the proportion of photopositive specimens rises somewhat more steeply than is the case for younger larvae of the same instar and does not flatten out but continues until all are photopositive (Fig. 33). During these experiments a large number of pupa-:tions took place. In experiment 16, the dishes containing the larvae were kept in absolute darkness between tests.

Similar tests were carried out on batches of fourth instar larvae of C. maritimus (Experiments 18 - 21). These showed a marked difference in behaviour towards light (Fig. 34). At first there was a rise in the proportion of photopositive larvae, but this was much more gradual than in the case of C. circum-:scriptus and did not reach the same high figures. Afterwards the proportions fluctuated to such an extent that when food was provided the drop in the curve could not be considered significant.

2) Conclusions and discussion

When the larvae of C. circumscriptus are extracted from the mud of their habitat the majority are negatively phototactic, but when kept in fresh water without food of any sort, many of them become positively phototactic. The percentage of larvae which exhibit this reversal increases rapidly for 6 - 7 days, thereafter, as a rule, remaining fairly constant at a level of somewhere between 70% and 90% of the total number. There is no

difference/

difference in this pattern when the larvae were kept in darkness between tests so that it is unlikely that the change in taxis of larvae exposed to light between tests can be explained as facilitation to light conditions. This is confirmed by the fact that reversion to photonegative behaviour can be brought about by feeding although the larvae are kept in the light.

When food is given to the larvae there is a rapid drop in the percentage of photopositive individuals. If the food consists of blow-fly larvae, as in experiments 5 - 10, this low proportion is not maintained but sooner or later begins to rise again, in spite of fresh supplies of food being given, until once again it reaches the level which existed prior to feeding. If, however, the larvae are kept in rearing medium between tests as in experiments 11 - 12, the percentage of photopositive larvae continues to fall until a level between 20% and 30% is reached and is maintained. In contrast, if larvae which have become photopositive are kept in mud containing no nutritive organic matter this does not occur.

It is apparent from the above experiments that the behaviour of the larvae of C. circumscriptus towards light stimuli is profoundly affected by hunger. This is borne out by the relative numbers of fed and unfed larvae in both photo-:positive and photonegative groups. In the experiments invol-:ving the use of culture medium the average proportion of the photopositive/

photopositive groups whose alimentary canals contained material at the time of testing (38.4%) was much lower than in the case of the photonegative groups in which 74.6% showed evidence of having fed. When the food consisted of blow-fly larvae the difference was not nearly so clear-cut (64.0% and 70.7% respectively) and it must be concluded from this and also from the fact that reversion to photonegative taxis was only tem-:porary, that the food involved was inadequate or at the most only temporarily adequate for the nutritional requirements of the majority of the larvae.

It will be noted, from the figures given above that, after feeding, a considerable proportion of the larvae which had material in their guts still remained photopositive and conversely, many of the apparently unfed larvae were photonega-:tive. A number of reasons may be suggested to explain this apparent anomaly. In the first place it is no doubt partly due to experimental error. Secondly, the material ingested by these photopositive larvae may not have been digested at the time of testing, or may not have contained enough nutritive matter to effect a reversion to photonegative behaviour, or may not have been of the type required for this to be achieved. Thirdly, the photonegative larvae which were apparently unfed may, in fact, have ingested colourless food or may have defaeca-:ted all waste material from a previous meal and were not yet hungry/

hungry enough to show photopositive reactions.

The negative phototaxis of the well-fed larvae and its reversal to a positive phototaxis when starved has a biological significance under natural conditions. Loeb (1889) has shown that the young caterpillars of the moth Porthesia (= Euproctis) chrysorrhoea exhibit a positive phototaxis before feeding, which is lost after they have fed. The larvae under natural conditions are directed by this initial taxis to the leaves at the top of the branches which constitute their food. Brandt (1937) demonstrated a similar phenomenon with the caterpillars of Lymantria monacha. Neither of these cases illustrate the complete and immediate reversion of taxis on feeding which has been shown to occur in C. circumscriptus and one must turn to the Platyhelminthes for a more analogous example. Beauchamp (1933) has shown that when Planaria alpina is developing sexually it is positively rheotactic and becomes negatively rheotactic on completing the sexual cycle. This change may also be brought about by starvation and if the starved animals find food they again become positively rheotactic.

The ecological significance of the behaviour of C. circumscriptus towards light can be appreciated by studying their feeding habits. Observation has shown that a large part of the larval life is spent burrowing below the surface of the mud where protection and some food may be had. It has

been shown by Megahed (1956a) and confirmed in the present work that feeding also occurs on the surface. Thus it would appear that while the larvae are generally photophobic and spend most of their time under the surface, the need to feed at the surface brings about a reversal of this reaction. When the larvae are fully fed they again become negatively phototactic and retreat to safety below the mud. The feeding of the larvae is further discussed in the following section.

When photopositive larvae were kept in the mixture of incinerated mud and "Dyox" it was found that eventually all of them ingested "Dyox" but, nevertheless, did not exhibit the reversion of taxis typical of fed larvae. It is evident from this that the mechanism controlling their reactions to light is not influenced by the mere presence or absence of material in the alimentary tract, but that reversion from photopositive to photonegative behaviour will only take place if the material ingested can be utilised as food by the larvae. Conversely, it may be assumed that the reversal from photonegative to photo-:positive behaviour is initiated not by an empty gut, but by what may be called 'physiological' hunger.

It is not fully understood why under starved conditions the proportion of photopositive larvae usually becomes stable between 70% and 90% and rarely approaches 100%. At present it can only be supposed that a small number of larvae may obtain food/

food by eating their dead or dying companions. The latter were removed at each test but mortality occurred continually and increased with the time of the experiment so that some food was always available.

In experiments 16 and 17, all the larvae eventually became photopositive and the rate of reversal was somewhat quicker than in other experiments (Fig. 33). The larvae used were in the latter stages of the fourth instar and pupation occurred con-:stantly throughout the experiment. In these cases, therefore, it must be assumed that as well as hunger another factor is also helping to bring about the change from negative to positive phototaxis. It has been observed that before the larvae pupate they come to the surface of the mud and lie in regions covered only by a thin film of water. It would appear that this is achieved by a reversal in phototaxis probably initiated by the physiological changes connected with pupation.

All the third instar larvae in experiment 3 and 4 became postively phototactic, but, because of the small numbers used in the experiment no reliable conclusions may be drawn from the data obtained. During the experiment, however, many became fourth instar larvae and the possibility that a temporary re-:versal of taxis may take place before ecdysis should not be overlooked. Up to the present it has not been possible to pursue this line of investigation further.

Considering/

Considering the conditions under which the larvae of C. circumscriptus live it must be assumed that, since the light could not penetrate more than a small fraction of an inch below the surface, their behaviour towards light stimuli can be nothing more than a maintenance reaction with the function of keeping them at the surface until they have obtained adequate nourish-:ment. The unfed larvae below the surface must initially be brought within reach of the light by some other tropism. While no attempt has been made to investigate this point, it has been noted during the experiments that the photopositive larvae not only swam towards the light but many of them also were found swimming upwards towards the surface of the water at the illumi-:nated end of the trough. It may be that the larvae can appreciate changes in pressure in the tracheae so that, when it is necessary for them to feed on the surface, they move upwards to levels where that pressure is at a minimum. Alternatively the reaction which brings them to the surface may be simply a negative geotaxis, though it is not known whether the larvae possess any sense organs which might receive gravitational stimuli. Brandt (op. cit.) has shown that the young unfed larvae of Lymantria monacha are negatively geotactic as well as positively phototactic. It may well be that the behaviour of the larvae of C. circumscriptus in relation to feeding is the result of a similar combination of reactions.

Finally,/

Finally, the differences in behaviour between C. circumscriptus and C. maritimus are worthy of mention. In tests made 24 hours after extraction from the mud only an average of 6.0% of the larvae of the latter species were photo-:positive compared with an average of 17.7% in the case of C. circumscriptus. The increase in the number of photopositive larvae is much slower and lasts longer after which the curve begins to fluctuate. Also, the proportion of photopositive larvae seldom becomes as large as was found in C. circumscriptus. This difference in behaviour may be correlated with feeding habits. The larvae of C. maritimus are much more plentiful in places where there are reeds whose roots make the ground much firmer than on the sites favoured by C. circumscriptus. They show, moreover, a greater tendency to burrow deeper into the mud, an average of only 51.2% occurring in the top inch as com-:pared with 78.5% in the case of C. circumscriptus. It may be the case, therefore, that under natural conditions a larger proportion of their food is found below the surface of the mud, and thus, their reactions to light stimuli are not so well-marked and consistent.

G. Feeding

1) Experiments and results

It has been shown in the preceding section that positive phototaxis in the larvae of C. circumscriptus is brought about by/

by starvation and that reversion to a photonegative taxis of most of the larvae may be effected by feeding with culture medium. This reversion was also demonstrated by feeding with the meat of blow-fly larvae, though in this case it was only temporary for the majority of the larvae, indicating that for them this type of food did not adequately provide the nutrition required.

In an attempt to establish the types of food taken by the larvae a further series of light experiments were conducted. In each of these a number of larvae which had previously been starved and found to be positively phototactic were put into various media. In every case the larvae were recovered at intervals and tested for response to light, after which they were returned to fresh medium.

In experiment 22, the larvae were kept in mud, newly re-:constituted from the powdered mud of the type used in the making of culture medium, without the addition of charcoal and yeast. During the tests most of the larvae maintained their photopositive behaviour. Very few showed any indications of having fed (Table VIII).

In experiment 23, the larvae were kept in the supernatant fluid which was decanted from 'mature' culture pots. Tests showed that there was initially a gradual drop in the proportion of photopositive larvae to 51.5% (Fig. 35). This was not

TABLE VIII

The percentages of larvae of C. circumscriptus which had visible material in their alimentary canals after being kept in various media between tests for reactions to light.

Medium	Photopositive Larvae	Photonegative Larvae
Water with stained blow-fly larvae	64.0	70.7
Culture medium	38.4	74.6
Incinerated mud	4.3	6.3*
Incinerated mud and "Dyox"	88.0+	83.0+
Newly reconstituted mud	0.6	4.2*
Newly reconstituted mud and supernatant liquid from culture pots	49.8	88.3
Supernatant liquid from culture pots	60.7	47.2
Surface of culture medium	71.8	72.2
Sub-surface culture medium	63.4	77.5

*Figures unduly high due to the small number of larvae which became negatively phototactic in these experiments.

+In the latter part of this experiment all the larvae in both groups had material (almost exclusively "Dyox") in their guts.

Figs 35 - 37. The reactions to light of groups of previously starved larvae of C. circumscriptus when placed in different media. The numbers of larvae at the beginning and end of each experiment are given.

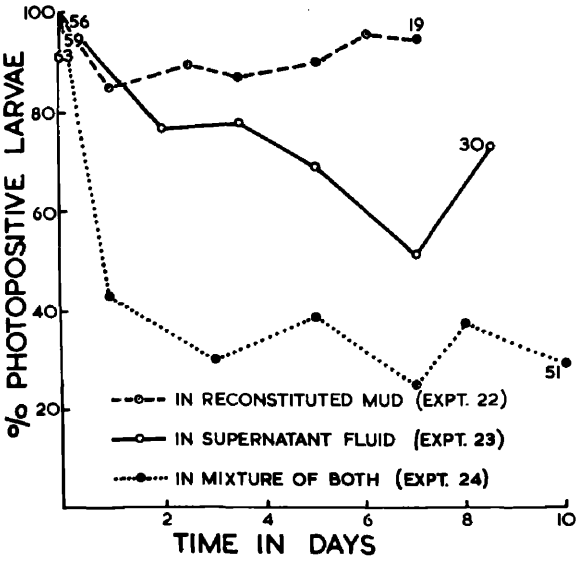


Fig. 35

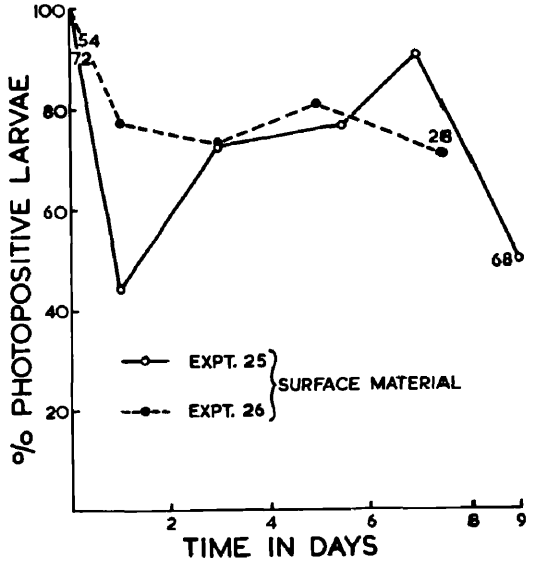


Fig. 36

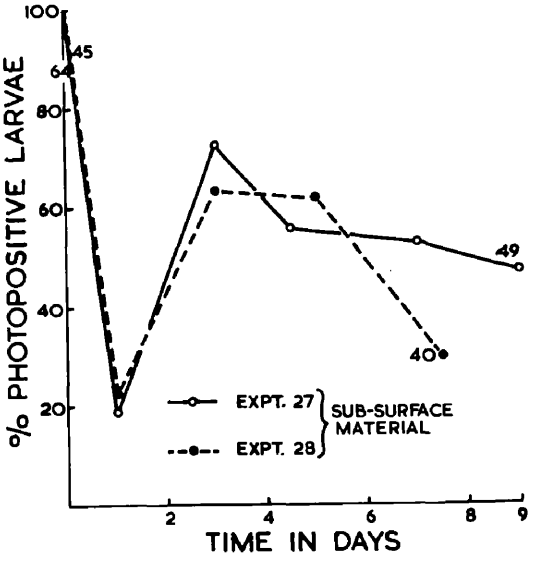


Fig. 37

maintained however, and at the end of the experiment this figure had risen again to 73.3%. Examination of the larvae showed rather surprisingly, that a larger percentage of the photopositive larvae had material in their alimentary canals than was the case among the photonegative larvae.

In experiment 24 powdered mud, reconstituted with the supernatant fluid from culture pots, was used. In this case the proportion of positively phototactic larvae fell quickly (Fig. 35) and fluctuated throughout the experiment between about 25% and 40%. There was a considerable difference in the pro-:portions of photopositive and photonegative larvae which had material in the alimentary canals.

In experiment 25 the larvae were given the opportunity to feed on material carefully scraped from the surface of 'mature' culture pots and mixed with a little of the supernatant fluid and in experiment 26, they were placed in a small sieve of 80-mesh wire gauze which was pressed on to the surface of the culture medium so that some of the material on the surface was squeezed through the mesh. Light tests showed that in the first case, though the percentage of photopositive larvae dropped initially (Fig. 36) it rose subsequently before dropping again. In experiment 26 the drop was not so great and the proportion of photopositive larvae maintained itself between about 70% and 80%. There was little difference in either experiment/

experiment in the percentage of photopositive and photonegative larvae with material in the alimentary canals, this being high in both cases.

In both experiments 27 and 28, the larvae were placed in mud taken from the bottom of the culture pots and mixed with tap water. In each the percentage of photopositive larvae dropped quickly only to rise and fall again. There was an appreciable difference in the percentage of larvae of both groups which had material in the alimentary canals but these percentages were nevertheless high in both photopositive and photonegative groups.

2) Conclusions and discussion of experiments

In the experiments described above and in the previous section only those in which the food offered was culture medium or powdered mud reconstituted with the supernatant fluid from 'mature' culture pots did the majority of the larvae revert to photonegative taxis which was maintained throughout the experiment. From this and the fact that there was a consider-:able difference in proportions of photopositive and photonega-:tive larvae with material in their guts, it may be assumed that these are the most adequate of the media which have been tested.

The larvae would not feed on newly reconstituted mud. It would thus appear that the ingredients on which they feed are present/

present or become edible only after the period of maturation to which the culture pots are subjected. When the mud powder was reconstituted with the supernatant liquid from culture pots, feeding and reversion of taxis took place. This would indicate that the food ingredients necessary for this to occur are present in the supernatant fluid, yet when photopositive larvae were put into this alone, only 50% of the larvae, at the most, became photonegative and only temporarily at that.

From the result of this last experiment it was thought that the food sought by photopositive larvae lay on the immediate surface of the mud. An attempt to demonstrate this was made in experiments 25 and 26, but here again, the majority of the larvae failed to show a permanent reversion to photonegative behaviour.

Paradoxically, when larvae were offered mud taken from the bottom of the culture pots, there was an immediate drop in the proportion of photopositive larvae after which fluctuations occurred. Since some of the material ingested consisted of green algae, it must be assumed that the medium, while being taken from the culture pots, became contaminated with material which had probably oozed from the surface beforehand, so that it retained, in part at least, the characteristics of 'whole' culture medium.

Megahed (1956a) has described the feeding of C. nubeculosus as seen on the surface and the behaviour of C. circumscriptus agrees in general with his description. However, he also states that the feeding of the larvae is concentrated on the surface or the top few millimetres of the mud. There seems little doubt, however, that a considerable amount of feeding also takes place below these levels. It has been shown by Kettle & Lawson (1952) and confirmed in the present work, that a substantial proportion of Culicoides larvae are found well below the surface of the mud. My own observations on the larvae of C. circumscriptus in culture pots indicate that at any given time only a small fraction of the total number of larvae in the pots occur on or just under the surface. Since the larval stage in insects is generally that in which feeding is almost constantly taking place it is reasonable to suppose that the midge larvae also do so while in the deeper levels of the mud. Larvae which have been newly recovered from marshes often have brown material in their alimentary canals and, while some of this may have been ingested on the surface, it is significant that those larvae which were kept in mud taken from the bottom of culture pots ingested more of this brown material than those kept in material taken from the surface only.

It has been suggested in the previous section that the positive phototaxis which occurs in starving larvae enables the larvae/

larvae in nature to supplement its diet with material found on the surface of the mud. Under natural conditions such larvae would have already fed on sub-surface material and after satisfying their requirements again revert to photonegative behaviour and descend to the lower levels of the substrate. Why then, do the larvae, having been given material taken from the surface, not show this change in taxis as clearly as those which were kept in complete culture medium?

It is suggested that the reason for this unexpected behaviour lies in the fact that the larvae used in the labora-:tory experiments had been starved completely and had no food at all in their alimentary canals. Such a contingency would not arise under natural conditions where they would have had available plenty of sub-surface food before becoming positively phototactic and would have reverted to negative phototaxis before this was expended. It would seem, therefore, that the need for surface food which brings about the positive phototaxis continues to operate because the larvae are also starved of sub-surface food. Although it would seem logical that such a need would be satisfied by a photonegative taxis this does not in fact occur since the circumstances do not apply in their natural habitat. Starvation of any kind, therefore, would bring about a positive phototaxis.

3) <u>General discussion on feeding</u>

When/

When examined immediately after the larvae are recovered from mud, their alimentary canals are seen to contain material which varies in colour from cream to dark brown. The alimentary canals of many specimens were dissected out and squashed on a slide, but in no case was it possible to recognise with any degree of certainty the material they contained.

It has been assumed that darkly coloured contents consisted of mud or the organic debris present in the habitat and this is borne out by the investigations of Mayer (1934a) who has shown that coarse detritus and diatoms comprise the main bulk of the food of one group of midge larvae.

When the larvae are kept in water which contains green algae the appearance of green matter in the gut shows that these are ingested. This is also seen in larvae reared in culture medium which has a growth of algae and is in this case mixed with brown material. Algae are reported as a constituent of the food of various species by Bequaert (1924), Painter (1926), Leathers (1922)*, Lang (1931), Mayer (1934a) and Séguy (1950). However, when larvae of C. circumscriptus feed on green algae, faeces of this colour may be seen in the rectum, as has been reported/

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*Leathers' identification of the larvae he studied is disputed by Mayer (1934a) who identifies them not as Culicoides sp. but Dasyhelia (= Tetraphora) sp.

reported in C. nubeculosus by Megahed (1956a), who concludes that some at least must be indigestible and is not used by the larvae. This view is supported by the facts that C. circumscrip-:tus does not thrive in a culture containing mostly green algae and that both this species and C. nubeculosus can develop nor-:mally in medium containing no algae.

It is also noteworthy that when starving larvae of C. circumscriptus were placed in the supernatant fluid of culture pots, which was rich in green algae and flagellates, it was in the photopositive groups that a greater percentage of larvae had ingested green material. As a rule, in the feeding experiments, the greater percentage of larvae which had swallowed material was found in the groups which had become photonegative (see Table VIII). This reversal of the usual state of affairs also indicates that algae are at least not an important part of the diet.

Carnivorous feeding by Culicoides larvae has been reported by Pratt (1907), who states that Ceratopogon (= Culicoides) guttipennis was seen feeding on cast skins, dead larvae of mosquitoes and Eristalis sp., and a dascyllid beetle. Lutz (1912) states that some species feed on the decaying food of crabs. Thomson (1937) and Hill (1947) are of the opinion that the larvae are cannibalistic.

Carnivorous/

Carnivorous feeding has also been observed in C. circumscriptus. For experimental purposes the larvae were kept for a varying number of days in dishes of mud together with larvae of other types. (See following section relating to predators.) On recovery many of the latter were found to be dead or missing altogether. Empty cuticles of the latter were recovered which were being gnawed by midge larvae. Dead Ephydrid and Limnobiid larvae were found with small holes in the body wall and in some cases midge larvae were seen with their heads thrust into the holes, vigorously attacking the internal tissues. In others, midge larvae had wholly entered the bodies of the larger larvae and could be clearly seen feeding on the tissues. Curiously, although a large proportion of Dolichopodid larvae were missing, midge larvae were never seen feeding on their remains.

Midge larvae have also been observed feeding on the dead remains of their companions and also on the pupae. They frequently attacked and devoured newly formed pupae.

Some doubt exists as to whether the larvae of Culicoides and other Ceratopogonidae are true predators. Many writers are of the opinion that they are detritus feeders which attack only dead or moribund insects, but Weerekoon (1953) has observed an undoubted case of true predatism in the larvae of Bezzia sp. which he saw attacking and feeding on larvae of Palpomyia quadrispinosa/

quadrispinosa. He is also of the opinion that animal matter plays a much more important role in the feeding of Ceratopogonid larvae than has generally been realised. The truth of this assumption is not borne out in the case of C. circumscriptus. The larvae do not appear to be true predators. Apart from the vulnerable and helpless new pupae no instance has been observed of midge larvae attacking and killing healthy insects. On many occasions they have been seen to nibble at their companions for a few moments with their man-:dibles but no case of penetration or killing has been observed. From the results of the experiments on phototaxis made on insects fed on insect flesh there is also reason to suppose that it does not constitute an adequate diet.

Observations also indicate that they will only eat insect flesh when no other is available. When pieces of blow-fly larvae were added to cultures of midge larvae containing plenty of other organic matter they eventually putrefied without apparently being eaten. As described in the section relating to predators, although a few of the Limnobiid larvae which had been added alive to such cultures could not be found later, there was no evidence, in these cases, in the form of empty skins, to indicate that they had been eaten by Culicoides. In the field the larvae may occasionally feed on the bodies of their own and other species, but this would not appear to be a major constitu-:ent of their diet, for when gauze bags containing pieces of blow-fly/

blow-fly larvae, which were laid in the mud of the natural habitat as traps and left for periods of up to a week, were found on recovery to contain only one or two Culicoides larvae if any at all.

The larvae appear to be primarily detritus feeders, feeding mainly on vegetable matter, both living and non-living. Although some of the green material from ingested algae and flagellate is voided in the faeces it is unlikely that they are completely devoid of food value since the alimentary canal is often full of them to the exclusion of other materials. Leathers (1921) and Lang (1931) state that Culicoides larvae also feed on bacteria and this has been confirmed with C. nubeculosus by Megahed (1956a) and in the present work with C. circumscriptus.

The question has arisen as to whether the larvae are selective or not in their feeding. Megahed states that in culture pots C. nubeculosus feeds with little discrimination on the available organic matter. Lang states that Culicoides larvae do not swallow mud and will grow and metamorphose in an aquarium lacking plants and animals, apparently living on bacteria and nannophytoplankton. He concludes from this that they are selective filter feeders. Mayer (1934a) was able to identify, among other things, detritus and sand in the gut contents. He rejects Lang's hypothesis of selection and accounts/

accounts for the differences in the constituents and particle size of the food in two groups of species by the variation in size and sucking strength of the pharyngeal skeleton.

The larvae of C. circumscriptus are certainly selective in their feeding. On the surface of culture pots their feeding activity appears to be very discriminatory. They nibble in one spot, leave it and go elsewhere, constantly changing their position as if searching for particular types of food. In spite of this, however, I feel that Lang's hypothesis of selection is based on false premises. The presence of detritus and comparatively large particles of material in the alimentary canal shows that the larvae do not confine their feeding to finely divided material. It has been shown earlier in this paper, that when the larvae of C. circumscriptus are kept in a medium similar to that described by Lang their rate of development is slowed down. Unless the species (unnamed) on which Lang based his conclusion has a diet radically different from that of other species it must be supposed that they were already well-advanced in maturity at the time of his observation.

Mayer's hypothesis of non-selective feeding, as applied to Culicoides larvae is likewise contradicted by observation and experiment. The indiscriminate ingestion of the substrate which he reports in Bezzia (Mayer, 1934b) does not occur in C. circumscriptus. It has been shown (Table VIII) that if the larvae are kept for as long as a week in mud containing no organic/

organic matter or in mud which has been freshly reconstituted from powder, hardly any of them ingest it.

It seems probable that at least three factors are involved in the choice of food. Firstly, the darting searching movements of the larvae on the surface of the culture pots indicate that the surrounding material is being tested for sapidity and that then the most acceptable constituents are ingested. That chemo-reception plays a part in food choice is also borne out by the fact that some substances such as insect flesh are not eaten when other food is available.

Secondly, the texture of the prospective food material seems to influence its selection. The larvae not only refuse to ingest incinerated mud - which is probably equally non-attractive to their chemo-receptors - but neither will they take any of the material available in newly reconstituted mud which contains plenty of organic matter. This would also explain why "Dyox" was ingested when mixed with incinerated mud. It was probably acceptable to their 'texture receptors' although their chemo-receptors would not register it as a sapid material.

The sensillae which control the choice of food are not known. It may be safely assumed that they are situated on the head. Lawson (op. cit.) and Kettle & Lawson (1952) have repor-:ted the presence of various sensillae on the labrum of C. nubeculosus/

C. nubeculosus and other species, but as yet we have no information regarding their function.

Lastly, as suggested by Mayer (1934a) there is reason to suppose that there is some mechanical selection with regard to particle size of the ingested material, carried out by the pharyngeal skeleton, in particular by the teeth borne on the epipharynx.

It is, of course, inevitable that particles of sand and mud would be ingested along with the food, hence Mayer's discovery of these materials in the larval guts. It is contended, however, that the ingestion of non-nutritive materials is accidental and incidental to the ingestion of food, and that it is not ingested merely that food may be extracted from it.

H. Predators of Larvae

1) Experiments and results

Early in the present work, in an attempt to discover indications of predation on Culicoides larvae, the numbers of Culicoides in a large number of mud samples were compared with those of other insect larvae also obtained. The resulting data, however, showed no correlation which might indicate predation.

In order to determine experimentally whether the larvae of Culicoides sp. were preyed upon by other insect larvae, some larvae of each type commonly found with them in the field, i.e. Chironomidae,/

Chironomidae, Ephydridae, Dolichopodidae and Limnobiidae, were first placed in separate dishes of water containing larvae of C. circumscriptus, and watched carefully for long periods. It became obvious, however, that, unlike the actively swimming midge larvae, the larger larvae were not suited to the medium. The Chironomids and Limnobiids were helpless and unable to make effective locomotory movements, while the Ephydrids and Dolicho-:podids climbed out of the water when they possibly could.

Accordingly, a thin layer of mud was put in the bottom of petri dishes and 24 Culicoides larvae and a small number of suspected predators introduced. These burrowed into the mud and the dishes were covered and left for 3 - 4 days. Two control dishes containing 24 Culicoides larvae only were similarly treated.

A further 12 petri dishes containing mud and only a surface layer of water were prepared and into each 30 Culicoides larvae and some of the suspected predators were introduced. Two con-:trols containing 30 midge larvae were also prepared. These were left for 6 - 8 days.

In a further experiment 60 Culicoides larvae were placed in 3 earthenware pots, containing culture medium. 20 Limnobiid larvae were added to two of the pots and the third kept as a control. These were left for 15 days.

TABLE IX

Results of predation experiments.

	No. of Culicoides	No. & type of other larvae	Time left together (Days)	No. of Culicoides recovered	No. of other larvae recovered
Thin layer of mud covered with water.	24	5 Chironomidae	3	24	3
	"	12 Ephydridae	"	23	9
	"	" "	4	24	10
	"	6 Limnobiidae	"	24	5
	"	" "	"	23	6
	"	" "	"	24	6
	"	6 Dolichopodidae	"	23	1
	"	Control	"	23	-
	"	"	3	24	-
Mud with thin surface film of water.	30	6 Dolichopodidae	6	26	1
	"	" "	6	23	1
	"	6 Chironomidae	"	26	5
	"	10 Ephydridae	"	23	9
	"	" "	"	26	9
	"	13 "	8	25	5
	"	8 Limnobiidae	6	24	7
	"	" "	"	30	7
	"	" "	"	30	7
	"	Control	"	26	-
	"	"	8	27	-
Larval culture medium.	60	20 Limnobiidae	15	48	17
	"	" "	"	45	16
	"	Control	"	42	-

The larvae were recovered at the end of the specified times by the magnesium sulphate flotation technique. The results are shown in Table IX.

98.2% of the Culicoides larvae were recovered from the first set of dishes as compared with 97.9% recovered from the control dishes. In the second experiment 3 of the dishes were found to be bad and foul-smelling and many of the larvae were dead or could not be found. From the remaining 9 dishes and the controls 86.3% and 88.3% respectively of the Culicoides larvae were recovered. In the third experiment 77.5% of the Culicoides larvae were recovered from the 2 dishes containing Limnobiid larvae and 70.0% from the control dish. These figures do not furnish any evidence of predation on Culicoides larvae by any of the species used in the experiment.

Some points of interest arise from the number of other species recovered from the dishes. In almost all cases these were less than the number originally introduced. In a few dishes dead remains were found, but in most cases the missing larvae could not be accounted for. As the dishes were covered there was no possibility of their escaping and, because of their large size, it was highly improbable that they could not have been overlooked during recovery. Culicoides larvae were found feeding on the dead remains which were recovered and it would seem likely that the disappearance of the others may be explained/

explained by the fact that the midge larvae had eaten their remains, if, in fact, they had not actually killed the larger larvae. This question is discussed further in the preceding section.

Also considered as possible predators were Nereis diversicolor, which were often found in samples of mud from which the midge larvae were obtained, and the stickleback (Gasterosteus aculeatus), found in the pools adjoining the midge breeding sites. 15 larvae were added to each of 4 dishes containing marsh water and a specimen of Nereis lying in an open-ended glass tube. 60 larvae were also placed in a dish con-:taining two sticklebacks. 8 sticklebacks caught in the pool on various occasions were dissected and the stomach contents examined.

On many occasions while the dishes were under observation midge larvae were seen being attacked and devoured by Nereis. Sometimes they were swept into the glass tube by the current set up by the undulations of the worm's body and on other occasions were caught when the worm was moving over the bottom of the dish. When a midge larva swam near the head of a worm the proboscis was extruded and the prey grasped and pulled into the mouth by the chitinous jaws. Occasionally the crushed and broken body of a larva was ejected from the mouth and abandoned but usually the larvae were permanently swallowed.

When/

When examined about 18 hours after the introduction of the larvae, only one larva remained alive in each of two dishes. The third contained two live larvae and the fourth none at all. In addition each dish contained the mangled remains of several other larvae.

When the larvae were put into the dish containing sticklebacks the fish fed voraciously on them. The stomach contents of other sticklebacks caught in the field yielded little that was possible to identify except several head-capsules of Chironomid larvae, which abound in the mud at the bottom of the ponds. No remains of Culicoides larvae were found.

2) Conclusions

There are few reports of predators of Culicoides larvae in previous literature. Thomson (1937) states that when placed in dishes along with larvae of Bezzia and Probezzia, Culicoides larvae are eaten by the other species. Mayer (1934b) gives a number of examples of Ceratopogonid larvae being eaten by other animals among which he reports having found the head of a Culicoides larva in the gut of a "Tanypinen-Larve".

In the present work no predators have been found among the insect larvae commonly living in the larval habitat of C. circumscriptus. The predation by sticklebacks observed in the laboratory would not be likely to occur often in the field since/

since the number of midge larvae found in the mud at the bottom of permanent pools is small. This is borne out by the absence of Culicoides remains in the stomachs of the sticklebacks caught in the field.

It seems very probable, however, that the larvae are preyed upon regularly by Nereis diversicolor. I am informed by my colleague, Dr. R.B. Clark, that during the day these worms are found at depths in the mud where few Culicoides larvae occur, but after dark they come to feed on or near the surface where the larvae would be available in large numbers.

I. Parasites of Larvae

1) Bacteria

While larvae were being recovered from mud obtained from a salt-marsh on the Firth of Tay, specimens were noticed which had a striking fluorescent blue coloration. The larvae were C. salinarius, which is closely related to C. circumscriptus. The blue coloration was mainly confined, as far as could be seen, to the fat-body and was particularly noticeable under ultra-violet light (Figs 38 and 39). Viewed by transmitted light (Fig. 40) the larvae presented a light brown granulated appea-:rance, and the thoracic pigmentation was obscured.

The blue colour of the larvae was found to be due to the presence in the body of fluorescent bacteria, identified by Dr R.B. Morrison/

Larvae of C. salinarius infected with the fluorescent bacteria, Pseudomonas sp.

Fig. 38. An infected specimen (above) and a non-infected specimen, photographed with reflected light of about 4700 Å.U. (x 14). (Photograph by J.M. M'Corquodale, A.R.P.S.)

Fig. 39. An infected specimen with reflected ultra-violet light, showing that much of the fluorescence is confined to the fat-body. (x 25).

Fig. 40. An infected specimen photographed with transmitted light, showing the granulated appearance and the obliteration of the thoracic pigment. (x 25).

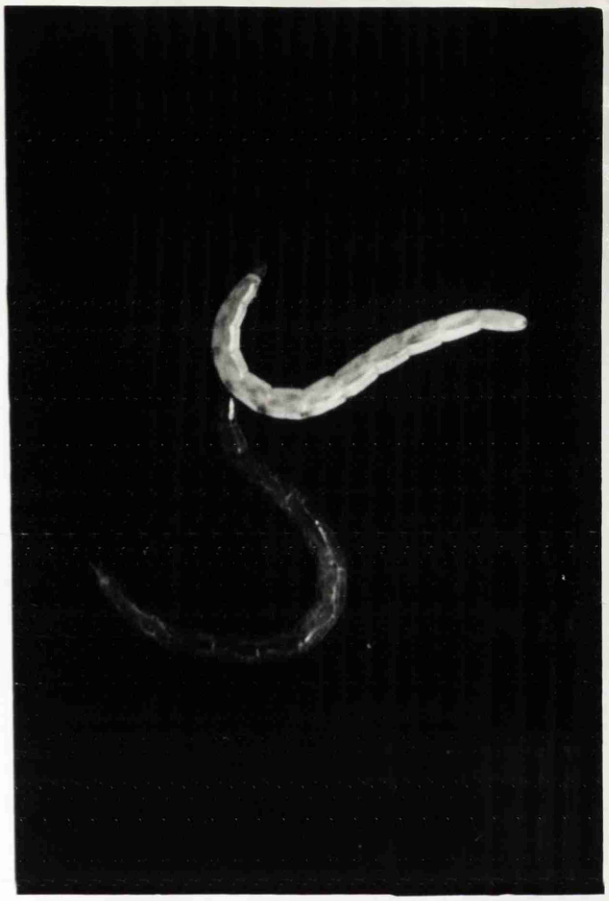


Fig. 38

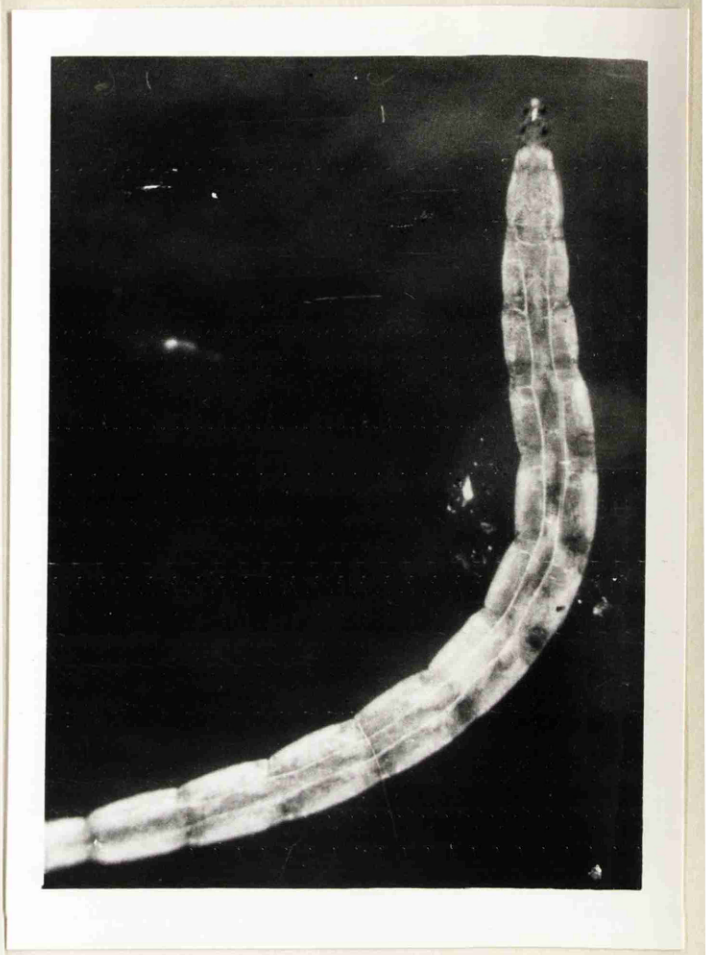


Fig. 39

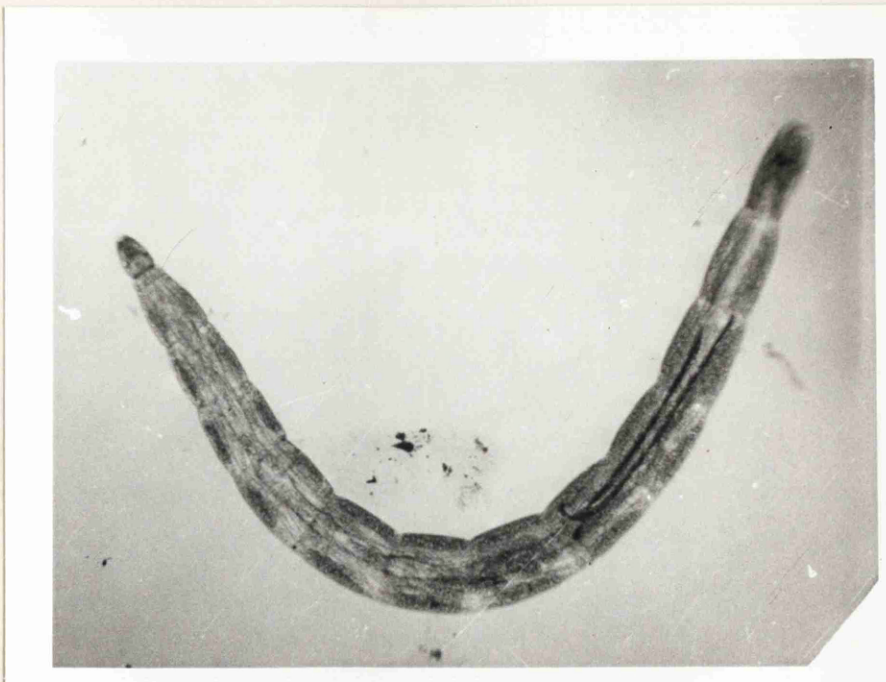


Fig. 40

Dr R.B. Morrison as Pseudomonas sp.. These were the predominant organisms found in cultures prepared from the crushed bodies of the fluorescent larvae. Similar preparations obtained from non-fluorescent larvae yielded considerably fewer organisms and no fluorescent bacteria.

In the absence of any evidence to the contrary it is pre-:sumed that the bacteria are symbiotic although Breed et al. (1948) report that some of the genus are pathogenic to the host.

An apparently non-fluorescent species, Pseudomonas fermen-:tans, has been obtained from Chironomus plumosus by von Wolzogen Kühr (1932). Lawson (1951) has reported the presence of motile particles, probably bacteria, but non-fluorescent, in the fat-body of C. nubeculosus. Other references to the presence of bacteria in larvae of Ceratopogonidae are given by Mayer (1934b) and Steinhaus (1946).

2) Ciliata

Also from mud taken from the Firth of Tay larvae of C. salinarius, C. odibilis and C. riethi were recovered which contained ciliates in their haemocoeles. From one sample approximately 20% of the larvae recovered were parasitised. The number of these protozoa varied in each larva and in some specimens they were packed closely together in the haemocoele (Fig. 41). Occasionally numbers of the ciliates could be seen to travel forwards towards the head. They did not appear to

be/

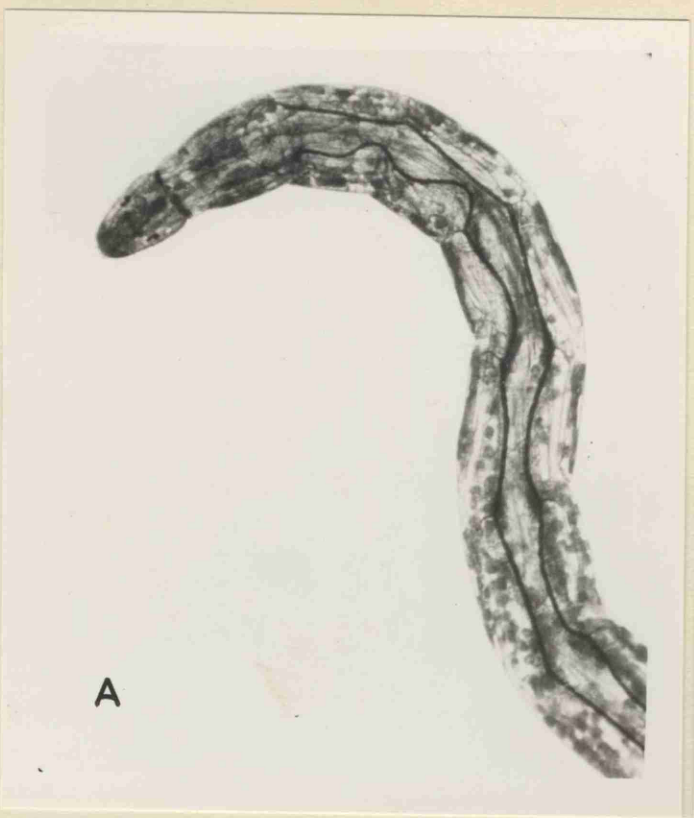
be moving under their own power. From the position of the moving individuals and from the fact that they tended to con-:gregate without further movement in the head and anterior part of the thorax, it may be presumed that they had entered the heart and were swept along with the bloodstream. When the body wall of the larva was broken, however, the ciliates moved around actively and quickly for a short time in the tap water in which the larvae were kept, and eventually became motionless and died. It seems evident that they were killed by the change of osmotic pressure.

The ciliates (Fig. 42) were identified by Mr J.B. Cowey of this Department as Perezella sp. (Perezellidae, Astomata). They are ovoid in shape and flattened ventrally. They vary in size, the largest being about 50μ in length and 30μ in breadth. The cilia are 4 - 5μ long and evenly and sparsely distributed over the whole body. The ectoplasm is very thin and appears to be almost reduced to the cuticle. Internally, the endoplasm is finely granular. When the ciliate is filled with food reserves the endoplasm is gorged with droplets of varying size, the smallest being refractile and composed of fat, while the largest ones appear to be glycogen. The macronucleus, as seen in fixed specimens, is central in position and ovoid in shape, measuring 20μ x 15μ. The micronucleus is spherical and about 2μ in diamter. It is situated close to the macronucleus. No contractile/

Fig. 41. The anterior (A) and posterior (B) parts of a larva of C. salinarius infected with the ciliate, Perezella sp. (x 50).

Fig. 42. Perezella sp. (x 1040).

Fig. 43. A rupture in the body-wall of a larva which was infected with Perezella sp. (x 50).



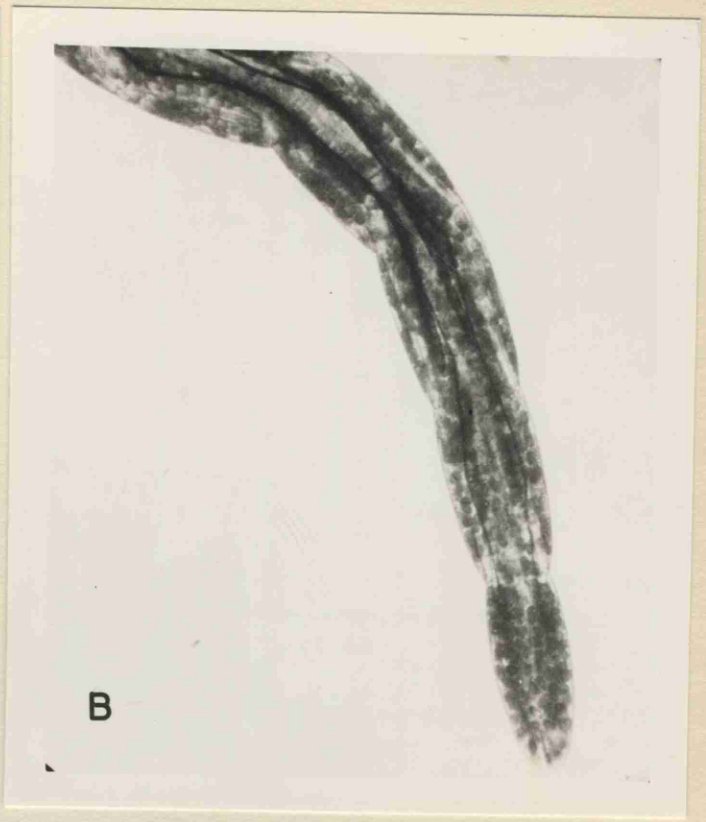


Fig. 41

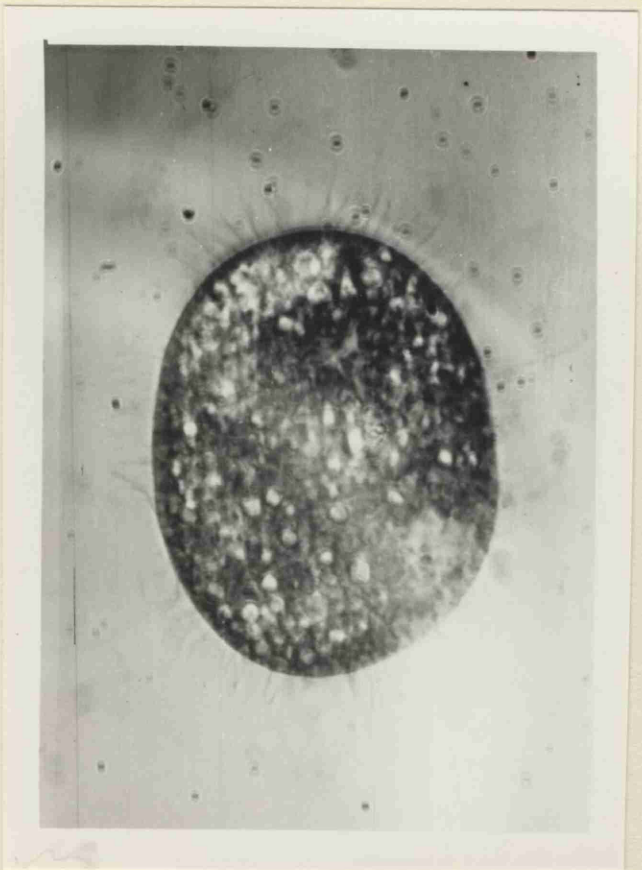


Fig. 42

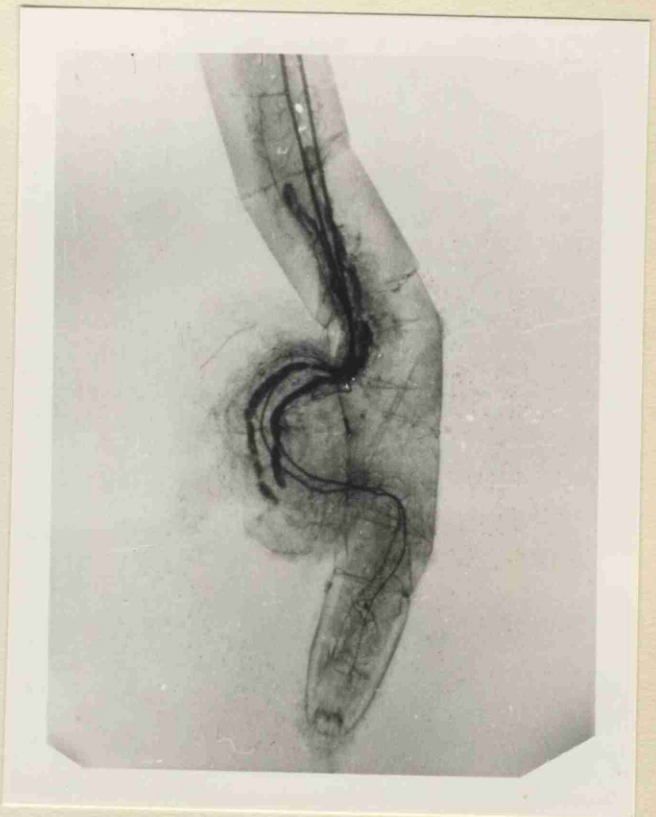


Fig. 43

Fig. 44. The anterior part (A) and the posterior part (B) of a larva of C. riethi with the haemocoele packed with cysts, which are responsible for the coarse granulated appearance. (x 50).

Fig. 45. Cysts from the haemocoele of C. riethi. (x 1040).

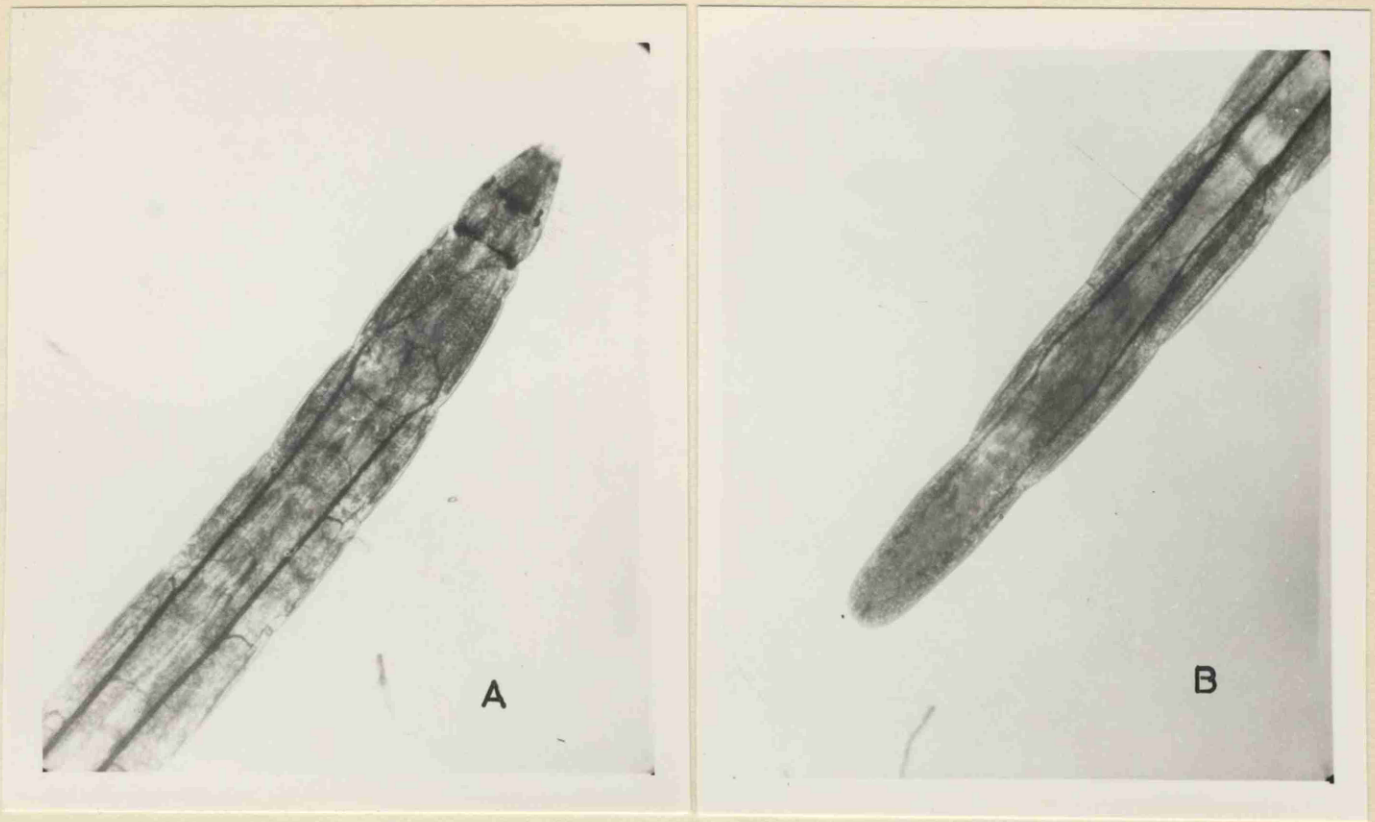


Fig. 44

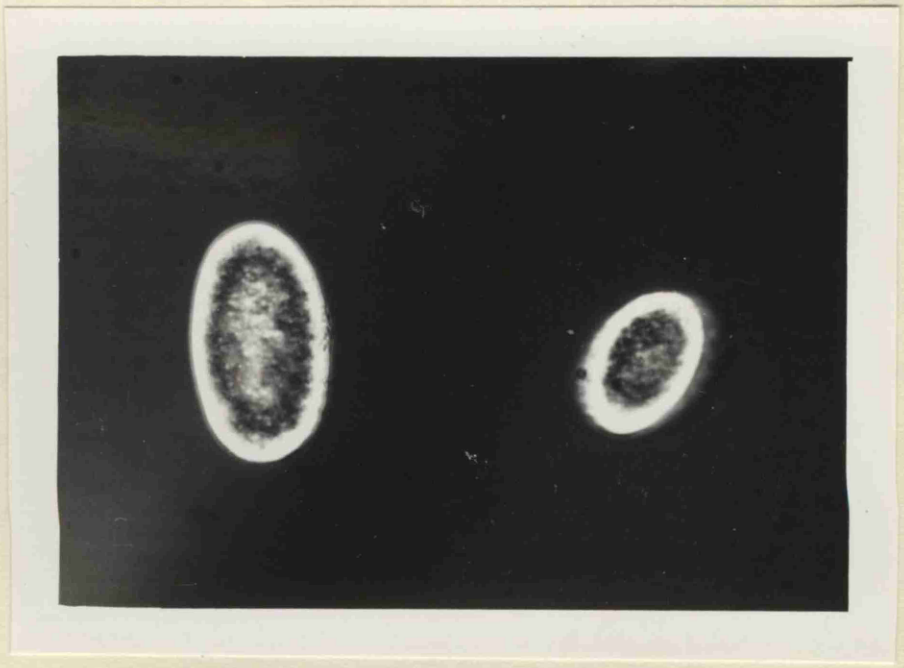


Fig. 45

contractile vacuole was observed.

Three larvae of C. riethi were also obtained in which the haemocoele was packed with small oval cysts, (Fig. 44). These varied in size, the largest measuring about 28μ x 18μ and had a very thick cuticle (Fig. 45). They were presumed to belong to Perezella.

The genus Perezella was first described by Cépède (1910) who found them present in the haemocoeles of several species of marine Copepoda.

Kettle (private communication) reports that he found in Angus one larva of C. pulicaris which was heavily infested with ciliates. Slides which were made of them show that they are undoubtedly of the same genus as those described above. Kettle also states that larvae of C. cubitalis and C. pallidicornis were frequently found at Drumchapel, Glasgow, bearing 'ectoparasitic cysts' (Kettle & Lawson 1952, Plate XVII), the nature of which was never properly resolved. No similar observations have been made in the present work, but Cépède describes cysts which he considered belonged to Perezella, on the appendages of Copepoda which were parasitised internally by these ciliates.

Hopkins (private communication) has also observed, in the British Cameroons, larvae of C. austeni which were infested with ciliates. These were never identified, however.

At the time of writing, there is little information available regarding the effects of the ciliates on the host larvae. In one dish containing infected specimens it was noted that the larvae died one by one, much more rapidly than was the case with non-infected larvae. In nearly every case the body wall had ruptured (Fig. 43) and Perezella were seen swimming in the surrounding water. Subsequent observations on the limited number of specimens available have not, however, resulted in a repetition of this phenomenon.

3) Nematodes

Kettle & Lawson (1952) found larvae of C. stigma parasitised by larvae of Gordiacea. No instance of parasitism by nematodes has been observed in the present work.

VIII. THE PUPA

A. Description

Kettle & Lawson (op. cit.) have already described the pupa of C. circumscriptus. Their description has been used successfully throughout the present work to identify the species and I have nothing to add to it.

B. Pupation

The first sign of impending pupation is the gradual disappearance of the deeply pigmented paired lateral bodies on the meso- and meta-thorax. These were completely replaced by the imaginal buds at least 48 hours before pupation takes place (Fig. 46). 10 hours prior to pupation the pupal prothoracic horns, as yet unpigmented, could be seen lying against the body (Fig. 47). At this time the caudal spines were also visible and slightly pigmented, folded under the larval skin with the points directed anteriorly (Fig. 48). Both prothoracic horns and caudal spines gradually became more deeply pigmented and about 5 hours before ecdysis takes place they are both easily seen under a low power lens (Figs 49 and 50).

In cultures kept in water and with an 'island' of plaster of paris it was observed that the prepupal phase were often to be found lying motionless on the plaster just below the surface of the/

Fig. 46. The fourth instar larva of C. circumscriptus prior to pupation. The paired lateral bodies on the meso- and meta-thorax have been replaced by the imaginal buds. (cf. Fig. 24) (x 50).

Fig. 47. Lateral view of the head and prothorax of a fourth instar larva prior to pupation, showing the unpig-:mented prothoracic horns (arrowed) of the pupa. (About 10 hours before pupation.) (x 150).

Fig. 48. The tail of a fourth instar larva prior to pu-:pation, showing the unpigmented caudal spines of the pupa (arrowed). (x 150).

Fig. 46

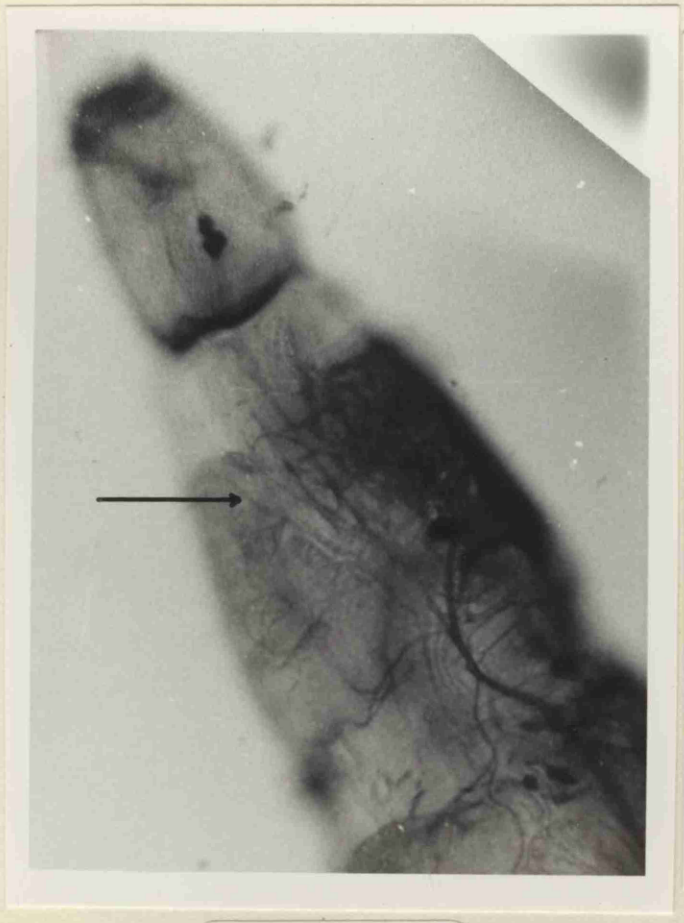


Fig. 47

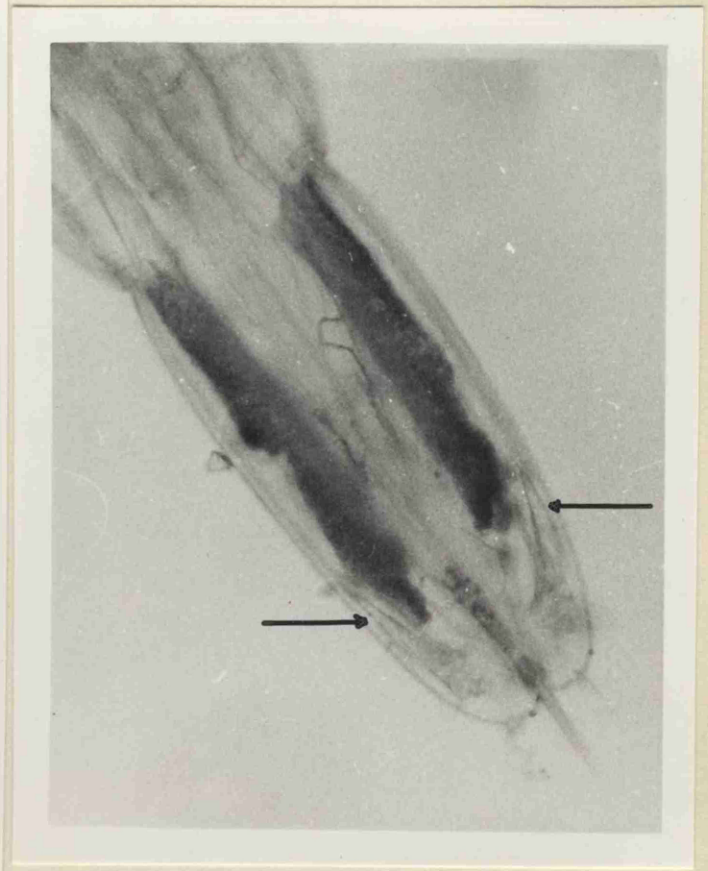


Fig. 48

Fig. 49. The tail of a fourth instar larva about 2 hours before pupation. The caudal spines of the pupa are pigmented. (x 150).

Fig. 50. Lateral view of the anterior end of a fourth instar larva shortly before pupation. The pro-:thoracic horns are deeply pigmented and the outlines of the appendages can be clearly seen. (x 50).

Fig. 51. A) Lateral view of a larva in the process of pupation. Due to the backward reflexion of the caudal spines the body has been pushed forward inside the cuticle which can be seen empty at the posterior end. The thorax is beginning to swell. (x 21).

B) Drawing to show the position of the caudal spines at the stage shown in A.

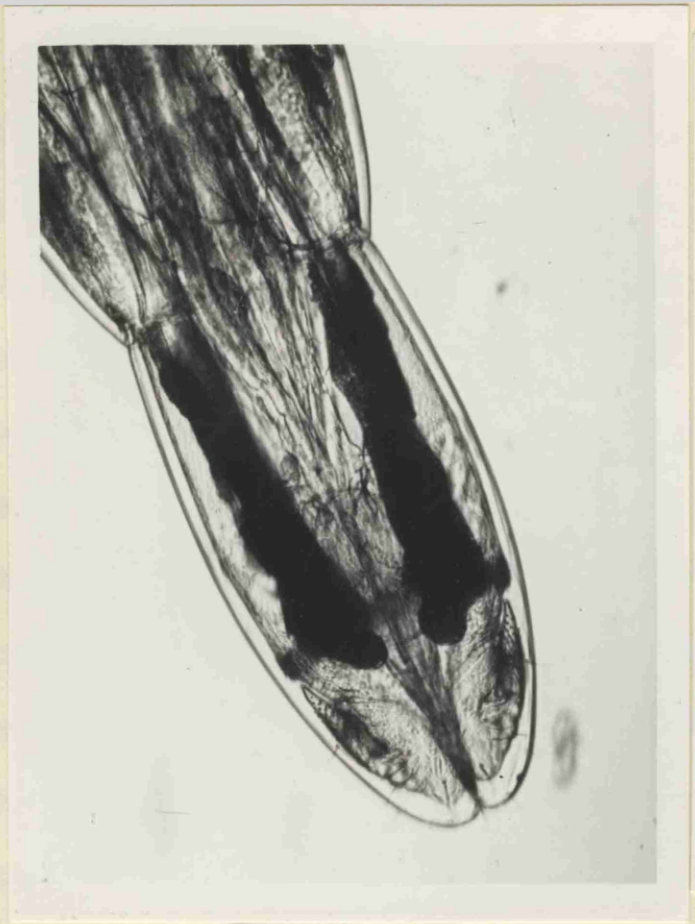
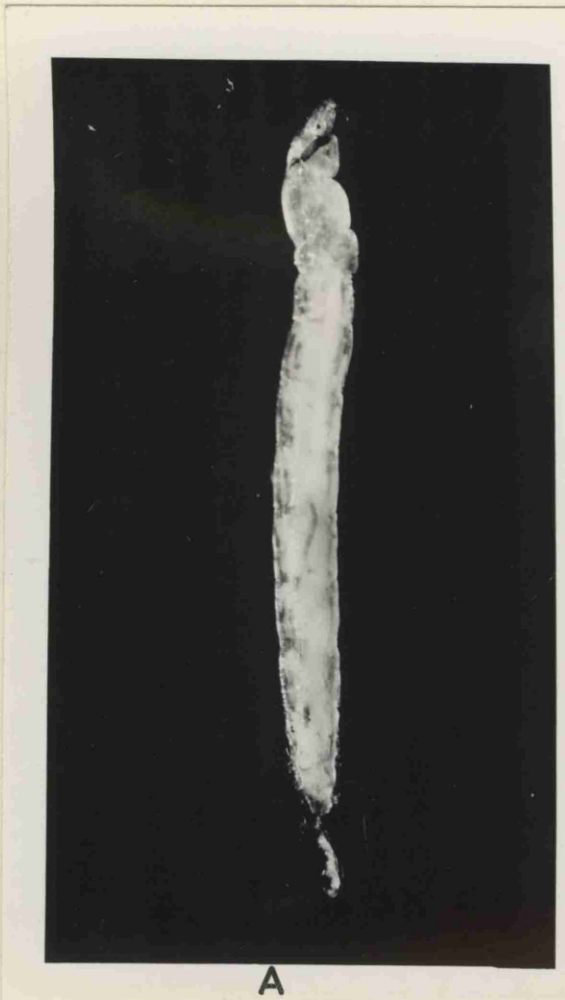
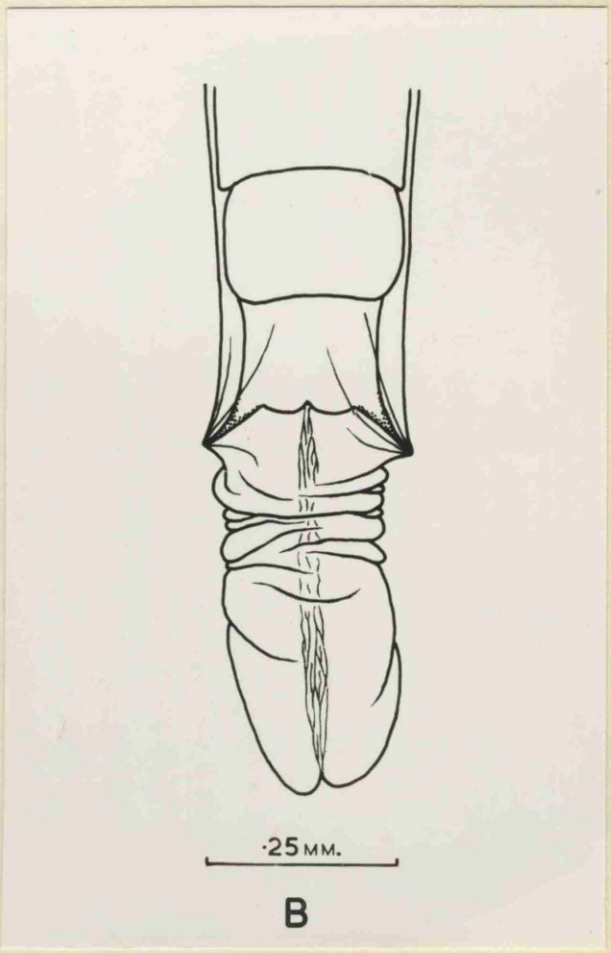


Fig. 49

Fig. 50



A



B

Fig. 51

the water. In the field they are always found in the top inch of mud. These observations, together with those made by Kettle & Lawson (1952) on the vertical distribution of other species, and Patel (1921) who noticed that advanced stages of C. oxystoma were to be found in recesses on the surface with their heads above the water, would make it appear improbable that migration to the surface takes place after pupation as Williams (1951) suggests is the case in C. tristriatulus. In C. circumscriptus and probably in other species the mature larvae come to lie, before pupation, on the drier parts of the surface where, as pupae, they will have easy access to the air. It is suggested by Hull et al. (1934) that some species are stimulated to pupate by the drying out of the habitat but this is unlikely to be the case in the species studied here as the mud in which they live practically never dries up.

The pupation of C. circumscriptus has been watched several times and, in general, is similar to that of C. albicans, described in detail by Lawson (op. cit.). The actual process of ecdysis was observed under a binocular microscope. It began with the posterior reflection of the caudal spines, by which the body is pushed forward in the loose larval cuticle (Fig. 51). This was done swiftly in some cases; in others it took a minute or two before the spines achieved the backwardly pointing position. At about the same time the thorax, already somewhat swollen,/

swollen, began to swell still further, no doubt due to the active swallowing of water which appeared to take place. Wave after wave of contractions of the abdominal segments, passing ante-:riorly along the body, and forcing it into the anterior segments, contributed to the pressure exerted on the old cuticle which lost all appearance of segmentation in that region. Ecdysis occurred instantaneously, the pupal thorax being thrust out of the ecdysial suture. By continued waves of contraction of the abdomen the old cuticle was gradually sloughed off posteriorly, being forced into concertina-like folds, possible due to the tension on the lining of the hind gut which was not finally pulled out until the exuviae had been almost completely shed.

After ecdysis the pupa was elongate, with a transparent cuticle and, apart from prothoracic horns and caudal spines, colourless. A gaseous bubble was then observed to appear around the base of the wing buds. This broadened out, inflating the cuticle slightly, until it covered the area over the limb and wing buds. The space thus formed, called the air-space by Lawson (1951) and Weerekoon (1953), is responsible for the buoyancy of the pupa in flooded conditions. In some pupae its formation was complete about 6 minutes after ecdysis. In other cases the initial bubble did not appear for 14 minutes after which nothing happened for a further 12 minutes. Then it began to/

to expand and was completely developed in 1 - 2 minutes. The time taken for the formation of the buoyancy space may be dependent on whether the pupa can squirm into a position whereby the tip of the prothoracic horns have access to the air.

The abdomen began to shorten some time after the space had formed. The time which elapsed before this happened varied considerably between 5 minutes and over half an hour. Pigmen-:tation began about 40 minutes after pupation and the pupa darkened very gradually, eventually becoming dark brown in colour in about 8 hours.

C. Origin of buoyancy space

There has been some uncertainty regarding the origin of the gaseous bubble which appears in the front and lateral regions of the thorax shortly after pupation, and which is responsible for the buoyancy of the pupa when in water.

Lawson (op. cit.) observed the pupation of C. albicans while it was submerged in water, indicating that the bubble was formed of gas either secreted in the body or extracted from the surrounding water. Weerekoon (op. cit.) disputed this implica-:tion and showed that in Palpomyia quadrispinosa and Bezzia sp. the gas is undoubtedly derived from the atmosphere via the pro-:thoracic horns, the larvae climbing out of the water before pupation.

In/

In order to test the findings of the latter with regard to Culicoides circumscriptus his experiment was repeated with minor variations. 8 glass dishes, 4 of which contained plaster of paris ramps sloping at an angle of about 30°, were filled with water to a depth of 1 inch, which allowed the upper part of the plaster to remain above the surface. The substitution of the plaster ramp for Weerekoon's sprig of Ceratophyllum was neces-:sary since the larvae of C. circumscriptus would not voluntarily climb steep surfaces.

Eight larvae in the prepupal phase were placed in each dish which were kept under observation until either pupation took place or the larva died.

TABLE X

Results of pupation experiment.

	Pupated successfully	Pupated unsuccessfully	Died
Plaster ramps	12	18	2
No ramps	0	25	7

The results (Table X) showed that out of the 32 larvae placed in dishes with plaster ramps, 12 (32.5%) pupated successfully and were found floating and fully tanned on the surface of the water. 2 died without pupating and the 18 remaining larvae pupated but were lying on the bottom of the dish. These were classed as unsuccessful pupations since they were unpigmented and the buoyancy space had not developed. In the other dishes no successful pupations occurred. 7 of the larvae died and the remaining 25 pupated unsuccessfully.

After having been left at the bottom of the dishes for some 18 hours, 20 of the unsuccessful pupae were transferred to wet filter paper. Of these 16 eventually shortened, developed buoyancy spaces and pigmentation, and produced adult flies. The other 4 became slightly tanned, but apart from that, showed no sign of life and did not develop further. None of the pupae left in the dishes developed further and none showed any sign of life after 48 hours.

The results of these foregoing experiments are not so clear-cut as those of Weerekoon. This is probably because of the fact that the experiments were necessarily carried out in water. This is not a natural medium for the larvae of C. circumscriptus which, unlike Palpomyia, normally live in mud which is covered most of the time only by a surface film of water, and whose pupation does not, under natural conditions, necessitate/

necessitate leaving the larval medium.

Nevertheless the results do indicate that the gas which fills the buoyancy space in the pupa of C. circumscriptus is derived from the atmosphere and that it is in all probability air. They also emphasise the statements made elsewhere in this paper that in order to pupate successfully under natural con-:ditions the larva must come to the surface. Only then can the buoyancy space be formed, enabling the pupa to maintain contact with the air by means of the prothoracic respiratory horns at all times.

Successful pupations have occasionally been noted in dishes containing only water. In these cases it must be assumed that the larvae managed to suspend themselves from the surface film long enough to pupate and for the buoyancy space to form.

The formation of a buoyancy space in itself is not neces-:sary for the successful development of a pupa provided it has access to air. In a few specimens, which had pupated under water and were subsequently laid on damp filter paper, the space failed to form. The pupae became fully tanned, however, and eventually adult flies emerged.

D. Behaviour

1) Laboratory observations

In culture pots the pupae are usually to be found buried in the/

the less saturated parts of the mud. The process of digging-in can be observed by placing a specimen on the surface where it may commence immediately or sometimes will move around as if searching for a suitable spot. This it does by contracting and expanding the abdomen, using the stout caudal spines to grip the substrate and push itself forward. At intervals it stops and thrusts the tip of the abdomen into mud. Then, presumably finding the spot unsuitable, it may move elsewhere. When a suitable site has been selected the tip of the abdomen is thrust downwards into the mud and by a corkscrew motion of the abdomen the hole is enlarged, after which the pupa backs into it. This is repeated over and over again until the pupa has buried itself in the mud and only the tips of the respiratory tubes are visible protruding above the surface water-film. Digging-in usually takes 2 - 3 minutes.

Newly pupated specimens whose integument is still colourless remain on the surface of the mud without much movement until tanning has taken place, after which they proceed to dig in as has been described. Pupae which are about to emerge will not bury themselves at all, but lie on the surface with little, if any, movement.

A quantity of soft mud was put into a glass dish and built up to form a slope of about 40°. Several pupae were then placed on the mud about halfway down the slope, and allowed to bury/

bury themselves. When this had been done the dishes were shaded from direct light and water was carefully decanted into the dish, disturbing the mud as little as possible, until the pupae were covered by about ½ inch of water.

After an interval of 5 - 10 minutes the pupae began to move. The head and thorax appeared and moved slowly with a corkscrew motion until the whole body was freed from the mud. As soon as this was accomplished the pupa floated to the surface of the water and the prothoracic horns pierced the surface film, re-:gaining contact with the air. The pupae remained floating on the surface, sometimes motionless, sometimes wriggling weakly, waving their abdomens back and forth. Most of them eventually reached the mud which had not been flooded and when this happened they quickly dug themselves in at the edge. The few pupae which did not reach the mud were caught on the side of the glass dish by capillary attraction, and when freed and pushed over near the mud, they also buried themselves when they reached it.

The pupae also show a distinct aversion to light. When buried specimens were flooded under the light of a bench lamp, they took much longer - an hour or more - to come out of the mud and float to the surface than when the dish was kept in shaded conditions. If the light was switched on while they were wriggling free the pupae usually buried themselves once more, and again/

again would remain so for about an hour or until the light was switched off.

Before the emergence of the adult the pupa wriggles out of the mud until the thorax is free. Thus the photophobic reac-:tions hitherto shown by the pupa are reversed and the attraction to light shown by the adult midge manifests itself sometime before ecdysis. In this way the adult is able to free itself easily from the exuviae and runs less risk of being trapped by the water film on the surrounding mud. Successful emergence may also take place while the pupa is floating on water but in many cases the adult is trapped on the surface before it is able to take wing.

2) Field observations

In the light of the flooding experiments carried out in the laboratory it was expected that, during the frequent innundations of the breeding areas by high tides the pupae would be found floating on the surface. This was, in fact, confirmed by sweeping the surface with a plankton net when the salt-marsh was flooded. Large numbers of pupae of C. circumscriptus, C. maritimus and Psychodidae were obtained in this way. It was found that they were most plentiful not in the vicinity of the larval breeding sites but some distance away to the east where they had been blown by the wind and stopped by clusters of reeds and grass stems which broke the surface of the water. In this way/

way the pupae are dispersed over areas much wider than those covered by the larval breeding sites.

Several wooden ramps were placed over the area sloping at an angle of about 30° to the mud so that the floating pupae might be caught as the tide receded. These ramps and also the vegetation where most of the pupae had been found floating were examined at low tide but none were found adhering to them. During the latter part of the breeding season large numbers of pupal exuviae have been observed adhering to the vegetation some way above the ground but complete pupae have never been found stranded in this fashion. Apart from those obtained with a net as described above, all pupae found in the field were recovered from samples of mud.

Discussion

Numerous authors, referring to different species of Culicoides, have given accounts of the behaviour of the pupae (Pratt, 1907; Lutz, 1912; Patton, 1913; Carter et al., 1920; Patel, 1921; Bequaert, 1924; Thomsen, 1937; Taylor, 1944; Dorsey, 1947; Séguy, 1950; Williams, 1951; Woke, 1954; and Megahed, 1956a). These accounts differ from one another to a greater or lesser extent and it seems likely that there are some specific variations in the pupal behaviour.

The pupae of many species have been found floating in pools, but/

but this has very seldom been observed in C. circumscriptus except when the marsh is inundated by high tides. Laboratory observations indicate that the pupae are photophobic and, whenever possible, bury themselves in the mud, where, in fact, they are easily obtained. Megahed states that in C. nubeculosus the burial of the pupae is only partial and Lutz has found the pupae of C. reticulatus and other species lying on the surface of the mud, but in C. circumscriptus burial is almost complete, only the tips of the prothoracic horns remaining above the surface However, in culture pots used by Megahed for C. nubeculosus and also in the present work, many pupae climb up the sides of the pots and attach themselves there. No similar behaviour has been seen in their natural habitat. Even immediately after high tides complete pupae have never been found adhering to vegetation or to the wooden ramps which were constructed on the marsh. It is probable that the pupae avoid such a situation by their own movements and are eventually deposited on the ground again by the receding water. The behaviour of the pupae in the pots, therefore, appears to be unnatural and the explanation may be that there was too much water present. The pupae would then be forced to climb to the drier sides of the pot where, of course, they would be unable to bury themselves. They do not become desiccated because of the moisture contained in the earthenware.

When/

When placed in water the pupae float with the tips of the breathing tubes above the surface film. They take up a position which varies in different specimens from vertical to almost parallel to the surface, this being probably dependent on the amount of air in the buoyancy space. While floating in this fashion the abdomen may be waved back and forth but never with the strong propelling movement seen by Megahed in C. nubeculosus. The mosquito-like movements described by Séguy have not been reported by any other author and it is improbable that such behaviour occurs in any species of Culicoides.

The mode of life led by both larvae and pupae necessitates repeated changes in their reactions towards light. It has been shown earlier that the larvae are normally photophobic, presumably for reasons of protection. They become photophilic when in need of food available on the surface of the mud and also prior to pupation since contact with the atmosphere is necessary for this to take place successfully. It is also suspected that the younger larval instars become photopositive before moulting but it is difficult to find a reason for this. After pupation the midge again becomes photophobic and buries itself in the mud for protection and to prevent desiccation. Shortly before emergence it comes some way out of its burrow, exhibiting a positive response to light which is retained, as far as is known, throughout adult life.

IX. THE ADULT

A. Description

A description of the adult has been given by Edwards (1939). Attention is drawn to the remarks made in Section X.

B. Flight and General Activity

As has been found generally among the genus by Parker (1949) and others, adults of C. circumscriptus are found flying in the evenings in sheltered localities or else when the weather is calm. They do not fly under windy conditions. Downes (1955a) states that flight by C. nubeculosus and C. riethi ceases when the wind is above an estimated 2 - 3 m.p.h.. For C. circumscriptus the figure is probably less. It has been found difficult to catch them by aerial sweeping even when the lightest of breezes is blowing.

Kettle (1955) has summarized the findings of a number of authors relating to the abnormal sex ratios often met with in collections of Culicoides made in the field. Similar anomalies are also apparent in the case of C. circumscriptus. The data in Table XI shows the sex ratios in a series of collections made over similar periods in the evening, compared with the ratio obtained from laboratory cultures.

TABLE XI

Sex ratios of C. circumscriptus obtained by different methods.

Method	Males	Females	Total	Percentage males
Light trap	3	110	113	3
Aerial sweeping	4	57	61	7
Vegetation sweeping	30	84	114	27
Laboratory rearing	1793	2059	3852	47

In the laboratory cultures, as Kettle also found, the ratios are nearly normal. In collections in the field the females generally outnumber the males considerably. There are, however, differences according to the method of collection used. The numbers of males in collections made by sweeping the vegetation are greater than in collections made either by aerial sweeping or by light trap.

Kettle has suggested that the explanation for the abnormal sex ratios of adult Culicoides caught in the field may be that the times of activity of the sexes may be different as was found in C. pallidicornis by Parker (1949). Another possible explanation/

explanation of the discrepancies, for C. circumscriptus at any rate, may be found in the figures given in Table XI. These figures indicate that the males fly less readily than the females and spend a far greater part of their time in the vege-:tation. It is also probable that the males are less positively phototactic than the females since the percentage caught by the light trap was appreciably lower than the percen-:tage caught on the wing, and this may have some connexion with their apparent preference for living among the vegetation.

C. Diurnal Incidence of Adults

It has been remarked earlier, in the description of the field location, that in spite of the fact that the larvae of C. circumscriptus were generally more numerous than the larvae of other species, this was not reflected in a superiority in the numbers of adults of the species. It was thought that a possible reason for this discrepancy might have been that it was nocturnal rather than crepuscular as is the case with most species. This idea was occasioned by an observation made at Beauly, Invernesshire, in August 1953. It was found that large numbers of Culicoides were attracted by a light in the tent between 11 p.m. and midnight. On examination it was noted that C. circumscriptus and the closely related C. salinarius were present in relatively greater numbers than had been obtained by sweeping earlier in the evening, this in spite of

the fact that the tent was at least 1/4 mile away from the nearest salt-marsh. C. salinarius outnumbered C. circumscriptus by about 3:1 but nevertheless the latter species formed a larger proportion of this catch than of any other collection made in the district.

Downes (private communication) has suggested the possibility that the light itself may have induced activity and attracted the midges at a time when they otherwise would have been inactive. He also cited cases where he found Culicoides attracted to light when they could not be caught by netting. My own observations agree with his but they do not adequately explain why the numbers of C. circumscriptus and C. salinarius on this occasion should have been so much greater relative to other species, which in all likelihood came from breeding grounds much nearer the tent.

On a number of occasions, endeavours were made to verify this observation experimentally and to sudy the diurnal fluctuations in the numbers of flying adults by aerial sweeping at intervals using Parker's (1949) standardized netting procedure. An investigation of this type, however, requires calm conditions where one can depend on obtaining a regular series of samples over a period of several hours at least. Unfortunately such conditions are seldom experienced at Dumbuck. Only on one occasion was it possible to obtain a series of samples/

samples of the flying population. These were taken over a period of about 4 hours in the evening around sunset. Collections were made from two sites, the first (Site A) being around the edge of the pool, and the second (Site B) on the area covered by reflexed poa about 20 yards west of the pool (see Fig. 1).

TABLE XII

The incidence of flying adults of C. circumscriptus during the evening of 8th September, 1956. (Sunset at 18.49 G.M.T.)

SITE A		SITE B	
Time (G.M.T.)	No. caught	Time (G.M.T.)	No. caught
17.30	1	17.35	0
18.00	3	18.05	0
18.30	3	18.35	2
18.50	16	18.55	11
19.10	12	19.15	6
19.25	13	19.30	15
19.40	22	19.45	14
20.00	6	20.05	3
20.15	2	20.20	1
20.30	0	20.35	0
20.45	0	20.50	0

Fig. 52. The incidence of adults of C. circumscriptus on two sites at Dumbuck (see Fig. 1) on the evening of 8th September, 1956.

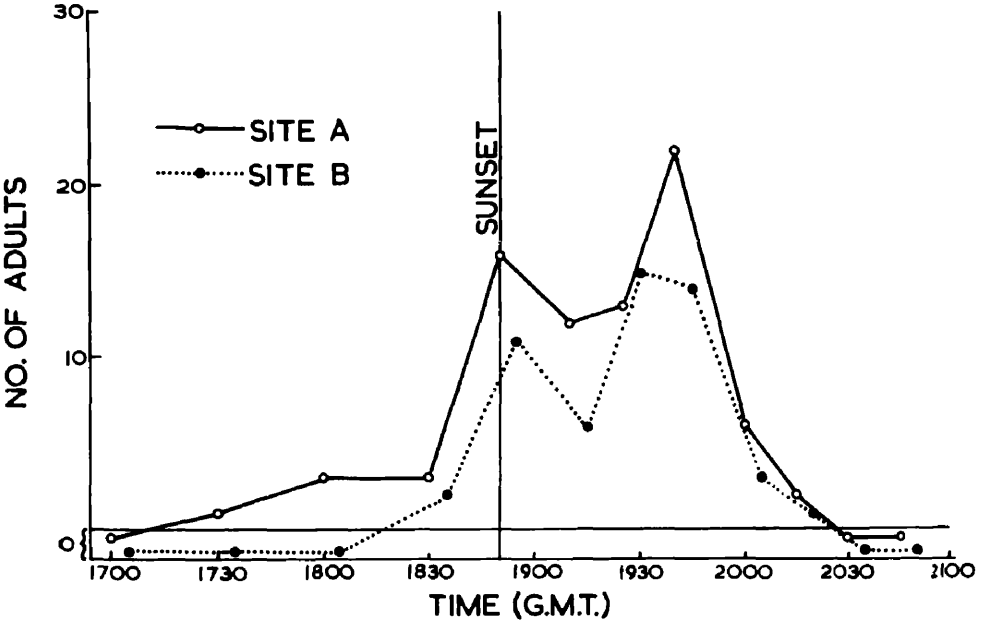

30
20
10
0
NO. OF ADULTS
SITE A
SITE B
SUNSET
1700
1730
1800
1830
1900
1930
2000
2030
2100
TIME (G.M.T.)

Fig. 52

The numbers of midges caught were small (Table XII) since the collections were made late in the season. In view of this and the fact that it was only possible to do the work on one evening one would not be justified in forming any definite conclusions regarding the diurnal incidence of C. circumscriptus. Wind prevented any flight before 6.30 p.m., and between 8 p.m. and 8.30 p.m. occasional puffs of wind caused a drop in the number of adults caught (Fig. 52). Nevertheless, it is safe to assume that the peak of flying acitivity occurs between sun-:set and one hour afterwards. The collections taken from the tent at Beauly were made 2 - 3 hours after sunset. It would appear, therefore, that the adults were, in fact, artificially induced by the light as Downes suggested. Why such an un-:usually large percentage of these should have been C. circumscriptus is still unexplained.

D. Mating and Feeding

1) Experiments

Since it was desirable that continuously breeding colonies of C. circumscriptus should be reared in the laboratory many attempts have been made to induce mating and to discover a suitable food which would enable fertilised females to develop their eggs.

Newly emerged adults were kept in "Perspex" cages, the humidity of which was maintained by dishes containing saturated cotton/

cotton wool covered with filter paper. Dampened broken raisins were supplied for carbohydrate requirements. Since Megahed (1956a) found that mating in C. nubeculosus occurred only occasionally in cages, his method was also attempted, and varying numbers of males and females were put into 3 inch by 1 inch tubes containing moistened plaster of paris and with a piece of raisin pinned to the cork. Neither of these methods met with success.

Mohan (1945), working on Anopheles fluviatilis and Muirhead-Thomson (1948), working on Anopheles gambiae, managed to induce mating by exposing these mosquitoes to blue and orange lights respectively. Bates (1949) also suggests using coloured lights for this purpose when mating does not occur naturally in the laboratory. Accordingly, cages and tubes containing laboratory-reared midges were kept under red, orange, green and blue lights. Again, however, no copulation occurred.

In an effort to activate the midges and cause them to fly about in the hope that pairing might occur, air was passed gently through the cage. The cage was also placed above a moving continuous band of black and white striped cloth. Dalmat (1950), succeeded in inducing mating and oviposition in Simulium sp. by exposing them to carbon dioxide. His method was repeated with the midges and on many occasions throughout this work carbon dioxide has been used as an anaesthetic. At

no/

no time, however, did mating occur as a result of these experiments.

In some species of blood-sucking Nematocera, it appears that the host animal exerts some attraction for the male as well as for the female and acts as a rendezvous for mating purposes. Pomerantsev (1932) has observed considerable numbers of male C. nubeculosus crawling on the skin of cows and copulating with the feeding females. Howard, Dyar & Knab (1913) have reported that males of Aëdes aegypti will land on a person's clothing and lie in wait for a female coming to feed, and Bates (1949) has observed males of Aëdes dominici hovering over men and animals for the same purpose. Another, though somewhat dif-:ferent, example of the food source performing this dual role is the case of Atrichopogon pollinivorus described by Downes (1955b) which copulates on the flower of the honeysuckle which provides food for both sexes. On the chance that a similar type of behaviour might also take place in C. circumscriptus, the feeding experiments were coupled with attempts to find evidence of mating.

Female midges, both wild and laboratory-reared, were put into 3 inch by 1 inch tubes which were inverted on the skin of the forearm. They were also kept in a glass-bottomed pill-box which was kept strapped to the arm for periods of up to 24 hours. The author's arm was also inserted into a cage through a muslin sleeve/

sleeve and kept there for long periods at all times of the day and night. On no occasion was any attempt made to bite.

Birds were also used as prospective hosts. The feathers of a pigeon were clipped to lay bare a small patch of the breast on which a tube containing females was inverted, but no biting occurred. Since those mosquitoes which suck avian blood generally attack the soft skin round the eyes and beak, an opportunity was given to the midges to feed on young chicks which were kept in a small wire cage inside a glass midge-rearing tank. On examination 12 hours later the midges showed no evidence of having had a blood meal.

Many authors have cited instances of attacks made by Ceratopogonidae, including one species of Culicoides (C. anophelis), on other insects. With this in mind, female midges were put into a cage with young cockroach nymphs and also into a cage containing a selection of insects (mostly Diptera) which were obtained by sweeping with a net the area where the midges occur. In neither case was feeding observed.

On no occasion during the feeding experiments was mating observed. Samples of female midges were also dissected and the spermathecae examined but no spermatozoa were found.

When the alimentary canal was dissected out of adult C. circumscriptus caught in the field it was often found to contain/

contain a clear fluid, colourless or slightly amber, and occurring both in the mid-gut and oesophageal diverticulum. Using the paper chromatography technique described earlier, it was found that the fluid in the alimentary canals of these midges usually contained fructose, glucose and sucrose (Fig. 53) i.e. the constituents of nectar. In some of the unfed controls very slight traces appear in the chromatograms at the level of glucose. If this is indeed glucose it must have been contained in the tissues of the alimentary canals and carried over from the larval stage, since the adult flies had no opportunity to feed at any time. In one batch of wild caught midges (B.4.), there appears to be no trace of fructose or sucrose. No explanation can be offered for this, other than that these two sugars may be absorbed and assimilated more quickly than glucose and in this batch were not present in sufficient quantity to show up on the chromatogram.

2) Conclusions and discussion

a) Feeding

There is no recorded instance of bloodsucking by C. circumscriptus, and the results of the experiments described above indicate thatm in fact, the females do not take a blood-meal. Observations made in the field support this view. Samples of midges taken while biting the author and while flying round grazing cattle has yielded none of this species. Further, no/

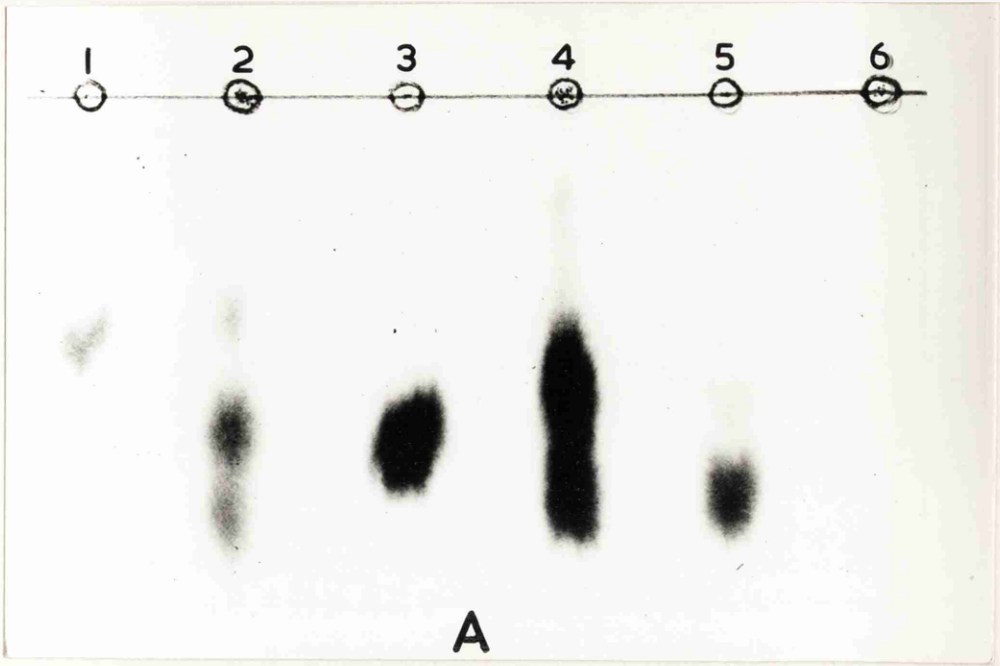
Fig. 53. Paper chromatograms obtained from the alimentary canals of wild-caught adults of C. circumscriptus, with unfed laboratory-reared adults as controls and solutions of sucrose, glucose and fructose as standards.

KEY

A) 1. Sucrose.
2. Wild-caught midges.
3. Glucose.
4. Midges fed with raisins
5. Fructose.
6. Control.

B) 1. Sucrose.
2. Wild-caught midges.
3. Glucose.
4. Wild-caught midges.
5. Fructose.

C) 1. Control.
2. Sucrose.
3. Control.
4. Glucose.
5. Control.
6. Fructose.



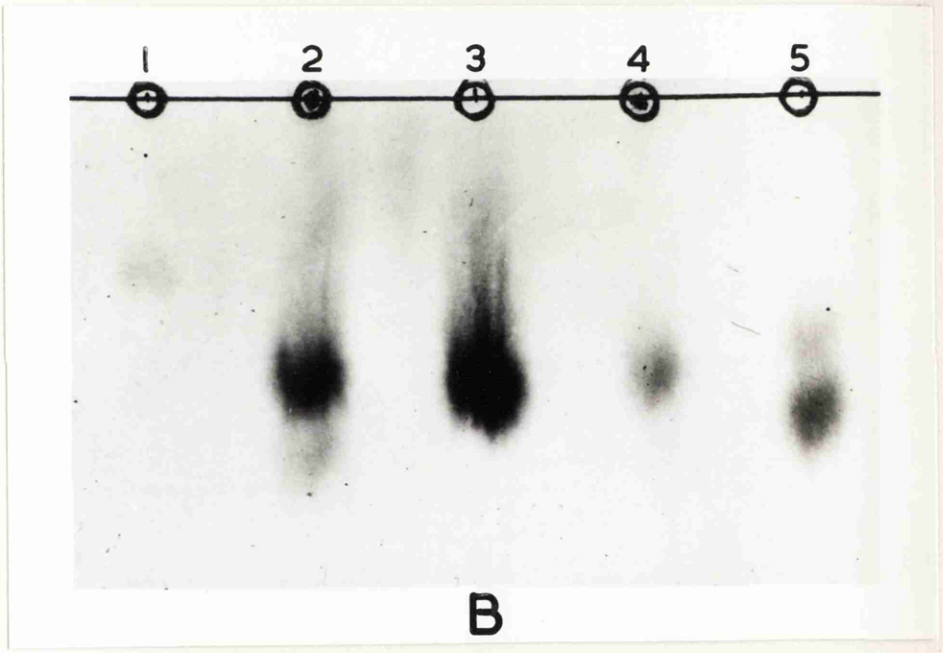
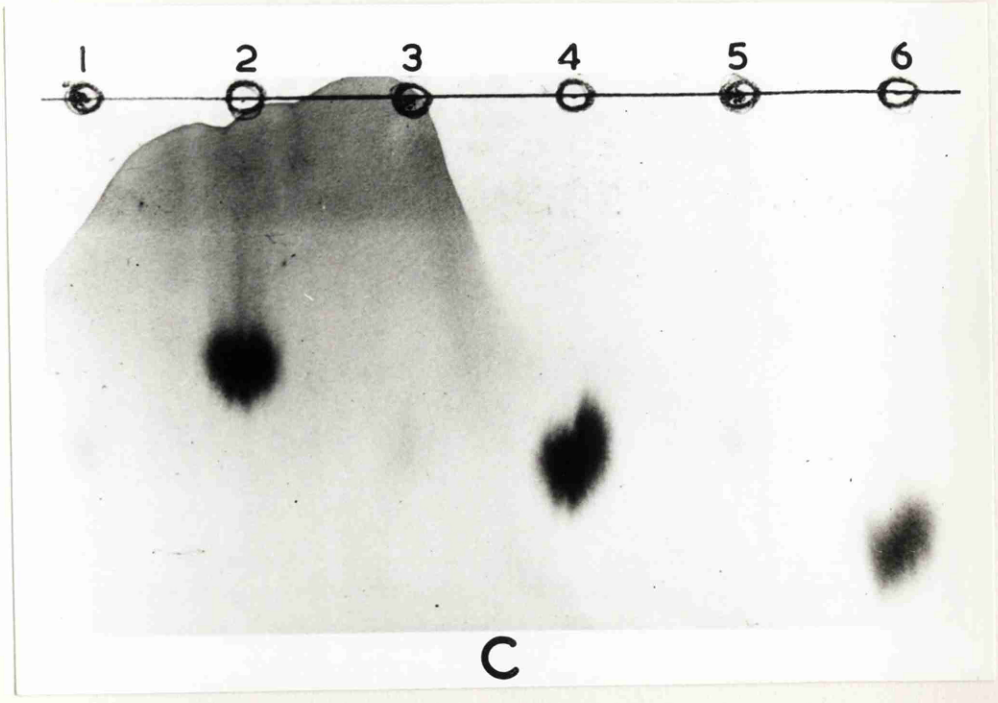


Fig. 53



no female circumscriptus captured in the field has shown any traces of having had a meal of vertebrate blood. Dissection has invariably revealed that the gut contained, if anything, only the clear fluid mentioned above.

It is possible that the species is autogenous, laying eggs without a blood meal. This phenomenon is well-known in the mosquito Culex molestus and has also been reported for Theobaldia subochrea and Aëdes concolor. The subject has been reviewed by Marshall (1938) and Bates (1949). There is no record, however, of autogeny among the blood-sucking Ceratopogonidae, and because of the failure to obtain eggs from laboratory-reared females of C. circumscriptus, conclusive evidence to support the hypothesis cannot easily be obtained for this species.

There is, in fact, a considerable amount of evidence which makes it difficult to support the suggestion that C. circumscrip-:tus is autogenous. This is linked with the question of where the female obtains the protein necessary for the development of her eggs. Marshall, Bates, and also Christophers (1945) report that Culex molestus, without a blood meal, can only lay a small number of eggs. The number of eggs laid by C. circumscriptus varies from 45 to 401 (average, 252). This is considerably greater than the numbers reported for other species of Culicoides which are known to take a blood meal (Table XIII) and must/

must necessitate the utilization of a large amount of protein in their development.

TABLE XIII

Comparison of the number of eggs laid by various species of *Culicoides*.

Species	Reference	No. of eggs
C. nubeculosus	Roberts (1950)	Av. 380*
" "	Lawson (1951)	More than 200
" "	Megahed (1956a)	Av. 135
C. pulicaris	Parker (1950)	Av. 93
C. impunctatus	Hill (1947)	30-65
C. obsoletus	" "	Av. 75
C. odibilis	" "	Av. 80
C. oxystoma	Patel (1921)	Up to 156
C. tristriatulus	Williams (1951)	42-81
C. austeni	Sharp (1928)	90-141
C. circumscriptus	This paper	Av. 252

*Megahed (1956a) suggests that this number may represent more than one batch of eggs.

In the case of Culex molestus it has been suggested that sufficient protein is carried forward from the larval stages to permit the development of a single small batch of autogenous eggs. This could hardly apply in the case of C. circumscriptus in view of the large number of eggs laid in comparison to blood-sucking species. Moreover, the larvae do not show signs of having stored in the fat-body any more material than other species.

It has recently been shown by Hocking (1952) that the necessary protein for the egg-development of an autogenous race of Aëdes communis in Manitoba is obtained from the autolysis of the flight muscles. The number of eggs laid is again small. This phenomenon does not occur in C. circumscriptus. Gravid females have often been caught on the wing and sections of the thorax of parous females, made by Mr H. Parsons of this Department, showed that all the flight muscles were still intact.

The source of the protein meal, therefore, still remains a mystery. It is unlikely that it is carried over from the larval stage. It is not obtained by autolysis of the flight muscles or by sucking vertebrate blood. There still remains the possibility, in spite of the negative results of the laboratory experiments, that, as is the case in many other genera of the Ceratopogonidae, C. circumscriptus attacks other insects, and that some, at least, of the clear fluid found in the alimentary/

alimentary canals is insect blood. Finally it is remotely possible that the necessary nutriment is obtained from a plant source. Downes (1955b) has shown that this is the case in Atrichopogon pollinivorus, the female of which pierces the pollen grains of honeysuckle and sucks out the sap. Both these suggestions, however, are the result of mere speculation and are not supported by any evidence whatsoever.

The results of chromatographic analysis of the contents of the alimentary canal strongly suggest that nectar is ingested by both sexes as a source of energy. This habit has been re-:ported in many blood-sucking Nematocera. Among Culicoides spp. it has been demonstrated by Downes (1955b), and Kieffer (1923), found C. parroti feeding on Umbelliferae. Knoll (1922) found that C. aricola and C. bromophilus were attracted to the flowers of Arum sp. The flowering plants in the vicinity of the breeding area at Dumbuck (see Table I) have all been examined carefully on many occasions but only seldom have midges been seen on them. At no time has feeding been observed. The numbers of midges caught by sweeping these flowers with a net did not differ markedly from the numbers taken on other vegeta-:tion in the vicinity. Apart from the imperfections of the observer, the explanation for the lack of observations of nectar-feeding, in spite of the positive evidence in the alimentary canals, may be that it possibly takes place largely after sunset,/

sunset, when the midges have ceased to fly and have settled on the vegetation.

Another likely source of carbo-hydrates is honey-dew deposited by aphids on the vegetation. Large numbers of aphids are found on the reeds on the marsh and in hot dry weather the leaves become thickly coated with honey-dew. No observations have been made which might support this suggestion, but it is unlikely that such an easily-won sugar meal would be ignored by the midges. Nielsen and Greve (1950) are of the opinion that Theobaldia morsitans feeds on honey-dew and it is possible that the females of Culicoides obsoletus observed by Gad (1951) 'on leaves and trunks of trees lapping drops of sap' were in fact feeding on this substance.

b) Mating

Copulation has never been observed in the laboratory, neither has any indirect evidence of copulation been observed in laboratory-reared flies.

In the field a close watch has always been maintained for indications of breeding behaviour, but none have been seen. Downes (1955a) has demonstrated swarming behaviour in eight species of Culicoides and has shown that in C. nubeculosus, C. riethi and, probably C. impunctatus and C. obsoletus mating occurs within the swarm. I have observed many such swarms on a/

a number of salt-marshes and have also induced their formation by means of artificial markers but on no occasion have they been found to consist of C. circumscriptus. Neither does Downes record any instance of swarming in this species, although many of his observations were carried out in areas where it is found. It is fair to assume, therefore, that the C. circumscriptus does not swarm, and that mating probably occurs while the flies are at rest among the vegetation. Although swarming is a wide-spread habit among the Nematocera, copulation has been observed to occur while at rest in Anopheles maculipennis var. atroparvus (Cambournac & Hill, 1940), Atrichopogon pollinivorus (Downes, 1955a) and in Culicoides nubeculosus and C. riethi (Pomerantsev, 1932; Downes, 1955a). When one considers that salt-marshes are, as a rule, without shelter from winds which make flight impossible for Culicoides spp., it will be realised that behaviour of this type would be distinctly advantageous for a species living in such a habitat. The fact that other salt-marsh species do form swarms does not invalidate this hypothesis. Considering the different types of mating behaviour found in C. nubeculosus and C. riethi it is not improbable that these other species may also mate without swarming, particularly when conditions are unfavourable for flight, though this has not been observed.

Cambournac and Hill (op. cit.) are of the opinion that the sexual/

sexual significance of swarming in Anopheles maculipennis var. atroparvus is only vestigial. There is some evidence also that in other species scattered throughout the Nematocera the habit has been lost or is in the process of dying out. Among those whose breeding habits are well-known, various species exhibit patterns of sexual behaviour which would appear to represent various stages in this process of evolution away from the typical swarm-mating behaviour. Thus, while in the majority of mosquitoes mating takes place in the swarm:

1) In Aëdes aegypti the males may seek out the females individually, though swarming is more common (Howard, Dyar & Knab, 1913).
2) Theobaldia morsitans forms swarms but mating seldom takes place in them. Mating takes place in solitary flight (Neilsen & Greve, 1950).
3) With Culicoides riethi mating usually takes place in swarms but on rare occasions it has been seen to copulate while resting (Downes 1955a).
4) Culicoides nubeculosus is able to mate equally easily either in swarms or at rest (Pomerantsev, 1932; Downes 1955a).
5) Anopheles maculipennis var. atroparvus mates, as a rule, while at rest. Swarming occurs, but only occasionally does mating take place in the swarm. (Cambournac & Hill, 1940).

6)/

6) Atrichopogon pollinivorus does not swarm. Mating occurs at rest on the food plant (Downes 1955a).

The mating habits of only 2 species of Culicoides have been observed in detail(by Downes 1955a.) It seems likely that the patterns of sexual behaviour among the genus may be more complex than has hitherto been realised and that these patterns may show specific variations. For example Downes (1955a) has established that C. impunctatus and C. obsoletus mate in flight, but has not observed swarming in these common species. He states that the copulating pairs seen were probably from unobserved swarms but it is equally possible that they may mate in solitary flight, as in the case of Theobaldia morsitans. It has been shown too, by Parker (1949), that in C. pallidicornis the females do not appear until the peak of flight activity of the males is passed, and that the peaks of activity of the two sexes are separated by about 2 hours. Although the males formed swarms it is doubtful if, under these circumstances, much mating would occur. Thus it is probable that in this species, as in Anopheles maculipennis var. atroparvus, the swarming habit is vestigial and that most mating takes place at rest on the vegetation. Likewise, it is suggested here that C. circumscriptus may belong to the category represented by Atrichopogon pollinivorus and mates while at rest in the vegetation, having lost completely the swarming habit. If this is the case, however, it is difficult to understand why they refuse to mate in cages.

E. Predators of Adults/

E. Predators of Adults

No attempt has been made in the present work to determine fully which animals might feed on adult midges. The following observations were made during the course of other investigations.

On two occasions Empidae have been caught on the Dumbuck salt-marsh carrying the bodies of Culicoides. In one case the predator was a female of the species Tachydromia minuta and the victim was a female C. circumscriptus which appeared to have been freshly killed. On the second occasion the Empid escaped and was not identified. It left behind the husk of its victim, a female C. maritimus.

It is highly probable that Culicoides adults form part of the diet of a number of birds, in particular swallows which are recorded by Witherby et al. (1938) as feeding on large numbers of Diptera while on the wing, including Chironomidae and Tipulidae. These birds are often seen flying and feeding over the marsh.

F. Parasites of Adults

1) Mites

On a number of occasions, while collecting adult midges, specimens have been caught which were parasitised by pinkish-red sub-spherical mites. Two types of these ecto-parasites have been collected and were identified by Dr G.O. Evans of the British/

British Museum.

Both species are larval forms. The first, of which only one specimen has been obtained, was found on the abdomen of a female C. heliophilus at Dingwall. It was, unfortunately, damaged in mounting but was identified as being probably the larva of Allothrombium sp. (family Trombidiidae).

The mites of the second type, which are more commonly found, have been obtained at Dumbuck on males and females of C. circumscriptus, C. maritimus and C. pulicaris, and at Loch Broom, Ross and Cromarty, on C. punctatus. They are the larvae of a hitherto undescribed genus of the family Trombiculidae. These mites were attached to the soft parts of the abdomen or thorax (Fig. 54). Usually only one of them is found on each host but sometimes midges were found carrying two and on one occasion three. It appears likely that their presence interferes with the midges' powers of flight, especially when the mites are attached to the thorax. In nearly all cases the parasitised specimens were obtained by sweeping the vegetation. Only twice were they recovered from aerial sweeps and on both of these occasions the midge carried only one mite attached to the abdomen.

According to Evans (private communication) the occurrence of the larvae of Trombiculidae on insects is relatively rare. This/

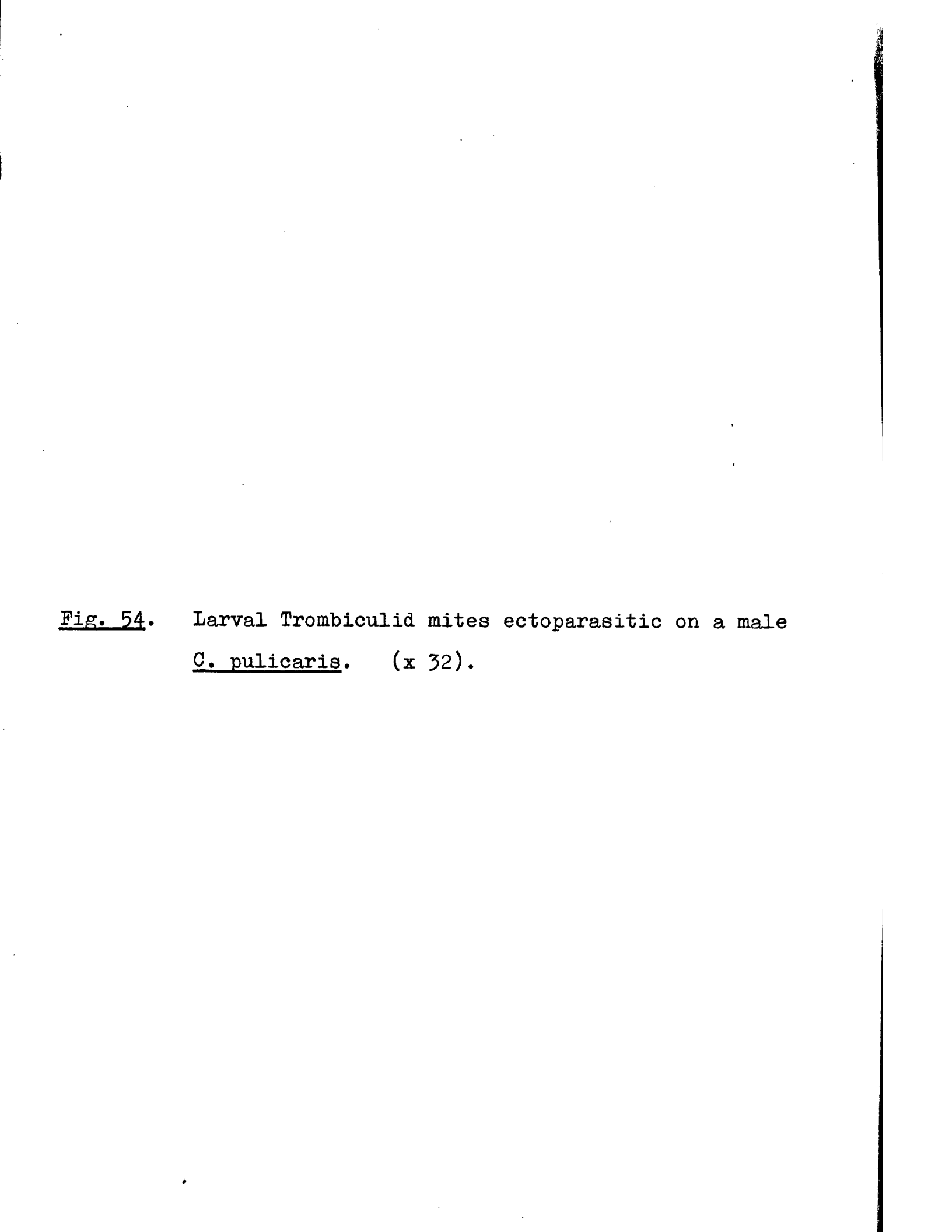


Fig. 54. Larval Trombiculid mites ectoparasitic on a male C. pulicaris. (x 32).

Fig. 54

This is the first record of the phenomenon in Britain. As a rule the larvae of this family, which includes the chigger mites and harvest mites, are parasitic on terrestial vertebrates. Only one species is recorded by Wharton & Fuller (1952) as being found on insects, the host in this case being Musca domestica. These authors are of the opinion that the association of the mite larvae with insects is accidental, but the frequency with which they are found on Culicoides spp. would indicate that they are mistaken in this assumption.

The specimens have been sent to Dr P.H. Vercammen-Grandjean for further examination.

2) Gregarines

During the dissection of specimens of C. circumscriptus Gregarines were found in the alimentary canals of some indivi-:duals.

3) General

In the course of their investigations other workers on Culicoides spp. must have observed parasitised specimens, in particular those with ecto-parasitic mites, yet only two references to the subject have been traced. Ghosh (1925) described a ciliate Balantidium knowlesii which was found in the coelomic cavity of C. peregrinus. Sharp (1928) also found ciliates in C. austeni as well as Acarids (unidentified) attached to the legs and bodies.

X. A NOTE ON THE TAXONOMIC STATUS OF C. CIRCUMSCRIPTUS

There is considerable doubt as to whether C. circumscriptus should be considered as a species to be separated from the closely related C. salinarius. The only features which Kettle & Lawson (1952) find to distinguish the larvae of the two forms from one another are the ranges in head measurements, which overlap slightly. It has been shown earlier, however, that the head measurements of Dumbuck larvae are smaller than those given by these workers for larvae of C. circumscriptus found in southern England. The heads were also shorter than in their specimens of C. salinarius obtained at Baauly, Inverness, and the head ratio differed considerably. Kettle and Lawson also found larvae at Chideock, Dorset, which had head sizes approa-:ching those of the Beauly C. salinarius but, which, when reared to the adult, gave rise to atypical C. circumscriptus. Thus it would appear that there is variation of head sizes and head ratios in different localities sufficient to render these criteria valueless as a means of distinguishing between the two forms. Indeed, when larvae which were obtained at Kincardine Bridge were separated according to these measurements and reared to the adult, it was found that most of the larvae with C. salinarius measurements gave rise to C. circumscriptus while one of the larvae with C. circumscriptus measurements turned

out/

out to be C. salinarius.

Kettle and Lawson have been unable to find any criteria whereby the pupae of the two forms may be distinguished from one another.

In the adults, Edwards (1939) makes it clear that the genitalia of the males are indistinguishable. He further states (pp. 135-136), "The breeding places also being similar, it may be that C. circumscriptus is merely a variety of C. salinarius; it is retained as distinct at present because the difference in markings (i.e. of the wings) seems fairly sharply defined and because the pattern in C. circumscriptus shows little variation as between specimens from Britain and from Palestine." The latter part of this statement is, as far as Britain, at least, is concerned, not correct.

The main differences in wing markings given by Edwards are as follows (see Fig. 55):-

1) C. circumscriptus - Small 'bulls-eye' spot (a) beside cross-vein. C. salinarius - No 'bulls-eye' spot.
2) C. circumscriptus - Pale streak on lower branch of cubital fork (b). C. salinarius - Lower branch of cubital fork entirely dark.
3) C. circumscriptus - Outer pale spot (c) in median fork more or less confluent with the spots above and below. C. salinarius - Pale spot (c) smaller and separate.

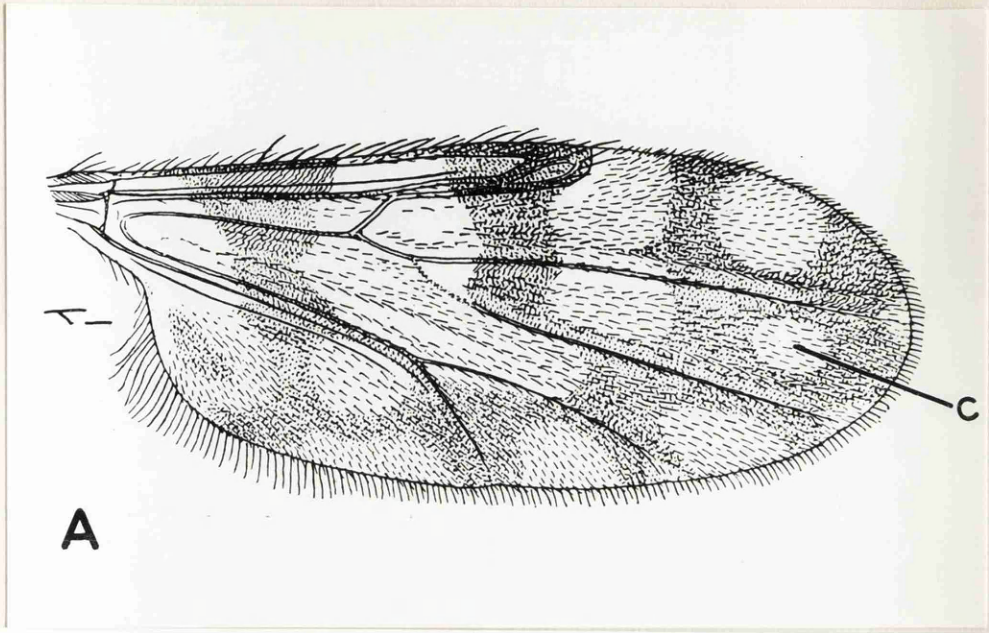
Far/

Fig. 55. A) The wing of C. salinarius.

B) The wing of C. circumscriptus.

C) The thorax of C. circumscriptus.

For lettering, see text. (From Edwards, 1939)



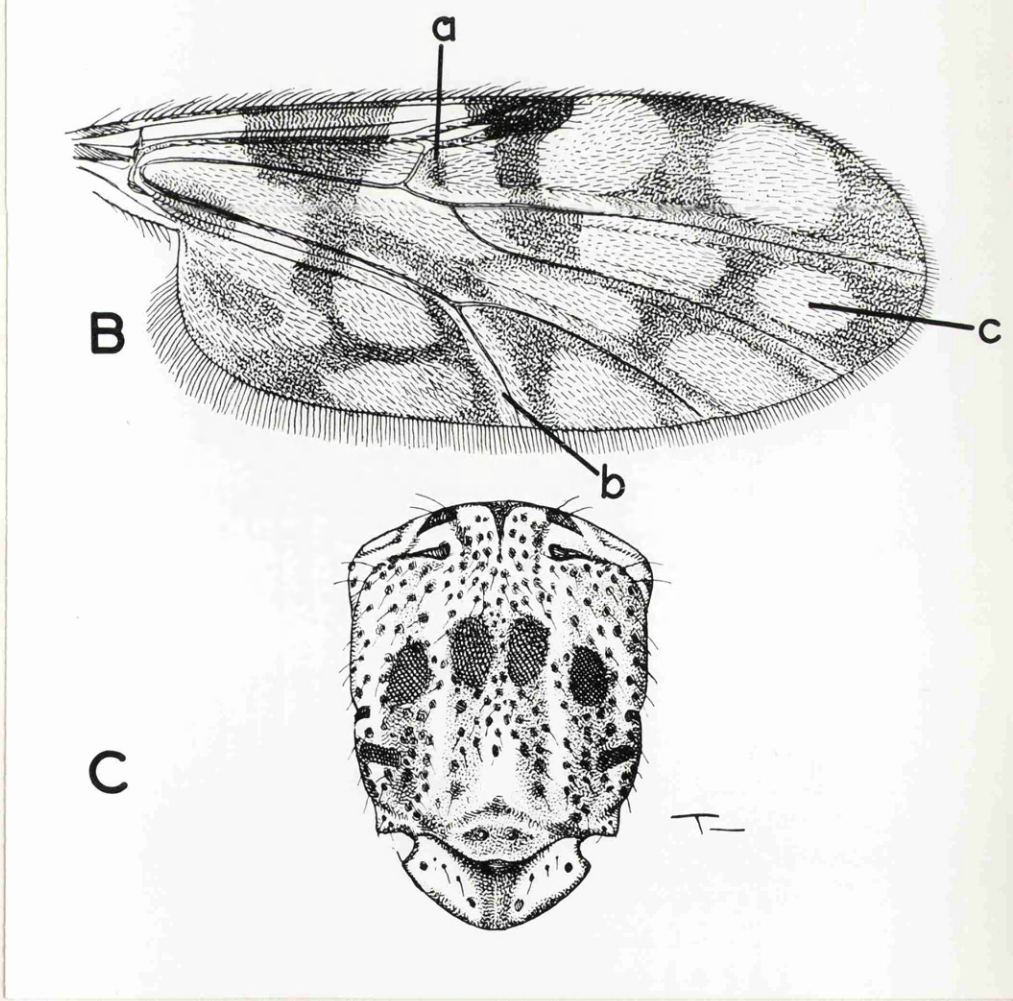


Fig. 55

Far from these differences being 'small and definite', it has been found that the shading of the wings of both forms varies greatly in distribution and intensity, and midges are often found with combinations of the characters of both forms.

The most reliable distinguishing feature of the wing mark-:ings is the presence or absence of the 'bulls-eye' spot. Although this varies in size its presence is, as a rule, clearly defined, and may be taken as an arbitrary distinguishing mark of C. circumscriptus.

The other characters are, however, very variable. The pale streak on the cubital fork of C. circumscriptus may vary between a broad well-defined mark and a thin, incomplete, and almost undetectable trace. In some, as Kettle and Lawson found in the atypical Chideock specimens, the cubital fork may be completely blacked over as in C. salinarius.

Similarly, the range in size of the pale spot (c) in the median fork of both forms makes it useless as a distinguishing mark.

In a specimen, identified as C. circumscriptus merely because of the presence of the 'bulls-eye' spot, the other wing characters may be either those described for C. circumscriptus or for C. salinarius or may be intermediate between the two forms, depending on the degree and intensity of the wing pigmentation./

pigmentation.

A further distinguishing mark given by Edwards for C. circumscriptus is the curved row of six large dark spots on the thorax of some specimens (Fig. 55, C). However, these have also been found to occur in some specimens of C. salinarius.

Thus we are left with only one distinguishing feature, namely the presence or absence of the 'bulls-eye' spot. The presence of this single feature, varying as it does in size, on a wing which is capable of considerable variations in the degree of pigmentation cannot be considered to justify the con-:tinued retention of C. circumscriptus as a separate species. It is suggested, therefore, that, in fact, C. circumscriptus is merely a variety of C. salinarius and should in future be considered as such.

Using the 'bulls-eye' spot to distinguish between the two forms, their geographical distribution so far determined in Scotland is as follows:-

Tynninghame (E. Lothian) - C. circumscriptus.

Kincardine Bridge (Stirling) - C. circumscriptus, and a few C. salinarius.

Guard Bridge (Fife) - C. circumscriptus.

Port Allen etc. (Perth) - C. salinarius

Montrose Basin/

Montrose Basin (Angus)	- Both forms in about equal numbers.
Beauly Firth (Ross & Cromarty)	- Some C. circumscriptus, but mainly C. salinarius.
Dingwall (Ross & Cromarty)	- Both forms in about equal numbers.
Torrisdale Bay (Sutherland)	- C. salinarius.
Loch Broom (Ross & Cromarty)	- C. salinarius.
Loch Torridon " " "	- C. salinarius.
Loch Laich (Appin, Argyll)	- C. circumscriptus.
Dumbuck (Dunbarton)	- C. circumscriptus and an occasional C. salinarius.

'Intermediate' forms were found in many of these localities, in particular Kincardine Bridge, Port Allen, Montrose Basin, Loch Laich and Dumbuck.

XI. SUMMARY

1. A description is given of the area of salt-marsh on the bank of the River Clyde, Dumbuck, Dumbartonshire, where most of the field studies on C. circumscriptus were carried out. Attention is drawn to the topographical differences found in various other Scottish salt-marshes where the species is found.

2. The methods and technique employed in the work are described.

3. An account is given of the life cycle of C. circumscriptus in the laboratory. The emergence of males from cultures is somewhat in advance of that of the females. The possibility of the occurrence of parthenogenesis is discussed. Data pertaining to the life cycle in the field, based mainly on the periodic sampling of the larval population, is also presented and discussed.

4. The eggs of C. circumscriptus are described. The sucker-like structures on the chorion are given the name of ansulae (singular : ansula) and it is shown that these serve to fix the egg to the substrate. Accounts are given of oviposition and hatching.

5./

5. Descriptions of the larval instars, supplementary to those found in other works, are given. It is suggested that the head sizes may vary in different localities.

6. A description is given of the type of habitat in which the larvae of C. circumscriptus are most likely to be found, but it is pointed out that they may be absent from such sites and conversely, may occur in places which differ considerably from the typical habitat.

7. The larvae of C. circumscriptus have considerable powers of osmoregulation, a function thought to be performed by the anal papillae.

8. The manner of locomotion of the larvae is described and it is shown that the proleg of the first instar larva may be extruded or invaginated at will.

9. During mud-sampling the numbers of larvae in the sites were found to fluctuate considerably. Experiments designed to show that this might be accounted for by migration from one part of the breeding area to another indicated, however, that the range of larval movements in the mud is very limited.

10. It is shown that larvae of C. circumscriptus which are extracted from mud and kept in water without food change their phototaxis from negative to positive. By supplying them with

suitable/

suitable food they can be made to revert to photonegative behaviour. The ecological significance of this phenomenon is discussed.

11. Experiments to determine the type of food taken by the larvae are described and discussed. The larvae appear to be detritus feeders but are nevertheless actively selective in the type of material they ingest.

12. Of a number of species cohabitant with the larvae of C. circumscriptus at Dumbuck only one, Nereis diversicolor, was proved to be a predator on the larvae.

13. Fluorescent bacteria and astomatous ciliates, found in the haemocoele of the larvae of C. salinarius and other species of Culicoides are described.

14. An account is given of the process of pupation. Experiments indicate that the gas inside the buoyancy chamber of the pupa is of atmospheric origin.

15. A description is given of the behaviour of the pupae as observed in the laboratory and in the field.

16. The diurnal incidence of the adults and the conditions of flight are discussed. It is suggested that the scarcity of males in the field may be due to their remaining deep in the vegetation.

17./

17. In spite of numerous attempts, no success has been had in inducing mating in the laboratory. Neither has it been observed in the field. C. circumscriptus does not form swarms. It is suggested that in a number of groups of the Nematocera the sexual significance of swarming is dying out and that in some species, among them C. circumscriptus, the habit of swarming has been completely lost.

18. C. circumscriptus does not suck vertebrate blood. The possibility of autogeny is discussed but the evidence is weighted against this conclusion. It is tentatively suggested that the female may obtain her protein meal from invertebrate or plant sources. While chromatographic analysis indicates that nectar is taken as a source of energy the midges have not been observed to feed on this in the field.

19. Empidae have been observed to prey on the adult midges and it is probable that the latter may constitute part of the diet of various birds, in particular, swallows.

20. Two types of mite larvae have been observed to parasitise adult midges. The type most commonly found is a member of the Trombiculidae. This is the first record in Britain of parasiti-:sation of insects by mites of this family.

21. In view of the great similarity of the larvae and pupae of C. circumscriptus to those of C. salinarius, and the existence of adult/

adult forms intermediate between the two species, it is suggested that the taxonomic status of C. circumscriptus be reduced to that of a variety of C. salinarius.

XII APPENDICES

Appendix A

1) The numbers of larvae of each instar and of pupae found in each sample of mud taken from the Dumbuck breeding area between May, 1954 and October, 1956.

a) Numbers of first instar larvae in each sample.

Date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total
1954							
17/5		0	0		0	0	0
30/5		2	4		3	4	13
10/6		4	0		2	0	6
26/6	0	1	9	1	0	0	11
13/7	3	0	0	0	0	0	3
29/7	3	0	2	1	3	0	9
24/8	0	0	8	8	32	0	48
2/9	3	1	10	6	22	4	46
13/9	1	0	2	1	5	0	9
27/9	1	0	1	2	0	0	4
8/10	0	0	0	1	1	0	2
22/10	0	0	0	0	0	0	0
1955							
27/6	0	0	0	0	0	0	0
19/7	0	0	0	0	0	1	1
2/8	4	0	0	0	1	0	5
14/8	6	1	0	17	7	1	32
28/8	5	0	0	15	5	0	25
14/9	2	0	0	5	4	2	13
26/9	3	1	0	2	5	1	12
9/10	0	0	0	0	0	0	0
1956							
24/5	0	0	0	0	0	0	0
16/6	2	0	2	0	2	0	6
29/6	0	0	0	0	0	151	151
18/7	0	0	0	0	1	0	1
2/8	0	0	0	0	0	6	6
23/8	0	1	1	0	0	0	2
20/9	0	0	0	0	0	0	0

b) Numbers of second instar larvae in each sample.

Date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total
1954							
17/5		0	0		0	0	0
30/5		20	8		13	19	60
10/6		4	0		61	3	68
26/6	8	8	130	19	75	13	253
13/7	19	0	0	17	8	0	44
29/7	9	1	5	17	50	7	89
24/8	3	8	23	73	140	5	252
2/9	8	6	53	90	111	35	303
13/9	16	2	33	27	211	2	291
27/9	8	18	23	24	46	3	122
8/10	6	1	31	16	21	0	75
22/10	5	2	4	5	6	3	25
4/11	2	5	1	24	0	0	32
18/11	3	0	0	0	0	0	3
9/12	2	0	0	0	0	0	2
27/12	0	1	0	0	5	0	6
1955							
14/1	1	1	0	4	10	1	17
28/1	0	0	0	0	1	0	1
17/2	0	0	0	1	0	0	1
3/3	0	0	0	0	2	0	2
17/3	0	0	0	0	0	0	0
		NOTHING FOR 3 MONTHS					
19/7	55	2	3	0	9	30	99
2/8	84	2	4	12	36	3	141
14/8	161	12	14	62	51	12	312
28/8	183	22	11	86	76	5	383
14/9	226	2	6	626	501	399	1760
26/9	75	4	19	202	212	178	690
9/10	35	3	4	2	2*	3*	49
25/10	5	1	2	7	4	2	21
29/11	7	3	4	1	9	0	24
1956							
2/1	5	2	0	2*	7*	1	17
2/2	4	0	2	0	2	1	9
2/3	4	3	0	3	0	0	10
3/4	0	3	3	1	1	0	8
21/4	0	0	0	0	0	0	0
7/5	0	0	0	0	0	0	0
24/5	0	0	0	0	0	0	0
16/6	8	0	2	0	1	2	13
29/6	5	1	3	17	0	459	485
18/7	5	2	0	0	1	1	9
2/8	0	0	1	0	10	45	56
23/8	10	13	2	0	13	2	40

Date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total	Mean of logs.
20/9	0	5	3	3	24	6	41	
10/10	0	2	0	11	50	18	81	

c) Numbers of third instar larvae in each sample and the mean of the logarithms of these numbers.

1954								
2/5		1	1		1	0	3	
17/5		1	0		0	0	1	
30/5		7	8		5	4	24	
10/6		5	1		15	2	23	
26/6	20	31	97	3	93	21	265	1.425
13/7	53	18	33	31	41	8	184	1.418
29/7	25	5	7	14	41	23	115	1.177
24/8	12	44	55	20	118	49	298	1.588
2/9	43	37	64	121	106	25	396	1.752
13/9	69	31	89	49	421	10	669	1.765
27/9	44	78	106	61	179	25	493	1.833
8/10	105	66	127	83	101	2	484	1.695
22/10	61	9	17	25	18	8	138	1.254
4/11	77	19	21	106	19	5	247	1.415
18/11	59	21	8	23	22	2	135	1.167
9/11	11	20	1	87	68	1	188	1.019
27/12	4	7	1	1	77	4	94	0.656
1955								
14/1	18	6	6	60	125	7	222	1.255
28/1	27	6	12	28	52	13	138	1.261
17/2	28	15	9	23	12	8	95	1.154
3/3	26	23	4	34	54	4	145	1.208
17/3	13	15	8	16	20	2	74	1.000
31/3	9	35	8	6	1	1	60	0.697
15/4	28	4	1	7	3	3	46	0.641
26/4	0	2	1	1	3	0	7	0.130
8/5	0	0	1	0	0	0	1	0.000
18/5	0	0	0	0	0	0	0	0.000
27/5	0	1	0	0	1	0	2	0.000
10/6	0	4	1	0	3	0	8	0.180
27/6	0	3	1	0	0	0	4	0.080
19/7	121	29	52	15	54	43	314	1.634
2/8	74	16	34	26	159	10	319	1.537
14/8	212	74	12	280	92	29	699	1.857
28/8	450	138	13	511	363	81	1556	2.181
14/9	494	23	29	865	704	553	2668	2.341
26/9	512	29	12	789	764	222	2328	2.229
9/10	589	46	53	289	527*	160*	1664	2.257
25/10	290	60	9	517	352	101	1329	2.077
29/11	523	40	776	16	609	133	2097	2.221

Date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total	Mean of logs.
1956								
2/1	146	28	12	40*	425*	199	850	1.870
2/2	402	60	19	73	15	8	577	1.601
2/3	53	46	29	207	115	10	460	1.704
3/4	86	62	11	101	120	34	414	1.731
21/4	6	14	5	11	9	2	47	0.820
7/5	7	3	5	3	3	0	21	0.496
24/5	1	4	5	0	2	1	13	0.267
16/6	4	1	0	0	0	1	6	0.100
29/6	45	3	3	31	5	200	287	1.183
18/7	30	15	4	16	12	185	262	1.301
2/8	16	15	4	18	143	77	273	1.380
23/8	22	4	11	1	79	557	674	1.272
20/9	3	10	21	14	112	114	274	1.342
10/10	12	5	0	126	316	54	513	1.352

d) Numbers of fourth instar larvae in each sample and the mean of the logarithms of these numbers.

1954								
2/5		108	115		18	13	254	
17/5		94	160		57	5	316	
30/5		29	15		18	30	92	
10/6		8	2		9	1	20	
26/6	26	73	70	0	31	12	212	1.282
13/7	31	11	79	81	61	66	256	1.490
29/7	10	9	18	13	22	44	116	1.218
24/8	21	96	42	37	73	51	320	1.678
2/9	22	21	41	129	143	64	420	1.725
13/9	104	97	85	62	139	15	502	1.841
27/9	59	64	50	76	124	22	395	1.766
8/10	72	66	38	160	59	4	399	1.639
22/10	106	6	13	39	18	3	185	1.207
4/11	111	31	42	69	48	17	318	1.652
18/11	59	38	24	14	48	5	188	1.376
9/12	10	37	15	119	172	36	389	1.602
27/12	92	29	12	13	184	26	356	1.550
1955								
14/1	21	24	26	83	132	61	347	1.657
28/1	49	61	33	88	131	89	451	1.901
17/2	72	48	24	62	28	6	240	1.489
3/3	48	63	25	44	181	29	390	1.707
17/3	25	28	24	34	67	17	195	1.469
31/3	30	38	12	28	22	6	136	1.284
15/4	95	34	33	64	41	24	291	1.638
26/4	79	26	33	28	55	10	231	1.503
8/5	14	13	33	18	35	6	119	1.276
18/5	23	26	42	9	3	0	103	0.972

Date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total	Mean of logs.
27/5	12	6	6	14	6	3	47	0.839
10/6	17	5	7	19	9	5	62	0.951
27/6	6	2	2	6	5	15	36	0.672
19/7	145	57	147	74	219	88	730	2.040
2/8	48	30	94	74	126	31	403	1.765
14/8	65	87	44	38	75	69	378	1.782
28/8	15	31	3	45	21	28	143	1.261
14/9	84	19	14	127	96	72	412	1.715
26/9	162	54	21	308	160*	256*	961	2.061
9/10	125	54	210	221	350*	394*	1354	2.273
25/10	92	160	29	85	298	247	911	2.071
29/11	70	66	211	21	260	83	711	1.941
1956								1.793
2/1	75	78	14	50*	141*	99	457	1.793
2/2	128	82	20	79	38	8	355	1.617
2/3	50	37	9	26	71	25	218	1.481
3/4	72	88	36	91	192	82	561	1.902
21/4	159	142	43	167	198	50	759	2.034
7/5	129	107	151	301	188	36	912	2.105
24/5	35	57	75	77	82	17	343	1.701
16/6	11	67	11	29	2	9	129	1.104
29/6	23	14	2	32	86	88	245	1.366
18/7	101	7	4	123	8	163	406	1.443
2/8	11	2	4	1	63	60	141	0.920
23/8	92	14	11	14	123	252	506	1.631
20/9	0	14	118	53	326	485	996	1.690
10/10	29	15	1	402	239	233	919	1.665

e) Numbers of pupae in each sample.

1954							
2/5		4	19		13	7	43
17/5		19	63		44	1	127
30/5		18	8		11	20	57
10/6		7	2		0	0	9
26/6	0	0	0	0	0	0	0
13/7	1	2	2	1	2	3	11
29/7	6	3	5	2	7	16	39
24/8	0	0	0	0	0	0	0
1955							
15/4	0	0	0	0	0	0	0
26/4	4	7	9	1	10	5	36
8/5	6	1	2	1	13	2	25
18/5	2	0	0	0	0	1	3
27/5	16	9	2	14	16	13	70
10/6	14	3	4	9	7	9	46
27/6	1	0	2	0	0	0	3
19/7	26	12	34	13	79	39	203
2/8	12	9	5	14	31	22	93

Date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total
14/8	14	2	7	9	44	22	98
28/8	10	1	0	2	2	6	21
14/9	0	0	1	0	0	0	1
26/9	0	0	0	0	0	0	0
1956							
3/4	0	0	0	0	0	0	0
21/4	1	0	0	0	0	1	2
7/5	3	1	2	2	4	0	12
24/5	0	1	9	44	80	38	172
16/6	0	0	3	10	1	20	34
29/6	0	2	0	7	0	40	49
18/7	1	0	0	0	22	18	41
2/8	2	1	0	1	23	32	59
23/8	0	0	1	1	12	3	17
20/9	0	0	0	0	0	0	0

*On these two occasions the larvae from two samples were accidentally mixed and the total was split arbitrarily to the numbers given.

2) Analysis of Variance Tables

a) Third instar

Variation due to	Degrees of Freedom	Sum of squares	Mean squares	F
Time	48	125.4723	2.6140	11.94 (S)
Sites	5	11.7406	2.3481	10.73 (S)
Residual	238	52.1098	0.2189	
Total	291*	189.3227		

b) Fourth instar

Variation due to	Degrees of Freedom	Sum of squares	Mean squares	F
Time	48	37.6399	0.7842	4.55 (S)
Sites	5	5.3807	1.0761	6.25 (S)
Residual	238	41.0140	0.1723	
Total	291*	84.0346		

S - Significant

*Two degrees of freedom have been removed since two counts had to be arbitrarily inserted in the data above.

Appendix B

1) The data obtained from Light Experiments Nos 1 - 28. The time at which tests were made is explained in the text. The larvae are divided into 4 groups, those which were photopositive at both tests (++), those which were photone-:gative at both tests (--), and 2 groups comprising those larvae which alternated in the sign of their taxis (+-) and (-+). In some of the later experiments (Nos 21 and 23 - 28) a second test was not made.

Time	++	--	+-	-+	Total
Expt 1 (Third instar)					
1 day	14	35	3	11	63
2 "	29	28	2	10	69
5 "	25	5	2	2	34
6 "	23	4	1	1	29
Expt 1 (Fourth instar)					
1 "	39	54	17	23	133
2 "	56	34	8	24	122
5 "	101	16	6	4	127
6 "	104	18	6	4	132
Expt 2 (Third instar)					
3 "	27	17	1	2	47
4 "	27	15	1	2	45
5 "	26	11	1	1	39
6 "	22	3	4	2	31
12 "	20	6	0	3	29
Expt (Fourth instar)					
3 "	162	87	31	19	299
4 "	145	71	14	24	254
5 "	148	36	13	9	206
6 "	151	19	10	10	190
12 "	89	35	8	8	140
Expt 3 (Third instar)					
1½ "	10	21	8	5	44
3½ "	19	5	7	2	33
4½ "	16	4	2	1	23
5½ "	14	3	0	2	19
6½ "	16	1	0	0	17

Time	++	--	+-	-+	Total
7½ days	16	1	0	0	17
8½ "	12	0	0	0	12
9½ "	10	0	0	0	10
Expt 3 (Fourth instar)					
1½ "	91	98	20	14	223
3½ "	108	70	18	10	206
4½ "	156	39	12	23	230
5½ "	147	31	14	8	200
6½ "	157	27	8	5	197
7½ "	160	24	4	4	192
8½ "	144	18	3	7	172
9½ "	151	21	7	5	184
Expt 4 (Third instar)					
4 hours	3	34	10	9	56
1 day	11	23	6	4	44
2 "	15	16	3	3	37
3 "	15	7	3	1	26
5 "	16	4	3	0	23
6 "	14	3	2	0	19
7 "	15	3	0	0	18
8 "	17	1	0	0	18
9 "	16	0	0	0	16
15 "	10	3	1	0	14
Expt 4 (Fourth instar)					
4 hours	18	141	61	41	261
1 day	70	114	30	16	230
2 "	100	83	28	13	224
3 "	121	46	21	14	202
5 "	124	25	16	6	171
6 "	125	18	11	6	160
7 "	122	16	7	4	149
8 "	124	12	9	2	147
9 "	120	10	8	1	139
15 "	65	21	5	6	97
Expt 5 (Fourth instar)					
12 hours	8	249	13	16	286
1½ days	53	142	17	50	262
3 "	64	117	18	57	256
5 "	135	48	44	36	263
6 "	144	41	14	44	243
7 "	167	41	17	14	239
8 "	159	27	16	27	229
9 "	111	42	32	8	193
11 "	124	18	14	16	172
12 "	113	22	7	8	150
13 "	95	19	6	9	129
14 "	86	16	5	8	115
15 "	14	24	6	5	49
18 "	43	35	2	3	83

Time	++	--	+-	-+	Total
Expt 6					
1 day	6	129	17	19	171
2 "	43	69	8	51	171
3 "	57	32	12	71	172
4 "	63	59	7	39	161
5 "	97	55	11	16	159
7 "	109	15	11	14	149
8 "	96	15	3	20	134
9 "	96	16	14	16	132
10 "	79	18	10	3	110
11 "	126	8	5	7	146
12 "	13	54	4	9	80
14 "	41	54	5	6	106
15 "	48	32	6	10	96
Expt 7					
1 "	37	133	15	45	230
2 "	48	98	40	31	217
4 "	96	81	12	19	208
5½ "	157	23	17	12	209
7 "	138	30	22	27	195
10 "	110	36	7	3	156
11½ "	132	7	5	3	147
13 "	34	66	0	13	113
16 "	25	65	2	9	101
17 "	60	16	4	2	82
Expt 8					
1 "	47	149	14	28	238
2 "	39	135	22	41	237
4 "	108	70	50	11	239
5½ "	184	15	21	2	222
7 "	155	19	13	4	191
10 "	126	14	10	7	157
12 "	96	10	8	1	115
13 "	55	30	2	7	94
14 "	58	24	4	1	87
16 "	31	8	2	2	43
Expt 9					
1 "	32	114	23	26	195
3 "	55	90	19	14	178
6 "	111	37	9	4	161
9 "	115	21	12	4	152
12 "	132	4	11	11	147
13 "	64	24	6	2	96
14 "	45	26	5	4	80
15 "	42	16	6	1	65
16 "	32	17	2	2	53
18 2	43	5	3	3	51

Time	++	--	+-	-+	Total
Expt 10					
1 day	18	130	12	44	204
3 "	79	89	13	11	192
6 "	94	56	17	4	171
9 "	123	26	9	2	160
12 "	136	2	8	0	146
13 "	69	29	9	4	111
14 "	68	23	8	1	100
15 "	74	10	10	0	94
16 "	48	18	6	2	74
18 "	57	7	6	1	71
Expt 11					
1 "	73	38	12	7	130
2 "	45	65	1	4	115
3 "	45	46	3	7	101
4½ "	19	48	4	3	74
6 "	16	38	1	5	60
7 "	13	31	5	2	51
8 "	9	28	2	1	40
Expt 12					
1 "	75	36	8	6	125
2 "	44	50	4	3	101
3 "	33	42	4	5	84
4½ "	27	29	3	6	65
6 "	16	30	2	2	50
7 "	8	25	4	1	38
8 "	6	18	2	2	28
Expt 13					
1 "	89	0	0	0	89
2 "	82	0	0	2	84
3 "	73	1	0	1	75
4½ "	61	0	0	0	61
6 "	43	2	1	1	47
7 "	34	1	0	0	35
8 "	29	0	0	0	29
Expt 14					
1 "	107	0	0	1	108
2 "	100	1	0	0	101
3 "	88	0	0	0	88
4½ "	76	2	1	0	79
6 "	63	0	0	1	64
7 "	55	0	0	0	55
8 "	44	3	0	0	48
Expt 15					
1 "	55	4	0	1	60
2 "	49	0	0	1	50
3½ "	35	1	0	0	36
5½ "	24	1	1	1	27
7 "	19	0	0	0	19

Time	++	--	+-	-+	Total
Expt 16					
2 hours	10	62	9	22	103
1 day	27	34	4	17	82
3 "	39	11	5	7	62
5 "	38	7	4	4	49
7 "	25	0	0	0	25
Expt 17					
4 hours	28	83	18	21	150
1 day	60	46	12	17	135
3 "	74	20	5	15	114
4 "	78	11	5	7	101
6 "	66	4	4	4	78
8 "	49	0	0	0	49
9 "	37	0	0	1	38
10 "	30	0	0	0	30
Expt 18					
2 hours	5	67	16	21	108
1 day	27	34	4	17	91
3 "	14	48	6	18	86
5 "	25	32	6	14	77
7 "	24	34	3	6	67
9 "	34	17	7	6	64
11 "	26	25	1	7	59
13 "	18	22	3	10	53
16 "	33	9	2	5	49
17 "	29	10	2	1	42
19 "	25	9	5	1	40
21 "	21	14	0	3	38
Expt 19					
1 "	1	171	4	6	182
2 "	4	134	6	25	169
3 "	9	135	1	17	162
4 "	1	132	2	19	154
5 "	7	106	14	9	136
7 "	29	48	11	40	128
8 "	47	35	8	32	122
9 "	58	29	2	19	108
10 "	50	23	12	5	90
11 "	18	39	2	11	70
12 "	5	35	0	2	42
14 "	12	30	0	14	56
15 "	26	16	0	10	52
Expt 20					
1 "	0	49	0	1	50
4 "	6	39	2	0	47
5½ "	9	31	3	3	46
7 "	9	23	4	4	40
8 "	8	19	3	7	37

Time	++	−−	+−	−+	Total
11 days	13	18	3	0	34
12½ "	18	6	6	1	31
14 "	8	11	3	3	25
Expt 21					
1 "	6	32			38
3 "	10	21			37
6 "	13	22			35
9 "	16	17			33
11 "	9	18			27
14 "	7	17			24
17 "	5	15			21
Expt 22					
1 "	50	6	0	3	59
2½ "	43	5	0	0	48
3½ "	34	4	1	0	39
5 "	27	2	0	1	30
6 "	23	1	0	0	24
7 "	18	0	0	1	19
Expt 23					
2 "	43	13			56
3½ "	39	11			50
5 "	31	14			45
7 "	17	16			33
8½ "	22	8			30
Expt 24					
1 "	27	36			63
3 "	18	42			60
5 "	24	38			62
7 "	14	42			56
8 "	20	33			53
10 "	15	36			51
Expt 25					
1 "	32	40			72
3 "	53	20			73
5½ "	53	16			69
7 "	65	6			71
9 "	34	34			68
Expt 26					
1 "	42	12			54
3 "	38	14			52
5 "	35	8			43
7½ "	20	8			28
Expt 27					
1 "	12	52			64
3 "	42	16			58
5½ "	33	26			59
7 "	28	25			53
9 "	23	26			49

Time	++	--	+-	-+	Total
Expt 28					
1 day	10	35			45
3 "	28	16			44
5 "	26	16			42
7½ "	12	28			40

2) The graphs which follow represent the results of those experiments on the reactions of Culicoides larvae to light stimuli which were not illustrated earlier in the text. In each case the numbers of larvae at the beginning and end of the experiments are given. Unless otherwise stated the larvae used were C. circumscriptus.

Experiments 1 - 4

Mixed batches of unfed third and fourth instar larvae after extraction from mud. In Experiments 2 and 4 pieces of blow-fly larvae were used at point F and a further test made 6 days later.

Experiments 5 - 10

Batches of unfed fourth instar larvae after extraction from mud. They were given fresh pieces of blow-fly larvae at point F and after each subsequent test.

Experiment 12

A batch of previously starved fourth instar larvae kept in rearing medium between tests.

Experiment 13

A batch of previously starved fourth instar larvae kept in incinerated mud between tests.

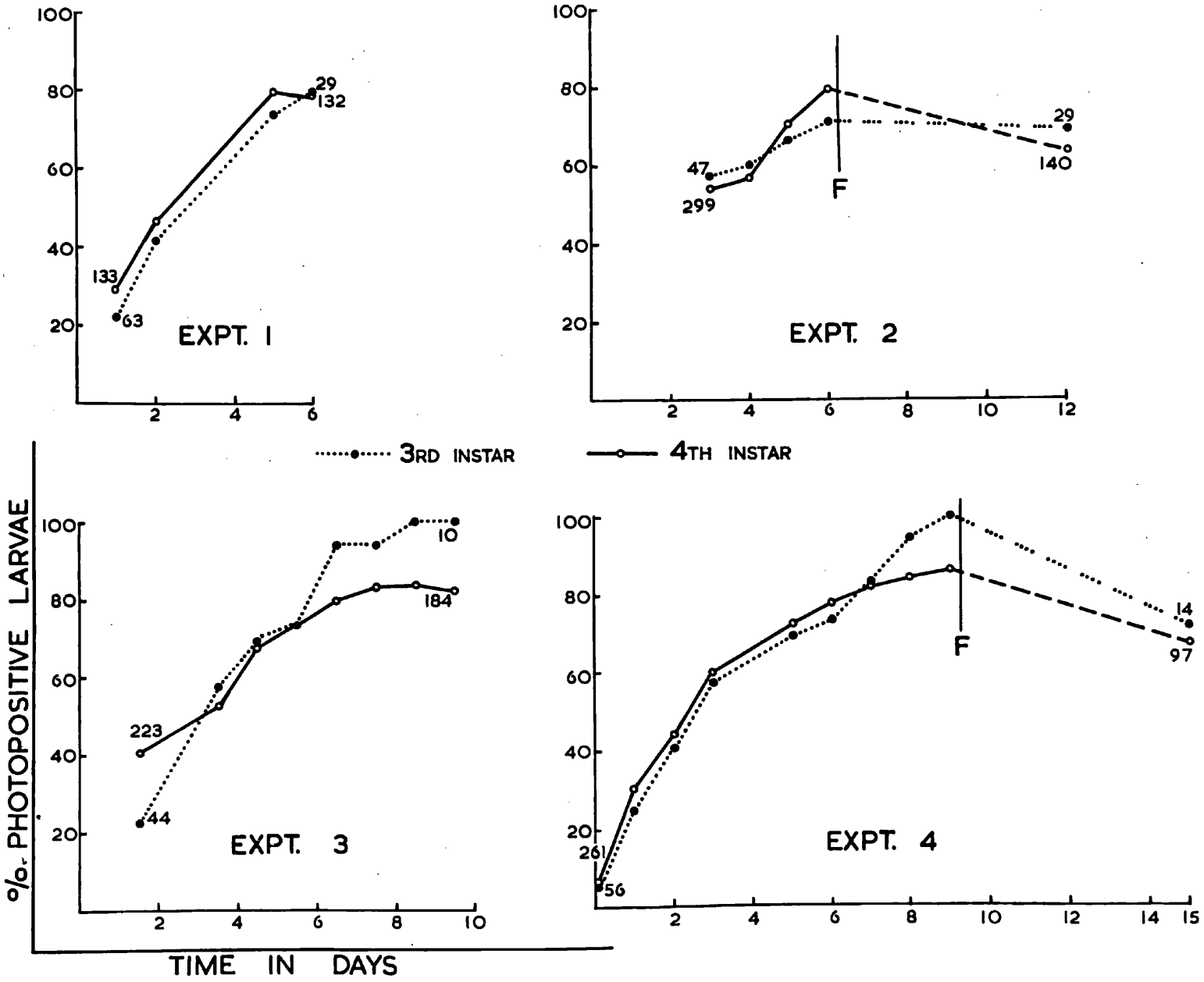
Experiment 16 - 17

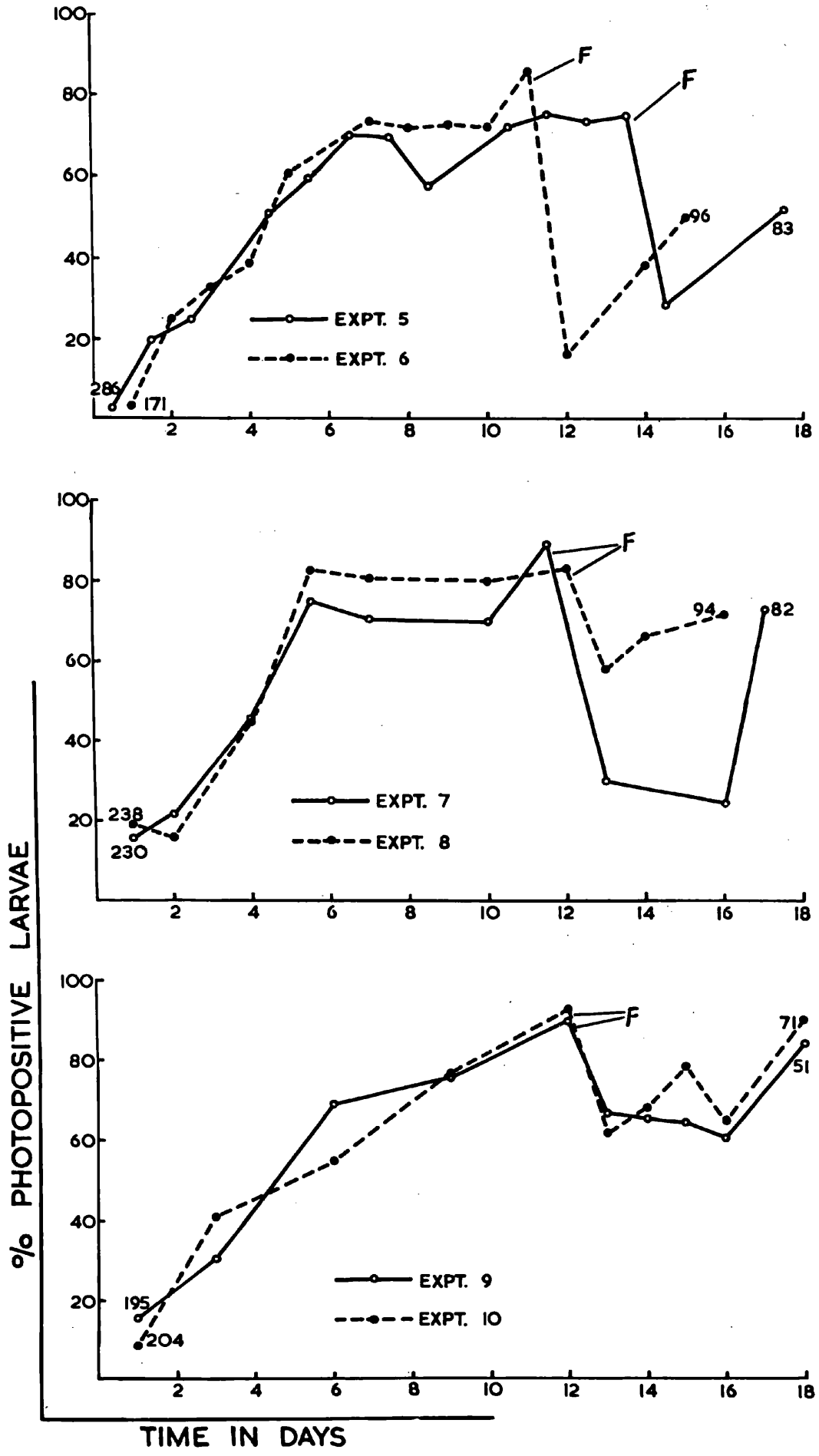
Batches of unfed late fourth instar larvae after extraction from mud.

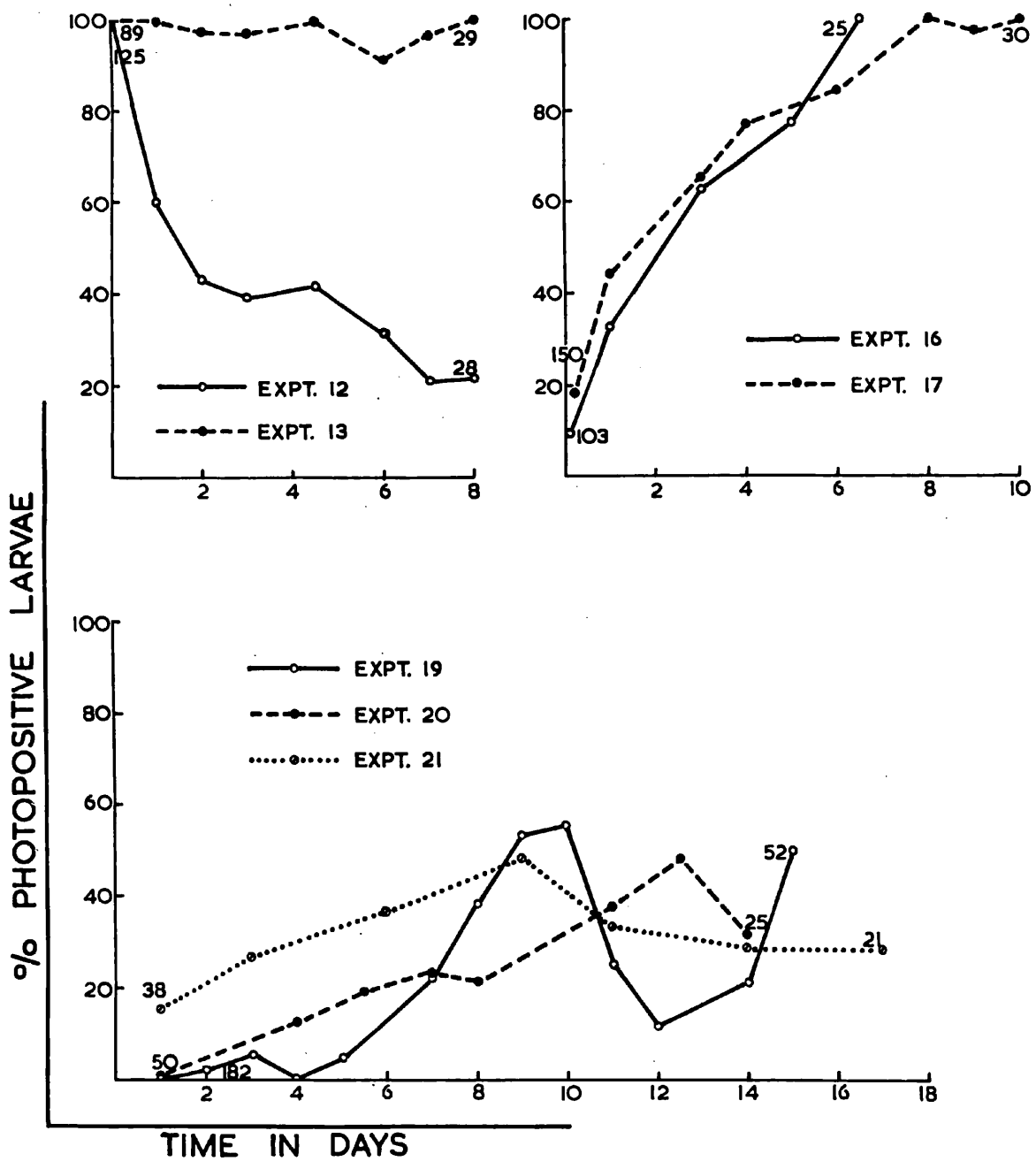
Experiments 19 - 21/

Experiments 19 - 21

Batches of unfed fourth instar larvae of C. maritimus after extraction from mud.


100
80
60
40
20
2
4
6
29
132
133
63
EXPT. 1
100
80
60
40
20
2
4
6
8
10
12
47
299
F
29
140
EXPT. 2
3RD INSTAR
4TH INSTAR
% PHOTOPOSITIVE LARVAE
100
80
60
40
20
2
4
6
8
10
10
184
223
44
EXPT. 3
100
80
60
40
20
2
4
6
8
10
12
14
15
261
56
F
14
97
EXPT. 4
TIME IN DAYS


100
80
60
40
20
F
F
96
83
EXPT. 5
EXPT. 6
286
171
2
4
6
8
10
12
14
16
18
100
80
60
40
20
F
94
82
238
230
EXPT. 7
EXPT. 8
2
4
6
8
10
12
14
16
18
100
80
60
40
20
F
71
51
195
204
EXPT. 9
EXPT. 10
2
4
6
8
10
12
14
16
18
% PHOTOPOSITIVE LARVAE
TIME IN DAYS


100
80
60
40
20
89
125
29
28
EXPT. 12
EXPT. 13
2
4
6
8
100
80
60
40
20
150
103
25
30
EXPT. 16
EXPT. 17
2
4
6
8
10
% PHOTOPOSITIVE LARVAE
100
80
60
40
20
EXPT. 19
EXPT. 20
EXPT. 21
38
50
182
52
25
21
2
4
6
8
10
12
14
16
18
TIME IN DAYS

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1947./

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