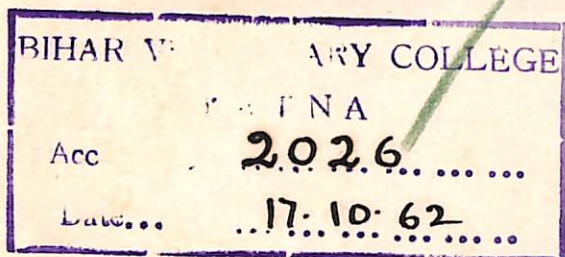THE UNIVERSITY OF ALBERTA

A STUDY OF THE BEHAVIOUR OF LARVAL TABANIDS

(DIPTERA: TABANIDAE)

IN RELATION TO LIGHT, MOISTURE AND TEMPERATURE.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ENTOMOLOGY

by

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The undersigned hereby certify that they have read and

recommend to the Faculty of Graduate Studies for acceptance,

a thesis entitled A STUDY OF THE BEHAVIOUR OF LARVAL

TABANIDS (DIPTERA: TABANIDAE) IN RELATION TO LIGHT,

MOISTURE AND TEMPERATURE submitted by MOHAMMAD

SHAMSUDDIN in partial fulfilment of the requirements for the

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ABSTRACT

The behaviour of larval tabanids (Tabanus reinwardtii Wied., Chrysops furcata Walk., and Chrysops mitis O.S.), in relation to light, moisture and temperature has been studied. Rate of movement, activity in time, aggregation, and localized movements of the head capsule were utilized as criteria for analyzing larval behaviour.

The effects of light on the behaviour of T. reinwardtii and C. furcata are described. The anterior region of the larval head capsule is shown to be sensitive to light. A pair of eye-spots on the head capsule is suggested as the photoreceptor. On illumination larvae are able to integrate light energy over periods of seconds and to utilize the effect to produce a directional response. Orientation mechanisms of larvae under various light sources are described.

Larval C. furcata and C. mitis do not show preference for the dry or the wet side in several different types of humidity-gradients. However, they show a complex of abnormal behaviour patterns to uniform conditions of low moisture content of the substratum. The mean water content of C. mitis larva was determined and the effects of desiccation in terms of survival time of larvae are discussed.

The reactions of C. furcata and C. mitis larvae in uniform temperatures and temperature-gradients are described. The speed of movement and percentage activity of larvae, though affected by temperature, are shown to be more affected by light. $21.42 \pm .81\,^{\circ}C$ has been suggested as the 'preferred temperature' of larval C. mitis. A temperature range of $37-40^{\circ}$ C is shown to be lethal to the larvae.

Results on the three physical factors in relation to the general behaviour of larvae under field conditions have been discussed and light and temperature are shown as the most important environmental factors.

ACKNOWLEDGEMENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION; BIOLOGY OF LARVAL TABANIDS

1.1. Introduction.

The family Tabanidae consists of blood-sucking flies which are mainly animal pests but many species both in larval and adult stages attack man inflicting painful bites (Miller, 1951; and Otsuru and Ogawa, 1959). The flies are of particular importance in connection with the transmission of Trypanosoma evansi, causing surra in horses, cattle, camels, dogs, etc., (Chandler, 1952). Since tabanids are important pests, any research that would elucidate the relation of the environmental factors to the behaviour of the fly at any point of its life-cycle would be valuable. A laboratory study of larval responses to light, temperature, humidity and moisture was therefore undertaken.

In the literature available, Cameron (1917) is the only investigator who has noted that larval tabanids, like other soil insects, are negatively photo-tactic. Other than this no work has been published on larval reactions to environmental factors. The chief aim of the present work has been to investigate the orienting reactions of larvae in relation to stimuli the larvae encounter in their natural environment. An attempt has been made to relate the results of this laboratory study to the activities of larval tabanids under natural conditions.

1.2. Notes on the Biology of larval Tabanids.

Review.

Detailed information concerning the morphology and biology of immature tabanids is included in papers by Hine, (1906); Cragg, (1912); Malloch, (1917); Marchand, (1920); Webb & Wells, (1924); Cameron, (1926); Stone,(1930); Philip, (1931); Schwardt, (1931 and 1932); Gjullin, (1945); Hatton, (1948); Miller, (1951); Lewis & Jones, (1955); Jamnback, (1959); and Ogawa, (1959).

Collection and rearing methods are reported in many papers important among which are those by Marchand, (1920); Jones & Bradley, (1923); Philip, (1928); Bailey, (1948 .); Jamnback, (1959); and Ogawa, (1959).

General description of tabanid larvae.

The larvae have 12 obvious segments, with a retractile head bearing well developed antennae and strong mandibles. The body is cylindrical, tapering at both extremities, and usually longitudinally striated; there is a circle of fleshy pseudopods around each abdominal segment.

Larvae of Tabanus and Chrysops are closely alike. A mature larva of T. reinwardtii Wied., (Fig. 1) is brownish grey and measures about 25-30 mm. Full grown larvae of C. mitis O.S., and C. furcata Walk., (Figs. 1a & 1b) are yellowish white to green in colour and
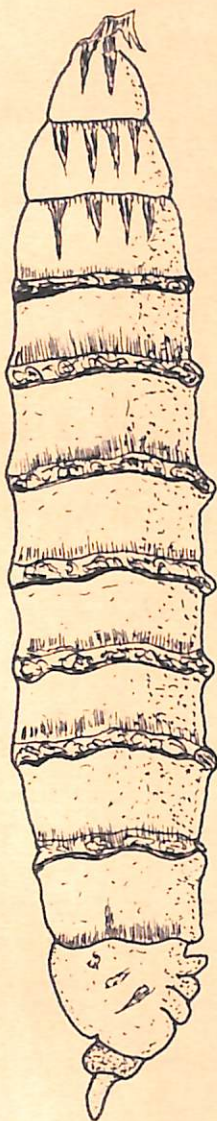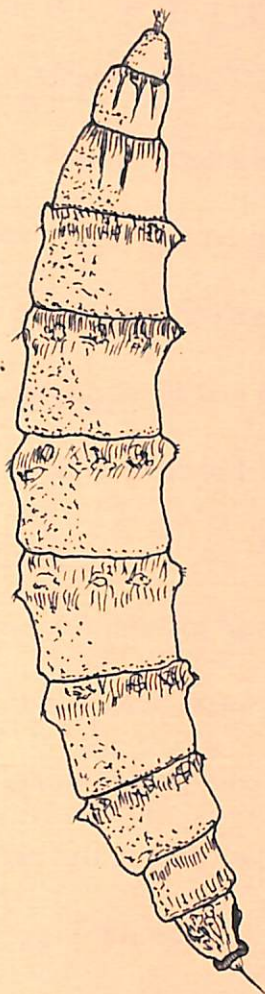
Fig. 1.   Fig. 1a.   Fig. 1b.

Fig. 1. Tabanus reinwardtii Fig. 1a. Chrysops-　　Fig. 1b. C. furcata Walk.,
Wied., larva x 6 .   mitis O.S., larva x 6. larva x 6.

measure 15-25 mm. Details of morphology of these 3 larvae are similar to those described by Marchand, (1920); Webb & Wells, (1924); Cameron, (1926); and Stone, (1930).

In the laboratory larval T. reinwardtii can be easily separated from C. mitis and C. furcata by their colour, large size, black mandibles, prominent thoracic rings and short conical siphon. Larval C. mitis are usually larger than C. furcata and have a dull greenish body colour. The most conspicuous character, however, which distinguishes larval C. mitis from C. furcata is the anal segment pattern. The anal siphon in C. mitis is long and pointed but short and blunt in C. furcata.

Collecting methods.

Larvae were obtained from the mud of irrigation ditches, along the banks of streams, pools and swamps. The method recommended by Marchand, (1920) of separating the larvae by washing the soil through a sieve was most effective in collection of C. mitis. T. reinwardtii and C. furcata could be easily obtained by turning over the soil with a garden fork.

Larval habitats.

The areas selected for collecting larvae (Fig. 2) were close to districts where cattle were numerous. The first collection was made on October 7, 1958 and was confined to Winterburn Swamp, about 8 miles west of Edmonton. This was the largest habitat exploited for
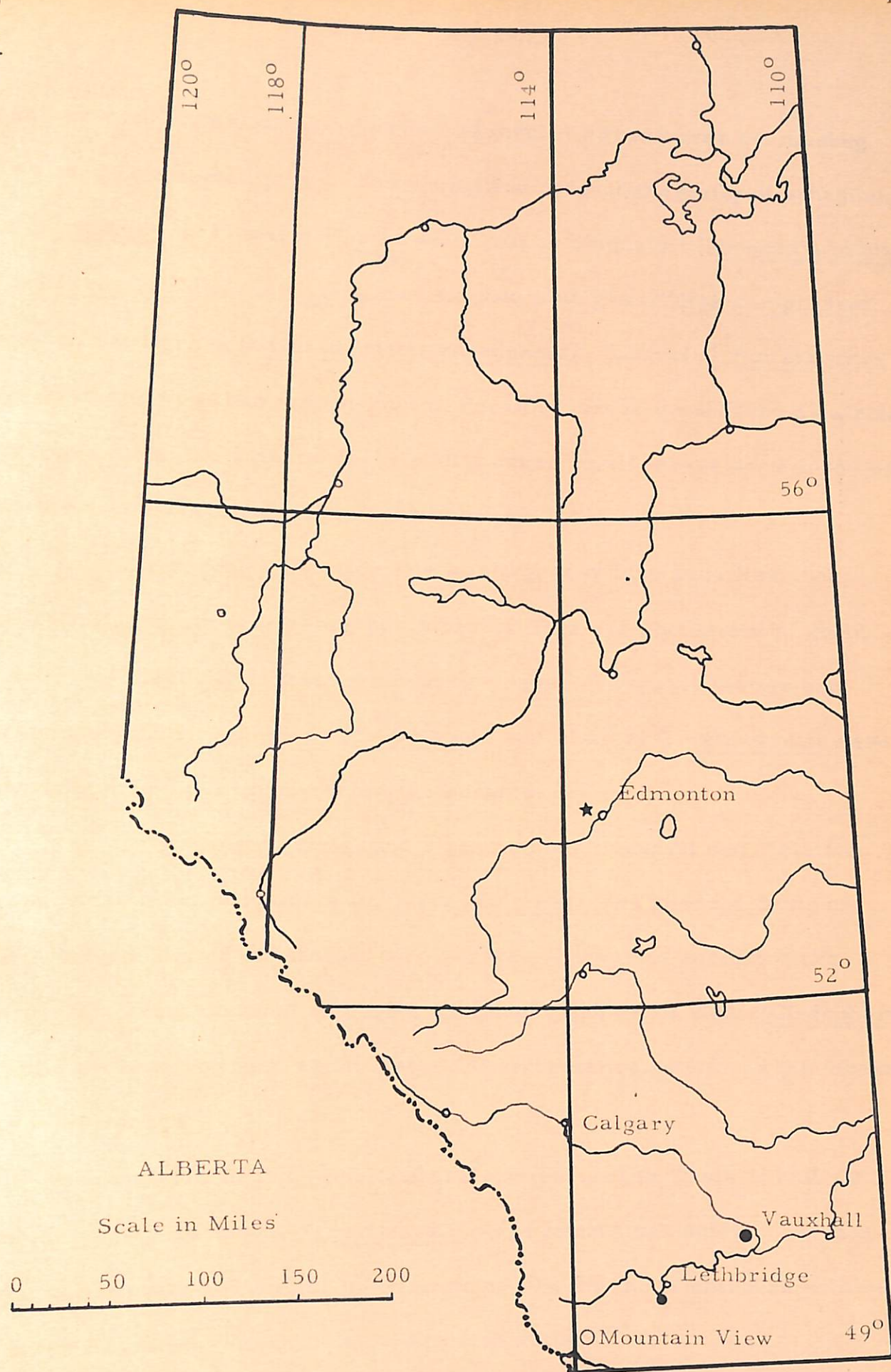
Fig. 2. Index map showing the areas searched for larval tabanids. The collection areas were close to Winterburn,✳; Vauxhall , ● ; Raymond, ● ; and Mountain View, O .

subsequent collections and consisted of several hundred acres of muskeg and two large connected pools. The vegetation consisted chiefly of sphagnum moss (_Shpagnum_ sp.), sedge (_Carex_ sp.), marsh cinquefoil (_Potentilla palustris_), spruce (_Picea_ sp.), larch (_Larix_ sp.), Canada blue grass (_Poa compressa_) and marsh reed grass (_Calamagrostis canadensis_). Larval _C. furcata_ were collected on the west bank of the pools. Pupating larvae were found as a rule 1-2 inches below the surface of the pool's edge. Small larvae were found deeper in the soil and almost submerged in water.

The second collection site was a grassy lake near Raymond, about 18 miles south of Lethbridge, Alberta. This area consisted of about 200 acres of clay soil covered with shallow layer of organic matter and interspered with slough grass (_Beckmannia_ sp.). Larval _T. reinwardtii_ were found at depths of 2-3 inches below the surface.

Two roadside irrigation streams near Vauxhall and Waterton, Alberta, were most productive for larval _C. mitis_. The average depth of water in the middle of the stream was 2-3 feet. Larvae of various sizes were obtained from the mud entangled with heavy growth of algae (_Cladophora_ sp.) and completely submerged under water. The vegetation bordering the stream banks were sparse.

Soil analyses of the 4 habitats are summarized in Table 1; only the Raymond soil where larval _T. reinwardtii_ were found, was acidic. Organic matter content was very high, an average of 69% for the Winterburn soil but only 42.5% for Raymond, Vauxhall and Waterton soils.

18.

The three species of tabanids described above were almost habitat-specific, for example larvae of C. furcata were never obtained from southern Alberta. Only larval C. mitis was collected from the irrigation ditches whereas T. reinwardtii larvae were obtained from comparatively dry soil around Raymond, Alberta. Larvae in each of the habitats were most abundant from June to October. Collection was difficult from November to March because of frozen soil.

Maintenance of larval stocks.

The method employed in maintaining larval stocks in the laboratory was similar to that recommended by Shemanchuk, (1959). Larvae were stored in 3 x 1 inch plastic vials provided with a soil medium rich in decaying organic matter. No other food was supplied. These larvae when kept at 5-10° C in a refrigerator, did not pupate. Room temperature of 21° C brought about pupation of mature larvae in a few days. The average pupal period determined from six specimens (5 female and 1 male) of C. mitis reared from larvae was 7 days. For C. furcata it was 11 days, based on 10 specimens (3 female and 7 male).

Larvae of Tabanus sp., occasionally struck each other when placed together in a dish. However, cannibalism was never observed. Larvae of Chrysops sp., showed least interest in fresh animal tissues even though they were kept together in vials with clean tap water and starved for a month or more. Greater activity amongst larval C. mitis than C. furcata was observed under laboratory conditions. Larval T.

reinwardtii did not survive such long periods in water as the larvae of Chrysops sp.

Mortality during maintenance of larval stocks ranged up to 40% chiefly due to fungus growth, a nematode (Bathymermis sp.) infestation, and inadequate ventilation. Few deaths occurred when the soil medium was changed once every 3 months and when the storage vials were provided with perforated caps to ensure proper ventilation.

2. BEHAVIOUR OF LARVAL TABANIDS IN RELATION TO LIGHT

2.1. Introduction and Review.

The extensive literature on the responses of insects to light has been reviewed in part by Cameron, (1938); Mast, (1938); Wigglesworth, (1953); Hollaender, (1956); Wulff, (1956); Carthy, (1958); and Milne and Milne, (1959). Since larval tabanids do not possess morphologically defined photoreceptors, the present review cites only such works which consider the eyeless forms both among insects and other animals.

The effect of light on the rate of locomotion of eyeless forms has been less studied than orientation to light. Welsh, (1932 and 1933), working with Unionicola (Arachnida), concludes that in a light sensitive organism the extent of muscular activity bears a very definite relationship to the intensity of illumination. Duggar, (1936); Jones, (1955); and Millott, (1957) give a good summary of photo-kinesis in eyeless forms such as certain insects, some echinoderms, and molluscs. Miller, (1929) has discussed the results obtained by Mast, (1911) and Herms, (1911) on the speed of crawling of fly larvae (Calliphora, Sarcophaga, and Musca sp.). Few of the above works provide anything definite concerning the quantitative relation between light intensity and response.

A considerable amount of information is included in the works of Holmes, (1905).; Herms, (1911); Mast, (1911); Patten, (1914, 1915 and 1916); Loeb, (1918); Crozier, (1927); Mitchell and Crozier, (1928); Ellsworth,

(1933); Fraenkel and Gunn, (1940); Bolwig, (1946); and Hafez, (1950 and 1953) on the photo-negative responses of muscoid larvae. There is a general agreement that fly larvae behave photo-negatively and that their mechanism of orientation to light represents a typical example of klino-taxis (Carthy, 1958). However, a wide diversity of opinion prevailed for a long time as to the true nature of photorecep- tors in fly larvae. Lowne, (1890-95) as quoted by Hollaender, (1956) described two pairs of small papillae on the apex of the larval head as being photosensitive. Ellsworth (1933), on the basis of histologi- cal findings in Lucilia sp., reported the presence of photoreceptors on the larval maxillary lobes. However, Welsh (1937) indicated that the sensory papillae of fly maggots previously credited as photorece- ptors proved to be gustatory in function. The photoreceptors of housefly larva were finally identified by Bolwig (1946) as two small groups of sense cells, situated on each side just above the anterior ends of the larval pharyngeal sclerites.

Important information has been obtained on the photosensitive organs of the eyeless forms by using localized stimulation through light patches (Harper, 1905; Herms, 1911; Hess, 1921, 1924, 1925; Ellsworth, 1933; Young, 1935; Hawes, 1945; Newth and Ross, 1955; Yoshida, 1956 and 1957; and Millott, 1957). Such a method has also been used extensively for investigation of dermal photosensitivity of forms ranging from invertebrates, through lower chordates to verte-

brates. Localization of sensitivity is, however, too ill-defined and an electrophysiological demonstration of photosensitivity is still needed.

The present study on larval C. furcata and T. reinwardtii deals with the investigation of photoreceptive organs; the effect of different light intensities on reaction time and speed of movement; and reactions to light-gradients and lateral light stimulation.

2.2. General Methods and Materials.

Experiments were done from May 1, 1959 to January 4, 1960 in a dark room at a temperature of $23.3 \pm 2.8^{\circ}$ C. Two glass plates, 35 x 70 cm. and 45 x 60 cm. in size and a photographic tray measuring 22 x 17 cm. were used. In all experiments the plates rested on black paper.

One of the light sources was a photographic enlarger with a 75-watt bulb. Low light intensities were obtained by using the enlarger lens which had a focal length of 150 mm. Light intensities of a high order were obtained from electric bulbs (100-150 watt) enclosed in a light-tight box.

Light intensities were measured with a photometer (GE, Type DP-9). The difficulty of observing in the dark room was overcome by using a photographic safe light which, according to the manufacturers, transmitted dominant wave lengths of 580 mμ or more.

Mature larvae used in the investigation were specimens of Tabanus reinwardtii and Chrysops furcata collected from the Raymond

and Winterburn areas. During the experiments the larvae were kept in 3 x 1 inch plastic vials with about 1/2 inch of tap water and stored in the dark room. If the larvae were exposed to light in an experiment they were allowed at least 1/2 hour rest in the dark before being used in another experiment.

Observations were usually made on single larvae. The sheet glass was treated with a water suspension of fine talc on which the larva crawled leaving a trail behind it. During each experiment, the time intervals were marked with a wax-pencil. The tracks were measured with a planimeter.

2.3. Preliminary Studies.

The activity of larvae in the dark.

118 larvae were watched singly for a duration of 3 minutes each in separate glass vials containing either clean moist sand or tap water under the red light. The results of these observations are summarized in Table 2. About 86% of the larvae were found to be active. The activity was characterised by general flexing of the body and occasional crawling. Such activities could be easily mistaken for definite response to light stimulation. It was, therefore, necessary to record each type of activity for a small group of 20 larvae with the help of a hand lens. These larvae were placed on the tray under the red illumination. All of them showed activity by crawling. However, a response characteristic of behaviour under the experimental white light i. e., withdrawal

Table 2.

Activity of larvae of <u>Chrysops</u> <u>furcata</u> Walk. recorded during different times. Each larva was placed at a distance of about 7 inches from the photographic safelight and observed for a period of 3 minutes.

Time during which observed	Number of larvae active during 3 minutes observation	Number of larvae inactive during 3 minutes observation	Percent Active
7 to 11 a.m.	47	3	94
3:45 to 5 p.m.	44	6	88
9 to 11 p.m.	14	4	77

of the head capsule into the cephalic collar preparatory to crawling, was never shown.

When 34 animals were put singly on the glass plate for 5 minutes, all responded by crawling. A mean speed of $1.6 \pm 1.1.$ cm/min was recorded for these larvae. It is suggested that this represents a basal rate of movement of larvae in the dark.

Response of larvae to general illumination.

If the experimental white light was turned on when the larvae were inactive or crawling they hesitated momentarily, raised the anterior tip, swung it violently from side to side in an exploratory fashion and then withdrew the head capsule into the cephalic collar. The sudden withdrawal of the head capsule was found to be a reliable indicator for photic response and henceforth will be referred to as the 'retraction reflex'.

The 'retraction reflex' was usually followed by a further series of head movements, then by turning movements involving the whole body, finally by crawling (For a detailed description of locomotion of/a dipterous larva see Fraenkel and Gunn, 1940; p60). Hence there are measureable time intervals between the onset of illumination and the 'retraction reflex' and crawling. The first time interval is referred to as the 'reaction time' of larvae which is the period from the time of illumination until the head capsule is withdrawn. Preliminary experiments showed that illumination must be continuous during the reaction time. The 2nd. time interval is the period elapsing from the end of the 'retraction

reflex' until crawling is started and is known as the 'crawling time'.

2.4. <u>Investigation of Reaction Time and Crawling Time.</u>

<u>Methods and Materials.</u>

The light sources used were the photographic enlarger and the electric bulbs as described earlier. To ensure that temperature did not affect the reaction time, glass heat filters were used in the intensity range of 100-1600 foot-candles.

The photographic tray was used as an experimental trough. This always contained at least 50 c.c. of tap water during each experiment. A rectangular blotting paper matting 19 x 14 cm., was provided in the tray.

Only larval <u>C. furcata</u> were used in these experiments. A glass vial containing one larva with about 1 cm. water was emptied over the tray and an interval of 1 minute was allowed. The light was then switched on and two stop watches started simultaneously. One of them was used to record the reaction time and the other the time to crawl.

<u>Results.</u>

The results obtained with 3 different groups of larvae are summarized in Table 3 and Figure 3. All larvae showed a great variation in reaction time even under the same light source and intensity. This was particularly true in the low light intensities.

Close statistical similarities are demonstrated by the data in-

Table 3.

Reaction times in seconds of 3 groups of larval <u>Chrysops</u> <u>furcata</u> Walk. to general illumination at different intensities.

Light intensity in foot-candles	A group of 5 larvae tested 10 times each			A group of 10 larvae tested 5 times each			A group of 55 larvae tested once each		
	RT*	SD	SE	RT*	SD	SE	RT*	SD	SE
0.5	17.3	9.2	1.29	13.5	5.9	0.85	15.4	8.9	1.19
5.0	10.8	4.7	0.67	6.9	2.9	0.41	8.0	3.2	0.43
30.0	7.9	3.6	0.50	4.8	1.8	0.25	6.1	2.7	0.36
100.0	5.6	2.5	0.35	3.9	1.4	0.19	4.7	2.1	0.28
500.0	4.0	1.5	0.21	3.6	1.2	0.17	3.9	1.9	0.26
1600.0	3.3	1.5	0.21	x	x	x	x	x	x

*RT = Mean Reaction time in seconds.

Fig. 3. The relation between reaction time and light intensity.

● Means of 5 larvae and 10 readings at each intensity.
○ Means of 55 larvae and 1 reading at each intensity.
★ Means of 10 larvae and 5 readings at each intensity.
◇ Means of 155 readings with SE.

Log. Light intensity in foot-candles

Reaction time in seconds
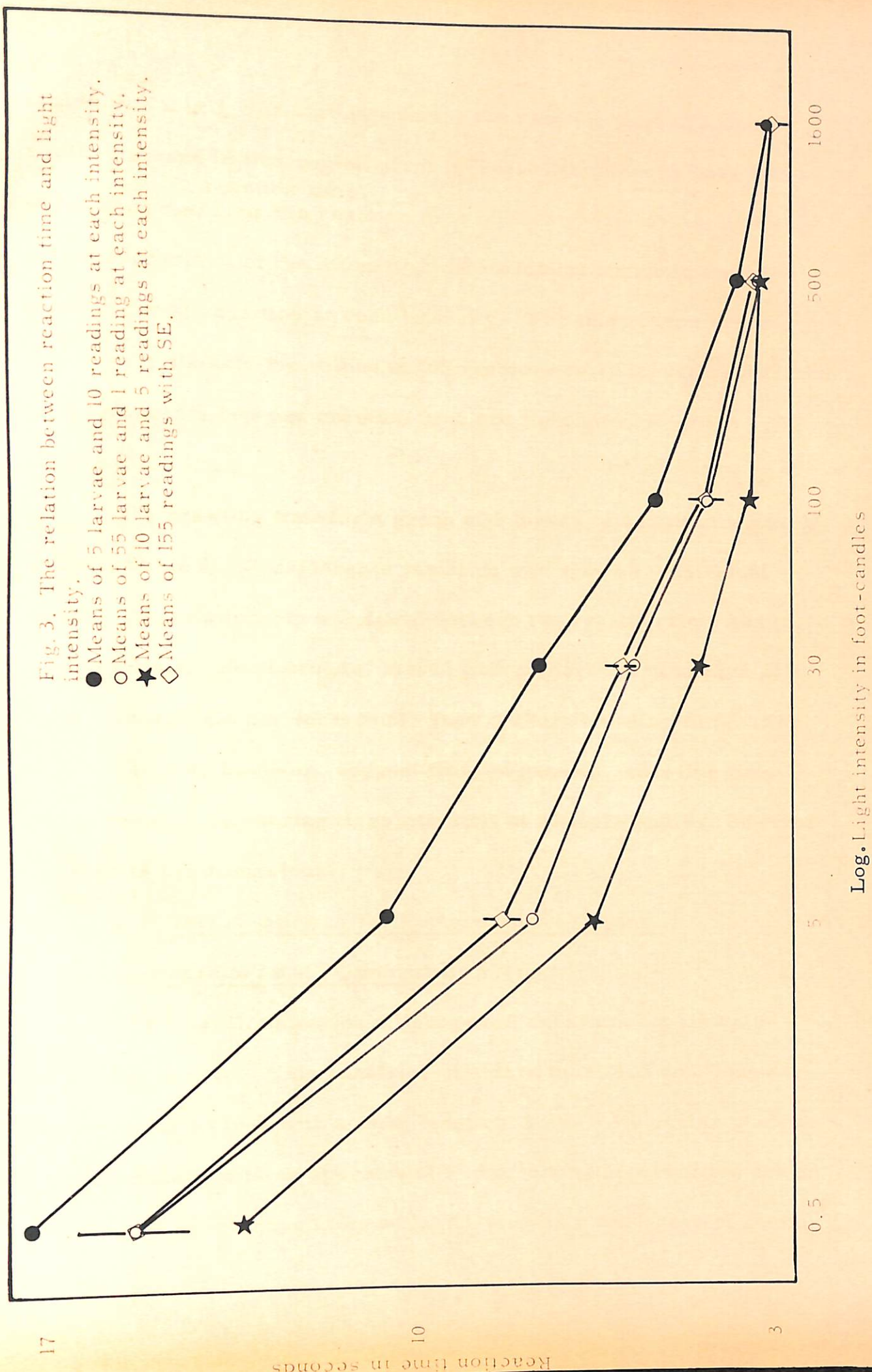
cluded in Table 3. At high intensities the reaction time approaches

a minimum and in this region great increases in intensity have little

effect upon the/Thus the reaction time of each group varies inversely
~~reaction time.~~

with the logarithm of the intensity, and maintains approximately the

same order of variation at each intensity. For this reason the line

in Figure 3 showing the values of 155 readings on 70 larvae represents

the relationship between reaction time and light intensity more

accurately.

The crawling time for a group of 5 larvae tested 10 times each

is given Table 4. Considerable variation was shown by individual

larvae. No relationship was found between the reaction time and the

crawling time. For example, larvae with a short reaction time at a

given intensity did not necessarily show a short crawling time. The

data in Table 4, however, suggest that, in general, crawling time

decreases with increasing light intensity; at 30 foot-candles, however

the results are anomalous.

2.5. Investigation of the Photoreceptive Organs.

Response to local illumination.

For local illumination a light pencil apparatus as shown in

Figure 4 was used. This consisted of a dark tube 24.5 cm. long hav-

ing a condensing lens with a focal length of 6 cm. A circular piece of

black cardboard with an aperture of 5 mm. diameter was fitted behind

the lens. A microscope lamp with a 15-watt bulb and a lens of 24 cm.

Table 4.

Time in seconds to start crawling of a group of 5 larval Chrysops
furcata Walk. tested 10 times each with general illumination at
different intensities.

Light intensity in foot-candles	Mean Crawling time (sec.)	SD	SE
0.5	38.8	39.8	5.6
5.0	25.0	24.2	3.4
30.0	62.0	42.2	5.8
100.0	17.5	11.0	1.6
500.0	11.3	10.0	1.4

1 inch = 1 cm

Scale

Transformer

Microscope lamp

Dark tube
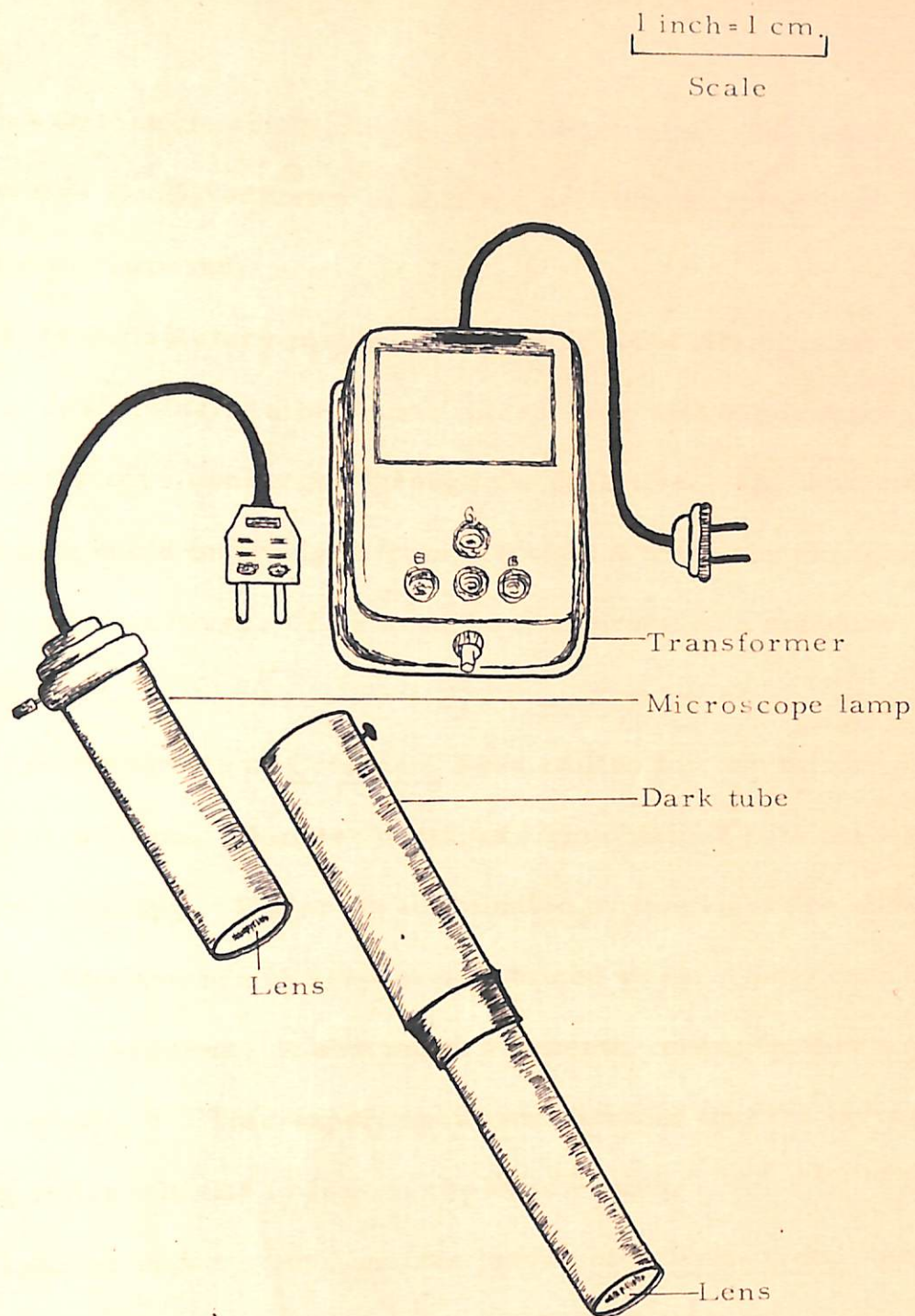
Lens

Lens

Fig. 4. The light pencil apparatus used for local illumination of larval tabanids.

focal length was then inserted into the cylindrical tube. The lens combination was finally adjusted to obtain a pencil of parallel light about 2 mm. in diameter.

A more satisfactory method of obtaining local illumination was by replacing one ocular of a binocular microscope with a microscope lamp so that the rays converged through the objective. The diameter of the light spot could be changed from 0.8 to 12.0 mm., by changing the microscope objectives. This arrangement provided a constant diameter of light.

20 mature larvae of C. furcata were chilled for one minute and examined with a 1 mm. diameter pencil of light obtained with the binocular microscope. The areas illuminated by this light are shown in Figure 5. The number of responses obtained were: head capsule, 20; 3rd thoracic segment, 1; abdominal segments, none; Graber's organ and siphon, 5. This experiment demonstrated that the larvae have maximum sensitivity to light in the head region.

In another experiment 3 mature larvae of T. reinwardtii were kept in a light of 7 foot-candles for an hour before the light pencil test started. All parts of the body of each animal were carefully searched with the light pencil apparatus giving a light spot 2 mm. in diameter. When the local light reached the pigmented spots[1] (Fig. 5)

1.   These pigmented spots have been described as eye-spots in the larva of Haematopota pulvialis L. (Tabanidae) by Cameron in 1934, and have been recorded in all species of larvae investigated in the present work.
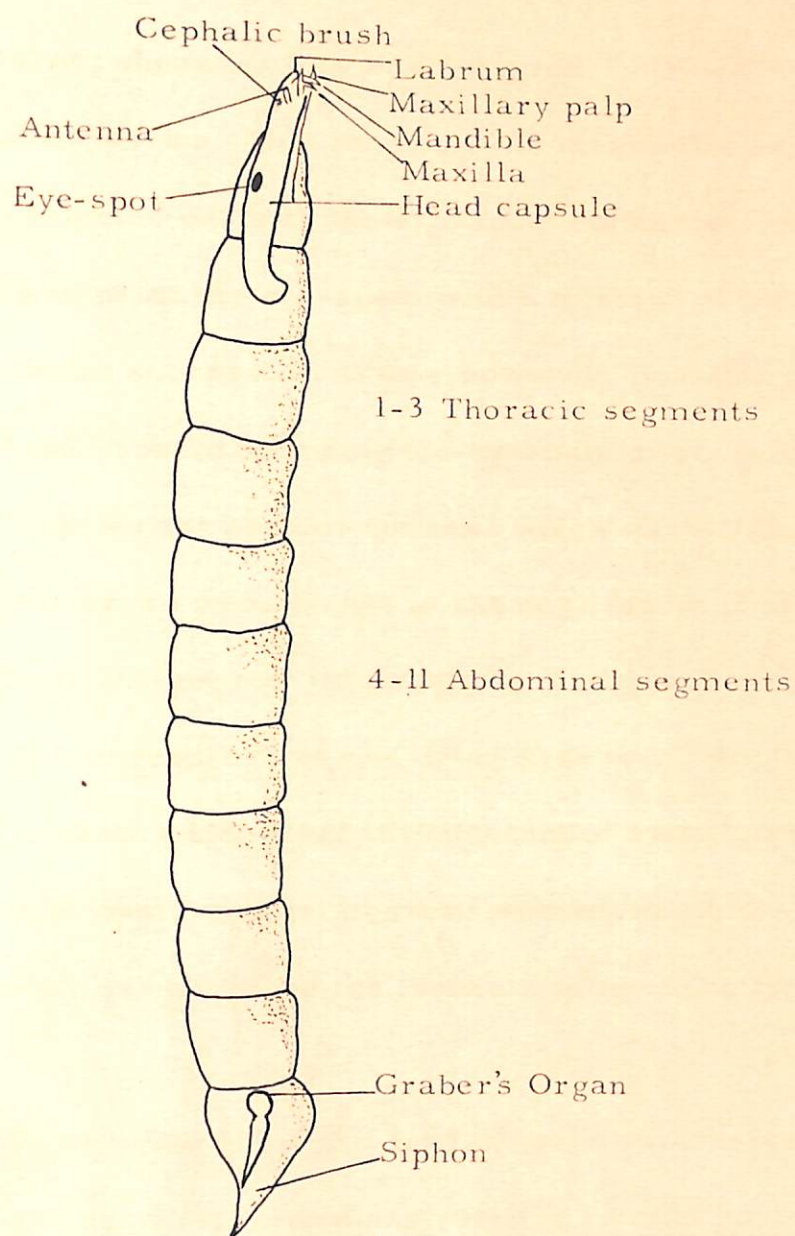
Fig. 5. General structure of a larval tabanid modified from Cameron, 1934.

situated latero-dorsally on the head capsule, the larvae responded by turning away from the light source. Localization of the light pencil on the eye-spots was a difficult task owing to the extremely mobile head capsule. However, whenever this was achieved, it caused violent head movements of the larvae. This reaction was maximal when the head capsule remained projected out with the eye-spots completely exposed.

Attempts to paint the eye-spots with a mixture of India ink and gum arabic were not successful. It was, however, possible to paint the anterior head capsule including the eye-spots of six larval Chrysops. Figure 6 shows the area of the head which was painted. After blackening, the larvae were stored in the dark for an hour and then examined by local and general illumination. The results are summarized in Table 5. The response varied from none to incomplete withdrawal of the head capsule under local illumination of 1mm. light spot. But under 10mm. light spot, the reaction was obvious in all the larvae. When the painted areas were washed the larvae displayed the typical 'retraction reflex'.

To see if the anterior tip of the head capsule was responsible for sensitivity to light as has been demonstrated in Lucilia sericata by Ellsworth (1933), 1 mm. of the anterior head tips were cut off from each of 7 larvae[2]. Reaction times for these were recorded one day

2.   Ten such operations were done; 3 died immediately but the remaining 7 were in healthy conditions for several months.
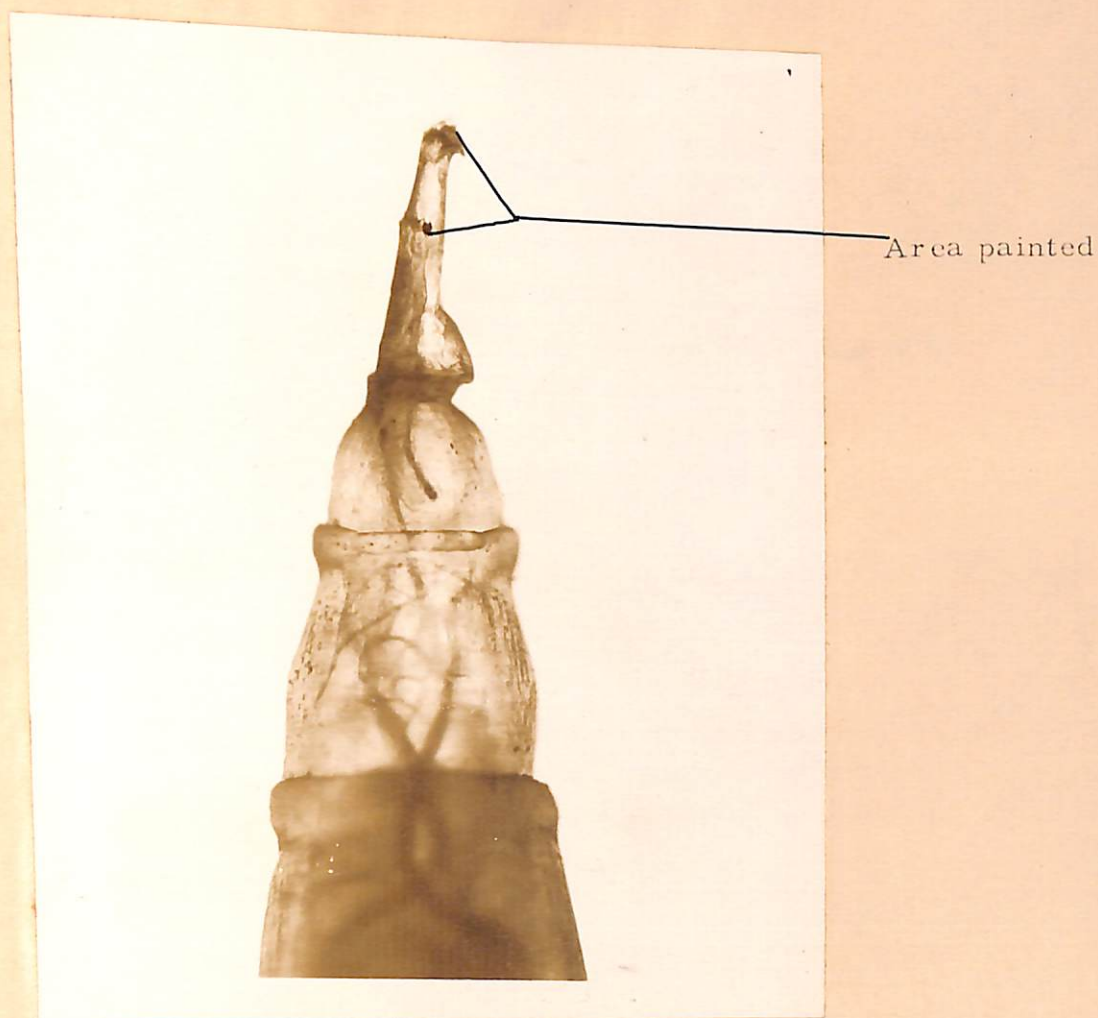
Fig. 6. Photomicrograph of the cephalic segments with the head capsule projected out, showing the area painted. Larva of C. furcata Walk.

Table 5.

Reactions obtained on painting the anterior head capsules of 6

larvae of <u>Chrysops</u> <u>furcata</u> Walk.

Larvae	Reaction to a 7948 F.C. light about 1 mm.* in dia.	Reaction to a 10150 F.C. light about 10 mm.* in dia.
A	Tremor in the blackened portion. No reaction in any other part of the body.	Rapid contraction and expansion of the body.
B & C	Sudden but incomplete withdrawal of head capsule.	Head movements activated.
D, E, & F	No reaction in any part of the body.	Rapid contraction and expansion of the body. Head movements sidewise.

* Light intensities were measured by the photometer and the
  light intensity recorded was multiplied by the ratio of the
  surface area of the photometer to the surface area of the
  light spot.

after the operation. The results are shown in Table 6. The reaction time under both local and general illumination varied considerably and ranged from 2.3 - 76.3 sec. This is a very wide range when compared with the data included in Table 3, at the given intensities of 5 and 100 foot-candles. For example, the mean value of reaction time of 28.7 sec. (Table 6) at 100 foot-candles is much higher than any of the mean values 5.6; 3.9; and 4.7 sec. of Table 3. This means that although the removal of the anterior tip does not alter the character of the 'retraction reflex', it does increase the reaction time considerably.

Anatomical and histological findings.

Since preliminary experiments indicated that larval tabanids have a light sense located in the eye-spots, several dissections were done to trace their innervation. Unstained dissections were of no help as nerves could not be easily identified. Fresh larvae, therefore, were injected in the thorax with about 0.5 c.c. of 1 part of 0.5% solution of Methylene Blue to 400 parts of water. They were then left in a physio-logical salt solution for an hour before the dissection was made. Similar treatments with Luxol fast blue of 1:1000 in 95 percent alcohol solution were also tried. No nerve connection was found to the eye-spots. Figure 7 shows a photomicrograph of a dissected head capsule.

The last step was to study serial sections of the head capsule for microscopic units suggestive of photoreceptors. Bouin's Picro-formal fixative with the usual paraffin embedding, was used with the

Table 6.

Reaction times in seconds of 7 larval Chrysops furcata Walk. to local and general illumination after removal of the anterior tip of head capsule.

Larvae #	RT.* in sec. to local illumination of 4 foot-candles				RT.* in sec. to general illumination of 100 foot-candles			
	1	2	3	Mean	1	2	3	Mean
1	2.0	3.0	3.5	2.8	5.0	12.0	15.0	10.6
2	1.0	2.0	4.0	2.3	13.0	16.0	7.0	12.0
3	35.0	60.0	32.0	42.3	55.0	52.0	17.0	41.3
4	20.0	45.0	52.0	39.0	62.0	75.0	92.0	76.3
5	2.0	1.5	3.5	2.3	5.0	7.0	4.0	5.3
6	17.0	31.0	26.0	24.7	23.0	34.0	18.0	25.0
7	4.0	17.0	13.0	11.3	25.0	25.0	41.0	30.0

Mean 17.8   Mean 28.7

$\pm$ 3.9 SE $\pm$ 5.3 SE

* RT. = Reaction time
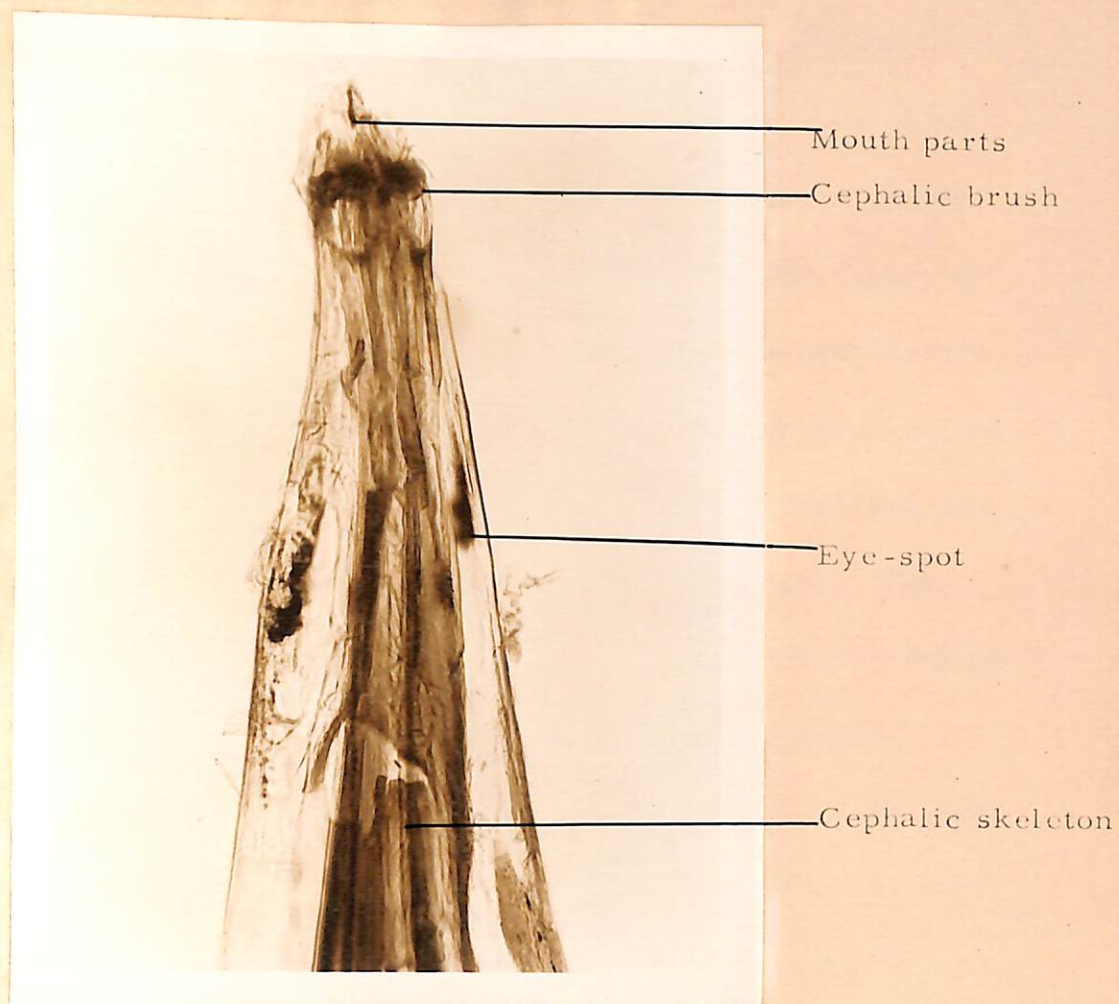
Fig. 7. Photomicrograph of dissected head capsule of a mature larva of

C. furcata Walk. The eye-spots were partly injured and therefore appear

elongated.

following difference as suggested by Ellsworth (1933): the animal, instead of being fixed and embedded entirely, was fixed and then cut into small pieces. The larval head and thorax were fixed, washed, dehydrated, cleared and embedded in the following manner: About 0.5 c.c. of a solution consisting of 15 c.c. of picric acid, 5 c.c. of formalin and 1 c.c. of acetic acid, was injected into the body cavity with a hypodermic needle. Sufficient pressure was exerted to extend the larva while injecting the solution. After two hours the larva was cut into small pieces and stored in the fixative overnight. The material was then washed in 1 percent solution of sodium bicarbonate and a short time in running water until the yellow disappeared. Dehydration was done with 30, 50, 70, 80, 95, and with two changes of 100% alcohol. This was followed by placing in xylol for 1-2 hours; in a solution of 1/2 xylol and 1/2 paraffin 1-2 hours and in 56-58° C paraffin for 4 hours. Finally, the material was sectioned at 8-10 μ, mounted and stained in haematoxyline and eosine. Microphotograph of a typical section passing through the region of the eye-spots is shown in Figure 8.

Discussion.

The results of the above experiments demonstrate the presence of photosensitive organs in the head capsule. The experiments on painting indicate that dermal sensitivity also exists in larval tabanids. The considerable increase in reaction times on removal of the

Tentorium

Eye-spot

Cuticle

Hypodermis

Pharynx

Salivary pump
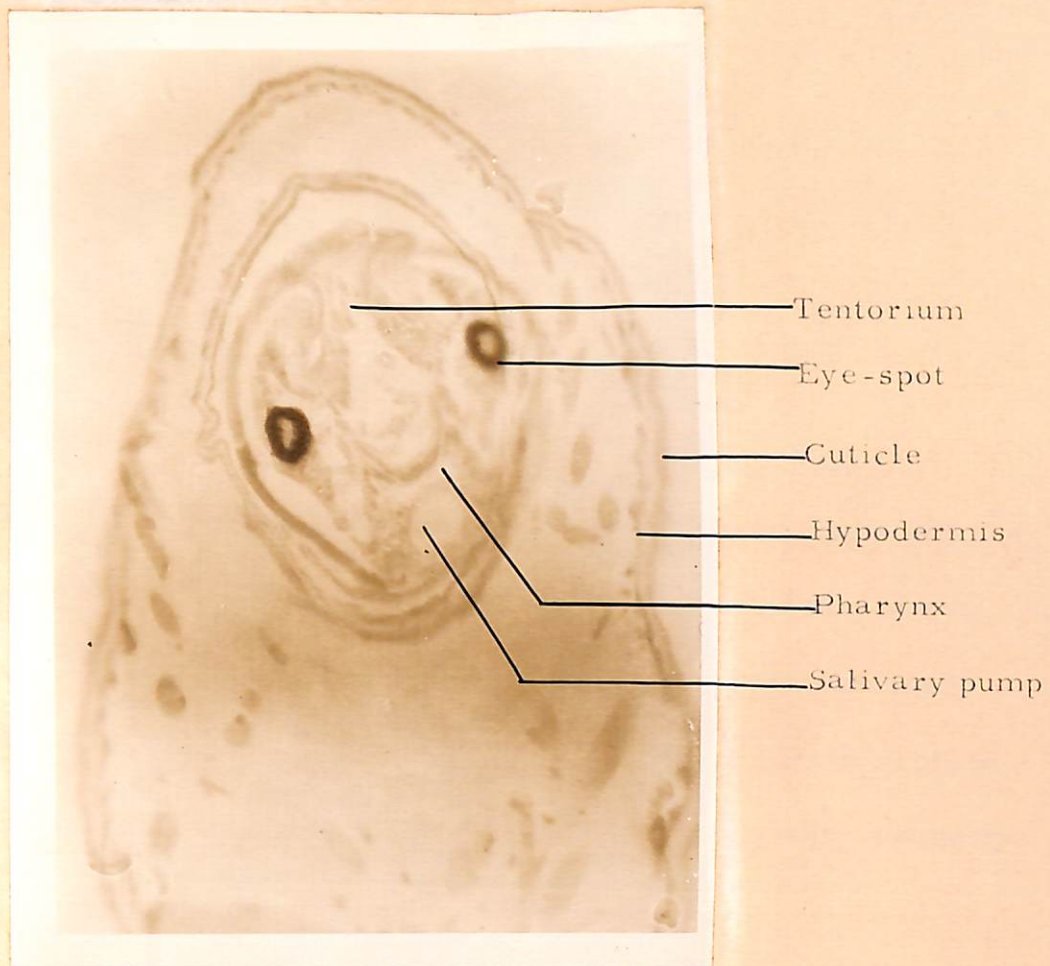
Fig. 8. Photomicrograph of a transverse section of C. furcata Walk.,
larva, through the head capsule showing the associated structures.

anterior tip suggests that photosensitivity is spread throughout the anterior region of the head capsule. The microscopic study shows gross concentration of pigments in the eye-spots. Since no nerve connections could be demonstrated in the present investigation, we cannot yet specify the nature of the photoreceptors in larval tabanids beyond stating that they appear to be contained in the anterior region of the head capsule, perhaps the eye-spots.

2.6. Reactions in a Dark-Light Alternative Chamber.

Although it is known that larval tabanids behave photo-negatively in nature (Cameron, 1917), no quantitative demonstration of this response has been made in the past. The experiments described under this section were conducted to ascertain if the extent of photo-negative behaviour varied directly with the increasing light intensity.

Methods and Materials.

Two petridish lids with a diameter of 15 cm., were placed one upon the other with the open edges in contact. A moistened disc of about 2 mm. thick brown cardboard divided the chamber into an upper and lower half. The lower half of the chamber was filled with water which remained in contact with the cardboard partition throughout the experiments. For each experiment a separate card was used. This arrangement kept the cardboard surface, on which the animals crawled, moist. One half of the chamber was covered with black cardboard to provide a choice of dark and light. The light source was

the photographic enlarger.

Fifty mature larval C. furcata were used. Ten larvae were put in the center of the choice chamber at a time and their positions were recorded after 15 minutes.

Results.

The results are summarized in Table 7. The intensity of light reaction is expressed as $100 \left(\dfrac{D - L}{N} \right)$, (Perttunen, 1959); where D represents the number of larvae on the dark side, L the number of larvae on the illuminated side and N the total number of position records.

At room temperature the larvae of C. furcata behave photo-negatively at all the intensities used. The intensity of reaction, how-ever, does not show a regular increase with increase in light intensity.

2.7. Rate of Movement in various Intensities of Dorsal Illumination.

It was anticipated that larval tabanids may move at different speeds at different light intensities, for light frequently has an effect on the rate of movement of animals, including dipterous larvae (Fraenkel and Gunn, 1940). Since the results in Section 2.6. did not provide anything definite concerning the quantitative relation between the light intensity and the amount of larval response, the following experi-ments were conducted.

Methods and Materials.

The light sources for dorsal illumination were the photographic

Table 7.

Intensity of light reaction of larval <u>Chrysops furcata</u> Walk. in choice-chambers of darkness and light. The mean represents the results of 5 experiments with the same group of 50 specimens at each light intensity.

Light intensity in foot-candles	Intensity of reaction, 100 $\frac{(D-L)*}{N}$ after 15 minutes for each experiment							
	1	2	3	4	5	Mean Intensity	SD	SE
0.25	50.0	66.6	55.5	75.0	40.0	57.4	12.3	5.5
0.50	40.0	20.0	50.0	60.0	66.6	47.3	16.3	7.3
1.00	80.0	77.7	100.0	25.0	33.3	63.2	29.0	12.9
2.00	11.1	71.4	75.0	33.0	71.4	52.4	25.8	11.5
5.00	100.0	71.4	20.0	80.0	80.0	70.3	26.8	11.9
10.00	66.6	50.0	80.0	100.0	60.0	71.3	17.3	7.7

Temp. $23.3 \overset{+}{-} 2.7^{\circ}C$.

* (Perttunen, 1959)

enlarger and the 100-watt electric bulb as mentioned in Section 2.2. A series of measurements were made with each of 7-10 larvae at each light intensity in the range of 0.03-500 foot-candles.

Larvae were placed in the centre of the glass plate. The light was switched on 30 seconds later. Each larva was allowed to crawl for 5 minutes, but only the track of the middle 3 minutes was measured. The average speed in cm/min was recorded.

Results.

The results are summarized in Table 8 and Figure 9. The mean values of speed cm/min, increase rapidly in the low light intensity range of 0.03-0.50 foot-candle. It appears therefore, that the larvae are more sensitive in this range. At high intensities the increase in speed is relatively less. The general picture is, however, the same throughout the range and any increase in the light intensity is accompanied by an increase in the rate of movement. The stimulus-response relationship, shown in Figure 9, suggests close agreement with the Weber-Fechner law (Patten, 1915), in the range of 10-500 foot-candles.

2.8. Experiments with Dorsal Light-Gradients.

The following experiments were done to analyse the effects of stimulation by light shining from above the animal, when all other sources of stimulation were excluded. The purpose was to determine whether the photo-negative behaviour of larvae involved complex

Table 8.

Speed of crawling of larval tabanids at various light intensities.

Number of Replicates	Light intensities in foot-candles	Mean speed in cm/min	SD	SE
7	0.03	2.6	1.34	0.51
7	0.06	3.4	1.05	0.40
7	0.12	4.1	0.94	0.36
7	0.25	4.7	1.26	0.48
7	0.50	5.1	0.93	0.35
7	1.00	5.1	1.14	0.43
7	2.00	5.5	1.13	0.43
8	10.00	5.6	0.98	0.34
8	20.00	5.8	0.63	0.22
10	50.00	6.5	0.94	0.29
10	100.00	6.6	0.94	0.29
10	500.00	7.6	0.72	0.23

Fig. 9. Effect of light intensity on the speed of movement of larval tabanids.

Y = 1.01 log. x + 4.80

● SE of the mean

Speed cm/min.

Log. light intensity in foot-candles
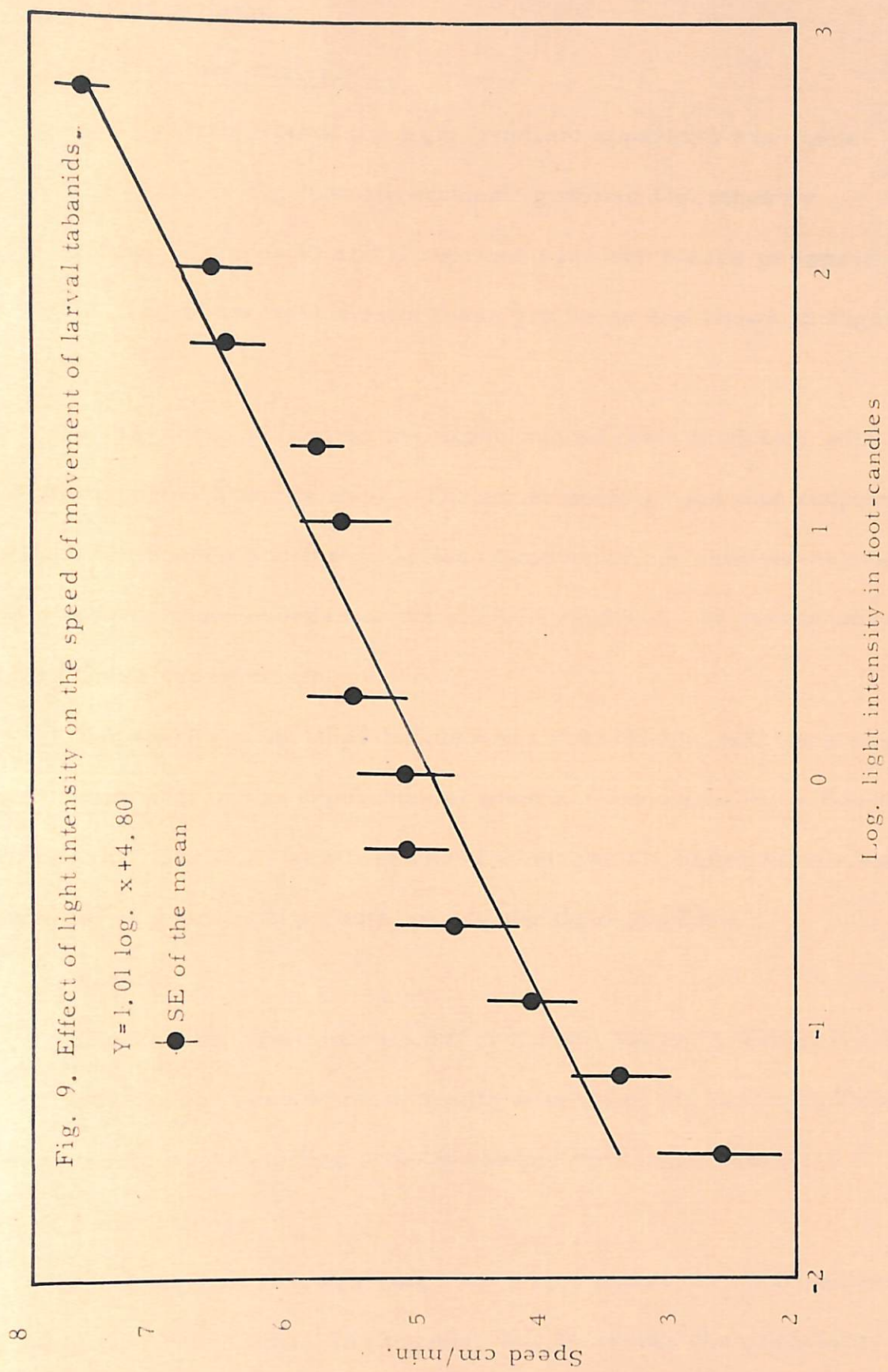
orienting mechanisms.

Methods and Materials.

The construction of the light-gradient apparatus was fundamentally similar to the 'non-directional' gradient described by Ullyott, (1936). Figures 10 and 11 represent the two pieces of apparatus used. The characteristics of these gradients are shown in Figure 12.

A larva was placed on the experimental plate at 15 cm. with its head directed towards East. The experimental light was switched on after 30 seconds and the direction of movement in relation to the light-gradient was recorded at the end of 5 minutes. 50 larvae were used in these experiments.

The tracks of 18 other larvae were recorded by placing a single larva in any part of the experimental plate and leaving it for 3 minutes in the dark. The light was then switched on and the observations made till the larva reached the glass edge of the light-gradient.

Results.

The results obtained with 50 larvae are shown in Tables 9 and 9a. If movements were at random with respect to the light-gradients then we would expect to find a mean number of 6.25 larvae going in each of 8 directions.

A chi-square test applying Yate's correction for small numbers was used for these data. The purpose was to assess the probability of

1 inch = 6 inches

Scale

150-Watt Lamp

Lamp housing

Diffusing plate

West

East

Experimental plate

Stand
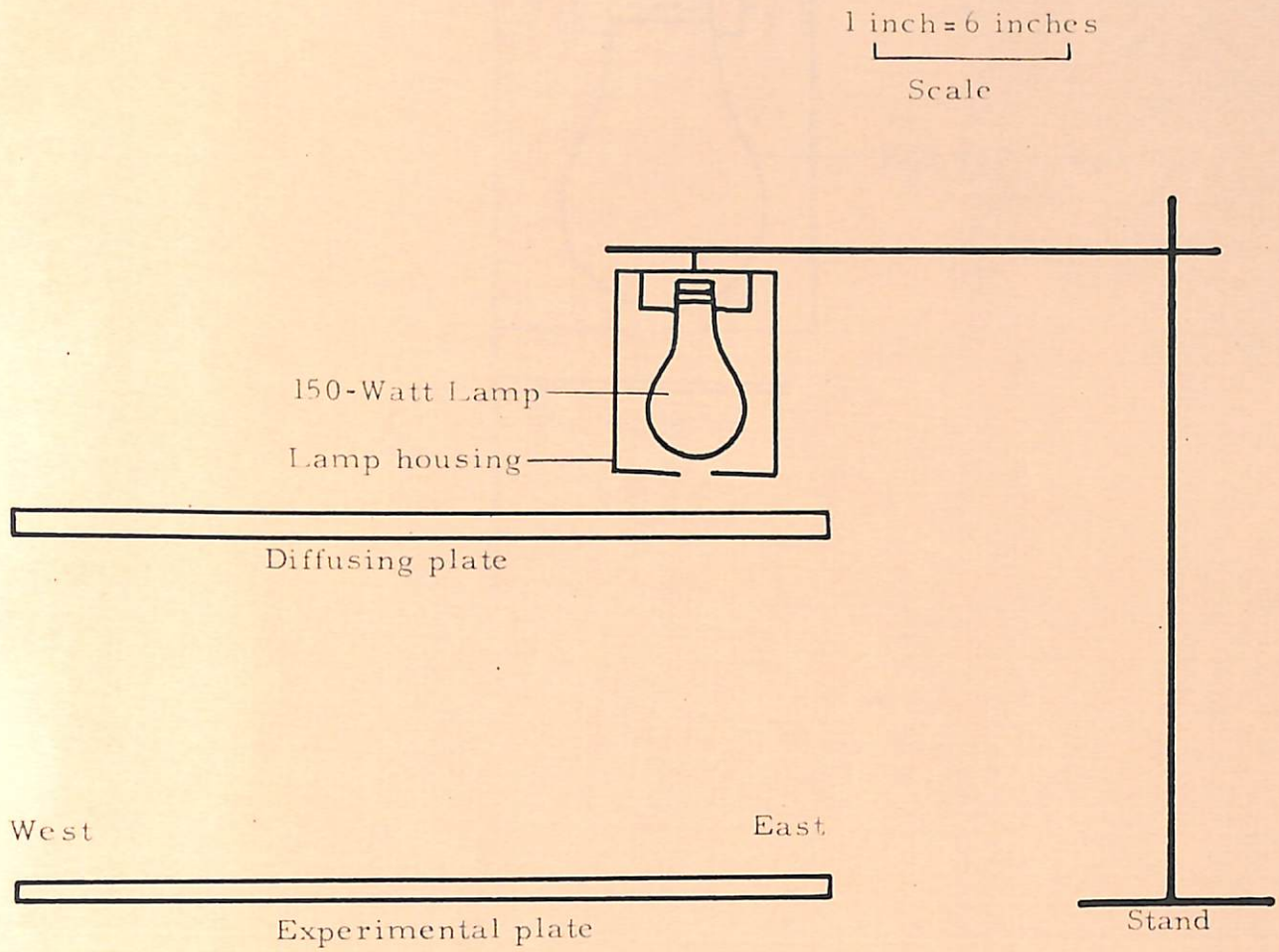
Fig. 10. Apparatus used to obtain the steep light-gradient.
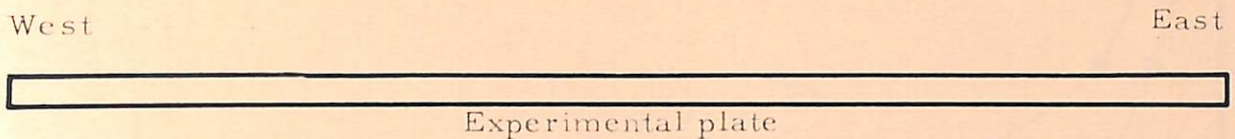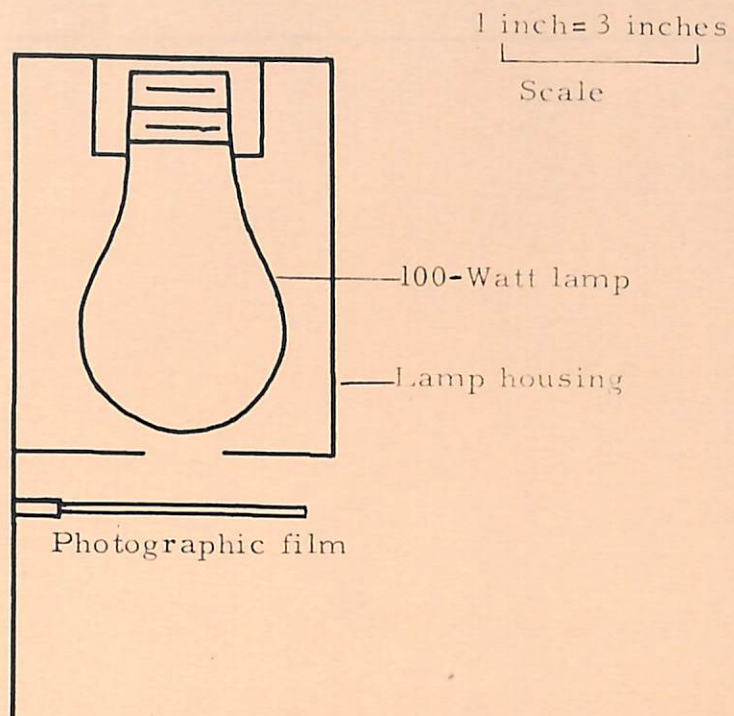
Fig. 11. Apparatus used to obtain the moderate light-gradient. The lamp was allowed to shine through a 9 x 6 cm. photographic sheet film. This film was transparent along one edge, but completely opaque along the opposite one with an even gradation of tone between the two extremes. By placing it nearer or farther from the lamp the steepness of the gradient could be altered.

Fig. 12. Light intensities obtained with the two apparatuses as shown in figs. 10 & 11.
● Steep-gradient.   ○ Moderate-gradient.
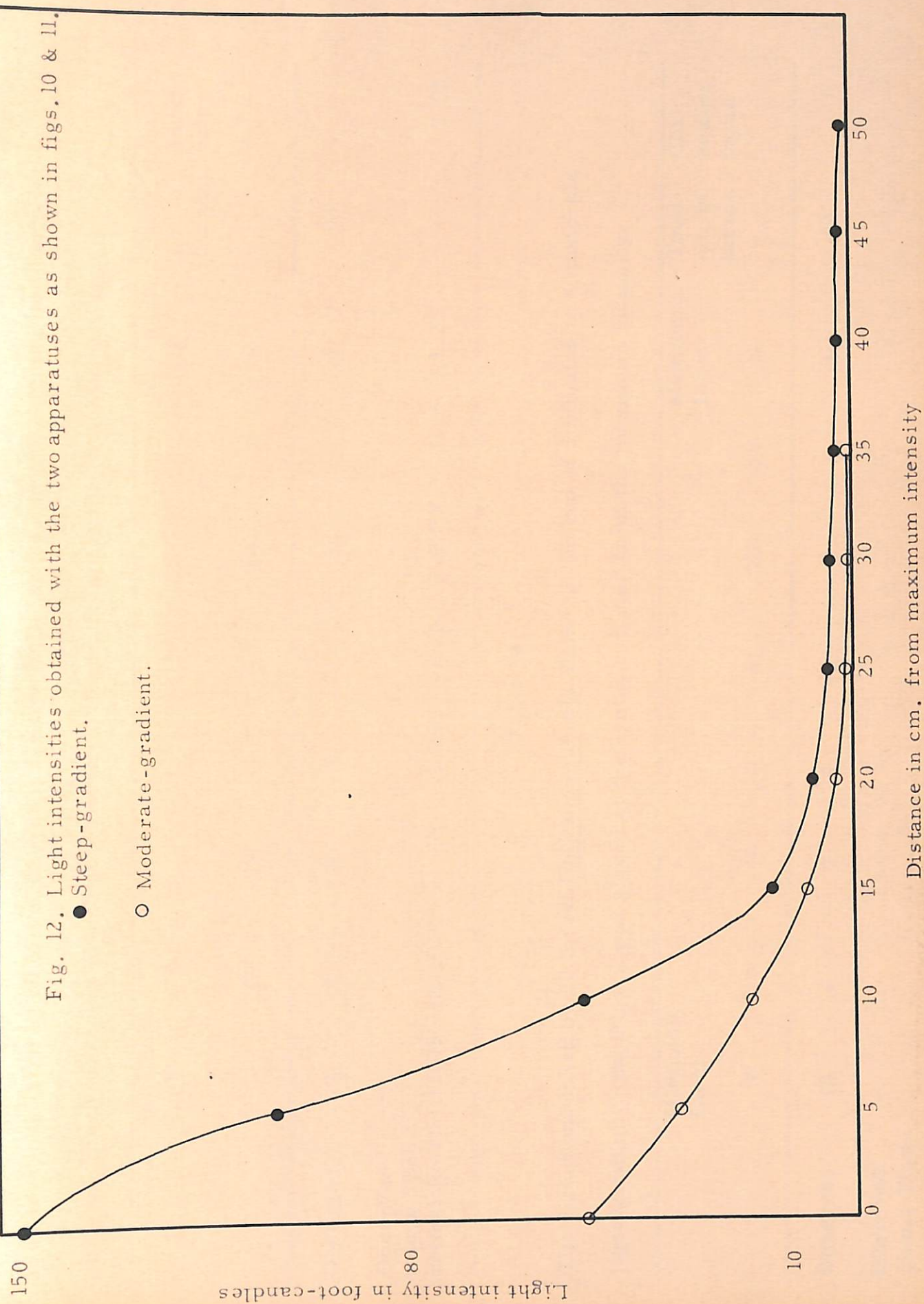
Table 9.

The directions taken by 50 larval <u>Chrysops furcata</u> Walk. at the end of 5 minutes in a steep light-gradient. Each larva was placed at the centre, 15 cm. from the maximum intensity.

	Minimum Light		SW	N	S	NE	SE	Maximum Light	Total no. of Larvae	Chi-square value
	W	NW						E		
Observed	12	9	5	5	3	6	5	5	50	9.52
Observed minus Theoretical (O-T)	+5.75	+2.25	-1.25	-1.25	-3.25	-0.25	-1.25	-1.25		

Table 9a.

The directions taken by 50 larval <u>Chrysops furcata</u> Walk. at the end of 5 minutes in a moderate light-gradient. Each larva was placed at the centre, 15 cm. from the maximum intensity.

	Minimum Light		SW	N	S	NE	SE	Maximum Light	Total no. of larvae	Chi-square value
	W	NW						E		
Observed	15	8	3	5	4	6	5	4	50	17.12
Observed minus Theoretical (O-T)	+8.75	+1.75	-3.25	-1.25	-2.25	-0.25	-1.25	-2.25		

any one direction being chosen by the larvae in the light-gradient.

The chi-square value obtained for moderate-gradient is significant

at the 2% level. It is, therefore, obvious that the larvae do not

move in all directions in equal numbers. The chi-square value for

the steep-gradient is not significant at the 5% level. The greatest

number of larvae, however, moved in the Westerly direction, where

the light intensity was the lowest.

On illumination all larvae, irrespective of their positions in

the light-gradient, exhibited the 'retraction reflex'. The reaction

time varied from 2 to 25 seconds. The least reaction time was

recorded in the high intensity zone of the steep-gradient. The time

between the 'retraction reflex' and crawling varied from 0 to 102

seconds. In low intensity zones crawling was usually inhibited by

a series of turning movements of the head and body which followed

the 'retraction reflex' as described in Section 2.4. Such inhibition

of crawling did not occur at very high intensity of light in either

gradient.

The speed of locomotion increased perceptibly when larvae

moved into the area of high intensity of each gradient.

Larvae which were placed in the gradients with their heads

facing the high intensity zone showed turning movements towards

the darker ends of either gradient. In fact, the longer time interval

between the first illumination and crawling favoured the chance of a

directed orientation in the low intensity region of the gradient.

2.9. Experiments using Lateral Light Stimulation.

Reactions to two equal lateral sources of light.

Loeb, (1905), has pointed out that "when two sources of light of equal intensity and distance act simultaneously upon a (negatively) heliotropic animal, the animal puts its median plane at right angles to the line connecting the two sources of light". We should expect, then, that a larva, subjected to the action of opposed beams of equal intensity, would continue crawling in a direction at a right angle to a line connecting the two sources. That such is the case with blowfly larvae has been demonstrated by Patten, (1915). Since larval tabanids in preliminary experiments showed a photo-negative reaction in a beam of horizontal light, it was thought necessary to check their reaction under balanced illumination.

Methods and Materials.

Three 25-watt lamps in light-proof cases with rectangular apertures 3 x 1 cm., cut in one face were used. The lights were placed in the centres of 3 sides of a 54 x 54 cm., wooden board with the bulbs facing the centre as shown in Figure 13.

14 larvae of C. furcata were used in these experiments. Each larva was put on the centre of a 23 cm., diameter glass plate, so that the axis of its body was directed towards the position of the light C and the head away from it. The lights A and B, were then
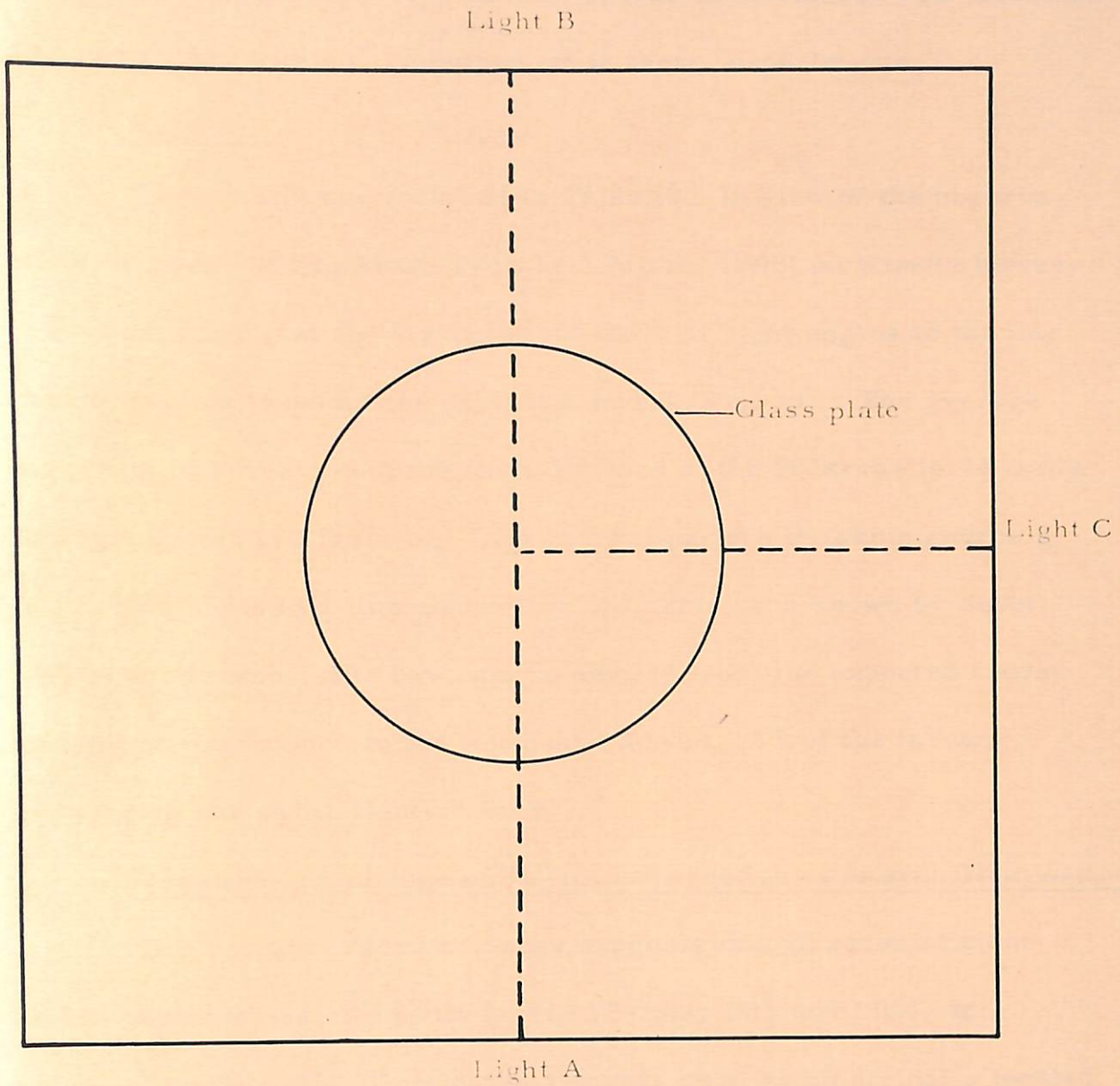
Fig. 13. Diagram to show arrangement of lights used in testing the photic reactions of larvae. For explanation see page

switched on simultaneously, and the course of movement was observed and recorded. Light C remained off in these experiments.

Results.

The results are included in Table 10. In view of the observations of Loeb, (1905); Mast, (1911); and Patten, (1915) on blowfly larvae, it was expected that the larvae would move at right angles to the line connecting the two sources of light A and B (Fig. 13). The average direction of several courses taken by each of the 14 larvae in 74 trails is represented in Figure 14. The general pattern in taking course 1 (at a right angle to a line connecting the lights) was shown by about 67% of the larvae. The tendency to deviate from the expected course was more pronounced in the immature larvae. 8% of the larvae crawled to one of the lights A or B.

Reaction to a change of 90° in the direction of lateral illumination.

The results described above suggests that (1) orientation of larval tabanids, as of blowfly larvae (Patten, 1915 and 1916), to balanced and opposed illumination depends upon symmetrically located sensitive areas operating bilaterally on the musculature; and that (2) such an orientation perhaps varies with the age of larvae. To further test these two points, the following experiments were conducted.

Methods and Materials.

Preliminary experiments were done to select larvae of uniform sensitivity as described by Patten, (1916). Nine mature and eight

Table 10.

Summary of the courses taken by 14 larval <u>Chrysops</u> <u>furcata</u> Walk.
under the influence of equal and opposite lateral sources of light
stimulation.

Stage	Number of times the types of courses taken by larvae			
	Course 1 Normal Direction	Course 2 Left or Right	Course 3 Towards Light	Total No. of Trails
Immature	2	1	2	5
"	3	2	0	5
"	3	1	1	5
"	4	1	0	5
Mature	5	0	0	5
"	4	0	1	5
"	4	2	0	6
"	2	4	0	6
"	4	0	1	5
"	5	0	1	6
"	3	3	0	6
"	4	1	0	5
"	3	2	0	5
"	4	1	0	5
Total	50	18	6	74
Percentage	67	24	8	
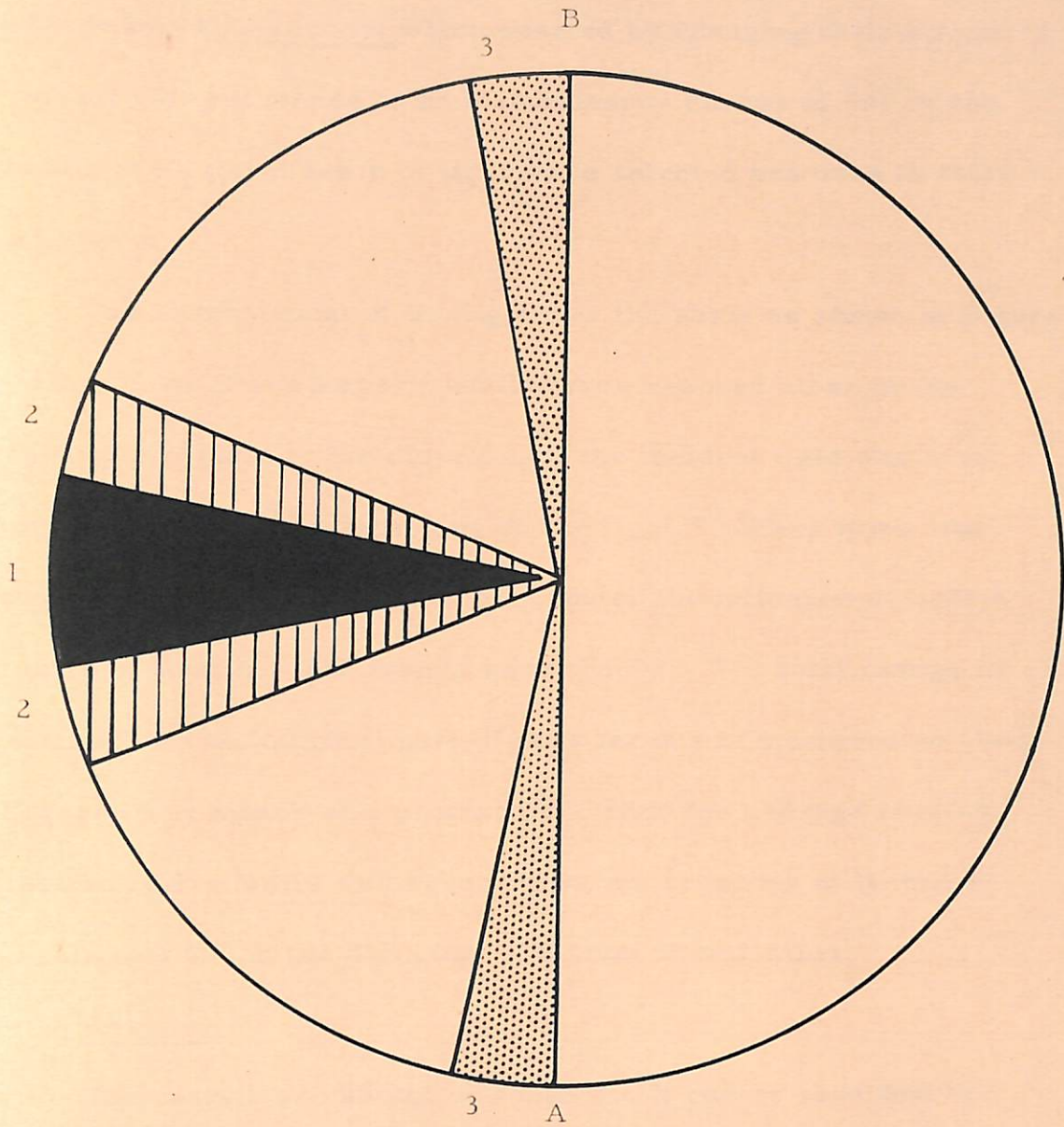
Fig. 14. Diagram to show the direction of movement of larvae in two beams of light from equal and opposite sources A and B. 1. Course of 67% trails. 2. Course of 24% trails and 3. Course of 8% trails. For explanation see page

immature larval C. furcata which reacted by changing their course of movement with reference to an instantaneous change of 90° in the direction of a lateral beam of light were selected and used in these experiments.

The arrangement of the light was the same as shown in Figure 13. Light A or B was kept on until larvae reached close to the circular glass plate. The direction of the incident light was then changed through 90° by switching on the light C. Each larva was allowed to crawl through twice, once under the influence of light A and once of light B. The tracks were traced. The total change in direction from the original path of each larva was measured as shown in Figure 15 by means of a protractor. Thus the average angular deflection of two trails was recorded as the response of larvae to the change of 90° in the direction of lateral illumination.

Results.

The results are shown in Table 11. It can be seen that the change in the direction from which the lateral light acts causes corresponding changes in the direction of locomotion of the larva. However, such a change is subject to considerable variation. The average values of two trails range from 4.5 to 60° in the immature and 41.5 to 90° in the mature larvae, so that a more accurate orientation to the change of 90° in the direction of illumination is shown by mature larvae. It seems, therefore, that a large part of the variation
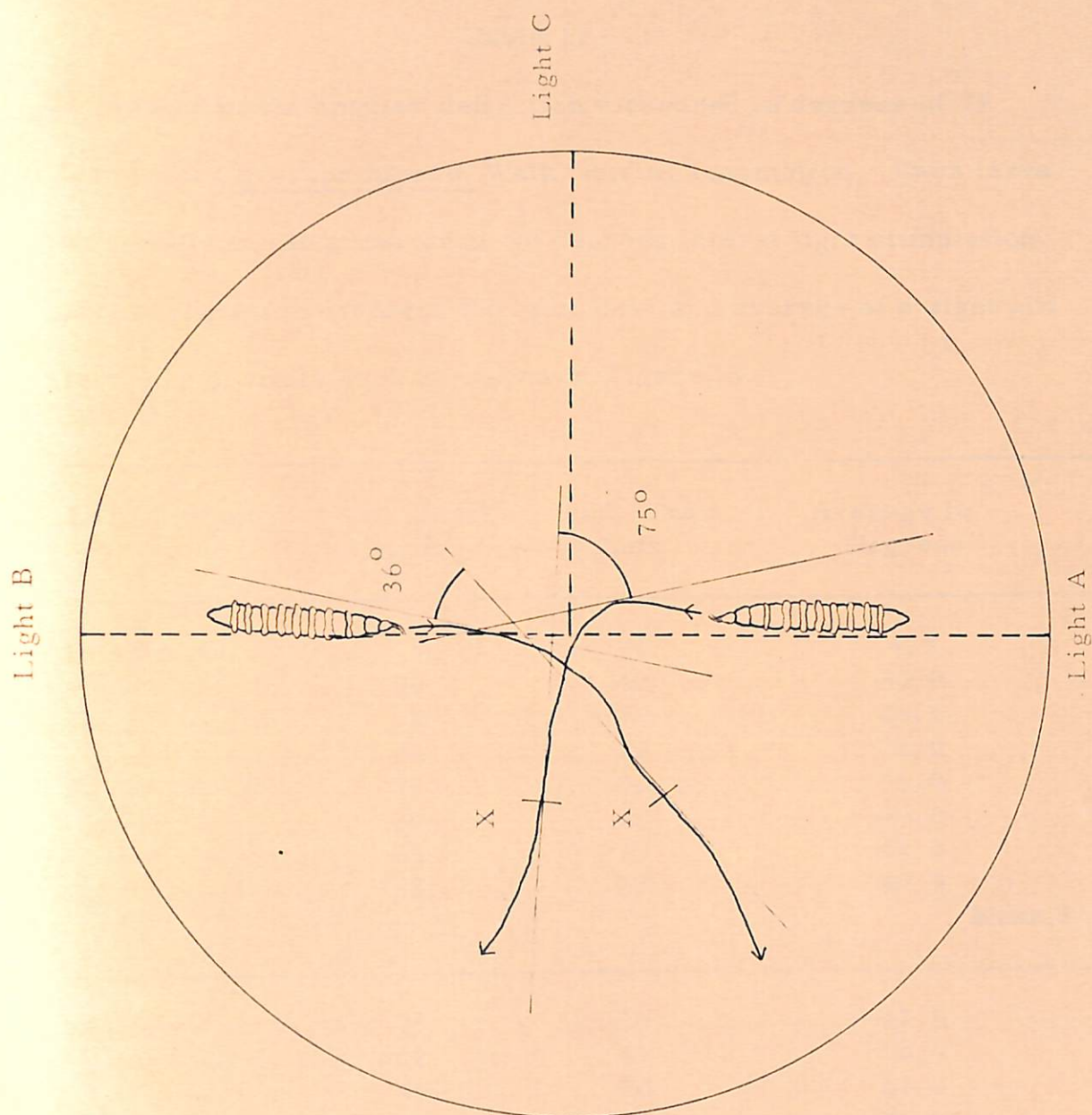
Fig. 15. Actual reproduction of the two trails of larva # 9. The starting lights A or B were shut off and light C was switched on when the animal reached close to the central point of the circular glass plate. The deflection up to a distance of 3 cm. (X) from the path at the time C was switched on was marked, the original and deflected paths were then projected and the resulting outer angles measured as shown above.

Table 11.

Summary of the angular deflection measured in degrees of 17
larvae of Chrysops furcata Walk. during locomotion. Each larva
was subjected to a change in 90 degrees lateral light stimulation.
Measurements represent the individual and average of a right and
left pair of trails such as shown in Figure 15.

Larval Stage	1st. Trail Right	2nd. Trail Left	Average in Degrees
Immature	5	4	4.5
"	80	40	60.0
"	8	45	26.5
"	34	10	22.0
"	75	36	55.0
"	30	68	49.0
"	55	29	42.0
"	58	23	40.5
			Mean 37.4°
Mature	75	20	47.5
"	82	45	63.5
"	65	50	57.5
"	58	110	84.0
"	53	64	58.5
"	100	56	78.0
"	87	68	77.5
"	90	91	90.5
"	45	38	41.5
			Mean 66.5°

in response to the lateral light stimulation is due to the difference
in age of the larvae. Such a variation has been reported in larvae
of several species of the family Calliphoridae (Patten, 1916).

    2.10. Discussion.

The larvae of Cyclorrhaphan Diptera have a pair of very sim-
ple photoreceptors which are probably degenerate lateral ocelli and
are situated in the extreme anterior end (Imms, 1957). In Musca
there is a small group of light-sensitive cells on each side of the
pharyngeal sclerites (Bolwig, 1946). It has been shown earlier that
the response to light in larval tabanids is probably due to the presence
of light-sensitive organs located in the anterior half of the head cap-
sule. On the basis of the histological findings and the experimental
results on local illumination, the eye-spots have been suggested as
the probable photoreceptors. In this regard it is interesting to re-
view Cameron's work (1934) concerning the life-history and morphology
of Haematopota pluvialis, (Tabanidae). He observed a progressive
posterior displacement of the lateral eye-spots before these were
transferred from the old to the new head capsule during each moult.
Such displacement of the eye-spots was further correlated with the
gradual concentration of the nervous ganglia anteriorly. These obser-
vations suggest that the eye-spots may function in part as precursors
of the compound eyes of the adult fly. The view (Snodgrass, 1935)
that lateral ocelli of holometabolous larvae are fore-runners of the

compound eyes seems to support this assumption indirectly.
Unfortunately, our present day knowledge of the larval eyes in
Diptera, and especially of larval tabanids, is clearly unsatisfactory
as it stands and the discussion of the exact organization of photo-
receptors only emphasizes our ignorance. Further embryological
work on this problem seems desirable.

Larval Chrysops react to light in two different ways. One is
by suddenly withdrawing the head capsule and the other by crawling.
Reactions similar to the first response have been recorded in wide-
ly different forms of animals such as hydroid polyps, sea-anemones,
tubicolous worms, several echinoderms and certain molluscs
(Hollaender, 1956) and are commonly known as the 'retraction reflex'.

The reaction times of 3 groups of larvae determined at differ-
ent intensities formed a hyperbolic relationship when plotted against
the logarithm of light intensity (Fig. 3). This suggests that the larva
roughly follows the Bunsen-Roscoe Law of reciprocity (Steven, 1950).
Close agreement to this law is not possible, since we know from the
works of Hecht (1918, 1919) on Ciona (Urochorda) and Mya (Mollusca);
Steven (1950) on Lampetra; and Newth and Ross (1955) on Myxine that
the reaction time is divisible into a 'sensitization period'[3] and a

3.   During which stimulating light must continue if a response
        is to be obtained.

'latent period'[4] of which only the 'sensitization period' varies with light intensity in Bunsen-Ruscoe manner. It is interesting to note that larval <u>Chrysops</u> are different from these animals as illumination is apparently necessary for the entire duration of the reaction time.

The data on crawling time (Table 4) are analysed to ascertain if the intensity of stimulus was related to the onset of locomotion. Although there is a clear indication of the crawling time having an inverse relationship at high intensities, the variations are too large and unexplainable. The crawling time, unlike the reaction time, does not appear to be concerned with the primary photochemical reaction. At this point it is worthwhile to mention that the nature of two responses in larval tabanids is very similar to those described in hagfish by Steven (1955).

The results in Section 2.7. have been considered from the point of view of the Weber-Fechner law as there are several noteworthy examples such as <u>Popillia</u> sp. (Moore and Cole, 1921); <u>Drosophila</u> sp. (Cole, 1922); <u>Dineutes</u> sp. (Clark, 1928); and <u>Unionicola</u> sp. (Welsh, 1932) in which the rate of locomotion is proportional to the log. of light intensity. The present data on intensity versus speed in cm/min, however, roughly follow this law over a given range only. Similar results

4.   During which the animal may remain in darkness and still respond.

have been reported with fly larvae by Mast (1911) and Herms (1911) who observed a higher rate of locomotion in strong than in a weak light but could not find any definite relation between intensity and speed. Unfortunately, there are no other data available on dipterous larvae with which a comparison of the present results could be made. Several reasons, however, can be given as to why the Weber-Fechner law cannot accurately describe the stimulus-response relationship as shown in Figure 9. According to Adrian (1928) this law represents only the mid-region of an integral distribution curve which is expected from the addition of more and more active receptors with increasing intensity of stimulus. Pirenne (1956) reports increased variation in the photic response of various groups of organisms as the threshold is approached. The relatively large deviations from the theoretical line (Fig. 9) in the low intensity region is apparently due to these two reasons. The deviations in the entire range seem possible because of an uncontrollable error, owing to a continuous change in the intensity of illumination of larval photoreceptors during movement. This can be easily brought about by the extension and retraction of the larval head capsule. Further, the works of Patten (1915), Hecht (1918), and Hartline and Graham (1932) point out the inefficiency of the Weber-Fechner law to describe responses in which several steps intervene between the stimulus and the production of a response. Since locomotion in the larval tabanids represents a complex of integrated

response which is brought about by two different reactions as shown earlier, the whole process cannot be properly described by this law.

Under the experimental conditions described in Section 2.6., the larvae tended to aggregate around the wall of the dark-light alternative chamber. The contact produced a slowing down of larval locomotion and also affected the direction of locomotion. This means that although larval <u>Chrysops</u> are photo-negative in the choice-chamber, their behaviour to light is subject to interference by the uncontrollable factor of thigmo-taxis. This is perhaps the reason why the intensity of reaction (Table 7) in the choice-chamber at a given time was not directly proportional to the log. of the light intensity.

The behaviour in dorsal light-gradients suggests that larvae react ortho-kinetically in the higher intensity zone. But at lower intensities they seem to react klino-tactically. Thus larvae left at the dark end of the gradient seldom moved up the gradient. They apparently showed directed reactions and turned back. It further seems that larvae arrive at the dark area as a result of 3 factors: (1) the 'retraction reflex'; (2) the local movement between the 'retraction reflex' and crawling; and (3) the increase in speed of locomotion due to an increase in light intensity. These observations and the data in Tables 9 and 9a suggest that the larval reactions were not truly random. Therefore the behaviour in a dorsal light-gradient can be satisfactorily described in terms of a combination of ortho-kinesis and klino-taxis.

The results described in Section 2.9. show that larval
tabanids, like <u>Musca</u>, <u>Calliphora</u>, and <u>Lucilia</u> larvae (Fraenkel and
Gunn, 1940), display a photo-negative reaction in a beam of horizon-
tal light.   The orientation away from the light source is chiefly
attained by klino-taxis.

These findings suggest that a negative photo-taxis coupled
with photo-ortho-kinesis would fit the larva well for its environment.
It will lie relatively inactive in the dark within the soil.   If exposed,
it will become active.   The negative photo-taxis would add an orient-
ing factor making the return to soil more rapid.   This could, there-
fore, explain the apparent absence of larvae from the surface of the
soil.

3. REACTIONS OF LARVAL TABANIDS TO MOISTURE

3.1. Introduction and Review.

Reviews of the literature on various aspects of chemo-and hygro-reception among arthropods may be found in articles and books by Snodgrass, (1935); Fraenkel and Gunn, (1940); Dethier and Chadwick, (1948); Buddenbrock, (1952); Wigglesworth, (1953); Perttunen, (1953); Dethier, (1953 and 1957); Hodgson, (1955); and Carthy, (1958). An extensive bibliography with abstracts dealing with the smell and humidity sense of insects has appeared recently (Hocking, 1960).

Our knowledge concerning the humidity sense of arthropods is primarily based on their locomotory reactions in different humidities or in humidity gradients. Such studies have been usually carried out by alternative chamber methods recommended by Gunn and Kennedy, (1936) and Wigglesworth, (1941). An olfactometer method (Willis and Roth, 1950) has also been employed. These methods not only provide valuable information on general humidity behaviour but have been further extended to localize or even identify the hygroreceptors of some arthropods. Noteworthy examples of arthropod hygroreceptors can be found in the works of Pielou, (1940); Wigglesworth, (1941); Lees, (1943 and 1948); Bentley, (1944); Begg and Hogben, (1946); Bursell and Ewer, (1950); Hafez, (1950 and 1953); Roth and Willis, (1951-52); Perttunen, (1951, 1955, and 1958); and Bursell, (1957).

Responses of arthropods to humidity are frequently determined by their state of water balance (see Dethier and Chadwick, 1948; and Perttunen, 1953). The responses may involve differences in activity and in rate of movement in moist and dry humidities, initial dry or wet reactions, reversal of initial reactions, and preference for inter-mediate humidity. Detailed informations on such responses are included in the works of Gunn, (1933 and 1937); Subklew, (1934); Kennedy, (1937); Thomson, (1938); Gunn and Cosway, (1938); Gunn and Pielou, (1940); Pielou and Gunn, (1940); Pielou, (1940); Walloff, (1941); Wigglesworth, (1941); Lees, (1943 and 1948); Dakshinamurty, (1948); Wellington, (1949-50); Bursell and Ewer, (1950); Jones, (1950); Willis and Roth, (1950); Hafez, (1950 and 1953); Perttunen, (1950-53, 1955-56 and 1958); Smith, (1951); Cloudsley-Thompson, (1951-52 and 1956); Roth and Willis, (1951-52); Dodds and Ewer, (1952); Green, (1954); Aziz, (1957); Bursell, (1957); Edney, (1957); El-Ziady, (1958); Lagerspetz and Jäyanäs, (1959); Riegert, (1959); and Syrjämäki, (1960).

Although soil moisture is an important environmental factor, the influence of water as distinct from air-humidity, on insect behaviour has not been widely investigated. Work on insect reactions to moisture has been reviewed by Lees, (1943). Since then, practically no work has been published in this regard.

Most of the works cited above deal with several groups of terrestrial arthropods and little is known about the humidity sense

and orientation in semi-aquatic insects. The humidity and moisture
reactions and data on water loss of the larvae of two species, C.
furcata and C.mitis are reported here.

3.2. Methods and Materials.

In most experiments the alternative chamber method as des-
cribed by Gunn and Kennedy, (1936); Wigglesworth, (1941); and Lees,
(1943) was used.

Three different types of humidity chambers were employed.
Type 1 consisted of/circular glass vessel, 15 cm. in diameter and
a
6 cm. deep. The chamber was enclosed by a glass plate with a seal-
able hole, 3 cm. in diameter in the middle. A vertical partition 2.5
cm. deep was attached to the glass roof to divide the chamber into
two halves. A petri dish, 14 cm. in diameter, which was divided in-
to two halves by a thin glass partition 2.5 cm. deep formed the floor
of the chamber. A false floor of wire gauze was supported from the
actual floor. In a few experiments layers of glass beads (average
diameter about 2 mm.) were introduced on the wire gauze tray to
facilitate the movement of the larvae. The fore-going apparatus
could also be used as a constant humidity chamber by removing the
partition from the glass roof and the petri dish.

Type 2 chamber consisted of two petri dish lids, 10 cm. in
diameter and 1 cm. deep, placed one upon the other with their well-
ground open edges in contact. A disc of 1 mm. mesh saran gauze

divided the chamber into an upper and a lower half.

Type 3 apparatus was essentially the same as in 2, except that the two lids had a diameter of 7.3 cm. and were 0.7 cm. deep and the lower lid was divided into two halves by a thin glass partition of 7.0 x 0.5 cm.

The chambers were made air-tight with vaseline and desired humidities were maintained by means of sulfuric acid-water mixtures (Wilson, 1921) placed on the floor of each chamber. A space of only about 2 mm. below the false floor remained empty. Thus the relative humidity just above the gauze must have been about the same as the theoretical value for each sulfuric-acid-water mixture.

Usually the humidity chambers were prepared on the evening prior to the experiments or were left undisturbed for at least 2-3 hours. Larvae were introduced and placed in the middle of each chamber either through the hole of the glass roof or by slightly lifting the cover of the humidity chambers. In similar experiments Hafez, (1950) reported that the disturbed humidity equilibrium is soon re-established.

Type 2 chambers were used to determine the rate of crawling of individual larvae in several relative humidities. After half an hour, movements of an animal for fifteen minutes were recorded on squared paper. The upper lids of the chambers were marked off into squares to facilitate this recording. A stop-watch was used to

record one-minute time marks on the tracks as well as the periods of inactivity lasting more than one minute. The distances were measured with a planimeter and the average speed in cm/min was recorded.

Alternative chambers of type 1 and 3 were used to determine the humidity preference of larvae. 5-20 animals were used in each experiment. The duration of each experiment was three hours. In order to eliminate any possible bias of the larvae toward any particular side due to light, the chambers were turned through 180° halfway through an experiment. Each experiment was repeated 5-10 times and a control (% R. H. 100:100) was used. The number of position records in each zone e.g. moister, drier and middle were noted. The excess percentage ratio $100 \left(\dfrac{W - D}{W + D} \right)$, (Gunn and Cosway, 1938) was employed to estimate the intensity of reaction. In this expression W and D are the numbers of the animals in the 'wet' and 'dry' sides respectively and the theoretical value for no reaction is 0.0%. For the purpose of comparison, the W/D ratio (Gunn, 1937) was also calculated. In this, the value for no reaction is 1.0. The animals recorded from the middle zone were omitted from the calculations.

For determining the differential activity of the larvae type 1 and 2 chambers were used. Ten animals were used in each experiment and the activity, either for 15 minutes or at different intervals

in the range of 0-60 minutes, were recorded.

Since certain soil insects under dry conditions are reported to lose water rapidly (Cameron, 1917; Subklew, 1934; and Lees, 1943), a few experiments were carried out to determine approximately the range of time over which larvae could survive in desiccated air. Small sulfuric acid desiccators (11 cm. deep and 11 cm. in diameter) at 21-22° C were used. Two batches of ten larvae each, were first washed in running water and then transferred to dry filter papers for 15 minutes prior to being weighed. During desiccation each batch was weighed at two-hour intervals. The loss of weight was used to represent the water loss (Gunn, 1933; and Syrjämäki, 1960).

Moisture experiments were carried out in the hope of finding certain definite larval reactions and to relate the results with those obtained on uniform relative humidities. These were conducted in a 9 cm. diameter petri dish. The bottom of the dish was covered with #1 Whatman filter paper which contained varying amounts of moisture. Percentage moisture was calculated from: $\frac{\text{Initial weight - Final weight}}{\text{Final weight}} \times 100$.

In each experiment one animal at a time was introduced into the petri dish for a period of 5 minutes. The following were calculated for each larva: (1) Average speed cm/min; (2) Average period of inactivity; (3) Average number of wall climbings; (4) Average number of burrowings; (5) Average number of head capsule elevations; (6) Number of

rollings.

In the above-mentioned studies larval C. furcata and C. mitis ranging from 15-20 mm. were most frequently used.

3.3. Preliminary Experiments.

In experiments with the constant humidity chambers both C. furcata and C. mitis show certain distinct reactions to low and high R.H. For example, in 50% R.H. and below the larvae remained strongly contracted for a period of 10-20 minutes. During contraction quick protrusion and retraction of the head capsule usually took place. The larvae seldom showed any crawling or burrowing movements although head movements were frequent. As a result dispersal from the center of the constant humidity chambers in the range of 0-40% R.H. was the least.

In chambers of 80-100% R.H., the larvae usually showed active movement and remained burrowed under the glass beads whenever these were provided. The larvae also crawled up on the roof of the chambers and tended to rest there in high relative humidities.

When larvae were observed over periods of 30 minutes in the choice-chamber apparatus (type 1), they showed a preference for one or the other side but did not remain in the moister side only. In most cases the larvae moved at random around the edge of the arena showing no behaviour suggestive of either a klino-kinesis or klino-taxis.

A few experiments were carried out in the constant humidity chambers of 0-100% R.H. to find the effect of desiccation. About 13 hours exposure at 24° C and approximately 0% R.H. was found to kill larval tabanids. It was also noticed that C. mitis was more active and sensitive to the effects of humidity than C. furcata.

3.4. Variation in Activity and Rate of Movement with Humidity.

Differential activity in constant humidities.

The results obtained from data on 100 larval C. mitis are summarized in Table 12. It can be seen that the activity increases as the relative humidity approaches 100%. This is in fair agreement with the observations made on preliminary experiments where the marked contraction of larvae has been pointed out as the cause of inactivity at low humidities.

A more detailed series of experiments was carried out to examine the activity under various relative humidities. Ten larval C. furcata were placed in the constant R.H. chambers and the number active and inactive were noted at 0, 10, 15, 20, 30, 40, 50, and 60 minutes. The data were obtained from 60 larvae which yielded 480 records. The results which are shown in Table 13, demonstrate that the activity reaches a basal level after 30 minutes and that the larvae are more active in wet air than in dry air.

The procedure for the experiments which are summarized in Tables 12 and 13, differed in only one respect e.g., the types of the

Table 12.

Activity of larval <u>Chrysops</u> <u>mitis</u> in uniform humidities after a 15-minute wait period.

% R.H. in the chambers:	% Active	% Inactive
0.0 (approx.)	43	57
10.0	42	58
30.0	38	62
60.0	70	30
90.0	82	18

Temp. 25° C; Light intensity, 85 F.C.; Type 1 chamber.

Table 13.

Activity of larval Chrysops furcata in uniform humidities. The proportion of larvae active or inactive at various lengths of time from the beginning of each experiment is shown.

Time in minutes from placement of larvae in the chamber

Percentage of larvae active and inactive: A = Active; and B = Inactive

% R.H.	0 A	0 B	10 A	10 B	20 A	20 B	30 A	30 B	40 A	40 B	50 A	50 B	60 A	60 B
0	17	83	11	89	6	94	11	89	8	92	7	93	4	96
10	20	80	21	79	19	81	18	82	16	84	7	93	11	89
30	22	78	18	82	21	79	20	80	17	83	22	81	14	86
60	29	71	27	73	27	73	27	73	28	72	28	69	30	70
90	37	63	37	63	39	61	40	60	36	64	38	62	38	62
100	25	75	35	65	42	58	38	62	45	55	34	65	41	59

Temperature 25.6±1.2° C; Light intensity 85 F.C.; Type 2 chamber.

humidity chambers used. However, if these tables are compared with reference to the activity records at 15 minutes the percentage activity for C. mitis is always found higher in the range of 0-90% R.H. than C. furcata.

Rate of movement under uniform humidities.

The results are shown in Table 14. The mean speed in cm/min increases with the increasing relative humidities and reaches a maximum at 100% R.H. Further, the mean period of inactivity decreases as the R.H. reaches saturation. The speed is almost constant in the range of 80-100% R.H. These results, however, present large individual variations. Another series of experiments was also conducted with five larvae which were selected on the basis of size and similarity of responses to various humidities. The results are given in Table 15. Omitting the individual variations, the speed and the mean period of inactivity of larvae in the range of 10-100% R.H., seem to be good examples of an orthokinetic orientation (Fraenkel and Gunn, 1940) in which the average velocity of locomotion or the frequency of activity depends on the intensity of stimulation.

3. 5. Rate of Moisture Loss.

Figure 16 shows the percentage loss of weight in C. mitis in successive hours during desiccation in dry air at $21^{o}C$. The weight of two batches showed almost no further change after desiccation for 18 hours. According to this the mean water content of larval C. mitis

Table 14.

The speed of movement of Chrysops furcata and periods of inactivity
in uniform humidities. 15 larvae in each test.

% R.H.	Mean speed in cm/min	SD	SE	Mean period of inactivity	SD	SE
10	0.55	0.55	0.14	6.7	6.1	1.6
40	0.61	0.70	0.18	7.2	5.4	1.4
80	1.20	1.17	0.30	4.1	5.1	1.3
90	1.24	0.78	0.20	2.9	3.7	1.0
100	1.64	1.20	0.31	1.7	2.4	0.6

Temp. 27.1±1.6°C; Light
intensity 85 F.C.; Type 2
chamber.

Table 15.

The speed of movement of larval C. furcata in uniform humidities;

five larvae were used.

Larva #	Mean speed in cm/min at different relative humidities				
	10	40	80	90	100
1	0.008	1.008	0.580	0.456	2.360
2	0.760	0.000	0.240	1.320	1.130
3	0.010	0.010	2.040	1.680	0.420
4	0.020	0.008	2.260	0.720	0.620
5	0.000	0.032	0.028	0.120	0.730
Mean	0.16	0.21	1.03	0.86	1.05
SD	0.34	0.45	1.04	0.64	0.77
SE	0.15	0.19	0.47	0.29	0.34

Temp. $26.4 \pm 1.1^\circ$ C; Light intensity 85 F.C.; Type 2 Chamber.

Fig. 16. Loss of weight in larval C. mitis in successive hours on desiccation at 0% R. H. and 21C.

○ Batch 1, with initial weight of 0.322 gm.
● Batch 2, with initial weight of 0.334 gm.
│ Loss of locomotion.
‖ 100% mortality.
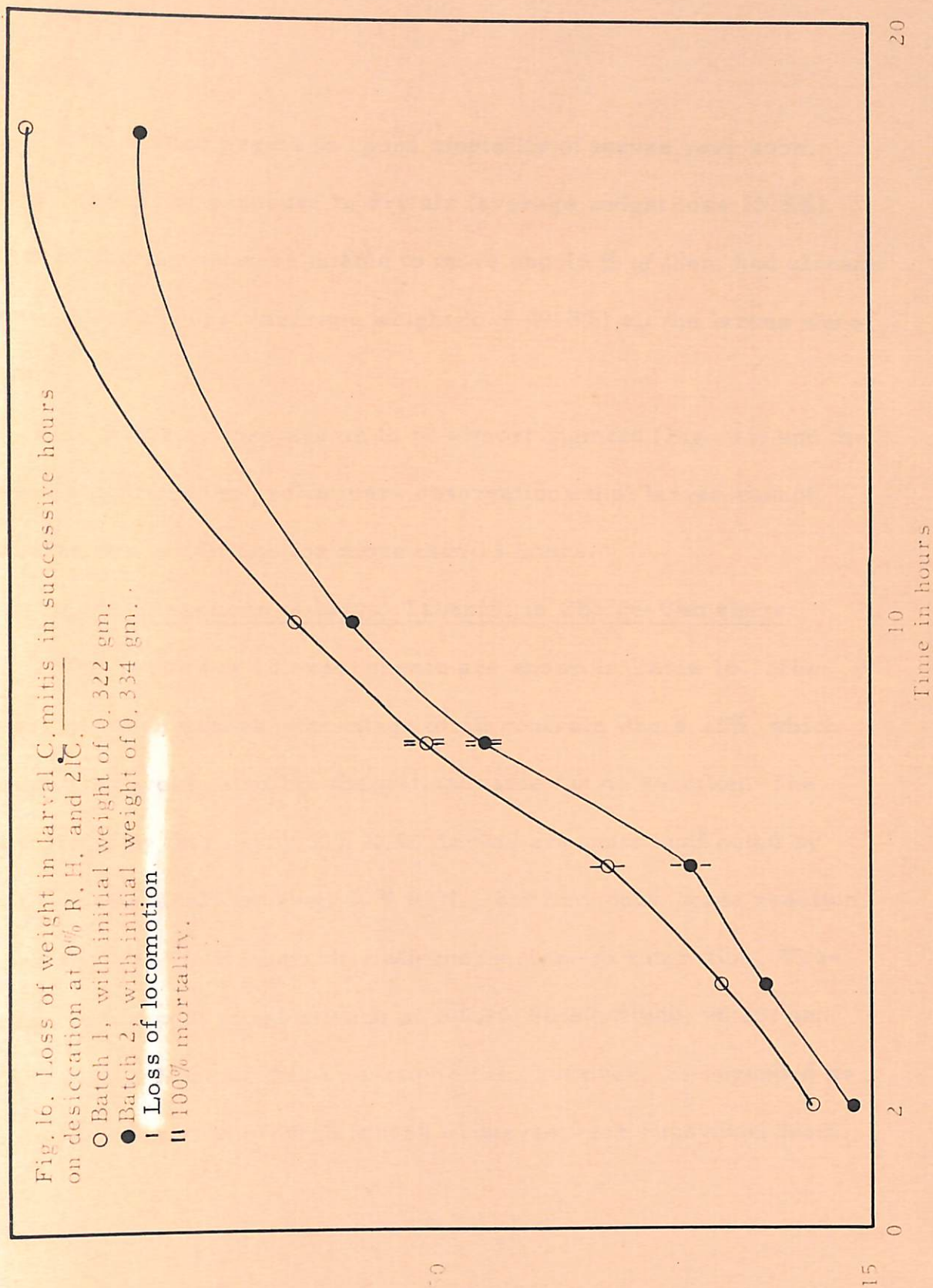
Time in hours

Loss of weight as % of the initial weight

is 79.5%.

Desiccation begins to cause mortality of larvae very soon. After 6 hours of exposure to dry air (average weight loss 33.5%) most of the larvae were unable to move and 15 % of them had already died. After 8hours' (average weight loss 49.5%) all the larvae were dead.

Rate of water loss seems to be almost sigmoid (Fig. 16) and the results confirm the preliminary observations that larvae cannot survive dry conditions for more than 13 hours.

3. 6. Reactions of larval Tabanids in Choice-Chmabers.

The results of 12 experiments are shown in Table 16. The average mean excess percentage of all controls was 4.15% which roughly approximated the theoretical value for no reaction. The results show that below 50% R.H. larvae are quite unaffected by differences of 30% or even 40 % R.H., but they show some reaction when the alternative humidity offered is close to saturation. Thus some reaction is found in each of 100:90; 90:60; 90:40; 90:30; and 70:30 R.H. None of these reactions can, however, be regarded as intense except that of 90:30 where 61 larvae were recovered from

Table 16.

Reactions of larval C. furcata to alternative relative humidities. Each figure represents 5 experiments involving 100 specimens.

	1	2	3	4	5	6	7	8	9	10	11	12
% R.H. IN THE CHAMBER												
a. Highest	100	100	90	90	90	80	90	70	50	50	50	20
b. Lowest	90	80	70	60	40	30	30	30	30	20	10	10
c. Difference (a-b)	10	20	20	30	50	50	60	40	20	30	40	10
NO. OF POSITION RECORDS IN EACH ZONE												
Moister (W)	53	44	53	52	56	61	54	54	47	38	44	47
Drier (D)	38	47	44	37	36	29	40	36	41	45	51	47
Middle	9	9	3	11	8	10	6	10	12	17	5	6
INTENSITY OF REACTIONS												
As excess %:	16.5	-3.3	9.3	16.9	21.7	35.5	14.9	20.0	6.8	-8.4	-7.4	-1.1
As ratio W/D:	1.4	0.9	1.2	1.4	1.6	2.1	1.4	1.5	1.1	0.8	0.9	1.0

Temp. $25.6 \pm .6°$ C; Light intensity 85 F.C.; Type 1 Chamber.

the wet side, 29 from the dry side and the remaining 10 position records from the middle zone. This partial avoidance of the dry side gives an excess percentage on the wet side of 35.5 and a W/D ratio of 2:1.

To check the above results it was thought necessary to use small alternative humidity chambers as described in type 3 for another set of experiments. Since the animals are small and sluggish, the use of such chambers would decrease the space and consequently increase the probability of larvae encountering the humidity boundry more frequently (Wellington, 1960). Further, in these experiments, light as a variable factor was controlled by covering the humidity chambers with a black piece of cloth and relatively more active larval C. mitis were used. It was expected that under these conditions the humidity reaction of larvae might prove to be an intense one. The results of 12 such experiments are summarized in Table 17. The average mean excess percentage of all controls was 1.3 which approximated the theoretical value for no reaction. With some exception, none of the results included in Table 17 show intense reaction of larvae to the wet side of the choice-chmaber. Thus neither the use of small alternative humidity chambers nor the control of light and the use of relatively active larvae improved the method for investigating the humidity reactions. However, still

Table 17.

Choice of alternative relative humidities by larval C. mitis. Each figure represents 10 experiments involving 50 specimens.

	EXPERIMENTS											
	1	2	3	4	5	6	7	8	9	10	11	12
% R.H. IN THE CHAMBER												
a. Highest	100	100	90	90	90	90	80	70	50	50	50	20
b. Lowest	90	80	70	60	40	30	30	30	30	20	10	10
c. (a-b)	10	20	20	30	50	60	50	40	20	30	40	10
# OF POSITIONS RECORDS IN ZONES												
Moister (W)	19	27	18	19	19	22	22	19	25	24	22	23
Drier (D)	23	21	28	18	20	21	25	18	20	23	20	11
Middle	8	2	4	13	11	7	3	13	5	3	8	16
INTENSITY OF REACTIONS												
As excess %	0.8	1.3	0.6	1.1	0.9	1.0	0.9	1.1	1.3	1.0	1.1	2.0
As ratio W/D	-9.5	12.5	-21.7	2.7	-2.6	2.3	-6.4	2.7	11.1	2.1	4.8	35.3

Temp. 26.1 $\pm$ 6° C; recorded with red light; Type 3 chamber.

another set of experiments was conducted with type 1 chamber in the hope of repeating the significant reaction in 90:30% R.H. The results of these experiments are given in Table 18. The data show that there is hardly any reaction over three hours in the dark.

Considering all the above results of the alternative humidity chamber experiments, it can be stated that with the exception of rather slight tendency to collect on the moister side, larval C. furcata and C. mitis show no response to humidity differences under the present experimental conditions.

3.7. Reactions of larval tabanids to the Moisture content of a substratum.

The results are included in Table 19. Examination of this table shows that larvae move faster with increasing moisture. Tendency to burrow and wall-climbing are also greater at higher percentage of moisture than at lower. On the other hand, period of inactivity, distinct elevation of the head capsule and inability to hold on the surface of the arena, e.g., rolling, are considerably higher at the lowest percentage moisture. Such behaviour as indicated by the longer periods of rest and consequently decreased rate of movement at low percentage moisture appears to be due to physiological instability, perhaps caused by loss of water through evaporation. Hence the injurious effects of unsuitable moisture conditions are reflected as distinct elevations of the head capsule and rolling which do not form a part of the normal larval behaviour. These results are consistent with those

Table 18.

Choice of alternative relative humidities of larval C. furcata in
the 90 : 30% R.H. Chamber.   Each experiment consisted of 5
replicates involving 100 larvae.

		Number of position records at the end of 1, 2 and 3 hours.		
	Zones	1.	2.	3.
	Moister	41	45	43
(IN THE DARK)	Drier	38	39	50
	Middle	21	16	7
Intensity of Reactions:				
As excess percentage		3.8	7.1	-7.5
As ratio W/D		1.1	1.2	0.9

	Number of position records at the end of 3 hours from each zone		
	Moister	Drier	Middle
(UNDER ROOM LIGHT OF 85 F.C.)	54	36	10
Intensity of Reactions:			
As excess percentage:	20		
As ratio W/D:	1.5		

Temp. $26.7 \pm .6^\circ$ C;
Type 1 Chamber.

Table 19.

Types of activities of larval C. mitis under various percentages of moisture. Each figure represents 10 observations involving 50 larvae.

% Moisture	Mean speed cm/min	SE	Mean PI in 5 mins.	SE	Mean WC	Mean EHC	Mean B	Mean R
2.3	1.99±.44	.13	52±34	10.7	0.0	15.3	0.0	7.4
12.0	1.95±.48	.15	76±31	9.6	0.0	16.4	0.0	1.9
24.4	2.32±.94	.29	50±23	7.1	0.2	6.2	0.0	0.0
41.5	3.90±1.10	.34	22±16	5.0	0.5	0.4	0.2	0.0
77.5	4.80±1.50	.47	18±11	3.5	1.4	0.1	0.1	0.0

PI - Period of inactivity in seconds

WC - Number of wall-climbings

EHC - Number of head capsule elevations

B   - Number of burrowings

R   - Number of rollings

±   - Value represents SD

obtained in experiments with constant humidity chambers, where it
has been shown that larvae seldom showed any rapid crawling or
burrowing movement under low humidities.

3.8. Discussion.

A brief knowledge of the life-cycle of a tabanid is essential
in understanding the larval reactions to moisture under laboratory
conditions. The two species studied are restricted to extremely
moist surroundings. Their eggs are deposited in compact masses
on the leaves and stems of plants growing in water or marshy places.
The newly hatched larvae fall into the water, move to the banks of
pools where they feed and grow. Usually in the spring they com-
plete their development and crawl out to damp ground to pupate.
In the laboratory larvae of both species show considerable activity
when immersed in water. They are, however, capable of living in
water without food for over two months although never completing
their life-cycle. They thrive best, pupate, and emerge as adults
when kept in moist soil rich in organic matter.

Most of the experiments were designed to find out whether
there were any klino-kinetic, klino-tactic, or ortho-kinetic reactions
(Fraenkel and Gunn, 1940) of larval tabanids to moisture. No informa-
tion on klino-kinetic and klino-tactic responses was provided. How-
ever, as the percentage activity and speed in cm/min of the larvae
increase with increasing moisture conditions and at the same time

the mean period of inactivity decreases, this would make the reaction at least an ortho-kinesis. A true ortho-kinesis is thus a major part of the orientation mechanism which, in larval C. mitis and C. furcata, would bring about aggregation in a dry place if the results are to be interpreted in a conventional way (see Fraenkel and Gunn, 1940). But such an explanation seems to be confusing in view of the following facts: (1) In nature neither larval nor pupal stages of the species studied are found in dry places. (2) In the choice-chamber apparatus (see Section 3.6.) larvae do not show any preference for the dry side; and (3) The inactivity and abnormal behaviour pattern of larvae in low R. H. and moisture percentage (see Section 3.3. & 3.7.) are obviously produced by the injurious effects of dry air. It follows therefore that the ortho-kinetic reaction of larvae to moisture would not possibly bring about larval aggregation to dry places. Now, moist soil is the ideal habitat of tabanid larvae and while the laboratory conditions included only moisture, the soil with its associated factors (thigmo-tactic, textural, food, etc.) were absent. Therefore, the moist substrate in the laboratory experiments would tend to produce an ortho-kinetic reaction, since in moist soil the larvae are relatively inactive. Possibly then, the larvae are stimulated under moist conditions to react ortho-kinetically until the ideal conditions of moist soil is reached. Although the significance of such an ortho-kinetic reaction in terms of behaviour under natural conditions is open to

question it seems likely that such a reaction would serve as a selective response during migratory phases of larvae from water to the bank of the pool and from the very moist soil to the slightly drier ground. Another possible explanation is that low moisture conditions would bring about temporary arrest of larval growth (aestivation) and consequently, no movement, while the activity of larvae could be brought on again by moist conditions. Such temporary arrest of activity during dry conditions and subsequently increased activity under moist conditions are quoted by Wigglesworth, (1953) amongst larval stages of some Diptera.

The experiments described in Section 3.5. show that the peril of desiccation is a very real one to larval tabanids. However, the initial contraction of the larval body wall in response to a continuous stimulus of low R.H. percentage as mentioned in Section 3.3. seems to be involved in the control of water loss at least for a short duration. These contractions do not last very long since the larva is incapable of maintaining the contracted condition after a certain amount of water is lost. These observations and the data on rapid loss of weight of C.mitis on desiccation indirectly support the view (Gunn, 1933; Palmen & Suomalainen, 1945) that the loss of weight of an arth.-ropod on desiccation is nearly entirely due to evaporation of water from the integument.

4. TEMPERATURE REACTIONS OF LARVAL TABANIDS.

4.1. Introduction and Review.

Much of the literature on various aspects of insect behaviour in relation to temperature has been summarized by Herter, (1953); Andrewartha and Birch, (1954); and Carthy, (1958). The important papers related to the present study and not included in the above works are reviewed here.

The works of Miller, (1929); Falconer, (1945); and Hafez, (1953) suggest that the rate of movement of larval insects is directly proportional to temperature over a wide range.

Omardeen, (1957) reported that when second-instar larvae of Aëdes aegypti were subjected to a temperature gradient of 8-42° C, the majority of the larvae aggregated over the range of temperature 23-32° C. Third and fourth-instar larvae and pupae showed a preference for 28-32° C.

To date most of the published works deal with the temperature reactions of terrestrial insects and literature pertinent to the behavioural work with aquatic insects or forms living in semi-fluid media is scarce. The present report describes the results on activity and rate of crawling of larval C.mitis and C.furcata in relation to temperature. The temperature preference of C.mitis was also studied.

In the past, three kinds of observations have been made on the

effects of temperature on the locomotory activity of insects. Shapely (1920) measured the speed of creeping of ants at various temperatures, the object being to find the 'normal range of temperatures for the locomotory activity'. Chapman et al. (1926), as quoted by Nicholson (1934), raised the temperature of a vessel containing various insects at a rate of 21° C per hour and recorded quantitatively the kinds of activity. Nicholson (1934) estimated the proportions of active or inactive in several batches of blowflies under given temperature conditions. Shapely's (1920) and Nicholson's (1934) methods have been adopted for the present experiments. Omardeen's (1957) method slightly modified as described below has been utilized for temperature preference experiment.

4.2. Methods and Materials.

Six temperatures: 5, 10, 15, 20, 25, and 30° C were obtained by working in constant temperature rooms. Higher temperatures (35, 37, 40 and 42° C) were obtained by the use of two water baths. The temperatures were always checked before and after each experiment through the use of a tele-thermometer with sensitive probes. Glass plate temperatures obtained by the use of the water baths varied $\pm 2°$ C in time and $\pm 0.8°$ C over the plate and hence the average temperatures for these were recorded.

The animals used for the activity records consisted of two batches of ten larvae of C. furcata. These were stored at 10° C for

twenty days in the dark. Before each experiment the larvae were transferred to a petri dish containing 1 cm. of tap water, and were then left at the constant temperature of the particular experiment for eight hours. For the higher temperature experiments the petri-dish was placed on a glass plate suspended over a water bath. The first reading on crawling (criterion for activity) was taken after seven hours and four further readings were made at fifteen minute intervals. These readings done during a period of 30 seconds each time on batches 1 and 2, were recorded with the help of a white diffuse light of 55 foot-candles and a red light of 2.5 foot-candles respectively.

Two batches of C. mitis of ten larvae each were used for determining the speed of movement under various temperatures. Observations were taken only after an animal had been at least two hours at any particular temperature. However, one batch of larvae was exposed to the experimental temperature for 8 hours before readings were taken on speed of movement. Several glass plates up to 132 x 100 cm. were used on which the larvae made their own trails. Again the glass plates were placed over a water bath for the high temperature experiments. Each larva was allowed to crawl for 15 minutes and the average speed in cm/min was recorded.

All experiments on the rate of movement were carried out in the dark. The only light used at the time of placing the larvae on the experimental glass plates was a red light of 2.5 foot-candles

intensity. Since differences in speed between individuals were considerable in the dark, larval photo-negative behaviour in a beam of horizontal light (see Section 2.9.) was utilized and a lateral light source of 55 foot-candles was used for one series of measurements to reduce the individual variations as well as to find a possible effect of light in combination with the experimental temperatures. Two batches of C. furcata larvae were used; #1 in the dark and #2 under a lateral light.

Temperature-gradient experiments were carried out in an apparatus fundamentally similar that as described by Omardeen (1957). This consisted of a thin metal sheet trough, 35.5 x 5 x 7 cm. with 1 cm. layer of 2-3 percent agar in tap water. The trough was fixed over a thick copper plate, 59.5 x 10 cm. An extended part, 7 x 5 cm., of the trough's floor was immersed in a freezing mixture of ice and salt. Streams of cold water were also circulated in the copper plate near the cold end. An electric flat immersion heater was used to heat the copper plate from the other end. Thus by continuous cooling of one end of the copper plate and simultaneous heating of the other it was possible to maintain a temperature-gradient in the agar solution ranging from $9.7-34.8^{\circ}$ C.

The floor of the trough was marked off into 14 sections of one inch each. The temperature at the middle of each section was measured with a tele-thermometer with sensitive probes as described

earlier. Convection currents were not of any particular importance and the temperature-gradient as shown in Figure 17 could be easily maintained over periods of two hours with slight variation.

A 47 x 10 cm. fluorescent lamp was constantly used overhanging the trough. The light intensity on the floor of the trough was 210 $\pm$ 5 foot-candles.

A total of 100 C. mitis larvae was used in the temperature preference experiments. These were stored at 21.5$\pm$.5° C for two weeks prior to experimentation. Only those larvae which showed the least ill effects of heat were used. In each experiment ten larvae were evenly spread over section seven (20.8^o C) of the trough and the number of larvae positions in each section was counted at 5-minute intervals for 30 minutes. Each series of experiments was repeated ten times.

4.3. Results.

Activity under uniform temperatures.

The results are summarized in Table 20. It will be seen that at any temperature from 10-37° C the percentage activity in batch 1 recorded under a 55 foot-candles light was always higher than that in batch 2, the average increase being 32%. This obvious difference is, therefore, caused by the effect of light. However, the two sets of data on the two batches are confirmatory to each other in so far as the activity under a normal range (15-25° C) of temperature is

Fig. 17. Temperature-gradient used in temperature preference experiments.
● Each point represents 27 readings with SD.

Table 20.

Activity of larval <u>Chrysops</u> <u>furcata</u> at several temperatures.   Each

figure represents five readings with ten larvae.

	Average Temperatures in 0° C						
BATCH 1,	Recorded under 55 F.C. Light						
	5	10	15	20	25	37	42
% Active	0	4	48	48	52	20	0
% Inactive	100	96	52	52	48	80	100
BATCH 2	Recorded under 2.5 F.C. red light						
% Active	0	0	8	14	14	4	0
% Inactive	100	100	92	86	86	96	100

concerned. Neither batch shows any appreciable activity up to

10° C. At 15° C the activity suddenly rises in each batch and remains

almost constant up to 25° C. Thus the degree of constancy with

reference to the activity in this temperature range (15-25° C) sugg-

ests that activity does not increase because of the harmful effects

of temperature. This temperature range of 15-25° C appears, there-

fore, to be the preferred zone of the larval C. furcata.

At 37° C an average of 30 percent mortality was noted for

each batch of larvae. The remaining 70 percent of the larvae af

the end of the experiment at 42° C were also dead. Thus the tempera-

ture range of 37-42° C is lethal to the larval C. furcata under the

present experimental conditions.

The rate of movement at constant temperatures.

The results on the two batches of C. mitis are included in Table

21. The mean values of speed cm/min increase very gradually in the

temperature range of 5-25° C. The speed is roughly constant at 25

and 30° C and is decreased at 35° C and 40° C. Thus the temperature

zone for maximum speed is 25-30° C.

The data obtained with the two batches with different pretreated

conditions prior to the experimental temperatures are closely similar.

It seems, therefore, that such preconditioning has no influence on the

rate of movement of the larval C. mitis.

Table 22, shows the results obtained with the two batches of

Table 21.

The speed of movement of larval <u>Chrysops mitis</u> at several constant temperatures. Each figure represents 30 readings i.e. 3 on each of 10 larvae.

Temperature (0°C)	(2 hrs. pretreated to the experimental temperature)		(8 hrs. pretreated to the experimental temperature)	
	Mean speed (cm/min)	SE	Mean speed (cm/min)	SE
5	0.06±0.05	0.05	0.22±0.05	0.01
10	0.27±0.12	0.04	0.23±0.15	0.05
15	0.36±0.12	0.04	0.51±0.27	0.09
20	0.75±0.23	0.08	0.82±0.27	0.09
25	1.13±0.24	0.08	1.14±0.20	0.07
30	1.06±0.27	0.09	1.01±0.41	0.14
35	0.71±0.33	0.11	0.99±0.38	0.13
40	0.32±0.11	0.04	0.15±0.18*	0.06

* This figure is based on only 10 readings since the animals were either dead or motionless after the first series of readings.

± Value represents SD.

Table 22.

The speed of movement of <u>Chrysops</u> <u>furcata</u> at several temperatures.

Each figure represents 30 readings. i.e. 3 on each of 10 larvae.

Temperature (0^oC)	BATCH 1 (Movement in the dark)		BATCH 2 (Movement under a lateral light source)	
	Mean speed (cm/min)	SE	Mean speed (cm/min)	SE
5	$0.10{\pm}0.02$	0.01	$0.34{\pm}0.09$	0.03
10	$0.16{\pm}0.05$	0.02	$0.72{\pm}0.01$	0.00
15	$0.17{\pm}0.03$	0.01	$0.71{\pm}0.20$	0.06
20	$0.36{\pm}0.12$	0.04	$1.60{\pm}0.25$	0.08
25	$0.73{\pm}0.22$	0.07	$2.50{\pm}0.44$	0.15
30	$0.40{\pm}0.18$	0.06	$3.95{\pm}1.23$	0.41
35	$0.29{\pm}0.11$	0.04	$2.69{\pm}1.25$	0.42
40	$0.14{\pm}0.08$	0.03	$2.01{\pm}0.60$*	0.20

* This figure is based on only 10 readings since the animals were either dead or motionless after the first series of readings.

± Value represents SD.

C.furcata. Here the mean values of speed at any temperature for batch 2 are always higher than batch 1. Since the experimental conditions were the same for either batch except that the lateral light source was used for batch 2, it is obvious that the difference in speed is caused by the influence of light. Leaving aside the difference produced by light, the two series of data are closely similar to each other in respect to minimum speed at 5° C, increasing speed with increasing temperature and by the amount of variation shown by the larvae. The temperature zones for maximum speed, however, are slightly different in each batch.

A comparison of the rate of movement between C. mitis and C.furcata (see batch 1 of Tables 21 and 22) shows a higher speed for C. mitis throughout the temperature range of 10-40° C. The rate of movement at 40° C does not diminish with extreme rapidity. However, all larvae of both species died at 40° C.

The distribution of larvae in the temperature-gradients.

The results of 40 experiments including the control and representing some 3000 position records are shown in Figure 18. Under conditions of uniform temperature and illumination the larvae moved freely along the trough aggregating at both ends. This 'end effect' is clearly shown in the histogram for the control experiments.

The temperature-gradient experiments (Fig. 18b and 18c) show that the larvae aggregate in the extreme cold end of the trough, the

Figure 18. The distribution of larvae in the temperature-gradients. 103

Fig. 18a. The distribution of larval C. mitis in control experiments with agar solution temperature constant at 21.5 ± .5°C and light intensity constant at 210 ± 5 foot-candles.

Fig. 18b. The distribution of larval C. mitis in a temperature-gradient of 9.7 - 34.8°C with light intensity constant at 210 ± 5 F. C.
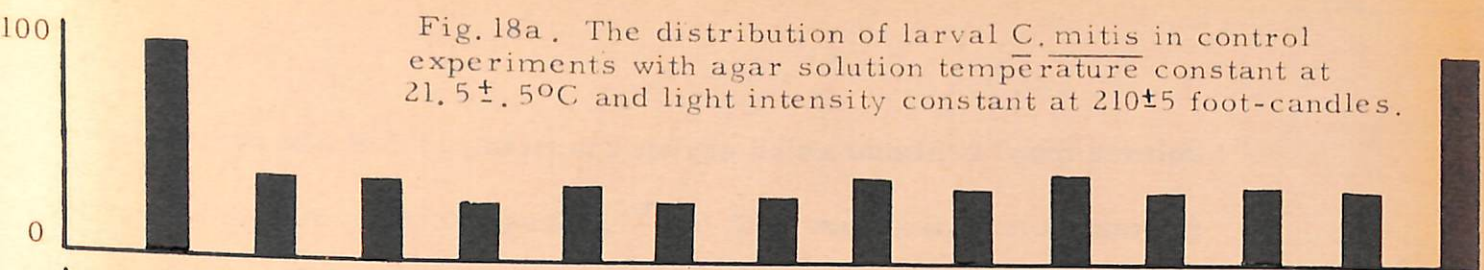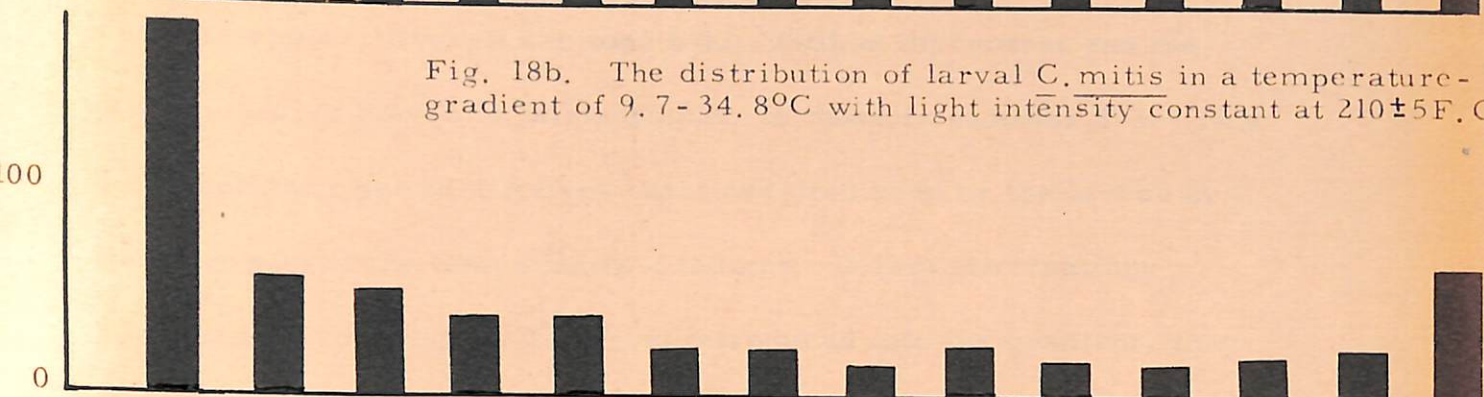
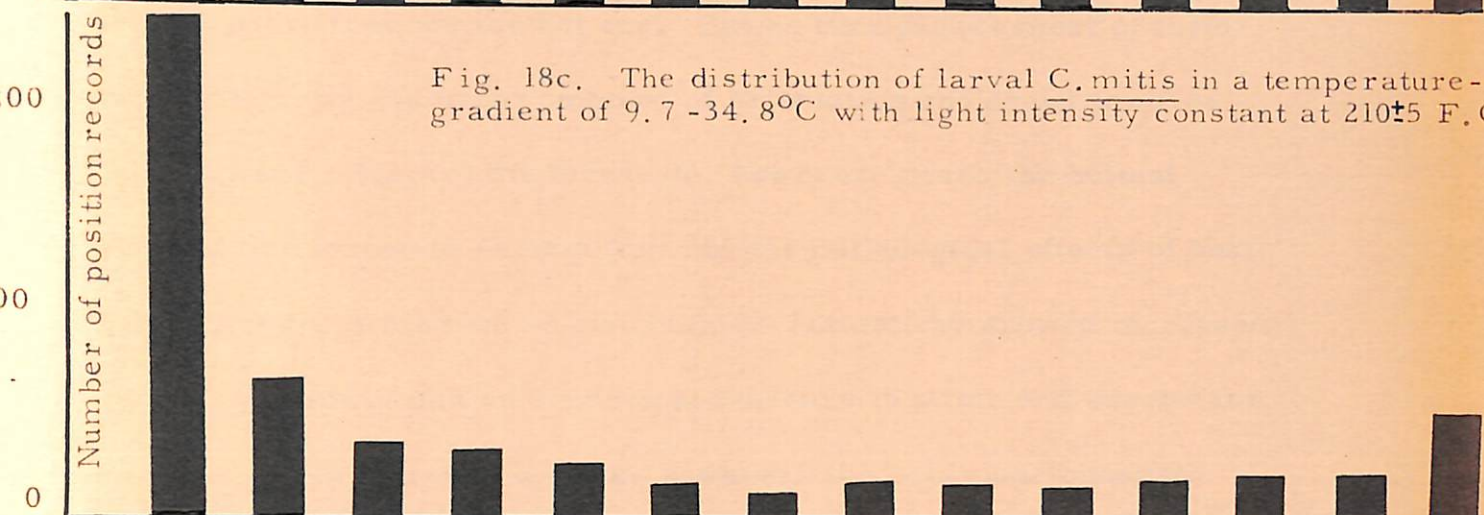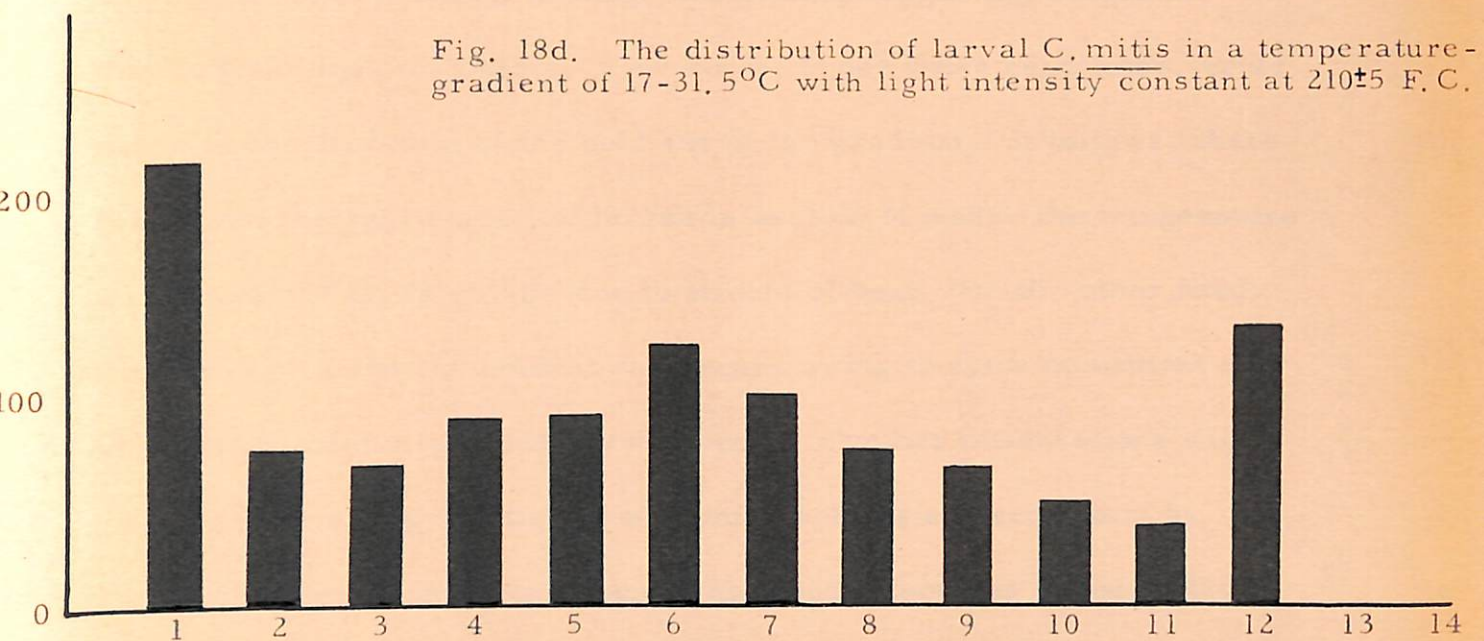Fig. 18c. The distribution of larval C. mitis in a temperature-gradient of 9.7 - 34.8°C with light intensity constant at 210 ± 5 F. C.

Fig. 18d. The distribution of larval C. mitis in a temperature-gradient of 17 - 31.5°C with light intensity constant at 210 ± 5 F. C.

Number of position records

Distance in inches from the coldest section.

maximum number of position records being obtained from section 1 where the temperature was 9.7° C. The least number of position records appear between sections 6-13, until at the hottest end the distribution of larvae is fairly similar to those in sections 2-3. This distribution is due to different reactions displayed by the larvae at the two ends of the temperature-gradient. Direct observations suggest that when larvae are first introduced into the gradient, they tend to move towards the hot end. But as time passes most of them turn by a 'trial and error' method of orientation and crawl towards the cold end. Some of the larvae do, however, reach the hottest end and are forced to remain there by the pathological effects of heat. For example, all larvae on reaching the hottest end showed increased rate of movement and an occasional tendency to climb and burrow for a while. With increase of time, however, the behaviour consisted mainly of rapid probing, coiling, and rolling suggesting stages of distress and loss of control. Few larvae under these conditions, could move in the direction of the cold end of the gradient. It follows, there-fore, that the aggregation of larvae in section 14 where the temperature was about 35° C, is chiefly due to effects of heat. On the other hand, the aggregation at the coldest end occurs owing to slow movement and increasing inactivity of larvae with time. Further observations sug-gest that larval aggregation is also influenced to a great extent by the 'end effect' at the coldest end. This end effect was different from

that of the hottest end since larvae here formed inactive groups
mainly in the corners of the trough. Thigmo-taxis, then appears
to be a potent factor in producing larval aggregations at the coldest
region. It is, therefore, possible that larvae are trapped at the
coldest end either due to the immobility forced upon them by the
physiological effects of cold or due to thigmo-taxis or both. The
former view, however, seems to be well supported by the results
as shown in Tables 20 and 21 where the least percentage activity and
speed in cm/min have already been demonstrated in the uniform
temperature range of 5-10° C.

The above explanation in connection with the larval aggrega-
tions at the two ends of the temperature-gradient and the absence of
any significantly well-defined aggregations in the temperature range
of 11.8-33° C point to the fact that larval C. mitis do not exhibit any
definite 'temperature preference' under the present experimental
conditions. It seemed likely that the failure of larval behaviour was
due to the steepness of the temperature-gradient. A few experiments
were, therefore, carried out with the same batch of larvae as des-
cribed in Section 4.2. but in a temperature-gradient where the tem-
perature ranged from 17-31.5° C. representing only twelve one-inch
sections of the metal trough. Ten larvae were evenly spread over
section six of the trough and the number of the larval positions
counted in each section at 5-minute intervals for 60 minutes. Such

an increase in recording time from 30 minutes to 60 minutes increased

the chances of a larva coming into contact with all the parts of the

temperature-gradient several times.   The results of ten such experi-

ments are summarized in Figure 18d.   The histogram for this series

of experiments is comparable with those given in Figures 18b and 18c

since Figure 18d is based on the same numbers of position records e.g.,

1200.   A comparison therefore shows: (1) a slight increase in the

number of position records at the hottest end (Fig. 18d); (2) but a

considerable decrease in the number of position records at the cold-

est end; and (3) the presence of a middle range of larval aggregation

between sections 6-7 with the temperature of 22-24° C.   These diff-

erences in the distribution pattern are accountable to a difference in

temperature range of the gradients used.   Larval C. mitis in the

temperature-gradient apparatus of 17-31.5° C showed continuous

activity at the coldest end of the gradient and there was no indication

that they were ever immobilised by the cold.   Further larval behav-

iour at the hottest end did not suggest stages of distress and loss of

control as described on page 104.   Observations, however, indi-

cate that aggregations at both ends occur mainly due to thigmo-taxis.

Thus among the larvae remaining outside the cold and hot sections

there is an indication of a well-defined aggregation in the sections

6-7 where the average temperature was 23° C.   This temperature

zone of 22-24° C, therefore, appears to be the preferred temperature

of larval C. mitis .

4.4. Discussion.

The results of all temperature experiments are summarized in Figures 19 and 20. It will be seen that all curves (1, 2, 3, 4, 5, and 6) show a very characteristic feature, e.g., indifference of larvae to lower and upper limits of 15 - 30°C. Thus larval C. mitis and C. furcata appear to exhibit a temperature preference in the range of 15 - 30°C.

Curves 1 and 6 show much greater activity and speed than curves 2, 3, 4, and 5, obviously because of the effect of light. Further, the slight irregularity in the shape of the curves 1 and 6 can be explained by a difference in the light exposure periods which were 30 seconds and 15 minutes respectively. However, both curves suggest that light is effective only within the ranges of 10 - 25°C (curve 1) and 15 - 30°C (curve 6). The mean value of these ranges, 12.5 - 27.5°C, therefore, seems to be the normal temperature range of larval tabanids in so far as the percentage activity and rate of crawling under conditions of light are concerned.

Various workers (Shapely, 1924; Crozier and Stier, 1925.; Bodenheimer and Klien, 1930; Falconer, 1945; and Hafez, 1950), have analyzed their data on the rate of movement of several insects on the

Fig.19. Effect of temperature on the activity of larval C. furcata.
O% Activity of larvae recorded under a red light source.
●% Activity of larvae recorded under a white light source.

Fig. 20. Effect of temperature on the rate of movement of larval tabanids.

Speed cm./min. of larval C. mitis in darkness.
Speed   "   "   "   "   "   "   under a lateral light source.
Speed   "   "   " C. furcata in darkness.
Speed   "   "   "   "   "   "   under a lateral light source.
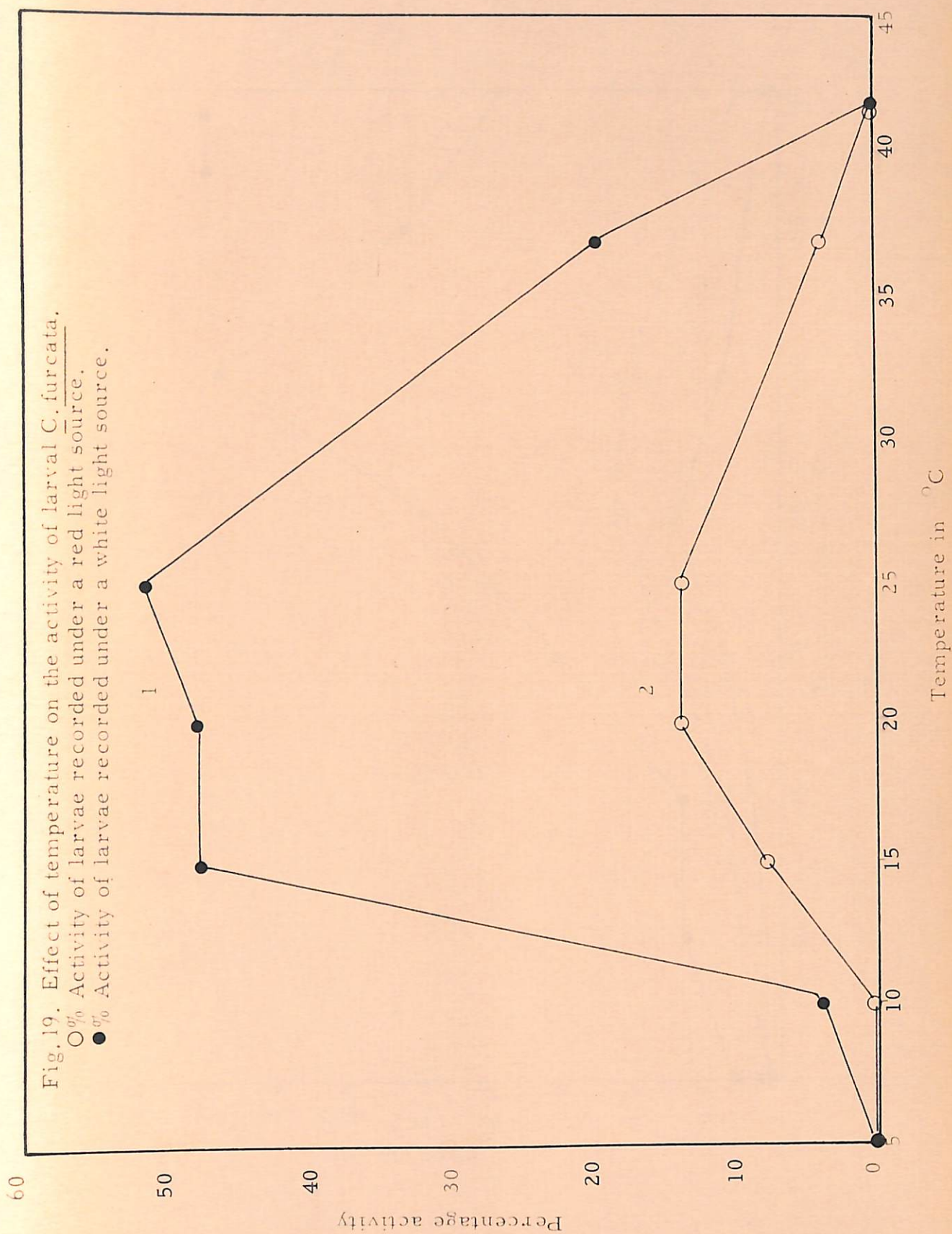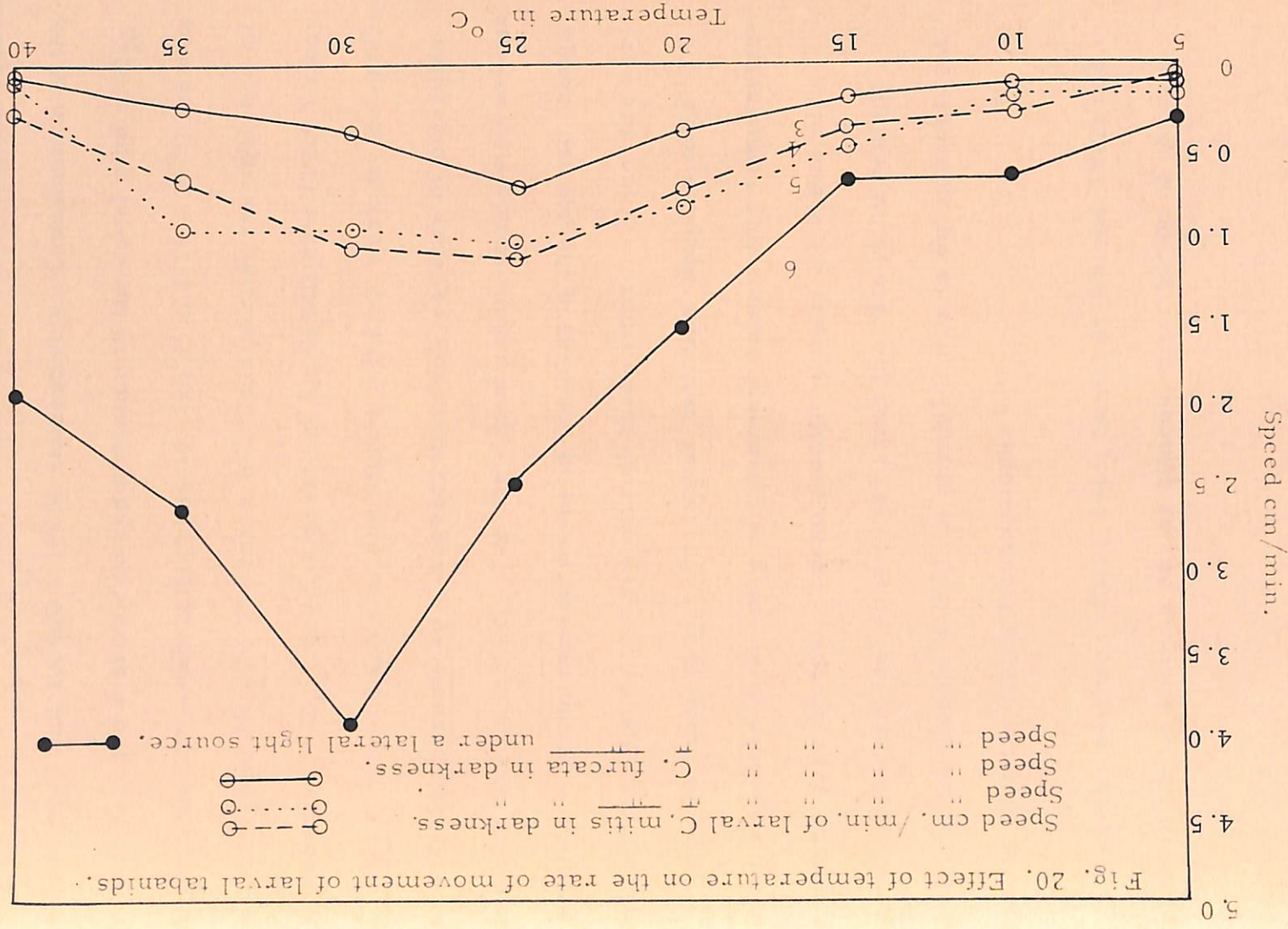
basis of the Q_{10} rule or the Arrhenius equation (Crozier, 1924). The values of Q_{10} and the critical increment (μ) for the Arrhenius equation are about 2 - 3 and 100000 - 18000 respectively. Examination of the curves 2, 3, 4 and 6 shows no resemblance to the usual type of Q_{10} or Arrhenius curve except between 10 - 15°C and 15 - 25°C (curves 2, 3, and 4) and 15 - 30°C (curve 6). This partial resemblance of the curves to the Q_{10} curve is possible since Miller's (1929) study on Lucilia larva and Crozier and Stier's (1925.) work on the cater-pillars of Malacosoma sp., suggest that the frequency of muscular contraction varies directly with the experimental temperatures but the amplitude of contraction waves is constant in the normal range of temperature and decreases outside these limits. Since the normal range of temperature of larval tabanids appears to be 15 - 30°C the decrease in the rate of movement outside this temperature zone seems quite logical. These results support the views of Uvarov (1931) and Mellanby (1939) that the rate of movement or activities of insects within the normal limits is not constant but increases with the rising temperature.

Several workers (Deal, 1941; Gunn and Walshe, 1942; and Lees, 1948) have shown a uni-or bimodal distribution of various

arthropods within a linear temperature-gradient with the largest

aggregation commonly occurring at the coldest end as a result of

trapping. The temperature-gradient results described in the

Section 4.3. show such trapping in an apparatus with a gradient

from 9.7-34.8º C. In another gradient from 17-31.5º C, the larval

aggregations at the two ends have been shown to occur chiefly due

to thigmo-taxis and not because of the ill-effects of cold or heat.

This view seems to be supported by the fact that in control experi-

ments (Fig. 18a) the 'end effects' were shown even during a short

period of 30 minutes. It seems that had the control experiments

been continued for a longer period all the larvae would have aggre-

gated at the two ends.

The larval aggregation in sections 6-7, which had a mean

temperature of 23º C (Fig. 18d) has been suggested as due to a

true temperature preference of the larvae. Since the various tempera-

ture results described in Section 4.3. suggest a number of normal

ranges of temperature for larval tabanids it was thought necessary to

calculate a 'temperature preference value' from the data obtained

with the two types of temperature-gradients. For such calculations,

the procedure recommended by Herter, (1953) was utilized. The num-

ber of larvae recorded from the extreme ends of the gradients were,

however, omitted from the calculations since we already know from

the control experiments that such larval positions were due to the 'end

effects'. Tables 23 and 24 include the values on preferred and maximum activity temperatures of larval tabanids under various sets of conditions. It will be seen that the maximum activity temperatures range from 21.86-28.4° C and that under laboratory conditions lateral light produces the most noticeable effect. The 'temperature preference' values vary little and range from 19.30-24.40° C. The calculated mean preferred temperature of 21.42 $\pm$ .81° C is in close agreement with the value, 23° C obtained from the temperature-gradient experiments (Fig. 18d). Thus 21.42 $\pm$ .81° C may be regarded as the preferred temperature of larval tabanids used in these experiments.

Table 23.

Temperature of maximum activity of larval tabanids under different conditions.

Maximum activity temperature in °C		Species and experimental conditions etc.
21.86$\pm$1.60 (cf. Table 20)	1.	C. furcata, % activity under a white light of 55 foot-candles; 20 days in the dark at 10°C prior to experimentation and recorded after 8 hours at constant temperatures.
22.45$\pm$2.88 (cf. Table 20)	2.	C. furcata, % activity under a red light of 2.5 foot-candles. Other conditions as in #1.
25.99$\pm$3.76 (cf. Table 21)	3.	C. mitis, Speed cm/min in the dark; 2 hours pretreated to experimental temperatures.
25.06$\pm$3.80 (cf. Table 21)	4.	C. mitis, Speed cm/min in the dark; 8 hours pretreated to experimental temperatures.
24.62$\pm$5.59 (cf. Table 22)	5.	C. furcata, Speed cm/min in the dark; 2 hours pretreated to experimental temperatures.
28.4$\pm$2.28 (cf. Table 22)	6.	C. furcata, Speed cm/min under a lateral light source; 2 hours pretreated to experimental temperatures.

Table 24.

'Preferred temperature' of larval C. mitis under different conditions.

'Preferred temperature' in °C	Species and experimental conditions etc.
1.* 24.40 ± .99	C. mitis, position records after 3 hours under a red light in the temperature-gradient (Fig. 17); storage temperature 5°C for 25 days; 1-2 hours pretreatment to 21°C prior to experimentation. Total # of position records 250 with 25 larvae in each experiment.
2. 22.58 ± .36 (cf. Fig. 18d)	C. mitis, position records under a uniform light intensity of 210 ± 5 F.C. from the moderate temperature-gradient; storage temperature 21.5 ± .5°C for 2 weeks.
3. 19.40 ± 1.10 (cf. Fig. 18b)	C. mitis, position records during 30 minutes in the steep temperature-gradient. Other conditions as in 2.
4. 19.30 ± .78 (cf. Fig. 18c)	C. mitis, conditions as in 3.

Mean 'preferred temperature' 21.42 ± .81

* The results of which are not included in Section 4.3.

5. GENERAL DISCUSSION AND CONCLUSIONS.

Although larval tabanids were exposed to simplified conditions of light, temperature, humidity, and moisture that do not occur separately in nature, the results obtained in the foregoing sections can be related to the ecology of the larvae in their normal environment.

The larvae exploit those habitats which are high in nutrient material and moisture. They pursue their activities in darkness at a depth not exceeding 15 cm. below the soil surface except perhaps during the winter months (Cameron, 1917 and 1926). Soil temperature data from Vauxhall (Oct. 1958 - Sept. 1959) and Fort Vermilion (Feb. 1959 - Jan. 1960), Alberta, included in Figures 20 and 21 show that larval tabanids are subject to extreme temperatures during the year. However, the mean maximum temperature for July from the surface to any depth of the soil down to 50 cm. does not exceed 23° C in either locations. Since the experimentally determined lethal temperature for larval tabanids is $37\text{-}40^{\circ}$ C, it must be concluded that extremely high temperature cannot be regarded as a factor of importance in limiting the distribution of larval tabanids in the soil, at least in the province of Alberta.

Concerning the minimum soil temperature it can be seen

Fig. 21. Soil temperature with time at various depths, at Vauxhall, Alberta, during October 1958 – September 1959. Data obtained from Dept. of Transport Meteorological Branch, Canada.

Surface
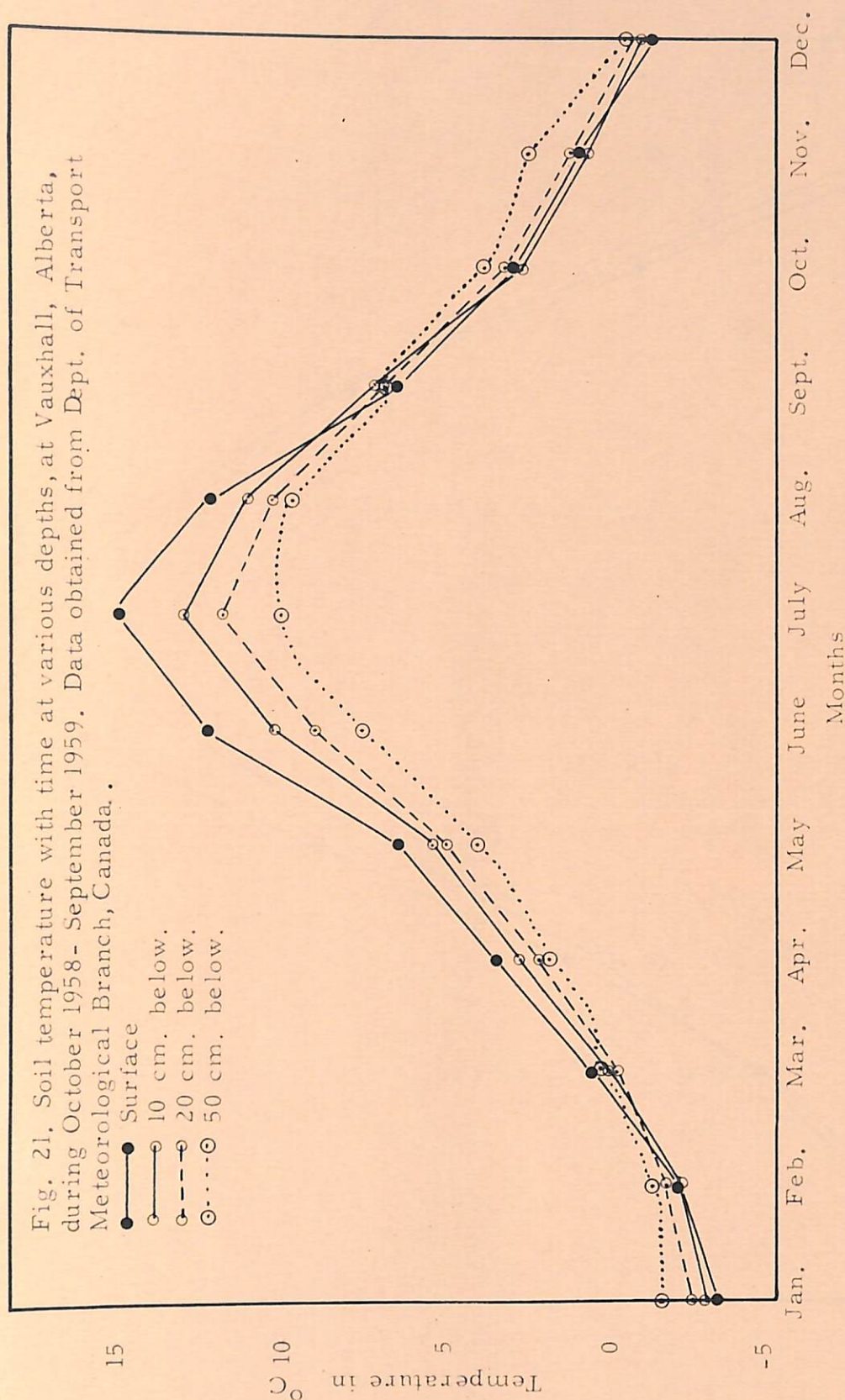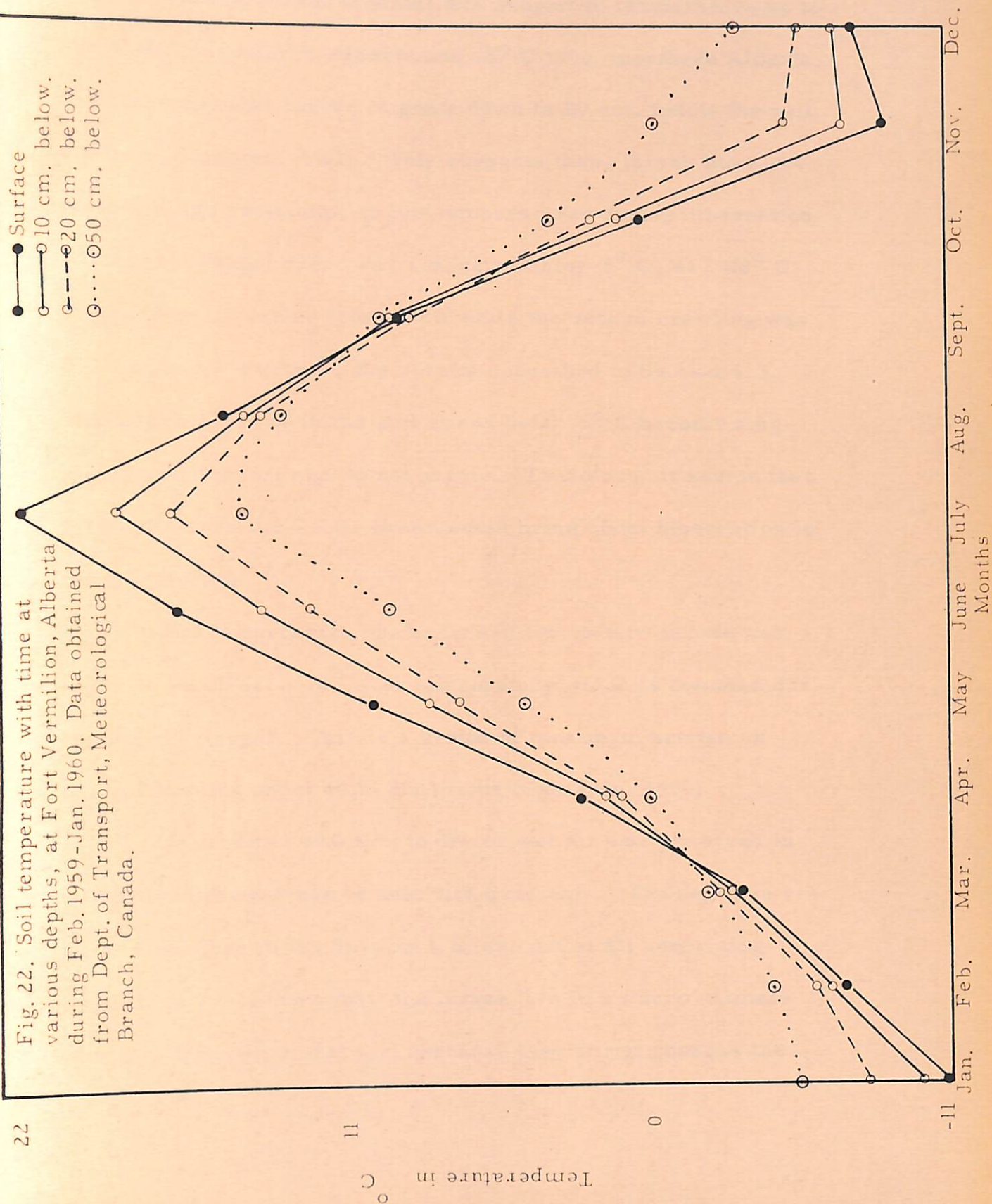○—● 10 cm. below.
⊙--⊙ 20 cm. below.
⊙····⊙ 50 cm. below.

Fig. 22. Soil temperature with time at various depths, at Fort Vermilion, Alberta during Feb.1959-Jan.1960. Data obtained from Dept. of Transport, Meteorological Branch, Canada.

that hibernating larval tabanids are subject to temperature up to
-3° C in   southern Alberta and -8° C in   northern Alberta,
if we assume that larvae migrate down to 20 cm. below the soil
surface (Cameron, 1917). This suggests then, larvae have ex-
tremely high resistance to low temperatures during hibernation.
Although no experiment was conducted below 5° C, at $5-10^\circ$ C
larval activity varied from 0-4% while the rate of crawling was
0.2 cm/min. Further, the results described in Section 4.3.
suggest that larvae in the soil at and below 5° C become slug-
gish and quiescent and do not pupate. Therefore, it seems that
a temperature of 5° C or lower would bring about hibernation in
larval tabanids.

The temperature preference of the mature larvae was
found to be $21.42 \pm .81^\circ$ C, a temperature which is common dur-
ing June - August. This is a period of maximum activity of
larval tabanids under field conditions (Cameron, 1926).

No directed reaction to dry or wet air was observed in
several different types of humidity-gradients. The negative re-
sults described in the Section 3.6. are not at all surprising
since it is well known that the larvae live in a micro-climate
which is typically moist and perhaps, they do not possess the

ability of hygro-tactic orientation. This statement then leads

us to assume that larvae show no preference for wet habitats

even in the nature. In fact, their presence in moist habitats

can be explained as a result of direct selection of such plates by

the ovipositing female tabanids.

A consistent inactivity under low percentage of R. H.

and moisture is shown by larval tabanids. Now, such a reac-

tion under natural conditions seems especially important for

aestivating larvae in which inactivity would be induced by the

dryness of the environment and greater activity by the increas-

ing moisture conditions of the soil. Variable activity of this

nature dependent on moisture percentage has been reported in

many soil insects (Uvarov, 1931).

                                    burrowing
Very few soil/insects are other than negatively photo-

tactic (Cameron, 1917). As described earlier larval tabanids

react to light either by crawling or by suddenly withdrawing

the head capsule. A general sensitivity to light resulting in

motor response would keep the larvae buried in the soil and

consequently protect them against wandering into illuminated

areas where they would be exposed to predators and desi-

ccation. This is perhaps the reason why even the pupae are

found covered under leaves or vegetation. This leads us

to believe then that a light sense of some kind is a practical necess-
ity to larval tabanids.

Reaction time experiments (Section 2.4.) have shown that
larvae are able to integrate light energy over periods of seconds and
to utilize the effect to produce a directional response. Several eye-
less forms have been shown to possess this ability (North, 1957).
The mechanism of integration of light might be of advantage to a
larva in the dark environment especially when it is migrating to the
soil surface for pupation.

On the basis of the laboratory findings it seems impossible
to assess the relative contribution made by each of the foregoing
physical factors to general behaviour of the larva in its normal habitat.
But it is highly probable that in swamps, pools and lakes, where
there is no risk of desiccation, light and temperature are the most
important environmental factors influencing the behaviour of the
larvae.

Variation in response of larval tabanids to physical factors
had been a common feature in the present study. It was not possi-
ble to use laboratory reared larvae since no suitable means of rear-
ing larvae from the eggs are known. Although it was possible to
collect larvae in adequate numbers, standardization of larvae for
testing in the laboratory was a problem. The response of larvae to

light varied somewhat with age (Section 2.9.); for example, immature ones may be indifferent to lateral light, mature ones show intense negative photo-taxis, whereas those which are about to pupate are more light tolerant. It was, therefore, possible to select larvae of almost uniform sensitivity for light experiments as described in Sections 2.4. and 2.9. But even in these experiments individual differences in efficiency of orientation, amount of activity and reaction time were common. Perhaps, such differences are simply reflections of deeper physiological differences among individuals as suggested by Wellington (1957).

Screening methods were not applicable in humidity, moisture, and temperature experiments since no directed reaction was obtained for these conditions on which to base the standardization test. Thus larvae which showed activity under normal laboratory conditions were used. The source of error in these experiments, besides the individual differences, could be due to age differences as well as to an uncontrollable factor of thigmo-taxis largely contributed by the shape of the humidity and temperature-gradient apparatuses. Collisions between individuals, when large numbers were used, also contributed to some error.

Species difference, in regard to the results of humidity and temperature experiments, were consistent throughout between C. furcata and C. mitis. Such variations may be explained as due to the

difference in size of the two species or to their habitat differences. For example, C. mitis is larger than C. furcata and is more active under normal laboratory conditions.

CONCLUSIONS

A. Light.

1. The illumination of larval tabanids produces general locomotory activity which always begins with a 'retraction reflex' of the head capsule. The reaction time is inversely proportional to the intensity of stimulus. The time interval between the 'retraction reflex' and crawling is an effective factor in the mechanism of klinotactic orientation of the larva in the regions of low intensities of dorsal light-gradients.

2. The anterior region of the larval head capsule is highly sensitive to light. A pair of eye-spots are suggested as the light-sensitive organs.

3. Larval C. furcata in a dark-light choice-chamber at six different light intensities were strongly photonegative.

4. The data on light intensity versus speed cm/min of larval tabanids have been analyzed in accordance with the Weber-Fechner Law, but no definite relationship between speed and intensity could be obtained.

5. The larvae do not move at random in a dorsal light-gradient. The mechanism of orientation involves a combination of ortho-

kinesis and klino-taxis.

6. The degree of photosensitivity in respect to lateral light stimulation has been shown to vary with the age of the larva. The process of orientation under a lateral light source is mainly achieved by a klino-tactic response.

B. Moisture.

1. Larval tabanids under conditions of low percentage moisture show an abnormal behaviour pattern which constitutes contraction, distinct elevation of the head capsule, rolling, and lack of crawling and burrowing movements. The amount of activity and the rate of movement of larvae increase with increasing percentage of moisture and at the same time the mean period of inactivity decreases. This reaction has been suggested as an example of ortho-kinesis. The significance of such a reaction, however, in terms of behaviour under natural conditions is open to question.

2. In the various types of choice-chamber apparatuses larvae do not show preference for dry or the wet side.

3. The mean water content of larval C. mitis is determined as 79.5%. Desiccation soon begins to cause the mortality of larvae and death occurs in about 8 hours.

C. Temperature.

1. The extent of activity shown by larvae is not a simple function of temperature alone but is considerably affected by light.

Between 15-25° C the rate of movement of larvae is directly proportional to temperature.

2.   In a temperature-gradient apparatus of 9.7-34.8° C, C. mitis larvae aggregate around 9.7° C largely due to the trapping effects of cold. In another gradient from 17-31.5° C two larval aggregations at 17 and 31.5° C have been shown to occur owing to thigmo-taxis and a third aggregation at 23° C due to the temperature preference of larvae. A value of 21.42 ± .81° C calculated from the data obtained with the two types of temperature-gradients was found in close agreement with the experimentally determined temperature preference value of 23° C and has been suggested as the 'preferred temperature' of larval C. mitis.

3.   The mechanism by which larvae react in a steep temperature-gradient (9.7-34.8° C) at the hot end is that of the nature of a 'shock reaction' suggesting stages of distress. They avoid the hot end by reversing their direction of movement in a 'trial and error' fashion. Avoiding reactions of larvae were apparently absent in the temperature-gradient of 17-31.5° C.

4.   The temperature range of 37-40° C is shown to be lethal to larval C. mitis and C. furcata.

D. General Discussion.

1. Results on the three physical factors in relation to the general behaviour of the larvae under field conditions have been discussed and light and temperature are shown as the most important environmental factors.

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