BEHAVIOUR OF LARVAL TABANIDS (DIPTERA : TABANIDAE) IN RELATION TO LIGHT, MOISTURE, AND TEMPERATURE

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The behaviour of larval Tabanus reinwardtii *Wied.,* Chrysops furcata *Walk., and* Chrysops mitis *O. S., in relation to light, moisture and temperature was studied. Rate of movement, aggregation, and localized movements of the head capsule were used as criteria for analyzing larval behaviour. The anterior region of the larval head capsule is sensitive to light; a pair of eye spots on the head capsule is suggested as the photoreceptors. On illumination larvae are able to integrate light energy over periods of seconds and to utilize this to produce a directional response. Larval* C. furcata *and* C. mitis *show no preference for the dry or the wet side in various humidity gradients. However, they show abnormal behaviour on a uniformly dry substratum. The mean water content of* C. mitis *larvae is 79.5% and the effects of desiccation on survival are discussed. The reactions of* C. furcata *and* C. mitis *larvae in uniform temperatures and in temperature gradients are described. The speed of movement and time percentage of activity, though affected by temperature, are shown to be more affected by light. 21.4–0.8C is suggested as the 'preferred temperature' of larval* C. mitis, *37 – 40C is lethal to the larvae. Light and temperature are the most important environmental factors.*

Cameron (1917) is the only investigator who has noted that larval tabanids, like other soil insects, are negatively phototactic. 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 the light, moisture, and thermal 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.

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 *Chrysops mitis* O.S. and *Tabanus reinwardtii* Wied. but *Chrysops furcata* Walk. were obtained by turning over the soil with a garden fork. The first collection was made on October 7, 1958 at Winterburn swamp, 8 miles west of Edmonton. The vegetation consisted chiefly of sphagnum moss and sedges, marsh cinquefoil, spruce, larch, Canada blue grass and marsh reed grass. Larval *C. furcata* were collected on the west banks of the pools. Pupating larvae were found as a rule 1 - 2 inches below the surface at the pool's edge. Small larvae were found deeper in the soil and often 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 a shallow layer of organic matter and interspersed

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 in water. The vegetation bordering the stream banks was sparse. Only the Raymond soil where larval *T. reinwardtii* were found, was acidic. Organic matter content was high, an average of 69% for the Winterburn soil and 42.5% for Raymond, Vauxhall and Waterton soils.

Maintenance of Stocks

Larval stocks in the laboratory were maintained as recommended by Shemanchuk. Larvae were stored in 3 x 1 inch plastic vials 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* was 7 days. For *C. furcata* it was 11 days, based on 3 females and 7 males.

Larvae of *Tabanus* sp., occasionally struck each other when placed together in a dish. However, cannibalism was never observed. Larvae of *Chrysops* spp., showed less 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* spp.

Mortality during maintenance of larval stocks ranged up to 40% chiefly due to fungus growth, a nematode identified by Dr. H. E. Welch, Belleville, Ontario as *Bathymermis* sp. (Shamsuddin 1966) and inadequate ventilation. Few deaths occurred when the soil was changed once every 3 months and when the storage vials were provided with perforated caps to ensure proper ventilation.

BEHAVIOUR OF LARVAL TABANIDS IN RELATION TO LIGHT

The effect of light on the rate of locomotion of eyeless forms has been less studied than orientation to light. Welsh (1932, 1933), working with *Unionicola* (Arachnida), concluded that in a light sensitive organism the extent of muscular activity bears a definite relationship to the intensity of illumination. Duggar (1936), Jones (1955), and Millott (1957) give good summaries of photokinesis in eyeless forms of insects, 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.).

Further information is included in the works of Holmes (1905), Patten (1914, 1915, 1916), Loeb (1918), Crozier (1927), Mitchell and Crozier (1928), Ellsworth (1933), Fraenkel and Gunn (1940), Bolwig (1946), and Hafez (1950, 1953) on the photonegative responses of muscoid

larvae. There is a general agreement that fly larvae behave photonegatively and that their mechanism of orientation to light represents typical klinotaxis (Carthy 1958). However, a wide diversity of opinion prevailed for a long time as to the true nature of photoreceptors 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 histological 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 regarded as photoreceptors are gustatory. The photoreceptors of housefly larva were finally identified by Bolwig (1946) as two small groups of sense cells, situated one 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, 1957, and Millott 1957). Such a method has also been used for investigation of dermal photosensitivity of forms ranging from invertebrates, through lower chordates to vertebrates. Localization of sensitivity is, however, too ill defined and an electrophysiological demonstration of photosensitivity is still needed.

Experimental Methods

Experiments were done from May 1, 1959 to January 4, 1960 in a dark room at a temperature of 23.3 ± 2.8 C. Two glass plates, 35 x 70 cm and 45 x 60 cm 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. High light intensities were obtained from 100 - 150 watt electric bulbs enclosed in a light tight box. The difficulty of observing in the dark room was overcome by using a photographic safe light which, according to the manufacturers, transmitted wave lengths beyond 580 mμ.

Mature larvae of *Tabanus reinwardtii* and *Chrysops furcata* 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 glass was treated with a water suspension of talc on which the larva crawled leaving a trail behind it. During each experiment, time intervals were marked with a wax pencil. The tracks were measured with a map measurer.

Preliminary Studies

Activity in the dark

One hundred and eighteen larvae in separate glass vials containing either clean moist sand or tap water were watched under the red light singly for 3 minutes each. About 86% of the larvae showed activity char-

acterised by flexing of the body and occasional crawling. Such activities could be easily mistaken for responses 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 crawled; however, a response characteristic of behaviour under the experimental white light i. e., withdrawal of the head capsule into the cephalic collar preparatory to crawling, was never shown. When 34 animals were put singly on the glass plate, each for 5 minutes, all responded by crawling. A mean speed of 1.6 ± 1.1 cm/min was recorded for these larvae. This is taken as a basal rate of movement of larvae in the dark.

Response 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. Hence there are measurable 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 referred to as the 'crawling time'.

Reaction Time and Crawling Time

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 experimental trough always contained at least 50 cc of tap water and a rectangular blotting paper matting 19 x 14 cm. A glass vial containing one *C. furcata* 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.

The results obtained with 3 different groups of larvae are summarized in fig. 1. 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. At high intensities the reaction time approaches a minimum and is little affected by great increases in intensity. The reaction time of each group varies inversely with the logarithm of the intensity.

The crawling time for a group of 5 larvae tested 10 times each is also given in fig. 1. Considerable variation was shown by individual

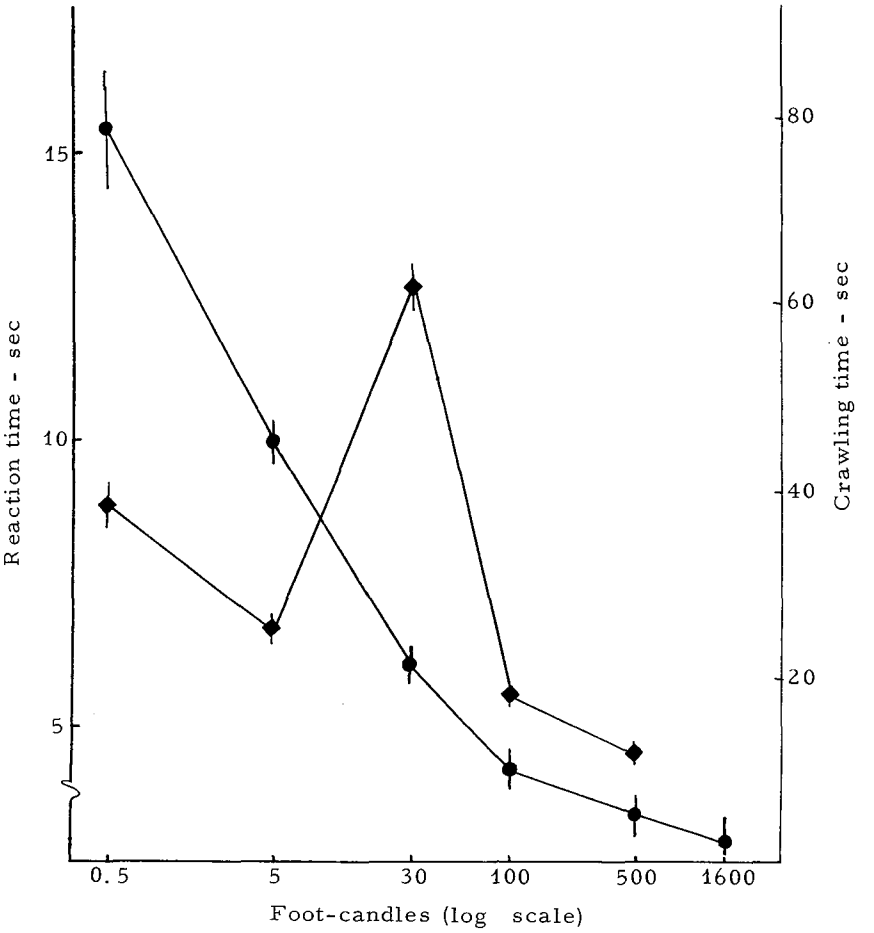

Fig. 1. The relation between reaction time (●155 readings with 70 larvae) and crawling time (◆50 readings with 5 larvae) and light intensity for *C. furcata*. Lines indicate standard errors.

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. However, apart from the anomalous data at 30 foot-candles, crawling time decreases with increasing light intensity.

Photoreceptive Organs

Structure

Preliminary experiments indicated that larval tabanids have a light sense located in the eye spots. Gross dissections, injection of vital stains, and serial sectioning all failed to reveal nerve connections to these although gross concentrations of pigment were found.

Experimental

A 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 precise diameter of light spot.

Twenty mature larvae of *C. furcata* were chilled for one minute and examined with a 1 mm diameter pencil of light. Responses were obtained as follows: 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 described as eye spots in the larva of *Haematopota pluvialis* L. (Tabanidae) by Cameron in 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 extreme mobility of the head capsule. However, whenever this was achieved, it caused violent head movements of the larva. 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*. Fig. 2 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 response varied from none to incomplete withdrawal of the head capsule under local illumination with a 1 mm light spot. But under a 10 mm light spot, the reaction was obvious in all the larvae. When the painted areas were washed and the tests repeated 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. Ten such operations were done; 3 died immediately but the remaining 7 were in healthy conditions for several months. Reaction times for these were recorded one day after the operation, under both local and general illumination. The mean reaction time at 100 foot-candles was 28.7 sec, much higher than any of the values of fig. 1. Thus although the removal of the anterior tip does not alter the character of the 'retraction reflex', it does increase the reaction time considerably.

The results of these 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 anterior tip suggests that photosensitivity is spread throughout the anterior region of the

head capsule. 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.

Reactions in a Dark-Light Choice Chamber

Two petri dish lids with a diameter of 15 cm, were placed one upon the other with the 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. The results are summarized in table 1. The intensity of light reaction is expressed as 100 (D-L)/N (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.

TABLE 1. Intensity of light reaction of larval *Chrysops furcata* Walk. in a choice chamber of darkness and light; mean of 5 experiments with the same 50 specimens at each light intensity, temperature 23.3 ± 2.7° C.

Light intensity in foot-candles	Mean intensity of reaction, 100 (D-L)/N after 15 min.	SD	SE
0.25	57.4	12.3	5.5
0.50	47.3	16.3	7.3
1.00	63.2	29.0	12.9
2.00	52.4	25.8	11.5
5.00	70.3	26.8	11.9
10.00	71.3	17.3	7.7

At room temperature the larvae of *C. furcata* behave photonegatively at all the intensities used. The intensity of reaction, however, does not show a regular increase with increase in light intensity.

Intensity of Illumination X Speed of Crawling

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). A series of measurements was 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 center of the glass plate. The light was switched on 30 seconds later. Each larva was allowed to crawl for 5 minutes, but

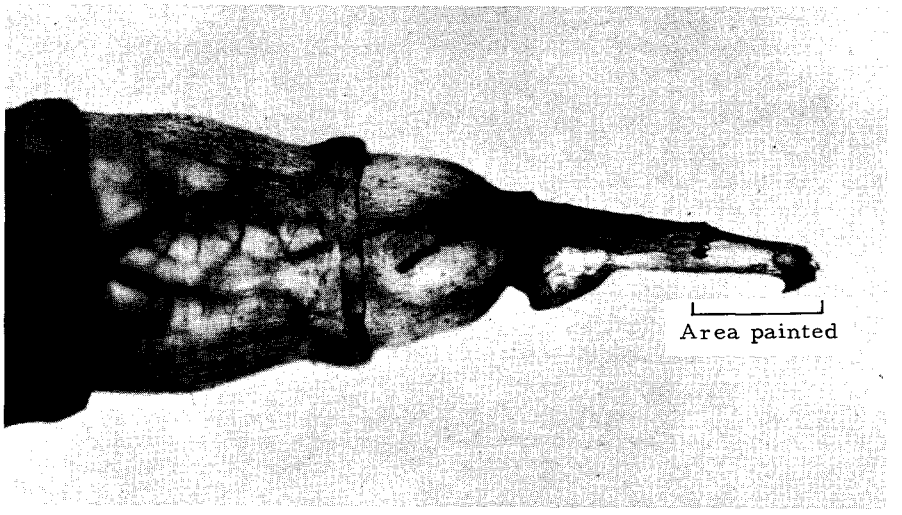

Fig. 2. Photomicrograph of the cephalic segments with the head capsule projected out, showing the area painted. Larva of *C. furcata* Walk.

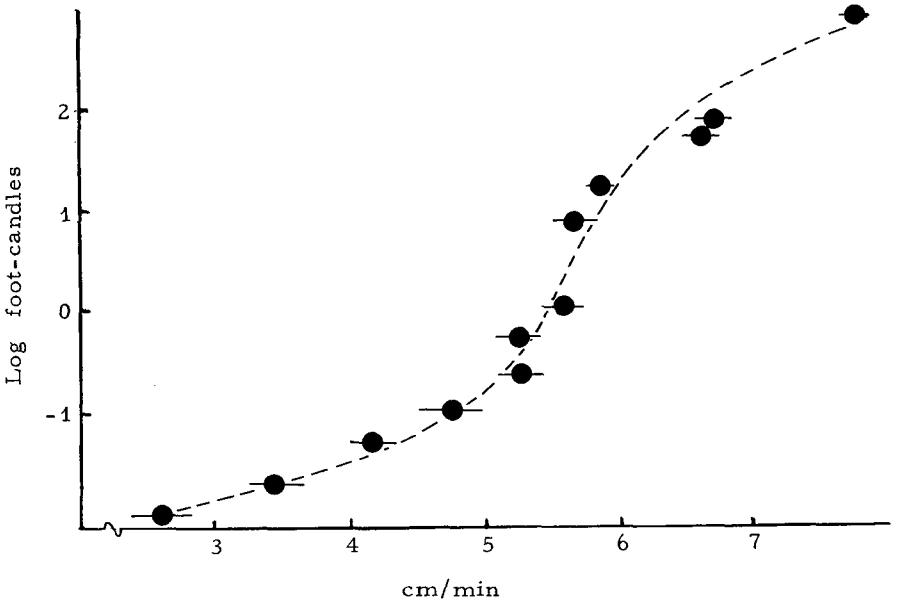

Fig. 3. Effect of light intensity on the speed of movement of larval *C. furcata*. Lines indicate standard errors.

only the track of the middle 3 minutes was measured. The average speed in cm/min was recorded. The results are summarised in fig. 3. The curve is sigmoid, but there is close agreement with the Weber-Fechner law (Patten 1915) in the range of 10 - 500 foot-candles.

Behaviour in a Light-Gradient

The construction of the light-gradient apparatus was fundamentally similar to the 'non-directional' gradient described by Ullyott (1936). A steep gradient was arranged with a glass diffusing plate and a more moderate gradient with a graded film in an enlarger. The former ranged from 150 to nearly zero foot-candles over 30 cm, the latter from about 50 foot-candles to zero over the same distance. Each gradient was oriented with the high intensity to the east.

A larva was placed in the center of the experimental plate 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. Fifty 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 edge of the glass.

The results obtained with 50 larvae are shown in table 2. 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 Yates' correction for small numbers was used for these data. The purpose was to assess the probability of any one direction being chosen by the larvae in the light-gradient. The chi-square value obtained for the 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, that is down the light intensity 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.

Lateral Light Stimulation

Reactions to two balanced lateral sources

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 photonegative reaction in a horizontal beam of light, it was thought necessary to check their reaction under balanced illumination.

TABLE 2. The directions taken by 40 larval *Chrysops furcata* Walk. at the end of 5 minutes in light-gradients. Each larva was placed at the center, 15 cm from the maximum intensity.

	Min. light							Max. light
STEEP GRADIENT	W	NW	SW	N	S	NE	SE	E
Observed	12	9	5	5	3	6	5	5
Observed minus random (O-R)	+5.75	+2.25	-1.25	-1.25	-3.25	-0.25	-1.25	-1.25
								$X^2 = 9.52$
MODERATE GRADIENT								
Observed	15	8	3	5	4	6	5	4
Observed minus random (O-R)	+8.75	+1.75	-3.25	-1.25	-2.25	-0.25	-1.25	-2.25
								$X^2 = 17.12$

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 centers of 3 sides of a 54 x 54 cm wooden board with the apertures facing the center. Fourteen larvae of *C. furcata* were used. With the lights switched off each larva was put on the center of a 23 cm diameter glass plate so that the axis of its body was at right angles to the line joining the balanced lights with its head pointing away from the unbalanced light. The balanced lights were then switched on simultaneously, and the course of movement was recorded. The unbalanced light remained off in these experiments.

The average direction of several courses taken by each of the 14 larvae in 74 trails is represented in fig. 4. The general pattern in taking course 1 (straight ahead) 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 or other of the

balanced lights.

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

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

Preliminary experiments were done to select larvae of uniform sensitivity as described by Patten (1916). Nine mature and eight 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 beam of light were selected and used in these experiments.

The arrangement of the lights was the same as before but the larvae were placed with heads pointing away from one of the balanced lights. The left or right balanced light was kept on until larvae reached close to the center of the circular glass plate. The direction of the incident light was then changed through 90° by switching on the unbalanced light instead. Each larva was allowed to crawl through twice, once under the influence of the left light and once under the influence of the right. The tracks were traced. The total change in direction of travel in 3 cm from the point at which the light was changed was measured as shown in fig. 5, by means of a protractor. Thus the average angular deflection of two trails was recorded as the response of a larva to the change of 90° in the direction of illumination.

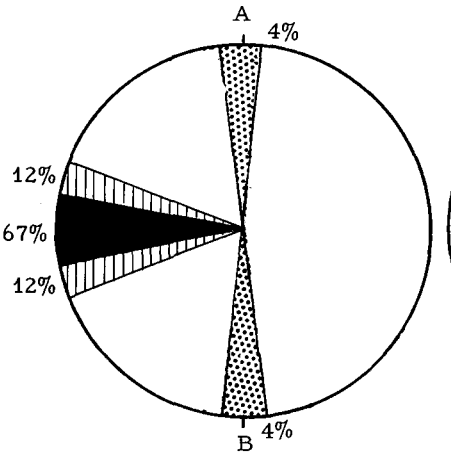

Fig. 4. The directions of movement of larvae in two beams of light from equal and opposite sources A and B.

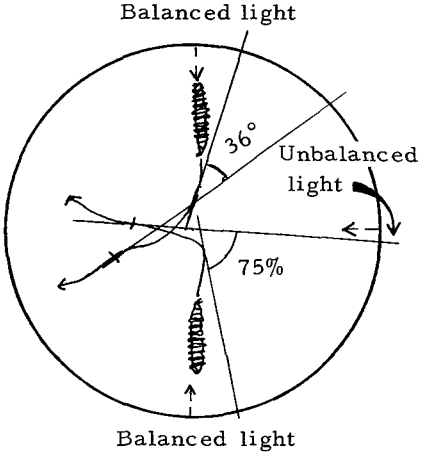

Fig. 5. Two trails of larva no. 9. The starting (balanced) light was switched off and the unbalanced light on when the animal reached close to the centre.

The results are shown in table 3. 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 51.5 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 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).

TABLE 3. The angular deflection measured in degrees of 17 larvae of *Chrysops turcata* Walk. subjected to a change of 90 degrees in the direction of light. Averages of a right and left pair of trails in fig. 5.

	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°

Discussion

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 widely 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 different intensities formed a hyperbolic relationship when plotted against the

logarithm of light intensity (fig. 1). This is roughly in agreement with the Bunsen-Roscoe Law of reciprocity (Steven 1950). 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. 3) in the low intensity region agree with these views. The deviations in the entire range may be due to 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 that the Weber-Fechner law cannot describe responses in which several steps intervene between the stimulus and the response. Since locomotion in the larval tabanids represents a complex or integrated response which is brought about by two different reactions as shown earlier, the whole process cannot be properly described by this law.

In the choice chamber experiments, the larvae tended to aggregate around the wall of the chamber. The contact produced a slowing down of locomotion and also affected the direction of this. This means that although larval *Chrysops* are photonegative in the choice chamber, their behaviour to light is subject to interference by the uncontrollable factor of thigmotaxis. This may be why the intensity of reaction (table 1) in the choice chamber was not directly proportional to the logarithm of the light intensity.

The behaviour in dorsal light gradients suggests that larvae react orthokinetically in the higher intensity zone. But at lower intensities they seem to react klinotactically. 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 table 2 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 orthokinesis and klinotaxis.

The experiments on lateral light stimulation show that larval tabanids, like *Musca*, *Calliphora*, and *Lucilia* larvae (Fraenkel and Gunn 1940), display a photonegative reaction in a horizontal beam of light. The orientation away from the light source is chiefly attained by klinotaxis.

These findings suggest that a negative phototaxis coupled with photoorthokinesis 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 phototaxis would add an orienting factor making the return to soil more rapid. Light reactions can, therefore, explain the apparent absence of larvae from the surface of the soil. Changes in them with age may also explain why larvae are found in different situations as they mature.

REACTIONS OF LARVAL TABANIDS TO MOISTURE

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. 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.

Experimental Methods

In most experiments the choice chamber method as described by Gunn and Kennedy (1936), Wigglesworth (1941), and Lees (1943) was used.

Three different types of humidity chambers were employed. Type 1 consisted of a cylindrical glass vessel, 15 cm in diameter and 6 cm deep. This was closed by a glass plate with a sealable 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 into 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. This was used as a constant humidity chamber by removing the partition from the glass roof and the petri dish. Type 2 consisted of two petri dish lids, 10 cm in diameter and 1 cm deep, placed one upon the other with their well-ground 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 was close to the theoretical value for the sulfuric - acid - water mixture used.

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 upper half of the chamber. 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 map measurer and the average speed in cm/min was recorded.

For determining specific differences in activity of 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, 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, Syrjämäki 1960).

Choice 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 due to light, the chambers were turned through 180° halfway through each 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 (W - D)/(W + D) (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.

Experiments on the moisture of the substratum were carried out in the search for 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 as: 100 x (wet weight - dry weight)/ wet weight. 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) Number of wall climbings; (4) Number of burrowings; (5) Number of head capsule elevations; (6) Number of rollings.

Preliminary Experiments

In experiments with the constant humidity chambers both *C. furcata* and *C. mitis* show distinct reactions to low and high R.H. For example, in 50% R.H. and below the larvae remained strongly contracted for periods 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 was least in the range of 0 - 40% R.H.

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 chamber 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 klinokinesis or klinotaxis.

A few experiments were carried out in 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*.

Variation in Activity and Rate of Movement with Humidity

Variation between species

The results obtained from data on 100 larval *C. mitis* are summarized in table 4. It can be seen that the activity increases as the relative humidity approaches 100%. This is in fair agreement with the observations made in preliminary experiments where the marked contraction of larvae at low humidities was noted.

TABLE 4. Activity of larval *Chrysops* in uniform humidities. The percentage of larvae active at various times. *C. mitis* at 25 C in type 1 chamber; *C. furcata* at 25.6 ± 1.2 C, in type 2 chamber; both at 85 foot-candles.

	Time in minutes from placement of larvae in the chamber								
	C. mitis (100)	*C. furcata* (6 x 10)							
	15	0	10	15	20	30	40	50	60
% R.H.	Percentage of larvae active								
0	43	17	11	6	11	8	7	7	4
10	42	20	21	19	18	16	7	7	11
30	38	22	18	21	20	17	22	19	14
60	70	29	27	27	27	28	28	31	30
90	82	37	37	37	39	40	36	38	38
100	-	25	35	42	38	45	34	35	41

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 4, 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 two species which are summarized in table 4 differed in only one respect e. g., the types of the humidity chambers used. However, the percentage activity in the range of 0 - 90% R. H. for *C. mitis* is always found higher than for *C. furcata*.

Variation in crawling rate with humidity

The results are shown in table 5. 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 5. 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 speed of locomotion or the frequency of activity depends on the intensity of stimulation.

TABLE 5. The speed of movement of *Chrysops furcata* and periods of inactivity in uniform humidities in type 2 chambers at 85 foot-candles.

15 larvae at 27.1 ± 1.6 C

% R. H.	Mean speed cm/min	SE	Mean period of inactivity	SE
10	0.55	0.14	6.7	1.6
40	0.61	0.18	7.2	1.4
80	1.20	0.30	4.1	1.3
90	1.24	0.20	2.9	1.0
100	1.64	0.31	1.7	0.6

5 larvae at 26.4 ± 1.1 C

% R. H.	Mean speed cm/min	SE
10	0.16	0.15
40	0.21	0.19
80	1.03	0.47
90	0.86	0.29
100	1.05	0.34

Rate of Moisture Loss

Figure 6 shows the percentage loss of weight in *C. mitis* in successive hours during desiccation in dry air at 21C. The weight of two batches showed almost no further change after desiccation for 18 hours. Accor-

ding to this the mean water content of larval *C. mitis* is 79.5%.

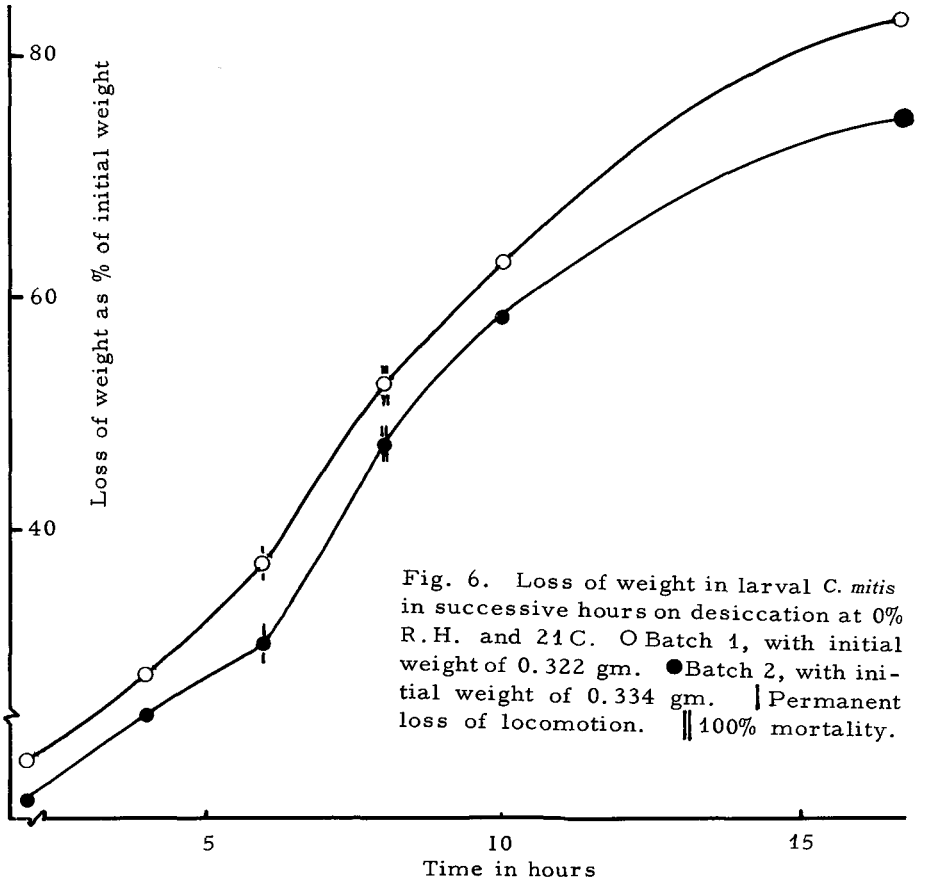

Fig. 6. Loss of weight in larval *C. mitis* in successive hours on desiccation at 0% R.H. and 21 C. O Batch 1, with initial weight of 0.322 gm. ●Batch 2, with initial weight of 0.334 gm. | Permanent loss of locomotion. ‖ 100% mortality.

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 8 hours (average weight loss 49.5%) all the larvae were dead. The rate of water loss follows a sigmoid progress and the results confirm the preliminary observations that larvae cannot survive dry conditions for more than 13 hours.

Reactions in Humidity Choice Chambers

The results of 12 experiments are shown in table 6. 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 the wet side, 29 from the dry side and the remaining 10 position records were 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.

TABLE 6. Reactions of larval *Chrysops* to alternative relative humidities.

% R.H.		Position Records			Intensity of Reaction as:	
High	Low	Wet	Dry	Middle	Excess%	Ratio W/D
C. furcata, means of 5 experiments with 100 larvae at 25.6 ± 0.6 C, type 1 chamber, 85 foot-candles.						
100	90	53	38	9	16.5	1.4
100	80	44	47	9	-3.3	0.9
90	70	53	44	3	9.3	1.2
90	60	52	37	11	16.9	1.4
90	40	56	36	8	21.7	1.6
* 90	30	61, 54, 43	29, 36, 50	10, 10, 7	35.5, 20, -7.5	2.1, 1.5, 0.9
80	30	54	40	6	14.9	1.4
70	30	54	36	10	20.0	1.5
50	30	47	41	12	6.8	1.1
50	20	38	45	17	-8.4	0.8
50	10	44	51	5	-7.4	0.9
20	10	47	47	6	-1.1	1.0
C. mitis, means of 10 experiments with 50 larvae at 26.1 ± 0.6 C, type 3 chamber, red light.						
100	90	19	23	8	-9.5	0.8
100	80	27	21	2	12.5	1.3
90	70	18	28	4	-21.7	0.6
90	60	19	18	13	2.7	1.1
90	40	19	20	11	-2.6	0.9
90	30	22	21	7	2.3	1.0
80	30	22	25	3	-6.4	0.9
70	30	19	18	13	2.7	1.1
50	30	25	20	5	11.1	1.3
50	20	24	23	3	2.1	1.0
50	10	22	20	8	4.8	1.1
20	10	23	11	16	35.3	2.0

* 90 : 30 experiment repeated and again repeated in the dark.

To check the above results it was thought necessary to use type 3 humidity choice chambers 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 frequency of larvae encountering the humidity boundary (Wellington 1960). Further, in these experiments, light as a variable factor was controlled by covering the humidity chambers with a piece of black cloth and the 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 also summarized in table 6. The average mean excess percentage of all controls was 1.3 which approximated the theoretical value for no reaction. 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 another set of experiments was conducted with the type 1 chamber in the hope of repeating the significant reaction in 90 : 30% R.H. The results of these experiments are also given in table 6. The data show that there is hardly any reaction over three hours in the dark. Clearly, larval *C. furcata* and *C. mitis* show only a rather slight tendency to collect on the moister side.

Reactions to the Moisture Content of the Substratum

The results are included in table 7, and show that larvae move faster with increasing moisture. The tendency to burrow and to climb the wall of the container 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 appears to be due to physiological instability, perhaps caused by loss of water through evaporation. These results are consistent with those obtained in experiments with constant humidity chambers, where larvae seldom showed any rapid crawling or burrowing movement under low humidities.

Discussion

Most of the experiments were designed to find out whether there were any klinokinetic, klinotactic, or orthokinetic reactions of larval tabanids to moisture. No information on klinokinetic and klinotactic responses was obtained. However, 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, a true orthokinesis is a major part of the orientation mechanism which would bring about aggregation of larval *C. mitis* and *C. furcata* in a dry place if the results are to be interpreted in the conventional way. Such an explanation is 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 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 of substrate are obviously produced by the injurious effects of dry air. It follows that the hygro orthokinesis

could not possibly bring about larval aggregation in dry places. Now, moist soil is the preferred habitat of tabanid larvae and while the laboratory conditions included moisture, the soil with its associated factors (thigmotactic, textural, food, etc.) were absent. Therefore, the moist substrate in the laboratory experiments would tend to produce an orthokinetic reaction, since in moist soil the larvae are relatively inactive. Possibly then, the larvae are stimulated under moist conditions to react orthokinetically until the ideal conditions of moist soil are reached. Although the significance of such an orthokinetic 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 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.

TABLE 7. 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
2.3	1.99 ± 0.44	0.13
12.0	1.95 ± 0.48	0.15
24.4	2.32 ± 0.94	0.29
41.5	3.90 ± 1.10	0.34
77.5	4.80 ± 1.50	0.47

The experiments on desiccation show that this is a real peril to larval tabanids. However, the initial contraction of the larval body wall in response to a continuous stimulus of low R.H. percentage seems to control water loss at least for a short time. 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 and Suomalainen 1945) that most of the loss of weight of an arthropod on desiccation is due to evaporation of water from the integument.

TEMPERATURE REACTIONS OF LARVAL TABANIDS

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 *Aedes aegypti* were subjected to a temperature gradient of 8 - 42 C, most larvae aggregated over the range of temperature 23 - 32 C. Third and fourth instar larvae and pupae showed a preference for 28 - 32 C. Literature pertinent to behavioural work with aquatic insects or forms living in semi-fluid media is scarce. I have studied 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. Shapley (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 individuals in several batches of blowflies under given temperature conditions. Shapley's and Nicholson's methods have been adopted for the present experiments. Omardeen's (1957) method slightly modified as described below was used for the temperature preference experiment.

Experimental Methods

Six constant temperature rooms at 5, 10, 15, 20, 25, and 20 C were used. Higher temperatures (35, 37, 40 and 42 C) were obtained by two water baths. Temperatures were checked before and after each experiment with a telethermometer. Glass plate temperatures obtained by the use of the water baths varied ± 2 C in time and ± 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 were recorded during a period of 30 seconds each time on batches 1 and 2 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 ten larvae each of *C. mitis* were used for determining the speed of movement at various temperatures. Observations were taken only after an animal had been at least two hours at the experimental 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 photonegative behaviour in a beam of horizontal light 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 seek possible effects of light in combination with the experimental temperatures. Two batches of *C. furcata* larvae were used; one in the dark and one under a lateral light.

Temperature gradient experiments were carried out in an apparatus similar that as described by Omardeen (1957). This consisted of a thin sheet metal 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, and cold water was circulated in the copper plate at the cold end. An electric flat immersion heater was used to heat the copper plate from the other end. Thus by cooling one end of the plate and heating the other a temperature gradient in the agar solution ranging from 9.7 - 34.8 C was maintained.

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 telethermometer and the slope of the temperature gradient was found to be uniform and maintained almost constant over periods of two hours. A 47 x 10 cm fluorescent lamp was constantly used overhanging the trough. The light intensity on the floor of the trough was 210 ± 5 foot-candles.

A hundred *C. mitis* larvae were used in the temperature preference experiments. These were stored at 21.4 ± .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 C) of the trough and the numbers of larval positions in each section were counted at 5 minute intervals for 30 minutes. Each series of experiments was repeated ten times.

Activity and Speed at Uniform Temperatures

The results are summarized in fig. 7. 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 attributed to the 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 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. The degree of constancy of activity in this temperature range (15 - 25 C) suggests that these temperatures are not harmful. This temperature range of 15 - 25 C appears to be the preferred zone of 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 at the end of the experiment at 42 C were also dead. Thus the temperature range of 37 -

42 C is lethal to the larval C. *furcata* under these conditions.

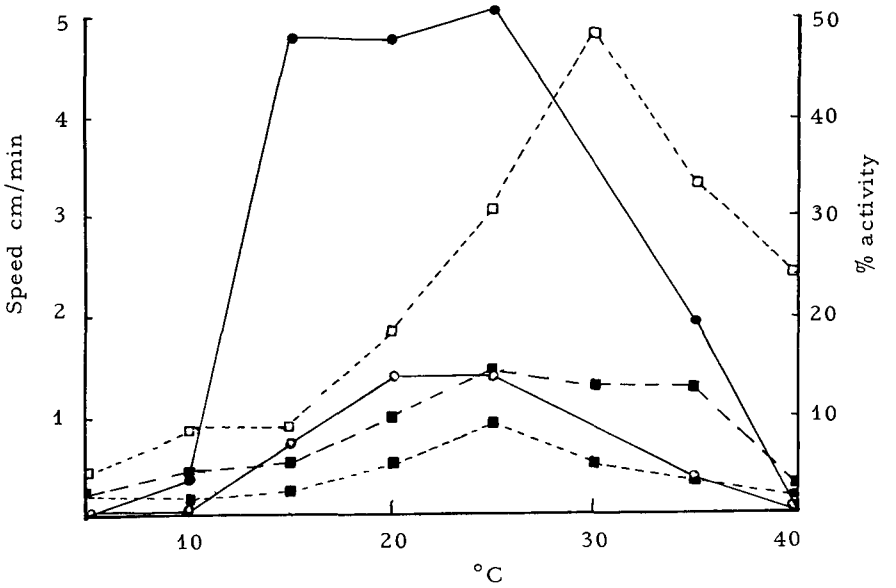

Fig. 7. Effect of temperature on the activity of larvae *C. furcata*, ○% activity under red light, ● % activity under white light, □ speed under lateral light, ■----speed in darkness; ■— larval *C. mitis* speed in darkness.

The mean values of speed cm/min of *C. mitis* increase very gradually in the temperature range of 5 - 25 C and are also included in fig. 7. 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 pretreatment conditions prior to the experimental temperatures are closely similar. It seems, therefore, that such preconditioning has little influence on the rate of movement of the larval *C. mitis*.

The mean values of speed of *C. furcata* 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 the amount of variation shown by the larvae. The temperature zones for maximum speed, however, are slightly different in each batch.

C. mitis shows a higher speed than *C. furcata* throughout the temperature range of 10 - 40 C. The rate of movement at 40 C does not diminish steeply. However, all larvae of both species died at 40 C.

The Distribution of Larvae in the Temperature Gradient

The results of 40 experiments including the control and representing some 3000 position records are summarized in fig. 8. 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.

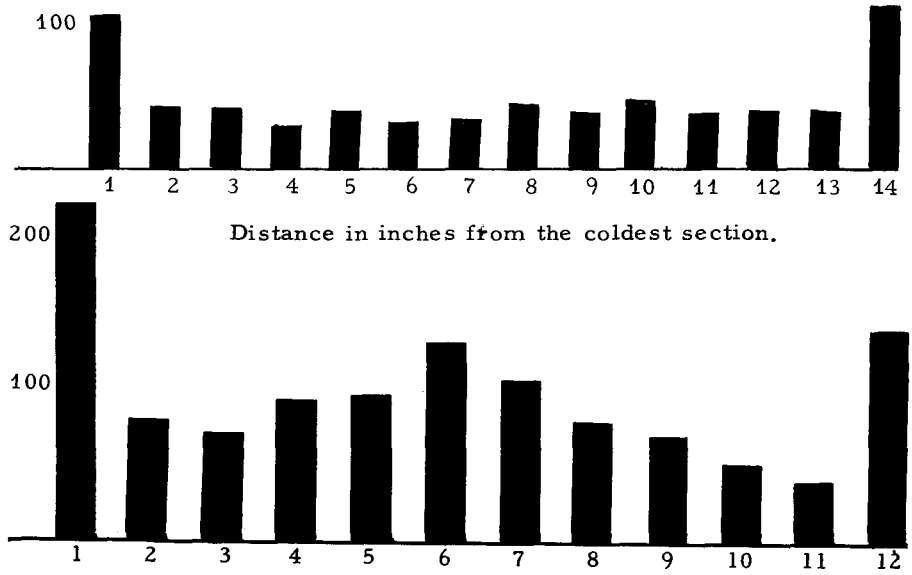

Fig. 8. The distribution of larval *C. mitis* in temperature-gradients; above, control, agar solution temperature constant at 21.5 ±0.5°C; below, temperature-gradient 17 - 31.5°C, light intensity 210 ± 5 foot-candles.

The temperature gradient experiments show that the larvae aggregate in the extreme cold end of the trough, the 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 many 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. All larvae on reaching the hottest end showed increased rate of movement and an occasional tendency to climb and burrow for awhile. With time, however, the behaviour changed to 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. On the other hand, the aggregation at the coldest end occurs owing to slow movement and decreasing activity of larvae with time. Further observations suggest 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; thigmotaxis appears to be a potent factor in producing larval aggregations at the coldest region. The importance of cold, however, is supported by the results as shown in fig. 7 where the least percentage activity and speed in cm/min have already been demonstrated in the uniform temperature range of 5 - 10 C.

Larval *C. mitis* do not exhibit any clear 'temperature preference' under these experimental conditions, perhaps because of the steepness of the temperature gradient. A few experiments were carried out with the same batch of larvae but in a temperature gradient where the temperature 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. The increase in recording time increased the chances of a larva coming into contact with all the parts of the temperature gradient several times. The histogram for this series of experiments is of comparable significance with the others since each is based on 1200 position records. A comparison shows: (1) a slight increase in the number of position records at the hottest end; but (2) a considerable decrease in the number of position records at the coldest end; and (3) the presence of a middle range of larval aggregation at a temperature of 22 - 24 C. These differences in the distribution pattern are attributable to the difference in temperature range and slope of the gradient. Larval *C. mitis* in the 17 - 31.5 C gradient showed continuous activity both at the cold and the hot end of the gradient. Observations, however, again indicated that aggregations at the ends are mainly attributable to thigmotaxis. Among the larvae remaining outside the cold and hot sections the temperature zone of 22 - 24 C appears to be preferred.

Discussion

Various workers (Shapley 1924, Crozier and Stier 1925, Bodenheimer and Klien 1930, Falconer 1945, and Hafez 1950) have analyzed their data on the rate of movement of insects on the 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 10,000 - 18,000 respectively. Examination of the curves in fig. 7 shows no resemblance to the usual type of Q_{10} or Arrhenius curve except between 10 - 30 C. This partial resemblance of the curves to the Q_{10} curve is to be expected since Miller's (1929) study on *Lucilia* larvae and Crozier and Stier's (1925) work on the caterpillars 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 for larval tabanid activity

appears to be 10 - 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 rising temperature.

The larval aggregation in sections 6 - 7 of the gradient, which had a mean temperature of 23 C has been suggested as due to a true temperature preference of the larvae. Since the other reactions to temperature described suggest a number of normal ranges of temperature for larval tabanids a 'temperature preference value' was calculated from the data obtained with the temperature gradients, using the procedure recommended by Herter (1953). The numbers of larvae recorded from the ends of the gradients were omitted since such larval positions were due to end effects. The maximum activity temperatures range from 21.9 - 28.4 C and, under laboratory conditions, lateral light produces a most noticeable effect. The 'temperature preference' values vary little and range from 19.3 - 24.4 C with a mean of 21.4 ± 0.8 C. This is in close agreement with the value 23 C obtained from the temperature gradient experiments directly.

GENERAL CONCLUSIONS

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

Very few soil burrowing insects are other than negatively phototactic (Cameron 1917). 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 desiccation. This may be why even the pupae are found covered under leaves or vegetation.

Reaction time experiments showed that larvae are able to integrate light energy over periods of seconds and to utilize the effect to produce a directional response. Several eyeless animals have been shown to posses 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.

No directed reaction to dry or wet air was observed in several different types of humidity gradients. These negative results are not at all surprising since it is well known that the larvae live in a microclimate which is typically moist and perhaps they do not possess the ability of hygrotactic orientation. Their presence in moist habitats can be explained as a result of direct selection of such places by the ovipositing female tabanids.

A consistent inactivity under low percentage of R.H. and moisture is shown by larval tabanids. Such a reaction 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 increasing moisture of the soil. Variable activity of this nature dependent on moisture percentage has been reported in many soil insects (Uvarov 1931).

Published data show that 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 any of my collecting sites. Since the experimentally determined lethal temperature for larval tabanids is 37 - 42 C, high temperature cannot limit the distribution of these larval tabanids in the soil in Alberta.

Hibernating larval tabanids are subject to temperatures down 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). Although no experiment was conducted below 5 C, at 5 - 10 C larval activity varied from 0 - 4% while the rate of crawling was 0.2 cm/min and larvae in the soil at and below 5 C become sluggish and quiescent and do not pupate. It seems likely that a temperature of 5 C or lower initiates hibernation. The temperature preference of the mature larvae was found to be 21.4 ± 0.8 C, a temperature which is common during June - August, the period of maximum activity of larval tabanids under field conditions.

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 was a common feature in this study. It was not possible to use laboratory reared larvae since no suitable means of rearing 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; for example, immature ones may be indifferent to lateral light, mature ones show intense negative phototaxis, 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. Screening methods were not applicable in humidity, moisture, and temperature experiments since no directed reaction was obtained for these conditions.

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