

THE ROLE OF RESPONSES TO LIGHT IN THE SELECTION AND MAINTENANCE OF MICROHABITAT BY THE NYMPHS OF TWO SPECIES OF MAYFLY

By D. A. HUGHES*

Department of Zoology, University of the Witwatersrand, Johannesburg

Introduction

In a field investigation of the role played by shade and direct illumination on the distribution of stream fauna (Hughes, in press), two species of mayfly nymph, *Baetis harrisoni* Barnard and *Tricorythus discolor* (Burm.), were found predominantly in reaches exposed to sunlight and reaches shaded by dense vegetation respectively. The results for *Baetis harrisoni* bear out the conclusions reached by Harrison & Elsworth (1958) who, during their Berg River survey, compared the faunas of a shady tributary and a physically similar open reach of the main Berg River at the same altitude and found higher incidences of this species at the open sampling station.

Light and shade are generally thought (Welch, 1952; Coker, 1954) to limit or favour the distribution of stream fauna through such indirect factors as branch and leaf debris, algal growth and temperature. In the case of these two species of mayfly, however, it is postulated that their distribution within the stream (with respect to regions of light and shade) is a function of the responses of the nymphs to light stimuli.

The following series of experiments was designed to investigate the responses of both species to light and thereby to determine the role played by these responses in the selection and maintenance of the microhabitat.

General Method

The experiments were conducted in an outdoor model stream, and in other apparatus used

*Present address: Institute of Marine Science, University of Miami, Miami, Florida, U.S.A.

both in the laboratory and out of doors. These will be described in detail when discussing the experiments in question.

The nymphs of both species were collected in large numbers from nearby streams and rivers. Nymphs of *Baetis harrisoni* were found in regions of faster current, predominantly on the upper surface of stones. *Tricorythus discolor* was also found in fast flowing water, but exclusively on the under surfaces of stones.

The initial series and several of the later experiments were performed in an artificial stream (Fig. 1). This was constructed from three 6-ft lengths of 6 in. diameter asbestos compound rain-water guttering. The 'stream' was fed by a short length of tubing led off a nearby tap (A). It was cradled on three concave concrete blocks (B) which rested on a wooden plank (C). Control of the rate of flow was thus possible by altering the slope of the 'stream bed'. The current speed was maintained at 30 cm/sec in all experiments. The depth of water was maintained at 5-6 cm and reasonably smooth flow was obtained by the insertion of two wire gauze partitions near the inflow between which an assortment of stones was placed.

The 18 ft stream was divided into three sections by wire gauze partitions (D) secured by putty. The sections are numbered from the inflow end. The substrate of the first section was painted uniformly white and the second uniformly black, while the third was painted with alternate black and white bands 10 cm wide. To enable the nymphs to cling to the substrate fine sifted sand was mixed with the paint. At the outflow end a weir (E) and an adjustable

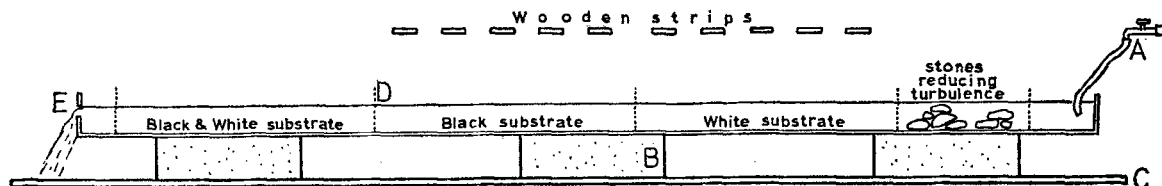


Fig. 1. Outdoor model stream.

V-notch further facilitated regulation of the depth and rate of flow.

The long axis of the stream was north-south, and as strong sunlight uniformly illuminating the entire stream bottom was necessary for many of the experiments, most observations and counts were carried out between 11.00 and 14.00 hours. This necessitated the collection of the nymphs in large numbers (300-400) during the early morning, introducing them into the stream as soon as possible and leaving them there for an hour or more before starting the experiments.

In all experiments the two species were tested separately, and at no time were both species in the stream together.

Results

1. Behaviour within the Artificial Stream Under Conditions of Uniform Illumination

Prior to experimentation, nymphs were introduced into section I of the 'stream' and their reactions were observed.

Baetis nymphs which are agile and stream-lined, moved about freely within the 'stream', progressing by means of darting swimming movements over short distances and by running and walking. The less motile *Tricorythus* nymphs made no swimming movements, and they progressed only by walking over the substrate.

In flowing water the nymphs were always orientated with their heads facing upstream. The maintenance of this position in similar mayfly nymphs is suggested by Neave (1930) to be controlled only by 'the shape of the animal rather than the rheotaxis previously postulated'. The result of this orientation was that, within a short period of time under conditions of uniform illumination, the majority of the nymphs

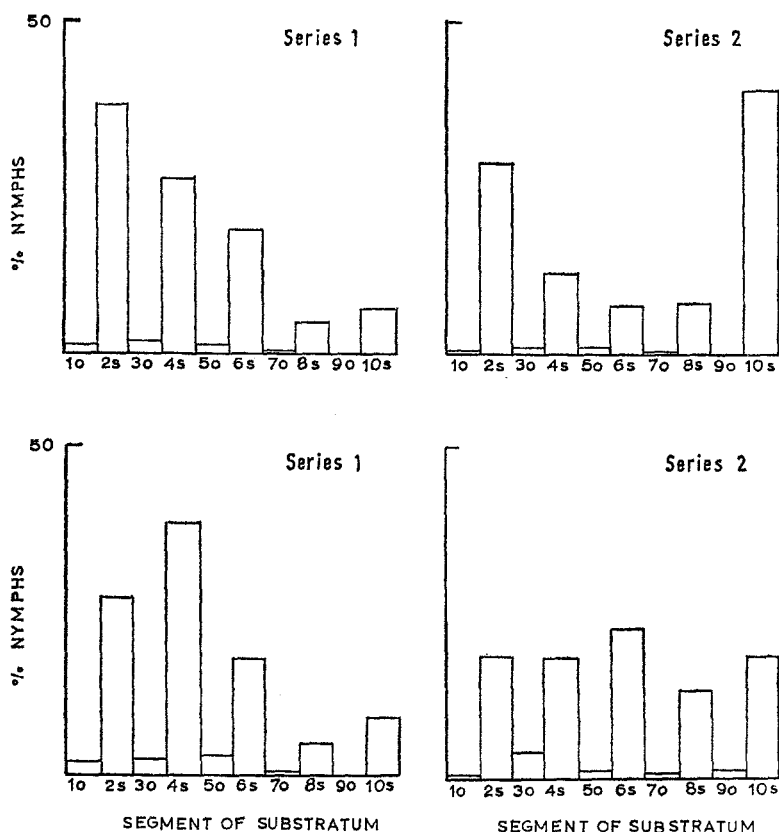


Fig. 2. The distribution of the nymphs of *Baetis harrisoni* (top) and *Tricorythus discolor* (bottom) with respect to regions of light and shade (white substrate). Odd numbered segments 10 to 90 were exposed to direct sunlight; even numbered segments 2s to 10s were shaded.

became aggregated at the upstream gauze partition of the section in which they were placed.

It was apparent that the nymphs behaved within this artificial stream in the same way as they are observed to do in natural streams, and it was obvious that they did not require a period of time in which to acclimatize to their new environment since they responded in precisely the same way to all stimuli regardless of how long they had been in the 'stream'.

2. Evidence for the Presence of a Response to Light or Shade, and the Nature of the Stimulus to which the Nymphs are Responding

(i) The reaction to regions of shade and direct illumination

A taut wire, strung between a series of metal stakes driven into the ground, flanked the 'stream' on both sides, at a height of 2 ft above

the 'stream' level. Five wooden strips, 10 cm wide, were placed across the two wires in such a way that the first section of stream (uniform white substrate) was divided into ten equal regions alternately shaded and illuminated. These regions were numbered 1 to 10 from the inflow end. The experiment was conducted under conditions of bright sunlight. The intensity of the light incident on the water of the open regions was approximately 8000 ft-candles, while that incident on the shaded regions was 150 ft-candles.

A number of individuals (100–200) were introduced into section I of the 'stream'. Their distribution with respect to shade and illumination was then observed and recorded numerically every 5–10 min. Typical results of two such series of counts for each species are shown in Fig. 2.

These results indicate the presence of a very marked light reaction.

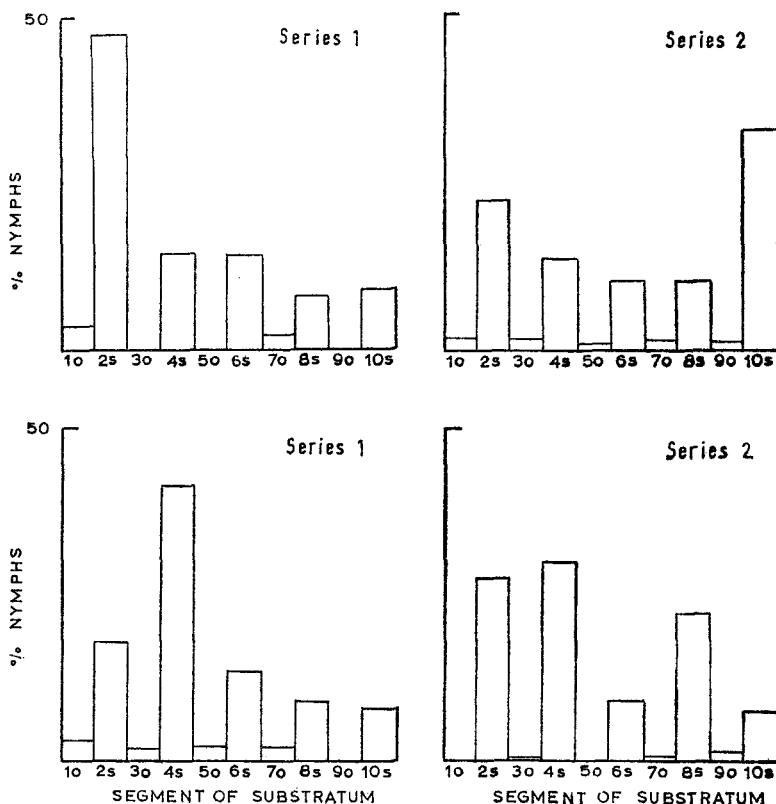


Fig. 3. The distribution of the nymphs of *Baetis harrisoni* (top) and *Tricorythus discolor* (bottom) with respect to regions of light and shade (black substrate). Odd numbered segments 1 to 9 were exposed to direct sunlight; even numbered segments 2s to 10s were shaded.

(ii) *The relative importance of incident and reflected light*

Test I. The previous experiment which was conducted on the white substrate of the 'stream', was repeated in section II, the substrate of which was black. In this way the strong contrast between the reflected light intensities from the substrate of the open and shaded strips was virtually eliminated.

The results (Fig. 3) indicate that some factor connected with incident illumination is the stimulus, or a major component of the stimulus, which elicits the observed photonegative behaviour but the nymphs could have been responding to reflected light as well.

Test II. This test was conducted in section III of the 'stream'. This section differed from the first two sections in that it was not shaded with

strips of wood, and the substrate was painted with ten alternate white and black bands, each 10 cm wide.

The same procedure was followed as in the previous two experiments. Nymphs were introduced into the stream, and the numbers found in each of the ten regions were recorded every 5–10 min. Two series of counts were conducted on both species. One series was under conditions in which the 'stream' was exposed to direct sunlight (intensity incident on water 8000 ft-candles) and in the other series the 'stream' was shaded from direct sunlight (intensity incident on the water 1000 ft-candles).

From the results (Fig. 4) it is not clear whether or not the nymphs are responding positively to the black regions of substrate. It appears from the histograms, however, that the incidence of

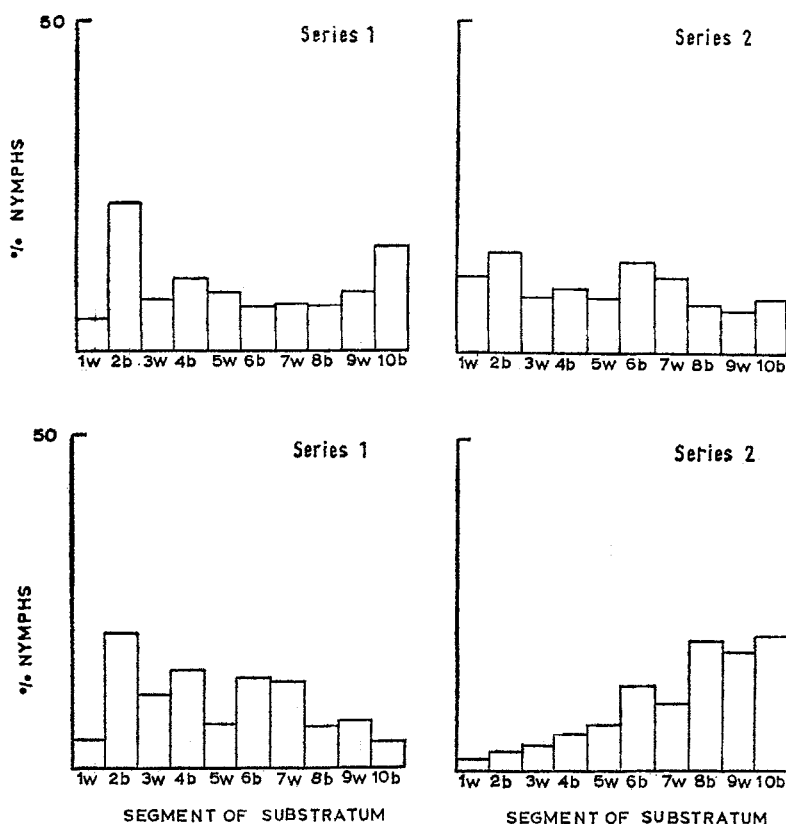


Fig. 4. The distribution of the nymphs of *Baetis harrisoni* (top) and *Tricorythus discolor* (bottom) with respect to black and white regions of substrate. Odd numbered segments 1w to 9w were painted white; even numbered segments 2b to 10b were painted black.

nymphs on each black region of substrate is more often higher than lower than their incidence on the adjacent white region.

It can be concluded from these two tests that the nymphs of both species are strongly influenced in their distribution with respect to regions of light and shade by some factor connected with the incident illumination, and that any response to the light reflected from the substrate represents only a minor component in the photonegative behaviour.

(iii) *The effect on the aggregation of nymphs in the shaded regions of lower light intensities on the open regions*

This experiment was designed to determine whether or not the observed distribution with respect to regions of light and shade arose solely from a non-tolerance of very high light intensities and were thus subject to a threshold effect, or whether aggregation in the shaded regions occurred even at very low light intensities. It was conducted in section I (white substrate) of the stream, which was again divided into alternately shaded and illuminated regions. The incident light intensities on the illuminated regions were varied from complete shade (900 ft-candles) to

complete exposure to midday sun (8000 ft-candles).

After each change of intensity, 10 min elapsed before the next count was taken. There was also a 5 min lapse between each count at any one intensity. Six series of readings were taken at each light intensity. These were taken in two separate series of three each. The different light intensities were applied in a random sequence (Fig. 5).

After each count any aggregations of the nymphs were dispersed and the nymphs were once more distributed evenly along the length of the 'stream'.

These results (Fig. 5) indicate that in the case of *Tricorythus* the tendency for nymphs to accumulate in the shade is present even when the light intensities incident on the open regions of the 'stream' are low (2000 ft-candles). *Baetis* nymphs did not react very strongly to intensities below 3500 ft-candles and the extent to which the species accumulated in the shade was less than that for *Tricorythus*.

3. Orthokinetic Response

Kineses have been defined as 'variations in generalized, undirected, random locomotory

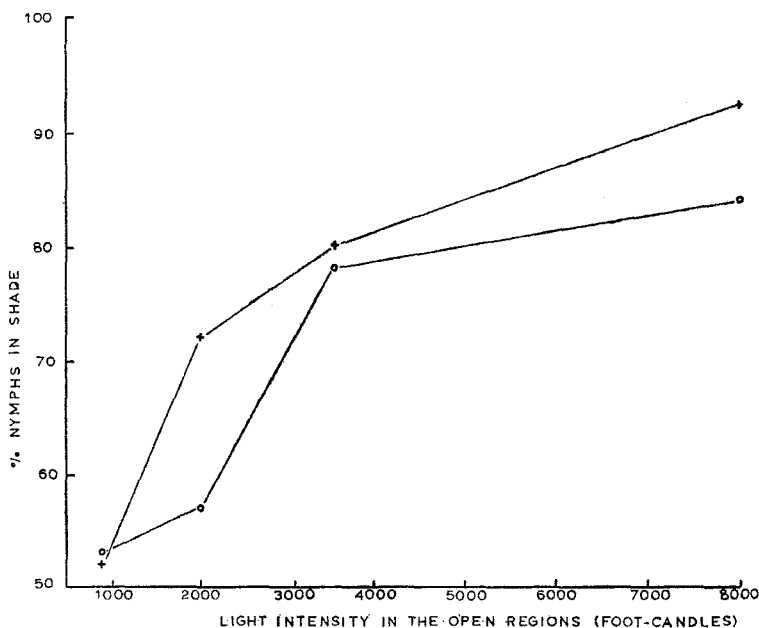


Fig. 5. The effect on the accumulation of nymphs in the shaded regions, of different intensities of light incident on the open regions. O, *Baetis harrisoni*; +, *Tricorythus discolor*.

activity due to variations in intensity of stimulus', by Gunn, Kennedy & Pielou (1937). They then defined orthokineses as responses in which the variation in intensity of stimulus resulted in variations in 'linear velocity'. Fraenkel & Gunn (1940) pointed out, and Ewer & Bursell (1951) have stressed, that orthokineses may be expressed by variations either in speed or activity or both.

(i) *The degree of locomotory activity of the nymphs under conditions of differing light intensities*

For the purposes of recording activity, the first section (uniform white substrate) of the stream was divided into eight segments by securing threads of cotton across the stream at 10 cm intervals. The light intensities incident on this entire section of 'stream' were uniform when it was fully exposed and were varied by shading in the same manner as in the previous experiment.

The unit for locomotory activity was taken as the number of nymphs which moved, per segment, during a 5 sec period, expressed as a percentage of the total number of nymphs in that segment.

The various light intensities were all applied twice, and on each occasion two readings were taken at 5 min intervals.

With the substrate and the intensity of illumination uniform in each case, all directional orientation except that imposed by the current is eliminated. The results (Fig. 6) can therefore be

interpreted in terms of a very marked orthokinetic reaction, in which locomotory activity virtually ceases under low light intensities whereas under high intensities a very high degree of locomotory activity occurs.

This reaction is sufficient to explain the distributions observed in the previous experiments. When moving upstream, the nymphs passed through regions of shade where activity was reduced, with consequent aggregations in these regions.

(ii) *The effect of reflected light intensity on the degree of locomotory activity*

Half of section I (uniform white substrate) and half of section II (uniform black substrate) of the 'stream' were divided by cotton threads into segments 10 cm in length. The intensity of illumination incident on these two sections was uniform (7500 ft-candles). Nymphs were introduced into both sections and their degree of locomotory activity measured as in the previous experiment. The results (Table I) have been examined using Student's *t* test. The difference between the activity on the black and on the white substrate was found to be statistically insignificant for both species.

(iii) *The efficiency of the orthokinetic response in aggregating the nymphs in an under-stone habitat*

'Stones' were made from curved guttering similar to that used for the stream. Each was

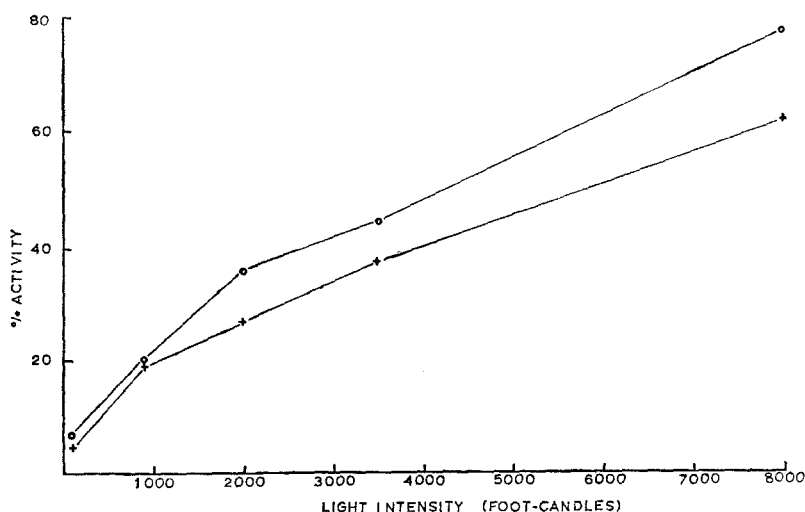


Fig. 6. The effect of different light intensities on the degree of locomotory activity shown by the nymph O, *Baetis harrisoni*; +, *Tricorythus discolor*.

Table I. The Effect of Reflected Light on the Degree of Locomotory Activity Shown by the Nymphs of *Baetis harrisoni* and *Tricorythus discolor* in Five Tests on each Type of Substrate

	White substrate	Black substrate
<i>Baetis harrisoni</i>		
Total no. moving	79	88
Total nymphs	112	135
Percentage activity	70.5	65.1
<i>Tricorythus discolor</i>		
Total no. moving	79	67
Total nymphs	122	114
Percentage activity	64.7	58.8

10 cm square and 2 cm thick and elevated 5 mm from the stream bed by putty placed only at the corners. They were painted white and the surface roughened like the 'stream'.

The experiment was conducted both in section I and in II (white and black substrates respectively). In each case four stones were introduced and readings taken of the numbers of nymphs under stones and not under them in alternate conditions of complete shade (incident light intensity: 100 ft-candles). After each reading, aggregations of nymphs were dispersed. Recordings were taken at 10 min intervals.

The results (Table II) show the effectiveness with which the nymphs are confined in an under-stone habitat. They do however also raise a problem. *Baetis* are not found in nature confined to an under-stone habitat to the extent that these results would lead us to expect. A smaller proportion of *Baetis* nymphs were found under stones, under shaded conditions, than *Tricorythus*. This is in keeping with the result expressed in Fig. 5 which indicate that *Baetis* accumulated to a lesser degree in the shaded strips under low light intensities than *Tricorythus*. In view of these results it is difficult to explain the similarity in the locomotory activity of both species at all light intensities (Fig. 6) unless an additional mechanism was responsible for the orientation of *Tricorythus*. Observations of the behaviour of this species within the 'stream' pointed to the existence of a directional response to shade or dark objects. The nymphs frequently turned away from the direction of current to move under a stone. This clearly directional

movement confirmed earlier observations of the reactions of nymphs to a thin strip of shade (2 cm wide) on the edge of the 'stream'. They were observed to turn into this strip and aggregate within it. Such a response was not observed in *Baetis*.

Table II. The Reaction of the Nymphs of *Baetis harrisoni* and *Tricorythus discolor* to Artificial Stones in the Stream. The results in each case indicate the means of at least three tests in which the direction of change was consistent for each test

	White substrate	Black substrate
<i>Baetis harrisoni</i>		
Mean percentage under stones in direct sunlight	83	87
Mean percentage under stones in shade	42	36
<i>Tricorythus discolor</i>		
Mean percentage under stones in direct sunlight	92	95
Mean percentage under stones in shade	86	73

4. Phototactic Response

Both Shelford (1945) and Moon (1940) have made general reference to the negative phototaxis exhibited by most stream forms. Referring more specifically to the Ephemeroptera, Wodsedalek (1911) reports on the negative phototaxis shown by most nymphs of *Heptagenia interpunctata* (Say); Percival & Whitehead (1926) record the same response to light in nymphs of *Ephemera danica* (Mull.) and Gros (1923) noticed that after the fifth ecdysis, the nymphs of *Ecdyonurus forcipula* (Pict.) avoided the light when not feeding.

(i) Responses within a light beam

In preliminary observations in which nymphs were placed midway along the gradient of a beam of light passed into an aquarium, a large proportion of *Baetis* swam directly towards the light and came to rest on the side of the aquarium nearest the light source. When the end of the aquarium towards the light was reversed, the tendency was, once again, to swim or crawl towards the light source.

Tricorythus, on the other hand, exhibited very little response within the beam except for

occasional nymphs which turned to face away from the light source.

The responses of the nymphs within a light beam were further investigated under conditions in which individual responses could be quantitatively estimated and in which handling of the nymphs could be reduced to a minimum by both housing the nymphs and conducting the investigation in the same apparatus.

Another artificial stream was constructed from a 6 ft long, square-bottomed asbestos compound guttering (Fig. 7), one end of which was enclosed (A) and the other fitted with a weir (B) leading to a drain. Two gauze partitions (C) prevented the nymphs from being washed over the weir and from getting among the stones (D) into which the water from the pipe (E) flowed and which prevented excessive turbulence. The water was maintained at 30 cm/sec and at a depth of 6–7 cm. A perspex window (F), 3.5 cm high and 8 cm long, was inserted in the side of the stream. A beam of light from a 100 W microscope lamp (G) was shone through the window. A plastic tube (H) of \square cross-section, open at both ends, was secured cross-wise on the stream substrate in such a way that the light beam shone directly down it. A hole (J) of 1.5 cm diameter, in the centre of the upper surface of the tube enabled the nymphs to be introduced at a fixed distance from the light source. The nymphs were introduced by means of a pipette, the enlarged end of which permitted the nymphs to be sucked into the bulb, thus avoiding unnecessary handling. The apparatus was housed in a room in which the light intensity incident on the 'stream' could be controlled.

Baetis harrisoni. Preliminary trials with *Baetis* indicated differences in the responses of different instars. The nymphs were therefore divided into four categories, and each tested separately.

- (a) Ultimate instar—characterized by their black wing buds.
- (b) Penultimate instar—characterized by large wing buds which were, however, not yet black. This stage probably comprised both genuine penultimate instars and nymphs of the final instar, recently moulted and therefore without black wing buds.
- (c) Middle stage—comprising those nymphs from approximately the twelfth to the sixteenth instar.
- (d) Early stage—nymphs of approximately the fourth to the eighth instar.

Points were awarded for the different responses within the beam as follows:

Direct forward movement towards the beam's source over a distance greater than 5 cm: 2

Indirect movement, backwards and forwards, but ultimately taking the nymph a distance greater than 5 cm towards the beam's source: 2^1

Direct forward movement towards the beam's source over a distance less than 5 cm: 1

Indirect movement, backwards and forwards, but ultimately taking the nymph a distance less than 5 cm towards the beam's source: 1^1

No movement whatsoever: 0

Indirect movement, backwards and forwards, but ultimately taking the nymph a distance less than 5 cm away from the beam's source: -1^1

Direct movement away from the beam's source over a distance less than 5 cm: -1

Indirect movement, backwards and forwards, but ultimately taking the nymph a distance greater than 5 cm away from the beam's source: -2^1

Direct movement of more than 5 cm away from the beam's source: -2

A hundred nymphs of each stage were placed individually in the beam and their responses recorded. The light intensity incident on the water was maintained at 100 ft-candles, and the

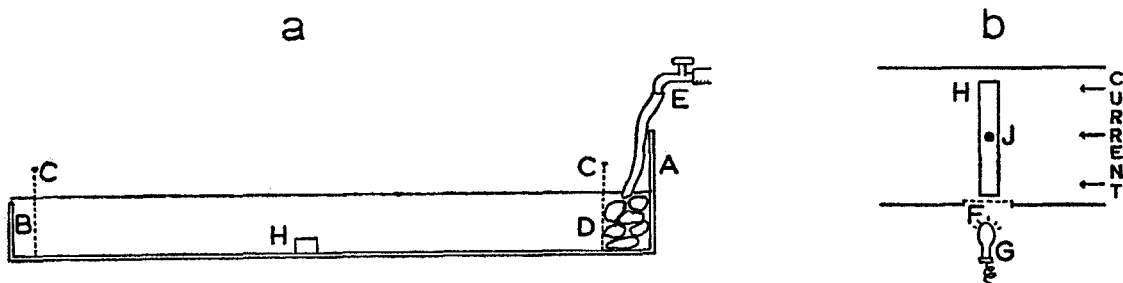


Fig. 7. Apparatus for investigation of response within a light beam. a, Longitudinal section; b, horizontal section.

intensity of the beam at the point at which it entered the perspex window was 1000 ft-candles.

Figure 8 shows a few typical tracks for each category. The loops in the figures indicate back somersaults executed in the vertical plane. This somersaulting was investigated and found to be caused by a dorsal light response present in *Baetis* but not *Tricorythus* (Hughes, 1966).

Tricorythus discolor. Although in a preliminary trial the different nymphal stages of *Tricorythus* did not appear to differ much in their responses within the beam, they were separated into three groups according to nymphal development, i.e. (i) ultimate instar; (ii) middle stages, and (iii) early stages. The responses of 100 nymphs from each category were tested in the

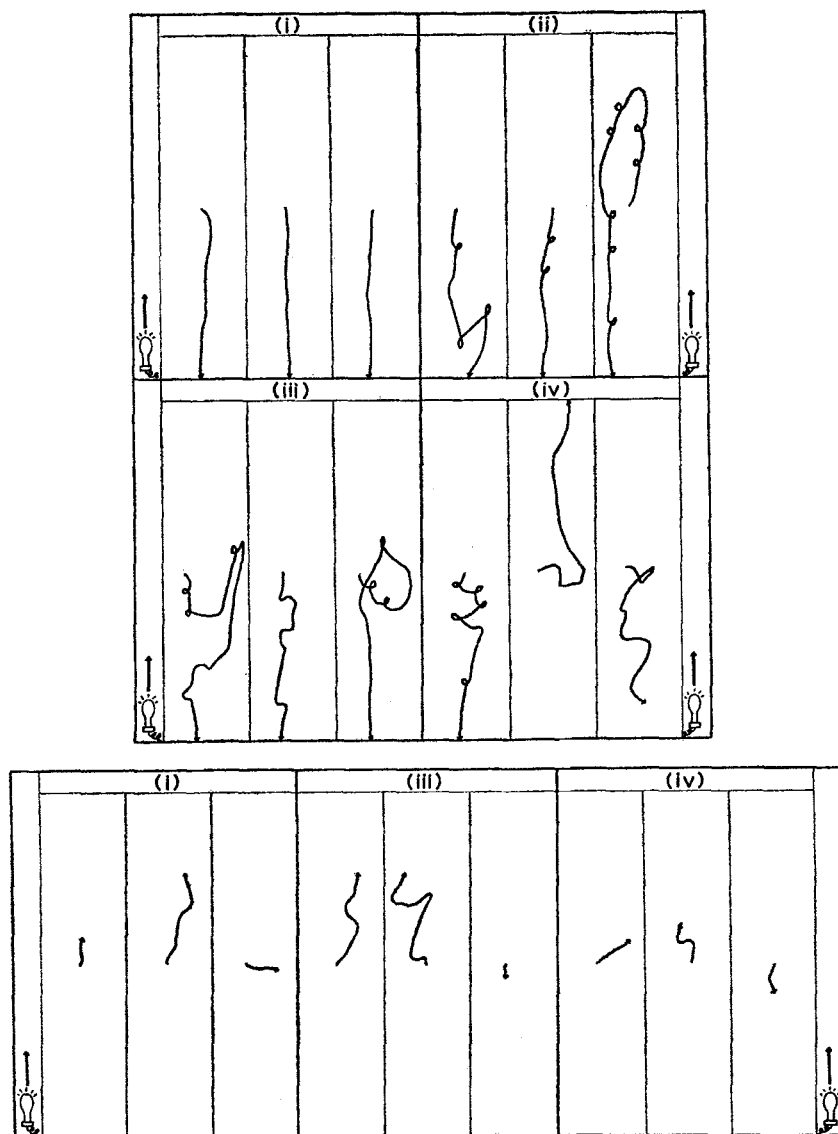


Fig. 8. Tracks of nymphs of *Baetis harrisoni* (top) and *Tricorythus discolor* (bottom) within a light beam. (i), Ultimate instar; (ii), penultimate instar; (iii), middle stages; (iv), early stages.

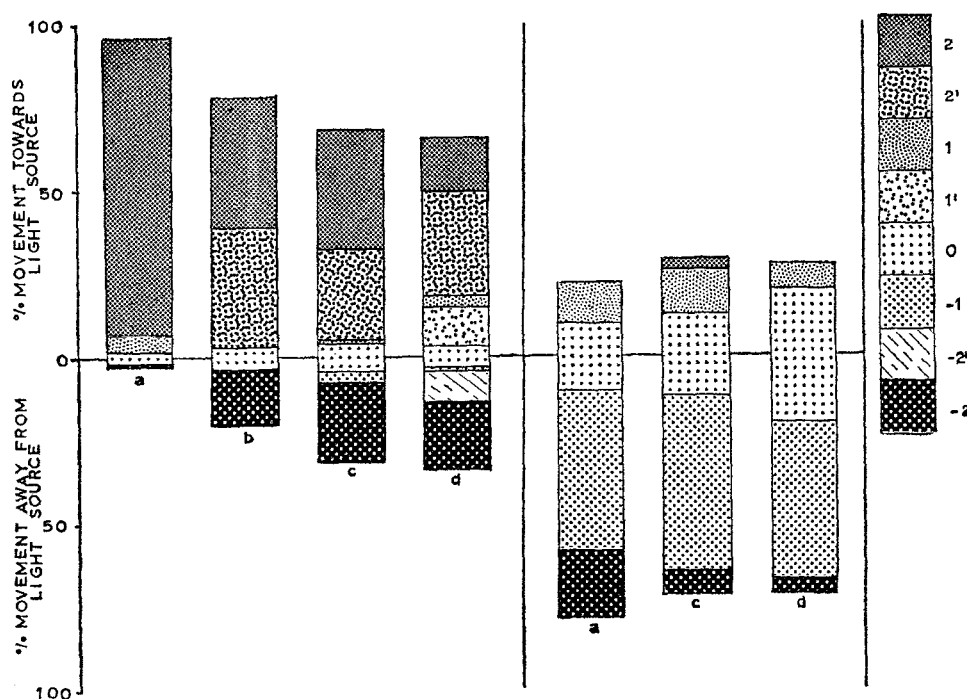


Fig. 9. The nature of the movements of the various nymphal stages of *Baetis harrisoni* (left) and *Tricorythus discolor* (right) in a light beam. For an explanation of the figures employed to record the movements see p. 24. a, Ultimate instar; b, penultimate instar; c, middle stages; d, early stages.

same manner as those of *Baetis*. A few typical tracks for each category are shown in Fig. 8. The histograms in Fig. 9 summarize the nature of the movements shown in the different nymphal developmental stages of both species.

In the case of *Baetis* all stages responded positively but there were quantitative and qualitative differences in the responses of the different stages. There was a decrease in 'somersaulting' and an increase in the 'strength' of the response with increasing nymphal development. The change in the response exhibited by the ultimate instar was particularly striking—all forms of 'vacillation' or 'delay' disappeared, and the nymphs responded rapidly and directly to the beam. Generally the nymphs walked up the beam and swimming movements decreased.

Tricorythus manifested a weak negative response with less quantitative and no qualitative differences between the groups. There was no 'somersaulting' and the nymphs always walked within the beam. The only quantitative difference was that, with increasing development, a greater number of nymphs responded negatively and obtained a score of -2 .

The possible significance of these differences between and within the species will be discussed later.

(ii) Consistency (in *Baetis*) of the negative light reaction

In most series tested there were always a small number of nymphs which exhibited a negative light response.

Nymphs which had at any time responded negatively when placed within the beam were isolated and tested repeatedly in the beam, and their successive tracks plotted. A few of the typical results obtained are recorded in Fig. 10. From these, it is evident that a number (e.g. A, B, C and D) reverted to a positive response in succeeding trials. This was generally the case. A few, however (e.g. E and G), showed a consistent negative response and an equally small proportion (e.g. F) did not appear to respond to the beam at all.

5. Response to Black Objects

The response to black objects was investigated quantitatively. This necessitated exposing

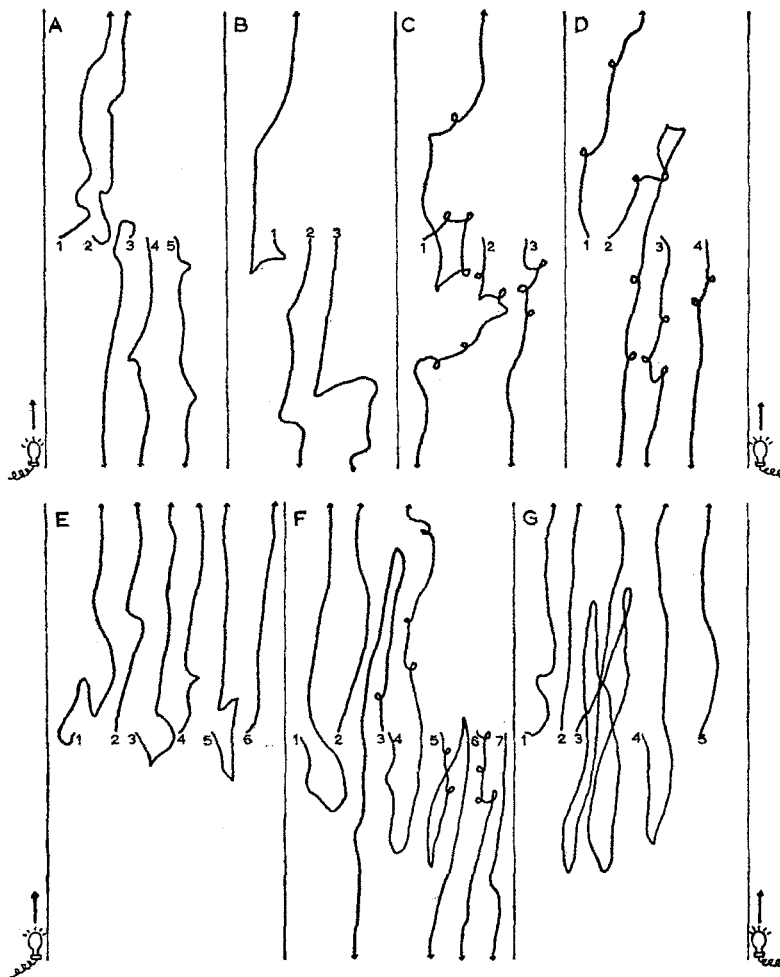


Fig. 10. Consecutive tracks of seven middle stage nymphs of *Baetis harrisoni* within a light beam.

a number of nymphs of both *Tricorythus* and *Baetis* to the same black object, and from the same distance in every case.

This experiment was conducted in a gently curved trough, 60 cm long and 30 cm wide (Fig. 11), through which a current of water (10 cm/sec) was flowing. Nymphs were introduced singly into a vertical glass tube (A), leading into a plastic 'tunnel' (B) open only at the end facing the current. The opposite end of the tunnel was covered with cloth gauze which prevented nymphs from leaving the tube, but which allowed a flow of water through the apparatus. A black object (C) 1.5 cm in height and

6 cm in length was placed 10 cm from the open end of the tunnel and to one side.

Fifty nymphs of *Baetis*, including all developmental stages, and fifty of each of the three stages (arbitrarily grouped into early stages, middle stages and late stages) of *Tricorythus*, were examined individually in this apparatus, with respect to their reaction to the black object. *Baetis* was tested only under conditions of high incident illumination (approximately 8000 ft-candles), whereas *Tricorythus* was tested under the same high light intensities, and also under low intensities (150 ft-candles) when the apparatus was completely shaded from direct sunlight.

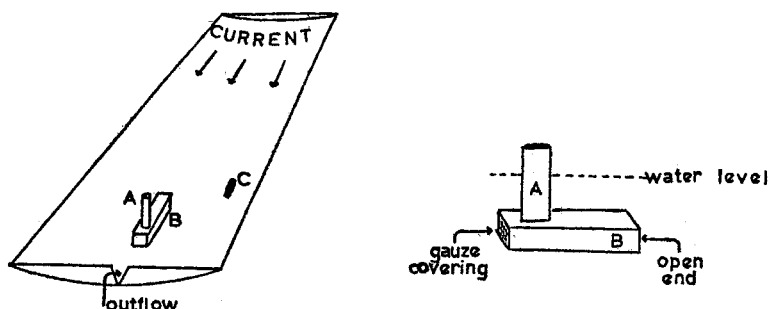


Fig. 11. Apparatus used for assessing the reaction of nymphs to black objects.

The results confirmed that nymphs of *Baetis* do not respond to black objects. Only 8 per cent made contact with the object and it is probable that they did so purely by chance.

Under high light intensities, *Tricorythus* orientated strongly with respect to the black object. Nearly all of them moved on an almost straight path from the tunnel mouth to the object: 100 per cent late stage, 92 per cent middle stage, and 96 per cent early stage nymphs made contact with it. There were, however, differences in the time required for the different nymphal stages to reach the object. The later stages generally moved without pausing towards the object, while the earlier instars spent long periods stationary either before moving or on the way to the object. Under high light intensities, both species were allowed 90 sec for each trial. When given unlimited time, under conditions of low light intensity (150 ft-candles), most nymphs of *Tricorythus* would eventually reach the black object, but when permitted only 90 sec, only seven late stage, four middle stage and five early stage nymphs of the fifty in each group had reached the object.

This can be explained primarily in terms of the considerable reduction in locomotory activity which accompanied the reduction in intensity of illumination. Contributing to this might be the decrease in contrast between substrate and object.

The nymphs of *Tricorythus* displayed only a weak negative response when placed in a light beam (Fig. 9) and yet their response to dark objects or regions of low light intensity was rapid and very marked. It was therefore evident that although negative phototaxis may be a component of the response to dark objects a further and more important component existed.

Skototaxis is the term that has been given to the response exhibited by a number of inverte-

brates towards black objects or regions of low light intensity within their field of vision. This term has given rise to much polemic as to the validity of distinguishing between negative phototaxis and *skototaxis*.

With this in mind the nymphs of *Tricorythus* were investigated firstly with respect to their responses within a light beam, and secondly with respect to a dark object placed within the beam without obscuring the light source.

The apparatus used in this investigation was the same as that described for the assessment of the nymphs' responses to black objects (Fig. 11). It was shielded from direct sunlight and a broad beam of light was reflected by a mirror from one side only. The beam was directed so as to strike the nymphs as they emerged from the plastic tunnel B.

The responses of the nymphs were recorded within the beam alone and then within the same beam when a black object (1.5 cm high and 6 cm long) was placed between the plastic tunnel and the mirror (Fig. 12). Fifty nymphs of each of the three nymphal categories were tested and the units employed to record the type of movements carried out were the same as those used in the investigation of phototactic responses (page 24).

It is evident from the results that a predominantly negative response was elicited by the beam. When the black object was placed between the nymphs and the light source (without obscuring the latter) a large proportion of the nymphs overcame the negative phototactic response and moved towards the object, up the light beam. (Note that, in this case, all nymphs scoring + 2 made contact with the object.)

Alverdes (1930) working with *Eupagurus bernhardus* and *Carcinus maenas* (Decapoda) and Dietrich (1931) working with *Oniscus asellus* and *Porcellio scaber* (Isopoda), both emphasized

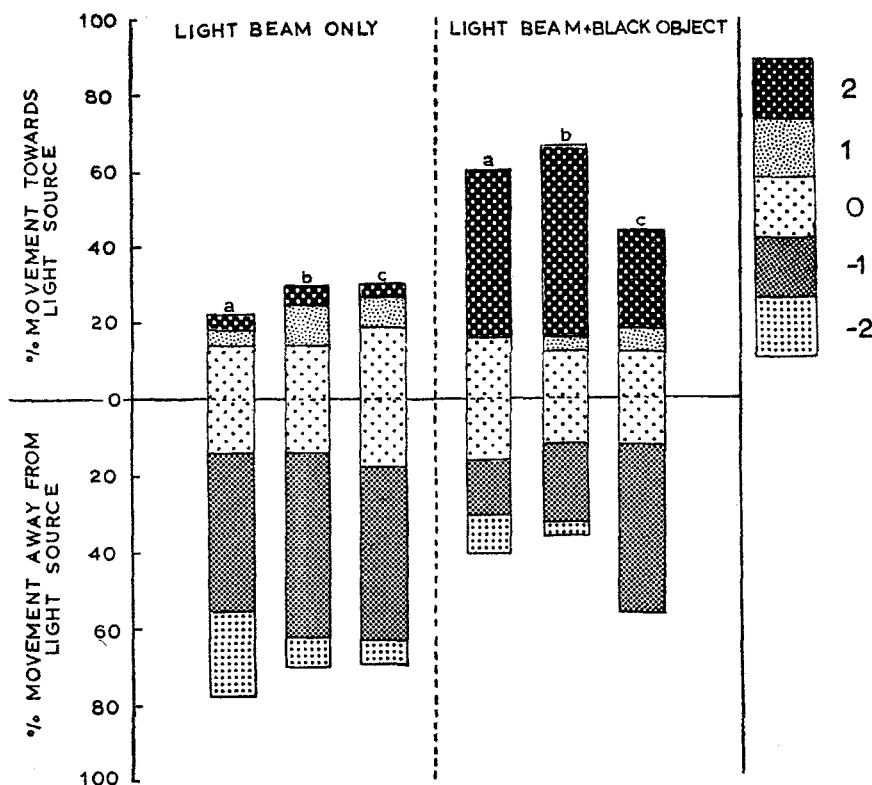


Fig. 12. The responses of *Tricorythus discolor* within a light beam alone and when a black object is placed within that beam. a, Final stages; b, middle stages; c, early stages. For an explanation of the figures employed to record the responses, see p. 24.

that negative phototaxis and *skototaxis* were separable in that they were responses to two separate stimuli. The former was a movement away from a light stimulus, while the latter was a movement towards a clearly defined dark area (*umgrenzten*). Other workers (among them Fraenkel & Gunn, 1940; Lees, 1948) contended that the so-called skototactic responses were not responses to the black regions themselves, but were generally negative phototropotactic responses and that instead of the black object being the attractant stimulus, the animals were orienting themselves with respect to the regions of higher light intensity flanking the black object or region. Ulyot (1936) showed that *Dendrocoelum lactum* orientated itself to intensities of lateral light stimulation of less than 7 ergs/cm²/sec, indicating that very low intensities of reflected light could be important as orienting stimuli. This supported the idea that *skototaxis* was, in fact, negative phototropotaxis. According to this theory, the contrast between the in-

tensities of reflected light from the black regions and the flanking lighter regions would be sufficient to guide the animals, by balancing the stimulation from the lighter regions, towards the dark object.

However, all *skototactic* responses investigated so far have been shown to be telotactic. Fraenkel & Gunn (1940) referring to such work as that of Dolley & Wierda (1929) invoked the idea of the differential turning power of certain ommatidia in a gradient over the eye, to indicate that 'a reaction having the appearance of skototelotaxis can be described quite easily in terms of negative tropotaxis, the light background acting like a constellation of lights'. There is, however, no evidence to support the existence of such gradients in the eyes of all the species exhibiting a skototactic response and, as all these species have been shown to respond telotactically to dark objects and regions, the argument for phototropotaxis can be given little support.

The precise nature of this very marked response to black objects and shade is not clear but it would certainly be incorrect to dismiss it simply as a negative phototaxis. Kennedy (1940) believed that those responses to black objects which cannot be explained solely in terms of negative phototaxis can be regarded as the interaction of phototactic and movement or form responses.

In the case of *Tricorythus* in which the animal showed a very weak orientation away from a source of high light intensity but a very strong one towards a region of low light intensity, and in which the response is not fully understood, the term skototaxis can best be used to describe it.

6. Responses of *Baetis harrisoni* Nymphs to Small Regions of Bright Illumination within Their Visual Field

The experiments so far showed both a strong orthokinetic reaction which is effective in maintaining *Baetis* in an under-stone habitat and a positive phototactic response which serves to attract the nymphs to brightly lit regions within their visual field. These two responses would in nature serve to draw the nymphs to different niches within the stream biotope.

An attempt was made to elucidate this paradoxical situation by observing the responses of nymphs to and within small regions of brightly illuminated substrate within the otherwise shaded artificial stream.

Section I (uniform white substrate) of the outdoor 'stream' was shaded from direct illumination and sunlight was directed onto a small area (diameter 5 cm) of the substrate with a mirror. The nymphs were observed to be attracted to and to swim into this area but seldom to remain within it. If however the light was reflected onto a portion of the gauze partition at the inflow end, or onto the substrate very near the gauze, the nymphs would swim into it and remain there for longer periods. It appeared that these nymphs are attracted by the brightly illuminated regions within the stream, and that certain features of current can override the orthokinetic response and cause cessation of the high degree of activity normally elicited within such high light intensities with the consequent aggregation of nymphs in these regions.

This was investigated by determining both (i) the extent to which brightly illuminated stones attracted nymphs, as opposed to shaded stones,

and (ii) the length of time spent on an illuminated stone as opposed to the length of time spent on an equal area of equally illuminated flat substrate. Both experiments were conducted in section I of the outdoor 'stream', in which were placed several hundred middle stage nymphs.

(i) Two equal-sized stones (approximately 5 cm diameter) were placed next to each other at the inflow end where turbulence was greatest. One of the stones was brightly illuminated by reflected sunlight, while the other stone and substrate were shaded. The number of nymphs on each stone was recorded ten times at intervals of 5 min. The total was 112 for the illuminated stone and fourteen for the shaded stone.

(ii) The length of time spent by nymphs on an illuminated stone was compared with the length of time spent on an equally illuminated equal sized region of substrate.

A round stone of diameter 5 cm placed near the inflow region and a circle of substrate 5 cm in diameter, approximately 1 m downstream from the inflow were both illuminated by reflected light. The length of time spent by twenty individuals on each of these was recorded. Individuals which spent periods of longer than 10 min, on the stone or substrate were removed and their time recorded as 10 +.

The time spent on the stone was considerably longer than that spent on the substrate, and of the twenty individuals landing on each, seventeen remained on the stone for periods of longer than 1 min as opposed to three on the substrate. The total time spent on the stone was >125 min as against 9 min 35 sec on the substrate.

Nymphs landing on illuminated stones seldom moved very far before coming to rest, frequently remaining stationary in that position for some time.

The results support the original supposition that *Baetis* nymphs are attracted to brightly illuminated regions or objects and that within such regions a position can be found in which the orthokinetic light response can be suppressed by some, as yet undefined, factor of current.

Discussion

The biological significance of the responses to light is thought to be as follows.

Tricorythus discolor is exclusively found beneath stones lying flush with the stream bed or below. It is slow moving and never swims but crawls over the bottom. Three responses which

contribute to guiding the nymphs to and maintaining them in this under-stone microhabitat have been demonstrated here.

(i) A strong orthokinetic response in which the correlation between locomotory activity and intensity of illumination was such that under high intensities movement was continuous and under low it practically ceased. Generally, kinetic displacement is non-directional, but in this case a directional quality is super-imposed on it by the strong rheotactic response. A kinetic response of such strength operating in the normal stony-bottomed stream environment, offering ample shade, would alone be sufficient to maintain the animal beneath stones. It is, however, reinforced by the other two directional responses.

(ii) The weak negative phototaxis would direct the animals away from regions of high light intensity.

(iii) The strong response towards dark objects (skototaxis) would serve to guide the nymphs directionally to dark regions. *Skototaxis* has not been previously recorded as such in freshwater invertebrates, but it is probable that its role in selecting the understone habitat frequented by many stream forms is important.

Baetis harrisoni is an agile streamlined nymph capable of darting about within the stream, and is predominantly found above the stones. The explanation of the mechanism whereby the microhabitat is selected is more difficult in view of the experimental findings. Again three light responses are operative.

(i and ii) The orthokinetic response, similar in all respects to that shown by *Tricorythus*, would tend to aggregate the nymphs in shaded regions. *Ex facie* this would conflict with the strong positive phototaxis which leads them to brightly illuminated areas. Aggregations beneath stones may theoretically be dispersed by the attraction to regions of high light intensity where it was shown that the orthokinetic response can be superseded by suitable current conditions. The precise conditions evade definition but it appears that certain positions, perhaps with respect to the thin layer of almost still water above stones (Ambuhl, 1959), satisfy the requirements. This is borne out while observing the nymphs within the natural habitat, where they are seen to be continuously active until a position with respect to the stone-current interface is either fortuitously reached or actively selected, when all movement suddenly ceases. This would explain how the nymphs are guided to their above stone

position in the stream by their positive phototactic response and how with the suppression of the orthokinetic light response they are maintained in this position where the current conditions are suitable.

(iii) The remaining response to light was the dorsal light response (Hughes, 1966) which contributes to the maintenance of the primary dorso-ventral orientation.

For both *Tricorythus* and *Baetis* there is a gap in our knowledge concerning the continuation of these responses to simple stimuli from one developmental stage to the next. There is some evidence both in the literature and from observation during these experiments that the phototactic and perhaps the *skototactic* response have some bearing on the time of emergence and the subsequent behaviour of the subimago and imago.

Baetis (strongly positively phototactic) emerged from the various experimental apparatus during the day, whereas *Tricorythus* (negatively phototactic) emerged during the late evenings and night. Both Crass (1947) and Barnard (1932) record instances of the emergence of species habitually found beneath stones and those habitually found in an above-stone habitat, at night and during the day respectively. They also mention several cases of apparent subimaginal and imaginal 'preferences' for shaded or sunny regions which can also be correlated with the habitat of the nymphs within the stream.

From the above it appears that the responses shown by the nymphs may be of significance both in water and to the imagos. If this is the case, selection of habitat and consequently oviposition site by the imagos would be another important factor maintaining the nymphs in shaded or open reaches.

Conclusions

The experiments reported here clearly support the claim that the responses of these nymphs to various properties of light are of importance as factors contributing to the nymphs' selection and maintenance of their respective microhabitats. And although this investigation has not been concerned with the exact positioning of the nymphs within their microhabitat, it appears that this, in the case of *Tricorythus discolor*, is the result of the interaction of their light responses, current requirements and marked thigmotaxis, and in the case of *Baetis harrisoni*, the interaction of the light responses and an undefined current

factor probably associated however with the boundary layer (Ambuhl, 1959).

Little is known of the movements of invertebrates within streams. Neave (1930) mentions the annual tendency for nymphs of the mayfly *Blasturus cupidus* Say, to move up small, newly formed tributaries, and Macan (1957) records, in connection with the nymphs of a species of mayfly, that 'the new generation was most abundant at a point further upstream than the old generation'. This, he states, 'Could have been due to movements of the adults before the eggs were laid or to movements of the nymphs after the eggs had hatched'. He describes, however, unpublished work carried out by Dr Janet Harker in which marked nymphs were shown to have moved upstream. Assuming therefore that nymphal movements do take place and that these are directed upstream, as a result of the rheotactic response, the nymphs could be expected to pass through alternately sunlit and shaded regions. When passing through regions of low light intensity, their activity would be considerably reduced resulting in the accumulation of nymphs within these regions. This is sufficient to explain the higher incidence of *Tricorythus* in shaded reaches.

The activity of *Baetis* would also decrease in the shaded regions and we would therefore expect accumulation in such regions, but whether this can be counteracted sufficiently by their attraction to regions of high light intensity and whether they would by virtue of the suppression of the orthokinetic response by the current factor remain in sunlit regions longer than in shaded is a moot point. That they do accumulate in areas of high light intensity is indisputable but at this stage it is impossible to be dogmatic about the mechanisms responsible.

Roos (1957) found that the direction of flight of egg bearing adult stream insects was generally upstream, and Muller (1954) who records the same phenomenon believed that this prevented upstream areas from being depleted by drifting of the nymphal and larval forms. It is here that our lack of knowledge of the imaginal responses is felt. If the imagoes of *Baetis* did respond like the nymphs towards regions of high illumination, the consequent effect on the oviposition site, coupled with the nymphal responses could adequately explain the apparent preferences for regions of high light intensity, even if the movements of the nymphs within the stream were limited. Similarly, if the imagoes of *Tricorythus* were attracted to regions of low light intensity,

this, too, would contribute greatly to the effect of the nymphal responses in accumulating them in shaded reaches.

Acknowledgments

I am grateful to Professor B. I. Balinsky for his guidance and for the provision of facilities in the Department of Zoology, University of the Witwatersrand, and to Professor B. R. Allanson, Department of Zoology, Rhodes University, for his helpful supervision throughout this work. I am also indebted to Dr W. Macnae, Department of Zoology, University of the Witwatersrand, for his suggestions and help in the preparation of the figures and to Dr J. D. Carthy, Department of Zoology, Queen Mary College, London, for his criticisms and advice.

Thanks are also due to the South African Council for Scientific and Industrial Research for the award of a research grant which enabled the completion of this work.

REFERENCES

- Alverdes, F. (1930). Tierpsychologische Analyse der intracentralen Vorgänge welche bei decapoden Krebsen die locomotorischen Reaktionen auf Helligkeit und Dunkelheit bestimmen. *Z. wiss. Zool.*, **137**, 403-475.
- Ambuhl, H. (1959). Die Bedeutung der Strömung als ökologischer Faktor. *Schweiz. Z. Hydrol.*, **21**, 133-264.
- Barnard, K. H. (1932). South African May-flies. *Trans. Roy. Soc. S. Afr.*, **20** (3).
- Coker, R. E. (1954). *Streams, Lakes, Ponds*. University of North Carolina Press.
- Crass, R. S. (1947). The mayflies (Ephemeroptera) of Natal and the Eastern Cape. *Ann. Natal Mus.*, **11** (1).
- Dietrich, W. (1931). Die lokomotorischen Reaktionen der Landasseln auf Licht und Dunkelheit. *Z. wiss. Zool.*, **138**, 187-232.
- Dolley, W. L. & Wierda, J. L. (1929). Relative sensitivity to light of different parts of the compound eye of *Eristalis tenax*. *J. exp. Zool.*, **53**, 129-139.
- Ewer, D. W. & Einar Bursell (1951). A note on the classification of elementary behaviour patterns. *Behaviour*, **3**, 40-47.
- Fraenkel, G. & Gunn, D. L. (1940). *The Orientation of Animals*. Oxford University Press.
- Gunn, D. L., Kennedy, J. S. & Pielou, D. P. (1937). Classification of taxes and kinesis. *Nature, Lond.*, **140**, 1064.
- Gros, A. (1923). Etudes sur les premières stades des Ephemeres du Jura français. *Annls. Biol. lacust.*, **12**, 49-74.
- Harrison, A. D. & Elsworth, J. F. (1958). Hydrobiological studies on the Great Berg River, Western Cape Province. I. *Trans. R. Soc., S. Afr.*, **35** (3), 125-225.
- Hughes, D. A. (1966). On the dorsal light response in a mayfly nymph. *Anim. Behav.*, **14**, 13-16.
- Kennedy, J. S. (1940). The visual responses of flying mosquitoes. *Proc. zool. Soc. Lond.*, **A 109**, 221-242.

- Lees, A. D. (1948). The sensory physiology of the sheep tick, *Ixodes ricinus*. *J. exp. Biol.*, **25**, 145-207.
- Macan, T. T. (1957). The life histories and migrations of the Ephemeroptera in a stony stream. *Trans. Soc. Br. Ent.*, **12**, 129-156.
- Moon, H. P. (1940). An investigation of the movements of freshwater faunas. *J. Anim. Ecol.*, **9**, 76-83.
- Muller, K. (1954). Investigations on the organic drift in north Swedish streams. *Rept. Inst. Freshwater Res. Drottningholm*, **35**, 133-148.
- Neave, N. (1930). Migratory habits of the mayfly *Blasturus cupidus* Say. *Ecology*, **11**, 568-576.
- Percival, E. & Whitehead, H. (1926). Observations on the biology of the mayfly *Ephemera danica* Mull. *Proc. Leeds Phil. Lit. Soc. (Sci. Sec.)*, **1** (3), 136-148.
- Roos, T. (1957). Studies on upstream migration in adult stream dwelling insects. *Rep. Inst. Freshwat. Res. Drottningholm*, **38**, 167-193.
- Shelford, V. E. (1945). Conditions of existence. *Freshwater Biology*, chap. II. Ward & Whipple.
- Ulliyott, P. (1936). The behaviour of *Dendrocoelum lacteum*. I and II. *J. exp. Biol.*, **13**, 253-264, 265-278.
- Welch, P. S. (1952). *Limnology*. New York: McGraw Hill.
- Wodsdalek, J. E. (1911). Phototactic reactions and their reversal in the mayfly nymphs *Heptagenia interpunctata* (Say). *Biol. Bull. Mar. biol. Lab., Woods Hole*, **21**, 265-271.

(Received 26th July 1965; Ms. number: 559B)