Strategies of host location employed by larval trematodes

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ABSTRACT

The behaviour of Echinoparyphium recurvatum and Plagiorchis elegans cercariae and Schistosoma mansoni miracidia were recorded in the unstimulated state and in response to environmental and host-derived stimuli. Quantative analyses of larval behaviour allowed the apparent strategies of host location to be described. Computer simulation models tested the hypotheses put forward.

E.recurvatum cercariae demonstrated active searching host location behaviour. In the vicinity of a snail, swimming speed was reduced and rate of turning increased. The cercariae appeared unable to determine the direction of the host at any distance from it. Computer models indicated that both reduced speed and increased rate of turning are important factors in host location in these cercariae.

P.elegans cercariae showed 'sit-and-wait' host location behaviour. In the vicinity of a chironomid larva, the active periods, swimming speed and rate of turning were increased. Host generated turbulence probably accounted for some of the increase in speed. These cercariae seemed unable to determine the direction of the host at any distance from it. Computer models of the behaviour of these cercariae indicated that increase in speed in the vicinity of the host is the most important factor in host location.

S.mansoni miracidia demonstrated active searching behaviour. Close to a snail, swimming speed was reduced and rate of turning increased. As with the cercariae studied, these miracidia were unable to determine the direction of the host. Miracidia responded to sharp changes in chemical stimulus concentration, the response to decreases being most marked. Computer models showed that both reduced speed and increased rate of turning are important in maximising the chances of host contact. Response time to a stimulus and sensory adaptation were also seen to be factors influencing the chances of host contact.

INTRODUCTION

The results presented in this study describe, for the first time, the host location behaviour of the cercariae of Echinoparyphium recurvatum and Plagiorchis elegans. In addition, techniques for quantitative description of the behaviour of trematode larva have been extended over those of previous workers (Wilson and Denison, 1970a, 1970b; Chapman, 1974; Mason and Fripp, 1976, 1977a, 1977b; Carter, 1978; Roberts et al, 1979). The use of these techniques has allowed a comprehensive investigation of the host-location behaviour of Schistosoma mansoni miracidia to be presented.

Data presented here on the behaviour of S.mansoni miracidia differs from the results of other workers in some important respects. Most significantly, in the present study miracidia have been seen to respond to both sharp increases and decreases in chemical stimulus concentration. Roberts et al. (1979) recorded no response to increases in stimulus concentration. The possible reason for this difference in observations is discussed in relation to the methods used to record the behaviour of miracidia in the two studies.

Increased knowledge of the behaviour of S.mansoni miracidia in the vicinity of a snail host and crossing stimulant boundaries has implicated the importance of response time and sensory adaptation in the host-location process. These new findings have been tested using simple computer simulation models.

CHAPTER 1 THE THEORY AND RECORDING OF HOST LOCATION BEHAVIOUR

1.1. INTRODUCTION TO THE THEORY OF HOST LOCATION STRATEGIES

Digenetic trematodes, in common with other parasites have pronounced multiplicative stages in their life cycles. In trematodes, miracidia and cercariae, the infective larvae, are produced in relatively large numbers. It is assumed that this production of large numbers of infective larvae maximises the chances of contact with the next host. The mortality rate of larvae is high and the probability of an individual larva successfully locating and penetrating its correct host is extremely small. Any behavioural response shown by miracidia or cercariae which enhances the chance of contact with the host is likely, therefore, to be of adaptive advantage. Such behavioural responses are investigated in the present study, the principal aims being to quantify the behaviour of some trematode larvae and attempt to assess the effect of changes in behaviour on the chances of host contact.

1.1.1. Characterisation of a Range of Behaviour Patterns

Direct observation of trematode larvae under the microscope allows subjective analysis of behaviour so that miracidia, for example, in the unstimulated state can be seen to swim in straight lines with

infrequent changes of direction. Using the same method of observation the behaviour in the vicinity of a snail host can be seen to be altered, there being a marked increase in the frequency of changes of direction. But, because direct observation is entirely subjective and non-quantitative, experimenters have arrived at diametrically opposed interpretations of the host location behaviour of Schistosoma mansoni miracidia (Sudds, 1960; MacInnis, 1965) using the classification of orientation behaviour of Fraenkel and Gunn (1940). A better approach is to quantify the behaviour of larvae, but to regard the patterns shown as a continuum rather than restricting it to one or more of the categories devised by Fraenkel and Gunn.

There is no disputing that many parasite larvae, digenetic trematodes included, have evolved behavioural responses which are elicited by environmental and host derived stimuli. It seems reasonable to assume that these responses are of adaptive advantage in that they persist and are found in so many species. Since the sole objective of miracidia and cercariae is to infect the next host it seems likely that observed behavioural responses in these larvae enhance the changes of their being successful. Cercariae locate such a wide variety of hosts it has been postulated that many different kinds of larval behaviour may be shown in different species. Accordingly, an aim of the present study was to investigate the behaviour of a number of different trematode larvae. This investigation generally fell into two parts, the study of responses to environmental stimuli, particularly directional light and gravity, and the study of responses to host-derived stimuli. As part of these studies, the movement of populations of larvae and

individuals within a responding population were recorded.

1.1.2. Evaluation of Behaviour Patterns as Strategies for Host Location

Schoener (1971), in looking at predator-prey interactions, classified predators into two categories, 'sit and wait' predators which expend no more energy in searching for prey than they would expend on other facets of their life, and 'active' predators which expend significant amounts of time and energy for the sole purpose of foraging for food.

By analogy, the host-location behaviour of larval trematodes can be classified into similar categories. Studies on the cercariae of S. mansoni (Carter, 1978) and Cryptocotyle lingua (Chapman, 1971) suggest the adoption of a 'sit and wait' strategy for host location. These cercariae minimise their energy expenditure in the absence of stimulation by using alternate vertical swimming and sinking periods to maintain a virtually stationary position in the water. When a potential host enters their vicinity, the cercariae respond by increasing their activity. It has been postulated that this change in behaviour enhances the chance of contact with the host during the brief period when it is near the cercariae. If no contact is made, and the host leaves the vicinity of the cercariae, their behaviour reverts to that of alternate swimming and sinking. By contrast, previous work on the miracidia of S.mansoni (Carter, 1978) and the cercariae of Himasthla secunda (Chapman, 1971) has indicated the use of an 'active' strategy for host location. These larvae swim

continuously and cover considerable distances in an apparently random search for their hosts. In the vicinity of their different molluscan hosts both S.mansoni miracidia and H.secunda cercariae respond so as to restrict the area covered. It is supposed that this change in behaviour increases their chance of locating their host by increasing the amount of time they spend in the vicinity.

Since trematode larvae do not feed but draw their energy from fixed food reserves, it seems likely that the two alternative strategies of host location have evolved as a response to differences in host behaviour. The energy expenditure of the larvae is minimised by adoption of one or the other host location strategy. Thus slow moving and stationary hosts, such as gastropod and lamellibranch molluscs, may be located by active, searching larvae. Hosts which move rapidly or are stationary only for transient periods, such as humans and fish, may be located by larvae displaying 'sit and wait' behaviour.

Although it has been suggested above that changes in behaviour elicited by the presence of a host serve to increase the probability of contact by trematode larvae, the assertion requires proof. In order to go some way towards satisfying this requirement computer simulation models of host-parasite interactions have been produced. A similar approach has been used to explain the aggregation behaviour of a variety of invertebrates (Patlak, 1953), and in a purely theroretical context (Rohlf and Davenport, 1969). The value of a model is that in a single system all the factors considered important can be combined in such a way as to represent their interaction in nature. In this way, manipulations of the system can

be made which would be impossible on the natural system. This in turn, allows investigation of the effect of individual parameters of behaviour and the cumulative effect of combinations of parameters on the chances of contact with the host. A model is, therefore, a test of the extent to which the biological processes of the natural system are understood.

1.2. TECHNIQUES FOR RECORDING THE MOVEMENT OF MICROSCOPIC ORGANISMS

The microscopic size of bacteria, protozoa and some metazoan larvae makes the recording of their movement very difficult. The major problem is designing an optical arrangement with sufficient magnification to resolve the organism studies yet still retain a field of view large enough to be of use. The need for high magnification also results in reduced depth of focus, so unless the recording system can be made to include a third dimensional or focussing component, the movement of the studied organism must be restricted in one plane to the focal depth of the optical system. In this event the movements of an organism are, effectively, recorded in two dimensions, but in reality this is a two-dimensional representation of a three-dimensional process. Artefacts are introduced, most notably in the calculation of the distance between two points. Any movement in the third, restricted plane results in an underestimation of the distance between two points.

The need to record the behaviour of microorganisms has led to the design of many systems, each with their good and bad points, some of

which are described below.

1.2.1. Sophisticated Designs

Davenport (1969) described the primary criteria for the investigation of the motile behaviour of microorganisms as the ability 'to observe and at the same time record quantitatively, the movements of a significant sample of individuals in an open, unrestricted preparation'. In order to achieve this aim he developed his 'Bugwatcher', basically a flying spot microscope connected to a digital computer via a preprocessing interface. Despite its technological complexity this apparatus suffered from a restricted depth of focus because of the high magnification required. As a result his records consisted of a two-dimensional representation of three dimensional behaviour and included the inherent artefacts.

Two systems for tracking microorganisms in three dimensions have been described. Berg (1971) used three pairs of fibre optics positioned so as to view each of the three axes in a test cell. Each pair was focussed at different distances withing its axis so that a cube in space was produced. A single bacterium within the cube of space would appear partially in focus to all of the fibre optics. When the bacterium moved, the balance of the degree of focus within fibre optic pairs was upset and a servo mechanism was electronically engaged to restore the balance by repositioning the cell. In this way, the movements of the cell represented a three dimensional record of the movement of the bacterium (see also Berg &

Brown, 1972).

Lovely et al (1974) used a similar system to that of Berg but used a human operator to reposition the test cell, by a joystick in the x and y axes and a foot control in the z axis or focus. Both three dimensional tracking systems suffered from problems of inertia such that exceptionally rapid changes in direction were not recorded accurately. Where human operators were used there was the additional problem of response time of the operator. Both systems were accurate to only one organisms' length, with the result that a microorganism rotating about its own axis appeared stationary. A major disadvantage of these systems and their possible application to studying trematode larvae is that the test cell has to be moved. Some cercariae are known to be stimulated by mechanical shock (Donges, 1964; Haas, 1969) and it would not be possible to record their behaviour without that response being elicited.

1.2.2. Previous Experimental Techniques with Trematode Larvae

Many attempts have been made to describe the behaviour of trematode larvae using a wide variety of techniques. At the simplest level these have included work by Faust (1924), Miller and McCoy (1930), Miller and Mahaffy (1930),Okamoto (1962), MacInnis (1965) and Chernin (1970) who all recorded the behaviour of trematode larvae in descriptive terms based on microscopic observations. Although these records are of value in indicating the type of behavioural responses which may occur in the studied species, there was no attempt to quantify them.

Campbell and Todd (1955) made an early attempt to track larvae when studying Fascioloides magna miracidia in the vicinity of their snail host. They plotted the position of miracidia at regular time intervals on a piece of graph paper below a petri dish containing the miracidia and a target snail in a suitable volume of water. Clearly accuracy was a problem and points could only be recorded at rather large time intervals. To overcome these problems Wilson and Denison (1970a, 1970b), Chapman (1974), Mason and Fripp (1976, 1977a, 1977b), Roberts et al (1979) and Plorin and Gilbertson (1981a) used long, single exposures in photographically recording the path of a variety of trematode larvae. Whilst accuracy and continuous recording were achieved by their techniques, the length of the recorded tracks was necessarily short and long periods of continuous observation were not possible.

An obvious development from long, single exposures is the use of cinematography to record multiple short exposures. Rather suprisingly there are few accounts in the literature of attempts to track trematode larvae by this technique. The assay system for miracidial behaviour described by Sponholtz and Short (1975), the track analysis of schistosome larvae by Carter (1978) and the study of body movements and ciliary action in schistome miracidial turning (Roberts et al, 1980) are notable exceptions. Cinematography has, however, been used by Haas (1974a, 1974b) to record the invasion mechanism of the cercariae of Diplostomum spathaceum, and by Chapman and Wilson (1973) to record the action of the tail of Himasthla secunda and Cryptocotyle lingua cercariae during swimming.

The above techniques have all been used to study the behaviour of particular trematode larvae by observing individuals. Another approach has been the interpretation of the behaviour of larvae based on the distribution of a population in an experimental system. The most documented of these techniques involves the use of 'choice chambers'. Yasuroaka (1953, 1954), Takahashi et al (1961), Etges and Decker (1963) and Roberts et al (1978) have all used variations on this technique. Generally trematode larvae were introduced into a central well in a test chamber bearing a number of side arms. Experimental variables such as target hosts or test chemicals were placed in the side arms and the distribution of the larvae was recorded after a suitable period of time. Problems arose, expecially when target hosts were used since the larvae disappear from the system if they penetrate a potential host or are predated upon. Shiff (1969, 1970) used the phenomenon of miracidial disappearance in studying the infection of Bulinus globosus snails by Schistosoma haematobium miracidia under various conditions. His major problem was that when the snails were sampled destructively at sufficient time after the experiments for development to have occurred,the extent of infection gave no reliable indication as to the number of miracidia which penetrated the snail or, indeed, the mechanism by which they achieved contact.

Nansen et al (1976) used radio-isotopically labelled Fasciola hepatica miracidia to determine their attraction to and ability to penetrate various aquatic snail species. Whereas the technique allowed the experimenters to determine relative extents of infection (or the lack of it) in the various snail species studies, again the mechanisms by which contact was established by the miracidia was not

described. In addition there was a possibility that the radio-labelling of the miracidia affected their behaviour and their ability to penetrate a host.

1.2.3. Methods Used in the Current Study

The techniques used in this study of trematode larval behaviour were extensions of those described by Carter (1978). The study used two main approaches, the cinephotography of populations of larvae from which individual tracks were analysed, and the recording of the movements of population of larvae using time lapse photography. Attempts have been made, wherever possible, to explain the distribution of a population of larvae in terms of the behaviour of individuals.

Confining larvae for study

In common with the techniques used by others in studying the behaviour of small motile organisms, the experiments described here used the principle of restricting movement in the third dimension. This was achieved by the use of specially constructed fused glass cells with internal dimensions of 70 x 50 x 1 mm (see Figure 1.1.) or by restricting water level so that the studied larvae could be maintained within the depth of field of the optical apparatus at the magnification used. By performing some experiments in two planes it has been possible to make inferences about the three dimensional behaviour of the studied larvae.

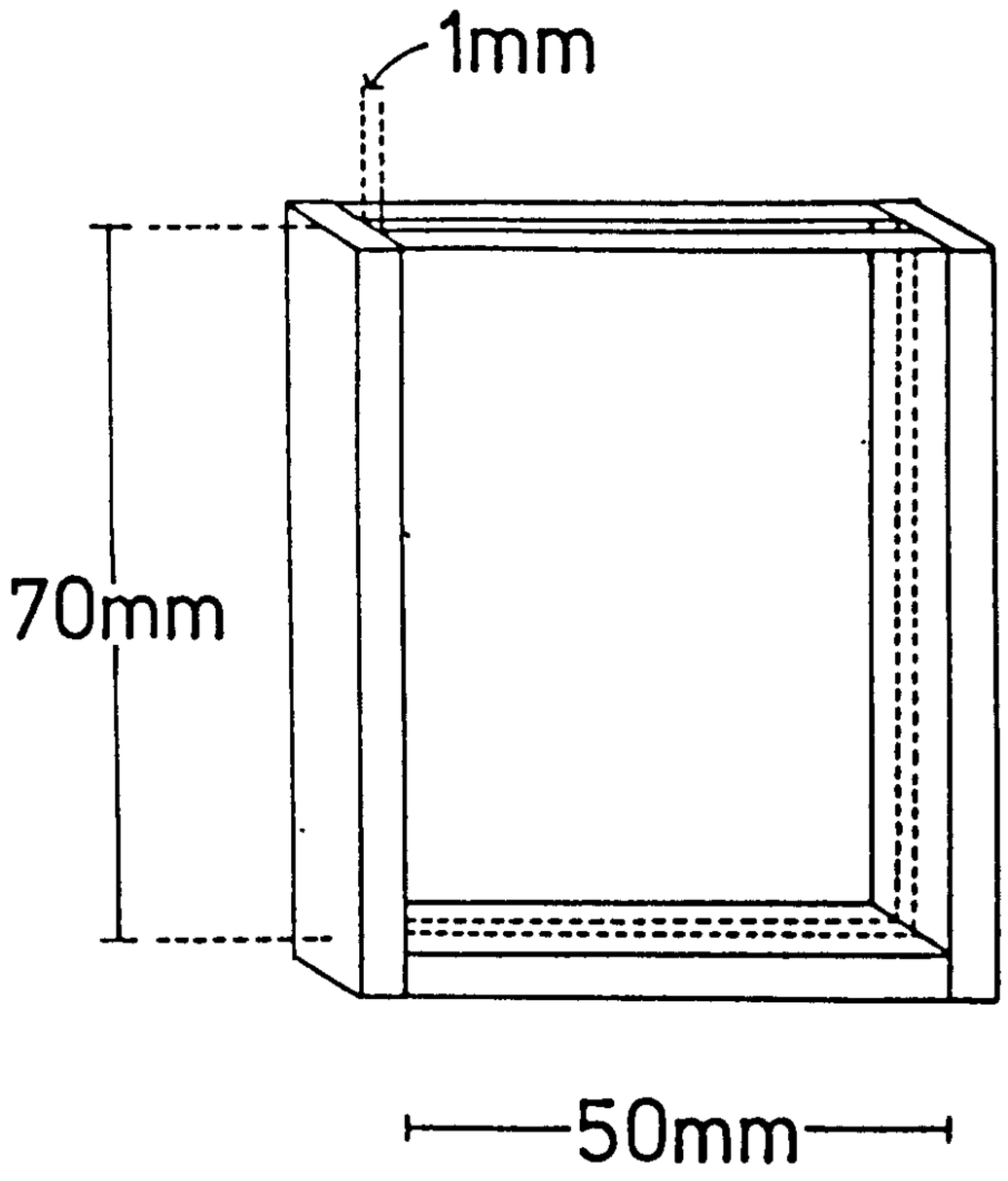


Figure 1.1.

The test cell, made up of fused glass blocks, used to confine larvae so as to limit their movement in the third dimension.

Figure 1.2.a

Experimental apparatus used for cinephotography, horizontal plane.

Key:

- c. cine camera
- c.l. condenser lens
- c.r. camera rostrum
- g.s. glass sheet
- m. motor for cine camera
- o.b. marble slab
- p.m. plane mirror
- p.s. power supply for cine camera
- r. rheostat
- r.c. remote control for cine camera
- t.c. test cell
- t.p. tungsten filament photoflood bulb

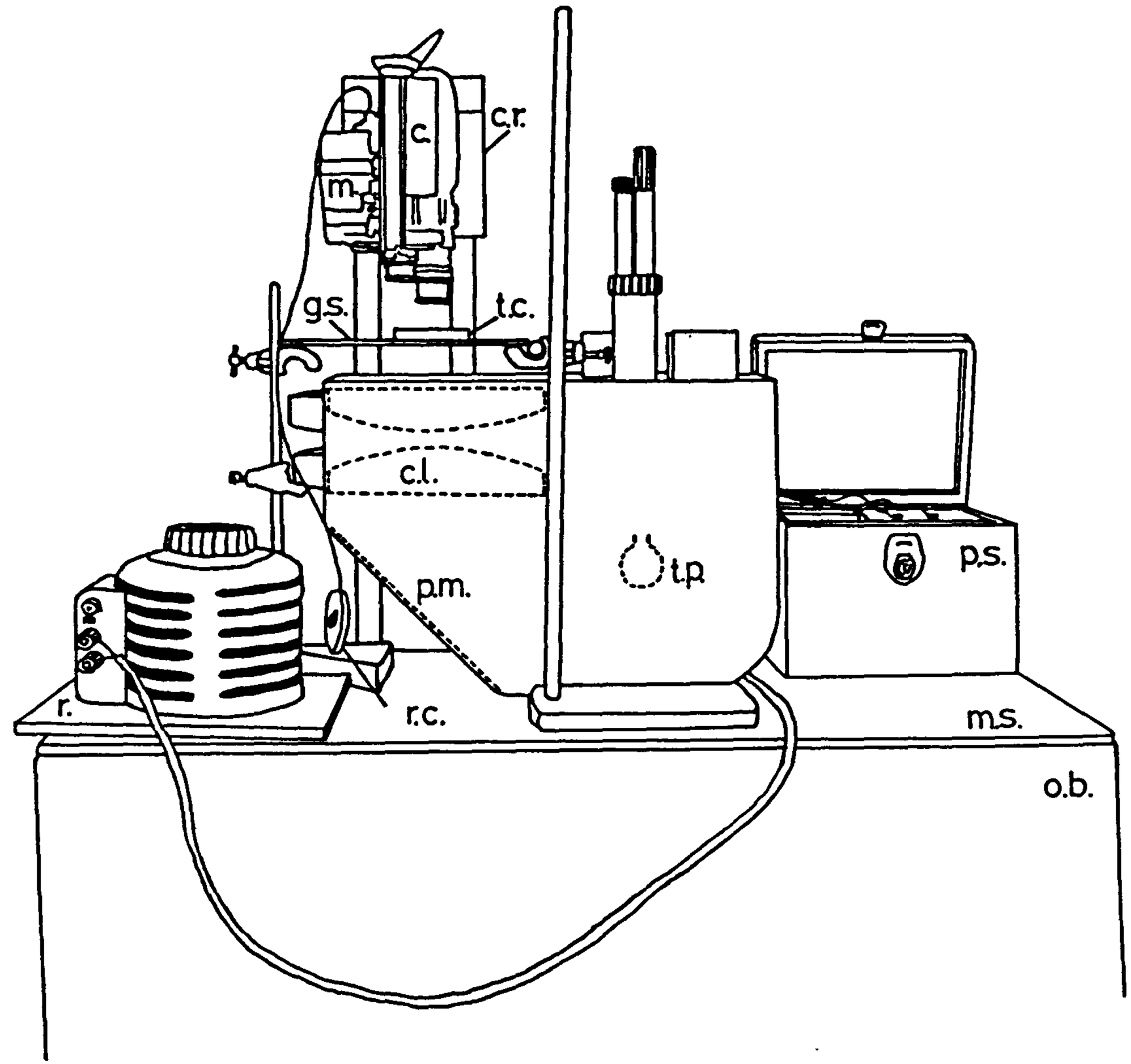
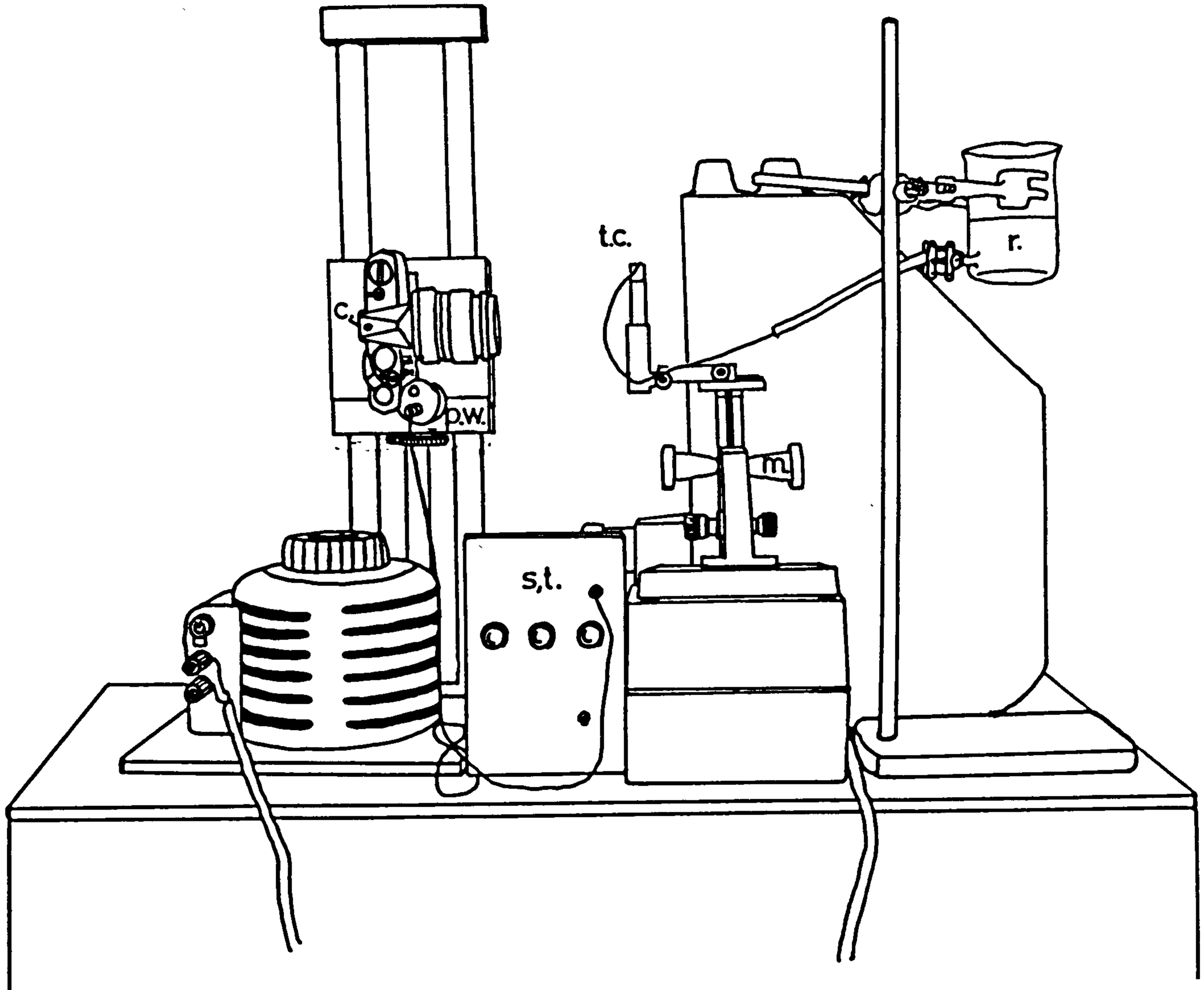

c
c.r.
m.
g.s.
t.c.
c.l.
p.m.
t.p.
p.s.
r.
r.c.
m.s.
o.b.

Figure 1.2.b.

Experimental apparatus used for time lapse photography, vertical plane.

Key:
- c. single lens reflex camera
- p.w. power winder
- r. reservoir system
- s.t. sequential timer
- t.c. test cell

(differences only from Figure 1.2.a. indicated)


t.c.
r.
C.
D.W.
m
s.t.

Optical apparatus

The most important requirement of the optical set up was a large area of even illumination. This was achieved by the use of a Durst Laborator 138S enlarger lighting hood. This compact piece of apparatus gave a field of even illumination of 190 by 150 mm. A 150 watt tungsten photoflood bulb was used with a variable voltage between 0 and 240 volts. In this way the light intensity could be varied as required. An anticipated problem was the transfer of heat from the lighting hood to the experimental test chamber. However, the tungsten photoflood bulb was rarely run at more than 110 volts, and with an air gap of 10 cm which was routinely used, heat transfer was not evident at all. The compactness of the whole lighting system enabled experiments to be carried out in the horizontal and vertical planes with equal ease (see Figures 1.2.a) and b)). All the experiments were carried out on an optical bench with a camera mount, and in a continuous temperature room. Details of temperature are given in the relevant sections later. The experiments were carried out in the absence of any light but that supplied by the experimental apparatus, except where the influence of a directional light source was being investigated.

There were some limitations of the experimental set up. Firstly, it was not possible to complete the full complement of experiments in the horizontal and vertical planes for logistic reasons. Principally these were the inability to introduce host animals into the fused glass cells because of their size. Ways of overcoming

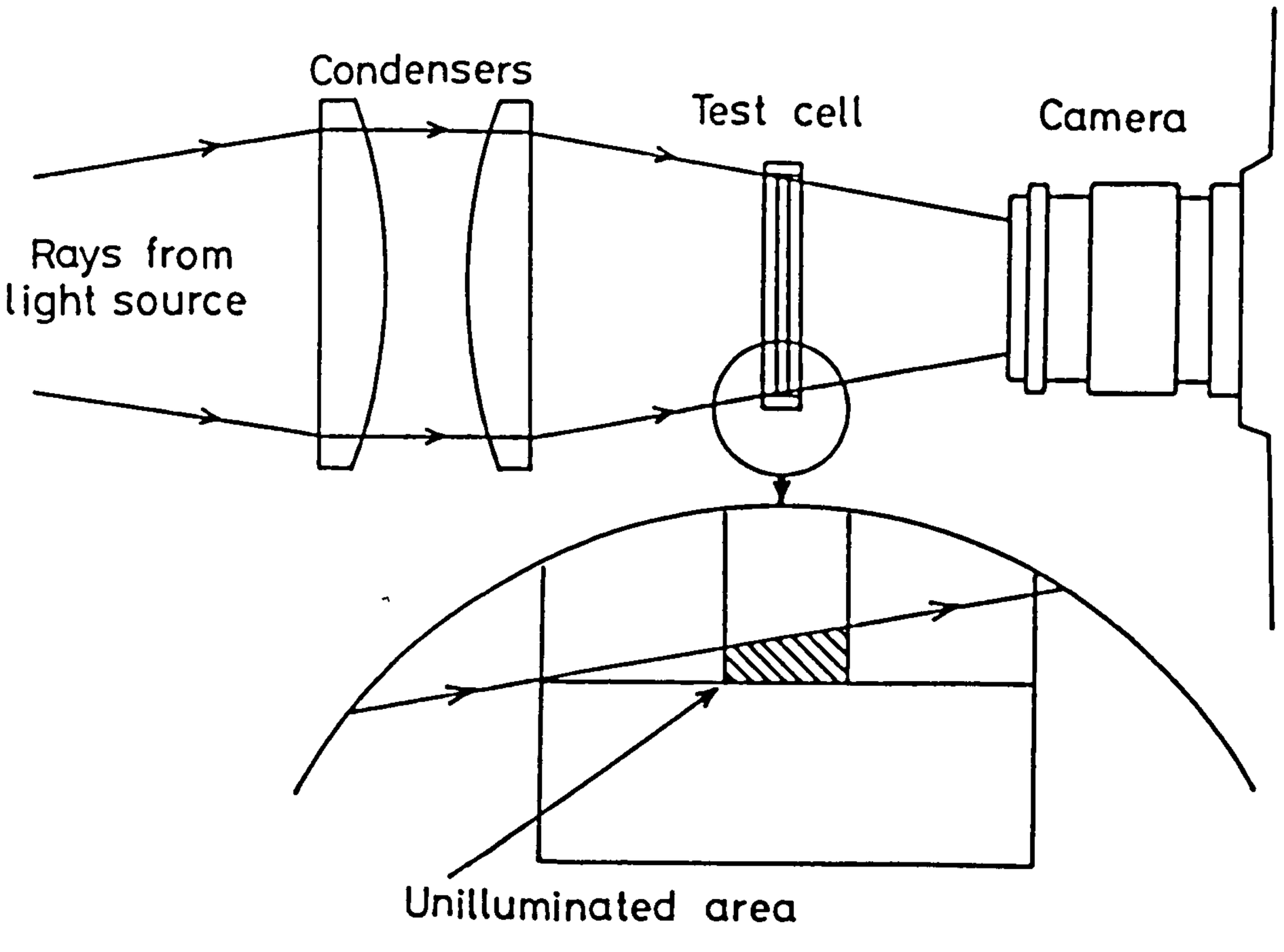


Figure 1.3.

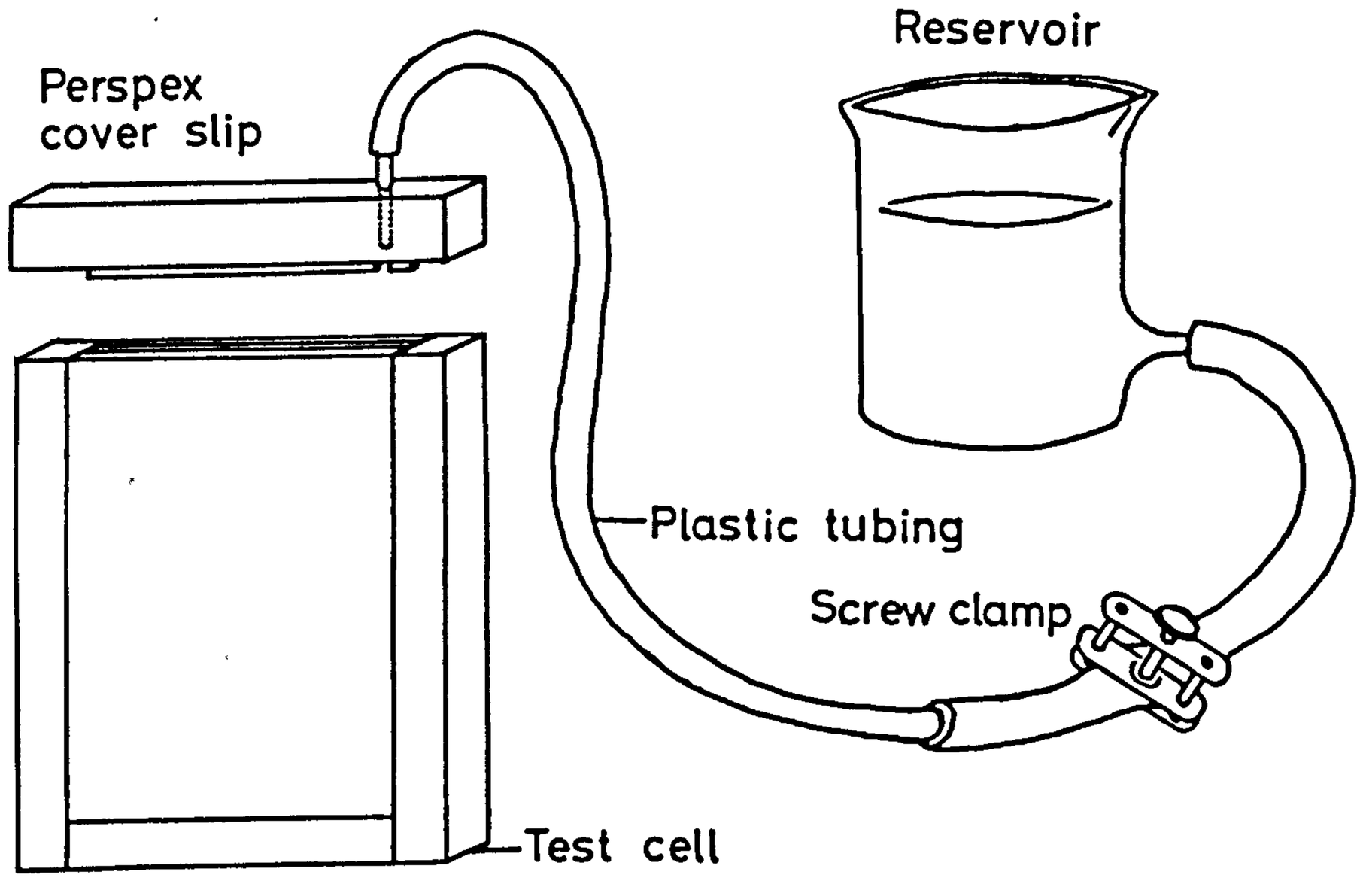
Diagram to illustrate the position of unilluminated areas in the test cell during experiments.

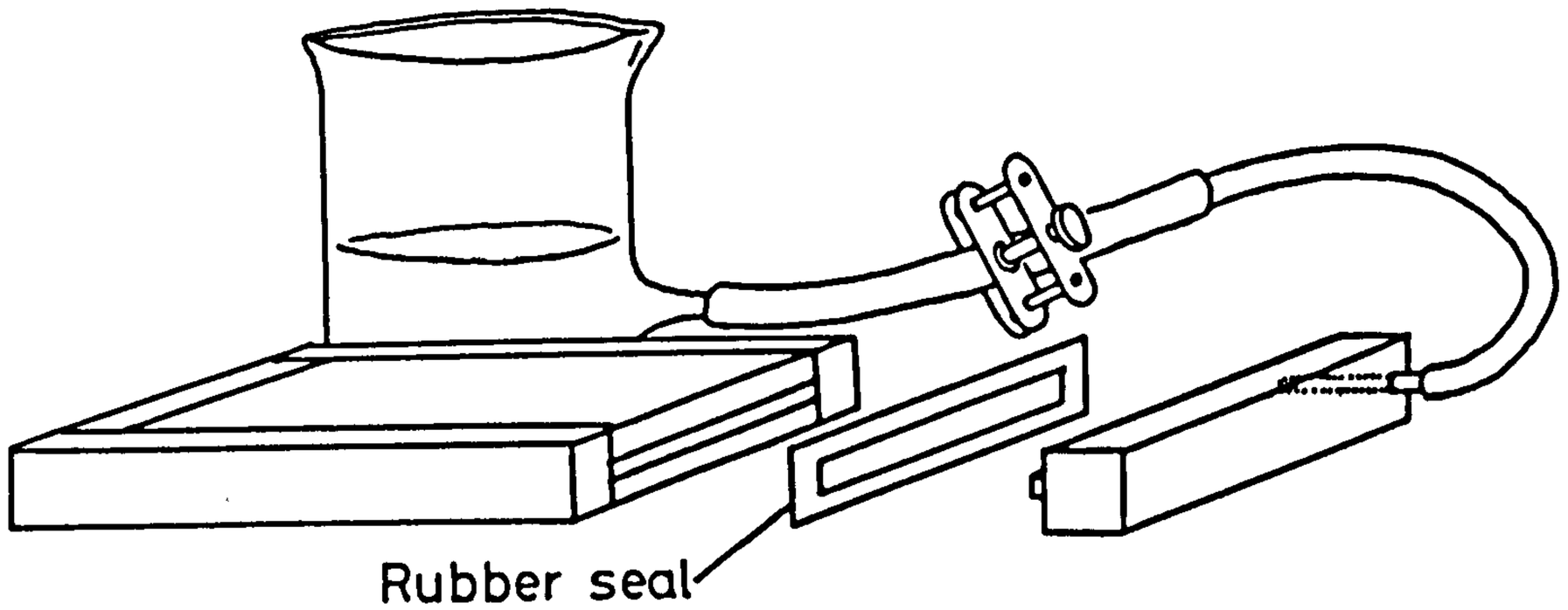
Figure 1.4.

The reservoir system used when confining larvae in the test cell for extended periods.

a) As used in the vertical plane

b) As used in the horizontal plane


Perspex
cover slip
Reservoir
Plastic tubing
Screw clamp
Test cell


Rubber seal

this problem are discussed as and when they arise later. Secondly, the construction of the fused glass cells and the way light passed through them led to small unilluminated areas around the edges (see Figure 1.3.). These 'blind spots' were very small but probably accounted for the disappearance of larvae in some of the studies of population movements. Thirdly, in those experiments which ran for more than a few minutes, essentially the single shot time lapse experiments, there was the need to compensate for evaporation of water from the fused glass cells. A reservoir system was devised in which water evaporating off was replaced with distilled water to prevent concentration of salts (see Figures 1.4a) and b)). In the vertical plane the cover piece of the reservoir system merely slotted into the top, open edge of the cell, but in the horizontal plane a rubber seal was used, the cover piece being held in position by a strong rubber band. Establishing the correct rate of flow from the reservoir was always difficult since the water evaporating off had to be replaced so as not to create an air bubble in the cell. Equally it was not desirable to have a continuous flow of water down the outside of the cell.

Time lapse camera equipment

For the time lapse experiments an Olympus OM1 camera fitted with a 50 mm f3.5 auto macro lens was used. In addition a range of extension tubes were available to increase magnification when desired. The camera was automated using an Olympus Winder 1 and a specially constructed sequential timer. The timer operated the winder by closing a circuit at a regular, predetermined time

interval. The time interval could be regulated in steps of one minute from sixty seconds to two hours. Accuracy was to within two seconds over one hour at 18°C. Kodak Plus-X Pan film rated at 125 ASA was used throughout. Since the photoflood bulb was run at 110 volts for most of the experiments the usual exposure was 1/125 at f11.

Development of time lapse exposures

Exposed films were developed using Kodak Microdol-X developer and fixed with Kodafix. For the earlier experiments each negative was printed on a 10 x 8 inch sheet of grade 3 Ilfoprint photographic paper. However, this became unnecessary as it was later possible to undertake analysis directly from the exposed developed films.

Analysis of time lapse photographs

The latterly developed means of analysis used a photographic enlarger mounted above a digitiser interfaced to Hewlett Packard Model 30 minicomputer (see Figure 1.5.). The digitiser and enlarger were enshrouded in a light proof hood which enabled details to be distinguished on negatives projected onto the top of the digitiser. The digitiser was capable of reading off cartesian co-ordinates from an origin which could be positioned as required. The special function keys on the Model 30 minicomputer were used for several purposes:

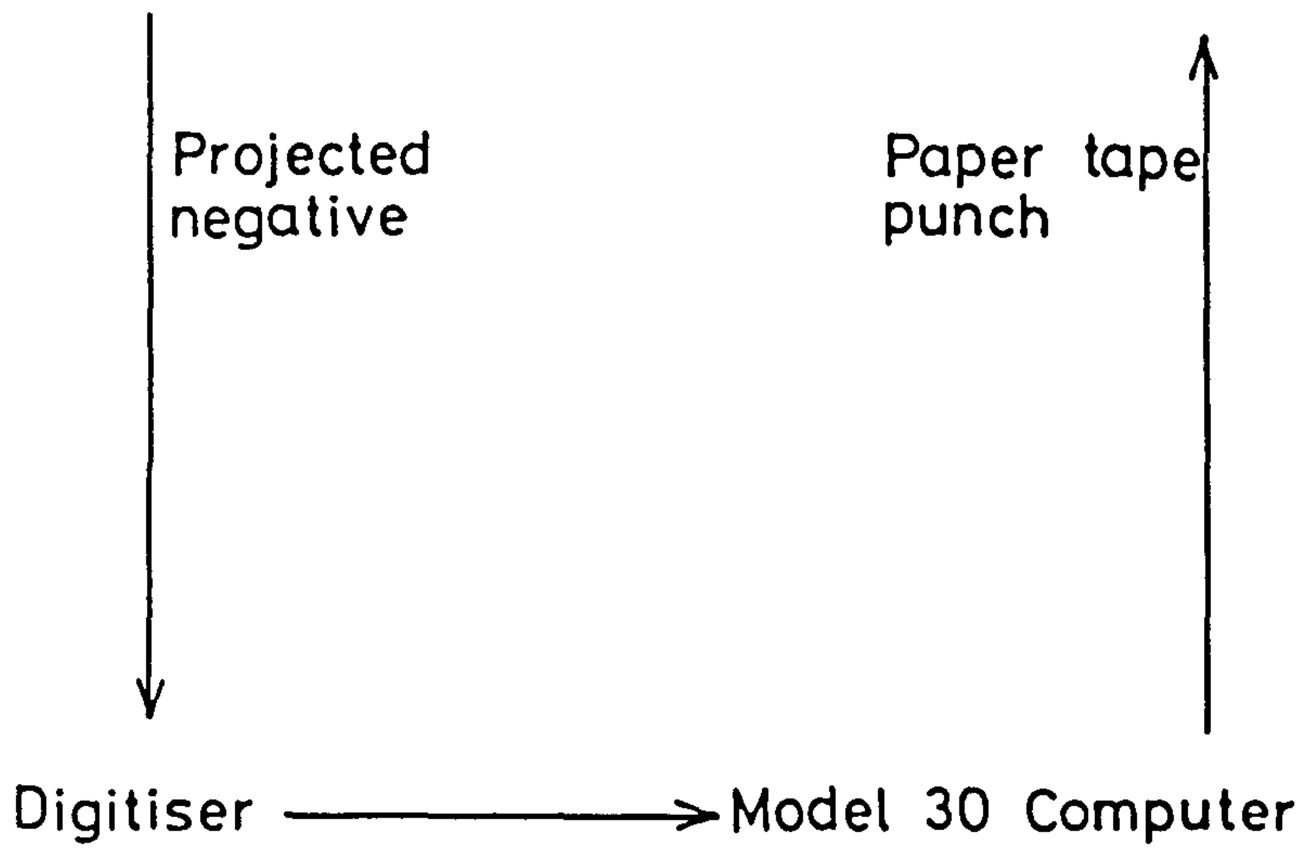


Figure 1.5

Schematic diagram to demonstrate the transfer of larval positions on paper to computer storage.

1) Aligning a perpendicular on the displayed negative to the y axis of the digitiser.

2) Reading off cartesian co-ordinates and converting them to integers. Since the digitiser reads, and is accurate to, one thousandths of an inch, all the data was multiplied by 1000.

3) Punching data onto paper tape.

4) Inserting markers to indicate the beginning and end of a data set.

When considering a negative from an experiment on population movements the following procedure was followed.

A) Display negative on digitiser board

B) Use align function to align upright of internal face of cell to y axis.

C) Set origin at bottom left corner of cell.

D) Read off the three other corners.

E) Insert character to denote commencement of dataset.

F) Plot positions of larvae.

G) Insert character to denote end of dataset.

The procedure was repeated for each negative in an experiment inserting characters to denote where new negatives were being considered. The resulting paper tapes were fed into the University's Dec System-10 Mainframe computer. Computer programs written in Algol were used to analyse the data. These were capable of counting up the number of larvae in each of the one centimetre bands comprising the fused glass cell. Depending on the type of experiment, the data was used in different ways. This will be explained in the relevant sections later.

Cine film camera equipment

Exactly the same apparatus was used for experiments involving cinephotography but the Olympus OM1 camera was replaced by a Paillard Bolex H16 Reflex 16 mm cine camera. This was powered by an electric motor from a remote battery source. A Pizar 25 mm lens was used throughout, and again extension tubes were available to increase magnification when required. Kodak Plus-X Negative 7231 film, rated at 64 ASA (tungsten) in one hundred foot lengths was used throughout. On each film the face of a digital watch was recorded to enable accurate calculation of film speed in frames per second. In addition, since the entire cell was not visible in the frame of the cine camera an eyepiece graticule was filmed to give accurate linear scaling. Exposed films were developed by Filmatic Laboratories Ltd, London prior to analysis.

Reconstructing tracks

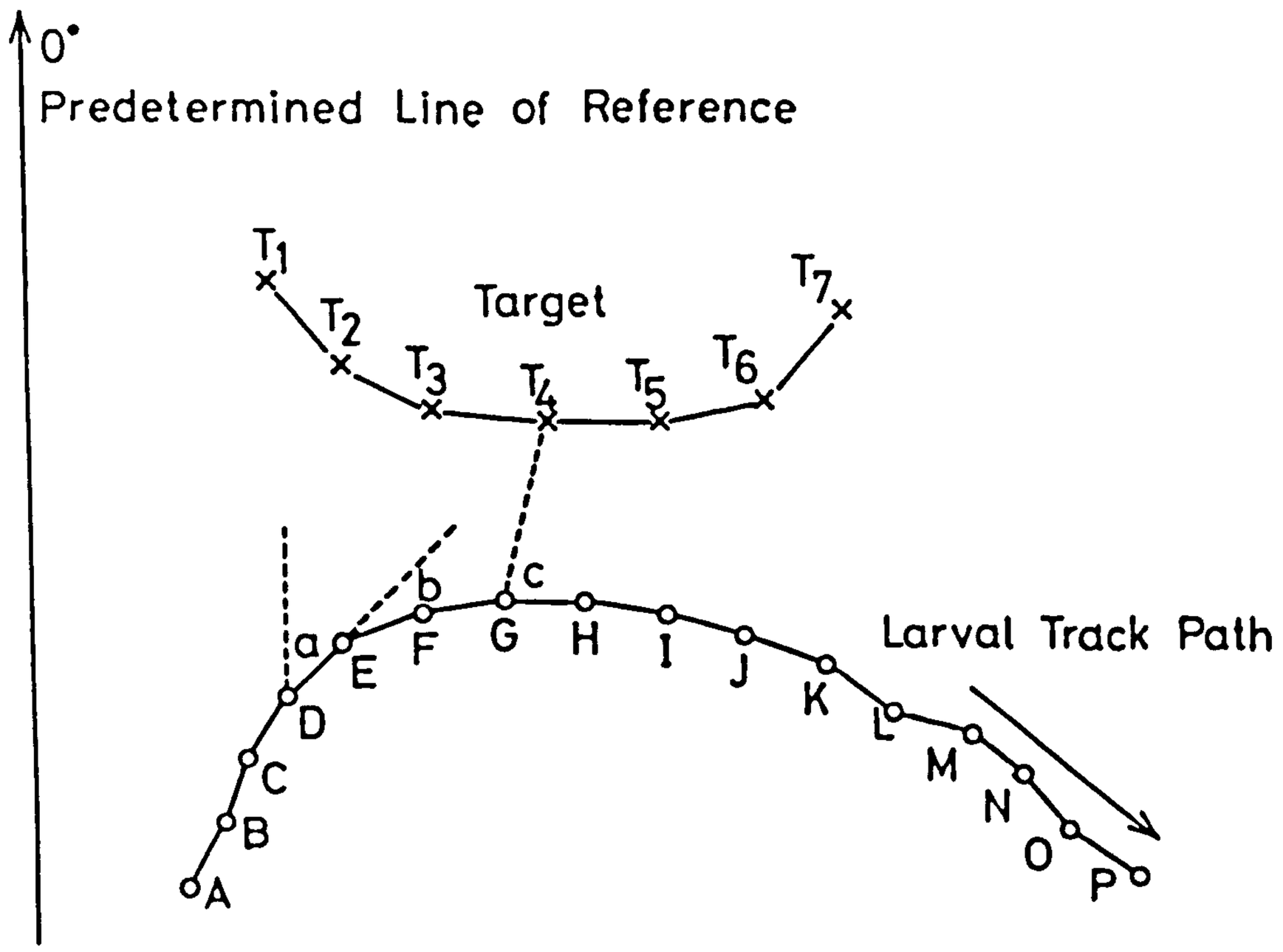
Processed films were analysed using a Spectro Mk III Motion Analysis Projector. This enabled the film to be advanced or reversed at required frame intervals. Tracks of larval swimming paths were plotted onto paper sheets at regular frame intervals. The tracks were selected by a number of criteria (see individual experiments) to ensure that representative samples of tracks were taken. The filmed eyepiece graticule gave accurate linear scaling factors for the x and y axes and the time between frames was calculated from observations on the watch face.

The Hewlett Packard Model 30 computer and digitiser was used to transfer the plotted data onto paper tape for input to the University mainframe computer. Scaling factors and code marks to indicate the beginning and end of individual tracks were included in the data.

Track analysis

Tracks were analysed in much the same way as Carter (1978) but with a number of important additions. Computer programs written in Algol were used to calculate four basic parameters for each 'step' between successive points of an observed track. These were: (see also Figure 1.6.)

1) 'Step length' - the distance between successive



	Example
Step Length	A-B
Angle of Direction	a
Angle of Turn	b
String Length	(B-L) 9 Right
Distance from Target	$G-T_4$
Angle of Direction Relative to Target	c

Figure 1.6.

The parameters of movement described by track analysis.

points of the track.

2) 'Direction' - the direction of the step relative to a predetermined point of reference.

3) 'Angle of turn' - the angle turned through between successive steps.

4) 'Sign of turn' - the turning of right or left (+ or -) relative to the previous step.

Where the movement of the larvae tracked was in the vicinity of a potential host two more parameters were considered, the host being recorded as an array of points to describe its outline, updated at regular frame intervals. The extra parameters are:

5) 'Distance from target' - the distance of the point at the end of the step from the nearest point of the target array.

6) 'Direction relative to target' - the deviation of a step from a path directly to the nearest target point.

In order to compare individual tracks with one another, and a series of tracks under two differing experimental conditions, the above parameters were averaged for the duration of each individual track, and for a group of tracks under the same experimental conditions.

Figure 1.7

Examples of the effect of plotting frequency on the accuracy of recording tracks:

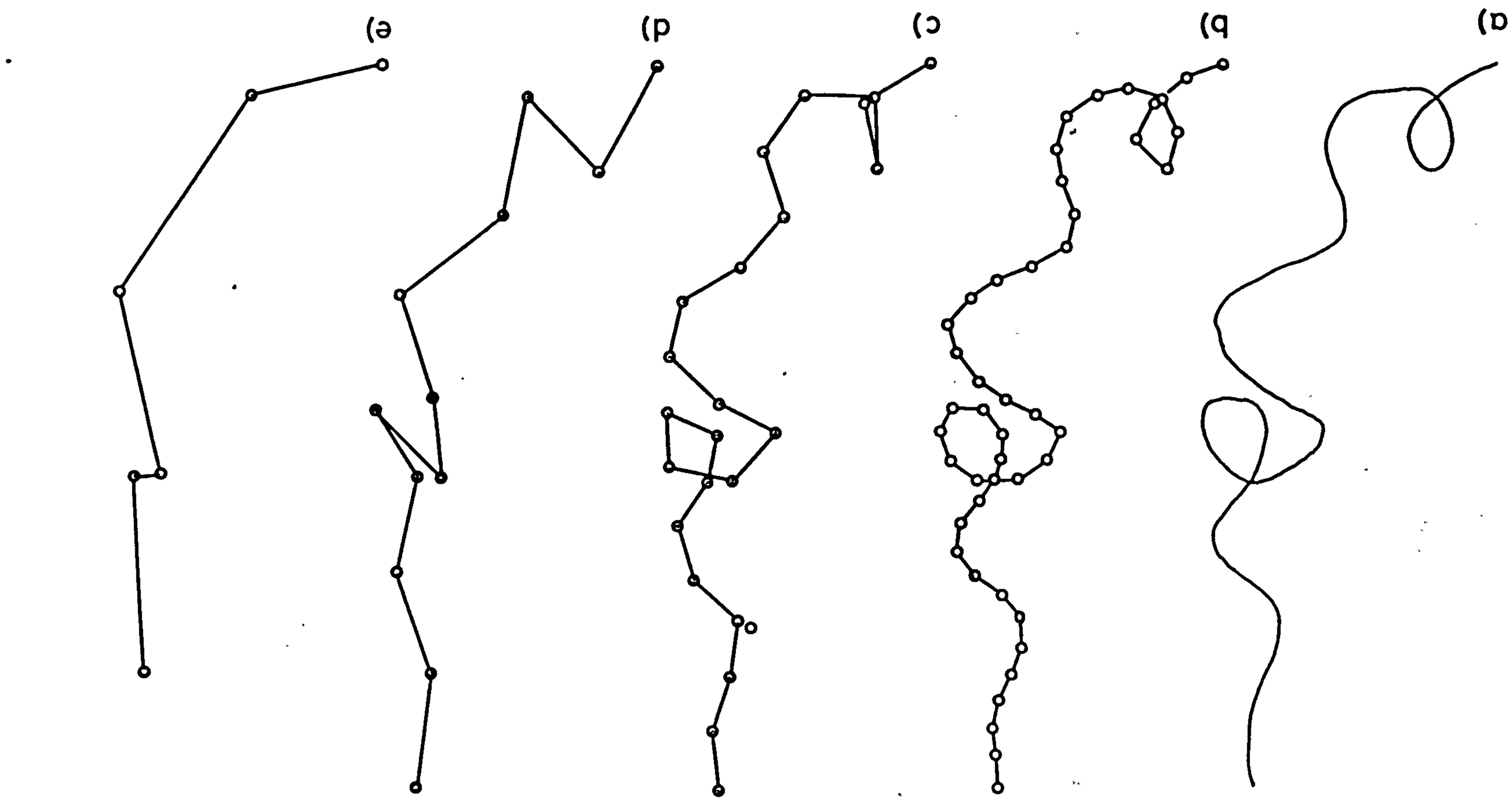
a) Actual track path

b) Adequate representation

c) Half frequency of b) some detail of tightest turns lost

d) Half frequency of c) much detail now lost

e) Half frequency of d), gross over-simplification


a)
b)
c)
d)
e)

Whilst these parameters can be calculated for any two sets of data they need to be standardised before they are directly comparable. In addition the calculation of some of the parameters was carried out so as to aid the identification of behavioural responses. When plotting tracks from film it was important to take enough data points per unit time to describe adequately the path of the larvae concerned. For example different plotting frequencies can give totally different impressions of reality (see Figure 1.7.). As a general rule it was decided that the plotting interval should be sufficiently small that the step length could not exceed half the radius of the tightest turn demonstrated. In this way, gross over-simplification of track paths was avoided.

Some of the parameters considered are time dependent and unless the time between plotted points is the same for two experiments, the results cannot be easily compared. This difficulty was avoided by creating new parameters of 'step speed' and 'rate of turning'. These quite simply were 'step length' and 'angle of turn' expressed per unit time. The mean values of these parameters are comparable when different plotting intervals have been used, provided in both cases over-simplification of track paths has not been allowed to occur.

Parameters involving direction are considered so as to identify behavioural responses to environmental or host derived stimuli. But, for individual steps the precise direction of movement needs to be described. Although the conventional method of representing direction as bearings satisfies this latter requirement, mean values for a track or series of tracks become useless (see Figure 1.8.).

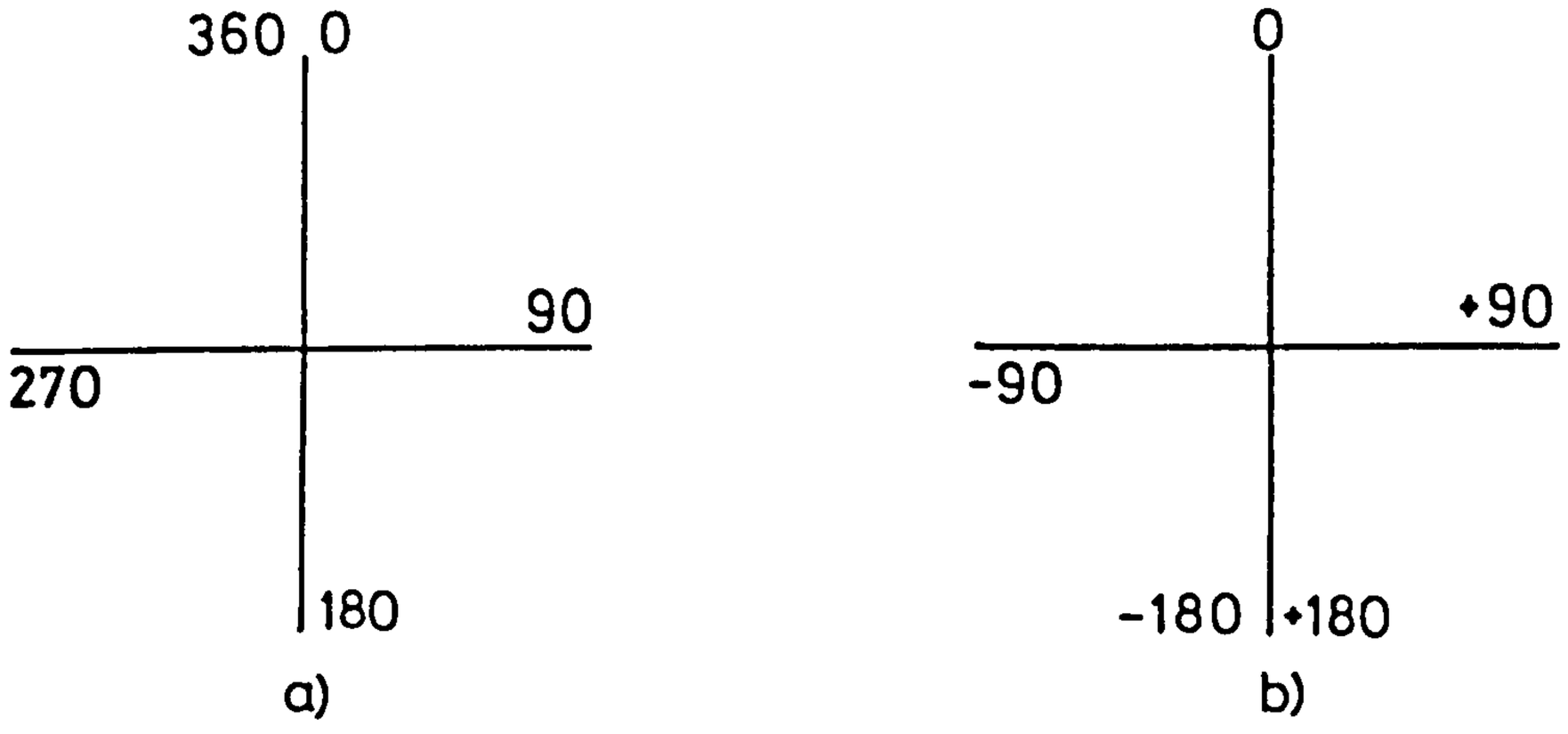


Figure 1.8

Two methods of representing direction:

a) As used when describing bearings

b) As used in track analysis

Method b) has the advantage in that absolute mean values for a series of observations can be used to identify directional responses to a stimulus.

For example

A response causing larvae to swim directly up the page here would give a theoretical mean value for direction of:

a) 180^{o} b) 0^{o}

The complete reverse of the above response would give theoretical mean values for direction of:

a) 180^{o} c)180^{o}

For this reason a system was devised to consider direction on a scale of 0 to 180 degress to the left (-) or right (+) of the predetermined line of reference (or in the case of 'direction relative to target', of the path to the nearest targer point). Using the means of absolute values then enables one to distinguish three behavioural responses in a population (see Figure 1.8. and explanation).

The track analysis programs used also calculated other parameters for a track on a step by step basis. The most important of these were string length (the number of consecutive turns of the same sign) and the duration of active and inactive periods.

CHAPTER 2: THE BEHAVIOUR OF THE CERCARIA OF ECHINOPARYPHIUM RECURVATUM

2.1. INTRODUCTION

Trematodes of the Family Echinostomatidae differ morphologically from other trematodes in the possession of a spiny head collar. Both cercariae and adults show this feature which is clearly visible using low power microscopy. Echinostomes are generally parasites of birds or mammals and often show low specificity of intermediate hosts. In many species miracidia will penetrate and develop in a wide range of molluscan hosts, with no apparent detriment to redial development. Cercariae typically show a similar lack of specificity for hosts and locate and penetrate a wide range of hosts including molluscs, aquatic insects and leeches. The resulting metacercarial cysts are taken up by the bird or mammalian definitive host when the primary host is ingested.

Because echinostome cercariae locate molluscan hosts, which are slow moving or sedentary, it was decided to analyse the cercarial host location behaviour of one species of this group to enable comparison with the host location behaviour of other trematode larvae which locate hosts with different life styles. Very few accounts of echinostome cercarial behaviour exist in the literature. In those that do exist observations are subjective and non-quantative (for example Khan 1961a). A population of snails, Lymnaea pereger, in a

drainage ditch adjacent to University playing fields (Map Reference 623500 -York extract) was found to have a high incidence of Echinoparyphium recurvatum infections. Evidence of the presence of infection was produced by 'shedding' - isolating snails in solid watch glasses for an hour and examining for the presence of free-swimming cercariae. Other infections were found in the snail population, but a population of snails shedding only E.recurvatum cercariae was eventually established in an aquarium kept in a continuous temperature room at 19°C on a 12 hours light, 12 hours dark cycle.

Freeswimming E.recurvatum cercariae were observed in the vicinity of a range of fauna found in the drainage ditch harbouring the L.pereger population. The snails themselves were found to be actively located by the cercariae which attached and formed metacercarial cysts. Accordingly it was decided to investigate the host location behaviour of E.recurvatum cercariae using infected L.pereger snails as target hosts.

2.2. THE BEHAVIOUR OF UNSTIMULATED ECHINOPARYPHIUM RECURVATUM CERCARIAE

2.2.1. Distribution of a Population of Cercariae with Time

As a means of evaluating how individuals are behaving, recording the distribution of a population of cercariae with time is a valuable

form of study. However, it is important to realise the limitation of this type of experiment, which in the present study was caused by the need to restrict the movement of the cercariae in the third dimension. Cercariae were kept for extended periods in a glass test cell during these experiments. The volume of water in the test cell was only 3.5 millilitres and the surface area over which oxygen could be absorbed was small. Since not less than 50 cercariae were used in each of these experiments on population movements it was estimated, using the known oxygen requirements of some trematode larvae (Bruce et al, 1971a, 1971b; Vernberg, 1961, 1963) and the described longevity of E. recurvatum cercariae (Evans and Gordon, 1983), that the cercariae were likely to suffer the physiological stress of oxygen deficiency during the experiments. Accordingly the population movement experiments were used only to give indications of behavioural responses which could be recorded over a short time span using cinephotography. Under these latter circumstances there would be no changes of the cercariae suffering physiological stress.

The population movement experiments were carried out in a continuous temperature room at 19°C using the apparatus described in Chapter One. The test cell was divided into seven one centimetre bands and the number of cercariae within each recorded for each of the exposures made. It should be noted that although a known number of cercariae were used in each experiment, the presence of small unilluminated areas in the test cell accounts for the 'disappearance' of a few cercariae in some exposures (see Section 1.2.3). At the beginning of each experiment all the cercariae were congregated at one end (horizontal plane) or the bottom (vertical plane) of the cell since the cercariae were put in first in a small

volume of water, and the cell subsequently topped with pond water.

The main purpose of these experiments was to detemine whether the cercariae would reach even distribution throughout the cell or whether some pattern of aggregation would emerge.

Horizontal Plane

The results of the experiment following the movements of cercariae in the horizontal plane are shown in Figure 2.1. Although the cercariae distributed themselves throughout the cell during the experiment, there seemed to be a preference for the closed end and an avoidance of the open end of the cell. Because of the problems of likely oxygen deficiency no attempt has been made to test the significance of this observation.

Vertical plane

The results of the experiment following the movements of cercariae in the vertical plane are shown in Figure 2.2. The result of this experiment was rather unexpected. The cercariae showed a strong preference for the bottom of the cell for a long period of time. Either the cercariae were demonstrating a positive geotaxis or they were unable to displace themselves vertically. Curiously, after five hours when the amount of oxygen in the cell must have been low, more cercariae found their way to the top of the cell. Again because of the problems of likely oxygen deficiency no attempts have

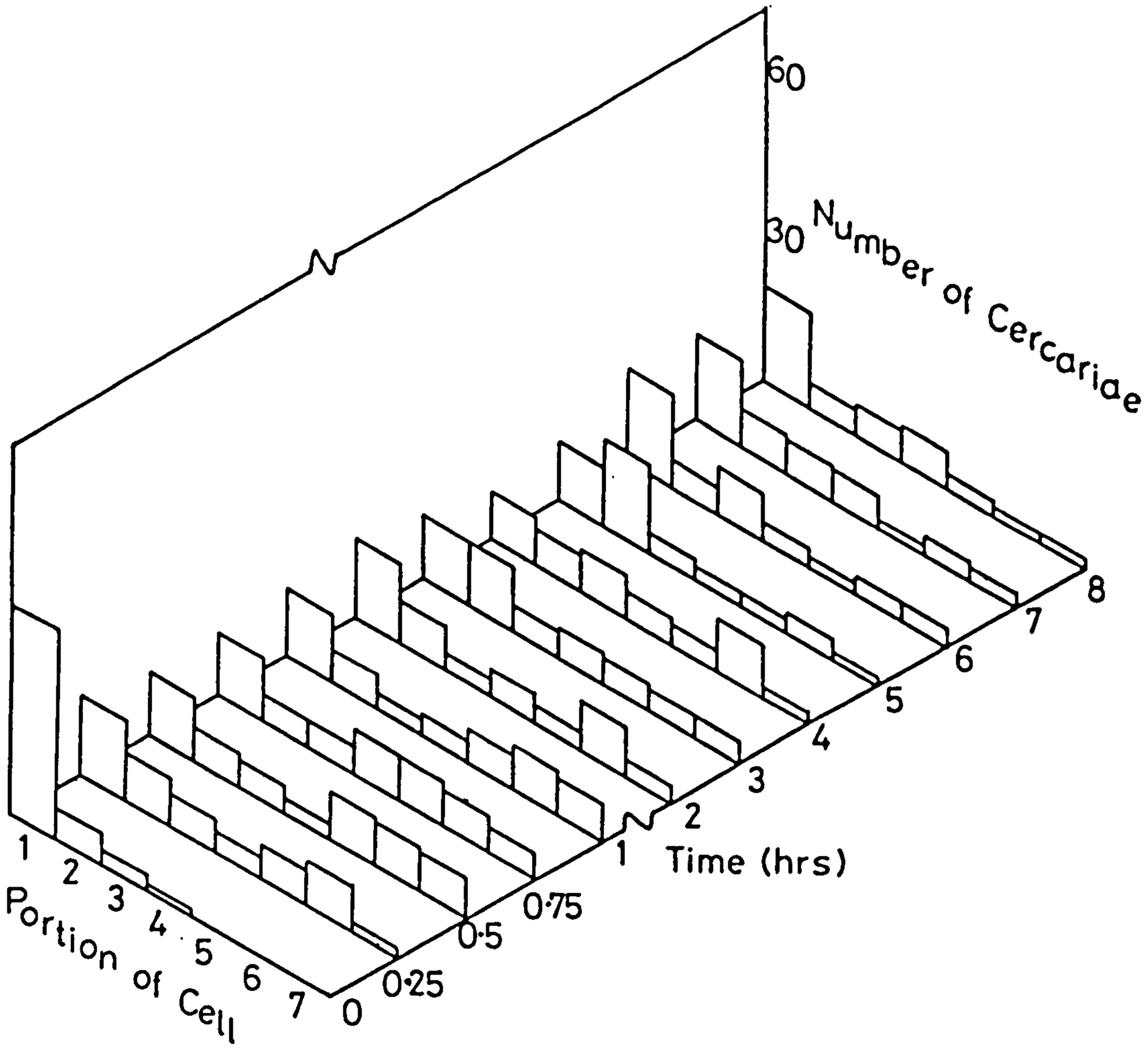


Figure 2.1.

The distribution of a population of unstimulated E.recurvatum cercariae in a test-cell over eight hours, horizontal plane.

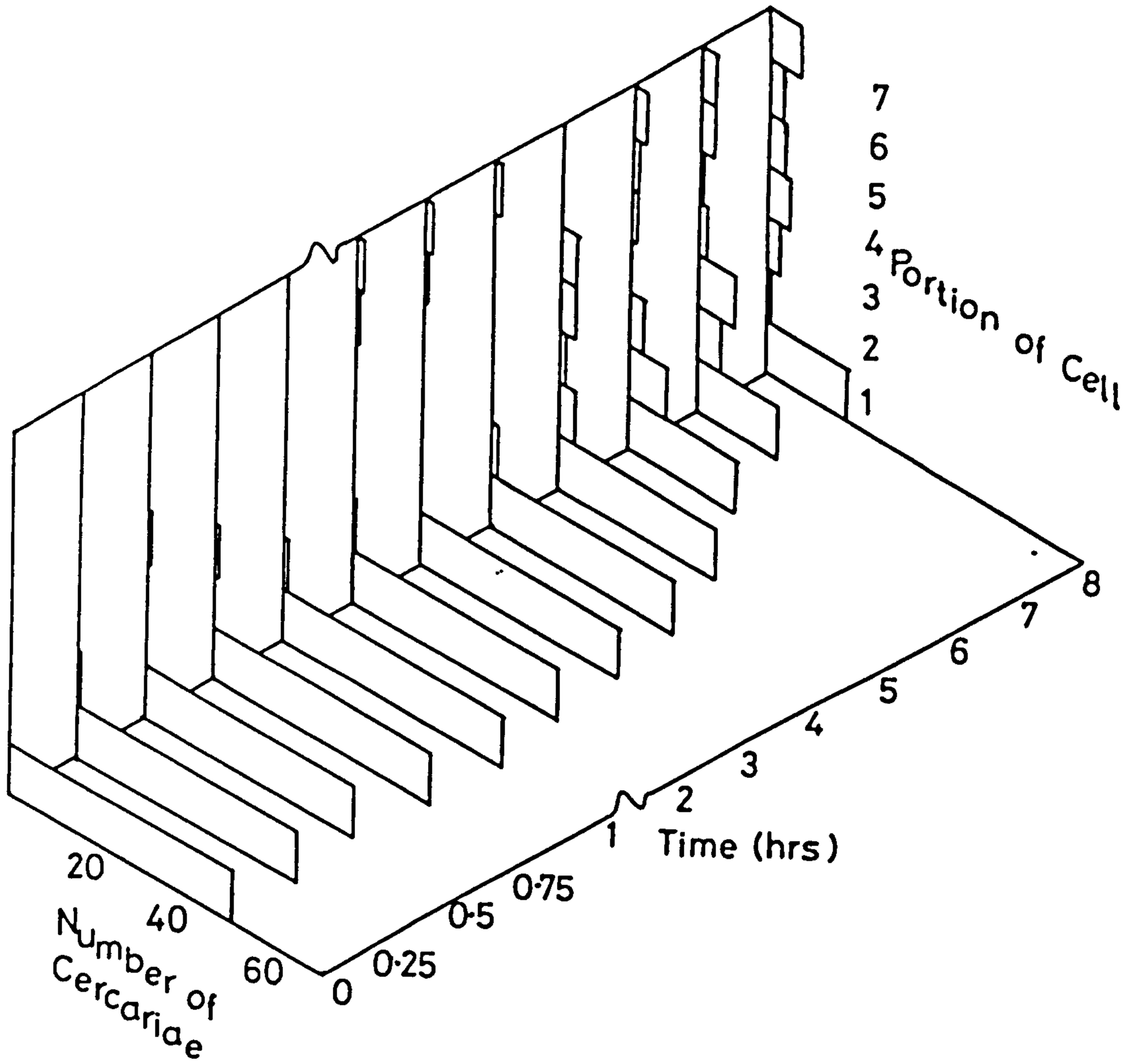


Figure 2.2.

The distribution of a population of unstimulated E.recurvatum cercariae in a test-cell over eight hours, vertical plane.

been made to test the significance of these results. However, this experiment did suggest that a study of the swimming speed of E.recurvatum against vertical direction might indicate whether the cercariae have difficulty in swimming vertically upwards.

2.2.2. Tracks of Unstimulated Cercarial Behaviour

Because the aim of track analysis was to follow the path of single individuals over a relatively short period of time, the restriction of the cercariae into 3.5 millilitres of water was calculated not to be important in these experiments. The cercariae were filmed for only a matter of a few minutes and this commenced almost immediately after the cercariae were placed in the cell. Approximately 100 cercariae were placed in the cell and their swimming behaviour was filmed with the cell in both the horizontal and vertical positions. The experiments were carried out under the same conditions as those in Section 2.2.1.

Tracks were reconstructed from the developed cine film using the methods described in Chapter One. For each experiment all the cercariae which passed through the field of view were numbered and tracks selected at random for analysis. Tracks of less than 20 steps were rejected and for those which were longer, only the first twenty steps were used. Accordingly for the analysis of unstimulated behaviour thirty tracks, each of twenty steps were analysed.

Horizontal plane

All the cercariae filmed in the horizontal plane swam continuously, so no patterns of activity and inactivity were recorded. The data for the parameters of movement described in Chapter One have been combined together for all the analysed tracks and are displayed in Table 2.1. For the most part these data represent the controls against which other data will be tested. The distributions of step speed, angle of direction, angle of turn and string length are shown in Figure 2.3.

Table 2.1. *Echinoparyphium recurvatum* cercariae - unstimulated behaviour, horizontal plane.

Number of observations	600
Step interval	0.342 (sec)
Mean step speed (± S.E.)	1.327 ± 0.007 (mm sec^{-1})
Mean angle of direction (± S.E.)	82.850 ± 1.871 (deg)
Mean rate of turning (± S.E.)	51.905 ± 2.167 (deg sec^{-1})
Number of left turns	307
Number of right turns	255

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Although the displayed data are controls for other experiments, two parameters can be studied in a little more detail. Firstly, for entirely random behaviour one would expect the mean angle of direction for the experiment to be 90°. In Appendix 2.1 the

Figure 2.3.

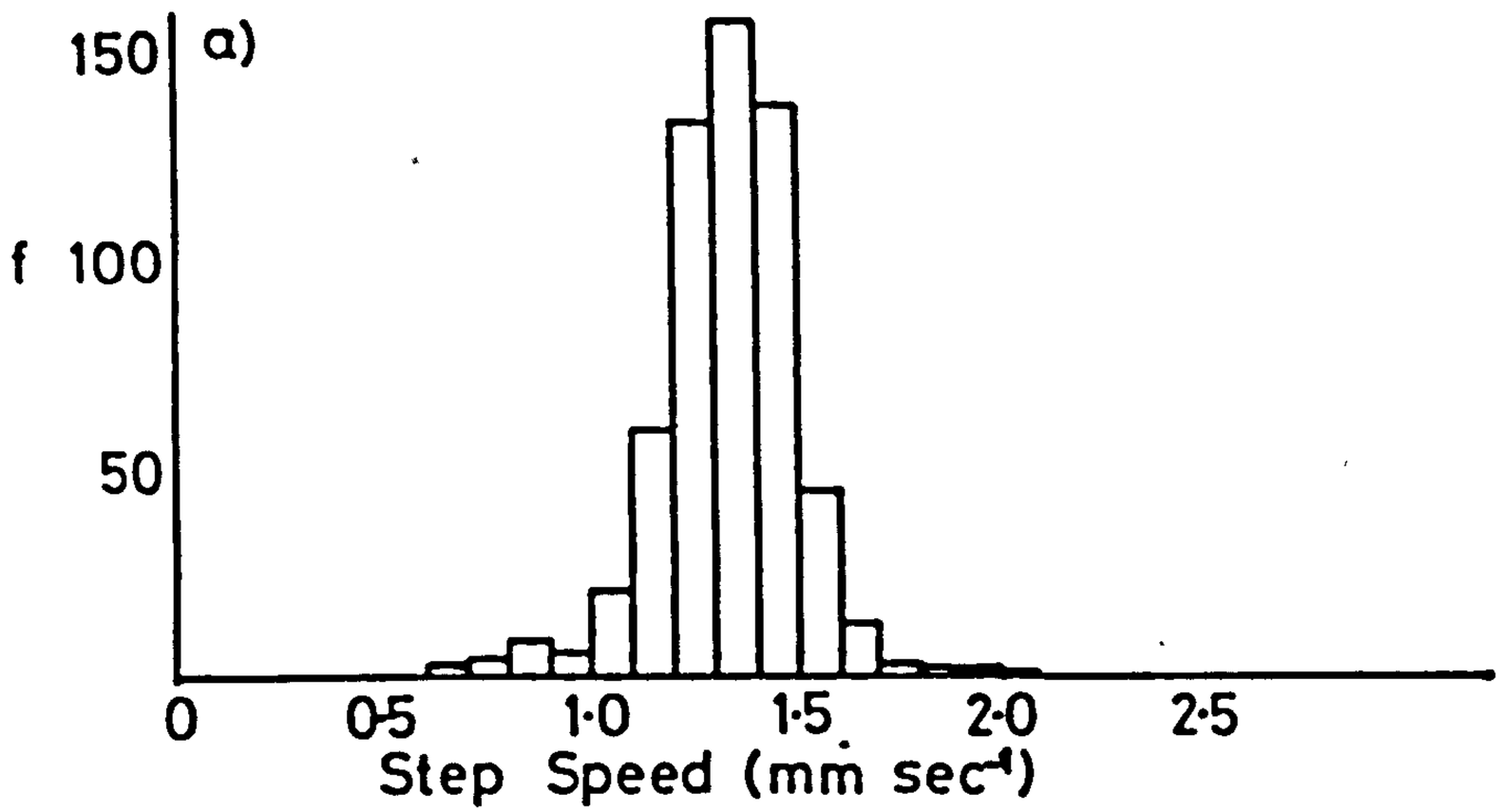
Distributions of parameters of movement for unstimulated E.recurvatum cercariae, horizontal plane:

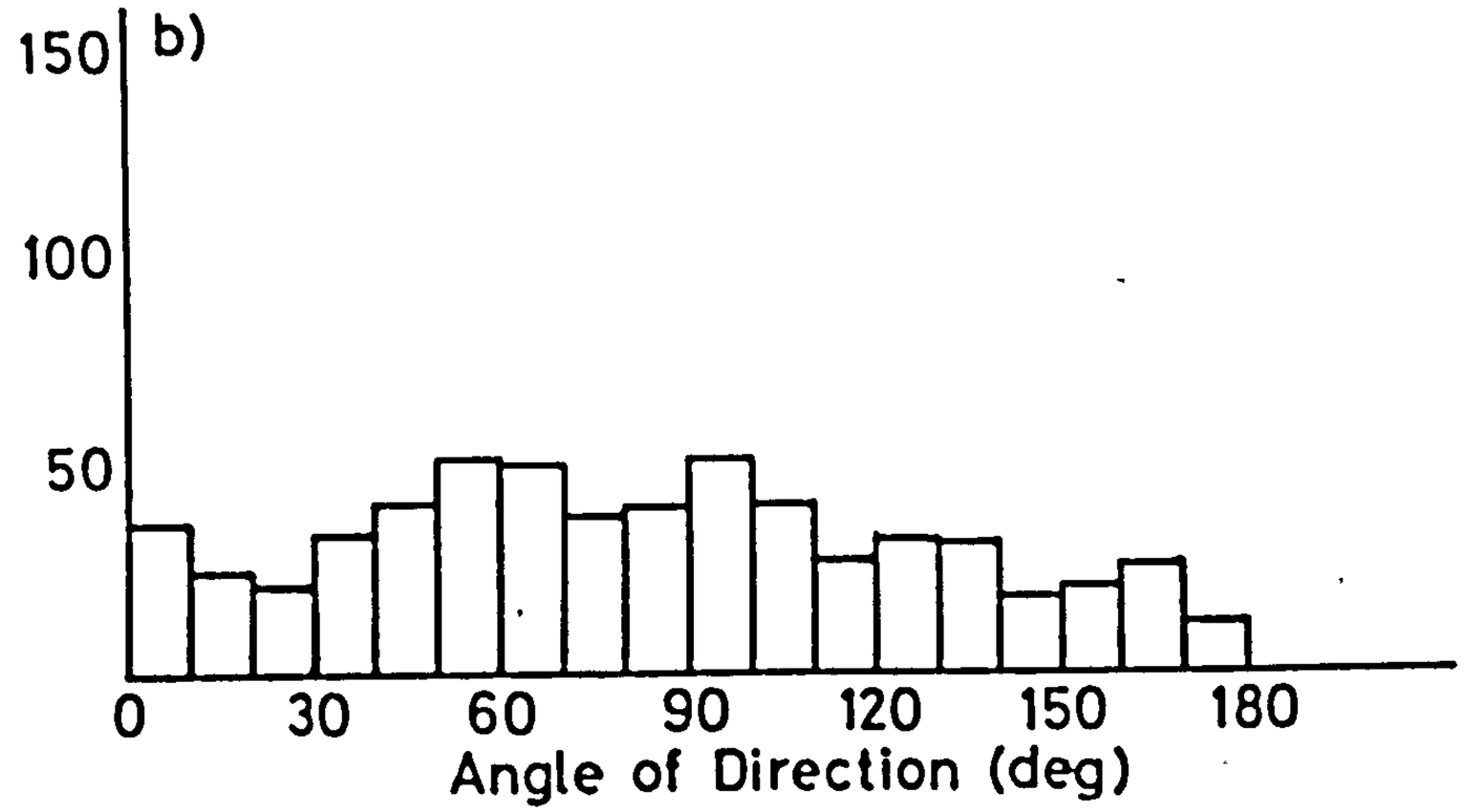
a) Step speed

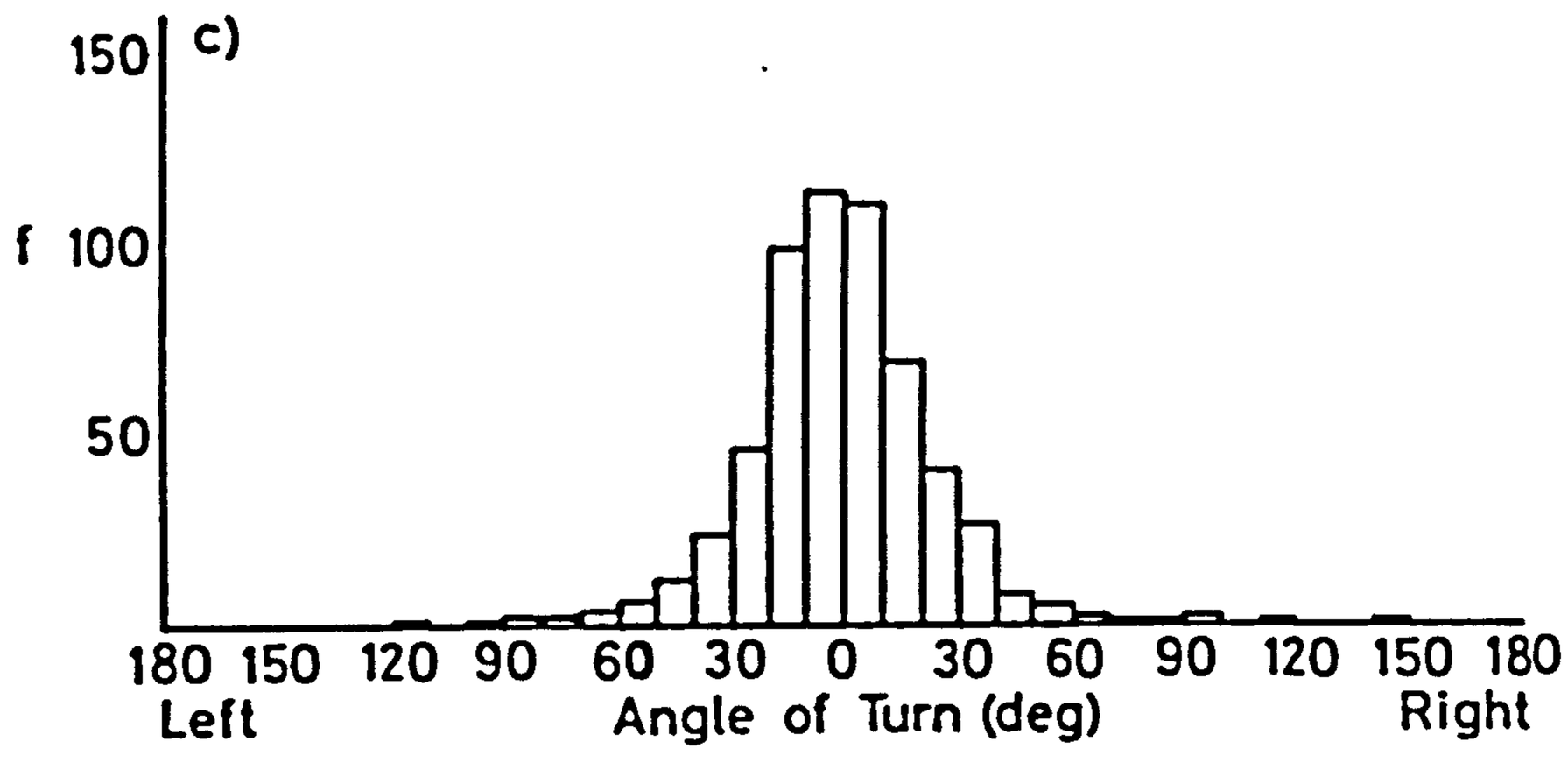
b) Angle of Direction

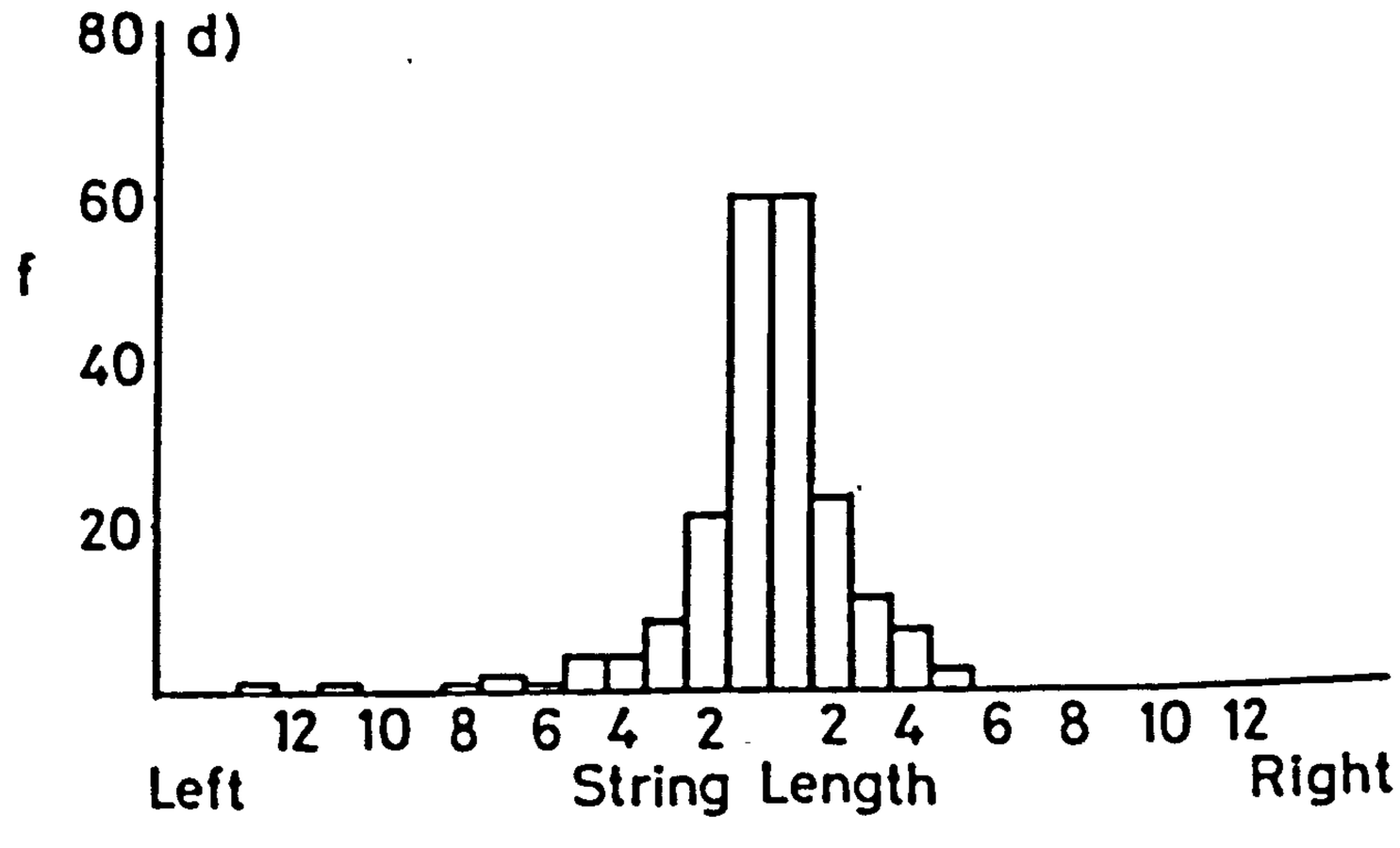
c) Angle of Turn

d) String Length


a)
150
f 100
50
0
0·5
1·0
1·5
2·0
2·5
Step Speed (mm sec⁻¹)


b)
150
100
50
0
30
60
90
120
150
180
Angle of Direction (deg)


c)
150
f 100
50
180 150 120 90 60 30 0 30 60 90 120 150 180
Left
Angle of Turn (deg)
Right


d)
80
60
f
40
20
12 10 8 6 4 2 2 4 6 8 10 12
Left
String Length
Right

distribution of direction is tested against random behaviour, when the number of observations in each class would be expected to be equal. The observed distribution differs from random behaviour significantly although at 82.85 $\pm$ 1.87 degrees the mean figure is not markedly different from 90 degrees. An explanation for this difference might be that although the test is based on 20 observations for each track, the initial direction to some extent determines the subsequent nineteen since the cercariae appear to swim in approximately straight lines with only infrequent turns.

Secondly, from the distribution of strings (as defined in Chapter One) it should be possible to determine whether the cercariae show random turning to right or left or whether there is a behavioural modification such that after a right turn the cercariae are more likely to turn left, and vice versa. The observed data cannot be tested against a binomial distribution since the number of 'choices' involved in a string length of, say, five does not equal that of a shorter or longer string length. However, assuming random choice of turn the probability of a string length n can be described as $1/2^n$. Accordingly, in an observed set of strings one half would be expected to be of length one, one quarter of length two, one eighth of length three and so on. In Appendix 2.2. the observed distribution of strings is shown not to differ significantly from that expected if the cercariae were showing random 'choice' of turn.

Vertical plane

In common with those filmed in the horizontal plane, the cercariae

filmed in the vertical plane all swam continuously, so no activity rhythm was recorded. Again data for the parameters of movement described in Chapter One have been combined for all the analysed tracks and are displayed in Table 2.2. These data represent the controls for vertical plane experiments with E.recurvatum cercariae. Distributions of step speed, angle of direction, angle of turn and string length are shown in Figure 2.4.

Table 2.2. Echinoparyphium recurvatum cercariae - unstimulated behaviour, vertical plane.

Number of observations	600
Step interval	0.353 (sec)
Mean step speed (± S.E.)	1.314 ± 0.0017 (mm sec^{-1})
Mean angle of direction (± S.E.)	82.492 ± 1.812 (deg)
Mean rate of turning (± S.E.)	113.119 ± 4.375 (deg sec^{-1})
Number of left turns	262
Number of right turns	301

As for the horizontal plane data, the distribution of angles of direction has been tested against random behaviour and again the difference is significant (Appendix 2.3.). In addition to the possible reason discussed previously, that for each track the initial direction to some extent determines that of the subsequent nineteen steps, another possible explanation exists in this case. From the movement of a population of cercariae in the vertical plane it was speculated that E.recurvatum cercariae may be relatively

Figure 2.4.

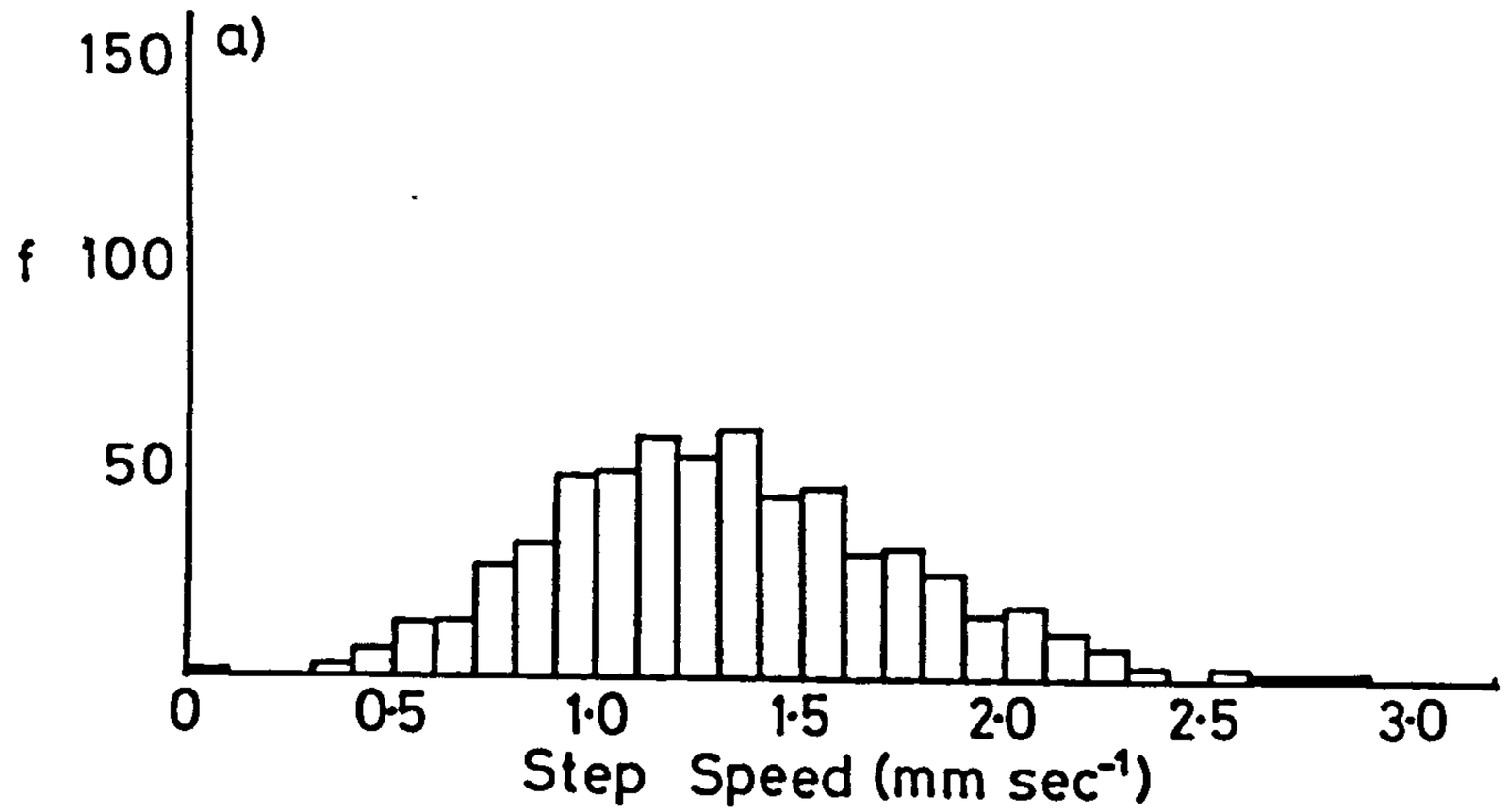
Distributions of parameters of movement for E.recurvatum cercariae, vertical plane:

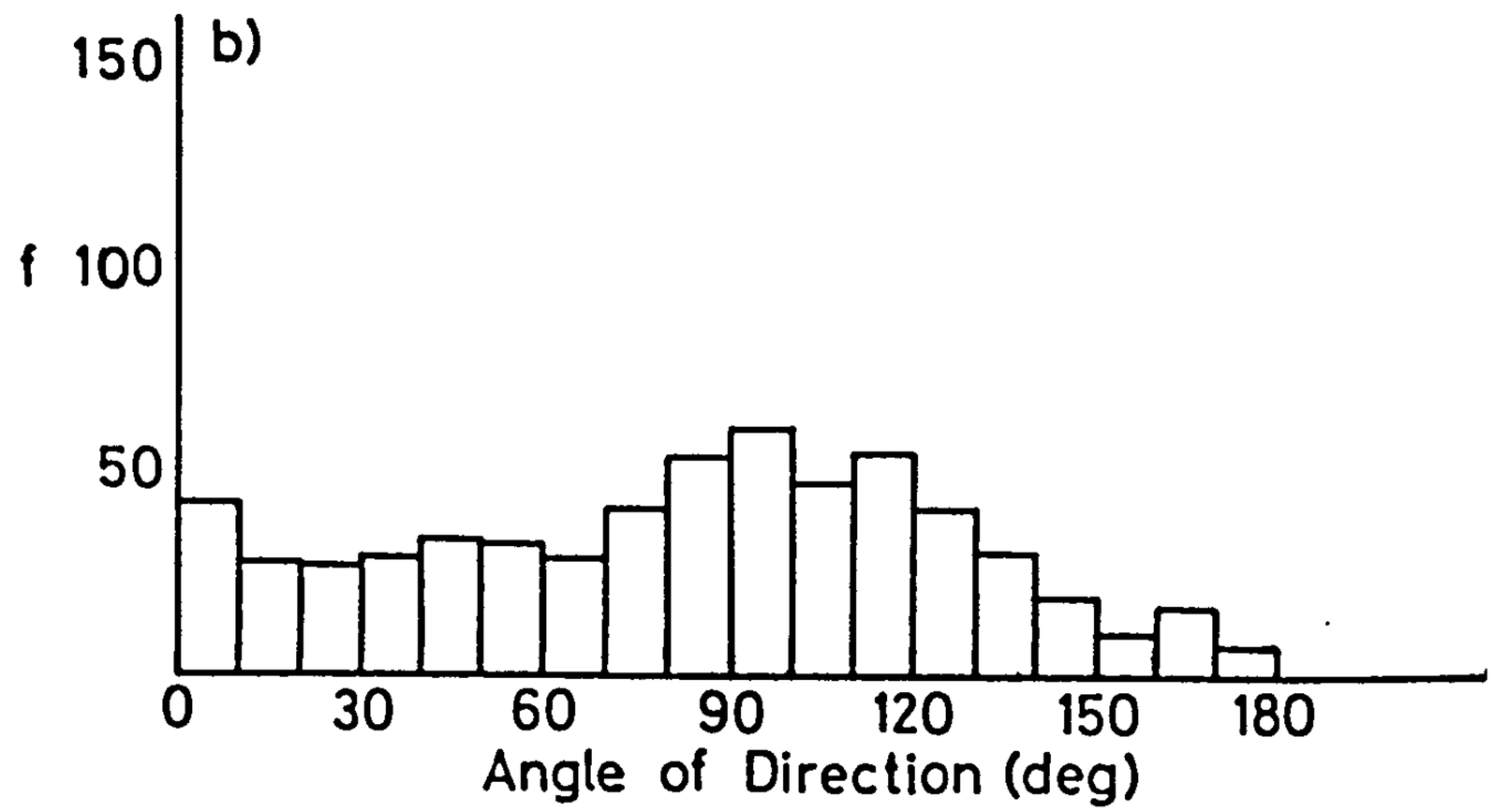
a) Step speed

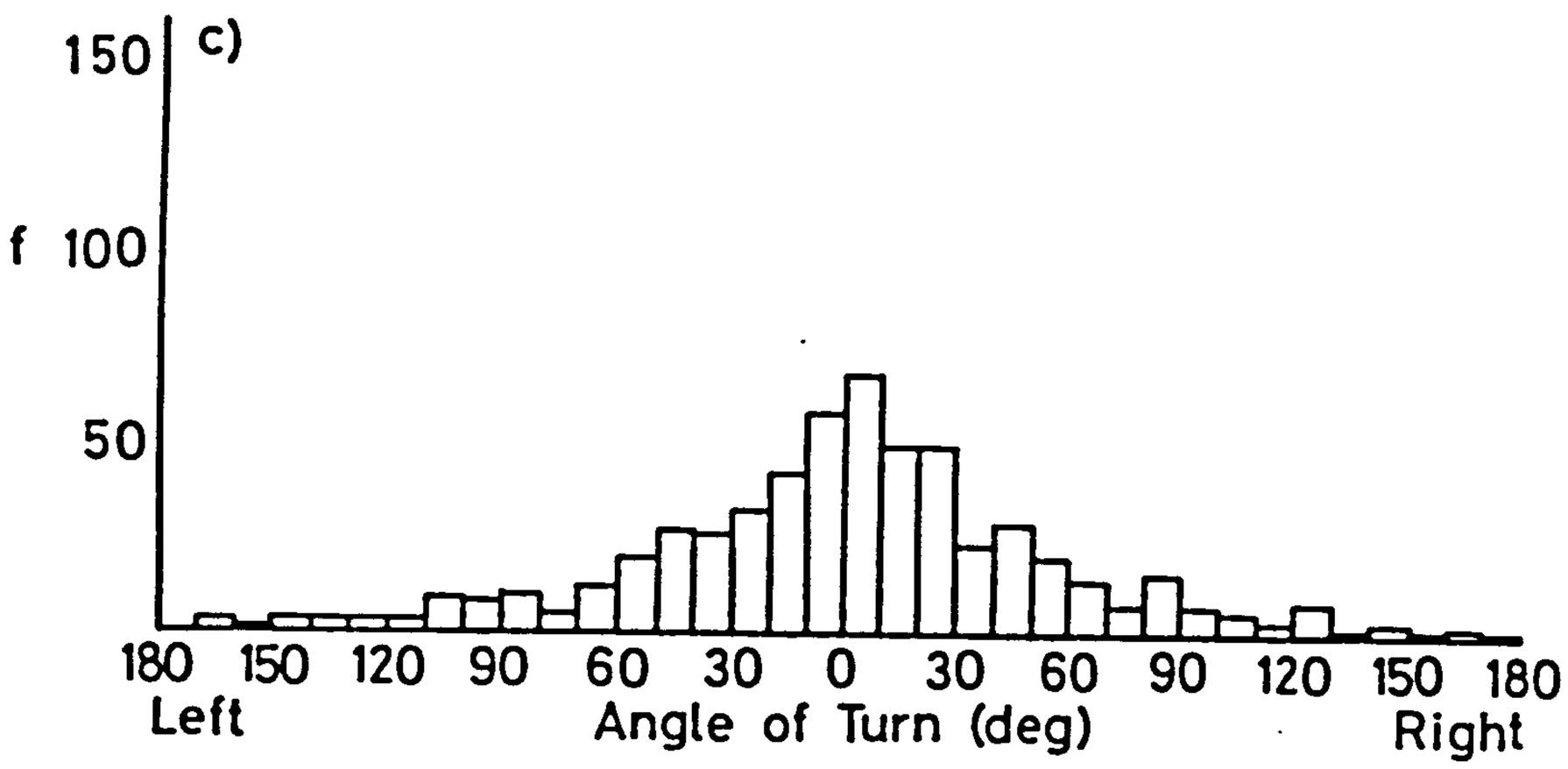
b) Angle of Direction

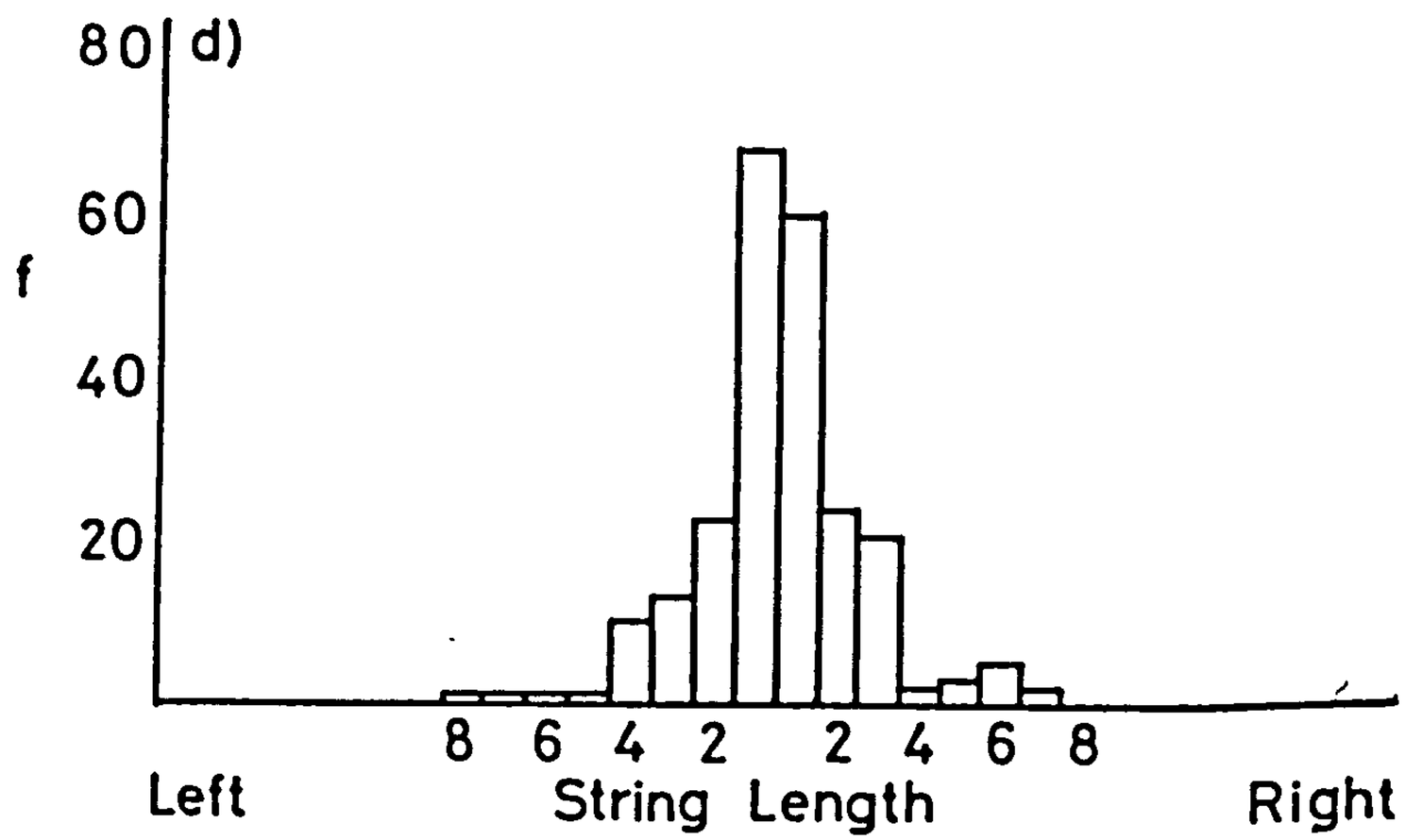
c) Angle of Turn

d) String Length


a)
150
f 100
50
0
0·5
1·0
1·5
2·0
2·5
3·0
Step Speed (mm sec⁻¹)


b)
150
f 100
50
0
30
60
90
120
150
180
Angle of Direction (deg)


c)
150
f 100
50
180 150 120 90 60 30 0 30 60 90 120 150 180
Left
Angle of Turn (deg)
Right


d)
80
f
60
40
20
8 6 4 2 2 4 6 8
Left
String Length
Right

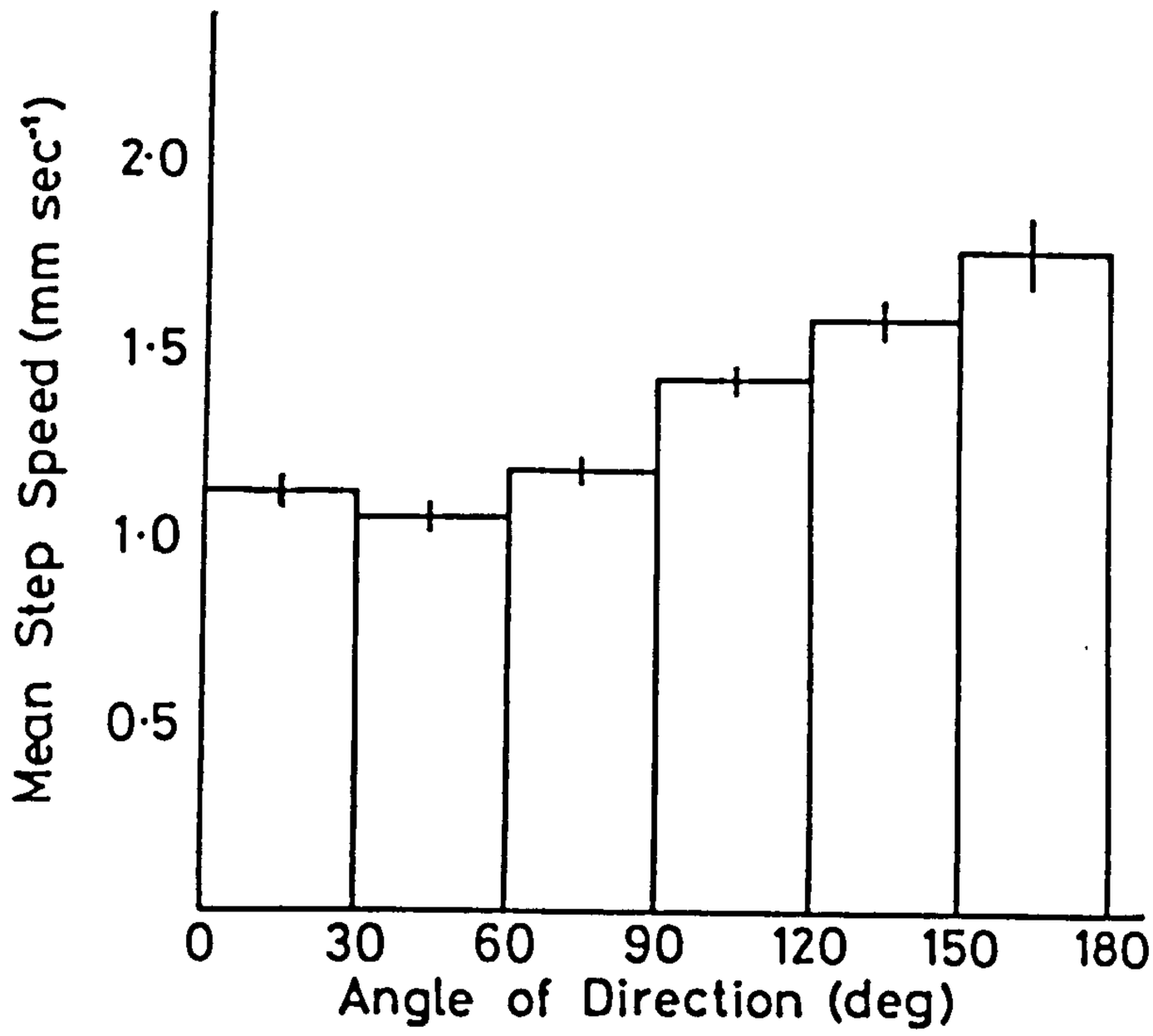


Figure 2.5.

The effect of vertical direction on step speed in E.recurvatum cercariae. (0^{o} is vertically upwards, 180^{o} is vertically downwards. Error bars indicate standard error of the mean).

inefficient at swimming vertically upwards. If this were true one might see fewer steps of direction 0 - 45° than expected, and more at angles of direction which are closer to horizontal (90°). In addition the attitude of cercariae in the water, tail and body in horizontal alignment, would make it relatively more difficult for cercariae to swim steeply downwards. This would produce fewer steps in the directions 140 - 180° than expected, and an excess in the more horizontal angles of direction, as appears to be the case. The ability of E.recurvatum cercariae to displace themselves vertically has been analysed by grouping step speeds into classes by angle of direction with a thirty degree interval, and comparing means. The data are shown in Appendix 2.4 and plotted in Figure 2.5.

Most of the comparisons are significant, some at the 0.1% level. These results support the theory that E.recurvatum cercariae are not able to swim as quickly vertically upwards as they are horizontally or vertically downwards. The data does not indicate that a gravitational taxis is being demonstrated, since there is no obvious bias towards low (negative geotaxis) or high (positive geotaxis) angles of direction.

The distribution of strings has been tested in the same manner as those for cercariae swimming in the horizontal plane. The data, shown in Appendix 2.5 and plotted in Figure 2.4.d indicate that, as in the horizontal plane, the cercariae are turning left and right between steps entirely at random.

2.2.3. Summary of the Behaviour of Unstimulated E. recurvatum Cercariae

Experiments on the behaviour of unstimulated E.recurvatum cercariae have led to the following observations:

1) The cercariae swim continuously.

2) The cercariae readily displace themselves in the horizontal plane but swimming speed is reduced when swimming vertically upwards.

3) Turns to the left or right are chosen at random and not affected by the sign of the previous turn.

4) Most turns are through small angles, with very few above 90^{o}.

Tentative assumptions about how this behaviour may be of advantage to the cercariae in locating their host can be made. Essentially the unstimulated behaviour of these cercariae would appear to lead to their large horizontal displacement from the point of shedding, but remaining close to the bottom of their habitat. As many molluscan species, which are potential hosts, share this bottom dwelling habit the unstimulated cercarial behaviour may act to increase the chances of entering the vicinity of a suitable host.

2.3. THE BEHAVIOUR OF ECHINOPARYPHIUM RECURVATUM CERCARIAE IN RESPONSE TO AN ORIENTATION LIGHT

Observation of E.recurvatum cercariae by binocular microscope revealed that, whilst they normally appear to swim around in a petri dish of pond water in all directions, when a directional light was introduced there appeared to be a strong preference to swim towards the source of the light. The speed of this apparent response was such that it seemed likely the cercariae were displaying a positive phototaxis. Accordingly a series of experiments were undertaken to investigate the behaviour of the cercariae in response to light, in both the horizontal and vertical planes, using time lapse photography to record the movement of a population of cercariae and cinephotography to record the behaviour of individuals.

In these experiments the only difference from the apparatus used to record the unstimulated behaviour of the cercariae was the presence of a tungsten light of approximately 2.5 times the intensity of the taking light at the top (vertical plane) or the open end (horizontal plane) of the test cell.

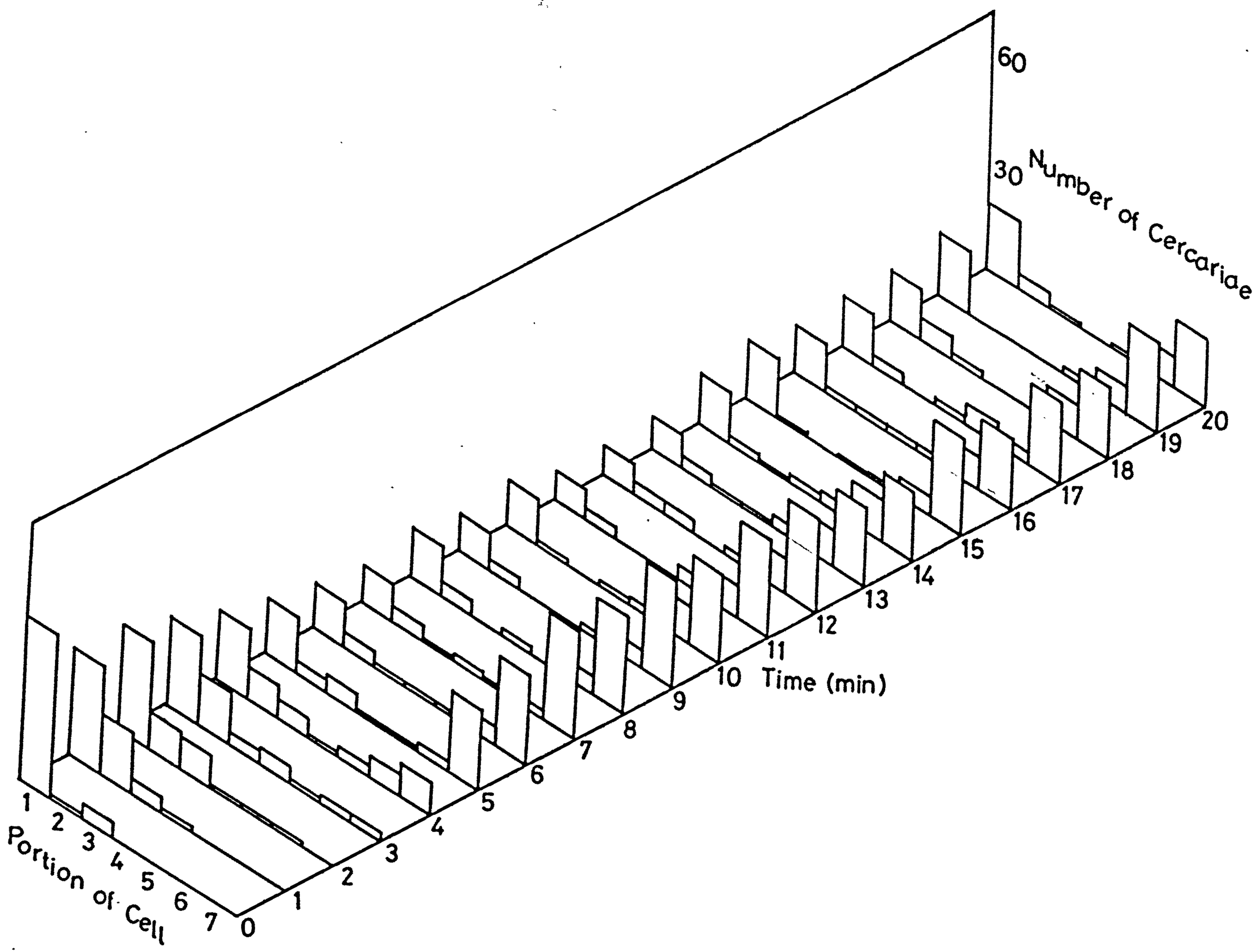
2.3.1. Distribution of a Population of Cercariae with Time in Response to an Orientation Light

Horizontal plane

Because the cercariae observed in a petri dish appeared to respond

Figure 2.6

The distribution of a population of E. recurvatum cercariae, stimulated by an orientation light, in a test cell over 20 minutes, horizontal plane. (The orientation light was adjacent to cell portion 7).


60
30
Number of Cercariae
20
19
18
17
16
15
14
13
12
11
10
Time (min)
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
Portion of Cell

very quickly in the horizontal plane to an orientation light, the movement of a population of cercariae was recorded at one minute intervals for twenty minutes. The results are shown in Figure 2.6. In comparison with the experiment with unstimulated cercariae, the population here more rapidly distributes itself throughout the cell. In this experiment cercariae apparently show preferences for both the open and closed ends of the cell, compared to the remaining 5 zones. The speed of the response by the population inferred that it might be possible to quantify the response in terms of the described parameters of movement in track analysis.

Vertical plane

The movement of a population of E.recurvatum cercariae responding in the vertical plane to a surface light was recorded. Since no response in the vertical plane was obvious by observation of individuals using a binocular microscope, exposures were taken at hourly intervals. The results, plotted in Figure 2.7, indicate that there may be a response to the surface light since more of the cercariae reached a higher point in the cell than unstimulated cercariae did. However, the long time which this apparent response took to manifest itself, and the doubts about keeping cercariae in the test cell over long periods, means that the results are rather inconclusive. Certainly no attempt could be made to record this vertical behaviour in the presence of a surface light using cinephotography.

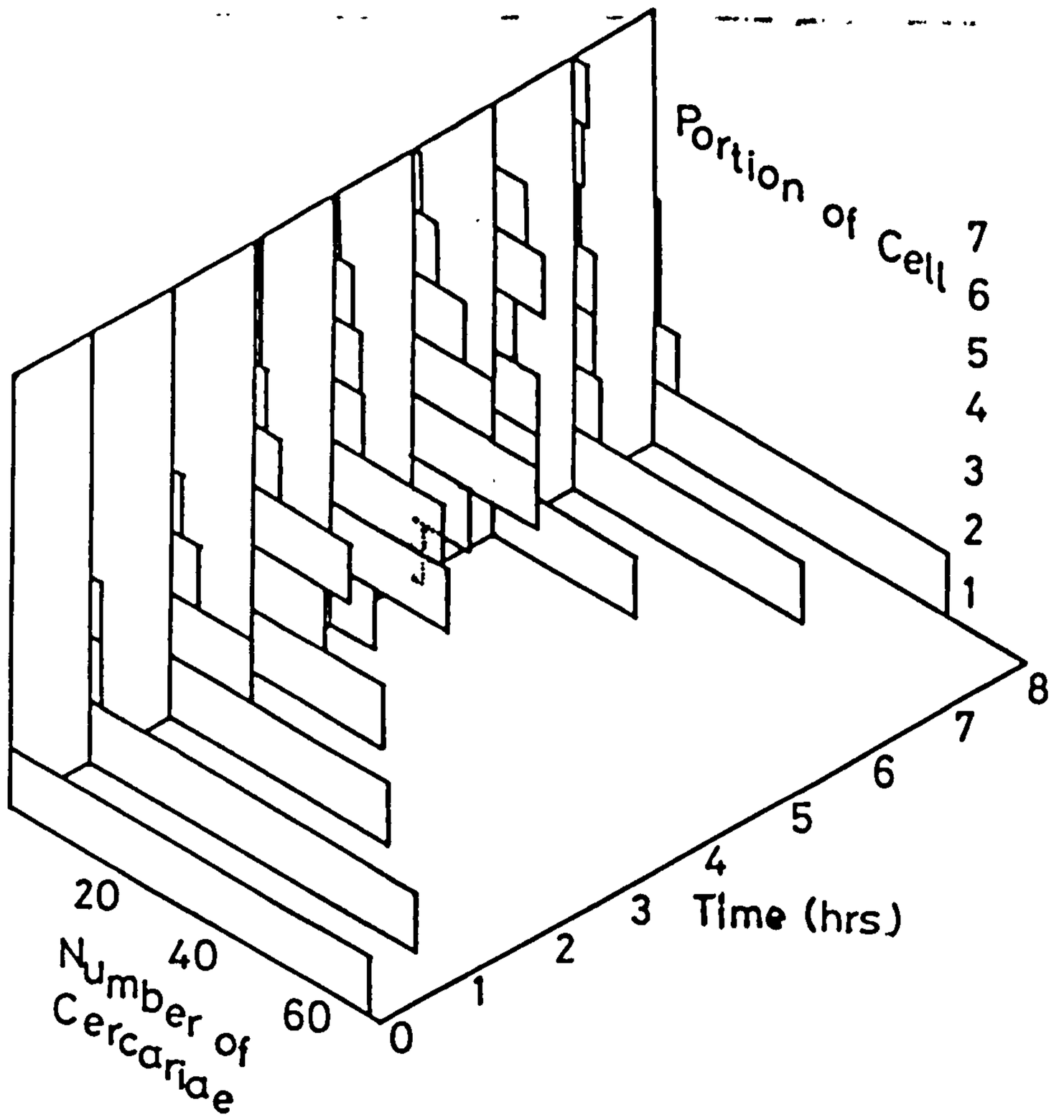


Figure 2.7.

The distribution of a population of E.recurvatum cercariae, stimulated by an orientation light, in a test cell over eight hours, vertical plane. (The orientation light was above cell portion 7).

2.3.2. Tracks of Cercariae Responding to an Orientation Light

The population movement experiments with an orientation light revealed that some E.recurvatum cercariae reacted in the horizontal plane to the presence of such a light, swimming towards it. However, the results of those population experiments were not as conclusive as had been suggested by microscopic observation of the cercariae in a petri dish. It was reasoned that the structure of the test cell, fused glass blocks, was not ideally suited to this type of experiment since much of the effect of the orientation light was dissipated by refraction within the glass blocks. This would make it much more difficult for the cercariae to identify the direction of a single light source. In an attempt to simplify the situation E.recurvatum cercariae were filmed in the horizontal plane in a petri-dish containing 2 mm depth of water, with and without an orientation light.

The combined data for all tracks with the orientation light on or with it off are shown in Table 2.3. The distributions of step speed, angle of direction, angle of turn and string length are shown in Figure 2.8. There are differences in each of the studied parameters of movement between the tracks of cercariae responding to the orientation light and the control. Each of these differences is investigated in turn.

Table 2.3. Echinoparyphium recurvatum cercariae in presence and absence of an orientation light, horizontal plane.

	Light on	Light off
Number of observations	900	900
Step interval (sec)	0.207	0.207
Mean step speed (mm sec^{-1})	1.607 ± 0.009*	1.493 ± 0.012*
Mean angle of direction (deg)	18.664 ± 0.589*	87.107 ± 1.732*
Mean rate of turning (deg sec^{-1})	91.909 ± 2.540*	109.715 ± 3.36*
Number of left turns	422	417
Number of right turns	435	443

(* ± standard error)

Cercariae responding to the orientation light showed a higher step speed than the control larvae and this difference has been tested (see Appendix 2.6) and found to be significant. These cercariae do swim faster when responding to an orientation light.

The distribution of angles of direction was markedly different when an orientation light was present. The orientation light was positioned at a direction of 0^{o} and the resulting distributions of angles of direction for the two conditions are shown in Figure 2.8. The difference between the means of angle of direction has been tested, using analysis of variance (Appendix 2.7). The difference between the means is significant suggesting that E.recurvatum cercariae are able to navigate relatively efficiently towards an

Figure 2.8.

Distributions of parameters of movement for <u>E. recurvatum</u> cercariae with and without an orientation light, horizontal plane:

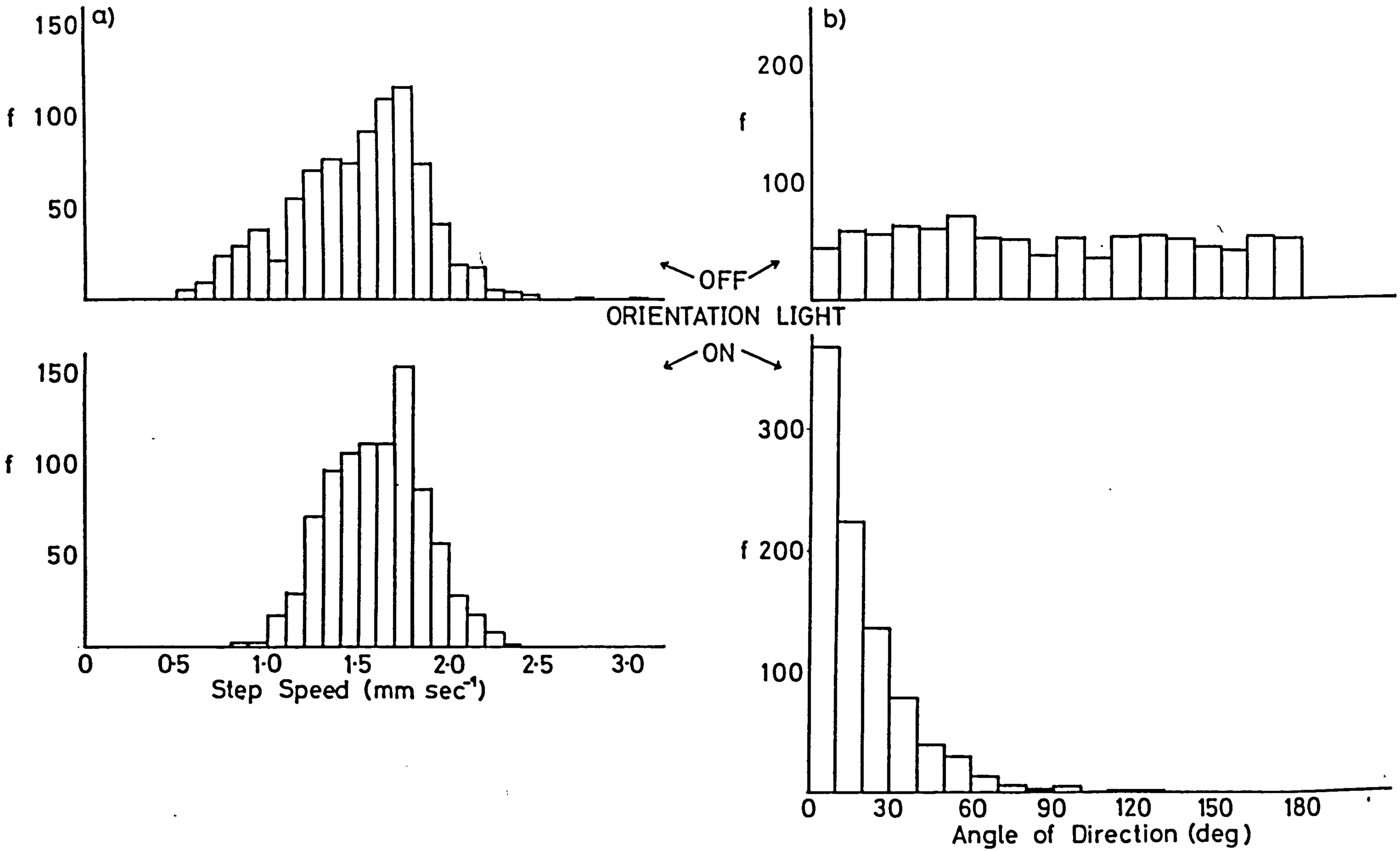
a) Step Speed

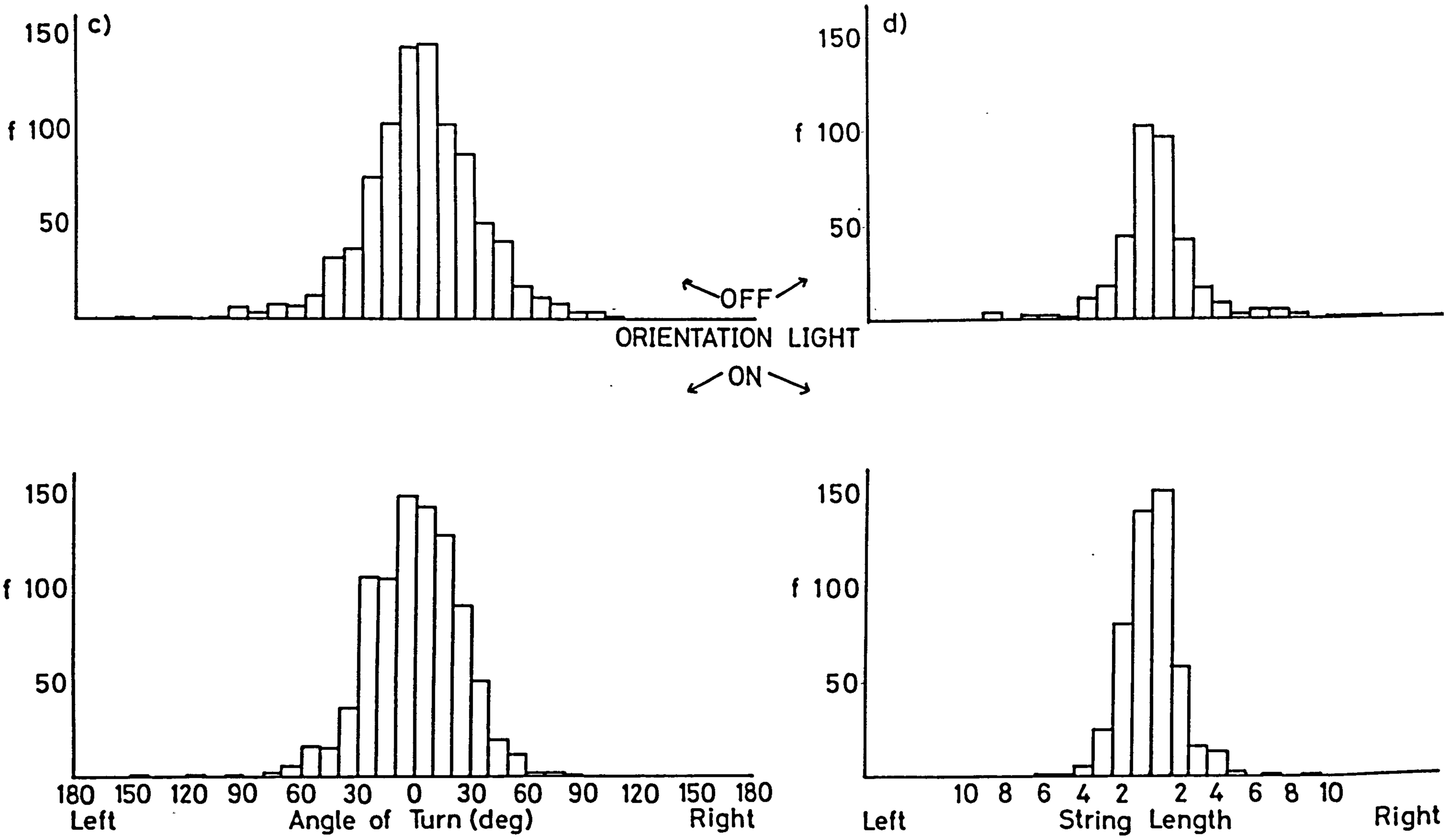
b) Angle of Direction

Overleaf

c) Angle of Turn

d) Angle of Direction


a)
150
f 100
50
0
0·5
1·0
1·5
2·0
2·5
3·0
Step Speed (mm sec⁻¹)
b)
200
f
100
OFF
ON
ORIENTATION LIGHT
300
f 200
100
0
30
60
90
120
150
180
Angle of Direction (deg)


c)
150
f 100
50
OFF
ORIENTATION LIGHT
ON
d)
150
f 100
50
150
f 100
50
180 150 120 90 60 30 0 30 60 90 120 150 180
Left
Angle of Turn (deg)
Right
10 8 6 4 2 2 4 6 8 10
Left
String Length
Right

orientation light.

An apparent difference in rate of turning has also been tested using analysis of variance (Appendix 2.8). Cercariae that are responding to an orientation light show a significantly lower rate of turning than those in the control experiment.

The distribution of strings in the two sets of data appears markedly different. Cercariae responding to an orientation light appear to show a higher number of short strings than expected. This would make their track paths considerably straighter than those of cercariae in the absence of an orientation light. In Appendix 2.9 the distribution of strings for both sets of data has been compared to that expected if cercariae turn left or right at random. Both sets of data differ from that expected with random choice of turn, those that are responding to the orientation light more significantly so. For those cercariae in the absence of an orientation light the difference is only just significant at the 5% level and this appears to be mainly due to an excess of string lengths of seven and upwards. If the cercariae responding to an orientation light really do swim straighter than those under control conditions then their ratio of net displacement to distance travelled will be significantly higher. This ratio has been calculated for each of the 30 tracks under the two experimental conditions and the differences compared (Appendix 2.10) by analysis of variance. The difference between means is significant at the 0.1% level and confirms that the cercariae responding to an orientation light swim straighter than those under control conditions.

2.3.3. Summary of the Behaviour of E.recurvatum Cercariae Responding to an Orientation Light

The experiments of the behaviour of E.recurvatum cercariae stimulated by an orientation light have revealed the following points:

1) The cercariae appear to show a weak phototaxis in the vertical plane.

2) The cercariae show a strong phototaxis in the horizontal plane.

3) The phototaxis in the horizontal plane is characterised by increased swimming speed, decreased rate of turning and tendency for swimming in a relatively straight line.

4) The cercariae are able to determine the source of an orientation light and navigate towards it relatively accurately.

The fact that a response to directional light exists in E.recurvatum cercariae suggests that it is an adaptative advantage. This being so, the response is most likely to result in the enhanced chances of an individual cercariae locating a snail host. Precisely how this response is of adaptive advantage is not known but two possibilities

can be put forward. Firstly, most snail species are herbivorous, feeding on aquatic plants and algal growth. Since plant growth is dependent on light, one might expect to see increased plant growth and higher snail density in well illuminated areas. A phototaxis shown by cercariae would perhaps lead them to these areas of higher snail density, improving their chances of a host contact.

Secondly, in deeper bodies of water the light intensity near the surface is greater than that near the bottom. If cercariae were able to orientate towards this higher light intensity they would reach the better illuminated areas where the increased plant growth and snail density, mentioned above, are likely.

2.4. THE BEHAVIOUR OF ECHINOPARYPHIUM RECURVATUM CERCARIAE IN THE VICINITY OF A SNAIL HOST.

Having studied the unstimulated behaviour of E.recurvatum cercariae, and the behaviour in response to one environmental stimulus, light, the culmination of the study of the behaviour of these cercariae was the investigation of their response to the presence of a snail host. Because of the dynamic nature of host location it was reasoned that for time-lapse photography to be any use it would have to be at very short time intervals. Under those conditions the time-lapse photography would merely become a poor substitute for cine photography. Accordingly, it was decided to concentrate entirely on cine photography.

Since the E.recurvatum cercariae did not seem able to reach the top of the glass test cell in previous vertical plane experiments it seemed likely that filming in the vertical plane, with a snail on top of the cell, would not be worthwhile. For this reason the host-locating behaviour has been analysed in the horizontal plane only.

The experimental arena was a small petri dish containing about 2 mm depth of water. A snail was introduced to the centre of the petri dish and allowed to attach itself to the bottom. About one hundred cercariae were added to the petri dish and filming commenced.

2.4.1. Tracks of E.recurvatum Cercariae in the Vicinity of a Snail Host.

All the tracks of E.recurvatum cercariae filmed in the vicinity of a snail were analysed. During the frame by frame reconstruction of the tracks as soon as a cercaria contacted the snail it was deemed to have successfully located the snail and reconstruction stopped, irrespective of whether the cercaria remained attached. A total of twenty-nine tracks were recorded and analysed, fourteen of which involved the cercaria successively locating the snail host. The combined data for the usual parameters of movement are shown in Table 2.4, except that since a target host was used the mean angle of direction has been replaced by the mean angle of direction relative to the host. Sample tracks are shown in Figure 2.9 with the host's start and end positions for the period of filming. Several points are immediately apparent. Firstly, the mean step speed is lower than previously recorded for unstimulated

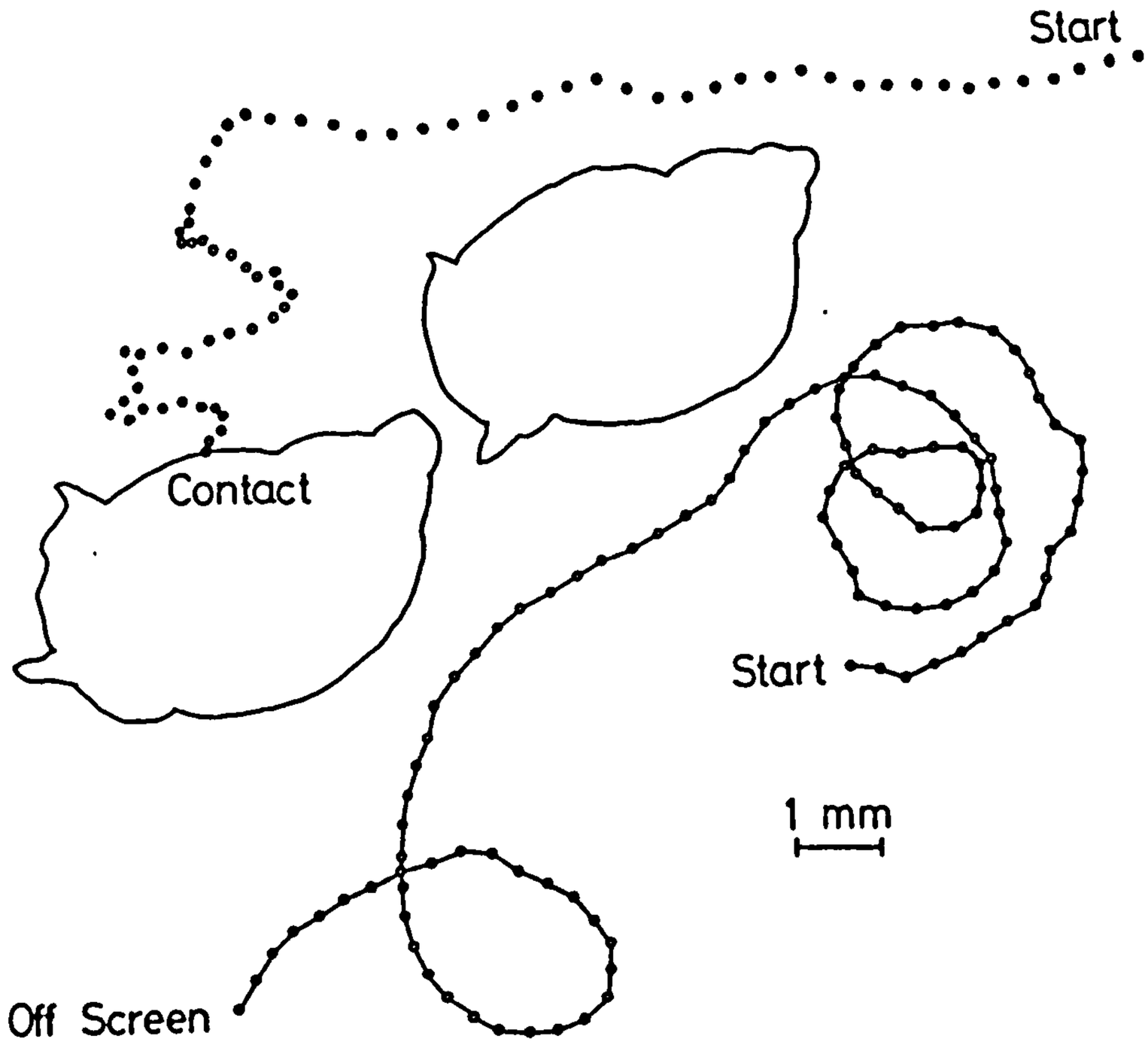


Figure 2.9.

Examples of tracks of <u>E.recurvatum</u> in the vicinity of a snail, <u>Lymnaea pereger</u>. (The two snail positions indicate the extent of movement for the duration of the longest track shown.

E.recurvatum cercariae. In this experiment the cercariae did show periods of inactivity, though not enough to describe an activity rhythm, but even when these are excluded the mean swimming speed for the cercariae was still only 0.951 $\pm$ 0.011 mm sec^{-1}. It seems that the swimming speed is significantly less than for unstimulated cercariae (see Table 2.1. Secondly, the mean rate of turning appears to be higher than that for unstimulated cercariae. Thirdly, the mean angle of direction relative to the host is close to 90°, the value one would expect if the cercariae were behaving randomly. This suggests that the cercariae cannot detect the direction of the host when in its vicinity. Finally, there appears to be a major difference between the number of left and right turns, more than might be expected by chance assuming equal probability. All these points will be returned to later.

Table 2.4. Echinoparyphium recurvatum cercariae in the vicinity of a snail host, horizontal plane.

Number of observations	3613
Step interval	0.347 (sec)
Mean step speed	0.919 (mm sec^{-1})
Mean angle of direction relative to host ($\pm$ S.E.)	85.151 $\pm$ 1.649 (deg)
Mean rate of turning ($\pm$ S.E.)	91.777 $\pm$ 3.004 (deg sec^{-1})
Number of left turns	1956
Number of right turns	1434

The distributions of step speed, direction relative to target, angle of turn and string length are shown in Figure 2.10. The distribution for step speed is not normal since the cercariae show some periods of inactivity. Inevitably in the process of stopping, or moving from rest there are also a number of observations in the very slow speed classes.

When tested against the distribution expected by random behaviour, the distribution for angle of direction relative to the host shows a highly significant difference (Appendix 2.11). It may well be that at different distances from the host, the behaviour of the cercariae changes and affects this parameter. Clearly consideration of the effect of distance from the host is necessary.

The distribution of turns shows that the major difference between the numbers of left and right turns occurred in the 0 to 60^{o} classes, turns of a higher magnitude occuring more or less equally right and left.

The distribution of strings has been tested against that expected by random choice of turn, as previously (see Appendix 2.12). The difference here is highly significant and appears to be due to the over-abundance of long strings. This suggest that the cercariae are tending towards swimming in arcs or circles rather than straight lines.

Clearly the extent of the analyses thus far leaves a lot of hypotheses untested. Since the distance from the host may be important in eliciting any behavioural response that the cercariae

Figure 2.10.

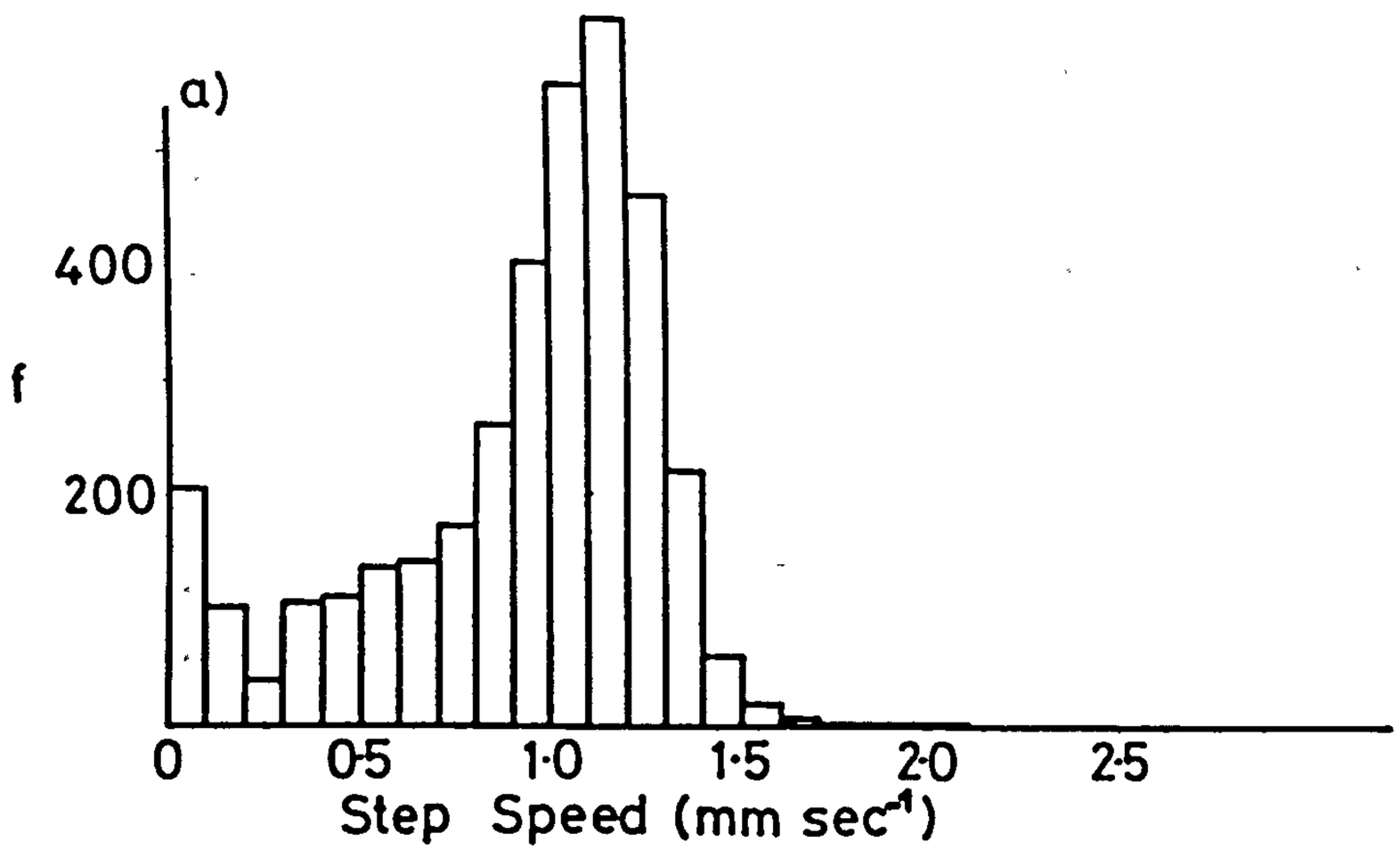
Distributions of parameters of movement for E.recurvatum cercariae in the vicinity of a snail, Lymnaea pereger, horizontal plane:

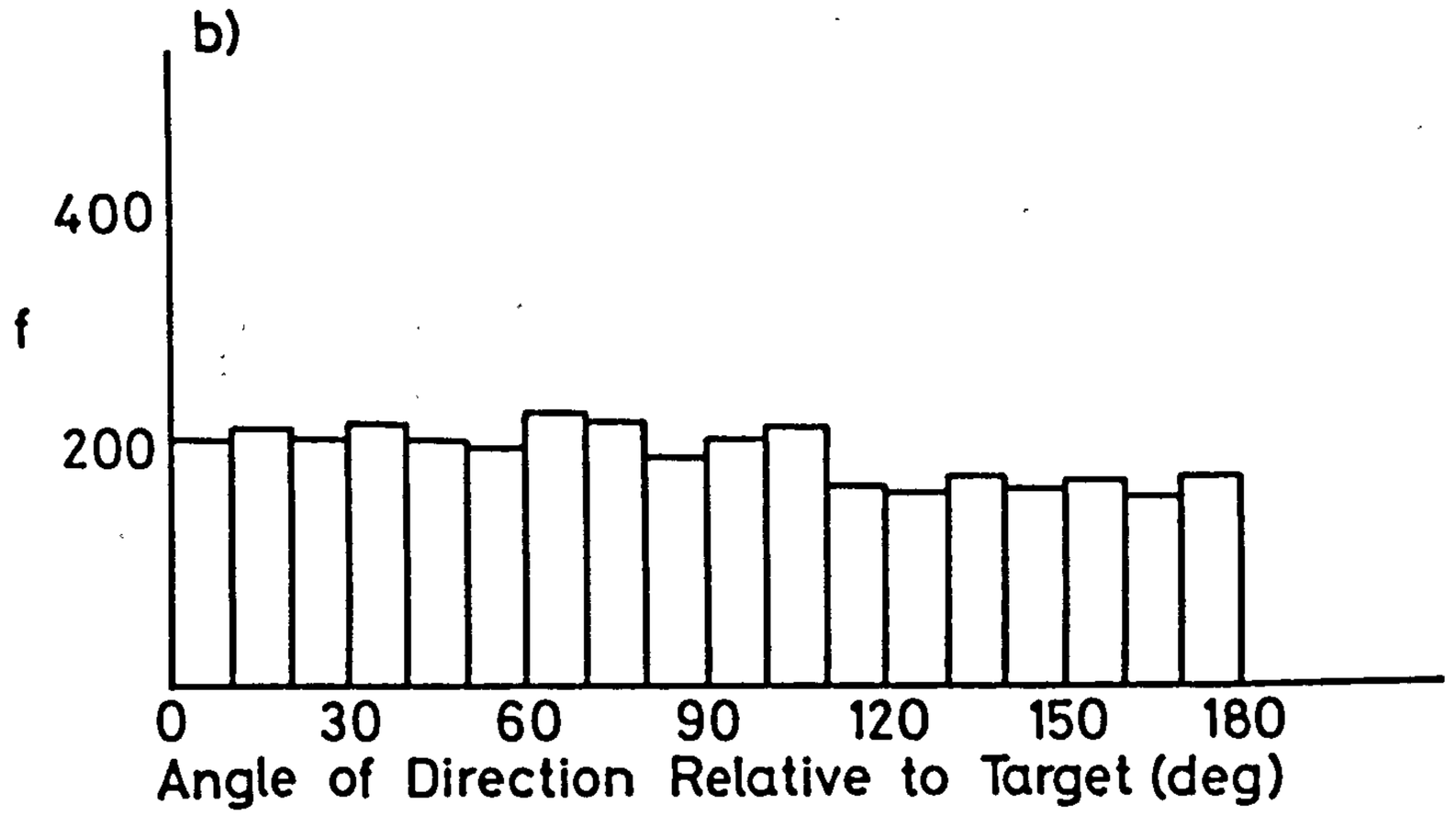
a) Step Speed

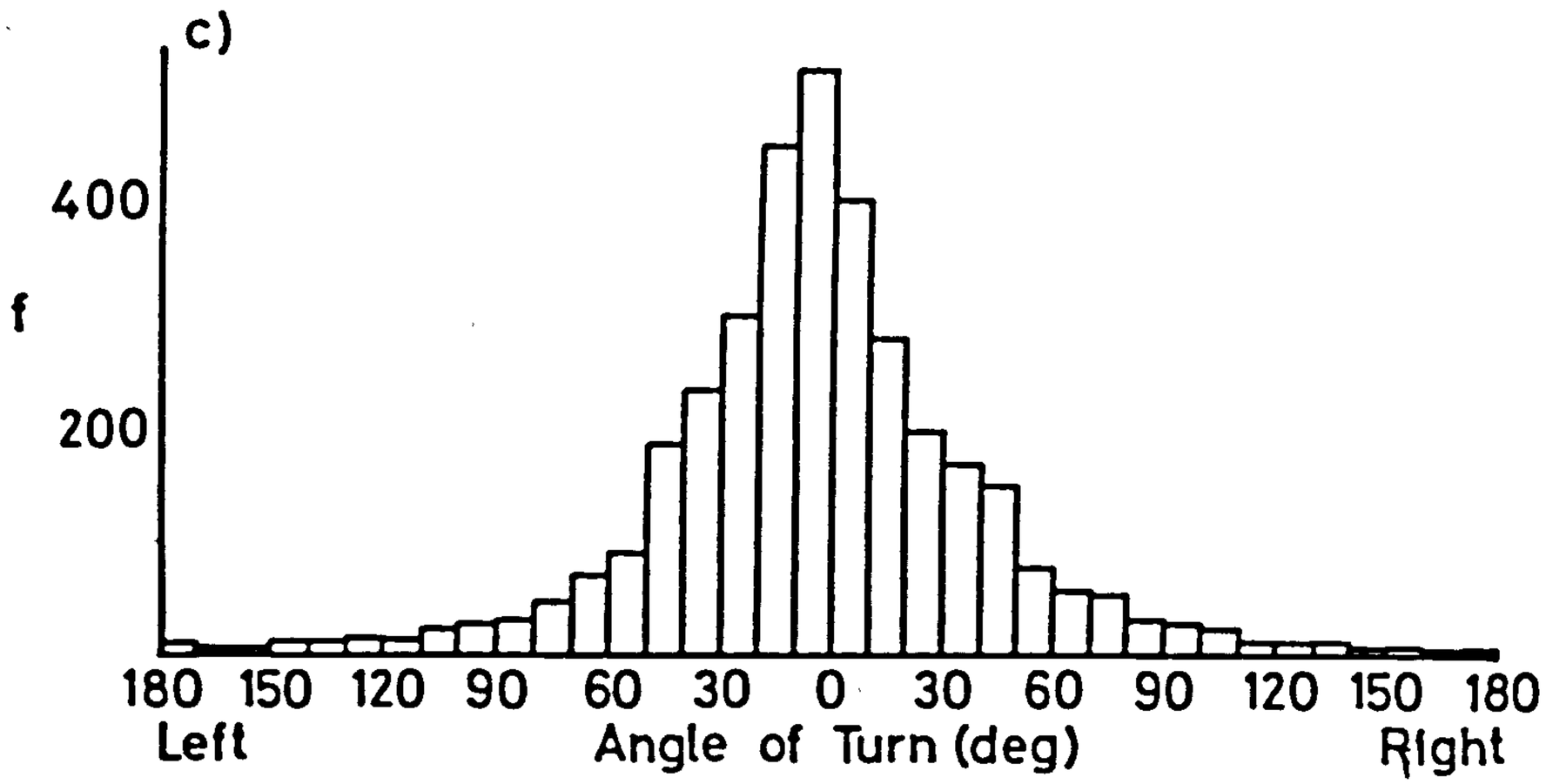
b) Angle of Direction Relative to Target

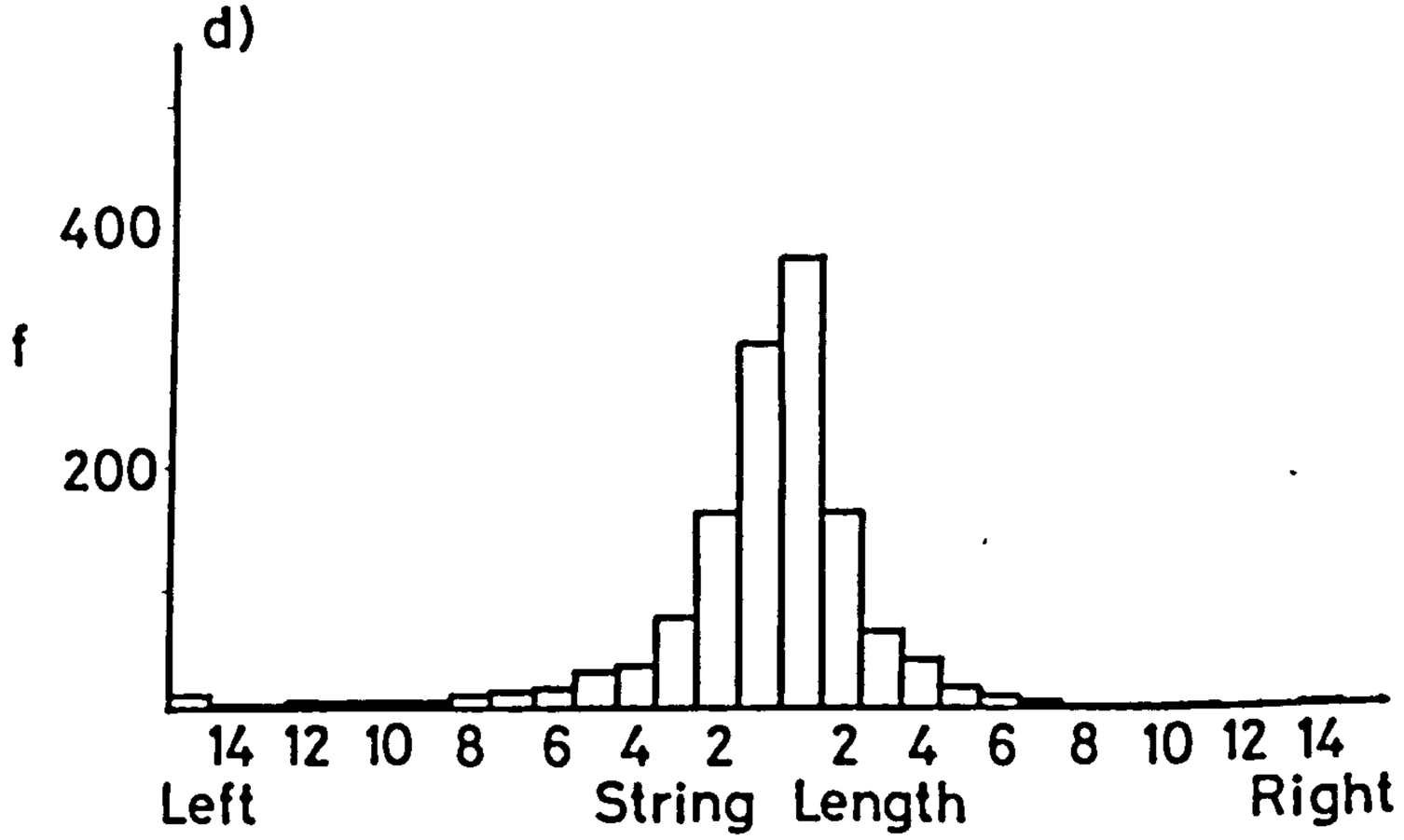
c) Angle of Turn

d) String Length


a)
400
200
f
0
0·5
1·0
1·5
2·0
2·5
Step Speed (mm sec⁻¹)


b)
400
200
f
0
30
60
90
120
150
180
Angle of Direction Relative to Target (deg)


c)
400
200
f
180 150 120 90 60 30 0 30 60 90 120 150 180
Left
Angle of Turn (deg)
Right


d)
400
200
f
14 12 10 8 6 4 2 2 4 6 8 10 12 14
Left
String Length
Right

may be displaying, the data has also been grouped together in classes of distance from the host, using one millimetre intervals. Eight classes have been created and for each the mean step speed, rate of turning and angle of direction relative to the host calculated. It is not possible to consider strings in this way since strings themselves are a collection of a number of observations which can cut across two or more of the created classes.

Means for step speed, rate of turning and angle of direction relative to the host have been plotted against distance from the snail host in Figure 2.11. The apparent differences in the values at the various distances from the host have been analysed in Appendix 2.13 - 2.15 using analysis of variance.

Appendix 2.13 confirms what appears to be the case in Figure 2.11.a, that the cercariae show a decreased step speed as they move successively nearer a target snail host. The mean values in each of the first 5 one millimetre zones are significantly different from each other. The analysis also indicates that the cercariae beyond seven millimetres from the snail swim significantly slower than those at five to seven millimetres away. A decrease in step speed close to a target host will increase the amount of time a cercaria spends in its vicinity and enhance the chances of contact, assuming that the host is stationary or moving at a slower rate than the cercaria. Since snails are slow moving or sedentary this observed decrease in step speed would appear to be of adaptive advantage.

The pattern for rate of turning shows an equally interesting result

Figure 2.11.

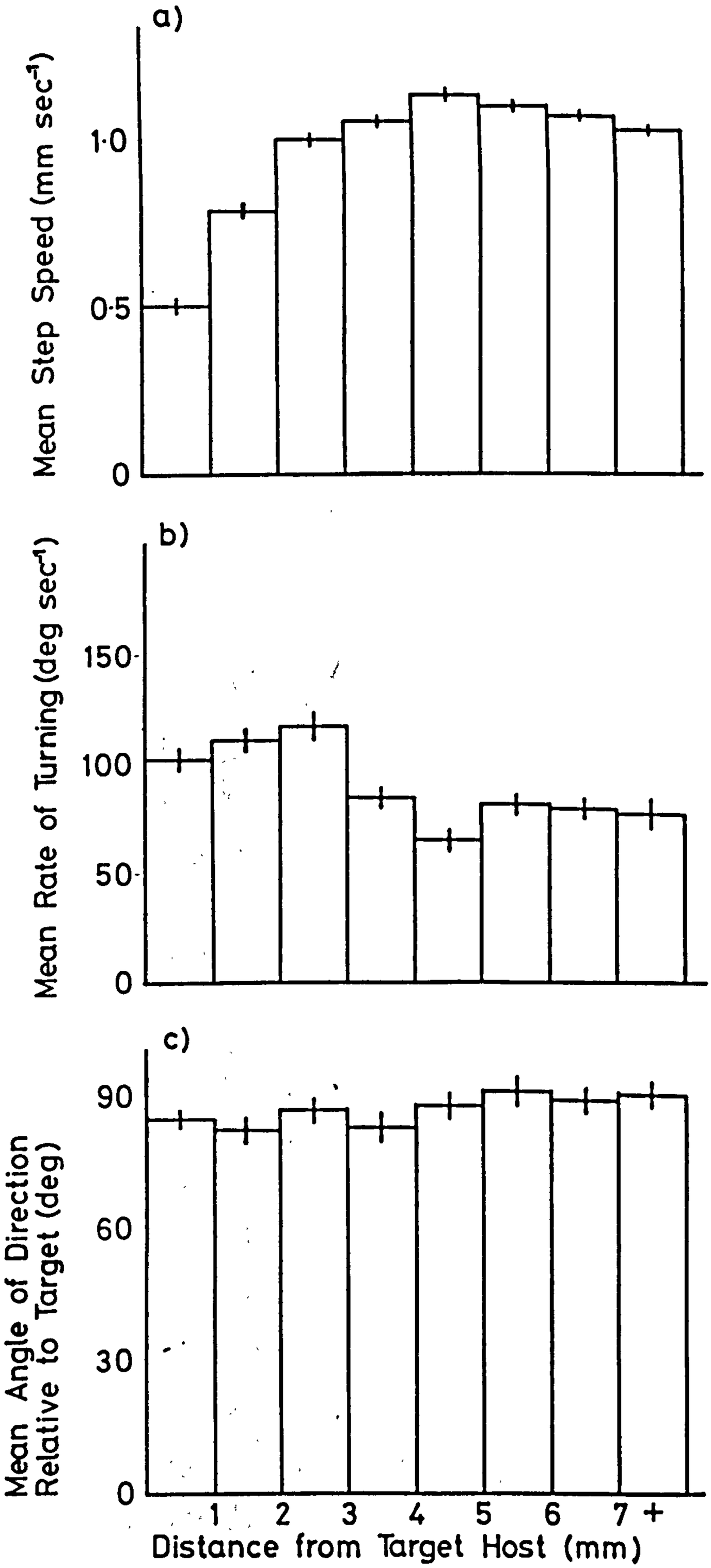
Changes in parameters of movement for <u>E.recurvatum</u> cercariae with distance from the target snail:

a) Mean Step Speed

b) Mean Rate of Turning

c) Mean Angle of Direction Relative to Target

(Error bars indicate standard error of mean).


a)
Mean Step Speed (mm sec⁻¹)
1·0
0·5
0
b)
Mean Rate of Turning (deg sec⁻¹)
150
100
50
0
c)
Mean Angle of Direction Relative to Target (deg)
90
60
30
0
1
2
3
4
5
6
7 +
Distance from Target Host (mm)

(see Figure 2.11.b and Appendix 2.14). Beyond four millimetres from the snail the rate of turning is the same, there being no significant difference in the means between classes. Between 4 - 5 and 3 - 4 millimetres from the snail there is a significant increase in rate of turning and a further significant increase between 3 - 4 and 2 - 3 millimetres from the snail. Means for the classes between 0 and 3 millimetres from the snail are not significantly different. It appears that in a zone between 3 and 5 millimetres from the snail the cercariae have responded to the host's'presence by an increase in rate of turning. Once within this zone of stimulation the response is maintained. Increased rate of turning reduces horizontal displacement and has much the same effect as decrease in step speed, it increases the amount of time spent in the vicinity of a slow moving or sedentary host. This response would also appear to be of adaptive advantage to the cercariae.

The nature of the stimulus which elicits the responses of reduced speed and increased rate of turning is probably a chemical emission from the snail. Snail-derived chemicals may diffuse out into the water around the host forming a gradient of chemical stimulus. If the cercariae respond only to the presence of the chemical stimulus they will not be capable of determining the angle of direction in which the host lies. If this is so, the angle of direction relative to the host will not differ with distance from the host. This is shown to be the case in Figure 2.11.c and Appendix 2.15. The cercariae are not capable of determining the position of the host, even when very close to it.

2.4.2. Summary of the Behaviour of E.recurvatum Cercariae in the Vicinity of a Snail Host

Study of the behaviour of E.recurvatum cercariae in the vicinity of a snail host has revealed that:

1) Cercariae decrease their speed successively as they approach the snail, starting at a distance of approximately five millimetres away from the snail.

2) Cercariae also increase their rate of turning as they approach a snail, starting at a similar point to that for decreased speed. However, the increase is not graded and within a zone of three millimetres around the snail remains the same.

3) Cercariae are not able to perceive the angle of direction of the host even when very close to it.

The observed responses shown by E.recurvatum cercariae in the vicinity of a snail host are consistent with a response to a chemical stimulus. If a gradation of chemical stimulus exists around a snail then the cercariae are responding to increased concentration by decreased speed. This behaviour would keep the cercariae in the zones of highest stimulation for relatively longer periods of time and increase the probability of contact, assuming random direction of swimming. The increased rate of turning within a zone of stimulation is a chemo-klinokinesis since the cercariae appear not to navigate directly towards the host. Given the

decrease in speed, an increase in rate of turning allows for greater exploration of a limited area and might enhance the chance of contact still further.

The extent to which the two facets of this change in behaviour are important in host contact cannot be studied with the cercariae themselves since the responses cannot be isolated. However, using computer simulation modelling the effects of each response can be studied independently. This study will be returned to in a subsequent chapter on computer modelling of trematode larval behaviour.

2.5. DISCUSSION

This chapter has involved the study of E.recurvatum cercariae in the unstimulated state, responding to an environmental factor, and to the presence of a snail host. In the process of host location it would appear that all three types of behaviour are important. Unstimulated behaviour leads to efficient displacement of cercariae from their point of shedding. Responses to environmental factors would seem to enhance the vicinity of potential snail hosts, and the behaviour in response to the presence of a potential host appears to increase the amount of time cercariae spend in the immediate vicinity. This in turn might lead to an increased probability of contact.

Parasite life cycles are characterised by the production of large numbers of infective larvae, and it is assumed that the chances of an individual larva successively locating and penetrating a host are very, very small indeed. If this is so then any behaviour which confers an increase in the chances of host contact is likely to be of adaptive advantage. Such a behavioural adaptation has been shown to occur in E.recurvatum cercariae, though the extent to which it increases the chances of contacting a host in nature cannot be ascertained. The fact that the responses recorded exist at all is evidence that the behavioural response may well be extremely important in the population dynamics of this parasite.

In this study, the host location behaviour of E. recurvatum cercariae was recorded within one hour of shedding. Subsequently, Evans and Gordon (1983) have investigated the transmission dynamics of these cercariae and recorded that maximal infectivity in an artificial situation is not reached until two hours after shedding. They speculated that reduced infectivity immediately after shedding may be an adaptation to ensure dispersal. However, it cannot be established from their experiments whether the observed reduced infectivity is a lowering of host location ability or of penetrative capacity.

CHAPTER 3 THE BEHAVIOUR OF THE CERCARIA OF PLAGIORCHIS ELEGANS

3.1. INTRODUCTION

Trematodes of the Family Plagiorchiidae include species which occur commonly as parasites of amphibians and reptiles, and less frequently as parasites of homiothermic animals, particularly rodents. All species have cercariae which bear a stylet on the oral sucker and because of this feature are termed xiphidiocercariae. Of the described species of xiphidiocercariae, most have been recorded as encysting in arthropods (usually insects) although some are known to encyst in vertebrates. (See Rees, 1932; Styczynska-Jurewicz, 1962; Bock, 1984).

Accounts of the behaviour of xiphidiocercariae are more numerous in the literature than those of echinostome cercariae. However, most are again purely subjective and non-quantatitive. Khan (1961b) described all the xiphidiocercariae found in his survey of freshwater snails in the London area as 'poor swimmers'. Most he recorded as aggregating near the bottom of a body of water though some had a 'weak tendency to hang near the surface'. Grabda-Kazubska (1969, 1970) recorded the behaviour of two species of Opisthoglyphe and of Haplometra cylindracea. O. ranae cercariae were said to swim persistently near the surface and occasionally attach themselves to the water surface film. O. rastellus cercariae were described as resting on the bottom but responding to mechanical shock, behaviour which was also shown by H. cylindracea cercariae.

Styczynska-Jurewicz (1961) supports Grabda-Kazubska's findings with O. ranae, describing a negative geotaxis. Because of the apparent differences between the behaviour of xiphidiocercariae and echinostome cercaraie in the literature it was decided to investigate the host-location behaviour of a xiphidiocercariae in the present study.

The species studied in the present investigation was found in a population of Lymnaea stagnalis at a site near Kirkstall Power Station, Leeds (Map Reference 185395 - Leeds/Bradfprd Extract) by workers in Dr R W Owen's laboratory at the Univesity of Leeds. The cercariae were originally named Cercaria kirkstallensis, but later identified as Plagiorchis elegans when a laboratory based life cycle was completed using chironomid larvae and white mice (Owen, personal communication). Previously, other workers had recorded finding (Tubangui, 1946) and rearing (Kavelaars and Bourns, 1968) plagiorchiids in laboratory rodents.

A population of laboratory kept Lymnaea stagnalis infected with Plagiorchis elegans was established following a number of trips to a collecting site on the Leeds-Liverpool Canal at Kirkstall Power Station, Leeds. The snails were kept in aquaria in a continuous temperature room at 19°C and with a light cycle of twelve hours on, twelve hours off. Cercariae used in the following expeiments were collected by removing a number of snails from their aquaria and isolating them in a litre beaker containing approximately 750 mls of pond water. Shed cercariae were recovered using a drawn out pipette and placed in a smaller volume of pond water. Cercariae in the following experiments were used during the first hour after

shedding.

Since Dr Owen's team had been able to infect chironomid larvae with P.elegans cercariae, investigation of the host location behaviour of these cercariae was possible in the present study. In addition, the differing capacity for movement of chironomid larvae, when compared to snails, led to speculation that the host location behaviour of P.elegans cercariae might show interesting differences to the recorded behaviour of Echinoparyphium recurvatum cercariae. Chironomid larvae, whilst sedentary for much of the time, are capable of quite rapid movement by a thrashing action of the entire body. This thrashing produces a large amount of turbulence in the surrounding water, far more than is evident in the vicinity of a snail.

3.2. THE BEHAVIOUR OF UNSTIMULATED PLAGIORCHIS ELEGANS CERCARIAE

3.2.1. Distribution of a Population of Cercariae with Time

The distribution of a population of Plagiorchis elegans cercariae was recorded in both the horizontal and vertical planes, in the unstimulated state, using the same techniques as described in the previous chapter. However, since a preliminary subjective study seemed to indicate that P.elegans cercariae quickly distributed themselves in the vertical plane, exposures were taken at shorter time intervals in that plane. The data for the distribution of

cercariae in the horizontal and vertical planes are shown in Figures 3.1 and 3.2 respectively.

Horizontal plane

It can be seen that the cercariae reached even distribution throughout the cell relatively quickly, indicating that they have little difficulty in horizontal displacement. By contrast to the cercariae of Echinoparyphium recurvatum, those of P.elegans remained at approximately even distribution throughout the remainder of the experiment.

Vertical plane

In the vertical plane, the P.elegans cercariae had, within 30 minutes, established two distinct populations, one near the surface and the other near the bottom. This situation persisted throughout the experiment. Since the number in each population did not remain constant it appears that some migration upwards and downwards was occuring throughout the experiment. It was decided that to fully investigate the vertical distribution of the cercariae it would be necessary to record the movements of individuals from both the surface and bottom populations using cinephotography,

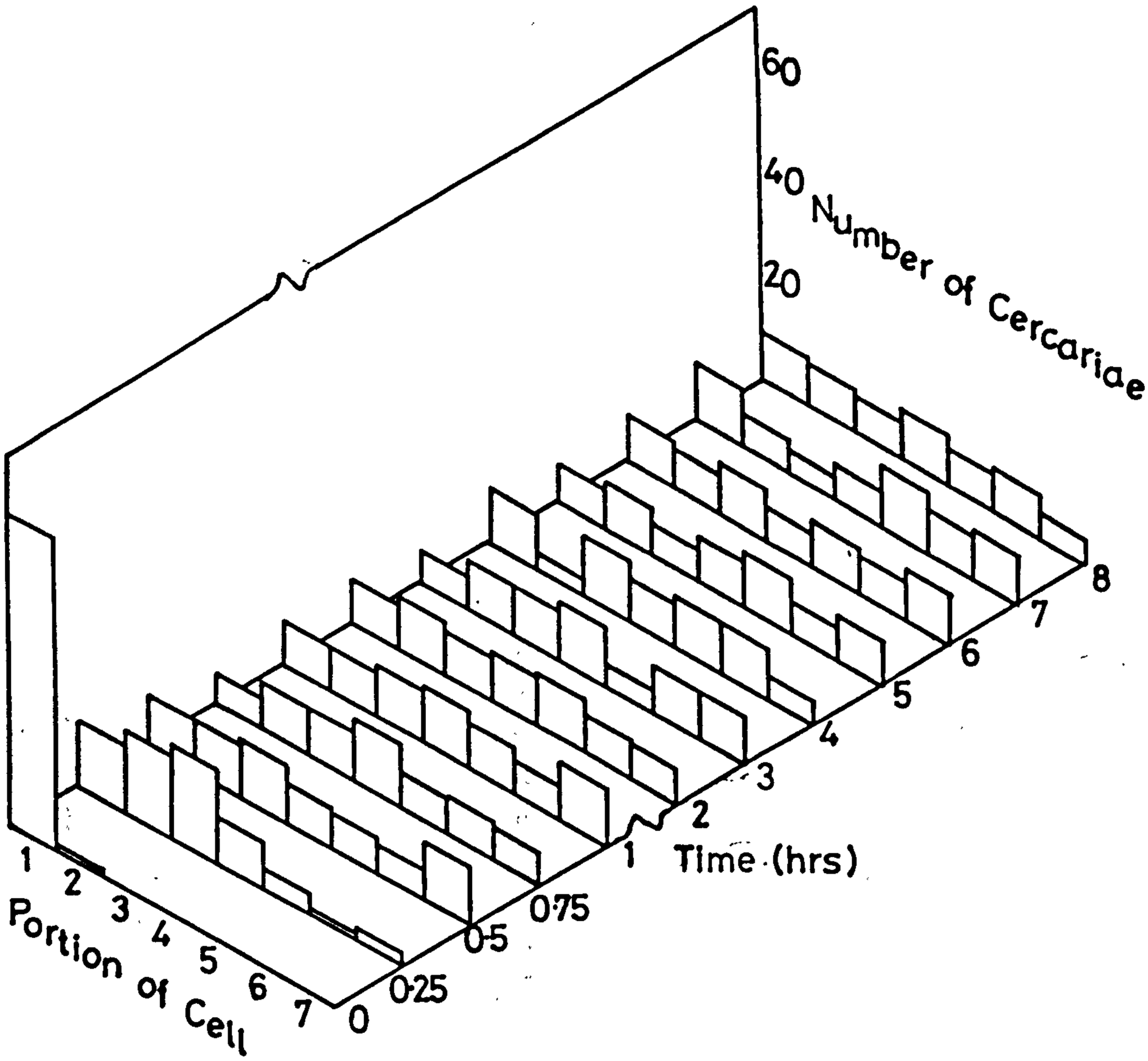


Figure 3.1.

The distribution of a population of unstimulated P.elegans cercariae in a test-cell over eight hours, horizontal plane.

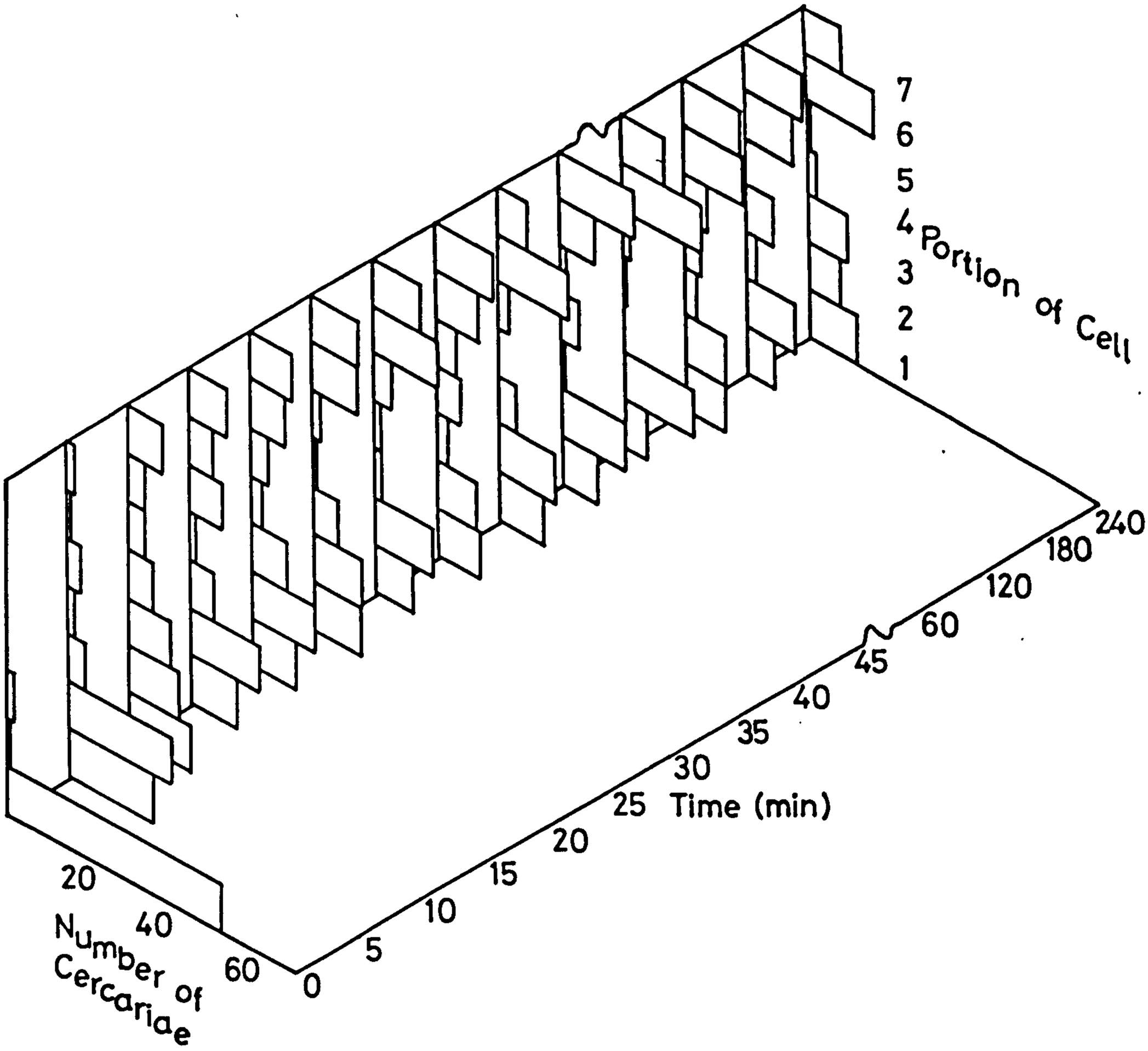


Figure 3.2.

The distribution of a population of unstimulated P.elegans cercariae in a test-cell over four hours, vertical plane.

3.2.2. Tracks of Unstimulated Cercarial Behaviour

Tracks of P.elegans cercariae swimming in the horizontal and vertical planes were filmed and analysed as described previously. Cercariae in the vertical plane were allowed to segregate into two populations, one near the surface of the test cell and the other near the bottom, as had been observed in the population movement experiments.

Horizontal plane

Combined data for the main studied parameters of movement are shown in Table 3.1. A difference from the observed behaviour of E.recurvatum cercariae is the presence of periods of inactivity in P.elegans cercariae. Hence two figures for speed are cited, mean step speed which indicates the rate of displacement, and mean swimming speed which represents the average speed of the cercariae when in motion. The angle of turn, distributions of step speed, angle of direction and string length are plotted in Figure 3.3.

As previously, the distribution of direction has been tested against random behaviour (see Appendix 3.1). When studying the cercariae of E.recurvatum it was remarked that the initial direction of a track might be expected to predetermine the subsequent nineteen observations and hence explain the significant difference from random behaviour. Here tracks of sixty steps each have been used. Despite this the distribution is significantly different to that

Figure 3.3.

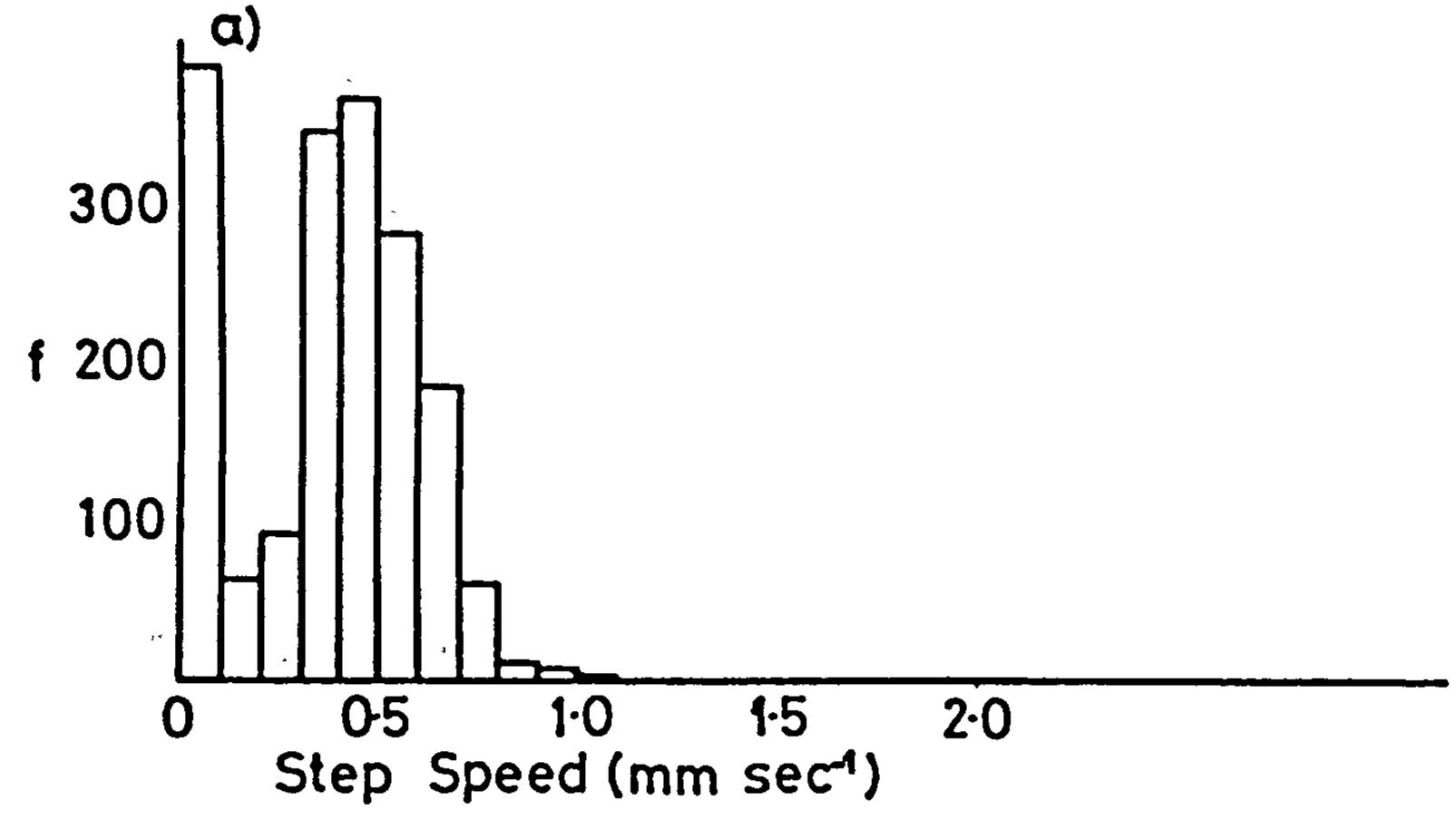
Distributions of parameters of movement for unstimulated P.elegans cercariae, horizontal plane:

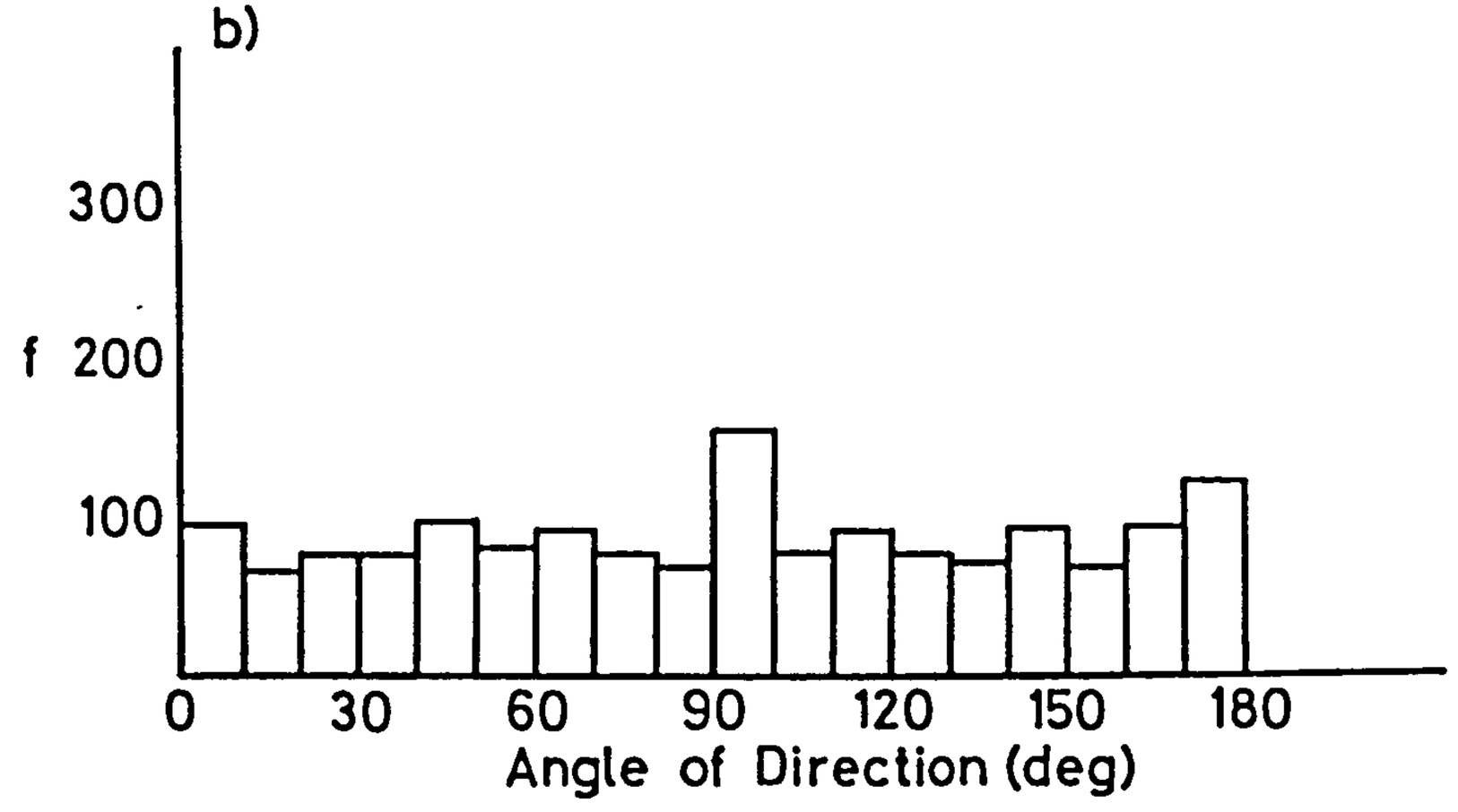
a) Step speed

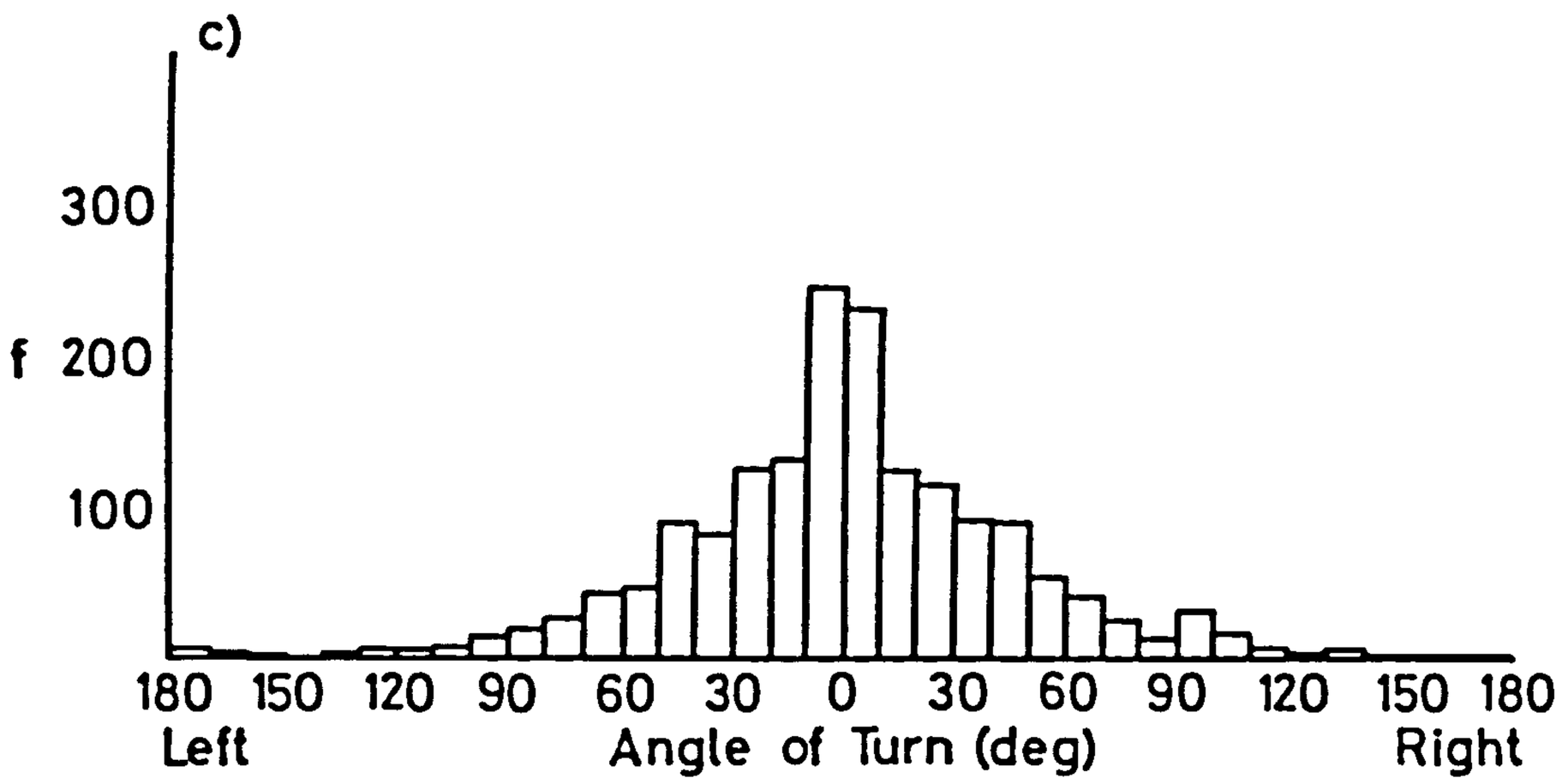
b) Angle of Direction

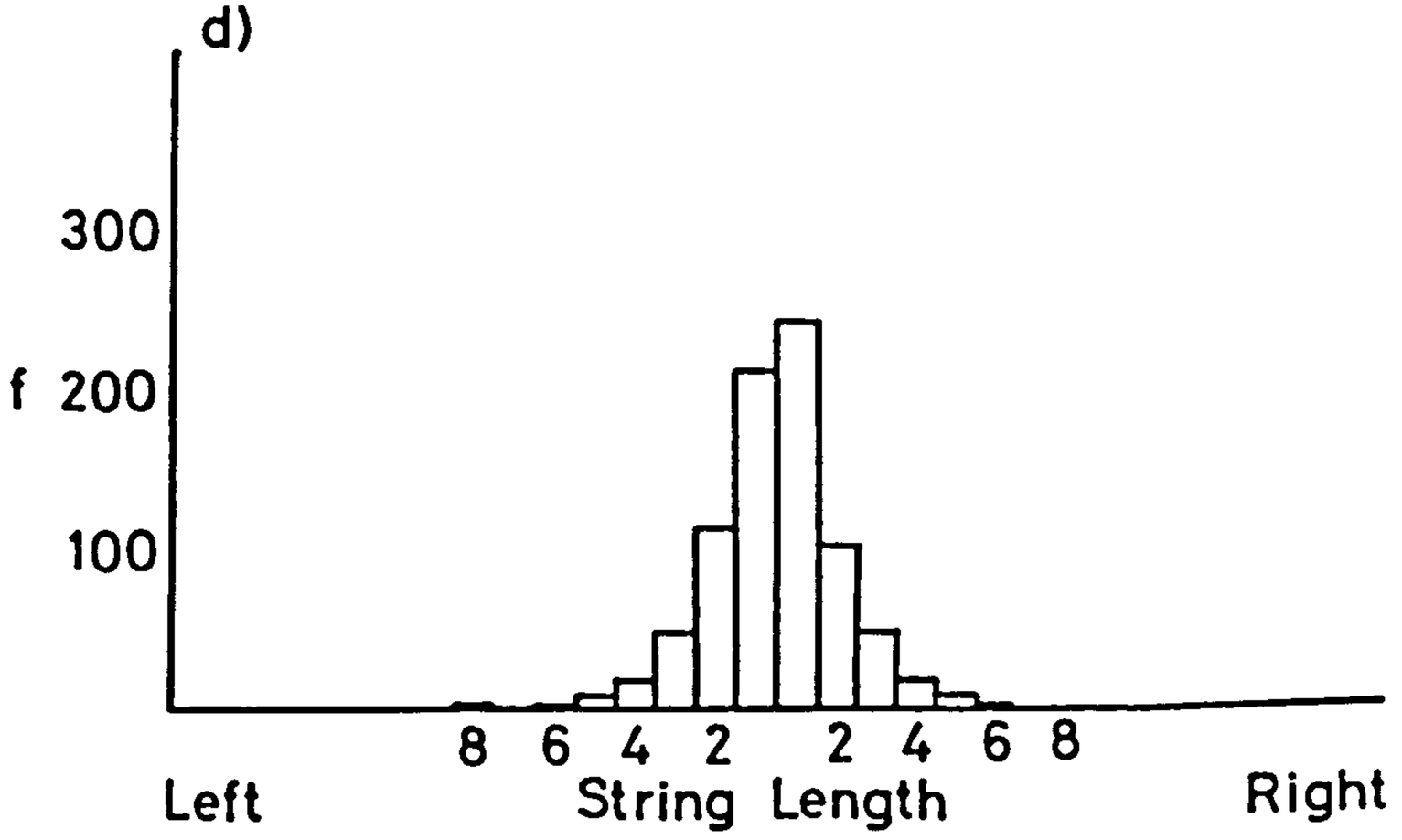
c) Angle of Turn

d) String Length


a)
300
f 200
100
0
0·5
1·0
1·5
2·0
Step Speed (mm sec⁻¹)


b)
300
f 200
100
0
30
60
90
120
150
180
Angle of Direction (deg)


c)
300
f 200
100
180 150 120 90 60 30 0 30 60 90 120 150 180
Left
Angle of Turn (deg)
Right


d)
300
f 200
100
8 6 4 2 2 4 6 8
Left
String Length
Right

expected if choice of direction was at random.

Table 3.1. Plagiorchis elegans cercariae - unstimulated behaviour, horizontal plane.

Number of observations	1800
Step interval	0.333 (sec)
Mean step speed (± S.E.)	0.365 ± 0.005 (mm sec^{-1})
Mean swimming speed (excludes periods of inactivity) (± S.E.)	0.430 ± 0.005 (mm sec^{-1})
Mean duration of active period (± S.E.)	7.163 ± 1.702 (sec)
Mean duration of inactive period (± S.E.)	1.333 ± 0.236 (sec)
Mean angle of direction (± S.E.)	92.131 ± 1.154 (deg)
Mean rate of turning (± S.E.)	92.223 ± 2.261 (deg sec^{-1})
Number of left turns	702
Number of right turns	711

Also as previously, the distribution of strings has been analysed to determine whether the cercariae show a behavioural preference for swimming in relatively straight lines (see Appendix 3.2). The difference between the observed distribution and that expected if left and right turns occur at random is highly significant. With an excess of short strings and a relative lack of long strings it seems that P.elegans cercariae are more likely to turn left after turning right, and vice versa, thus keeping their path relatively straight. This might account for the observed distribution of angles of

direction. If the track paths are relatively straight, the initial direction of a track will, to some degree, predetermine the subsequent ones. Under these circumstances it is the number of tracks, which was small, rather than the number of observed steps, which was large, that is important.

Vertical plane

Data for P.elegans cercarial swimming in the vertical plane has been displayed in two sets, representing the surface and bottom populations, as described previously. Combined data for all tracks recorded in the vertical plane are shown in Table 3.2. Some differences between the sets of data are evident. Whilst the mean step speed of the cercariae in the two populations is identical, the mean swimming speed in the surface population is significantly higher than that in the bottom population. This is only possible if the cercariae in the bottom population are active for more of the time than are those in the surface population. Comparing the mean duration of the active and inactive periods for the two populations reveals that this is the case, and that the proportionately greater time spent swimming is due to a longer acive period, the inactive periods being much the same in the two populations. The shape of the distributions of step speed are also slightly different in the two populations (see Figure 3.4.a).

Figure 3.4.

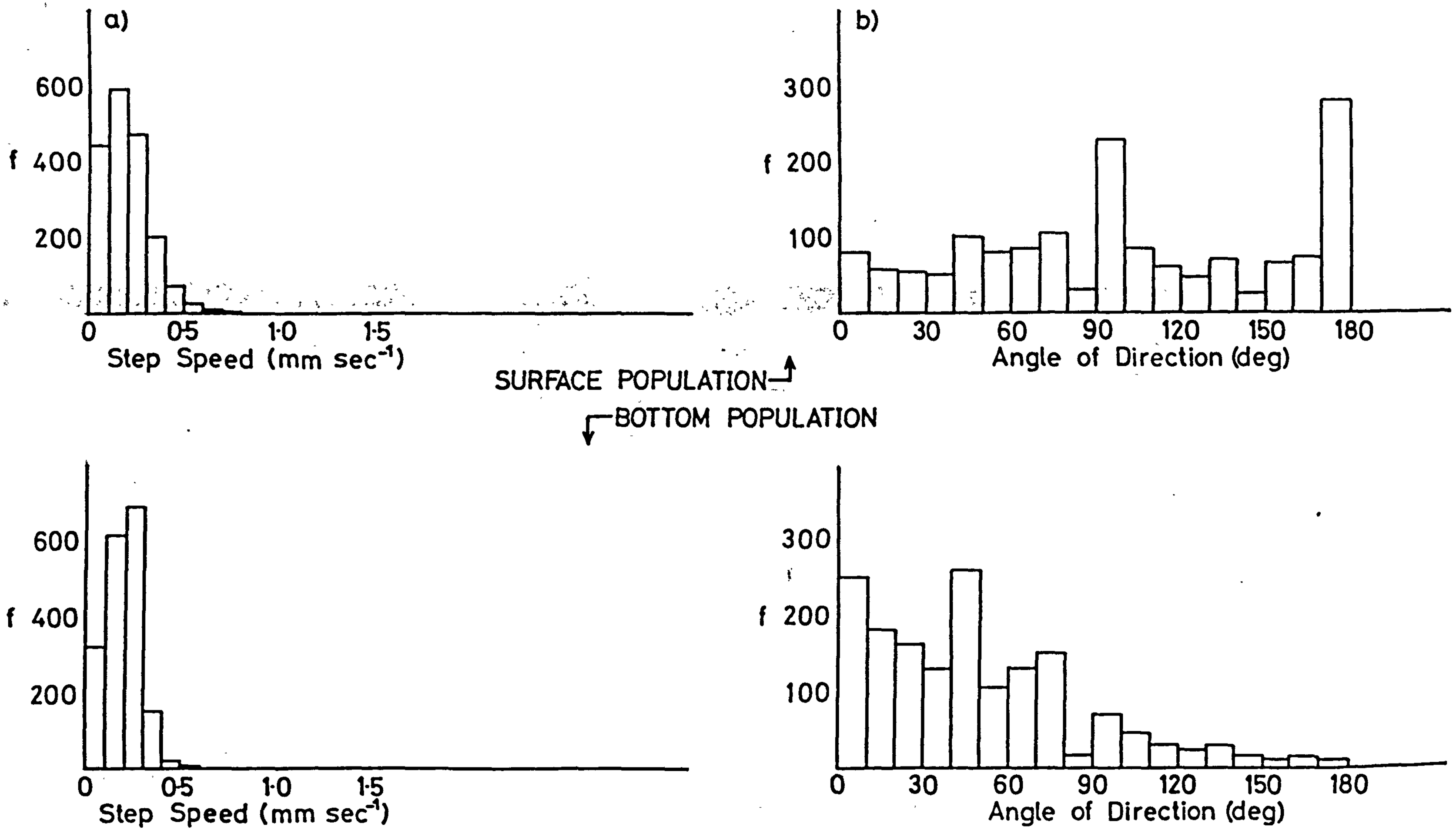
Distributions of parameters of movement for unstimulated P.elegans cercariae, vertical plane, surface and bottom populations:

a) Step speed

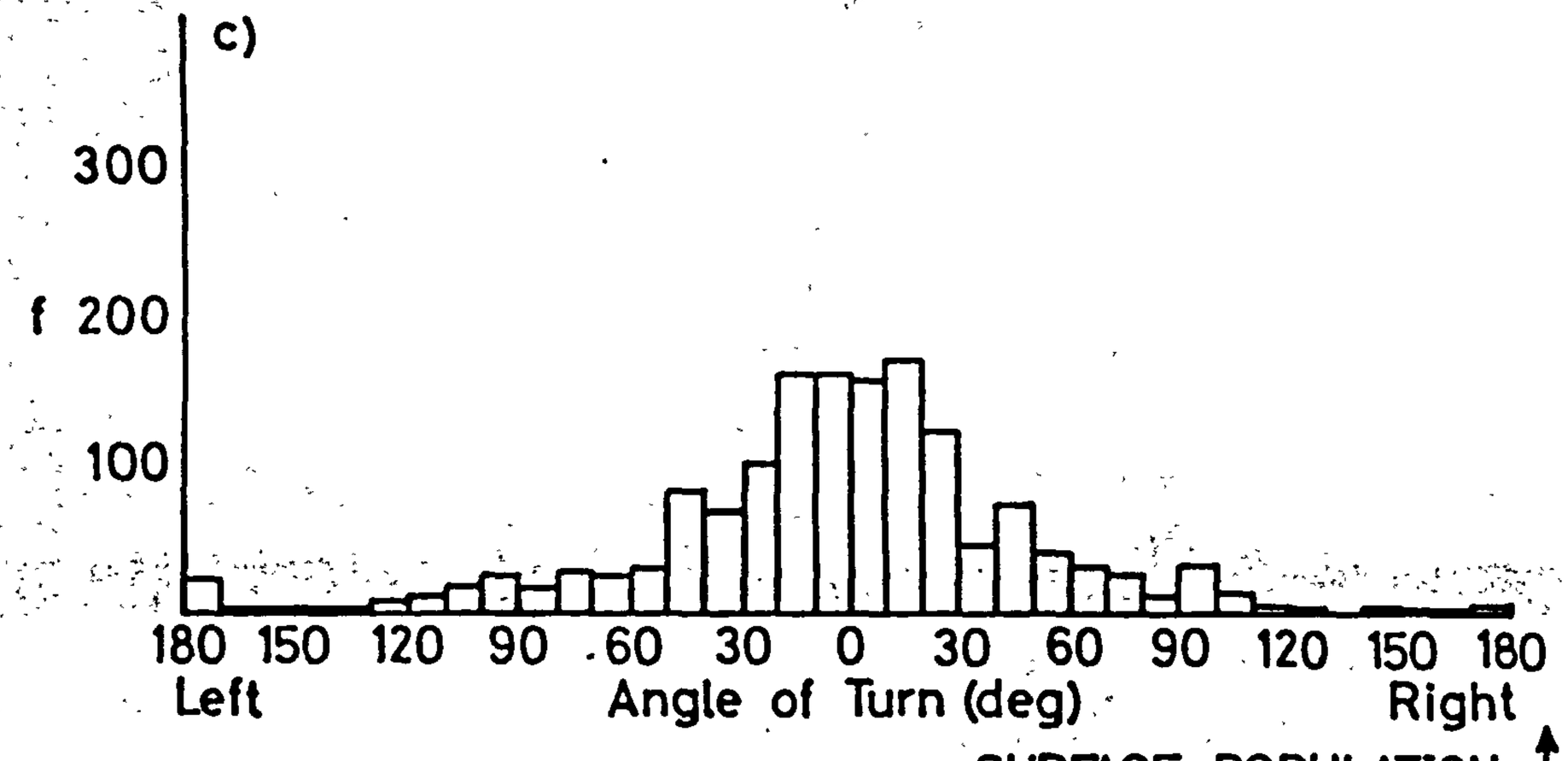
b) Angle of Direction

c) Angle of Turn

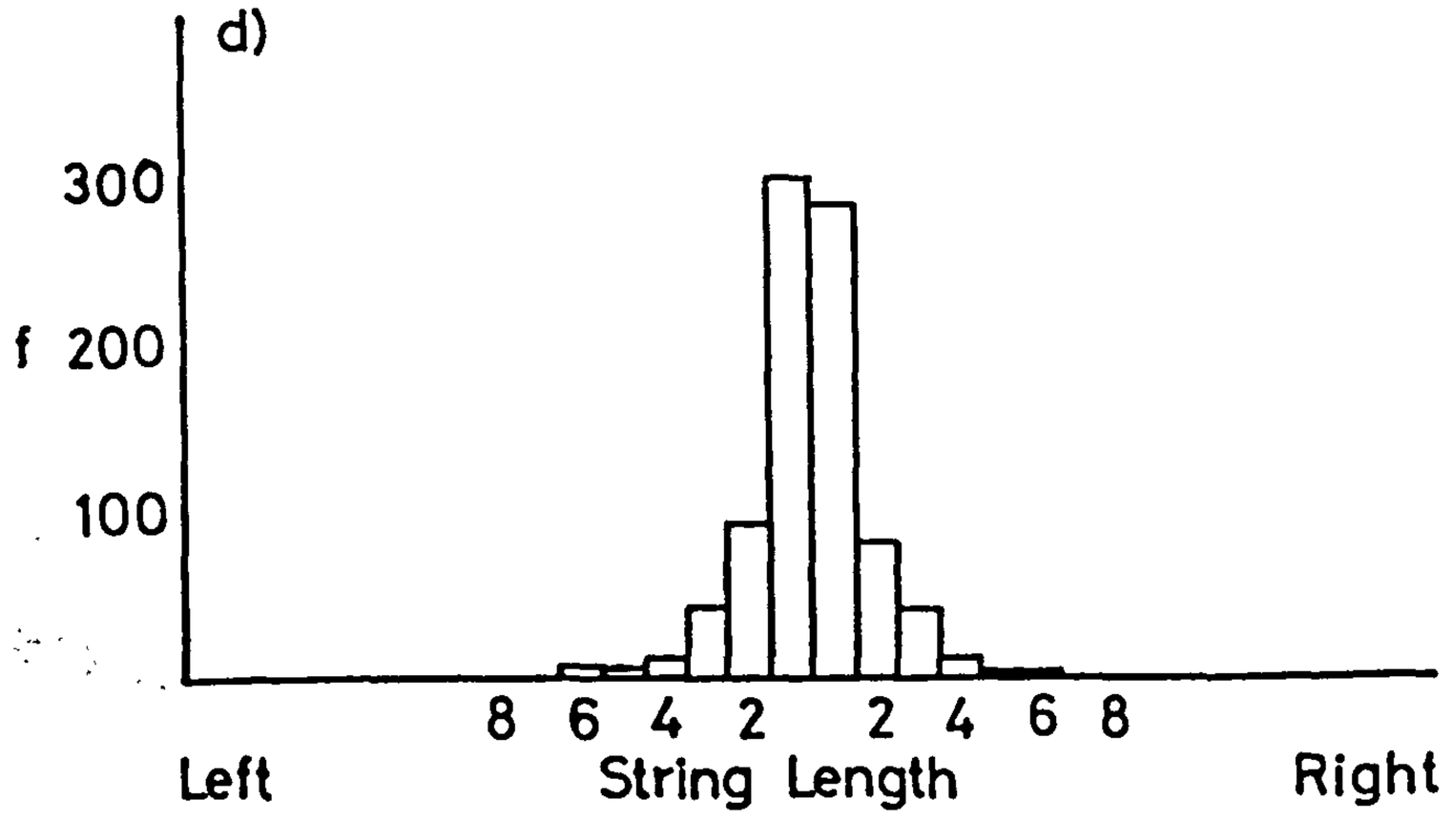
d) String Length


a)
600
f 400
200
0
0·5
1·0
1·5
Step Speed (mm sec⁻¹)
b)
300
f 200
100
0
30
60
90
120
150
180
Angle of Direction (deg)
SURFACE POPULATION
BOTTOM POPULATION
600
f 400
200
0
0·5
1·0
1·5
Step Speed (mm sec⁻¹)
300
f 200
100
0
30
60
90
120
150
180
Angle of Direction (deg)

c)

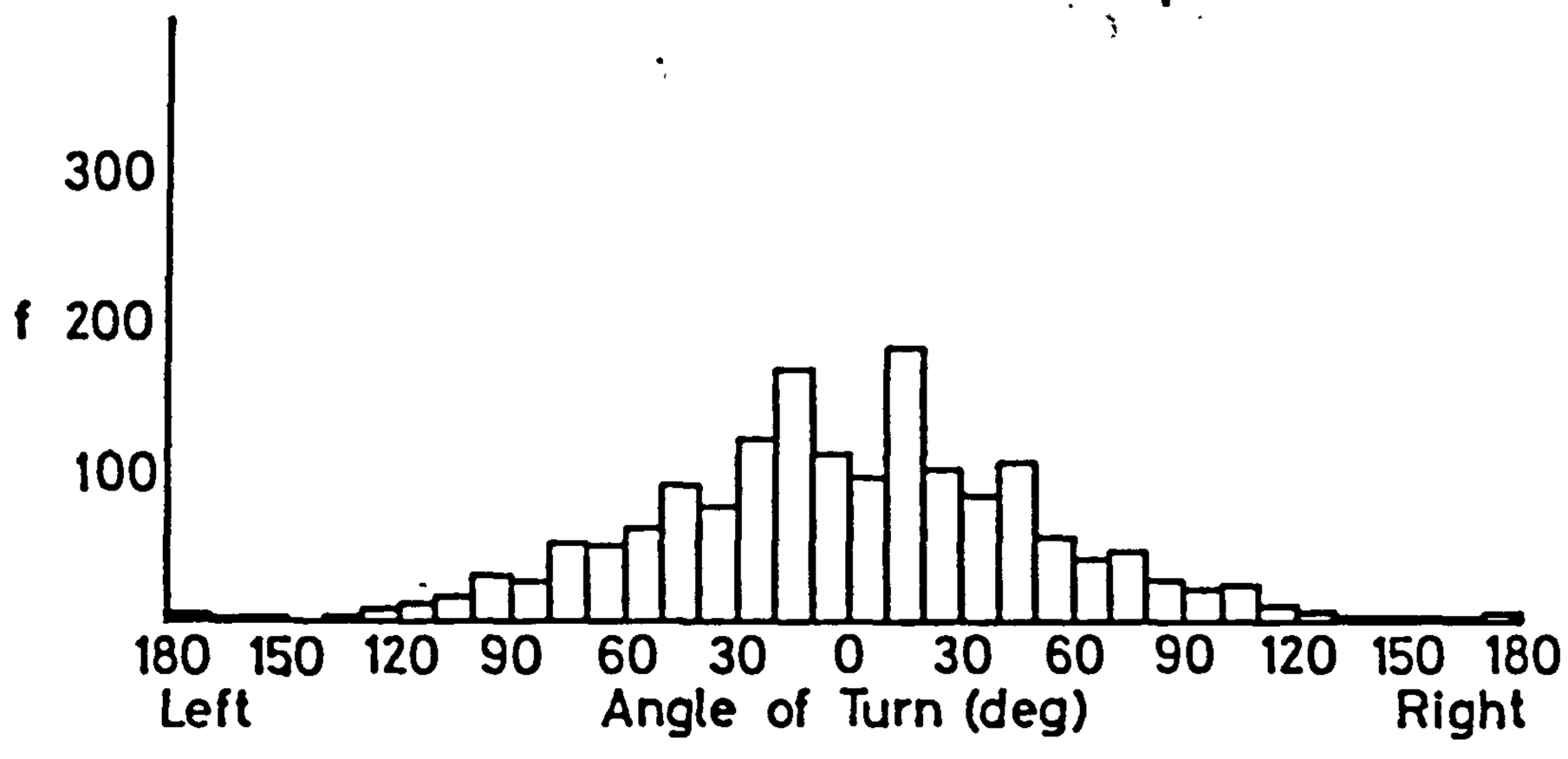


d)



SURFACE POPULATION

BOTTOM POPULATION



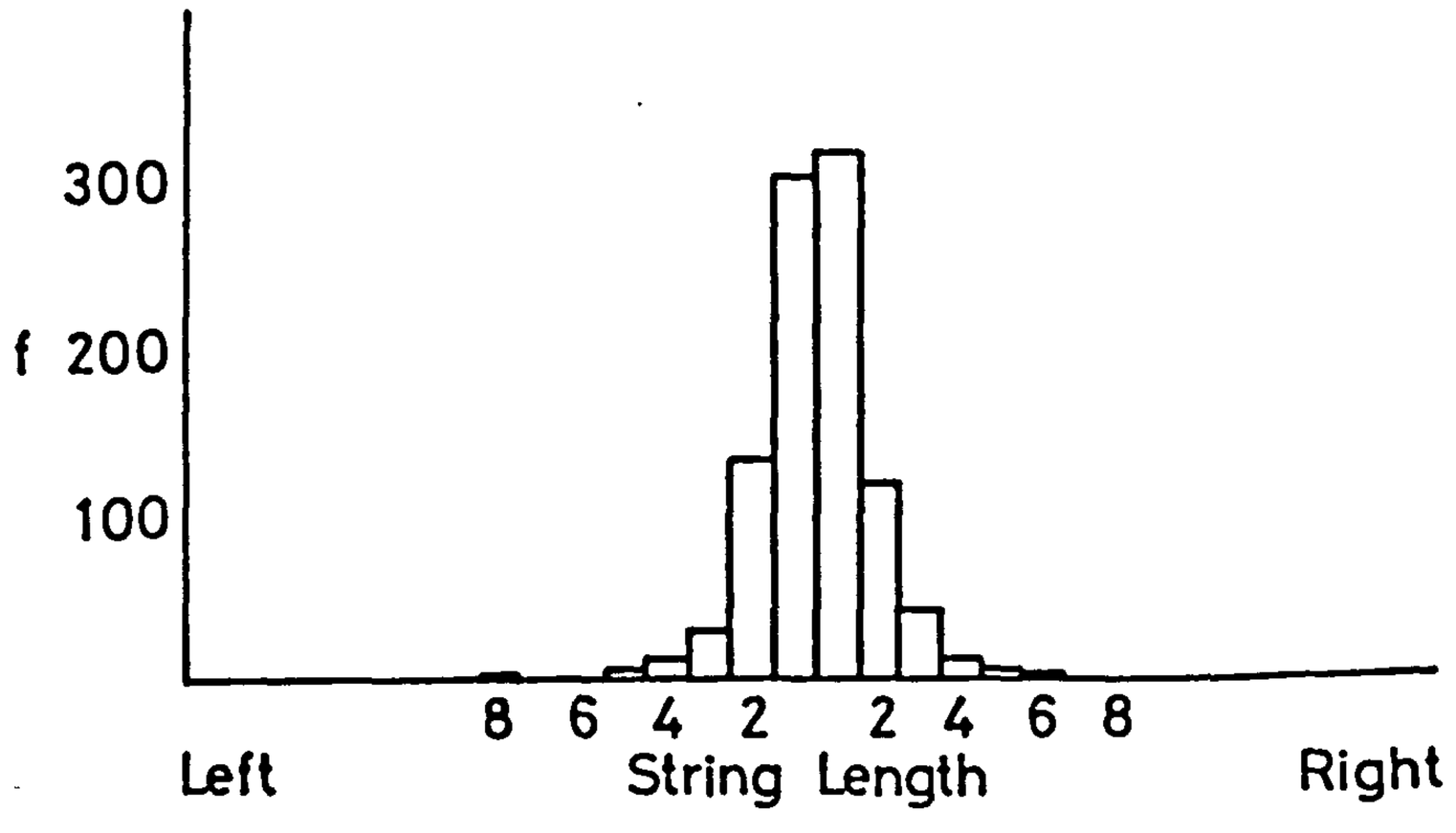


Table 3.2. Plagiorchis elegans cercariae - unstimulated behaviour, vertical plane.

	Surface popn.	Bottom popn.
No. of observations	1800	1800
Step interval (sec)	0.333	0.333
Mean step speed (mm sec^{-1})	0.191 ± 0.003*	0.191 ± 0.002*
Mean swimming speed (mm sec^{-1})	0.213 ± 0.003*	0.198 ± 0.002*
Mean duration of active period (sec)	6.671 ± 1.732*	18.192 ± 5.072*
Mean duration of inactive period (sec)	0.925 ± 0.156*	0.708 ± 0.120*
Mean angle of direction (deg)	98.501 + 1.228*	52.458 ± 0.915*
Mean rate of turning (deg sec^{-1})	88.923 ± 2.481*	110.995 ± 2.312*
Number of left turns	658	782
Number of right turns	638	766

(* ± standard error of the mean)

The mean angle of direction in the surface and bottom populations is markedly different. The plots of the distribution of angles of direction in the two populations indicate why this is so (see Figure

3.4.b). In the surface population the distribution is relatively even, with two pronounced peaks, in the 90 - 100 and 170 - 180 degree classes. The latter peak is easily explained. Cercariae near the surface were observed to demonstrate a 'sink-swim' pattern of behaviour. The large excess of observations in the 170 - 180 degree class of angle of directions is due to this. Cercariae which are sinking are subject to the effects of gravity and descend almost exactly vertically. This also highlights a feature of the periods of inactivity recorded by computer analysis of tracks. Where cercariae are not active, but are sinking due to the effects of gravity, they are recorded as active during track analysis by computer. Accordingly further evaluation of the comparison of active and inactive periods is necessary.

The 90 - 100 degree peak in angle of direction is less easily explained. Whilst cercariae which are not moving are assigned an angle of direction of 90^{o} during track analysis (they are neither responding directionally to or against a stimulus), these observations are not plotted in the distribution of angles of direction. It is possible that there is a preference for swimming horizontally, but this is hard to substantiate. Similarly it is possible that cercariae very close to the surface, being unable to swim vertically upwards further, are merely forced to swim more or less horizontally.

Cercariae in the bottom population demonstrate a strong bias towards swimming vertically upwards (see Figure 3.4.b). There are very few observations in the classes between 80 - 90 and 170 - 180 degrees. Since there are very few observations in the 170 - 180 degree class,

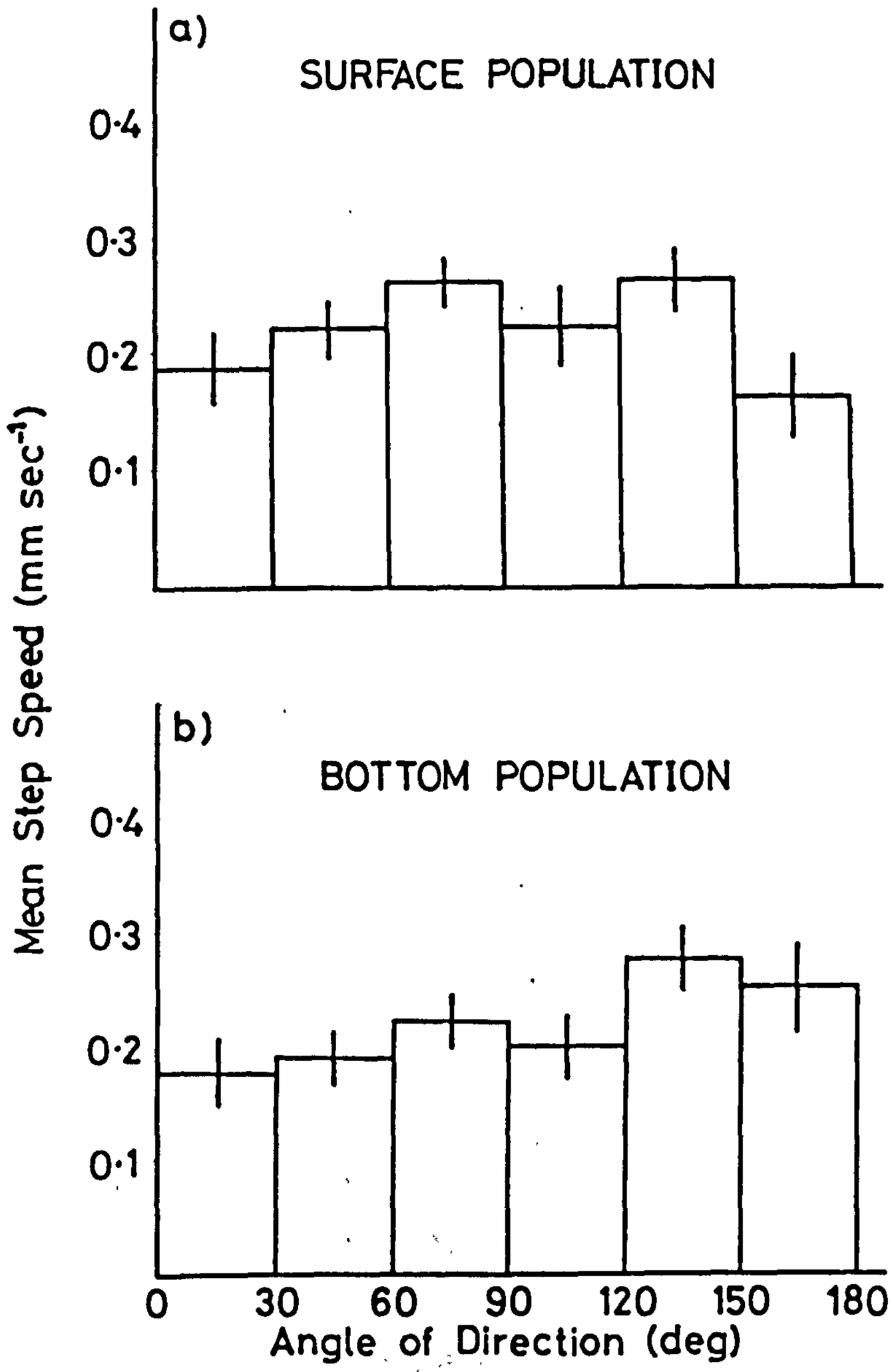
Figure 3.5.

The effect of vertical direction on step speed in P.elegans cercariae:

a) Surface Population

b) Bottom Population

(0^{o} is vertically upwards, 180^{o} is vertically downwards. Error bars indicate standard error of the mean).


a)
SURFACE POPULATION
0·4
0·3
0·2
0·1
b)
BOTTOM POPULATION
0·4
0·3
0·2
0·1
Mean Step Speed (mm sec⁻¹)
0
30
60
90
120
150
180
Angle of Direction (deg)

little sinking is occuring. This infers that the observed similarity in inactive periods in the two populations is an observation created by track analysis. Since sinking is recorded as movement and not inactivity, the cercariae in the bottom population probably have longer active periods and shorter inactive periods than their counterparts in the surface population.

In Figure 3.5 the mean step speed has been plotted against angle of direction for the two populations. Broadly the histograms support the theory that the cercariae displace themselves more slowly as they swim more directly upwards. However, the situation is complicated by the presence of 'sink-swim' rhythms. This in particular explains the low value for mean step speed in the 150 - 180 degree class for the surface population.

The distributions of angles of turn for the two populations are shown in Figure 3.4.c. There was an observed difference in mean rate of turning between the two populations (Appendix 3.3.) but the distributions of angles of turn indicate that the two populations are behaving very similarly in terms of their turning.

In Figure 3.4.d the distribution of strings has been plotted for each of the two populations. There appears to be little difference in the distributions and in both cases there is an over abundance of short strings (see Appendix 3.4). As seen previously, this is consistent with swimming in relatively straight lines with only infrequent changes of direction.

3.2.3. Summary of the Behaviour of Unstimulated P.elegans cercariae

Experiments on the behaviour of unstimulated P.elegans cercariae have led to the following observations:

1) The cercariae do not swim continuously in either the vertical or the horizontal plane but show periods of inactivity at intervals. The effect of these periods of inactivity in the vertical plane is a 'sink-swim' cycle of activity.

2) The cercariae readily displace themselves in the horizontal plane but in the vertical plane two distinct populations are established. In the surface population cercariae roughly maintain their position by a 'sink-swim' cycle of activity. In the bottom population cercariae mostly attempt to swim vertically upwards. The cercariae do seem to swim more slowly when moving vertically upwards, and there is a strong probability that although two separate populations persist in the vertical plane, there is movement between the two populations.

3) Turns to the left or right are to some extent predetermined by prior behaviour, since there is an overabundance of short strings and very few long strings.

4) Most turns are through relatively small angles, with increasingly few at successively larger angles.

The above observations enable speculation as to the preliminary process of host location in these cercariae. Since the cercariae are relatively slow moving, and their hosts, including chironomid larvae, capable of moving much faster, a great deal of energy would be expended in adopting an active searching strategy. In addition the chances of contact would be small. These cercariae appear to have evolved an activity rhythm as a means of preserving energy reserves, using energy only to displace themselves from the point of shedding, and to maintain themselves in a position where the chances of coming into contact with a potential host are increased.

When compared to the unstimulated behaviour of E.recurvatum cercariae, the behaviour shown by P.elegans cercariae differs mainly in the speed and amount of time active. Assuming that the two species are using fixed food reserves as an energy source, one would expect a 'lie in wait' strategy in P.elegans compared to the 'active searching' strategy in E.recurvatum.

3.3. THE BEHAVIOUR OF PLAGIORCHIS ELEGANS CERCARIAE IN THE VICINITY OF A CHIRONOMID LARVA

3.3.1. Tracks of Cercariae in the Vicinity of a Chironomid Larva

By a series of brief experiments it was established that P.elegans would locate and penetrate a number of aquatic insect species. However, since Dr Owen's team in Leeds had been able to complete the life cycle of P.elegans using chironomid larvae, it was decided to use these insect larvae as target hosts in the following experiments.

A chironomid larva was introduced into a small petri dish containing pond water and a large number of cercariae added. The level of the water in the petri dish was then reduced to approximately two millimetres by removal with a drawn out teat pipette. The petri dish was placed in the experimental apparatus for filming in the horizontal plane and one hundred feet of film exposed. A total of thirty tracks were recorded and analysed, twelve of which involved the cercaria successfully locating the target host. Combined data for the usual parameters of movement are shown in Table 3.3. Sample tracks are shown in Figure 3.6 with the host's start and end positions indicated for the duration of the longest track shown. By comparison with the data for unstimulated P.elegans cercariae, also in the horizontal plane (see Table 3.1), it can be seen that there are very few differences here. Step speed and rate of turning are

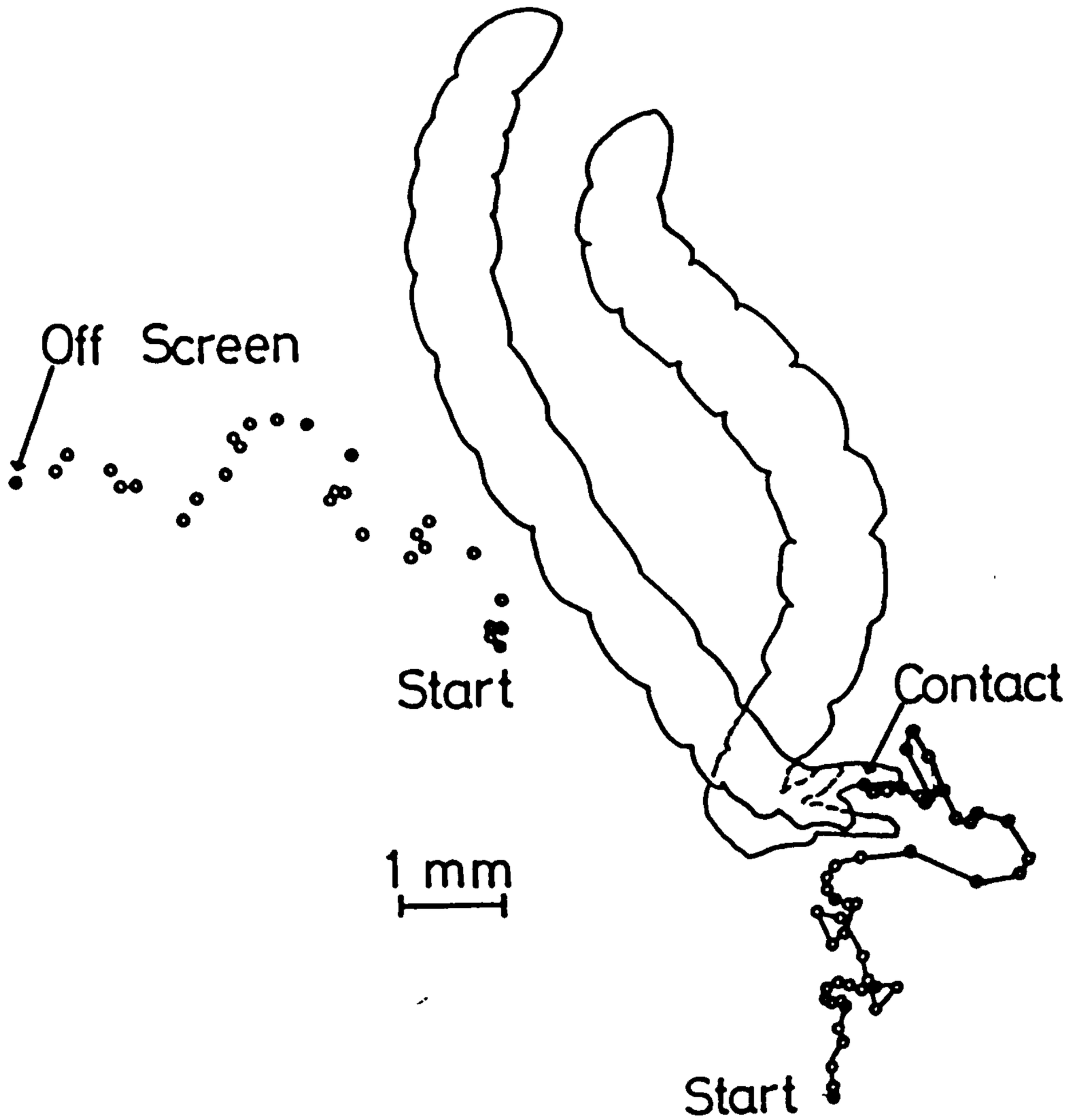


Figure 3.6.

Examples of tracks of <u>P.elegans</u> cercariae in the vicinity of a chironomid larva. (The two positions of the chironomid larva indicate the maximum extent of its thrashing for the duration of the longest track shown).

very similar, with the mean duration of the active period slightly reduced, and the mean duration of the inactive period slightly increased. The distributions of step speed, angle of direction relative to the host, angle of turn and strings are shown in Figure 3.7. The distributions plotted give more information allowing further comparison with the results for unstimulated cercariae.

Table 3.3. Plagiorchis elegans cercariae in the vicinity of a chironomid larvae.

Number of observations	3073
Step interval	0.345 (sec)
Mean step speed (± S.E.)	0.312 ± 0.006 ($mm\ sec^{-1}$)
Mean angle of direction relative to host (± S.E.)	91.042 ± 0.810 (deg)
Mean rate of turning (± S.E.)	93.845 ± 2.022 ($deg\ sec^{-1}$)
Number of left turns	1056
Number of right turns	1138
Mean distribution of active period (± S.E.)	6.563 ± 1.060 (sec)
Mean duration of inactive period (± S.E.)	1.880 ± 0.555 (sec)

The distribution of step speeds here is very much the same shape as for unstimulated cercariae over the range 0 to 1.1 mm sec^{-1}, but in addition here there are observations up to 3.6 mm sec^{-1}. By

Figure 3.7.

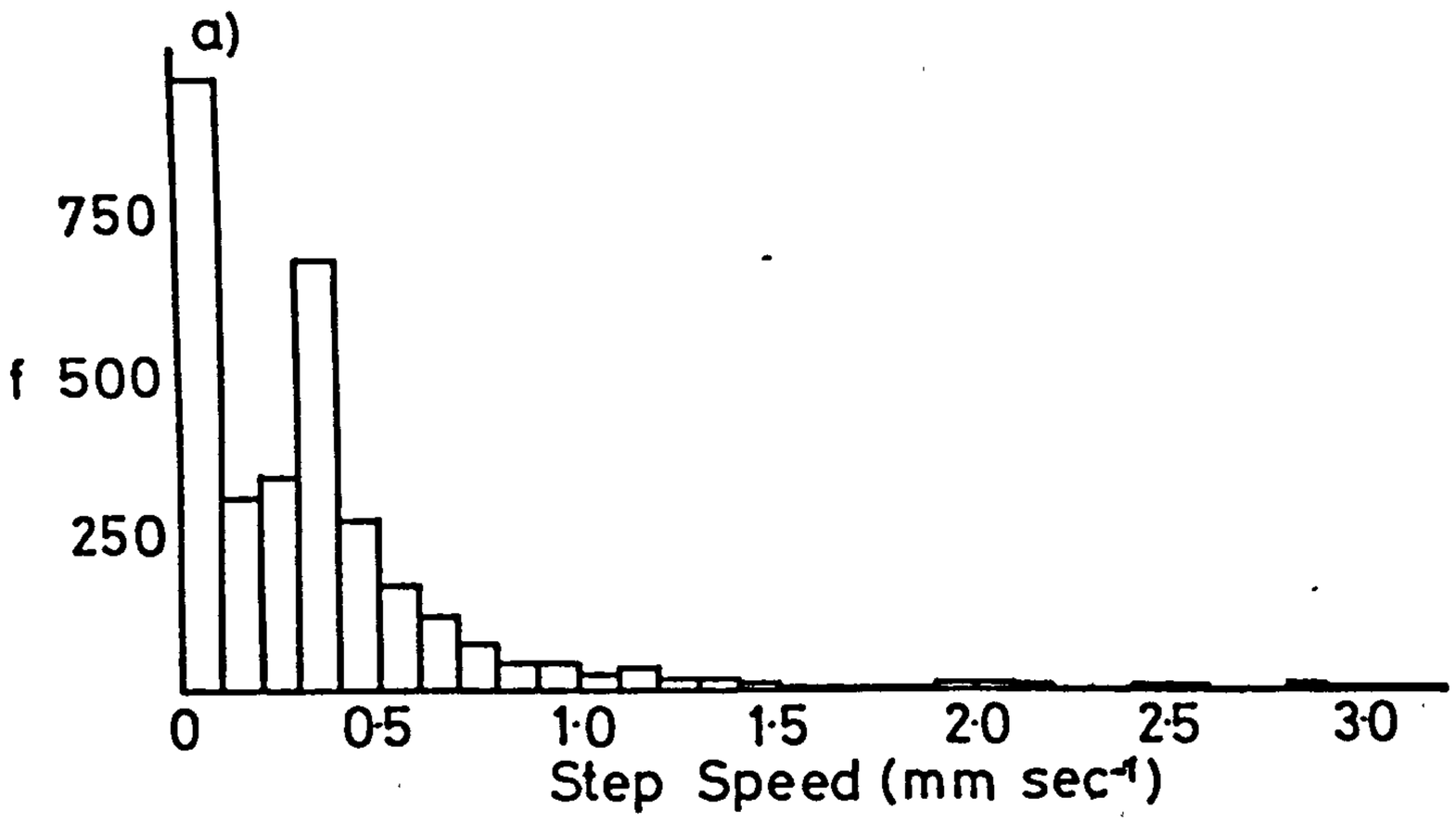
Distributions of parameters of movement for P.elegans cercariae in the vicinity of a chironomid larva, horizontal plane:

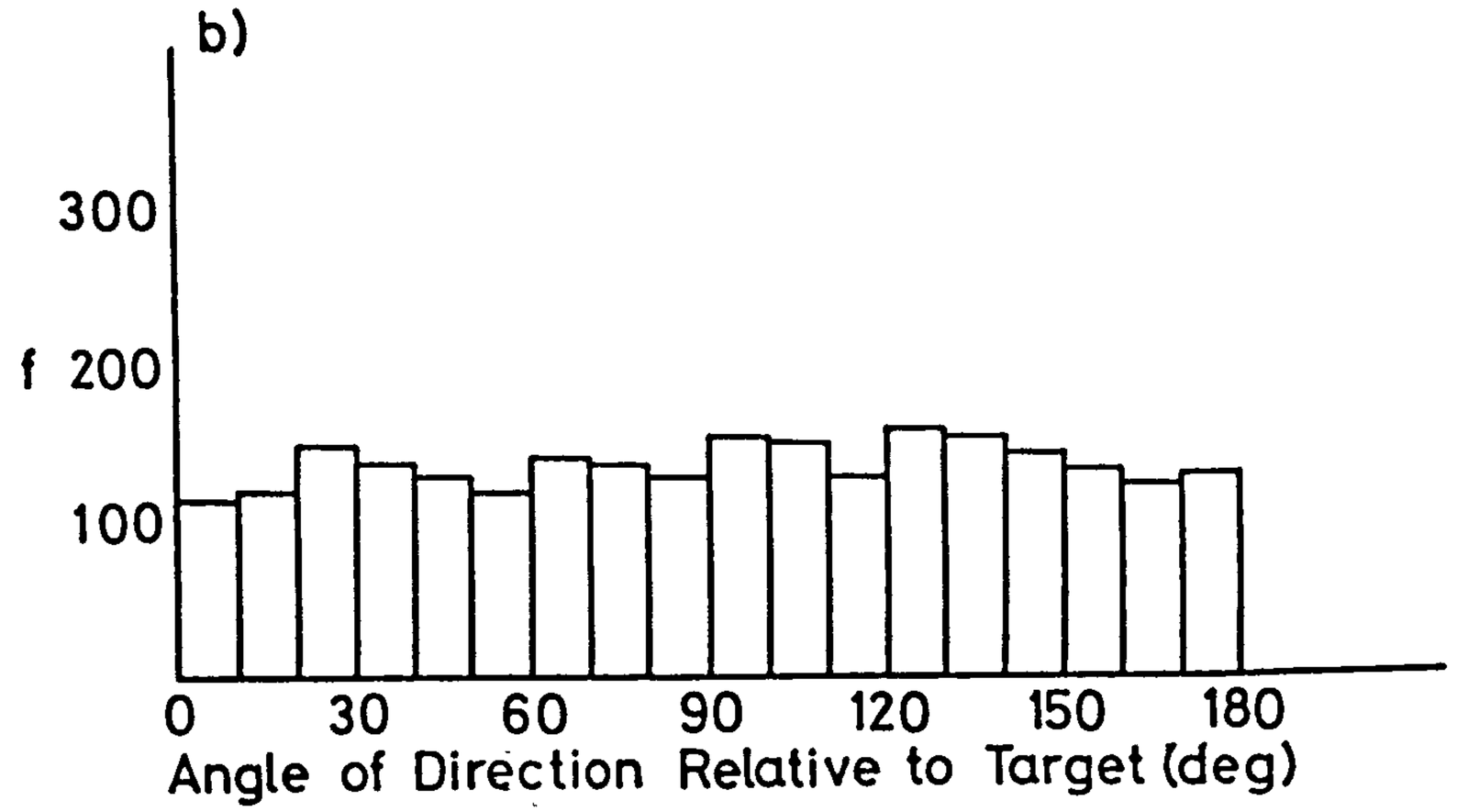
a) Step Speed

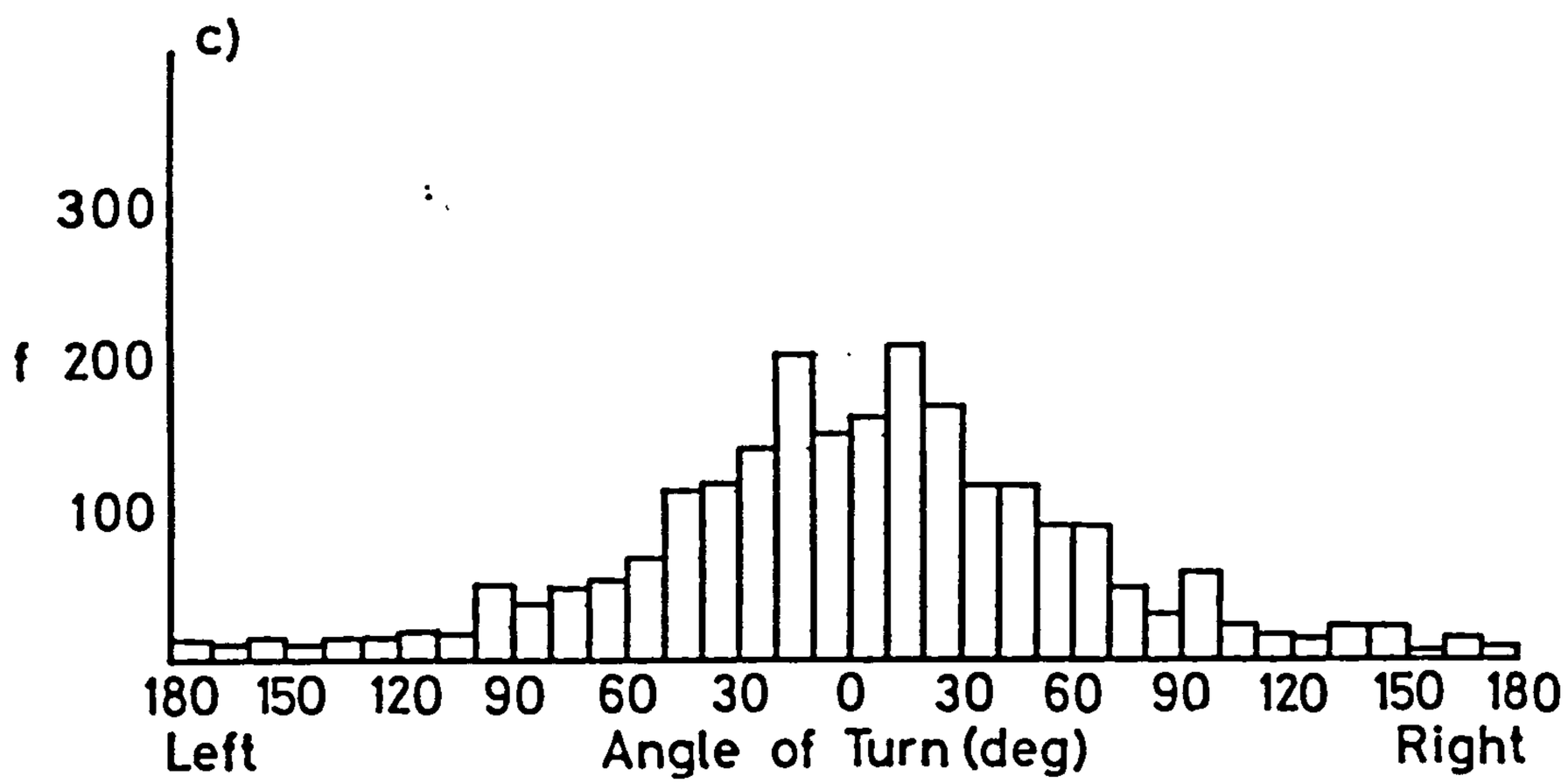
b) Angle of Direction Relative to Target

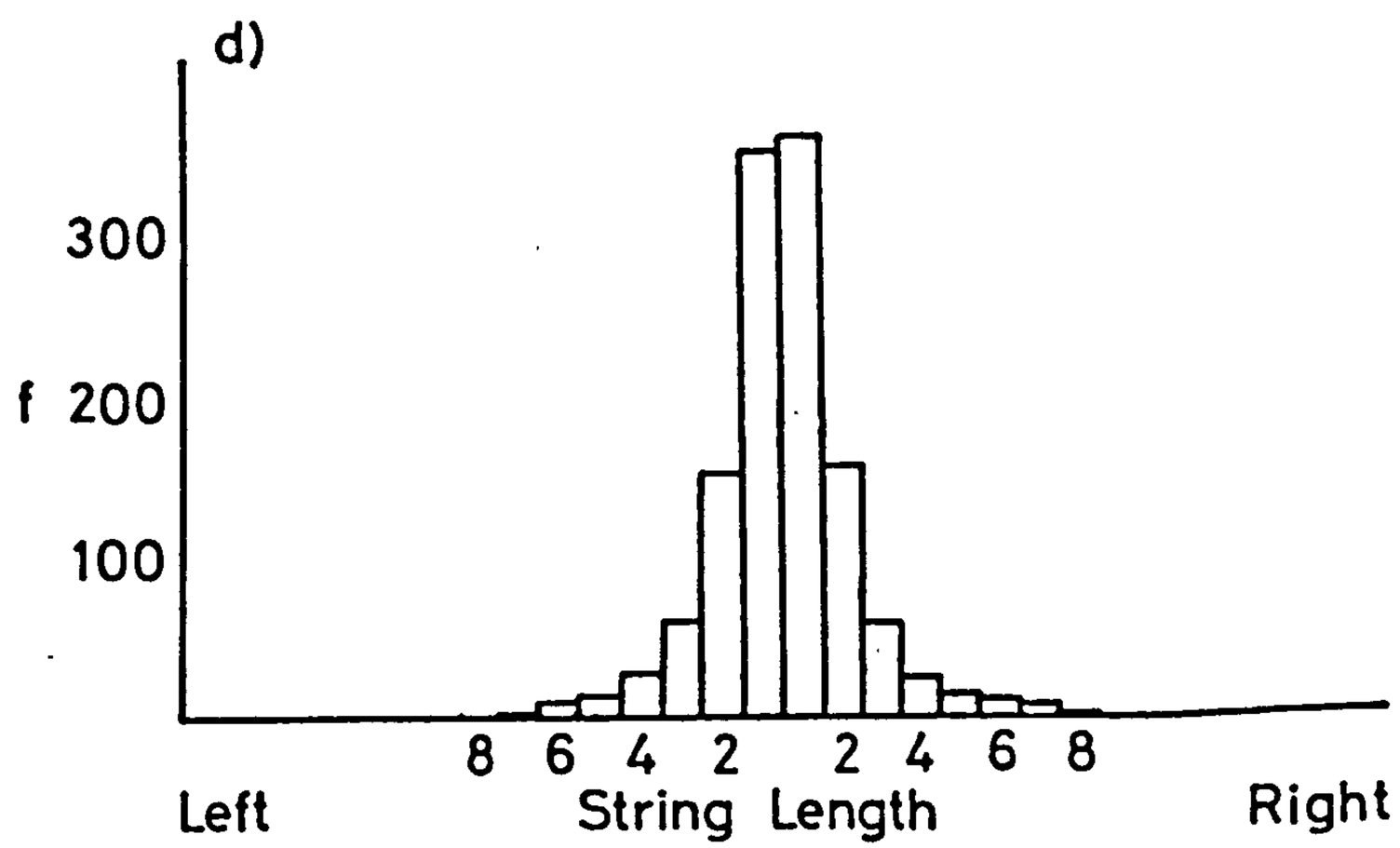
c) Angle of Turn

d) String Length


a)
750
f 500
250
0
0·5
1·0
1·5
2·0
2·5
3·0
Step Speed (mm sec⁻¹)


b)
300
f 200
100
0
30
60
90
120
150
180
Angle of Direction Relative to Target (deg)


c)
300
f 200
100
180 150 120 90 60 30 0 30 60 90 120 150 180
Left
Angle of Turn (deg)
Right


d)
300
f 200
100
8 6 4 2 2 4 6 8
Left
String Length
Right

contrast, in the unstimulated state the maximum speed recorded was in the 1.0 to 1.1 mm sec^{-1} class. Study of the film revealed that cercariae are displaced at these high speeds when the bodies of the chironomid larvae rapidly curl and uncurl. This activity causes turbulence in the water into which the cercariae, being extremely small and relatively poor at displacing themselves, are swept.

The distribution of angles of direction relative to the target has been tested against that expected if the cercariae showed no orientation towards the host, or any other stimulus (see Appendix 3.5). There is no significant difference between the observed and expected distributions, indicating that the cercariae do not seem to be able to determine the host's position.

The distributions of angle of turn, and of strings are very similar in the unstimulated and host-stimulated cercariae. This appears to infer that turning is relatively unimportant in the host-locating behaviour of these cercariae.

Consideration of the above parameters, taking means for all observations, makes no allowance for any changes with distance from the target host. Accordingly the data for step speed, angle of direction relative to host and rate of turning have been reclassified, by distance from the target host. The mean values for each class, for each of the three parameters of movement are shown in Figure 3.8. Apparent differences between means in the different classes have been tested using analysis of variance (Appendices 3.6 - 3.8).

Figure 3.8

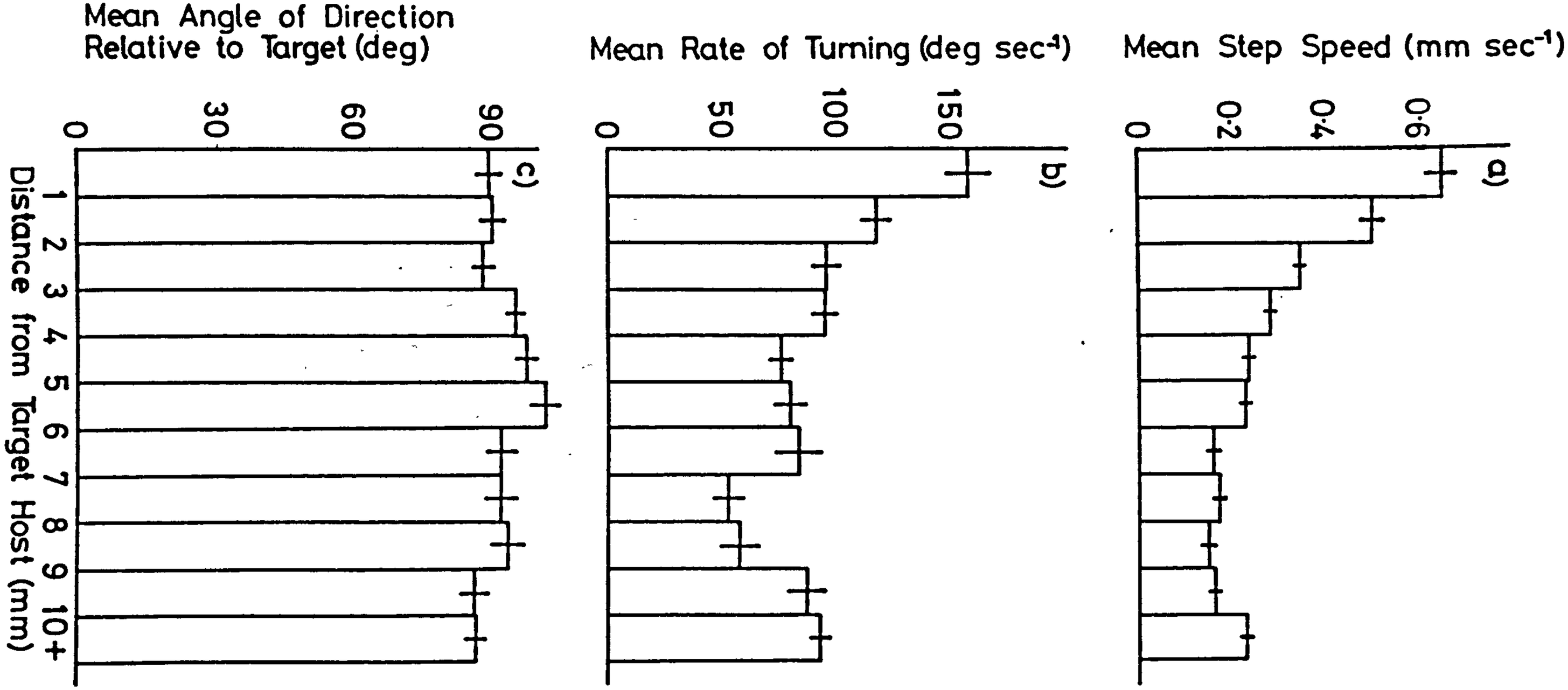
Changes in parameters of movement for P.elegans cercariae with distance from the target chironomid larva:

a) Step Speed

b) Mean Rate of Turning

c) Mean Angle of Direction Relative to Target

(Error bars indicate standard error of mean)


Mean Angle of Direction Relative to Target (deg)
0
30
60
90
c)
Distance from Target Host (mm)
1
2
3
4
5
6
7
8
9
10+
Mean Rate of Turning (deg sec-1)
0
50
100
150
b)
Mean Step Speed (mm sec-1)
0
0·2
0·4
0·6
a)

Step speed can be seen to increase significantly at successively shorter distances from the host. This effect seems to start at about 5 millimetres distance from the host. Speeds achieved in close proximity to the host are almost exactly twice that of unstimulated cercariae swimming in the horizontal plane. Between five and ten millimetres from the host, speed is depressed and significantly lower than for unstimulated cercariae. It would appear that a response to the presence of the host is occuring, and in two stages. The higher speed in close proximity to the host may be as a result of turbulence caused by the host's movement. Whilst considering this it should be borne in mind that this may be an important factor in host location by these cercariae. A slowing down between five and ten millimetres from the host would have the effect of increasing the amount of time in the host's vicinity.

The mean rate of turning also alters significantly at different distances from the host. At very small distances (less than two millimetres) from the host, rate of turning is significantly increased. This may in part be as a result of turbulence created by the host. If the observed increase in rate of turning is a true response by the cercariae, it would result in an increase in the amount of time spent in the vicinity of the host, enhancing the chances of contact.

Between four and ten millimetres from the host, rate of turning is significantly reduced from that demonstrated by unstimulated cercariae. If cercariae in this zone are able to detect the presence of the host, by whatever means, reduced rate of turning would increase the area of searching.

As with step speed, there appears to be a two step response occurring. The changes in rate of step speed and rate of turning are dissimilar to those demonstrated by E.recurvatum in the vicinity of a snail host and it can be speculated that this may be an adaptation to the different nature of host being sought. Since P.elegans cercariae seem able to respond in two ways, at different distances from the host, one might expect that they are capable of determining the direction in which the host lies. However, analysis of mean angle of direction to the host dispels this suggestion. At no distance from the host is the mean value significantly less that 90 degrees. Indeed in the region four to nine millimetres from the host the mean is consistently more that 90 degrees, though not substantially so.

3.3.2. Summary of the Behaviour of P.elegans Cercariae in the Vicinity of a Chironomid Larva

Experiments on the behaviour of P.elegans cercariae in the vicinity of a chironomid larva have led to the following observations;

1) Cercariae in close proximity to a host are capable of much increased rate of displacement. There is evidence to suggest that turbulence created by the host may be a contributory factor in this.

2) Between five and ten millimetres from the host

these cercariae show a reduced rate of displacement.

3) Cercariae in the vicinity of a host also increase their rate of turning though host created turbulence may again be a contributory factor. It appears sensible to assume from the magnitude of increase, and the fact that the chironomid larva moved only intermittently, that this was a real response by the cercariae.

4) Between four and nine millimetres from the host the cercariae demonstrate a reduced rate of turning.

5) These cercariae are apparently not able to determine the angle of direction of the host at any distance from it.

3.4. DISCUSSION

This chapter has described the behaviour of P.elegans cercariae in the unstimulated state, and in the presence of a chironomid larva. Despite the fact that a chironomid larva has been used as a target host, it is important to remember that P.elegans cercariae have been shown to locate, penetrate and develop in a wide range of aquatic insects.

The unstimulated behaviour of P.elegans cercariae appears to be an adaptation to dispersal from the point of shedding at minimal energy expenditure, whilst enhancing the chances of coming into the vicinity of a potential host. Both horizontal and vertical displacement have been shown to be slow, but relatively efficient. The extent to which energy expenditure is reduced in P.elegans cercariae, when compared to those of E.recurvatum, can be estimated by their differing life spans. The cercariae of these species are similar in size yet P.elegans cercariae have been seen to survive for up to 72 hours in the laboratory. By contrast, very few E.recurvatum cercariae are still active after 12 hours. If E.recurvatum cercariae can be considered to display 'active searching' host location behaviour, then the behaviour shown by P.elegans cercariae can be considered to be a 'lie in wait' strategy.

The 'lie in wait' strategy of host location shown by P.elegans cercariae is characterised by a suppression of activity until in close proximity to the host. Then a burst of activity in terms of greatly increased speed of movement and rate ofturning takes place. This is somewhat paradoxical since increased speed theoretically increases the area searched in a given time and increased rate of turning reduces it. However, since the response is only observed at a very short distance from the host, and even at that distance the cercariae appear unable to determine the direction of the host, the responses elicited ensure that a small area is searched in a short period of time. If cercariae cannot match the speed of movement of their host then the response recorded in P.elegans, increased activity and localised searching when a host is very close, might

well confer a significantly increased probability of host contact. The fact that the response persists suggest that it may be of adaptive advantage.

Much use has been made in this study of the analogy between host location behaviour and predation tactics. Considering host location at the species rather that the individual level opens up a new area of speculation. 'Active searching' predators more often than not hunt alone but predators employing a 'lie in wait' strategy, where speed of movement is less than the prey is capable of, often hunt in packs. To some extent 'lie in wait' predators succeed by weight of numbers.

Anecdotal evidence exists to suggest that xiphidiocercariae also succeed in host location by weight of numbers (Stycznska-Jurewicz, 1962). Indeed their whole life cycle is geared to maximal coverage of restricted areas by infective stages. Plagiorchids, which account for many of the known xiphidiocercariae, produce eggs which are passed out in faeces from their definitive hosts. The eggs are ingested by snails and miracidia are not released from the eggs until this ingestion has taken place. The absence of a free living miracidium prevents wide dispersion and ensures that any snails grazing in the area of egg deposition have a high probability of becoming infected. This may account for observations that where xiphidiocercariae are being shed from a population of snails, a high percentage of the snail population are harbouring infections.

Since snails have not been shown to displace themselves over large distances it seems likely that cercarial shedding is in much the

same location as infection occured. Whilst P.elegans cercariae have been shown to be capable of dispersion within a small experimental arena, it has also been shown that by periods of activity and inactivity they maintain station with minimal energy expenditure. Many workers have observed xiphidiocercariae in the field, and recorded their occurence at high density in restricted areas. If this aggregation occurs commonly then it must be due to lack of dispersion since cercariae have insufficiently developed perceptive powers to bring it about by their own devices. It is not hard to accept that whilst the behaviour of P.elegans cercariae might not lead to a high probability of an individual cercaria contacting a passing host, a host passing through a dense population of cercariae has a high probability of being located by at least one cercariae. Unfortunately, this hypothesis is not one that can be tested experimentally with the techniques used in this study.

CHAPTER 4 THE BEHAVIOUR OF THE MIRACIDIUM OF SCHISTOSOMA MANSONI

4.1. INTRODUCTION

The economic importance of schistosomiasis as a tropical parasitic disease has led to more work being published on schistosomes than any other trematodes. Extensive studies have been undertaken on all stages and aspects of the life cycle, including the subject of this chapter. However, to date no satisfactory quantitative description of schistosome miracidial host location has been published.

Many workers have reported on factors affecting the hatching of schistosome eggs and the motility, longevity and infectivity of miracidia (Maldonaldo et al, 1948, 1950; Oliver and Short, 1956; Kusel, 1970; Morgan, 1972; Bair and Etges, 1973; Kassim and Gilbertson, 1976; Prah and James, 1977; El Ridi et al, 1983; Donnelly et al, 1984). It is generally agreed that the main stimulus for hatching of schistosome eggs is a decrease in the osmotic pressure of the surrounding medium caused by dilution (Faust and Meleney, 1924; Okamoto, 1962; Chernin and Bower, 1971; Donnelly et al, 1984). There is also general agreement on the longevity of schistosome miracidia, a half-life of 5-9 hours and a small percentage surviving until 12 hours after hatching (Faust, 1924; Faust and Hoffman, 1934; Penner, 1939; Schreiber and Schubert, 1949; Singh, 1950; Sugiura et al, 1954; Farley, 1962).

Extensive studies on the response of schistosome miracidia to environmental stimuli have been published. Carter (1978) and Plorin

and Gilbertson (1981) have recorded the behaviour of S. mansoni miracidia when contacting solid surfaces, reaching different conclusions. Carter observed a wide variation around the general rule that miracidia hitting a solid surface left at an angle of reflection similar to the angle of incidence. Some miracidia showed 'contact with return'. Plorin and Gilbertson reported that miracidia hitting a surface with an incident angle of less than 14.7^{o} left at a slightly larger angle, whilst those hitting at greater than 14.7^{o} left at a smaller angle. They recorded no evidence of attraction to solid surfaces.

The relationship between temperature and miracidial swimming speed has been recorded as linear in Schistosoma japonicum (Takahashi et al, 1961) and in S.mansoni (Mason and Fripp, 1976). In both studies it was suggested that increased swimming speed was the direct result of increased metabolic activity at higher temperatures.

Differences in responses to light and gravity have been recorded in different schistosome miracidia. The miracidia of S.haematobium have been recorded as negatively phototactic and positively geotactic (Faust, 1924; Shiff, 1969, 1974), whilst miracidia of S.mansoni and S.japonicum have been described as positively phototactic and negatively geotactic (Takahashi et al, 1961; Chernin and Dunavan, 1962; Upatham, 1972a, 1972b; Sturrock and Upatham, 1973; Chernin, 1975; Mason and Fripp, 1976, 1977a). It has been postulated that these responses are adaptations which enhance the chances of host contact since the intermediate snail hosts of S.haematobium are most commonly found on the bottom of ponds, whilst those of S.mansoni and S.japonicum are most commonly found near the

surface. Further similarities between miracidial and snail behaviour have been recorded. In S.haematobium phototactic responses have been seen to change from negative to positive with lowered temperature (Shiff, 1974). The same author reported that although the snail host, Bulinus globosus, is found on the bottom of ponds in summer, in winter these snails are very frequently found in sunlight 'basking at the upper surface of a pond'. Miracidia of S.japonicum have also been seen to reverse a phototactic response with decreased temperature (Takahashi et al, 1961). Wright et al (1972) and Wright (1974) have investigated the responses of Schistosomatium douthitti and Schistosoma mansoni miracidia to monochromatic light. They concluded that both species respond maximally in a directional manner to wavelengths which make up the greatest percentage composition of spectral intensity in clear freshwater and in muddy waters.

Studies on the host location behaviour of schistosome miracidia have used a variety of approaches to study responses to the presence of a potential snail host and of snail-derived or other 'attractive' chemicals. Repeatedly throughout the literature some form of chemical attraction has been implicated in schistosome miracidal host-location behaviour.

Some workers have used direct, subjective observation to describe the responses of miracidia to snails or to test chemicals (Faust, 1924; Kloetzel, 1958, 1960; Sudds, 1960; MacInnis, 1965; Wright, 1966; Chernin, 1970, 1972; Etges et al, 1975). Because these studies were subjective, various conclusions have been drawn and several terms used to describe the nature of the miracidial

responses recorded. These terms include 'tropism', 'chemotaxis' and 'chemokinesis', terms which are not synonymous, indicating the problem of drawing accurate conclusions from subjectve analysis.

Other studies have used the recording of miracidial aggregation, under control and test conditions, in experimental designs where a 'choice' or 'preference' must be shown by miracidia (Etges and Decker, 1963; Shiff and Kriel, 1970; Wright, 1972; Wright and Ronald, 1972; MacInnis et al, 1974; Etges et al, 1975; Roberts et al, 1978). Whilst under these conditions a statistically significant 'preference' can be demonstrated for numbers of miracidia aggregating around a particular snail species or test chemical, the dynamic process by which this occurs (host location behaviour) remains a mystery. A logical extension of these experiments was to allow miracidial infection of snails under varying experimental conditions and compare infection rates of the snails by destructive sampling after sufficient time for sporocyst development had passed (Chernin, 1968a, 1968b; Shiff, 1968, 1969, 1970; Wadji, 1972; and Etges et al, 1975). The same lack of information on host location behaviour is also inherent in these experiments.

A fourth group of studies have attempted to describe the behaviour of schistosome miracidia in precise terms from photographic recordings of active miracidia. Mason and Fripp (1976, 1977), Mason (1977) and Plorin and Gilbertson (1981a, 1981b) described the use of long single exposures of miracidia with dark ground illumination to record short tracks of miracidial swimming. Roberts et al (1979) used stroboscopic lighting to record multiple exposures on single

negatives using a similar illumination system. Each group of workers has described miracidial behaviour under different conditions. Cinephotography has been used to study schistosome miracidial swimming rather infrequently (Sponholtz and Short, 1975; Carter, 1978; Roberts et al, 1980), but has the advantage that long periods of miracidial activity can be recorded and the light intensity of the experimental conditions kept constant.

Despite the inadequacies of many of the above studies of schistosome miracidial behaviour, a number of consistent and interesting observations have been described. Responses to chemicals have long been implicated in miracidial host location behaviour and Chernin (1970) was the first to suggest that, since responses he recorded were independent of the source of stimulation, it is more likely to be a chemokinesis that is operating than a chemotaxis. Mason and Fripp (1976), studying rate of turning of S.mansoni miracidia in snail-conditioned water, reinforced this observation and suggested that the response was a 'klinokinesis with adaptation'. Observations of miracidia in the immediate vicinity of potential snail hosts (MacInnis, 1965) indicate it is possible that chemotaxes take place over a very short distance.

Many chemicals have been shown to elicit responses from schistosome miracidia and the term 'miraxone' has been put forward as a generic term to encompass those which are snail-derived (Chernin, 1970). Test chemicals shown to change miracidial behaviour include amino acids (Wright and Ronald, 1972; MacInnis et al, 1974), fatty acids (MacInnis, 1965) Calcium, Magnesium and Sodium ions(Sponholtz and Short, 1976; Stibbs et al, 1976; Knabe et al, 1982) and peptides

(Mason, 1977). Roberts et al (1978) tested behavioural responses of S.mansoni miracidia to a number of chemicals and concluded that, although several inorganic cations have 'miraxone-like' activity for S.mansoni miracidia, none of these ions was as effective a stimulant as Mg^{2+}, a known component of snail-conditioned water (Stibbs et al, 1976). Roberts et al (1978) also found that schistosome miracidia are stimulated by 5-hydroxytryptamine (5-HT) and other neurotransmitters, as well as by some acetylcholine antagonists. The authors remarked that since Mg^{2+} contamination of these substances could not be ruled out, assertions about their activity should be guarded.

Carter (1978) attempted to describe schistosome miracidial host location behaviour more accurately in quantitative terms and tested his findings using computer simulation modelling. However, his models indicated that increased rate of turning by miracidia in the vicinity of a snail host should decrease the chances of contact. He speculated that since miracidia show a chemoklinokinesis it is likely to be of adaptive advantage and concluded therefore that the parameters programmed into his models were insufficiently close to the situation in nature. Subsequent experiments using interfaces of pond water and snail-conditioned water (Roberts, 1979) have led to further inferences about miracidial host location behaviour, so an aim of the present study was to expand on this area of experimentation with a view to describing miracidial host-location behaviour more precisely.

4.2. THE BEHAVIOUR OF UNSTIMULATED SCHISTOSOMA MANSONI MIRACIDIA

Since so much documentation on the behaviour of S.mansoni miracidia exists, it was decided here to record only unstimulated behaviour in the horizontal plane. However, a population of miracidia were filmed over eight hours at hourly intervals to record the effects of ageing on the miracidia, and the effect of temperature on miracidial swimming were investigated. The miracidia used in all the described experiments were of a Puerto Rican strain of Schistosoma mansoni maintained in the laboratories of Dr. R.A. Wilson (For techniques used in the maintenance of the parasite life cycle, see Lawson, 1977). Miracidia were hatched from eggs by placing in well-illuminated pond-water. Eggs were obtained from the livers of infected laboratory mice by enzyme digestion.

4.2.1. Tracks of Unstimulated Miracidial Behaviour

Thirty tracks of miracidial swimming were selected at random from film of a population of miracidia swimming in pond water at 26°C. The combined data for parameters of movement are shown in Table 4.1. The mean step speed here is higher than observed by Carter (1978) but filming was carried out at a higher temperature than in his experiments. The distribution of step speeds (see Figure 4.2.a) is interesting in that it shows a pronounced skew. Despite a mean speed of 2.424 mm sec^{-1}, the largest number of observations are in the range 2.4 - 2.7 mm sec^{-1}.

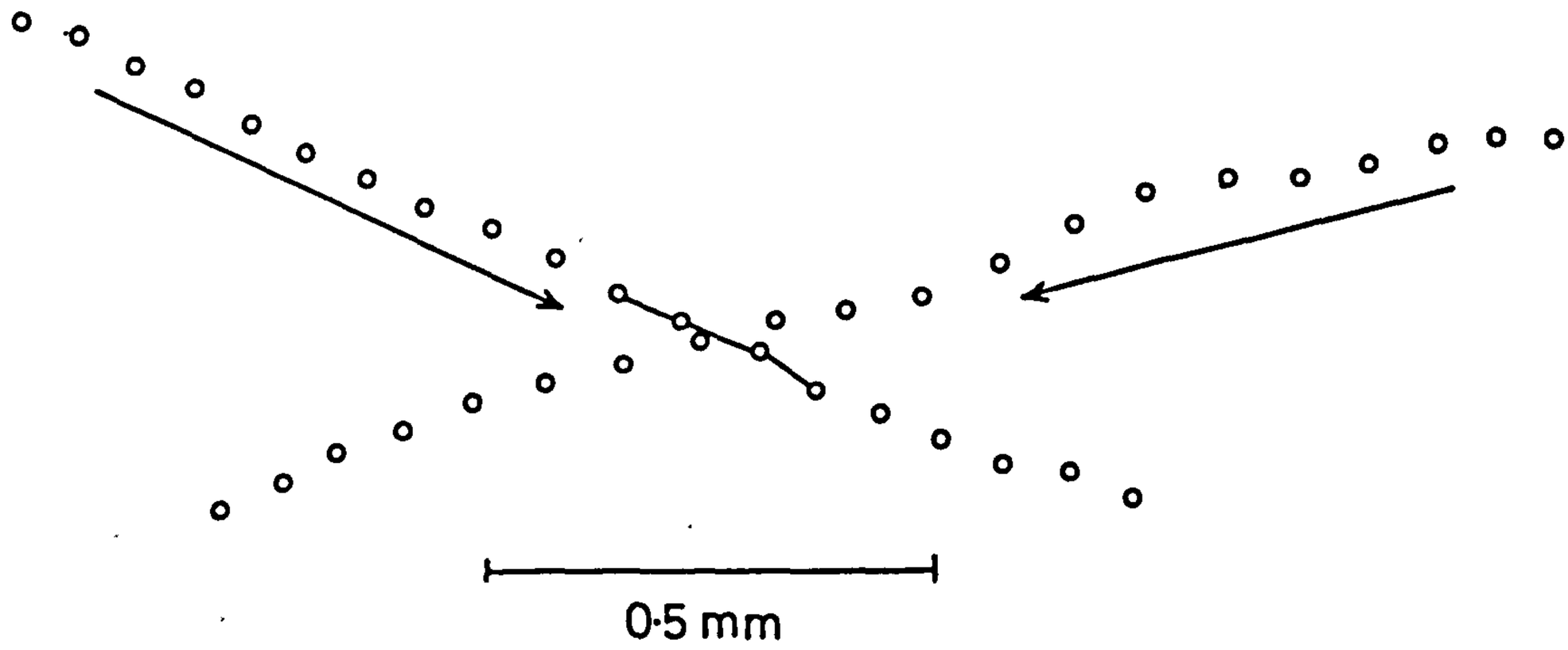


Figure 4.1.

Examples of tracks of unstimulated

S.mansoni miracidia.

The distribution of angle of direction (Figure 4.2.b) is similar to that observed by Carter (1978) in that it is bimodal. Since the 0° angle of direction was aligned with the short side of the cell it appears that miracidia were most frequently swimming approximately along the diagonals of the cell (i.e. the longest straight lines).

Table 4.1. Schistosoma mansoni miracidia unstimulated behaviour, horizontal plane

No. of observations	366
Step interval	.263 (sec)
Mean step speed (± S.E.)	2.424 ± .015 (mm sec^{-1})
Mean angle of direction (± S.E.)	96.009 ± 2.85 (deg)
Mean rate of turning (± S.E.)	27.966 ± 2.47 (deg sec^{-1})
Number of left turns	170
Number of right turns	165

The distribution of angles of turn is very much as expected, but with one very high observation in the 100 - 105° right turn class (see Figure 4.2.1.c). The distribution here is similar in shape to that observed by Carter (1978) but with fewer turns recorded above a magnitude of 40°. A difference in step interval can easily explain this inconsistency, since angle of turn is dependent on the frequency of plotting (see Chapter 1).

Strings are distributed here (Figure 4.2.d) in a similar way to that

Figure 4.2.

Distributions of parameters of movement for unstimulated S.mansoni miracidia horizontal plane:

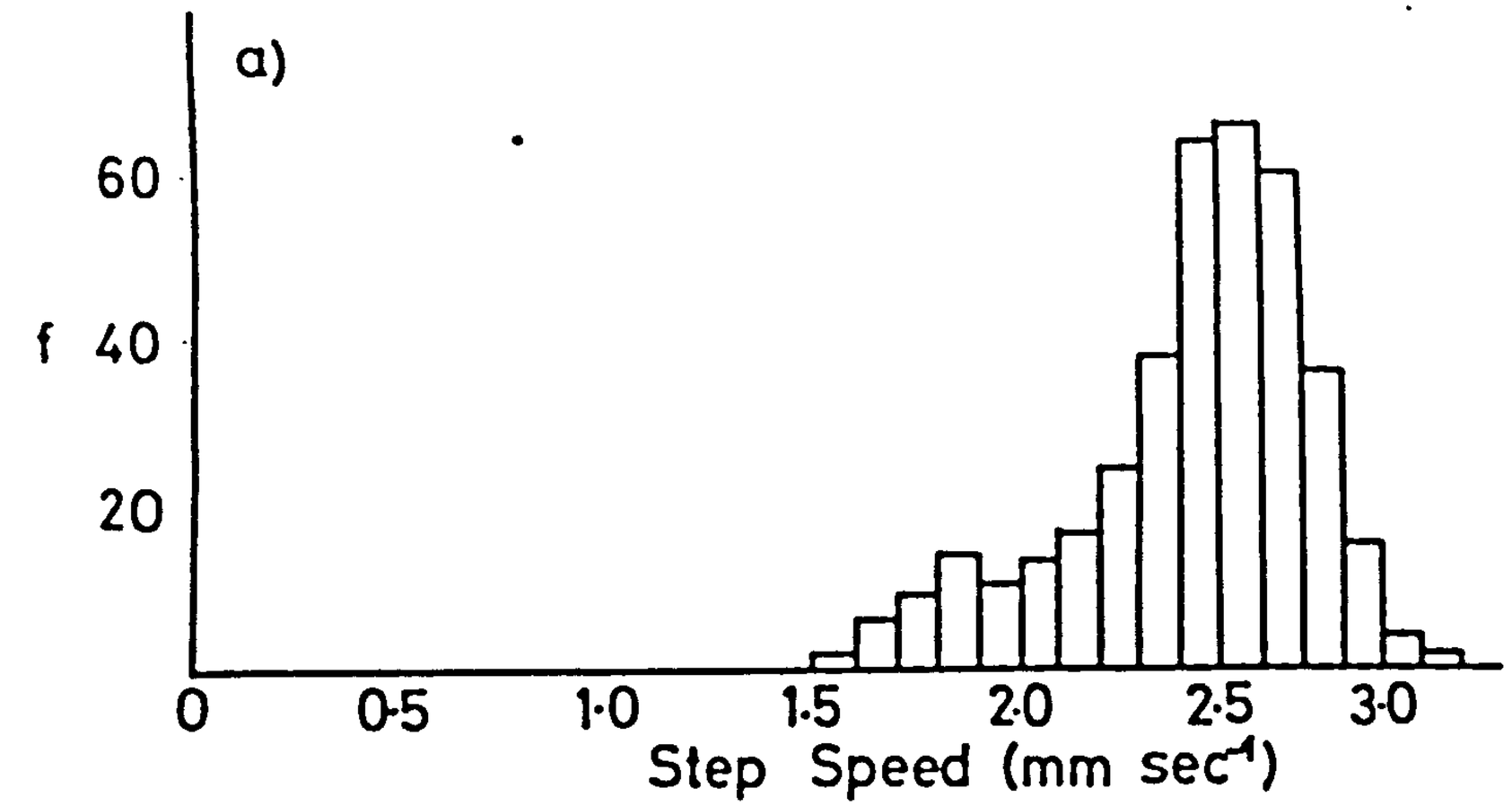
a) Step speed

b) Angle of Direction

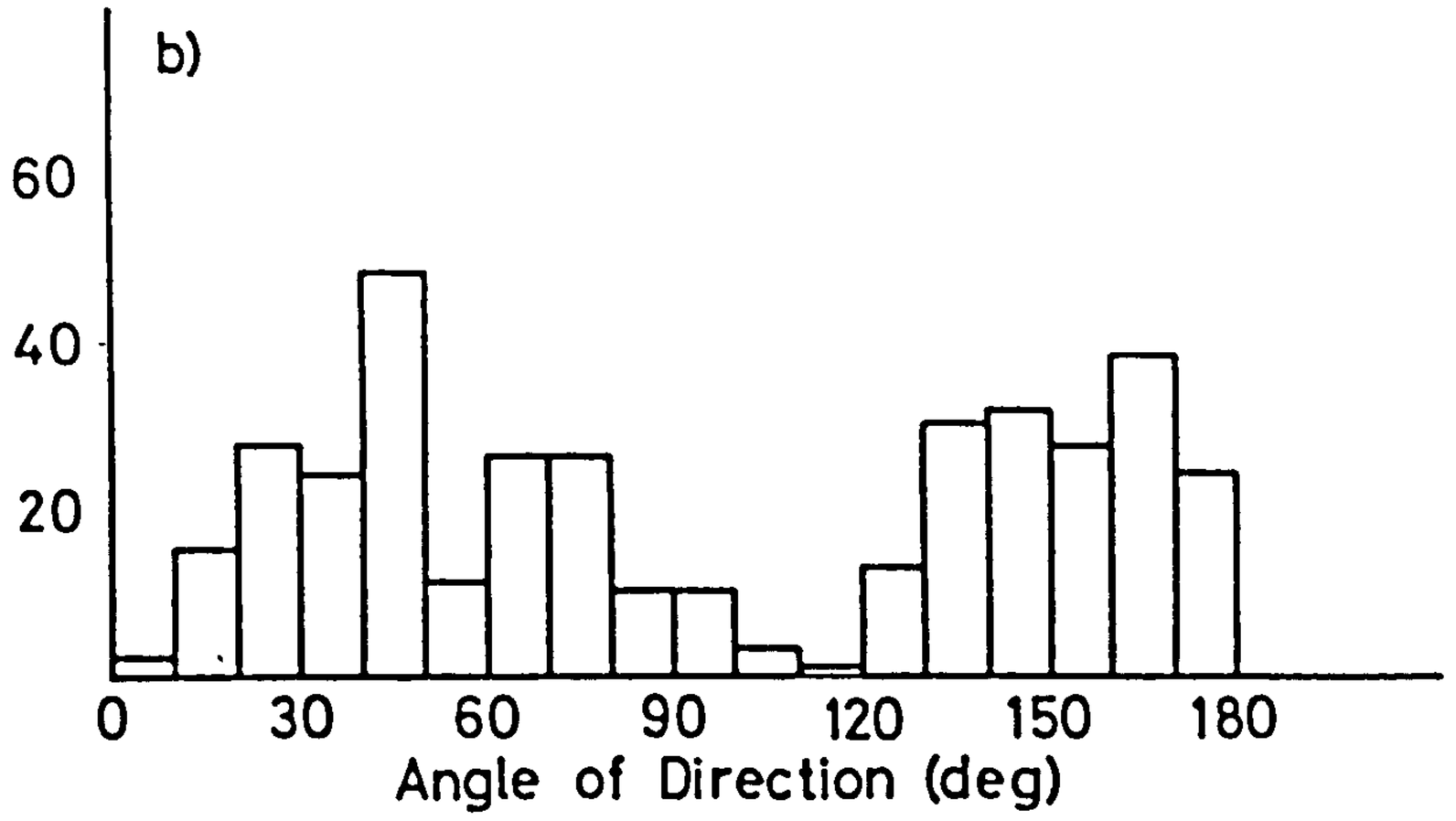
c) Angle of Turn

d) String Length

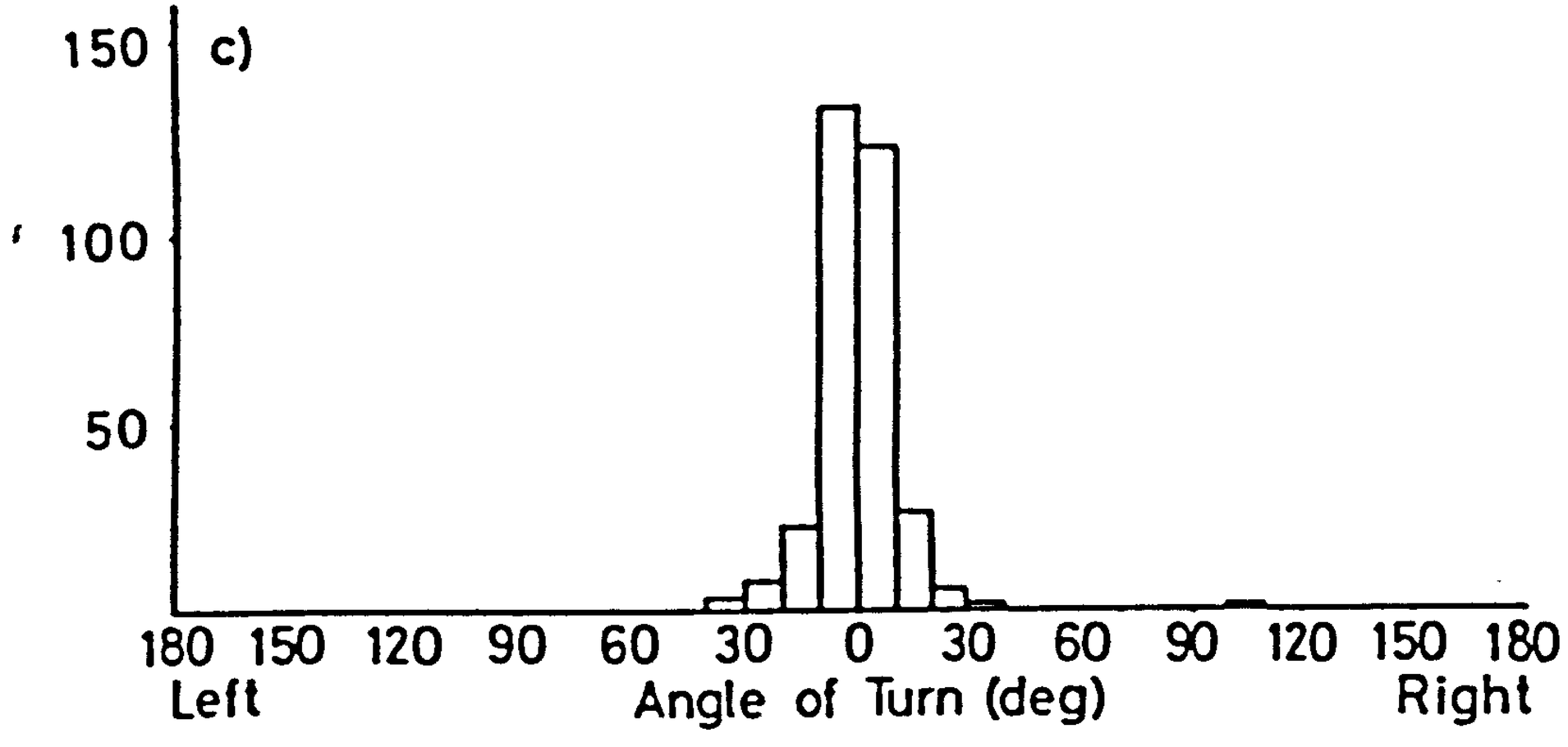
a)


60
f 40
20
0
0·5
1·0
1·5
2·0
2·5
3·0
Step Speed (mm sec⁻¹)

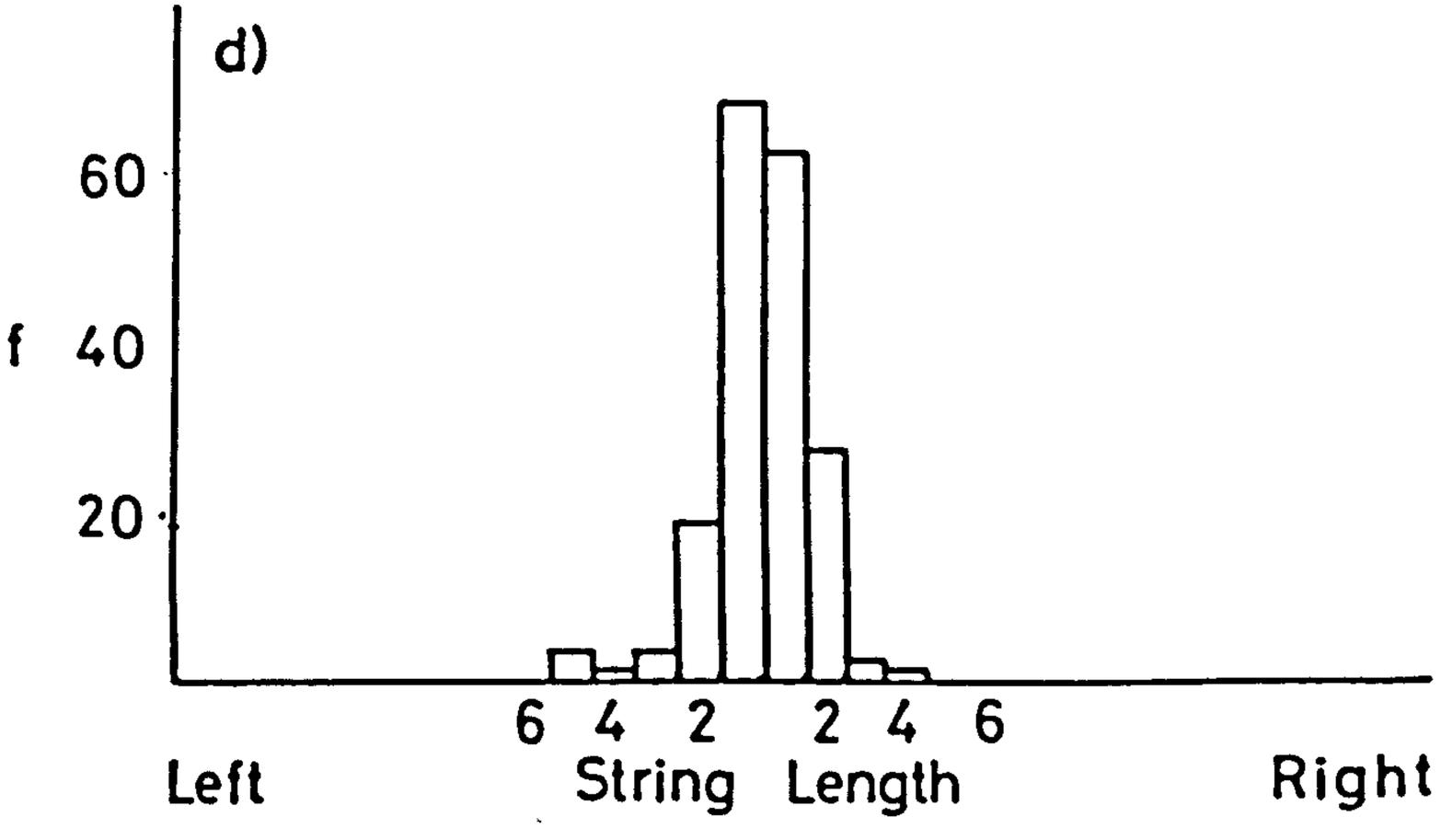
b)


60
f 40
20
0
30
60
90
120
150
180
Angle of Direction (deg)

c)


150
100
50
180 150 120 90 60 30 0 30 60 90 120 150 180
Left
Angle of Turn (deg)
Right

d)


60
f 40
20
6 4 2 2 4 6
Left
String Length
Right

described by Carter (1978), but with slightly fewer long strings. Again a difference in step interval can explain this observation. In Appendix 4.1 the distribution of strings has been tested against that expected if miracidia turned left or right independently of previous turns. There is a highly significant difference between the observed and expected distributions, with an excess of short strings. This appears to indicate that S.mansoni miracidia in the unstimulated state have a behavioural mechanism which results in swimming in approximately straight lines. A displacement to the right or left is corrected by a displacement to the opposite side almost immediately.

4.2.2. The Effects of Ageing on Unstimulated Miracidial Behaviour

The effects of ageing on miracidial swimming were investigated using a large population of miracidia in a large volume of pond water kept at a constant temperature of 26°C. At hourly intervals a teat pipette full of water was taken from each of five levels within the vessel containing the miracidia. Each pipette full of water was placed into the same petri dish from which miracidia were recovered and placed into the glass test cell for filming. This sampling method was used to negate the possible effect of selecting miracidia which were active and near the surface of the water in the experimental container. Filming took place after allowing two minutes to elapse in each case. Nine separate bursts of film were used to record the behaviour of miracidia at the start of the experiment, and at hourly intervals for eight hours. Tracks were plotted and analysed in the normal way.

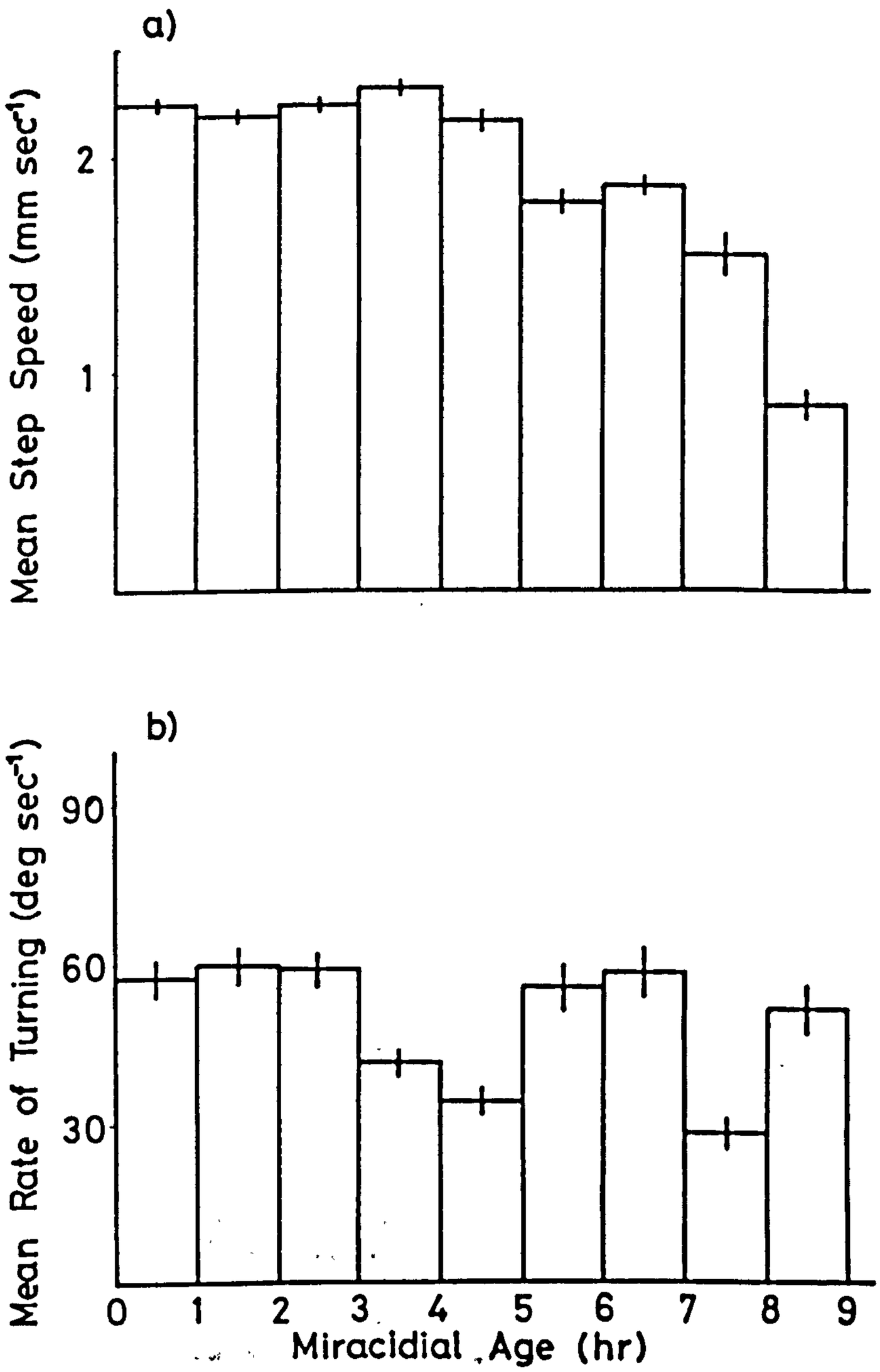
Figure 4.3.

The effect of ageing on <u>S.mansoni</u> miracidial behaviour:

a) Mean Step speed

b) Mean Rate of Turning

(Error bars indicate standard error of the mean).


a)
Mean Step Speed (mm sec⁻¹)
2
1
b)
Mean Rate of Turning (deg sec⁻¹)
90
60
30
0
1
2
3
4
5
6
7
8
9
Miracidial Age (hr)

Appendix 4.2. shows figures for mean swimming speed and rate of turning and their change with time. The data are shown graphically in Figure 4.3. It has already been noted (Section 4.1.) that S.mansoni miracidia have a half life of five to nine hours. The data for swimming speed first shows a significant decrease after five hours (when compared to that at the beginning of the experiment). After eight hours the surviving miracidia are swimming at less that half their original rate. The mean rate of turning varies quite erratically after three hours, having remained constant prior to this time. If swimming speed is a measure of miracidial fitness then the observed decrease in rate of turning at three and four hours is not due to miracidial deterioration. This behaviour would allow a greater area to be searched in a given time but I think it unlikely that this is a real response. More likely is increasing erratic behaviour with deterioration in miracidial condition due to expenditure of fixed energy reserves.

4.2.3. The Effects of Temperature on Unstimulated Miracidial Behaviour

Takahashi et al (1961) and Mason and Fripp (1976) have described a linear relationship between temperature and miracidial swimming speed in S.japonicum and S.mansoni respectively. It was decided to investigate this relationship with S.mansoni miracidia using cine-photography, and in addition study any changes in rate of turning. A population of miracidia was chilled to 10°C in a container using a surrounding bath of iced water. A glass test-cell was similarly

chilled to the required temperature. Miracidia were pipetted into the glass test-cell for filming in the horizontal plane and left for two minutes before filming commenced. At lower temperatures the mass of the glass test-cell compared to that of the water in it prevented sufficient heat transfer to raise the temperature of the water, which was monitored, during filming. Subsequently the process was repeated for five other, higher temperatures. All filming was completed within one hour to ensure that the effects of miracidial ageing were negligible. Data for mean swimming speed and rate of turning are shown in Appendix 4.3. The relationship between swimming speed and temperature is linear (at least over the range of temperatures tested) and a regression line has been fitted to the data in Figure 4.4.a. If the relationship were completely linear, miracidial death is likely to occur at 5°C. However, conclusions from extrapolation of this type would need to be confirmed by further experiments.

Figure 4.4.b shows the observed data for mean rate of turning with temperature change. There is no obvious relationship and no line has been fitted to the data. However standard errors are small and whilst rate of turning is relatively constant in the range 10 - 20°C, it sharply decreases with increasing temperature thereafter. This may well be a true response. At lower temperatures, when speed is low and ability to be displaced over large distances impaired, it may well be a better strategy for a miracidium to spend relatively longer searching its immediate vicinity for a host. Increased rate of turning would form part of this process. However, this hypothesis is not tested here. Mean rate of turning at 26°C in this experiment was 27.9 deg sec^{-1}, as compared to an observed maximum of

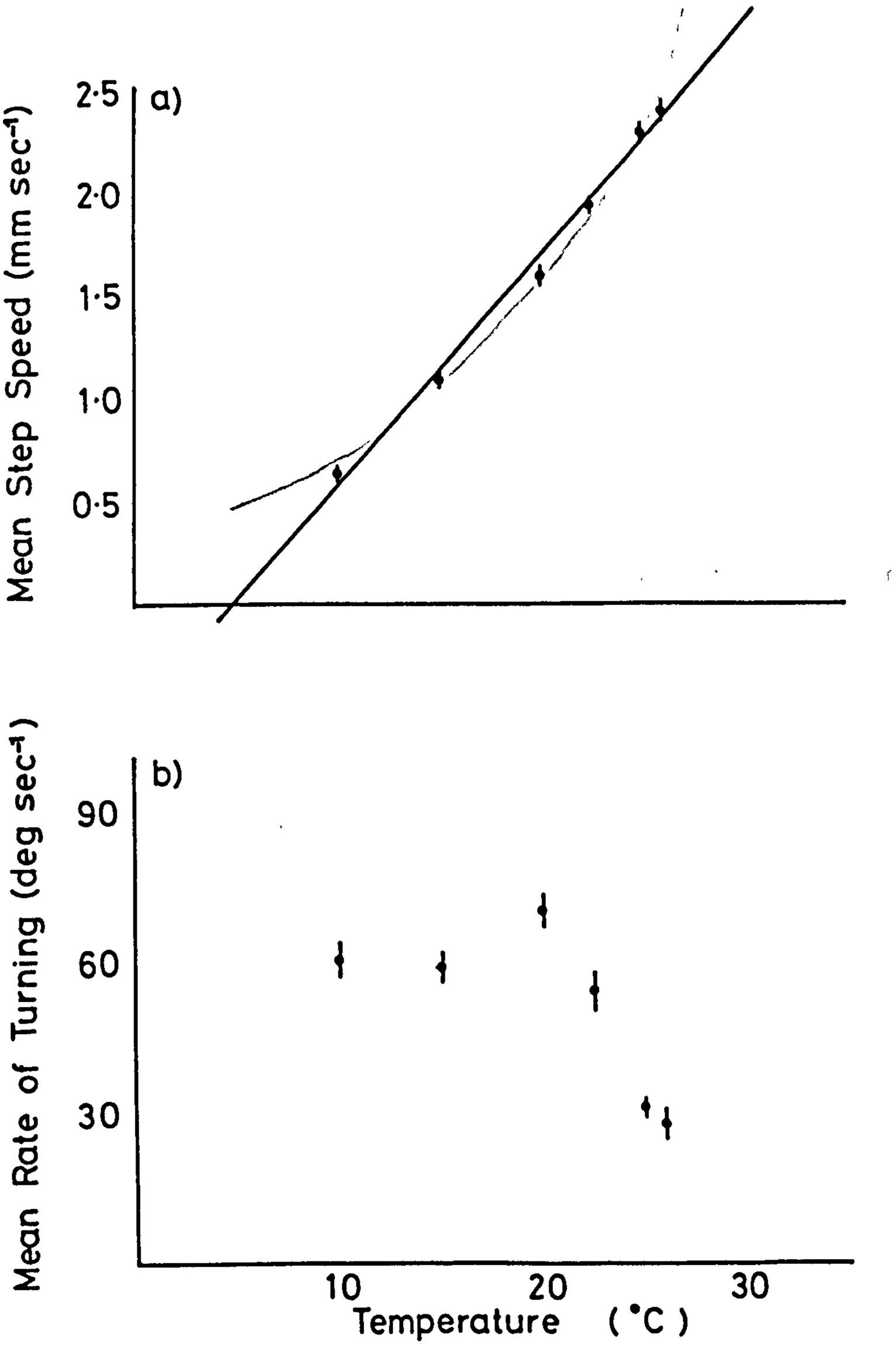
Figure 4.4.

The effect of temperature on S.mansoni miracidial behaviour:

a) Mean Step Speed

b) Mean Rate of Turning

(Error bars indicate standard error of the mean).


a)
Mean Step Speed (mm sec⁻¹)
2·5
2·0
1·5
1·0
0·5
b)
Mean Rate of Turning (deg sec⁻¹)
90
60
30
10
20
30
Temperature (°C)

70.5 deg sec^{-1} at 20°C. Some inferences as to the effect of temperature on miracidial searching ability could be gained by comparing infection rates in an artificial arena at different temperatures.

4.2.4. Summary of the Behaviour of Unstimulated S.mansoni Miracidia

In this section the unstimulated behaviour of S.mansoni miracidia has been recorded and the effects of ageing and of temperature described. The following points can be regarded as a summary of the section:

1) Unstimulated S.mansoni miracidia swim at approximately 2.2 mm sec^{-1} at 25°C.

2) There is a linear relationship between swimming speed and temperature (over the observed range 10 - 26°C).

3) Miracidia swim in relatively straight lines with only infrequent changes of direction. There appears to be a behavioural mechanism which operates to ensure that this occurs in the unstimulated state.

4) Rate of turning is low at 25°C but is apparently increased at lower temperatures.

5) Miracidia start to slow down 5 hours after hatching (at 26°C) and their behaviour (in terms of rate of

turning) becomes erratic.

4.3. THE BEHAVIOUR OF SCHISTOSOMA MANSONI MIRACIDIA IN THE VICINITY OF A SNAIL HOST

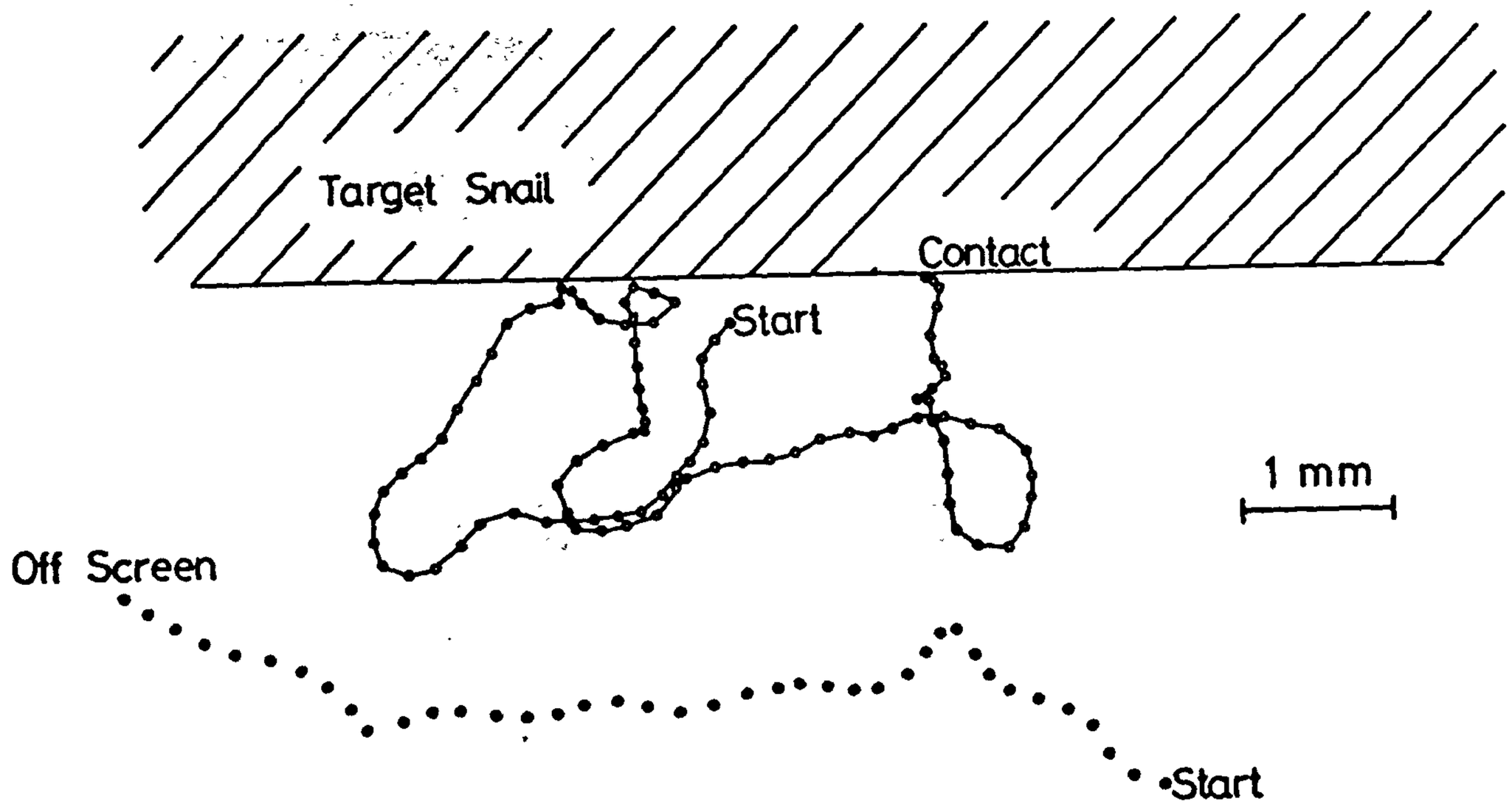
Following experiments carried out in order to quantify the unstimulated behaviour of S.mansoni miracidia, the response to the presence of a snail was investigated. The methods used in filming such behaviour were very similar to those of Carter (1978) in that miracidia were filmed in a fused glass block cell with a 5 mm snail (Biomphalaria glabrata) attached by the foot to the open end. Filming commenced after a period of three minutes. Tracks were subsequently analysed as described previously. For examples of plotted tracks see Figure 4.5.

4.3.1. Tracks of Miracidia in the Vicinity of a Snail Host

In analysing tracks of miracidial swimming in the vicinity of a snail Carter (1978) assigned individual tracks into one of three categories depending on the presence and duration of periods of stimulation (defined as increased rate of turning). Whilst there were clearly defined reasons for this categorisation, the subsequent analysis only of tracks of 'miracidia showing prolonged periods of turning' left much recorded data unstudied. Miracidia in the other two categories were excluded 'as their behaviour patterns were

Figure 4.5.

Examples of tracks of <u>S.mansoni</u> miracidia in the vicinity of a snail, <u>Biomphalaria glabrata</u>.


Target Snail
Contact
Start
1 mm
Off Screen
Start

thought unlikely to increase the chance of an individual contacting the host'. I believe this exclusion was unjustified since it has never been satisfactorally established that increased rate of turning leads to increased chances of host contact. In the present study data for all recorded miracidial tracks in the vicinity of a snail have been analysed. Combined data for the studied parameters of movement are shown in Table 4.2. The distributions of step speed, angle of turn, direction relative to target and strings are shown in Figure 4.6.

Table 4.2. Schistosoma mansoni miracidia in the vicinity of a snail, horizontal plane.

No. of observations	5591
Step interval	.089 (sec)
Mean step speed (± S.E.)	1.896 ± 0.012 (mm sec^{-1})
Mean angle of direction relative to target (± S.E.)	94.199 ± 1.22 (deg)
Mean rate of turning (± S.E.)	205.581 ± 6.98 (deg sec^{-1})
No. of left turns	2644
No. of right	2764

The distribution of step speeds (Figure 4.6.a.) is skewed to the left, as noted previously with unstimulated miracidia. The skew is more pronounced here than previously and it suggests that whilst some miracidia are swimming at close to their maximum speed, others are responding to some factor or other by reduction in speed. For

Figure 4.6.

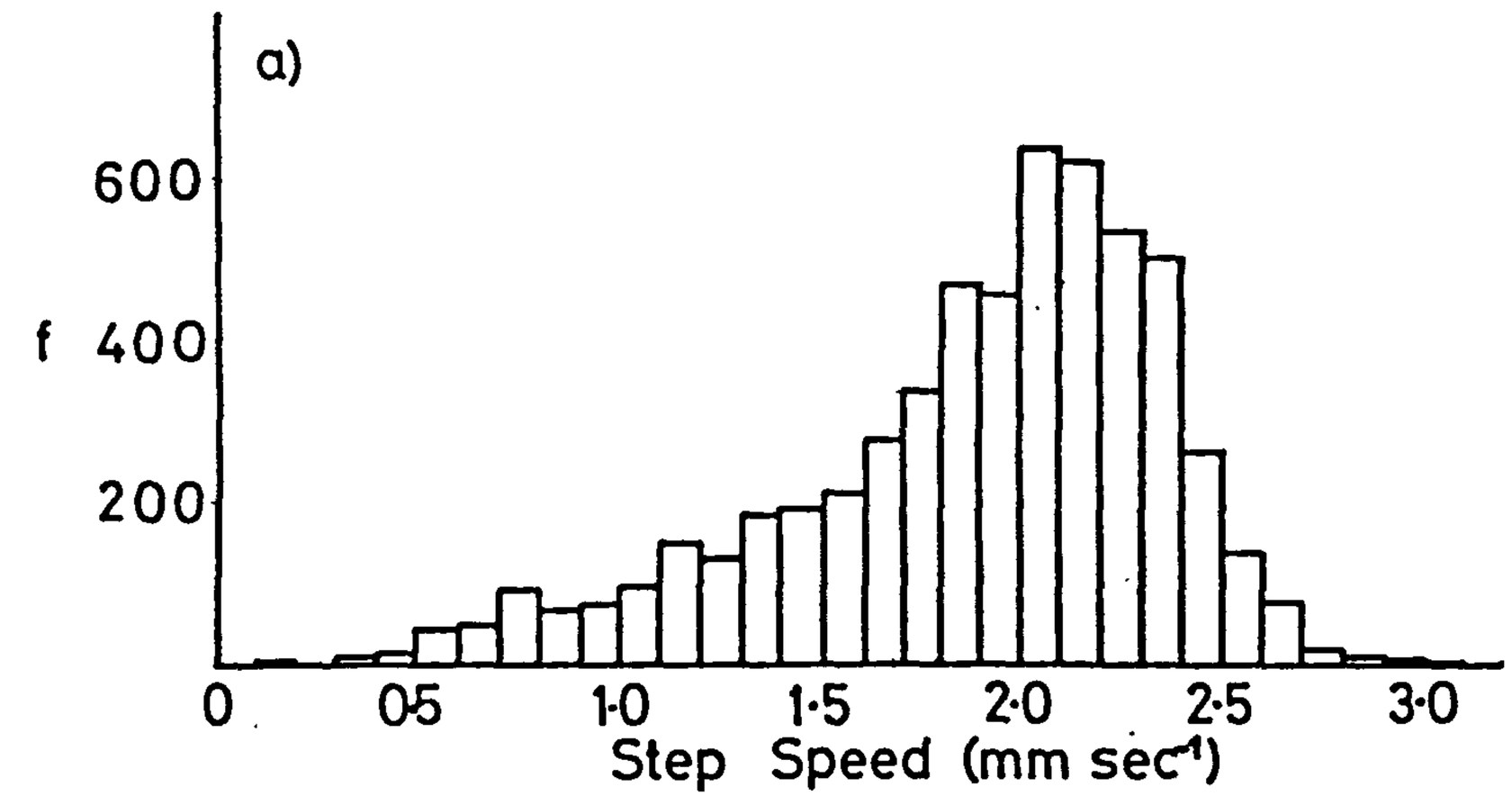
Distributions of parameters of movement for S.mansoni miracidia in the vicinity of a snail, B. glabrata, horizontal plane:

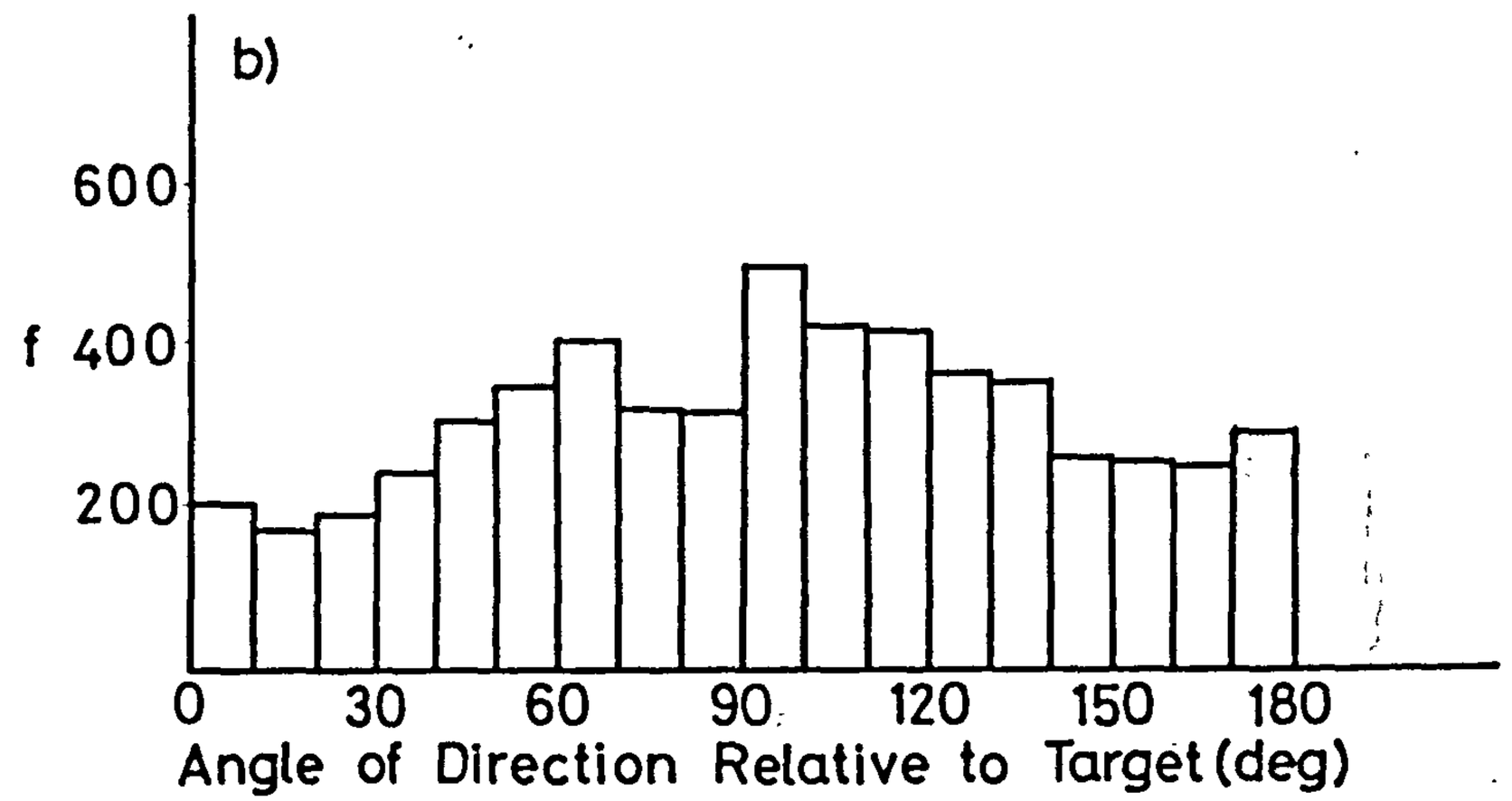
a) Step Speed

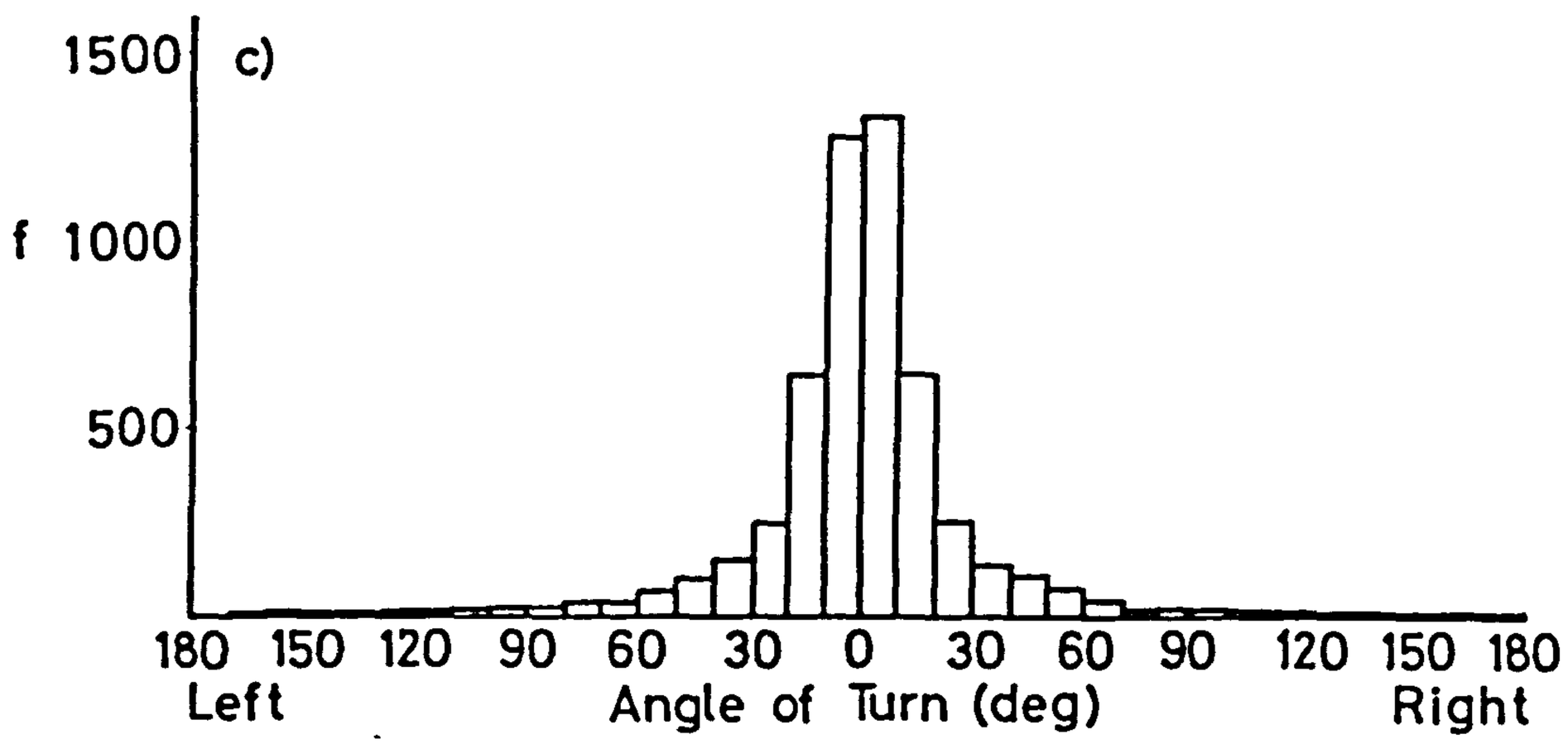
b) Angle of Direction Relative to Target

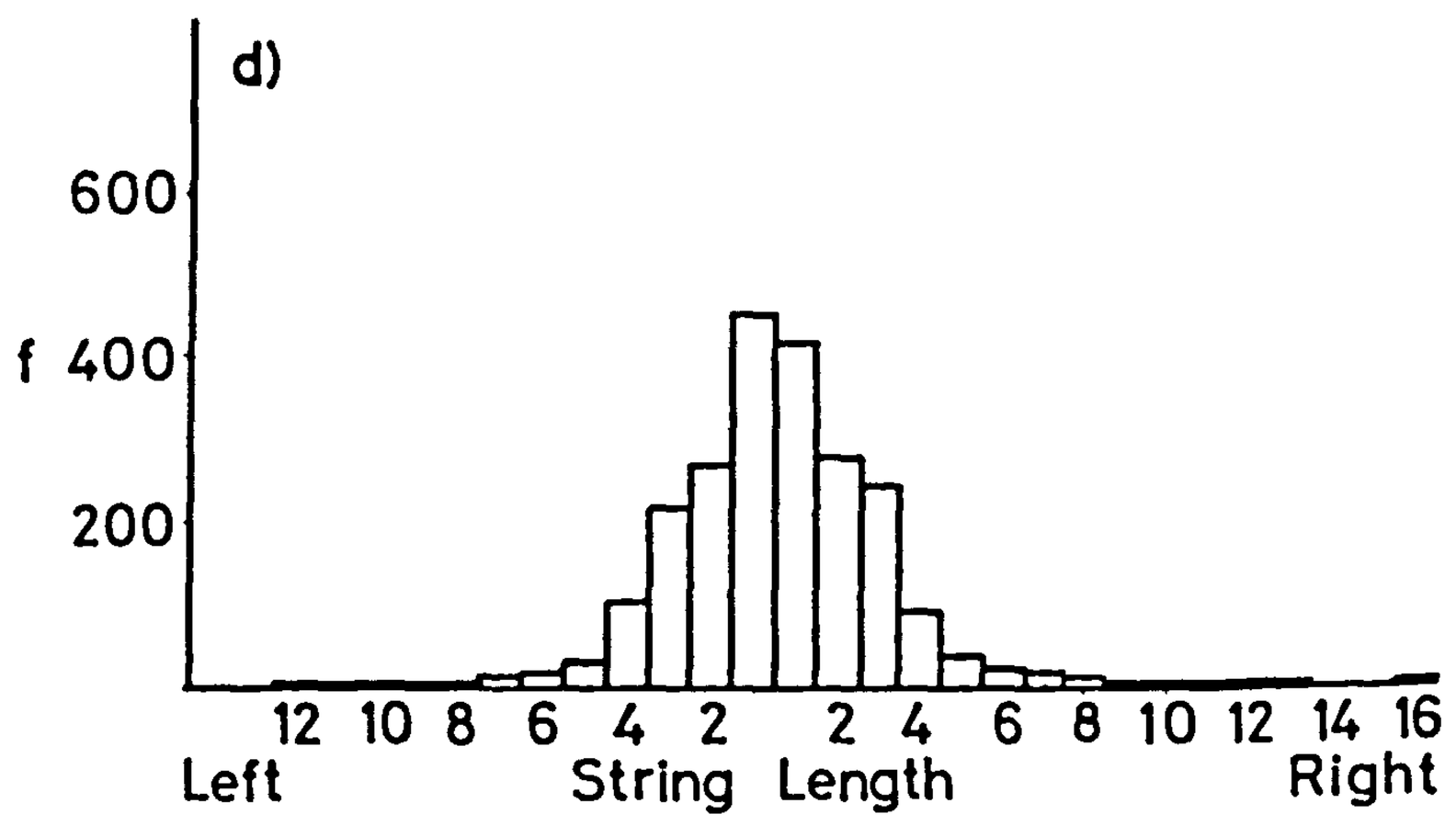
c) Angle of Turn

d) String Length


a)
600
f 400
200
0
0·5
1·0
1·5
2·0
2·5
3·0
Step Speed (mm sec⁻¹)


b)
600
f 400
200
0
30
60
90
120
150
180
Angle of Direction Relative to Target (deg)


c)
1500
f 1000
500
180 150 120 90 60 30 0 30 60 90 120 150 180
Left
Angle of Turn (deg)
Right


d)
600
f 400
200
12 10 8 6 4 2 2 4 6 8 10 12 14 16
Left
String Length
Right

unstimulated miracidia the lower end of the distribution of step speeds was 1.5 mm sec^{-1}. Here miracidia are displacing themselves as slowly as 0.4 mm sec^{-1} for a significant amount of time.

The distribution of turns (Figure 4.6.c.) is very similar in shape to that shown by unstimulated miracidia. However, miracidia in the vicinity of a snail show turns in every class from 180° left to 180° right. The limits observed for unstimulated miracidia were 40° left and 105° right (one observation in the 100 - 105° class and none in the range 35 - 100°). Miracidia in the vicinity of a snail do show increased magnitude of turns.

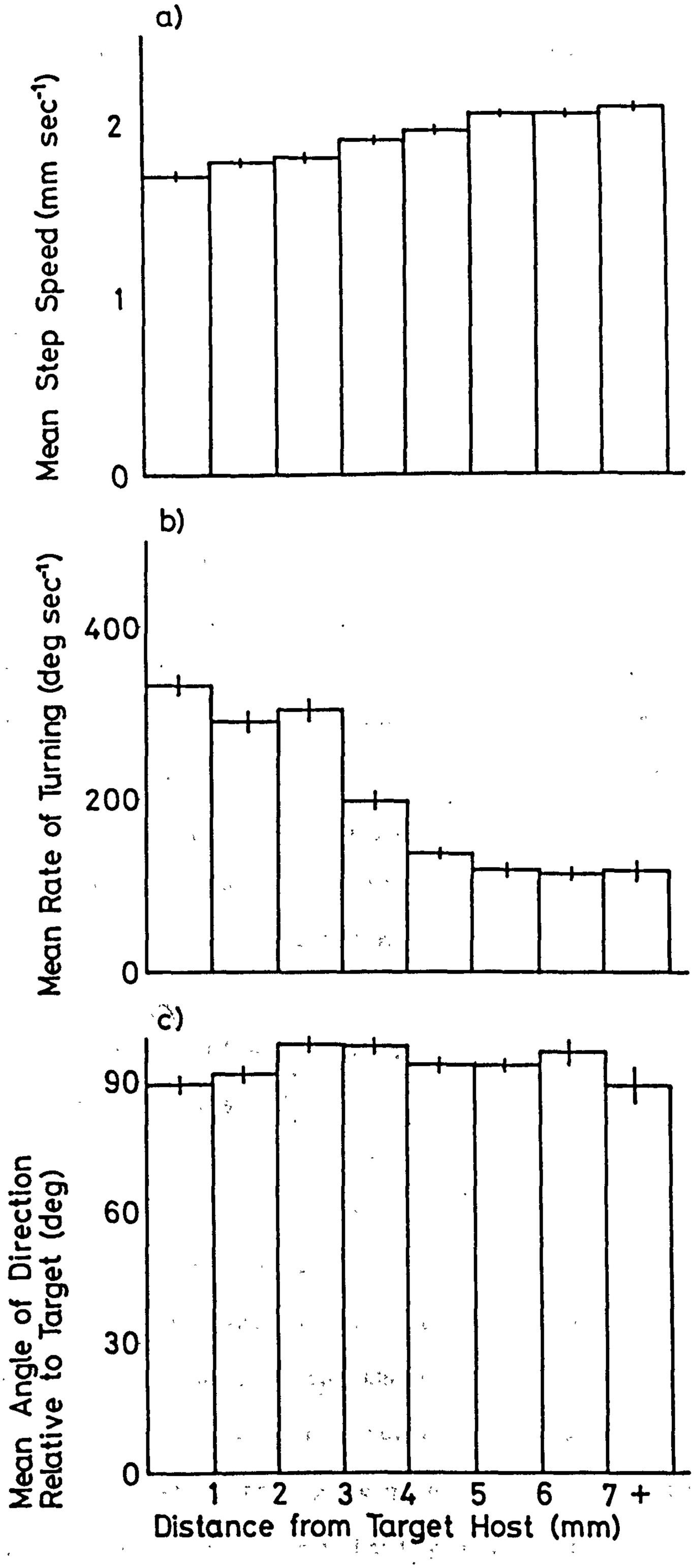
Angles of direction relative to the target are apparently unevenly distributed (Figure 4.6.b.). A test against even distribution is shown in Appendix 4.4. If miracidia were behaving independently of the presence of the snail one would expect to see an even distribution. The observed distribution is significantly different to that expected, inferring that miracidia are responding to a directional stimulus, possibly the presence of the snail. However, the pattern of the distribution indicates that there is no taxis operating (a directional movement towards or away from the target) since the classes in the ranges 0 - 30° and 150 - 180° have the least number of observations. This result supports the general theory that miracidia do not exhibit a chemotaxis, but gives no evidence to demonstrate the existence of a klinokinesis.

The distribution of strings (see Figure 4.6.d) is markedly different to that shown by unstimulated miracidia. Here there are observations of up to 12 successive left turns, and up to 17

successive right turns. The peak of small strings is also reduced for miracidia in the vicinity of a snail. Testing for random turning (i.e. 50-50 probability of left or right irrespective of previous events) reveals a significant difference between the observed and expected distributions (Appendix 4.5.). In this case there is a lack of single strings and an overabundance of longer strings. It seems that miracidia under these conditions are abandoning straight line swimming in favour of turning in arcs or circles. This observation lends support to the theory that miracidia exhibit a klinokinesis.

Whilst the data presented thus far gives some indications as to how S.mansoni miracidia behave in the vicinity of a snail, it does not give an indication as to the distances from the host over which these responses are elicited. In order to investigate this aspect of miracidial behaviour the data for step speed, rate of turning and direction relative to the host have been divided up into one millimetre zones from the snail's surface.

The data for step speed are shown in Figure 4.7.a. There is apparently a consistent decrease in step speed with decreasing distance from the snail. Investigation of the difference between means by analysis of variance (Appendix 4.6.) reveals that not all differences are significant. In the range 5 - 7 mm from the snail miracidia swim at approximately 2.05 mm sec $^{-1}$. There are successive decreases in speed from 5 to 3 mm from the snail and a more substantial decrease in the range of 1 - 3 mm from the snail. Under 1 mm from the snail, speed is at its lowest observed level. The observed data suggest a series of separate responses rather than


a)
Mean Step Speed (mm sec⁻¹)
2
1
0
b)
Mean Rate of Turning (deg sec⁻¹)
400
200
0
c)
Mean Angle of Direction Relative to Target (deg)
90
60
30
0
1
2
3
4
5
6
7 +
Distance from Target Host (mm)

a single graded response by miracidia and may be the result of encountering more than one type of stimulation or several sharp changes in the strength of a single type of stimulus.

The data for rate of turning (Figure 4.7.b and Appendix 4.7) reinforce the above observation. There are three significant increases in rate of turning, at approximately 4 mm, 3 mm and 1 mm from the snail's surface. The magnitude of increase is almost three-fold from 7 mm to less than 1 mm from the snail. In some ways this result is unexpected. Carter (1978) shows using simulation models that increased rate of turning with decreasing distance from the host is not in itself a good strategy for host location since probability of host contact is not increased over random behaviour. In addition, subjective observation and description of miracidial behaviour (Faust, 1924; Sudds, 1960; and others) has consistently included descriptions of miracidia in the immediate vicinity of a snail 'making a beeline drive' (or similar) for the snail's surface.

If miracidia are capable of determining the direction of a host over extremely short distances and of navigating towards it, then they would show a mean direction relative to the target significantly less than 90 degrees. The data for mean direction relative to the target are shown in Figure 4.7.c and analysed in Appendix 4.8. Whilst there are significant differences between means in the data there is no evidence to support the theory that over short distances miracidia can perceive the direction of their host and navigate towards it. Over most of the range of distances the data suggest that miracidia spend more time swimming away from their host than towards it. This observation goes some way to supporting Carter's

(1978) assertion that increased rate of turning alone is not a good host location strategy.

4.3.2. Summary of the Behaviour of S.mansoni Miracidia in the Vicinity of a Snail Host

In this section the behaviour of S.mansoni in the vicinity of a snail has been investigated. The following points represent a summary of the observations made:

1) In the vicinity of a snail a proportion of miracidia, or all miracidia for a proportion of the time, show reduced swimming speed. This response is seen to be the result of three successive zones of stimulation at different distances from the snail's surface.

2) Miracidia exhibit more turns of a higher magnitude in the vicinity of a snail such that their rate of turning is much increased. This response is also seen to be the result of successive zones of stimulation around a snail.

3) Miracidia are unable to detect the direction of a host snail and navigate towards it, even when stimulated by its presence and at very short distances from it. This infers that no taxis is in operation.

4) In the vicinity of a snail, miracidia show extended

strings. The mechanism by which miracidia swim in straight lines in the unstimulated state is absent and there is a tendency for turning in arcs or circles. This lends support to the theory that a klinokinesis is taking place.

4.4. THE BEHAVIOUR OF SCHISTOSOMA MANSONI MIRACIDIA IN RESPONSE TO SNAIL CONDITIONED WATER

In the previous section the behaviour of S.mansoni miracidia in the vicinity of a snail host was investigated. The observed data gave an indication that a klinokinesis was in operation, consistent with previous workers speculation of a chemoklinokinesis (Mason and Fripp, 1976). However, in the present study no evidence to implicate a chemical stimulus has yet been produced.

Roberts et al (1979) used a series of 'coverslip chambers' to investigate the behaviour of S.mansoni miracidia in several concentrations and gradients of snail-conditioned water (SCW). A number of single 5 second exposures with stroboscopic lighting were used to record the behaviour of the miracidia, and tracks were analysed from the projected negatives. Roberts et al found that miracidia in a uniform concentration of SCW swam at the same speed and with the same rate of turning as those in pond water. Miracidia in gradients of SCW exhibited a three-fold increase in rate of turning without altering their swimming speed. In addition, miracidia ascending gradients of SCW did not increase their rate of turning and failed to orient to the gradient, whilst miracidia

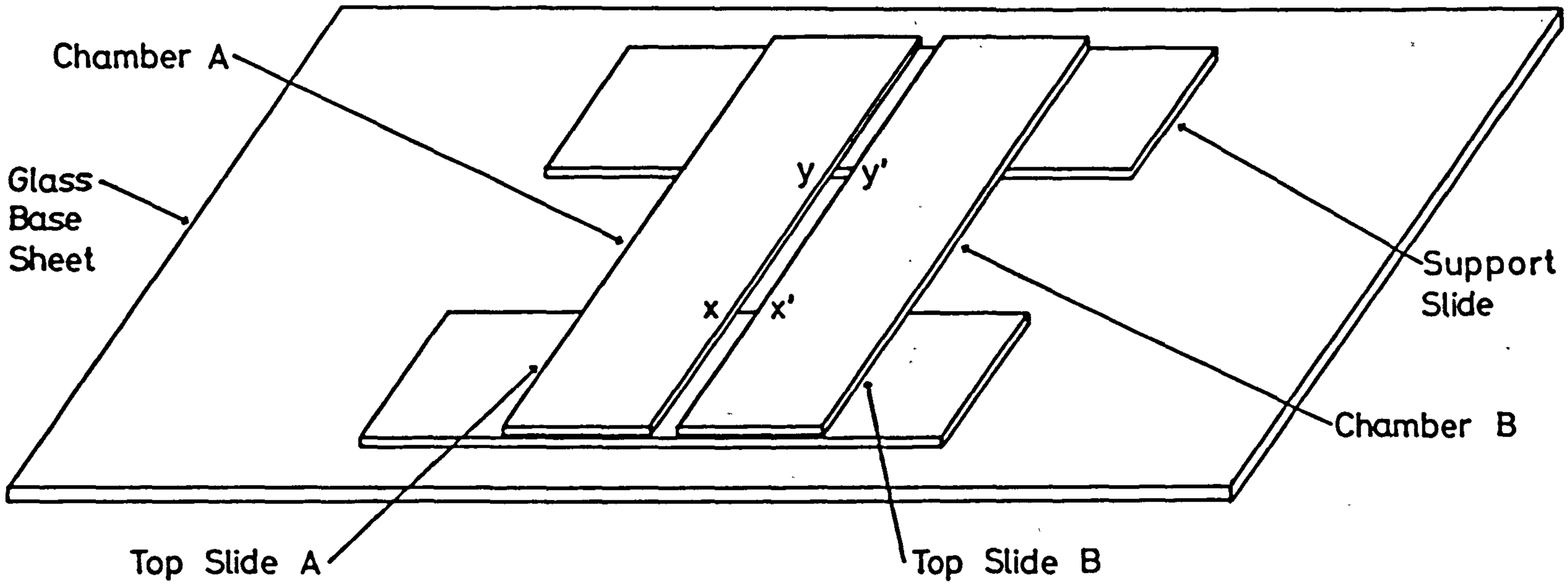
experiencing a sharp decrease in concentration of SCW sharply increased their rate of turning. Roberts et al concluded that the miracidial response is a 'boundary - reaction', a form of chemokinesis, and not a chemotaxis. It was decided to investigate the behaviour of miracidia encountering a sharp boundary of pond-water (PW) and snail-conditioned water in the present study for several reasons. Firstly, it would establish the role of chemical stimulation in miracidial host location behaviour. Secondly, I had reservations about the experimental procedures used by Roberts et al (1979). For example, using their system of recording tracks, individuals could only be tracked for 5 seconds at a time. In addition, the side walls of their 'coverslip chambers' were constructed of Vaseline, a substance not ruled out as a miracidial stimulant.

4.4.1. Tracks of Miracidia Crossing a Stimulant Boundary

In the present study, an experimental design was evolved to record the behaviour of miracidia swimming across an interface. A glass sheet and numerous microscope slides were used to construct a 'canal' over which two further slides could be placed at right angles to form open ended chambers (see Figure 4.8). Small amounts of water were used to 'stick' slides together by surface tension, in addition preventing seepage of water from the sides of the open-ended chambers. In an experiment, a small volume of pond water containing miracidia was introduced into Chamber A and a test solution was introduced into Chamber B. By manoeuvring top slide B against top slide A (bringing x' and y' against x and y

Figure 4.8.

Apparatus used in experiments with pond water - snail conditioned water interfaces.


Chamber A
Glass
Base
Sheet
y
y'
x
x'
Support
Slide
Chamber B
Top Slide A
Top Slide B

respectively) the solutions were joined. With practice the 'interface' between the solutions could be created with very little disturbance to the solutions in each chamber. As with Roberts et al (1979), methylene blue was used to distinguish the 'test' solution (Chamber B) in each case. A preliminary series of experiments established that both the stain and any physical effects created by the apparatus at the interface had no effect on the unstimulated behaviour of miracidia.

To investigate the behaviour of miracidia crossing a pond water - snail-conditioned water interface, a large number of miracidia were introduced into Chamber A which was then topped up with pond water. Chamber B was then filled with snail-conditioned water. The SCW was prepared by confining twenty snails (Biomphalaria glabrata) in ten millilitres of water for three hours before the experiment. The SCW was filtered through Whatman No.1 filter paper before use and 5 drops of methylene blue stain added to aid recording of the subsequent interface position. The entire preparation process was carried out in a constant temperature room at 26°C.

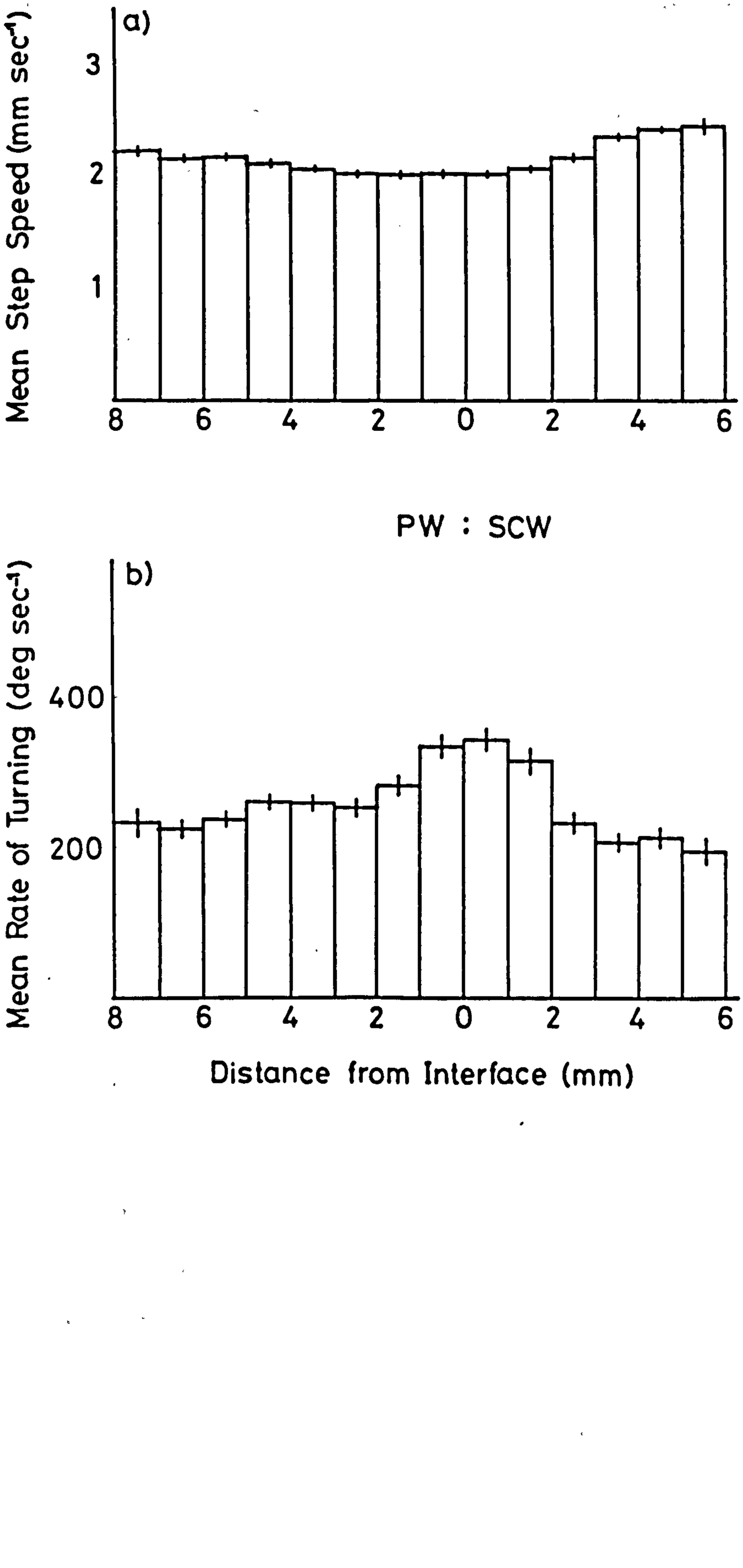
The final part of preparation of the interface was carried out in position under the cine camera so filming could commence immediately the interface was set up. However, it should be noted that it often took five or six attempts to produce an acceptably straight interface, and each attempt meant starting completely from the beginning. Once an acceptable interface was obtained, filming commenced and one hundred feet of film were exposed in one continuous burst. A total of sixty-one tracks were subsequently plotted and analysed in the normal way.

Figure 4.9.

Changesin S.mansoni miracidial behaviour around a pond water - snail conditioned water interface:

a) Mean Step Speed

b) Mean Rate of Turning


a)
Speed (mm sec⁻¹)
Mean Step S
3
2
1
8
6
4
2
0
2
4
6
PW : SCW
b)
sec⁻¹)
Mean Rate of Turning (deg s
400
200
8
6
4
2
0
2
4
6
Distance from Interface (mm)

Since the position of the interface and its effect on miracidial behaviour was the factor under consideration here, primary analysis is categorised as step speed and rate of turning by distance from the interface. These data are plotted in Figures 4.9.a. and b respectively. An important factor to consider in this analysis is that although all the miracidia started in the pond water (and therefore initially crossed from PW to SCW) the time period of filming was long enough for miracidia to cross the interface in both directions. Accordingly Figures 4.9.a and b include data for all possible responses shown by miracidia.

Mean step speed is significantly reduced in a zone around the interface, from 5 mm into the pond water side to 3 mm into the snail-conditioned water side. In addition, miracidia were swimming significantly faster in snail-conditioned water than in pond water (see Appendix 4.9. for significance tests). Mean rate of turning is significantly increased in a small zone around the interface, from 1 mm into the pond water to 2 mm into the snail-conditioned water.

The data as presented thus far merely represent the behaviour of all the miracidia, crossing the interface in both directions. Since individuals within a population can reasonably be expected to respond quite differently to the same stimulus, further analysis is necessary, especially to make a comparison with the results of Roberts et al (1978). Accordingly tracks have been categorised subjectively based on their start position and outcome. The six categories involved, shown in Figure 4.10, are defined as follows:

Figure 4.10

Examples of the six categories of miracidial behaviour around a PW - SCW interface.

1 - 2 Category A

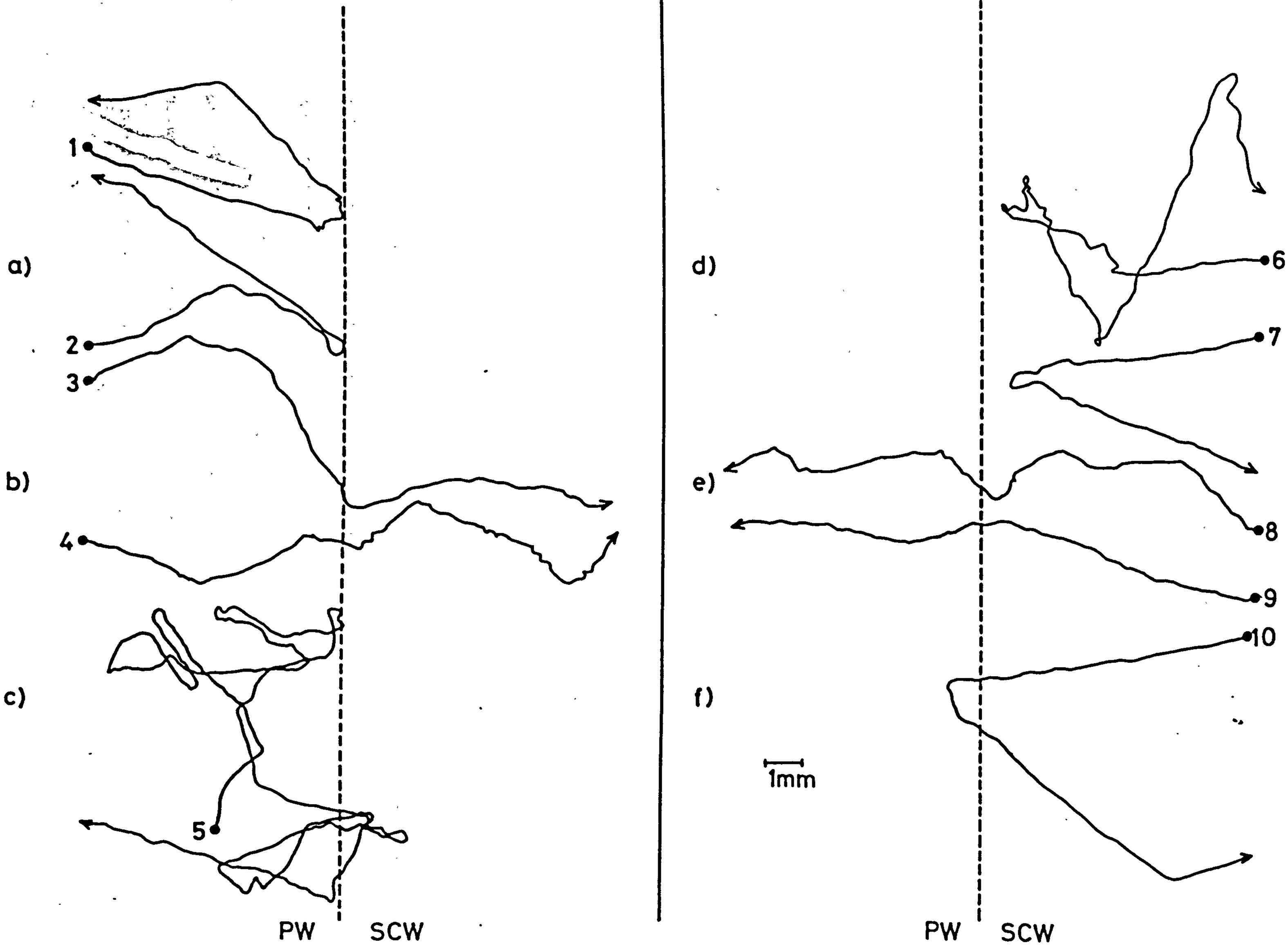
3 - 4 Category B

5 Category C

6 - 7 Category D

8 - 9 Category E

10 Category F


a)
1
2
3
b)
4
c)
5
PW
SCW
d)
6
7
e)
8
9
10
f)
1mm
PW
SCW

A Miracidia starting and remaining in pond water.

B Miracidia starting in pond water, crossing into the snail-conditined water and remaining there.

C Miracidia crossing from pond water to snail-conditioned water and eventually returning (the interface possibly having been crossed on numerous occasions).

D Miracidia starting and remaining in snail conditioned water.

E Miracidia crossing from snail-conditioned water to pond water and remaining there.

F Miracidia crossing from snail-conditioned water to pond water and eventually returning (possibly crossing the interface on many occasions.)

For each of the above categories, mean step speed and rate of turning have been compared in zones by distance from the interface. Apparent differences in means have been tested using analysis of variance (see Appendices 4.10 - 4.15) and the data presented graphically in Figures 4.11 and 4.12. Where significant differences between mean values occur from one zone to the adjacent one, arrows have been included in the histograms.

Category A miracidia show a reduction in mean step speed at both 5

Figure 4.11

Changes in <u>S.mansoni</u> miracidial behaviour around a PW - SCW interface, Categories A, B and C:

a) Mean Step Speed

b) Mean Rate of Turning

Category A

Category B

Category C

Mean Step Speed ($mm\ sec^{-1}$)

0 1 2 3

8 6 4 2 0

8 6 4 2 0 2 4 6

8 6 4 2 0 2 4

PW : SCW

PW : SCW

PW : SCW

Mean Rate of Turning ($deg\ sec^{-1}$)

0 200 400 600

8 6 4 2 0

8 6 4 2 0 2 4 6

8 6 4 2 0 2 4

Distance from Interface (mm)

and 1 mm from the start position of the interface, and a large increase in rate of turning at 1 mm from the interface (Figure 4.11). Obviously an interface presents a sharp change in stimulant concentration as soon as it is set up, but immediately diffusion of molecules occurs in both directions causing the interface to be less sharply defined. It seems that miracidia in this category respond to a small change in stimulant concentration as they approach the boundary and their resultant behaviour prevents them from crossing the interface.

Category B miracidia show significant changes in step speed and rate of turning on both sides of the interface (Figure 4.11), but no clear pattern emerges as to where the major effects are. It would appear that the bulk of miracidia in this category are not responding at all to the changes in stimulant concentration.

Miracidia in Category C show significant reductions in step speed at 5 and at 2 mm from the start position of the interface, and a much larger reduction 2 mm into the snail-conditioned water (Figure 4.11). Rate of turning is significantly increased at successively short distances from the start position of the interface, from 3 to 1 mm in distance. It appears that these miracidia respond to a small increase in stimulant concentration as they approach the interface, and exhibit a more marked response to a higher concentration of stimulant when they enter the snail-conditioned water. This observation differs from data presented by Roberts *et al* (1978) who recorded no behavioural change in *S.mansoni* miracidia experiencing a sharp increase in stimulant concentration.

Figure 4.12

Changes in S.mansoni miracidial behaviour around a PW - SCW interface, Categories D, E and F:

a) Mean Step Speed

b) Mean Rate of Turning

Category D

Category E

Category F

Mean Step Speed (mm sec^{-1})

3
2
1
0

0 2 4 6

8 6 4 2 0 2 4 6

0 2 4 6

PW : SCW

PW : SCW

PW : SCW

Mean Rate of Turning (deg sec^{-1})

600
400
200
0

0 2 4 6

8 6 4 2 0 2 4 6

0 2 4 6

Distance from Interface (mm)

Category D miracidia respond like those of Category A, before reaching the interface. Mean step speed is reduced significantly at four points successively close to the interface. Mean rate of turning significantly increases at 2 and 1 mm from the interface (Figure 4.12). As with Category A miracidia the responses elicited prevent the miracidia in this category from crossing the interface.

Category E miracidia show no significant changes in step speed as they approach and cross the interface but rate of turning alters significantly in the snail-conditioned water between 1 and 3 mm from the interface (Figure 4.12). However, as with Category B miracidia, it seems that the bulk of miracidia in this category are not responding at all to changes in stimulant concentration.

Miracidia in Category F show a very marked change in behaviour at short distances (up to 1 mm) either side of the interface (Figure 4.12). On crossing into the pond water, step speed is significantly reduced. At 1 mm from the interface in the snail-conditioned water and on crossing the interface, rate of turning is also significantly increased. The change in behaviour is enough to prevent miracidia from passing more than 1 mm into the pond water before returning to the snail-conditioned water. The response is much more marked than the reverse situation (Category C) and may account for the reason why Roberts et al (1978) observed this response but not the converse one.

4.4.2. Summary of The Behaviour of S. mansoni Miracidia in Response to Snail-Conditioned Water

From the data presented in this section a number of points can be made about the behaviour of S.mansoni miracidia when experiencing changes in the concentration of stimulants produced by their snail host, Biomphalaria glabrata:

1) There is a pronounced variation in the way in which individual miracidia respond to the same stimulus.

2) Miracidia respond to both increases and decreases in stimulant concentration, though more quickly and more markedly to decreases.

3) The responses elicited are increased rate of turning and reduced step speed.

The above observations lend further support to the theory that S.mansoni miracidia respond to chemicals produced by their snail hosts by exhibiting a klinokinesis. Since behaviour in pond water and snail-conditioned water does not differ significantly in terms of rate of turning and step speed it would appear that it is changes in stimulant concentration that are important and not absolute concentration.

4.5. DISCUSSION

In this chapter the behaviour of S.mansoni miracidia has been

recorded under a number of experimental conditions. In addition the results of other studies on schistosome miracidial behaviour have been considered. Drawing all the available information together, a convincing account of the host location behaviour of S.mansoni miracidia can be put forward.

In the unstimulated state miracidia swim in relatively straight lines at a fairly constant speed. In the absence of responses to environmental stimuli this would lead to wide dispersal and no exploration of any discrete area. Responses to light and gravity vary with schistosome species, but S.mansoni are said to be positively phototactic and negatively geotactic. By responding to these environmental stimuli S.mansoni miracidia tend to be found at the surface of ponds, where the vegetation on which their snail hosts graze occurs most frequently. The unstimulated behaviour of miracidia and their responses to environmental stimuli would seem to enhance the chances of coming into the vicinity of potential snail hosts.

Once in the vicinity of potential snail hosts other responses are elicited, most significantly changes in speed of swimming, rate of turning and the length of strings. Swimming speed is reduced, rate of turning increased and strings lengthened, thereby increasing the amount of time spent in a discrete area. These responses appear to be elicited by sharp changes in concentration of snail derived chemicals, but do not persist for more than a few seconds. This is an important fact, bearing in mind miracidia appear not to be able to perceive the position of a host even when very close to it. By responding to snail derived chemicals for only a short period of

time, and to sharp changes in concentration rather than absolute concentration, miracidia do not spend long periods searching small areas, but search only briefly before moving on. This type of response has the advantage that each new stimulation will result in a brief period of searching in that vicinity and, since it is likely that many sharp changes in stimulant concentration are found around a snail, many of such brief searches will take place.

Brief periods of altered behaviour in response to a change in stimulus are consistent with the possession of rapidly adapting sensory apparatus. It seems possible that S.mansoni miracidia have chemosensors of this kind which are constantly 'sampling' the chemical nature of their immediate vicinity. Behavioural responses are elicited by a change in stimulus concentration identified by the chemosensors. The detection of a change in stimulus concentration can be simplistically viewed as a perceived change in a 'sample' with reference to the previous 'sample.

There are many examples of adaptation in invertebrate behaviour in the literature. Of particular interest, when compared to the recorded behaviour of S.mansoni miracidia here, are the experiments of Ullyott (1936a, 1936b) on the behaviour of the planarian Dendrocoelium lacteum at light and dark boundaries, and in response to non-directional gradients of light. Careful and complete observation of these flatworms was possible because they move very slowly and the exact nature and position of the light stimuli could be accurately recorded. Ullyott noted that stimulation of D. lacteum by non-directional light produced an increase in the rate of change of direction and he was able to demonstrate that this could

be expressed as a function of stimulus intensity. However, in constant stimulation the rate of change of direction decreased as the animals became adapted. He concluded that alternate stimulation and adaptation has an effect on the rate of change of direction so as to lead the animal to the place of minimal intensity.

The behaviour of S.mansoni miracidia recorded above is strikingly similar to that for D.lacteum as described by Ullyott. However, the results are less conclusive here than in Ullyott's experiments because the nature and position of the stimuli (particularly in the vicinity of a snail) are less clearly defined. S.mansoni miracidia do appear to adapt following a change in stimulus concentration, but they do so more quickly to a decrease than to an increase. Using Ullyott's reasoning, for S.mansoni miracidia this would tend to result in their aggregation in the zone of highest stimulant concentration. Further experiments with S.mansoni miracidia in which they would encounter both sharp changes and gradients of stimulant chemicals would be valuable in confirming this hypothesis.

An important factor which should not be overlooked is that individual miracidia vary in their response to the same stimulus. Accordingly, not all of a population crossing a stimulant interface will respond, some will continue to show unstimulated behaviour. This heterogeneity may constitute a very important part of the host location success of miracidia at the species level, though it cannot disguise the fact that certain types of response which have been recorded in the vicinity of a snail are key factors in the host location success of individual miracidia. Clearly the changes in behaviour and their effect on the chances of individual miracidia

successfully locating a snail can be evaluated by computer simulation modelling and the effects of heterogeneity of response can be ignored for this purpose.

CHAPTER 5 SIMULATING THE BEHAVIOUR OF TREMATODE LARVAE USING COMPUTER MODELS

5.1. INTRODUCTION

In previous chapters, conclusions have been drawn as to which aspects of trematode larval behaviour appear to be important in host location. However, it has not been possible to study the effects of one behavioural response, such as increased rate of turning or decreased swimming speed, in the absence of others since it has been shown that responses are elicited concurrently in the vicinity of a host. The inability to study individual responses in isolation has meant that no assessment of the modification of chances of host contact can be made for single types of behavioural change.

Computer simulation modelling is often used to represent and manipulate a complex system comprised of a number of inter-related factors. The essence of computer simulation modelling is that models are open to manipulations which are impracticable, expensive, time-consuming or even impossible of the real system which the model represents. Carefully constructed computer models combine in a single system all the factors which are considered important in such a way that they interact as they do in the real system. Any model which closely resembles a real system must, therefore, be constructed using information gained from a precise study of the system under consideration. Once constructed a model can be run and its predictions compared against the real system. The most useful

form of computer model is one which enables it's constituent parts to be modified independently. In this way the contribution of each of the constituent parts to the whole system can be inferred.

Very often computer models are a gross over-simplification of the systems they represent. This in itself is important since failure of the model's predictions to compare with the real system can suggest further investigative experimentation to understand better the factors important in the real system. For example, a model's poor prediction may indicate the importance of an unconsidered factor, or of a factor previously considered as relatively unimportant. Also a model's results may cause suspicion as to the accuracy of some of the data used in it's construction, leading to further experimentation to quantify more accurately a range of parameters.

The most outstanding use of computer simulation modelling of invertebrate behaviour has been that of Rohlf and Davenport (1969). They studied behavioural responses as categorised by Fraenkel and Gunn (1961), simulating kineses and taxes as tracks consisting of straight lines interspersed with 90 degree changes in direction. Variation in the frequency of changes of direction was according to the type of response being simulated, and its magnitude. Of particular interest in view of the current study were their results of simulating klinokineses. They found that simple klinokineses produced no net displacement in a concentration gradient and klinokineses with sensory adaptation resulted in displacement down the gradient. As an overall conclusion, Rohlf and Davenport stated that more frequent turning at higher levels of stimulation decreases

the efficiency of target finding.

In this chapter computer simulation models of the host location behaviour of the cercariae of Echinoparyphium recurvatum and Plagiorchis elegans and of the miracidia of Schistosoma mansoni have been constructed. The models have been manipulated in a number of ways to assess the contribution of individual responses to modification of the chances of host contact.

5.2. METHODS USED IN THE SIMULATION MODELLING

A single basic model was constructed and used to simulate the behaviour of the larvae studied in Chapters 2,3 and 4. The model used data generated in the track analysis of the relevant larvae in the vicinity of their target hosts to construct simulated tracks. Since it was not possible to describe changes in any of the parameters of movement by any mathematical relationship, this form of model construction was entirely necessary and, arguably desirable since the simulated tracks should bear a similarity to those originally recorded. This form of constructing the models was previously used by Carter (1978) when simulating the host location behaviour of Schistosoma mansoni miracidia and cercariae.

Whereas track analysis has been used to study a number of parameters of movement, only three of those parameters are necessary to generate simulated tracks. These are:

i) String length

ii) Distance moved per step

iii) Angle turned between steps.

However, each of these three parameters of movement have been shown to change in a variety of ways for the larvae studied in the vicinity of their hosts. Accordingly the model had to be capable of representing these changes. Of the three parameters listed above two, string length and angle turned between steps, are segregated during track analysis into left and right components. For simulation modelling the left and right components were combined, creating single frequency distributions for string length and angle of turn.

For use in the models, the frequency distributions were all treated in the same way, constructing a form of distribution which was used to select values for the parameters when the model was run. In devising this form of distribution an important factor was taken into consideration. Reference to the distribution of angles is made here to illustrate how important this factor is. All the observed data of angles turned for a number of larvae in the vicinity of their host can be plotted as a frequency distribution. Ignoring sign of turn (combining data for left and right turns) it is possible to say that when selecting a turn at random from that distribution, it has a certain probability of occuring in a certain range of angles. For example, if there are 258 observations in the class 0 - 10 degrees, from a total of 1000 observations, the probability that a turn selected at random will be in this class is 258/1000. Whilst merely selecting turns from this type of distribution would create tracks with a similar distribution of turns it was reasoned that this would be inefficient in simulating

the actual behaviour of the larvae studied. It is reasonable to assume that the angle of turn between steps may be predetermined to some extent by the preceeding angle. For example, unstimulated larvae swim in relatively straight lines with infrequent changes in direction, inferring a number of successive small angles of turn. Conversely, some larvae when stimulated increase their rate of turning, inferring several successive large angles of turn. The important random elements are when unstimulated larvae show large turns and stimulated larvae, small turns.

Since at any time, the angle turned may be predetermined by the magnitude of the previous one, a complex two dimensional array was constructed from which parameter values were selected during simulation. Real track data was taken in sequence and turns allocated to a column of the array depending on the magnitude of the previous turn. In this way every turn succeeding one in the class 0-10 degrees was recorded, and similarly for every class up to 170-180 degrees. The parameter values in each of these eighteen columns were then sorted into ascending order to create a frequency distribution for that class. In the first row of each column the total number of parameter values in that distribution was recorded. During simulation, the initial angle turned for any track was selected at random from the entire distribution. For subsequent turns the magnitude of the previous turn determined the column of the data array from which the next value should be randomly selected. Creation of data arrays and the selection of parameter values during simulation were similarly carried out for step length and strings.

Simulation of a single track took place in the following way. Initially a start position (X_1, Y_1,) and direction (Z^o) were selected at random. Next a string was selected randomly from the entire string distribution array and assigned as right or left by random choice. The final parameter selection for calculation of the first step was random choice of a step distance from that entire array. Calculation of successive points used selection of values from the relevant angle and step distance distributions, with new strings, each the opposite sign of the previous one, being selected as necessary.

Successive positions were calculated using simple trigonometry, much the same as in track analysis though in reverse. However, since there was no need to consider direction in any other way than in reference to the relationship of one position to the next, direction could be calculated in the manner of bearings. This simplified the programming required in the trigonometric calculations and helped to shorten the execution time of the program. A new position for a larva at the point (x_1, y_1) and travelling in the direction (d_1) was calculated in the following series of steps:

i) Continue existing string or select new one.

ii) Select angle of turn (a) and step distance (sd)

iii) Calculate new direction (d_2) by:

$$d_2 = d_1 + a \text{ (during right strings)}$$

or $$d_2 = d_1 - a \text{ (during left strings)}$$

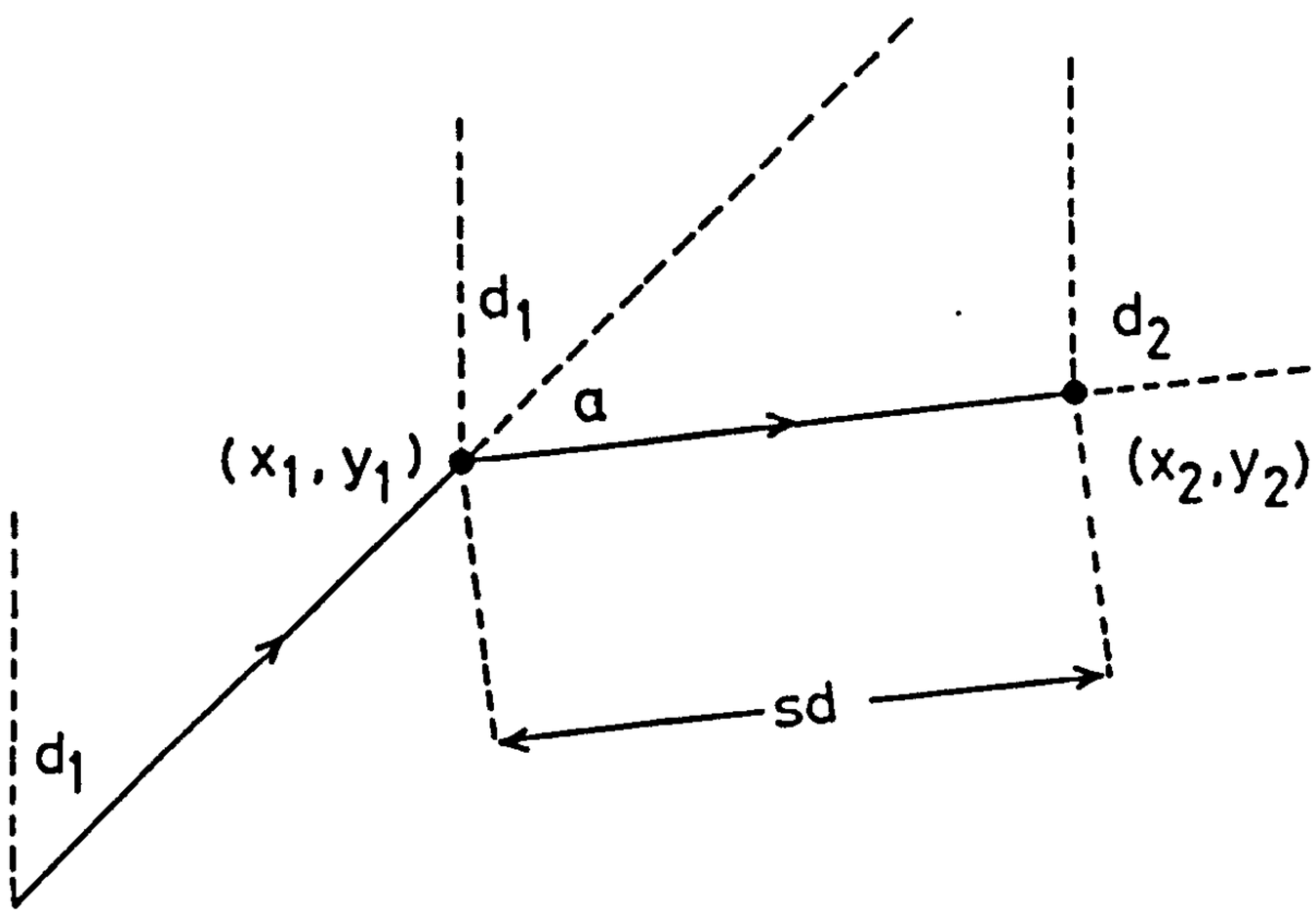


Figure 5.1.

Method of calculating new track position from angle of turn (a) and step distance (sd).

(See text for explanation)

(should d_2 be negative, $d_2 = 360 + d_2$)

iv) Calculate new position (x_2, y_2) by:

$$(x_2, y_2) = (x_1 + sd \sin d_2, y_1 + sd \cos d_2)$$

(see Fig. 5.1)

v) Check new position against:

a) boundaries of arena

b) target position

c) stimulation zones

If the new position was found to be outside the boundaries of the arena it was recalculated such that the larval path was bounced off the wall at the same angle of reflection as the incident angle. The new direction was amended accordingly. If the new position was within the target boundary the track was deemed a 'hit', the number of steps recorded and a new track commenced. Depending on the conditions being simulated, crossing into a different zone of stimulation required selection of parameters from a different data set.

This basic form of the model was used in the subsequent sections, with additions or amendments as indicated.

5.3. SIMULATING THE HOST LOCATION BEHAVIOUR OF THE CERCARIA OF ECHINOPARYPHIUM RECURVATUM

In chapter 2 the host location behaviour of the cercaria of Echinoparyphium recurvatum was investigated. The main conclusions drawn were that the cercariae seem unable to perceive the direction of a target snail even when very close to it, and that they show increased rate of turning and decreased swimming speed at different distances from the snail. To simulate this behaviour, data arrays for step distance and angle of turn were created for each of eight zones around the target snail. These were 0 - 1 mm, 1 - 2 mm constant throughout the zones.

Tracks were simulated as in a 100 mm square arena with the target snail represented as a circle of 5 mm in the centre. No movement of the target snail was simulated. The first run of the model simulated behaviour as observed in Chapter 2, increased rate of turning and decreased step speed in the different zones around the snail, and consisted of 500 tracks of approximately 30 minutes real time (5400 steps). The cumulative hits curve for this simulation is shown in Figure 5.2. and can be regarded as a control for subsequent runs of the simulation model.

The principal reason for using simulation models is the manipulation of the studied system in a way that is not possible in nature. Whilst it is not possible to assess the efficiency of E.recurvatum cercariae locating a target snail in the absence of any behavioural responses, because the presence of the snail elicits such response, such a manipulation is possible using computer simulation of the

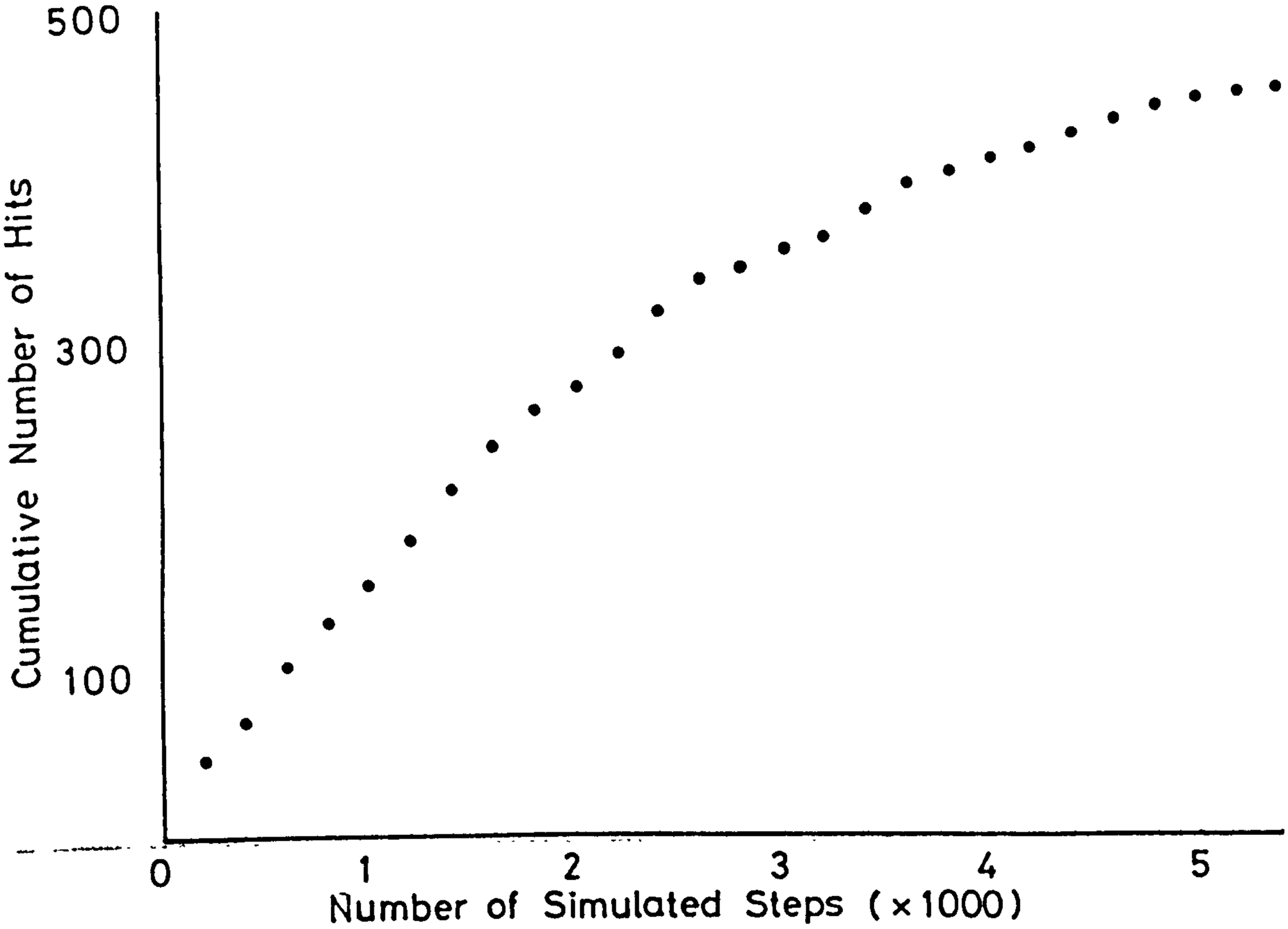


Figure 5.2.

Plot of cumulative number of hits for simulation of E.recurvatum cercariae locating a snail.

Run 1: Control - behaviour as recorded in Section 2.4.

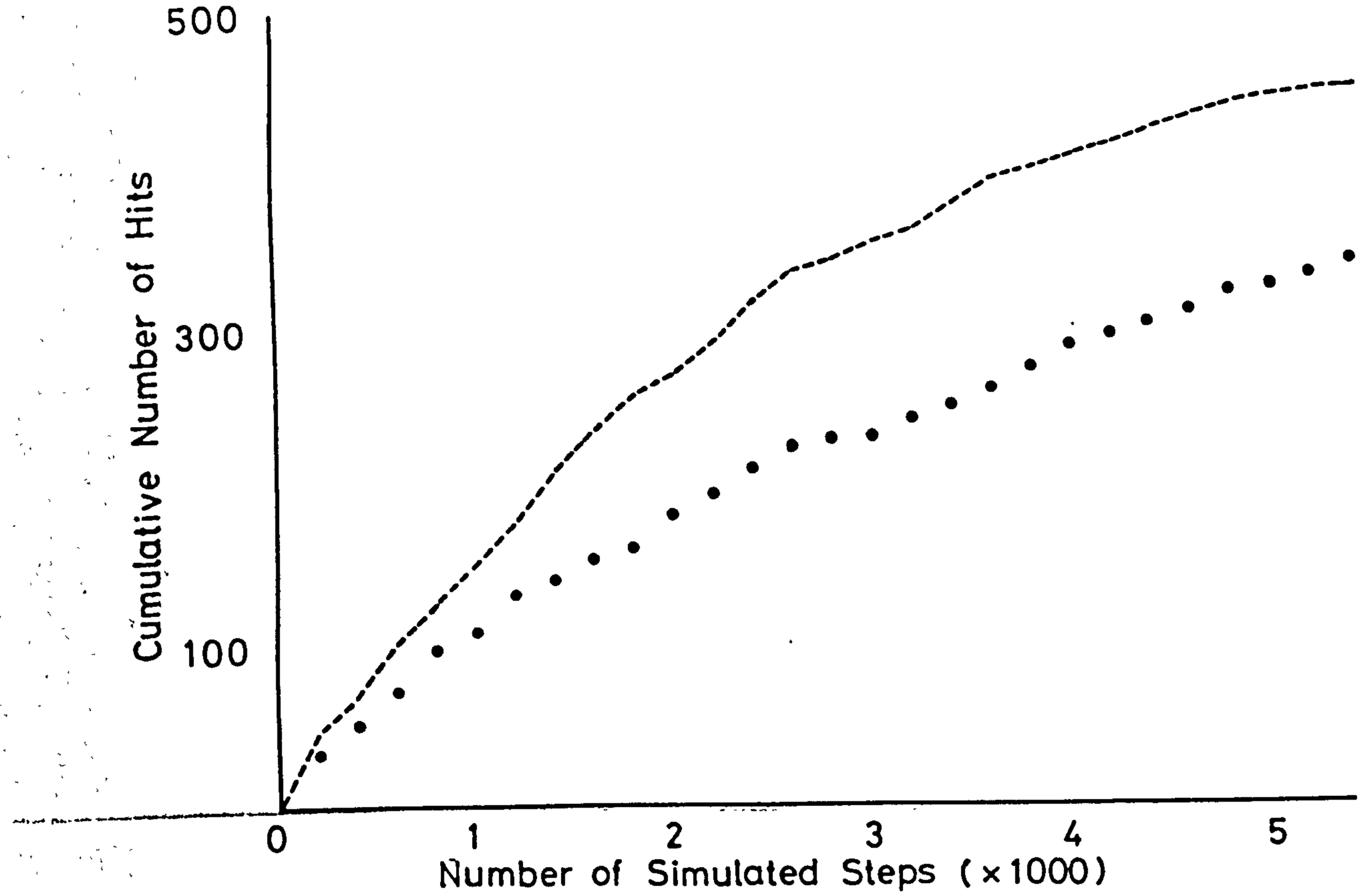


Figure 5.3.

Plot of cumulative number of hits for simulation of E.recurvatum cercariae locating a snail.

Run 2: Cercariae show no response to presence of the snail.

(The broken line represents the control simulation).

system. Accordingly the next run of the model used data only from the 7mm + zone and can be regarded as entirely unstimulated behaviour. As previously, 500 tracks over 5400 steps were simulated. The cumulative hits curve is shown in Figure 5.3., the broken line being the control derived from the first run of the model. The result of this run implies that the behavioural responses shown by E.recurvatum in the vicinity of a snail confer a 33 % higher chance of contact than if no responses are elicited.

Since the model had predicted that increased rate of turning and decreased swimming speed, when acting together, conferred a higher probability of target contact it was decided to run the model twice more, eliciting only one of the two responses on each occasion. Firstly, the model was run with only increased rate of turning in the zones successively close to the target snail. As previously, 500 tracks over 5400 steps were simulated. The cumulative hits curve is shown in Figure 5.4. The inference of these is results that increased rate of turning is an important part of the location process since the curve observed in this case is very similar to that of the control. Indeed over the first approximately 11 minutes real time (2000 steps) the model predicts that this behaviour would lead to a greater frequency of hits.

Given the results of the first three runs of the model, it was speculated that the fourth run would produce a low hit rate since the track paths would be relatively straight and, because of the slow speed, take longer to move around the arena. The actual cumulative hits curve for the fourth run is shown in Figure 5.5. The fact that this curve was similar to the control was a surprise.

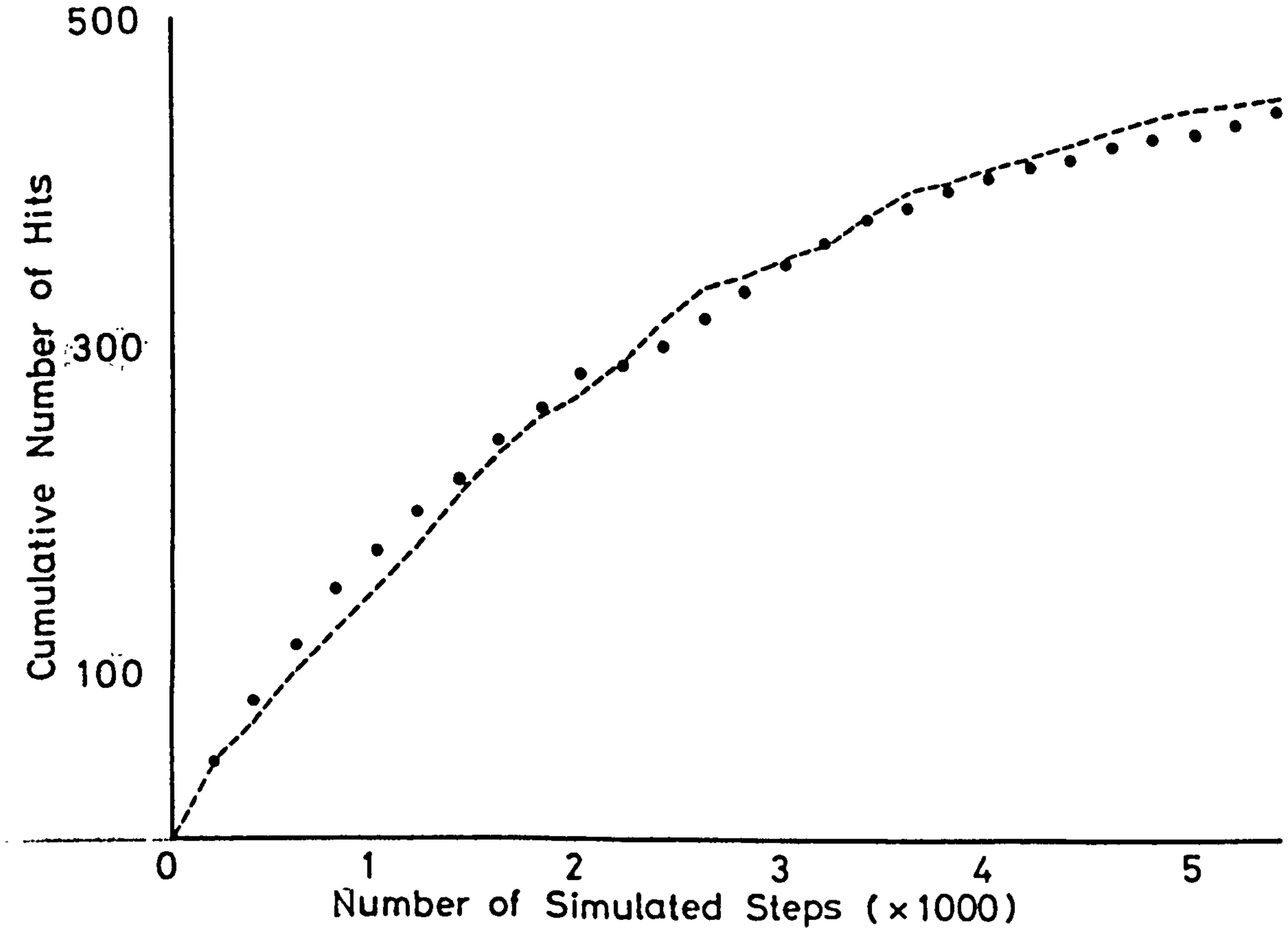


Figure 5.4

Plot of cumulative number of hits for simulation of E.recurvatum cercariae locating a snail.

Run 3: Cercariae respond by increased rate of turning only

(The broken line represents the control simulation)

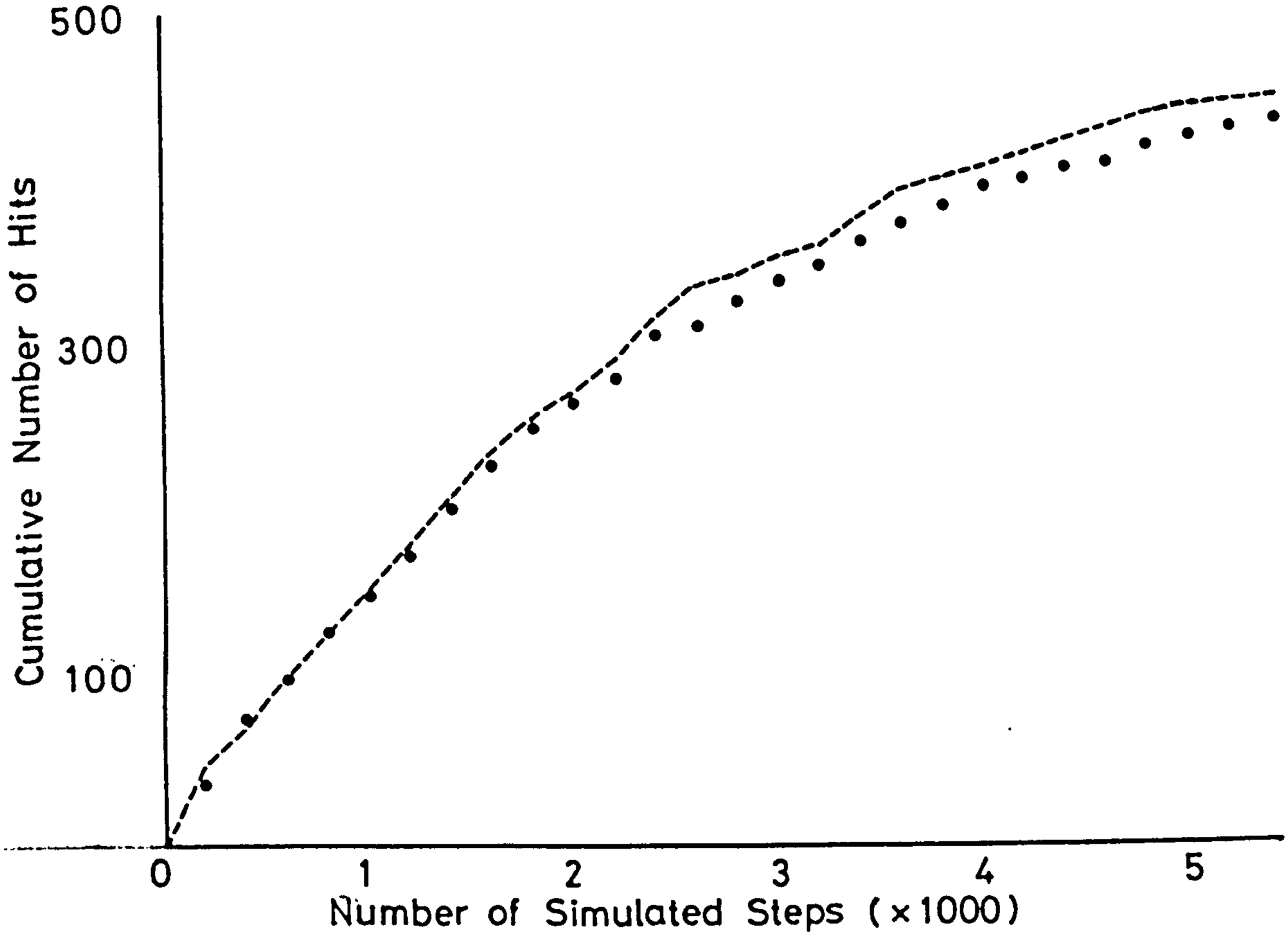


Figure 5.5.

Plot of cumulative number of hits for simulation of E.recurvatum cercariae locating a snail.

Run 4: Cercariae respond by decreased step speed only.

(The broken line represents the control simulation)

However, further consideration of the effects of manipulating step speed and angle of turn introduced a possible reason for this result. It was supposed that searching efficiency was closely related to increased rate of turning and that rate of turning in this run would be low as in run number 2. This supposition was based on rate of turning being expressed in terms of time. In this run, simulated tracks in the vicinity of the target snail were subject to reduced step speed but turns were still being introduced at the same time interval as in the previous runs of the model. The result was that although rate of turning was not increased per unit time, it was increased per unit distance. The effect of this would be increased time spent in the areas of stimulation, and hence increased chances of contact over unstimulated behaviour (Run 2).

It was possible to study this effect only by looking at visual presentation of simulated tracks. This presented no great difficulty except that the models, when run as described, were capable of producing over two and a half million simulated larval positions. The models required large amounts of computer run time (just under one hour per run) and enormous amounts of memory storage. In constructing the model, a Tektronix 4010 Storage Oscilloscope had previously been used to display the paths of simulated tracks and ensure that all aspects of the model were performing correctly. Using the same system, simulated tracks could be followed from each of the four runs and their paths compared in the vicinity of the target. Unfortunately, this analysis was quite subjective and because it was the time spent in the vicinity of the host that was of prime importance, not the path of the track, graph plotter output was not a suitable substitute.

Summary

Using a simulation model constructed from real track data it has been shown that increased rate of turning and decreased step speed (as described in Chapter 2), both together and combined, appear to confer on E.recurvatum cercariae similar, increased chances of host contact in an experimental arena when compared to the absence of these responses (unstimulated behaviour).

5.4. SIMULATING THE HOST LOCATION BEHAVIOUR OF THE CERCARIA OF PLAGIORCHIS ELEGANS

In chapter 3, the host location behaviour of the cercaria of Plagiorchis elegans was investigated. It was shown that in the vicinity of a chironomid larva, these cercariae swim significantly faster and demonstrate much increased rate of turning, as compared to unstimulated behaviour. However, it was speculated that movement of the host might be a contributory factor to these responses, principally by creating turbulence in the surrounding water. As in the previous section, data arrays for step distance and angle of turn were created for a number of zones around the target host, in this case a chironomid larva. However, here eleven zones each representing one millimetre were used. As previously, string data remained constant throughout the zones.

Tracks were simulated for a 100 mm square arena as before, but here the host was represented initially as a stationary oblong 10 millimetres long and 1 millimetre across. The first run of the

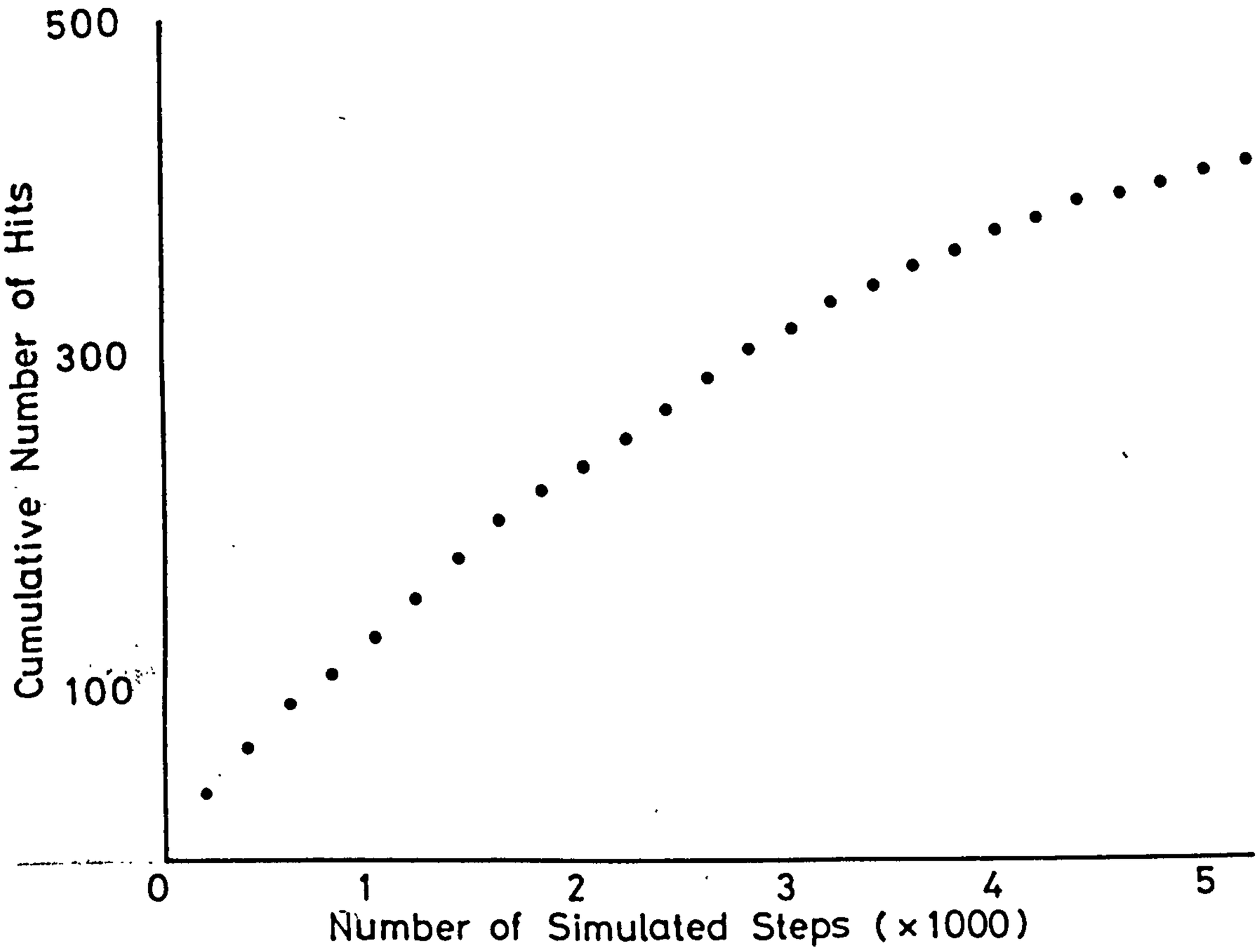


Figure 5.6.

Plot of cumulative number of hits for simulation of P.elegans cercariae locating a chironomid larva.

Run 1: Control - behaviour as recorded in Section 3.3.

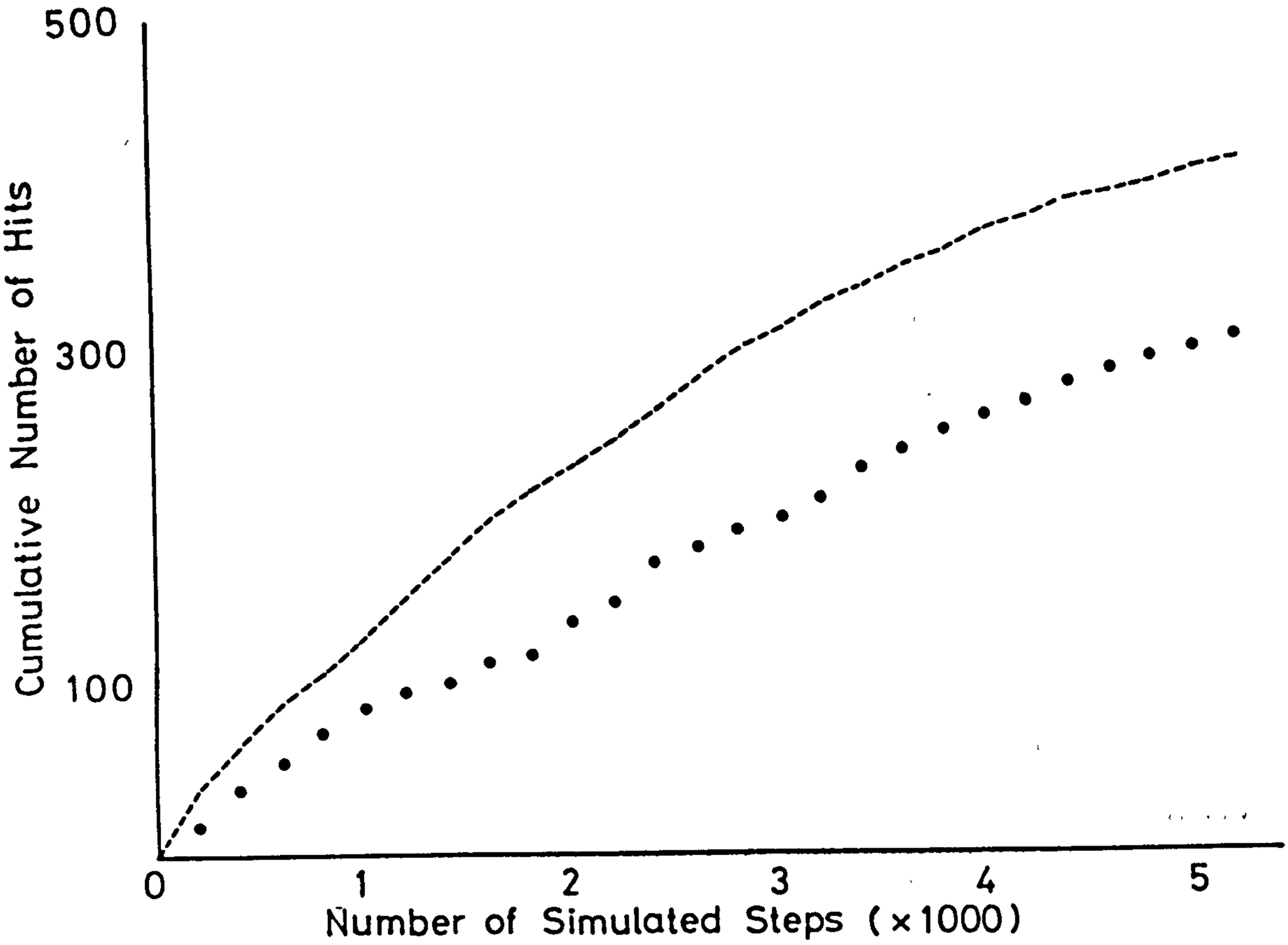


Figure 5.7.

Plot of cumulative number of hits for simulation of P.elegans cercariae locating a chironomid larva.

Run 2: Cercariae show no response to presence of the chironomid larva

(The broken line represents the control simulation)

model simulated behaviour as described in Section 3.3., and consisted of 500 tracks of approximately 30 minutes real time (in this case 5200 steps). The cumulative hits curve for this simulation is shown in Figure 5.6. This was regarded as the control for subsequent runs of the model. Whilst comparison between the results of simulations for different species was not a primary aim of this Chapter, it is interesting to note that the control results for P.elegans indicate a less efficient host location strategy than that for E.recurvatum.

For the second run of the model, data was drawn entirely from the 10 + mm data arrays and the behaviour simulated can be regarded as entirely unstimulated. The results are plotted in Figure 5.7. with the control (broken line). The results indicate that the responses shown by P.elegans do confer an advantage in host location since the cumulative hits curve here is much depressed compared to the control. Curiously, it is however not too dissimilar to the corresponding curve for E.recurvatum (Figure 5.3.). This was somewhat of a surprise since in the unstimulated state P.elegans cercariae are less efficient at displacing themselves that are those of E.recurvatum.

In the third run of the model, rate of turning only was varied with distance from the chironomid larva. Selection of values for step speed was entirely from the 10 + mm data array. Since rate of turning, expressed per unit time and per unit distance, appears to be an important factor in enhancing the chance of host contact in E.recurvatum cercariae, the result here was somewhat of a surprise. Increased rate of turning conferred only a small advantage over

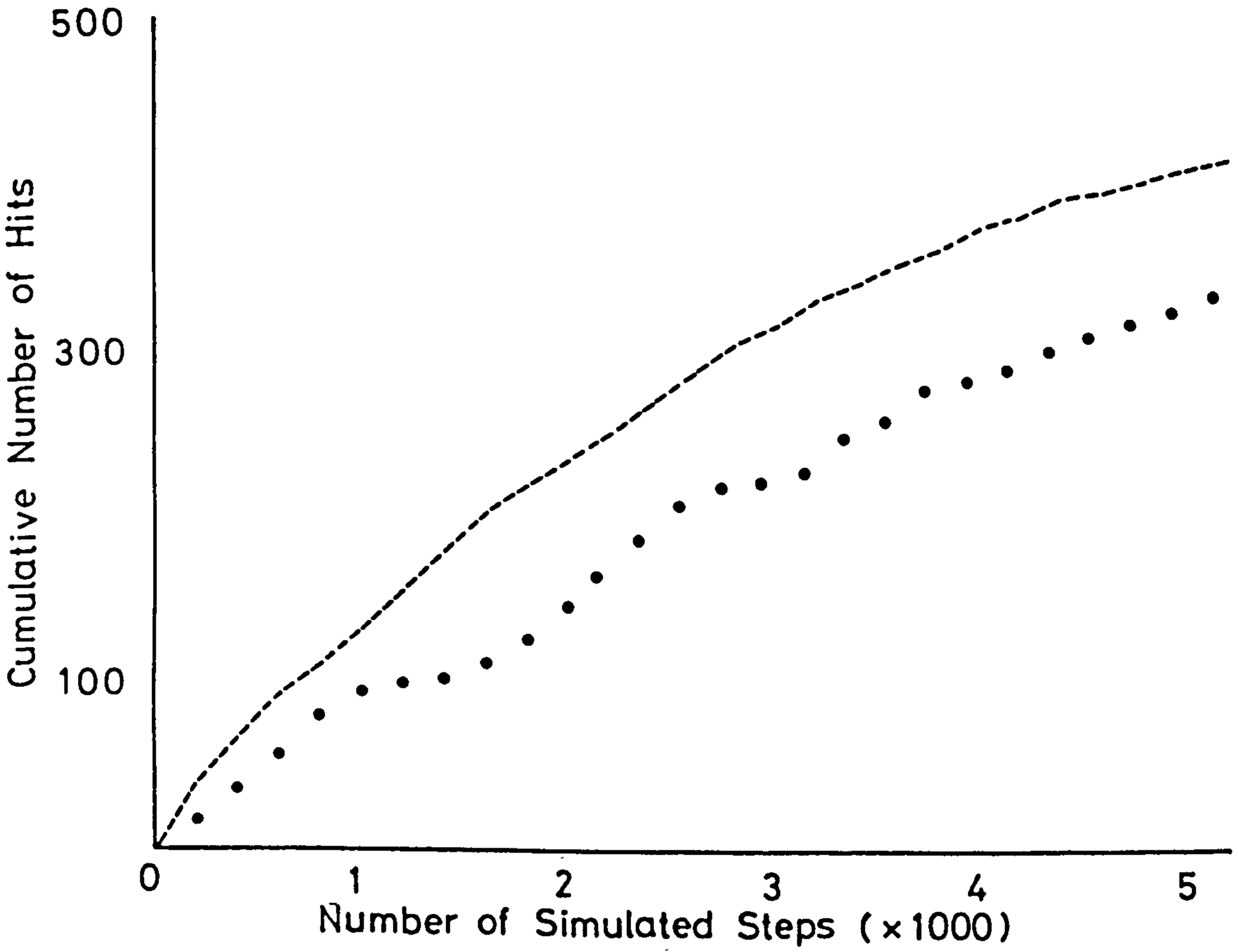


Figure 5.8.

Plot of cumulative number of hits for simulation of P.elegans cercariae locating a chironomid larva.

Run 3: Cercariae respond by increased rate of turning only.

(The broken line represents the control simulation)

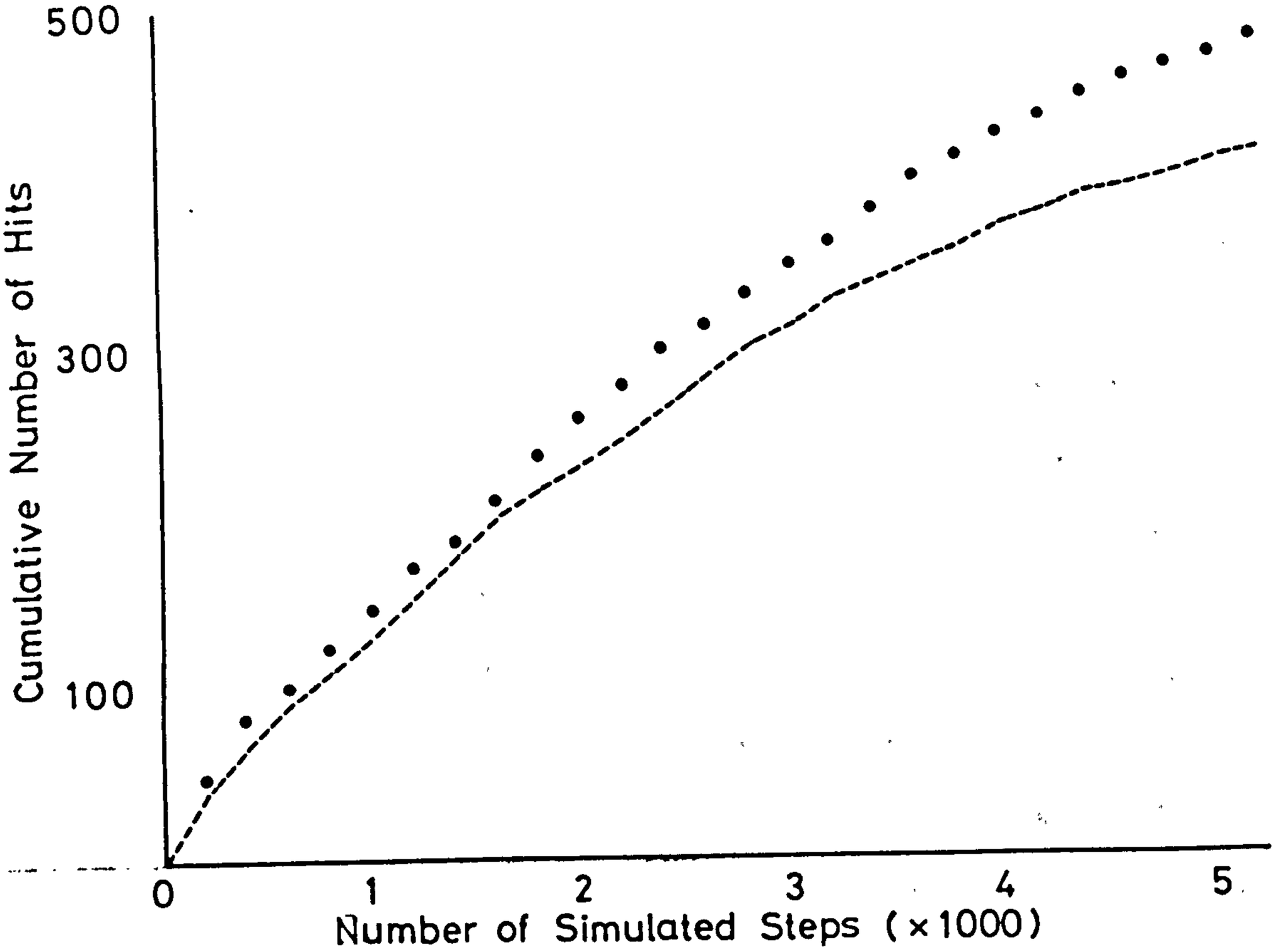


Figure 5.9.

Plot of cumulative number of hits for simulation of P.elegans cercariae locating a chironomid larva.

Run 4: Cercariae respond by increased step speed only.

(The broken line represents the control simulation)

unstimulated behaviour and the rate of hits was considerably lower than for the control simulation. The new curve is plotted in Figure 5.8. The result implies that increased rate of turning is not the major factor in host location in P.elegans cercariae.

For the fourth run of the model, only step speed was varied. Selections of angle of turn were from the 10+ mm data array. As before 500 tracks of a maximum of 5200 steps were simulated. The result also provided somewhat of a surprise since the rate of hits was higher than that for the control simulation (see Figure 5.9.). Consideration of how behaviour predicted by the model varied from the filmed behaviour of P.elegans cercariae in the vicinity of a chironomid larva leads to a hypothesis as to why this might be. Film of cercariae in the vicinity of a chironomid larva clearly showed that thrashing movements of the chironomid larva produced turbulence in the surrounding water into which cercariae were occasionally drawn. This turbulent water gave the cercariae greater momentum than they themselves were capable of producing. In some cases cercariae in this turbulent water were drawn towards the chironomid larva, but in just as many cases the cercariae were swept away. In using observed data for cercarial movement in the model, the momentum generated by movement of the chironomid larva has been programmed in. However, movement of the chironomid larva has not. The net effect of this appears to have been artificially increasing the speed of the cercariae in a situation where this would not occur in nature. Increased speed in close proximity to a target host could result in rapid movement away from the target or, as appears to have happened here, an increased rate of hits due to faster searching of the host's vicinity.

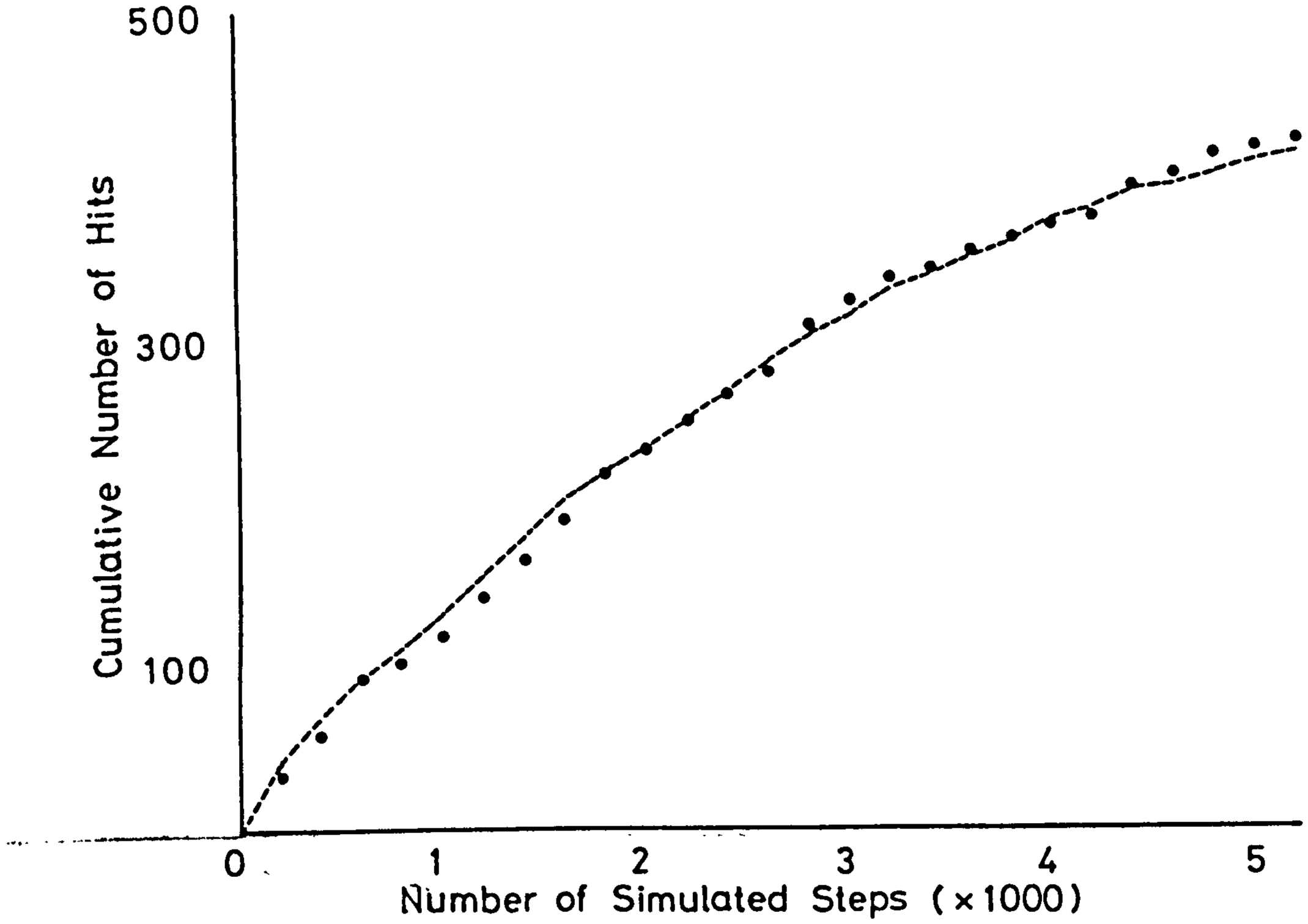


Figure 5.10.

Plot of cumulative number of hits for simulation of P.elegans cercariae locating a chironomid larva.

Run5: Cercarial behaviour as control, chironomid larva thrashing at random intervals

(The broken line represents the control simulation)

An attempt to program in movements of the chironomid larva has been made in a fifth run of the model. Here at random intervals the host was bent about its mid point in a series of steps until its two halves formed an angle of 30 degrees. The process was then reversed. The entire process was completed in eight steps (approximately 2.75 seconds real time) and was a crude attempt to recreate the thrashing movement seen in filmed chironomid larvae. Cercarial behaviour was simulated as in the control. The results of this fifth run of the model are plotted in Figure 5.10. The curve is very similar to that of the control simulation. This result was not altogether unexpected. Apparently movements of the chironomid larva which brought it into contact with cercariae were counteracted by just as many instances where the movement took the chironomid larva away from cercariae which would otherwise have hit it. This highlights a failure of this run of the model. The model does not adequately simulate the effect of movement of the chironomid larva on the movement of cercariae. In order to program this element into the model it would be necessary to reanalyse film of P.elegans cercariae in the vicinity of a chironomid larva and find a way of quantifying the effect of chironomid movement on cercariae.

Summary

The host location behaviour of P.elegans cercariae has been simulated using computer modelling. Two components appear to be important in enhancing the chances of host contact. Increased rate of turning in close proximity to a host appears to confer a small increase in the chances of host contact over unstimulated behaviour.

A further increase in the chances of host contact is predicted by the model when step speed is increased at small distances from the host. Increased step speed alone appears to confer the highest probability of host contact. It is recognised that a major failing of this model is its inability to describe fully the effects of movement of the host on cercarial movement by the creation of local turbulence.

5.5. SIMULATING THE HOST LOCATION BEHAVIOUR OF THE MIRACIDIUM OF SCHISTOSOMA MANSONI

In chapter 4, the host location behaviour of the miracidium of Schistosoma mansoni was investigated. By contrast to the investigations of the behaviour of Echinoparyphium recurvatum and Plagiorchis elegans cercariae, the study of S.mansoni miracidia included not only the recording of behaviour in the vicinity of a host, but also investigation of the responses to sharp changes in concentration of a stimulus. The two parts of the study have led to the use of two types of model, the first as used in previous sections. The second model was constructed to test hypotheses derived from the interface experiments, the findings of Roberts et al (1978) and conclusions drawn by Carter (1978) from his simulation models of S.mansoni miracidial host location behaviour.

Model 1

The first model was the basic model used in previous sections. The

data from track analysis of S.mansoni miracidia in the vicinity of a snail host was used to construct data arrays for step speed and angle of turn for eight zones, each of one millimetre, from the target's surface. As previously string length data was constant across these zones.

Major differences between the track analysis of S.mansoni miracidial host location and that of E.recurvatum and P.elegans cercariae were necessary because of the difference in size and speed of movement of the miracidia. In the miracidial host location experiments miracidia were effectively locating a flat surface (the foot of the snail) and were, therefore, approaching the snail from one direction. For the purposes of the model it has been assumed that miracidia would show the same behaviour from whatever direction they approached the snail. The accuracy of this assumption has to be questioned since the part of the snail accessible to the miracidia in the experiments carried out in the present study, the base of the foot, is perhaps unlikely to be accessible to miracidia in nature. A further difference in the present model is the step interval. S.mansoni miracidia swim relatively quickly at 26°C and it was necessary to record their behaviour at a frequent step interval (0.089 second) to ensure an accurate representation of their tracks. Simulation at the same step interval resulted in the need to simulate nearly four times as many steps as the models for E.recurvatum and P.elegans cercariae, to recreate the same amount of real time.

The first run of this model represented the control as previously, and used data arrays for step speed and angle of turn for all of the

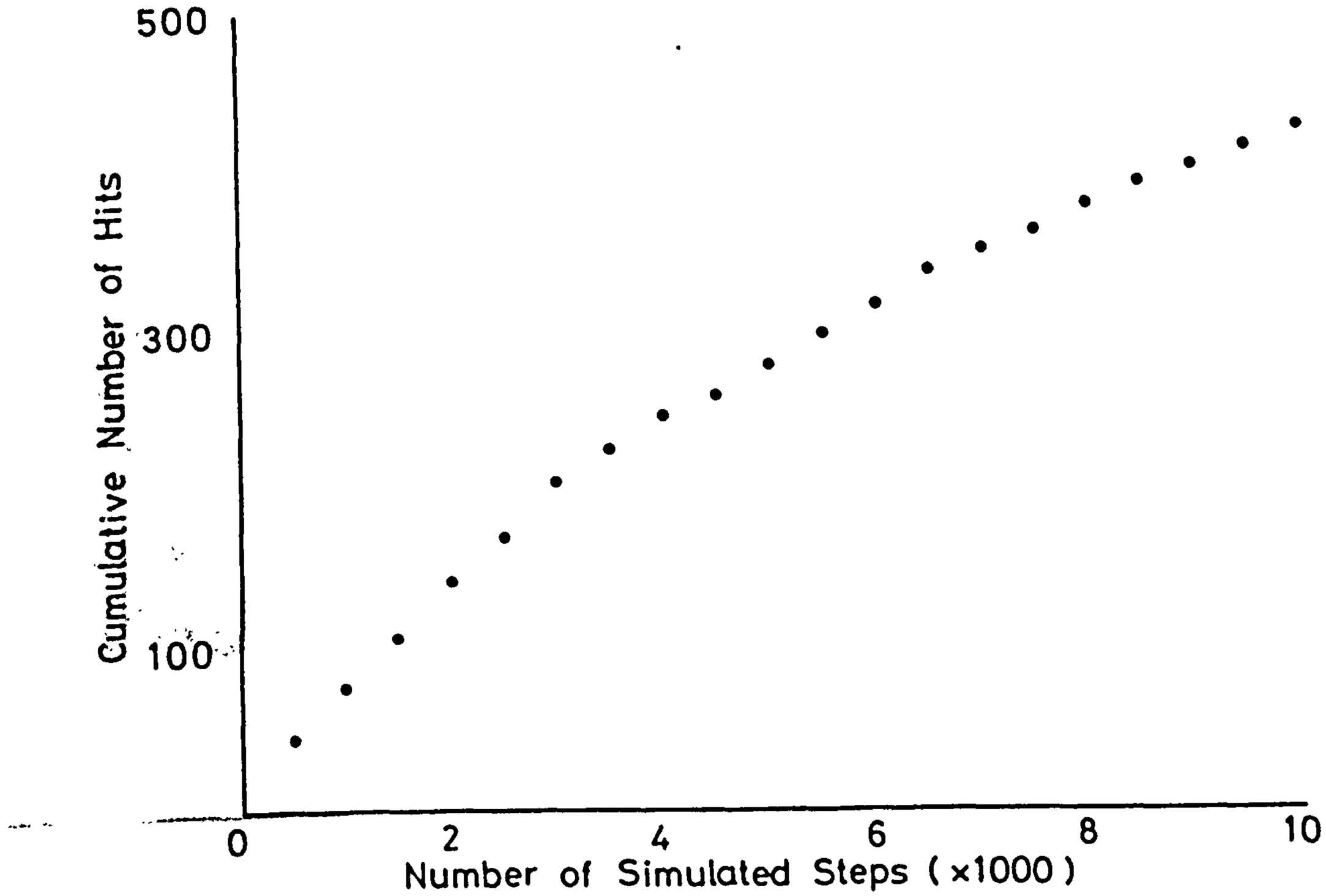


Figure 5.11.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 1, Run 1: Control behaviour as recorded in Section 4.3.

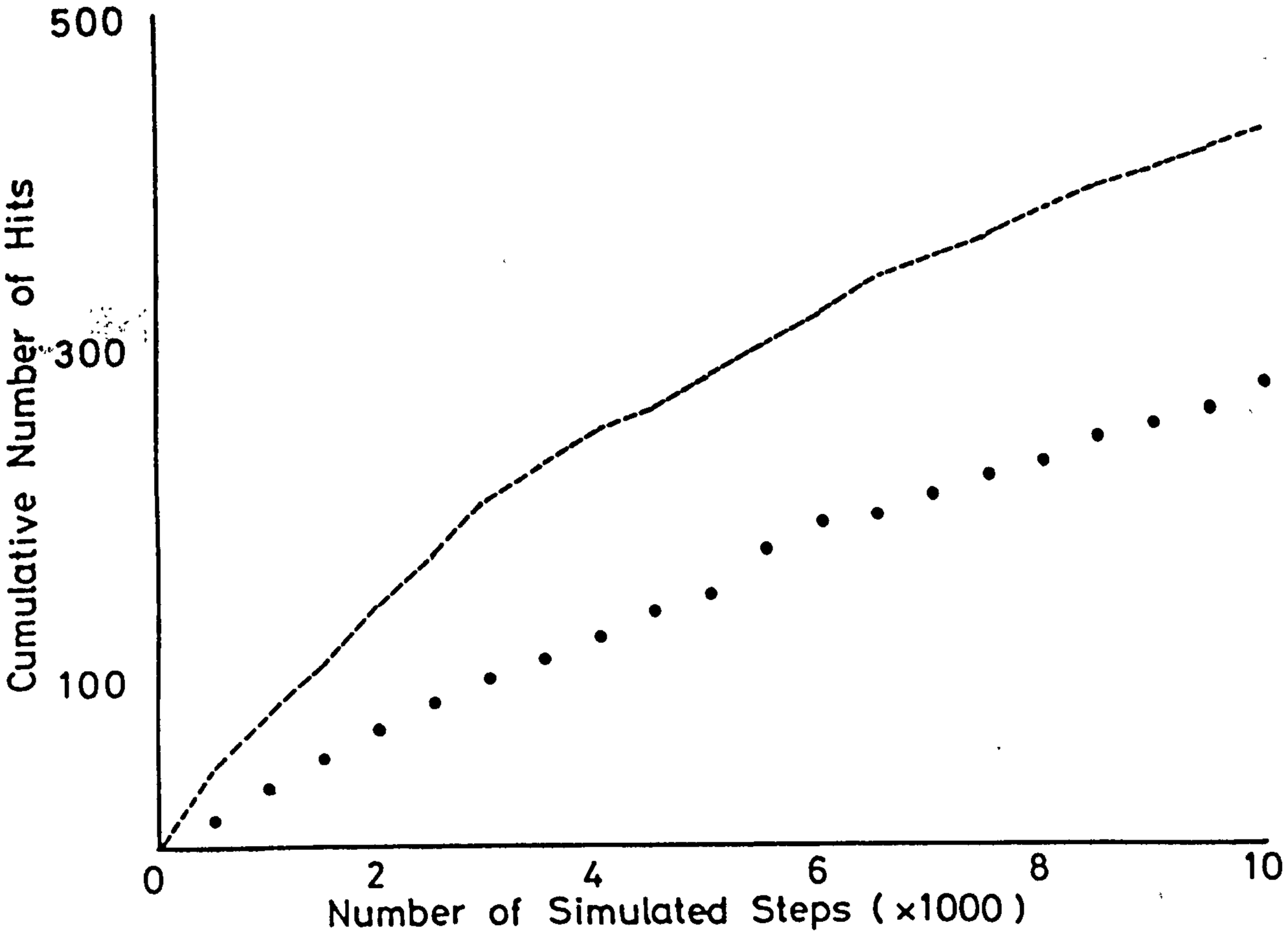


Figure 5.12.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 1, Run 2: Miracidia show no response to presence of the snail.

(The broken line represents the control simulation)

zones around the snail host. For economies of computer run time 500 tracks each of 10 000 steps, representing only approximately 15 minutes real time, were simulated. The arena size was set at 100 mm square, and the snail size at 5 mm for two reasons. Firstly, this was consistent with simulations carried out in previous sections. Secondly, Carter (1978) used the same values and it seemed sensible to try and facilitate comparison with his results. The cumulative hits curve for the first run (the control) is shown in Figure 5.11.

The second run of the first model simulated unstimulated miracidial behaviour at all distances from the target snail. The same number and length of tracks was used and the size of snail and arena were identical to the control run. The cumulative hits curve is compared to the control simulation in Figure 5.12. It can be seen that the model predicts a lower hit rate than for the control situation, ~~inferring~~ imply that increased rate of turning and decreased step speed together enhance the chances of host contact.

As previously the third run of this model simulated miracidial response to the presence of the snail host as increased rate of turning only. The cumulative hits curve is plotted with that of the control in Figure 5.13. As with E.recurvatum cercariae the curves are very similar, although here the curve under consideration never crosses the control curve. The result would appear to confirm that increased rate of turning is an important factor in the host location behaviour of S.mansoni miracidia.

The fourth run of this initial model simulated only reduced step speed in close proximity to the snail host. Bearing in mind the

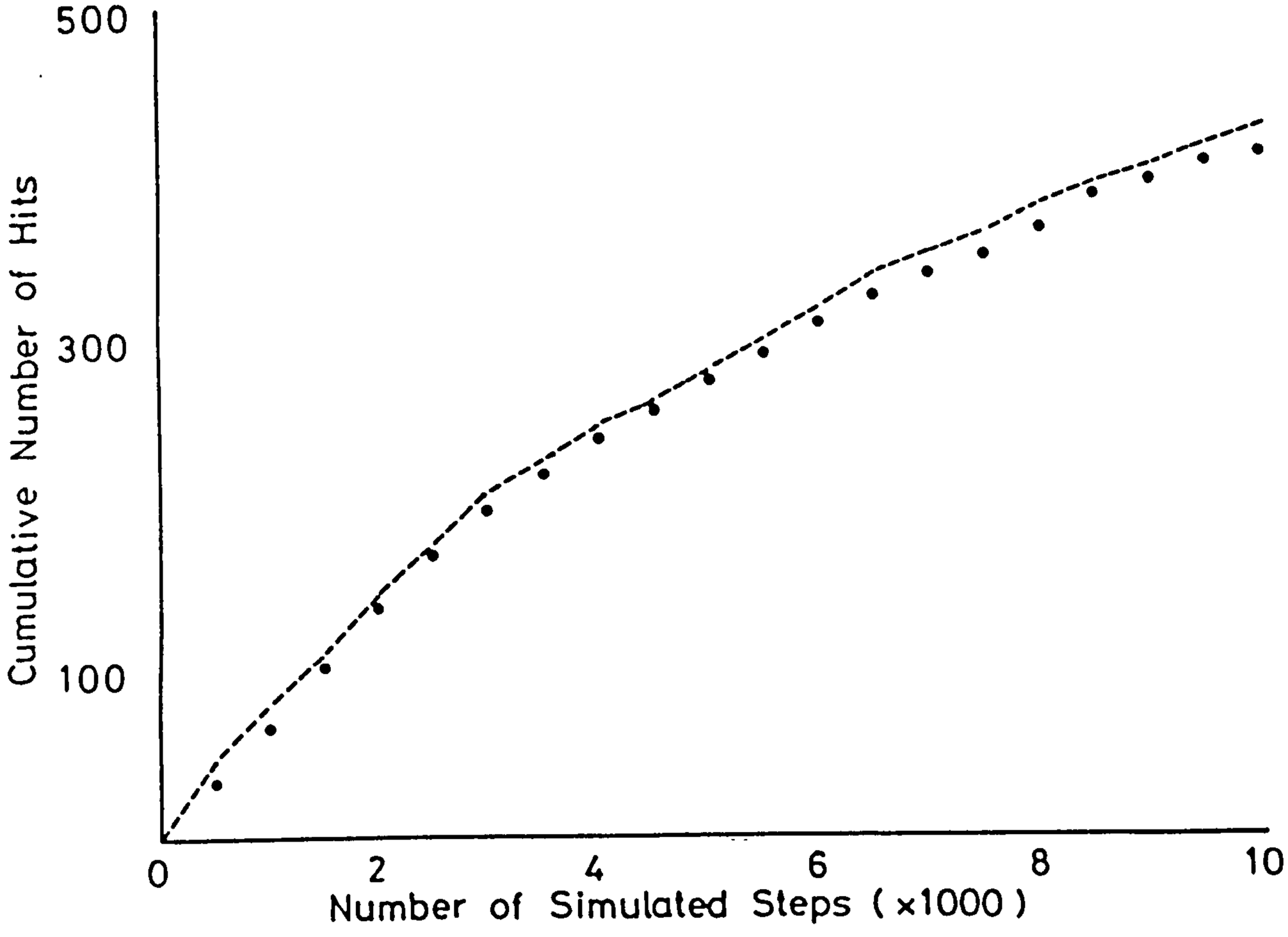


Figure 5.13.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 1, Run 3: Miracidia respond by increased rate of turning only.

(The broken line represents the control simulation)

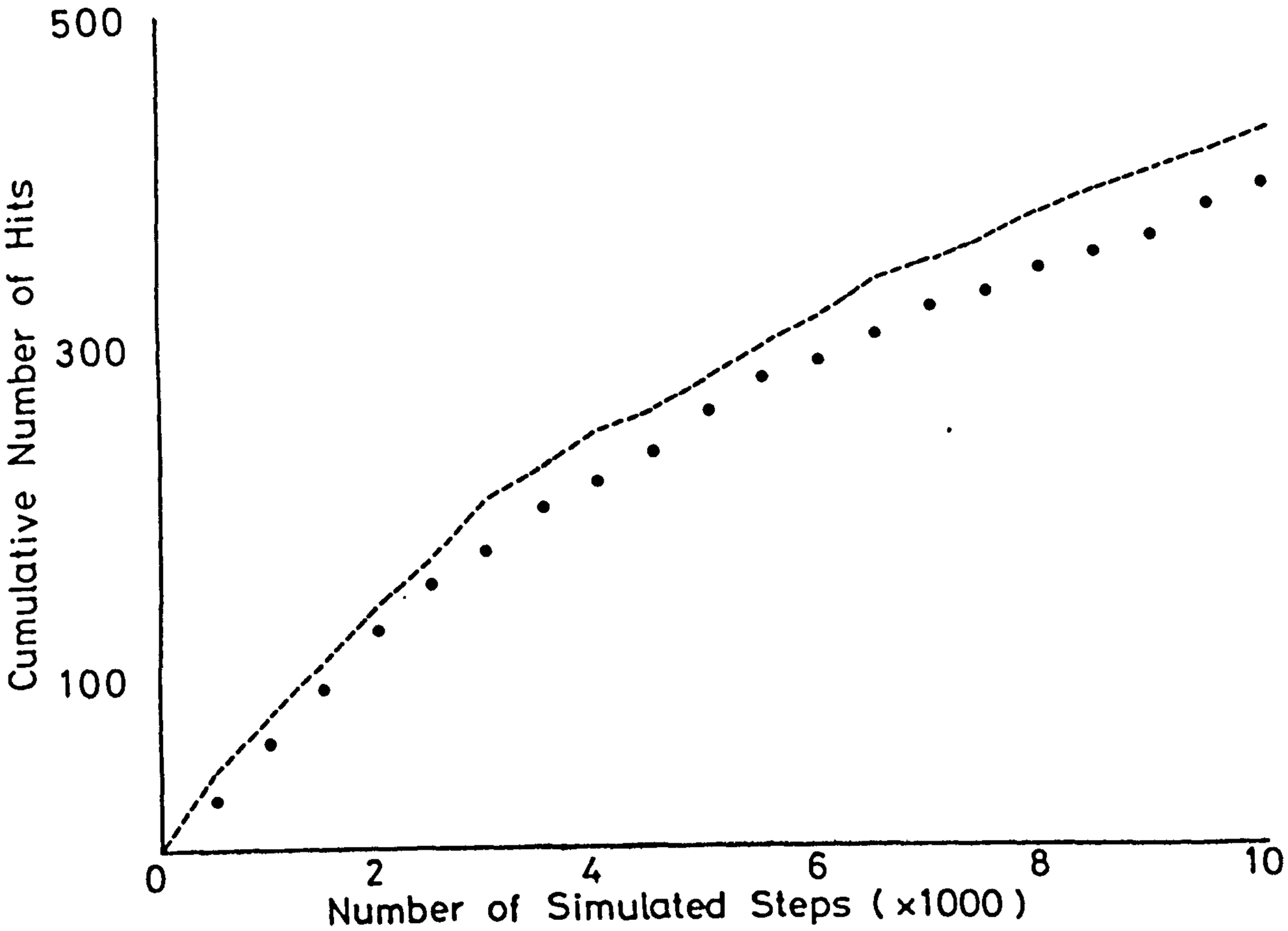


Figure 5.14.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 1, Run 4: Miracidia respond by decreased step speed only.

(The broken line represents the control simulation)

results obtained for E.recurvatum cercariae and the suggestion as to why this should be, the results, plotted with the control curve in Figure 5.14, were not altogether surprising. The cumulative hits curve is once again similar to the control curve, though slightly depressed in comparison to it and to the previous run where only increased rate of turning was considered.

Summary

The results obtained using this initial model suggest that both increased rate of turning and decreased step speed are important in the miracidial host location process. These results are not entirely consistent with those of Carter (1978) but there are important differences in the construction of the models which may account for this. Carter used observed track analysis data and segregated it into two sets subjectively. One data set was regarded as 'stimulated' parameters, the other as 'unstimulated'. In his model a 5 mm snail in a 100 mm square arena had a 5 mm zone of stimulation around it. Miracidia inside the zone of stimulation were allocated parameters from the 'stimulated' data set and those outside the zone were allocated parameters from the 'unstimulated' data set. In the model used here no such hard and fast assumptions have been made. In the control simulation real data have been used from the the correct zones and no attempt has been made to characterise the simulated behaviour of the miracidia. In subsequent manipulations of the model, changes in simulated behaviour have been modified in a carefully controlled manner, but still rely as much as possible on real data from the correct zones

around the target.

Model 2

The purpose of the second model was to evaluate a number of hypotheses which were derived from experiments on the behaviour of S.mansoni miracidia crossing interfaces and the findings of other workers, most notably Carter (1978) and Roberts et al (1979). An important point to consider here is that the purpose of this model was to compare different types of miracidial behaviour and assess the effects on chances of host contact, not to accurately predict the contact rate of a population of miracidia where heterogeneity in the response to a stimulus has been observed (Section 4.4.).

The basis of the model was data derived from Category C and F miracidia in Section 4.4. These miracidia exhibited responses when crossing a pond water - snail-conditioned water interface in both directions. Tracks of Category C and F miracidia were reanalysed and the data classified into two data sets representing stimulated and unstimulated behaviour. This segregation of data was based upon the significant changes in step speed and rate of turning observed when Category C and F miracidia encountered a pond water (PW) - snail-conditioned water (SCW) interface (see Figures 4.11 and 4.12). Since miracidia were observed to travel only 3 millimetres into SCW when crossing from PW, and only 1 millimetre into PW when crossing from SCW, a basic assumption of the initial run of this model was that miracidia on average reponded in the time taken to travel half those distances. Accordingly, tracks simulated by the model had

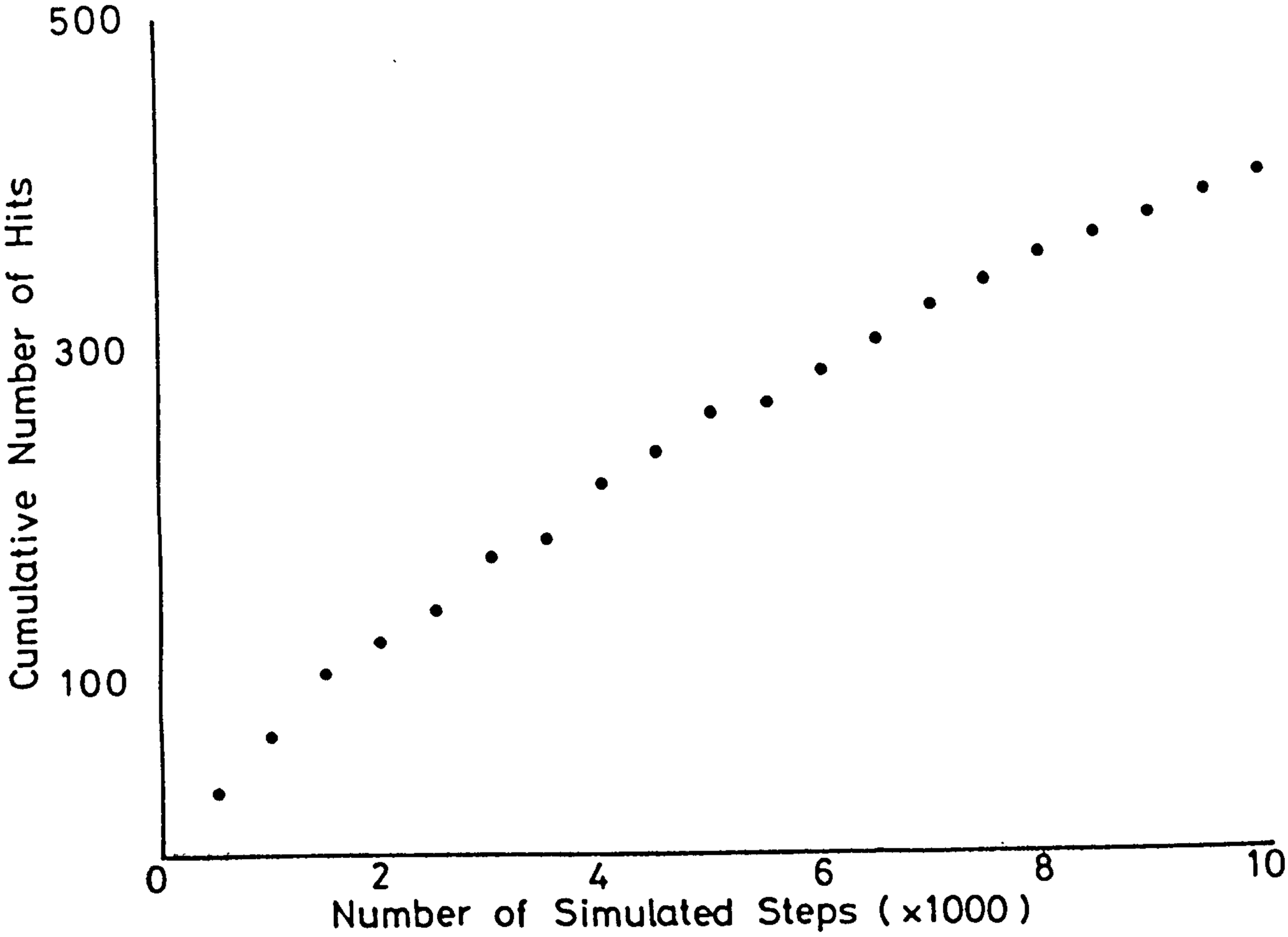


Figure 5.15.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 2, Run 1: Control (5mm snail, 5mm stimulation zone, 9 step delay in adopting stimulated behaviour and 3 step delay in revertingto unstimulated behaviour)

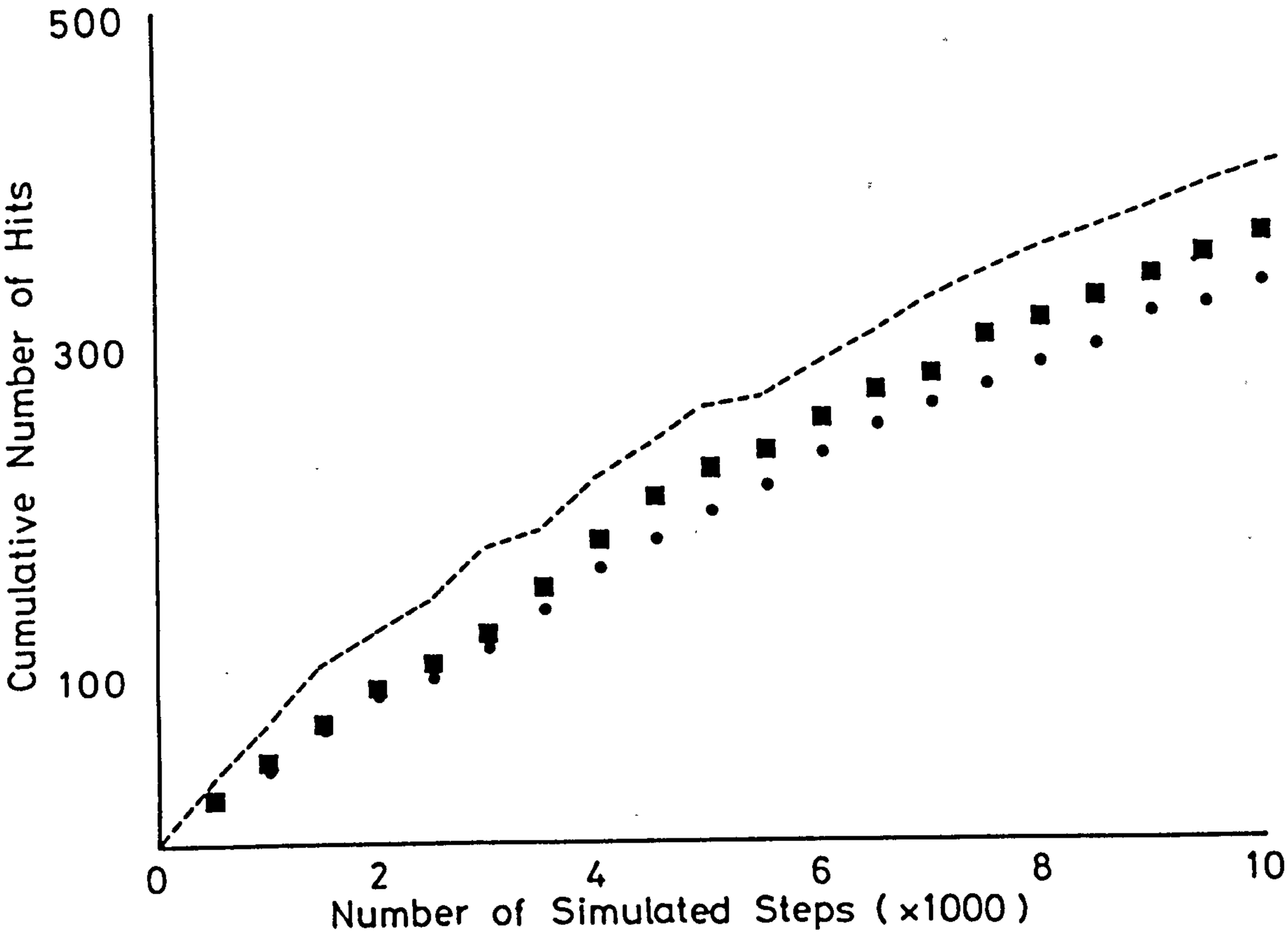


Figure 5.16.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 2, Runs 2 and 3

The effects of change in response time before adopting stimulated behaviour:

● No delay

■ 3 step delay

(The broken line represents the control simulation)

delays of 9 steps before adopting stimulated behaviour when entering an area of stimulation, and of 3 steps before returning to unstimulated behaviour when leaving an area of stimulation.

As previously, tracks were simulated in an arena of 100 mm square with the target snail represented as a circle of 5 mm diameter. For the first run of this model the snail was surrounded by a zone of stimulation 5 mm in width. The initial run of the model simulated 500 tracks each of 10 000 steps and the cumulative hits curve, the control or reference for other runs, is shown in Figure 5.15. Against this control it was decided to compare runs of the model with the following parameter changes:

i) response time for adoption of stimulated behaviur
ii) response time for adoption of unstimulated behaviour
iii) stimulation zone size

Two runs of the model were undertaken, each simulating 500 tracks of 10 000 steps. The first of these simulated an instantaneous adoption of stimulated behaviour when miracidia passed into the zone of stimulation, the second a time delay of three steps. The effects of these changes are shown with the control curve in Figure 5.16. It can be seen that prompter response to the zone of stimulation has been predicted by the model to be a less efficient strategy for searching.

Another two runs of the model were undertaken, again simulating 500 tracks of 10, 000 steps. In these cases the response time in adopting unstimulated behaviour when leaving the zone of stimulation

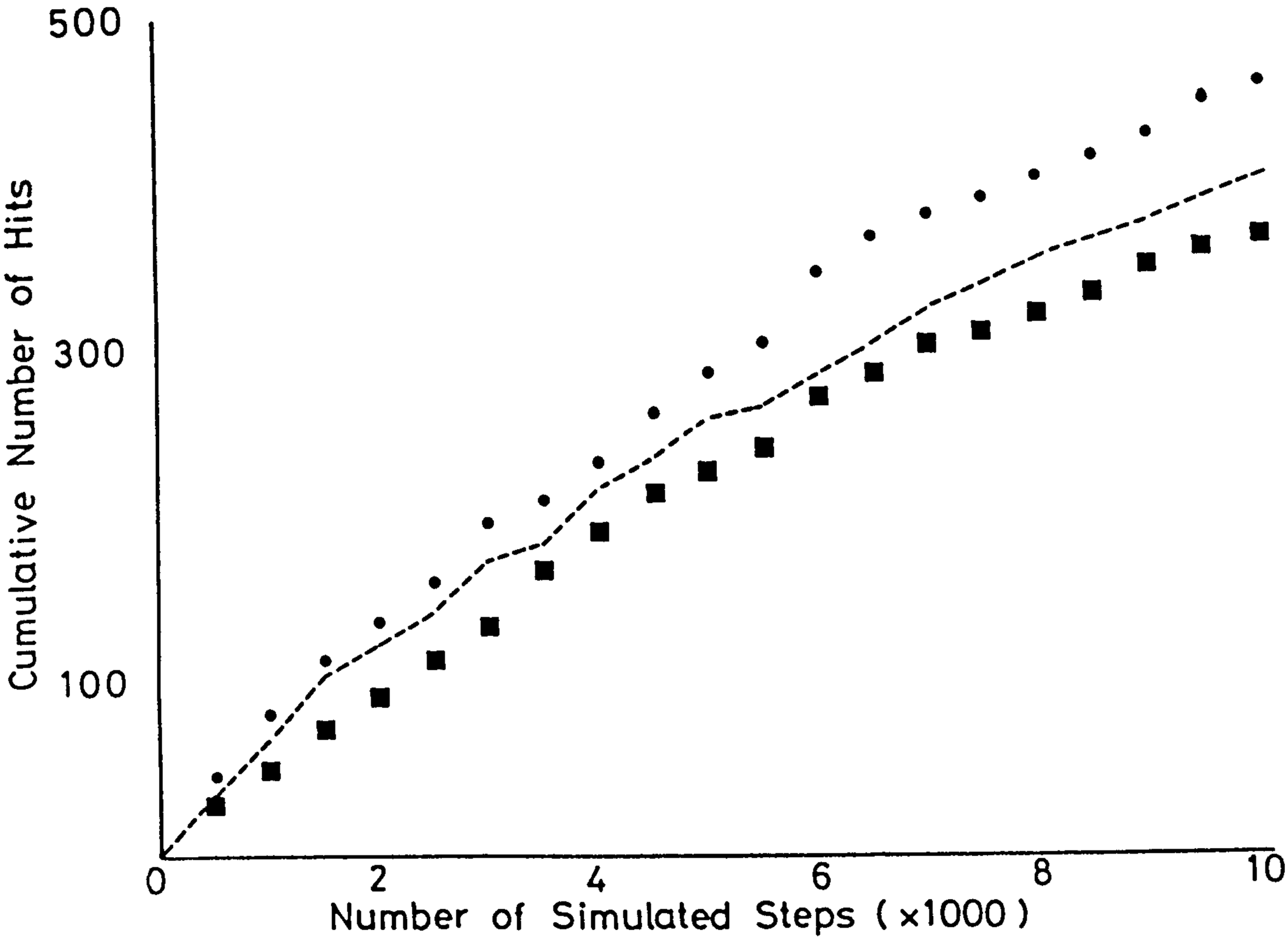


Figure 5.17.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 2, Runs 4 and 5

The effects of change in response time before reverting to unstimulated behaviour:

■ 9 step delay

● No delay

(The broken line represents the control simulation)

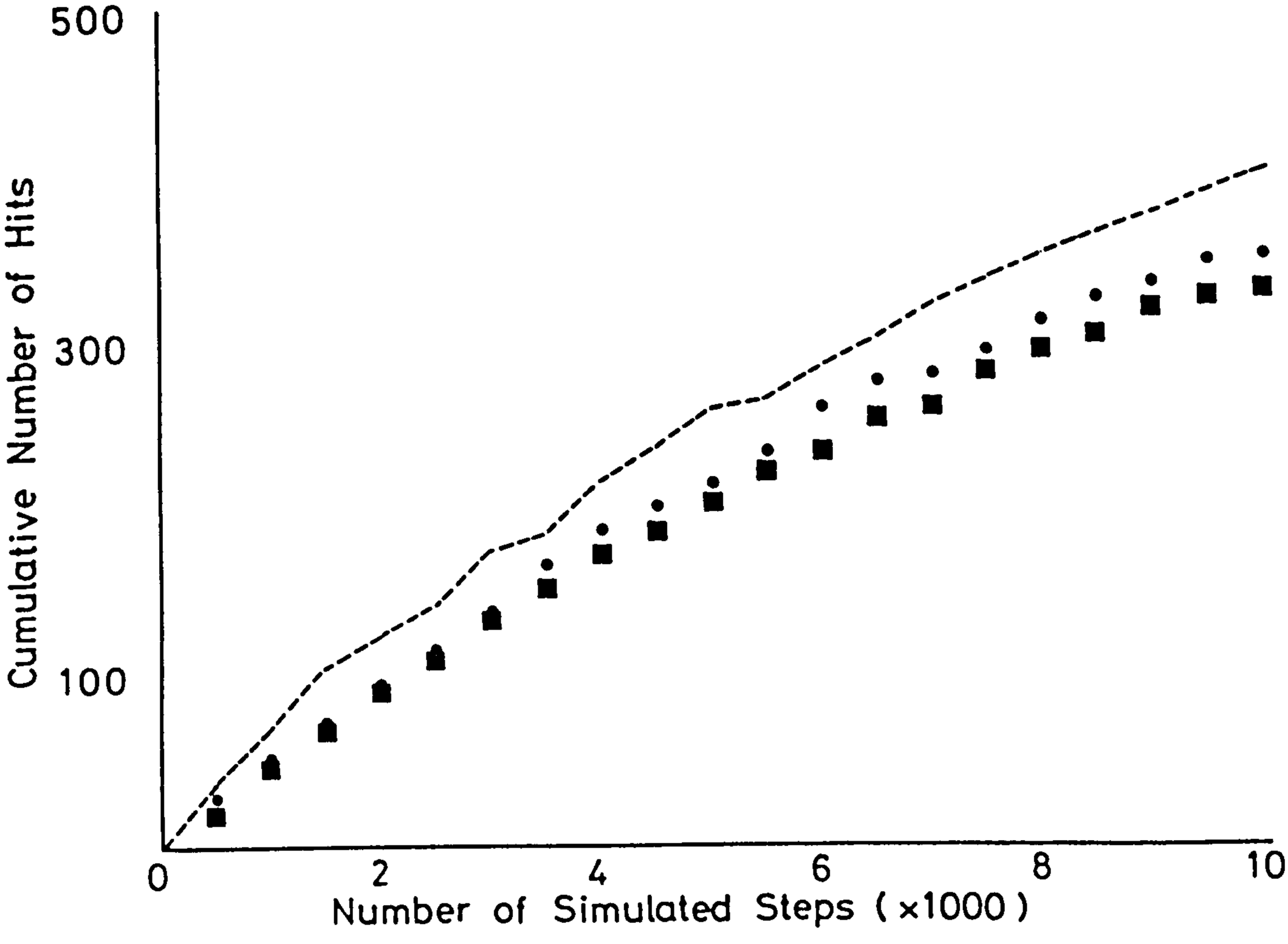


Figure 5.18.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 2, Runs 6 and 7

The effects of change in simulation zone size:

- 2.5 mm
- 7.5 mm

(The broken line represents the control simulation)

was first of all extended to 9 steps, and then removed completely. The results are plotted with the control curve in Figure 5.17. As can be seen, the model predicts that reduced reaction time to the change in stimulus enhances the chances of host contact.

The size of the zone of stimulation was varied in a further two runs of the model. In the first case the zone of stimulation was reduced to 2.5 mm, and in the second increased to 7.5 mm. The cumulative hit curves are shown in Figure 5.18 with the control. Somewhat surprisingly both runs gave lower predicted hit rates. This result can be explained if the benefits conferred by reduced stimulation zone size are negated by the difficulty in locating the zone in the first place. That is, there is probably an optimum size of stimulation zone, apparently between 2.5 and 7.5 mm, for the parameters programmed into the model.

All of the runs of the second model presented thus far have a major fault when compared to the observed behaviour of S.mansoni miracidia, there is no adaptation to the presence of a stimulus. In Section 4.5. summarising the results of the interface experiments, it was noted that miracidia respond to sharp changes in concentration of snail-derived chemicals but only for a few seconds. Miracidia filmed in pond water and in snail-conditioned water did not show significantly different behaviour. Accordingly, further runs of the model were undertaken where changes in behaviour when crossing the stimulant zone boundary were only maintained for fixed periods of time. For all of these runs simulated behaviour was derived from the unstimulated data set except for a period of time after crossing the stimulant zone boundary in either direction. The

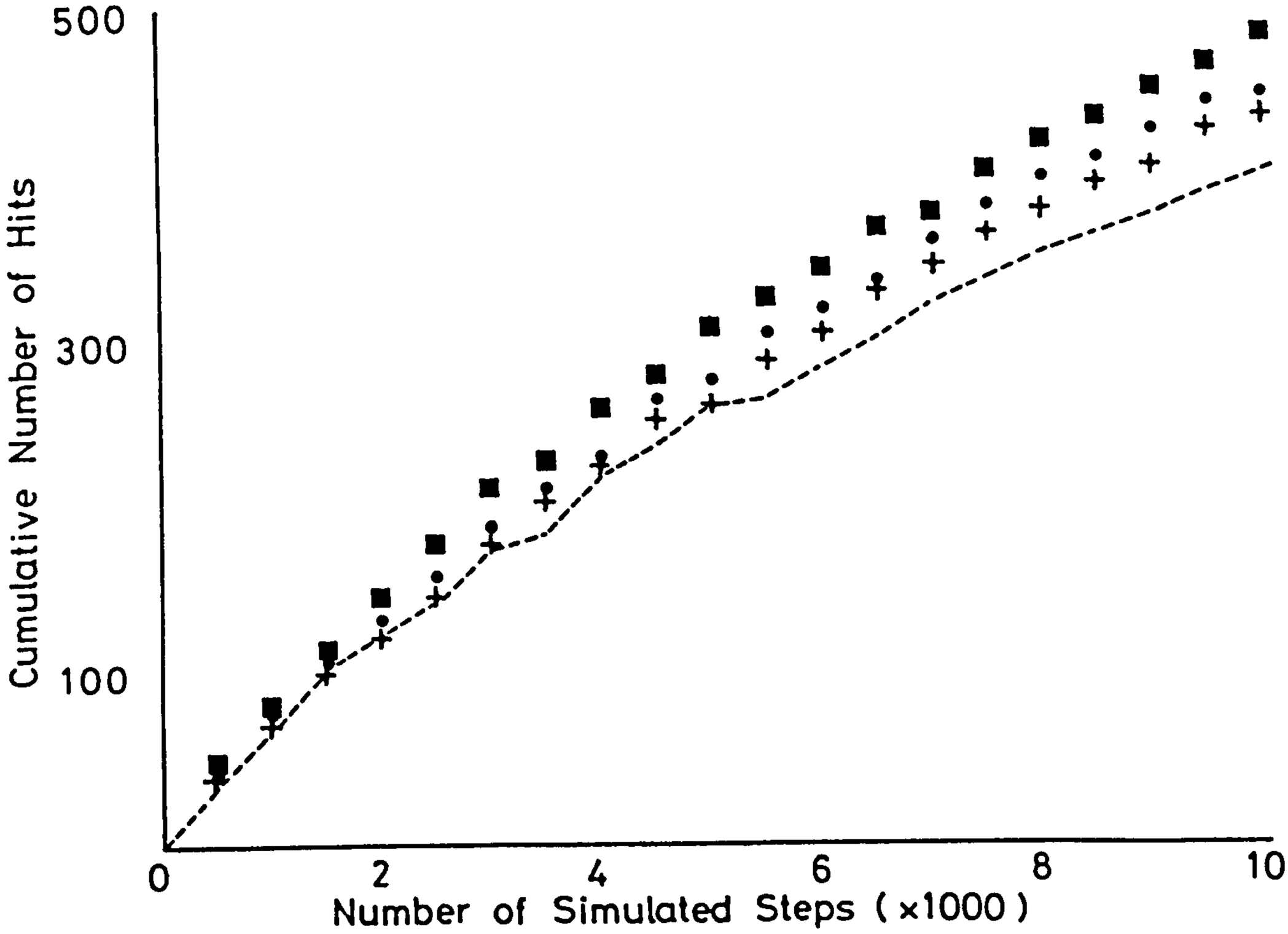


Figure 5.19.

Plot of cumulative number of hits for simulation of S.mansoni miracidia locating a snail.

Model 2, Runs 8, 9 and 10

The effects of change in periods of stimulation when encountering the stimulation zone boundary.

■ 20 steps • 40 steps + 80 steps

(The broken line represents the control simulation)

period of stimulation was delayed by 9 steps when entering the zone of stimulation and by 3 steps when leaving the same zone. These delays were as programmed into the control run of this model. Runs with stimulation times of 20, 40 and 80 steps were computed and the results plotted in Figure 5.19. The results differ from the control to varying extents and the model predicts that shorter stimulation times confer the greatest chances of host contact.

Summary

The second model has been used to investigate the effects of certain aspects of S.mansoni miracidial behaviour on the chances of host contact. The two most important of these, discussed in Section 4.5., are response time and sensory adaptation. This model has predicted that delays in response to a change in concentration of stimulus confer enhanced prospects of host contact on miracidia. The effect is most marked when the response time is faster for a reduction in stimulus concentration than for an increase. This agrees with observed miracidial behaviour crossing interfaces as described in Section 4.4. A further significant finding in the present study is that adaptation following the eliciting of a response could be an important factor in determining the chances of host contact. Once a response is elicited, the simulation model used has predicted that quicker miracidial adaptation to the new surroundings, in terms of reversion to unstimulated behaviour, will enhance the chances of host contact.

Whilst progress has been made here in describing the important

factors in schistome miracidial host location behaviour, three major difficulties obscure a clear understanding of the proces. Firstly, heterogeneity in miracidial response to the same stimulus confuses the distinction between the presence and absence of a response. Secondly, the exact chemical nature of the substances responsible for eliciting miracidial responses is unknown. It is likely that there are numerous chemicals produced by snails which are attractive to miracidia, and that their relative concentrations vary greatly under natural conditions. This means that investigation of absolute concentrations of stimuli can only be carried out for single chemical attractants, as for example by Roberts et al (1980) in studying the effects of magnesium ion concentration changes in S.mansoni miracidial behaviour. Thirdly, the size and shape of the zone of stimulation is largely unknown. It is likely to be highly complex because of the currents of water which snails induce around themselves (see Carter, 1978).

In conclusion, the most likely prospects for further understanding of the schistosome miracidial host-location process would seem to lie in further investigation of behaviour on crossing stimulant boundaries. In particular, delays in response time when encountering sharp changes in stimulant concentration and the duration of periods of stimulation would appear to be factors worthy of closer scrutiny.

CONCLUDING REMARKS

In chapter 1, the analogy between larval host location behaviour and predator-prey interactions was introduced. This was subsequently referred to in later chapters when the host location strategy of a particular larva was investigated.

It has been shown that Echinoparyphium recurvatum cercariae and Schistosoma mansoni miracidia, which can move more quickly than their hosts, show an active searching strategy. This is characterised, in the unstimulated state, by swimming in relatively straight lines with infrequent small turns. In reponse to the presence of a host, the behaviour is altered by an increase in rate of turning and a decrease in swimming speed. One would expect this type of behaviour to increase the amount of time spent in the vicinity of a host and improve the chances of contact. Simulation models presented in Chapter 5 confirm that the change in behaviour close to a host does enhance the chances of contact.

The host location behaviour of Plagiorchis elegans cercariae has been described as a 'sit-and-wait' strategy. Since the cercariae are incapable of moving as fast as their host can, they do not attempt to locate by active searching. Instead, by reduced activity, energy expenditure is minimised until such time as the host is present. In response to the host's proximity, activity is increased in terms of both the speed of and amount of time spent swimming. Theoretically, this behaviour maximises the chances of cercariae contacting the host during the brief period in which it is

in the vicinity. The results of simulation models presented in Chapter 5 confirm that this behaviour enhances the chances of host contact over that of unstimulated behaviour.

In the studies of E.recurvatum and P.elegans cercariae, no attempt has been made to investigate the nature of the stimuli eliciting changes in behaviour in the vicinity of a host. Observations of these cercariae and their hosts by binocular microscope suggest that the stimulus is chemical in E.recurvatum and mechanical, by host created turbulence, in P.elegans. Further experiments to confirm these assertions should form part of any future investigation of the host location behaviour of these cercariae.

For S.mansoni miracidia, it has been seen that a chemical stimulus elicits changes in behaviour identical to those observed in the vicinity of a snail. Because under artificial conditions, the position and shape of the chemical stimulus can be carefully controlled and recorded, it has been possible to record very accurately the nature of the behavioural responses elicited. A significant observation is that S.mansoni miracidia respond more quickly to a decrease in chemical stimulus concentration than to an increase. This would tend to keep miracidia in or close to a zone of stimulation around a target host and presumably enhance the chances of contact. Computer simulation models have confirmed that this is the case.

Experiments with stimulus boundaries have also revealed that miracidia respond only to changes in chemical stimulus concentration and revert relatively quickly to 'unstimulated' behaviour even if

the absolute concentration of stimulus is high. It appears that sensory adaptation is occuring. The restriction of behavioural changes to a brief period after a sharp change in chemical stimulus concentration has been shown by computer simulation models to enhance the chances of host contact over entirely unstimulated behaviour and over prolonged periods of stimulated behaviour. Rohlf and Davenport (1969) and Carter (1978) concluded that increased rate of turning at successively higher levels of stimulation reduces the efficiency of target finding. Since they did not consider the possibility of sensory adaptation occuring in the larval behaviour they simulated, their results can now be put into perspective.

Two factors combine to prevent complete understanding of the host location behaviour of the larvae studied. Firstly, the nature of the stimulus which elicits behavioural changes in the larva while in the vicinity of a host cannot at present be adequately described. The effect is to prevent standardisation of experimental conditions and make results from repeated experiments difficult to interpret. Secondly, larvae show heterogeniety in responses to the same stimulus. In order to compare the efficienty of searching strategies it is, therefore, necessary to categorise behaviour according to some subjective criterion as has been done in Section 4.4.

Further understanding of trematode host location strategies will be achieved when techniques have been developed to record the shape and position of zones of stimulation around target hosts and to minimise the effects of the behavioural heterogeneity of larvae.

APPENDICES

Appendix 2.1.
Test of distribution of angles of direction against that expected if behaviour is random.
E.recurvatum cercariae - horizontal plane.

Dir.	f	Exp	$(O-E)^2/E$
0–10	36	33.33	0.21
10–20	24	"	2.61
20–30	21	"	4.56
30–40	34	"	0.01
40–50	41	"	1.76
50–60	52	"	10.45
60–70	51	"	9.36
70–80	38	"	0.65
80–90	40	"	1.33
90–100	52	"	10.45
100–110	41	"	1.76
110–120	27	"	1.20
120–130	32	"	0.05
130–140	32	"	0.05
140–150	19	"	6.16
150–160	21	"	4.56
160–170	26	"	1.61
170–180	13	"	12.40
	600		Chisq= 69.18
			(17 d.f.)
			p<0.001

Appendix 2.2.
Test of distribution of strings against that expected if sign of turn is chosen independently of previous events. E.recurvatum cercariae - horizontal plane.

String Length	Left	Right	Both	Exp.	$(O-E)^2/E$
1	60	60	120	103.00	2.81
2	21	23	44	51.50	1.09
3	8	11	19	25.75	1.76
4	4	7	11	12.88	0.27
5	4	2	6	6.44	0.03
6	1	0			
7	2	0			
8	1	0			
9	0	0			
10	0	0			
11	1	0	6	6.44	0.03
12	0	0			
13	1	0			
14	0	0			
15+	0	0			
	103	103	206	206.00	Chisq= 5.99
					(5 d.f.)
					0.3>p>0.2

Appendix 2.3.
Test of distribution of angles of direction against that expected if behaviour is random.
E.recurvatum cercariae - vertical plane.

Dir.	f	Exp	$(O-E)^2/E$
0–10	42	33.33	2.25
10–20	28	"	0.85
20–30	27	"	1.20
30–40	29	"	0.56
40–50	33	"	0.00
50–60	32	"	0.05
60–70	29	"	0.56
70–80	41	"	1.76
80–90	53	"	11.60
90–100	60	"	21.33
100–110	47	"	5.60
110–120	54	"	12.81
120–130	41	"	1.76
130–140	30	"	0.33
140–150	19	"	6.16
150–160	10	"	16.33
160–170	17	"	8.00
170–180	8	"	19.25
	600		Chisq= 110.40
			(17 d.f.)
			p<0.001

Appendix 2.4.
Analysis of step speed v. angle of vertical direction.
E.recurvatum cercariae - vertical plane.

Class	N	Mean	Variance
0– 30	97	1.109	0.0927154
30– 60	94	1.053	0.0738396
60– 90	123	1.185	0.1265193
90–120	161	1.424	0.1431426
120–150	90	1.593	0.2301595
150–180	35	1.778	0.2202288

d-tests for significance between means.

		d	
0– 30 ct	30– 60	1.360	NS
30– 60 ct	60– 90	3.109	0.01>p>0.001
60– 90 ct	90–120	5.466	p<0.001
90–120 ct	120–150	2.869	0.01>p>0.001
120–150 ct	150–180	1.973	p<0.05

Appendix 2.5.
Test of distribution of strings against that expected if sign of turn is chosen independently of previous events. E.recurvatum cercariae - horizontal plane.

String Length	Left	Right	Both	Exp.	$(O-E)^2/E$
1	67	59	126	115.00	1.05
2	22	23	45	57.50	2.72
3	13	20	33	28.75	0.63
4	10	2	12	14.38	0.39
5	1	3	4	7.19	1.41
6	1	5			
7	1	2			
8	1	0			
9	0	0			
10	0	0	10	7.19	1.10
11	0	0			
12	0	0			
13	0	0			
14	0	0			
15+	0	0			
	116	114	230	230.00	Chisq= 7.30
					(5 d.f.)
					0.2>p>0.1

Appendix 2.6.
Comparison of step speed distributions by analysis of variance. E.recurvatum cercariae in the presence and absence of an orientation light.

Class	N	Sum	Mean	Sumsq
Light off	900	1343.7	1.493	2122.65
Light on	900	1446.3	1.607	2381.74
	1800	2790		4504.39

Total SS	4512.39	-	4324.5	=	187.89
Treatment SS	4330.348	-	4324.5	=	5.848
Error SS					182.042

Source	D.F.	SS	MS	F
Treatment	1	5.848	5.848	57.76
Error	1798	182.042	.1012469	p<0.001
Total				

t-tests for difference between means

$$\frac{|1.607-1.493|}{\sqrt{0.1012469(1/900+1/900)}} = 7.600$$

(897 d.f)
p<0.001

Appendix 2.7.

Comparison of angle of direction distributions by analysis of variance.

E.recurvatum cercariae in the presence and absence of an orientation light - horizontal plane.

Class	N	Sum	Mean	Sumsq
Light off	900	78396.3	87.107	9256013
Light on	900	16797.6	18.664	594203.8
	1800	95193.9		9850217.

Total SS 9850217 - 5034372. = 4815845.

Treatment SS 7142366. - 5034372. = 2107993.

Error SS 2707852.

Source	D.F.	SS	MS	F
Treatment	1	2107993	2107993	699.07
Error	1798	2707852	1506.036	p<0.001
Total	1799	4815845		

t-test for difference between means

$$\frac{|87.107-18.664|}{\sqrt{3015.4244(1/900+1/900)}} = 26.439$$

(897 d.f)

p<0.001

Appendix 2.8.

Comparison of rate of turning by analysis of variance.

E.recurvatum cercariae in the presence and absence of an orientation light - horizontal plane.

Class	N	Sum	Mean	Sumsq
Light off	870	95452.05	109.715	19007793
Light on	870	79960.8	91.909	12226718
	1740	175412.9		31234511

Total SS 31234511 - 17683678 = 13550833

Treatment SS 17821631 - 17683678 = 137953

Error SS 13412880

Source	D.F.	SS	MS	F
Treatment	1	137953	137953	17.876
Error	1738	13412879	7717.422	p<0.001
Total	1739	13550833		

t-test for difference between means

$$\frac{|109.715-91.909|}{\sqrt{7717.4217(1/870+1/870)}} = 4.227$$

(867 d.f)
p<0.001

Appendix 2.9.
Test of distribution of strings against that expected if sign of turn is chosen independently of previous events. E.recurvatum cercariae - horizontal plane.
a). Orientation light off.

String Length	Left	Right	Both	Exp.	$(O-E)^2/E$
1	102	95	197	177.00	2.26
2	42	41	83	88.50	0.34
3	17	16	33	44.25	2.86
4	10	8	18	22.13	0.76
5	1	2	3	11.06	5.87
6	2	4	6	5.53	0.04
7	2	4			
8	0	2			
9	3	0			
10	0	1	14	5.53	12.97
11	0	1			
12	0	1			
13	0	0			
14	0	0			
15+	0	0			
	179	175	354	354.00	Chisq= 25.10 (6 d.f.) 0.05>p>0.02

b). Orientation light on.

String Length	Left	Right	Both	Exp.	$(O-E)^2/E$
1	139	150	289	239.00	10.46
2	69	57	126	119.50	0.35
3	24	15	39	59.75	7.21
4	5	13	18	29.88	4.72
5	1	2	3	14.94	9.54
6	1	0	1	7.47	5.60
7	0	1			
8	0	0			
9	0	1			
10	0	0			
11	0	0	2	7.47	4.00
12	0	0			
13	0	0			
14	0	0			
15+	0	0			
	239	239	478	478.01	Chisq= 41.88 (6 d.f.) p<0.001

Appendix 2.10.
Comparison of the ratio of net displacement per distance travelled.
E.recurvatum cercariae in the presence and absence of an orientation light - horizontal plane.

Class	N	Sum	Mean	Sumsq
Light off	30	17.314	0.577	11.713
Light on	30	27.726	0.924	25.707
	60	45.04		37.42

Total SS	37.42	-	33.81	=	3.61
Treatment SS	35.617	-	33.81	=	1.807
Error SS					1.803

Source	D.F.	SS	MS	F
Treatment	1	1.807	1.807	58.29
Error	58	1.803	.0310862	p<0.001
Total	59	3.61		

t-test for difference between means

$$\frac{|0.924-0.577|}{\sqrt{0.031\ (1/30+1/30)}} = 7.633 \quad (27\ d.f)$$

p<0.001

Appendix 2.11.
Test of distribution of angles of direction relative to target against that expected if behaviour is random.
E.recurvatum cercariae in the vicinity of a host.

Dir.	f	Exp	$(O-E)^2/E$
0-10	206	193.66	0.79
10-20	217	"	2.81
20-30	208	"	1.06
30-40	220	"	3.58
40-50	207	"	0.92
50-60	199	"	0.15
60-70	226	"	5.40
70-80	220	"	3.58
80-90	189	"	0.11
90-100	206	"	0.79
100-110	217	"	2.81
110-120	167	"	3.67
120-130	162	"	5.18
130-140	175	"	1.80
140-150	164	"	4.54
150-160	171	"	2.65
160-170	156	"	7.33
170-180	176	"	1.61
	3486	3486	Chisq= 48.78 (17 d.f.) p<0.001

Appendix 2.12.
Test of distribution of strings against that expected if sign of turn is chosen independently of previous events. E.recurvatum cercariae in the vicinity of a host.

String Length	Left	Right	Both	Exp.	$(O-E)^2/E$
1	295	370	665	666.50	0.00
2	159	158	317	333.25	0.79
3	72	62	134	166.63	6.39
4	34	39	73	83.31	1.28
5	29	16	45	41.66	0.27
6	15	10	25	20.83	0.84
7	12	6	18	10.41	5.52
8	10	4	14	5.21	14.85
9	5	1			
10	6	3			
11	4	2			
12	5	2	42	5.21	259.98
13	2	0			
14	2	0			
15+	9	1			
	659	674	1333	1333.01	Chisq= 289.92

(8 d.f.)
p<0.001

Appendix 2.13.
Tests of significance for mean step speed at different distances from the target host.
E.recurvatum cercariae in the vicinity of a host.

Class	N	Sum	Mean	Sumsq
0-1	663	325.355	0.491	262.445
1-2	380	298.195	0.785	276.646
2-3	337	332.507	0.987	350.246
3-4	260	272.317	1.047	299.132
4-5	264	299.218	1.133	348.558
5-6	290	315.949	1.089	355.956
6-7	382	410.479	1.075	459.769
7-8	401	411.359	1.026	454.847
	2977	2665.379		2807.599

Total SS	2807.599	-	2386.377	= 421.222
Treatment SS	2553.375	-	2386.377	= 166.998
Error SS				254.224

Source	D.F.	SS	MS	F
Treatment	7	166.9976	23.8568	278.616
Error	2969	254.224	.0856261	p<0.001
Total	2976			

t-tests for differences between means

	t	
0-1 ct 1-2	15.615	p<0.001
1-2 ct 2-3	9.226	p<0.001
2-3 ct 3-4	2.484	0.05>p>0.02
3-4 ct 4-5	3.360	p<0.001
4-5 ct 5-6	1.721	NS
5-6 ct 6-7	0.614	NS
6-7 ct 7-8	2.342	0.05>p>0.02

Appendix 2.14.
Tests of significance for mean rate of turning at different distances from the target host.
E.recurvatum cercariae in the vicinity of a host.

Class	N	Sum	Mean	Sumsq
0-1	661	69304.67	104.848	15768367
1-2	376	41776.55	111.108	8294947.
2-3	336	39172.18	116.584	8743325.
3-4	259	21831.25	84.291	3715676.
4-5	263	17329.99	65.894	2343986.
5-6	288	23334.74	81.023	3658776.
6-7	377	29669.09	78.698	4258001.
7-8	399	30508.44	76.462	4516134.
	2959	272926.9		51299212

Total SS	51299212	-	25173707	= 26125505
Treatment SS	26015416	-	25173707	= 841709
Error SS				25283796

Source	D.F.	SS	MS	F
Treatment	7	841709	120244.1	14.034
Error	2951	25283796	8567.874	$p<0.001$
Total				

t-tests for differences between means

	t	
0-1 ct 1-2	1.047	NS
1-2 ct 2-3	0.788	NS
2-3 ct 3-4	4.219	$p<0.001$
3-4 ct 4-5	2.270	$0.05>p>0.02$
4-5 ct 5-6	1.916	NS
5-6 ct 6-7	0.321	NS
6-7 ct 7-8	0.336	NS

Appendix 2.15.
Tests of significance for mean angle of direction relative to target at different distances from the target host.
E.recurvatum cercariae in the vicinity of a host.

Class	N	Sum	Mean	Sumsq
0-1	663	55977.46	84.431	6071377.
1-2	380	30807.90	81.073	3453497.
2-3	337	28576.42	84.797	3259143.
3-4	260	21123.33	81.244	2339593.
4-5	264	22622.74	85.692	2581407.
5-6	290	26098.44	89.995	3258100.
6-7	382	33339.58	87.276	4053376.
7-8	401	35470.29	88.455	4281537.
	2977	254016.2		29298028

Total SS	29298028	-	21674235	=	7623793
Treatment SS	21697805	-	21674235	=	23570
Error SS					7600223

Source	D.F.	SS	MS	F
Treatment	7	23570	3367.143	1.311
Error	2969	7623793	2567.798	NS
Total	2976			

Appendix 3.1.
Test of distribution of angles of direction against that expected if behaviour is random.
P.elegans cer-carie - horizontal plane.

Dir.	f	Exp	$(O-E)^2/E$
0-10	96	84.89	1.45
10-20	66	"	4.20
20-30	74	"	1.40
30-40	74	"	1.40
40-50	94	"	0.98
50-60	78	"	0.56
60-70	89	"	0.20
70-80	75	"	1.15
80-90	42	"	21.67
90-100	156	"	59.57
100-110	75	"	1.15
110-120	91	"	0.44
120-130	75	"	1.15
130-140	68	"	3.36
140-150	93	"	0.77
150-160	67	"	3.77
160-170	93	"	0.77
170-180	122	"	16.22
	1528		Chisq= 120.20

(17 d.f.)
$p<0.001$

Appendix 3.2.
Test of distribution of strings against that expected if sign of turn is chosen independently of previous events.
P.elegans cercariae - horizontal plane.

String Length	Left	Right	Both	Exp.	$(O-E)^2/E$
1	210	239	449	394.50	7.53
2	109	99	208	197.25	0.59
3	44	44	88	98.63	1.14
4	14	13	27	49.31	10.10
5	6	7	13	24.66	5.51
6	1	2	3	12.33	7.05
7	0	0	0	6.16	6.16
8	1	0			
9	0	0			
10	0	0			
11	0	0	1	6.16	4.33
12	0	0			
13	0	0			
14	0	0			
15+	0	0			
	385	404	789	789.00	Chisq= 42.40

(7 d.f.)
$p<0.001$

Appendix 3.3.

Test of difference between means of rate of turning in the surface and bottom populations.

P.elegans cercariae - vertical plane.

Class	N	Sum	Mean	Sumsq
Surface	1770	15393.71	88.923	3487652
Bottom	1770	196461.2	110.995	4512268
	3540	211854.9		7999920

Total SS	21940093	-	12678644	=	9261449
Treatment SS	20678564	-	12678644	=	7999920
Error SS					1261529

Source	D.F.	SS	MS	F
Treatment	1	7999920	7999920	22442.38
Error	3539	1261529	356.4648	p<0.001
Total				

t-tests for difference between means

$$\frac{|110.995-88.923|}{\sqrt{356.4648(1/1770+1/1770)}} = 34.778 \quad (1767 \text{ d.f})$$

p<0.001

Appendix 3.4.

Test of distribution of strings against that expected if sign of turn is chosen independently of previous events.
P.elegans cercariae - vertical plane.
a). Surface population.

String Length	Left	Right	Both	Exp.	$(O-E)^2/E$
1	302	283	585	423.50	61.59
2	92	79	171	211.75	7.84
3	27	38	65	105.88	15.78
4	10	8	18	52.94	23.05
5	1	2	3	26.47	20.81
6	3	2	5	13.23	5.12
7	0	0			
8	0	0			
9	0	0			
10	0	0			
11	0	0	0	13.23	13.23
12	0	0			
13	0	0			
14	0	0			
15+	0	0			
	435	412	847	847.00	Chisq= 147.42 (6 d.f.) p<0.001

b). Bottom population.

String Length	Left	Right	Both	Exp.	$(O-E)^2/E$
1	302	316	618	485.00	36.47
2	130	117	247	242.50	0.08
3	42	38	80	121.25	14.03
4	8	11	19	60.63	28.58
5	2	2	4	30.31	22.84
6	0	1	1	15.16	13.22
7	0	0	0	7.58	7.58
8	1	0			
9	0	0			
10	0	0			
11	0	0	1	7.58	5.71
12	0	0			
13	0	0			
14	0	0			
15+	0	0			
	485	485	970	970.00	Chisq= 128.51 (7 d.f.) p<0.001

Appendix 3.5.

Test of distribution of direction relative to target against that expected if behaviour is random.
P.elegans cercariae in the vicinity of a host.

Dir.	f	Exp	$(O-E)^2/E$
0–10	113	132.89	2.98
10–20	118	"	1.67
20–30	146	"	1.29
30–40	134	"	9.27
40–50	124	"	0.59
50–60	115	"	2.41
60–70	137	"	0.13
70–80	133	"	9.11
80–90	126	"	0.36
90–100	150	"	2.20
100–110	148	"	1.72
110–120	126	"	0.36
120–130	155	"	3.68
130–140	151	"	2.47
140–150	140	"	0.38
150–160	131	"	0.03
160–170	121	"	1.06
170–180	124	"	0.59
	2392		Chisq= 40.30

(17 d.f.)
$p<0.05$

Appendix 3.6.
Tests of significan ce for mean step speed at different distances from the target host.
P.elegans cercariae in the vicinity of a host.

Class	N	Sum	Mean	Sumsq
0-1	249	160.736	0.646	178.523
1-2	403	202.258	0.502	187.139
2-3	440	151.869	0.345	98.093
3-4	385	107.826	0.280	54.854
4-5	359	84.421	0.235	36.856
5-6	191	43.669	0.229	17.399
6-7	136	21.727	0.160	7.253
7-8	147	25.292	0.172	8.614
8-9	112	16.548	0.148	5.689
9-10	173	27.732	0.160	10.539
10+	460	107.907	0.235	37.861
	3055	949.985		642.82

Total SS	642.82	-	295.408	=	347.412
Treatment SS	357.748	-	295.408	=	62.34
Error SS					285.072

Source	D.F.	SS	MS	F
Treatment	10	62.34	6.234	66.57
Error	3044	285.07	.0936498	p<0.001
Total	3054	347.41		

t-tests for differences between means

	t	
0-1 ct 1-2	5.838	p<0.001
1-2 ct 2-3	7.441	p<0.001
2-3 ct 3-4	3.044	0.01>p>0.001
3-4 ct 4-5	2.004	0.05>p>0.01
4-5 ct 5-6	0.219	NS
5-6 ct 6-7	2.010	0.05>p>0.01
6-7 ct 7-8	0.330	NS
7-8 ct 8-9	0.625	NS
8-9 ct 9-10	0.323	NS
9-10 ct 10+	2.748	0.01>p>0.001

Appendix 3.7.
Tests of significan ce for rate of turning at different distances from the target host.
P.elegans cercariae in the vicinity of a host.

Class	N	Sum	Mean	Sumsq
0-1	247	38603.88	156.291	11291909
1-2	394	45946.47	116.615	11584532
2-3	437	41036.35	93.950	9129130
3-4	379	35355.52	93.286	7828854
4-5	353	26531.65	75.160	4713621
5-6	190	14793.82	77.862	3148686
6-7	135	11036.37	81.751	2666029
7-8	147	7714.61	52.450	1145873
8-9	112	6344.22	56.645	1151250
9-10	173	14879.59	86.006	3401104
10+	460	41021.87	89.178	8050593
	3027	283264.4		64111581

Total SS	64111581	-	26511397	= 37600184
Treatment SS	28297461	-	26511397	= 1786064
Error SS				35814120

Source	D.F.	SS	MS	F
Treatment	10	1786064	178606.4	15.04091
Error	3016	35814117	11874.71	p<0.001
Total	3026	37600183		

t-tests for differences between means

	t	
0-1 ct 1-2	4.486	p<0.001
1-2 ct 2-3	2.994	0.01>p>0.001
2-3 ct 3-4	0.087	NS
3-4 ct 4-5	2.249	0.05>p>0.01
4-5 ct 5-6	0.276	NS
5-6 ct 6-7	0.317	NS
6-7 ct 7-8	2.253	0.05>p>0.01
7-8 ct 8-9	0.305	NS
8-9 ct 9-10	2.222	0.05>p>0.01
9-10 ct 10+	0.326	NS

Appendix 3.8.
Tests of significan ce for angle of direction relative to target at different distances from the target host.
P.elegans cercariae in the vicinity of a host.

Class	N	Sum	Mean	Sumsq
0-1	249	22104.23	88.772	2552642.
1-2	403	36094.35	89.564	4061625.
2-3	440	38434.29	87.351	4197374.
3-4	385	36534.93	94.896	4206226.
4-5	359	34794.21	96.920	4016207.
5-6	191	19366.88	101.397	2334319.
6-7	136	12457.27	91.598	1338173.
7-8	147	13432.91	91.380	1508417.
8-9	112	10415.14	92.992	1124803.
9-10	173	14840.62	85.784	1567622.
10+	460	39518.21	85.909	4528063.
	3055	277993.0		31435471

Total SS	31435471	-	25296268	=	6139203
Treatment SS	25360418	-	25296268	=	64150
Error SS					6075053

Source	D.F.	SS	MS	F
Treatment	10	64150	6415	3.214
Error	3044	6075053	1995.747	p<0.001
Total	3054	6139203		

t-tests for differences between means

	t	
0-1 ct 1-2	0.220	NS
1-2 ct 2-3	0.718	NS
2-3 ct 3-4	2.420	0.05>p>0.01
3-4 ct 4-5	0.618	NS
4-5 ct 5-6	1.119	NS
5-6 ct 6-7	1.955	NS
6-7 ct 7-8	0.041	NS
7-8 ct 8-9	0.288	NS
8-9 ct 9-10	1.330	NS
9-10 ct 10+	0.031	NS

Appendix 4.1. Schistosoma mansoni miracidia unstimulated behaviour, horizontal plane. Test of observed string distribution against that expected if sign of turn is independent of previous turns.

String Length	Left	Right	Both	Expected	$(O - E)^2/E$
1	68	62	130	92.5	15.203
2	18	27	45	46.25	0.034
3	3	2	5	23.125	14.206
4	1	1	2	11.563	7.908
5	3	0	3		
6	0	0	0	11.563	6.34
				CHISQ =	43.692
				(4 df)	p<0.001

Appendix 4.2. Schistosoma mansoni miracidia - change in mean step speed and rate of turning with age (± standard error) with age.

Age (hrs)	N	Mean step speed ($mm\ sec^{-1}$)	Mean rate of turning ($deg\ sec^{-1}$)
0	728	2.266 (±0.015)	56.956 (±3.39)
1	548	2.203 (±0.019)	59.563 (±3.55)
2	666	2.266 (±0.015)	59.199 (±3.58)
3	561	2.321 (±0.015)	41.126 (±2.29)
4	479	2.154 (±0.019)	34.663 (±2.19)
5	422	1.784 (±0.026)	55.228 (±4.38)
6	277	1.831 (±0.022)	58.293 (±4.68)
7	64	1.553 (±0.067)	24.119 (±2.85)
8	130	0.848 (±0.044)	52.415 (±4.44)

Appendix 4.3. Schistosoma mansoni miracidia - change in mean step speed and rate of turning (± standard error) with temperature.

Temp. (^{o}C)	N	Mean step speed ($mm\ sec^{-1}$)	Mean rate of turning ($deg\ sec^{-1}$)
10	600	0.628 (±0.008)	60.681 (±3.06)
15	600	1.096 (±0.008)	59.342 (±2.43)
20	600	1.606 (±0.011)	70.551 (±3.14)
22.5	600	1.967 (±0.015)	54.001 (±3.44)
25	600	2.318 (±0.011)	30.913 (±1.67)
26	600	2.424 (±0.011)	27.966 (±2.47)

Appendix 4.4 Schistosoma mansoni miracidia in the vicinity of a snail. Test of angles of direction relative to host against even distribution.

Class (deg)	Observed	Expected	$(O - E)^2/E$
Ø - 1Ø	2Ø3	31Ø.611	37.282
1Ø - 2Ø	17Ø	31Ø.611	63.653
2Ø - 3Ø	192	31Ø.611	45.293
3Ø - 4Ø	242	31Ø.611	15.156
4Ø - 5Ø	3Ø6	31Ø.611	Ø.Ø68
5Ø - 6Ø	344	31Ø.611	3.589
6Ø - 7Ø	4Ø6	31Ø.611	29.294
7Ø - 8Ø	317	31Ø.611	Ø.131
8Ø - 9Ø	32Ø	31Ø.611	Ø.284
9Ø - 1ØØ	495	31Ø.611	1Ø9.459
1ØØ - 11Ø	42Ø	31Ø.611	38.524
11Ø - 12Ø	415	31Ø.611	35.Ø83
12Ø - 13Ø	365	31Ø.611	9.524
13Ø - 14Ø	348	31Ø.611	4.5Ø1
14Ø - 15Ø	258	31Ø.611	8.911
15Ø - 16Ø	251	31Ø.611	11.44Ø
16Ø - 17Ø	248	31Ø.611	12.621
17Ø - 18Ø	291	31Ø.611	1.238
		CHISQ =	627.133
		(17 df)	p<Ø.ØØ1

Appendix 4.5. Schistosoma mansoni miracidia in the vicinity of a snail, horizontal plane. Test of observed string distribution against that expected if sign of turn is independent of previous turns.

String Length	Left	Right	Both	Expected	$(O - E)^2/E$
1	45Ø	412	862	1Ø97.5	5Ø.533
2	264	276	54Ø	548.25	Ø.124
3	222	238	46Ø	274.375	125.58
4	1Ø1	9Ø	191	137.188	21.1Ø8
5	31	35	66	68.594	Ø.Ø98
6	11	13	24	34.297	3.Ø91
7	8	12	2Ø	17.148	Ø.474
8	3	7	1Ø	8.574	Ø.237
9	3	2	5		
1Ø	4	3	7		
11	1	4	5		
12	2	1	3		
13	Ø	1	1		
14	Ø	Ø	Ø		
15	Ø	Ø	Ø		
16+	Ø	1	1	8.574	8.28Ø
				CHISQ =	2Ø9.529
				(8 df)	p<Ø.ØØ1

Appendix 4.6 Schistosoma mansoni miraicida in the vicinity of a snail, horizontal plane. Significance tests for differences between means of step speed at different distances from the snail.

Distance from host (mm)	N	Mean	SUM	SUMSQ
0 - 1	645	1.694	1092.662	2023.764
1 - 2	864	1.764	1524.378	2896.592
2 - 3	709	1.781	1262.913	2445.685
3 - 4	802	1.907	1529.683	3072.781
4 - 5	941	1.964	1847.951	3791.469
5 - 6	901	2.048	1845.446	3902.357
6 - 7	593	2.043	1211.735	2546.526
7+	135	2.082	281.061	598.645
	5590		10595.83	21277.82

Total SUMSQ = 1192.562
Treatment SUMSQ = 93.578
Error SUMSQ = 1099.984

Source	df	SUMSQ	MeanSQ	
Treatment	7	93.578	13.368	
Error	5582	1099.984	0.197	
Total	5589	1193.562		
			F =	67.839
				p<0.001

Significance tests for difference between means by Student's t-test

	t	p
0 - 1 ct 1 - 2	3.030	0.01>p>0.001
1 - 2 ct 2 - 3	0.756	NS
2 - 3 ct 3 - 4	5.506	p<0.001
3 - 4 ct 4 - 5	2.672	0.01>p>0.001
4 - 5 ct 5 - 6	4.060	p<0.001
5 - 6 ct 6 - 7	0.213	NS
6 - 7 ct 7+	0.922	NS

Appendix 4.7 Schistosoma mansoni miracidia in the vicinity of a snail, horizontal plane. Significance tests for differences between means of rate of turning at different distances from the snail.

Distance from host (mm)	N	Mean	SUM	SUMSQ
Ø - 1	635	331.61	210572.5	1.441E8
1 - 2	849	289.166	245501.9	1.503E8
2 - 3	698	304.082	212249.5	1.492E8
3 - 4	791	197.635	156329.3	75737914
4 - 5	935	135.23	126439.8	44378425
5 - 6	888	114.515	101689.6	25246140
6 - 7	576	112.478	64787.31	18807003
7 +	106	114.415	12128.00	3218929

Total SUMSQ = 3.8153E8
Treatment SUMSQ = 40703000
Error SUMSQ = 3.4083E8

Source	df	SUMSQ	MEANSQ
Treatment	7	40703000	5814714.2
Error	5470	3.4083E8	62309.14
Total	5477	3.8153E8	

F = 93.32041
p<Ø.ØØ1

Significance tests for difference between means by Student's t-test

	t	p
Ø - 1 ct 1 - 2	3.241	Ø.Ø1>p>Ø.ØØ1
1 - 2 ct 2 - 3	1.17Ø	NS
2 - 3 ct 3 - 4	8.212	p<Ø.ØØ1
3 - 4 ct 4 - 5	5.175	p<Ø.ØØ1
4 - 5 ct 5 - 6	1.771	NS
5 - 6 ct 6 - 7	Ø.153	NS
6 - 7 ct 7+	Ø.Ø73	NS

Appendix 4.8 Schistosoma mansoni miracidia in the vicinity of a snail, horizontal plane. Significance tests for differences between means of direction relative to the snail at different distances from it.

Distance from host (mm)	N	Mean	SUM	SUMSQ
Ø - 1	645	88.922	57354.65	6540688.
1 - 2	864	91.423	78989.40	8822329.
2 - 3	709	97.948	69445.43	8347316.
3 - 4	802	97.824	78454.48	9375204.
4 - 5	941	93.358	87849.74	10166268.
5 - 6	901	93.922	84623.45	9930784.
6 - 7	593	96.534	57244.84	7001251.
7+	135	88.393	11933.04	1411711.
	5590		525895.0	61595549.

Total SUMSQ = 12120594
Treatment SUMSQ = 53609
Error SUMSQ = 12066985

Source	df	SUMSQ	MEANSQ
Treatment	7	53609	7658.4285
Error	5582	12066985	2161.762
Total	5589	12120594	

F = 3.542670
p<Ø.ØØ1

Significance tests for difference between means by Student's t-test

	t	p
Ø - 1 ct 1 - 2	1.03372	NS
1 - 2 ct 2 - 3	2.76951	Ø.Ø1>p>Ø.ØØ1
2 - 3 ct 3 - 4	Ø.Ø517362	NS
3 - 4 ct 4 - 5	1.9987	Ø.Ø5>p>Ø.Ø2
4 - 5 ct 5 - 6	Ø.26Ø25	NS
5 - 6 ct 6 - 7	1.Ø6239	NS
6 - 7 ct 7 +	1.83612	NS

Appendix 4.9.

S.mansoni miracidia crossing a PW:SCW interface - Step speed.

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	189	2.214	418.511	979.848
-7 to -6	590	2.177	1284.696	2966.789
-6 to -5	743	2.168	1625.564	3761.53
-5 to -4	824	2.075	1709.506	3783.015
-4 to -3	986	2.052	2023.181	4429.969
-3 to -2	997	2.024	2017.737	4417.492
-2 to -1	743	1.979	1470.038	3130.411
-1 to 0	664	2.017	1339.262	2892.763
0 to 1	616	2.015	1263.597	2782.03
1 to 2	574	2.081	1194.604	2689.723
2 to 3	522	2.157	1125.891	2606.69
3 to 4	537	2.373	1274.095	3194.438
4 to 5	515	2.406	1239.258	3134.829
5 to 6	152	2.429	369.205	952.624
	8652		18355.15	41752.15

Total sumsq = 2811.906
Treatment sumsq = 131.966
Error sumsq = 2671.94

Source	D.F.	Sumsq	Meansq	
Treatment	13	139.966	10.76662	
Error	8638	2671.94	.3093239	F= 34.80693
Total	8651	2811.906		

p<0.01

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	.7959471	NS
-7 to -6 ct -6 to -5	.3586671	NS
-6 to -5 ct -5 to -4	4.016021	p<0.001
-5 to -4 ct -4 to -3	.8761637	NS
-4 to -3 ct -3 to -2	1.120928	NS
-3 to -2 ct -2 to -1	1.669455	NS
-2 to -1 ct -1 to 0	1.279410	NS
-1 to 0 ct 0 to 1	1.092805	NS
0 to 1 ct 1 to 2	.9298029	NS
1 to 2 ct 2 to 3	2.239437	0.05>p>0.02
2 to 3 ct 3 to 4	6.318635	p<0.001
3 to 4 ct 4 to 5	.9620402	NS
4 to 5 ct 5 to 6	.4480068	NS
-7 to -6 ct 4 to 5	6.827770	p<0.001

S.mansoni miracidia crossing a PW:SCW interface - Rate of turning.

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	168	226.602	38069.1	17783580
-7 to -6	584	221.036	129085.1	67446145
-6 to -5	743	224.467	166778.6	1.0146E8
-5 to -4	824	248.739	205002.4	1.1253E8
-4 to -3	985	266.743	262742.1	1.6350E8
-3 to -2	997	251.319	256565.1	1.7092E8
-2 to -1	743	277.173	205939.5	1.3633E8
-1 to 0	664	324.351	215368.9	1.5828E8
0 to 1	616	331.409	204148.3	1.3867E8
1 to 2	573	305.754	175196.8	1.1598E8
2 to 3	522	246.607	128728.7	78566342
3 to 4	536	209.394	112235.4	53681216
4 to 5	504	205.591	103618.1	45241070
5 to 6	143	198.433	28375.9	11506718
	8602		2251854.	1.9292E8

Total sumsq = 1.3689E9
Treatment sumsq = 18771000
Error sumsq = 1.3501E9

Source	D.F.	Sumsq	Meansq	
Treatment	13	18771000	1443923	
Error	8588	1.3501E9	157206.6	F= 9.18468
Total	8601	1.3689E9		

p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	.1604587	NS
-7 to -6 ct -6 to -5	.1565864	NS
-6 to -5 ct -5 to -4	1.213365	NS
-5 to -4 ct -4 to -3	.959824	NS
-4 to -3 ct -3 to -2	.8665219	NS
-3 to -2 ct -2 to -1	1.346367	NS
-2 to -1 ct -1 to 0	2.229064	0.05>p>0.02
-1 to 0 ct 0 to 1	.3184336	NS
0 to 1 ct 1 to 2	1.115622	NS
1 to 2 ct 2 to 3	2.467213	0.02>p>0.01
2 to 3 ct 3 to 4	1.527346	NS
3 to 4 ct 4 to 5	.1546947	NS
4 to 5 ct 5 to 6	.1906737	NS
-7 to -6 ct 4 to 5	.6412062	NS

Appendix 4.10. Schistosoma mansoni miracidia interface experiments - Miracidial Category A.

S.mansoni miracidia crossing a PW:SCW interface - Step speed - Category A

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	82	2.333	191.321	462.009
-7 to -6	316	2.26	714.285	1677.622
-6 to -5	430	2.233	960.137	2299.687
-5 to -4	398	2.092	832.551	1846.488
-4 to -3	400	2.032	812.975	1759.496
-3 to -2	386	1.973	761.506	1638.065
-2 to -1	195	1.923	375.079	777.214
-1 to 0	60	1.766	105.978	202.069
0 to 1				
1 to 2				
2 to 3				
3 to 4				
4 to 5				
5 to 6				
	2267		4753.83	10662.65

Total sumsq = 694.0135
Treatment sumsq = 41.0125
Error sumsq = 653.001

Source	D.F.	Sumsq	Meansq	
Treatment	7	41.0125	5.858929	
Error	2259	653.001	.2890664	F= 20.26845
Total	2266	694.0135		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	1.095553	NS
-7 to -6 ct -6 to -5	.6777584	NS
-6 to -5 ct -5 to -4	3.770356	p<0.001
-5 to -4 ct -4 to -3	1.576243	NS
-4 to -3 ct -3 to -2	1.538036	NS
-3 to -2 ct -2 to -1	1.058510	NS
-2 to -1 ct -1 to 0	1.977990	0.05>p>0.02
-1 to 0 ct 0 to 1		
0 to 1 ct 1 to 2		
1 to 2 ct 2 to 3		
2 to 3 ct 3 to 4		
3 to 4 ct 4 to 5		
4 to 5 ct 5 to 6		

S.mansoni miracidia crossing a PW:SCW interface - Rate of turning - Category A

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	72	214.396	15436.52	6125517.
-7 to -6	308	194.807	60000.43	28448878
-6 to -5	412	226.15	93173.91	49525379
-5 to -4	393	243.162	95562.61	52421323
-4 to -3	390	245.282	95659.85	53957725
-3 to -2	347	276.911	96087.97	63401934
-2 to -1	178	302.034	53762.03	32039012
-1 to 0	42	468.475	19675.94	18322896
0 to 1				
1 to 2				
2 to 3				
3 to 4				
4 to 5				
5 to 6				
	2142		529359.3	3.0424E8

Total sumsq = 1.7342E8
Treatment sumsq = 4011000
Error sumsq = 1.6941E9

Source	D.F.	Sumsq	Meansq	
Treatment	7	4011000	573000	
Error	2134	1.6941E8	79385.66	F= 7.217928
Total	2141	1.7342E8		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	.531118	NS
-7 to -6 ct -6 to -5	1.476819	NS
-6 to -5 ct -5 to -4	.8563105	NS
-5 to -4 ct -4 to -3	.1052719	NS
-4 to -3 ct -3 to -2	1.521170	NS
-3 to -2 ct -2 to -1	.9671538	NS
-2 to -1 ct -1 to 0	3.443599	p<0.001
-1 to 0 ct 0 to 1		
0 to 1 ct 1 to 2		
1 to 2 ct 2 to 3		
2 to 3 ct 3 to 4		
3 to 4 ct 4 to 5		
4 to 5 ct 5 to 6		

Appendix 4.11. Schistosoma mansoni miracidia interface experiments - Miracidial Category B

S.mansoni miracidia crossing a PW:SCW interface - Step speed - Category B

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	16	2.052	32.834	68.768
-7 to -6	37	2.006	74.239	156.619
-6 to -5	38	1.808	68.721	134.446
-5 to -4	30	2.048	61.452	133.521
-4 to -3	31	2.108	65.352	143.953
-3 to -2	94	1.766	166.005	316.38
-2 to -1	69	1.758	121.328	228.782
-1 to 0	84	2.032	170.651	366.106
0 to 1	112	1.932	216.39	450.203
1 to 2	118	1.998	235.765	515.125
2 to 3	88	1.946	171.274	377.319
3 to 4	63	2.191	138.058	320.251
4 to 5	87	2.145	186.587	433.899
5 to 6	26	2.48	64.503	167.749
	893		1773.159	3813.121

Total sumsq = 270.4417
Treatment sumsq = 21.8587
Error sumsq = 248.583

Source	D.F.	Sumsq	Meansq	
Treatment	13	21.8587	1.681438	
Error	879	248.583	.282802	F= 5.945638
Total	892	270.4417		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	.2890945	NS
-7 to -6 ct -6 to -5	1.612081	NS
-6 to -5 ct -5 to -4	1.847861	NS
-5 to -4 ct -4 to -3	.4405422	NS
-4 to -3 ct -3 to -2	3.105105	0.01>p>0.001
-3 to -2 ct -2 to -1	.0948951	NS
-2 to -1 ct -1 to 0	3.171234	0.01>p>0.001
-1 to 0 ct 0 to 1	1.302806	NS
0 to 1 ct 1 to 2	.940782	NS
1 to 2 ct 2 to 3	.6942423	NS
2 to 3 ct 3 to 4	2.791571	0.01>p>0.001
3 to 4 ct 4 to 5	.5228793	NS
4 to 5 ct 5 to 6	2.818463	0.01>p>0.001

S.mansoni miracidia crossing a PW:SCW interface - Rate of turning - Category B

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	15	163.168	2447.826	507534.6
-7 to -6	36	185.793	6688.551	2713431.
-6 to -5	38	312.799	11886.38	7706612.
-5 to -4	30	180.932	5427.971	1322770.
-4 to -3	31	198.453	6152.029	1613828.
-3 to -2	94	248.108	23322.17	13111361
-2 to -1	69	291.664	20124.78	11426994
-1 to 0	84	229.389	19268.70	8442735.
0 to 1	112	297.535	33323.91	17915744
1 to 2	118	286.754	33836.96	21221608
2 to 3	88	276.825	24360.58	15626668
3 to 4	63	296.959	18708.41	11027850
4 to 5	81	285.926	23160	14403333
5 to 6	25	114.122	2853.043	539538.8
	884		231561.3	1.2758E8

Total sumsq = 66923213
Treatment sumsq = 1847103
Error sumsq = 65076110

Source	D.F.	Sumsq	Meansq	
Treatment	13	1847103	142084.8	
Error	870	65076110	74800.13	F= 1.899527
Total	883	66923213		p<0.05

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	.268946	NS
-7 to -6 ct -6 to -5	1.996643	0.05>p>0.02
-6 to -5 ct -5 to -4	1.974163	0.05>p>0.02
-5 to -4 ct -4 to -3	.2501406	NS
-4 to -3 ct -3 to -2	.8766002	NS
-3 to -2 ct -2 to -1	1.064596	NS
-2 to -1 ct -1 to 0	1.401461	NS
-1 to 0 ct 0 to 1	1.726274	NS
0 to 1 ct 1 to 2	.2988092	NS
1 to 2 ct 2 to 3	.2577526	NS
2 to 3 ct 3 to 4	.4460689	NS
3 to 4 ct 4 to 5	.2401452	NS
4 to 5 ct 5 to 6	2.748263	0.01>p>0.001

Appendix 4.12. Schistosoma mansoni miracidia interface experiments - Miracidial Category C

S.mansoni miracidia crossing a PW:SCW interface - Step speed - Category C

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	71	2.116	150.271	345.718
-7 to -6	184	2.098	385.973	886.246
-6 to -5	224	2.165	484.998	1110.036
-5 to -4	325	2.044	664.237	1461.322
-4 to -3	469	2.051	961.771	2122.946
-3 to -2	462	2.105	972.548	2175.262
-2 to -1	430	2.025	870.9	1896.675
-1 to 0	404	2.069	835.971	1847.706
0 to 1	287	2.015	575.657	1319.288
1 to 2	100	1.995	199.506	434.544
2 to 3	22	1.46	32.13	54.498
3 to 4				
4 to 5				
5 to 6				
	2978		6133.962	13654.24

Total sumsq = 1019.759
Treatment sumsq = 13.754
Error sumsq = 1006.005

Source	D.F.	Sumsq	Meansq	
Treatment	10	13.754	1.3754	
Error	2967	1006.005	.3390647	F=4.0564
Total	2977	1019.759		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	.2212582	NS
-7 to -6 ct -6 to -5	1.155485	NS
-6 to -5 ct -5 to -4	2.392903	0.02>p>0.01
-5 to -4 ct -4 to -3	.1665619	NS
-4 to -3 ct -3 to -2	1.414772	NS
-3 to -2 ct -2 to -1	2.050325	0.05>p>0.02
-2 to -1 ct -1 to 0	1.090572	NS
-1 to 0 ct 0 to 1	1.201287	NS
0 to 1 ct 1 to 2	.295784	NS
1 to 2 ct 2 to 3	3.901621	p<0.001
2 to 3 ct 3 to 4		
3 to 4 ct 4 to 5		
4 to 5 ct 5 to 6		

S.mansoni miracidia crossing a PW:SCW interface - Rate of turning - Category C

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	65	282.186	1832.089	10500001
-7 to -6	182	268.826	48926.38	30654188
-6 to -5	224	216.803	48563.77	29566794
-5 to -4	325	262.077	85174.93	51042944
-4 to -3	468	269.002	126172.9	84502883
-3 to -2	462	223.609	103307.2	51414421
-2 to -1	430	268.255	115349.6	79554875
-1 to 0	404	320.987	129678.8	1.0145E8
0 to 1	286	337.518	96530.14	68756265
1 to 2	100	389.016	38901.59	33307275
2 to 3	22	535.31	11776.81	12238497
3 to 4				
4 to 5				
5 to 6				
	2968		822724.3	5.5299E8

Total sumsq = 3.2493E8
Treatment sumsq = 6825000
Error sumsq = 3.1811E8

Source	D.F.	Sumsq	Meansq	
Treatment	10	6825000	682500	
Error	2957	3.1811E8	107578.3	F= 6.344217
Total	2967	3.2493E8		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	.2818954	NS
-7 to -6 ct -6 to -5	1.589387	NS
-6 to -5 ct -5 to -4	1.589520	NS
-5 to -4 ct -4 to -3	.2924054	NS
-4 to -3 ct -3 to -2	2.065377	0.05>p>0.02
-3 to -2 ct -2 to -1	2.031389	0.05>p>0.02
-2 to -1 ct -1 to 0	2.320352	0.05>p>0.02
-1 to 0 ct 0 to 1	.6522078	NS
0 to 1 ct 1 to 2	1.351504	NS
1 to 2 ct 2 to 3	1.894068	NS
2 to 3 ct 3 to 4		
3 to 4 ct 4 to 5		
4 to 5 ct 5 to 6		

Appendix 4.13. Schistosoma mansoni miracidia interface experiments - Miracidial Category D

S.mansoni miracidia crossing a PW:SCW interface - Step speed - Category D

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7				
-7 to -6				
-6 to -5				
-5 to -4				
-4 to -3				
-3 to -2				
-2 to -1				
-1 to 0				
0 to 1	76	1.825	138.706	273.542
1 to 2	239	2.074	495.68	1116.668
2 to 3	303	2.23	676.33	1486.009
3 to 4	301	2.394	864.156	2198.055
4 to 5	357	2.478	884.519	2285.276
5 to 6	107	2.414	258.328	667.818
	1443		3317.719	8027.368

Total sumsq = 399.3299
Treatment sumsq = 46.5858
Error sumsq = 352.7441

Source	D.F.	Sumsq	Meansq	
Treatment	5	46.5858	9.31716	
Error	1437	352.7441	.2454725	F=37.956023
Total	1442	399.3299		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6		
-7 to -6 ct -6 to -5		
-6 to -5 ct -5 to -4		
-5 to -4 ct -4 to -3		
-4 to -3 ct -3 to -2		
-3 to -2 ct -2 to -1		
-2 to -1 ct -1 to 0		
-1 to 0 ct 0 to 1		
0 to 1 ct 1 to 2	3.816355	p<0.001
1 to 2 ct 2 to 3	3.639520	p<0.001
2 to 3 ct 3 to 4	4.248496	p<0.001
3 to 4 ct 4 to 5	2.271461	p<0.001
4 to 5 ct 5 to 6	1.172050	NS

S.mansoni miracidia crossing a PW:SCW interface - Rate of turning - Category D

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7				
-7 to -6				
-6 to -5				
-5 to -4				
-4 to -3				
-3 to -2				
-2 to -1				
-1 to 0				
0 to 1	76	473.103	35955.82	31431598
1 to 2	237	344.741	81703.58	51412604
2 to 3	303	228.809	69329.10	28500539
3 to 4	360	219.092	78873.13	38252115
4 to 5	350	206.945	72430.75	29156275
5 to 6	101	242.348	24477.16	10732742
	1427		362769.6	1.8949E8

Total sumsq = 97262856
Treatment sumsq = 7019920
Error sumsq = 90242936

Source	D.F.	Sumsq	Meansq	
Treatment	5	7019920	1403984	
Error	1421	90242936	63506.64	F= 22.10767
Total	1426	97262856		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6		
-7 to -6 ct -6 to -5		
-6 to -5 ct -5 to -4		
-5 to -4 ct -4 to -3		
-4 to -3 ct -3 to -2		
-3 to -2 ct -2 to -1		
-2 to -1 ct -1 to 0		
-1 to 0 ct 0 to 1		
0 to 1 ct 1 to 2	3.863988	P<0.001
1 to 2 ct 2 to 3	5.305085	P<0.001
2 to 3 ct 3 to 4	.4945821	NS
3 to 4 ct 4 to 5	.642119	NS
4 to 5 ct 5 to 6	1.243761	NS

Appendix 4.14. Schistosoma mansoni miracidia interface experiments - Miracidial Category E

S.mansoni miracidia crossing a PW:SCW interface - Step speed - Category E

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	13	2.048	26.622	58.113
-7 to -6	41	2.003	82.11	177.663
-6 to -5	36	2.134	76.808	170.694
-5 to -4	37	2.202	81.461	185.43
-4 to -3	41	2.055	84.237	177.211
-3 to -2	43	2.083	89.586	196.062
-2 to -1	49	2.097	102.771	223.338
-1 to 0	64	1.953	125.008	259.686
0 to 1	58	2.082	120.781	267.971
1 to 2	62	2.108	130.71	292.301
2 to 3	48	2.099	100.77	233.467
3 to 4	45	2.344	105.488	256.956
4 to 5	44	2.204	96.968	228.03
5 to 6				
	581		1223.32	2726.922

Total sumsq = 151.1702
Treatment sumsq = 5.4831
Error sumsq = 145.6871

Source	D.F.	Sumsq	Meansq	
Treatment	12	5.4831	.456925	
Error	568	145.6871	.2564913	F= 1.781444
Total	580			
				p NS

S.mansoni miracidia crossing a PW:SCW interface - Rate of turning - Category E

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7	14	159.595	2234.328	618125.7
-7 to -6	38	301.296	11449.25	5188650.
-6 to -5	36	236.422	8511.194	2723225.
-5 to -4	37	221.065	8179.403	2506376.
-4 to -3	41	253.036	10374.48	3862357
-3 to -2	43	290.024	12471.04	5345346.
-2 to -1	49	378.684	18555.52	12061551
-1 to 0	64	299.314	19156.12	10606620
0 to 1	58	346.853	20117.46	12381363
1 to 2	62	243.274	15082.99	7631327.
2 to 3	48	388.302	18638.51	16847134
3 to 4	45	161.254	7256.418	2010220
4 to 5	41	240.335	9853.731	4236621.
5 to 6				
	576		161880.4	86018914

Total sumsq = 40523776
Treatment sumsq = 2556566
Error sumsq = 37967210

Source	D.F.	Sumsq	Meansq	
Treatment	12	2556566	213047.2	
Error	563	37967210	67437.32	F=3.1591879
Total	575	40523776		
				p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6	1.745327	NS
-7 to -6 ct -6 to -5	1.074107	NS
-6 to -5 ct -5 to -4	.2526079	NS
-5 to -4 ct -4 to -3	.5429396	NS
-4 to -3 ct -3 to -2	.6525251	NS
-3 to -2 ct -2 to -1	1.633864	NS
-2 to -1 ct -1 to 0	1.610108	NS
-1 to 0 ct 0 to 1	1.009774	NS
0 to 1 ct 1 to 2	2.183438	0.05>p>0.02
1 to 2 ct 2 to 3	2.904838	0.01>p>0.001
2 to 3 ct 3 to 4	4.213597	p<0.001
3 to 4 ct 4 to 5	1.410493	NS
4 to 5 ct 5 to 6		

Appendix 4.15. Schistosoma mansoni miracidia interface experiments - Miracidial Category F

S.mansoni miracidia crossing a PW:SCW interface - Step speed - Category F

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7				
-7 to -6				
-6 to -5				
-5 to -4				
-4 to -3				
-3 to -2				
-2 to -1				
-1 to 0	53	1.945	103.094	221.21
0 to 1	84	2.304	193.544	473.231
1 to 2	55	2.417	132.948	331.084
2 to 3	61	2.383	145.366	356.517
3 to 4	68	2.447	166.393	418.897
4 to 5	53	2.486	131.589	335.589
5 to 6	20	2.43	48.061	122.061
	394		921.708	2258.589

Total sumsq = 102.3819
Treatment sumsq = 10.8818
Error sumsq = 91.5001

Source	D.F.	Sumsq	Meansq	
Treatment	6	10.8818	1.813633	
Error	387	91.5001	.2364343	F= 7.670771
Total	393	102.3819		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6		
-7 to -6 ct -6 to -5		
-6 to -5 ct -5 to -4		
-5 to -4 ct -4 to -3		
-4 to -3 ct -3 to -2		
-3 to -2 ct -2 to -1		
-2 to -1 ct -1 to 0		
-1 to 0 ct 0 to 1	4.208789	p<0.001
0 to 1 ct 1 to 2	1.339791	NS
1 to 2 ct 2 to 3	.3760462	NS
2 to 3 ct 3 to 4	.7463626	NS
3 to 4 ct 4 to 5	.4377335	NS
4 to 5 ct 5 to 6	.438859	NS

S.mansoni miracidia crossing a PW:SCW interface - Rate of turning - Category F

Analysis of variance and test for significance between means.

Class	No.	Mean	Sigma x	Sigma x2
-8 to -7				
-7 to -6				
-6 to -5				
-5 to -4				
-4 to -3				
-3 to -2				
-2 to -1				
-1 to 0	53	438.564	23243.91	18676925
0 to 1	84	236.28	19847.54	81714165
1 to 2	55	157.802	8679.13	2449753.
2 to 3	61	149.66	9129.275	2356075.
3 to 4	68	145.501	9894.058	2391486.
4 to 5	52	159.969	8318.406	1741392.
5 to 6	17	103.299	1756.087	234939.7
	390		80868.41	36021986

Total sumsq = 19253551
Treatment sumsq = 3802242
Error sumsq = 15451309

Source	D.F.	Sumsq	Meansq	
Treatment	6	3802242	633707	
Error	383	15451309	40342.84	F= 15.70804
Total	389	19253551		p<0.001

Student's t-test for significance between means.

Comparisons	t	p
-8 to -7 ct -7 to -6		
-7 to -6 ct -6 to -5		
-6 to -5 ct -5 to -4		
-5 to -4 ct -4 to -3		
-4 to -3 ct -3 to -2		
-3 to -2 ct -2 to -1		
-2 to -1 ct -1 to 0		
-1 to 0 ct 0 to 1	5.741106	p<0.001
0 to 1 ct 1 to 2	2.252570	0.02>p>0.01
1 to 2 ct 2 to 3	.2180043	NS
2 to 3 ct 3 to 4	.1174167	NS
3 to 4 ct 4 to 5	.3910126	NS
4 to 5 ct 5 to 6	1.009884	NS

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