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ABSTRACT

The morphology of the rays of the primary fans, the main food collecting organ, of 25 species of black-fly larvae (Diptera: Simuliidae) were studied using the scanning electron microscope. The form, arrangement, spacing and dimensions of the microtrichia on the rays of these species was recorded. The flicking action of the primary fan was found to be frequent and irregular. The actions of the primary fan and mandible were also studied and their mode of action outlined.

Experiments on black-fly larvae of four species (Simulium ornatum Mg., S. variegatum Mg., S. monticola Fried. and S. reptans (L.)) were conducted in an artificial environment in which current velocity, food concentration and light level could be controlled. Each species was found to differ from the others in its rate of food intake over a range of current velocities. Species with similar patterns of microtrichia on their head fans had comparable rates of food intake at the same current velocities.

Experiments were done, using a dye-tracer technique, on S. ornatum, S. variegatum, S. reptans, S. venustum and S. pictipes-longistylatum in the natural stream habitat of these larvae to determine the rate of intake of natural food. Intake rates varied widely from species to species and within a species depending on current velocity and probably the amount of suspended matter in the stream.

Larvae of P. ferrugineum (Wahlb.) from Eastern Norway were found to predate on other aquatic arthropods including other species of black-fly larvae.

Experiments were conducted on the space requirements of S. ornatum larvae in an artificial environment. Densities of up to 141 larvae per sq. cm. were obtained. These densities were much higher than those found in nature. Densities in nature varied widely. Analysis was made of the movements of larvae on an attachment site of limited area.

STUDIES ON THE BIOLOGY OF BLACK-FLY LARVAE (DIPTERA:SIMULIIDAE) WITH REFERENCE TO THE STRUCTURE AND FUNCTION OF THE FEEDING ORGANS

by

ALLAN FREDERICK JOHNSON
B.S.A.(Toronto), M.Sc.(Guelph)
(Graduate Society)

... being a thesis presented in candidature for the degree of Doctor of Philosophy in the University of Durham, 1970.

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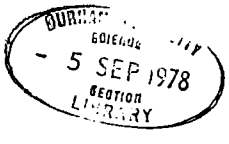

ACKNOWLEDGMENTS

The author wishes to express his deep appreciation to Dr. L. Davies for his guidance and assistance. Dr. Davies has also provided most of the specimens used in the morphological studies and has been generous in his loans of scientific papers. Thanks are due to Professor D. Barker for the provision of facilities in the Department of Zoology, Durham and for financial support for the use of a scanning electron microscope.

The author also wishes to thank the following people:

Mr. E. Boult and Mr. P. Reavey of the University of Newcastle-upon-Tyne for their technical assistance in scanning electron microscopy.

Dr. D. M. Davies of McMaster University for the loan of specimens and laboratory facilities.

Professor A. M. Fallis of the University of Toronto for financial support during the summer of 1967.

The Rev. Dr. C. M. K. Hewitt for help in translation.

"There is no department of human enquiry with which I am even slightly acquainted which presents so many pitfalls as the interpretation of natural contrivances." "Still we do not taste the full delight of Natural History unless we attempt to walk where no one has walked before."

L. C. Miall (1895)

"It is not only the great works, - those vast planets, - not those gargantuan animals on the land as well as in the sea which declares the majesty of the Almighty. No! It is the most minute which reflect most vividly the perfection of their Creator. May I go even further and say the latter even more than the former show this perfection. A large church-clock is, to be sure, a most marvellous invention; but a pocket-watch set in a fine case is certainly even more marvellous and redounds to the greater honour and fame of its maker."

J. C. Eichhorn (1781)

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INTRODUCTION

The members of the family Simuliidae are a widely distributed group of flies chiefly known for the bites inflicted by the females on man and animals. Fallis (1964) has listed the world-wide biting records of the Simuliidae (commonly known as black-flies or buffalo gnats). Wilhelmi (1920) summarized the early research on black-flies and their depredations.

In the British Isles, the larvae of the Simuliidae are probably more often encountered than the adults. Black-fly larvae generally resemble the species shown in Fig. 1 and are common inhabitants of flowing water which is not highly polluted.

Black-fly larvae have a characteristic dumb-bell-like shape and possess a single ventral proleg on the prothorax. This proleg together with an anal disc located on the posterior end of the abdomen are provided with numerous rows of minute hooks which aid the attachment of a larva to stones, sticks or stream vegetation in moving water. Larvae are capable of spinning silk, either in the form of a thread (used as a safety line), or as a small blob which acts as a substrate into which the hooks of the anal disc can grip. The pupal cocoon is also spun from silk. General accounts of the habits of the larvae are given by Miall (1895), Hynes (1966) and Macan and Worthington (1962).

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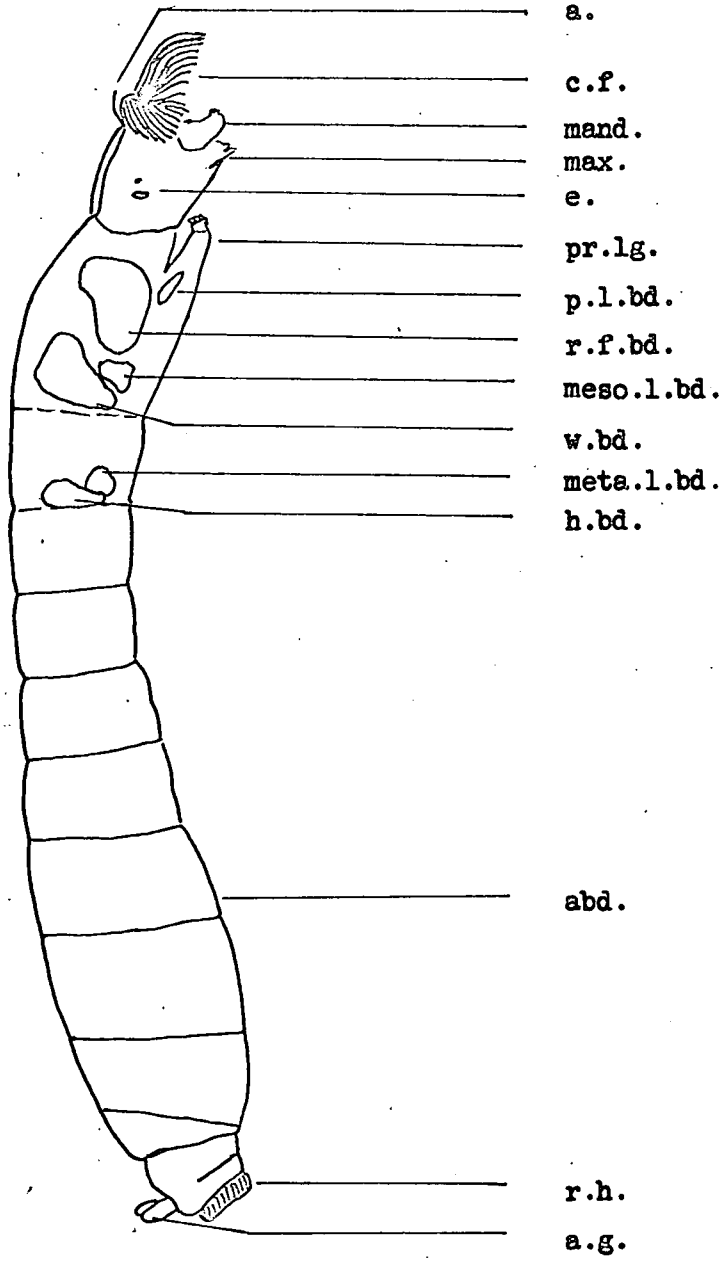
Fig. 1. A mature larva of Simulium ornatum Mg. X18 (after Smart) (a., antenna; abd., abdomen; a.g., anal gills; c.f., cephalic fan; e., eyespots; mand., mandible; max., maxilla; pr.lg., pro-leg; p.l.bd., prothoracic leg bud; meso.l.bd., mesothoracic leg bud; meta.l.bd., metathoracic leg bud; r.f.bd., respiratory filament bud; w.bd., wing bud; h.bd., haltere bud; r.h., ring of hooks on "posterior sucker")


a.
c.f.
mand.
max.
e.
pr.lg.
p.l.bd.
r.f.bd.
meso.l.bd.
w.bd.
meta.l.bd.
h.bd.
abd.
r.h.
a.g.

The scope of this study of the ecology of black-fly larvae has been necessarily restricted by the size of the subject. The habitat of larvae is affected indirectly by environmental factors which do not operate on the larvae themselves but on another stage (eggs, pupae or adults). Such factors are: 1) the presence of suitable oviposition sites, 2) conditions suitable for mating and 3) the presence of an appropriate host (for anautogenous species). Such factors, while they may considerably affect the distribution and abundance of black-fly larvae, are more properly part of a study of the biology of the adult flies.

The portions of the larval ecology which have been considered in this study are: 1) the relationship of black-fly larvae to the water currents in which they live, 2) the amounts of food obtained by feeding larvae, 3) the method by which larvae obtain food, and 4) the densities to which black-fly larvae can be crowded.

Other facets of the ecology of black-fly larvae such as water temperature, phenology, stream bottom type, downstream migration and the number of larval instars have been investigated by other workers (Davies and Smith 1958), (Davies and Syme 1958), (Ussova 1964), (Johnson 1966), (Ulfstrand 1967), (Terterjan 1957), (Harrod 1965), (Johnson and Pengelly 1970) and (Rubtzov 1964).

The previous research conducted in the four above-mentioned areas which are the subject of this thesis will now be considered separately.

1. LITERATURE REVIEW

a) Current and black-fly larvae

Early workers (Eichhorn, 1781; Verdat, 1822) merely stated that black-fly larvae were inhabitants of running water. Riley (1886) noted differences in the speed of current preferred by two species. The idea that different species had different current preferences was expanded by Wu (1931). Wu conducted a number of "transplantation" experiments in which cat-tail leaves bearing larvae and pupae were moved carefully to areas of the same stream having slower current velocity. Both plankton-rich and plankton-poor streams were used. In the plankton-rich stream, larvae transplanted from a velocity of 46 cm./sec. to 18 cm./sec. found the lower velocity suitable enough to remain for at least 20 days. Transplantation to a velocity of 12 cm./sec. resulted in larvae disappearing within 4 days. In the plankton-poor stream, larvae transplanted from 84 cm./sec. to 38 cm./sec. remained on the leaves but when transplanted from 84 cm./sec. to 30 cm./sec. they disappeared within 4 days. Wu attributed the difference in minimum current rates to which larvae could be transplanted to a difference in the amount of food material available in the two streams. After admitting that food and current were linked in the ecology of the larvae, Wu concluded that they responded to current pressure on their bodies and that black-fly larvae had an inherent requirement for current due to the fact that in another experiment larvae endured starvation in filtered flowing lake water for 7 to 9 days.

Fortner (1937) using an unidentified species stated that the optimum current required by larvae was 0.6 - 0.7 m./sec.

Grenier (1949) noted that, "In mountain areas most species inhabit rapid or tumultuous streams, whose current speeds reach 1 - 2 m./sec. even in summer. On the other hand, lowland species are found in moderate or slow-running waters (0.20 - 0.60 m./sec.). It must be pointed out that some lowland species when found in hilly regions are confined to small brooks ecologically very similar, especially in respect of current, to lowland streams." The above statement suggests strongly that current velocity rather than the location of the stream is important to larvae.

Zahar (1951) measured stream current with a water-wheel type of velocity gauge and listed the current velocities at which he found each species abundant. He felt that current velocity affected larvae through the quantity of food made available to them. He said, "... since at any given point in a fast current a larger quantity of plankton will pass in a given time than at a comparable point in a slow current in the same period of time." Fredeen (1964) also made a similar statement to Zahar's.

Both these authors did not explain why some species apparently prefer a slower current. This would, on the basis of the above statement, mean that such species would have access to a smaller amount of food in a given time than if they were in a faster current. Species found in slower water would thus seem (by these authors' statements) to have put themselves at a disadvantage by choosing a habitat low in food particles.

Phillipson (1956) (1957), in laboratory experiments on preferred current velocities, found S. ornatum larvae to aggregate at 80 to 90 cm./sec. and S. variegatum larvae to aggregate at 1.0 to 2.0 m./sec. S. ornatum larvae occurred from 50 to 120 cm./sec. while S. variegatum occurred from 50 to 250 cm./sec. He suggested that current velocity was important to the distribution of different species in a single stream and might cause isolation of species in different parts of a watercourse. Harrod (1965) found S. nitidifrons larvae to aggregate at velocities of 50 to 60 cm./sec.

Johnson (1966) in studies on the North Madawaska River in Ontario found that the densities of S. rugglesi were highest at current velocities of 0.61 - 1.1 m./sec. while the densities of S. venustum-verecundum larvae were highest at velocities from 1.1 - 1.34 m./sec.

Davies, Peterson and Wood (1962) found S. pictipes and S. longistylatum larvae to be restricted in habitat to the fastest parts of waterfalls, where they occurred in dense masses.

Such observations as those of Phillipson (1956) (1957), Johnson (1966) and Davies, Peterson and Wood (1962) suggest that aggregation of a particular species of larvae to a certain current speed range denotes an adaptation of that species to those particular conditions. Zahar (1951) and Fredeen (1960) (1964) have suggested that food availability may be the reason for this aggregation in a particular current range.

b) Food and black-fly larvae

The food of filter-feeding simuliid larvae has been found to consist mainly of algae, diatoms, microscopic particles of organic matter and tiny crustaceans (Planchon, 1848; Puri, 1925; Anderson and Dicke, 1960). Also present are quantities of microscopic inorganic particles.

Puri (1925) found head capsules of chironomids in the mid-guts of black-fly larvae and Serra-Tosio (1967) observed predation on chironomid larvae by P. inflatum.

Anderson and Dicke (1960) analysed the mid-gut contents of a number of Wisconsin species and concluded that larvae were only selective in feeding in that they were capable of rejecting large soil particles or detritus too large for larvae to ingest. The predominant organisms in a water sample from a particular stream were also the predominant organisms in the intestinal contents of larvae from that stream. All larvae studied were considered to be unselective when feeding on suspended solids.

Williams et al. (1961) measured the particle size in the intestines of larvae from various habitats and found the longest axis of particles to range from 15.4μ ± 2.1μ to 11.3μ ± 1.8μ and the shortest axis from 8.0μ ± 1.8μ to 6.6μ ± 1.6μ. These particles were not identified.

Fredeen (1960)(1964) reared larvae on suspensions of bacteria, pointing out that this was probably their natural food in the drainage ditches of western Canada. Wood and Davies (1966) reared larvae on a suspension of brewer's yeast.

Chance (1970) examined the intestinal contents of larvae from natural habitats and found that, "most particles were from 20μ to 100μ long and 10μ to 60μ wide" but that particles smaller than 0.5μ were abundant. In her artificial feeding experiments, Chance (1970) found that S. decorum and S. vittatum larvae tended to select "Sephadex" beads of the smallest size offered (25μ diameter). The size of particles offered in these experiments ranged from 25μ to 405μ. Chance did not state whether or not the artificial beads of various diameters were circulated with equal frequency in her apparatus. If the smaller beads, having a larger surface to weight ratio were circulated more rapidly than beads of larger diameter, the smaller beads would have been more frequently exposed to capture by larvae.

Both Fredeen's (1960)(1964) and Chance's (1970) work suggest that black-fly larvae can ingest very small particles such as bacteria. Williams et al. (1961) are not clear in the graph in their paper which suggests that there were numerous particles smaller than the size ranges given in the table in the same paper.

Maciolek and Tunzi (1968) found that loss of cellular microseston in a small fast-flowing mountain stream was due mostly to removal by simuliid larvae. These larvae were capable of removing 60% of the suspended algae within a 0.4 km. section of the stream studied.

Summarizing the above mentioned results, it appears that black-fly larvae are capable of ingesting particles with a wide size range (even up to the size of small insect larvae) but that the sizes of particles generally found in the intestines of individual larvae

reflect the sizes available in the water. Results such as those of Williams et al. (1961) are likely due to the presence of large numbers of particles in a specific size range. The ability to rear larvae on bacterial cultures (Fredeen 1960, 1964) and the apparent preference of larvae for particles of 25μ diameter over those of larger diameter (Chance 1970) suggests that black-fly larvae may be adapted to feeding on smaller particles than other aquatic insects (Jørgensen 1966).

c) The feeding apparatus and its actions

A characteristic feature of many species of black-fly larvae (not those in the genera Twinnia Stone and Gymnopais Stone or the first instar larvae of the genus Prosimulium Roubaud) is the presence, on the anterio-lateral margins of the head, of a pair of complex cephalic fans (also called head fans or mouth brushes). These cephalic fans are usually regarded as premandibular organs (Grenier 1949). Each fan consists of a basal stalk upon which is arranged, as in a lady's fan, a number (20-50 in mature larvae) of sclerotized curved rays. The internal margins of these curved rays bear numbers of very fine microtrichia which are arranged in a single row from a point near the base of each ray to its tip. This structure is known as the principal fan (Grenier 1949).

Grenier (1949) illustrates two secondary fans which together with the principal fan make up what is generally known as the cephalic fan. These two secondary fans are called the accessory fan and the marginal fan. The accessory consists of a number of simple rays, each

with a single row of fine hairs, lying in a semi-circular shape near the base of the principal fan and attached to the basal stalk. The marginal fan consists of a few rays projecting medially from the basal stalk. Sommerman (1953) and Wood et al. (1963) called the principal fan the primary fan and the accessory fan the secondary fan and noted that the secondary fan in the genus Prosimulium is of a much simpler pattern than that found in members of the genera Cnephia and Simulium.

The method by which this cephalic fan complex gathers food particles and the action of each part of the fan complex in feeding has been given various interpretations. Riley (1886) stated that the fans created currents of water towards the mouth and that the curved rays directed particles into the mouth. Strickland (1911) considered the fan as a strainer and that the flicking motion of each fan caused food to be brushed into the mouth. Wilhelmi (1920) stated that there was much conjecture about the way food was collected but considered the cephalic fans as "Strudelapparat" or whirlpool organs. Nauman (1924) and Puri (1925) rejected this idea and considered that feeding was by passive straining, although Puri (1925) suggested that when a fan was folded it swept particles into the mouth.

Fortner (1937), in an experimental study of larvae with complete and amputated cephalic fans in standing water, found the larvae with normal fans to use them as whirlpool-creating organs. The larvae with amputated fans were noted to lose food particles from the amputated stumps as the fans closed. The flicking of the cephalic fans was 15-17 per minute in standing water and up to four times that rate in moving water.

Grenier (1949) considered that the cephalic fans acted as strainers.

Harrod (1965) stated that S. nitidifrons required a minimum current of 19 cm./sec. to hold their cephalic fans open for any length of time.

Jørgensen (1966) classified black-fly larvae as non-selective passive filter feeders.

Chance (1970) studied the rate of fan flicking and found a mean rate of 0.81 per second with a standard deviation of 0.33. She considered that fan flicking was an irregular occurrence. Empty fans were often flicked and fans containing food particles were held open and not flicked. She concluded that the rate of fan flicking could not be used to represent a feeding rate. Instead she estimated feeding rate by studying ingestion. Chance mentioned fan flicking but illustrated the transfer of food particles from the primary fan to the mouth by the cleaning of the fans when they are completely closed and retracted by bristles on the mandibles. In suggesting that the mandibles scrape food particles from the closed fan she appears to consider that the cleaning action of the fans by larvae (wherein the fans are stroked by the mandibles, an action which goes on at infrequent intervals) is the real food particle transfer action and not the fan flicking motion considered as feeding by other authors (Fortner, 1937; Grenier, 1949). Chance (1970) did not propose any role for the flicking action.

It remains to be discovered what the motions of the mouthparts of black-fly larvae are in the food-gathering process and the resulting

path of the water-borne food particles. The controversy of whether simuliid larvae are active or passive filter feeders reflects the small amount of experimental data which exists to support either contention.

d) Population density

Black-fly larvae are often found concentrated in large numbers, on a small area of suitable habitat. A number of workers have recorded population densities for a given area of stream bottom or aquatic vegetation. Zahar (1951) found larvae on vegetation in densities of 2.1-3.2 larvae per sq. cm. with one record at 5.5 per sq. cm. Anderson and Dicke (1960) found 2,000 to 4,000 larvae on a stone 10 to 20 inches in diameter.

Carlsson (1962) determined the numbers of simuliid larvae per 1,000 sq. cm. of stream bottom. In 64 samples from 19 localities, he found from 1 to 9,600 larvae per 1,000 sq. cm. (ie. up to 9.6 larvae / sq. cm. or an average of .35 per sq. cm. for all collections). Maitland and Penney (1967) obtained densities from 0 to 228 larvae per sq. m. (14 samples) in the River Endrick or an average area of 36 sq. cm.

No study appears to have been done to determine if there is any sort of maximum density to which larvae will aggregate. Carlsson's (1962) observations show that larval densities may occasionally be very high but do not suggest that this occurs very often.

2. THE ABSOLUTE DENSITIES OF VARIOUS POPULATIONS OF BLACK-FLY LARVAE

As previously noted, several authors (Zahar, 1951; Anderson and Dicke, 1960; Carlsson, 1962; and Maitland and Penney, 1967) have recorded population densities of larvae. The author has occasionally noticed in general collecting, high concentrations of larvae such as that illustrated in Fig. 2 (taken of a stone in the Klara R. in eastern Norway.) The larvae shown are Gnus rostratum Rubtzov.

The following work is in two sections. The first is an analysis of population densities of larvae from the North Madawaska River, Ontario, Canada, and from Allerton Beck near Stanhope in Co. Durham, England. The second section deals with experiments in crowding a commonly occurring English species, Simulium ornatum Meigen.

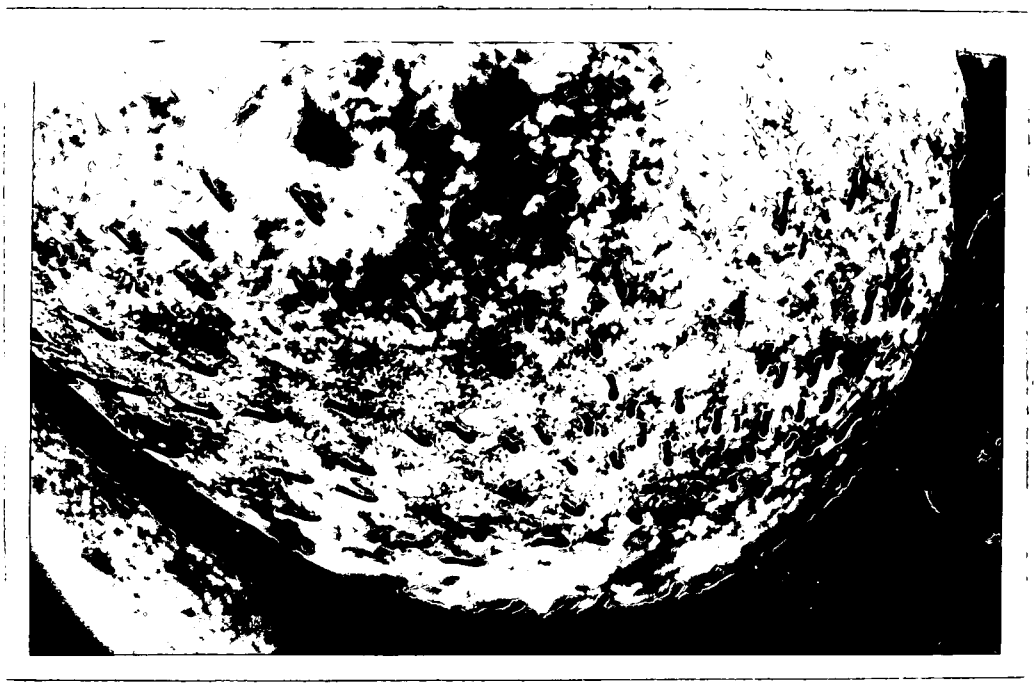
a) Methods and Materials for Field Studies

The collections from natural habitats in Canada and England were made by selecting blades of grasses trailing in the stream; these were either leaves of aquatic weeds or leaves of grasses from the river bank which were hanging in the water. The area of the substrate in each sample was calculated by measuring the length and width of the leaf or leaves and multiplying by two to give the total leaf surface. The attached larvae were preserved in 95% ethyl alcohol and counted. The number of larvae per sq. cm. was calculated by division.

b) Results of Stream Observations

Seventy-eight collections of larvae from the North Madawaska River during May and June, 1965 are tabulated in Table 1. The area of

Fig. 2. Larvae of Gnus rostratum Rubtz. on a stone in the Klara R., Eastern Norway.



vegetation sampled and the density of larvae per sq. cm. is given. These larvae were a mixture of two species - S. venustum Say and S. rugglesi N.&M. Eighteen of these samples contained more than one larva /sq. cm., the greatest density being 19.8 larvae /sq. cm. Four samples showed densities below 0.1 larvae /sq. cm. The average leaf area per collection was 226 sq. cm. with an average density of 1.0 larvae /sq. cm. The North Madawaska River (in the 100 metre distance in which the collections were made) was a stream 4-6 metres in width with a rubble bottom of stones 10-15 cm. in diameter. There were frequent patches of eelgrass (Vallisneria americana Michx.) which was the vegetation usually sampled in this study.

Forty-seven samples of vegetation from Allerton Beck, Co. Durham were collected on two dates. Twenty-five were made on March 30, 1967 and twenty-two on January 21, 1968. The results of each of these collections are presented in Table 2. The average leaf area sampled in 1967 was 25.7 sq. cm. and there was an average larval density of 7.4 larvae /sq. cm. In 1968 the average area sampled was 9.6 sq. cm. and the average density was 2.5 larvae /sq. cm. In the 400 metres of Allerton Beck sampled the width of the beck rarely exceeded 1 metre and was often less than 0.5 metre. Most of the vegetation sampled was grass which was trailing in the water. The species of larvae were S. spinosum D.&D. and S. brevicaule D.&G.

Table 1

The densities of larvae and areas of vegetation from which they were collected.

Samples from the North Madawaska River, Ontario - May - June, 1965.

Area of vegetation (sq. cm.)	Density of larvae / sq. cm.
140	0.04
214	0.1
98	0.1
70	4.1
70	2.8
90	4.1
90	1.3
220	0.2
380	0.2
180	0.5
120	0.3
128	1.2
210	1.5
110	0.9
112	1.4
116	1.8
120	0.6
228	0.4
152	0.6
156	0.5
144	0.8
164	1.3
158	0.2
118	1.1
126	0.4
180	0.02
156	0.1
194	0.4
140	0.5
80	0.4
45	2.7
8	19.8
250	0.7
320	0.6
178	0.5
214	1.3
214	0.3
172	0.4
250	0.3
340	0.2

Table 1 cont'd.

Area of vegetation (sq. cm.)	Density of larvae / sq. cm.
302	0.2
276	0.1
340	0.1
198	0.5
150	0.4
312	0.4
266	0.6
322	0.3
290	0.8
210	0.6
320	0.9
224	0.9
430	0.2
360	0.02
165	0.2
200	0.6
410	0.4
354	0.8
394	0.1
380	0.7
338	0.7
280	2.1
430	0.9
290	0.6
266	0.04
288	0.2
92	0.7
420	0.4
308	1.2
306	0.3
340	1.0
322	0.4
204	2.2
398	0.6
326	0.5
158	3.6
320	2.0
242	0.1
Sum 17,606	81.
Average 226	1.

Table 2

The densities of larvae and the areas of vegetation from which they were collected.

Samples taken March 30, 1967 and January 21, 1968 from Allerton Beck, Co. Durham, England.

March 30, 1967

Area of vegetation (sq. cm.)	Density of larvae / sq. cm.
3.9	1.5
1.0	6.0
2.8	2.5
7.0	0.7
10.0	0.6
1.0	16.0
6.6	1.3
9.5	4.6
5.4	2.1
3.6	0.6
8.0	0.1
3.4	4.1
3.0	22.0
3.2	15.9
4.8	21.6
24.2	0.1
18.0	2.0
10.4	0.9
2.8	10.7
1.8	20.0
4.0	2.2
3.6	7.5
1.5	0.8
2.0	8.0
1.2	34.5
Sum 141.7	186.2
Average 5.7	7.4

January 21, 1968

Area of vegetation (sq. cm.)	Density of larvae / sq. cm.
5.5	1.3
17.6	3.9
4.5	4.9
2.4	6.7

Table 2 cont'd

	Area of vegetation (sq. cm.)	Density of larvae / sq. cm.
	7.0	4.9
	17.6	3.5
	12.0	7.4
	3.6	10.6
	10.0	0.2
	15.0	0.1
	18.0	0.1
	3.6	1.1
	3.6	1.9
	7.8	0.4
	3.2	0.6
	30.0	1.3
	15.0	0.7
	15.0	0.5
	3.2	0.7
	8.0	0.9
	3.2	2.5
	4.8	1.3
Sum	211.6	55.5
Average	9.6	2.5

c) Discussion of Stream Observations

The average densities of larvae in collections on both dates from Allerton Beck were notably higher than that recorded for larvae in the North Madawaska River. The overhanging grass of Allerton Beck, it was noted, provided far fewer attachment sites than the plentiful aquatic vegetation in the North Madawaska. The areas of vegetation sampled in the first stream were much smaller than in the second. While the two streams differ considerably in physical size and in availability of larval habitat, comparison between their larval populations on the basis of density can give some food for further studies.

In both streams there were samples where the larvae were crowded quite densely. In Allerton Beck, with its paucity of attachment sites such behaviour would seem logical; but even in that stream larval densities as low as 0.1 larva /sq. cm. were found. In the North Madawaska, with numerous attachment sites with a density below 1.0 larvae /sq. cm., nine samples had larval densities over 2.0 /sq. cm. These results would seem to suggest that there is no strong pressure on larvae in crowded conditions to seek locations with a lower number of larvae per sq. cm. The lack of such a response to the presence of other larvae could well be responsible for the occasional high densities found in nature.

The results from the Allerton Beck samples of 1967 suggest that high densities may be much more common than previously reported. Seven of the twenty-five samples had larval densities above 10.0 larvae /sq. cm. Whether any response to crowding appears at very high densities

is difficult to determine in the field. The following experiments on crowding in the laboratory were an attempt to provoke the appearance of such a response.

d) Methods and Materials for the Laboratory Experiments

A trough 60.0 cm. long by 3.0 cm. wide by 3.0 cm. deep was constructed of transparent plastic sheet 3 mm. thick. At one end a header box 10 cm. by 10 cm. by 10 cm. was constructed and joined such that the box emptied into the trough. The box was fed via a stainless steel header tank supplied with tap water. Suspended by a 4 mm. diameter stainless steel rod just so it touched the surface of the water in the trough was a piece of 2 mm. thick flexible plastic strip 5 mm. wide and 40 mm. long. This strip was divided by painted black lines every 5 mm. of its length. The tip of the steel rod was glued to the upper side at one end of the strip and a bent insect pin inserted into the other end to provide an anchorage point. To this anchorage point was attached a length of monofilament fishing line (see Fig. 3). By pulling on the line the flexible plastic strip can gradually be lifted out of the water, thereby reducing the area available for attachment by the larvae. Figures 4-8 show the progressive reduction of the area of the strip during Experiment D.

The larvae used in these experiments were Simulium ornatum larvae collected from a stream near Shadforth, County Durham (Map Reference NZ 347 410). They were collected, from vegetation trailing in the water of this small stream, into 9.5 cm. diameter by 8 cm. deep

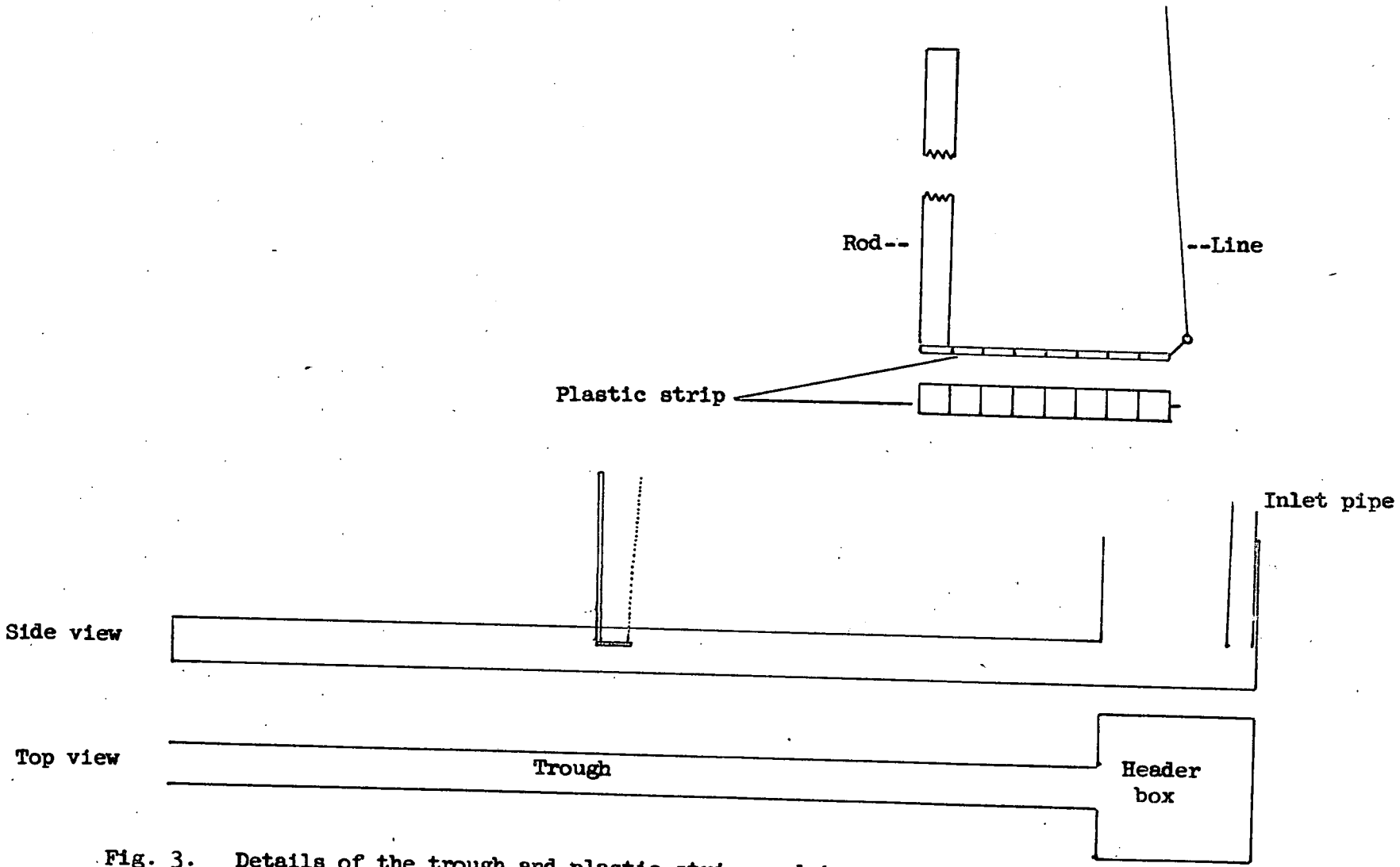


Fig. 3. Details of the trough and plastic strip used in microdistribution studies. (Trough x 1/4, strip actual size).

screw top jars which were then kept in the laboratory at 12°C. with an air stone connected to an aquarium pump supplying air (and agitation) to each jar. The larvae were kept in these jars overnight before being used for an experiment. In one experiment (Experiment D) larvae which had been kept in such jars for six days were used.

Experiments were conducted by adjusting the strip so that the entire length of its lower surface was just under the surface of the water. The rigid end of the strip (that held by the rod) was placed downstream for Experiments A to J and upstream for Experiments 1 to 3.

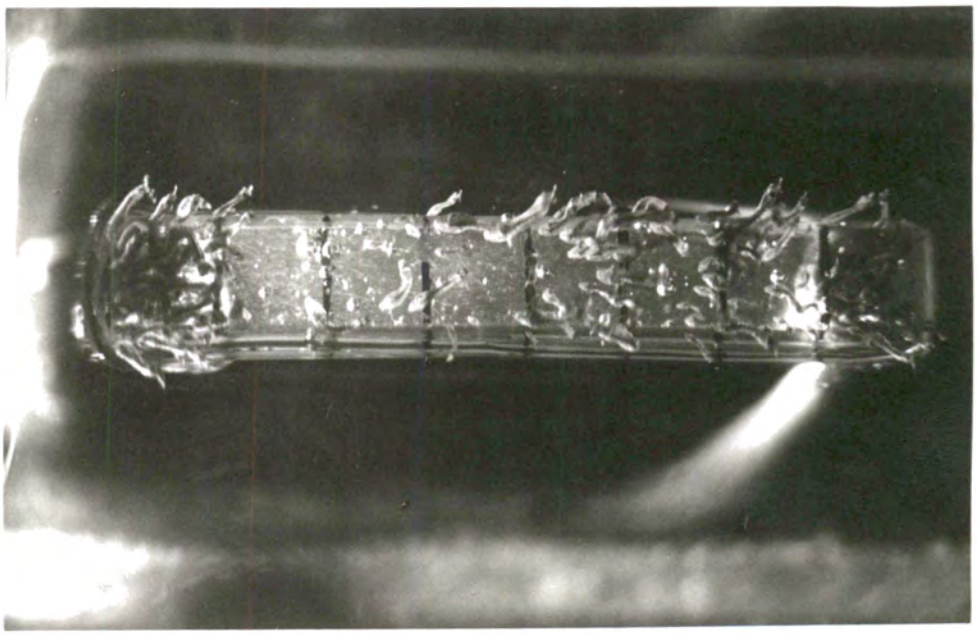
The velocity of the current in the trough was measured by means of a pitot tube gauge with the top positioned midway down the length of the plastic strip and as close to its lower surface as possible. The construction and callibration of the pitot tube gauges is given in Appendix I.

In Experiments A to J the larvae were first crowded down the plastic strip and then later allowed to recolonise it by moving upstream. In Experiments 1 to 3 larvae were first crowded upstream and could then recolonise the empty portion of the strip by moving downstream.

Larvae were established by carefully lifting them with fine forceps into the water and allowing them to spin out a length of silk thread which was then used to position the larvae next to the plastic strip so that they could attach themselves to it. When the desired concentration of larvae was obtained on the total strip (area 2 sq. cm.) the flexible end of the strip was then raised gradually from the water.

Fig. 4. S. ornatum larvae on the plastic strip, area 2 sq. cm.

Fig. 5. S. ornatum larvae on the plastic strip, area 1.75 sq. cm.



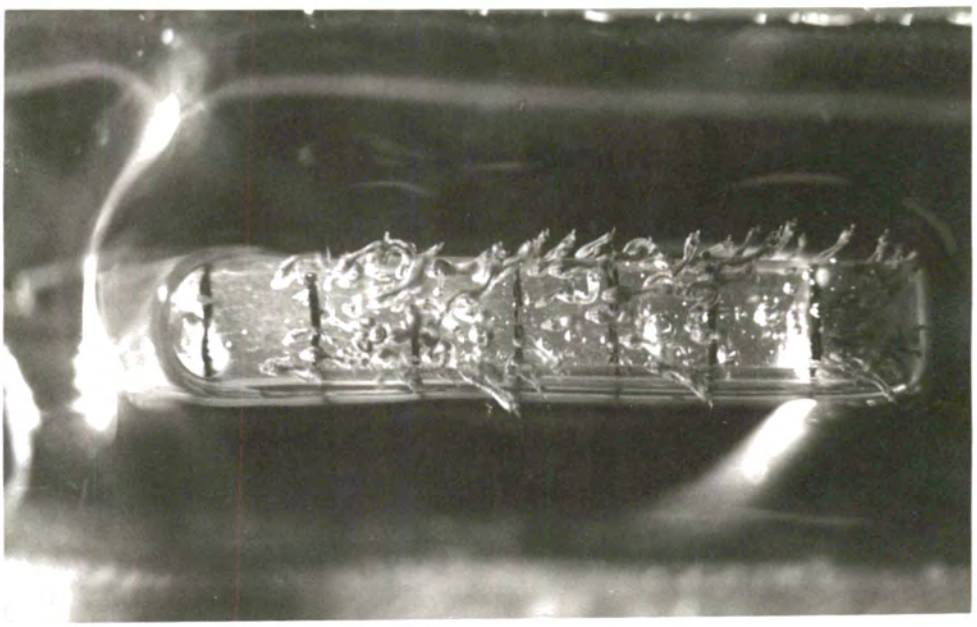
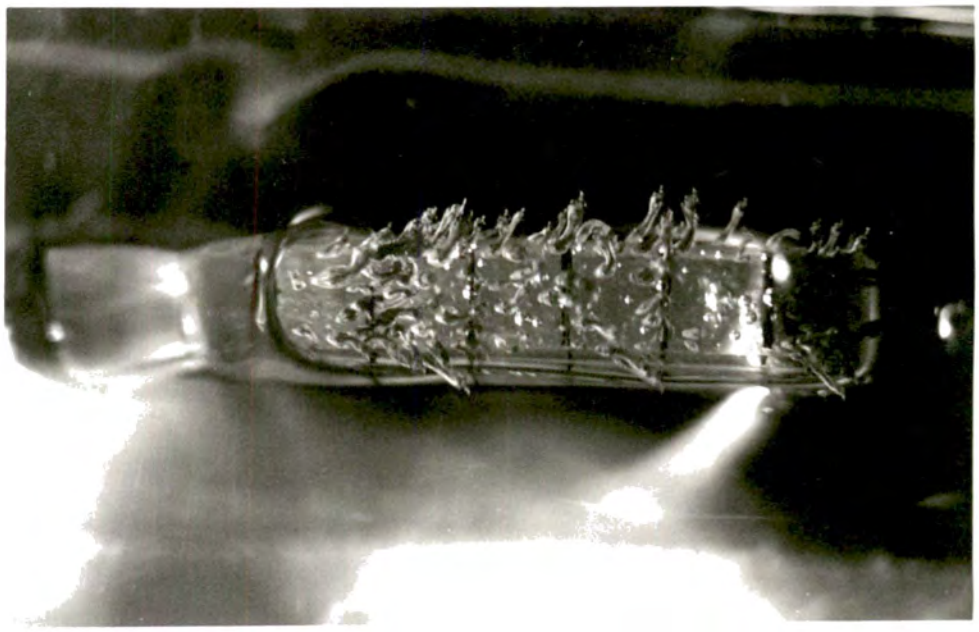


Fig. 6. S. ornatum larvae on the plastic strip, area 1.5 sq. cm.

Fig. 7. S. ornatum larvae on the plastic strip, area 1.25 sq. cm.



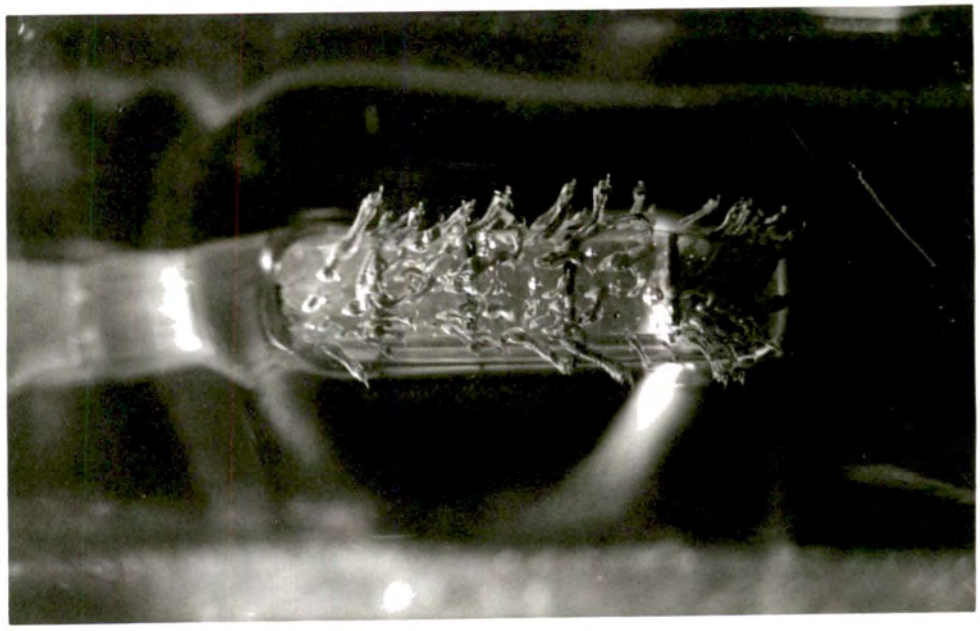
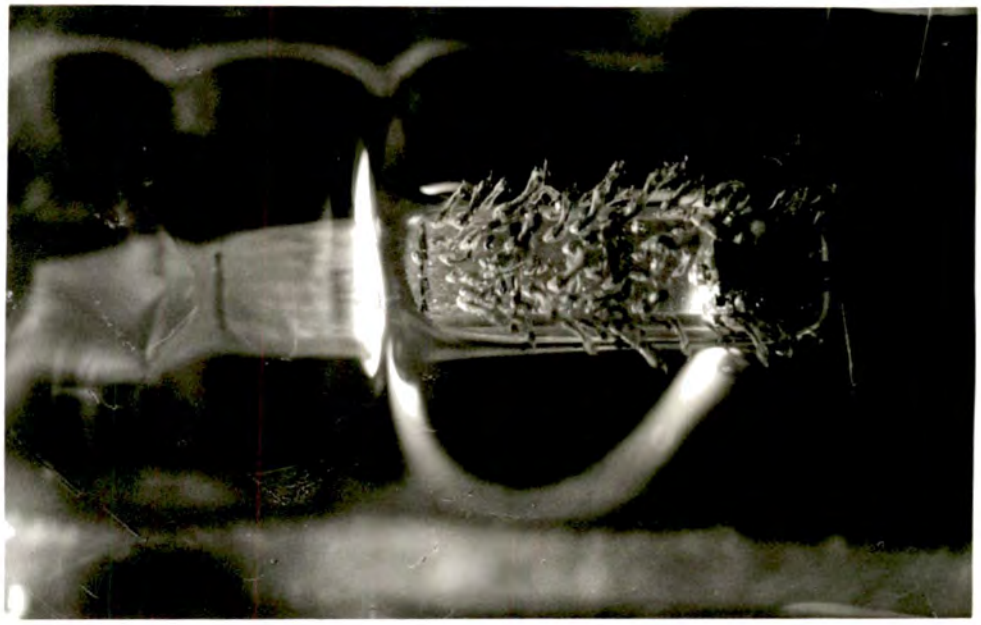


Fig. 8. S. ornatum larvae on the plastic strip, area 1 sq. cm.



This was done so that the larvae were never lifted out of the water but were only buffeted by the flow of the current past them. On being buffeted in this fashion larvae would either move to a less buffeted area of the plastic strip or abandon it altogether, allowing the current to carry them downstream.

After the larvae had been crowded onto an area of 1 sq. cm. and counted, the part of the plastic strip which had been lifted from the water was allowed to re-enter. The numbers of larvae that re-established on the area formerly denied them was recorded. The illumination over the larvae which had been maintained at a level approximating that of noon on a dull cloudy day at Durham was reduced during a period of 40 minutes to total darkness. The larvae were then observed under the illumination of a red photographic dark room safelight.

e) Results of the Laboratory Experiments

Table 3 presents the water temperature and current velocity under which each experiment was conducted.

Each experiment was divided into two parts. The first part considered was the reactions of the larvae to being crowded (i.e. whether they allowed themselves to be crowded together or whether they abandoned the plastic strip). The second part dealt with the movements of the larvae after the area of the plastic strip previously denied to them was lowered back into the water.

Table 4 presents an analysis of the first part of Experiments A to J (i.e. crowding). The time between 2 sq. cm. of plastic being available and only 1 sq. cm. being available is given (col.2). This

time period was to some extent determined by the larvae themselves, as the area available for attachment was reduced by .25 sq. cm. segments only when all the larvae had moved to such a position that they would not be lifted out of the water when such a reduction was made. The need for a longer time in Experiment C was due to the larger number of larvae taking more time to move themselves down the strip. The numbers of S. ornatum larvae on 2 sq. cm. at the start of the crowding and on 1 sq. cm. at the end of the crowding period are given. The "percent crowded" figure is the percent increase in numbers of larvae per sq. cm. at the beginning and the end of the crowding period. From this figure it can be seen that an increase in larval density was obtained at the end of each crowding experiment ranging from 128.2% to 180.5% of the original densities. A maximum density of 141 larvae per sq. cm. was obtained.

TABLE 3

The Temperatures and Current Velocities at which each Crowding Experiment was Conducted

Experiment	Temperature °C.	Current Velocity cm./sec.
A	12.5	44
B	13.0	62
C	11.0	44
D	12.0	44
E	10.5	44
F	10.5	44
G	11.5	44
H	11.5	44
J	9.0	44
1	12.5	44
2	11.5	44
3	14.0	62

TABLE 4

The results of crowding experiments on S. ornatum

Experiment	Time larvae crowded Hr.	Min.	No. on 2 sq. cm.	No. on 1 sq. cm.	% crowded
A	3	00	82	74	180.5
B	1	50	99	89	179.8
C	3	50	220	141	128.2
D	1	15	109	100	183.5
E	1	35	105	89	169.5
F	2	35	148	119	160.8
G	1	05	104	83	159.6
H	1	00	167	128	153.3
J	1	30	105	70	133.3

Table 5 presents an analysis of the numbers of larvae abandoning the plastic strip during crowding. The number of larvae abandoning the plastic strip varied from 8 to 79. (This last figure was obtained in Experiment C, in which 220 larvae were on the strip at the start of the experiment).

TABLE 5

Numbers of Larvae Dropping Off During Crowding

Experiment	Time Larvae Crowded Hr.	Min.	No. Dropping Off	No. at Start	% Dropping Off
A	3	00	8	82	9.8
B	1	50	10	99	10.1
C	3	50	79	220	35.9
D	1	15	9	109	8.3
E	1	35	16	105	15.2
F	2	35	29	148	19.6
G	1	05	21	104	20.2
H	1	00	39	167	23.4
J	1	30	35	105	33.3
Average	1	58	27.3	126.5	19.5

The second part of the experiment attempted to study the recolonisation of the empty upper half of the plastic strip. The results are given in Table 6.

TABLE 6

Number of Larvae Returning up the Strip when not Crowded

Experiment	Time to move back Hr.	Min.	No. moved back	% moved back	No. available to move back
A	3	00	5	6.8	74
B	9	50	25	28.8	89
C	3	20	13	9.2	141
D	2	05	49	49.0	100
E	2	50	5	5.6	89
F	2	30	7	5.9	119
G	2	40	17	20.5	83
H	3	00	13	10.2	128
J	3	00	5	7.1	70
Average	3	36	15.4		99.2

Only one experiment was left longer than 3 hours 20 minutes in this stage. Experiment B was left for 9 hours 50 minutes to see if larval movements were greater during a much longer period of time. More larvae moved up the strip in Experiment B than in any of the other experiments except D. The increased number moving up the strip in Experiment B was only 28.8% of the total number of larvae at the start, even allowing them 9 hours 50 minutes to do it. The large percentage

moving up in Experiment D can perhaps be explained by the fact that the larvae in this experiment had been kept in the laboratory for 6 days previous to the experiment. The larvae used in all other experiments were only kept one day in the laboratory before use. The number of larvae moving up the plastic strip are lower than the numbers originally there before crowding took place (see Table 7).

TABLE 7

The number of larvae originally on the upstream half of the plastic strip and the number moving up to it again

Experiment	No. of larvae moving up	No. before crowding
A	5	55
B	25	42
C	13	148
D	49	54
E	5	49
F	7	96
G	17	63
H	13	85
J	5	57

Only in Experiment D, presumably for the reason given above, did the number of larvae returning approach that of the number originally resident there.

Table 8 shows the number of larvae in the second part of each experiment that abandoned the plastic strip rather than moving up it or remaining on the lower half. These numbers are generally quite low - only in Experiment C did an appreciable number of larvae abandon the strip, and these are only 23.3% of the total larvae on the strip.

TABLE 8

Numbers of larvae dropping off when not crowded

Experiment	Time to leave Hr.	Min.	No. abandoning strip	% abandoning strip	No. available to abandon strip
A	3	00	10	13.5	74
B	9	50	5	5.5	89
C	3	20	33	23.3	141
D	2	05	14	14.0	100
E	2	50	7	7.9	89
F	2	30	4	3.4	119
G	2	40	3	3.6	83
H	3	00	3	2.3	128
J	3	00	1	1.4	70
Average	3	36	8.8		99.2

Three experiments were conducted in which S. ornatum larvae were crowded up the plastic strip and later given the opportunity to move down it again. The results of the crowding portion of the experiment

are given in Table 9. They show that some degree of crowding took place.

TABLE 9

Experiments in crowding S. ornatum larvae in an upstream direction

Experiment	Time crowded Hr.	Min.	No. on 2 sq. cm.	No. on 1 sq. cm.	% crowded
1	1	00	62	40	129.0
2	1	20	47	42	178.7
3	3	25	79	52	131.6

Table 10 gives the numbers of larvae abandoning the plastic strip during crowding. The percentages of larvae abandoning the plastic strip during crowding in an upstream direction do not differ markedly from those obtained in the experiments where larvae were crowded in a downstream direction.

TABLE 10

Number of larvae abandoning the plastic strip during crowding in an upstream direction

Experiment	Time crowded Hr.	Min.	No. abandoning	No. at start	% abandoning
1	1	00	22	62	35.5
2	1	20	5	47	10.6
3	3	25	27	79	34.2

Table 11 gives the number of larvae which either recolonised the part of the plastic which was returned to the water after crowding or abandoned the plastic strip entirely.

TABLE 11

Number of larvae changing positions after the area of the plastic strip downstream was returned to the water

Experiment	Time observed Hr.	Min.	No. of larvae moving down	No. of larvae abandoning strip	No. available to move or abandon
1	13	05	3	1	40
2	7	00	1	1	42
3	6	55	0	3	52

Very few larvae moved downstream on the plastic strip during these experiments as opposed to larvae moving upstream in Experiments A to J, especially considering that much more time was allowed in Experiments 1 to 3. Also, very few larvae in Experiments 1 to 3 abandoned the plastic strip as compared with Experiments A to J.

f) Discussion of the Laboratory Experiments

The most significant result of these experiments is the fact that densities of black-fly larvae much higher than those found in the field experiments were obtained. Such results must further reinforce the thesis that black-fly larvae do not show much tendency to disperse from a substrate on which they occur at a high density.

It should also be noted that S. ornatum larvae will move away from an area where they are being buffeted (ie. the water flow is turbulent) to an area where the flow of water is more laminar. This observation agrees with Maitland and Penney (1967).

During crowding both in upstream and downstream directions, numbers of larvae abandoned the plastic strip (see Tables 5 and 10). It is not clear whether these larvae abandoned the strip in preference to being crowded more closely together with other larvae or whether they simply lost their secure hold on the substrate and were swept downstream. Whatever the reason, the effect of the reduction of habitat space resulted in the majority of larvae preferring to be crowded more closely together while a minority abandoned that substrate.

The numbers of larvae moving upstream after having been crowded downstream (Experiments A-J) were low in all experiments except one (see Table 7). However, the numbers of larvae moving downstream after having been crowded upstream (Experiments 1-3) were lower still (see Table 11). There is a suggestion here that S. ornatum larvae, when they move under their own volition, prefer to move against the current rather than away from it. They were, however, quite capable of moving either upstream or downstream as shown by their responses to crowding in both directions.

Black-fly larvae, while amenable to forced movement and to a great degree of crowding, do not appear to exist in nature at anything like the possible densities at which they can be forced to exist in the laboratory. At the densities found in the field samples taken from

Allerton Beck and the North Madawaska River, interaction between individual larvae would be much less than in the far more crowded laboratory experiments. It is likely that at the densities which larvae are found in nature movement of larvae from an area of high larval density to an area of lower larval density would not be caused by population pressure.

Further, it is possible that crowding of larvae, resulting in a patchy distribution when large areas of a stream are concerned, is not an unusual or detrimental phenomenon.

3. THE MEANS AND MECHANISM OF FOOD COLLECTION

a) The fine structure of the rays of the primary fan

The arrangement and general morphology of the various mouthparts of black-fly larvae have been discussed quite thoroughly by several authors (Puri 1925, 1926; Grenier 1949; Davies 1965; Chance 1970). Davies (1966) has shown the taxonomic significance of the variations in the morphology of the mandible tip. Puri (1926), Grenier (1949) and Chance (1970) have noted variations in the morphology of the rays of the cephalic fans.

Chance (1970) stated that the primary fan was the chief food collecting organ. Her observations of the trichiation of the rays of the primary fans suggested that there were specific differences but that these had no effect on the varying rates of ingestion of food by various species.

Since the exact fine structure of a primary ray is difficult to determine under the light microscope due to the fact that the spacing of microtrichia on a ray is often in the 1 micron range, the rays of the primary fan of twenty British species, three Canadian species, one species from material collected in Norway and one species (Crozetia crozetense) collected by Dr. L. Davies in the Crozet Islands were examined and photographed using the scanning electron microscope. The British species studied were as follows:

Genus Prosimulium Roubaud
Prosimulium hirtipes (Fries)
Prosimulium inflatum Davies
Prosimulium arvernense Grenier

Genus Simulium Latreille
Simulium ornatum Meigen
Simulium nitidifrons Edwards
Simulium spinosum Doby and Deblock
Simulium monticola Friedrichs
Simulium variegatum Meigen
Simulium reptans (Linnaeus)
Simulium tuberosum (Lundstroem)
Simulium argyreatum Meigen
Simulium erythrocephalum Degeer
Simulium latipes Meigen
Simulium brevicaule Dorier and Grenier
Simulium armoricanum Doby and David
Simulium costatum Friedrichs
Simulium angustitarse Lundstroem
Simulium equinum (Linnaeus)
Simulium salopiense Edwards
Simulium subexcisum Edwards

The following other species were examined:

Prosimulium (Helodon) ferrugineum (Wahlberg)
Simulium venustum Say
Simulium rugglesi Nicholson and Mickel
Simulium longistylatum Shewell
Crozetia crozetense (Womersley)

The actual measurements, as determined from the photographs of the various parts of the ray of each species studied, are given in Table 12. The photographs are presented in the actual size as displayed on the cathrode ray tube of the scanning electron microscope. Magnifications are given in the captions to each photograph. In the following text the photographs of each species studied will be described. These descriptions are intended only to supplement the photographs and draw attention to the outstanding features of each specimen.

In the tables, tip length refers to the length of the tip of a ray from the start of the microtrichia to the tip of the ray. The thickness of a ray is the depth in the plane of the microtrichia. The thickness of the primary and secondary microtrichia was measured at their bases as was the spacing between the secondary microtrichia.

TABLE 12

The dimensions of the microtrichia on the rays of the cephalic fans of 24 species of black-fly larvae

Species	Tip length (μ)	Ray thickness (μ)	Length of primary micro-trichia (μ)	Thickness of primary micro-trichia (μ)	Spacing of primary micro-trichia (μ)
P. ferrugineum	116	6-17	11-16	1-2	7
P. hirtipes	34	3-9	13-15	1	13
P. inflatum	64	5-9	6-8	1	18
P. arvernense	46	2-15	13	1	19
S. ornatum	28	1-4	13-15	0.5	9
S. nitidifrons	17	1-6	7-8	0.5	11
S. spinosum	13	1-4	8-9	0.5	8
S. variegatum	7	2-10	7-11	1	8
S. monticola	9	1-7	9-10	1	11
S. reptans	11	1-7	5-6	0.5	13
S. tuberosum	10	1-3	8-9	0.8	13-14
S. equinum	11	1-2	6-8	0.5	8
S. salopiense	16	1-5	6-7	0.5	13-17
S. erythrocephalum	18	1-3	5-6	0.3	4-6
S. argyreatum	17	1-17	10-11	0.5	9-11
S. subexcisum	16	1-2	No change	-	-
S. angustitarse	14	1-3	6-7	0.3	9
S. latipes	10	1	7-9	0.3	8
S. brevicaule	14	2-3	6-10	0.6	8

TABLE 12 (CONT'D.)

Species	Tip length (μ)	Ray thickness (μ)	Length of primary micro-trichia (μ)	Thickness of primary micro-trichia (μ)	Spacing of primary micro-trichia (μ)
S. armoricanum	15	1-2	8	0.9	6
S. costatum	9	1-3	7-8	0.3	5
S. venustum	22	2-3	9-11	0.6	12
S. rugglesi	10	1-3	2-5	0.3	5
S. longistylatum	25	2-6	31-33	0.8	20

Species	Thickness of secondary micro-trichia (μ)	Number of secondary micro-trichia (μ)	Spacing of secondary micro-trichia (μ)	Length of secondary micro-trichia (μ)
P. ferrugineum	1-2	3-5	0.3	11-12
P. hirtipes	0.7	8-9	0.1	8-10
P. inflatum	0.8	17	0.2	3-5
P. arvernense	0.8	11	0.2	7-9
S. ornatum	0.3	9-11	0.1	6-7
S. nitidifrons	0.3	24-25	0.1	3-5
S. spinosum	0.3	9-12	0.2	1-5
S. variegatum	0.5	8-11	0.2	4-8
S. monticola	0.5-1	9-12	0.1	2-8

TABLE 12 (CONT'D.)

Species	Thickness of secondary micro-trichia (μ)	Number of secondary micro-trichia (μ)	Spacing of secondary micro-trichia (μ)	Length of secondary micro-trichia (μ)
S. reptans	0.4	15-18	0.1	3-5
S. tuberosum	0.3	12-16	0.2	3-6
S. equinum	0.3	10-11	0.1	3-7
S. salopiense	0.3	32-43	<0.1	4-5
S. erythroce-phalum	0.2	13-21	<0.1	4
S. argyreatum	0.3	7-16	0.1	2-4
S. subexcisum	0.2	-	0.1	5-6
S. angustitarse	0.2	15-16	0.1	3-5
S. latipes	0.2	13-14	<0.1	3-6
S. brevicaule	0.4	9	0.1-0.2	1-8
S. armoricanum	0.5	8	0.1	1-5
S. costatum	0.3	8-10	0.1	4-5
S. venustum	0.4	8-12	0.2	4-7
S. rugglesi	0.2	9	0.1	3-4
S. longistyla-tum	0.6	13-19	0.1	5-15

Primary microtrichia are considered as the long and strong setae which occur at intervals along a ray. Crosskey (1960) called these socketed macrotrichia. Secondary microtrichia are defined as those finer and shorter setae which occur in varying numbers between the primary microtrichia. These were thought to be microtrichia by Crosskey (1960). However, no evidence of the socketed macrotrichia was found on the rays of the species studied and all the setae depicted here are considered to be microtrichia.

When held extended, the primary fan of a larva appears in the form depicted in Fig. 9. The orientation of the rays with their tips pointed posteriorly can be clearly seen. In a higher magnification of the same primary rays (Fig. 10) the orientation of the rays around their basal stalk and their general shape (approaching that of a farmer's scythe) is apparent. The microtrichia can be seen on the interior margin of each flattened curved ray.

In the following, photomicrographs and descriptive text outline the variations from species to species of the fan rays and their microtrichia.

b) Observations on individual species under the scanning electron microscope

1. Prosimulium (Helodon) ferrugineum (Wahlberg)

Figures 11-14

This species has the heaviest and thickest ray of any species examined. It varies in thickness from 6 microns at the base of the tip to 17 microns at the middle of its length. The surface of the ray

shows definite striations. The microtrichia show only a very slight cyclic pattern (3 to 5 in number) of varying lengths. They are at least one micron in diameter and 11 microns in length. None of the microtrichia appear to be socketed. All the microtrichia are quite stout and the spacings between them 0.3 microns.

2. Prosimulium hirtipes (Fries)

Figures 15-18

The structure of the ray of a P. hirtipes larva is somewhat finer than that of P. ferrugineum. The tip length is much shorter at 34 microns while the ray thickness is 3-7 microns. The microtrichia are nearly as long as those of P. ferrugineum but occur in a cyclic pattern of 8-9 secondary microtrichia. The spacing of the microtrichia is .1 microns.

3. Prosimulium inflatum Davies

Figures 19-21

The ray of this species appears intermediate between that of P. ferrugineum and that of P. hirtipes. The ray is nearly as thick at the tip as in P. ferrugineum but towards the base is only as thick as P. hirtipes. The length of the tip is midway between the two other species. The microtrichia are much shorter (3 microns to 8 microns) and the secondary microtrichia occur in a cyclic pattern of 17. The spacing of the secondary microtrichia is intermediate to P. ferrugineum and P. hirtipes.

4. Prosimulium arvernense Grenier

Figures 22-24

P. arvernense, the third of the three British species in the genus Prosimulium, has a rather slender ray and rather long secondary microtrichia in a cyclic pattern of 11. The spacing of the secondary microtrichia is similar to that of P. inflatum while the microtrichia approach those of P. hirtipes in length.

5. Simulium ornatum Meigen

Figures 25-27

S. ornatum, one of the commonest species in Britain, has a fine ray (1-4 microns) in depth, surmounted by long slender microtrichia. The primary microtrichia are double the length of the secondary microtrichia. The latter show only a small variation in length and are in a cyclic group of 9 to 11. They are spaced only 0.1 microns apart. The tip of the ray is slender and twice the length of the primary microtrichia.

6. Simulium nitidifrons Edwards

Figures 28-30

This species has a somewhat thicker ray (1-6 microns) than that of S. ornatum. The tip of the ray is also shorter (17 microns). The primary microtrichia are half as long as those of S. ornatum but the secondary microtrichia are proportionately longer and occur in a cyclic pattern of 24 to 25. The spacing of the secondary microtrichia is the same (0.1 microns) as for S. ornatum.

7. Simulium spinosum Doby and Deblock

Figures 31 and 32

This species has a ray of similar depth to S. ornatum. The tip of the ray is shorter than both S. ornatum and S. nitidifrons. The primary microtrichia are similar in dimensions to S. nitidifrons although more closely spaced. What sets this species apart from S. ornatum and S. nitidifrons is the regular variation in length of the secondary microtrichia (1-5 microns). These are arranged in a cyclic group of 9 to 12 microtrichia and vary in length in a manner resembling an ascending scale of organ pipes. The spacing between the secondary microtrichia is much coarser (0.2 microns) than the other two species.

8. Simulium variegatum Meigen

Figures 33-36

The rays of this species are notable for the shortness of their tip. The ray is somewhat deeper than S. ornatum and the primary microtrichia are thicker. An "organ-pipe" cyclic arrangement occurs as with S. spinosum, but the number of secondary microtrichia per cycle is 8-11. The spacing of the secondary microtrichia is 0.2 microns.

9. Simulium monticola Friederichs

Figures 37-39

This species has rays very similar to those of S. variegatum. The "organ-pipe" cycle of the secondary microtrichia is similar but

9 to 12 microtrichia in number. On the whole, the microtrichia are slightly shorter and broader in diameter than is the case with S. variegatum.

10. Simulium reptans (Linnaeus)

Figures 40-41

This species has a delicately tapered ray with a short tip only slightly longer than that of S. monticola. The cyclic arrangement of the secondary microtrichia resembles those of S. ornatum and S. nitidifrons but numbers 15 to 18 microtrichia. Their spacing is 0.1 microns. The primary microtrichia are only 5-6 microns long. There is very little "organ-pipe" variation in the lengths of the secondary microtrichia.

11. Simulium tuberosum (Lundstroem)

Figures 42-44

The ray of S. tuberosum resembles that of S. reptans with the exception of the longer and thicker primary microtrichia and the generally smaller number of secondary microtrichia (12-16) in a cycle. There is also a more pronounced "organ-pipe" variation in the secondary microtrichia similar to S. monticola and S. variegatum.

12. Simulium equinum (Linnaeus)

Figures 45 and 46

This species has a thin and shallow ray (1-2 microns) with a short tip (11 microns). The secondary microtrichia show an "organ-pipe" variation with a cycle of 10 or 11. The spacing between secondary microtrichia is quite small at 0.1 microns.

13. Simulium salopiense Edwards

Figures 47-49

This species has an even finer ray than S. equinum (1-5 microns). There is a little variation in the lengths of the secondary microtrichia and they are arranged in a cycle of 32 to 43. The spacing of the secondary microtrichia is very small (less than 0.1 microns).

14. Simulium erythrocephalum DeGeer

Figures 50-52

This species has a very slender ray (1-3 microns) with very delicate microtrichia. The tip of the ray is 18 microns long. The secondary microtrichia are nearly as long as the primary microtrichia. The cyclic arrangement of secondary microtrichia numbers 13-21. Their spacing is very fine (less than 0.1 microns).

15. Simulium argyreatum Meigen

Figures 53 and 54

This species has a thin ray with long and stout primary microtrichia. Contrasting this are the much shorter and thinner secondary microtrichia. The arrangement of the microtrichia in this species has some resemblance to those of S. ornatum. The secondary microtrichia have a spacing of 0.1 microns and a cyclic arrangement of 15-16.

16. Simulium subexcisum Edwards

Figures 55-57

In this species we find a very delicate ray form without primary microtrichia. All the microtrichia appear to be nearly the

same length (5-6 microns) and a spacing of 0.1 microns. The tip of the ray is 16 microns long and diverges from the end of the ray at a large angle.

17. Simulium angustitarse (Lundstroem)

Figures 58-61

The ray of S. angustitarse is only slightly less delicate than that of S. subexcisum and has a tip 14 microns long. The secondary microtrichia show a definite "organ-pipe" arrangement numbering 15-16 microtrichia. Their spacing is 0.1 microns.

Two views of the broken end of a ray demonstrate that it appears to be a hollow structure somewhat rectangular in cross-section.

18. Simulium latipes (Meigen)

Figures 62-64

S. latipes has a very slender ray carrying slender microtrichia; the secondary microtrichia are in a cycle of 13-14. These secondary microtrichia show an "organ-pipe" variation in their lengths and are spaced at intervals of less than 0.1 microns. The tip of the ray is 10.0 microns long and quite slender.

19. Simulium brevicaule Dorier and Grenier

Figures 65 and 66

S. brevicaule has a much stouter ray than S. latipes and a slightly longer tip (14 microns). The microtrichia are much thicker and slightly longer. The "organ-pipe" variation in the secondary

microtrichia is quite pronounced and the cyclic variation is 9. Their spacing is larger (.1-.2 microns) than in S. latipes.

20. Simulium armoricanum Doby and David

Figures 67 and 68

This species has a ray which in thickness is intermediate between S. latipes and S. brevicaule. The tip is longer (15 microns) than that of S. brevicaule. A very pronounced "organ-pipe" variation of the secondary microtrichia is an outstanding feature. The spacing of these microtrichia is 0.1 microns.

21. Simulium costatum Friederichs

Figures 69 and 70

S. costatum has a very short tip to its ray. (The tip on the photographed specimen is curved because of electron beam pressure.) The ray itself is slender and the microtrichia arranged in a similar form to the three previous species but without a highly pronounced "organ-pipe" variation.

22. Simulium venustum Say

Figures 71 and 72

This species, from North America, has a ray of average thickness and a tip 22 microns in length. The secondary microtrichia are arranged in cycles of 8 to 12 and show some degree of "organ-pipe" variation. The spacing of the secondary microtrichia is 0.2 microns.

23. Simulium rugglesi Nicholson and Mickel

Figures 73 and 74

This species has a very fine ray with a short (10 microns) tip. The microtrichia are all quite short. The secondary microtrichia are in cycles of 9 and show only a slight "organ-pipe" variation, and are spaced 0.1 microns from one another.

Two of the photographs of S. rugglesi are of general views of the rays of the primary cephalic fan demonstrating the curvature of the rays and the way they fit together when closed.

24. Simulium longistylatum Shewell

Figures 75-77

This species has a tip to the ray 25 microns in length. The chief peculiarity of the ray is the long length of the microtrichia, especially the primary microtrichia. The secondary microtrichia, in cycles or groups of 13 to 19, show a very marked "organ-pipe" variation in length from 5 to 15 microns.

25. Crozetia crozetense (Womersley)

Figures 78 and 79

C. crozetense has fan rays of a very short and peculiar form which resemble to some degree toothbrushes with a single row of bristles.

c) Discussion of scanning electron microscope results

While the primary rays of the above species have a basically similar structure (a sclerotized curved ray, rectangular in cross-section

and carrying a single row of microtrichia), they vary considerably in detailed structure. In all but one species studied the primary fan is used to filter food particles from flowing water. The one exception, Crozetia crozetense, apparently does not filter feed but used its short primary rays as rakes to browse on the substratum of the stream (Dumbleton 1962, Davies 1965). This unusual structure of the cephalic fans together with the larval labrum, has led Davies (1965) to propose that Crozetia crozetense is primitive with respect to these structures and that the cephalic fan of this species represents an early stage in the evolution of cephalic fans which are now used as filtering organs by later Simuliidae. In connection with this view, it is interesting to note the similarity of the peg-like microtrichia of the rays of P. ferrugineum to those of Crozetia crozetense. P. ferrugineum is placed in the genus Helodon by Rubtzov (1959) and is considered a relict genus by him and Carlsson (1962).

If we accept the view that the filtering forms of cephalic fans evolved from raking brushes or combs similar to those of Crozetia crozetense, we can then postulate that the wide variety of patterns of microtrichia on filtering cephalic fans have arisen as modifications of a simple row of peg-like microtrichia. Three basic patterns can be seen in the filtering species studied. The first has primary microtrichia at intervals between which are a number of secondary microtrichia which are of more or less the same length (eg. P. hirtipes, S. ornatum, S. nitidifrons, S. angustitarse, S. salopiense, S. reptans and S. erythrocephalum). The second has microtrichia which are all apparently

the same size (eg. S. subexcisum). The third form has primary microtrichia at intervals along a ray with secondary microtrichia which vary in length in a fashion similar to organ pipes. They increase in length in a regular series towards the tip of a ray (eg. S. brevicaule, S. armoricanum, S. venustum, and S. longistylatum).

This wide variety of forms, even falling into three general patterns as they do, suggests that filter-feeding black-fly larvae may be a complex matter. Rubtzov (1959) claimed that the microtrichia on the primary rays of larvae of anautogenous species are spaced 10 to 20 microns apart while on autogenous species they are 1 micron apart. The wide range of patterns of microtrichia suggest that Rubtzov's view is an oversimplification. The various modified rays are more likely to be morphological evidence of adaptation to a particular environment.

It is unlikely that the rays of the cephalic fans are adapted to feeding on a particular segment of the microseston of a stream as Anderson and Dicke (1960) found Wisconsin species unable to feed selectively. It is more probable that the various patterns of microtrichia on the rays of the primary fans of black-fly larvae are an adaptation to food gathering in various rates of water current for which many species have been found to have preferences (see introductory section on Current and black-fly larvae). It is difficult to explain how these different patterns of microtrichia would affect food intake. The following section reports a study of the feeding action of a live larva.

Fig. 9. Cephalic fan of S. rugglesi

X 190

Fig. 10. Rays of the primary fan of S. rugglesi.

X 950

Fig. 11. Side view of a ray of P. ferrugineum.

X 2,000

Fig. 12. Tips of the rays of P. ferrugineum.

X 470

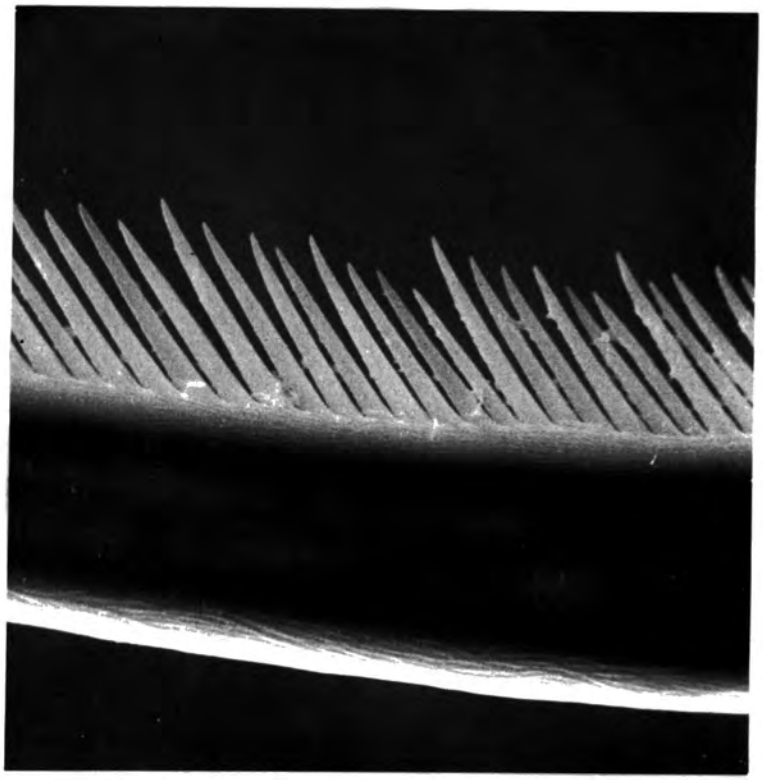


Fig. 13. View of the interior edge of a ray of P. ferrugineum.

X 4,700

Fig. 14. Side view of the ray of P. ferrugineum.

X 2,000

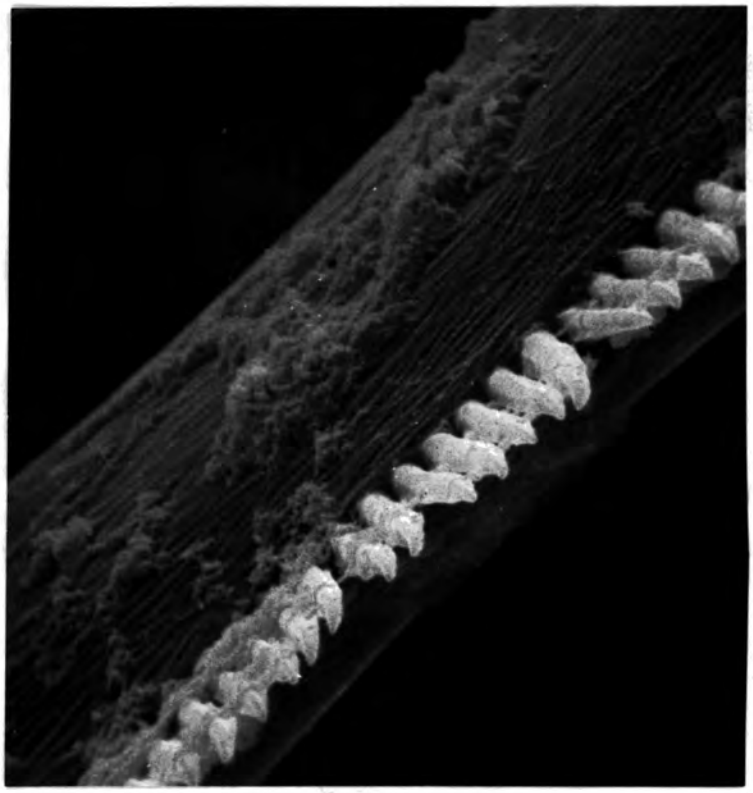


Fig. 15. The tip of a ray of P. hirtipes.

X 2,000

Fig. 16. Side view of a ray of P. hirtipes.

X 2,000

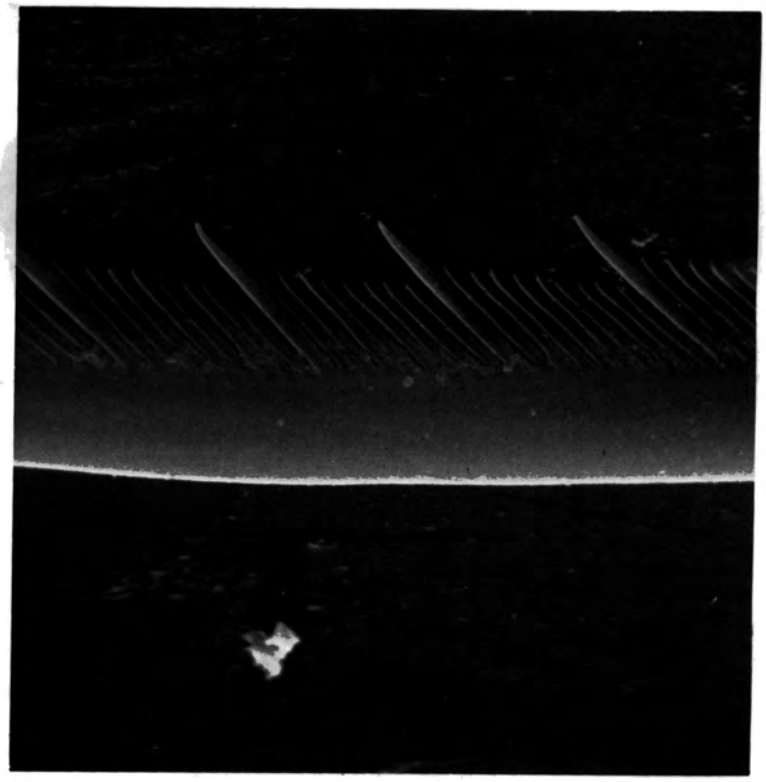


Fig. 17. Side view of a ray of P. hirtipes.

X 5,000

Fig. 18. Side view near the base of a ray of P. hirtipes.

X 5,000

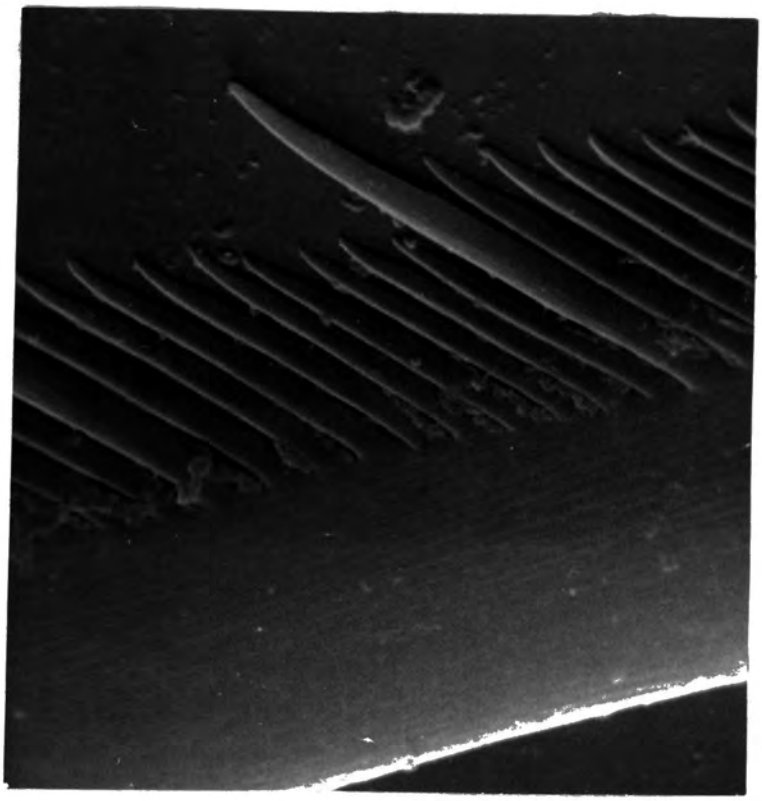


Fig. 19. Tips of the rays of P. inflatum.

X 500

Fig. 20. Side view of a ray of P. inflatum.

X 2,000

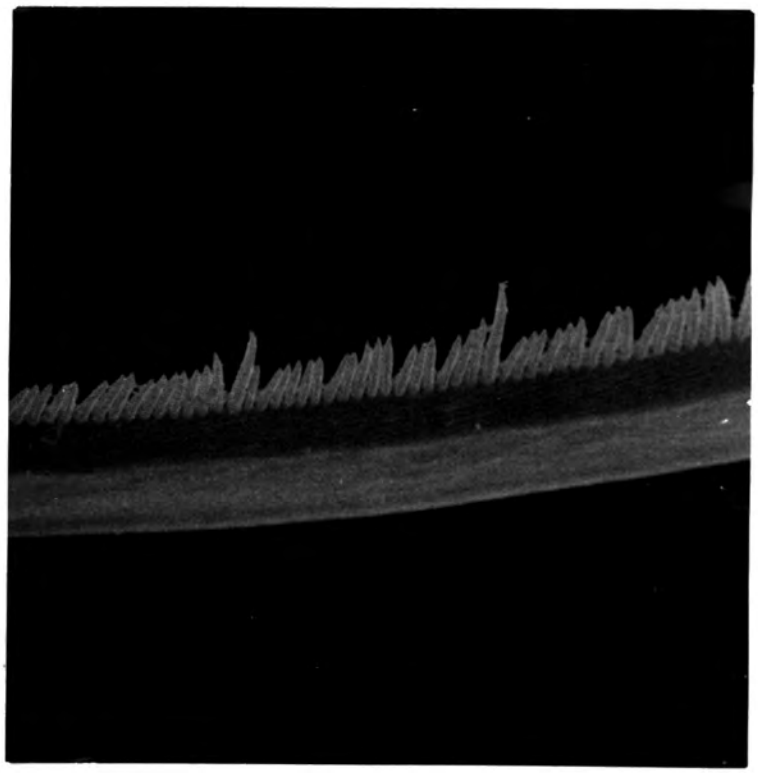
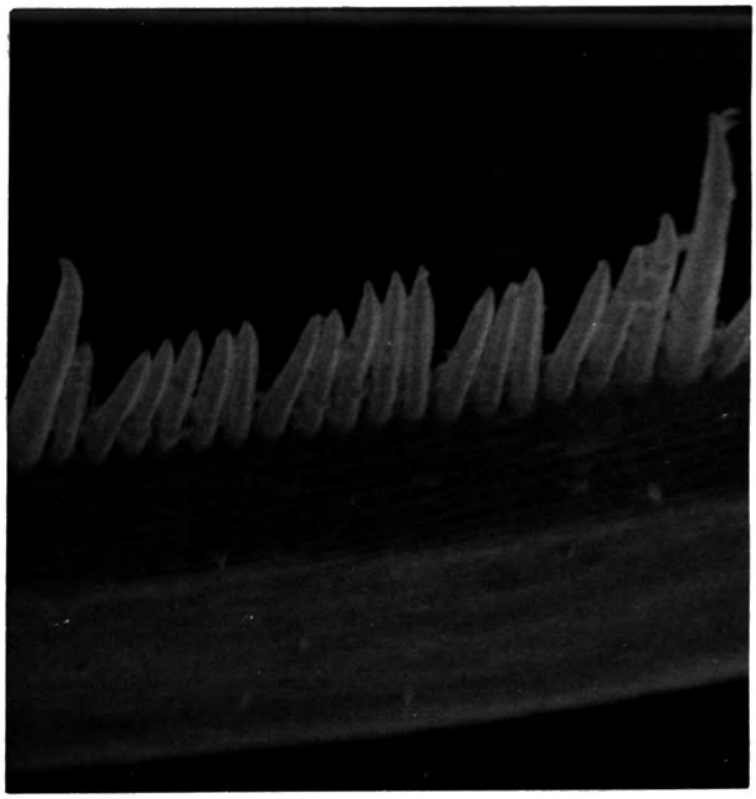


Fig. 21. Side view of a ray of P. inflatum.

X 5,000

Fig. 22. Tip of a ray of P. arvernense.

X 1,050



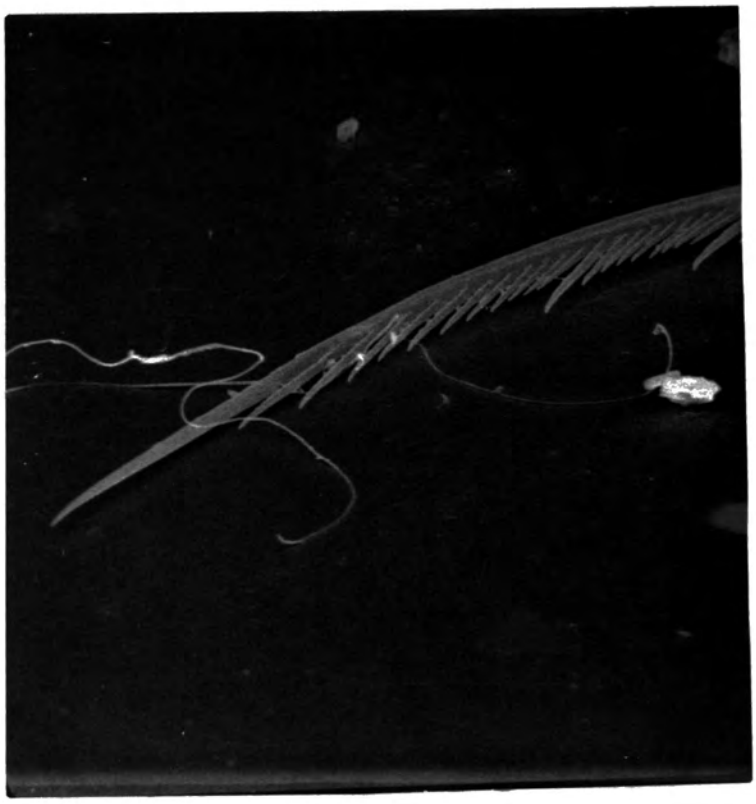
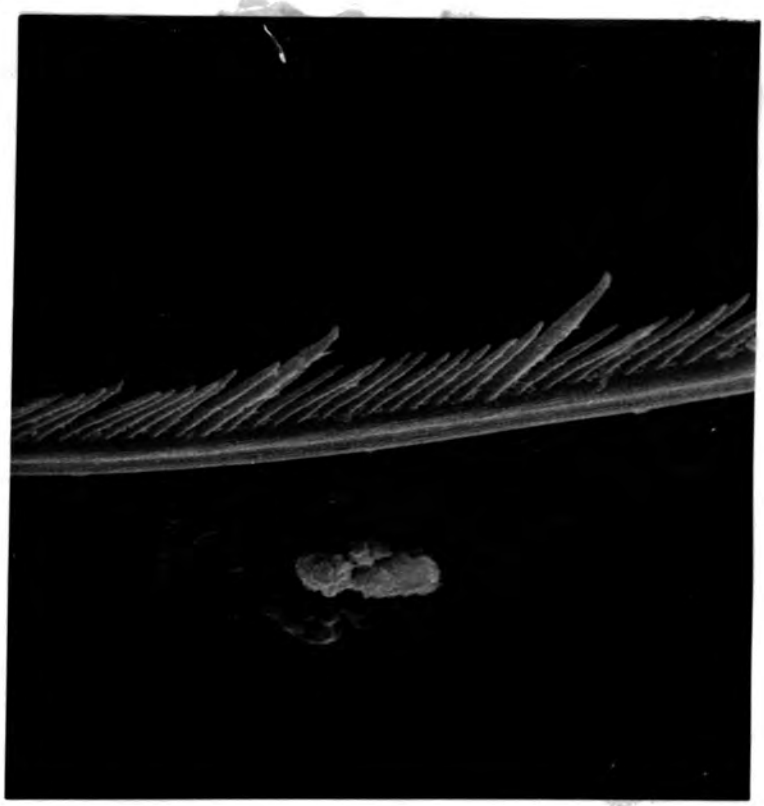


Fig. 23. Side view of a ray of P. arvernense.

X 2,100

Fig. 24. Side view of a ray of P. arvernense near its base.

X 2,100



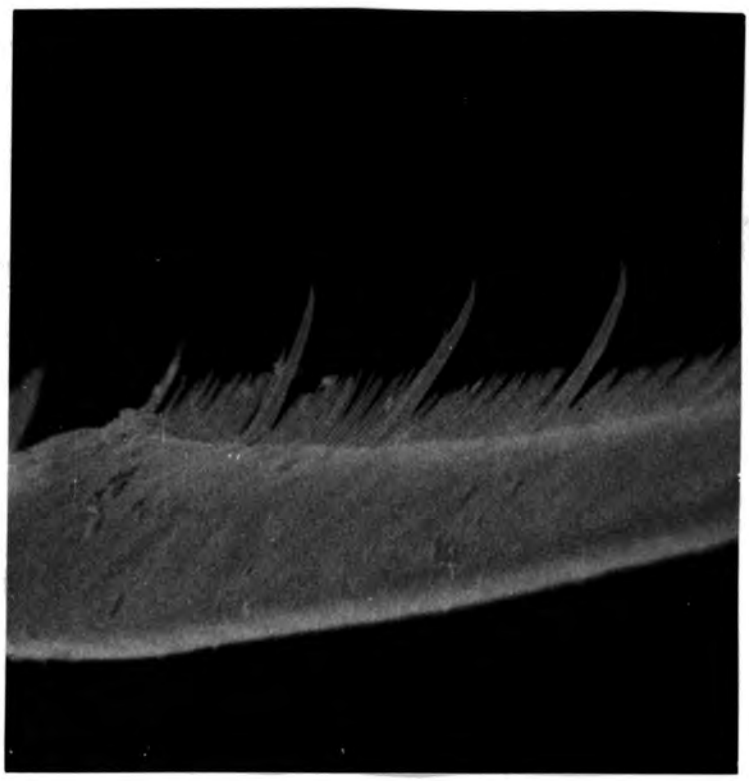


Fig. 25. Tip of a ray of S. ornatum.

X 2,000

Fig. 26. Side view of a ray of S. ornatum.

X 2,000

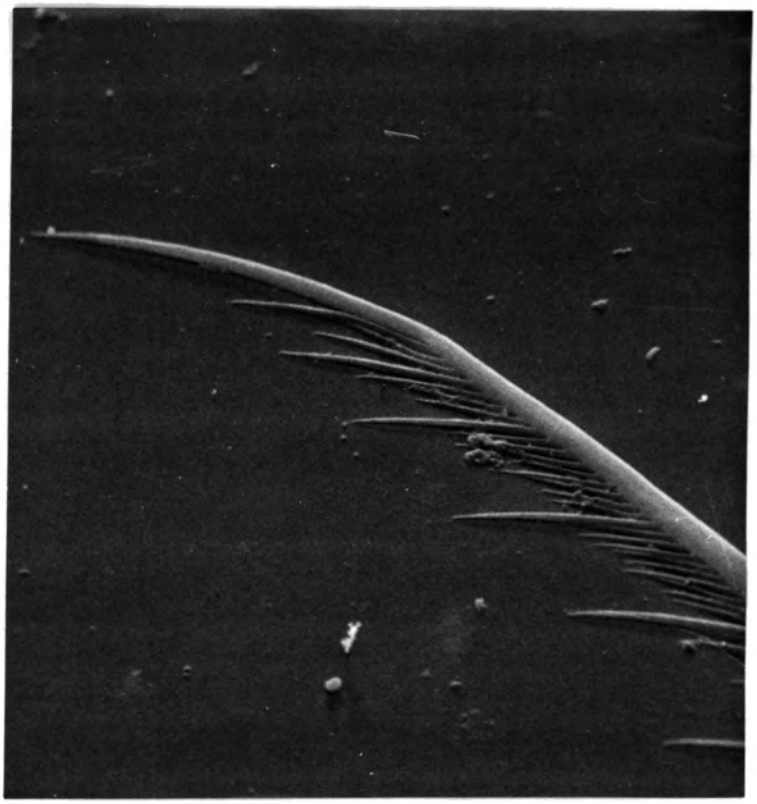
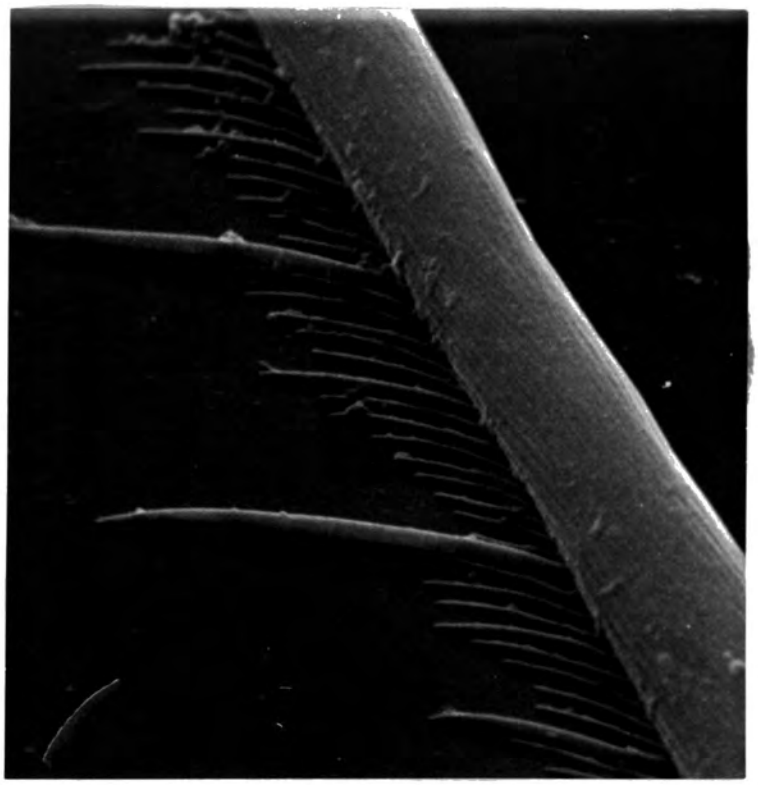


Fig. 27. Side view of a ray of S. ornatum.

X 5,000

Fig. 28. Tip of a ray of S. nitidifrons.

X 2,000



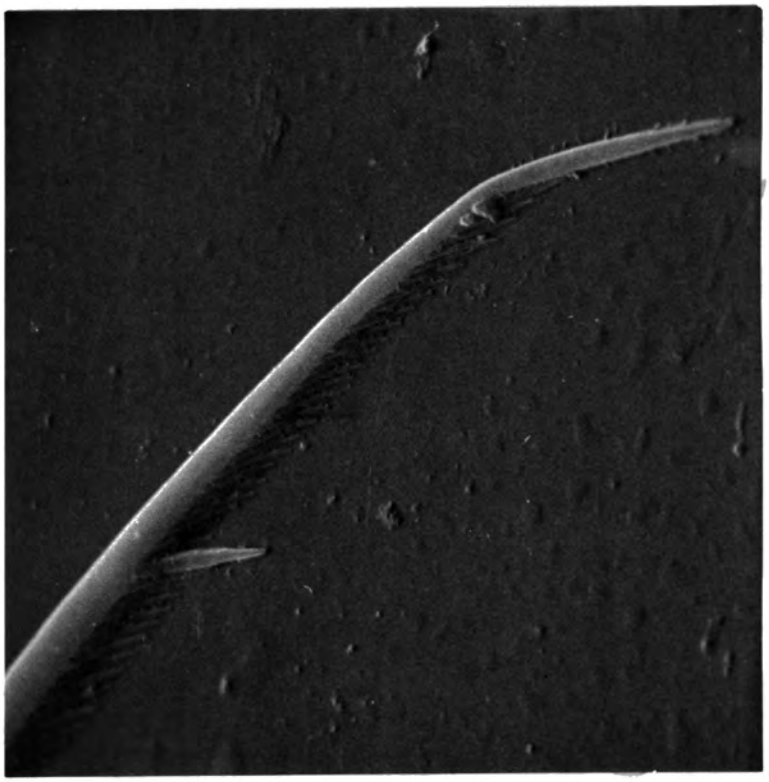
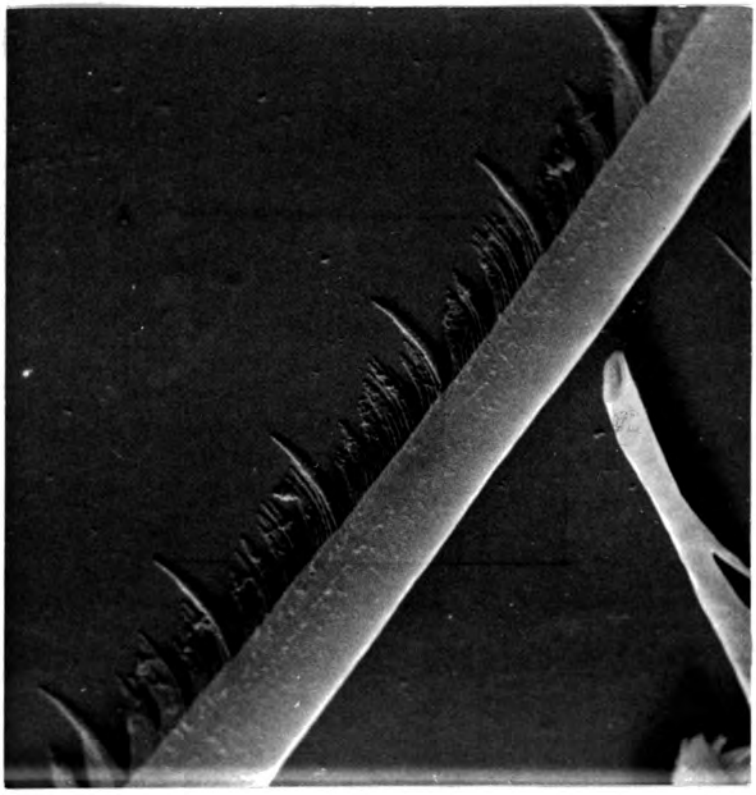


Fig. 29. Side view of a ray of S. nitidifrons.

X 2,000

Fig. 30. Side view of a ray of S. nitidifrons.

X 5,000



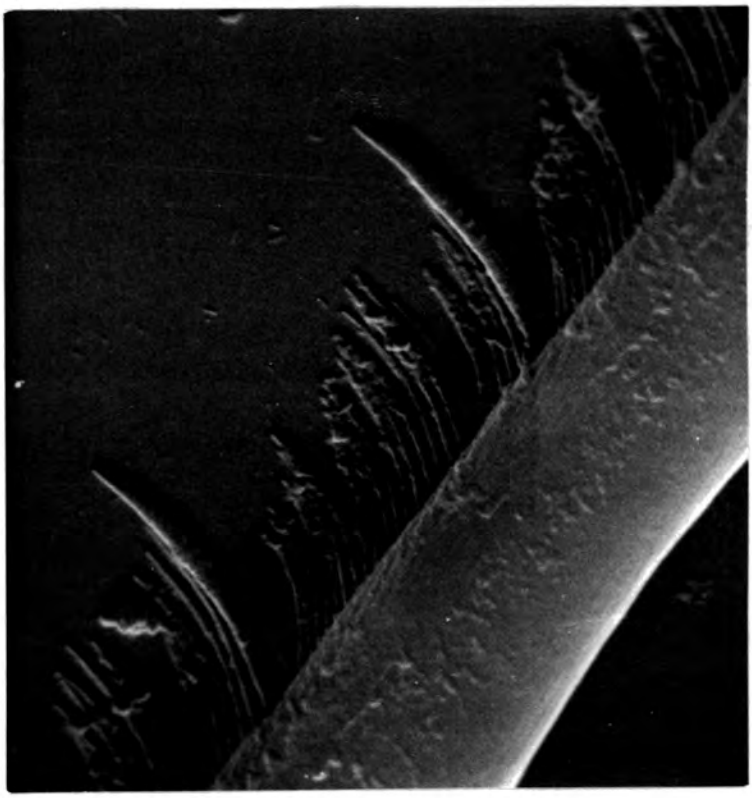
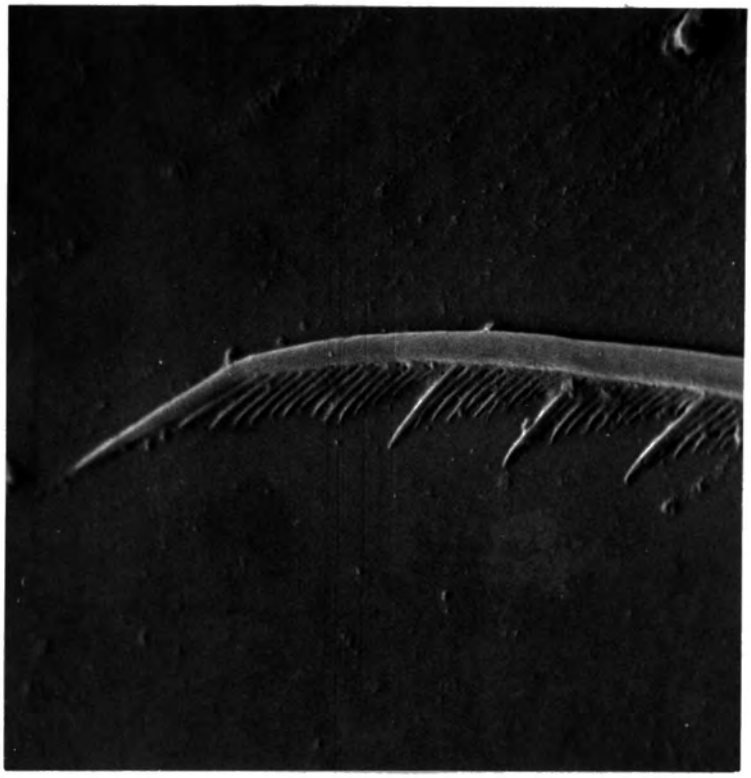


Fig. 31. Tip of a ray of S. spinosum.

X 2,000

Fig. 32. Side view of a ray of S. spinosum.

X 2,000



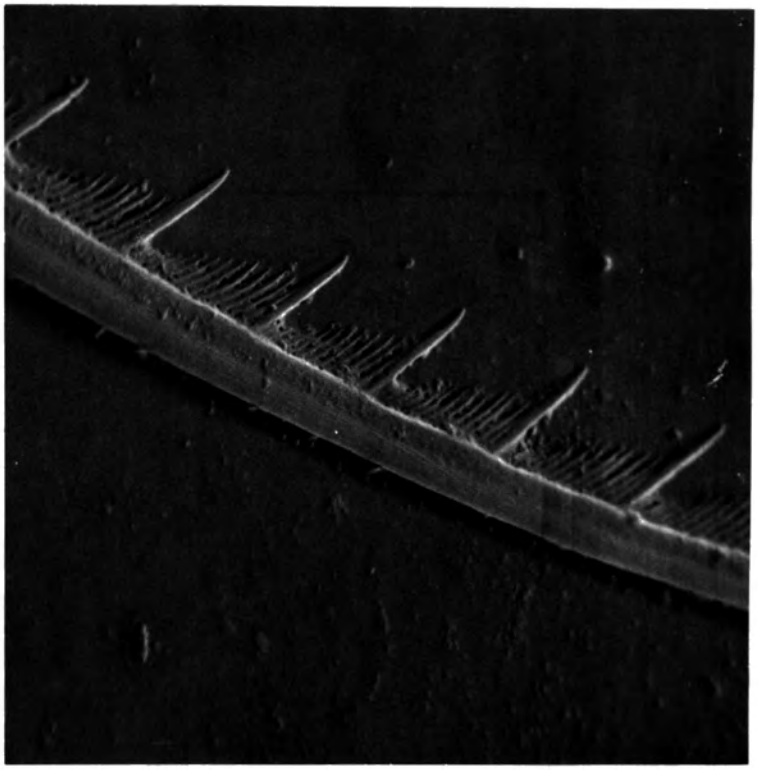


Fig. 33. Tip of a ray of S. variegatum (tip slightly deformed by the beam of the microscope).

X 2,000

Fig. 34. A number of rays of S. variegatum in side view.

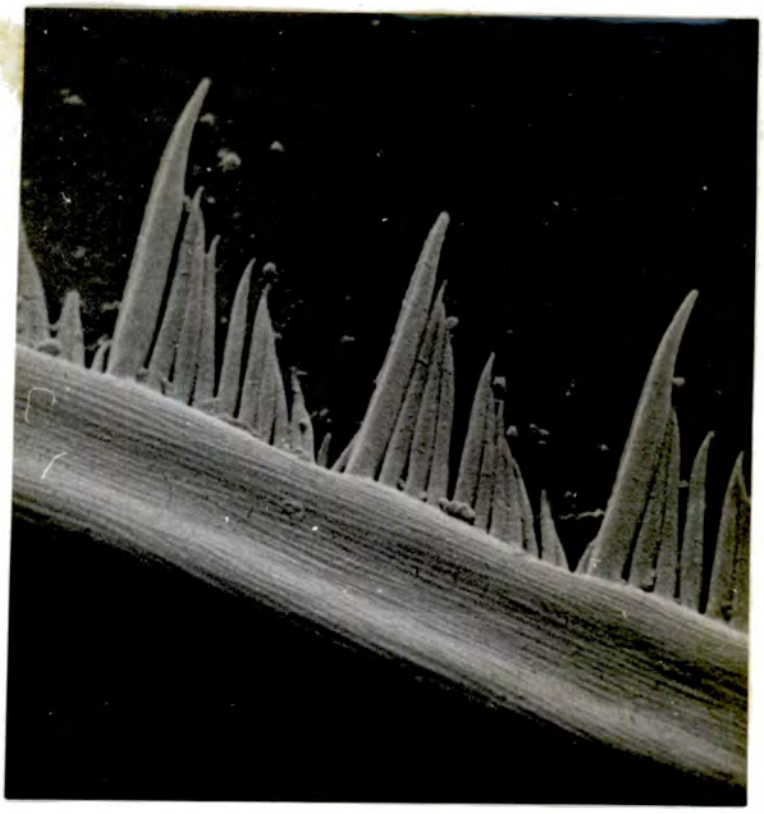
X 210

Fig. 35. Side view of a ray of S. variegatum.

X 5,000

Fig. 36. Side view of a portion of a ray of S. variegatum showing the spacing of the microtrichia.

X 20,000



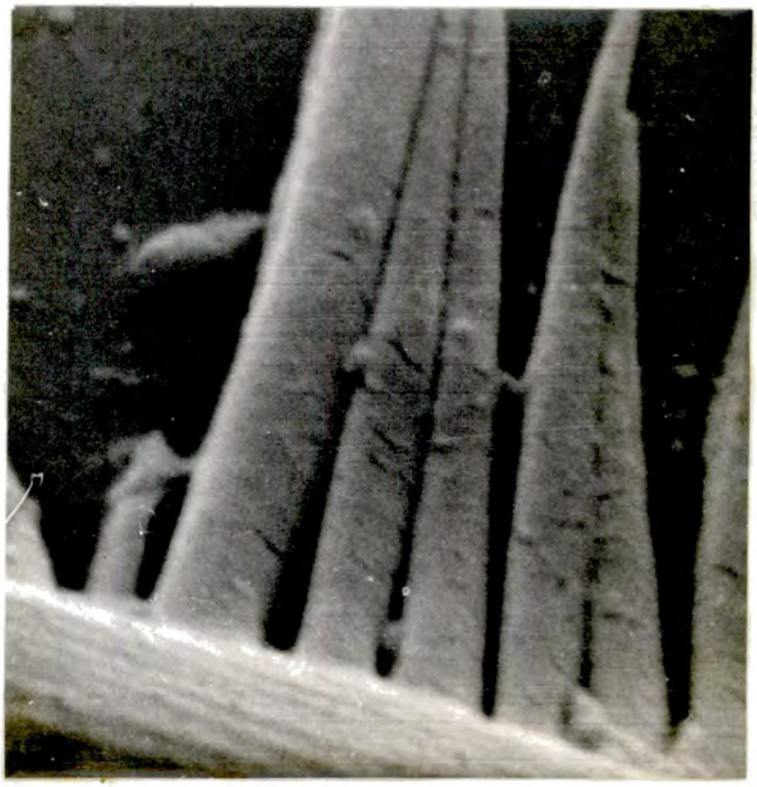


Fig. 37. Tip of a ray of S. monticola.

X 2,200

Fig. 38. Side view of a ray of S. monticola.

X 2,200

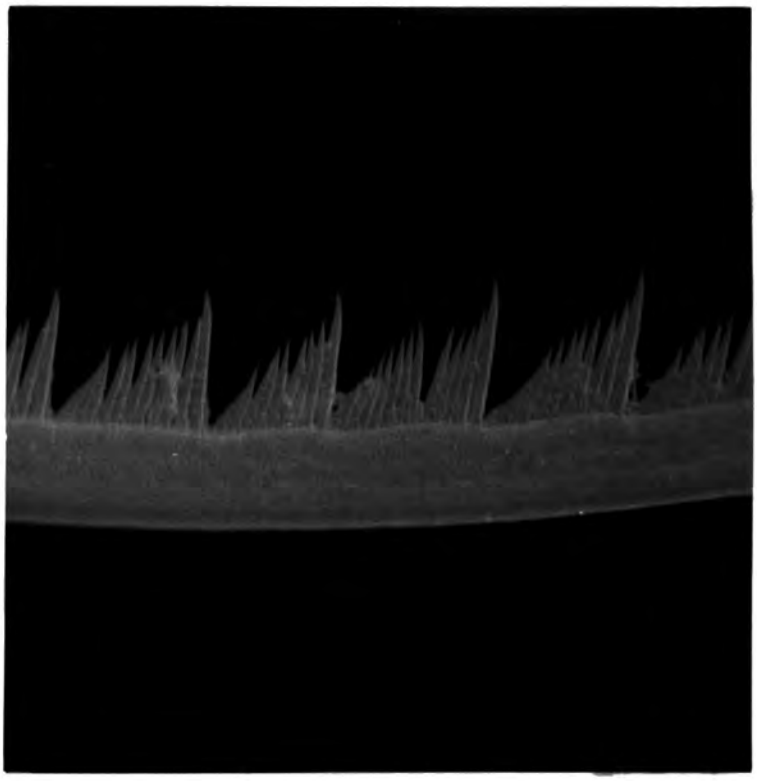


Fig. 39. Side view of a ray of S. monticola.

X 5,500

Fig. 40. Tip of a ray of S. reptans.

X 2,080

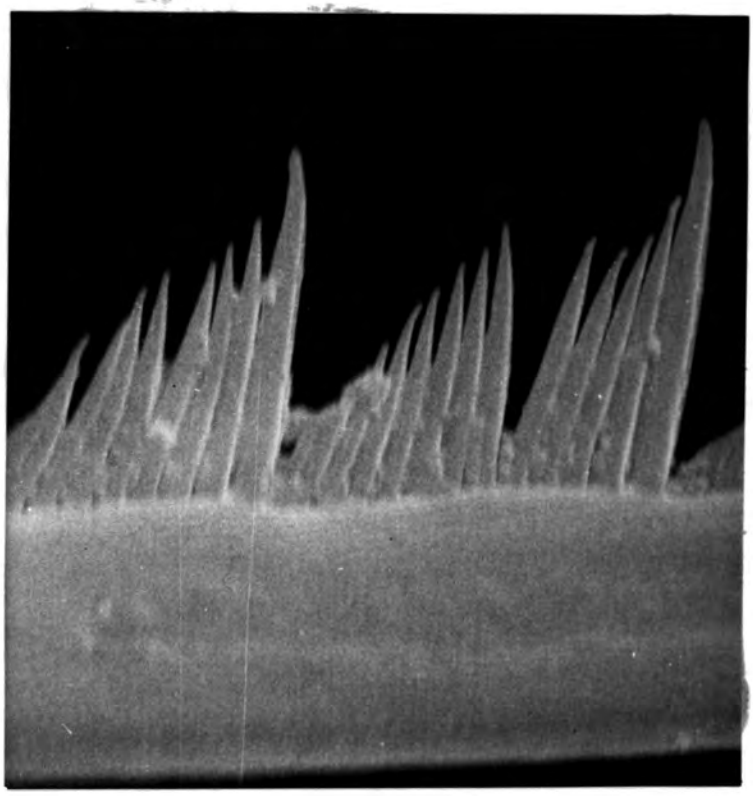


Fig. 41. Side view of a ray of S. reptans.

X 5,200

Fig. 42. Tip of a ray of S. tuberosum.

X 1,980

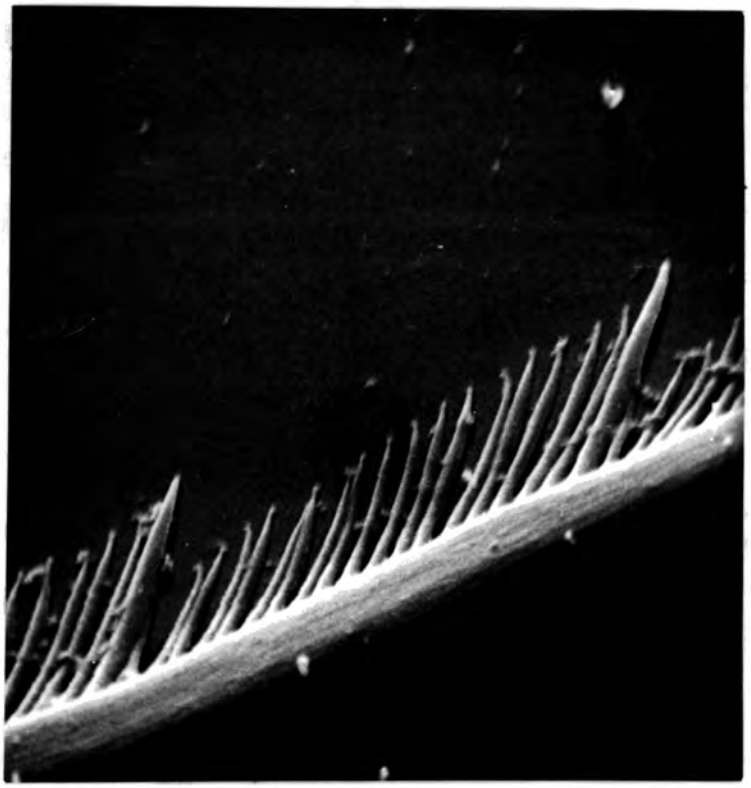


Fig. 43. Side view of a ray of S. tuberosum.
X 1,950

Fig. 44. Side view of a ray of S. tuberosum.
X 4,900

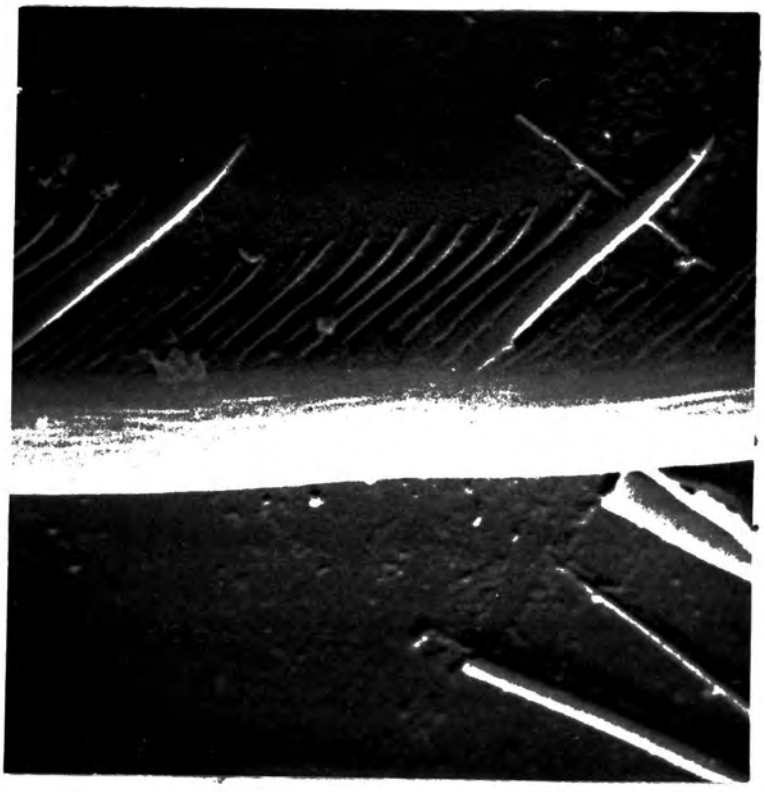


Fig. 45. Tip of a ray of S. equinum.

X 5,400

Fig. 46. Side view of a ray of S. equinum.

X 5,500

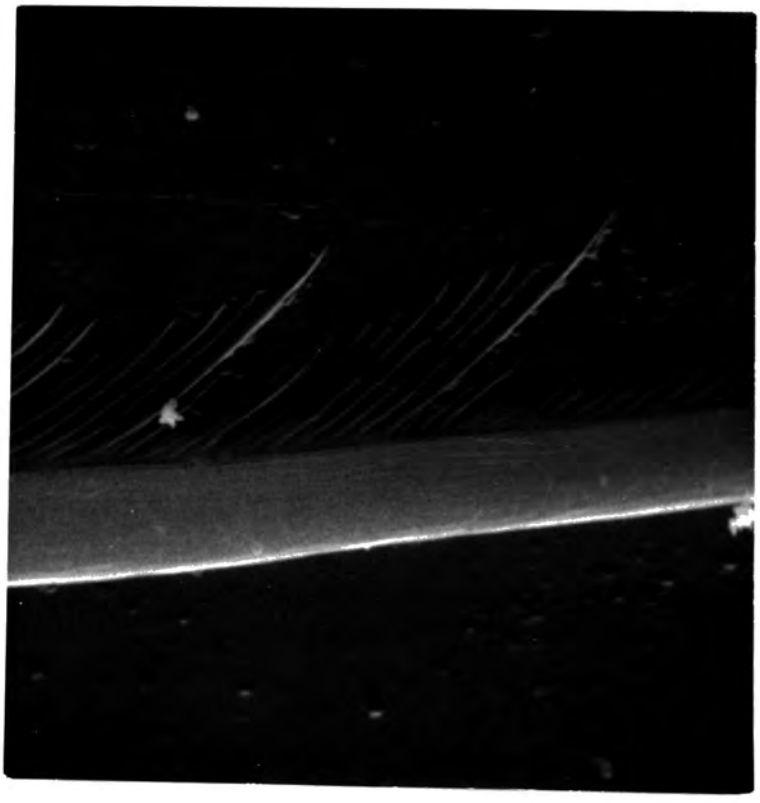
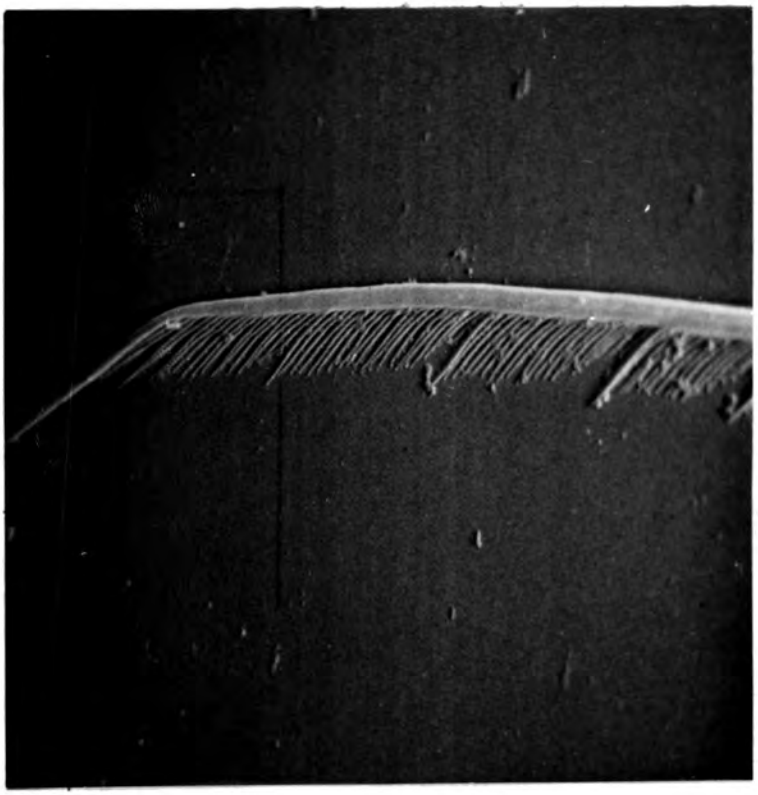


Fig. 47. Tip of a ray of S. salopiense.

X 2,200

Fig. 48. Side view of a ray of S. salopiense.

X 2,200



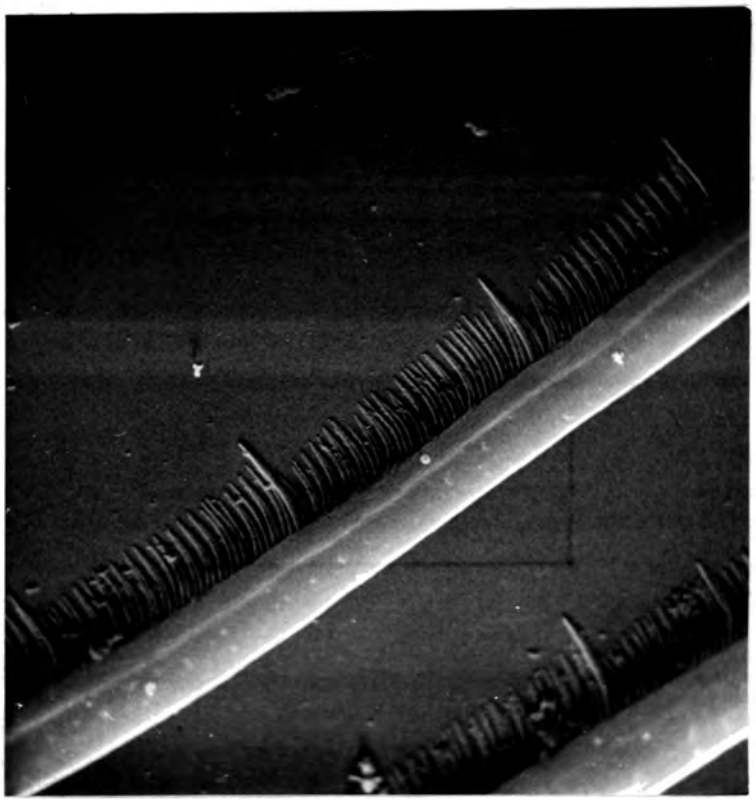


Fig. 49. Side view of a ray of S. salopiense.
X 5,500

Fig. 50. Tip of a ray of S. erythrocephalum.
X 2,200

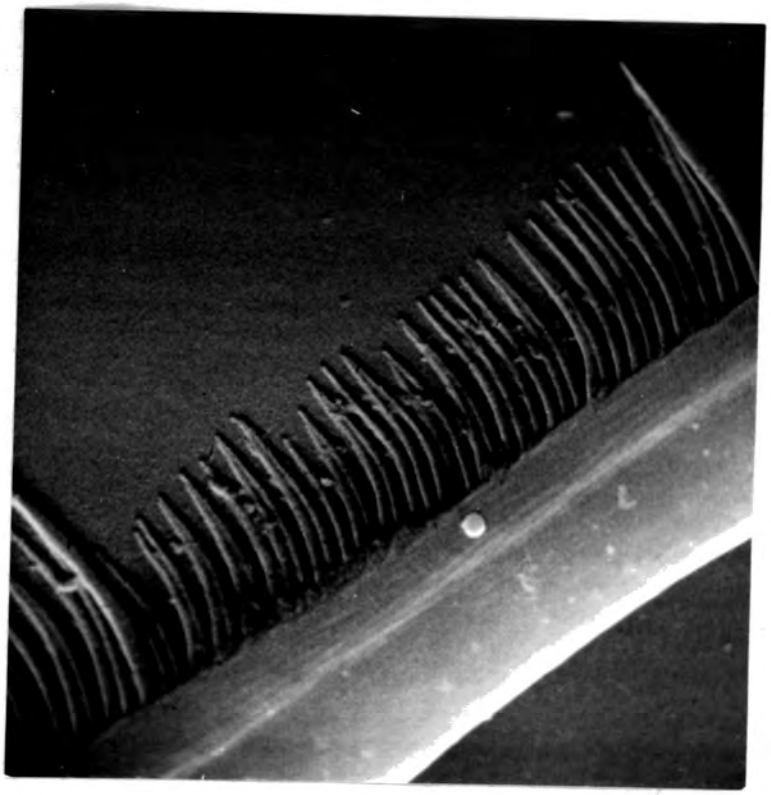
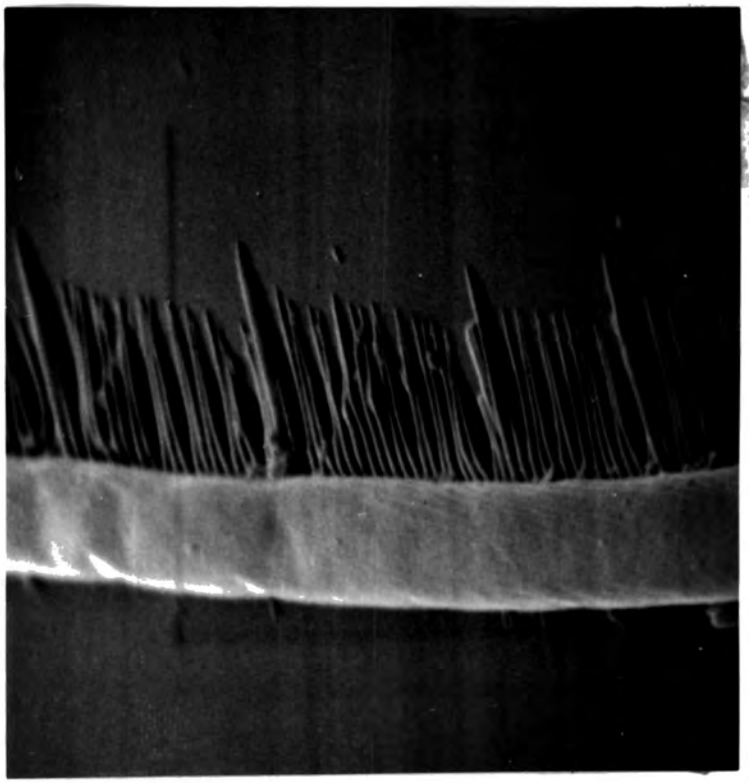


Fig. 51. Side view of a ray of S. erythrocephalum.

X 5,500

Fig. 52. Side view of a ray of S. erythrocephalum.

X 11,000



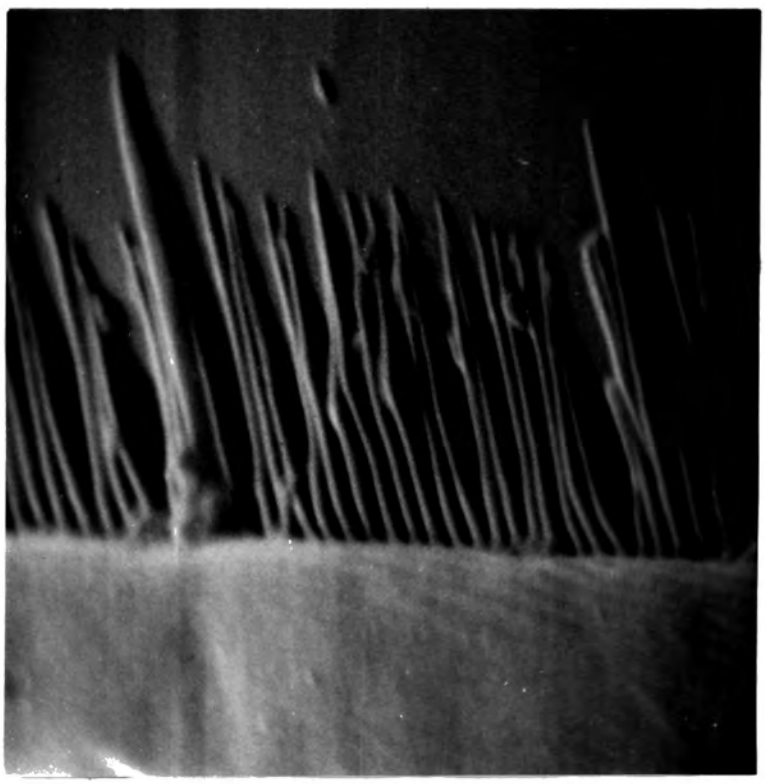


Fig. 53. Tip of a ray of S. argyreatum.

X 2,200

Fig. 54. Side view of a ray of S. argyreatum.

X 5,500

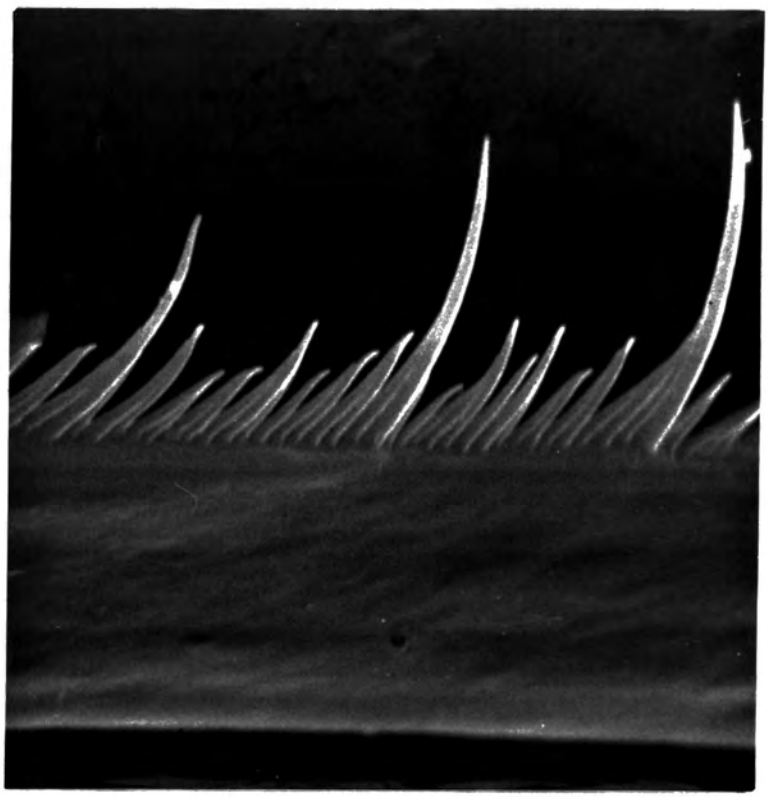


Fig. 55. Tip of a ray of S. subexcisum.

X 5,500

Fig. 56. Side view of a ray of S. subexcisum.

X 5,500

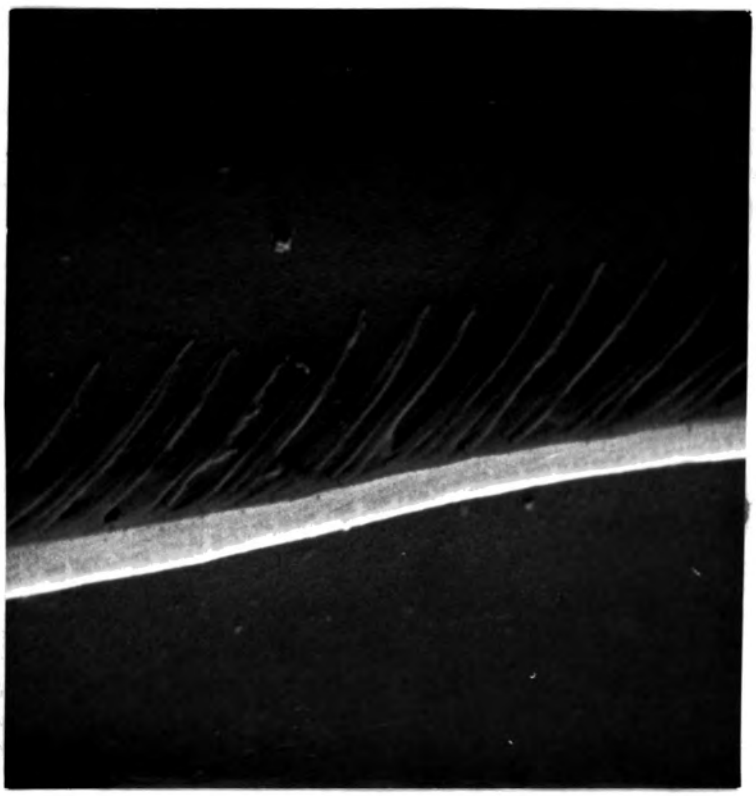
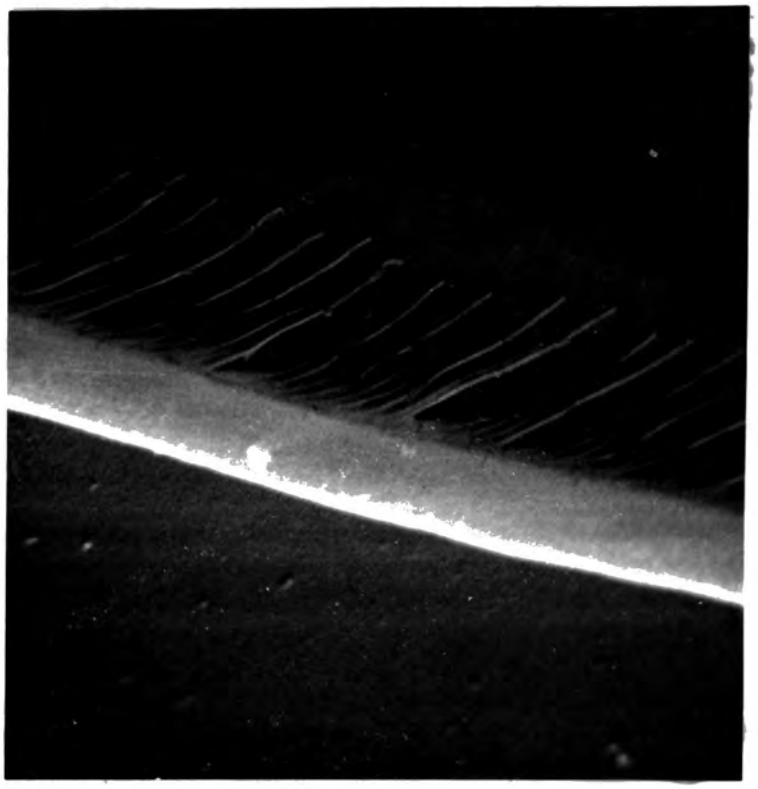


Fig. 57. Side view of a ray of S. subexcisum near the base.

X 5,500

Fig. 58. Tip of a ray of S. angustitarse.

X 5,000



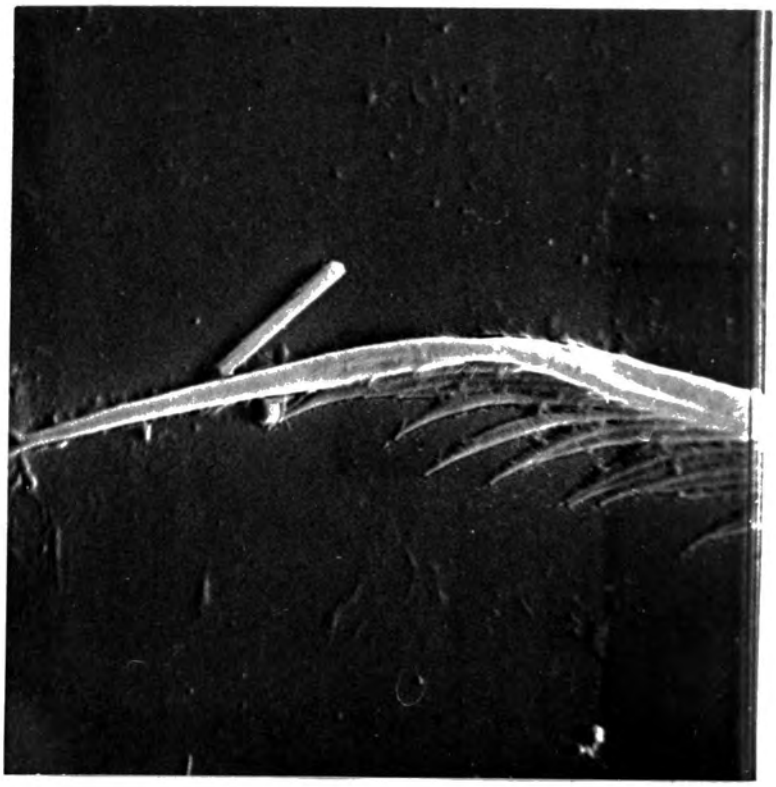


Fig. 59. Side view of a ray of S. angustitarse.

X 5,000

Fig. 60. View of the broken end of a ray of S. angustitarse.

X 11,000

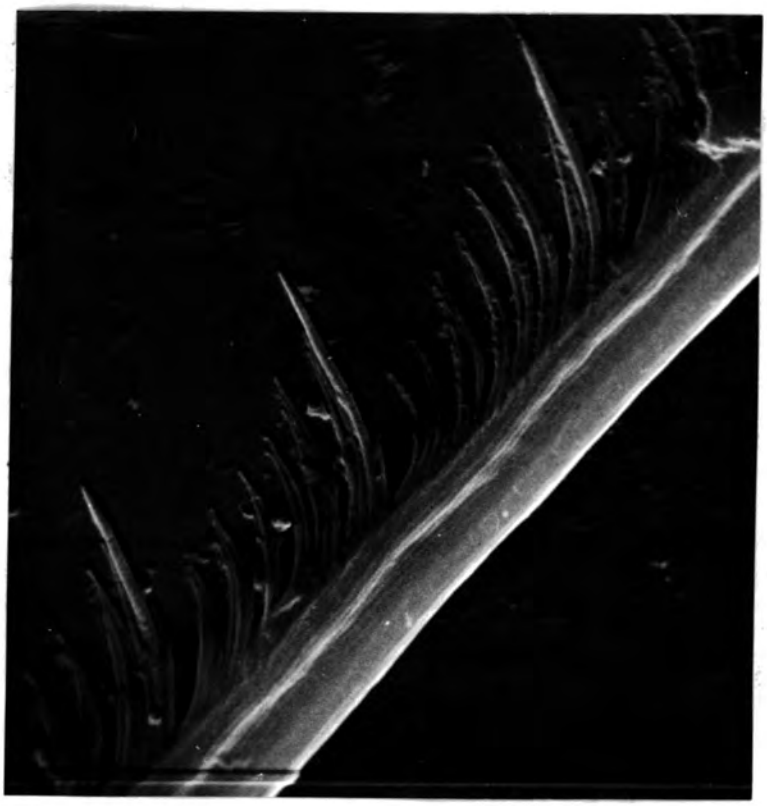


Fig. 61. View of the end of a broken ray of S. angustitarse.

X 22,000

Fig. 62. Tip of a ray of S. latipes.

X 2,000

Fig. 63. View of the side of a ray of S. latipes near the tip.
X 4,750

Fig. 64. Side view of a ray of S. latipes.
X 5,250

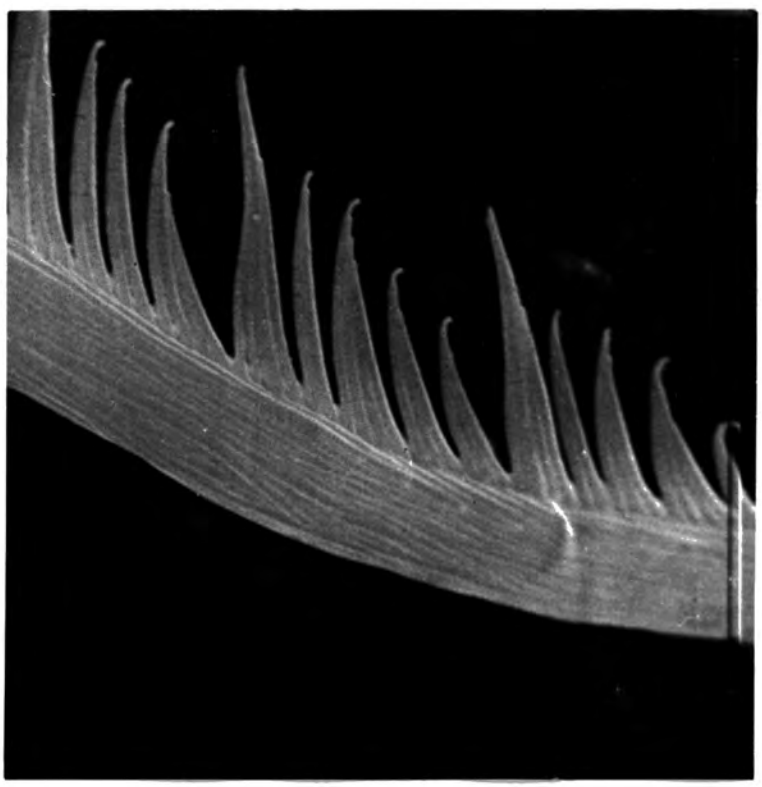


Fig. 65. Tip of a ray of S. brevicaule (tip bent due to the force of the electron beam).

X 2,000

Fig. 66. Side view of a ray of S. brevicaule. (The serrated edge on the microtrichia is an artifact.)

X 5,000

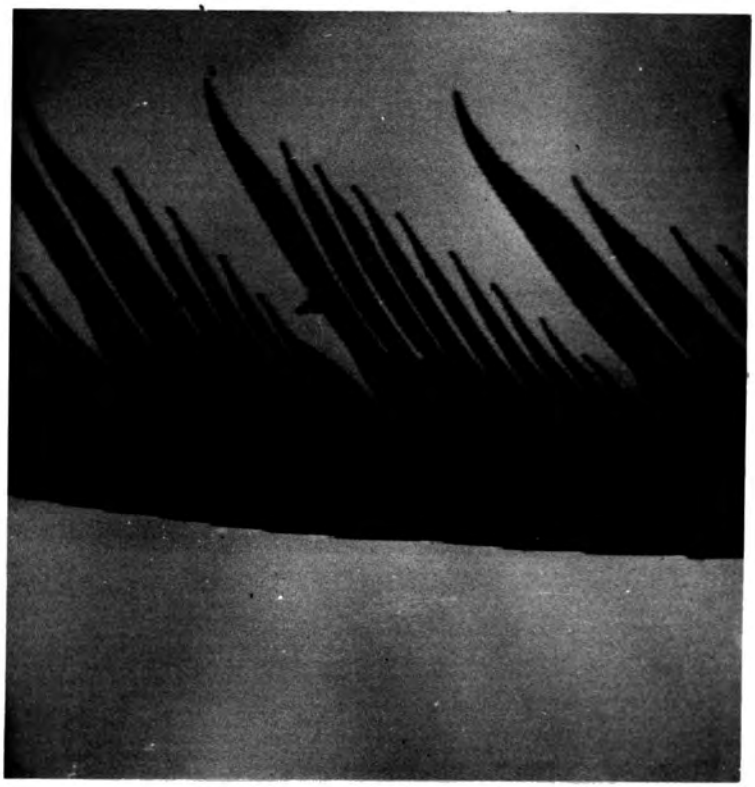


Fig. 67. Tip of a ray of S. armoricanum.

X 2,200

Fig. 68. Side view of a ray of S. armoricanum.

X 5,500

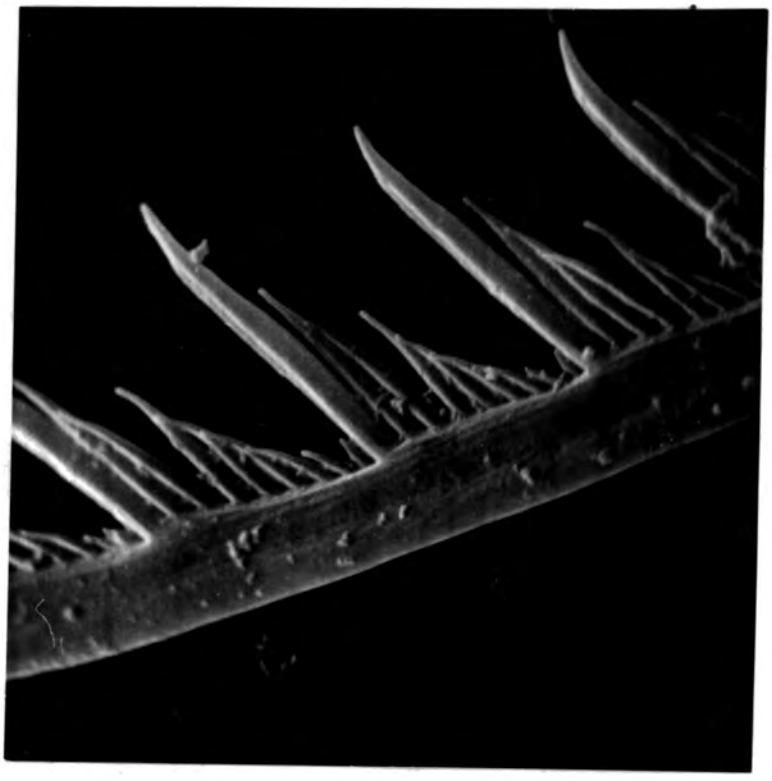


Fig. 69. Tip of a ray of S. costatum. (The tip has been bent by the electron beam.)

X 5,000

Fig. 70. Side view of a ray of S. costatum near its base.

X 5,000

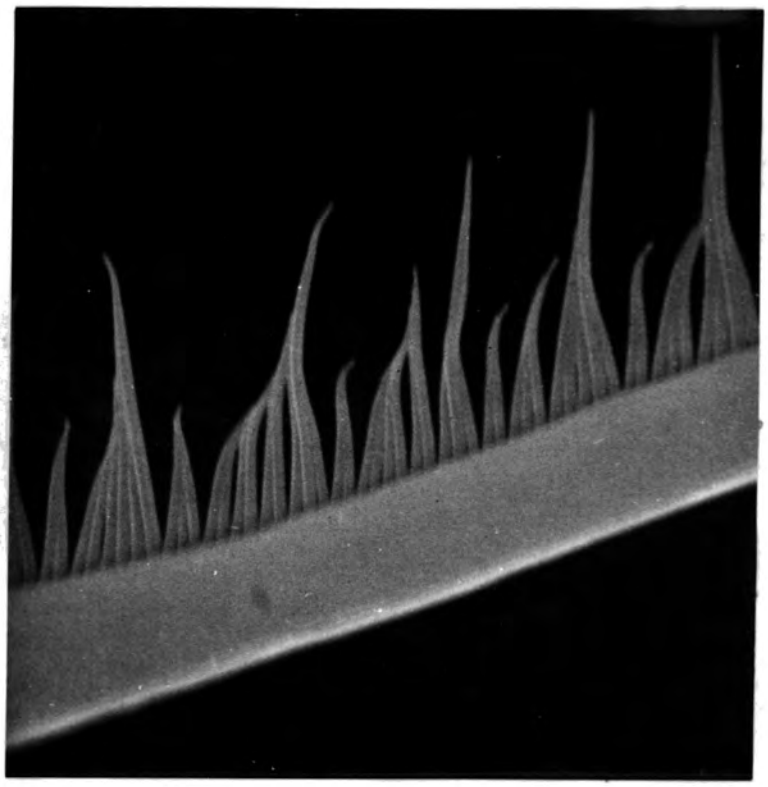
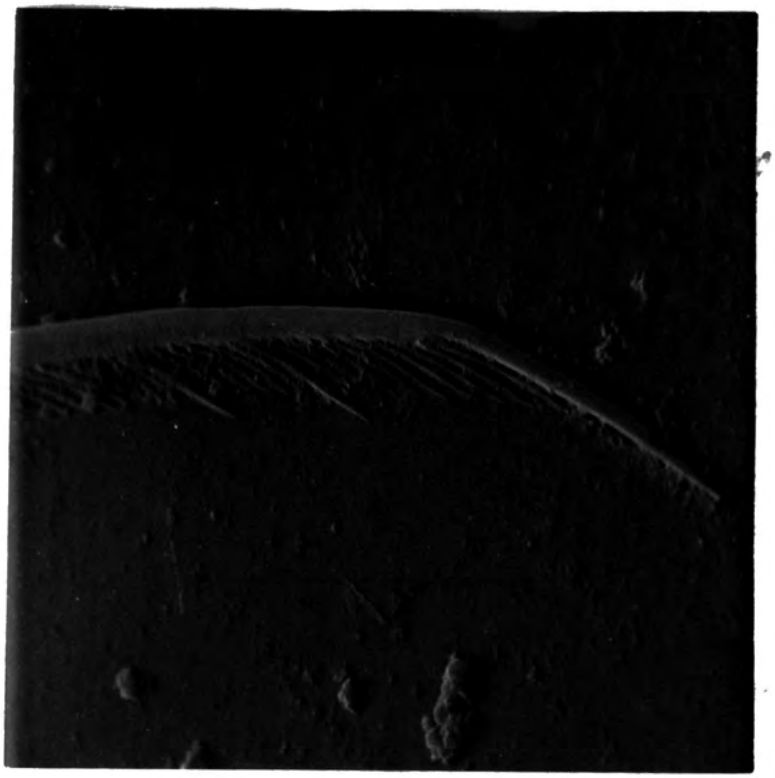


Fig. 71. Tip of a ray of S. venustum.

X 2,000

Fig. 72. Side view of a ray of S. venustum.

X 5,000



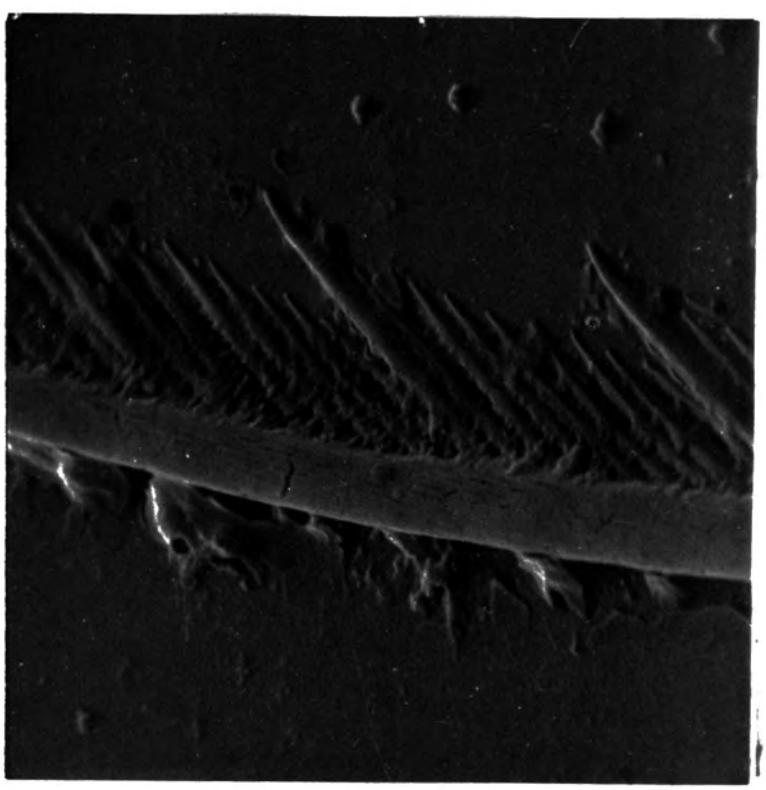


Fig. 73. Tip of a ray of S. rugglesi.

X 5,000

Fig. 74. Side view of a ray of S. rugglesi.

X 5,000

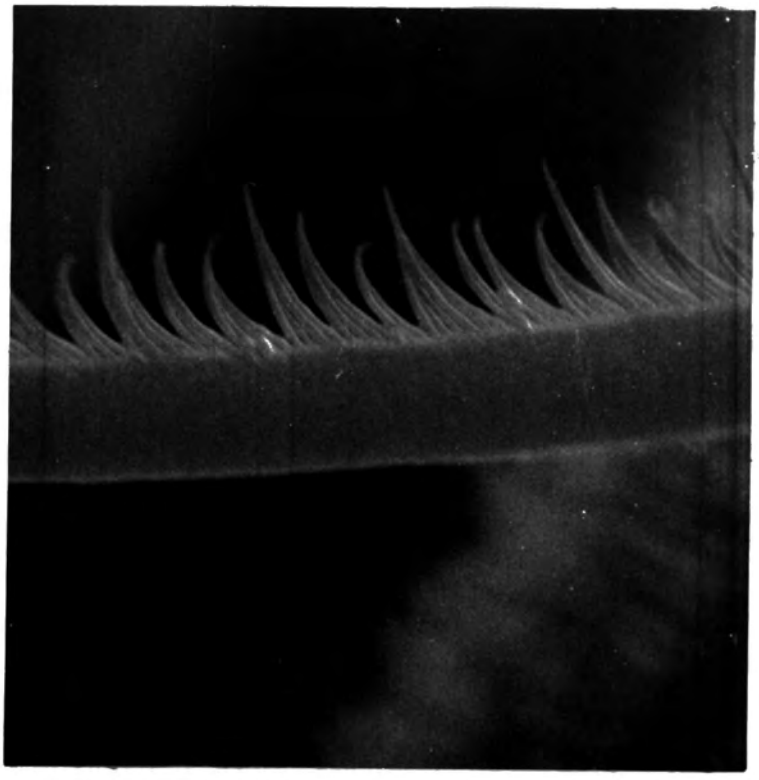


Fig. 75. Tip of a ray of S. longistylatum.

X 1,000

Fig. 76. Side view of a ray of S. longistylatum.

X 2,000

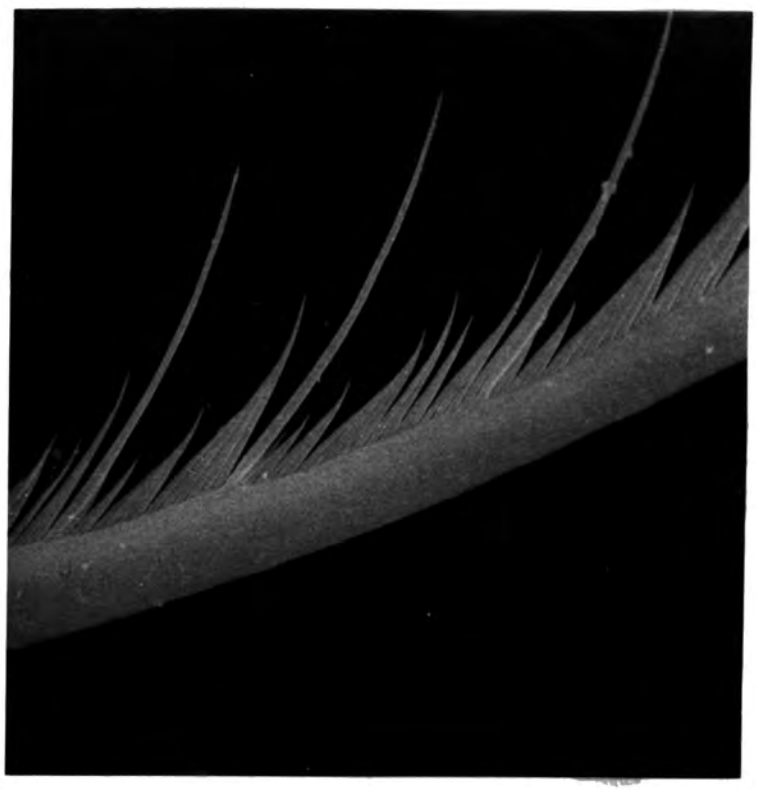


Fig. 77. Side view of a ray of S. longistylatum.

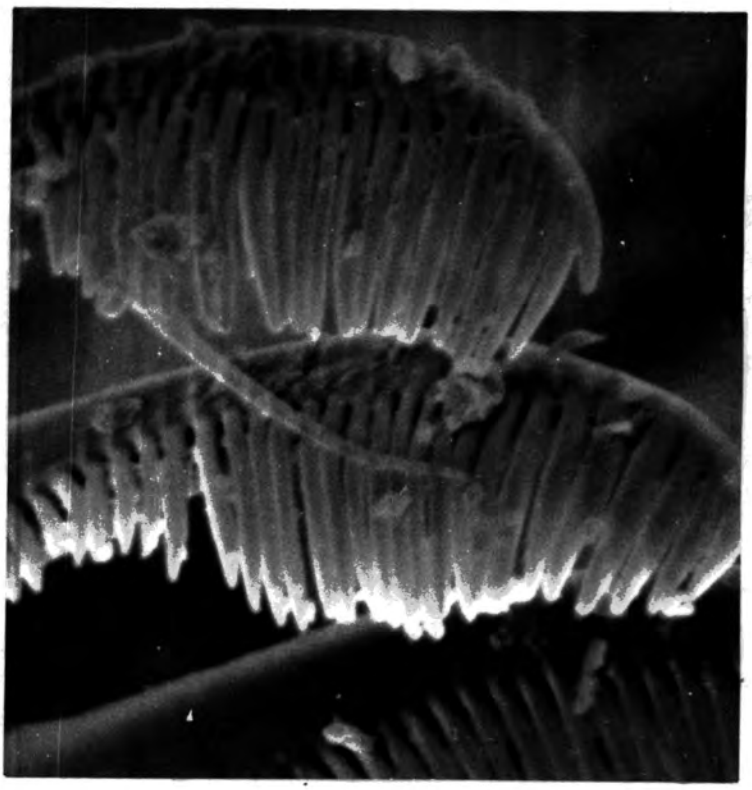
X 5,000

Fig. 78. Four rays of Crozetia crozetense.

X 2,000

Fig. 79. Rays of *Crozetia crozetense*.

X 5,000



d) The actions of the cephalic fans and mouthparts of filter-feeding black-fly larvae.

The major difficulty in studying the actions of the cephalic fans and mouthparts is the problem of observing the very rapid action of such extremely small structures. While the opening action of the cephalic fans can be induced in preserved larvae by squeezing the thorax with forceps (Wood 1963), the mandibles and maxillae do not respond to changes in internal body pressure and remain motionless.

Observation of live larvae in a small trough of slowly flowing water with the aid of a low powered stereoscopic microscope was similarly fruitless. While the opening and closing motions of the head fans could be seen, the motion of the mandibles and maxillae occurred too rapidly for their coordination with the cephalic fan movements to be observed. Even when small particles of carmine were added to the water the only certain observation was that these particles were trapped by the extended rays of the cephalic fans and then disappeared from the rays when the fan was flicked (rapidly closed and opened).

This rapid flicking action was considered by Fortner (1937) to be the feeding action. Chance (1970) found that the frequency of flicking was irregular and did not vary either between late or early instar larvae or between larvae with full or empty guts. The following study was performed to obtain cephalic fan flicking rates.

1) Observations on larvae in flowing water

Two experiments to study head fan movements in Simulium ornatum were conducted in the feeding apparatus (see Fig. 86).

In the first, the larvae were kept at a temperature of 10.5°C. and the water velocity was 31.3 cm./sec. After being established for ten minutes, the number of head fan movements per minute for two consecutive minutes was recorded for five larvae. Then enough yeast-carmine mixture was added to the water to give a standard concentration of .375 gm. yeast and .0375 gm. carmine per 6000 ml. of water. After ten minutes to allow the larvae to become accustomed to the changed conditions, the number of head fan movements per minute for two minutes was recorded for eight larvae. (Several more larvae had moved within range of the microscope used to watch the fan movements). The number of fan flicks per minute for larvae in distilled water and in distilled water with food are given in Table 13.

TABLE 13

Results of experiment showing the rate of head fan flicking of S. ornatum larvae in the presence and absence of food at a temperature at 10.5°C. and a current velocity of 31.3 cm./sec.

	Larva No.	Flicks in 1st. minute	Flicks in 2nd. minute
No food	1	30	29
	2	21	7
	3	21	24
	4	5	10
	5	23	26
	Total	100	96
	Mean of each min.	20	19.2
	Overall mean		19.6 ± 8.58
With food	1	33	31
	2	19	14
	3	25	33
	4	43	43
	5	20	23
	6	30	23
	7	43	35
	8	21	25
	Total	234	227
	Mean of each min.	29.3	27.5
	Overall mean		28.8 ± 8.78

It will be observed that the number of beats per minute is quite variable both in the absence and presence of food.

A second experiment was conducted where the rate of head fan movements was recorded in both distilled water and in distilled water with the standard yeast-carmine mixture at velocities of 31.3 cm./sec., 54 cm./sec. and 70 cm./sec. The water temperature was 11.0°C. The results of this experiment are presented in Tables 14 and 15.

TABLE 14

Rate of fan flicking of S. ornatum larvae in distilled water at a temperature of 10.5°C. and at three current velocities

Velocity cm./sec.	Larva No.	Flicks in 1st. minute	Flicks in 2nd. minute
31.3	1	13	11
	2	20	26
	3	9	11
	4	11	14
	5	19	14
	Total	72	76
	Ave. of each Min.	14.4	15.2
	Overall Mean		14.8 ± 5.016
54	1	36	22
	2	14	10
	3	3	9
	4	3	2
	5	15	6
	Total	71	49
	Ave. of each Min.	14.2	9.8
	Overall Mean		12.0 ± 10.0
70	1	15	15
	2	30	25
	3	14	8
	4	19	26
	5	16	19
	Total	94	93
	Ave. of each Min.	18.8	18.6
	Overall Mean		18.7 ± 6.26

TABLE 15

Rate of fan flicking of S. ornatum larvae in distilled water containing yeast and carmine particles at a water temperature of 10.5°C. and at three current velocities

Velocity cm./sec.	Larva No.	Flicks in 1st. minute	Flicks in 2nd. minute
31.3	1	12	2
	2	5	7
	3	3	0
	4	3	7
	5	3	6
	Total	26	22
	Ave. of each Min.	5.2	4.4
	Overall Mean		4.8 ± 3.209
54	1	11	13
	2	2	3
	3	36	35
	4	22	25
	5	11	19
	Total	82	95
	Ave. of each Min.	16.4	19.0
	Overall Mean		17.7 ± 10.87
70	1	45	31
	2	16	18
	3	20	14
	4	1	20
	5	25	11
	Total	107	94
	Ave. of each Min.	21.4	18.8
	Overall Mean		20.1 ± 11.26

It will be noted that the standard deviation from the mean in these experiments was quite large. This suggests that there was considerable individual variation in the cephalic fan flicking rate. Chance's (1970) contention that flicking rate is irregular was borne out by these observations. This irregularity of flicking has led Chance (1970) to propose that food particles are scraped from the closed cephalic fans by the mandibles when the fans are closed for extended periods of time.

If food transfer from the cephalic fans to the mandibles occurs as Chance (1970) has proposed there is then no explanation for the flicking action. In the views of Fortner (1937) and Grenier (1949) the flicking of the cephalic fan is the feeding action. Chance (1970) observed particles being moved by the closing part of the flicking action as did this author (see above).

In order to analyse the exact motions of the cephalic fans and mouthparts during the flicking action, high speed motion pictures were taken of S. longistylatum larvae.

ii) Methods and materials for photographic observations

S. longistylatum larvae (which were chosen because of their large size) were placed in a sheet glass trough 60 cm. long, 3 cm. wide and 6 cm. deep and closed at one end. Tap water at 10°C. was pumped in at the closed end of the trough at 50 cm./sec. Larvae were kept at 10°C. in tap water in a container provided with bubbling compressed air until placed in the flowing water of the trough with fine curved forceps.

Larvae were photographed at 64 frames/sec. with a camera equipped with an automatic exposure meter and extension tubes between the lens and camera. Two 500 watt floodlights, each a distance of 1 metre from the observation trough, were used. Due to the intense heat from the lamps, exposures were limited to 10 to 15 seconds running time.

iii) Results of photographic analysis

Analysis of the films of S. longistylatum larvae a single frame at a time under ten power magnification revealed that the cephalic fans were found in three characteristic positions or states. These were:

1. Cephalic fans closed and retracted.
2. Cephalic fans opened or extended fully.
3. Cephalic fans engaged in a flicking action.

Calculating from the number of motion picture frames per second (in this case 64), it was possible to calculate the time required for actions of the cephalic fans and mandibles. The following descriptions of mouthpart movements during each of the three characteristic positions are composite observations of a number of larvae.

1. Cephalic fans fully closed and retracted.

In this position the rays of the cephalic fans were brought together so that each fan appeared as a single scythe-like "blade". This blade was held so that the congregated tips of the rays were inserted into the mouth and the fan stalks and the bases of the rays appeared as a pair of protuberances on the head. The congregated rays or "blades"

were drawn tightly into the spaces between the labrum and each mandible. The mandibles moved along a line of articulation between the fan stalks and the hypostomium. The closed and retracted fans lay along these lines and just medially to them.

When the cephalic fans were in this position larvae were either searching for a new position, their head weaving from side to side with the maxillae extended, or were engaged in passing the mandibles over the convex or outer surface of the "blades" of fan rays. Silk spinning was not observed but the extrusion of a small blob of silk as a hold fast for the looping method of locomotion was noticed. Larvae placed this blob of silk and then grasped it with the maxillae and released their hold on the substrate with the anal disc. After moving the anal disc to a new attachment site on the same blob of silk, the maxillae were withdrawn from the silk. This use of the maxillae for locomotion may explain Chance's (1970) suggestion that the maxillae do not assist in filtering or combing food but have a similar function in all species including those without head fans.

The mandibles were, as noted above, occasionally passed to and fro over the convex or outer surface of the congregated rays or "blades" of the cephalic fans. Chance (1970) considers this to be a combing action to remove food particles from the rays. It seems unlikely that food particles would collect on the smooth convex surface of the rays but much more likely that food particles would collect on the microtrichia on concave surfaces of the rays. Brushing the "blades" away from the mouth would move any food particles away from it. Any

other purpose for the to and fro brushing of the convex surface of the retracted rays of the cephalic fans was not observed. However, if this action is a food gathering one, it goes on at very infrequent intervals.

2. Cephalic fans fully extended.

In this position the rays of the primary fans were spread out in an arc at either side of the head. Each arc varied from approximately one half to three quarters of a complete circle; the plane of the circle was roughly perpendicular to the direction of the current flow. In the observation trough such an angle was difficult to determine exactly due to the effect of the side walls.

The mandibles opened and closed frequently while the fans were held extended. The closing or opening action each required about .04 seconds. When closed, the mandibles met each other medially over the mouth; when opened, each mandible was drawn back close to the base of the fan stalk. The mandibles appeared to always work simultaneously. When closed, they appeared to be forcing food into the mouth. During this motion the maxillae do not appear to make any discernible movement (definition of them on the film was not as good as for the mandibles). However, the maxillary palps were noted to move quite rapidly in a dorso-ventral direction.

3. Cephalic fans engaged in a flicking action.

Just before the start of a fan flick it was noted that both mandibles were held open in a position at the base of the cephalic fan

stalk. They were held quite stationary in this position for several seconds, whereas if the fan was not to be flicked when a mandible was extended, then it was quickly closed again.

Cephalic fans were closed singly although the mandibles moved together. A single fan was often flicked several times in succession before the other fan was used.

A cephalic fan first began to close with a drawing together of the rays. The rays at each end of the fan closed with the rays at the centre of the fan. When these rays were closed together to the extent that the fan appeared as a solid scythe-like "blade" the tips of the rays were brought towards the mouth. The rays continued to be drawn together as the inward motion of their tips began. The rays when closed together formed a curved trough-like blade due to the placement of the bases of the rays around the fan stalk. The concave surface of this trough contained the interior edge of the rays upon which are found the microtrichia. About 0.05 of a second after closing of the fan rays began the rays had completely closed into troughs and their tips had reached the mouth. The fans remained in this position in which only the tips of the fan rays touched the mouth until about 0.13 seconds after closing started. During this period the maxillae were held close to the hypostomium and ventral to the mouth so that the tips of the rays passed dorsally to them.

At about 0.11 seconds after closing started the mandible began to move from its original position at the base of the fan stalk.

It travelled in a ventral and medial direction along the line of the inside of the trough formed by the closed rays of the cephalic fan. The cover bristles of the mandible appeared to brush the inside of this trough. This movement of the mandible was very rapid; it took only 0.03 seconds. Just before the mandible closed, the tip of the fan "blade" began to move away from the mouth. The mandible began to close about 0.02 seconds before the tip of the fan blade began to move away from the mouth.

The opening of the cephalic fan required a much shorter time than the closing action. (The opening action is performed by the internal body pressure of the larva and is no doubt aided by the water currents). The closing action of the cephalic fan required 0.05 seconds while the opening action took about 0.03 seconds. The entire flicking action lasted about 0.15 sec. After the fan had opened fully the mandibles were noticed to open and close rapidly several times as if forcing food particles into the mouth.

The cephalic fan was never fully retracted in this action; the tips only of the rays were brought down to touch the mouth. This holding of the rays in an elevated way allowed the cover bristles on the mandible to reach the concave side of the "blade" of rays and scrape them once in a closing movement.

iv) Discussion of photographic analysis

The described coordinated action of the cephalic fans and mandibles suggests very strongly that flicking is indeed the food-

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gathering action. Chance's (1970) proposal that retracted fans are cleaned on their convex surfaces by the mandibles would appear now to be incorrect. The cleaning of the concave surfaces of the fan rays with their large numbers of microtrichia by a single mouthward sweep of a mandible seems a much more logical feeding action. The microtrichia of the rays of most species are very closely spaced (as we have seen) and certainly are capable of retaining particles as small as bacteria (Fredeen 1960,1964).

The variations in the patterns of microtrichia were discussed earlier in this section of the thesis. It was suggested there that the various patterns of microtrichia on cephalic fan rays of different species might have ecological significance.

If one particular pattern was more effective at retaining particles as the cephalic fan closed than another pattern, then the species with the first pattern would be at an advantage and would eventually supplant the other species. While we have species of black-flies whose larvae are widely distributed we also find many species whose larvae have more restricted habitats. Black-fly workers have long known that particular types of stream are suitable for a particular species.

If these patterns of microtrichia have any effect on determining the habitat of larvae it is certainly through their action on food-gathering.

The very strong rays and microtrichia of the larva of P. ferrugineum prompted a study of the contents of the mid-guts of a

number of larvae of this species.

The remainder of this thesis deals with the rate of food-gathering by larvae of several species.

4. THE FOOD OF Prosimulium (Helodon) ferrugineum

During general collecting for black flies in Eastern Norway, numbers of larvae of Prosimulium (Helodon) ferrugineum Whalb. were collected. Puri (1926) noted that the rays of the cephalic fans of this species were quite heavy in structure and had thickened spear-shaped tips. This characteristic was most noticeable in scanning electron micrographs.

a) Methods and Materials

In order to determine if the unusual cephalic fan ray form was reflected in the diet of P. ferrugineum, a number of larvae from various collecting sites were dissected. The mid-gut of each larva was removed, its contents gently teased apart and then mounted in Euparal mountant on a standard 3" x 1" microscope slide. Slides were examined under a compound microscope.

The animal contents of each gut were classified as far as possible into Chironomid larvae, Simuliid larvae, water mites and mayflies or stoneflies (i.e. legged insects). The classification "insect tissue" includes remains not identifiably belonging to the other classifications. Fifty-one P. ferrugineum larvae were selected from six samples taken from five locations. Since it was possible that other species of black-fly larvae from the same habitats were feeding on other insects, Cnephia spp. larvae from samples number four and six were examined also.

b) Results

The results, giving the numbers of animals ingested by P. ferrugineum larvae are given in Table 16. The mid-gut contents of each larva are given in Appendix II.

Sample 1, collected from the Renåa River above Renåvangen Motel (Map Reference 32VPP276430) was made on the 18 June, 1967. Ten P. ferrugineum larvae were examined. Of these ten larvae, all ten contained insect tissue - nine contained midge larvae, two contained mites and one contained a black-fly larva.

Sample 2 was collected from the Flena River (Map Reference 32VPP177386) near its mouth on the 27 June, 1967. Of five P. ferrugineum larvae examined, all five contained midge larvae, four contained simuliid larvae (one Cnephia spp., one Gnus spp., two unidentifiable) and two contained may-flies or stoneflies.

Sample 3 was collected from the Flena River (Map Reference 32VPP263303) near Flenbua, on the 27 June, 1967. Of ten P. ferrugineum larvae examined, all ten contained insect tissue, six contained midge larvae, ten contained simuliid larvae and one contained the remains of either a mayfly or stonefly.

Sample 4 was collected from the same location as Sample 3 but on the 3 July, 1967. Of ten P. ferrugineum larvae examined, all contained insect tissue, seven contained midge larvae, eight contained midge larvae, eight contained simuliid larvae and one contained a mite.

Sample 5 was collected from the Flena River (Map Reference 32VPP286271) near Flendammen on the 18 July, 1967. Of six P. ferrugineum

TABLE 16

The number of P. ferrugineum larvae examined and the number of arthropods found in their mid-guts

Sample	1	2	3	4	5	6	Totals
P. ferrugineum examined	10	5	10	10	6	10	51
Chironomid Larvae	23	11	13	16	2	25	90
Simuliid Larvae	1	4	15	15	5	3	43
Insect Tissue	18	9	3	14	3	10	57
Mites	2	0	0	1	2	1	6
Ephemeroptera or Plecoptera	2	3	2	3	3	3	16

larvae examined, four contained insect tissue, two contained mites, two contained midge larvae, two contained either mayflies or stoneflies and three contained simuliid larvae.

Sample 6 was collected from a tributary of the Flena R. called Bekkevodbekken at Map Reference 32VPP258309 on the 18 July, 1967. Of ten P. ferrugineum larvae examined, ten contained insect tissue, eight contained midge larvae, three contained simuliid larvae and one contained a mite.

None of the ingested black-fly larvae were of the species P. ferrugineum. The intestines of most of the P. ferrugineum larvae examined also contained quantities of filamentous algae. In addition, Cnephia spp. larvae from sample 4 were examined and no insects of any sort found. Five Cnephia spp. larvae from Sample 6 were examined and one small piece of otherwise unidentifiable insect cuticle was found.

Fifty-one P. ferrugineum larvae contained remains of 212 aquatic animals. This is an average of 4.2 animals per predator.

c) Discussion

A number of other workers have noted the presence of insect remains in the intestines of black-fly larvae (Puri, 1925; Badcock, 1949; Peterson and Davies, 1960; Serra-Tosio, 1967). These workers referred to feeding on chironomid larvae or cannibalising of simuliid larvae of the same species. Feeding of one species of simuliid larvae on another does not appear to be a common occurrence. In the case of P. ferrugineum, predation upon other species of simuliid is perhaps more likely than cannibalism, as cited for S. venustum (Peterson and

Davies, 1960), because of the larger size of P. ferrugineum larvae in relation to the other species present in the stream (i.e. Cnephia spp., Gnus spp.), and the smaller intraspecific size range. In collections made on 18 June, P. ferrugineum larvae were nearly full grown and were thus much larger than the other species in the stream. In view of Serra-Tosio's paper (1967) on predation on chironomid larvae by P. inflatum, it is interesting to note the similarities in structure of the cephalic fan rays of P. inflatum to those of P. ferrugineum.

5. LABORATORY AND FIELD EXPERIMENTS ON THE FEEDING OF VARIOUS SPECIES OF BLACK-FLY LARVAE

a) Experiments in an environment of unknown current velocity

i) Introduction

After deciding to study food intake by black-fly larvae, the first problem was to develop a technique which would allow the determination of feeding rates. Another problem was to develop apparatus in which the environmental variables affecting larvae could be controlled. The first problem was solved by using powdered carmine as a tracer dye in food consisting of a dilute suspension of yeast cells.

The second problem, that of suitable apparatus, was not immediately tackled. A pilot apparatus in which the current speed of the water past the larvae was not controlled was used. This simple and inexpensive apparatus was used to test the yeast-carmine technique and to gain a qualitative assessment of larval feeding. The lessons learned from this apparatus were of great assistance in the design and operation of the trough apparatus used for the experiments in section (b) of this chapter.

ii) Methods and Materials

The apparatus used consisted of three 3 1/2" x 1 1/4" glass tubes fixed upright in a culture dish (which served as a temperature control bath). Each glass tube was fitted with an air hose so that the contents could be kept agitated. Dilute mixtures of a yeast-carmine mixture in the ratio 10 to 1 were placed in the tubes. A total volume

of 40 ml. was maintained in each tube. The larvae were first established in the tubes with distilled water only in the tubes. At the start of the experiment the appropriate amount of a standard yeast-carmine mixture (1 gm. yeast and .1 gm. carmine in 400 ml. distilled water) was added to each tube to give the three concentrations as outlined in Table 17. This was the procedure in all experiments except D where a single yeast-carmine mixture was used against an algal culture of unknown concentration.

TABLE 17

Yeast-Carmine Concentrations used for experiments in glass tubes

I - 6.9×10^{-5} gm./ml.

II - 34.5×10^{-5} gm./ml.

III - 69.0×10^{-5} gm./ml.

Five larvae were removed from each tube at 5 minute intervals until 30 minutes had elapsed and then at 10 minute intervals. Each experiment was concluded at the end of one hour.

The larvae thus collected were dissected and the percentage of the mid-gut which contained the food being fed was recorded. The values obtained for each of the larvae in each sample were averaged and plotted.

S. variegatum larvae were used for all experiments except Experiment 1, where S. spinosum larvae were used. Experiments 4, 5

and 6 were conducted with mature S. variegatum larvae, some of which may have been entering the prepupa stage and therefore not feeding.

iii) Results

Figure 80 shows the results of feeding each of three concentrations of the yeast-carmine mixture to S. spinosum larvae over a period of 45 minutes at 12.5°C. There was considerable irregularity in feeding at the highest level of food concentration. Such an irregularity is probably due to the small sample of larvae investigated at each time interval.

Figure 81 shows a similar experiment carried out using S. variegatum larvae and an experimental time of 60 minutes. Comparing Experiments 1 and 2, it would seem that S. variegetum larvae obtain more food from a denser concentration of it.

The amount of food ingested does not appear to be directly related to the concentration of the food, for the amount ingested in a concentration of 69.0×10^{-5} gm./ml. was less than double that ingested in a concentration of 6.9×10^{-5} gm./ml., one-tenth that of the former.

Figure 82 gives the results of Experiment 3 in which two groups of S. variegatum larvae were fed at 12.5°C. over a period of one hour, one on a yeast-carmine suspension at 6.9×10^{-5} gm./ml. and the other in distilled water containing 1 ml. of a culture of the alga Chlorogloea fritschii Mitra. The concentration of this culture was not known.

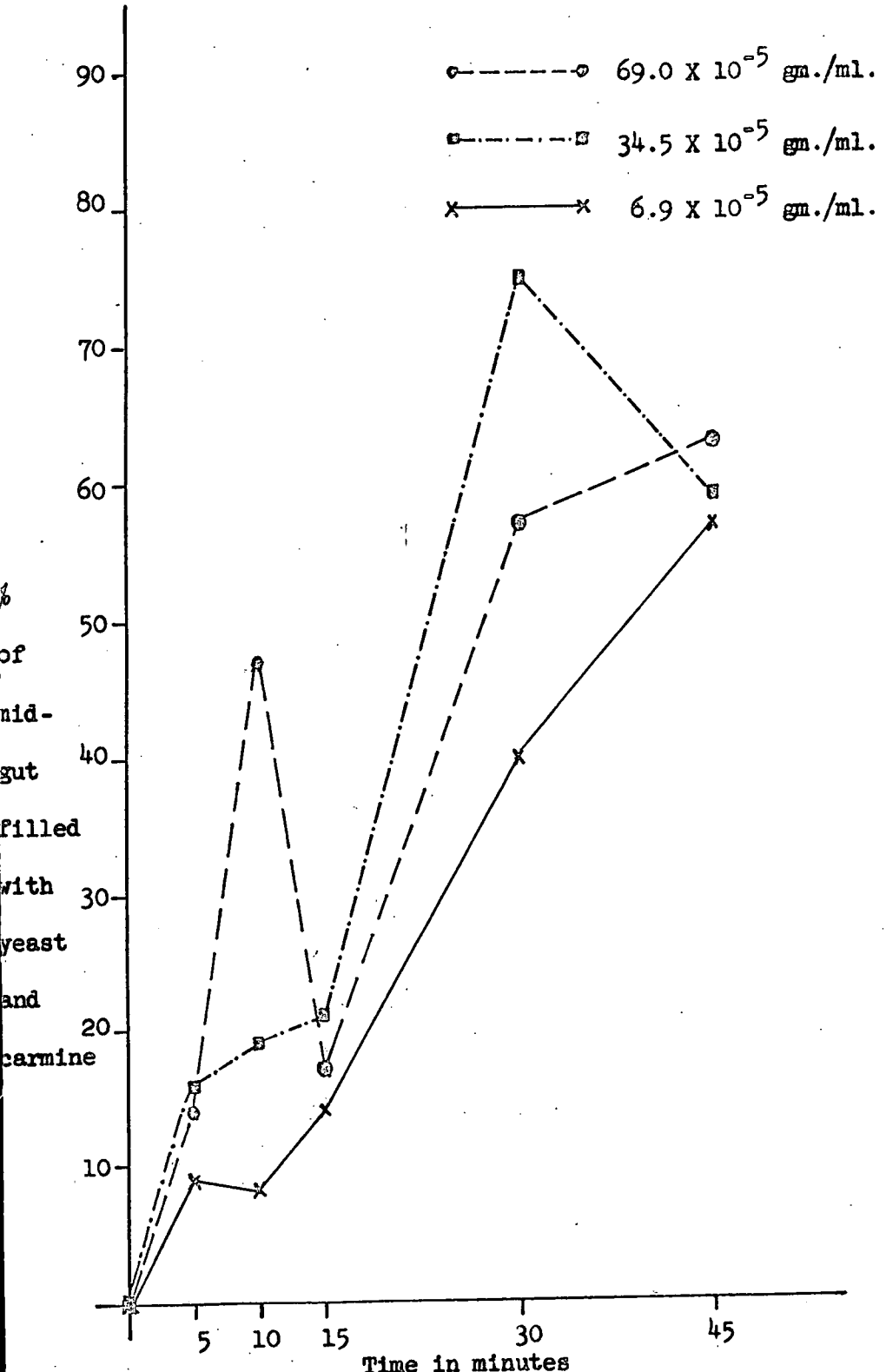

Fig. 80. S. spinosum larvae fed on 3 concentrations of yeast-carmine mixture at a temperature of 12.5°C. (Each point is the mean for 5 larvae.)

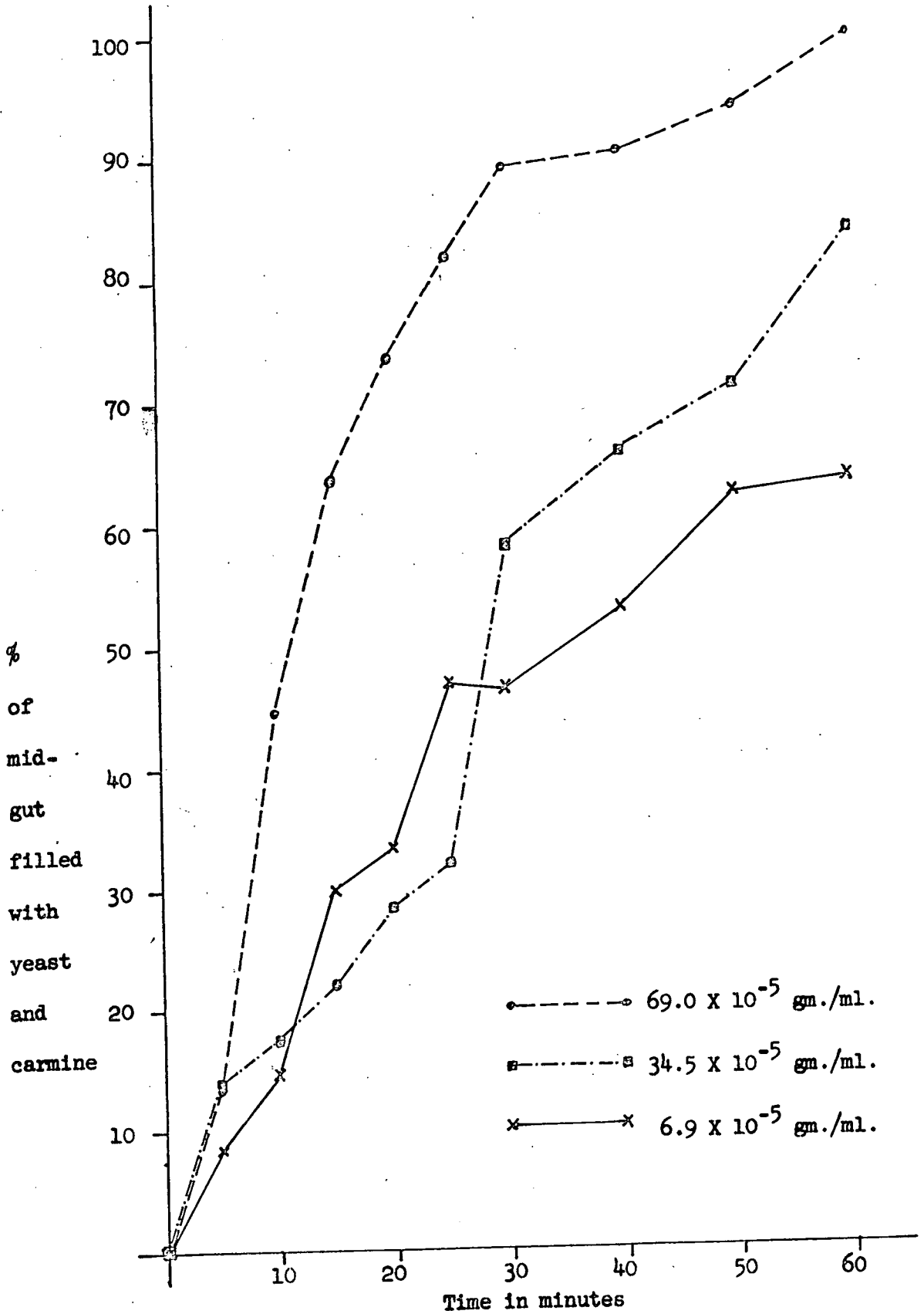

Fig. 81. S. variegatum larvae fed on 3 concentrations of yeast-carmine mixture at a temperature of 11-14°C.

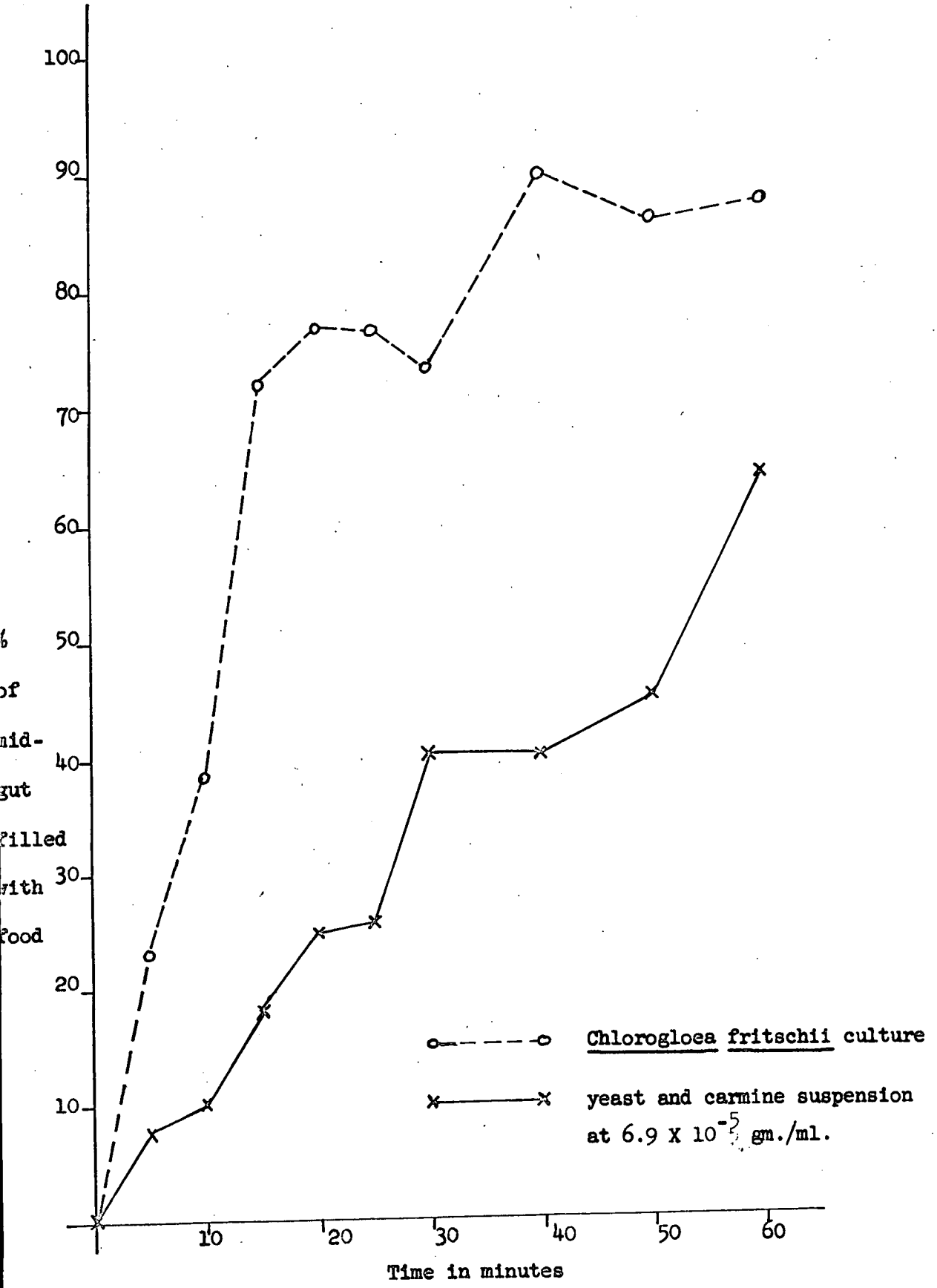

Fig. 82. S. variegatum larvae fed on two different diets as outlined below at a temperature of 12.5°C.

The results show that the larvae took in considerably more from the algal culture than from the yeast-carmine suspension. The amount of the algal culture in the guts of the larvae was much more difficult to assess than with the yeast-carmine mixture because of the lack of a tracer colour. This experiment did show, however, that both an alga and a yeast were acceptable food for black-fly larvae.

Experiments 4, 5 and 6 studied mature S. variegatum larvae at the three concentrations of yeast-carmine suspension previously used and at three different water temperatures - 8°C., 15°C. and 22°C. The results are presented in Figures 83, 84 and 85. Feeding by the larvae used in these experiments was at a much lower level than in Experiment 2. This can perhaps be explained by the fact that the larvae in Experiments 4, 5 and 6 were nearly all in their last larval instar and approaching the pre-pupal stage when feeding is known to cease.

Feeding at the lowest concentration was very slight at 15 and 22°C. and non-existent at 8°C. At the intermediate concentration, food gathering appears to have been greater through most of the time of the experiment (until the last sampling at 30 minutes) at 15°C. The values obtained at 30 minutes in all three experiments at this concentration suggest that there is no difference. Obviously a larger sample size would have changed these results.

At the high concentration of yeast (69.0×10^{-5} gm./ml.) more food was taken in at 22°C. than at 15°C. or 8°C. At the last two temperatures feeding took place at approximately the same rate.

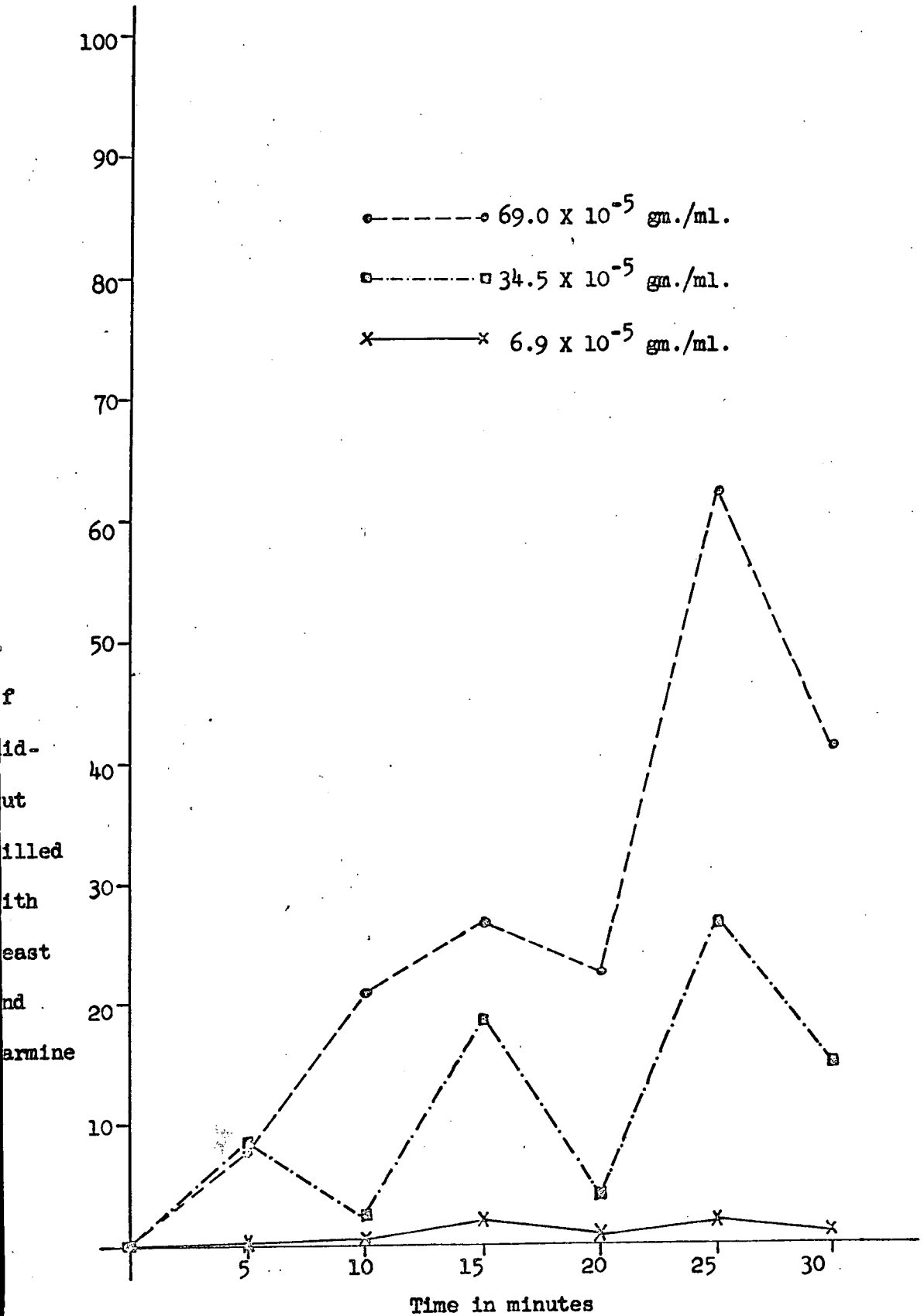

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Fig. 83. S. variegatum larvae fed on 3 concentrations of yeast-carmine mixture at a temperature of 22°C.

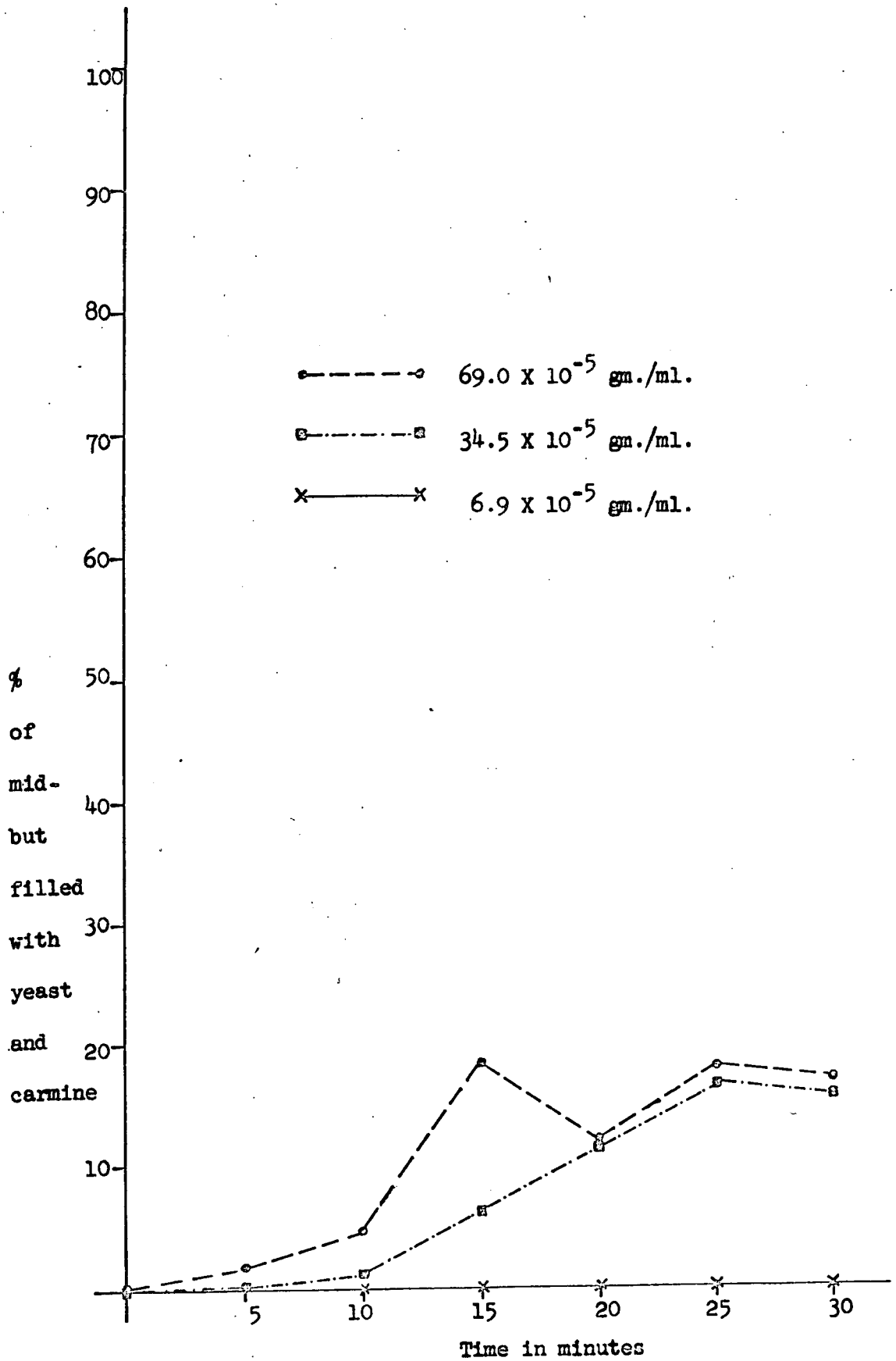

Fig. 84. S. variegatum larvae fed on 3 concentrations of yeast-carmine mixture at a temperature of 8°C.

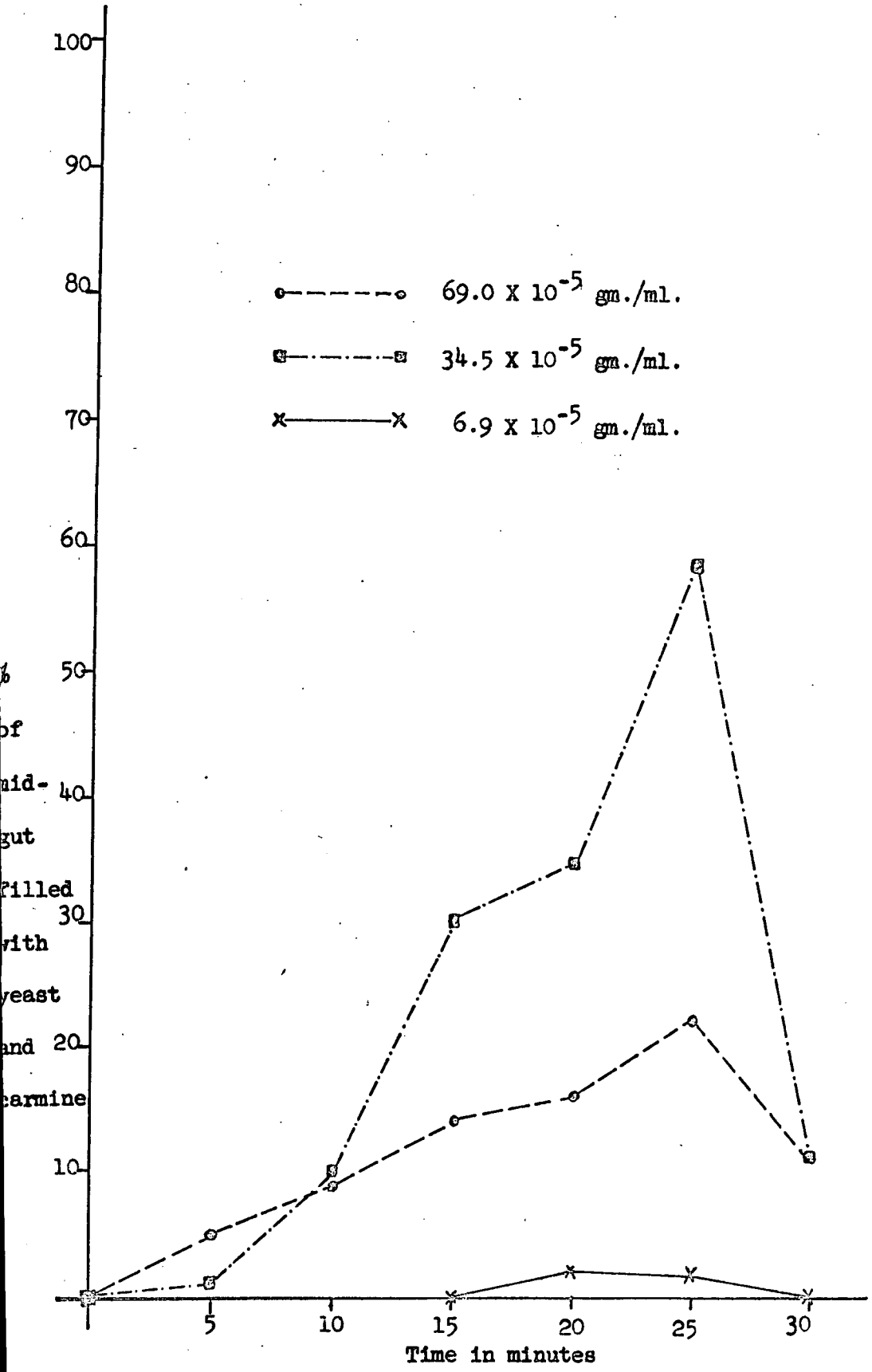

Fig. 85. S. variegatum larvae fed on 3 concentrations of yeast-carmine mixture at a temperature of 15°C.

iv) Discussion

There are several obvious failings with the experiments in this section, most of them due to the method employed. The current velocity past the larvae was not capable of being measured. It was quite likely that the current velocity varied from one part of each chamber to another. The interruption of the air flow to allow the removal of a group of five larvae from each chamber every few minutes would surely upset the feeding patterns of the larvae. A sample of five larvae was really insufficient when such apparent variability in feeding rate occurred. (The physiological state of the larvae was undetermined). However, a sample of five was taken because this had been the maximum number of larvae which could be extracted from each chamber in a period of one minute.

What positive things were learned from these experiments? First, the rate of intake of artificial and natural food suspensions occurred quite rapidly. The rates of ingestion bore some relationship to the rate suggested by Nauman (1924) for an unnamed species. No comparison could be made with the rates of Sephadex bead ingestion given by Chance (1970).

The rate of ingestion by S. variegatum larvae appears to be affected by the concentration of food available (Experiment 2). This result was not obtained in Experiment 1 for S. spinosum larvae. It must be considered possible that there is a variation amongst species in their feeding behaviour. The lower feeding rate of the later instar S. variegatum larvae suggests that differences in feeding rate may be present in larvae of different instars.

b) Experiments on food intake by black-fly larvae in an environment of known current velocity

i) Introduction

The studies in the preceding section suggested that experiments where more of the components of the environment were controlled and where large numbers of larvae were used would yield useful results. In view of the work by previous authors (Wu, 1931; Zahar, 1949; Grenier, 1949; Phillipson, 1956 and 1957) it was decided that one of the variables which must be capable of precise variation would be current speed. Coupled with controlled food density this would enable study to be made of the importance of the relationship of current velocity and food to black-fly larvae.

ii) Methods and Materials

Larvae of four species, S. ornatum, S. variegatum, S. monticola and S. reptans, were used in these experiments. S. ornatum larvae were collected from a stream near Sherburn Hill, County Durham at map reference NZ 333413. S. variegatum larvae were obtained from the Belah R., Westmorland at map reference NY 824118. The S. monticola larvae used were collected from Swindale Beck, Westmorland at map reference NY 691283. The S. reptans larvae were from a stream unnamed on the one inch to the mile ordnance survey map, flowing into the River Tees. The collecting site on this stream was at map reference NY 850308 near the bridge carrying the road to Upper Teesdale.

Larvae from these sites were collected into jars 9.5 cm. in diameter and 8.0 cm. deep and about a quarter full of stream water

containing a small amount of grass stems and blades. It was found that these gave the larvae positions to attach to and reduced the tendency for large numbers of larvae to clump into a ball. (Such an action resulted in high larval mortality).

After transportation to the laboratory, larvae were kept at 10°C. in a constant temperature room. An air stone connected to an aquarium pump provided aeration and motion to the water. The jars were kept filled with water, tap water being used in addition to the river water into which the larvae had been collected. The jars were kept under illumination equal to noon on an overcast dull day in Durham. The day length was 12 hours. This light regimen was followed for the experiments; only one experiment was conducted in the dark.

Larvae were first used for experiments on the day following collection from the field. Each series of experiments (e.g. Series I, II, III etc.) denotes larvae from one collection. They were used consecutively for three days only.

Larvae used in these experiments were most numerous at the velocity ranges given in Table 18.

TABLE 18

Location and current habitat of larvae used in Experiments of Series I-VII

Species	Map reference	Velocity/range cm./sec.
S. ornatum	NZ 333413	44-62
S. variegatum	NY 824118	62-99
S. monticola	NY 691283	44-108(est.)
S. reptans	NY 850308	54-70

The food used in the experiments was a yeast-carmine suspension. A stock solution was made up by adding 1.000 gm. (air dried weight) common general purpose yeast and .100 gm. powdered carmine to 400 ml. distilled water. This mixture was stirred and left at 20°C. for 1 hour when it was then mixed thoroughly by stirring and cooled at 10°C.

Four concentrations of yeast-carmine were used. The one used for most experiments was .375 gm. yeast and .0375 gm. carmine in 6,000 litres of water. This concentration, given the name "N" concentration, was obtained by adding 150 ml. of stock suspension to 5.850 l. of distilled water in the apparatus. The other three concentrations were .1875 gm. yeast and .01875 gm. carmine in 6.000

litres of water ("1/2N"), .0938 gm. yeast and .00938 gm. carmine in 6.000 litres of water ("1/4N"), and .750 gm. yeast and .0750 gm. carmine in 6.000 litres of water ("2N").

One experiment was conducted without the addition of carmine as a tracer. Examination of the larvae was made quite difficult by the lack of colour but results were obtained.

The water used in the trough system was distilled water held at 10°C. All experiments were conducted at this temperature.

The artificial stream was constructed of transparent, colourless plastic sheet, 3 mm. in thickness. A header box 10 cm. by 10 cm. by 10 cm. led into two flat-bottomed troughs (called trough A and trough B) 60.0 cm. long by 3.0 cm. wide by 3.0 cm. deep (see Fig. 86). These troughs and box were held in a wooden frame which allowed the slope of the troughs to be varied.

The troughs emptied into a stainless steel tank, the water flowing off their lower lips and falling into the tank. This waterfall provided aeration of the water. The water was pumped from the tank by means of an "H-R Flow-Inducer" Type HRSR (Watson Marlow Ltd.) to the header box at the upper end of the troughs. This pump operated on the peristaltic principle by compressing 2.5 cm. (inside diameter) rubber tubing between a curved plate and three rotating rollers. This type of pump is intended for use with a special polythene pipe which, however, has a short working life expectancy. It was found that red rubber tube was more satisfactory from the point of view of working life and cost.

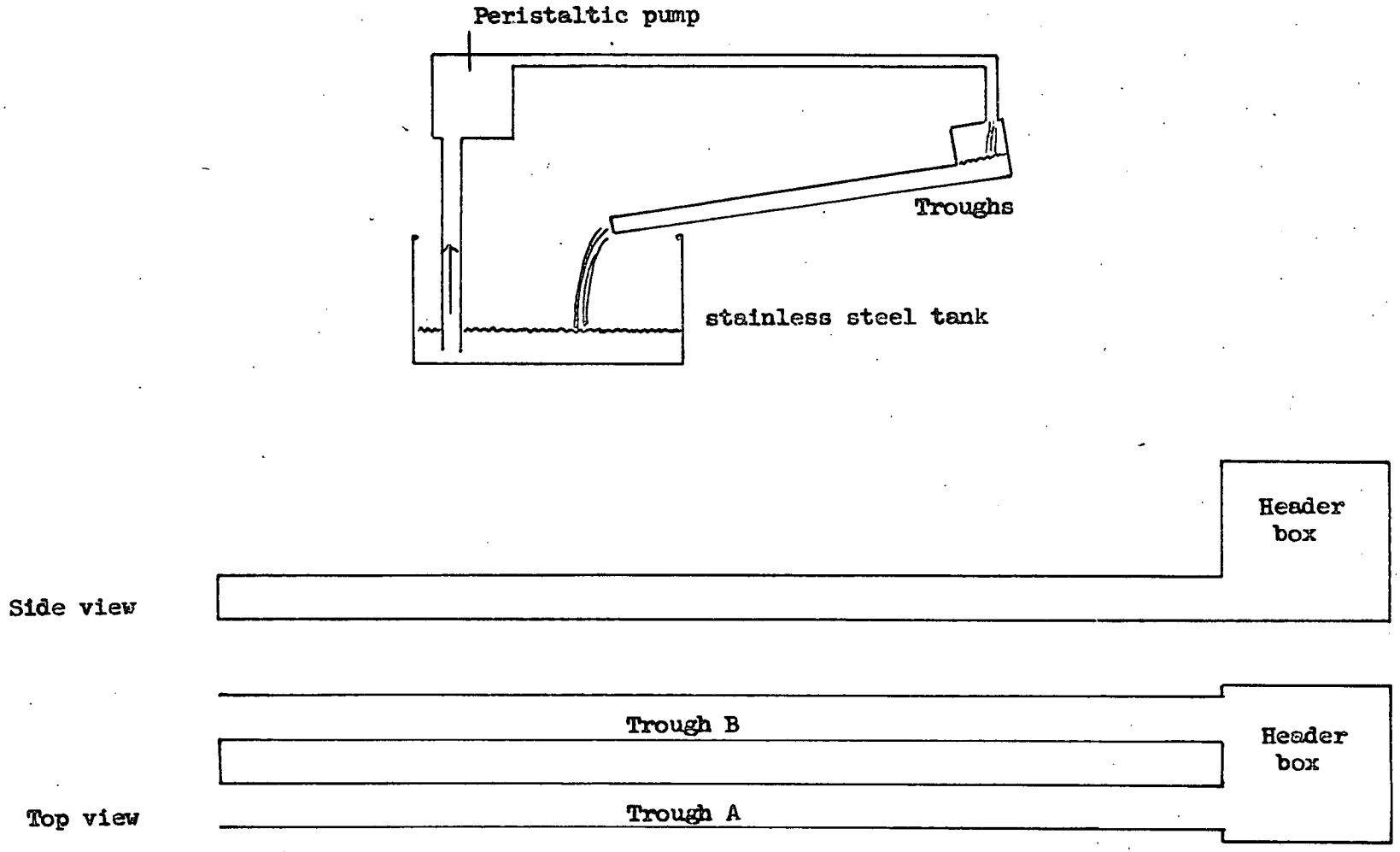

Fig. 86. The trough system used for the feeding experiments of part 5(b).
(Plan of trough x 1/4 Diagram of apparatus x $\frac{1}{12}$).

The following was carried out in the performance of each experiment. Since the total volume of water circulating around the system was 6.0 litres, a slightly smaller quantity of distilled water was placed in the tank. For an experiment where a weight of yeast per litre of .0625 gm. and a weight of .00625 gm. of carmine per litre was used, 5.85 litres of distilled water were placed in the tank (addition of 150 ml. of the stock mixture referred to above would bring the volume up to 6.00 litres and the weights of yeast and carmine to the figures given above). The pump was then started and the water circulated for several minutes while the current velocity in the troughs was set at 40 cm./sec. by adjusting the slope of the troughs. Current velocity was measured by means of a glass pitot tube (Grenier, 1949). After the current velocity had been adjusted, the lower ends of the troughs were covered with a piece of fine net from a nylon stocking, held in place with a rubber band. Larvae of the particular species being studied were then placed in each trough by means of fine forceps. They were allowed to spin a silk thread and eventually to attach themselves to the bottom and side walls of the troughs. Care was taken to ensure that the larvae in each trough of an experiment were as alike in size as possible. The nylon mesh prevented larvae from being lost into the tank. When 40 to 50 larvae were established in the mid-region of each trough, the slope of the troughs was adjusted slowly to provide the current velocity at which the experiment was to be conducted. Care was taken not to cause larvae to release themselves due to abrupt

changes of current velocity. When the desired velocity was obtained, the apparatus was left for five minutes. Then the nylon mesh screening at the lower end of the troughs was removed.

The actual experiment was then begun by adding the appropriate amount of stock mixture of yeast and carmine by pouring it slowly into the header box within a period of one minute. Care was taken to see that the stock mixture was well dispersed into the distilled water in the steel tank. The experiments were timed from the first introduction of food into the system. The experimental period was two hours for S. ornatum, S. variegatum and S. monticola and one hour for S. reptans. At the end of the experimental period the larvae from each trough were removed and preserved in 95% ethyl alcohol.

Larvae were examined under a low power stereoscopic dissecting microscope. The length of the larva, the width of its frons-clypeus or cephalic apotome between the sutures and the percentage of the mid-gut containing yeast-carmine suspension was recorded. Thirty larvae from each trough of each experiment were examined. Care was taken to examine only larvae which appeared to be in a healthy, actively feeding condition. The average percentage of the thirty mid-guts filled with the yeast-carmine suspension was considered as the result for each trough.

iii) Results

Nine experiments, each over a timed period of two hours, were done using larvae of S. ornatum. The results of these experiments are given in Table 19.

TABLE 19

The results of 9 experiments on food intake by S. ornatum larvae (Temp. 10°C.) (See Appendix III, Table III-1 for values of Student's T between troughs)

Experiment No.	Trough	Current velocity cm./sec.	Food concentration	Light on or off	Average % of mid-gut filled for 30 larvae
I-7	A	31	N	On	41.2
	B	31	N	On	49.2
I-8	A	54	N	On	78.0
	B	54	N	On	68.5
I-9	A	70	N	On	43.5
	B	70	N	On	39.8
I-4	A	70	N	On	24.5
	B	70	N	On	28.0
I-5	A	94	N	On	9.0
	B	94	N	On	8.3
I-6	A	112	N	On	12.5
	B	112	N	On	15.3
II-1	A	54	N	On	81.3
	B	54	N	On	74.9
II-2	A	54	1/2 N	On	54.1
	B	54	1/2 N	On	53.9
II-3	A	54	N	Off	91.0
	B	54	N	Off	85.5

Fig. 87 presents Experiments I-4, 5, 6, 7, 8 and 9 as a graph of mean percentage food intake at various current velocities for two

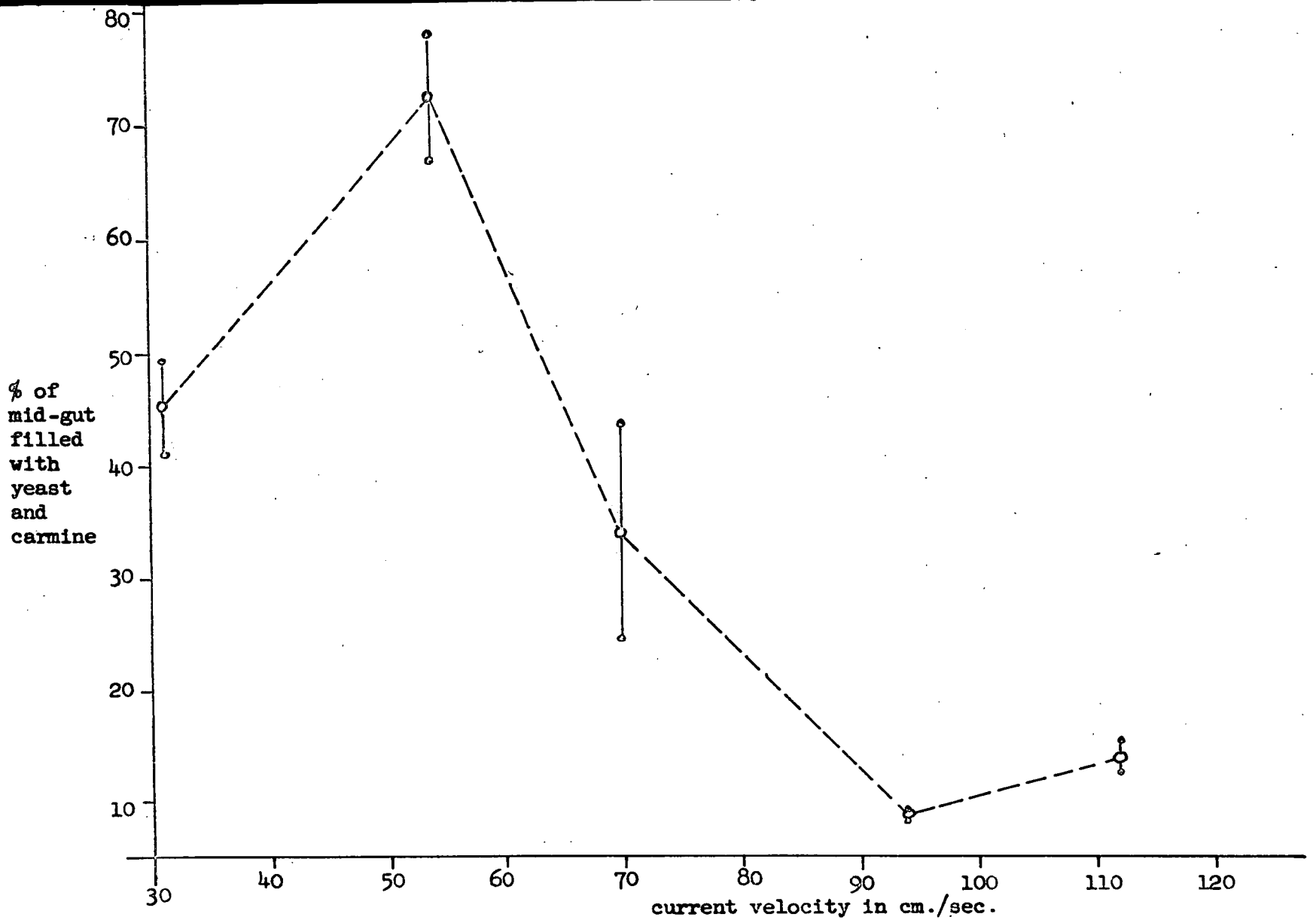

Fig. 87. Rate of intake of yeast and carmine suspension by S. ornatum larvae at various current velocities (Temp. 10°C.)

hours. Appendix III, Table III-2 presents the Student's T values and levels of significance between experiments. The highest rate of food intake occurred at a velocity of 54 cm./sec. Rate of food intake at the higher velocities was significantly lower. No experiment could be conducted at a water velocity higher than 112 cm./sec. because larvae would not remain in the troughs in sufficient numbers above this current speed.

Table 20 presents the data from Experiments II-1 and II-2 and shows the Student's T values and levels of significant difference between each trough result. A significantly lower mean percentage of food was taken in by larvae in the experiment where the food concentration was "1/2N" than in the experiment where it was "N".

TABLE 20

The rate of intake of yeast and carmine by S. ornatum larvae at 54 cm./sec., 10°C. and 2 food concentrations

Experiment	Food Conc.	%	T value	Significance
II-1A	N	81.3	4.000	.01
II-2A	1/2N	54.1		
II-1A	N	81.3	3.7203	.01
II-2B	1/2N	53.9		
II-1B	N	74.9	5.6319	.01
II-2A	1/2N	54.1		
II-1B	N	74.9	5.2048	.01
II-2B	1/2N	53.9		

Table 21 presents the data from Experiments II-1 and II-3 with Student's T values and levels of significant difference between each trough result. Only in one of the four comparisons (II-1B against II-3A was there a significant difference at the 1% level. Three of the

TABLE 21

The rate of intake of yeast and carmine food by S. ornatum larvae under two conditions of illumination at 10°C. and 54 cm./sec. (Food "N")

(T for 1% level of significance = 2.5758,
N.S.= not significant)

Experiment	Light	%	T value	Significance
II-1A	On	81.3	0.5805	N.S.
II-3A	Off	91.0		
II-1A	On	81.3	0.7047	N.S.
II-3B	Off	85.5		
II-1B	On	74.9	3.0225	.01
II-3A	Off	91.0		
II-1B	On	74.9	1.8187	N.S.
II-3B	Off	85.5		

comparisons suggest that there was no significant difference in the rate of food intake under lighted conditions and in the total darkness of a normal night period for the larvae under test.

Table 22 gives the results of Experiments II-1 and II-4 with Student's T values and levels of significant difference between each trough result. In one comparison there was no significant difference,

in one there was significance at the 2% level and in two there was significance at the 1% level.

TABLE 22

The intake of yeast and yeast and carmine food by S. ornatum larvae at 54 cm./sec. and 10°C.

Experiment	Food Type	%	T value	Significance
II-1A	Yeast & carmine	81.3	3.6793	.01
II-4A	Yeast only	96.4		
II-1A	Yeast & carmine	81.3	1.6686	N.S.
II-4B	Yeast only	90.0		
II-1B	Yeast & carmine	74.9	4.3112	.01
II-4B	Yeast only	96.4		
II-1B	Yeast & carmine	74.9	2.5447	.02
II-4B	Yeast only	90.0		

The results suggest that yeast alone was ingested more rapidly than when in combination with carmine. However, all rates of intake were quite high and the colour provided by the carmine made detection of yeast in the mid-guts of the larvae a much easier task.

Seven experiments, each lasting two hours, were conducted using S. variegatum larvae. The results are given in Table 23. All these experiments were carried out with illumination.

TABLE 23

The results of 7 experiments on food intake by *S. variegatum* larvae (Temp. 10°C.) (See Appendix III, Table III-3 for values of Student's T between troughs of the same experiment)

Experiment No.	Trough	Current Velocity cm./sec.	Food Concentration	Ave. % of mid-gut filled for 30 larvae
IV-1	A	54	N	0.2
	B	54	N	0.33
IV-4	A	80	N	49.2
	B	80	N	48.6
IV-2	A	108	N	40.0
	B	108	N	39.7
IV-3	A	140	N	21.46
	B	140	N	21.27
V-1	A	80	N	50.73
	B	80	N	48.0
V-2	A	80	1/2N	33.7
	B	80	1/2N	32.8
V-3	A	80	2N	78.6
	B	80	2N	82.26

Fig. 88 is a graph of Experiments IV-1, 2, 3 and 4 showing the mean percentage food intake at velocities from 54 cm./sec. to 140 cm./sec. The highest rate of food intake was at 80 cm./sec., although intake at 108 cm./sec. was also high. Appendix III, Table III-3 presents the

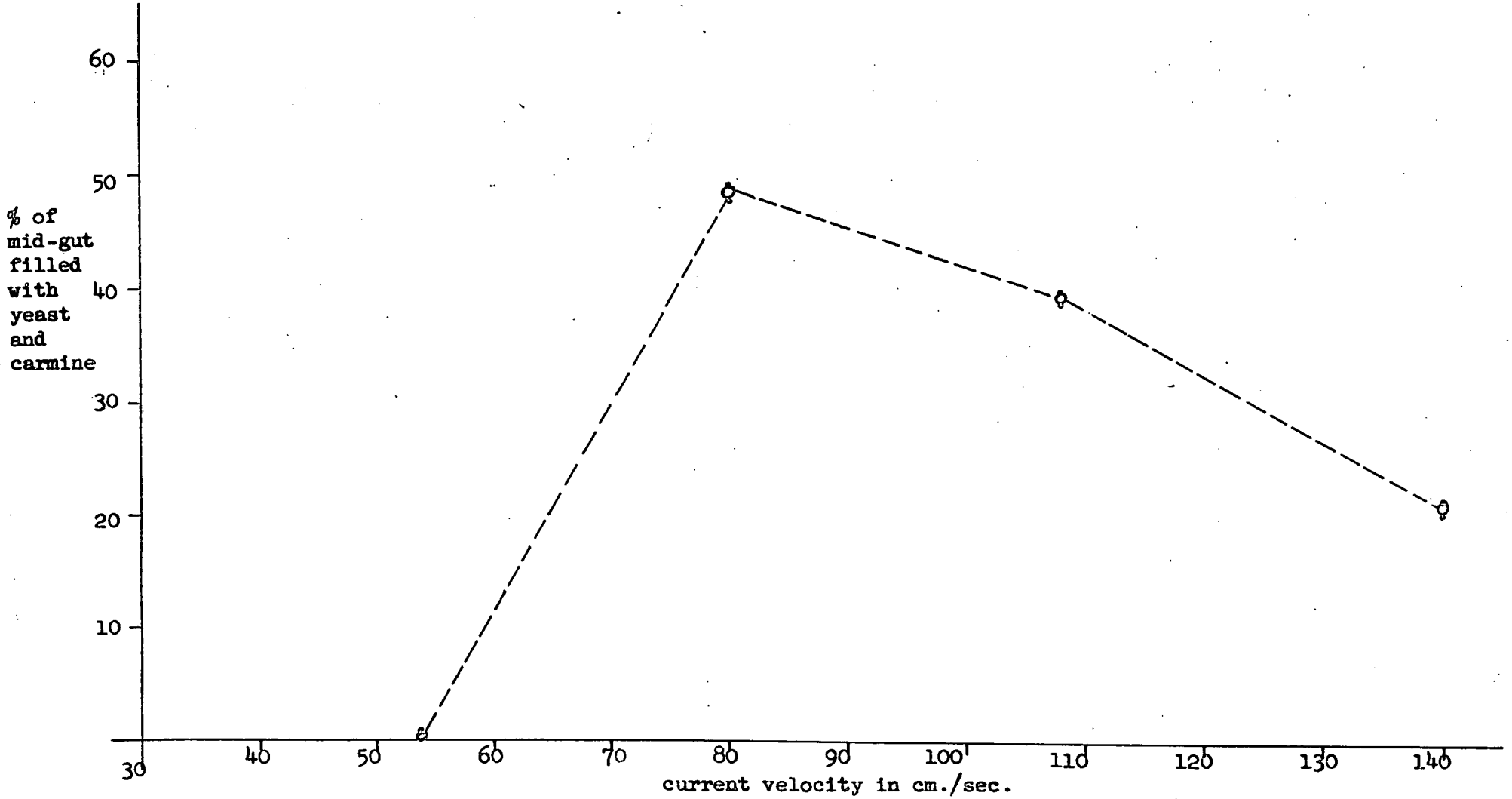

Fig. 88. The intake of yeast and carmine food by S. variegatum larvae at various current velocities. (Temp. 10°C. and food concentration "N").

Student's T values and levels of significance between experiments.

Table 24 shows the results of Experiments V-1, 2 and 3. All these experiments were conducted under the same conditions of current velocity and light. They varied only in the concentration of food available.

TABLE 24

The intake of yeast and carmine food by S. variegatum larvae at three food concentrations. (Temp. 10°C. and current velocity 80 cm./sec.) (T.01 = 2.5758, T.02 = 2.3263, T.05 = 1.9599)

Experiment No.	Food Concentration	Ave. % of midgut filled for 30 larvae	T value	Level of significance
V-1A	N	50.73	2.5698	.02
V-2A	1/2N	33.7		
V-1A	N	50.73	2.6442	.01
V-2B	1/2N	32.8		
V-1B	N	48.0	2.1327	.05
V-2A	1/2N	33.7		
V-1B	N	48.0	2.1327	.05
V-2B	1/2N	32.8		
V-1A	N	50.73	4.1703	.01
V-3A	2N	78.6		
V-1A	N	50.73	4.5260	.01
V-3B	2N	82.26		
V-1B	N	48.0	5.1773	.01
V-3A	2N	78.6		
V-1B	N	48.0	5.5482	.01
V-3B	2N	82.26		

The differences between food intake at the "1/2N" and "N" levels are significantly different at the 5% level of confidence in two comparisons, the 2% level in one comparison and the 1% level in one comparison. The differences between food intake at the "N" and "2N" levels are significantly different at the 1% level of confidence for all comparisons.

The results of six experiments done with S. monticola larvae are given in Table 25. All these experiments were at the same food concentration (N) and under the same illumination (light on).

TABLE 25

The results of 6 experiments on food intake on S. monticola

Experiment No.	Trough	Current Velocity cm./sec.	Ave. % of midgut filled for 30 larvae
VI-6	A	31	48.1
	B	31	35.9
VI-1	A	54	36.6
	B	54	37.5
VI-2	A	70	57.7
	B	70	56.0
VI-3	A	94	37.6
	B	94	42.8
VI-4	A	108	34.5
	B	108	35.6
VI-5	A	140	21.1
	B	140	15.5

Appendix III gives the Student's T values and levels of confidence between troughs of each experiment (Table III-4) and between experiments (Table III-5).

Figure 89 presents the results of Table 23 as a graph. There is no significant difference between the results of troughs A and B of Experiment VI-6. There is a significant difference at the 1% level of confidence between Experiments VI-1 and VI-2.

Due to a lack of available larvae, experiments were not done on the reactions of S. monticola larvae to various food concentrations.

The results of 9 experiments using S. reptans larvae are given in Table 26. These experiments used a timed feeding period of only one hour instead of the two hour period given to all other species and were all carried out with illumination as given in the Methods. Appendix III gives the Student's T values and levels between troughs of each experiment (Table III-6) and between experiments (Table III-7).

Figure 90 presents graphically the results of experiments VII-1, 2, 3, 4, 6 and 8. The maximum intake of food during the one hour period was at 54 cm./sec. current velocity. Food intake at higher and lower velocities was at a much lower level than at 54 cm./sec.

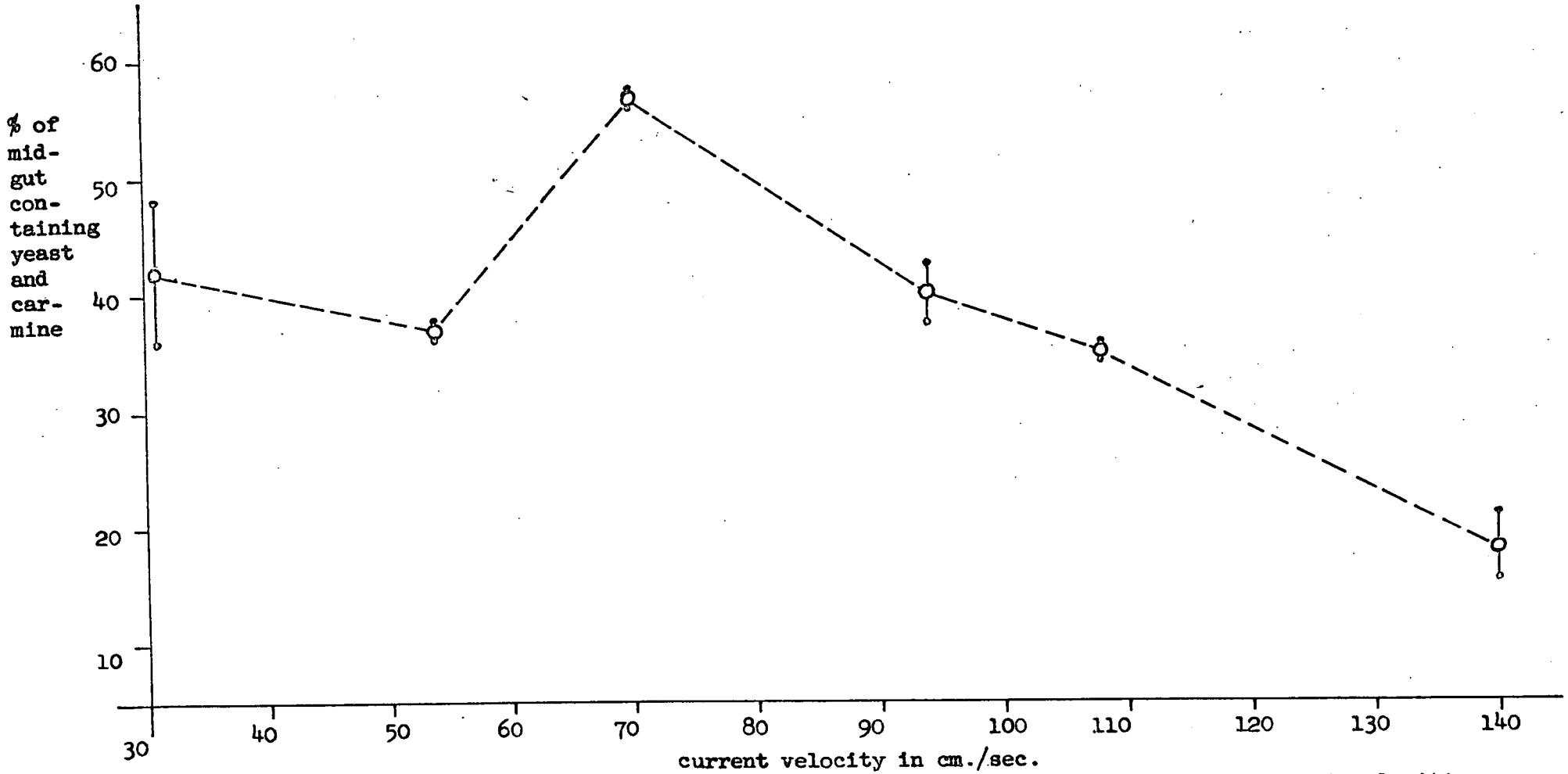

Fig. 89. The intake of yeast and carmine (food) by S. monticola larvae at various current velocities (Temp. 10°C. and food concentration "N")

TABLE 26

The results of 9 experiments on food intake by S. reptans larvae

Experiment No.	Trough	Current velocity cm./sec.	Food concentration	Ave. % of midgut filled for 30 larvae
VII-3	A	31	N	8.87
	B	31	N	10.3
VII-1	A	54	N	66.9
	B	54	N	64.4
VII-2	A	70	N	21.5
	B	70	N	25.8
VII-4	A	88.5	N	19.5
	B	88.5	N	14.5
VII-6	A	108.5	N	15.7
	B	108.5	N	17.67
VII-8	A	140	N	15.0
	B	140	N	14.4
VII-9	A	54	1/2N	42.9
	B	54	1/2N	38.0
VII-10	A	54	2N	68.9
	B	54	2N	55.4
VII-12	A	54	1/4N	10.13
	B	54	1/4N	10.3

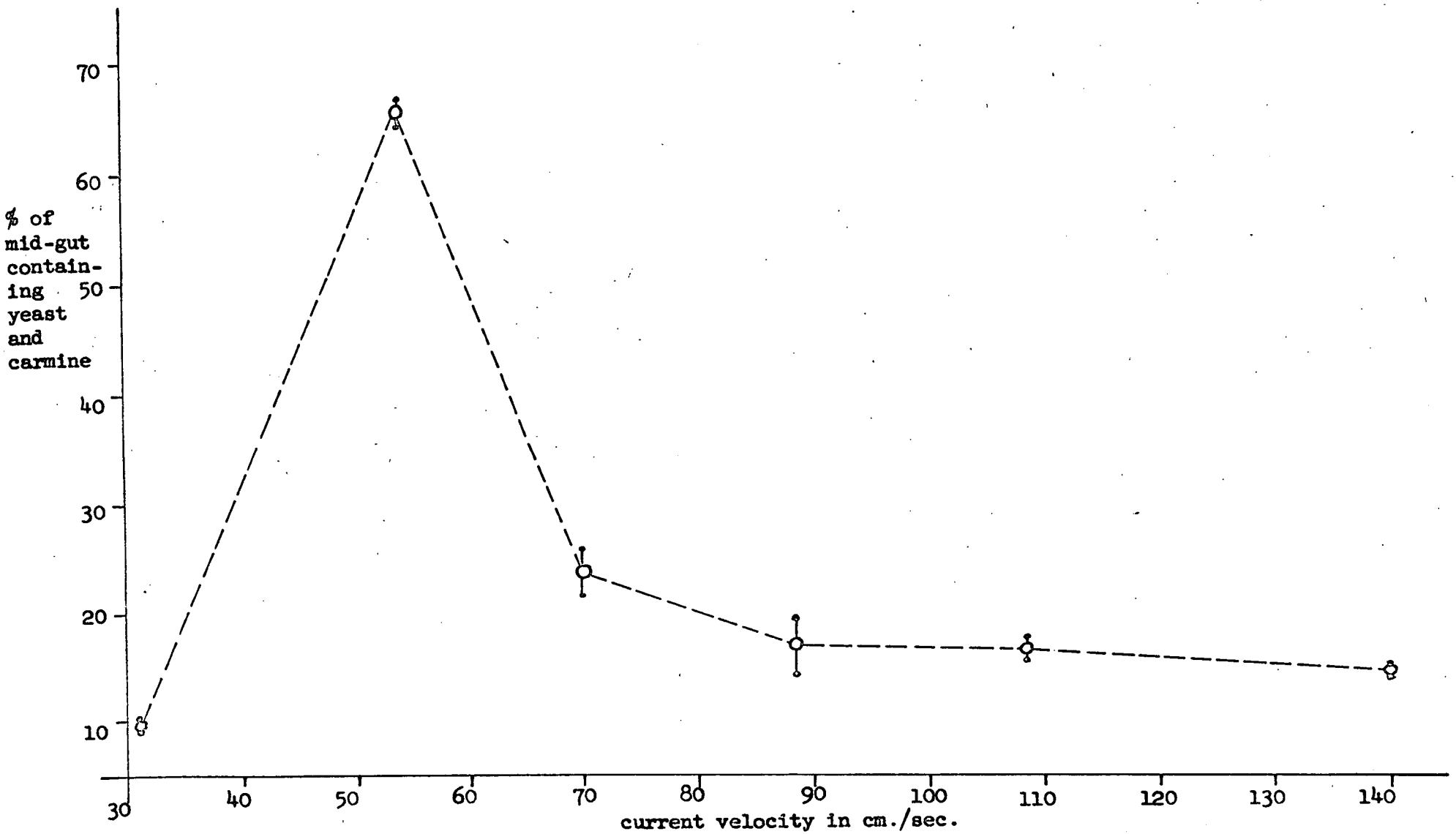

Fig. 90. The intake of yeast and carmine food by S. reptans larvae at various current velocities (Temp. 10°C. and food concentration "N")

Table 27 presents graphically the results of experiments VII-1, 9, 10 and 12. These experiments are at the same current velocity but at four food concentrations. These results suggest that for S. reptans, the doubling of the food concentration from a low level ("1/4N") to a higher one ("1/2N") may result in a much greater rate of increase than would be expected. (In fact, food intake was quadrupled.) This effect was not continued upon further doubling the food concentration (to "N"). Only an increase in food intake of about 40% was obtained. Further doubling of the food concentration to "2N" resulted in a slight decrease which was not significantly different from the result at food concentration "N" for three comparisons and significantly different at the 5% level of confidence for one comparison.

TABLE 27

The intake of yeast and carmine food by S. reptans larvae at 4 food concentrations for a period of one hour. (Temp. 10°C. and current velocity 54 cm./sec.)
(T.01 = 2.5758, T.02 = 1.9599, N.S. = not significant)

Experiment	Food Concentration	Ave. % of mid-gut filled for 30 larvae	T value	Level of significance
VII-1A VII-9A	N 1/2N	66.9 42.9	3.7999	.01
VII-1A VII-9B	N 1/2N	66.9 38.0	4.7463	.01
VII-1B VII-9A	N 1/2N	64.4 42.9	3.1604	.01
VII-1B VII-9B	N 1/2N	64.4 38.0	4.0042	.01
VII-1A VII-10A	N 2N	66.9 68.9	0.3418	N.S.
VII-1A VII-10B	N 2N	66.9 55.4	2.0751	.05
VII-1B VII-10B	N 2N	64.4 68.9	0.7060	N.S.
VII-1B VII-10B	N 2N	64.4 55.4	1.4778	N.S.
VII-9A VII-12A	1/2N 1/4N	42.9 10.13	6.1148	.01
VII-9A VII-12B	1/2N 1/4N	42.9 10.3	5.9381	.01
VII-9B VII-12A	1/2N 1/4N	38.0 10.13	5.4378	.01
VII-9B VII-12B	1/2N 1/4N	38.0 10.3	5.2984	.01

iv) Discussion

If we compare the food intake curves for the four species studied in these experiments (Figures 87-90), we can see that they all differ to some extent from one another. (It must be remembered that the experiments with S. reptans, Figure 90, only lasted one-half as long as for the other species. While this fact should alter the height of the curve it should not alter the shape). There are some similarities, however. The curves for S. ornatum and S. reptans both peaked at the second lowest test velocity, 54 cm./sec., and showed a decreasing intake of food above this velocity. S. reptans showed a very low intake at the lowest test velocity, 31 cm./sec., while the intake for S. ornatum at this velocity was higher but still significantly lower than the intake by that species at 54 cm./sec.

The curves for S. variegatum and S. monticola both peak at higher velocities than those for S. ornatum and S. reptans. Also S. variegatum and S. monticola larvae show a much greater ability to feed effectively at higher velocities than the other two species. S. variegatum seems to be almost incapable of feeding at 54 cm./sec. while S. monticola feeds at that velocity and at 31 cm./sec. as well but at a significantly lesser rate than at 70 cm./sec.

Phillipson (1956) found larvae of S. ornatum to aggregate at velocities of 80 to 90 cm./sec. and to prefer velocities of 50 to 120 cm./sec. in their natural habitat. Since food concentration affects the intake rate also (see Table 104), it is possible that S. ornatum larvae, although not at their optimum current speed for

efficient feeding, may be able to get enough food to live due to the high concentration in the water. A similar situation in nature would account for his slightly higher aggregation velocities for S. monticola and S. variegatum.

The velocity at which food intake in the experiments was found to be at a maximum for each species lay within the velocity range in that species' natural habitat at which it was most commonly found. This suggests that larvae aggregate within a range of current velocities at which they can most easily get food. Many authors (Wu, 1931; Zahar, 1949; Grenier, 1949; Phillipson, 1956 and 1957; Johnson, 1966) have noted that various species were found to aggregate at certain current speeds. The results given here would suggest that those workers found larvae which were aggregated at their most efficient velocities in terms of food-gathering ability.

Further, these results suggest that larvae are not capable of adjusting their food intake rate by any other means than by moving themselves to a region of different velocity.

The experiment on feeding S. ornatum larvae under day and night conditions shows that feeding goes on at a slightly faster rate at night. Johnson (1966) found that the numbers of black-fly larvae colonising habitats at night were greater than the numbers colonising the same habitats during the day. It was suggested that the larvae were more active during the night. Perhaps such an increase in activity is reflected in an increased feeding rate; such an indication was not definitely proven in this study.

The experiments comparing yeast as a food both with and without carmine particles as a tracer dye showed that the feeding rate of S. ornatum larvae was slightly inhibited by the presence of carmine.

The experiments at different food concentrations with larvae of S. variegatum and S. reptans gave somewhat similar results to the concentration experiments with S. ornatum. A variation in the concentration of the food results in a variation in the amount of food obtained. This relationship is not strictly linear. With S. variegatum larvae the increase in food taken when the concentration was "2N" rather than "N" was significantly greater by about 30%, while in the experiments with S. reptans intake, the decrease at "2N" over that at "N" concentration was only statistically significant in one of the four comparisons. At all concentrations of food S. reptans was the more efficient food collector since the experiments with it lasted only one hour instead of the two hours for those with S. variegatum. S. reptans was certainly a more efficient collector of food particles when the concentration was "1/2N".

The control of variables in experiments such as these is often very difficult. While the current velocity, level of illumination, water temperature and concentration and quality of the food were all controlled, there was still some degree of variation in experimental results. Most of this variation must be put down to the range of physiological states in a population of larvae. Unless larvae could be obtained which were exactly uniform in all respects then we must accept

such variation. If reared larvae were used for experiments, we would be faced with the problem of whether the rearing conditions were those which the larvae would be found in in their natural habitat. The experiments given here will no doubt be useful to those workers who wish to rear larvae of the four species studied, since the environmental requirements of these species have now been more closely defined.

c) Field Studies on the Rate of Food Intake by Various Species of Simuliid Larvae

i) Introduction

Maciolek and Tunzi (1968) suggested that black-fly larvae were very efficient removers of cellular microseston from a small mountain stream. The experiments in the preceeding section have shown that larvae could readily ingest an artificial food suspension fed to them in an artificial environment.

Finding that an artificial food suspension was acceptable to larvae in their natural surroundings, a technique for studying the rate of ingestion of naturally occurring particulate matter from the stream by larvae was developed. This technique relied on the ingestion by larvae of a small quantity of coloured artificial food suspension over a brief period of time. This ingested coloured food appeared as a visible tracer, eaten at a known time, in the mid-gut of each larva. The amount of natural food eaten in a given time after the tracer was ingested was measured as a percentage of the total gut contents.

ii) Methods and Materials

A mixture of 5.0 gm. dried granulated yeast and 0.5 gm. powdered carmine was placed in 5.0 litres of stream water. This mixture was agitated thoroughly and allowed to stand for 30 minutes before being used.

Populations of larvae used in experiments were selected from microhabitats of uniform current velocity where there was a concentration of at least 150 larvae. Where more than one experiment was conducted on a given day in the same stream, care was taken to prevent tracer from a previous experiment from contaminating larvae of a subsequent experiment.

After a suitable site was found, the experiment was begun by allowing the coloured food mixture to be run into the stream at a point just upstream from the selected group of larvae. The 5.0 litres of mixture was run into the stream in 2 to 3 minutes. (The faster rate occurred in faster currents.) Immediately after the mixture was run into the water a collection of 20 to 30 larvae was taken from the treated area. This collection was considered to mark time zero in that experiment. Further collections were made at 10, 20 and 30 minutes after the first collection. All collections were made directly into 95% ethyl alcohol.

Collected larvae were dissected in the laboratory and the position of the band of coloured particles in the mid-gut was measured as a percentage of the total mid-gut length. Measurement of these percentages was accurate to 2%. The length of each larva was also recorded.

Six experiments were carried out in England and sixteen in Ontario. Tables 28 and 29 give the species studied, location of stream, current speed and water temperature for each experiment.

TABLE 28

Data on the experiments conducted in England on food intake in nature by black-fly larvae.

Expt. No.	Species Studied	Map Reference (Britain)	Current Speed cm./sec.	Water Temp. °C.
B1	*S. ornatum*	NZ 333413	54	12.0
B2	*S. ornatum*	NZ 333413	47	3.0
B3	*S. ornatum*	NZ 333413	63	8.8
B4	*S. variegatum*	NY 824118	91	7.0
B5	*S. reptans*	NY 850308	54	16.5
B6	*S. reptans*	NY 850308	54	13.7

TABLE 29

Data on the experiments conducted in Ontario on food intake in nature by black-fly larvae.

Expt. No.	Species Studied	Stream Location	Current Speed cm./sec.	Water Temp. °C.
C1	S. venustum	7 mi. N. of Fenelon Falls, Ont. on Hwy. 121	70	12.0
C2	S. venustum	"	89	12.0
C3	S. venustum	"	70	13.0
C4	S. venustum	"	63	18.0
C5	S. venustum	"	77	18.0
C6*	S. venustum	"	63	15.0
C7*	S. venustum	"	54	15.0
C8	S. venustum	"	63	21.0
C9	S. longistyatum	Marsh's Falls Oxtongue River nr. L. of Bays	88	16.5
C10	"	"	108	16.5
C11	"	"	108	21.0
C12	"	"	140	16.5
C13	"	Elliot Falls Gull River nr. Norland, Ontario	44	16.0
C14	"	"	44	16.0
C15	"	"	54	15.0
C16	"	"	77	15.0
C17	"	"	77	16.0
C18	"	"	83	15.0
C19	"	"	99	15.0

*These experiments were carried out at night.

Fig. 91. Site of the field experiments B1, B2 and B3 on *S. ornatum* larvae.

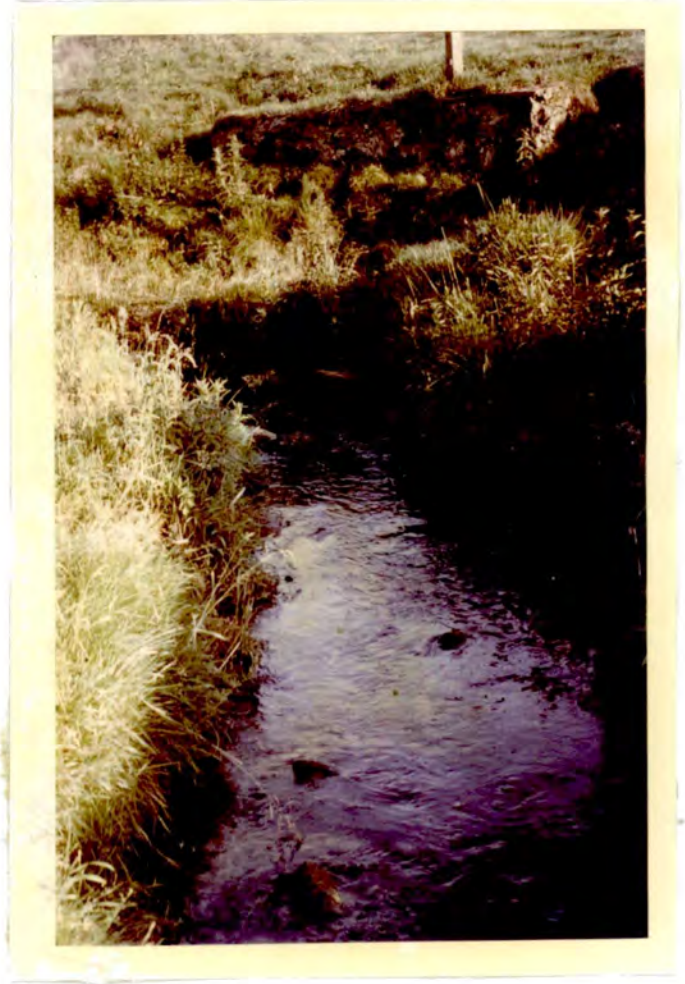

Fig. 92. Site of the field experiment B4 on S. variegatum larvae.

Fig. 93. Site of the field experiments B5 and B6 on S. reptans larvae.

The sites of experiments B1 - B6 are illustrated in Figs. 91-93.

iii) Results of English Experiments

The results of Experiment B1 are given in Table 30 and as a graph in Figure 94. The percent of the mid-gut which has filled with natural food at a given time during the experiment is that part of the mid-gut between the "From" percentage and zero (the start of the mid-gut). The figures for each larva are given in Appendix IV.

TABLE 30

Experiment B1: The intake of food by S. ornatum larvae at 54 cm./sec. and 12.0°C.

Time in minutes	No. of larvae examined	Ave. % position in mid-gut of coloured band	
		From	To
Zero	15	0	4
5	10	8	16
10	10	12	16
20	10	52	62
30	10	84	94

The rate of intake of natural food material by S. ornatum larvae in this experiment was very rapid, with an average intake of 84% of the total gut contents in 30 minutes. The coloured band of yeast and carmine was easily found in the mid-gut.

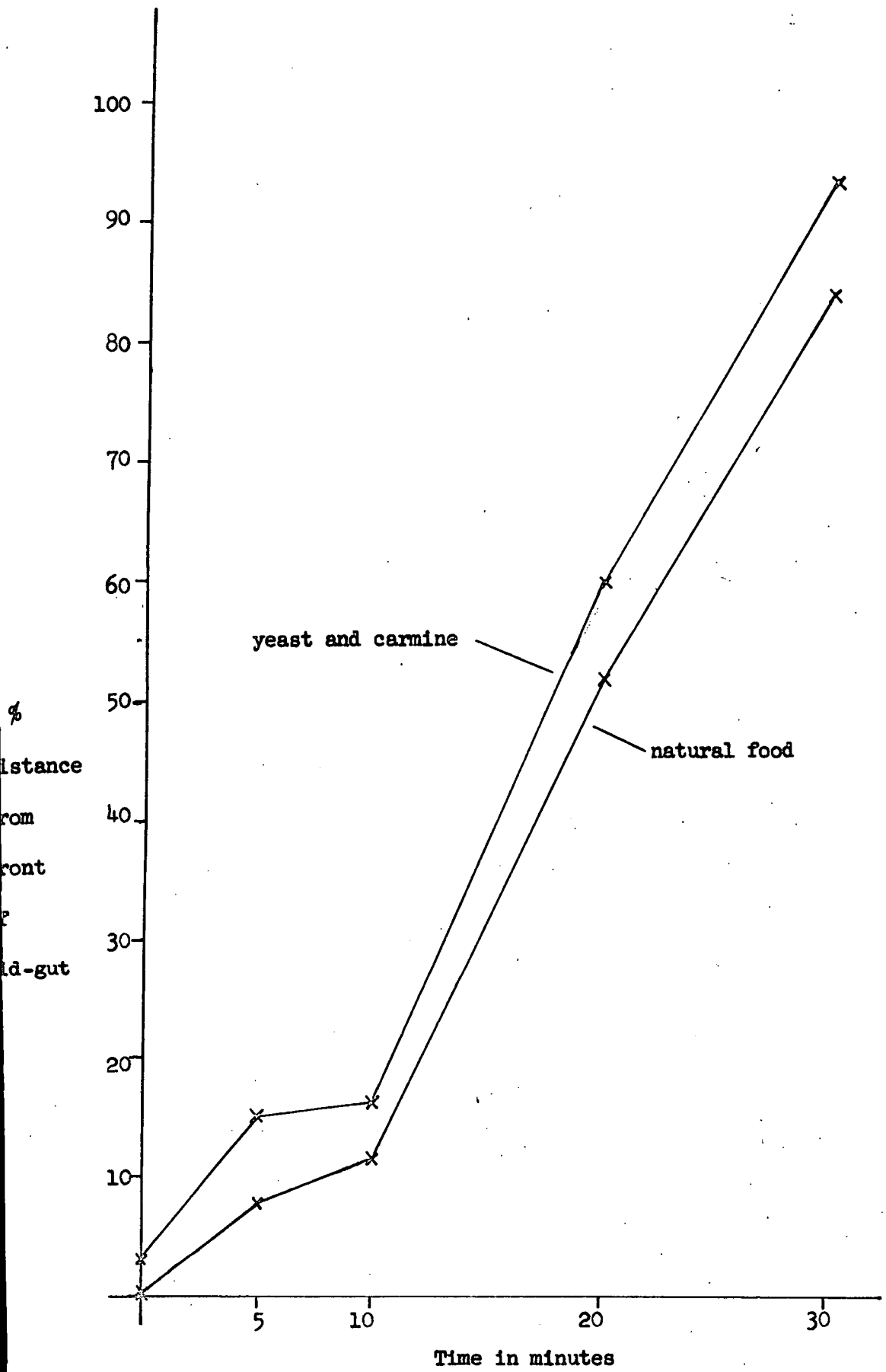

Fig. 94. The intake of yeast and carmine and natural food by S. ornatum larvae in nature. (Expt. B1)

Experiment B2, done when the water temperature was 3.0°C., achieved very poor results. Most of the larvae (S. ornatum) in the site selected did not feed on the yeast and carmine suspension although it was offered in the same manner as with Experiment B1. Table 31 gives the results of this experiment.

TABLE 31

Experiment B2: The rate of food intake by S. ornatum larvae at 44-48.5 cm./sec. and 3.0°C.

Time in minutes	No. of larvae examined	No. feeding on yeast and carmine	Ave. % position in mid-gut of colour
Zero	10	3	0
5	10	3	2
15	10	3	16
20	33	5	42
30	30	10	48

The coloured band of yeast and carmine in this experiment was in many larvae reduced to a few coloured grains. It is probable that feeding was proceeding at a low rate due to the low water temperature.

Experiment B3 (see Table 32 and Figure 95), also conducted on S. ornatum larvae, gave results indicating a lower rate of intake than Experiment B1.

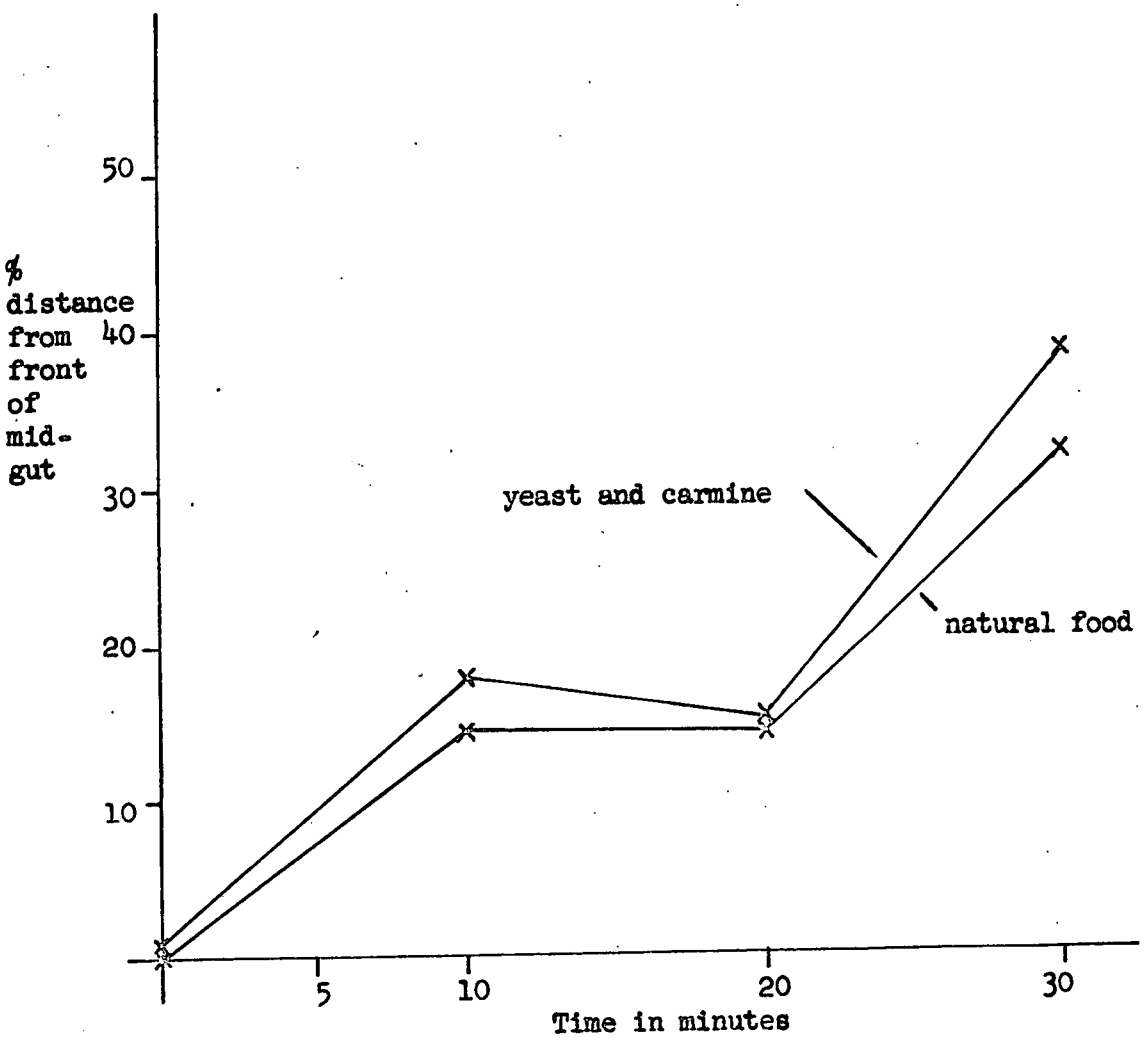

Fig. 95. The intake of yeast and carmine and natural food by S. ornatum larvae in nature. (Expt. B3)

TABLE 32

Experiment B3: The rate of food intake by S. ornatum larvae at 62.6 cm./sec. and 8.8°C.

Time in minutes	No. of larvae examined	Ave. % position in mid-gut of coloured band	
		From	To
Zero	10	0	1
10	6	14	18
20	5	14	16
30	9	32	40

This experiment was conducted at a higher current speed than Experiment B1, and one which, from the evidence of the trough feeding experiments, is not ideal for this species of larva. A lower intake rate would be expected at velocities above 54 cm./sec. with S. ornatum. Also, the density of food matter in the stream may have been less than during the first experiment.

Experiment B4, which was done on larvae of S. variegatum, yielded inconclusive results (see Table 33).

TABLE 33

Experiment B4: The rate of food intake by S. variegatum larvae at 82.8-98.9 cm./sec. and 7.0°C.

Time in minutes	No. of larvae examined	No. feeding on yeast and carmine	Ave. % position in mid-gut of colour
Zero	10	2	0
10	11	4	6
20	16	8	20
30	16	3	22

Very few larvae ingested the yeast and carmine suspension. It was thought that the dilution of the suspension by the faster current and the fact that the location of the experimental site was in deeper water than Experiments B1 and B3 may have prevented much of the coloured suspension from reaching the larvae on the stream bottom. Perhaps if it had been possible to use a larger volume of suspension over the same period of time, more larvae would have ingested the colour.

The results from Experiments B5 and B6 using S. reptans larvae proved the opposite of the experiment with S. variegatum. The results of these two experiments are given in Tables 34 and 35 and Figures 96 and 97. The full data of each of these experiments is given in Appendix IV. The coloured band was quite readily determined in dissections and nearly all the larvae collected had ingested some of the yeast and carmine suspension.

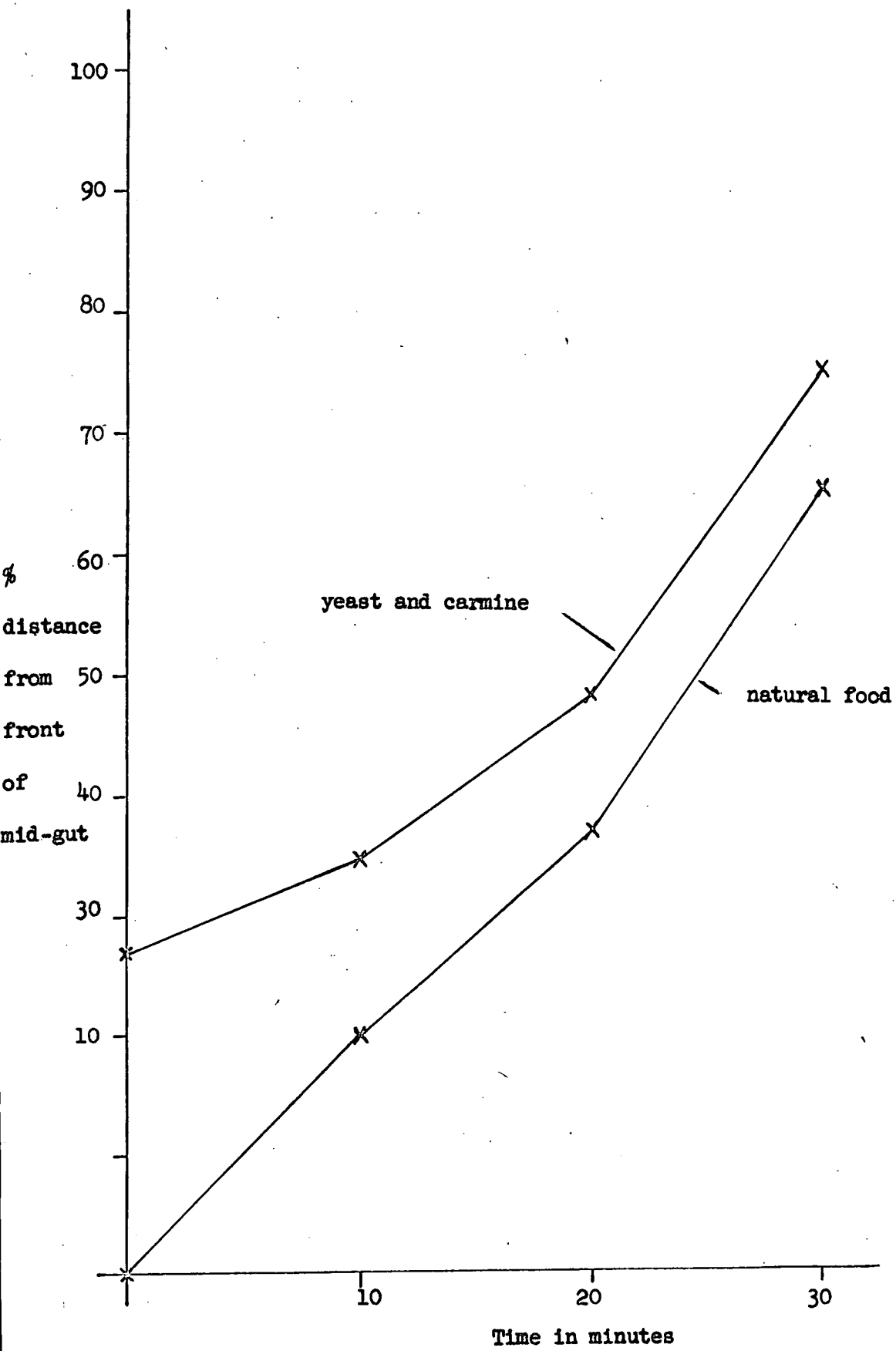

Fig. 96. The intake of yeast and carmine and natural food by S. reptans larvae in nature. (Expt. B5)

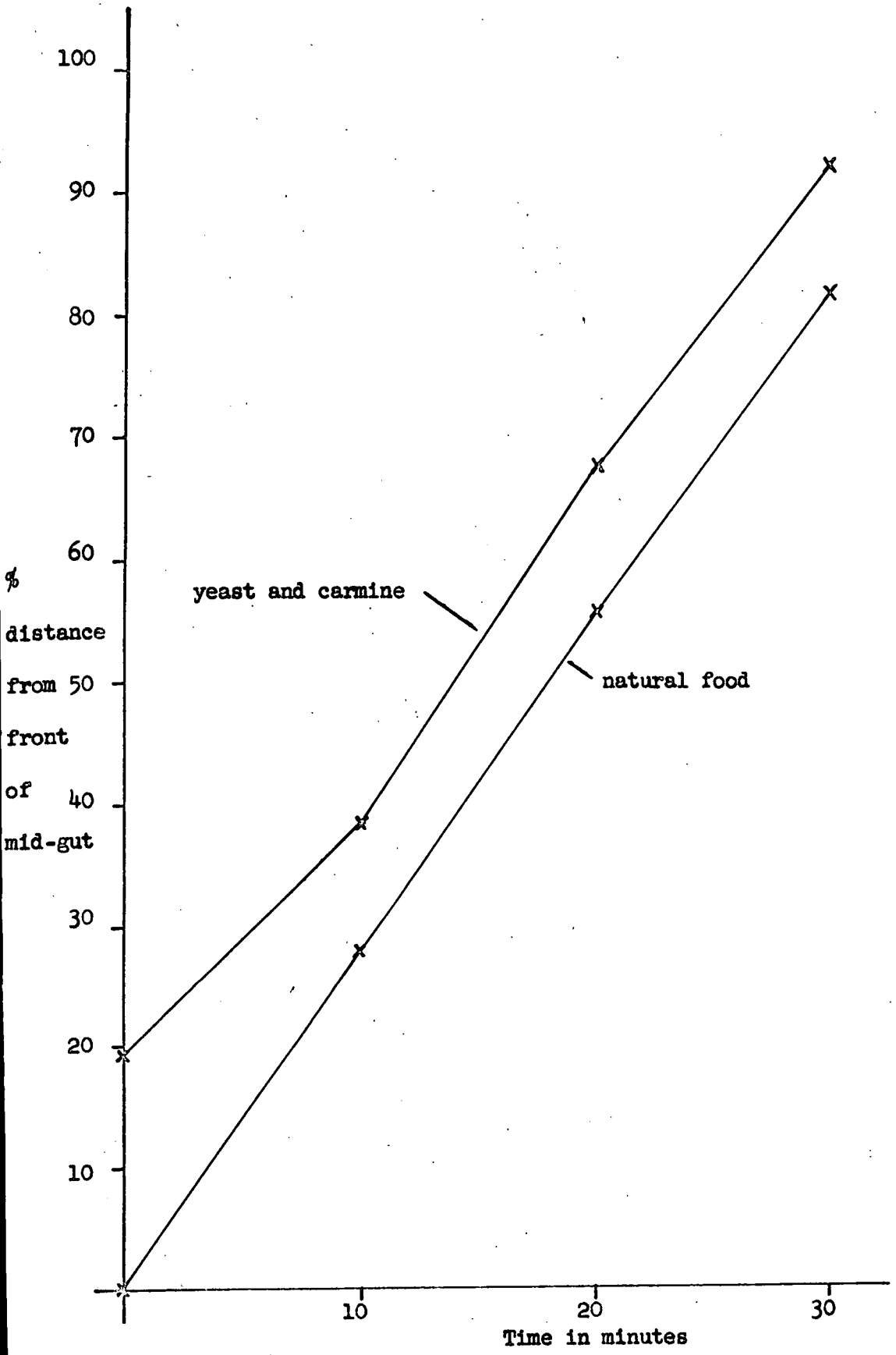

Fig. 97. The intake of yeast and carmine and natural food by S. reptans larvae in nature. (Expt. B6)

Table 34

Experiment B5: The rate of food intake by S. reptans larvae at 54 cm./sec. and 16.5°C.

Time in minutes	No. of larvae examined	Ave. % position in mid-gut of coloured band	
		From	To
Zero	10	0	28
10	10	20	36
20	10	38	48
30	10	66	76

TABLE 35

Experiment B6: The rate of food intake by S. reptans larvae at 54 cm./sec. and 13.7°C.

Time in minutes	No. of larvae examined	Ave. % position in mid-gut of coloured band	
		From	To
Zero	10	0	20
10	10	28	40
20	10	56	68
30	10	82	92

Both these experiments show a rapid rate of intake of natural food by S. reptans larvae. The higher rate for Experiment B6, which was conducted one week after Experiment B5 but at the same location, is difficult to explain. A small difference in the concentration of natural food particles could make such a difference.

iv) Results of Ontario Experiments

The Ontario experiments utilized three common species; S. venustum, a well known pest of man and large mammals, passes the larval stage in small to medium-sized streams while S. pictipes and S. longistylatum characteristically spend their larval stage in the rapids and waterfalls of large rivers. These two species cannot be taxonomically separated as larvae except when fully mature. Even then, separation of many individuals of mixed populations is impossible as there is a gradation of taxonomic characters (D.M. Davies, personal communication). These two species have not been separated from one another in these experiments. The results of each experiment are presented separately both in tabular and graphic form.

TABLE 36

Experiment C1: The intake of food by medium-sized S. venustum larvae at 70 cm./sec. current velocity and 12.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	16
10	10	26	36
20	10	58	66
30	10	86	94

TABLE 37

Experiment C2: The intake of food by medium-sized S. venustum larvae at 89 cm./sec. current velocity and 12°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	20
10	10	32	40
20	10	58	66
30	10	78	98

TABLE 38

Experiment C3: The intake of food by medium-sized S. venustum larvae at 70 cm./sec. current velocity and 13.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	20
10	10	30	40
20	10	56	66
30	10	76	94

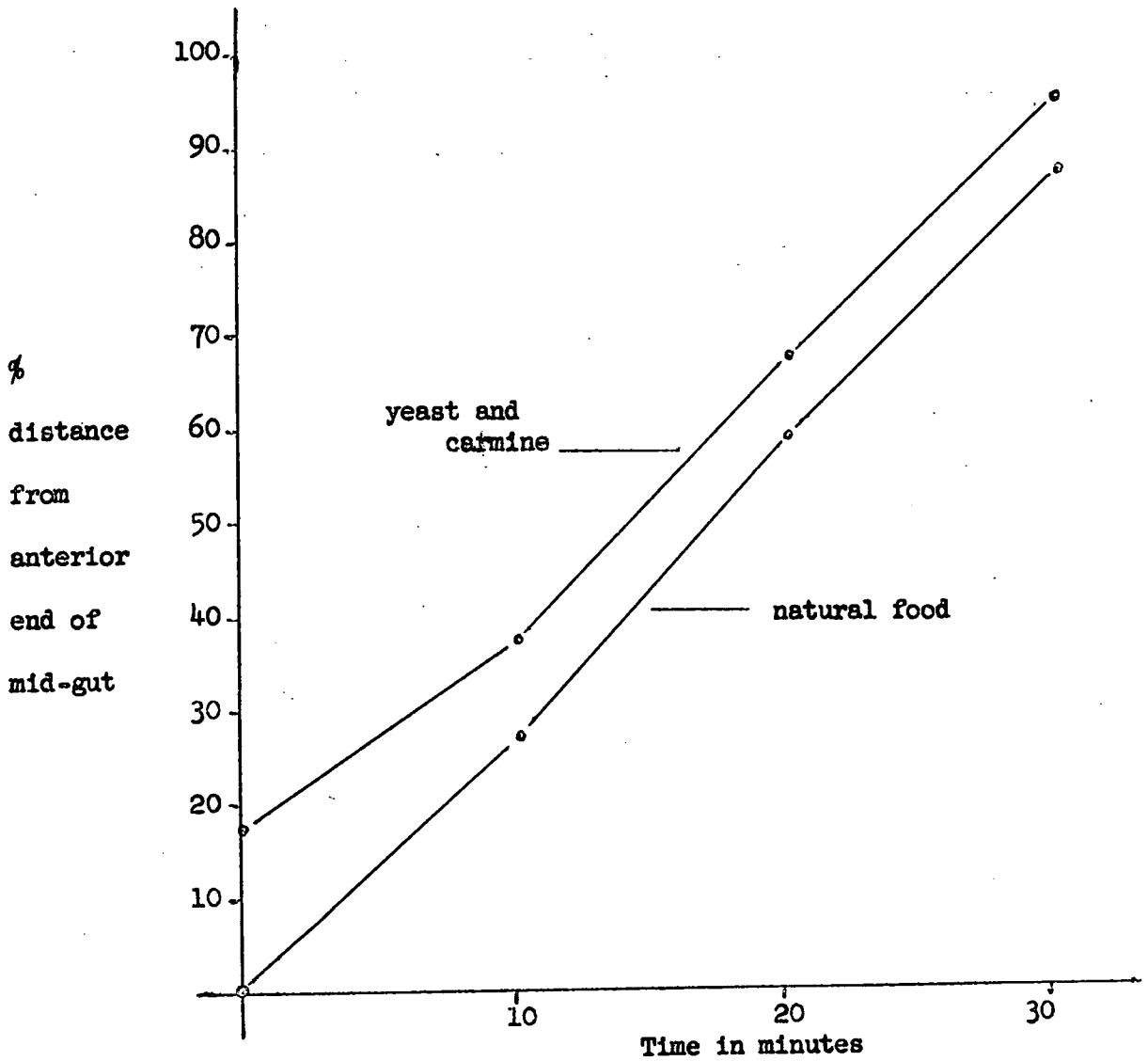

Fig. 98. Expt. C1: The intake of food by medium-sized S. venustum larvae at 70 cm./sec. current velocity and 12.0°C.

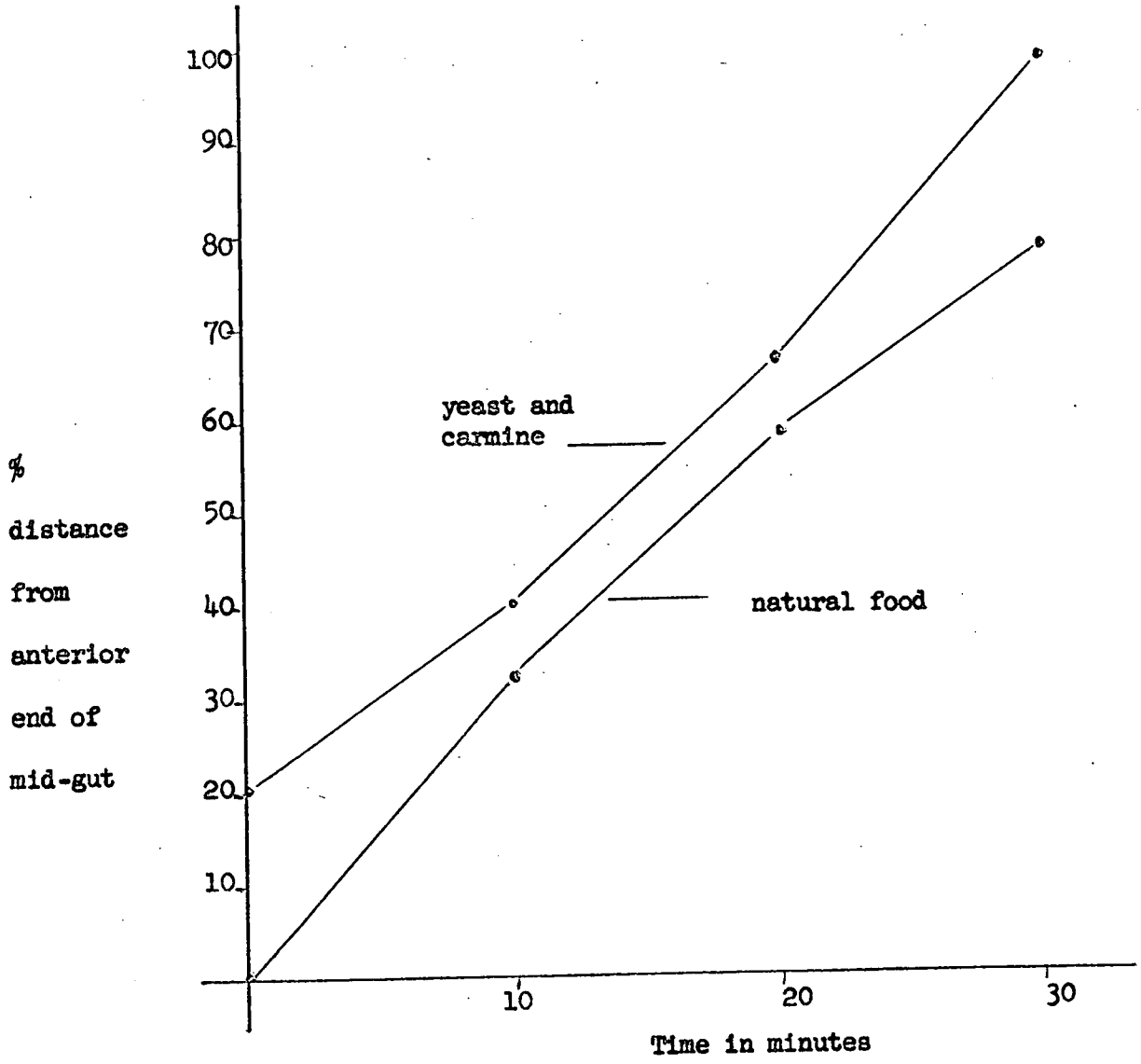

Fig. 99. Expt. C2: The intake of food by medium-sized S. venustum larvae at 89 cm./sec. current velocity and 12.0°C,

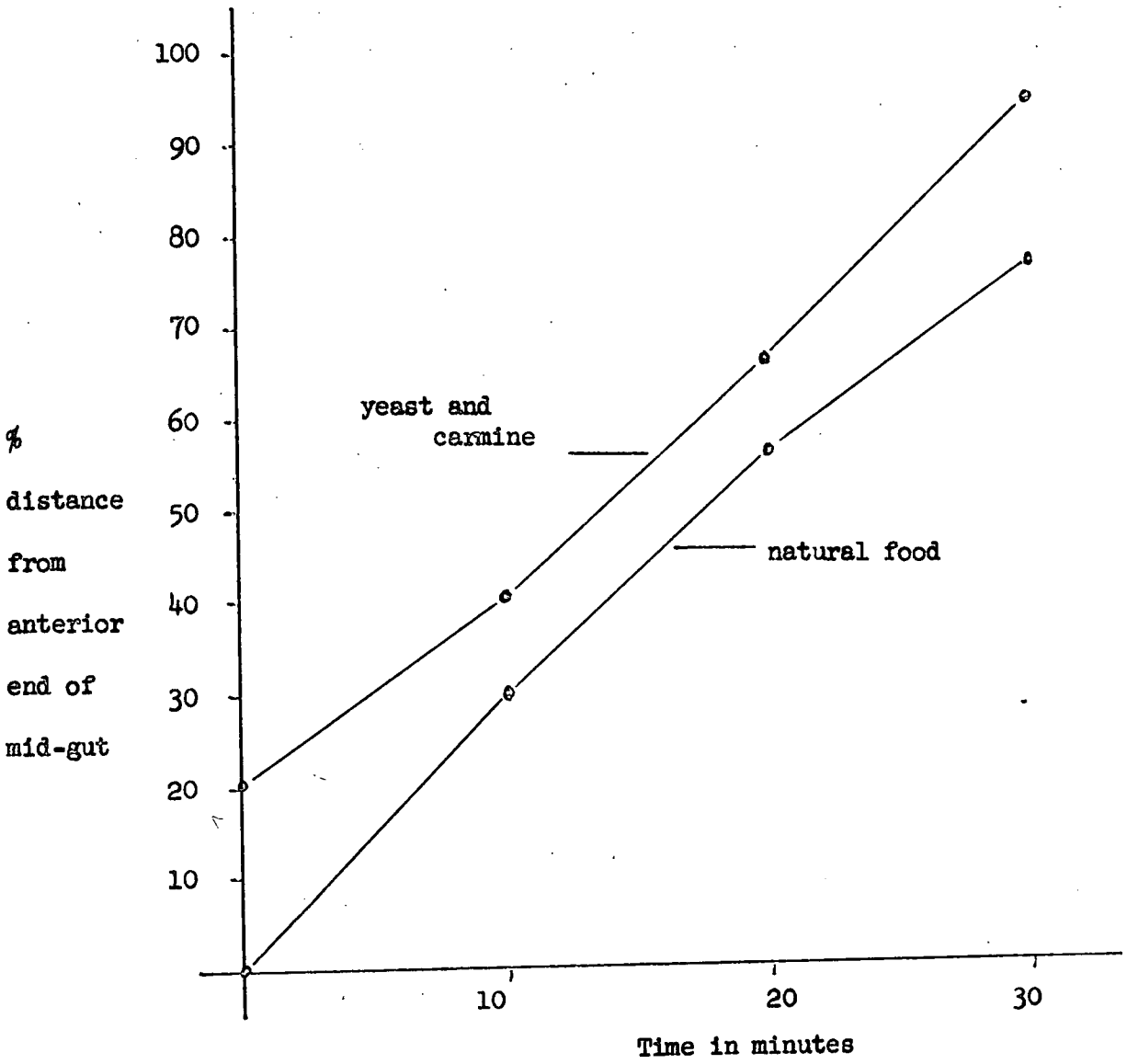

Fig. 100. Expt. C3: The intake of food by medium-sized S. venustum larvae at 70 cm./sec. and 13°C.

Experiments C1, C2 and C3 were all carried out on May 9, 1970 on a population of medium-sized S. venustum larvae. The rate of food intake (Figs. 98, 99 and 100, and Tables 36, 37 and 38) is quite rapid in all cases. The limited range of velocities at which larvae were found in the stream precluded tests of food intake at lower or higher current velocities than those recorded here.

The larvae in all three of these experiments (C1, C2 and C3) were feeding at a rapid rate, which was similar to the results obtained for S. ornatum and S. reptans larvae. S. venustum larvae of this size range (2.5 - 4 mm. in length) were feeding at a rapid rate.

Experiments C4, C5, C6 and C7 were performed on the same population of S. venustum larvae but on May 23, 1970. The larvae were larger than when studied two weeks previously. The volume and rate of flow of the stream were reduced while stream temperature was higher.

Experiments C4 and C5 were carried out during daylight hours while C6 and C7 were performed at night before moonrise in order to determine whether there was any change in feeding rate at night. These results are presented in Tables 39-42 and Figures 101-104.

TABLE 39

Experiment C4: The intake of food by large S. venustum larvae during daylight at 63 cm./sec. current velocity and 18°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	12
10	10	16	24
20	10	32	42
30	10	52	58

TABLE 40

Experiment C5: The intake of food by large S. venustum larvae during daylight at 77 cm./sec. current velocity and 18°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	12
10	10	10	20
20	10	26	36
30	10	50	56

TABLE 41

Experiment C6: The intake of food by large S. venustum larvae at night and at 63 cm./sec. current velocity and 15°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	14
10	10	12	26
20	10	26	34
30	10	46	56

TABLE 42

Experiment C7: The intake of food by large S. venustum larvae at night and at 54 cm./sec. current velocity and 15°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	12
10	10	12	24
20	10	26	38
30	10	38	50

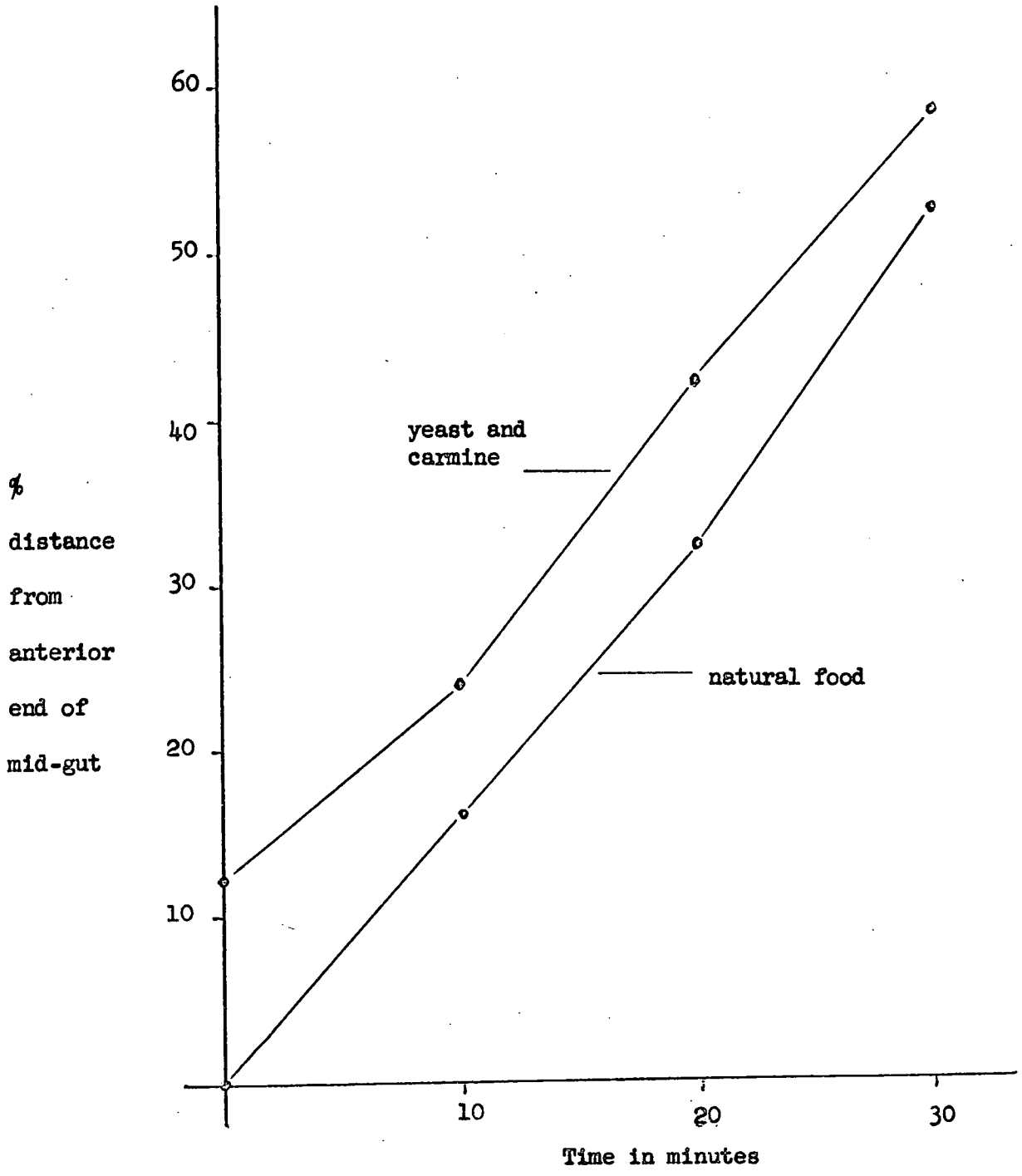

Fig. 101. Expt. C4: The intake of food by large S. venustum larvae during daylight at 63 cm./sec. current velocity and 18°C.

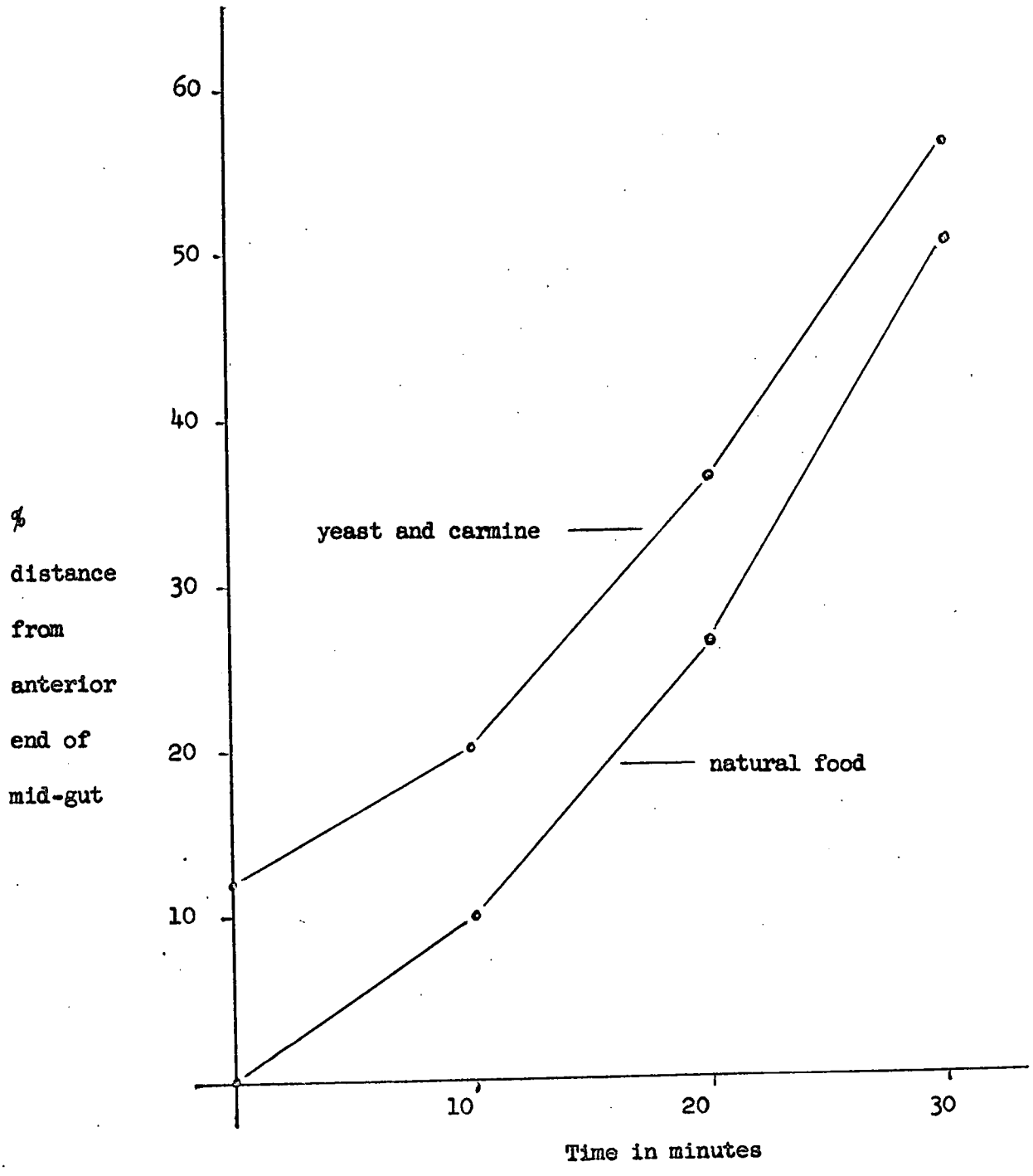

Fig. 102. Expt. C5: The intake of food by large S. venustum larvae during daylight at 77 cm./sec. current velocity and 18°C.

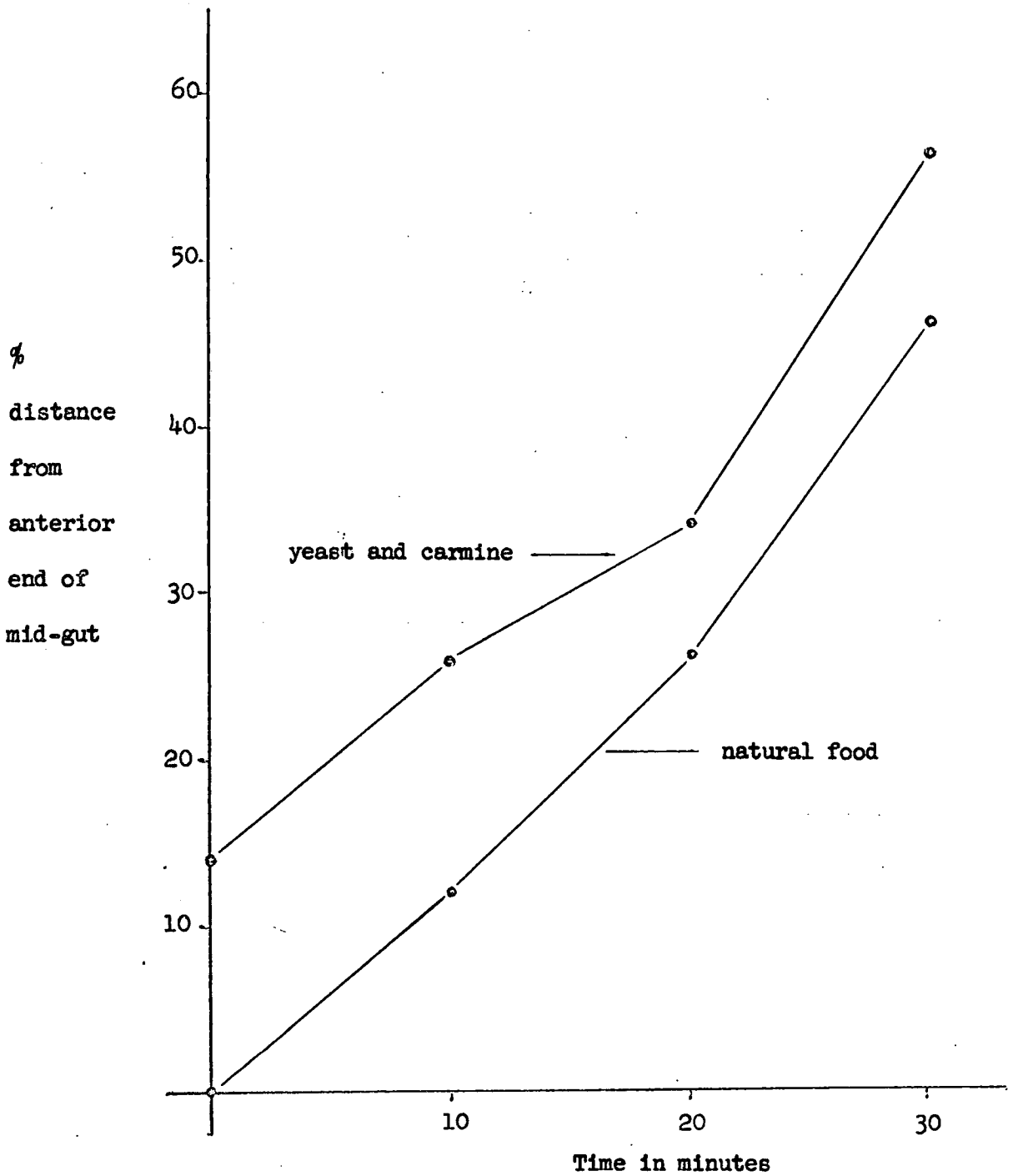

Fig. 103. Expt. C6: The intake of food by large S. venustum larvae at night and at 63 cm./sec. and 15°C.

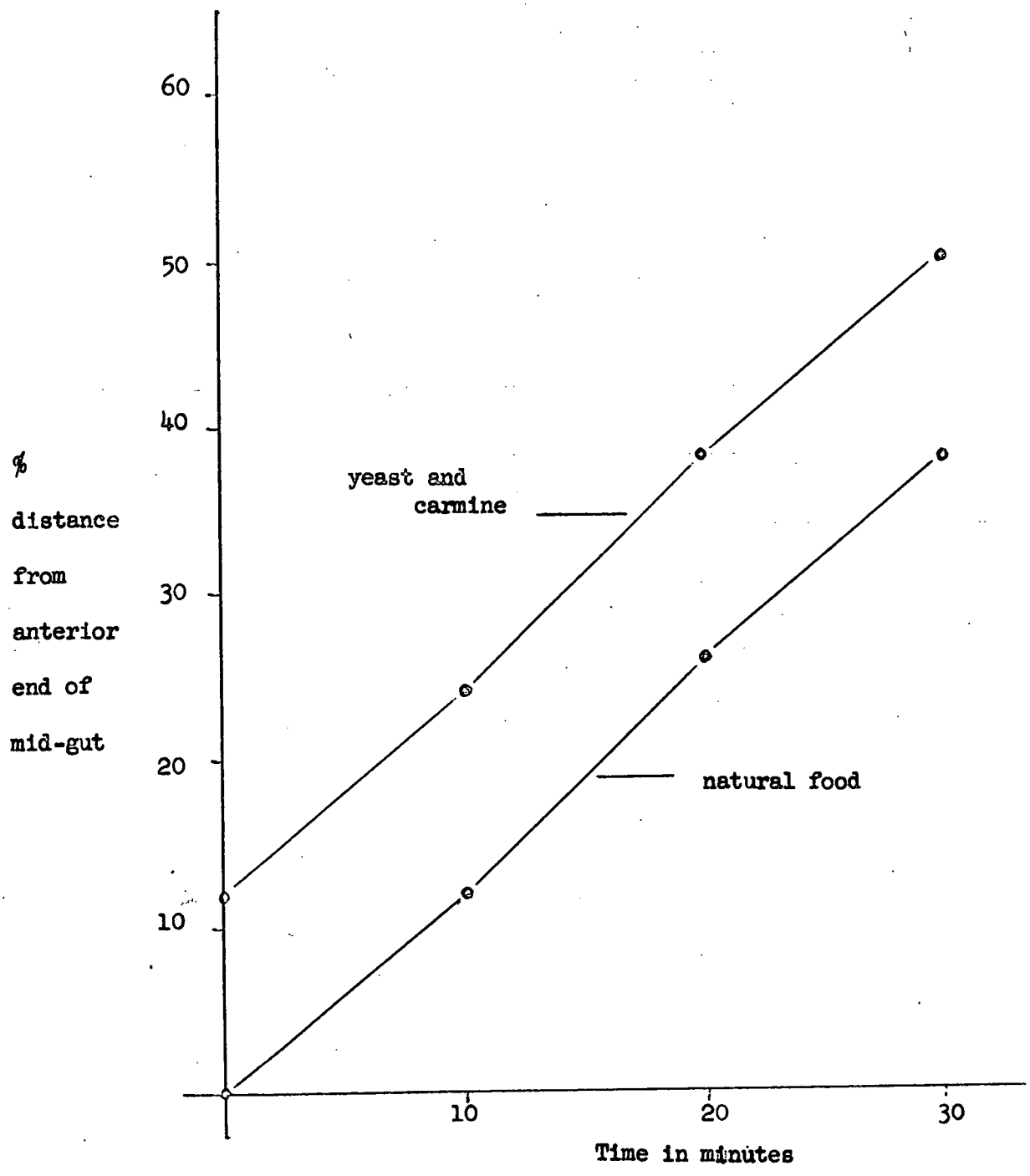

Fig. 104. Expt. C7: The intake of food by large S. venustum larvae at night and at 54 cm./sec. current velocity and 15°C.

Experiments C4 and C5 showed that larger larvae from the same population as experiments C1, C2 and C3 were feeding at a much lower rate. It was not determined if this reduction was due to a sparser population of microseston being carried by the stream but it was noted that the volume of flow of the stream was reduced so that the stream was approximately one half its former width. The ability of the stream to carry heavier segments of the microseston would have been curtailed.

The slightly lower rates of intake for Experiments C6 and C7 when compared with C4 and C5 may not be due to night conditions but rather a result of the lower water temperatures at night. There appears to have been no drastic cessation or other change in feeding rates of the black-fly larvae studied at night as opposed to daylight conditions.

Experiments C8 - C12 were done on a population of S. pictipes and S. longistylatum larvae at Marsh's Falls in the Oxtongue River near Dorset, Ontario. These larvae were located in the rapidly moving water near the edge of the falls. The results of these experiments are given both in tabular and graphic form.

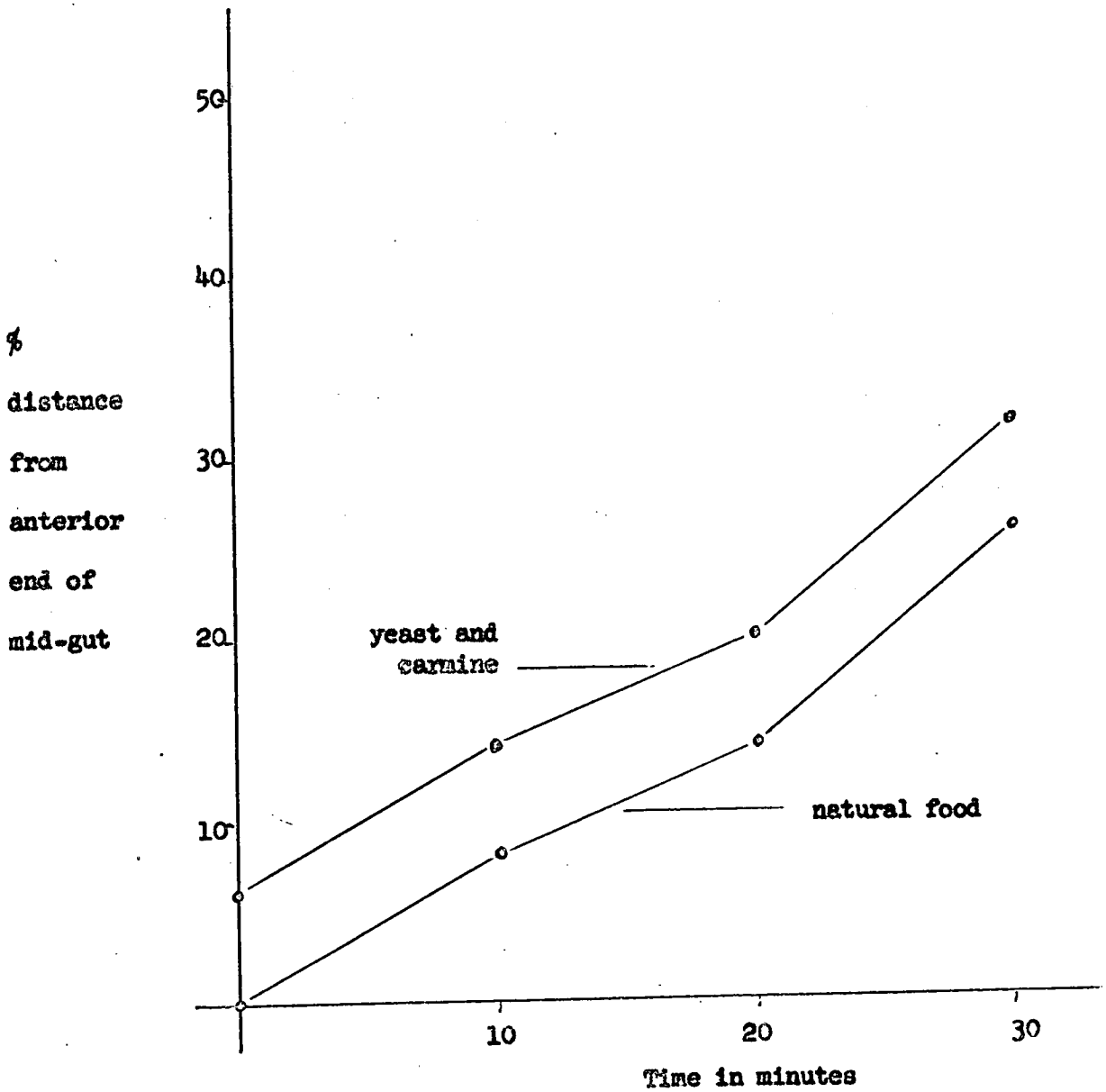

Fig. 105. Expt. C8: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 63 cm./sec. current velocity and 21.0°C.

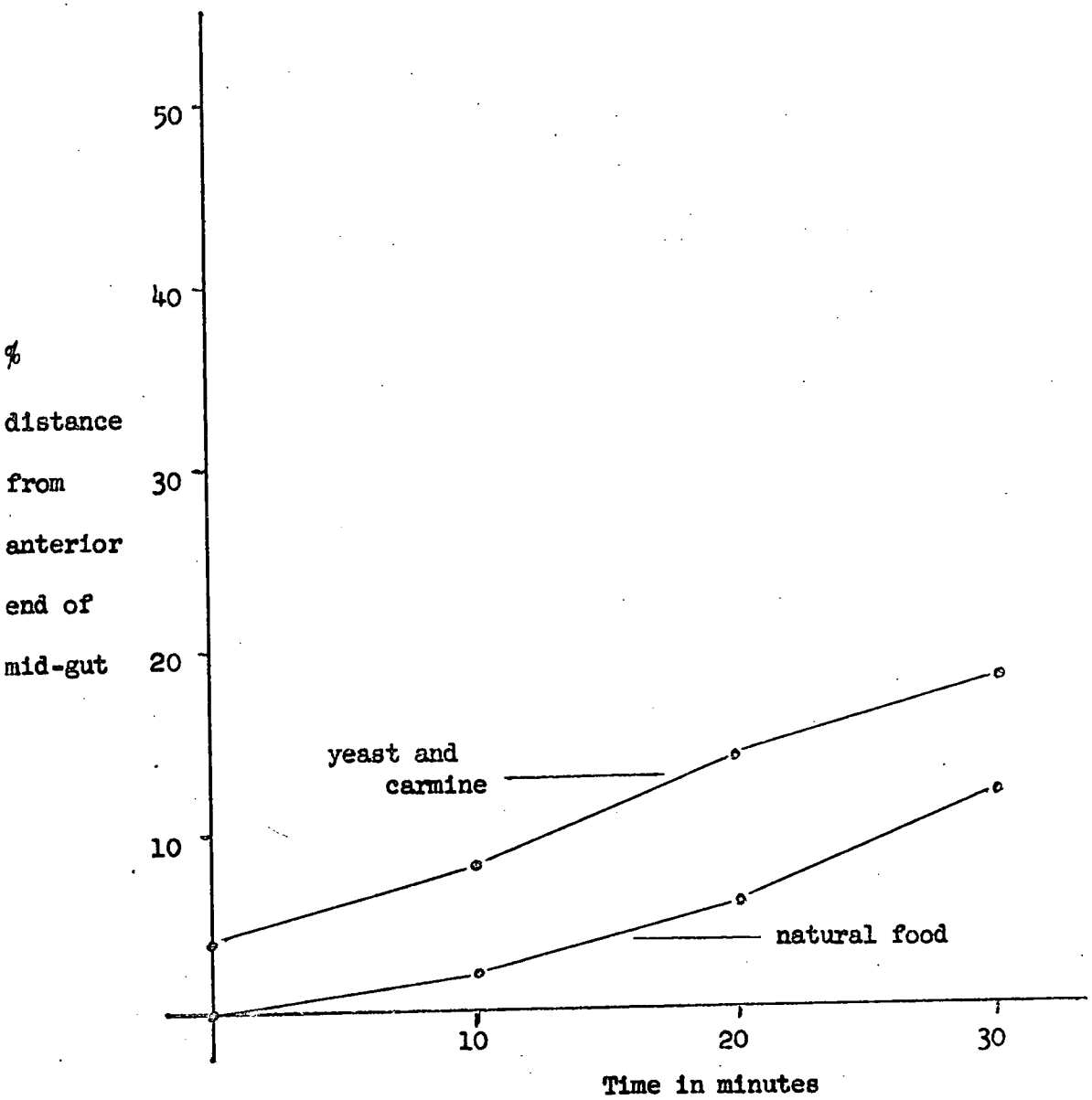

Fig. 106. Expt. C9: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 89 cm./sec. current velocity and 16.5°C.

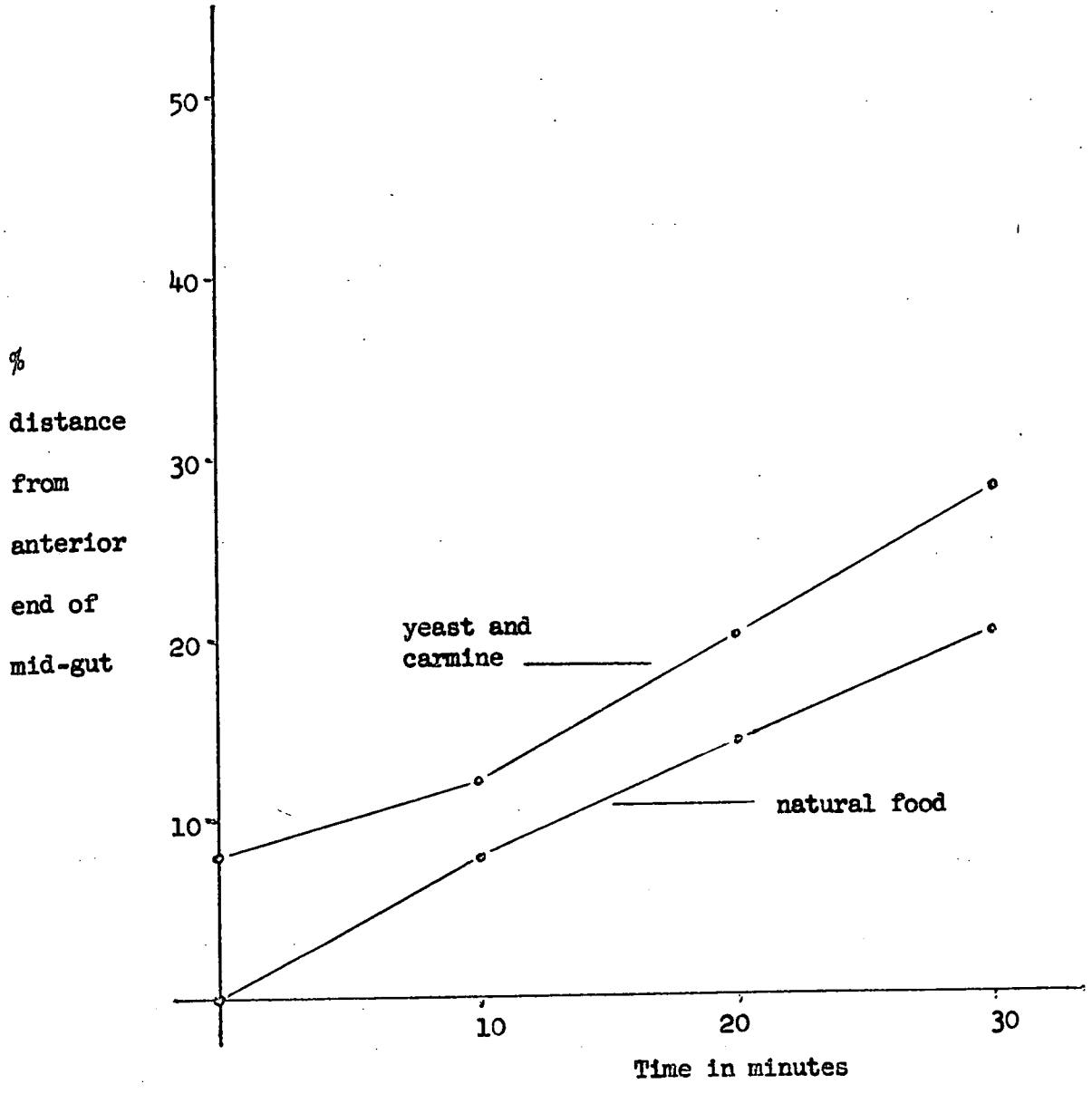

Fig. 107. Expt. C10: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 108 cm./sec. current velocity and 16.5°C.

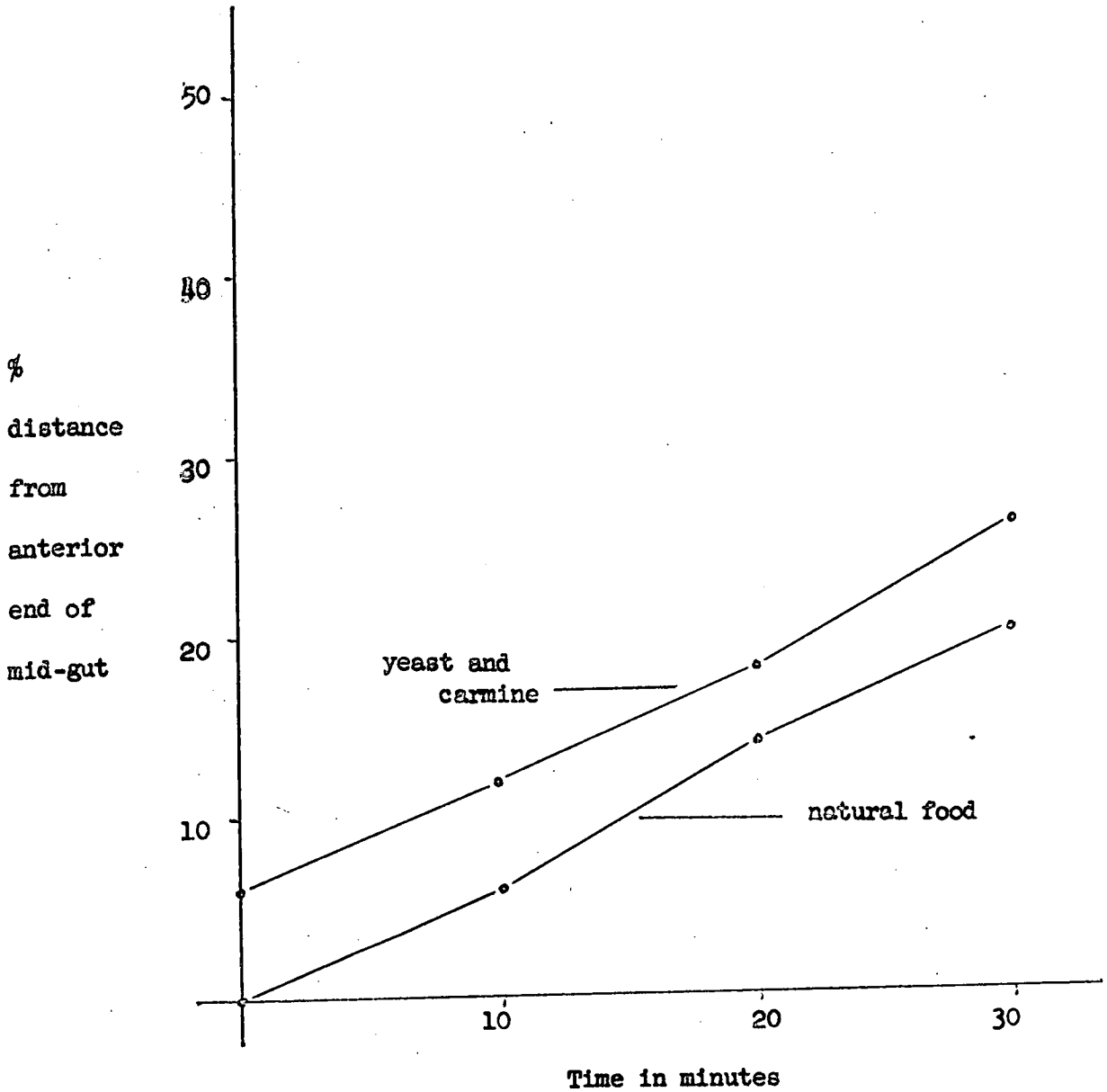

Fig. 108. Expt. C11: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 109 cm./sec. current velocity and 21.0°C.

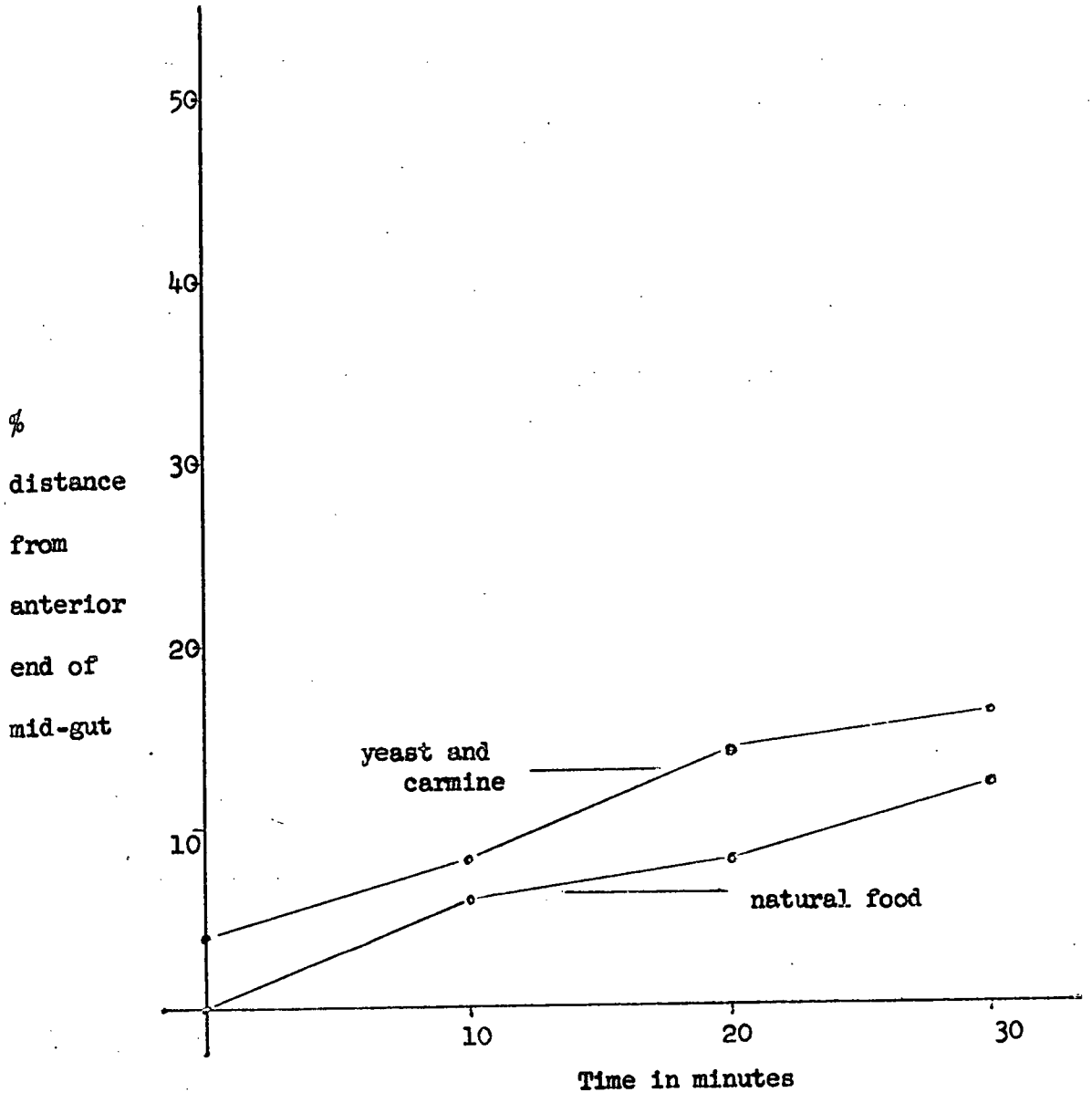

Fig. 109. Expt. C12: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 140 cm./sec. current velocity and 16.5°C.

TABLE 43

Experiment C8: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 63 cm./sec. current velocity and 21.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	6
10	10	8	14
20	10	14	20
30	10	26	32

TABLE 44

Experiment C9: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 89 cm./sec. current velocity and 16.5°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	4
10	10	2	8
20	10	6	14
30	10	12	18

TABLE 45

Experiment C10: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 108 cm./sec. current velocity and 16.5°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	8
10	10	8	12
20	10	14	20
30	10	20	28

TABLE 46

Experiment C11: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 109 cm./sec. current velocity and 21.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	6
10	10	6	12
20	10	14	18
30	10	20	26

TABLE 47

Experiment C12: The intake of food by S. pictipes and S. longistylatum larvae from Marsh's Falls at 140 cm./sec. current velocity and 16.5°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	4
10	10	6	8
20	10	8	14
30	10	12	16

The results of experiments C8 - C12 showed that S. pictipes and S. longistylatum larvae from Marsh's Falls were feeding at different rates under different current velocities. Fig. 110 gives the percent intake of natural food after thirty minutes at four velocities. Intake was low at 89 and 140 cm./sec., higher at 108 cm./sec. and highest at 63 cm./sec. There is some similarity to the experimental results obtained with S. monticola in the feeding experiments.

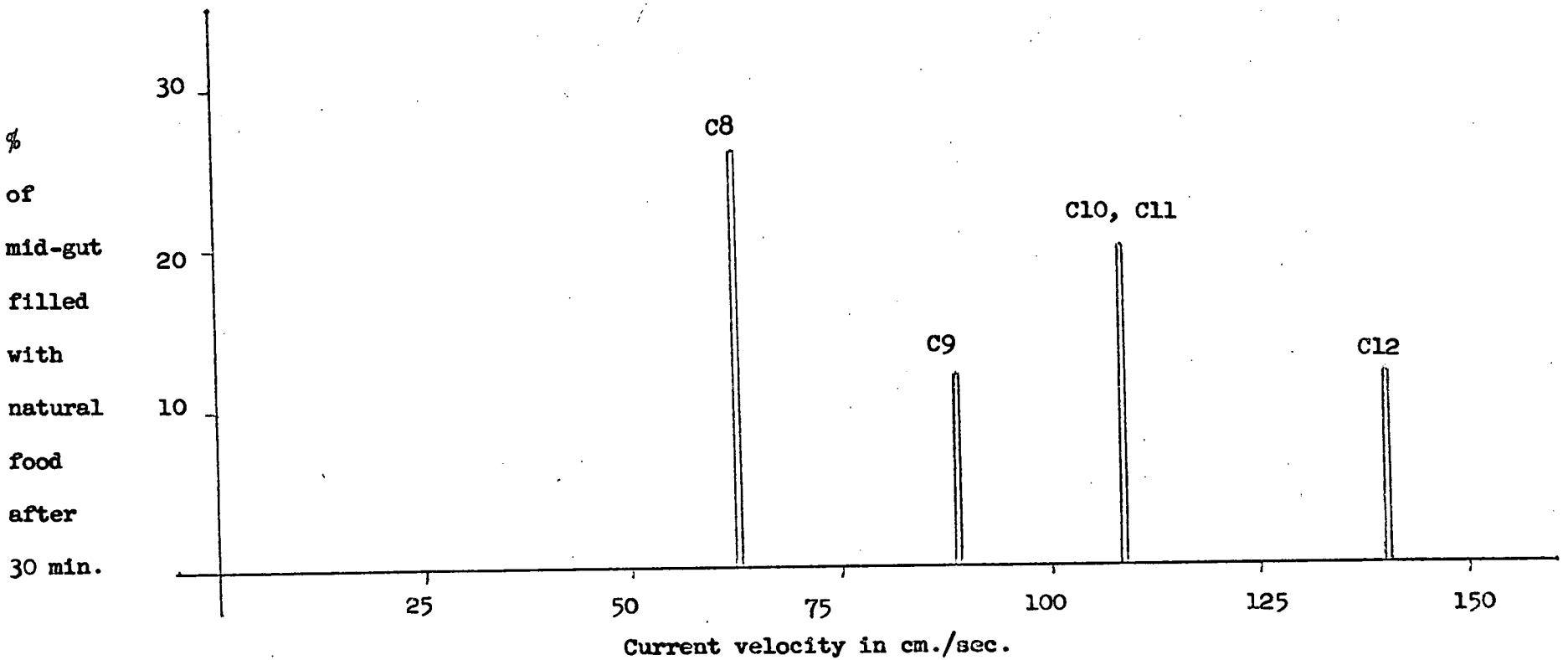

Fig. 110. Expts. C8 - C12: Intake of natural food at various current velocities after 30 minutes by S. pictipes and S. longistylatum larvae at Marsh's Falls, Ontario.

TABLE 48

Experiment C13: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 44 cm./sec. current velocity and 20°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	4
10	10	8	12
20	10	18	26
30	10	26	32

TABLE 49

Experiment C14: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 44 cm./sec. current velocity and 20.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	6
10	10	6	14
20	10	16	22
30	10	24	30

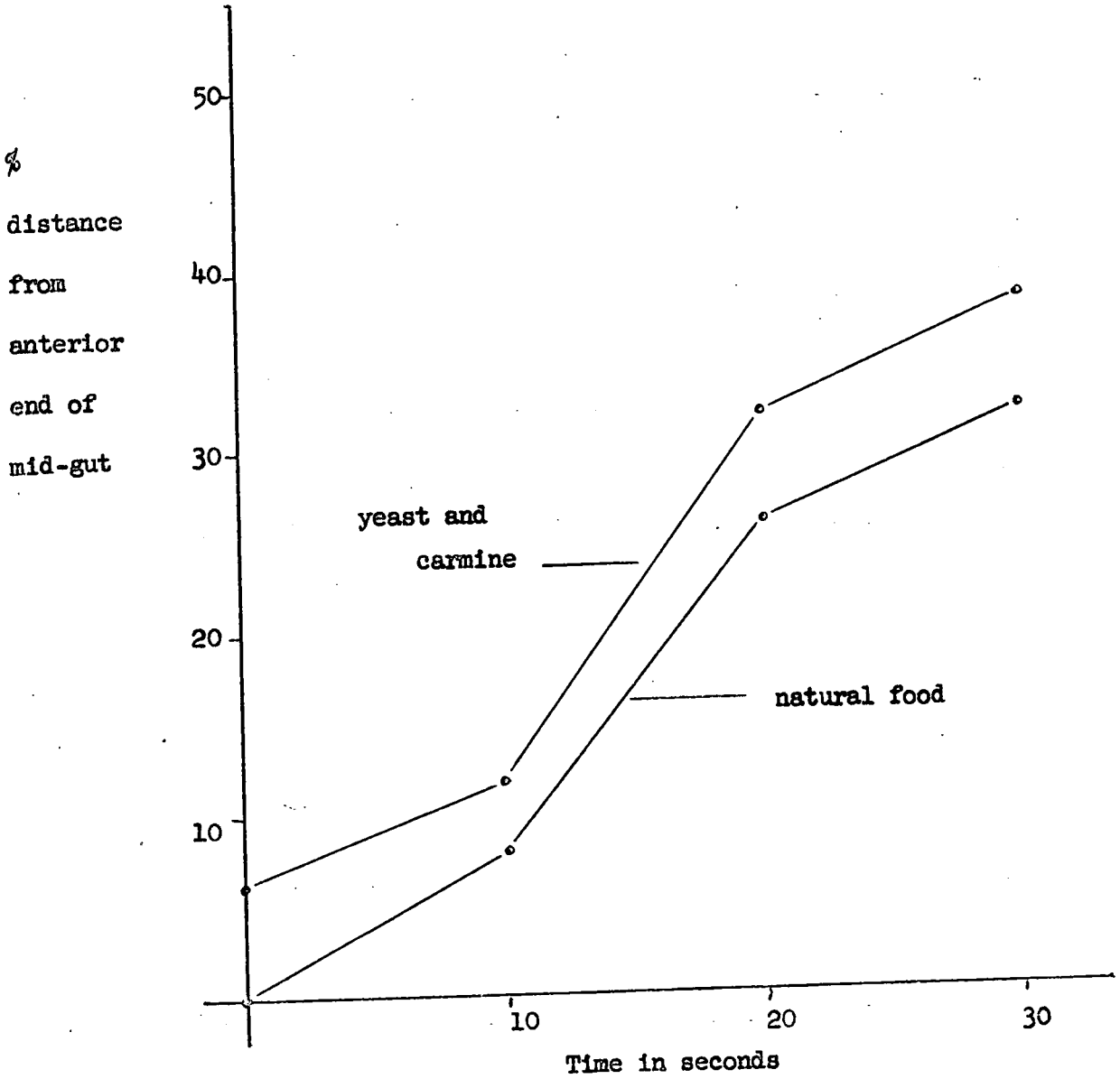

Fig. 111. Expt. C13: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 44 cm./sec. current velocity and 20.0°C.

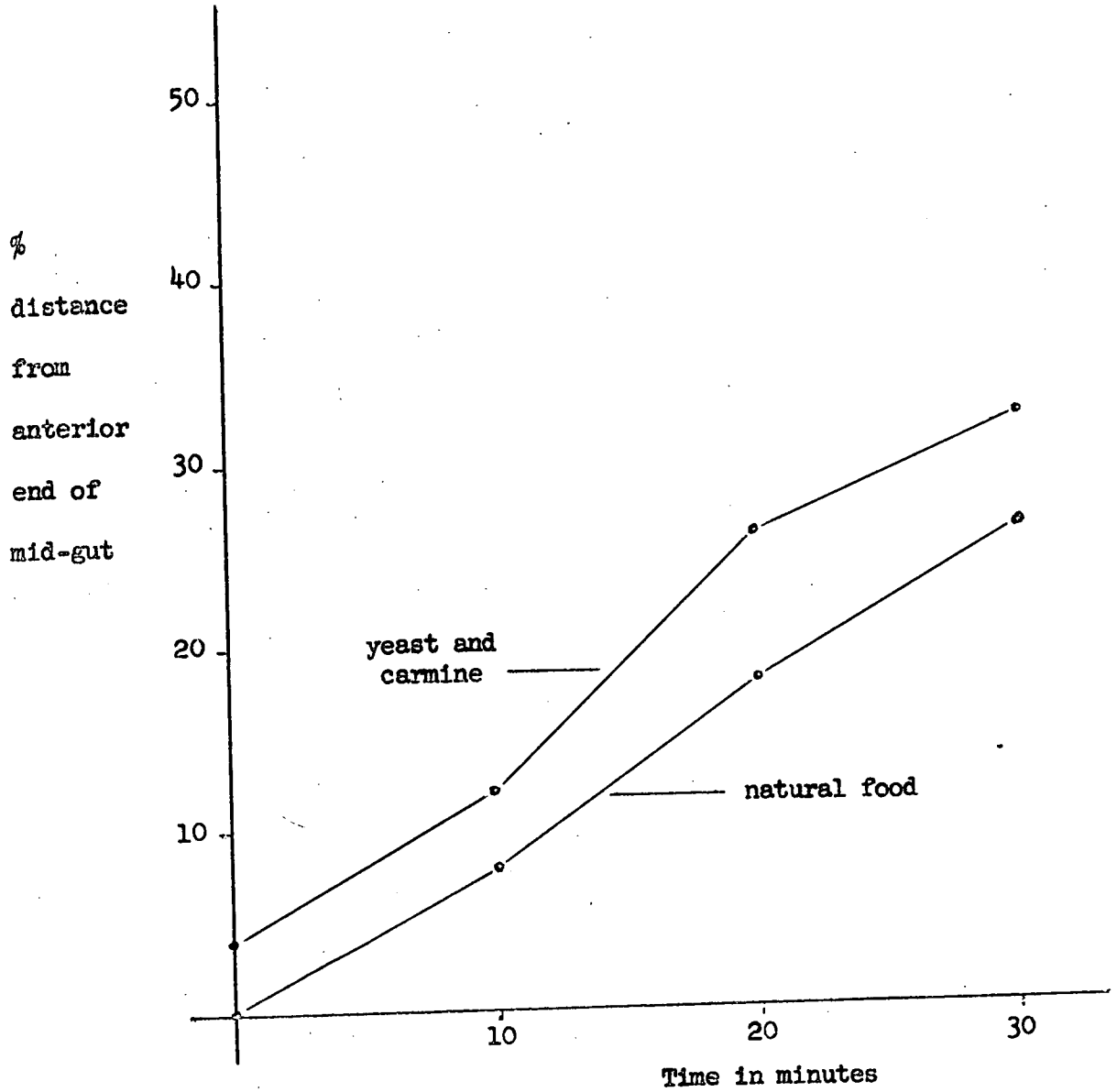

Fig. 112. Expt. C14: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 44 cm./sec. current velocity and 20.0°C.

TABLE 50

Experiment C15: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 54 cm./sec. current velocity and 15.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	6
10	10	8	12
20	10	26	32
30	10	32	38

TABLE 51

Experiment C16: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 77 cm./sec. current velocity and 15.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	2
10	10	12	16
20	10	16	18
30	10	20	22

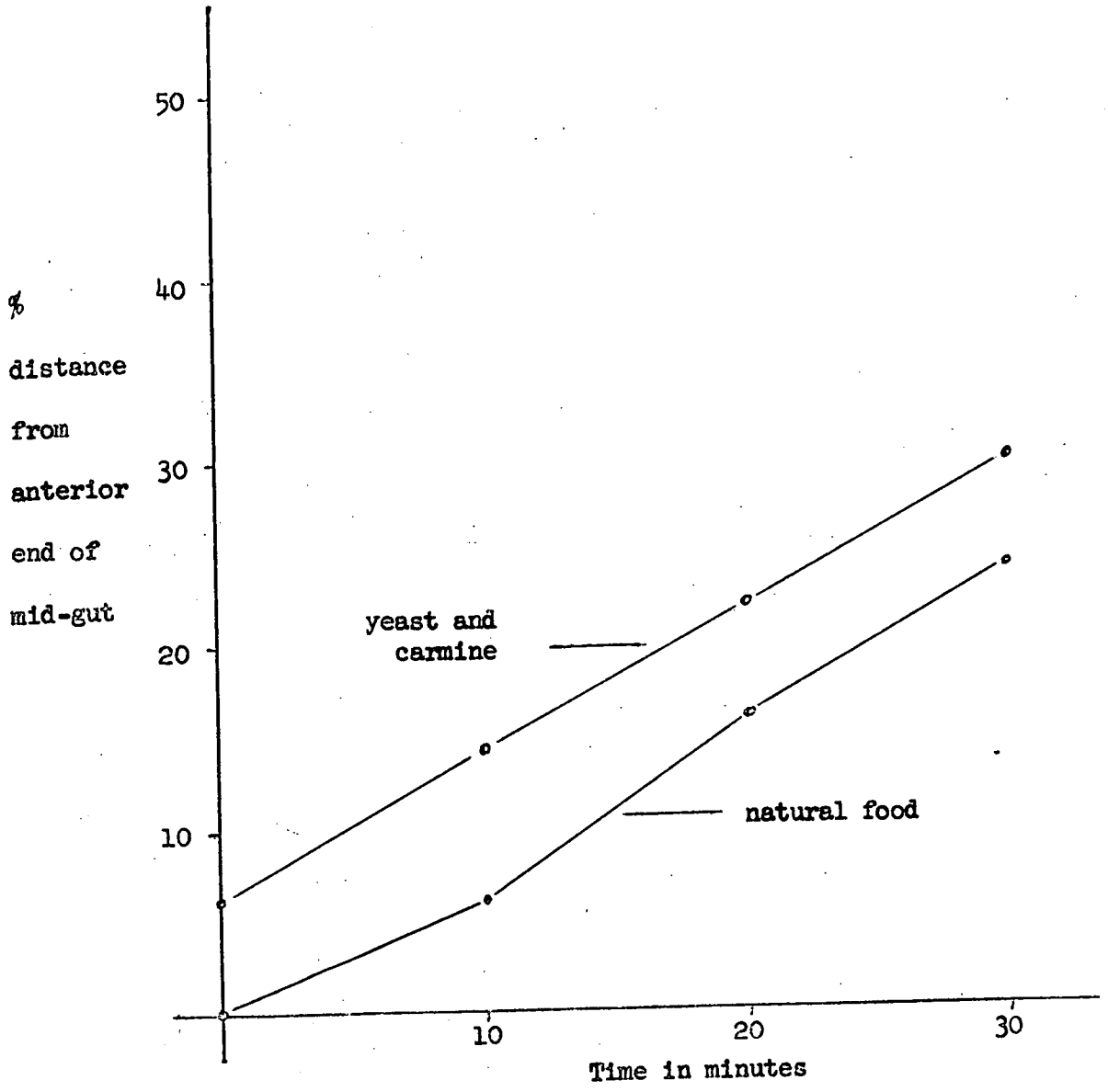

Fig. 113. Expt. C15: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 54 cm./sec. current velocity and 15.0°C.

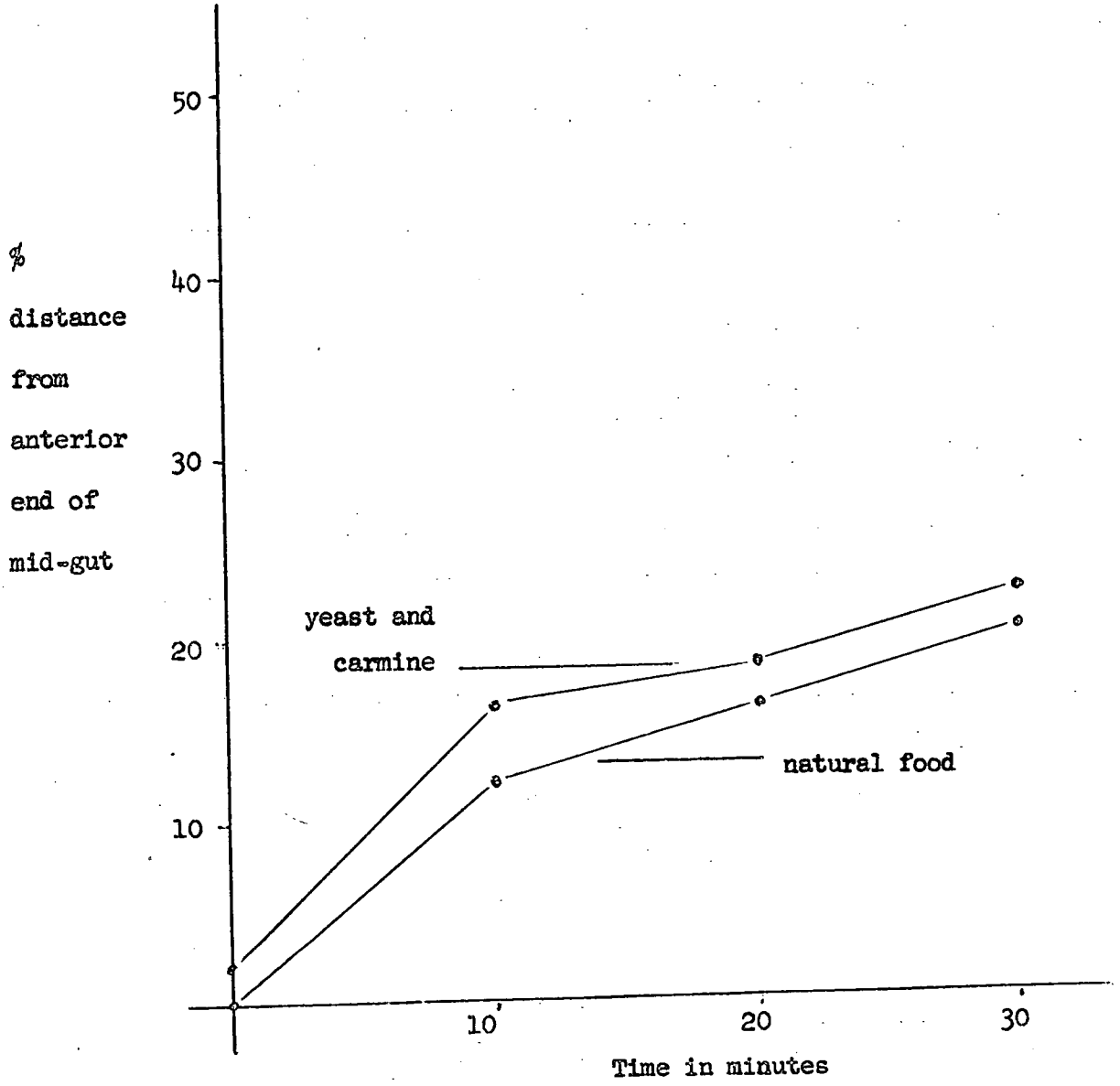

Fig. 114. Expt. C16: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 77 cm./sec. current velocity and 15.0°C.

TABLE 52

Experiment C17: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 77 cm./sec. current velocity and 20.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	2
10	10	4	6
20	10	14	16
30	10	18	22

TABLE 53

Experiment C18: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 83 cm./sec. current velocity and 15.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	4
10	10	16	20
20	10	26	32
30	10	38	46

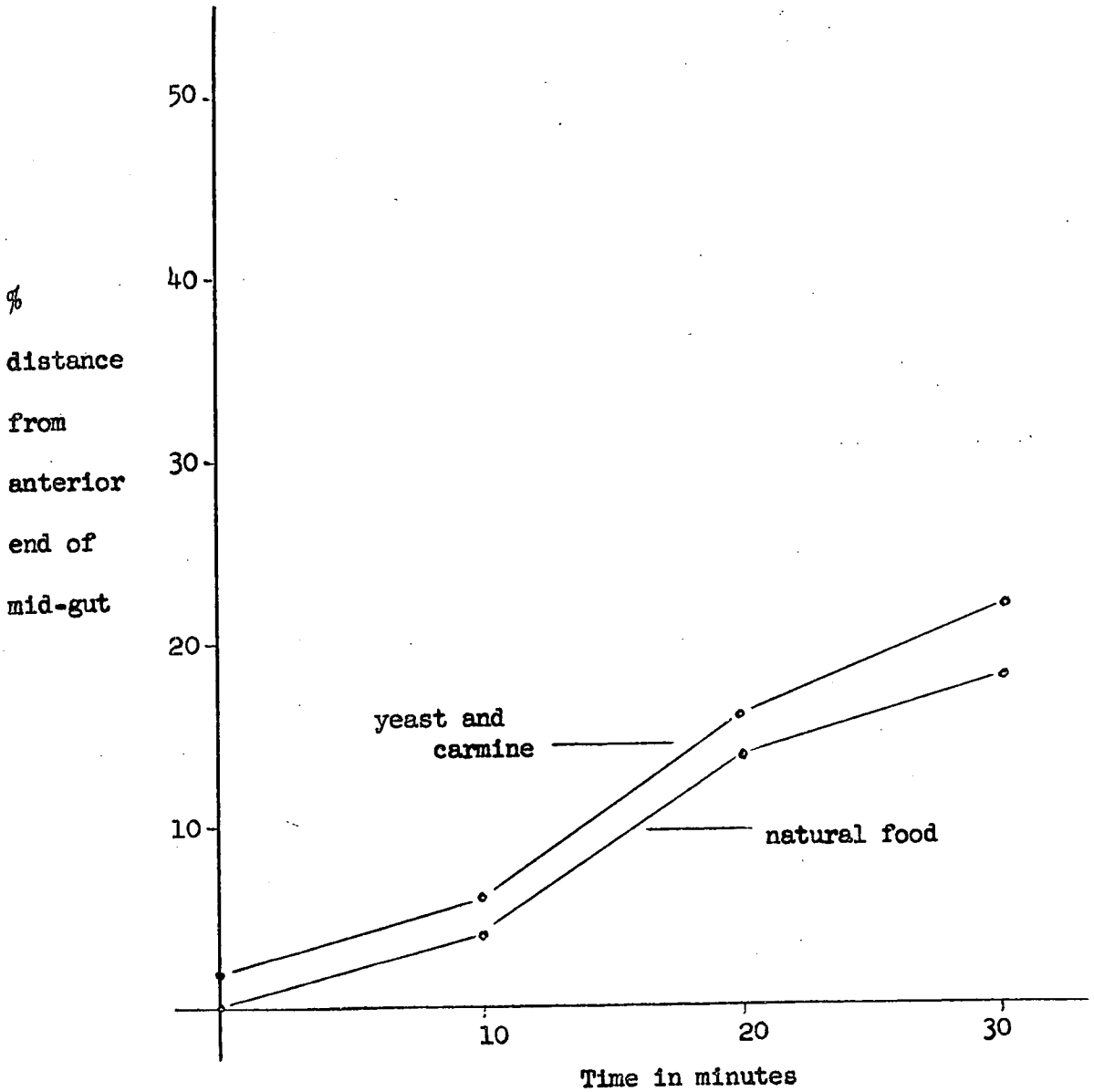

Fig. 115. Expt. C17: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 77 cm./sec. current velocity and 20.0°C.

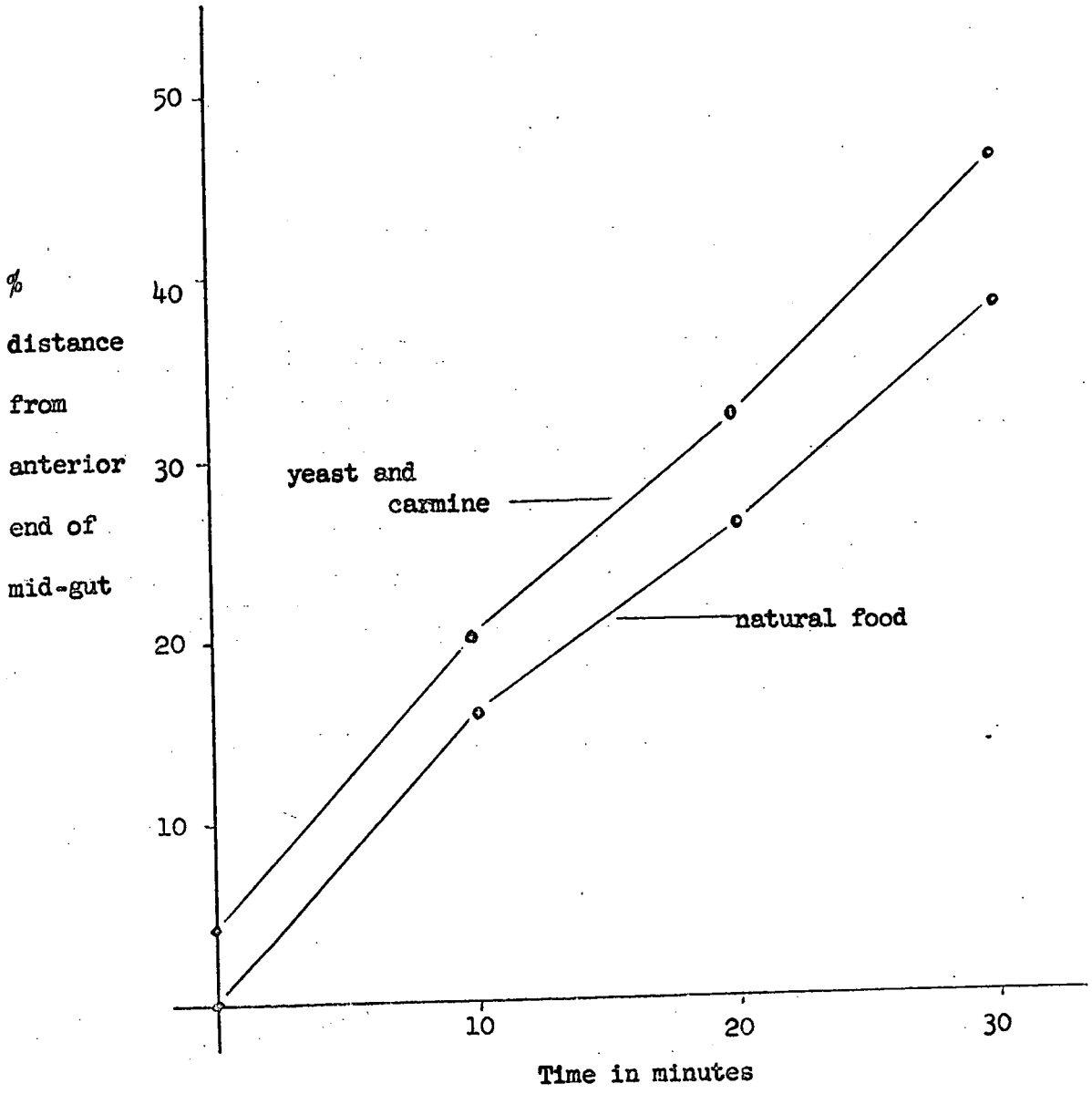

Fig. 116. Expt. C18: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 83 cm./sec. current velocity and 15.0°C.

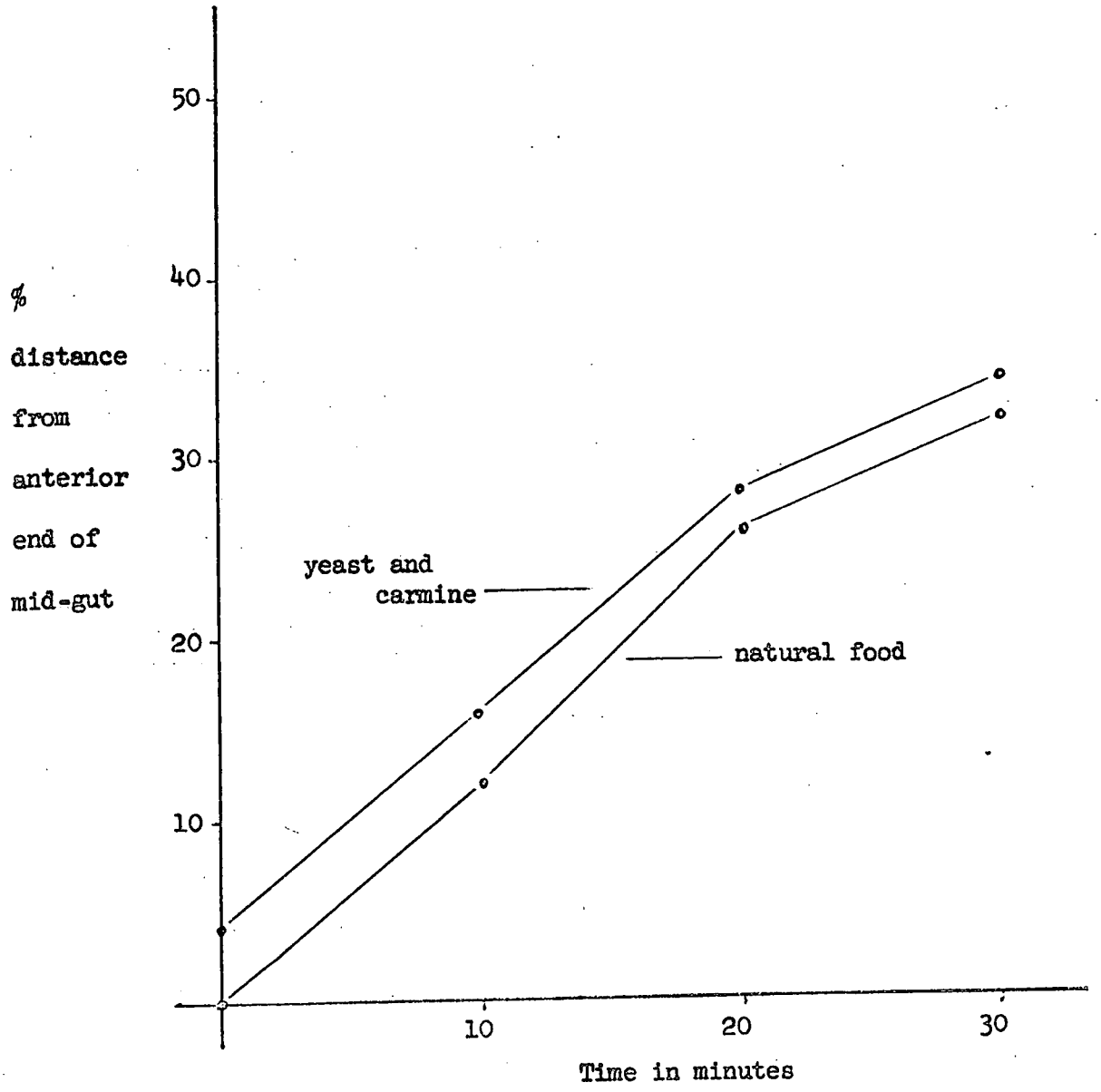

Fig. 117. Expt. C19: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 98 cm./sec. current velocity and 15.0°C.

TABLE 54

Experiment C19: The intake of food by S. pictipes and S. longistylatum larvae from Elliot Falls at 99 cm./sec. current velocity and 15.0°C.

Time (min.)	No. of larvae examined	Ave. % position in mid-gut of coloured food	
		From	To
0	10	0	4
10	10	12	16
20	10	26	28
30	10	32	34

The experiments on S. pictipes and S. longistylatum larvae at Elliot Falls (Tables 48-54 and Figs. 111-117) showed (as did experiments C8 - C12 at Marsh's Falls) variations in the rate of food intake at different current velocities. A pattern of varying intake rates (Fig. 118) similar to that for larvae from Marsh's Falls can be seen. Larvae at Elliot Falls were living in a habitat with generally lower current velocities and no microhabitats with current faster than 1 m./sec. were found.

Larvae from Marsh's Falls were least efficient at collecting food at 89 cm./sec. while larvae from Elliot Falls were most efficient at 83 cm./sec. Both populations showed high rates of intake at a lower and higher velocity with a lower intake rate at an intermediate velocity.

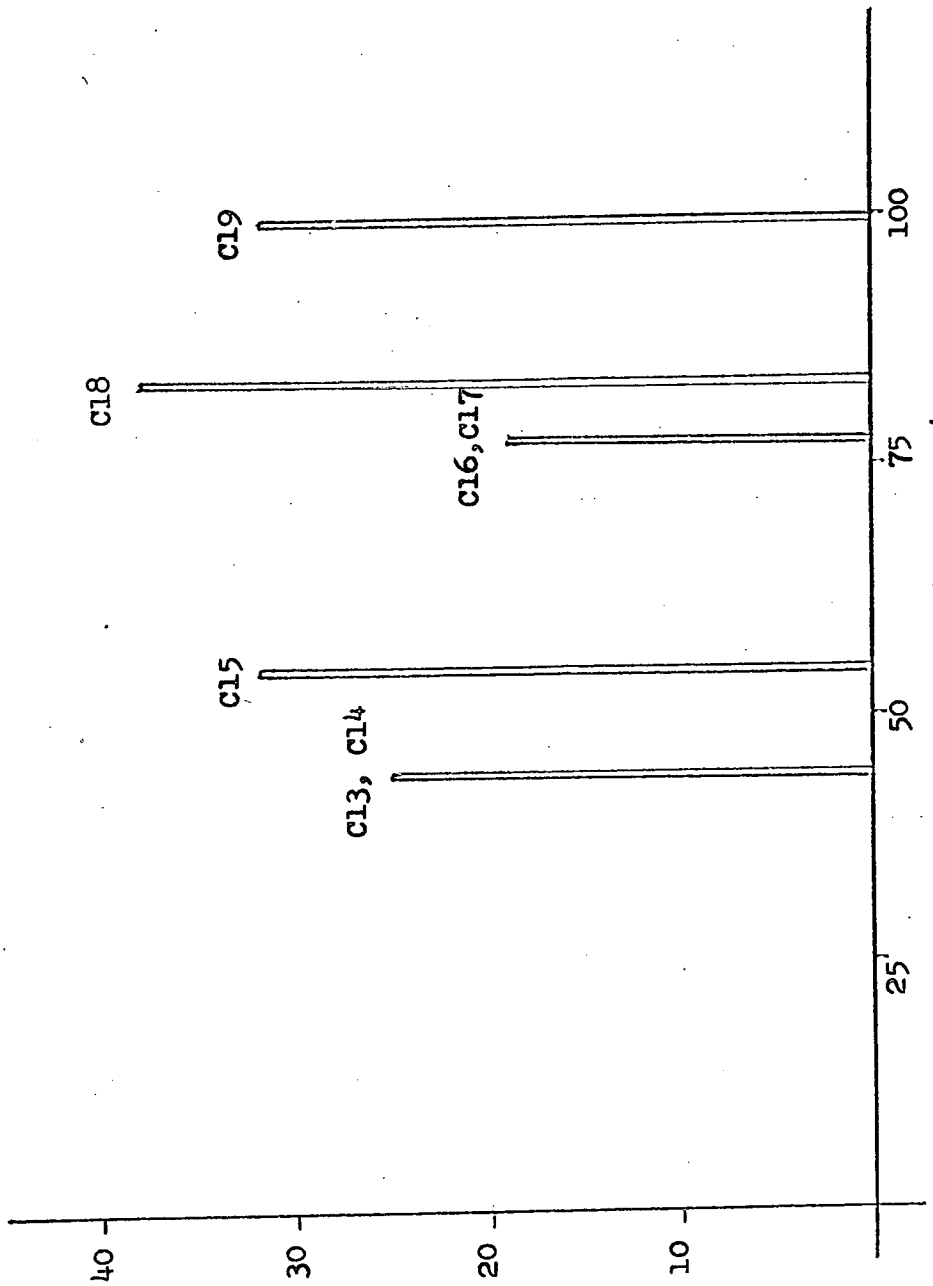

Fig. 118. Expts. C13 - C19: Intake of natural food at various current velocities after 30 minutes by S. pictipes and S. longistylatum larvae at Elliot Falls, Ontario.

v) Discussion

Nauman (1924) mentioned that larvae filled their intestines in thirty minutes with the unspecified food he gave them. It is perhaps not surprising to find that feeding rates for several species on two continents are not very far removed from this observation. Neither is it surprising that some intake rates are much lower than this. Changes in intake rate were observed in the trough experiments with changes in the concentration of artificial food. Concentrations of microseston must vary widely in nature and, as Maciolek and Tunzi (1968) have pointed out, black-fly larvae are efficient removers of the cellular portion of it. In field experiments then, the microseston available to any of the populations studied here had undoubtedly been reduced by larvae further upstream. Carlsson (1962) pointed out that larvae are often heavily concentrated in areas where a river flows out of a lake. Larvae in such a location would not, therefore, suffer competition for food.

It would be very difficult to show definite differences in rates of ingestion for a given species of larva encountered in its natural habitat. Larvae encountered in a particular location in a stream have presumably attached themselves in a suitable location. Any population existing in a microhabitat in the numbers required in the experiments discussed here would certainly have been in a location suitable to them. These locations were usually areas with a restricted amount of current speed variation.

One exception was at Marsh's Falls where current velocity varied from 63 cm./sec. to 140 cm./sec. and larvae were present throughout that current range. S. pictipes and S. longistylatum larvae are normally associated with a waterfall habitat and appear to be less restricted in current preference than other species such as S. venustum and S. reptans. It is also possible, since S. pictipes and S. longistylatum larvae are so difficult to separate taxonomically, that one of these species is prevalent in the slower water on the lip of the falls and the other is more common in the more rapid water. There would then be some degree of separation into different ecological niches. If this is not the case and both species share the same physical habitat, food and predators and parasites, we would be confronted with the problem of two species sharing the same niche.

6. SUMMARIZING DISCUSSION

The field studies on streams both in England and Ontario showed that black-fly larvae were found at a wide range of population densities. Carlsson (1962) found large variations in population densities from locality to locality and from sample to sample in Scandinavia. The densities found in this study exceeded the highest found by him. Densities in Allerton Beck, a small moorland stream in County Durham, England, were on the average much higher than those found in a larger Ontario stream. Very low densities were found at both locations, however.

Such a wide variation in population densities in a single stream raise the question of whether or not larvae on a particular piece

of substrate are aware of the presence of other larvae in close proximity to them.

The laboratory experiments recorded densities of larvae as high as 141/sq. cm. After being crowded to higher densities than those at which they were first established, larvae generally showed little inclination to disperse when adjacent unoccupied substrate was made available. It was also found that the majority of larvae being crowded together on a limited area of substrate preferred to remain on that substrate rather than abandon it. Releasing hold of a substrate and dropping downstream on the end of a silk thread would expose a larva to considerably more buffeting from the current and to the danger of being swept into an unsuitable habitat. The apparent reluctance of larvae to abandon the crowded substrates used in the laboratory experiments may simply indicate that they were not really crowded, although these densities were higher than those found during the field studies.

The larva of Crozetia crozetense has been considered by Davies (1965) as primitive in regard to the labrum and the cephalic fan. The cephalic fan rays of this species have a raking function rather than a filtering one and represent an early stage in the evolution of the simuliid cephalic fan.

Similarities in the appearance of the microtrichia of primary fan rays of P. ferrugineum to those of Crozetia crozetense were noted. Rubtzov (1959) and Carlsson (1962) consider P. ferrugineum to be a relict species. Larvae of this species were found (in several

Norwegian collections) to be feeding on other stream insects including black-fly larvae of other species. Serra-Tosio (1967) has recorded P. inflatum as a predator on chironomid larvae. Similarities can be seen between the primary fan rays of this species and P. ferrugineum. S. venustum larvae have been noted to be cannibalistic (Peterson and Davies, 1966); this behaviour may not naturally occur. The role of the primary cephalic fan in predation has not been observed; the long heavy tips of the rays of the primary fan of P. ferrugineum larvae may play some part in this mode of feeding.

Perhaps predation is an attempt to obtain an increased amount of food. Other species of larvae found in the same streams as P. ferrugineum apparently had no need to practice predation on other stream animals. If P. ferrugineum larvae were not as effective in filter-feeding as other species, then to obtain sufficient food they would be required to change their feeding habits. Adoption of a predatory habit may well have enabled P. ferrugineum larvae to survive in environments where filter-feeding alone would not provide enough food. The larva of Crozetia crozetense uses its cephalic fans as rakes rather than as a filter-feeding organ (Davies 1965). P. ferrugineum larvae have been found to predate on other stream insects, perhaps using their strong spear-pointed cephalic fan rays in this action.

The filter-feeding species of larvae studied here (ie. those other than Crozetia crozetense and P. ferrugineum and P. inflatum to some extent) have cephalic fan rays with varying patterns of microtrichia.

Laboratory experiments showed for four species of larva that the rate of food intake for each species varied with the current speed. Further, species were most efficient at gathering food at current velocities at which they were found in nature. The plotted curves of food intake against current velocity were different for each species, thereby suggesting that an environment with a current speed favourable to one species for food-gathering would not be as favourable to another species because of its inability to extract as much food in a given time. This latter species would therefore be at a disadvantage in competing with the former species. Phillipson (1957) stated that current speed was important in determining the distribution of black-fly larvae and possibly isolating different species in streams. It seems probable that it is the ability to gain food from a particular current velocity range that determines the distribution of a species.

S. ornatum larvae and S. reptans larvae both were efficient collectors of artificial food at 54 cm./sec. and less efficient at higher and lower current velocities. The primary fan rays of both species possess microtrichial patterns with secondary microtrichia of nearly equal length interspersed with primary microtrichia of greater length. S. monticola and S. variegatum larvae were found to be efficient particle collectors at 70 and 80 cm./sec. current velocity respectively. These two species both have primary fan rays with secondary microtrichia in an "organ-pipe" varying pattern of increasing length. Apart from these variations in species tested during the studies reported here, it

is difficult to associate a particular microtrichial pattern with a particular current environment. Grenier (1949) associated the degrees of chitinization of the rays of the primary fan with current velocity. He stated that species with heavily chitinized rays such as P. hirtipes, S. monticola and S. variegatum were always found in rapidly moving water. Species found in slowly moving waters and bearing weakly chitinized primary fan rays were S. angustitarse, S. equinum, S. salopiense, S. costatum and S. aureum. Species with primary fan rays of intermediate chitinization were S. ornatum and S. latipes. They were considered to inhabit currents of intermediate velocity around 60 cm./sec.

On Grenier's (1949) classification there would seem to be an indication that species with "organ-pipe" secondary microtrichial patterns are found in faster flowing water. S. monticola and S. variegatum support this idea. Also, species with less variation in the lengths of their secondary microtrichia are found in slower currents (S. angustitarse, S. salopiense and S. costatum). There are numerous exceptions to this idea, however.

If it were possible to determine the way in which the microtrichia on the rays of the primary fan function during fan movements, it might be possible to explain their morphology more fully.

In the three species in the species-group of S. ornatum, (Davies 1966) each species has a separate pattern of microtrichia on its primary fan rays. S. spinosum larvae have the "organ-pipe" pattern of secondary microtrichia while S. ornatum and S. nitidifrons possess

secondary microtrichia which have less variation in length. S. nitidifrons has a greater number of secondary microtrichia between each primary microtrichium than does S. ornatum. Davies (1966) points out that S. nitidifrons larvae replace S. ornatum larvae in upland and hill areas and also in poorer habitats at all altitudes in western and northern Britain. S. spinosum larvae are sometimes found with S. nitidifrons larvae but are much less common than the other two species. These three species were previously considered as one species with S. nitidifrons and S. spinosum treated as varieties. Whether or not the differing patterns of microtrichia on the primary fan rays of these three species have an ecological significance, it is certain that they are characteristic morphological differences in three closely related species whose larvae would appear to have different ecological requirements.

The actual process of food-gathering occurs by flicking the primary fan to a partially closed position where it is then cleaned by the cover bristles of the mandible. This flick of the primary fan takes place in only 0.15 seconds and is repeated very frequently (see Tables 11 and 12). Several other authors (Fortner, 1937; Grenier, 1949) have considered flicking of the cephalic fans to be the feeding action. The co-ordination of the primary fan with the mandible in the flicking action suggests very strongly that there is a very rapid transfer taking place. The rapid action of the mandibles after a flick of a fan confirms that they have collected food particles and are engaged in forming them into a bolus.

Chance (1970) stated that food transfer occurred when the convex surface of the closed and retracted fan was scraped by the external bristles of the mandible. The infrequent occurrence of this action by larvae and the fact that the convex surface of the rays of a primary fan is unlikely to have collected any food are serious objections to this theory of feeding. Furthermore, Chance's theory does not give any explanation for the flicking action.

The results of the food intake studies of black-fly larvae in their natural habitats showed that all species were taking in food when studied. Two night experiments showed that S. venustum larvae were still feeding in total darkness thus confirming the dark experiments carried out on S. ornatum larvae.

There was considerable variation in the rates of intake by different species under various conditions. In one experiment S. venustum larvae were feeding at a rate which would mean that their entire mid-gut contents would have been changed every 35 minutes. At the other extreme, larvae of the S. pictipes-S. longistylatum complex in two experiments would have completely changed their mid-gut contents in 4 hours and 10 minutes. This wide variation meant that S. venustum larvae under certain conditions were ingesting food more than seven times as rapidly as larvae of the S. pictipes-S. longistylatum complex.

The range of intake rates found in single species also showed considerable variation. S. ornatum larvae in three experiments would have completely changed their mid-gut contents in from 36 to 94 minutes.

S. reptans larvae in two experiments would have required 37 and 45 minutes. Seven experiments on S. venustum larvae would have given figures from 35 to 79 minutes. S. pictipes-S. longistylatum larvae, feeding at slower rates, would have exchanged their mid-gut contents in 79 to 250 minutes. The laboratory experiments showed that food intake rate was affected by current velocity and food concentration. Food concentration will be to some extent be affected directly by current velocity since heavier water-borne particles (especially inorganic material) will settle to the bottom in slower currents. Microseston levels in a stream will certainly vary over a period of time. (Such a variation may explain why later experiments on S. venustum larvae gave lower intake rates.)

Chance (1970) stated that larvae feed automatically and at times ingest more than they require. The nutritional requirements are not known, but from the experiments here it appears that larvae feed continuously at a rate determined by current velocity and food concentration. Davies and Syme (1958) found that starved P. fuscum and P. mixtum larvae required two weeks to empty their digestive tracts. It is probable that either food utilization is poor or that the great majority of the material ingested under natural conditions is inorganic and therefore of no food value to larvae.

These studies have shown something of the densities of population attained by black-fly larvae and their reactions when crowded onto a limited space or when offered an increased amount of space.

Both the rate and irregularity of primary fan action and its detailed co-ordination with the mandibles were studied. Investigation was made of the fine structure of the rays of the primary fan in a number of species. Also studied was the curious flicking action of the cephalic fan.

Discovery of such wide and species-specific variation in the microtrichia on the primary fan rays of 24 species led to speculation that there might be differences in the rate of food intake by different species. It had long been known that larvae of certain species were restricted to habitats in certain current ranges and it had occasionally been suggested that current preferences were connected with food-gathering. Laboratory experiments performed in this study showed that black-fly larvae were not equally efficient food gatherers at all the current velocities in which they would exist. Rather they were most efficient at collecting food from current velocities which formed their normal habitat and much less efficient at current velocities abnormal to them.

Field experiments further showed that the rate of intake of natural food may vary quite widely from species to species and from habitat to habitat. Larvae in natural habitats were not usually found at current velocities disadvantageous to them and so the influence of current velocity on rate of food intake was somewhat obscured compared to the laboratory results. Nevertheless, the field studies on natural food intake showed that larvae fed continuously, often at very high rates.

The casual observer may wonder at the energy expended on such minute animals and question the eventual importance of such work when viewed against larger questions of this age. These studies have attempted to clarify the biology of larval simuliids in relation to food and living space, two important components of their environment. Black-fly larvae are common and important members of flowing fresh-water invertebrate communities. They are usually equipped with special adaptations for food-gathering which make them unique in these communities.

The simuliids are not merely worthy of study just because of their own importance. They are also of considerable importance to man for two other reasons.

Adult female black-flies are important as pests of man and animals. One of the chief methods of control of black-flies is by the use of larvicides poured into their larval habitats. However, larvicides (such as DDT) are now being prohibited due to concern over environmental pollution. Basic research such as that performed here may eventually lead to the development of larvicides which would have to be ingested by the larvae before they exhibited any toxic effect. Such larvicides would pose no problem to the environment if dispersed into the stream in particle sizes only acceptable to black-fly larvae.

The ability of black-fly larvae to extract minute organic and inorganic particles from flowing water brings into focus another reason for this study. Much of the work done here illuminates the importance of black-fly larvae as part of the river ecosystem. Their

place is that of a primary converter of organic debris and vegetable matter into animal tissue. They are one of the chief forms of river life, along with caddis and chironomid larvae, to feed on particles in suspension. Their immense numbers in many rivers suggest their importance to the ecosystem in the conversion of microseston. The control of water pollution and the desire of man to retain unspoiled natural environments receives much attention to-day. Perhaps it would be well to further determine the importance of such a basic member of the aquatic food web as the black-fly larva. It is possible that these insects, acting as filtering mechanisms in streams, may in fact be helpful to man in his attempts to combat organic pollution of streams and rivers.

7. CONCLUSIONS

The Absolute Densities of Various Populations of Black-Fly Larvae

1. Larvae were found in nature at a wide range of larval densities. In seventy-eight collections from the North Madawaska River in Ontario the density of black-fly larvae varied from 19.8/sq. cm. to less than 0.1/sq. cm. with an average density of 1.0/sq. cm. Twenty-five collections from Allerton Beck, Co. Durham, on March 30, 1967 gave densities of larvae from 34.5/sq. cm. to 0.1/sq. cm. with an average of 7.4/sq. cm. Twenty-two collections made on January 21, 1968 from Allerton Beck showed larval densities to vary from 10.6/sq. cm. to 0.1/sq. cm. with an average of 2.5/sq. cm.

2. Simulium ornatum larvae were crowded in the laboratory to densities of 70 to 141 larvae per sq. cm. These densities were higher than found in naturally occurring populations.

3. S. ornatum larvae preferred to be crowded closely together on an attachment site of limited area rather than abandon that substrate.

4. Larvae were reluctant to disperse to attachment sites previously occupied by them before crowding.

5. Larvae were found to move (by looping) more readily in an upstream direction than in a downstream direction.

The Means and Mechanism of Food Collection

1. The microtrichial patterns on the rays of the cephalic fans of 25 species of black-fly larvae were studied using the scanning electron microscope. Species-specific variations in these patterns were demonstrated.

2. Fan flicking rates were determined for S. ornatum larvae and found to be irregular but frequent as stated by Fortner (1937).

3. The actions of the primary fans and mouthparts of S. longistylatum larvae were photographed with a ciné camera. The flicking action of the primary fan was seen to be co-ordinated with the mandible so as to provide for effective transfer of food particles from the primary fan to the mouth. Fan flicking has been considered to be the actual feeding action.

The Food of Prosimulium (Helodon) ferrugineum

1. Fifty-one larvae of P. ferrugineum were found to contain the remains of 212 aquatic arthropods of other species. The larvae of Cnephia spp., from some of the same habitats as the larvae of P. ferrugineum, contained only one small piece of unidentifiable insect tissue. It is suggested here that the larvae of P. ferrugineum, found in several rivers of eastern Norway, obtain part of their diet by predating upon other aquatic arthropods.

Laboratory Experiments on the Feeding of Various Species of Black-Fly Larvae

1. Larvae of S. ornatum, S. variegatum, S. monticola and S. reptans were fed artificial food, consisting of a suspension of yeast cells and powdered carmine, at various current velocities in a recirculating trough apparatus. Each of these species was found to differ from the others in its rate of food intake over a range of current velocities.

2. S. variegatum and S. monticola larvae showed similarities in their feeding rates at the higher current speeds.

3. S. ornatum and S. reptans larvae were both more efficient at gathering food at 54 cm./sec. than at higher and lower current velocities.

4. S. ornatum larvae were not found to feed at a significantly greater rate in total darkness than under illumination.

5. The presence of carmine particles in the yeast suspension significantly reduced the rate of food intake by S. ornatum larvae.

6. The rate of food intake by larvae of S. ornatum, S. variegatum and S. reptans was found to be affected by the concentration of food presented to them.

Field Experiments on the Rate of Food Intake by Various Species of Simuliid Larvae

1. A number of species of black-fly larvae, both in England and Ontario, were found to ingest an artificial food suspension composed of yeast and powdered carmine when this was added to the river water just above the larvae studied.

2. Using the ingested artificial food as a timed tracer, the average rate of food ingestion for three English species (S. ornatum, S. variegatum and S. reptans) and two Canadian species (S. venustum and the S. pictipes-S. longistylatum complex) were determined. A wide range of rates of intake occurred, not only between species but between larvae of the same species living at different current velocities.

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APPENDICES

APPENDIX I

The Pitot-tube velocity gauge

The pitot tube method has been used by several black-fly workers and is a cheap and convenient method of gauging the velocity of running water. Its advantages for the present study were that it is capable of measuring current velocity in very close proximity to larvae either in an experimental apparatus or on a stone or piece of vegetation in nature.

The pitot tubes used in this study were made of glass tubing with a 4 mm. inside diameter and 1 mm. wall thickness. The tip of the horizontal arm was reduced to an opening of about 2 mm. and rounded by fire-polishing. Next to the vertical arm of the pitot tube a wooden rule marked in millimetres was fixed so as to provide an easily read scale. On the top of the wooden scale was a small glass spirit level, as used in carpenter's levels. This provided a check that the vertical arm was, in fact, being held vertical while measurements were being taken.

The formula used for conversion of the difference in height of the water column inside and outside the glass tube to the current velocity was $V = \sqrt{2gh}$ where V is velocity in cm./sec., h is the height of the water column in cm., and g is the force of gravity (980 cm./sec.)

It was not known whether variations in the bore of tubing used or the degree of restriction of the tip of the horizontal arm would affect the readings of a pitot tube gauge. Accordingly, the pitot tube described above was compared with tubes constructed from glass of several sizes of bores and with both restricted and unrestricted tips. The following pitot tubes were used:

1. Tubing of 4 mm. inside diameter with 1 mm. thick wall, tip reduced to 2 mm. inside diameter.
2. Tubing of 4 mm. inside diameter, with 1 mm. thick wall, tip not reduced but fire-polished.
3. Tubing of 5 mm. inside diameter, with 1 mm. thick wall, tip reduced to 1 mm. inside diameter.
4. Tubing of 5 mm. inside diameter, with 1 mm. thick wall, tip not reduced but fire-polished.
5. Tubing of 3 mm. inside diameter with a .5 mm. thick wall, tip not reduced but fire-polished.

These five pitot tubes were compared in the trough apparatus illustrated in Figure 101 at four velocities. The velocities recorded by each tube are given in Table I-1.

TABLE I-1

Velocity readings given by five different pitot tubes at four current velocities

Velocity	1	2	3	4	5
1	31.3	31.3	31.3	31.3	34.3
2	44	44	44	44	44
3	62.6	62.6	62.6	62.6	62.6
4	76.7	76.7	76.7	76.7	79.2

The results of these tests suggest that small-bore pitot tubes may give a slightly higher reading than larger bore tubes but that the restricted opening of a tip does not affect the readings to a discernible degree.

APPENDIX II

TABLE II-1

The contents of the mid-gut of each P. ferrugineum larva examined

Sample No.	P. ferrugineum larva	Chironomid larvae	Simuliid larvae	Insect tissue	Mites	Ephemeroptera or Plecoptera
1	1	2		1		1
	2	7		2		1
	3	2		3		
	4	2			1	
	5			1		
	6	2		2		
	7	1				
	8	1	1	3	1	
	9	3		3		
	10	3		3		
2	1	1	1	1		2
	2	1	1			
	3	5	1	1		1
	4	3	1	4		
	5	1		3		
3	1		2			
	2	1	2			
	3		1			
	4		1			
	5	4	4			
	6	1	1	1		
	7	3	1			
	8	3	1			2
	9	1	1	1		
	10		1	1		
4	1	2	3	1		1
	2	3	1	2		1
	3	3		1	1	1
	4	1	2	2		
	5		4	2		
	6	2	1	1		
	7	3	2	2		
	8	1	1	1		
	9	1		1		
	10		1	1		

TABLE II-1 (CONT'D)

Sample No.	P. ferrugineum larva No.	Chironomid larvae	Simuliid larvae	Insect tissue	Mites	Ephemeroptera or Plecoptera
5	1			1	1	1
	2	1	1	1		
	3					
	4					
	5		3			
	6	1	1	1	1	2
6	1	5		1		1
	2	2		1		
	3	2		1	1	
	4	3		1		
	5	1	1	1		
	6	1	1	1		
	7	8		1		1
	8			1		1
	9			1		
	10	3	1	1		

APPENDIX III

TABLE III-1

The values of Student's T between troughs A and B of the experiments in Series I and II (T for 1% level of significance = 2.5758, T for 5% level of significance = 1.9599)

Experiment No.	Trough	Ave. % of mid-gut filled	T value
I-7	A	41.2	1.1687
	B	49.2	
I-8	A	78.0	1.2864
	B	68.5	
I-9	A	43.7	.5524
	B	39.3	
I-4	A	24.5	0.5524
	B	28.0	
I-5	A	9.0	.4217
	B	8.3	
I-6	A	12.5	1.2121
	B	15.3	
II-1	A	81.3	1.0526
	B	74.9	
II-2	A	54.1	0.0248
	B	53.9	
II-3	A	91.0	0.0717
	B	85.5	
II-4	A	96.4	1.6478
	B	90.0	

APPENDIX III

TABLE III-2

The values of Student's T between experiments in Series I
(T for 1% level of significance = 2.5758,
for 2% level of significance = 2.3263
and for 5% level of significance = 1.9599.)

Comparison between Experiment No.	Experiment No.	T value
I-7A	I-8A	5.2243
I-7A	I-8B	3.7593
I-7B	I-8A	4.1296
I-7B	I-8B	2.6820
I-8A	I-9A	5.0248
I-8A	I-9B	5.4501
I-8B	I-9A	3.5256
I-8B	I-9B	3.9696
I-9A	I-4A	3.1741
I-9A	I-4B	2.2562
I-9B	I-4A	3.3008
I-9B	I-4B	3.1085
I-4A	I-5A	3.8644
I-4A	I-5B	4.1200
I-4B	I-5A	3.6266
I-4B	I-5B	3.8046
I-5A	I-6A	1.4125
I-5A	I-6B	3.2651
I-5B	I-6A	1.7895
I-5B	I-6B	3.9112

APPENDIX III

TABLE III-3

The values of Student's T between troughs A and B of the experiments in Series IV and V (T for 1% level of significance = 2.5758

Experiment No.	Trough	Ave. % of mid-gut filled	T value
IV-1	A	0.2	0.3302
	B	0.33	
IV-4	A	49.2	0.1507
	B	48.6	
IV-2	A	40.0	0.0362
	B	39.7	
IV-3	A	21.46	0.0474
	B	21.27	
V-1	A	50.7	0.3713
	B	48.0	
V-2	A	33.7	0.1483
	B	32.8	
V-3	A	78.6	0.6831
	B	82.3	

APPENDIX III

TABLE III-4

The values of Student's T between troughs A and B of experiments in Series VI

(T for 1% level of significance = 2.5758
T for 5% level of significance = 1.9599)

Experiment No.	Trough	Ave. % of mid-gut filled	T value
VI-6	A	48.1	1.6243
	B	35.9	
VI-1	A	36.6	0.2056
	B	37.5	
VI-2	A	57.7	0.2447
	B	56.0	
VI-3	A	37.6	1.2164
	B	42.8	
VI-4	A	34.5	0.2064
	B	35.6	
VI-5	A	21.1	1.8182
	B	15.5	

APPENDIX III

TABLE III-5

The values of Student's T and the levels of significant difference between experiments in Series VI

(T for 1% = 2.5758, T for 2% = 2.3263, T for 5% = 1.9599, N.S. = not significant.)

Comparison between		T value	Level of significance
Experiment No. and trough	Experiment No. and trough		
6A	1A	1.6947	N.S.
6A	1B	1.5593	N.S.
6B	1A	0.1292	N.S.
6B	1B	0.2901	N.S.
1A	2A	3.5732	.01
1A	2B	3.4569	.01
1B	2A	3.3700	.01
1B	2B	3.2422	.01
2A	3A	3.3787	.01
2A	3B	2.5353	.02
2B	3A	3.2509	.01
2B	3B	2.3647	.02
3A	4A	0.6201	N.S.
3A	4B	0.4211	N.S.
3B	4A	1.6897	N.S.
3B	4B	1.5457	N.S.
4A	5A	2.8596	.01
4A	5B	4.4351	.01
4B	5A	3.2813	.01
4B	5B	5.0363	.01

APPENDIX III

TABLE III-6

The values of Student's T between troughs A and B of the experiments in Series VII

(T for 1% level of significance = 2.5758,
T for 5% level of significance = 1.9599)

Experiment No.	Trough	Ave. % of mid-gut filled	T value
VII-3	A	8.9	0.5267
	B	10.3	
VII-1	A	66.9	0.4263
	B	64.4	
VII-2	A	21.5	0.7923
	B	25.8	
VII-4	A	19.5	1.0493
	B	14.5	
VII-6	A	15.7	0.6143
	B	17.7	
VII-8	A	15.0	0.1579
	B	14.4	
VII-9	A	42.9	1.8431
	B	38.0	
VII-10	A	68.9	2.2208
	B	55.4	
VII-12	A	10.13	0.0749
	B	10.3	

APPENDIX III

TABLE III-7

The values of Student's T and the levels of significant difference between experiments in Series VII

(T for 1% level of significance = 2.5758)

Comparison between Experiment No.	Experiment No.	T value	Level of significance
1A	2A	9.9758	.01
1A	2B	6.7811	.01
1B	2A	8.2405	.01
1B	2B	5.8779	.01
1A	3A	13.9894	.01
1A	3B	13.3901	.01
1B	3A	11.4292	.01
1B	3B	10.9848	.01
2A	4A	0.5051	N.S.
2A	4B	1.9977	.05
2B	4A	1.1206	N.S.
2B	4B	2.1237	.05
1A	9A	3.7999	.01
1A	9B	4.7463	.01
1B	9A	3.1604	.01
1B	9B	4.0042	.01
1A	10A	0.3418	N.S.
1A	10B	2.0751	.05
1B	10A	0.7060	N.S.
1B	10B	1.4778	N.S.
9A	12A	6.1148	.01
9A	12B	5.9381	.01
9B	12A	5.4378	.01
9B	12B	5.2984	.01

APPENDIX IV

TABLE IV-1

The position of the coloured band of food in the mid-guts of S. ornatum larvae examined as part of field experiment B1

Time in mins.	Specimen No.	Length in mm.	% position in mid-gut of coloured band	
			From	To
Zero	1	5.5	0	0
	2	5	0	2
	3	5.5	0	0
	4	6	0	2
	5	6.5	0	2
	6	5	0	0
	7	5	0	0
	8	6	0	2
	9	5.5	0	8
	10	4.5	0	6
	11	5.5	0	2
	12	5	0	4
	13	8	0	2
	14	6	0	8
	15	6	0	4
5	1	8	2	4
	2	7.5	2	4
	3	4.5	6	10
	4	4	8	10
	5	4	0	12
	6	3.5	12	22
	7	3	10	20
	8	2.5	16	24
	9	3	10	20
	10	2.5	12	24
10	1	7	10	12
	2	5	14	16
	3	4.5	18	26
	4	3	20	24
	5	9	8	10
	6	6	2	10
	7	8	8	12
	8	5.5	12	14
	9	4.5	14	20
	10	6	10	20

TABLE IV-1 (Contd.)

Time in mins.	Specimen No.	Length in mm.	% position in mid-gut of coloured band	
			From	To
20	1	4.5	28	44
	2	3.5	58	70
	3	5	40	48
	4	5	46	56
	5	5.5	44	48
	6	5	66	70
	7	4	62	70
	8	4	68	72
	9	4	44	50
	10	3.5	64	78
30	1	5	68	74
	2	3	80	90
	3	4	96	100
	4	3.5	90	100
	5	4.5	74	86
	6	3.5	74	86
	7	4	88	100
	8	3.5	98	100
	9	4	88	100
	10	4	84	100

TABLE IV-2

The position of the coloured band of food in the mid-guts of *S. reptans* larvae examined as part of field experiment B5

Time in mins.	Specimen No.	Length in mm.	% position in mid-gut of coloured band	
			From	To
Zero	1	5	0	22
	2	4	0	50
	3	5	0	18
	4	5	0	24
	5	4	0	22
	6	4.5	0	18
	7	4	0	30
	8	4.5	0	18
	9	4.5	0	48
	10	4.5	0	20
10	1	5	14	24
	2	5	26	40
	3	5	18	32
	4	5	18	30
	5	5	24	34
	6	5	20	28
	7	5	20	38
	8	5	30	56
	9	5	10	20
	10	4	20	46
20	1	5.5	46	60
	2	4.5	40	54
	3	4.5	44	56
	4	5	30	40
	5	5.5	38	52
	6	5	36	48
	7	4.5	36	44
	8	4.5	40	50
	9	5	30	40
	10	5	30	40
30	1	5.5	46	54
	2	4.5	54	62
	3	5	66	76
	4	5	70	80
	5	5	60	68
	6	5	72	80
	7	5	64	74
	8	4.5	58	72
	9	4.5	70	82
	10	4.5	90	100

TABLE IV-3

The position of the coloured band of food in the mid-guts of S. reptans larvae examined as part of field experiment B6

Time in mins.	Specimen No.	Length in mm.	% position in mid-gut of coloured band	
			From	To
Zero	1	5	0	16
	2	4.5	0	24
	3	4.5	0	18
	4	4.5	0	18
	5	4.5	0	18
	6	5	0	24
	7	5	0	12
	8	4.5	0	18
	9	5	0	22
	10	4	0	22
10	1	5	28	40
	2	5	38	48
	3	4.5	26	42
	4	4.5	24	30
	5	5	30	40
	6	5	32	42
	7	4	28	42
	8	4.5	22	32
	9	4.5	30	40
	10	5	22	30
20	1	4.5	64	84
	2	4	60	74
	3	4	58	72
	4	5	52	62
	5	4.5	58	68
	6	4.5	48	58
	7	5	56	64
	8	5	60	72
	9	5	52	60
	10	5	50	62
30	1	5	66	78
	2	5	86	100
	3	4.5	94	100
	4	4.5	84	96
	5	5	72	80
	6	4.5	90	100
	7	4	94	100
	8	5	74	86
	9	5	78	90
	10	5	80	92