

**Bangor University**

## **DOCTOR OF PHILOSOPHY**

**A comparative study of morphology, behaviour and ecology of Chaetogaster Limnaei (von Baer) from several host species**

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*Award date:*  
1968

*Awarding institution:*  
Bangor University

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A COMPARATIVE STUDY OF THE MORPHOLOGY, BEHAVIOUR AND ECOLOGY  
OF CHAETOGASTER LIMNAEI (von Baer) FROM SEVERAL HOST SPECIES.

A THESIS

submitted to the University of Wales by

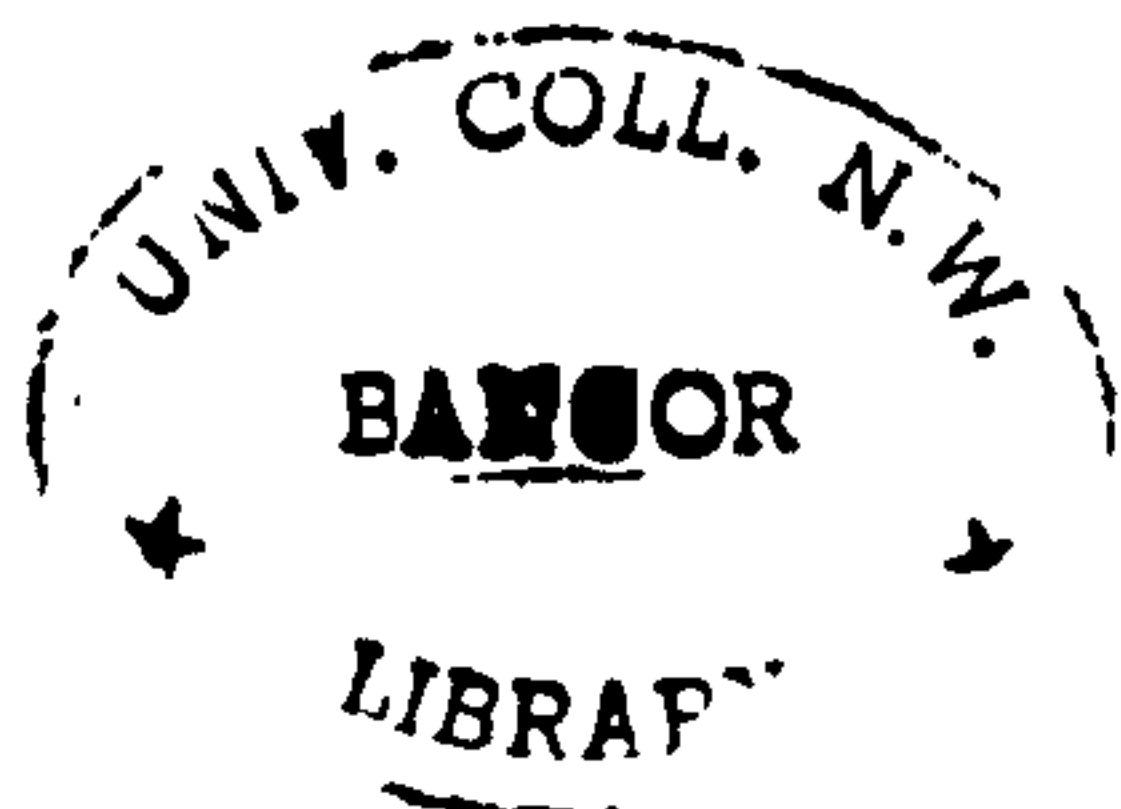
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in candidature for the degree of

PHILOSOPHIAE DOCTOR

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September, 1968.



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## Section A. INTRODUCTION.

Chaetogaster limnaei (Oligochaeta:Naididae) is a small worm, approximately 3mm. in length, with setae on the ventral surface only. It is found in association with many species of fresh-water gastropods. Two subspecies of Ch. limnaei are recognised (Gruffydd, 1965a), namely Ch. limnaei limnaei living on the outer surface of the snail and Ch. limnaei vaghini in the kidney.

Previous work on Ch. limnaei has either been descriptive or concerned with the possible economic importance of the worm in controlling trematode infection by feeding on emerging cercaria larvae. The only population study on this species (Gruffydd, 1965b) examined the dynamics of Ch. l. limnaei and Ch. l. vaghini in the snail Lymnaea pereger (Mull.). The size of the Ch. l. vaghini population was small, so it was decided to study the ecology of a large population in Lymnaea stagnalis (Linn.), a common snail inhabiting a small artificial pond in the grounds of the University College of North Wales, Bangor. Samples of snails for the examination of the Chaetogaster population were taken each month from October, 1965 to December 1967.

Interest in the symbiotic relationships (de Bary, 1879) between animals has increased in recent years. The relationships between commensals and their hosts have been studied mainly in marine organisms (Davenport, 1950 et seq.; Johnson, 1952; Gage,

1966a,b,c), and those between parasites and their hosts mainly in parasitic mites (Welsh, 1930, 1931), parasitic Hymenoptera (e.g. Laing, 1937; Thorpe and Jones, 1937; Edwards, 1954; Wylie, 1958) and Trematoda (e.g. Faust, 1924; Barlow, 1925; Wright, 1959b; Etges and Decker, 1963; MacInnis, 1965).

As the previous work on Ch. limnaei had been limited to one host species, it was decided to examine Chaetogaster from a wide range of gastropod hosts. The morphology and behaviour of Ch. limnaei were therefore investigated in relation to the host from which they had been obtained. This was to determine the nature of attraction to the host and whether Ch. limnaei was specific to its own host species. A preliminary investigation indicated that host-specificity might be present, in that in the College pond, Ch. l. limnaei was found almost exclusively on L. pereger and Ch. l. vaghini solely in L. stagnalis.

## Section B. LITERATURE REVIEW.

### 1. Description of Chaetogaster limnaei and its location in the host.

The anatomy of the Naididae has been described by Sperber (1950). In the classification of this group, the setal bundles are important. The genus Chaetogaster differs from other Naid genera in that only ventral setae are present. These, as in



other Naididae, have two teeth, are hooked, and have a nodulus. The setae of each bundle of Chaetogaster limnaei are arranged in a semi-circle. In segment II there are 5-20 setae per bundle, each seta being 53-123  $\mu$  in length, from segments III-V the setae are absent, and posterior to segment V there are 4-20 of them per bundle, each having a length of 42-84  $\mu$ . In Ch. limnaei, the teeth of the setae are very strongly curved, the distal tooth being almost as long as the proximal (Plate 1).

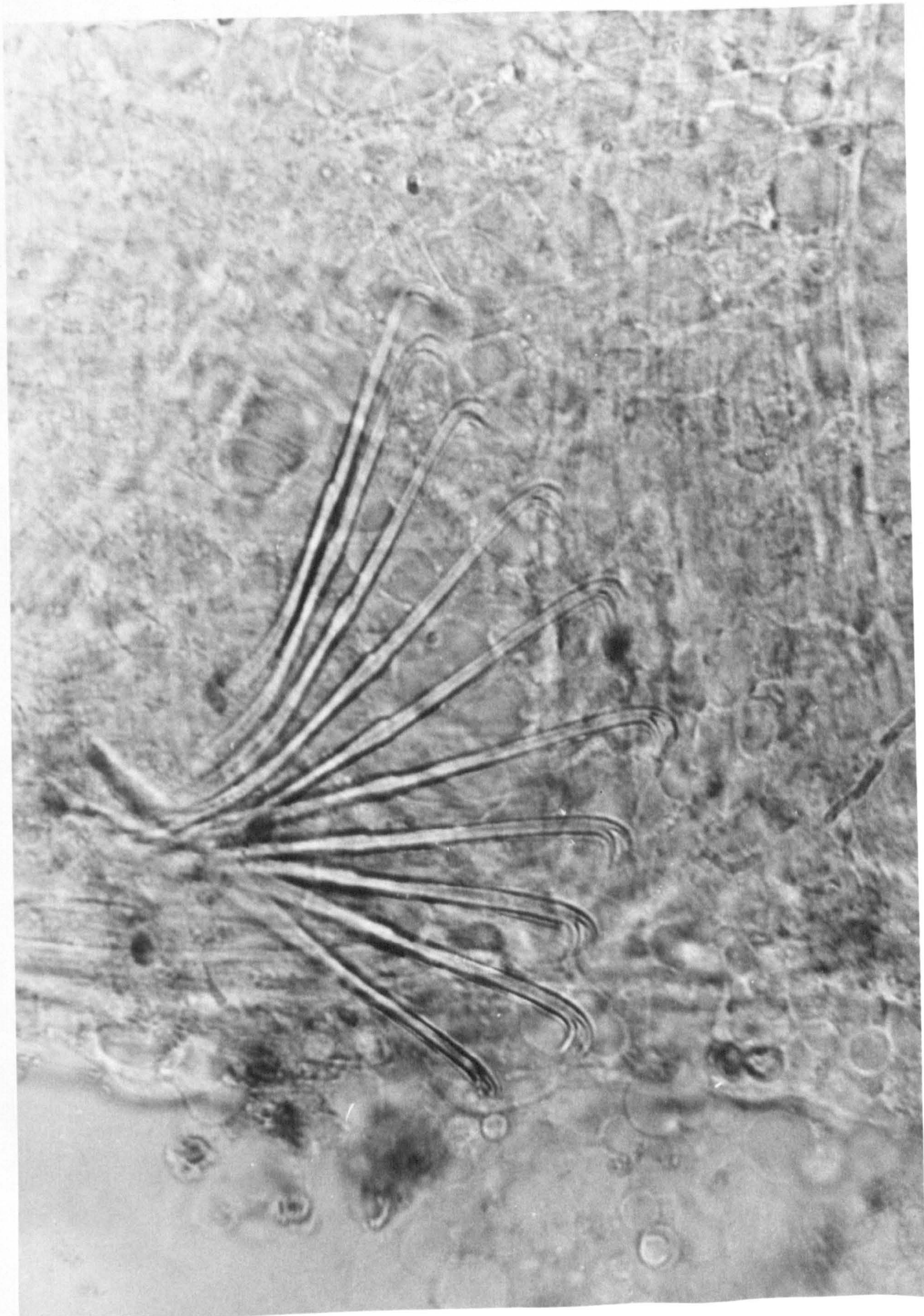
Chaetogaster limnaei was originally described by von Baer in 1827 as having tufts of bristles in pairs on the under surface. On either side at the anterior end were a number of short, stiff bristles, which were partly retracted and drawn together. The distances between the bristle tufts in the hinder region of the worm were irregular.

The majority of workers have observed the position of Chaetogaster limnaei in the snail as either on the external surfaces, in particular the head and the foot, or in the mantle cavity. It has been found occupying the mantle cavity by von Baer(1827), Willcox(1901), Wagin(1931), Chen(1940), Wallace (1941), Vaghin(1946), Backlund(1949), Bayer(1955), and Khalil (1961).

Chaetogaster limnaei has been observed on the outer surfaces of the snail by Lankester(1869a), Willcox(1901), Wesenberg-Lund(1934), Krasnodebski(1936), Wallace(1941) and Gruffydd(1965b). In cases of extreme infection, it inhabited



Plate 1. One setal bundle of Chaetogaster limnaei limnaei.





the external surface of the snail shell (Vaghin, 1946).

The worms have been found free-living in water inhabited by pulmonate snails by von Baer(1827) and Wolf(1928). Krasnodebski(1936) and Wallace(1941) also reported finding Chaetogaster away from its host. Annandale(1905) described Ch. bengalensis as leaving the host when the population became very large and the water became foul. Wagin(1931), however, never found Ch. limnaei free-living.

A few authors have observed Chaetogaster limnaei in the kidney of fresh-water gastropods. Von Baer(1827) described Chaetogaster as being present in the kidney of Lymnaea stagnalis and Lankester(1869a) also found them, gorged with kidney cells, in the same species of snail. Michelson(1964) found Chaetogaster in the kidney of Physa heterostropha and they again contained kidney concretions in their guts. Michelson was of the opinion that the snail could be free of external Chaetogaster and still have the renal phase. Vaghin(1946) suggested that the kidney form might be a different biological species from the outer form and Gruffydd(1965a) proposed that the kidney form should be considered as a different subspecies, Chaetogaster limnaei vaghini, the outer subspecies being known as Ch. l. limnaei.

Chaetogaster l. vaghini differed from Ch. l. limnaei in a variety of features (Gruffydd, 1965a). It inhabited only the kidney of fresh-water gastropods. The number of setae in each bundle varied from 5-7, except in the mature form which

occasionally had 8. The number of setae in each bundle of Ch. l. limnaei, however, was constantly between 7 and 12. The setal lengths in Ch. l. vaghini were in segment II, 51-68  $\mu$  and in segments VI, VII and VIII, 41-49  $\mu$ , whereas in Ch. l. limnaei their lengths were in segment II, 72-96  $\mu$  and in segments VI, VII and VIII from 47-54  $\mu$ . A difference between the subspecies was also shown in the structure of the gut wall, it being thicker in the outer form than in the kidney form. The feeding habits of the subspecies also differed, Ch. l. limnaei ingesting diatoms and small planktonic animals and Ch. l. vaghini feeding solely on the kidney cells of the host. Behavioural differences were apparent in the reaction of the subspecies to the mucus of the host. Ch. l. limnaei was able to follow a mucous trail and find the host, whereas Ch. l. vaghini was not.

Various theories have been proposed as to how Chaetogaster l. limnaei might benefit by its association with fresh-water snails. It has been suggested that protection and shelter from predators was obtained (Vaghin, 1946; Khalil, 1961; Gruffydd, 1965b), that there was a better food supply (Vaghin, 1946; Gruffydd, 1965b) and that protection from desiccation was conferred (Gruffydd, 1965b). Andrée (1893) stated that it was not a parasite and Wagin (1931) that it did not damage the host in any way.

Chaetogaster l. vaghini in the kidney of gastropods has

generally been considered to be parasitic (Stephenson, 1930; Vaghin, 1946; Gruffydd, 1965b).

## 2. The life-cycle of Chaetogaster limnaei.

In both Chaetogaster limnaei limnaei and Ch. l. vaghini asexual reproduction by budding occurs. The sequence in which the buds arise has been described by von Baer(1827), Claus (1860) and Gruffydd(1965b). Budding began posterior to segment VIII of the parent worm. Segment VIII of the new worm was the first formed, followed by segment VII and so on. The order of age of the buds in an individual with 5 buds was therefore 0, 4, 2, 1, 3, 5 where 0 was the parent and 5 was the youngest bud.

The mature form of Chaetogaster l. limnaei has rarely been observed. Lankester(1869b) and Vaghin(1946) both reported finding the sexual form in low numbers and Wagin(1931) also described the maturing of the gonads in Ch. l. limnaei. Gruffydd(1965b), however, found no mature worms.

The mature form of Chaetogaster l. vaghini has been observed more frequently. Von Baer(1827) found its cocoons in the late Autumn. Vaghin(1946) reported the appearance of mature forms at the end of August, the total population becoming mature by the beginning of November. Cocoons were then found containing developing eggs, the adults perishing



after their production. The reproductive cycle of Ch. l. vaghini has also been described by Gruffydd(1965b), who observed that sexual reproduction began in November or December and continued through the winter. The highest proportion of mature forms amounted to only 4 per cent of the population. He also found cocoons in the kidneys of the snails.

A description of the mature form of Chaetogaster species was given by Stephenson(1930). The testes were diffuse and arose before the ovaries. As regression of the former occurred, they were absent in the fully mature individual. No seminal vesicles were found. The female ducts degenerated or were absent. The ova were large and took up the whole diameter of the body cavity, expulsion of them taking place by rupture of the body wall. Penial setae were present, these being larger and simpler than the normal ventral setae.

Lipps(1920) studied the factors influencing the development of sexual organs in the Naididae. In the majority of species, a rise in temperature induced the development of sexual organs, whereas in Chaetogaster cold caused their development.

### 3. Gastropods infected with Chaetogaster limnaei.

Chaetogaster limnaei limnaei has been described by many workers from a wide range of gastropod species, as shown in Table 1(pp. 8 and 9). Those genera which occur in Britain and

British gastropod species

Reference	<i>Ancylus fluviatilis</i>	<i>A. lacustris</i>	<i>Lymnaea</i> sp.	<i>L. auricularia</i>	<i>L. ovata</i>	<i>L. palustris</i>	<i>L. pereger</i>	<i>L. stagnalis</i>	<i>Physa fontinalis</i>	<i>Planorbis</i> sp.	<i>Pl. carinatus</i>	<i>Pl. corneus</i>	<i>Pl. planorbis</i>	<i>Pl. vortex</i>
von Baer(1827)		x										x		
Lankester(1869a)		x								x				
Willcox(1901)										x				
Annandale(1905)		x												
Wagin(1931)		x				x	x			x	x	x		
Krasnodebski(1936)		x		x	x	x		x	x			x	x	x
Chen(1940)										x				
Wallace(1941)														
Vaghin(1946)	x			x				x	x			x	x	
Backlund(1949)				x				x						
Bayer(1955)														
Geldiay(1956)	x													
Coelho(1957)														
Khalil(1961)														
Michelson(1964)														
Maitland(1965)	x													
Gruffydd(1965a, b)								x						

Table 1. Species of mollusc infected by Ch. l. limnaei. [Over/...

Other gastropod species.Lamellibranchs

Reference	<i>Australorbis glabratus</i>	<i>Biomphalaria glabratus</i>	<i>B. pfeifferi</i>	<i>Bulinus natalensis</i>	<i>B. tropicus</i>	<i>B. truncatus</i>	<i>Helisoma trivolvis</i>	<i>Lymnaea acuminata</i>	<i>L. natalensis</i>	<i>Melanoïdes tuberculata</i>	<i>Physa heterostropha</i>	<i>Physopsis africana</i>	<i>Segmentina</i> sp.	<i>Pisidium</i> sp.	<i>Sphaerium</i> sp.
von Baer(1827)															
Lankester(1869a)															
Willcox(1901)											x				
Annandale(1905)															
Wagin(1931)															
Krasnodebski(1936)															
Chen(1940)															
Wallace(1941)							x								
Vaghin(1946)														x	x
Backlund(1949)															
Bayer(1955)			x	x	x					x		x	x		
Geldiay(1956)															
Coelho(1957)	x														
Khalil(1961)		x			x	x		x	x						
Michelson(1964)	x										x				
Maitland(1965)															
Gruffydd(1965a, b)															

Table 1. Continued.

in which the worm has been found are Lymnaea, Planorbis, Ancylus and Physa. Vaghin(1946) also described Ch. l. limnaei as occurring in Lamellibranchs of the family Sphaeriidae. It has also been found in many gastropod species in other parts of the world.

The subspecies Chaetogaster l. vaghini, although not recognised as such, was studied by Lankester(1869a) in Lymnaea stagnalis, Vaghin(1946) in L. ovata and L. stagnalis, and Michelson(1964) in Physa heterostropha. It was also found by Gruffydd(1965a,b) in Lymnaea pereger.

The number of Ch. l. limnaei found by various authors on individual snails varies. Von Baer(1827) found 40-50 of them on Lymnaea sp. In L. stagnalis, 10 worms per snail were found by Backlund(1949), whereas Krasnodebski(1936) found up to 300 worms on each snail. Krasnodebski also found up to 60 worms on Physa fontinalis and a mean of 1.3 on Ancylus lacustris.

Khalil(1961) observed that both young and old snails had fewer Chaetogaster present than half-grown snails. The number of worms found on a particular species of snail thus varies according to the habitat examined and the stage in the life-history of the snail.

The percentage infection of various snail species has been examined by both Krasnodebski(1936) and Vaghin(1946). The proportion of L. stagnalis infected was 80-100 per cent (Vaghin) and 85 per cent (Krasnodebski). Planorbis corneus was found to have a percentage infection of 80-100 (Vaghin) and



83 (Krasnodebski). On Physa fontinalis a 3-15 per cent (Vaghin) and a 100 per cent (Krasnodebski) infections were found. The proportion of the snail population infected thus varies in different localities and probably at different times of the year.

#### 4. The food of Chaetogaster limnaei limnaei.

The food of Chaetogaster limnaei limnaei has been described by various authors (Table 2, p. 12) and according to them it consisted of unicellular and filamentous algae, decaying leaves, Protozoa, Crustacea, Rotifers and cercaria and miracidia larvae. Krasnodebski(1936) observed that although he found plant material in the gut, the majority of the food was animal in origin.

The presence of trematode larvae in the gut of Chaetogaster has created interest in the economic importance of the worm. This is especially true when the worm is present in snails which act as intermediate hosts of those trematodes which infect man or agricultural animals. The importance of Ch. limnaei in the control of trmatode infections has been discussed by various authors.

Krasnodebski(1936) observed that at the time of year when the cercariae were leaving the snails, they formed the most important food of Chaetogaster. Backlund(1949) also reported



Food in gutReference

<u>Reference</u>	Diatoms	Unicellular x algae	Filamentous algae	Decaying leaves	Protozoa	Crustacea	Rotifers	Cercariae	Miracidia
Lankester(1869a)		x			x	x	x	x	
Willcox(1901)	x								
Mrazek(1917)								x	
Wagin(1931)	x				x	x	x	x	
Wesenberg-Lund(1934)								x	
Krasnodebski(1936)			x	x	x	x		x	
Wallace(1941)								x	
Vaghin(1946)					x		x		
Backlund(1949)								x	
Ruiz(1951)								x	
Bayer(1955)								x	
Coelho(1957)									x
Khalil(1961)									x
Michelson(1964)								x	x
Gruffydd(1965a)	x		x		x		x	x	

Table 2. The food of Chaetogaster limnaei limnaei.

that the worms took large numbers of cercariae, especially in the warm conditions of the laboratory.

Both Mrazek(1917) and Wallace(1941) suggested that the feeding of Ch. limnaei on the cercariae might serve as one of the factors controlling trematode infection, and Michelson (1964) stated that Chaetogaster gave some degree of protection to the snails from miracidia.

Wagin(1931) observed that only moving cercariae were taken and that some Chaetogaster contained as many as 7-10 cercariae in the gut. As the snails were 100 per cent infected, and each had 70-100 individuals on them, he argued that Chaetogaster were not an insignificant factor in the destruction of cercariae. As the worm could curb the dispersal of trematodes, he suggested that it would be a useful measure to colonise ponds with Ch. limnaei.

Bayer(1955) differed in opinion from the other authors in that he considered the suggestion of control of the cercariae by Chaetogaster was not justified, as these oligochaetes were possibly rare. Also, the cercariae were produced in very large numbers. The majority of authors, however, have concluded that Chaetogaster limnaei does exert an effect on the population of trematodes in the host snail and that they are of economic importance in the control of trematode infection.

## Section C. DESCRIPTION OF HABITATS.

1. The College pond habitat.

The population of Lymnaea stagnalis inhabiting the College pond at the Zoology Department of the University College of North Wales (Grid Ref. SH 577719) was used as the host material in the study of the population dynamics of Ch. l. vaghini. The pond was constructed in 1961 (Plate 2). It is oval in shape, as shown in Figure 1 (p. 15), with two circumjacent troughs, the water of which is continuous with that in the central pond by a series of openings in the trough walls. The pond measures 9.6 metres by 7.9 metres. The depth of water in the trough is between 0.3 and 0.4 metre and in the central pond it varies from 0.5 metre on the circumference to 1 metre in the centre. The west shore of the pond slopes from the inlet to the centre. The outlet leaves the central pond at surface level and so a constant level is maintained. The inlet stream flows throughout the year.

The substratum of the pond is soft, being composed of decomposing vegetable material and faecal material of the snails.

The vegetation of the pond and its position was as in Figure 1. The central area of the pond was largely covered in the summer by Nymphaea alba. Potamogeton crispus was the most



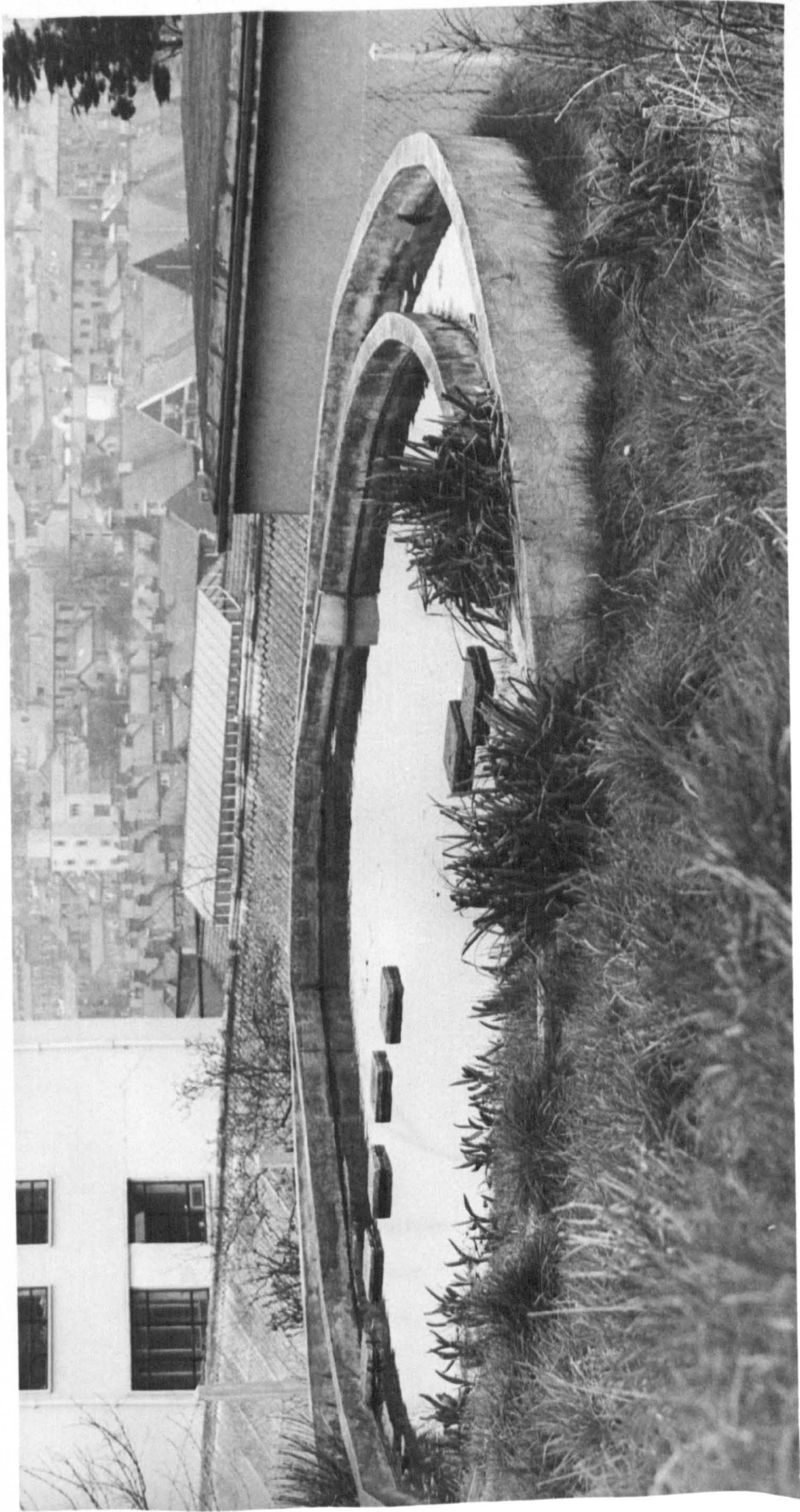
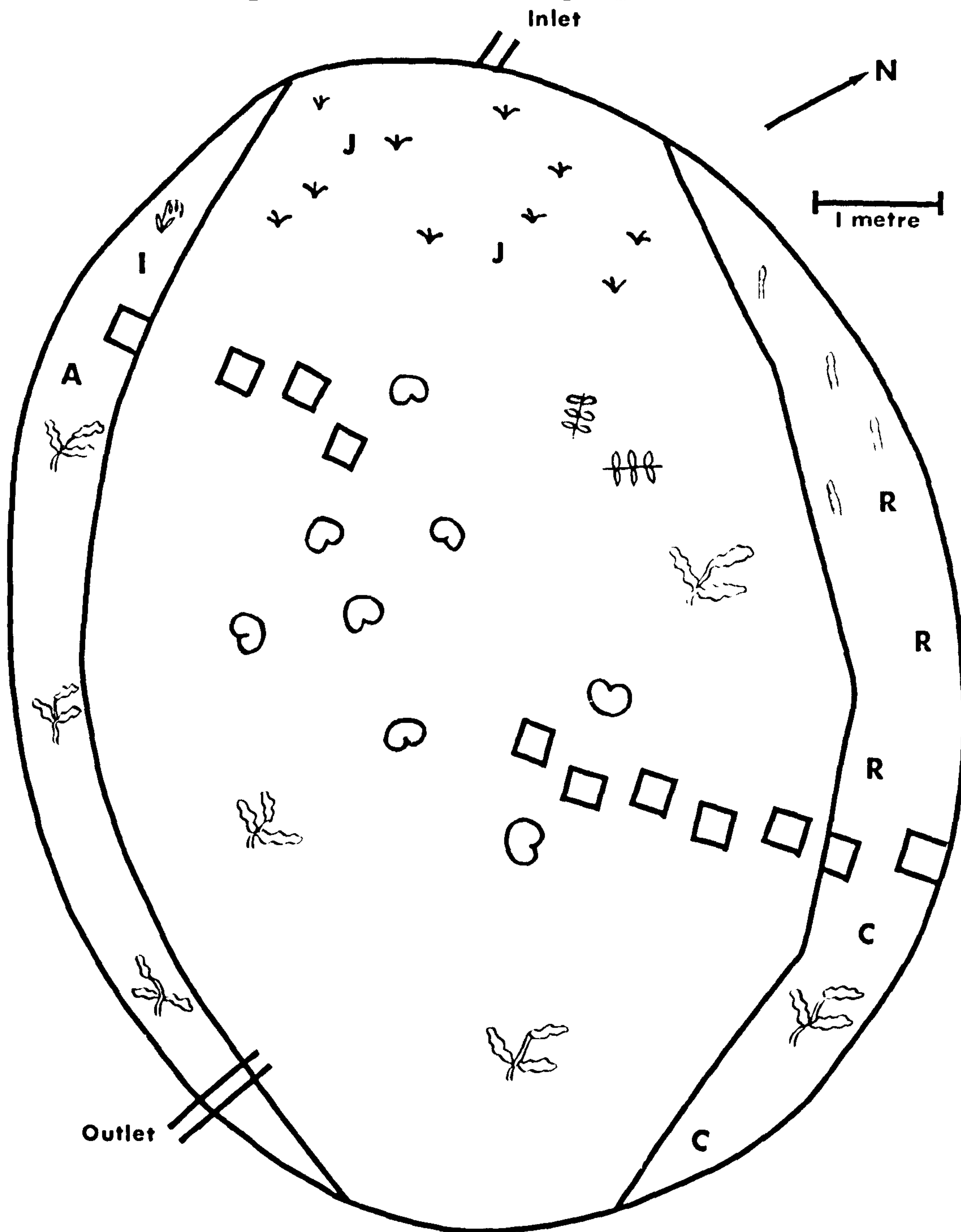


Plate 2. The College pond.



Figure 1.

## Diagram of the College pond.



Y *Agrostis stolonifera*

A *Apium nodiflorum*

C *Callitriche verna*

↘ *Carex pendula*

⊕ *Elodea canadensis*

⊥ *Equisetum limosum*

| *Iris pseudacorus*

J *Juncus* sp.

⊕ *Nymphaea alba*

⊕ *Potamogeton crispus*

R *Ranunculus lingua*



common submerged plant in the central pond. In the summer months, a mat of green algae was present in the troughs and around the edges of the central pond.

The gastropod species found in the pond are shown in Table 3 (p. 17). The population of L. stagnalis was very heavy, in the summer months there being over 5,000 individuals in the trough on the south side. The ratio L. stagnalis : L. pereger was approximately 100 : 1. The four other species of gastropod present were found in smaller numbers. Both L. stagnalis and L. pereger were obtained from the College pond for experimental purposes. The other main animals found in the pond are also shown in the table.

Details of the water chemistry of the pond are given in Table 4 (p. 18). The calcium content and pH are relatively high for the area. This is due to the inlet stream draining from a limestone outcrop above the pond.

## 2. Habitats used as sources of experimental animals.

### Mill pond.

The mill pond on the College Farm at Aber, Caernarvonshire (Grid Ref. SH 652726) was used as a source of Potamopyrgus jenkinsi. The pond is rectangular and measures approximately 42 metres by 21 metres. The floor of the pond shelves from the inlet stream to the outlet. The pond is situated in

<u>Platyhelminthes</u>	Turbellaria	Dugesia lugubris (Schmidt) Polycelis tenuis (Ijama) Polycelis nigra (Müll.)
<u>Annelida</u>	Oligochaeta	Chaetogaster diaphanus (Guith.) Chaetogaster limnaei (v. Baer) Lumbriculus variegatus (Müll.)
	Hirudinea	Glossiphonia complanata (Linn.) Glossiphonia heteroclita (Linn.) Helobdella stagnalis (Linn.)
<u>Mollusca</u>	Gastropoda	Ancylus lacustris (Linn.) Lymnaea pereger (Linn.) Lymnaea stagnalis (Linn.) Planorbis albus (Müll.) Planorbis carinatus (Müll.) Potamopyrgus jenkinsi (Smith)
	Lamellibranchia	Sphaerium sp.
<u>Insecta</u>	Coleoptera	Corixa sp. Dytiscus marginalis (Linn.)
	Odonata	Coenagrion sp.
	Trichoptera	Plectonemia sp.
<u>Vertebrata</u>		Gasterosteus aculeatus Rana temporaria Triturus helveticus

Table 3. Main fauna of the College pond.



Calcium	38 mg./litre
Calcium and magnesium	63 mg./litre
Chloride	28 mg./litre
pH	8.0
Total conductivity	381 micromhos

Table 4. Water chemistry of the College pond.

agricultural land. Most of the vegetation was at the shallow end of the pond.

The only other gastropod present was L. pereger.

Beumaris reservoir.

Physa fontinalis was obtained from the reservoir at Beumaris, Anglesey (Grid Ref. SH 584750). The gastropods were collected from an area at the inlet, cut off from the main reservoir by a sluice gate. The area had thick vegetation and, as it was surrounded by woodland, contained much decaying organic matter.

Six other species of gastropod were present, in very low numbers, as shown in Table 5 (pp. 20 and 21).

Wirral: Pond 1 and Pond 2.

Planorbis corneus was obtained from two ponds (Pond 1, Grid Ref. SJ 325814 and Pond 2, Grid Ref. SJ 330804) on the Wirral, Cheshire. Both ponds are in agricultural land. The substratum is soft and the vegetation dense.

The other species of gastropod present are shown in Table 5 (pp. 20 and 21).

Llanllechid: Lake 1.

Lake 1 (Grid Ref. SH 636693) above Llanllechid was used as a source of L. pereger with no other gastropod species



HabitatsGastropods present

	College pond	Mill pond	Llanllechid	Waynol 1	Waynol 2	Llyn Sisi	Ecaumaris reservoir	Llyn Coron	Llyn Hendref	Llangefni reservoir
<i>Ancylastrum fluviatile</i> (Mull.)									x	
<i>Ancylus lacustris</i> (Linn.)	x									
<i>Bithynia leachii</i> (Sheppard)										
<i>B. tentaculata</i> (Linn.)										
<i>Lymnaea auricularia</i> (Linn.)										
<i>L. palustris</i> (Mull.)							x	x	x	
<i>L. pereger</i> (Mull.)	x	x	x	x	x	x		x	x	
<i>L. stagnalis</i> (Linn.)	x									
<i>L. truncatula</i> (Mull.)							x		x	
<i>Physa fontinalis</i> (Linn.)							x	x		
<i>Planorbis albus</i> (Mull.)	x			x			x			
<i>Pl. carinatus</i> (Mull.)	x									
<i>Pl. complanatus</i> (Linn.)										
<i>Pl. contortus</i> (Linn.)							x	x		
<i>Pl. corneus</i> (Linn.)										
<i>Pl. crista</i> (Linn.)							x			
<i>Pl. planorbis</i> (Linn.)										
<i>Pl. vortex</i> (Linn.)										
<i>Potamopyrgus jenkinsi</i> (Smith)	x	x		x				x	x	x
<i>Succinea</i> sp.							x			
<i>Valvata cristata</i> (Mull.)				x						
<i>V. piscinalis</i> (Mull.)				x				x		
<i>Zonitoides nitidus</i> (Mull.)										

Table 5. The gastropod species present in the habitats examined.

Over/.....

<u>Gastropods present</u>	<u>Habitats</u>									
	Holland Arms	Cole Mere	The Mere	Newton Mere	Blake Mere	White Mere	Birchgrove Pool	Croose Mere	Wirral 1	Wirral 2
Ancylastrum fluviatile		x	x		x					
Ancylus lacustris		x	x							
Bithynia leachii		x	x				x			
B. tentaculata		x	x		x	x	x	x		
Lymnaea auricularia			x			x				
L. palustris										
L. pereger	x	x	x	x		x	x	x		x
L. stagnalis		x					x		x	x
L. truncatula						x				x
Physa fontinalis	x	x		x				x		
Planorbis albus	x	x	x	x	x		x			x
Pl. carinatus		x	x	x			x	x		
Pl. complanatus	x							x		
Pl. contortus	x									
Pl. corneus									x	x
Pl. crista										
Pl. planorbis						x				
Pl. vortex		x	x				x	x		
Potamopyrgus jenkinsi		x	x	x	x	x		x		
Succinea sp.										
Valvata cristata										
V. piscinalis		x						x		
Zonitoides nitidus						x				

Table 5. Continued.



present. The lake is 1,000 feet above sea level and is formed by an earth dam on the side of the hill. It is very shallow and there is almost no vegetation present. The substratum is soft with only a few isolated rocks.

### Cole Mere.

Cole Mere, Shropshire (Grid Ref. SJ 435329) was used as a source of L. pereger from a lake with many other species of gastropod present. It is a large mere with a gravel substratum and a stony shore. Eleven other gastropod species were found in Cole Mere (Table 5, pp. 20 and 21).

### 3. Other habitats examined.

A list of the other habitats examined is given in Table 6 (p. 23). The gastropods in these habitats were investigated to determine the incidence of Chaetogaster in them.

## Section D. MAINTENANCE OF FRESH-WATER SNAILS IN THE LABORATORY.

To culture snails which were not infected with Chaetogaster, their egg masses were collected in the field, examined under the binocular microscope and any Chaetogaster removed. These egg masses were then introduced into plastic bowls, 30 cm. in

<u>Location</u>	<u>Grid Reference</u>	<u>County</u>
Llyn Sisi	SH 640693	Caernarvonshire
Vaynol, Lake 1	SH 541694	"
Vaynol, Lake 2	SH 541693	"
Llyn Coron	SH 378700	Anglesey
Llyn Hendref	SH 398766	"
Holland Arms Pool	SH 462723	"
Llangefni Reservoir	SH 442774	"
Birchgrove Pool	SJ 435233	Shropshire
Blake Mere	SJ 418338	"
Crose Mere	SJ 431307	"
Newton Mere	SJ 424344	"
The Mere, Ellesmere	SJ 404348	"
White Mere	SJ 418329	"

Table 6. Location of the other habitats examined.



diameter, kept in the laboratory. Up to 50 snails were kept in one bowl, depending on snail size. Water was obtained from the College pond and filtered before filling each bowl to a depth of 5 cm. To prevent the snails leaving the water and drying up, the water was continuously aerated through a diffuser block. This was especially important with the young. The water was changed each week and all faecal material removed from the bowls.

The snails were fed on an artificial food. The preparation of this food was described by Standen (1951) and modified by Ollerenshaw (Gruffydd, 1963). A mixture of 8 gm. of dried, powdered grass, 8 gm. of Froment and 4 gm. of dried milk was made in 800 ml. of boiling water. Five gm. of sodium alginate were stirred in and the mixture poured into a shallow container and allowed to cool. A solution of 16 gm. of dried calcium chloride in 800 ml. of water was poured over the mixture in the container and left overnight. The gel, containing the powdered grass, was then separated from the plain gel, washed and kept in a refrigerator. Fresh food had to be prepared each week. The snails were fed twice a week with small pieces of food and those not eaten were removed from the bowls before adding fresh food.

Other snails brought in from the field and not cultured from egg masses were kept under the same conditions as above.

Section E. THE POPULATION BIOLOGY OF CHAETOGASTER LIMNAEI  
VAGHINI IN LYMNAEA STAGNALIS.

1. Introduction.

Monthly samples of the population of Ch. l. vaghini in the kidney of L. stagnalis were taken to determine the life-cycle and population dynamics of the oligochaete. Factors which appeared to have a major influence on the life-cycle of Chaetogaster were examined experimentally. These included both abiotic factors and biotic factors such as the host snail.

2. Sampling procedure.

Monthly collections of Lymnaea stagnalis for the examination of the Ch. l. vaghini population were made from October, 1965 to December, 1967. At each sample, estimates of the number of snails present and the size-structure of the snail population were made. The samples were taken in the central area of the College pond. Two methods of collection, hand and net, were employed to give a representative sample of the population of L. stagnalis throughout the whole pond. Most of the young snails were found at first on the vegetation



and were thus taken by the net collection. Many of the older snails were found on the outer wall of the pond and were sampled by hand. The net collection gave a better indication of the number of snails in the population than the hand collection in a situation such as the College pond where a large population of snails was present. This was due to the maximum number of snails obtained being restricted by the rate at which they could be collected, there being a physical limit to the number that could be collected per unit time. Thus the validity of the hand collection method deteriorated as the snails' numbers increased in the pond.

At each monthly sample, a 10 minute collection was made by hand on the outer walls of the central pond. A 6 minute collection was then taken by sweeping with a net in the vegetation of the pond. The times allotted for collection by each method were based on those necessary to obtain an equal number of snails in a preliminary sample in October, 1965. The greatest length of each snail collected was measured from the apex of the spire to the lower edge of the aperture, and the snails separated into size-groups of 5mm. (Appendix 1). A total of 50 snails was examined from these, the number examined in each size-group being in proportion to the number of snails of that size collected. Bias by the choice of the larger size snails for examination was therefore prevented.

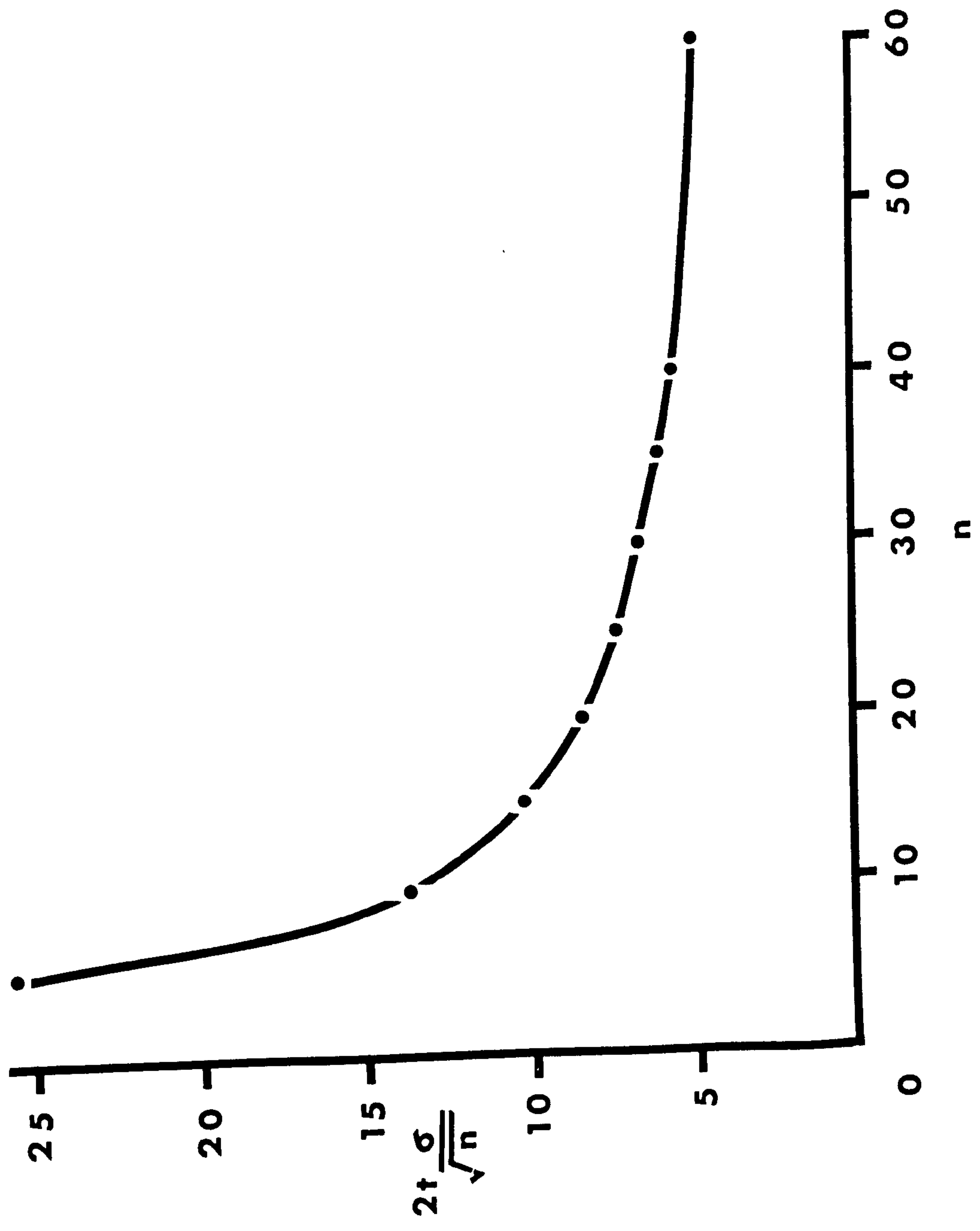
The size of the sample for examination was determined by

making a preliminary sample to obtain a rough estimate of the minimum number of snails necessary to give a reliable indication of changes in the Chaetogaster population. The confidence limits of the mean number of Chaetogaster per snail were calculated for increasing numbers of individual snails examined, using the formula  $t \frac{\sigma}{\sqrt{n}}$ . The total confidence limits, i.e.  $2t \frac{\sigma}{\sqrt{n}}$ , were plotted against the number of snails in the sample (Fig. 2, p. 28). The larger the sample taken, the greater the accuracy obtained. However, the labour involved must be balanced against the accuracy and, where the graph levels off, a reasonable sample size is indicated. Thus, a sample size of approximately 40 would give a reasonable estimate of the population and would be an economical number of snails to examine. A sample size of 50 was used to allow for the effect on the Chaetogaster population of the variation in the size of the snails in other seasons of the year.

The variation in the Chaetogaster population in the samples was high, due partly to a wide range of snail size and consequently variation in the numbers of Chaetogaster in each snail, and also to some snails having no infection. It was therefore decided to calculate the confidence limits for each monthly sample. The significance of any apparent differences between the Chaetogaster populations of successive months could then be determined.



Figure 2. The relationship between the total confidence limits of the mean number of Ch. l. vaghini and the sample size of L. stagnalis.



$n$  = no. of snails in preliminary sample, October, 1965

Each of the 50 snails in the sample was kept in a separate container until examined to prevent the migration of Chaetogaster between the snails. When examining a snail, the water in the container was also searched for any worm which may have left the snail after collection.

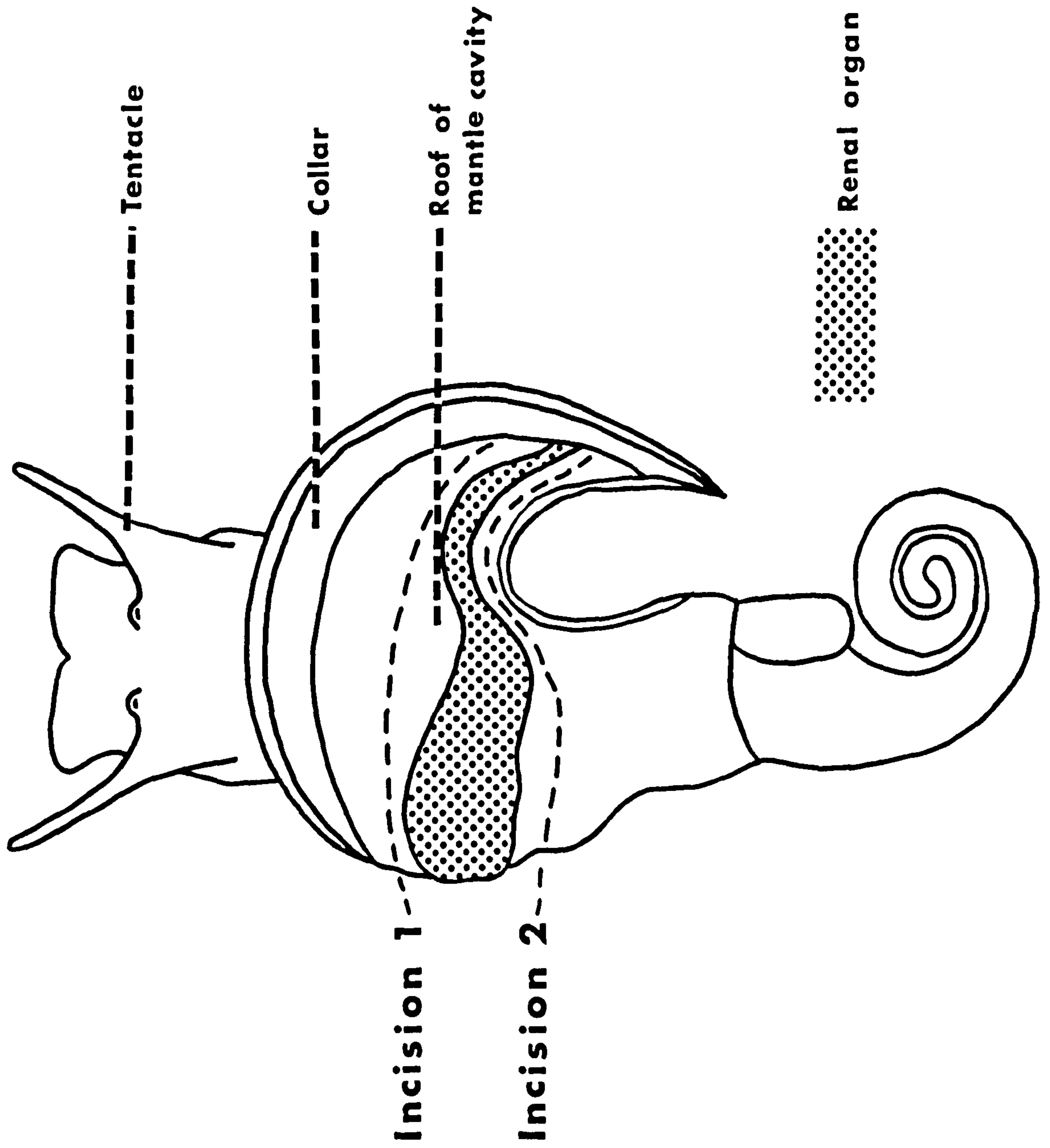
The examination of the snails for Chaetogaster was carried out as by Gruffydd (1963). The outside of the body of each snail was first examined for Ch. l. limnaei. The shell was then removed, exposing the renal organ which in L. stagnalis lies transversely in the mantle roof (Fig. 3, p.30). The snail was pinned on wax in a dish and covered with water. An incision was made anterior to the kidney and a cut made outside its edge from the level of the pericardium to the ureter (Incision 1, Fig. 3). The roof of the mantle cavity was then turned back and the inside of the mantle examined for Chaetogaster. The renal organ was removed in one piece by cutting outside its posterior edge (Incision 2, Fig. 3) and placed in water in a petri-dish. The kidney tissue was teased apart and the number of mature and immature worms, and viable and non-viable cocoons were recorded.

### 3. Population dynamics.

The population of Ch. l. vaghini in L. stagnalis was studied for two consecutive years. The seasonal changes in



Figure 3. The dissection of the kidney of L. stagnalis.



population size observed were similar in both years. The variation in the mean number of Ch. l. vaghini per snail each month is shown in Figure 4c (p. 32) for 1966 and in Figure 5c (p. 33) for 1967.

The population mean was at its minimum in January, the mean number of Chaetogaster per snail being only 0.16 (Confidence limits  $\pm 0.1$ ) in 1966 and 0.5 ( $\pm 0.3$ ) in 1967. January represents the period between the reduction in number of the mature individuals of Chaetogaster following cocoon production and the build up of the immature worm population by asexual reproduction later in the year.

The average number of Ch. l. vaghini per snail increased from January to a peak of 24.0 ( $\pm 9.3$ ) in May in 1966 and 20.8 ( $\pm 4.5$ ) in June in 1967. The graphs of the size-frequency structure of the L. stagnalis population at each monthly sample (Fig. 4a and Fig. 5a) show that these months were the first in which the young snails appeared in the population. The Chaetogaster population continued to increase in the older snails until May and June, but any further increase was concealed by the average number per snail being reduced by the dilution effect of the young uninfected snails entering the population. A further reduction in the average occurred in both years until August due to the addition of further young to the snail population and the earlier snails with small infections growing in size and replacing the older, more

Figure 4. Samples from the College pond in 1966.

- a) Size frequency distri<sup>n</sup> of L. stagnalis.
- b) Percentage infection of L. stagnalis with Ch. l. vaghini.
- c) Mean number of Ch. l. vaghini in each snail.

■ 50%

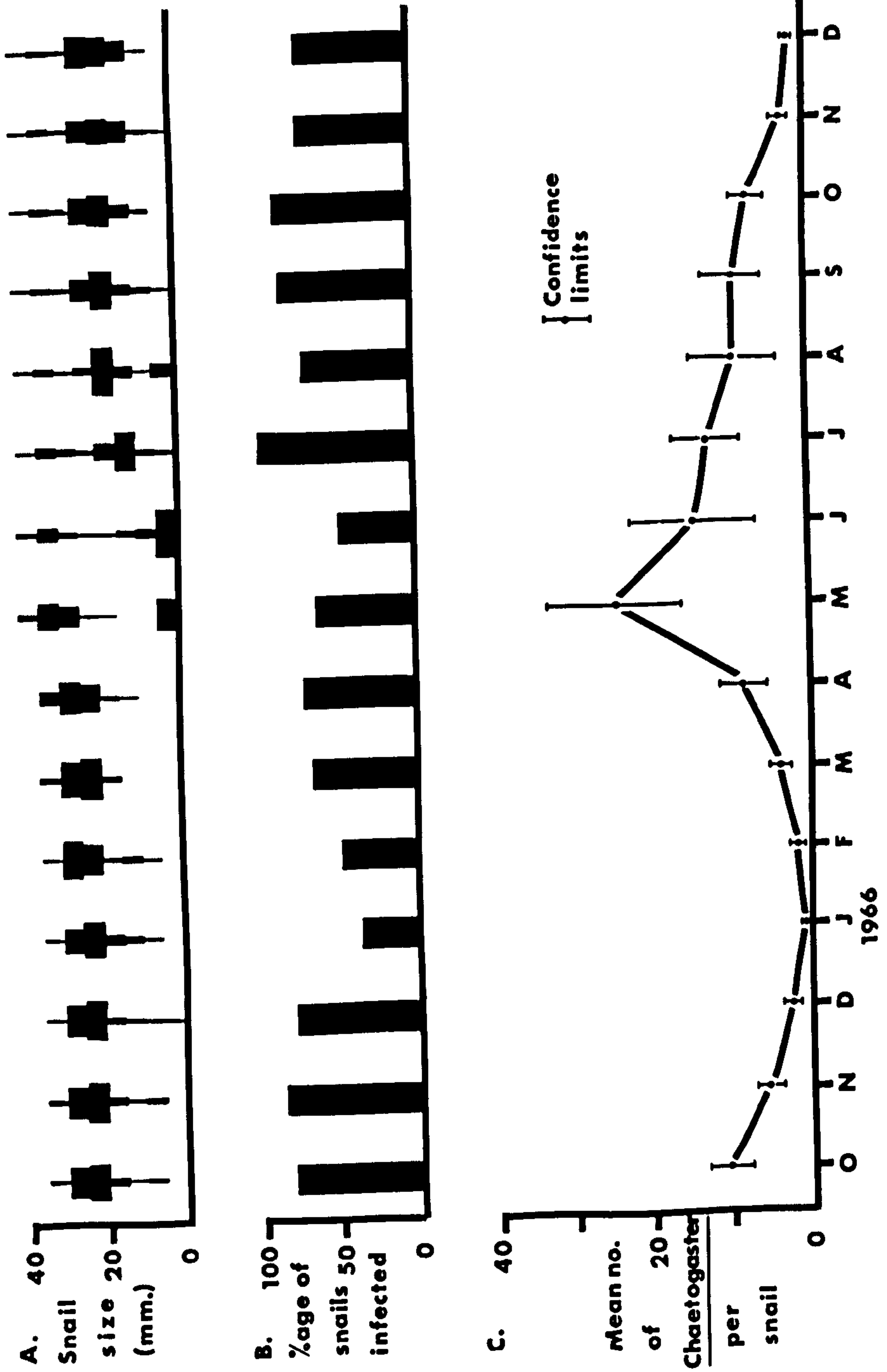
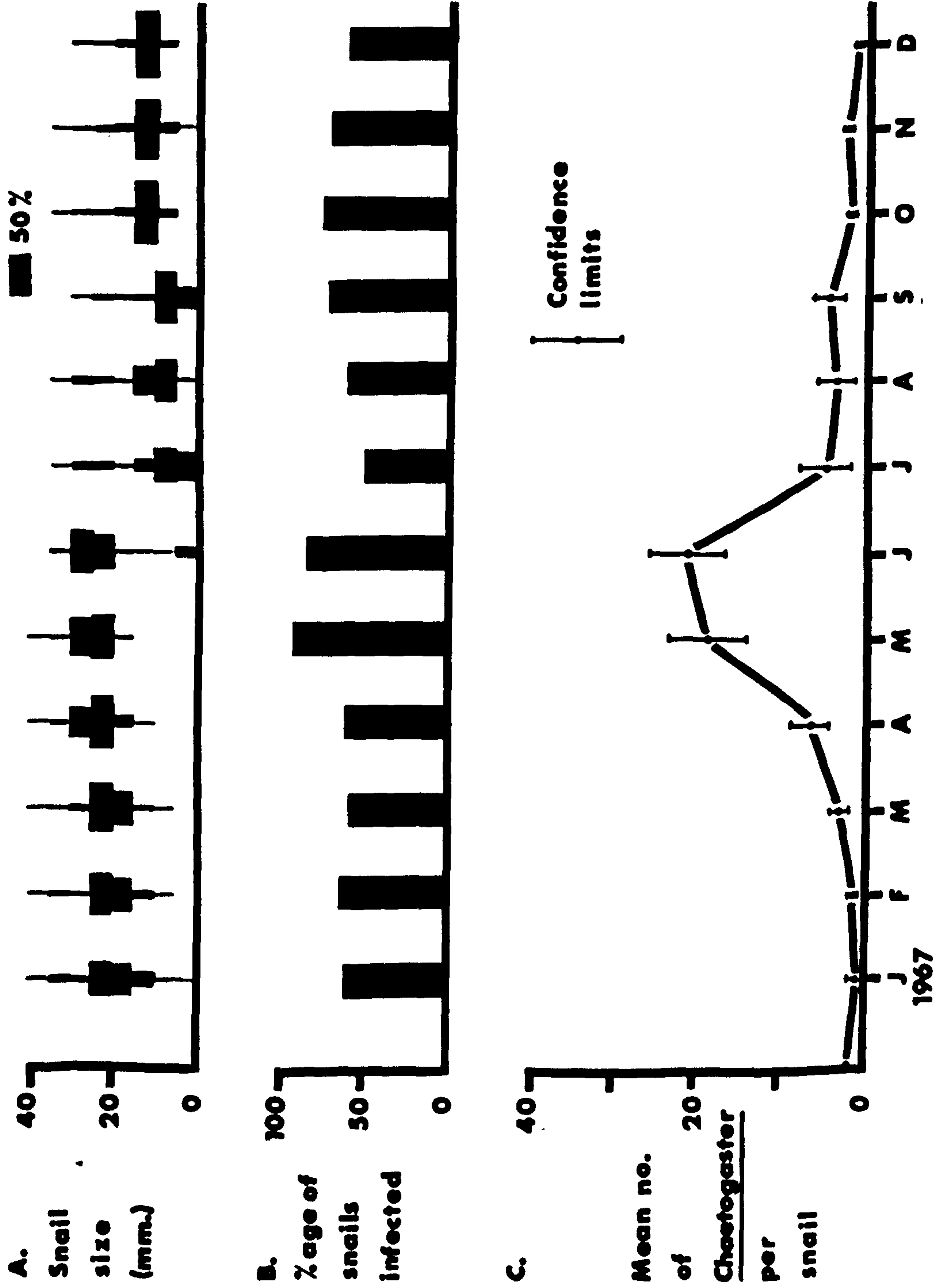




Figure 5. Samples from the College pond in 1967.

- a) Size frequency distribution of L. stagnalis.
- b) Percentage infection of L. stagnalis with Ch. l. vaghini.
- c) Mean number of Ch. l. vaghini in each snail.



heavily infected snails of the larger size groups. There was a slight increase in the population level in September due to more young snails becoming infected and an increase by asexual reproduction in the Chaetogaster population of those infected earlier in the year. From October to December, the average number of Chaetogaster gradually decreased, due to the production of cocoons and the death of the mature Chaetogaster after breeding.

The percentage of snails infected also varies through the year (Fig. 4b and Fig. 5b). In January, it was higher than expected from the values for the mean number of Chaetogaster per snail. This was due to the percentage infection including those snails which contained cocoons, but no mature or immature Chaetogaster. The percentage infection was highest in July, 1966. This was when the first peak in young snails, which occurred in May, had begun to grow in size and had become infected with Chaetogaster. In 1967, when the young snails appeared in the population in June, the highest percentage infection was in May, this apparently being due to the longer time available for the snails to become infected before the young snails entered the population. There was a drop in the proportion infected in June, 1966 and July, 1967 when the majority of young snails entered the population. There followed a steady increase as the young snails became infected. This continued until November when the worm

population became mature and cocoons were produced.

Using the mean number of Chaetogaster per snail as an indication of changes in the population has inherent disadvantages. When the young snails appear, the mean number of Chaetogaster in each L. stagnalis is reduced, whereas the total Ch. l. vaghini population might in fact be increased. To obtain an idea of the changes in the total Ch. l. vaghini population during the year, the product of the average number of Chaetogaster per snail and the number of snails collected per minute by the net collection was calculated for each sample and plotted against time (Fig. 6, p. 36). The net collection data were used rather than those for the hand collection, because they give a better indication of the relative population size. The graph shows a similar fluctuation to that for the mean number of Ch. l. vaghini per snail. However, for both years the largest population was found in June. This coincided with the largest snail population in 1966 and preceded it in 1967. The occurrence of the maximum total population of Ch. l. vaghini at this time seems to be due to a build up in the number of worms by asexual reproduction since the early spring, and to the death in the following months of many of the older snails which contained the largest populations of the worm.

It seemed likely that there was a correlation between the size of the snail and the number of Ch. l. vaghini found



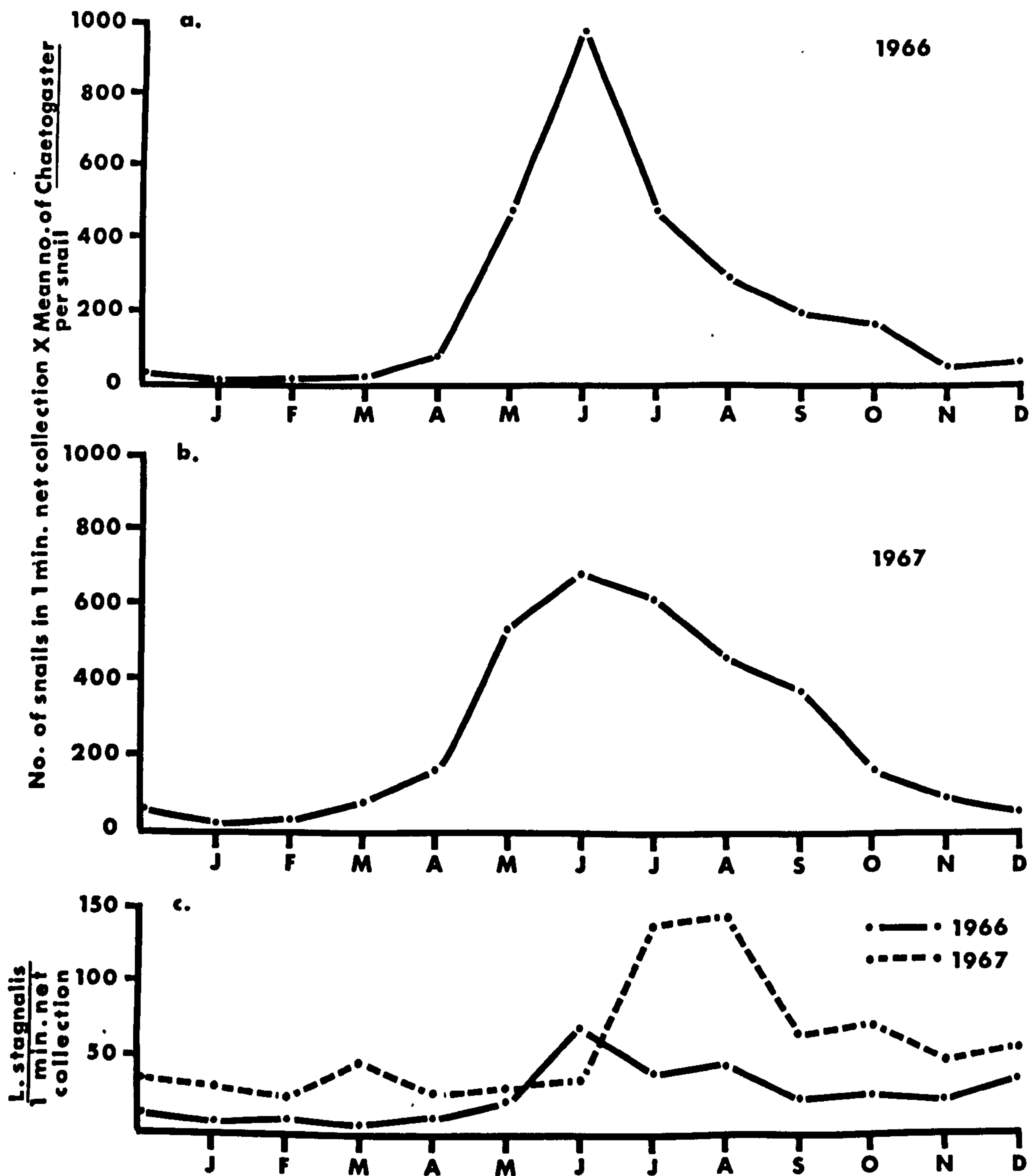
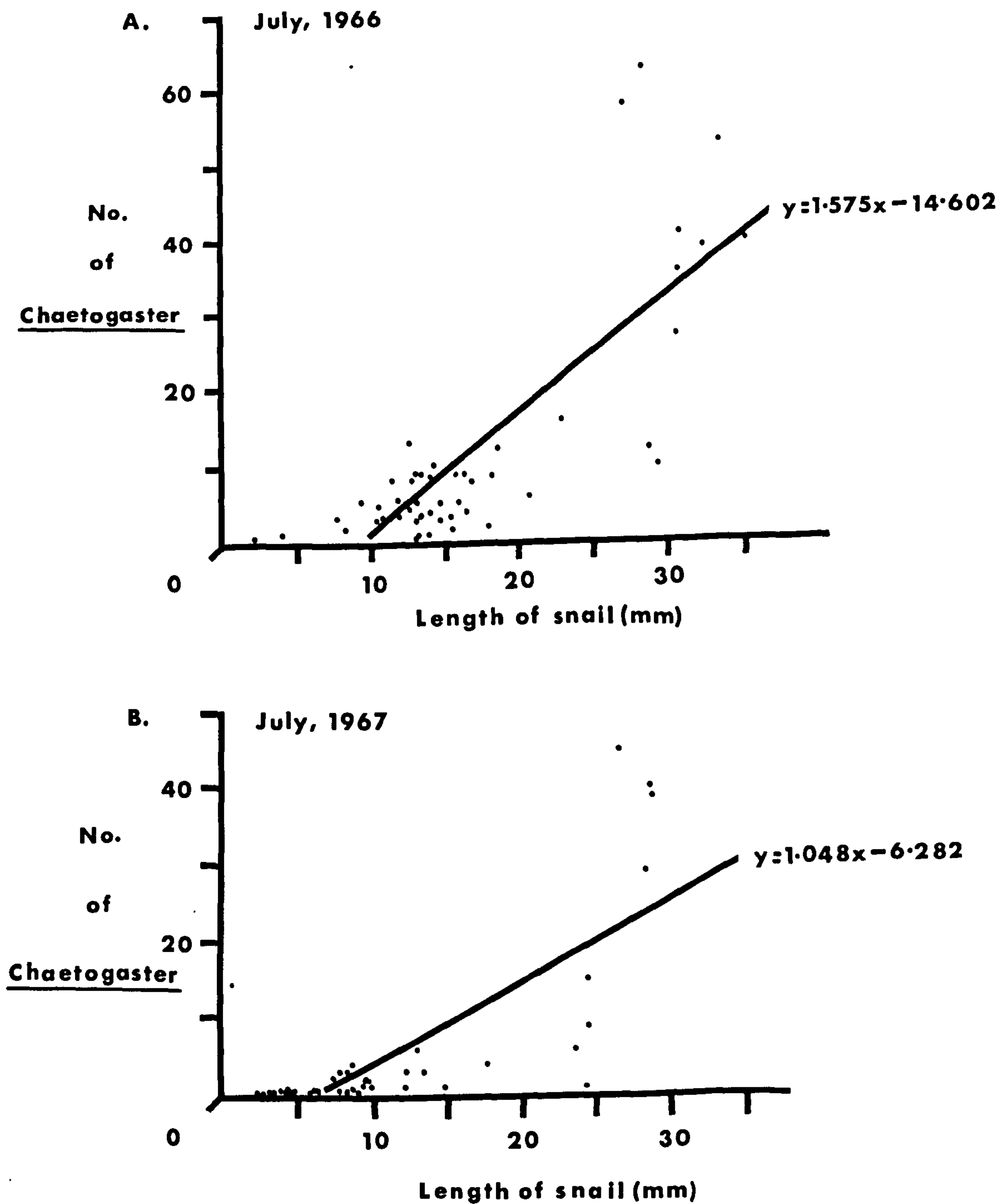


Figure 6. a) A comparison of changes in the total population of *Ch. l. vaghini* in 1966.  
 b) A comparison of changes in the total population of *Ch. l. vaghini* in 1967.  
 c) The number of *L. stagnalis* collected by net per minute in each sample.

in it. The length of the snail was taken as an indication of snail size. A month when the range of snail size was at its greatest was necessary to test this hypothesis, and so July was chosen in each year. The number of Ch. l. vaghini in the kidney was plotted against snail size (Fig. 7, p. 38). The regression coefficient was calculated for each case and the regression lines drawn. A positive correlation, significant at 0.1 per cent (Fig. 7), between the Ch. l. vaghini population and snail size was shown, the larger snails supporting the greater population.

There was also the possibility that the size of the kidney of L. stagnalis limited the size of the population of Ch. l. vaghini which could live in it. To test this, a certain size of snail would have to be investigated for a number of months. It was necessary to examine the Ch. l. vaghini population in the snails from the beginning of the year when only immature Ch. l. vaghini were present and during a period when numbers were increasing. For the percentage infection of Ch. l. vaghini to be high, snails of one of the larger size groups would have to be examined. To satisfy these requirements, the size group 20-25 mm. was used, from January to June in 1967. The Ch. l. vaghini population was large enough during these months for any differences in the numbers found to be significant. After June, the number of snails in this size group was too few to provide adequate samples for statistical purposes. The mean number of Chaetogaster per snail for this

Figure 7. The relationship between the number of Ch. l. vaghini in the kidney and the size of L. stagnalis.



For 49 degrees of freedom and  $P = 0.001$ ,  $r = 0.354$ .  
 For July 1966,  $r = 0.802$  and for July 1967,  $r = 0.778$ .  
 Both are significant at 0.1 per cent level.



size group is plotted against time in Figure 8 (p. 40). It is seen that the population increases exponentially from February to May and the logarithmic plot (Fig. 9, p. 41) confirms this. The population then levels off (Fig. 8). This suggests that the Ch. l. vaghini population in L. stagnalis was being limited by the size of the kidney.

#### 4. The life-cycle of Ch. l. vaghini in the field.

##### 4.0. Introduction.

Three stages in the life-cycle of Ch. l. vaghini can be distinguished; the immature individuals which do not show any sign of reproductive organs when examined under the microscope, the mature individuals in which such structures are visible and the cocoons. The number in each of these stages has been observed in all the monthly samples to provide a picture of the life-cycle of this oligochaete. Changes in water temperature were compared with events in the life-cycle to look for possible correlation.

##### 4.1. The mature form and the cocoons.

An examination of the mature form of Ch. l. vaghini showed that it had two bundles of penial setae. These were

Figure 8. The increase in number of immature Ch. l. vaghini in the kidney of snails of size 20-25 mm.

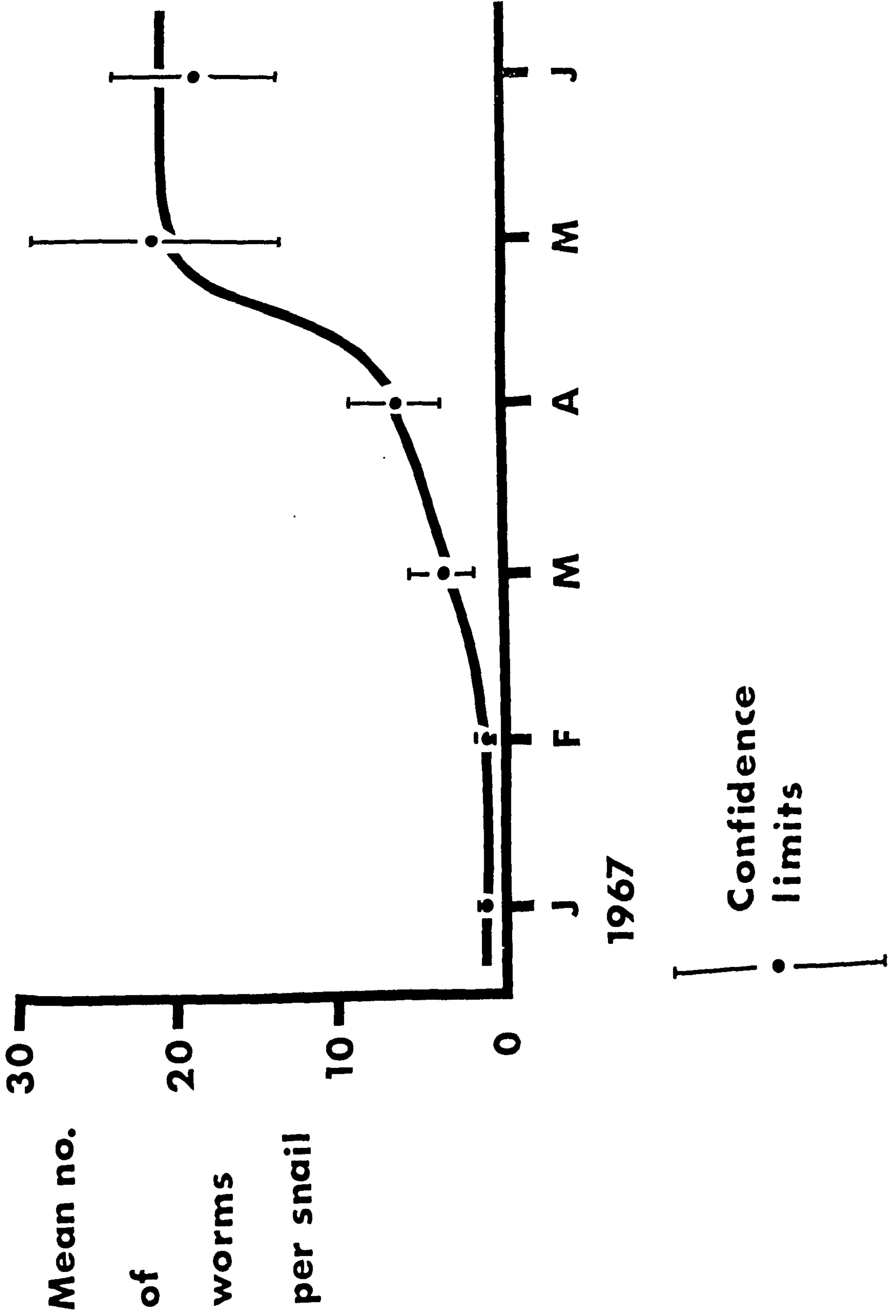
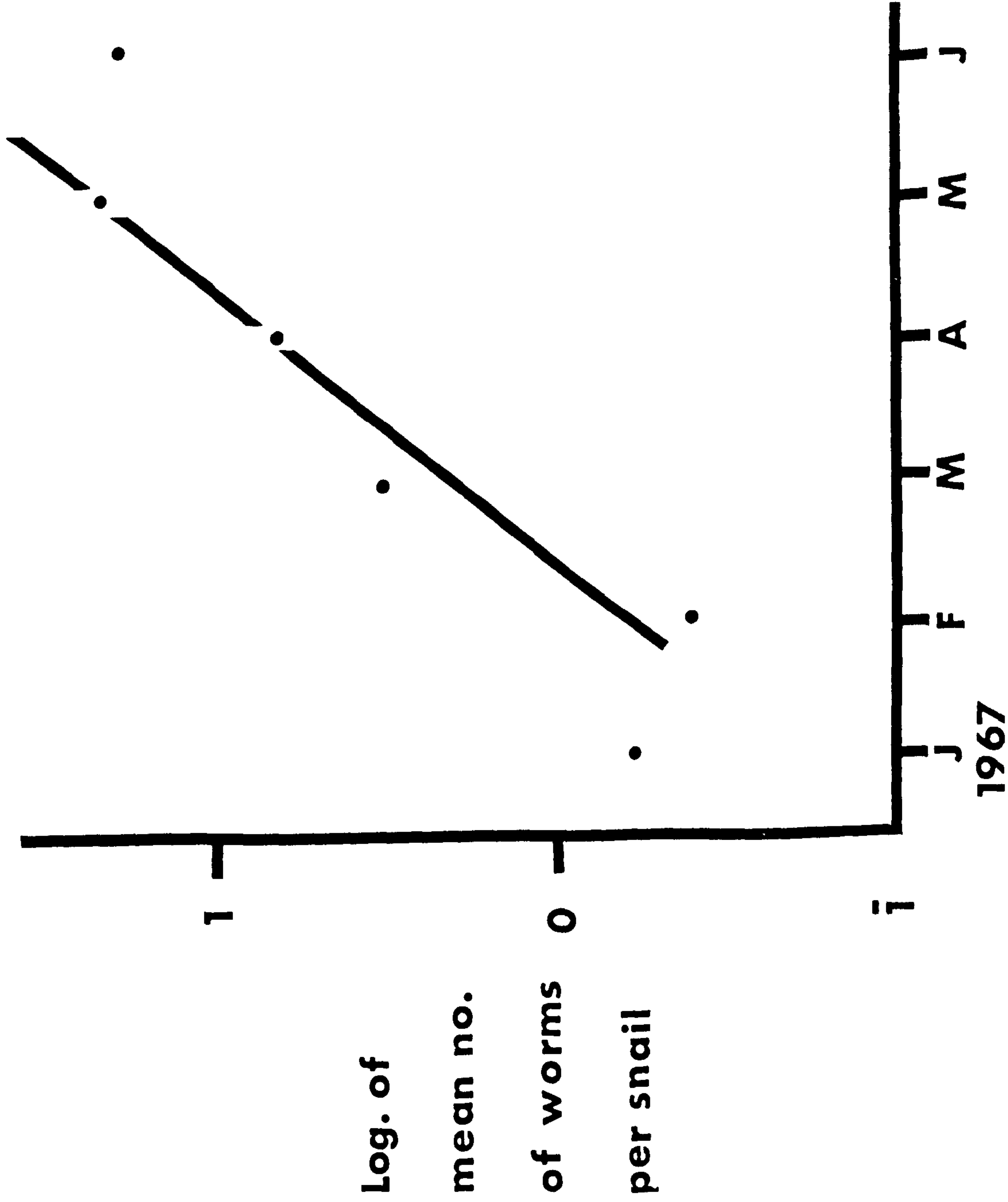


Figure 9. The log. of the number of Ch. l. vaghini found in the kidney of 20-25 mm. snails from January to June, 1967.





present in segment 6 and were found just posterior to the normal setal bundles of that segment. There were 3 penial setae in each bundle, as shown in Plate 3. The setae were approximately the same length as the normal setae of segment 6, being 50-60  $\mu$  in length. On two of the setae in each bundle, two teeth were present as on the normal setae. However, those of the penial setae were less recurved. The third seta of each bundle was simple. The nodulus of the penial setae was one third along the length of the seta from the tip, whereas in the normal seta it was half way along. The diameter of the penial setae was twice that of the ordinary setae, measuring approximately 3.4  $\mu$  as compared with 1.7  $\mu$ .

Microscopic examination of the mature Ch. l. vaghini showed that only one ovary was present. Two or three ova were found in it at the early stages of maturation, but only one large ovum was found in the fully mature worm (Plate 4). Thus only one cocoon will be produced by each worm.

The cocoons of Ch. l. vaghini were oval in shape (Plate 5). Their size varied from 291-481  $\mu$  in length and from 219-461  $\mu$  in width. The ratio of length to breadth varied from almost 1:1 to 1.8:1 in different cocoons. Only one embryo developed in each cocoon. Thus each mature Ch. l. vaghini can produce only 1 worm by sexual reproduction.

The position of the cocoons in the kidney of L. stagnalis was examined to see if they were evenly distributed. An



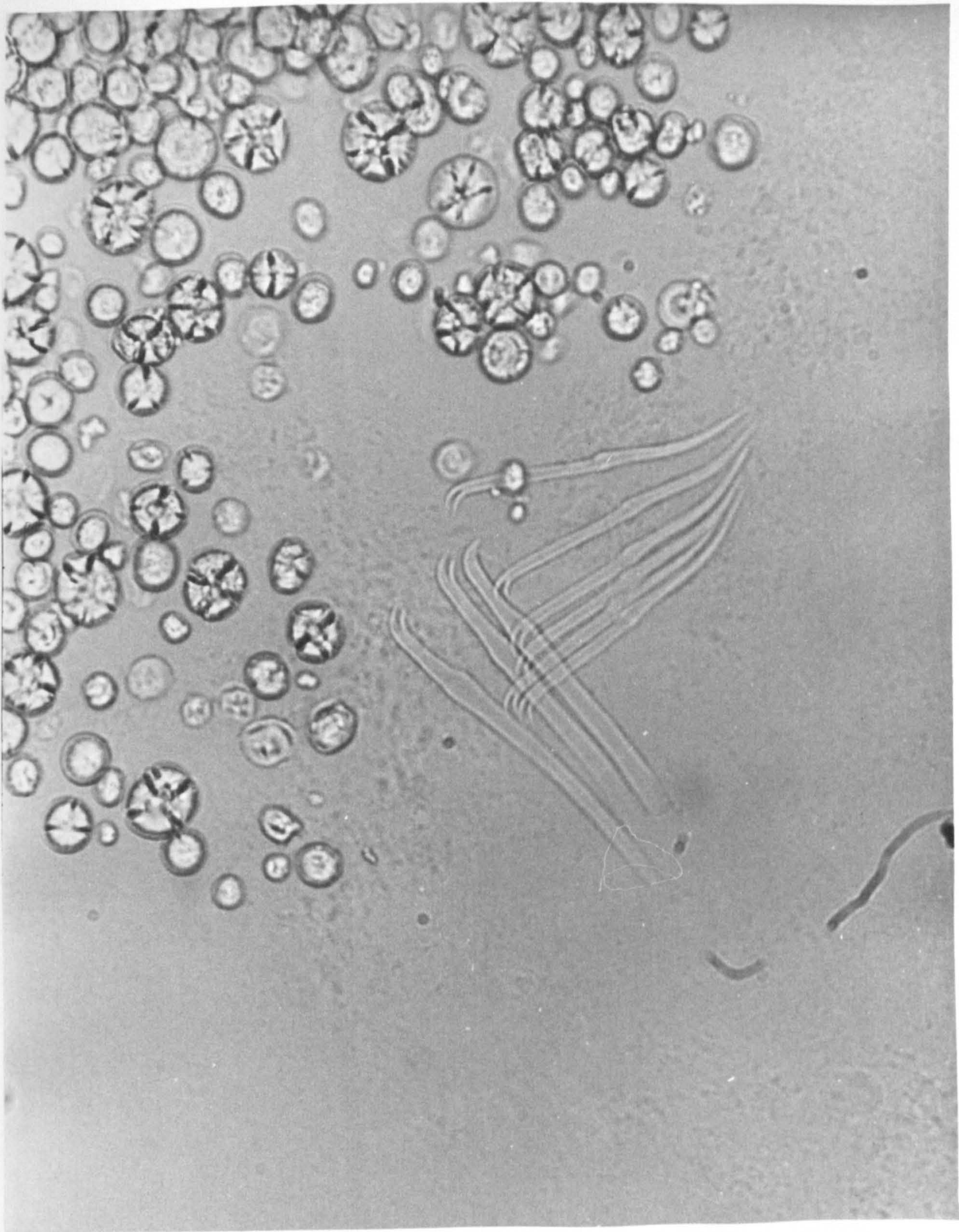


Plate 3. The penial setae and normal setal bundle, in segment 6 of a mature Ch. l. vaghini.



Plate 4. The ovum in a mature Ch. l. vaghini.

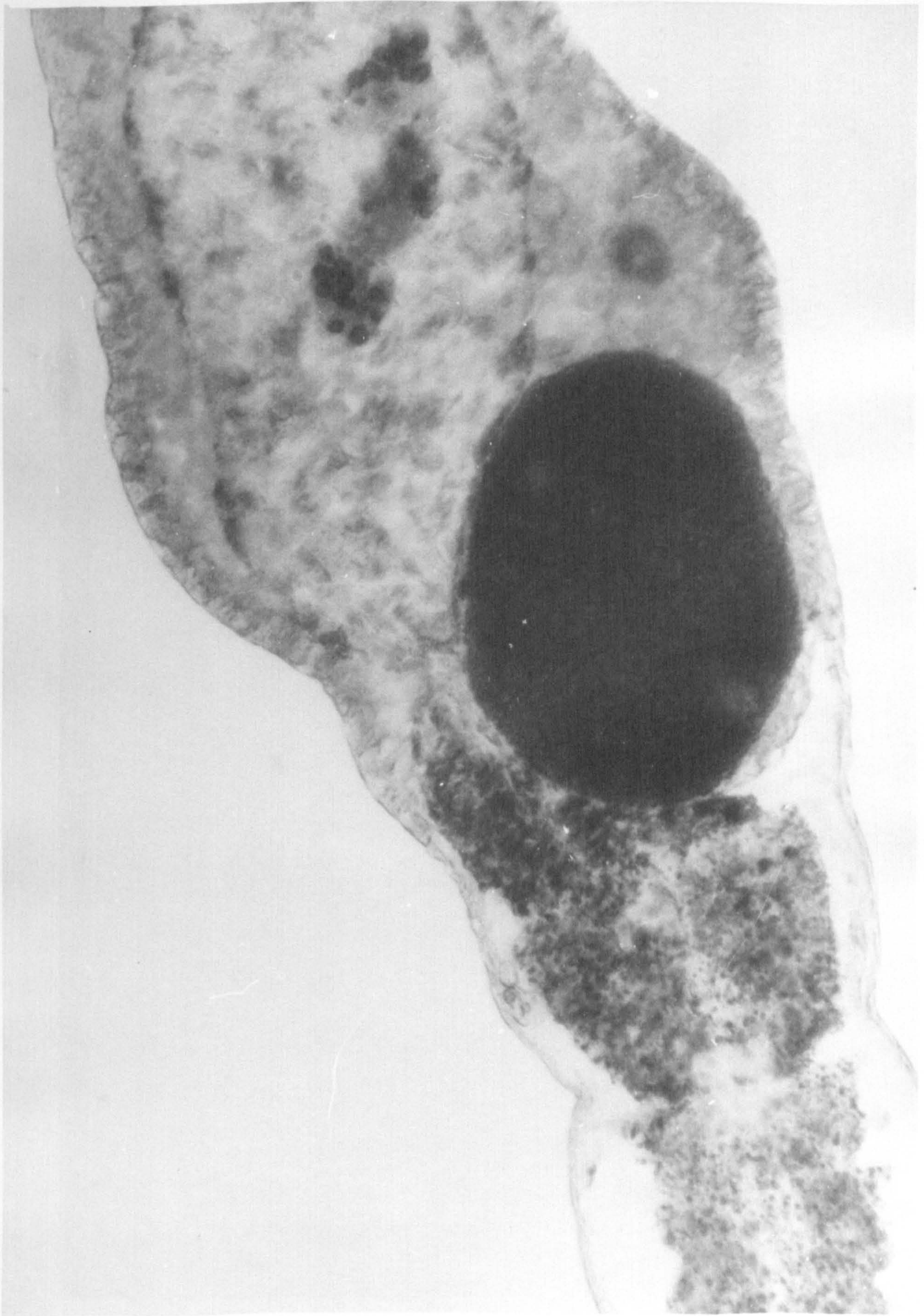
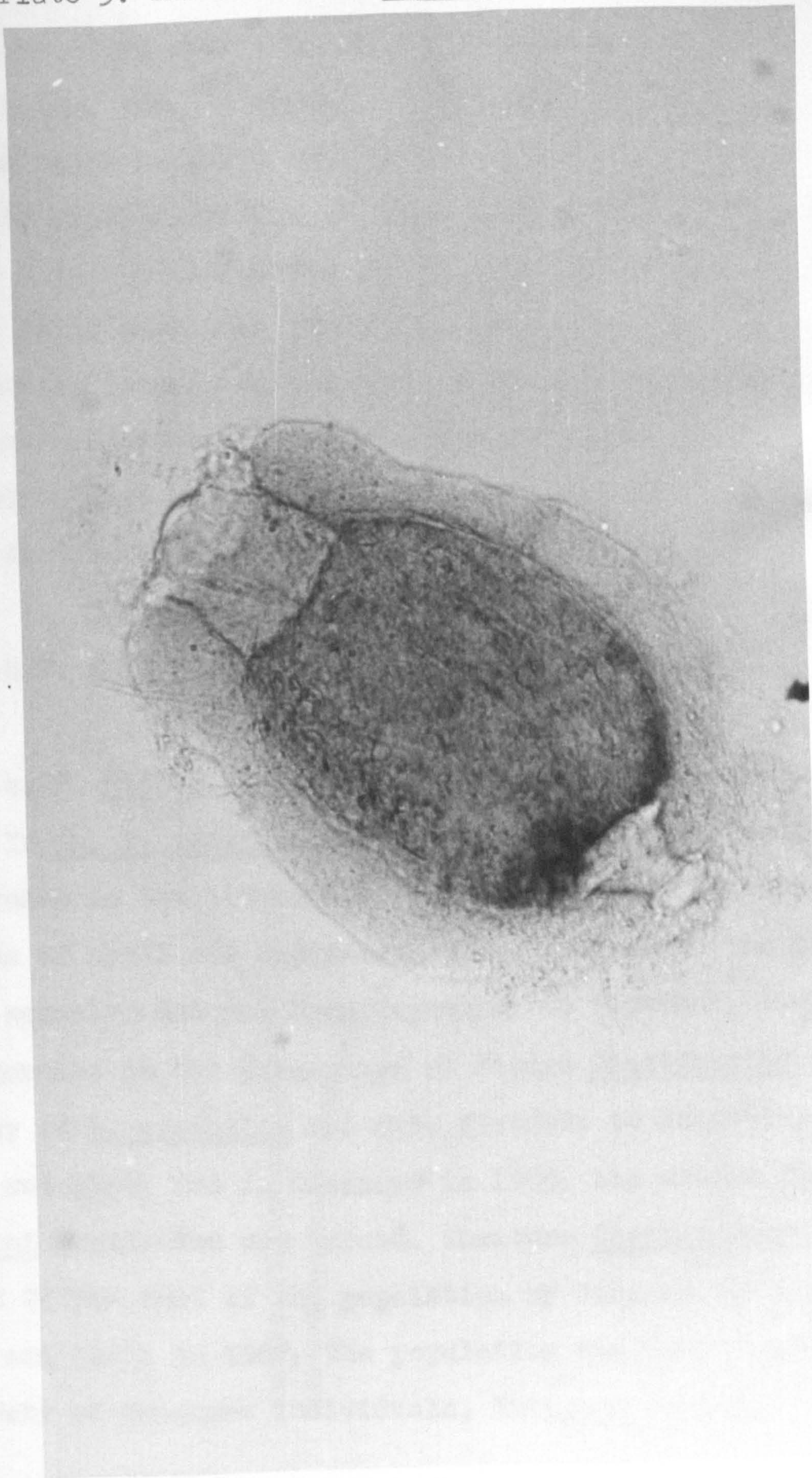




Plate 5. The cocoon of Ch. l. vaghini.



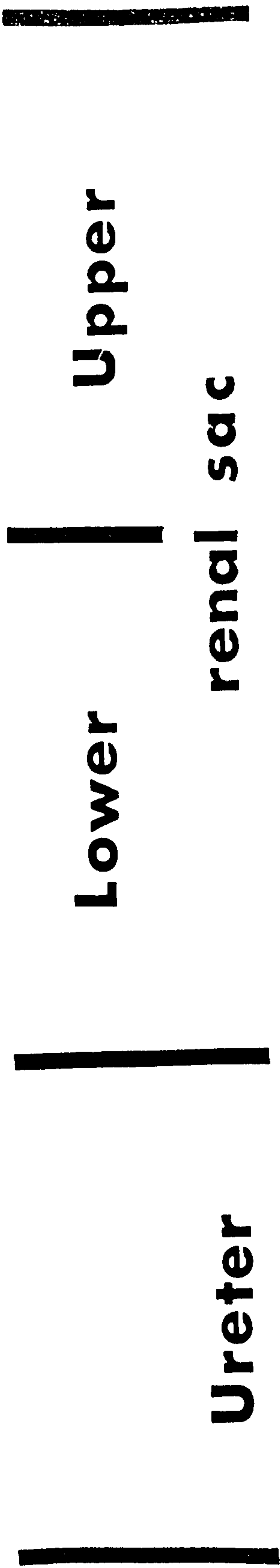
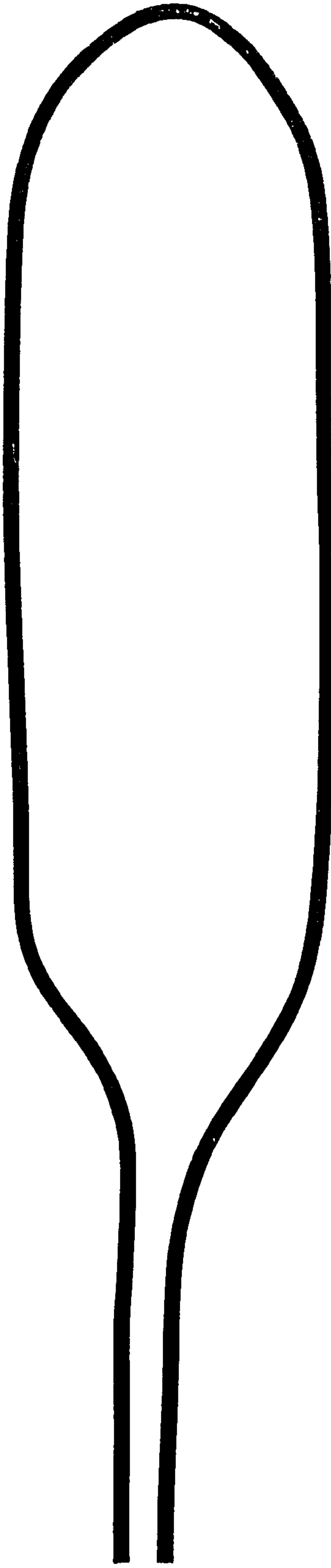


arbitrary division of the kidney into three equal lengths, i.e. the ureter, the lower part of the renal sac and the upper part of the renal sac, was made (Fig. 10, p. 44). The number of cocoons present in each of these divisions was recorded and Table 7 (p. 45) shows the distribution of viable cocoons in them. It is seen that there were significantly more cocoons in the ureter than in either section of the renal sac. This contrast is enhanced if the number per unit volume is considered, the size of each section of the renal sac being much greater than that of the ureter.

#### 4.2. Reproductive cycle.

##### 4.20. Mature and immature stages.

In Ch. 1. vaghini, asexual and sexual reproduction alternate in the life-cycle (Fig. 11, p. 46). Between the months of April and September no individuals in the population were sexually mature. From September to November, there was an increase in the percentage of mature Chaetogaster in the kidney of L. stagnalis and from November to December, in 1966 and 1967, and in December in 1965, the entire Ch. 1. vaghini population was mature. Immature Chaetogaster formed about 25 per cent of the population by January. By March in 1966 and April in 1967, the population was again composed entirely of immature individuals. This was the position until



**Upper**

**renal sac**

**Lower**

**Ureter**

Figure 10. Divisions of the kidney of L. stagnalis.



	Ureter	Lower part of renal sac	Upper part of renal sac
Number of cocoons found	111	61	24

$$\chi^2 = 39.2$$

Highly significant

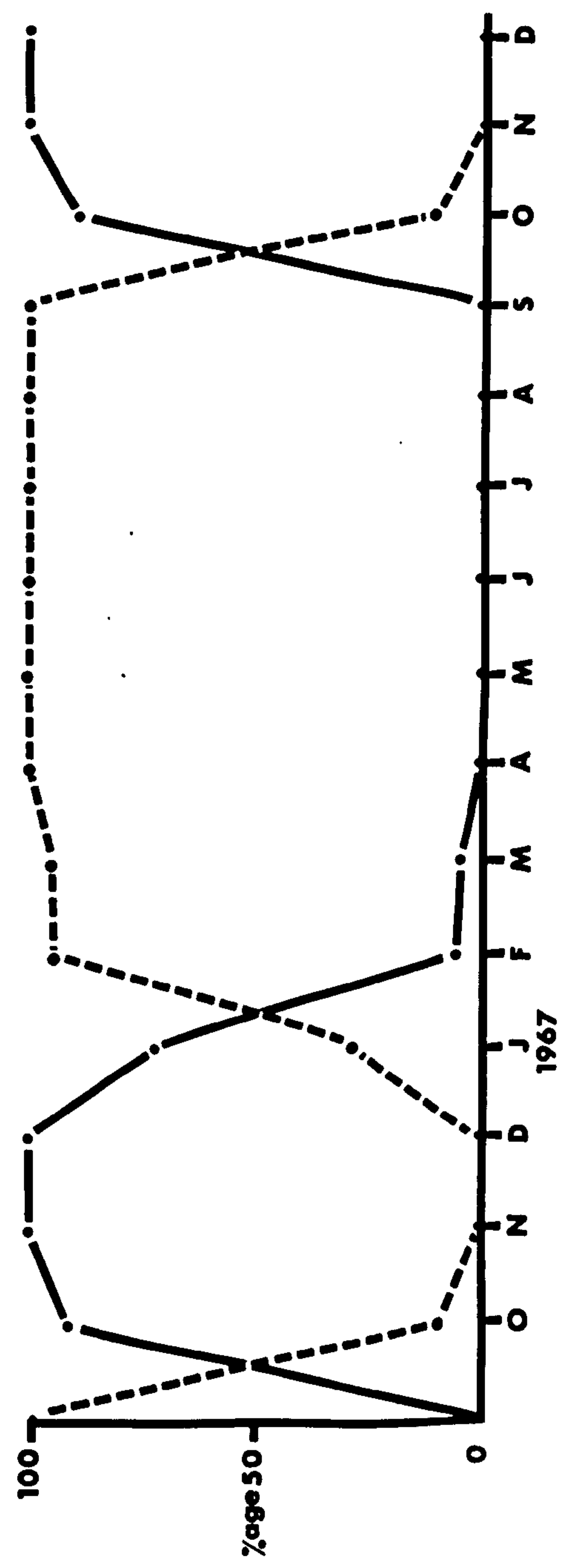
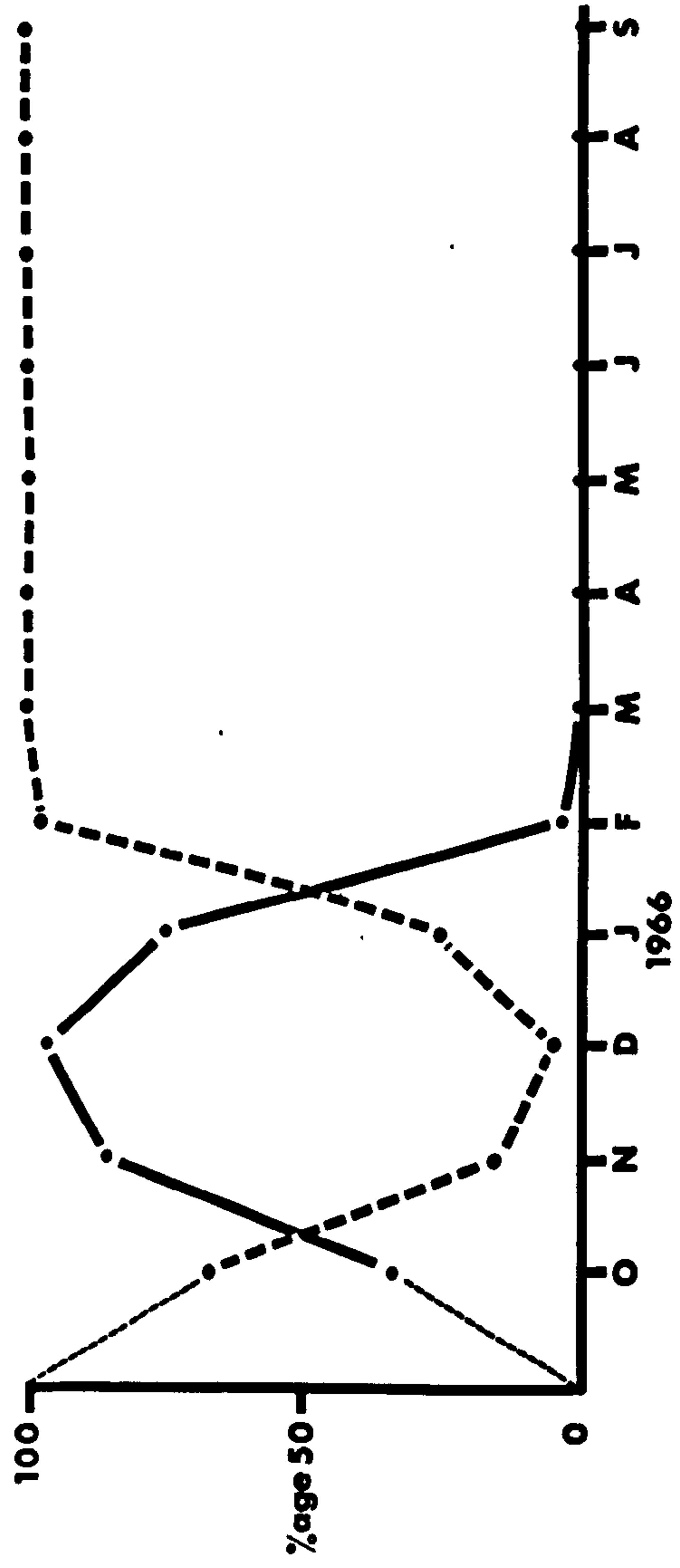
(For 2 degrees of freedom and  $P = 0.05$ ,  $\chi^2 = 5.99$ )

Number of L. stagnalis kidneys examined = 57.

Table 7. Position of the cocoons of Ch. l. vaghini in the kidney of L. stagnalis.

Figure 11. The percentage maturity and immaturity of the Ch. l. vaghini population.

●—● Mature  
 ●- - -● Immature





September, from which time the proportion of immature specimens began to decline.

In 1966, the mean number of mature Chaetogaster per snail dropped from 6.28 in October to 0.36 in January (Fig. 12, p. 48). Similarly in 1967, the mean fell from 1.5 in October to 0.88 in December and in 1965 from 4.56 in November to 0.12 in January.

The population of immature Ch. l. vaghini began to increase from January onwards. In 1966, the mean number of immature Chaetogaster per snail in January was 0.04, increasing to 3.58 in March and to a peak of 24.0 in May. In 1967, the number rose from 0.14 in January to 2.58 in March and to a maximum of 20.8 in June. The mean number of immature worms then fell in both years to 8.9 (1966) and to 3.2 (1967) in August. There was a slight increase in September, followed by a fall due to the onset of maturation.

#### 4.21. Cocoon production.

Figure 13 (p. 49) shows the mean number of viable cocoons of Ch. l. vaghini found in the kidney of L. stagnalis at different times of the year. The mean number of mature Chaetogaster is also shown. The first cocoons were found in October and the mean increased until December. The peak in mature Ch. l. vaghini was in November in 1965 and 1967, and in October in 1966. The peak in the number of cocoons present

Figure 12. The mean number of mature and immature Ch. l. vaghini in each L. stagnalis.

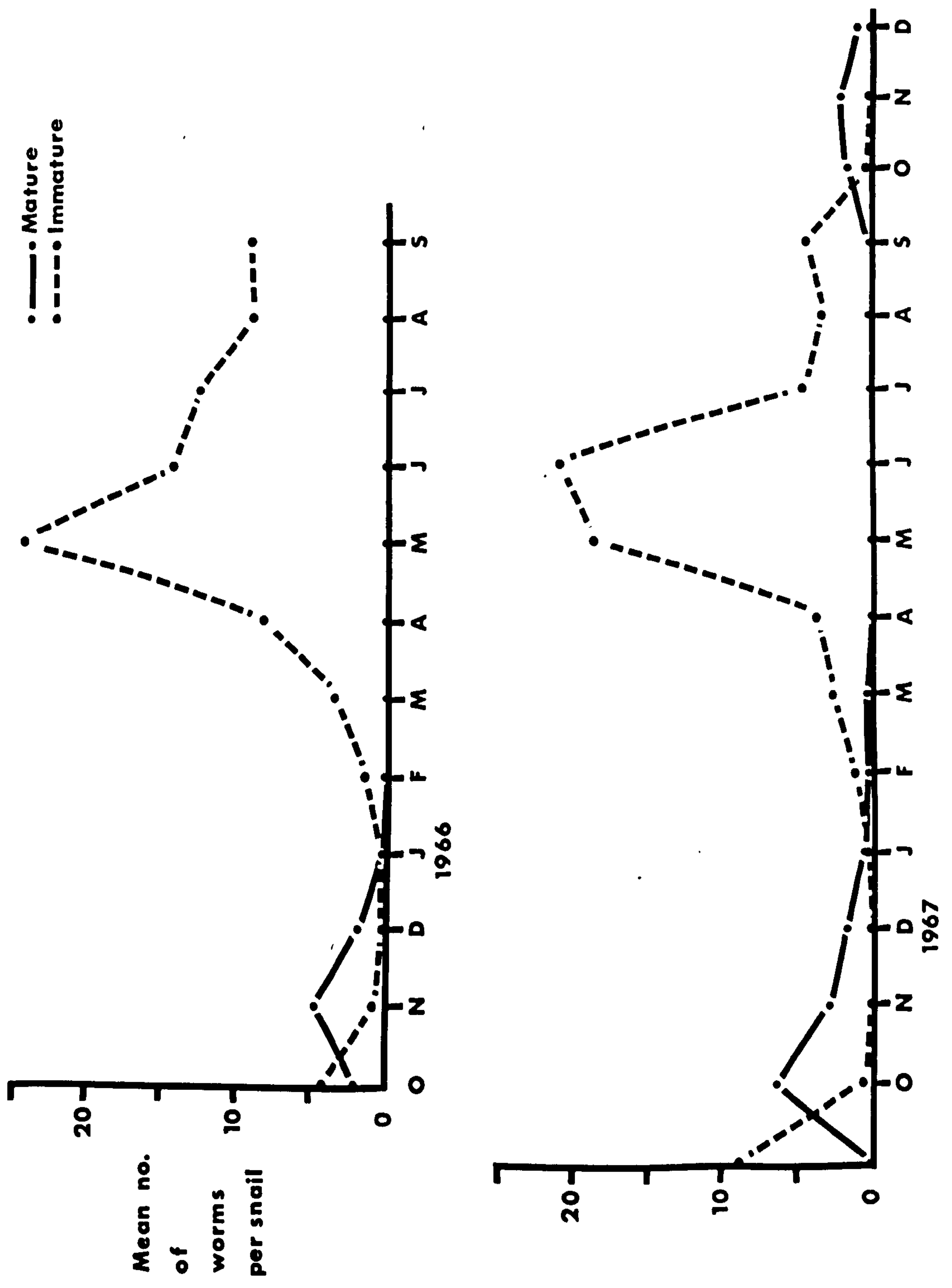
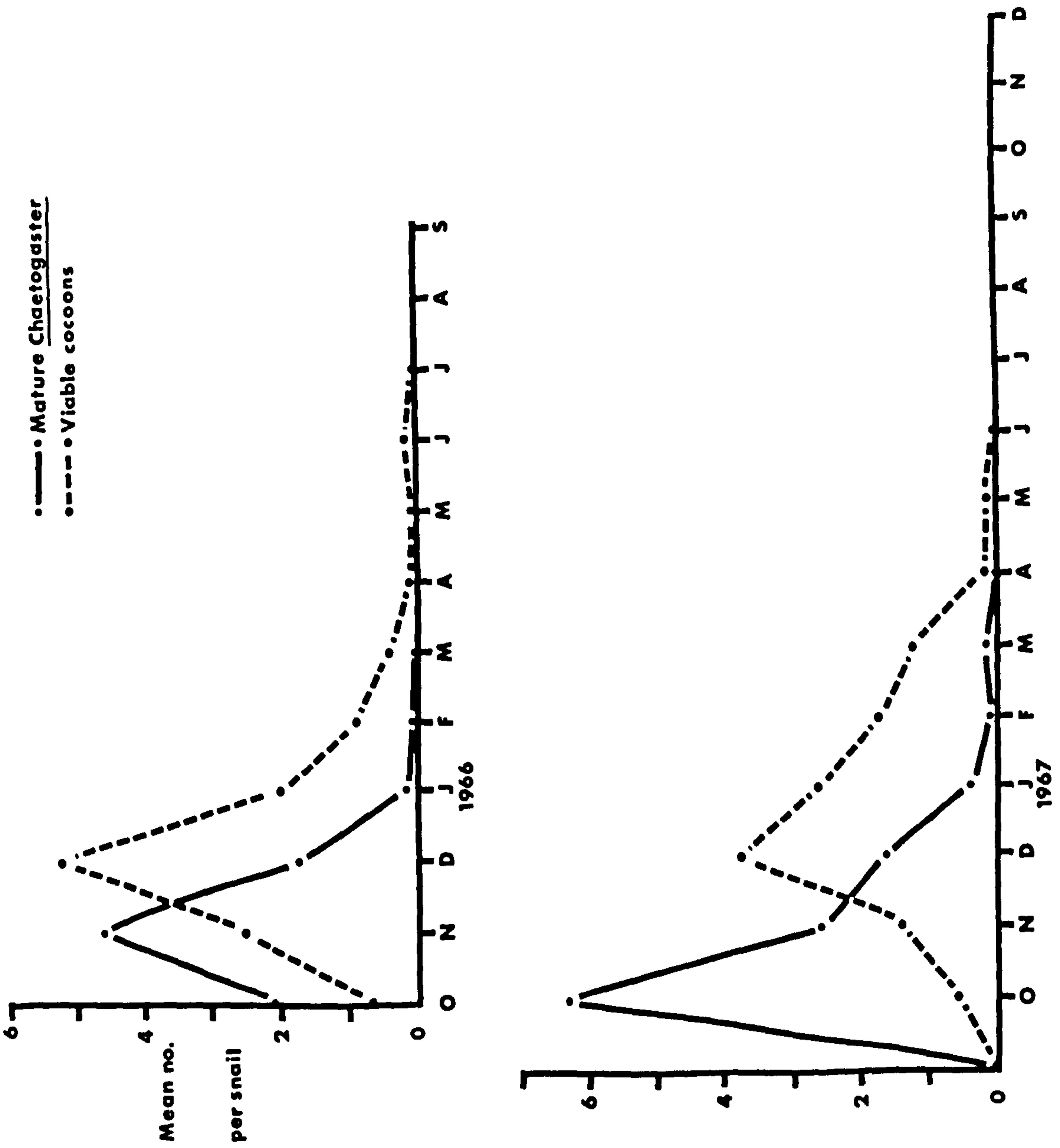




Figure 13. The mean number of cocoons and mature Ch. l. vaghini in L. stagnalis.



followed that of the mature Chaetogaster by a month in 1965 and 1967 and by two months in 1966. Expulsion of the egg was by rupture of the body wall, and each worm died after cocoon production. The remains of these worms could be found in the kidney. This explains why the peak in cocoon production followed closely the peak in the number of mature individuals. As one ovum develops in each worm, only one cocoon was produced. The level of the maxima in the number of mature worms and the number of cocoons were therefore almost identical in the winters of 1965-1966 and 1967-1968, but not in the winter of 1966-1967.

Figure 14 (pp. 51 and 52) shows the mean number of viable cocoons and empty cocoons found in the kidney at different times of the year, together with the number of mature and immature Chaetogaster. Between December and January in both 1966 and 1967, there was approximately a 50 per cent reduction in the number of viable cocoons present. This drop in number could have been due to the cocoons hatching, or to their leaving the kidney either as viable cocoons or as empty cocoons and immature Chaetogaster. A combination of these possibilities may have occurred. The low number of empty cocoons in the kidney in January suggests there was little hatching of cocoons there, unless the empty cocoons were being eliminated from it. However, the very few immature Chaetogaster present shows that hatching could not have been occurring to any

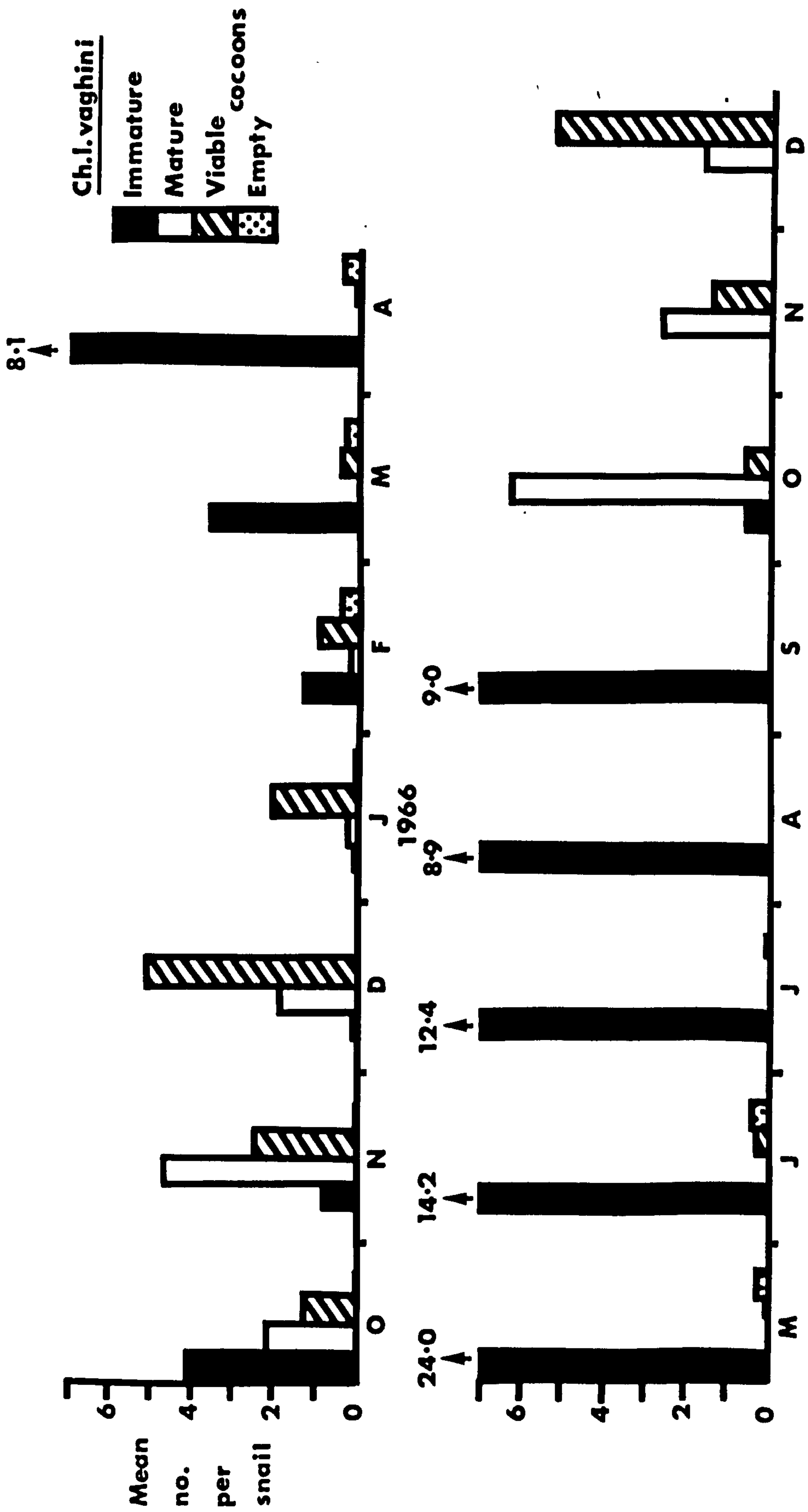


Figure 14a. Comparison of the stages in the life-history of Ch. l. vaghini during 1966.



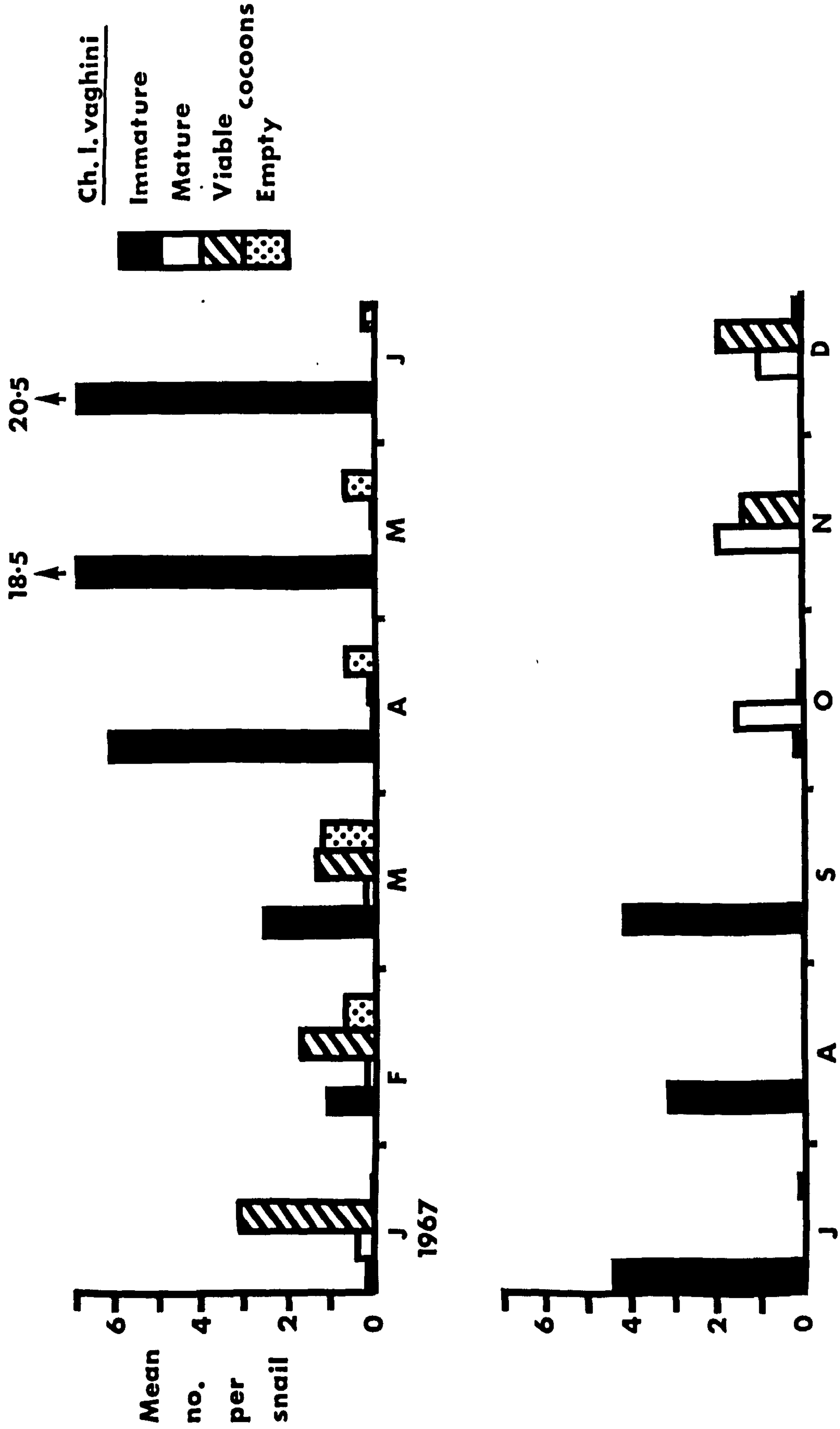


Figure 14b. Comparison of the stages in the life-history of Ch. l. vaghini during 1967.

extent. The other possibility that the cocoons hatched and both the Chaetogaster and the empty cocoons were washed from the kidney is unlikely as no specimens of snail were found with large numbers of either of these present, as would occur if this was happening. The majority of the cocoons therefore left the kidney before hatching. As some empty cocoons were found in the kidney in later months, some of them evidently did hatch in the kidney. The immature Chaetogaster in the kidney rapidly increased in number by asexual reproduction from February onwards.

The cocoons were therefore part of a dispersal stage in the life-cycle of Ch. l. vaghini. When the cocoons hatch outside the snail, the immature Chaetogaster will have to find a new host snail for the life-cycle to continue.

#### 4.3. Elimination of cocoons from the kidney.

It was suggested in Section 4.2 that the cocoons left the kidney of L. stagnalis before hatching. To verify this, detritus from bowls containing L. stagnalis kept at winter temperatures in the laboratory during the winter of 1966-1967 was searched under the binocular microscope for Ch. l. vaghini cocoons. The detritus was composed of faecal material and uneaten food particles. No cocoons were found, and this was thought to be due to their destruction by the

snails in such crowded conditions.

In the winter of 1967-1968, an experiment was set up such that L. stagnalis would not have the opportunity of destroying the cocoons. An outer bowl of diameter 30 cm. contained a less deep bowl supported on its rim, so that its base was 4 cm. above the base of the outer bowl. The base of the inner bowl was perforated with holes of 4 mm. diameter. The total depth of water was 8 cm. Twenty L. stagnalis were kept in the inner bowl and the faecal material and other particles, which fell through the holes into the larger bowl, were collected at regular intervals and examined. Forty ml. of water containing the faecal material were searched on each occasion. Only 2 viable and 2 empty cocoons were found (Table 8, p. 55). Three immature Ch. l. vaghini were also found, suggesting that hatching of the cocoons had taken place outside the snail. It is possible that the low numbers of cocoons recovered was due to their sticking to the mucous trail produced by the snail and being destroyed by the movement of the L. stagnalis in the inner bowl. The experiment shows that some viable cocoons leave the kidney. The empty cocoons found may have hatched either before or after leaving it. No cocoons were found in a cage set up in the field.

#### 4.4. Correlation of the reproductive cycle with temperature.



Date	Viable cocoons	Empty cocoons	Immature <u>Chaetogaster</u>
10 Nov., 1967	1		
6 Dec.			
21 Dec.			
21 Jan., 1968	1	2	3

Table 8. Cocoons of Ch. l. vaghini found after leaving the kidney of L. stagnalis.

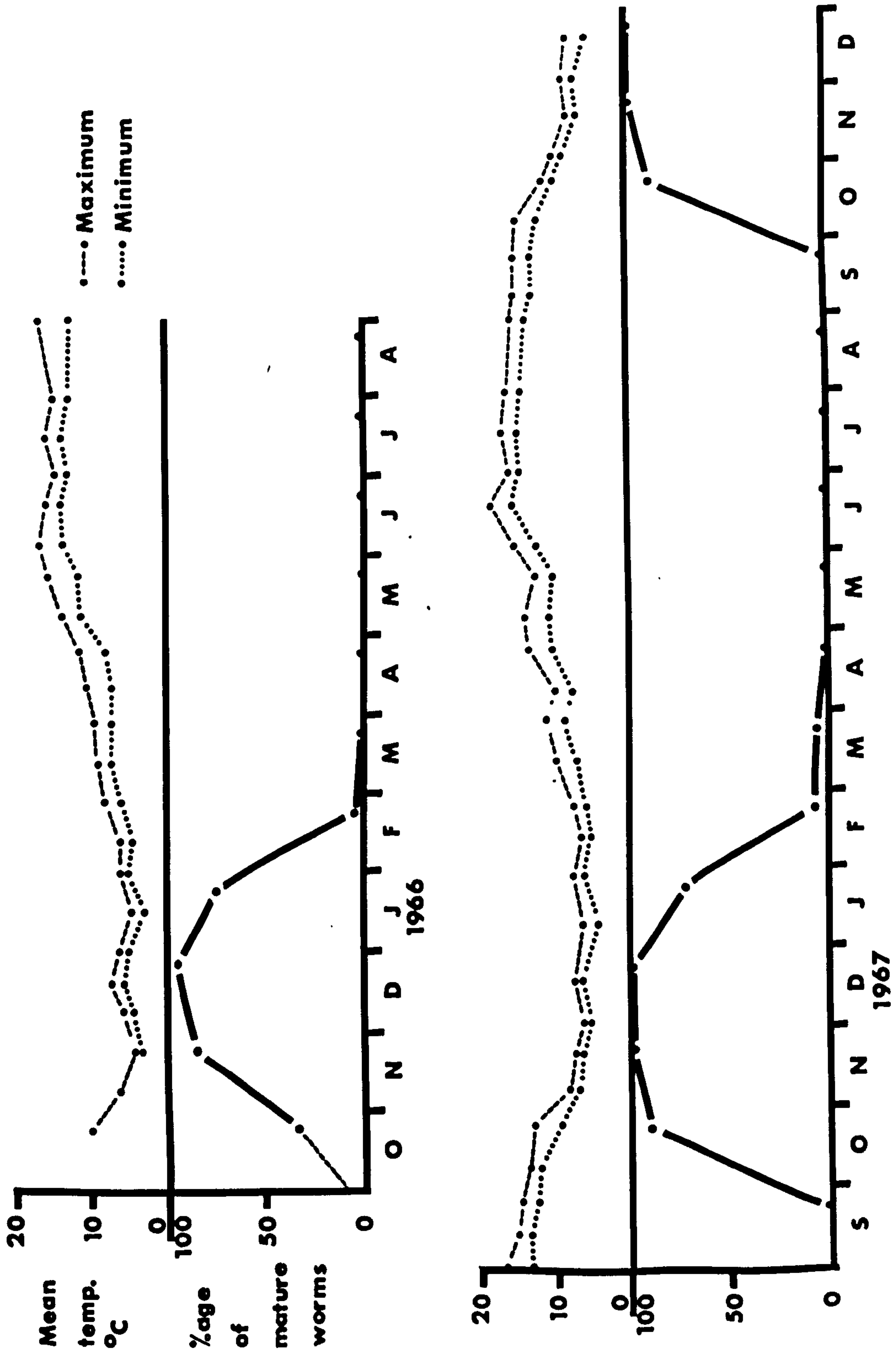
Daily readings of maximum and minimum temperatures in the College pond were taken during the two years of sampling. The average maximum and minimum temperatures for each fortnight are shown in Figure 15 (p.57) for comparison with the percentage of mature worms present at each sample. The rapid increase in maturity from 0 to approximately 90 per cent in October occurred at the same time as, or slightly behind, the fall in temperature from September onwards. In both years, the mature Chaetogaster were found for the first time in October, the temperature being by this time below 10°C. A slightly higher temperature during the preceding weeks may have been involved in the causation of maturation of the Chaetogaster, the data suggesting a temperature of about 13°C. If temperature was concerned in the maturation of the Chaetogaster it could either have a direct or indirect effect on this process.

#### 4.5. The infection of Lymnaea stagnalis.

The behavioural aspects of the infection of Lymnaea stagnalis by Chaetogaster l. vughini are dealt with in Section F.

To determine whether the size of the L. stagnalis, and therefore the size of its kidney, was critical for infection to occur, large numbers of young snails were examined in June,

Figure 15. The relationship between temperature and percentage maturity in Ch. l. vaghini.



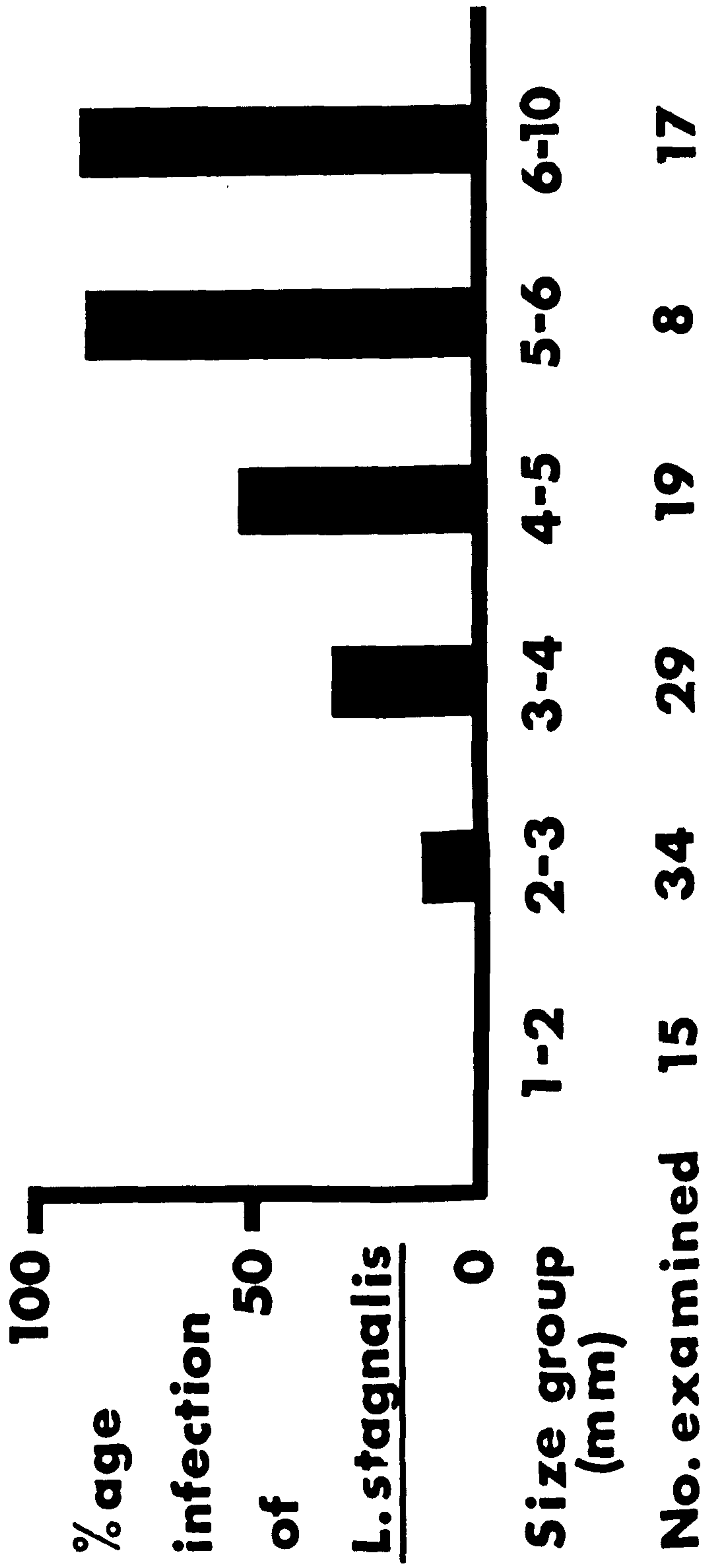


1966. The size groups examined were 1-2 mm., 2-3 mm., 3-4 mm., 4-5 mm., 5-6 mm. and 6-10 mm. The results in Figure 16 (p. 59) show 50 per cent of the snails to be infected when they had reached the 4-5 mm. size group. No infection was found in the 1-2 mm. size group, and only low infection in the 2-3 mm. group. Thus the size of the renal opening does not prevent the infection of L. stagnalis in the 2-3 mm. group. The lack of infection in the 1-2 mm. group could be due either to the insufficient time since hatching for Chaetogaster to find the host, or to the kidney opening being too small. A microscopic examination suggested the latter to be true, but, due to the elastic nature of the ureter opening, infection was not precluded.

As the time taken for a snail to reach the size at which it had a 50 per cent chance of being infected was important, the growth rate of the snail was examined in the field. A perspex cage was constructed, measuring 30 cm. by 30 cm. by 8 cm. All the sides were covered with fine nylon mesh. Twenty-five newly hatched snails, marked with cellulose paint in different colour combinations so that each individual could be identified, were introduced into the cage. The cage was fixed vertically in the College pond, with the top of the cage 5 cm. out of the water to allow the snails to respire. The snails were measured each week. Figure 17 (p. 60) shows the rate of growth of the snails. The death rate was high, only 2 of the

Figure 16. The percentage infection of young snails with Ch. l. vaghini.

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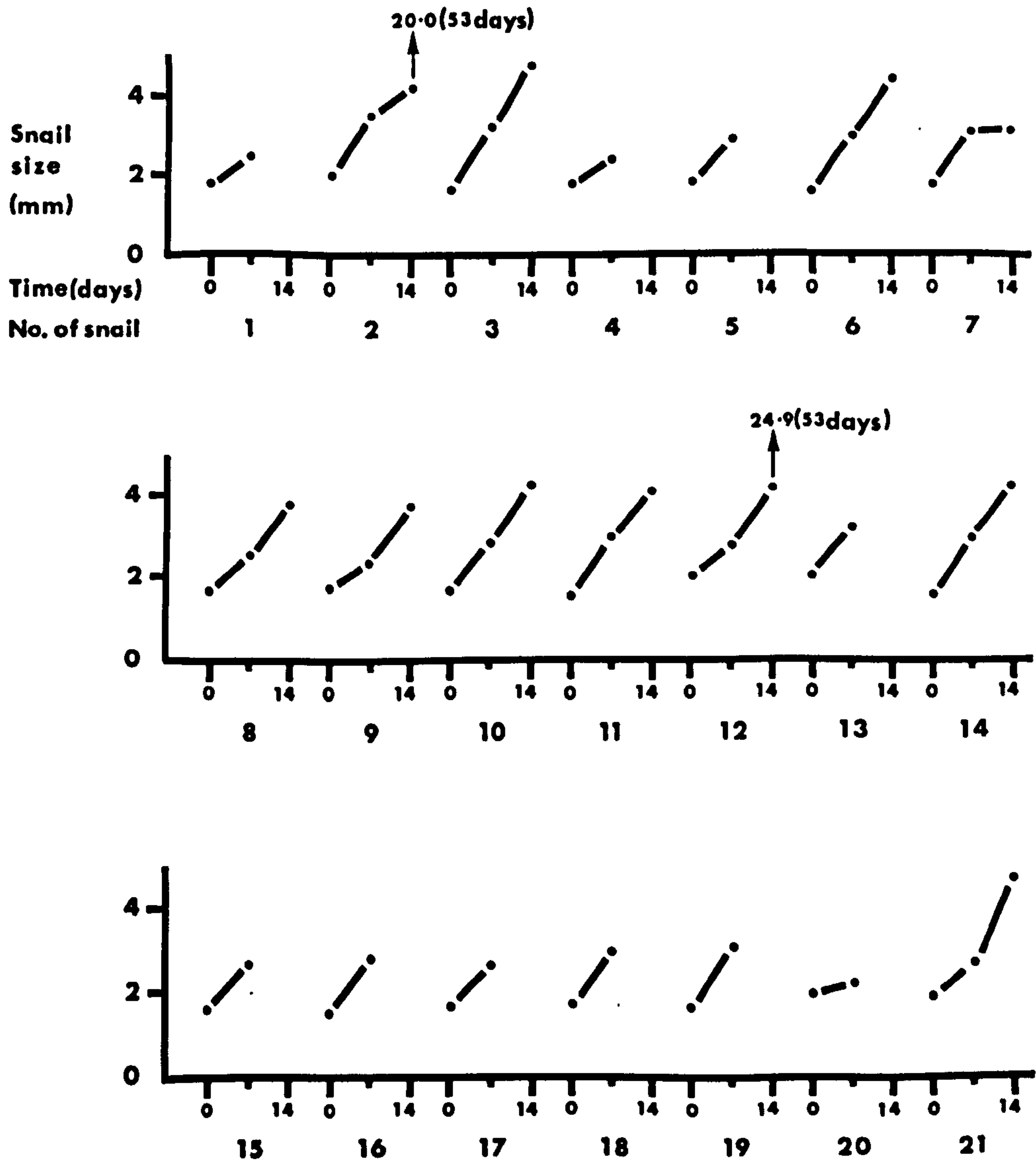


Figure 17. The rate of growth of L. stagnalis.



25 snails surviving for 53 days. The graphs for the individual snails show that a size of 4-5 mm. was reached within two weeks.

Thus a fortnight after hatching, 50 per cent of the L. stagnalis became infected with Ch. l. vaghini. This shows that the Chaetogaster were very efficient in infecting the newly hatched snails and suggests that infection was a positive process rather than just due to chance.

## 5. An experimental study of the life-cycle of Chaetogaster limnaei vaghini.

### 5.0. The onset of maturation.

#### 5.01. Introduction.

Most of the Naididae become mature during the summer months, whereas Chaetogaster species mature in the winter (Stephenson, 1930). The few mature specimens of Ch. l. limnaei that have been reported were found in mid-August (Vaghin, 1946) and for two weeks in October (Lankester, 1869). During the present study, mature specimens of Ch. l. limnaei were only found once, this being at Cole Mere, in November, 1965, in the snails Bithynia tentaculata and Physa fontinalis. Gruffydd (1965b) never found mature specimens of Ch. l. limnaei. Due to the very low numbers maturing in Ch. l. limnaei, it was not

possible to examine the causative factors involved in maturation in this subspecies. However, Ch 1. vaghini provided ample opportunity to determine why maturation only occurred in the winter months and whether it was brought about by external factors, innate factors, or a combination of both.

#### 5.02. The factors involved.

If the time of the onset of maturation was not determined solely by genetic control, some factor(s) in the environment must have been effective. Such a factor could either influence the Chaetogaster l. vaghini directly, or indirectly via the snail. As it must show seasonal variation, light, temperature, or food seemed to be the most likely candidates. In the experiments, two contrasted values of each factor were used, corresponding to summer and winter régimes respectively (Table 9, p. 63).

The experiments were carried out in circular, plastic containers of 30 cm. diameter, in which the depth of water was 5 cm. and constant aeration was supplied. In each experimental bowl there were 30 Lymnaea stagnalis, half of which were in the 15-20 mm. size group and half in the 20-25 mm. group. Eight experiments were set up, with the various factors which were being examined in all possible combinations. Light was supplied by 2 foot long, 20 watt fluorescent strip lights, suspended above the bowls. Fluorescent light was used because of its even light distribution and its cool working

Factor.	Summer régime.	Winter régime.
Light	Constant	12 hours (reducing)
Temperature	20°C	Pond (15°C reducing to 7.5)
Food	Surplus	Restricted

Table 9. Factors used in examining the cause of the onset of maturation in Ch. l. vaghini.



temperature. The light in the summer régime was 500 foot-candles, which was equivalent to a cloudy, summer's day. The winter régime had a light of 130 foot-candles, equivalent to a cloudy, winter's day. The intensity of light in both régimes could be regarded as average for the time of year. A variable time switch was used to keep the experimental winter's day length the same as the natural winter's day length.

The summer temperature of  $20^{\circ}\text{C}$  was that of the laboratory in which the experiments were performed. The bowls at winter temperature were kept in a cold room in which the temperature was adjusted daily to that of the College pond from which the Lymnaea stagnalis were obtained. The temperature decreased from  $15^{\circ}\text{C}$  at the beginning of the experiment to  $7.5^{\circ}\text{C}$  at the end.

The snails were fed on artificial food (see p. 24) throughout the experiment. Those in the summer régime were supplied with surplus food, twice a week, at the rate of 0.25 gm. of food, per snail, per day. The snails under winter conditions were fed once a week at a rate of 0.05 gm. of food, per snail, per day.

The duration of the experiment was from 28 September, 1966 to 11 November, 1966, i.e. it was concluded two weeks after the mature Chaetogaster had appeared in the field population. The conditions of each experimental bowl are shown in Table 10 (p. 65).

Exp. no.	Conditions			Results	
	Temp.	Light	Food	Mean no. per snail	Percentage mature
1.	20°C	C.	S.	27.1	0
2.	20°C	C.	R.	6.0	0
3.	20°C	12 hr.	S.	5.8	0
4.	20°C	12 hr.	R.	7.9	0
5.	P.	C.	S.	9.9	60.5
6.	P.	C.	R.	6.5	81.6
7.	P.	12 hr.	S.	6.1	96.5
8.	P.	12 hr.	R.	4.4	100.0

P = Pond  
 C = Constant  
 S = Surplus  
 R = Restricted

Table 10. The effect of various factors on the maturation of Ch. l. vaghini.

The results (Table 10) show that the maintenance of a high temperature of 20°C prevented the development of mature individuals, whereas at pond temperatures, maturation of the worms had occurred. This indicates that temperature was the most important factor affecting the onset of maturation. The other factors appear to have affected the time taken for the whole population to become mature. The percentage maturation was less at the time of examination where there was constant light, being only 60.5 and 81.6 per cent as compared with 96.5 and 100 per cent respectively for winter daylength. The amount of food provided exerted an effect in that a greater percentage of the Chaetogaster were mature when food was restricted, being 60.5 compared with 81.6 per cent and 96.5 compared with 100 per cent mature. The summer level of both light and food may have delayed the time of maturation of the population, so that it was not completely mature at the time of examination.

Inspection of the seasonally variable factors showed that it was a drop in temperature which was correlated with the onset of maturation.

### 5.03. The innate component of maturation.

An innate mechanism could also be involved in the onset of maturation. To determine whether maturation was only delayed by high temperature and not prevented, the régime of



experiments 1 and 4 (Table 10, p. 65) were continued with the remaining 5 snails in each experiment under the same conditions. These were examined at intervals until March, 1967. Throughout the period, the Ch. l. vaghini population remained immature. Thus a low temperature was essential for the population to mature and there was no innate mechanism which had an effect independently of temperature.

Maturation in the field occurred when the temperature fell in October to about 9-10°C. However, in the early part of the year, the temperature was far below this and yet the Ch. l. vaghini population was immature. It was possible that maturation could only take place during one period of the year, due to an innate factor.

Nine snails remained from those kept at summer temperatures in the experiments investigating the factors involved in the onset of maturity, and an examination of other specimens had shown that the Ch. l. vaghini population in these had remained immature. On various dates, some of these snails were transferred to a temperature of 8-10°C. The results are shown in experiments 1 - 4 (Table 11, p. 68). The last worms to have matured were found on 1 February. At later dates, the population remained immature despite being kept for a longer time at 8°C.

Snails were also brought in from the College pond in May and kept at 8°C in experiment 5 (Table 11). A small proportion

EXP. TEMP.

NO. (°C) Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov

1 10 | 1 9 13  
m 0 14

2 10 | 1 22 2  
m 0 6

3 8 | 1 0 20 1 34  
m 3 0 0

4 8 | 1 56 1  
m 0 0

5 8 | 1 20 23 28 61 19 12 15 0 0  
m 0 0 4 9 0 7 13 2

6 8 | 1 12 12 0 20 11 17  
m 0 0 0 0 0 0 0

Source | Summer régime of experiment | College pond  
in Table 10

| = Date exp. begun.  $\frown$  = Date snail examined. i = immature. m = mature.

Table 11. The effect of the season on the onset of maturation in Ch. l. vaghini.

i 9 = No. of immature } in 1 snail  
m 0 = No. of mature }

became mature in August, but 100 per cent maturation only occurred at the end of October. Similarly, in snails brought into the laboratory at the end of August and kept at 8°C (exp. 6), the Chaetogaster population became totally mature at the end of October.

It therefore appears that maturation can only occur in the autumn and that the total population matured only after the middle of October. There was therefore an innate mechanism determining the time of year at which the Chaetogaster matured, but even during this period of the year, a low temperature was essential.

#### 5.04. The temperature necessary for maturation to occur.

Snails were kept at temperatures of 10°C, 8°C and 5°C. At the first two, maturation occurred in the autumn (Section 5.03). At 5°C, the Chaetogaster population was eliminated from the kidney of the fifteen snails examined in various experiments.

The Chaetogaster l. vaghini population thus became mature at temperatures as high as 10°C. Since temperatures below 5°C destroyed the Chaetogaster population, this might explain why the cocoons were formed at this time of the year, since they could then sustain the population over the time of year when the immature worms were vulnerable. Experiments in Section 5.1 showed that the cocoons remained viable after



being kept at 4°C.

### 5.1. The cocoons of Chaetogaster limnaci vaghini.

#### 5.10. Introduction.

A consideration of the reproductive cycle of Ch. l. vaghini showed that the cocoons were leaving the kidney before hatching (p. 47). The factors influencing the hatching of these cocoons were investigated and the relationship between the length of time for which the cocoons were in the kidney and hatching success was determined.

#### 5.11. The factors affecting the hatching of cocoons.

It seemed likely that temperature, osmotic concentration or light were the most likely factors to exert an effect on the hatching of the cocoons.

The osmotic concentration of the water which the cocoons entered on leaving the kidney was much lower than that of the kidney. Sodium chloride solution was used to give an osmotic concentration the same as that in the kidney. Picken (1937) found that a solution of 0.3 per cent saline was equivalent to the osmotic concentration of the urine of Lymnaea pereger. To determine whether this was the correct value for L. stagnalis, immature Chaetogaster l. vaghini were kept in sodium chloride solution at a range of concentrations 0.0,

0.1, 0.2, . . . . ., 1 per cent. Ten Chaetogaster were kept at each concentration and the time taken for 50 per cent to die was determined (Table 12, p. 72). The 50 per cent survival time for the Chaetogaster was longest in 0.2 per cent saline solution. This solution was therefore used to represent the kidney osmotic concentration. Cocoons for the experiments were obtained by dissection of the L. stagnalis kidney.

The first experiments were performed to determine whether light and osmotic concentration had an effect on the hatching of cocoons. The experiment was carried out at 20°C, with 18 cocoons kept under each experimental régime. The embryo of each cocoon showed movement and was therefore at a late stage of development and almost ready to hatch. Four combinations of light and dark, and saline and water were used. The experiment was performed in January, 1967. The results (Fig. 18, p. 73) show that light had no effect on the hatching of the cocoons, whereas water stimulated their hatching. The cocoons in saline solution hatched less readily, or not at all. When some of these were transferred to water, a few hatched. Many of the remaining embryos died after attempting to leave the cocoons in saline solution. The lowered osmotic concentration of the water to which the cocoons were exposed on leaving the kidney thus appears to have stimulated hatching.

A similar experiment had previously been performed in November, 1966, but no hatching occurred. The effect of the

Sodium chloride solution. ....	50 per cent survival time (hrs.) of 10 <u>Ch. l. vaghini</u> .	
(Percentage concentration)		
1.0	< 3	
0.9	< 3	
0.8	< 3	
0.7	< 2	
0.6	> 2	< 5
0.5	> 21	< 45
0.4	> 21	< 46
0.3	> 21	< 46
0.2	> 46	< 69
0.1	> 8	< 20
0.0	> 8	< 20

Table 12. The survival of Ch. l. vaghini at various osmotic concentrations of sodium chloride solution.



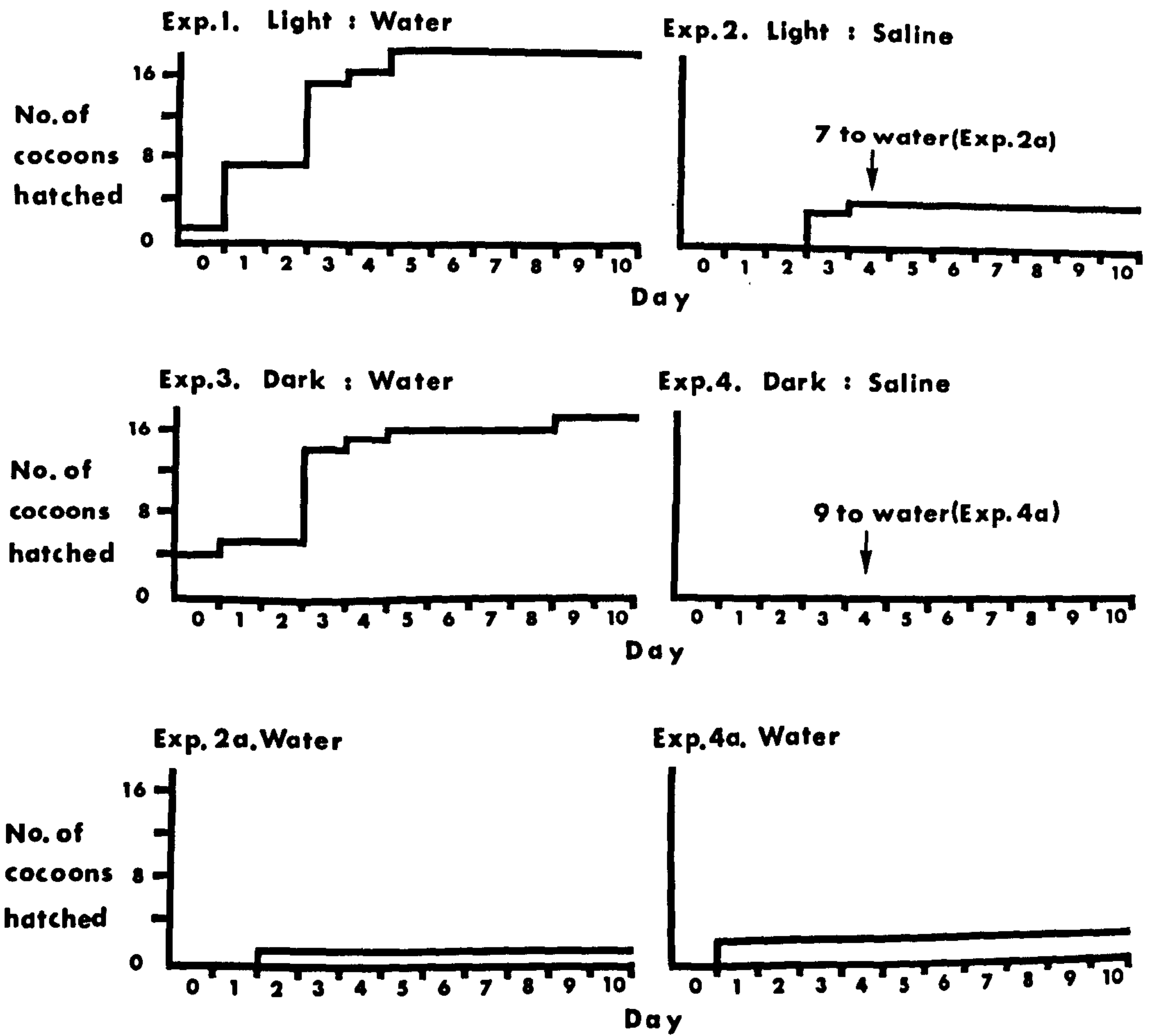


Figure 18. The effect of light and osmotic concentration on the hatching of the cocoons of Ch. l. vaghini.

time of year on the hatching of the cocoons was therefore examined in the winter of 1967-1968. The influence of different temperatures on the hatching of the cocoons was included.

Cocoons were collected at fortnightly intervals from November to January, after which too few were found for experimentation. At each collection, they were divided into 6 experimental groups, three in water and three in 0.2 per cent saline, and one of each was kept at 4°C, pond temperature and 20°C respectively. The cocoons were examined at 7 or 14 day intervals for hatching and for disintegration of the embryo. Disintegration was recorded rather than death, as the breaking up of the embryonic mass was the first indication of death having occurred.

The results (Fig. 19, pp. 75 and 76) show that hatching was influenced by the osmotic concentration of the surrounding medium. Only 1 cocoon hatched in the 0.2 per cent saline, whereas many hatched in water. This may be a mechanism to prevent hatching from occurring within the kidney of the host snail. It was unlikely that the death of the embryos in the cocoons in saline was due to an effect of the medium, as a few cocoons hatched after being removed from saline. It was more likely that when the embryos had reached the stage at which they should hatch, if this did not occur they were unable to obtain food and therefore died.

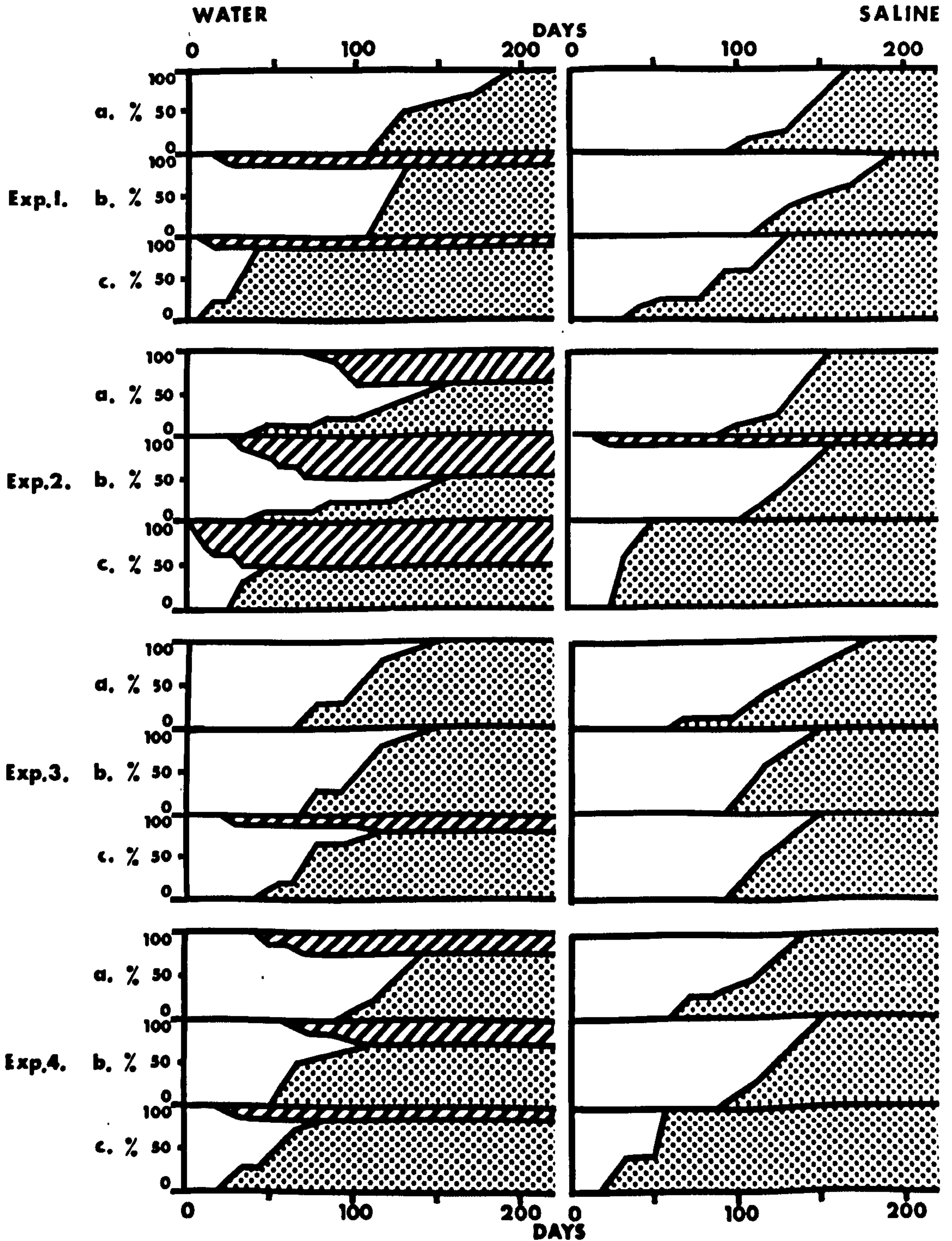


Figure 19. The effect of the date of collection, osmotic concentration and temperature on the hatching of the cocoons of Ch. l. vaghini.

Over/.....



The effect of temperature on the hatching of cocoons was examined. The results of experiments 5, 6 and 7 (Fig. 19) showed that a greater number hatched at 20°C than at the pond temperature or 4°C. Very few cocoons hatched at 4°C, which suggests that low temperatures retarded the development and hatching of Chaetogaster from the cocoons.

Due to an apparent difference in the proportion hatching in different experiments, the effect of the date of collection of the cocoons from the kidneys of L. stagnalis on hatching success was investigated. From experiment 1 in November to experiment 4 in early December, excluding experiment 3, the percentage of cocoons hatching was very low. The percentage increased from experiment 5, in mid-December, onwards. There may therefore be a diapause before the embryos develop, or development might be obligatory for a length of time in the kidney for hatching to take place when the cocoons are expelled. Thus, if the cocoon was removed before the embryo reached a certain stage of development in the kidney, hatching did not occur. This indicates that 0.2 per cent saline was not a suitable, long term substitute for the snail kidney and explains the low percentage of cocoons hatching during these experiments.

## 5.2. Conclusion.

Maturation of Chaetogaster limnaei vaghini only occurred if there was a drop in temperature. An innate mechanism controlled the time of year at which maturation occurred.

The cocoons of Chaetogaster were stimulated to hatch by a lowered osmotic concentration, and the number hatching was greater at higher temperatures. The cocoons must develop for a time in the kidney for them to hatch when they leave the snail.

Section F. THE OCCURRENCE OF CHAETOGASTER LIMNAEI ON  
OTHER GASTROPOD SPECIES.

1. Introduction.

Chaetogaster limnaei was obtained from a wide variety of species of gastropod. The morphology of the worms was investigated to determine whether any differences could be detected in the Chaetogaster from different habitats and from different snail species. The behaviour of Chaetogaster in the presence of its host was examined and experiments performed to determine how the worms find their hosts and whether they are host-specific.

2. The incidence of Chaetogaster limnaei.

Most of the species of gastropods examined were found to be infected with Chaetogaster l. limnaei (Table 13, p. 80). It was found in 18 species out of the 21 examined; the uninfected species were available only in small numbers. The highest percentage infection was 100 per cent in both Planorbis corneus and Physa fontinalis. The percentage found in the Lymnaea sp. was approximately 75. The variation between species in percentage infection may reflect the different habitats of the snails and also the various times of the year at which collections were made.

The number of Chaetogaster found in each snail varied greatly in the different species examined. The greatest number, 144, was found in a specimen of Lymnaea pereger, the next being 83 in a specimen of Bithynia tentaculata. An examination of Table 13 shows that the larger snails, such as Lymnaea sp., Bithynia sp. and Planorbis corneus, supported the greatest average Chaetogaster populations. However, in spite of its smaller size, Physa fontinalis had a large mean population of Chaetogaster. None of the bivalves examined contained Ch. l. limnaei.

The percentage infection of Chaetogaster l. limnaei in each snail varied with the locality from which the snails were collected. Table 14 (pp. 81 and 82) shows the percentage infection of the various species of snails in the localities examined. The infection of any particular species with Ch. l.



Species	No. examined	Percentage infected	Mean no. per snail	Range of nos.
<i>Ancylastrum fluviatilis</i>	41	20	0.8	0-10
<i>Ancylus lacustris</i>	4	0		
<i>Bithynia leachii</i>	4	50	6	0-23
<i>B. tentaculata</i>	22	59	16.9	0-85
<i>Lymnaea auricularia</i>	4	0		
<i>L. palustris</i>	4	75	14.8	0-32
<i>L. pereger</i>	89	67	15.2	0-144
<i>L. stagnalis</i> (ex. Coll. pond)	8	75	19.3	0-63
<i>L. truncatula</i>	4	50	4.5	0-17
<i>Physa fontinalis</i>	38	100	9.9	1-26
<i>Planorbis albus</i>	20	15	0.3	0-2
<i>Pl. carinatus</i>	29	48	2.4	0-12
<i>Pl. complanatus</i>	3	67	0.3	0-1
<i>Pl. contortus</i>	14	21	0.3	0-2
<i>Pl. corneus</i>	9	100	19.2	1-36
<i>Pl. planorbis</i>	2	0		
<i>Pl. vortex</i>	33	9	0.2	0-3
<i>Potamopyrgus jenkinsi</i>	181	23	0.2	0-3
<i>Succinea</i> sp.	1	100	1	1
<i>Valvata cristata</i>	14	7	0.7	0-1
<i>V. piscinalis</i>	37	32	2.4	0-11
<i>Anodonta</i>	3	0		
<i>Dreissena polymorpha</i>	4	0		
<i>Sphaerium</i> sp.	10	0		

Table 13. The incidence of Chaetogaster l. limnaei in various molluscs.

	College pond	Mill pond	Llanllechiâ	Waynol 1	Waynol 2	Llyn Sisi	Beumaris	Llyn Coron	Llyn Hendref	Llangefnj
<i>A. fluviatilis</i>									50	
<i>A. lacustris</i>										
<i>B. leachii</i>										
<i>B. tentaculata</i>										
<i>L. auricularia</i>										
<i>L. palustris</i>						100		0		
<i>L. pereger</i>	93	100	100	100	66			33	25	
<i>L. stagnalis</i>										
<i>L. truncatula</i>						*100		*100		
<i>P. fontinalis</i>						100	*100			
<i>Pl. albus</i>			0							
<i>Pl. carinatus</i>										
<i>Pl. complanatus</i>										
<i>Pl. contortus</i>						67	0			
<i>Pl. corneus</i>										
<i>Pl. planorbis</i>										
<i>Pl. vortex</i>										
<i>P. jenkinsi</i>	0	37	0					0	0	0
<i>Succinea sp.</i>						*100				
<i>V. cristata</i>			7							
<i>V. piscinalis</i>			0				86			

\* = Only 1 snail examined

Table 14. The percentage of snails infected with Chaetogaster limnaei limnaei in various localities.

Over/....

	Holland Arms	Cole Mere	The Mere	Newton Mere	Blake Mere	White Mere	Birchgrove Pool	Croze Mere	Wirral 1	Wirral 2
<i>A. fluviatilis</i>		0	75							
<i>A. lacustris</i>		0	0							
<i>B. leachii</i>		0	*100				*100			
<i>B. tentaculata</i>		80	100		0	*100	100	17		
<i>L. auricularia</i>			0			0				
<i>L. palustris</i>										
<i>L. pereger</i>		100	80	100		14	100	0		
<i>L. stagnalis</i>		0					*100		100	
<i>L. truncatula</i>						0				
<i>P. fontinalis</i>		100		100				*100		
<i>Pl. albus</i>	0	*100	0	17			67			
<i>Pl. carinatus</i>		63	13			0	100	40		
<i>Pl. complanatus</i>	50					0				
<i>Pl. contortus</i>	0			33						
<i>Pl. corneus</i>									100	100
<i>Pl. planorbis</i>						0				
<i>Pl. vortex</i>		0	0				50	0		
<i>P. jenkinsi</i>		50	0	0		9		6		
<i>Succinea</i> sp.										
<i>V. cristata</i>										
<i>V. piscinalis</i>		100						0		

\* = Only 1 snail examined.

Table 14. Continued.



limnaei varied from one habitat to another, in the case of L. pereger varying from 0-100 per cent infection. Physa fontinalis is of interest in that in each locality in which it was found, every specimen was infected. It is possible that the habitat preference of P. fontinalis for running water and water weed (Hunter, 1961) was also an optimal habitat for the Chaetogaster. The time of year of sampling may also affect the percentage infection of the snails.

The percentage infection of each of the various species of snail in one locality also varied. At Cole Mere, for example, only half of the species were found to be infected. Thus, Bithynia tentaculata, Lymnaea pereger, Physa fontinalis, Planorbis albus, Pl. carinatus, Potamopyrgus jenkinsi and Valvata piscinalis were infected, whereas Ancylastrum fluviatilis, Ancylus lacustris, Bithynia leachii, Lymnaea stagnalis and Planorbis contortus were not. In the College pond, only 2 per cent of the population of L. stagnalis was infected with Ch. l. limnaei, whereas 93 per cent of the L. pereger population was infected.

Thus one species of snail could have a different percentage infection in different localities, and the infection of different species in one locality was also variable.

Chaetogaster limnaei vaghini was found in four species of gastropod (Table 15, p. 84), but it was only common in the kidney of Lymnaea sp. Table 16(p. 85) shows that the highest

	No. examined	Percentage infection	Mean no. per snail	Range of nos.
<i>L. pereger</i>	89	16	0.8	0-8
<i>L. stagnalis</i> (except College pond)	8	63	37	0-116
<i>Pl. carinatus</i>	29	3	0.3	0-1
<i>P. jenkinsi</i>	181	0.6	0.06	0-1

Table 15. The incidence of *Chaetogaster l. vaghini* in various gastropods.

Gastropod species

<u>Habitats</u>	L. pereger	L. stagnalis	Pl. carinatus	P. Jenkinsi
College pond	0	68		0
Mill pond	0			0
Llanllechid	0			
Vaynol 1				0
Vaynol 2	69			
Llyn Sisi	0			
Beaumaris				
Llyn Coron	0			0
Llyn Hendref	0			0
Llangefni				0
Holland Arms				
Cole Mere	25	0	0	0
The Mere	0		0	0
Newton Mere	0			*100
Blake Mere	0			
White Mere				
Birchgrove Pool	0		0	0
Crosc Mere	0	0	0	
Wirral 1	0		20	0
Wirral 2		83		

\* = Only 1 snail examined.

Table 16. The percentage of gastropods infected with

Chaetogaster l. vaghini in various localities.



percentage infection was recorded in L. stagnalis from the Wirral and the College pond. Only one infected specimen of each of Pl. carinatus and P. jenkinsi was found.

### 3. The morphology of Chaetogaster limnaei.

The most important features in differentiating between the two subspecies of Ch. limnaei are the length and the number of setae in each bundle (Gruffydd, 1965a). An examination of both these features was made in Ch. l. limnaei and Ch. l. vaghini from a variety of habitats but the same species of snail, and between worms from the same habitat but different gastropod species. The number of setae in the setal bundles of segments II, VI, VII and VIII was determined and the length of the setae in one bundle of each segment was measured in 12 specimens of Chaetogaster from each source. Observations on the length of setae in Chaetogaster indicated that they maintained a constant length once they had been formed. No regeneration or replacement of broken or torn out setae was apparent, the only developing setae being found in the buds of the worm.

#### 3.1. The setae of Chaetogaster limnaei limnaei.

##### 3.10. The length of the setae.

The greatest length of the setae of Chaetogaster was measured (Sperber, 1950), as shown in Figure 20 (p. 88), in specimens from each species of snail and habitat shown in Table 17 (p. 89). Each mean was based on data from approximately 100 setae. A comparison of the means of the setal lengths shows that variation occurred between Chaetogaster from different snail species in the same habitat and in the same species from different habitats. To determine whether this difference was real, the variance was obtained for each mean and the means compared by the calculation of 'Student's' t (Bailey, 1959). Table 18 (p. 90) compares the mean of the setal lengths in segment 2 of Chaetogaster from each snail species and habitat. The setae of segments VI, VII and VIII were similar in length and number, and so those of segment VI were used for a comparison of the setal lengths, as shown in Table 19 (p. 91). It is clear that a greater number of the differences between the setal lengths were significant for segment VI than for segment II and that the value of 't' was greater for the significant differences of the setae of segment VI.

The data in Tables 18 and 19 were examined to see whether there was any indication of the cause of the differences between the setal lengths. The likely causes were that the Chaetogaster of different gastropod species were morphologically different, that differences in habitat were

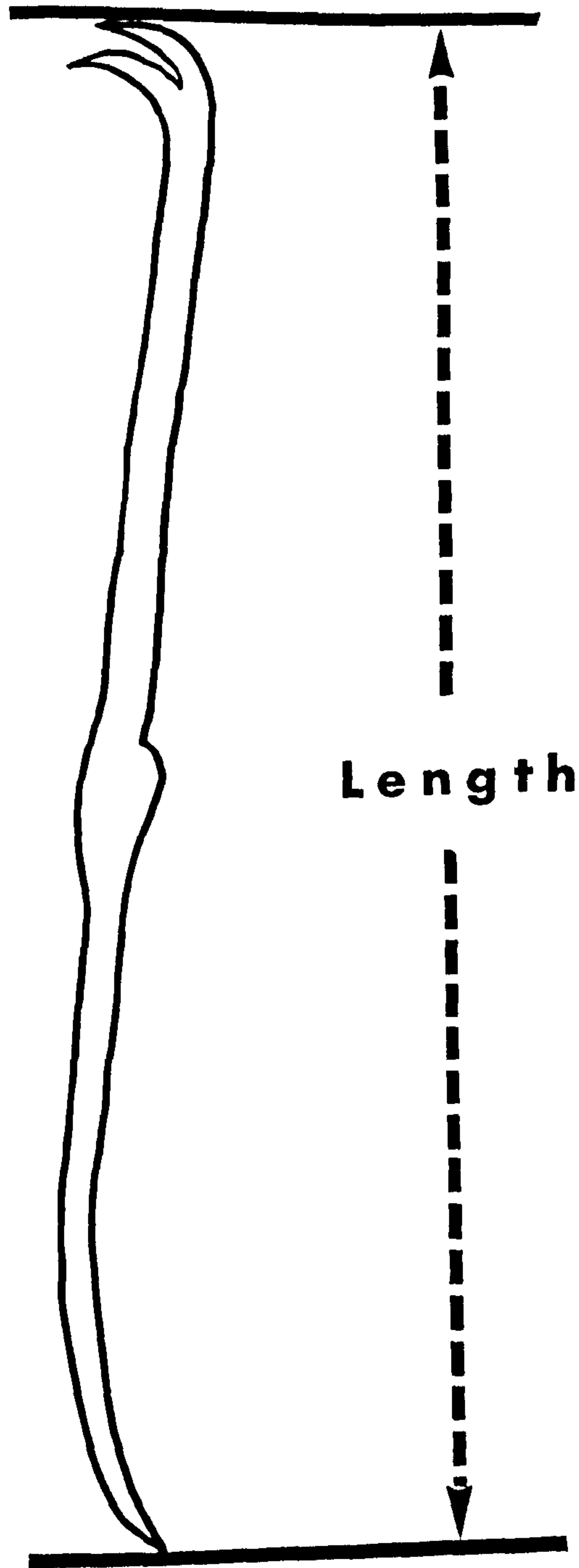


Figure 20. Measurement of a seta of Ch. limnaei.



<u>Gastropod</u>		<u>Number of segment</u>			
<u>species</u>	<u>Source</u>	<u>II</u>	<u>VI</u>	<u>VII</u>	<u>VIII</u>
L. natalensis	Laboratory	75.1	46.8	46.6	47.3
L. pereger	College pond	76.4	50.2	50.5	49.5
L. pereger	Llanllechid	83.2	52.7	53.2	53.3
L. pereger	Cole Mere	82.0	54.4	54.5	54.1
L. stagnalis	Wirral	76.8	54.4	54.0	53.1
L. truncatula	Beaumaris	78.4	50.4	49.5	49.5
P. fontinalis	Beaumaris	78.3	51.6	50.6	50.1
Pl. corneus	Wirral	74.5	52.6	51.0	47.9
P. jenkinsi	Mill pond	70.6	47.4	47.4	47.5

Length of setae in  $\mu$ .

Table 17. The mean length of setae in segments II, VI, VII, and VIII of Chaetogaster l. limnaei.

<u>Species of gastropod</u>	<u>P. jenkinsi</u>	<u>Pl. corneus</u>	<u>P. fontinalis</u>	<u>L. truncatula</u>	<u>L. stagnalis</u>	<u>L. pereger (Cole Mere)</u>	<u>L. pereger (Llanllechid)</u>	<u>L. pereger (College pond)</u>
<u>L. natalensis (Laboratory)</u>	- 1.6	- 0.2	- 1.3	- 1.3	- 0.5	+ 2.5	+ 2.6	- 0.5
<u>L. pereger (College pond)</u>	+ 3.4	- 1.1	- 1.3	- 1.3	- 1.7	+ 3.4	+ 3.3	
<u>L. pereger (Llanllechid)</u>	+ 5.5	+ 3.9	+ 2.4	+ 2.2	+ 2.4	- 0.5		
<u>L. pereger (Cole Mere)</u>	+ 6.0	+ 4.1	+ 2.3	+ 2.0	+ 2.3			
<u>L. stagnalis (Wirral)</u>	+ 2.6	- 1.0	- 0.8	- 0.8				
<u>L. truncatula (Beaumaris)</u>	+ 4.3	+ 2.2	- 0.08					
<u>P. fontinalis (Beaumaris)</u>	+ 4.6	+ 2.4						
<u>Pl. corneus (Wirral)</u>	+ 2.1							

+ = Significant difference

- = Not significant

No. = Value of 't'

Table 18. A comparison of the means of the setal lengths of segment II in Chaetogaster l. limnaei from various snail species.

<u>Species of gastropod</u>	<u>P. jenkinsi</u>	<u>Pl. corneus</u>	<u>P. fontinalis</u>	<u>L. truncatula</u>	<u>L. stagnalis</u>	<u>L. pereger (Cole Mere)</u>	<u>L. pereger (Llanllechid)</u>	<u>L. pereger (College pond)</u>
<u>L. natalensis (Laboratory)</u>	- 1.3	+ 10.5	+ 9.0	+ 16.4	+ 13.1	+ 13.1	+ 13.1	+ 6.9
<u>L. pereger (College pond)</u>	+ 10.2	+ 6.5	+ 4.9	- 0.9	+ 14.9	+ 10.5	+ 9.4	
<u>L. pereger (Llanllechid)</u>	+ 23.2	- 0.3	+ 5.0	+ 13.3	+ 7.0	+ 5.0		
<u>L. pereger (Cole Mere)</u>	+ 19.5	+ 3.8	+ 7.8	+ 13.1	- 0.09			
<u>L. stagnalis (Wirral)</u>	+ 28.9	+ 5.2	+ 11.6	+ 21.4				
<u>L. truncatula (Beaumaris)</u>	+ 16.9	+ 8.0	+ 6.3					
<u>P. fontinalis (Beaumaris)</u>	+ 17.5	+ 3.2						
<u>Pl. corneus (Wirral)</u>	+ 15.7							

+ = Significant difference

- = Not significant

No. = Value of 't'

Table 19. A comparison of the means of the setal lengths of segment VI in Chaetogaster l. limmaei from various snail species.



the causative factors or a combination of both.

The fact that the Chaetogaster from the various host species of gastropod usually had setae which differed significantly in mean length, points to the likelihood of different biological races of Chaetogaster associated with each host species. If the races differed in the various species of snail it would be expected that the snails in one habitat would contain Chaetogaster with differing means of setal length. In Table 19, Physa fontinalis and Lymnaea truncatula were both obtained from Beaumaris, and a significant difference was shown between their means. Similarly, for L. stagnalis and Pl. corneus from the Wirral, the means of the setae of segment VI were shown to be different. A difference was also apparent for segment II in the case of Beaumaris, but not for the two snail species in the Wirral. Thus in most cases there was a difference in the Chaetogaster from different snail species in the same habitat.

The mean length of the setae of Chaetogaster from the same species of snail from different habitats might have been expected to be the same. A comparison of the means of the setal lengths of segment VI of Chaetogaster from L. pereger from three different habitats shows them all to be different. Although Chaetogaster in Lymnaea pereger from Llanllechid and Cole Mere did not show a significant difference in the length of setae from segment II, this is not surprising since

almost half of such comparisons from other host species were not significant.

It therefore appears that in each habitat, the Chaetogaster tended to form races in the different snail species, suggesting that the worms were host-specific. The Chaetogaster from the same snail species from different habitats did not have the same setal lengths. This might be expected from the isolation of the different habitats, if the tendency to form races was a continuation from the division of Ch. limnaei into its two subspecies.

### 3.11. The number of setae.

The number of setae in each bundle of Ch. l. limnaei was counted from segment II to segment VIII of the worm. Table 20 (p. 94) shows a comparison of the means of the number of setae found in each segment in Chaetogaster examined from each site. The range of the number of setae found in each bundle is also shown. The significant differences between the number of setae found in various species were not calculated as there was often a loss of setae from the bundles. A comparison of the number of setae in all of the bundles of Chaetogaster from various sources showed that the number on formation of the bundle was either 10 or 11. As the bundles were used for locomotion, many of the setae were torn out and this was especially apparent in segment VI, which was the main

<u>Gastropod</u> <u>species</u>	<u>Number of segment</u>			
	<u>II</u>	<u>VI</u>	<u>VII</u>	<u>VIII</u>
L. natalensis	9.4 (8-10)	8.3 (8-9)	9.7 (9-10)	10.0 (10)
L. pereger (College pond)	9.6 (7-10)	8.2 (4-11)	9.3 (5-11)	10.3 (9-11)
L. pereger (Llanllechid)	10.2 (10-11)	9.8 (4-11)	10.5 (5-11)	10.4 (9-11)
L. pereger (Cole Mere)	9.3 (6-11)	8.8 (7-11)	9.8 (7-11)	10.8 (10-12)
L. stagnalis	10.0 (9-11)	7.8 (4-9)	9.7 (7-11)	9.9 (9-11)
L. truncatula	9.7 (9-10)	8.7 (8-10)	10.5 (10-11)	10.5 (10-11)
P. fontinalis	9.4 (8-10)	8.5 (5-11)	10.2 (6-11)	10.5 (9-11)
Pl. corneus	8.6 (4-10)	7.6 (3-10)	8.4 (5-10)	7.8 (6-10)

( ) = Range of number of setae.

Table 20. The mean number of setae found in the bundles of segments II, VI, VII and VIII of Chaetogaster l. limnaei from various gastropod species.



bundle by which the worm clings to the host (Gruffydd, 1963). The number of setae left in one of these bundles was, in the case of a specimen of Chaetogaster from Pl. corneus, as low as three.

The number of setae in the bundles of Ch. l. limnaei from different hosts and habitats thus appears to be the same.

### 3.2. The setae of Chaetogaster limnaei vaghini.

#### 3.20. The length of the setae.

Chaetogaster l. vaghini was collected from L. stagnalis in two habitats only. Table 21 (p. 96) shows the mean of the setal length from the two sources, and Table 22 (p. 97), a comparison of the means. These show that differences were again apparent in segment VI, suggesting that there was variation in Ch. l. vaghini from different habitats. There was no significant difference in segment II.

#### 3.21. The number of setae.

The number of setae found in each bundle of segments II, VI, VII and VIII of Ch. l. vaghini from the Wirral and the College pond are shown in Table 23 (p. 98). The variation in the number found is also shown. As in Ch. l. limnaei, variation due to the loss of setae prevented a comparison of significance from being made. The most common numbers of setae in the

<u>Gastropod</u> <u>species</u>	<u>Source</u>	<u>Number of segment</u>			
		<u>II</u>	<u>VI</u>	<u>VII</u>	<u>VIII</u>
L. stagnalis	College pond	60.3	46.2	47.5	47.4
L. stagnalis	Wirral	60.3	42.4	41.9	42.3

Table 21. The mean length of setae in segments II, VI, VII and VIII of Chaetogaster l. vaghini.

	Number of segment	
	<u>II</u>	<u>VI</u>
Difference between means of setal lengths of <u>Ch. l. vaghini</u> from <u>L. stagnalis</u> from the Wirral and the College pond.	- (0.03)	+ (5.6)

+ = Significant difference

- = Not significant

( ) = Value of 't'

Table 22. A comparison of the means of the setal lengths  
in segments II and VI of Chaetognaster l. vaghini  
from different sources.



Gastropod species	Source	Number of segment			
		<u>II</u>	<u>VI</u>	<u>VII</u>	<u>VIII</u>
<i>L. stagnalis</i>	College pond	5.9 (5-7)	4.0 (3-6)	5.1 (5-7)	5.7 (4-7)
<i>L. stagnalis</i>	Wirral	6.4 (4-7)	6.0 (4-7)	6.2 (6-7)	6.4 (5-7)

( ) = Range of number of setae

Table 23. The mean number of setae found in the bundles of segment II, VI, VII and VIII of Chaetogaster l. vaghini from L. stagnalis from various sources.

bundles were 6 and 7.

4. The behaviour of *Chaetogaster limnaei* - the reaction to its host.

4.0. Introduction.

The behaviour of *Chaetogaster limnaei* was investigated to determine by what method the worms found a new host during the dispersal phase. Dispersal would occur when snails die in the winter, the *Chaetogaster* then leaving their hosts, following the death of snails after breeding in the spring and when the young worms hatch from the cocoons. When the young population of snails appeared during the summer, it would be of advantage to the *Chaetogaster* to infect it rapidly for them to make most efficient use of the available resources.

The possibility that host-specificity might occur was also examined. Its operation had previously been inferred from the morphology of the *Chaetogaster* (p. 86ff.). It was considered that host-specificity would be most apparent during dispersal and so various experiments were performed to investigate this stage in the life-cycle of the worm.

The effect of the habitat from which the *Chaetogaster* and the snails were obtained on the behaviour of the worms was also examined. A range of habitats was chosen with one

species of snail, a few species of snail and many species respectively.

#### 4.1. The general behaviour of Chaetogaster limnaei.

The reaction of Chaetogaster to light and to water current was observed, to determine whether the behaviour of the worms was affected by them. These factors were considered to be those most likely to affect the results of later behaviour experiments.

#### 4.10. The reaction of Chaetogaster limnaei to light.

The apparatus used to investigate the effect of light on Chaetogaster is shown in Figure 21 (p. 101). A petri-dish 85 mm. in diameter was used for the experiments and a black paper shield was made to cover half of the dish. This was then placed beneath a rectangular trough containing 3 cm. of water. Cold tap water was passed through the trough during the experiment to prevent heat from the lamp from raising the temperature in the experimental container. Light was supplied by a 60 watt tungsten bulb above the water and the intensity of the light reaching the petri-dish was approximately 2,000 foot candles. The Chaetogaster to be tested were introduced in equal numbers on either side of the petri-dish and the experiment allowed to continue for 2 hours.



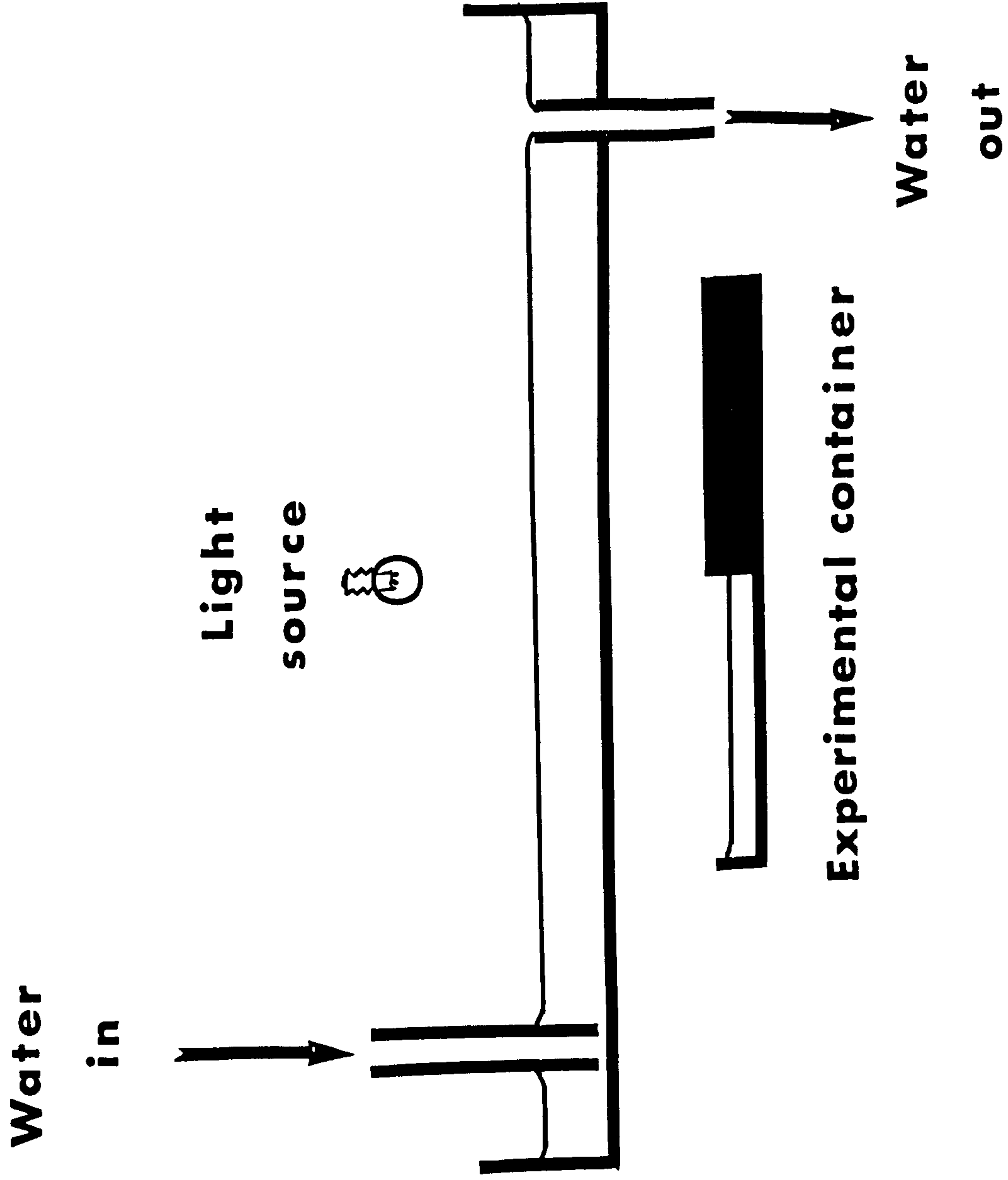


Figure 21. Apparatus used to determine the effect of light on Ch. limmaei.

The effect of light on Chaetogaster limnaei limnaei, obtained from L. pereger from the College pond, was determined. The results of the experiments (Table 24, p. 103) show that light affected the behaviour of Ch. l. limnaei, causing the worms to accumulate on the dark side of the dish.

Chaetogaster l. vaghini were obtained from L. stagnalis from the College pond and similar experiments were performed, the results of which are also shown in Table 24. It was found that light had no effect on the distribution of the Chaetogaster in the petri-dish.

Light, therefore, affected the two subspecies of Ch. limnaei in different ways. Ch. l. limnaei was shown to be negatively photo-tropo-tactic (Fraenkel and Gunn, 1940), whereas Ch. l. vaghini was not affected by light. All later experiments were therefore carried out in the dark to preclude the interference of light on the results.

#### 4.11. The reaction of Chaetogaster limnaei to water current.

To determine the effect of a current of water on Chaetogaster, a trough, with a central compartment 64 mm. square and separated from the ends by fine nylon mesh, was used as shown in Figure 22 (p. 104). The trough was constructed of perspex and the base roughened to enable the Chaetogaster to obtain purchase on the substratum. A current

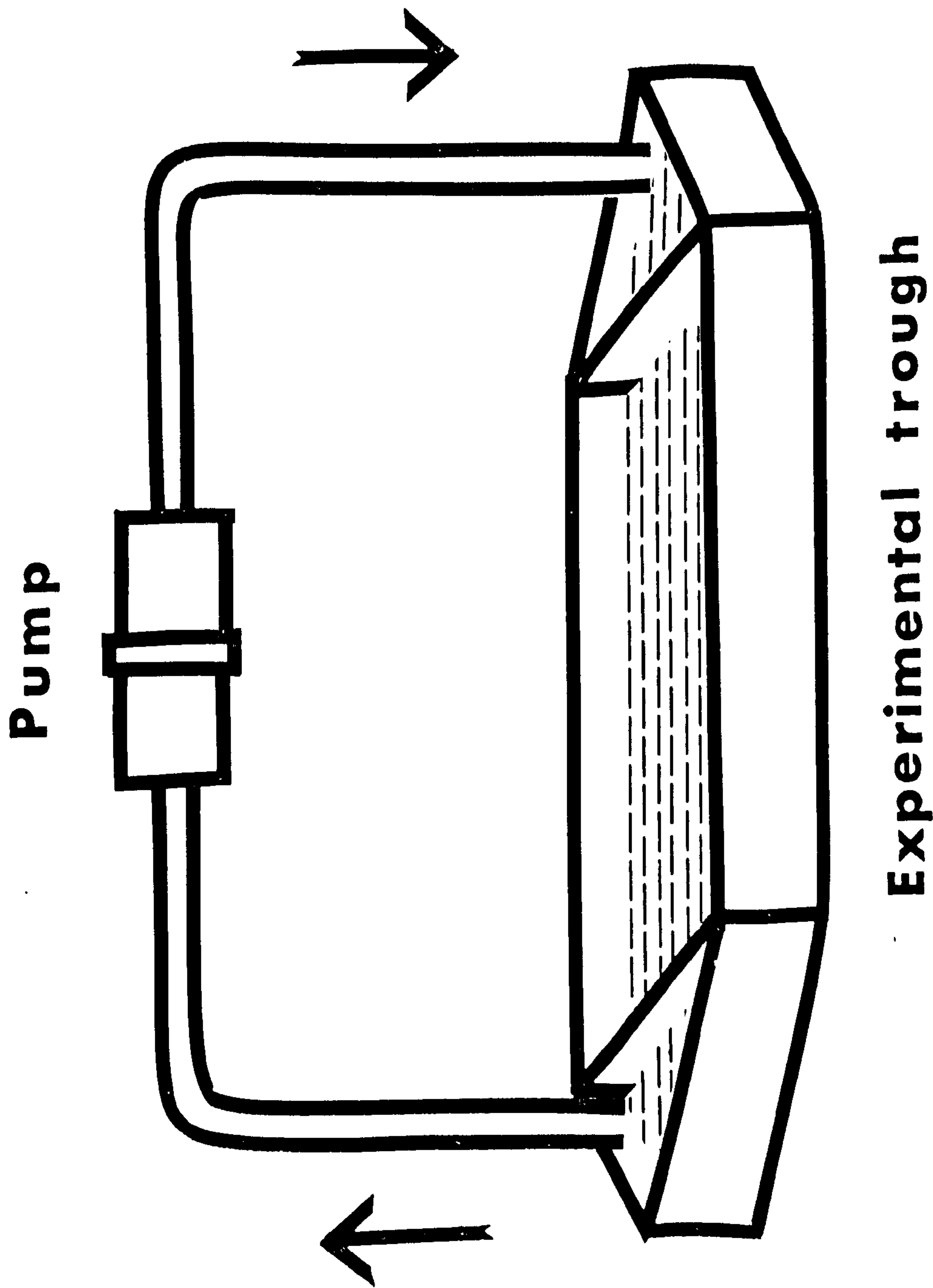
<u>Chaetogaster</u> subspecies	No. of expts.	Result		$\chi^2$	Significance
		Light	Dark		
<u>Ch. l. limnaei</u>	7	96	224	50.12	Highly significant
<u>Ch. l. vaghini</u>	5	92	117	2.99	Not significant

At  $P = 0.05$  and 1 degree of freedom,  $\chi^2 = 3.84$

Table 24. The effect of light on Chaetogaster limnaei.



Figure 22. Apparatus used to determine the reaction of Ch. limnaei to a current of water.



of water through the apparatus was obtained by the use of a miniature electric pump to circulate the water. The depth of water was 4 mm. and the rate of flow in the central compartment was approximately 0.22 cm. per second.

In the first experiment, pond water was circulated through the apparatus and the Chaetogaster introduced in equal numbers upstream and downstream from the centre of the experimental compartment. After 5 minutes the number of Chaetogaster upstream and downstream from this point were counted. The results (Table 25, p. 106) show that the Chaetogaster tended to move downstream in a current of pond water.

The experiment was then repeated, using water taken from a dish containing L. pereger. The use of the pump to circulate the water ensured that any chemical substance from the snail would be equally distributed through the apparatus and any effect observed would be due to the water current. The Chaetogaster were introduced as before and their position recorded after 5 minutes. The results in Table 25 show that the Chaetogaster tended to move upstream under these conditions.

The reaction of Chaetogaster to a stream of water was thus reversed by the presence of a snail 'factor' in the water. After the addition of such a factor, the worms became positively rheotactic. This was important for later experiments, since the worms were not washed out of the

Water used	No. of expts.	Result		$\chi^2$	
		No. upstream	No. downstream		
Pond	2	13	45	17.65	S.
Pond + snail 'factor'	2	70	29	16.98	S.

At  $P = 0.05$  and 1 degree of freedom,  $\chi^2 = 3.84$

S. = Significant difference

Table 25. The effect of water current on Ch. l. limnaei.



apparatus under these conditions.

#### 4.2. The source of Chaetogaster for the experiments.

The habitats from which the snails and Chaetogaster used during the behaviour experiments were obtained are shown in Table 26 (p. 108). Where more than one source is indicated, the additional sources were used for a comparison of the same species of snail from different habitats.

#### 4.3. Methods and apparatus used.

##### 4.30. Experiments with non-infected snails.

These experiments were performed in plastic bowls, 30 cm. in diameter and with a depth of water of 5 cm. Aeration was supplied constantly. Three species of snail, L. pereger, L. stagnalis and Pl. corneus, were cultured from egg masses, as described earlier (p. 22), to ensure that they were not infected by Chaetogaster.

The experiments were carried out by introducing into the experimental bowls the required species of snail, with artificial food, and leaving the bowl for 7 days. The Chaetogaster to be tested were then added and, after an interval of normally 7 days, the snails were examined to determine which species had been infected.

Species of snail	Main source	Supplementary source
<i>L. pereger</i>	College pond	Cole Mere, Llanllechid
<i>L. stagnalis</i>	College pond	Wirral
<i>P. fontinalis</i>	Beaumaris	
<i>Pl. corneus</i>	Wirral	
<i>P. jenkinsi</i>	Mill pond	

Table 26. The source of the gastropods used during the behaviour experiments.

#### 4.31. Experiments using egg masses.

The discovery of Chaetogaster on egg masses in the laboratory suggested that the worms might be attracted to them.

Plastic petri-dishes of 85 mm. diameter were used for the experiments, with a depth of water of 8 mm. Four egg masses were normally used in each experiment, two from each of the snail species being tested. The two types of egg mass being tested were arranged alternately around the petri-dish in each experiment. The experiments were performed under an overhead light, and the position of the Chaetogaster on either egg mass recorded for 24 hours, at intervals of approximately 2 hours. Each experiment was carried out in duplicate.

To investigate the attraction of Chaetogaster to egg masses of their own host, artificial egg masses were made of calcium alginate. These were prepared from a solution of 0.08 gm. of sodium alginate in 5 ml. of boiling water by allowing it to cool and pouring 10 drops into a petri-dish containing a solution of 2 gm. of calcium chloride made in 100 ml. of water. Cylindrical gels were formed, approximating to the shape of the egg masses. A preliminary experiment was performed to determine whether the artificial egg masses had an effect on Ch. limnaei. The base of a plastic petri-dish was cut into sections and gels were made of the same size and



shape. Three sections of petri-dish and three gels were placed in a petri-dish and 20 Ch. l. limnaei from L. pereger introduced. The results in Table 27 (p. 111) show no significant difference in the choice of the worms between the gels and the petri-dish sections. Greater numbers did, however, accumulate on the gels, which indicates that these may have been slightly attractive to Chaetogaster.

#### 4.32. Experiments using mucous trails.

It was shown by Gruffydd (1965a) that Ch. l. limnaei were attracted to and followed the mucous trails left by Lymnaea pereger. Experiments using mucous trails from various species of snail were therefore set up to determine whether the Chaetogaster accumulated on the mucus of their respective hosts and whether, if many types of mucous trail were available, they would show specificity to the host.

A petri-dish divided into two areas was used to test a single pair of mucous types. A perspex separator was placed across the dish and the two species of snail to be tested were placed one on either side of the separator and allowed to crawl around for approximately 30 minutes. The snails and the separator were then removed and the Chaetogaster introduced in equal numbers on either side of the apparatus. To test the reaction of Chaetogaster to many types of mucus, the petri-dish was divided into 6 sections, as shown in

Experiment number	Number on gel	Number on petri-dish sections
1	20	10
2	16	13
Total	36	23

$\chi^2 = 3.34$  Not significant

[ At P = 0.05 and 1 degree of freedom, = 3.84 ]

Table 27. The accumulation of Chaetogaster l. limnaei on gels and petri-dish sections.

Figure 23 ( p. 113). With the perspex separator in place, the 5 different snail species, L. pereger, L. stagnalis, Pl. corneus, P. fontinalis and P. jenkinsi were placed in different sections, one section being left empty. The position of each snail species in the apparatus was determined by the use of random number tables (Fisher and Yates, 1957), their position being different in succeeding experiments.

It was attempted to determine the area of mucus deposited by each snail species by the use of finely powdered carmine. This stained the mucus immediately after the snails had been removed, but not after the 18 hours of the experiment. As it was therefore not possible to measure the area of mucus in each section of the apparatus, an equivalent amount of mucus was obtained in each section by using more of the small snails than the large snail species.

#### 4.33. Chemical factor 'choice' experiments.

The apparatus used to investigate possible attraction to chemical factors emanating from the snail was adapted from an apparatus used by Gage (1966) to investigate the behaviour of the bivalves, Montacuta sp., in a current of water coming from its spatangoid host.

The apparatus is shown in Figure 24 (p. 114). The trough was 15 cm. long by 7.7 cm. wide and was constructed of perspex. The internal measurements of the choice compartment were



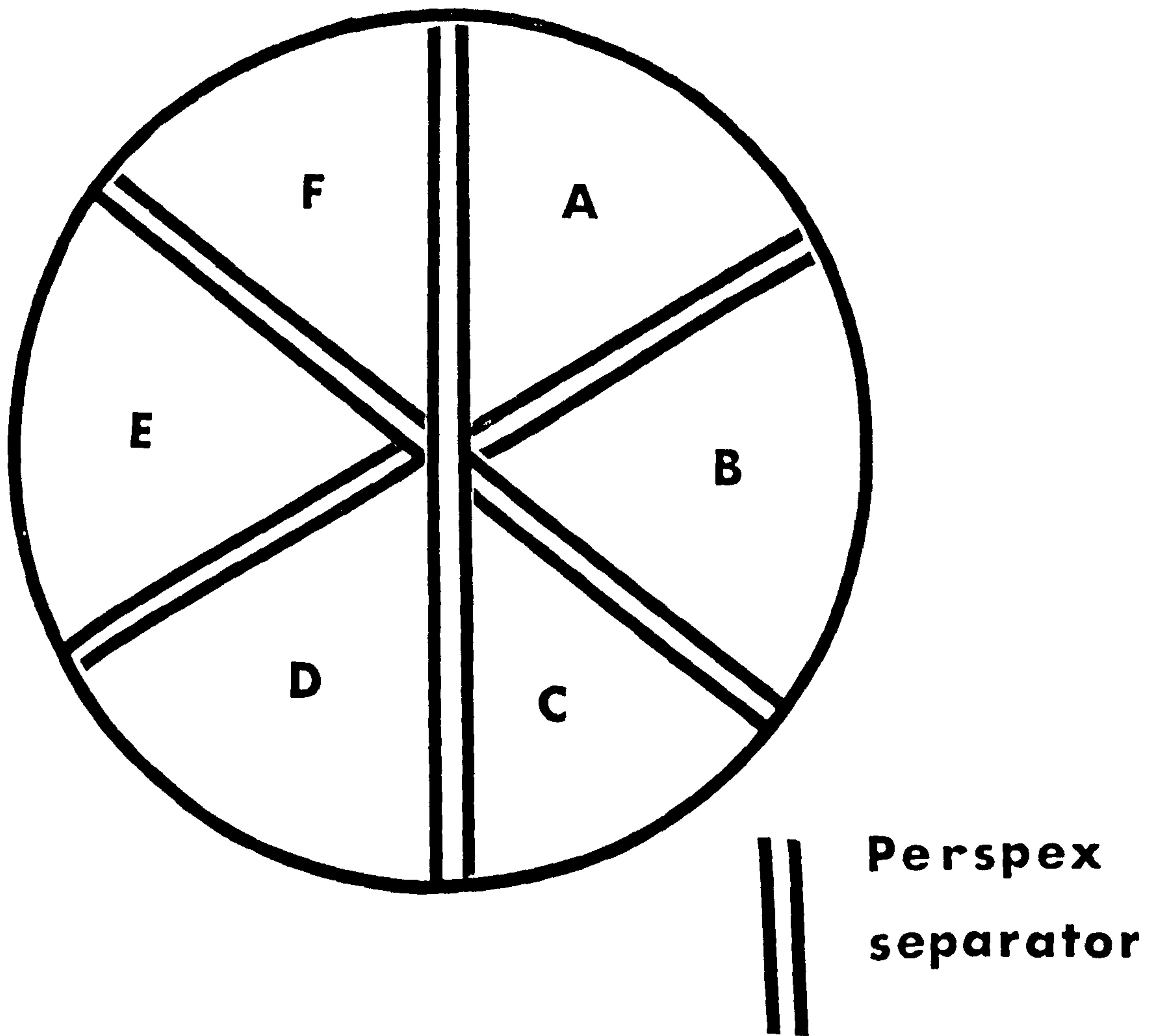
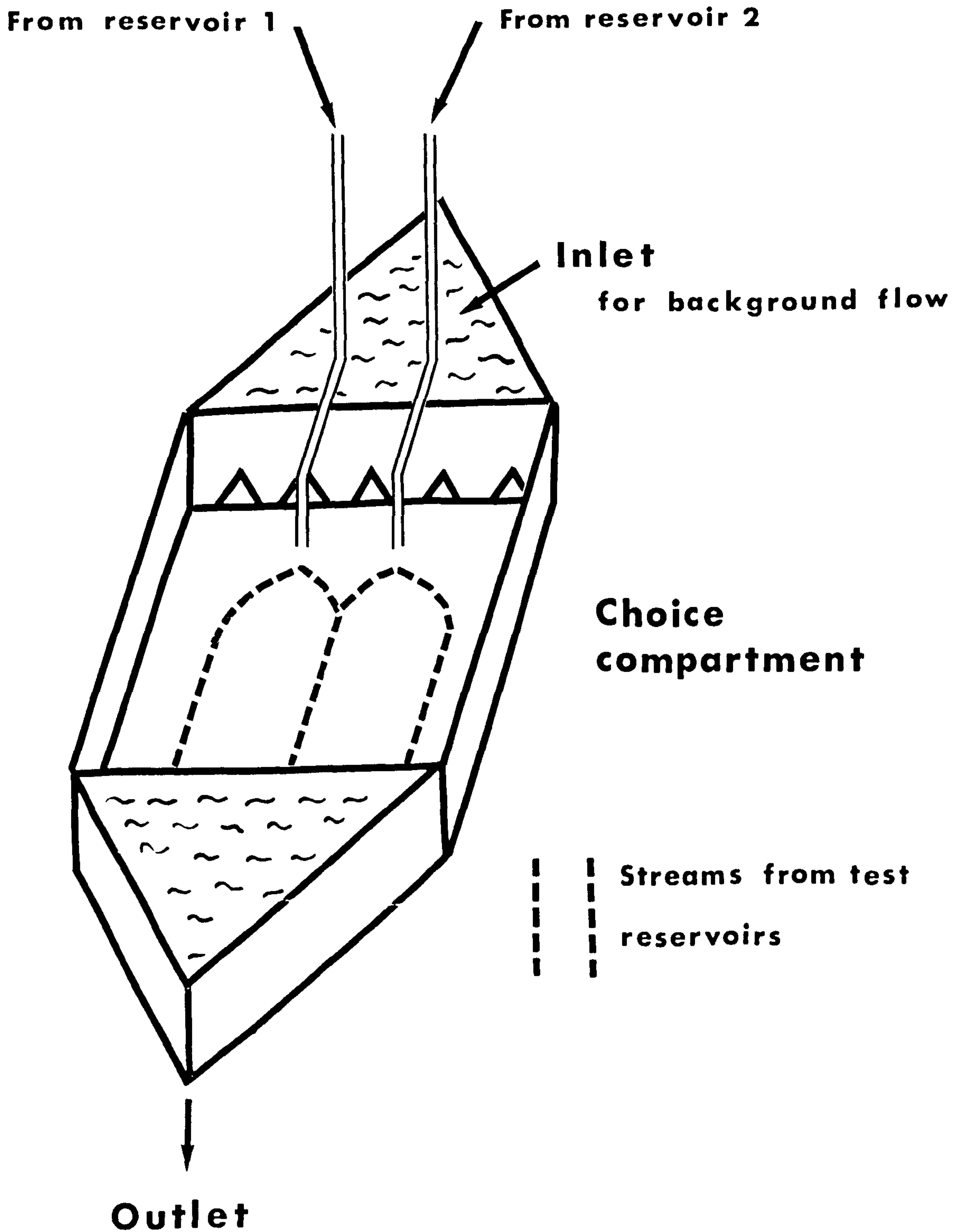


Figure 23. Apparatus used to test the reaction of Ch. limnaei to a variety of mucous trails.

Figure 24. The trough apparatus for the chemical factor 'choice' experiments.



6.4 cm. square by 1 cm. deep. The two internal walls at either end of the choice compartment had  $\wedge$ -shaped slots cut in them to allow water to flow through, and they were covered on the inside by fine nylon mesh. The triangular inlet and outlet area had a thin layer of glass wool to smooth out the flow of the water current. A roughened piece of perspex was fitted into the base of the choice compartment, to enable the Chaetogaster to move in either direction in the apparatus. Pond water was used in all of the experiments. The inlet for the background flow of water came from a large reservoir, 30 cm. in diameter, in which the head of water was kept constant. Water from the two test reservoirs, in each of which four snails of the species being investigated were placed, was brought into the apparatus by two drip tubes, of internal diameter 1.5 mm., as shown. To obtain a non-turbulent flow of water in the two streams, as shown in the diagram, fluorescein dye was added to one of the reservoirs and the rate of flow of the background current and from the drip tubes adjusted for optimum results. The best results were obtained by having as slow a background current as possible, so that the two test streams would be wide, without having the current slow enough for the two streams to intermingle. The overall rate of the background flow was 0.065 cm./second and that from each drip tube, 0.009 cm./second. The same flow of water was used in each experiment. The rate of flow would be greatest at the



inlet of the drip tubes.

Preliminary experiments showed that the Chaetogaster could withstand the flow of water being used. It was originally intended to colour one of the streams during the experiments with fluorescein, for the purpose of observation of its limits. An experiment was therefore set up in which fluorescein was in one stream and only water in the other. The results in Table 28 (p. 117) show that Chaetogaster were repelled by the dye.

In the experiments, 30 Chaetogaster, introduced in equal numbers on either side of the trough, were normally used. Each experiment was performed in the dark and allowed to continue for 3 hours. The Chaetogaster were then removed from either side of the apparatus and counted. An equal number of experiments was performed with the position of the reservoirs one way and with them transposed.

As a control experiment, before the Chaetogaster from a particular species of snail were used in the trough experiments, an experiment was performed with pond water only in both the experimental reservoirs. The results of these experiments are shown in Table 29 (p. 117). In all cases, the Chaetogaster were equally distributed between the two streams.

#### 4.4. The attraction of Chaetogaster limnaei to the host.

Expt. no.	Choice	
	Fluorescein	Water
1	0	22
2	0	29

Table 28. The accumulation of Chaetogaster l. limnaei from L. pereger when given the choice of water and fluorescein labelled water.

<u>Chaetogaster</u> subspecies	Source	Choice		
		Water 1	Water 2	
Ch. l. limnaei	L. pereger	26	26	-
Ch. l. vaghini	L. stagnalis	17	14	-
Ch. l. limnaei	P. fontinalis	53	52	-
Ch. l. limnaei	Pl. corneus	52	60	-

+ = Significant difference - = Not significant

Table 29. Control trough experiments, giving Chaetogaster limnaei a choice of pond water in both streams.

The possibility that Chaetogaster limnaei was attracted to the host from which it was removed was tested using the egg mass and the trough experiments.

The egg mass experiments were performed, as described earlier (p. 109), using two of the host egg masses and two gels of similar size and shape. The results in Table 30 (p. 119) show that in the case of Ch. l. limnaei from both L. pereger and Physa fontinalis, accumulation occurred on the egg masses of their respective hosts. Chaetogaster l. vaghini also aggregated on the egg masses of its host, L. stagnalis. Chaetogaster l. limnaei from Pl. corneus, however, became equally distributed on the egg masses and the gels. This was probably due to the similar nature of the Pl. corneus egg mass and the gel, both having a hard outer coating as compared with the egg masses of other species.

To determine whether the Chaetogaster were attracted to a chemical factor from their host snail, the trough experiment was set up as described above with the host snail in pond water in one reservoir and pond water only in the other reservoir. The results in Table 31 (p. 119) show that Ch. l. limnaei from L. pereger, Pl. corneus and Physa fontinalis all accumulated in the stream of water from their hosts in preference to the stream containing pond water only. Ch. l. vaghini also accumulated in the current from its own host.

The results of the egg mass experiments and the trough



<u>Chaetogaster</u> subspecies	Source of	No. of worms on		
	<u>Chaetogaster</u> and egg mass	Egg mass	Gel	
Ch. l. limnaei	L. pereger	33	1	+
		55	0	+
Ch. l. limnaei	P. fontinalis	17	0	+
Ch. l. limnaei	Pl. corneus	10	9	-
Ch. l. vaghini	L. stagnalis	20	0	+
		72	0	+

+ = Significant difference      - = Not significant

Table 30. The accumulation of Chaetogaster limnaei on the egg masses of its host and on gels.

<u>Chaetogaster</u> subspecies	Source	No. of expts.	Choice		
			Host	Pond water	
Ch. l. limnaei	L. pereger	3	45	6	+
Ch. l. vaghini	L. stagnalis	6	99	45	+
Ch. l. limnaei	P. fontinalis	7	89	59	+
Ch. l. limnaei	Pl. corneus	4	74	36	+

+ = Significant difference      - = Not significant

Table 31. The choice of Chaetogaster limnaei between a stream of water from its host and a stream of pond water.

experiments indicate that both Ch. l. limnaei and Ch. l. vaghini were attracted to their respective hosts. In all of the trough experiments, the Chaetogaster tended to move upstream and to accumulate under the inlet of the drip tube coming from the reservoir containing the host species of snail.

#### 4.5. The specificity of Chaetogaster limnaei to its own host.

##### 4.50. Introduction.

As the previous experiments showed that Chaetogaster limnaei was attracted to its host, it was of interest to investigate the possibility that Ch. limnaei from a particular species of snail could detect and accumulate on its own host species in preference to other gastropod species. Four types of experiment were used to determine whether any degree of host specificity occurred, these being experiments with non-infected snails, egg mass experiments, mucous trail experiments and trough 'choice' experiments.

##### 4.51. Experiments with non-infected snails.

These experiments were performed with various species of snail and Ch. limnaei from one of these species, to determine whether Chaetogaster would accumulate on its own host species or would be distributed randomly between the species of snail

present. In the first experiment Ch. l. limnaei from L. pereger was used and four non-infected specimens of each of L. pereger, L. stagnalis and Pl. corneus were presented. The results in Table 32 (p. 122) show that Ch. l. limnaei became equally distributed between L. pereger and Pl. corneus, but did not infect L. stagnalis to any extent in either experiment. To determine whether L. stagnalis somehow repelled these Ch. l. limnaei, the experiment was repeated with only non-infected L. stagnalis present. Table 33 (p. 122) shows that L. stagnalis was infected by Ch. l. limnaei and so the previous result was not due to repulsion of the Chaetogaster by L. stagnalis.

Similarly, Ch. l. limnaei from Pl. corneus was introduced into a bowl containing non-infected L. pereger, L. stagnalis and Pl. corneus. The results in Table 34 (p. 123) indicate a similar situation to that of Ch. l. limnaei from L. pereger. Both L. pereger and Pl. corneus became heavily infected, whereas L. stagnalis had only a very low infection.

At the end of the corresponding experiments with Ch. l. vaghini from L. stagnalis, 1 worm was found in L. pereger and 2 in L. stagnalis, as shown in Table 35 (p. 123). The number of Ch. l. vaghini which succeeded in reinfecting other snails was much lower than in the experiments with Ch. l. limnaei.

The results of these experiments show that there was differential infection of some of the species of snail.



Expt. no.	No. of each snail species	No. of <u>Chaetogaster</u> found on			
		<u>L. pereger</u>	<u>L. stagnalis</u>	<u>Pl. corneus</u>	
1.	4	18	0	11	+
2.	4	15	1	14	+

Table 32. The infection by Chaetogaster l. limnaei from L. pereger of non-infected specimens of L. pereger, L. stagnalis and Pl. corneus.

Expt. no.	No. of snails	No. of <u>Chaetogaster</u> on <u>L. stagnalis</u> after 7 days.	
1.	4		39
2.	3		6

Table 33. The infection of L. stagnalis by Chaetogaster l. limnaei from L. pereger.

No. of each snail species	No. of <u>Chaetogaster</u> found on			
	<u>L. pereger</u>	<u>L. stagnalis</u>	<u>Pl. corneus</u>	
2	19	1	23	+

+ = Significant difference

Table 34. The attachment to non-infected L. pereger, L. stagnalis and Pl. corneus by Chaetogaster l. limnaei from Pl. corneus.

No. of expt.	No. of each snail species	No. of <u>Ch. l. vaghini</u> on		
		<u>L. pereger</u>	<u>L. stagnalis</u>	<u>Pl. corneus</u>
1.	4	1	0	0
2.	4	0	2	0

Table 35. The attachment to non-infected L. pereger, L. stagnalis and Pl. corneus by Ch. l. vaghini from L. stagnalis.

However, the results of these experiments did not suggest that Ch. limnaei was host-specific.

#### 4.52. Experiments using egg masses.

The specificity of Chaetogaster limnaei to its own host egg mass was studied in experiments using 2 egg masses from the host species and 2 from a different species of snail. The results are shown in Table 36 (p. 125). A significant accumulation occurred on the host egg masses in some cases, for example Ch. l. limnaei from L. pereger (exp. 3 and 4, Table 36). Similarly, for Ch. l. limnaei from Pl. corneus, aggregation occurred on the host egg masses when presented together with L. pereger egg masses. Ch. l. vaghini from L. stagnalis when offered the egg masses of L. stagnalis and L. pereger accumulated significantly on the former. In the other experiments, there was no significant difference shown, apart from in the accumulation of Ch. l. limnaei from L. pereger in experiment 2.

The egg mass experiments therefore showed some degree of host specificity with some of the Chaetogaster and hosts used.

#### 4.53. Experiments using mucous trails.

The mucous trail experiments offered 6 alternative areas on which the Chaetogaster could accumulate. Each experiment was performed 8 times with the snails in different sections



Expt. no.	<u>Chaetogaster</u> subspecies and source	Egg masses presented	No. of worms on egg masses	
1.	Ch. l. limnaei	L. pereger	25	
	L. pereger	L. stagnalis	29	-
2.	"	"	18	
			82	+
3.	"	L. pereger	65	
		Pl. corneus	27	+
4.	"	"	46	
			8	+
5.	"	L. pereger	17	
		P. fontinalis	15	-
6.	Ch. l. limnaei	Pl. corneus	26	
	Pl. corneus	L. pereger	3	+
7.	"	"	28	
			19	-
8.	Ch. l. limnaei	P. fontinalis	12	
	P. fontinalis	L. pereger	15	-
9.	Ch. l. vaghini	L. stagnalis	22	
	L. stagnalis	L. pereger	10	+
10.	"	"	26	
			12	+

+ = Significant difference      - = Not significant

Table 36. The accumulation of Chaetogaster limnaei on the egg masses of the host species and other species of snail.

of the apparatus, as previously described (p. 110).

The results of the selection by Ch. l. limnaei and Ch. l. vaghini are shown in Table 37 (p. 127). In each case, the difference in the accumulation on the mucus of the different snail species was significant. To examine the results for aggregation of the Chaetogaster on the mucus of their own host,  $\chi^2$  was calculated for the number accumulated on the host mucus and the number on the mucus of another species of snail. This comparison was made for all the experiments and the significant differences are shown in Table 38 (p. 128).

Chaetogaster l. limnaei from L. pereger did not aggregate on the mucus of L. pereger in significantly greater numbers than on that of Pl. corneus. This agrees with the result of the experiments with whole snails, where Ch. l. limnaei was equally distributed on the L. pereger and Pl. corneus. An experiment was therefore performed giving Ch. l. limnaei from L. pereger a choice of mucus from L. pereger and Pl. corneus only. Table 39 (p. 129) shows that there was then a significant accumulation on the mucus of L. pereger under these conditions.

In the comparison of the aggregation on the mucus of the host and of other species of snail by Ch. l. limnaei from Physa fontinalis, the accumulation on the host's mucus was significant in all cases. The same was true for Ch. l. limnaei from Pl. corneus which also accumulated on the mucus of its host when presented together with that of other species of

Exp. no.	<u>Chaetogaster</u> subspecies	Accumulation on mucus types						
		L. pereger	L. stagnalis	Pl. corneus	P. fontinalis	P. jenkinsi	None	
1.	Ch. l. limnaei L. pereger	119	50	101	89	42	51	+
2.	Ch. l. limnaei P. fontinalis	89	70	80	135	67	40	+
3.	Ch. l. limnaei Pl. corneus	50	30	88	24	40	52	+
4.	Ch. l. vaghini L. stagnalis	112	106	77	62	51	54	+

+ = Significant difference.

Each result is the total of 8 experiments.

Table 37. The accumulation of Ch. limnaei from various sources on 5 types of mucus.



Exp. Chaetogaster L. pereger L. stagnalis Pl. corneus P. fontinalis P. jenkinsi None  
 no. subspecies

1.	Ch. l. limnaei L. pereger	+	-	+	+	+
2.	Ch. l. limnaei P. fontinalis	+	+	+	+	+
3.	Ch. l. limnaei Pl. corneus	+	+	+	+	+
4.	Ch. l. vaghini L. stagnalis	-	+	+	+	+

+ = Significant difference, i.e. host mucus chosen  
 - = No significant difference

Table 38. Significant differences of the accumulation on the host mucus and the mucus of other gastropods by Ch. limnaei.

<u>Chaetogaster</u> subspecies	Source	Mucous types presented and results		
Ch. l. limnaei	L. pereger	L. pereger 140	Pl. corneus 99	+
Ch. l. vaghini	L. stagnalis	L. stagnalis 194	L. pereger 191	-

+ = Significant difference

- = Not significant

Table 39. Comparison of the accumulation of Ch. limnaei on mucus from the host and from another species of snail.

snail.

In the experiments with Chaetogaster l. vaghini from L. stagnalis, a significant difference between the accumulation on the host mucus and on the mucus of other gastropod species was shown in all cases except for the mucus of L. pereger. A comparison of the accumulation of Ch. l. vaghini on the mucus of its host L. stagnalis and that of L. pereger was made in a separate experiment. The results in Table 39 (p. 129) again show that there was no selection for the mucus of L. stagnalis. This may be due to the two species of snail being closely related.

The results of the mucus experiments show that in the majority of comparisons, accumulation of the Chaetogaster occurred on the mucus of the host snail rather than on the mucus of other snail species. This shows that Ch. limnaei tended to show some host-specificity in the presence of a variety of mucous trails.

#### 4.54. Trough experiments.

The trough for testing the reaction of Ch. limnaei to chemical factors from the host snail was set up as described previously (p. 112), but with the host snails in one reservoir and another species of snail in the second test reservoir.

The results (Table 40, p. 131) show that Ch. l. limnaei from L. pereger, L. stagnalis, and Pl. corneus each accumulated



<u>Chaetogaster</u> subspecies	Source	No. of expts.	Stream chosen	
Ch. l. limnaei	L. pereger	7	L. pereger 110	L. stagnalis 52 +
Ch. l. limnaei	P. fontinalis	7	P. fontinalis 103	L. pereger 59 +
Ch. l. limnaei	Pl. corneus	4	Pl. corneus 97	L. pereger 21 +
Ch. l. vaghini	L. stagnalis	7	L. stagnalis 93	L. pereger 53 +

+ = Significant difference

Table 40. The choice of Ch. limnaei between a stream containing the 'host-factor' and a stream from another snail species.

in significantly larger numbers in the stream containing the chemical factor from the host snail, indicating a preference for the host stimulus over that of the other snail species present. Thus, the Chaetogaster from each source showed a degree of host-specificity when given a choice of a stream from the host and one from another species of snail.

It was shown in Section 4.4 that, given the choice of a stream of water from the host snail and a stream of pond water, the Chaetogaster accumulated in the former. It was therefore decided to determine how Chaetogaster from various sources reacted to a stream of water from a species of snail other than the host and a stream containing water only. Table 41 (p. 133) shows that in the absence of the host species of snail, Chaetogaster were attracted to chemical factors from the alternative species. However, when the choice was between a stream containing chemical substances from the host and another species of snail, the worms showed a degree of host-specificity and accumulated in the stream from the host.

#### 4.6. The effect of the source of Chaetogaster limnaei and of the snails on host selection.

##### 4.60. Introduction.

It might be expected on 'a priori' grounds that in habitats with several species of snail Chaetogaster limnaei

<u>Chaetogaster</u> subspecies	Source	No. of expts.	Stream chosen		
Ch. 1. limnaei	L. pereger	3	L. stagnalis	Water	
			89	17	+
Ch. 1. limnaei	P. fontinalis	3	L. pereger	Water	
			83	15	+
Ch. 1. limnaei	Pl. corneus	4	L. pereger	Water	
			54	22	+
Ch. 1. vaghini	L. stagnalis	5	L. pereger	Water	
			48	27	+

+ = Significant difference

Table 41. The choice of Ch. limnaei between a stream of pond water and a stream containing chemical substances from a snail species other than the host.



would have a greater degree of host-specificity than in habitats with only a single species of snail and it would tend to form biological races in each host species. Where only one species was present, host-specificity would not be necessary, a response being developed only to gastropods in general.

Similarly, the same species of snail from different habitats will vary to some degree. Thus, if given a choice of the host species and the same species of snail from a different habitat, it might be expected that Chaetogaster would show preference for snails from their own habitat.

#### 4.61. Comparison of the host-specificity of Chaetogaster limnaei.

To determine whether host-specificity varied with the number of species of snail in a habitat, experiments were carried out using L. pereger from habitats with from one to many species of gastropod present.

Experiments with non-infected snails were performed using Ch. l. limnaei from L. pereger from Llanllechid, where L. pereger was the only species of snail present, from the College pond with two dominant species of snail and from Cole Mere with many species of snail. Non-infected L. pereger, L. stagnalis and Pl. corneus were presented to Ch. l. limnaei from L. pereger collected in each of the three habitats.

The results in Table 42 (p. 135) show that no Chaetogaster

Source of <u>Ch. 1. limnaei</u>	No. of snail species at source	Choice of snails		
		<u>L. pereger</u>	<u>L. stagnalis</u>	<u>Pl. corneus</u>
Llanllechid	1	29	2	35
College pond	2	33	1	25
Cole Mere	Many	15	0	5

Table 42. Accumulation on non-infected snails by Ch. 1. limnaei from L. pereger from 3 different sources.

accumulated on the L. stagnalis. Between the accumulation on L. pereger and Pl. cornus by Ch. l. limnaei from either Llanllechid or the College pond, there was no significant difference, although a greater number of worms from the latter accumulated on L. pereger. The Ch. l. limnaei from L. pereger at Cole Mere showed significant aggregation on L. pereger. These results indicate that the specificity for the host species of snail was greater when the Ch. l. limnaei were from a habitat containing a greater number of gastropod species.

Mucous trail experiments were also performed to investigate the degree of specificity present. This was again done for Ch. l. limnaei from L. pereger from Llanllechid, College pond and Cole Mere. The results in Table 43 (p. 137) show that there was a significant difference in the distribution of Ch. l. limnaei between the types of mucus present. Table 44 (p. 138) shows that no selection was shown for the mucus of L. pereger by the Chaetogaster from Llanllechid, the other mucus types having an equal accumulation of Chaetogaster on them. The Chaetogaster from the other two sources showed a significant accumulation on the mucus of L. pereger in most of the comparisons made.

In both types of experiment, the results indicate that Chaetogaster l. limnaei from a source with only one species of snail were less specific to their host species than were those



Mucous types chosen

<u>Source</u>	<u>L. pereger</u>	<u>L. stagnalis</u>	<u>Pl. corneus</u>	<u>P. fontinalis</u>	<u>P. Jenkinsi</u>	<u>None</u>
Llanllechid	24	20	29	15	21	15 +
College pond	119	50	101	89	42	51 +
Cole Here	31	20	17	18	11	13 +

+ = Significant difference

Results are the total of 4 experiments.

Table 43. The accumulation on the mucus of various snails by Ch. l. limnai  
from L. pereger from three sources.

Source of L. pereger and that of other snail species

Significant difference between the accumulation on the mucus of

Ch. L. limnaei L. stagnalis Pl. corneus P. fontinalis P. jenkinsi None

Mandlechid	-	-	-	-	-
College pond	+	+	-	+	+
Cole Mere	+	+	-	+	+

+ = Significant difference, i.e. L. pereger mucus chosen  
 - = No significant difference

Table 44. A comparison of the significant differences between the accumulation of Ch. L. limnaei from L. pereger of various sources on the mucus of L. pereger and other snail species.

taken from a habitat with many species of gastropod present.

4.62. Variation in the gastropods.

Experiments with mucous trails were performed using mucus from L. pereger from Llanllechid, College pond and Cole Mere. Chaetogaster l. limnaei from L. pereger from each of these habitats were tested against the three types of mucous trail.

The results in Table 45 (p. 140) show selective accumulation in the choice between the mucus from the three sources in the case of Ch. l. limnaei from Cole Mere only. This would suggest that only in Cole Mere, where there were many species of snail was host-specificity of the worms sufficiently discriminating to detect differences between the three habitat types of L. pereger.

Trough experiments were also performed, giving a choice between a stream of water from a reservoir containing the host L. pereger and a reservoir containing L. pereger from another habitat. The results, in Table 46 (p. 141), show that there was no preference shown by Ch. l. limnaei from L. pereger at Llanllechid between a stream of water from L. pereger from Llanllechid and L. pereger from the College pond. For both Ch. l. limnaei from the College pond and Cole Mere, a significant preference was shown for the stream of water from the reservoir containing their own hosts, and this again indicates variation in the attraction of distinct snail



Source of	Mucous type chosen			
	<u>Ch. 1. limnaei</u>	Llanllechid	College pond	
Llanllechid	39	45	39	-
College pond	33	31	33	-
Cole Mere	31	27	61	+

+ = Significant difference

- = No significant difference

Table 45. The accumulation on L. pereger mucus from 3 habitats by Ch. 1. limnaei from each of these habitats.

Source of <u>Ch. l. limnaei</u>	Source of <u>L. pereger</u> and result of choice		
Llanllechid	Llanllechid	College pond	
	70	79	-
College pond	College pond	Llanllechid	
	87	21	+
Cole Mere	Cole Mere	College pond	
	32	8	+

Table 46. Trough 'choice' experiments by Ch. l. limnaei from various sources between a stream from L. pereger from its own habitat and L. pereger from a different habitat.

populations to the Chaetogaster.

The Ch. l. limnaei from Cole Mere accumulated on L. pereger from its own habitat, and this indicates that the quality of the L. pereger population varied between the different habitats. The experiments also showed that the degree of host-specificity of the Ch. l. limnaei was most highly developed at Cole Mere, where a large number of snail species was present. The Ch. l. limnaei from L. pereger at Llanllechid, where there was only one snail species, did not 'select' L. pereger from its own habitat.

#### 4.7. The nature of the reaction of Chaetogaster limnaei to its host.

The nature of the specificity of Chaetogaster limnaei to its host could be due either to conditioning or to genetic factors. To determine which of these was involved, Ch. limnaei were kept on snails of a different species from the host for long periods of time. When the Chaetogaster were introduced initially into a bowl containing a species of snail different from that of its natural host, a 'non-infected snail experiment' was set up at the same time, to determine the existing reaction of the worms to the host and test species of snail. After a period of time, either 1 week or 1 month, the Chaetogaster were removed from the experimental snail species



and the 'non-infected snail experiment' repeated. If accumulation continued to occur on the original host, specificity would be genetic, whereas if it occurred on the new host, it would be due to conditioning.

The results (Table 47, p. 144) show that Ch. l. limnaei from L. pereger still accumulated on L. pereger after 7 days on Pl. corneus. However, after 1 month on Pl. corneus, the Chaetogaster accumulated significantly on Pl. corneus in the non-infected snail experiment. This suggests that conditioning had taken place, but a longer time than 1 week was necessary for it to occur.

The results with Ch. l. limnaei from Pl. corneus are inconclusive in that on being taken from Pl. corneus, accumulation occurred chiefly on L. pereger, and after 7 days on L. pereger accumulation occurred on Pl. corneus.

The previous 'non-infected snail' experiments (p. 134) also did not show specificity of the Ch. l. limnaei to their own hosts, whereas the other types of experiment did. It is possible that, if the differential accumulation of the Chaetogaster was due to conditioning, the specificity would be apparent only in the short term experiments. In the longer experiments, the other species of snail could also become infected. The infection of other species of snail by Chaetogaster in these experiments may have been due to the crowded conditions of the snails compared with the situation

Host of Source

Accumulation after 7 days

Ch. l. limnaei

		<u>L. pereger</u>	<u>Pl. corneus</u>	
1. <u>L. pereger</u>	Direct from			
	<u>L. pereger</u>	129	29	+
	After 1 week			
	in <u>Pl. corneus</u>	87	25	+
		<u>L. pereger</u>	<u>Pl. corneus</u>	
2. <u>L. pereger</u>	Direct from			
	<u>L. pereger</u>	129	29	+
	After 1 month			
	in <u>Pl. corneus</u>	6	33	+
		<u>L. pereger</u>	<u>Pl. corneus</u>	
3. <u>Pl. corneus</u>	Direct from			
	<u>Pl. corneus</u>	31	19	+
	After 1 week			
	in <u>L. pereger</u>	15	29	+

+ = Significant difference

Table 47. Accumulation on non-infected snails after the conditioning of Ch. l. limnaei.

in their natural habitats.

5. The sensory cells of Chaetogaster limnaei.

As Chaetogaster limnaei was able to detect the differences in the trough experiments between the chemical stimuli from different species of snail and even between variations in the same species, it was decided to examine the sensory apparatus of the worms.

Specimens of Chaetogaster l. limnaei were stained with 0.25 per cent methylene blue for 2 minutes, and examined microscopically. Many single-cell receptors were stained in the epidermis, particularly in the prostomial region of the worm. Plate 6 shows some of these cells, which are typical, flask-shaped, single-cell receptors. In Plate 7, the sensillae can be seen to project from these sensory cells. The majority of the sensillae were found in the region of the prostomium. Subepidermal nerve cells could be observed, to which nerve fibres from the sensory cells were traced.

The function of these sensory cells cannot be experimentally ascertained, but it is suggested that they are able to detect chemical materials in the surrounding water and that they might also detect tactile stimuli. Chaetogaster has, therefore, a sensory system capable of 'evaluating' both chemical stimuli and water movement.



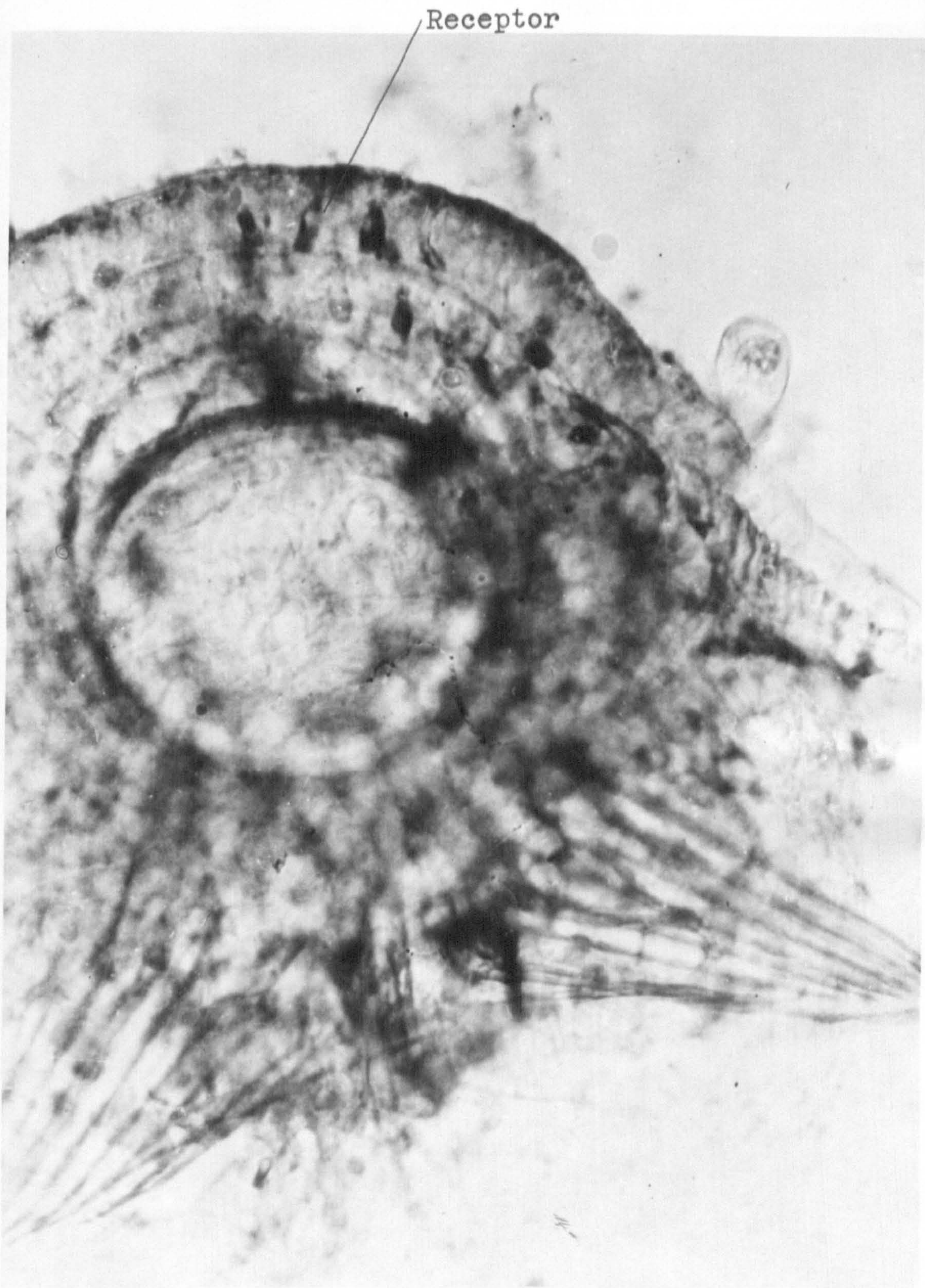


Plate 6. The prostomial region of Ch. l. limnaei, showing single-cell receptors.



Sensillae

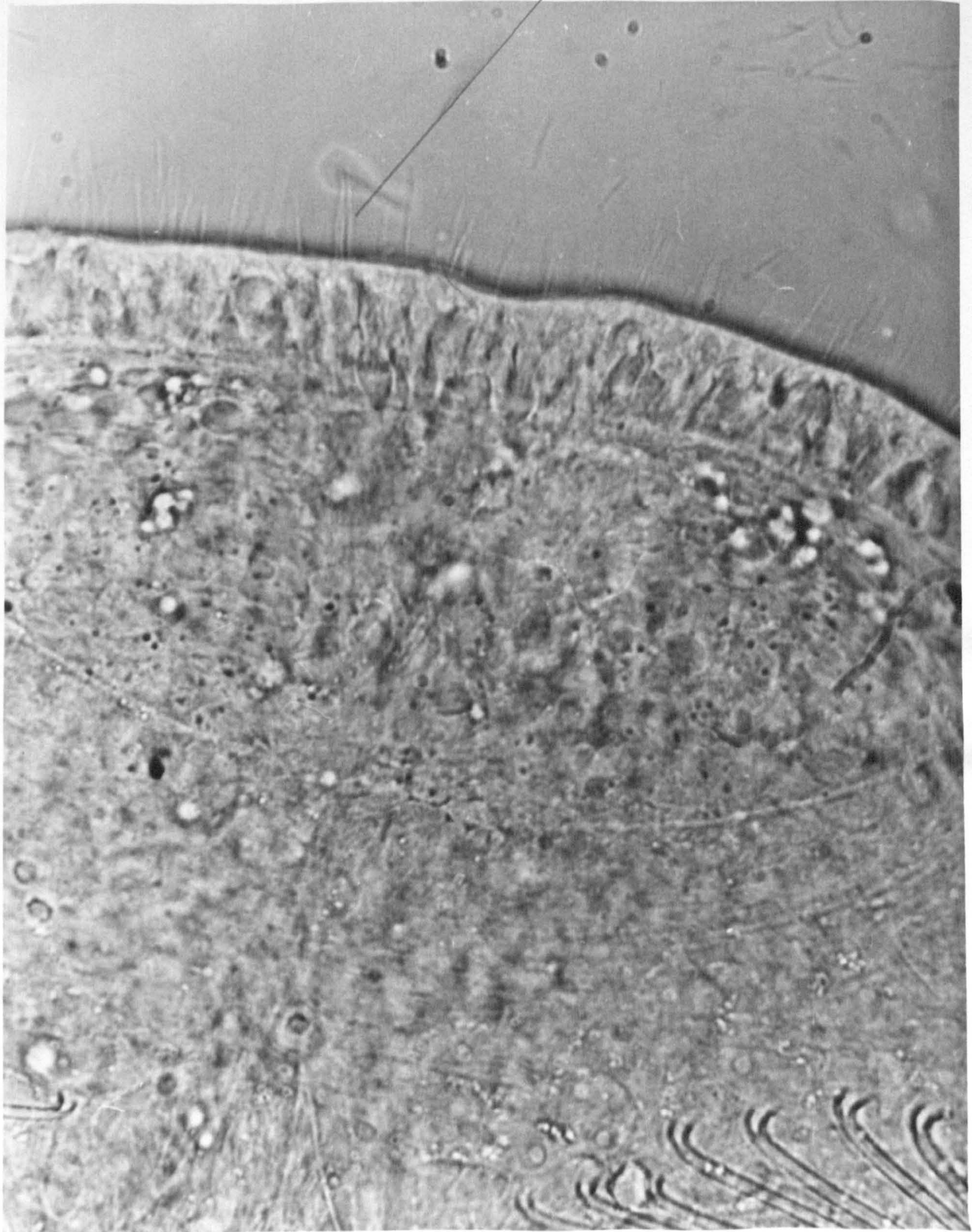


Plate 7. The prostomium of Ch. l. limnaei, showing sensillae.



## Section G. DISCUSSION.

1. The relationship between Chaetogaster limnaei and its host.

Symbiosis was originally defined by de Bary (1879) as a relationship which must be constant, intimate and between dissimilar species. This includes various types of association, such as mutualism, commensalism and parasitism. Other definitions of symbiosis which have been used described a more restricted association than that suggested by de Bary. For instance, Rogers (1962) defined symbiosis as an intimate association in which both partners benefit. Henry (1966), however, considered the definition given by de Bary as being the original and the more correct, and this definition will be used in this discussion.

The symbiotic relationships of Chaetogaster l. limnaei and Ch. l. vaghini with their hosts appear to be different. The association between Ch. l. limnaei and its host is apparently commensalism. This type of partnership was originally defined by van Beneden (1886), who described the participants of such an association as messmates, receiving <sup>food</sup> at the table of a neighbour, but not living at the expense of the host. Henry (1966) defined commensalism as an association in which members



are 'at table' together, and he pointed out that it is often used in situations in which only one partner profits. Smyth (1962) observed that in commensalism neither partner is metabolically dependent on the other.

Chaetogaster limnaei limnaei obtains its food supply from the current of water which occurs around the head and tentacles of the snail. The worms are transported from one place to another by the snail, enabling them to obtain their food from a wider area with less expenditure of energy. They are also protected by their position in the snail from small predators. All these observations suggest that Ch. l. limnaei is a commensal. No advantage is apparent for the snail from the association, but some protection may be conferred from the penetration of the head and foot of the gastropod by miracidia since the Chaetogaster feed on these.

The relationship of Chaetogaster limnaei vaghini with its host is of a different nature. Nitrogen excretion in Lymnaea stagnalis is in the form of uric acid (Potts, 1967). The gut of Ch. l. vaghini is full of concretions of uric acid in the form of granules or crystals. Whether the kidney cells containing these are ingested when they break away in the kidney, or whether the cells are taken directly from the living kidney tissue cannot be determined. However, a comparison of the sizes of the concretions in the gut of

Chaetogaster and of those free in the kidney lumen, shows them to be the same, suggesting that the uric acid containing cells are sloughed off before they are ingested by the Chaetogaster. Organic compounds, such as glucose, and inorganic salts enter the kidney (Potts, 1967) and these may be absorbed by the Chaetogaster.

The definition of parasitism given by Rogers (1962) stated that parasitism is an association between two organisms, in which one is dependent on and lives at the expense of the other. The host provides the environment and, in using this, the parasite injures the host. A simpler definition, suggested by Smyth (1962), was that a parasite is metabolically dependent on its host. Under the latter definition, Ch. l. vaghini is definitely a parasite. The worms are apparently not harmful to their host, although they are metabolically dependent on it. However, in absorbing some compounds that the snail would otherwise have used, this subspecies can be regarded as 'harmful'. Gruffydd (1963) also regarded Ch. l. vaghini as being parasitic.

## 2. The life-cycle of Chaetogaster limnaei vaghini.

The timing of the occurrence of maturation, cocoon production and the reappearance of the immature stages in the



life-cycle of Ch. l. vaghini are important in its continuance as a parasite in Lymnaea stagnalis. A comparison of the seasons at which these occur in Ch. l. vaghini and closely related species might indicate the advantage of the timing found in the parasite.

The first mature forms of Ch. l. vaghini were found in the population in September or October, and by December each individual of the population was mature. Von Baer (1827), Vaghin (1946) and Gruffydd (1965b) reported that mature worms were present in the winter from the month of October to November and December. Stephenson (1930) in a summary of data given by earlier workers, reported that in most other Naididae, maturation occurs during the summer months, although a few species such as Nais elinguis and Stylaria lacustris become mature during the autumn. All observations on the various Chaetogaster species have been of maturation occurring in the autumn or winter. Experimental work by Lipps (1920) showed that a rise in temperature above 18°C caused the formation of sexual organs in Stylaria lacustris. He stated that in Chaetogaster, however, cold provokes the development of the gonads. Stolte (1921) in experiments on Nais variabilis and N. elinguis showed that high temperature indirectly causes the sexual form to develop due to high acidity and high food concentrations in the surrounding medium. Piguet (1906) observed that some species of Nais, namely N. variabilis



and N. obtusa became mature in the autumn, whereas N. elinguis and N. communis matured in June and July. He stated that Stylaris lacustris matured in the autumn and winter, but as Stolte showed that high temperature caused the development of maturity in S. lacustris, this must have a delayed effect for Piguet's observation to be true.

The cause of the onset of maturation in Ch. l. vaghini was shown by experiment to be due to a drop in temperature below 13°C. Maintenance of the snails and their Chaetogaster population at high temperatures during the winter prevented the onset of maturation. The time of year of the fall in temperature was important since, if this occurred during the spring or summer, maturation did not ensue.

The effect of temperature on the Chaetogaster could either operate directly on the worm or indirectly by exerting an effect on the snail. These two possibilities could not be separated in the present study. In-vitro experiments might have determined which was involved, but these were unsuccessful due to the specialised nature of the food of Ch. l. vaghini and the problem of the concentration necessary. Preparation of an artificial medium in which the worms could be kept indefinitely would resolve this problem. If maturation was due to a lowering of the snail's temperature, it could be due to the reduced metabolism and the subsequent effect on the

contents of the snail's kidney. Potts (1967), describing excretion in the molluscs, stated that ultrafiltration occurs directly from the blood into the lumen of the kidney sac, and that resorption also takes place from the kidney sac. Thus the kidney will be rich in glucose and other organic substances. A fall off in the amount of excretion into the kidney due to a lowering of the metabolic activity of the snail in the autumn could effect the onset of maturation.

Neurosecretion by the snail is another factor that might cause maturation of the parasite. The annual variation in the neurosecretion of the snail coincides with the sexual and asexual reproductive cycles of Ch. l. vaghini. Joosse (1964) observed that in the spring, neurosecretory substances were being produced in the mediodorsal and laterodorsal cells of the cerebral ganglia of Lymnaea stagnalis. By the autumn, production was very low and during the winter, the cells were inactive. Joosse also pointed out that neurosecretion may effect the reproductive cycle of L. stagnalis, spermatogenesis coming to a peak in March. The reduction of neurosecretory activity in L. stagnalis coincides with the onset of maturation in Ch. l. vaghini and so could be a causatory factor.

It is therefore not possible to decide whether the effect of temperature is direct on the Chaetogaster or indirect



through its effect on the metabolism or the neurosecretion of the snail.

It was observed that the majority of the cocoons left the kidney before hatching, few hatched or unhatched cocoons remaining in it. This was also shown by a comparison of the number of cocoons and newly hatched Chaetogaster in the kidney at one time, with the number of cocoons and mature Chaetogaster present in the previous month. The presence of the majority of the cocoons in the ureter, rather than in the renal sac, also suggested that the cocoons were eliminated from the kidney before they hatched.

The stimulation of the cocoons to hatch, by the lowered osmotic concentration of the surrounding medium on leaving the kidney, ensures that dispersal from the old host occurs before the Ch. l. vaghini hatch. Due to the chemical attraction to the snails, they would otherwise tend to remain in the same host. A similar situation has been described by Davis (1959) in copepods, where hatching was prevented by their maintenance in concentrated sucrose solution. Faust and Meloney (1924) also observed that isotonicity tended to prevent hatching, whereas hypertonicity caused the hatching of the miracidia of Schistosoma japonica. This was also found to occur in the hatching of the eggs of Trichostrongylus by Wilson (1958). In all cases, the correct environment for



hatching was determined by a lowered osmotic pressure being necessary in the surrounding medium.

The production of cocoons during the winter may be a means whereby the Chaetogaster population can survive through adverse conditions. The observation that in L. stagnalis kept at 5°C all the Ch. l. vaghini population died, suggests that it is unable to survive at low temperatures. The fact that cocoons hatch more readily at high temperatures than at low temperatures suggests that this is another mechanism which prevents the Chaetogaster from hatching during an unfavourable period. That those cocoons which were collected from the kidney of the snail early in the winter did not hatch, whereas later ones did, suggests that the cocoons must develop for a time in the kidney of the snail. This will prevent the Chaetogaster from hatching early in the winter and later being killed by low temperatures.

The percentage of immature worms in the Chaetogaster l. vaghini population increased from January to March or April, when the population became completely immature. The number of Chaetogaster in each snail also increased, so that when the young snails entered the population in May or June, infection of them occurs rapidly. In fact, 50 per cent of the young snails became infected within two weeks of hatching. The number of worms present in the population during the immature

phase, when asexual reproduction was taking place was much greater than after hatching from the cocoons. This may explain why maturation occurs during the winter months, since it allows asexual reproduction to occur at the time when young snails are available for re-infection.

### 3. The infection of the host and host-specificity.

The location of and the attachment to a host can be divided into several stages. Laing (1937) divided these into finding the area in which the host is and finding the host when in the area. Similarly, Reynoldson (1956) divided the establishment of Urceolaria mitra on its host into three stages, namely, contact with the host, maintenance of the contact and the ability to grow in situ. A combination of the first statement of Laing with those of Reynoldson defines all the stages involved.

The general behaviour of Chaetogaster limnaei is important in the maintenance of contact with the host. In the experiments determining the reaction of Ch. l. limnaei to light, the worms moved into the dark. As the reaction of the worms to light caused them to move away from the source of stimulation, this, by the definition of Fraenkel and Gunn (1940), is a directed reaction and the behaviour involved is a



taxis. As the movement is without deviation, orientation being obtained directly, the reaction can be described as a tropotaxis. Chaetogaster l. limnaei is therefore negatively photo-tropo-tactic. Due to this reaction, the worms will tend to stay under the collar and tentacles of the snail.

Chaetogaster l. vaghini showed no reaction to light. As the habitat of this subspecies is in the kidney, a reaction to light would not be necessary, as long as chemical attraction was involved in the worms finding their position in the host.

A number of behavioural adaptations were used by Chaetogaster limnaei to find the area that the host was in and to make contact with the host. The reaction of Chaetogaster to a water current is important. When the water current did not contain chemical 'host-factor', the Chaetogaster were washed down with the water and could therefore be considered as either insensitive to the water current or negatively rheotactic (Fraekel and Gunn, 1940). When the 'host-factor' was present in the water, the worms moved upstream. As the chemical substances were evenly distributed throughout the water in the apparatus, the experiment showed that rheotaxis is reversed in the presence of the 'host-factor'. A similar observation was made by Welsh (1930, 1931), who showed that the phototaxis of the parasitic mites of fresh-water mussels was reversed on the addition of the 'host-factor' to water



containing them. The positive rheotaxis of Ch. limnaei is of importance as they are then able to move in the direction of the host when the chemical stimuli are present. The importance of rheotaxis in other cases appears to vary. Davenport et al. (1960) showed that for a polychaete and its crab host, rheotaxis did not play a part in finding the host. In experiments on bivalves of the species Montacuta, a commensal in spatangoids, Gage (1966b) regarded rheotaxis as providing the directional component of chemo-kinesis. The miracidia of Fasciola hepatica were also shown to be positively rheotactic by Yasuraoka (1953).

Chaetogaster limnaei was shown in the experiments to react to its host by contact with the egg masses and the mucous trail, although the results of these experiments may also be due to the diffusion of a chemical substance(s) from these. They were also attracted by a chemical 'host-factor' coming directly from the snail.

Selection of habitat is made by various means in animals. The choice of the substratum by contact was shown to be made by a preference for a certain size and shape of sand grain in the case of Ophelin bicornis larvae (Wilson, 1952). Reaction also occurs to other individuals of the same species, as was observed in the case of barnacles, the larvae of which only settle where barnacles have previously been attached (Knight

Jones, 1953).

Attraction to the mucus of the host snail has been shown to occur in the case of Trematodes in a similar way to that observed in Ch. limnaei. Faust (1924) and Faust and Meleney (1924) described how Schistosoma japonica miracidia were attracted to the mucous trail of their host species of snail, Katyama. They suggested that this was a chemotactic reaction.

The accumulation of Chaetogaster on egg masses is probably due to reaction to the various chemical substances in them. In the field, very few Chaetogaster were found on the egg masses examined, so this situation may be artificial, due to the crowded conditions in the laboratory. However, accumulation on the egg masses of the host will enable the worms to be in the right area to infect the young snails when they hatch. The experiments of MacInnis (1965) on the behaviour of miracidia in the presence of gels impregnated with various chemical substances showed that attraction and stimulation of miracidia by certain of these occurred. In these experiments, both butyric and glutamic acids were found to be attractants.

The trough experiments showed that Chaetogaster limnaei were attracted to chemical substances originating from the host. Davenport (1950) called this substance from the host in a commensal union a 'host-factor'. He showed that various



polynoid species were attracted to their respective hosts by means of this 'host-factor' (Davenport, 1950, 1953a, 1953b, 1955; Davenport and Hickok, 1951).

Chemotropisms were observed by Kloetzel (1958) in the reaction of the miracidia of Schistosoma mansoni to Australorbis glabratus. Campbell and Todd (1955) and Abdel-Malek (1950) found no evidence of chemotaxis in the behaviour of the miracidia they examined.

The study of the microscopic structure of Ch. limnaei showed that it had sense cells which could be responsible for detecting chemical substances in the surrounding medium, and also cells which could detect water currents.

Host-specificity is complete when a commensal or parasite will only attach to its own host species and to no other. Welsh (1930, 1931) showed that in three species of mite from three species of mussel, reversal of phototaxis only occurred when the 'host-factor' from the respective host species was present. Similarly, Barlow (1925) observed that the miracidia of Fasciolopsis buski would only choose two species of snail from a large number of species.

In the case of Chaetogaster limnaei, a high degree of specificity to the host species from which the worms were obtained was shown particularly in the mucous trail experiments and in the trough 'choice' experiments. However, if Ch. limnaei from any snail species was given a choice



between 'host-factor' from a different species of snail and water, the Chaetogaster were attracted by the chemical stimuli.

For the Chaetogaster to be able to select different gastropod species, there must be some difference between the chemical substances produced by the snails. Those involved must be diffusing through the water and coming either from the snail or from the mucus secreted by the snail. Wright (1959) in a study of the chromatographic patterns of the mucus of various species of the genus Lymnaea, discovered that there were species-specific substances in the mucus of the body surface of the snail. He suggested that snails, being non-visual, use chemotactic methods of species recognition. The use of such contrasts in the identification of different host species by the parasites or commensals of fresh-water snails would be a method whereby such specific host detection could be made.

There was also variation in the degree of specificity shown by Chaetogaster limnaei from habitats containing different numbers of snail species. Where only one snail species was present in a habitat, the Chaetogaster from that habitat could not detect the differences between various host species to the same degree as Chaetogaster from a habitat with many gastropod species. The fact that Ch. l. limnaei

from the habitat with many snail species could detect variation between L. pereger from different sources and choose the snail from its own habitat shows that it has a greater degree of specificity. Wright (1964) showed by chromatographic techniques that there was variation between the mucus of L. pereger from different habitats, and this could explain how such detection is achieved.

It is possible that in both the mucous trail experiments and the trough 'host-factor' choice experiments it is differences in the chemical composition of the mucus that are being detected, by diffusion of chemical substances from it. As mucus is being continually secreted by the snail, this would be a constant source of chemical stimuli for species detection. If, as suggested by Wright, recognition between snails is by this method, this is the most likely means by which Chaetogaster can distinguish between prospective host species.

The method of detection between the egg masses of different hosts could be due to the mucus on the egg masses when they are laid. This would explain why in the laboratory, where the eggs were laid during experiments, Chaetogaster were found accumulated on them whereas very few were found on egg masses in the field. Other differences between the egg masses of different snail species have been described, and



these might be involved. For example, electrophoretic studies on the egg proteins of Planorbid snails showed differences to be present (Wright, 1965). Bondesen (1950) described the differences in the physical nature of the egg masses of different species, which could also account for selection between egg masses.

The specificity shown by Chaetogaster suggests that the worms tend to vary on different species of snail. The morphological differences found also indicate that there is a separation of the Chaetogaster population into different biological races. For this to occur, there must be an advantage to the Chaetogaster species in remaining on one species of snail. In those snails which are ciliary feeders, such as Bithynia (Gruffydd, 1963), a definite advantage can be seen in remaining on one species rather than infecting any species of snail. However, since the ciliary action of the snail causes water currents in its immediate vicinity, food particles will be brought to the Chaetogaster in all gastropod species. It would also be of advantage for the Chaetogaster to be specific to the larger species of snail in any particular habitat, these being able to maintain a larger population of the commensal. For Ch. l. vaghini, it was shown that the size of the kidney limited the population which it could hold, and so it would be of particular advantage to infect the large species of snail.



It is possible that, just as Ch. l. limnaei and Ch. l. vaghini have evolved into separate subspecies, so Chaetogaster l. limnaei may evolve into biological races and eventually subspecies. As Chandler (1923) pointed out for parasites in general, there are slight physiological contrasts in the different environments of the various hosts and it would therefore be expected that different races of the parasite would occur due to the isolation of the environments. In the case of Chaetogaster, its isolation on different species of snail in a habitat would tend to promote the formation of races.

#### 4. The status of the subspecies of Chaetogaster limnaei.

Gruffydd (1965a) gave reasons, described in Section B, why the two types of Ch. limnaei should be regarded as the subspecies Ch. l. limnaei and Ch. l. vaghini.

It is suggested that these two subspecies should, in fact, be considered as species. A species is defined as a group of actually or potentially interbreeding natural populations, which are reproductively isolated from other groups and have a common gene pool (Mayr, 1963). In the case of Chaetogaster limnaei it is not possible to determine whether the two subspecies could interbreed. Due to the fact that the two populations are completely isolated from each other and

mature Ch. l. limnai are of rare occurrence, it is suggested that these two subspecies should be promoted to species level. Differences in morphology, food and behaviour support their being species rather than subspecies.

#### Section H. SUMMARY.

1. The greatest number of Ch. l. vaghini and the highest percentage infection of the snails were in May and June. The larger snails supported the greatest populations of the worm, and the volume of the kidney limited the size of the population.
2. Maturation began in September when the temperature fell below 13°C, and the population became completely mature by December. High temperatures prevented the onset of maturation. Due to an innate mechanism, low temperatures were only effective in the production of maturation during the winter.
3. The peak in cocoon production was in December, and the cocoons left the kidney before hatching. A medium hypotonic with that of the kidney was necessary for the cocoons to hatch. Obligatory development for a time in the kidney and the greater number of cocoons hatching with increased temperature prevented the death of the Chaetogaster by the hatching of



cocoons under adverse conditions.

4. The timing of the life-cycle with asexual reproduction in the summer months enabled rapid infection of the newly-hatched snails.

5. The relationship of Ch. l. limnaei to its host was commensalism, whereas that of Ch. l. vaghini was parasitism.

6. The greatest number of Ch. limnaei were found on the largest snail species.

7. The formation of biological races was suggested by the setal lengths differing in Chaetogaster from different snail species in the same habitat. Worms from the same species of snail from various habitats did not have the same setal lengths.

8. Ch. l. limnaei was photo-tropo-tactic, enabling the worm to remain in contact with the host by accumulation under the collar and the tentacles. Ch. l. vaghini showed no response to light.

9. Positive rheotaxis was shown to occur in Ch. limnaei, but only when the 'host-factor' was present. This would enable the snail to be located.

10. A high degree of specificity was shown by the worms to their respective host species. Species-specific substances in the mucus may enable the Chaetogaster to differentiate between different species of snail.

11. By its specificity, Chaetogaster would be able to infect



- the species of snail which was most beneficial to it.
12. A comparison of the selectivity of Chaetogaster from one habitat with many snail species and another with few snail species showed that those from the former were more specific to the host, selecting the host species from its own habitat in preference to the same species from another habitat.
13. Ch. limnaei had sensory cells of the type capable of detecting chemical stimuli and water currents.
14. It was suggested that, due to the isolation of the two subspecies and the rare occurrence of mature Ch. l. limnaei, the two subspecies of Chaetogaster limnaei should be promoted to species level.

## ACKNOWLEDGMENTS.

I would like to thank Dr. T.B. Reynoldson for his advice and encouragement during the supervision of this work.

I am indebted to Professor F.W.R. Brambell for his continued interest and support, and to the technical staff of the Zoology Department, in particular Mr. A. Thomas for the construction of the apparatus and Mr. B. Gower for assistance in the preparation of the photographic material.

I am also grateful to Mr. D. Machin for his statistical aid and to Mr. J.F. Wright for supplying details of the water chemistry of the College pond.

The work was carried out during the tenure of an award from the Natural Environment Research Council.

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## APPENDIX 1

The number and size range of *Lymnaea stagnalis* collected in each monthly sample.

<u>Size group</u>	<u>Length (mm.)</u>	
	Greater than	Less than
a		5
b	5	10
c	10	15
d	15	20
e	20	25
f	25	30
g	30	35
h	35	40

Date	<u>No. collected</u>		<u>No. in each size group</u>							
	Net	Hand	a	b	c	d	e	f	g	h
Oct 1965	43	133		5	4	18	76	67	6	
Nov	94	76		9	7	11	77	61	5	
Dec	69	164	1	3	15	21	101	87	5	
Jan 1966	37	63		4	10	10	42	32	2	
Feb	49	77		2	9	6	47	59	3	
Mar	28	84				5	55	48	4	
Apr	54	118			2	8	54	81	27	

Over/.....

Date	<u>No. collected</u>		<u>No. in each size group</u>							
	Net	Hand	a	b	c	d	e	f	g	h
May	118	139	98			1	9	60	78	11
Jun	409	196	355	63	33	7	4	37	88	18
Jul	228	188	17	24	187	83	15	31	54	5
Aug	262	155	63	2	48	235	24	12	31	2
Sep	131	248	11	13	45	166	99	19	25	1
Oct	143	205		12	53	126	108	15	30	4
Nov	119	136	1	12	65	75	66	13	19	4
Dec	196	280		2	103	159	167	16	25	4
Jan 1967	176	205	1	10	61	134	143	10	17	5
Feb	134	124		2	21	98	114	9	12	2
Mar	265	319		3	27	197	308	40	8	1
Apr	153	351			3	57	298	128	15	3
May	172	383				12	280	240	22	1
Jun	196	318	52	3	1	6	201	239	12	
Jul	822	332	391	415	149	15	79	100	5	
Aug	857	345	29	590	406	23	59	90	5	
Sep	533	307		197	491	52	42	53	4	

Over/.....



Date	<u>No. collected</u>		<u>No. in each size group</u>							
	Net	Hand	a	b	c	d	e	f	g	h
Oct	565	319		76	639	78	44	43	4	
Nov	271	165	1	43	319	32	26	13	2	
Dec	336	158		37	384	40	16	17		

## APPENDIX 2

The mean number of Chaetogaster limnaei per snail at each stage of the life-cycle found in the monthly samples of 50 Lymnaea stagnalis.

Date	Mean no. of <u>Ch. l. limnaei</u> per snail	Mean no. of <u>Ch. l. vaghini</u> per snail			
		Immature	Mature	Viabile cocoons	Empty cocoons
Oct 1965	0.0	10.30	4.15	0.65	0.0
Nov	0.0	0.78	4.56	2.44	0.02
Dec	0.0	0.08	1.72	5.18	0.0
Jan 1966	0.08	0.04	0.12	2.00	0.06
Feb	0.06	1.36	0.04	0.86	0.30
Mar	0.12	3.58	0.0	0.36	0.20
Apr	0.06	8.08	0.0	0.46	0.26
May	0.20	24.04	0.0	0.02	0.14
Jun	0.0	14.20	0.0	0.14	0.30
Jul	0.0	12.40	0.0	0.0	0.06
Aug	0.04	8.90	0.0	0.0	0.0
Sep	0.10	9.00	0.0	0.0	0.0
Oct	0.06	0.62	6.28	0.54	0.0

Over/.....

Date	Mean no. of <u>Ch. 1. limnaei</u> per snail	Mean no. of <u>Ch. 1. vaghini</u> per snail			
		Immature	Mature	Viable cocoons	Empty cocoons
Nov	0.0	0.0	2.58	1.36	0.0
Dec	0.0	0.0	1.60	3.82	0.0
Jan 1967	0.02	0.14	0.36	2.60	0.06
Feb	0.02	1.04	0.06	1.70	0.52
Mar	0.0	2.58	0.12	1.20	0.62
Apr	0.0	6.20	0.0	0.18	0.58
May	0.0	18.50	0.0	0.08	0.60
Jun	0.0	20.80	0.0	0.0	0.16
Jul	0.0	4.48	0.0	0.0	0.12
Aug	0.0	3.20	0.0	0.0	0.0
Sep	0.0	4.20	0.0	0.0	0.0
Oct	0.0	0.18	1.50	0.04	0.0
Nov	0.0	0.0	1.90	1.30	0.0
Dec	0.0	0.0	0.88	1.88	0.14



## APPENDIX 3

The mean number of Chaetogaster l. vaghini per snail at each stage of the life-history, in each snail size group and at each monthly sample.

[For lengths of the snails in each size group see Appendix 1.]

Mean no. per snail

Date	Size group	No. examined	Mean no. per snail		Viabile cocoons	Empty cocoons
			Immature	Mature		
Oct	b	2			0.0	0.0
1965	c	3	Not differentiated		0.30	0.0
	d	12	between in first		1.50	0.0
	e	15	half of sample.		0.46	0.0
	f	11			0.27	0.09
Nov	b	3	0.0	0.33	0.33	0.0
	c	2	0.0	1.50	2.50	0.0
	d	3	0.0	6.33	8.00	0.0
	e	23	0.65	4.57	2.56	0.04
	f	18	1.11	4.11	2.22	0.0
	g	1	0.0	30.0	0.0	0.0

Date	Size group	No. examined	Mean no. per snail			
			Immature	Mature	Viable cocoons	Empty cocoons
Dec	b	1	0.0	0.0	0.0	0.0
	c	3	0.0	0.0	0.66	0.0
	d	4	0.0	0.25	2.25	0.0
	e	22	0.05	1.46	8.59	0.0
	f	19	0.16	1.89	3.59	0.0
	g	1	2.00	15.00	7.00	0.0
	Jan	b	2	0.0	0.0	0.0
1966	c	5	0.20	0.20	0.80	0.0
	d	5	0.20	0.0	5.60	0.60
	e	21	0.0	0.14	2.72	0.0
	f	16	0.0	0.12	0.25	0.0
	g	1	0.0	0.0	7.00	0.0
	Feb	b	1	0.0	0.0	0.0
	c	3	0.0	0.0	0.0	0.0
	d	2	0.0	0.0	0.0	0.0
	e	18	3.00	0.0	1.06	0.72
	f	25	0.56	0.08	0.92	0.08
	g	1	0.0	0.0	1.00	0.0

Date	Size group	No. examined	Mean no. per snail			
			Immature	Mature	Viable cocoons	Empty cocoons
Mar	d	2	8.50	0.0	0.0	0.0
	e	25	4.64	0.0	0.04	0.0
	f	21	1.86	0.0	0.38	0.48
	g	2	3.50	0.0	0.0	0.0
Apr	e	1	0.0	0.0	0.0	0.0
	d	2	0.50	0.0	0.0	0.0
	e	16	4.25	0.0	3.23	0.06
	f	23	11.52	0.0	0.44	0.44
	g	8	8.50	0.0	1.00	0.25
May	a	19	0.0	0.0	0.0	0.0
	e	2	53.50	0.0	0.0	0.0
	f	12	41.33	0.0	0.08	0.08
	g	15	37.60	0.0	0.0	0.40
	h	2	17.50	0.0	0.0	0.0
Jun	a	29	0.17	0.0	0.0	0.0
	b	5	3.60	0.0	0.0	0.0
	c	3	5.00	0.0	0.0	0.0
	d	1	8.00	0.0	0.0	0.0



## Mean no. per snail

Date	Size group	No. examined	Mean no. per snail		Viable cocoons	Empty cocoons
			Immature	Mature		
	f	3	85.66	0.0	0.66	1.33
	g	7	43.28	0.0	0.71	1.43
	h	2	52.50	0.0	0.0	0.0
Jul	a	2	0.50	0.0	0.0	0.0
	b	3	3.33	0.0	0.0	0.0
	c	22	5.23	0.0	0.0	0.0
	d	10	6.30	0.0	0.0	0.0
	e	2	11.00	0.0	0.0	0.0
	f	4	35.75	0.0	0.0	0.0
	g	6	38.17	0.0	0.0	0.50
	h	1	40.00	0.0	0.0	0.0
Aug	a	8	0.0	0.0	0.0	0.0
	b	6	3.00	0.0	0.0	0.0
	d	28	5.68	0.0	0.0	0.0
	e	3	3.66	0.0	0.0	0.0
	f	1	37.00	0.0	0.0	0.0
	g	4	55.00	0.0	0.0	0.0

Date	Size group	No. examined	Mean no. per snail			
			Immature	Mature	Viable cocoons	Empty cocoons
Sep	a	1	0.0	0.0	0.0	0.0
	b	2	0.50	0.0	0.0	0.0
	c	6	0.83	0.0	0.0	0.0
	d	22	4.64	0.0	0.0	0.0
	e	13	6.93	0.0	0.0	0.0
	f	3	38.66	0.0	0.0	0.0
	g	3	45.33	0.0	0.0	0.0
Oct	b	2	0.0	0.50	0.0	0.0
	c	8	0.0	2.00	0.13	0.0
	d	18	0.11	4.56	0.50	0.0
	e	15	0.44	7.60	0.94	0.0
	f	2	1.50	22.00	0.0	0.0
	g	4	3.00	12.52	0.0	0.0
	h	1	6.00	6.00	0.0	0.0
	Nov	b	2	0.0	0.0	0.0
c	13	0.0	0.85	0.69	0.0	
d	15	0.0	2.60	2.40	0.0	
e	13	0.0	2.77	0.77	0.0	
f	2	0.0	2.50	0.50	0.0	

Date	Size group	No. examined	Mean no. per snail			
			Immature	Mature	Viable cocoons	Empty cocoons
	g	4	0.0	8.50	2.75	0.0
	h	1	0.0	4.00	2.00	0.0
Dec	c	11	0.0	0.82	1.91	0.0
	d	17	0.0	2.29	7.58	0.0
	e	17	0.0	1.18	2.23	0.0
	f	2	0.0	0.0	2.50	0.0
	g	3	0.0	4.00	1.66	0.0
Jan	b	1	0.0	0.0	0.0	0.0
1967	c	8	0.0	0.38	1.38	0.0
	d	18	0.0	0.39	2.78	0.11
	e	19	0.0	0.58	4.21	0.05
	f	1	0.0	0.0	1.00	0.0
	g	2	0.0	1.00	0.0	0.0
	h	1	0.0	2.00	7.00	0.0
Feb	c	4	0.75	0.0	1.75	1.0
	d	19	1.95	0.11	1.58	1.05
	e	23	0.38	0.0	1.70	0.09
	f	2	0.50	0.50	4.00	0.50
	g	2	0.50	0.50	0.50	0.0



Date	Size group	No. examined	Mean no. per snail			
			Immature	Mature	Viable cocoons	Empty cocoons
Mar	c	2	0.0	0.0	0.0	0.0
	d	17	2.24	0.0	1.47	0.46
	e	26	3.00	0.19	1.15	0.88
	f	4	1.50	0.25	0.50	0.00
	g	1	5.00	0.0	2.0	1.00
Apr	d	6	3.33	0.0	0.33	0.50
	e	30	6.33	0.0	0.20	0.60
	f	13	5.94	0.0	0.08	0.80
	g	1	0.0	0.0	0.0	0.0
May	d	1	5.00	0.0	0.0	0.0
	e	25	21.24	0.0	0.16	0.76
	f	22	17.50	0.0	0.0	0.64
	g	2	4.00	0.0	0.0	0.0
Jun	a	5	0.20	0.0	0.0	0.0
	d	1	21.00	0.0	0.0	0.0
	e	20	18.70	0.0	0.0	0.30
	f	23	27.09	0.0	0.0	0.09
	g	1	21.00	0.0	0.0	0.0

Date	Size group	No. examined	Mean no. per snail			
			Immature	Mature	Viable cocoons	Empty cocoons
Jul	a	17	0.0	0.0	0.0	0.0
	b	18	1.17	0.0	0.0	0.0
	c	6	2.50	0.0	0.0	0.0
	d	1	4.00	0.0	0.0	0.0
	e	4	7.75	0.0	0.0	0.0
	f	4	38.25	0.0	0.0	0.0
Aug	a	1	0.0	0.0	0.0	0.0
	b	25	0.92	0.0	0.0	0.0
	c	17	2.00	0.0	0.0	0.0
	d	1	7.00	0.0	0.0	0.0
	e	3	17.66	0.0	0.0	0.0
	f	3	14.66	0.0	0.0	0.0
Sep	b	12	1.25	0.0	0.0	0.0
	c	29	2.31	0.0	0.0	0.0
	d	3	0.66	0.0	0.0	0.0
	e	3	15.66	0.0	0.0	0.0
	f	3	26.66	0.0	0.0	0.0

Date	Size group	No. examined	Mean no. per snail			
			Immature	Mature	Viable cocoons	Empty cocoons
Oct	b	4	0.50	0.75	0.0	0.0
	c	36	0.03	1.39	0.03	0.0
	d	4	0.0	1.50	0.25	0.0
	e	3	0.33	2.33	0.0	0.0
	f	<del>3</del>	2.00	2.33	0.0	0.0
Nov	b	5	0.0	1.20	1.50	0.0
	c	37	0.0	1.51	1.30	0.0
	d	4	0.0	2.25	0.25	0.0
	e	3	0.0	7.66	0.33	0.0
	f	1	0.0	1.00	0.0	0.0
Dec	b	4	0.0	0.25	0.25	0.0
	c	39	0.0	0.79	1.87	6.15
	d	4	0.0	2.00	3.25	0.00
	e	1	0.0	3.00	7.00	1.00
	f	2	0.0	0.50	0.0	0.0