

**Bangor University**

## **DOCTOR OF PHILOSOPHY**

### **An ecological study of *Chaetogaster limnaei*( von Baer)**

Gruffydd, Llyr Dafis

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Bangor University

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AN ECOLOGICAL STUDY  
OF CHAETOGASTER LEHMANI (von Baer).

A THESIS

submitted to the University of Wales by

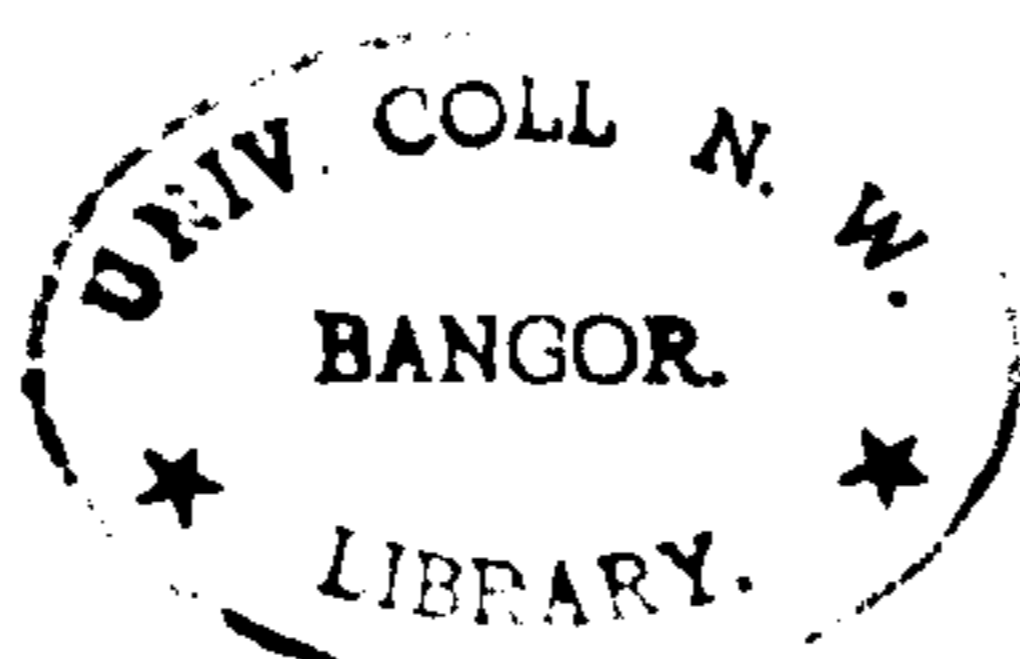
LLYR DAFIS GRUFFYDD

in candidature for the degree of

PHILOSOPHIAE DOCTOR

Department of Zoology,  
University College of North Wales,  
Bangor.

April 1963



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Poor text in the original  
thesis.

Some text bound close to  
the spine.

Some images distorted

Errata.

Details of setal lengths on Pages 23 and 24 should read :-

	Segment 2	Segments 6, 7 & 8.
Length of setae of outer form	From 72 to 96 $\mu$	From 47 to 54 $\mu$
Average length	85 $\mu$	50 $\mu$
Length of setae of kidney form	From 51 to 68 $\mu$	From 41 to 49 $\mu$
Average length	60 $\mu$	45 $\mu$
Length of setae of a mature <u>Chaetogaster</u> (Kidney form). (average)	49 $\mu$	42 $\mu$

*L.D. Grant*

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## Section 1.

## INTRODUCTION.

Chaetogaster limnaei (v. Baer) is one of very few Oligochaeta that have formed associations with other animals. Its relationship with freshwater pulmonates is usually described as commensalism (p.138), but this has by no means been universally accepted. The discovery of the worm in the snail's kidney led some to believe that it could adopt a parasitic mode of life. Vaghin (1946) went further and suggested that the commensal form living on the outer surface of the snail and the form found in the kidney belonged to two distinct populations. The main aim of this work was to study the ecology and population dynamics of these two forms and it was hoped that the results would help to explain the relationships that exist between these two forms of Ch. limnaei and their host.

Lymnaea pereger (Müll.), being common and found in fairly large numbers in North Wales, was chosen as the source of Chaetogaster limnaei used in this investigation. Two large populations of this snail were selected for study and were sampled for a period of two years between January 1960 and February 1962. Experimental work was carried out in the field and in the laboratory to test and extend ideas derived from the field data. In all cases L. pereger was used as the host.

The response of Ch. limnaei to various stimuli associated with discovery of the host was observed, and a brief report on the morphology and anatomy of the sexual form is presented. This part of the work however can only be considered as an introduction to the investigation of



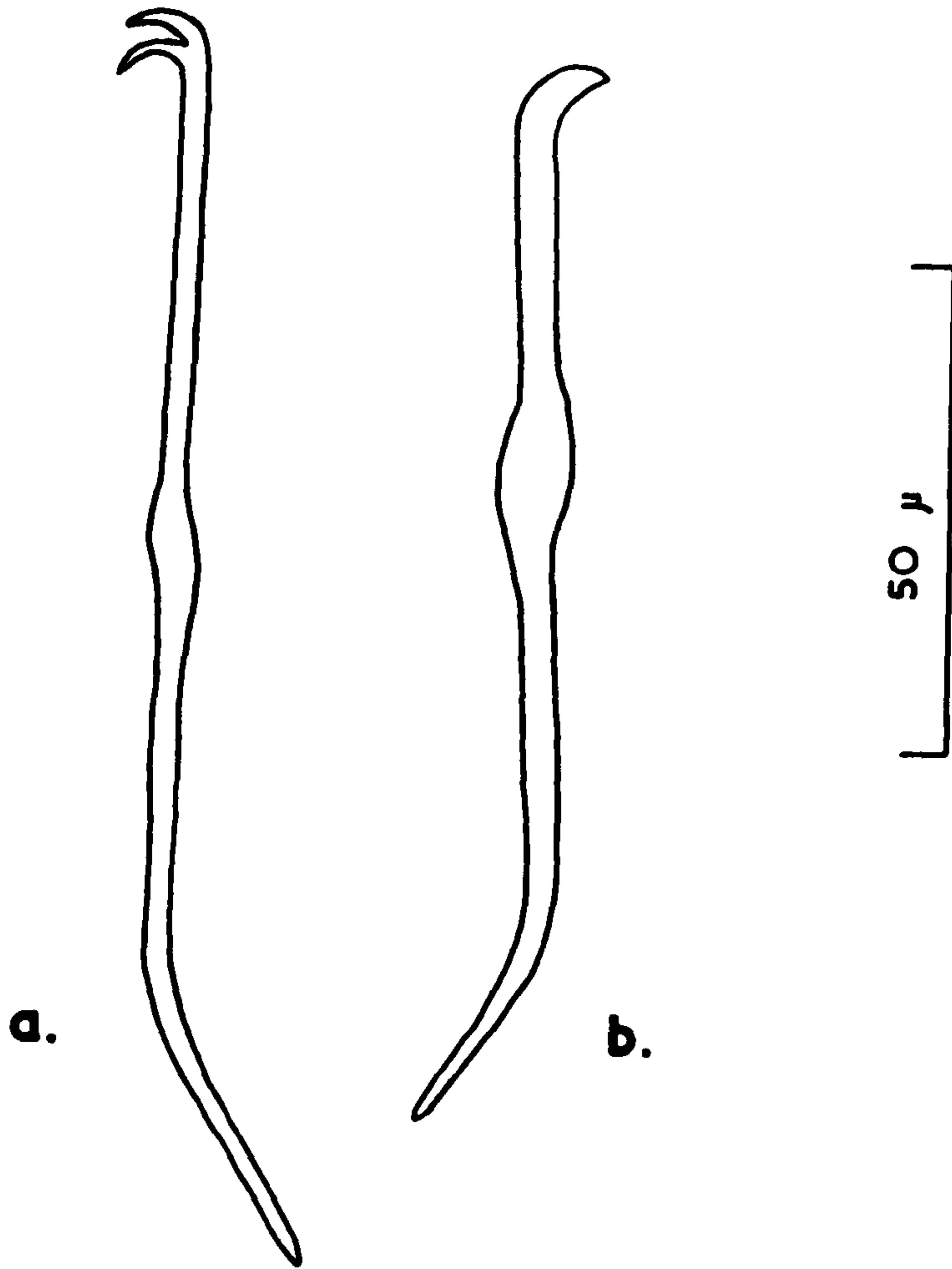


Fig. 1. (a) Ordinary seta from segment 2 of an outer form.  
(b) Genital seta of a kidney form.

a complex problem that has hitherto remained unexplored.

## Section 2.

### REVIEW OF THE LITERATURE.

Chaetogaster limnaei is an oligochaete worm belonging to the family Naididae. The genera Chaetogaster and Amphichaeta are grouped together by Sperber (1948) in the subfamily Chaetogastrinae because they are distinguished from all other Naididae by a number of characters. These are a lengthened pharynx without a dorsal diverticulum and without any gland cells connected to the body wall by numerous radial muscular strands, a short narrow oesophagus, a stomach sharply marked off from both oesophagus and intestine, a strongly reduced vascular system and closed nephridia. No hair setae or eyes are present in either of the genera. In the genus Chaetogaster the dorsal setae are wholly absent and the ventral setae of segments 3 to 5 also have been lost. The septa are strongly perforated and there is often a statocyst present in the brain. The setae of this genus are fairly straight with strongly curving teeth at the distal end. This curve is accentuated in the setae of Chaetogaster limnaei (Fig. 1) and the number of setae here is high compared with that of other Chaetogaster species. Sperber states that segment 2 has 5 to 20 setae per bundle and the following setal bearing segments 4 to 20 per bundle. She also records Ch. limnaei as having no statocyst in the brain.

Chaetogaster limnaei was first described by von Baer in 1827. He described it as an annulated worm possessing rows of setae in pairs

along its ventral surface. One pair was situated at the anterior end of the worm and the posterior end he described as carrying setal bundles at varying distances from each other. He found no eyes. The worms used the setae in crawling and no swimming movements were observed. The buccal aperture when open had the shape of a sucker, and leading from this to the prestomach was a narrow oesophagus. Posterior to this prestomach was the part of the gut he named the true-stomach. The prestomach and stomach described by v. Baer seem to correspond to what are generally considered to be the stomach and the intestine respectively (Sperber 1948). Von Baer reported finding Ch. limnaei in the mantle cavity and in the kidney of Lymnaeidae as well as in the mantle cavity of Planorbis cornuus. He also found the worm living free in water which was inhabited by pulmonate snails. He was not sure whether or not these were forms that had originated in the kidney and had been liberated from the kidney with the urine. He was certain however that one could not regard the presence of the worm in the kidney as a chance occurrence, or as a temporary sojourn necessary to complete its development.

Lankester (1869a) described Chaetogaster limnaei as a minute whitish creature living on the surface of the body and in the kidney of freshwater snails. He failed to find the worms during the winter months, but in the summer they were plentiful.

Willcox (1901) reported finding the worm living on the head and in the respiratory cavity of Physa heterostropha. He states that a few of the worms had anchored themselves to the host snail by embedding their posterior end in the snail tissue.

In 1905, Annandale described a worm closely resembling

Ch. limnaei (Ch. limnaei bengalis) living on the outer surface of lymnaeids. He maintained that when the Chaetogaster population grew excessively large or when the water became warm or foul, the worms left their host and became free-living.

Michaelsen (1926) had reason to believe that Ch. limnaei also lived as a commensal on crayfishes. He found the worm in the bottom of a bottle containing alcohol in which he had preserved a crayfish.

Welf (1928) reported finding Ch. limnaei free-living in a habitat in which Lymnaea and Planorbis lived in large numbers. He said that they were only found thus during the aestivation period of the host snails.

Wagin (1931) found Ch. limnaei in great numbers in the mantle cavities of pulmonates. He adds that he never found the worm in the free-living state.

In a report on the varieties of Lymnaea pereger of Irish lakes, Boycott, Oldham and Waterston (1932) mention that only one of eighteen populations examined was infested with Ch. limnaei. In a later paper Boycott (1936) discusses the causes of mortality in the young forms of freshwater molluscs and was of the opinion that Ch. limnaei is not a contributory cause of this mortality.

Krasnodebski (1936) investigated feeding in Ch. limnaei. He found the worm usually crawling on the head and body of aquatic molluscs. Table 1, (p. 5) reproduced from his paper, shows the degree of infestation by Ch. limnaei on various species of freshwater snails. He also found Ch. limnaei living away from its host on the leaves of Elodea canadensis and other aquatic plants.

Chen (1940) reported finding Ch. limnaei and Ch. limnaei

Table 1.

Species	% infestation
<i>Lymnaea stagnalis</i>	85
<i>Radix auricularia</i>	77
<i>Radix ovata</i>	76
<i>Stagnicola palustris</i>	88
<i>Coretus corneus</i>	83
<i>Planorbis planorbis</i>	46
<i>Spiralina vortex</i>	67
<i>Ancylus lacustris</i>	30
<i>Physa fontinalis</i>	100

Table 2.

Species	% infestation
<i>Lymnaea stagnalis</i>	80 - 100
<i>Lymnaea ovata</i>	100
<i>Lymnaea pereger</i>	50 - 70
<i>Planorbis corneus</i>	80 - 100
<i>Planorbis marginatus</i>	50 - 70
<i>Ancylus fluviatilis</i>	5 - 7
<i>Physa fontinalis</i>	3 - 15
<i>Sphaerium</i> sp.	1 - 2
<i>Pisidium</i> sp.	1 - 2
Unionidae (all sp.)	0

bengalis as commensals in the mantle cavity of freshwater snails in China.

Wallace (1941), whilst working on the life history of a trematode parasite of Ch. limnsei, found the oligochaete occasionally feeding on the substratum some distance away from its host. The trematode in question, Tricpanodistomum gutabile (Cort), can cause the death of Chaetogaster by becoming so large in the coelom of the worm that it prevents the passage of food through the gut.

Vagin (1946) while studying the biological cycle of Ch. limnsei obtained the values presented in Table 2 (p. 5) for the percentage infestation of various molluscs by the worm. He concentrated his studies on the Chaetogaster populations of Lymnaea ovata (L) and Lymnaea stagnalis (L). He found the worm on the external surface, in the mantle cavity and also in the kidney of these molluscs. The Chaetogaster living in the kidney he considered as being an endoparasite; and the others inhabiting the mantle cavity and the outer surface of the snail as commensals. His reasons for saying this will be presented later in this section. He said that the commensal form obtained only shelter from the host, and attached itself to the snail by clinging to its surface layer of mucus. In cases of severe infestation some of these forms appeared on the external surface of the mollusc's shell.

Bayer (1955) found Ch. limnsei in the respiratory chamber and along the outer lip of the shell of the South African snails Biomphalaria Pfeifferi, Physopsis africana, Bulinus tropicus and Melanooides tuberculata. He did not find the worms living on any of the South African Lymnaeidae.

a. Life cycle and reproduction of Chaetogaster limnasi.

Von Baer (1827) noticed that Chaetogaster limnasi, in common with all other Naididae, multiplied asexually by producing a chain of buds at its posterior end. He observed individuals possessing up to three budding zones and remarked that all the buds were at different stages of development. In late autumn he found the eggs of Ch. limnasi which he described as having a thick transparent cover open at both ends. Inside this was another layer surrounding the embryo and projecting into both the ends to form plugs thus sealing the openings. He does not state where he found the cocoons but the inference is that they were found in the kidney.

Claus (1860) described the sequence of budding in Ch. limnasi. The series of numbers below represent his description of (a) a three bud form and (b) a seven bud form, the numbers representing the buds in order of appearance, 0 being the parent animal.

(a)        0    2    1    3

(b)        0    4    2    6    1    5    3    7

Lankester (1869a) observed Ch. limnasi possessing chains of 16 buds or sooids, the first zone of division occurring behind the third abdominal setal bundle (segment 8). In his papers (1869 b,c) he describes the sexual form of the worm. Compared with the immature form this had approximately double the number of setae in each bundle, i.e., instead of 12 in the first bundle it had between 20 and 30, and instead of 8 in each of the other bundles it had 16. Four club shaped setae were also seen in front of each of the first setal bundles of the abdominal region (i.e., those of segment 6) in the mature specimens. This mature form, which was observed

in October, was not producing buds.

Whereas Lankester found differences between the setae of mature and immature forms, Piguet (1906) found two types of immature forms differing in respect of the number and length of their setae. One form had about 6 to 8 setae per bundle and the other had about 8 to 20 in each bundle. The former also differed from the latter in that it had shorter setae.

The worms observed by Wilcox (1901) were all actively budding and there were no sexual forms among them. Stephenson (1915) also states that sexual forms of Naid worms are very rarely seen and in some worms are never seen.

Wagin (1951) found mature gonads in the autumn but he did not find any cocoons.

A considerable amount of light was thrown on the breeding activity of Ch. lignesi by Vaglin in 1946. He treated the kidney and outer populations separately because he believed that they consisted of separate 'biological species' of Ch. lignesi. He based this belief on the fact that their habits were different and that their life cycle also differed slightly. He found the outer form reproducing asexually throughout the summer, often forming chainlets of upto 11 individuals. Towards late November the number dropped to about five and this condition prevailed until the early summer of the following year. In August he observed sexually mature individuals to the extent of 12 to 15 per cent of the population. No cocoons were found. Thus he maintained that the main mode of reproduction of this outer form was asexual and that the numerical relationship between the asexual and sexual individuals was similar to that of most Naididae. Vaglin found the



kidney form indistinguishable morphologically from the outer form. He found, however, that the percentage of worms that became sexually mature was far greater here than in the outer population. In July, the kidney forms each possessed between 4 and 5 buds and asexual reproduction continued until the end of August when between 4 and 10 per cent of the population became mature. During the autumn the percentage of mature worms increased, and by the beginning of November 100 per cent of the population was sexually mature. No individuals with buds were present. Cocoons appeared in the kidney of the molluscs and embryonic development proceeded in the kidney. The breeding worms, after depositing their eggs apparently died in the kidney and remains of some of these were found in the kidney. At the end of December nothing but cocoons could be found in the kidney on dissection. Young worms hatched from the cocoons in May and soon after hatching propagation by way of budding began. So here Vaghin maintained there was complete alternation of generations. He suggested that transfer from one mollusc to another occurred at the time of copulation during the summer, the worms crawling from the kidney into the mantle cavity and then crossing to the other snail.

Some mollusc populations Vaghin studied were often subjected to severe drought conditions and to escape desiccation the snails buried themselves in sand. This resulted in the death of large numbers of the outer forms of Ch. linnaei. He does not state whether or not the kidney forms survive.

Vaghin believed that the two forms of Ch. linnaei had diverged in fairly recent times and that the outer form was ancestral. He also believed that the kidney form showed a recapitulation of the ancestral mode

of life when it temporarily left the kidney in order to move onto a new host. The absence of morphological differences between the two forms led him to believe that this was the very beginning of the divergence of two species.

Bayer (1955) noticed that mechanical stimulation of a budding individual caused the separation of fully developed buds from the parent worm and that further stimulation resulted in the breaking off of partly developed buds.

b. Feeding in *Chaetogaster limnaci*.

Most reports on feeding in *Chaetogaster limnaci* are concerned with the significance of the fact that *Chaetogaster* will destroy trematode cercaria. Lankester (1869a) mentioned that the outer form fed on cercaria as well as on rotifers and Protozoa while the kidney forms fed on cells derived from this organ. Wilcox (1901) however reported finding only diatoms in the gut of *Ch. limnaci*.

Krasek (1917) found cercaria in the gut of *Ch. limnaci* and this find prompted his investigation of the feeding behaviour of the worm. He states that the worm on detecting the presence of a moving cercaria in the immediate vicinity makes a quick movement and engulfs it. He seems convinced that *Ch. limnaci*, in swallowing great numbers of cercaria play an important part in the control of trematode larvae.

Wagin (1931) states that *Ch. limnaci* feeds on various Protozoa, Rotifera and young Cladocera as well as on diatoms. He found that when the host snail was infected by cercaria many of the *Chaetogaster* had

these in their gut. The worm only attacked the cercaria if the latter moved and resting cercaria were not touched. He said that Ch. limnaei must be considered as a significant factor in controlling trematode infections since the oligochaete is found on snails in very large numbers. He also thought that it would be advisable to introduce Ch. limnaei into ponds where it was not found, in order to attempt to control the spread of parasitic trematodes.

Krasnodabski (1936) thought it worth while to verify Wagin's observations. He found that the principal food of Ch. limnaei consisted of animal material and that plant material was only taken in large quantities when the former was not available. Protozoa were eaten consistently and Cladocera, Ostracoda and Copepoda were frequently found in the gut. He was fairly certain that Ch. limnaei could become cannibalistic at times and he based his theory on the finding of Ch. limnaei setae in the gut of a few of the worms. He found, as Wagin did, that at the time when cercaria leave the host snail many of the worms had cercaria in their gut. Approximately 72% of the worms found on trematode - infested snails had eaten cercaria. Krasnodabski agreed with Wagin that Ch. limnaei could consume a considerable number of cercaria if it were present in large numbers, but on the other hand he thought that the number of cercaria escaping from a snail is so large that probably only a small percentage of them is eaten.

Krasnodabski, having found Ch. limnaei living away from the host, e.g., on Flodas, decided to attempt to keep Ch. limnaei alive under laboratory conditions in the absence of the host snail. He kept five worms in each of many small vessels and fed them on ciliates. He succeeded in keeping the worms alive in one vessel at 18°C for 63 days. In vessels which

were kept at 24 to 26°C the worms did not live for more than 7 days. During the course of these experiments he noticed that many of the worms divided, causing their number in each dish to fluctuate a great deal. Chen (1940) also succeeded in keeping free-living Ch. limnaei alive for a period of time which he does not specify.

Vaghin (1946) mentions the fact that the Chaetogaster inhabiting the kidney of molluscs feed on cells derived from the kidney. He also states that these kidney forms are able to adopt a temporarily free-living mode of life whilst feeding on small organisms but he does not provide any evidence to support this statement. He found various small organisms including Rotifera and Protozoa in the gut of the outer form of the worm.

Ruis (1951) also believed that Ch. limnaei could be an important factor in the control of trematode infections. He found that mollusc infestation by larval forms of trematodes was connected inversely with infestation by Ch. limnaei. Populations of Australorbis glabratus heavily infected with trematode larvae harboured few or no Ch. limnaei, and conversely populations heavily infested with Ch. limnaei were not heavily infected with the trematode parasite. He thought that this was due to the predatory action of Ch. limnaei on the miracidia as well as on the cercaria themselves.

Bayer (1955) reporting on the presence of Ch. limnaei on South African snails felt that although the worm helped to combat trematode infections it was not present in sufficient numbers to play a major part in controlling the parasite.

Hunter (1960 p.e.) put forward the theory that under certain circumstances the outer form of Ch. limnaei could become a symbiont or even

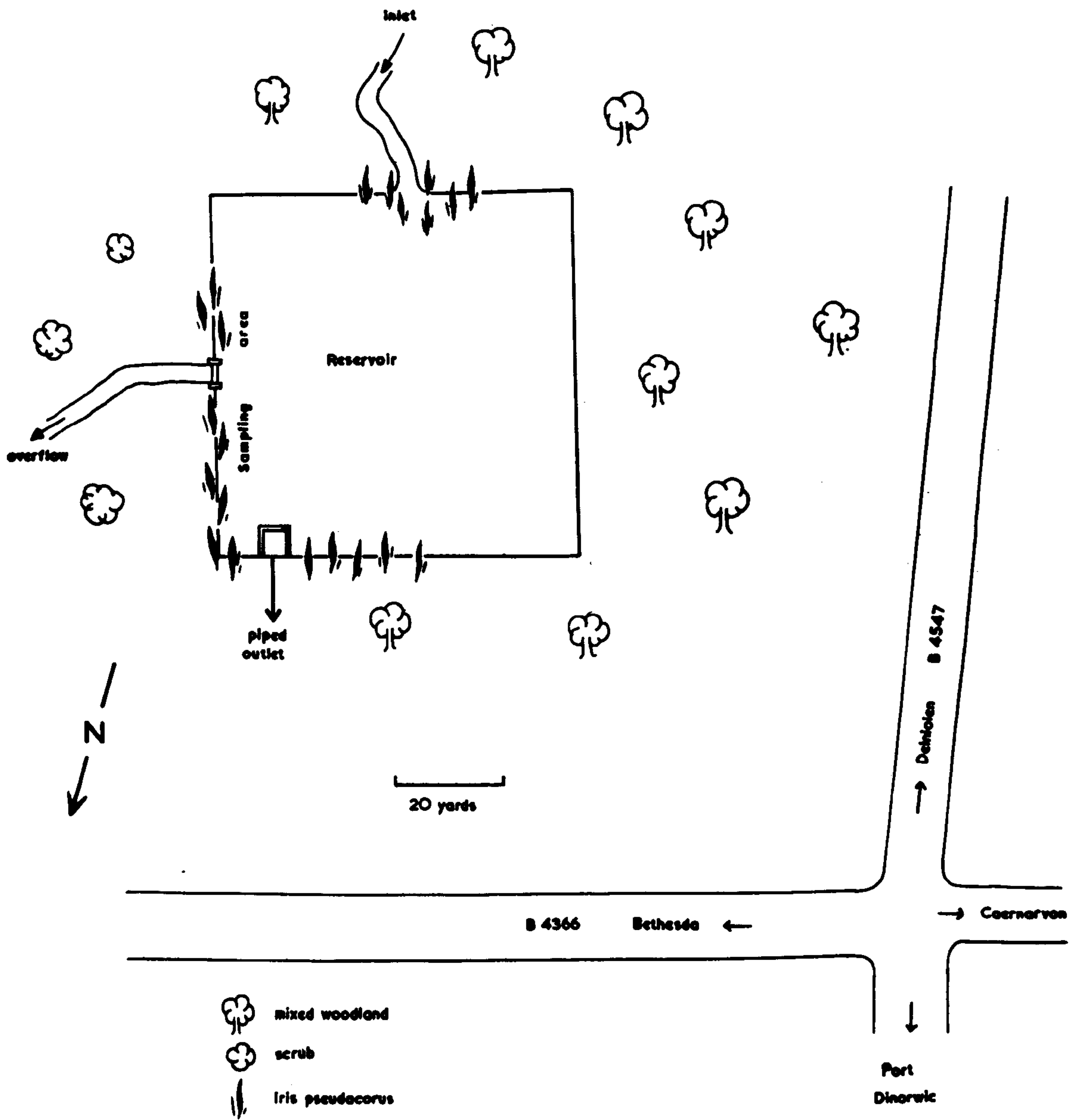


Fig. 2. Plan of the reservoir and its surroundings.

a parasite because of its feeding habits. Although the Chaetogaster - host relationship is clearly an example of commensalism in populations of Lymnaea pereger, he believes that it can be of some significance as a symbiont in certain populations of Ancylus fluviatilis where it assists in cleaning the poorly ciliated mantle structures. On the other hand, in populations of Bithynia tentaculata where a ciliary feeding mechanism is important, the worm can be almost parasitic as it ingests pieces of the 'food string' formed by the snail.

### Section 3.

#### MATERIALS AND METHODS.

##### a. Habitats and field work.

The host snail used throughout this work was Lymnaea pereger. Chaetogaster limnaii was found in the kidney and on the outer surface of the snail. A population of this snail was sampled at fortnightly intervals from January 1960 to January 1961, and at monthly intervals from February 1961 to February 1962 in a reservoir which is situated at the crossing of the main Caernarvon - Bethesda and the Port Dinorwic - Deiniolen roads.

The reservoir is about one acre in area and square in outline. It is fed by a stream entering half way along one side and an overflow leaving half way along an adjacent side controls the maximum level of the water. It was only during very dry periods that the level fell below the maximum. Sampling was carried out along the outlet side of the reservoir only (Fig. 2). The shore here shelves steeply to a depth ranging between

one and two feet but thereafter the gradient is much less severe. The bottom is covered by a deep layer of mud. Elodea and Potamogeton grow in large quantities in the water and it was on these water plants that the L. peregrin occurred. Apart from a few Planorbis albus and Lymnaea palustris, no other molluscs were found. On p. 15 are listed the animals commonly found in this habitat and the results of the chemical analysis of the water.

Monthly samples were also taken between February 1961 and January 1962 from a slow moving lowland stream at Coed Mawr, near Bangor. This stream is about four feet wide and one to two feet deep. The banks are steep and here again the bottom is of mud. The dominant plants are Potamogeton and Elodea, and as in the reservoir L. peregrin was found in large numbers on these aquatic plants. The level of the water in the stream fluctuated a great deal, rising after heavy rain and falling <sup>in</sup> dry periods, but it was not observed to dry up completely at any time during this sampling period. On page 16 is a list of the animals found in the stream and the results of the chemical analysis of the water.

Sampling, in both habitats, was carried out using a sweep net. A nylon material with a mesh of 40 strands to the inch was used for the net. This was fine enough to prevent the escape of very small snails during sampling. The time taken to obtain a sample was used to provide a rough indication of fluctuations in snail population size (Hairston et al 1958). During the snail's breeding season, all vegetation brought out of the water by the net was searched in the laboratory for young snails.

b. Sampling.

## Reservoir fauna.

Annélida.Oligochaeta

- : *Lambriculus variegatus* (Mull.)
- Stylaria lacustris* (L.)
- Gnathogaster limnaei* (v. Baer)

Hirudinea

- : *Glossiphonia heterocolita* (L.)
- Glossiphonia complanata* (L.)
- Hellobdella stagnalis* (L.)
- Erpobdella octulata* (L.)
- Theromyzon tessulatum* (Mull.)

Platyhelminthes.Turbellaria

- : *Polycolis tenuis* (Ijima)
- Polycolis nigra* (Mull.)

Mollusca.Gastropoda

- : *Lymnaea pereger* (Mull.)
- Lymnaea palustris* (Mull.)
- Planorbis albus* (Mull.)

Pelecypoda

- : *Sphaerium* sp.

Insecta.Trichoptera

- : *Polycentropid* sp.
- Triacnodes* sp.
- Limnophilus* sp.

Neuroptera

- : *Nepa cinerea* (L.)

Odonata

- : *Coenagruid* larvae (2 sp.)

Coleoptera

- : *Gyrinus* sp. larva

Diptera

- : *Culex* sp. larva
- Chaoborus* sp. larva

Arachnida.Acarina

- : *Hydracoid* mite

Vertebrata.Amphibia

- : Smooth newt
- : Spawning toads

Pisces

- : 3 spined stickleback
- Trout (reported to be present)

## Analysis of water.

Total hardness of water in terms of Calcium	=	18.24 mgms. per litre.
Calcium	=	10.00 mgms. per litre.
Chloride	=	13.80 mgms. per litre.



## Stream fauna.

Annelida.

Oligochaeta  
Hirudinea

- : Chaetogaster limnaci (v. Baer)  
: Erpobdella octulata (L.)  
Glossiphonia complanata (L.)

Platyhelminthes.

Turbellaria

- : Polycolis tenuis (Ijima)

Mollusca.

Gastropoda

- : Lymnaea pereger (Mull.)  
Physa fontinalis (L.)  
Planorbis albus (Mull.)

Pelecypoda

- : Sphaerium sp.

Insecta.

Hemiptera  
Coleoptera

- : Corixa falleni (Fieb.)  
: Haliphus lineaticollis (Marsh)  
Dytiscid sp. (prob. Platambus maculatus (L.) )

Crustacea.

Isopoda

- : Asellus sp.

Vertebrata.

Amphibia

- : Smooth newt  
Spawning toads

Pisces

- : 3 spined stickleback

## Analysis of water.

Total hardness in terms of Calcium = 43.52 mgms. per litre.  
Calcium = 25.20 mgms. per litre.  
Chloride = 23.60 mgms. per litre.

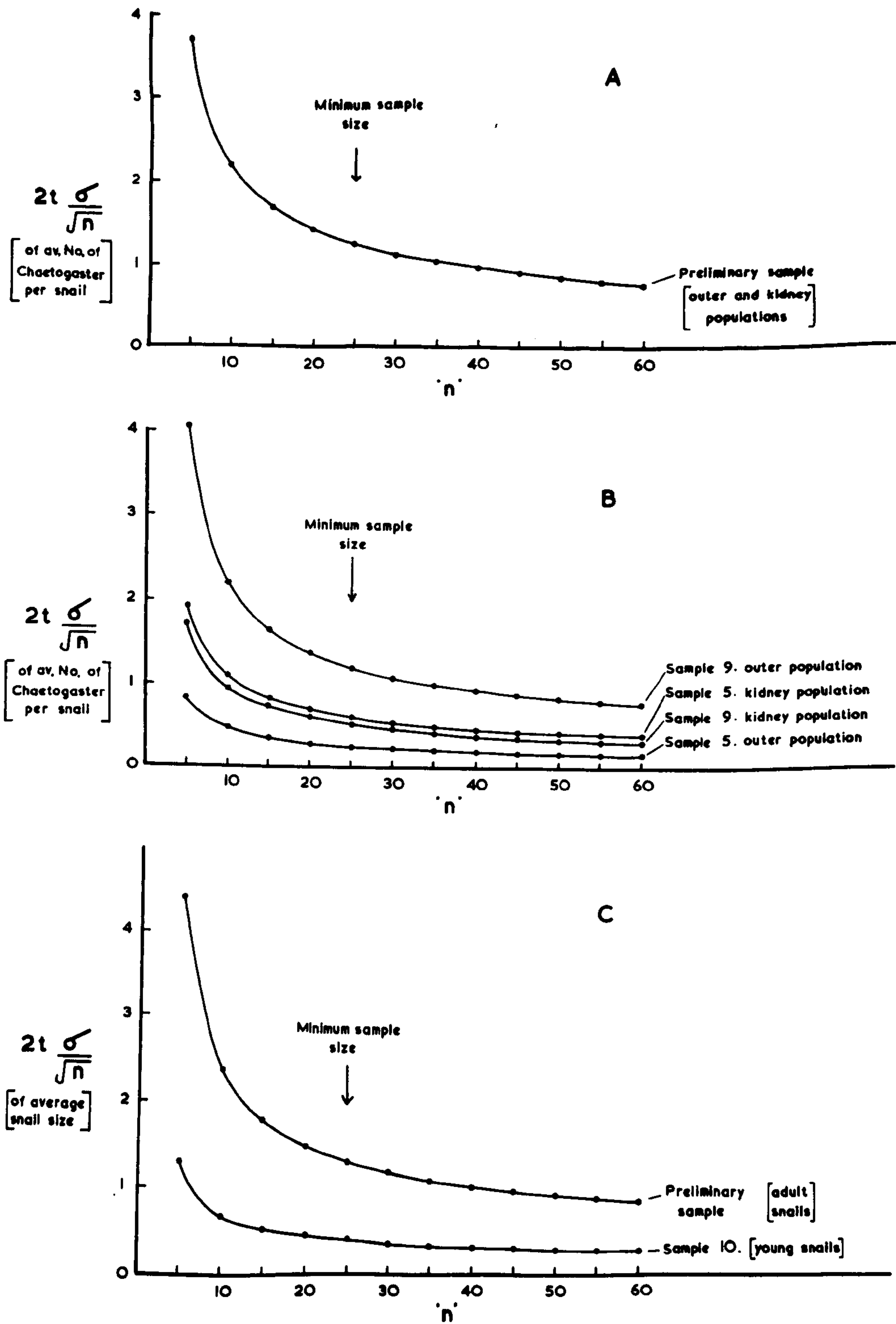


Fig. 3. The relation between fiducial limits and sample size for (A) average total number of Chaetogaster per snail, (B) average number of outer and kidney Chaetogaster per snail in samples 5 & 9, and (C) snail size in a sample of adult snails and a sample of young snails.

In any sampling scheme it is necessary to balance labour in dealing with samples against the reliability of the information it provides. As a guide to the appropriate sample size bearing these two aspects in mind, the fiducial limits of the mean values for the Ghaetogaster population were calculated from a preliminary sample (Fig. 3A). This shows that below a sample size of about 25 snails the reliance which can be placed upon the mean deteriorates rapidly while above 25 it improves relatively slowly. That is to say, there was little return for time spent above this size of sample. However, to provide some safety margin for variation in proportion and intensity of host infestation a sample size of 45 snails was decided upon. Later samples (samples 5 & 9) were also examined in this way, treating the kidney and outer forms of Ghaetogaster separately (Fig. 3B). In sample 5 the population was small, whilst in sample 9 it was large. Nevertheless, it is seen that in both cases a minimal sample size of 25 snails is suggested as appropriate.

Figure 3C shows that the minimal desirable size of sample for estimating snail size was also around 25. For a sample of young snails this dropped to 15 because of uniformity. However, since this parameter is quickly measured, samples taken from the reservoir during 1960 usually consisted of about 100 individuals. As far as could be foreseen at the time it was considered that this number of snails removed once a fortnight from the population would only deplete this by a negligible amount. Samples taken from the reservoir and from the stream during 1961 usually exceeded 25 but were considerably smaller than those of the previous year in order that more time could be devoted to experimental work.

Analysis of samples of different mean values were also made

in respect of Chaetogaster to study the distribution of the individual values around the mean. These (Figs.17,18 p.37 ) showed that at low mean values the distribution approximated to the Poisson type, at high mean values to the Normal type while at middle values it was intermediate. Since it was desirable to evaluate the relative variance at different population levels the coefficient of variation was calculated on transformed data. An appropriate transformation to an approximate Normal distribution was obtained by using the term  $\sqrt{x + 24}$ , where  $x$  is the actual value recorded. The coefficient of variation is an index of relative or proportional variability expressed as a percentage and calculated by expressing the standard deviation as a percentage of the mean. The results are shown in Table 3 (p. 19) and it is seen that all coefficients lie between 4 and 18%. Since the range of these limits is small and the upper limit is not excessively high, the use of the mean to represent the size of the population was considered justifiable. A general characteristic of the coefficient here is that it tends to increase with the mean.

c. Laboratory treatment of samples.

The measurement taken to represent the size of the snail was the length of the shell from the tip of the spire to the leading edge of the aperture. This was estimated to the nearest 0.1 mm. using a micrometer screw gauge. After being measured, all the snails were then placed in separate containers. This was necessary to prevent the migration of Chaetogaster from one snail to another which would occur if the snails were kept together in one dish for the four or five days taken to investigate

Table 3.

Sample	Outer forms			Kidney forms		
	Mean No. of worms per sn'l.	Mean of $\sqrt{x + 2}$	Coeff. of var.	Mean No. of worms per sn'l.	Mean of $\sqrt{x + 2}$	Coeff. of var.
5	4.29	5.31	5.49	8.07	5.62	12.40
9	42.64	8.04	18.12	15.27	6.24	9.82
14	8.80	5.67	13.81	1.13	5.01	4.06
20	8.24	5.64	12.17	6.24	5.46	12.62

--- = incision

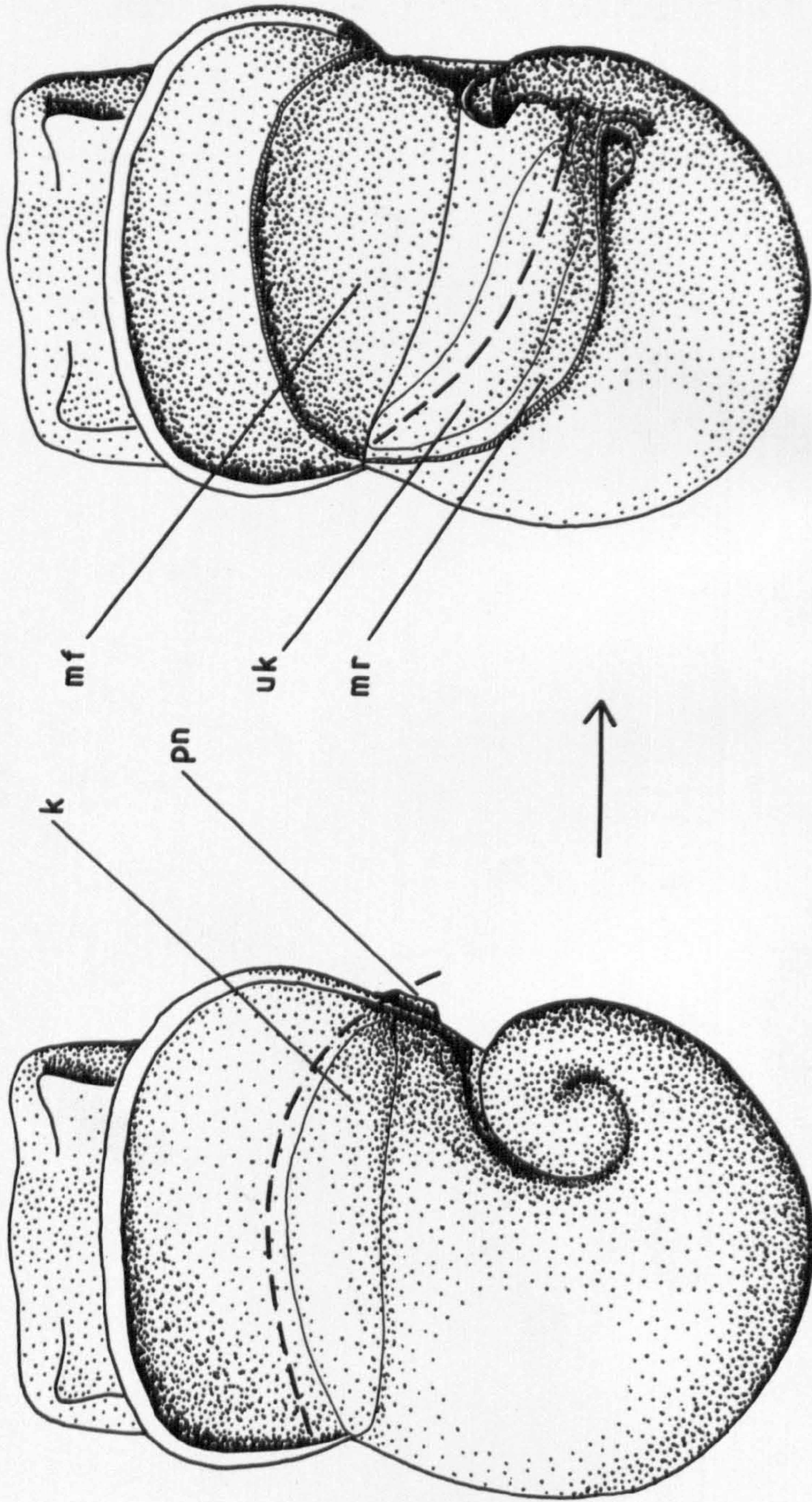


Fig. 4. Showing the process of dissection of *L. pereger* after removal of shell. k, kidney. mf, floor of mantle cavity. mr, roof of mantle cavity. pn, pneumostome. uk, under side of kidney.

one sample.

From each sample, 45 snails were selected at random and each snail was searched for Gh. lignaei in the following way. The snail's shell was removed and the body pinned down under water. All Gh. lignaei found on the outer surface of the snail were removed using a test pipette. This was done quickly before the Ghaetogaster became entangled in mucus secreted by the snail. An incision was then made across the roof of the mantle cavity immediately in front of the anterior border of the kidney. This enabled the kidney to be turned back to expose the mantle cavity and any worms dwelling here could be removed. Finally, the kidney was split open and the Ghaetogaster therein removed. This whole process is summarised in Fig. 4. The number of worms found in each of these three sites was recorded.

The number of buds per individual was also recorded for Ghaetogaster obtained from a sub-sample of 25 snails randomly selected from the original 45. In the treatment of the samples of the second year at the reservoir and those from the stream at Coed Mawr, the observations on budding in Ghaetogaster were omitted. These samples, being fairly small, were not subdivided and all the snails brought in were dissected.

Records were kept of the number of Glossiphonia heteroclita found in the mantle cavity of the snails.

The water temperature was always recorded at the time of sampling.

#### d. General.

Lymnaea peregra not infested with Ghaetogaster lignaei were

obtained for use in experiments by breeding from eggs in large tanks in the laboratory. The snails were fed throughout on an artificial food prepared according to the following method described by Standen (1951) and modified by Ollerenshaw (pers. comm.). Sixteen gms. dried powdered grass, 16 gms. Froment and 8 gms. dried milk were mixed in 1600 mls. of hot water. Ten gms. of sodium alginate were added and the mixture was poured into a shallow container and left to cool. A solution of 32 gms. calcium chloride in 1600 mls. of water was then added slowly to one corner of the container so that it ran underneath the mixture; this was then left overnight. The resultant gel of calcium alginate containing the Froment, powdered grass and dried milk was insoluble in water. The gel was washed thoroughly and stored in a refrigerator. Small pieces of the gel were fed to the snails every few days and any stale food in the tanks that had not been eaten was always removed. After about a week the food becomes sour and so fresh food had to be prepared once a week.

The pond water in the tanks was constantly aerated and changed once a fortnight. Daphnia were kept in the tanks to prevent excess bacterial growth (Standen 1949).

Non-infested L. pereger from Bardsey Island were also used in experiments. These were collected from a small shallow stream on the island and were to be found crawling on water plants and on the muddy bottom in this stream. These snails were collected in the spring and at that time they were plentiful. It has been reported that this stream often dries up completely in summer and so it seems that this population of L. pereger is often subjected to severe drought conditions. Such conditions may be the reason why Ch. limnaei was not present. Drought conditions can



cause a high rate of mortality amongst outer forms (Vaghin 1946), and if drying up occurred during the period when both forms of the worm were leaving a dying generation of snails, the kidney population could also be similarly affected.

In all experiments snails were kept in filtered pond water in glass dishes at 7°C and fed on the artificial food.

Cocoons of Gh. limnesi were incubated either at 7°C or  $15 \pm 2^\circ\text{C}$  in water, as well as in 0.3% saline and in snail kidney extract. This strength of saline is isotonic with the urine of L. pereger (Picken 1937). The kidney extract was prepared by crushing large numbers of L. pereger kidneys with a little 0.3% saline, and filtering to remove the kidney tissue.

Sections of mature kidney forms, immature kidney forms and immature outer forms of Gh. limnesi were cut in order to ascertain the position of the reproductive organs and to compare the internal anatomy of the kidney and outer forms. The worms were anaesthetised using chlorotone in distilled water and fixed in Bouin's solution. Longitudinal and transverse sections of the worm were cut at  $7.5\mu$  and stained with Haematexylin and Eosin. Sections of infested and non-infested L. pereger kidney were also prepared by this method, the kidney having first of all been removed from the snail and fixed in Bouin's solution.

## Section 4.

THE MORPHOLOGY AND ANATOMY OF THE TWO TYPES OF CHAETOGASTER LINNAEI.

This section deals with the differences that were found in the morphology and anatomy of the outer and kidney forms. The differences found were in the number and size of setae, and in the thickness of the gut endothelium.

a. Length of setae.

Chaetogaster linnaei has no dorsal setae and the ventral setae are absent in segments 3 to 5. The setae are double pronged, the teeth being strongly hooked (Fig. 1. p. 2).

The lengths of the setae from one bundle in each of segments 2, 6, 7 and 8 of five randomly selected outer forms and five randomly selected kidney forms were measured. The results are presented below.

## Outer form.

	Segment 2.	Segments 6, 7 & 8.
Length of setae.	From 145 $\mu$ to 192 $\mu$	From 95 $\mu$ to 108 $\mu$
Average length.	170 $\mu$	100 $\mu$

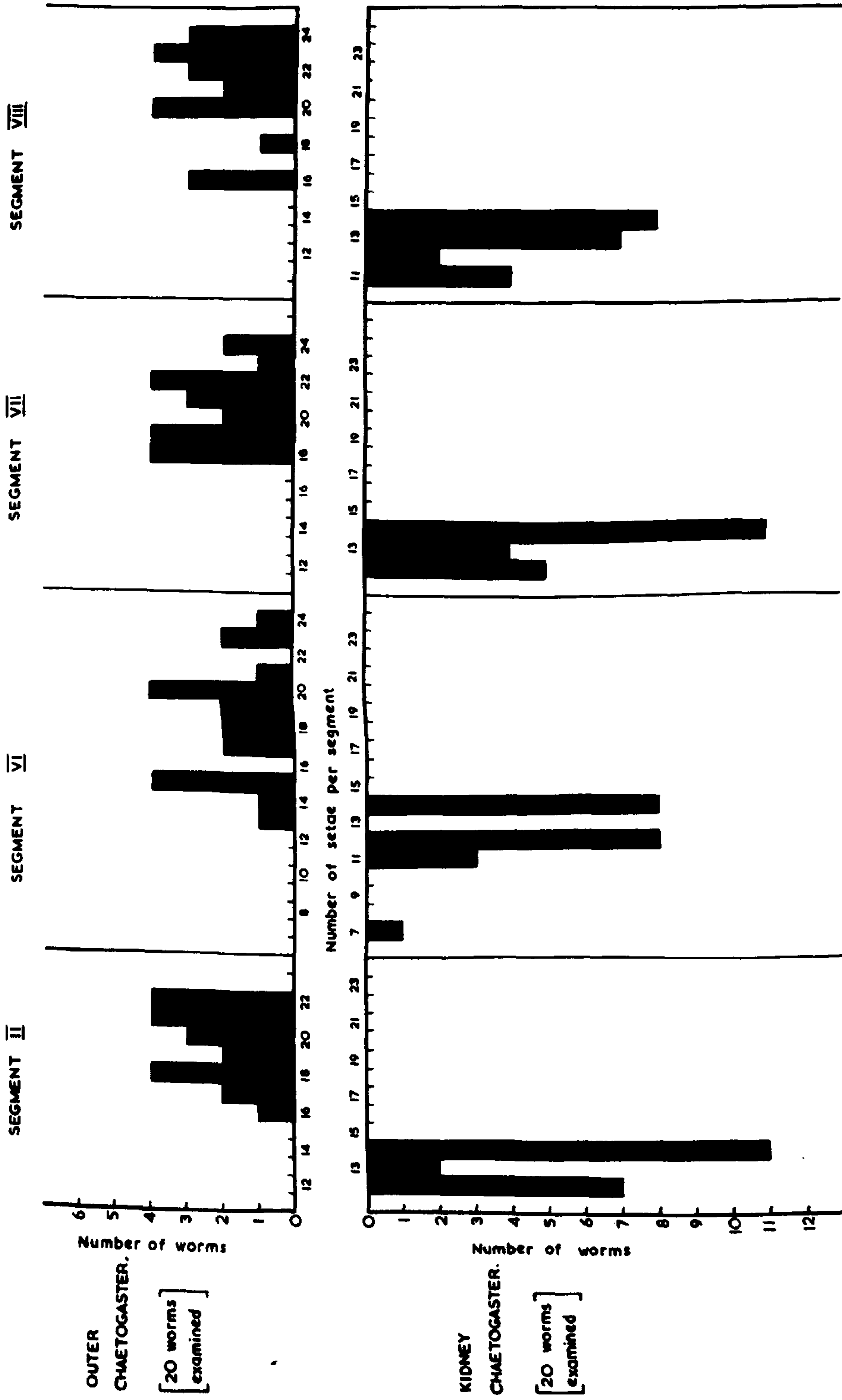


Fig. 5. The frequency distribution of setal numbers in outer and kidney forms of Ch. limnei obtained from the reservoir.

**Kidney form.**

	Segment 2.	Segments 6, 7 & 8.
Length of setae,	From 103 $\mu$ to 136 $\mu$	From 83 $\mu$ to 98 $\mu$
Average length.	120 $\mu$	91 $\mu$

Since the lengths of the setae in segments 6, 7 and 8 were very similar, they were grouped together as shown.

The average lengths of the setae in one mature kidney Chaetogaster were as follows.

Segment 2.	97 $\mu$
Segments 6, 7 & 8.	83 $\mu$

It seems that the setae of outer forms are slightly but consistently longer than the corresponding setae of kidney forms. The average length of the setae in the one mature kidney form examined corresponded roughly to those of immature kidney forms. The shape of the setae of the outer and kidney forms is indistinguishable.

b. Number of setae per bundle.

(1) Reservoir population.

A random sample consisting of 20 outer and 20 kidney Chaetogaster were squashed under a coverslip and the number of setae in each bundle in segments 2, 6, 7 and 8 were counted. These results are presented in Fig. 5., as the total number of setae per segment, i.e., the sum of the number of setae in both bundles, and this is plotted against the

number of animals. It is seen that the kidney forms never had more than 14 setae per segment, i.e., 7 per bundle. The outer forms however, in the vast majority of cases had between 15 and 24 setae per segment. Odd numbers of setae per segment are due to the fact that the two bundles in the same segment were not always made up of the same number of setae. Occasionally, an outer form was found that had as few as 13 setae in one segment. In such cases it was usually the sixth segment that had this low number of setae, all the other segments having between 15 and 24. This contrast in setal numbers affords a means of distinguishing between outer and kidney forms. Any worm having 14 setae or less in all segments would be a kidney form, and any worm having more than 14 in any one or all of its segments would be an outer form. In practice, the outer forms had more than 14 setae in at least three of the segments in question. The exception to this rule is the case of mature kidney forms which often have an additional seta in some bundles giving a total of 15 or 16 setae per segment (see p.27 ).

These results were examined statistically by comparing the mean number of setae per segment in outer and kidney forms for each segment. The method used to compare the means was that for comparing the means of small samples using tests given in Bailey (1959). The following table shows that in all segments the means were significantly different at the 1% level.

Segment.	Values obtained for 'Student's' t. Degr. of freedom	
2	13.462	18
6	7.318	17
7	14.872	18
8	12.374	18

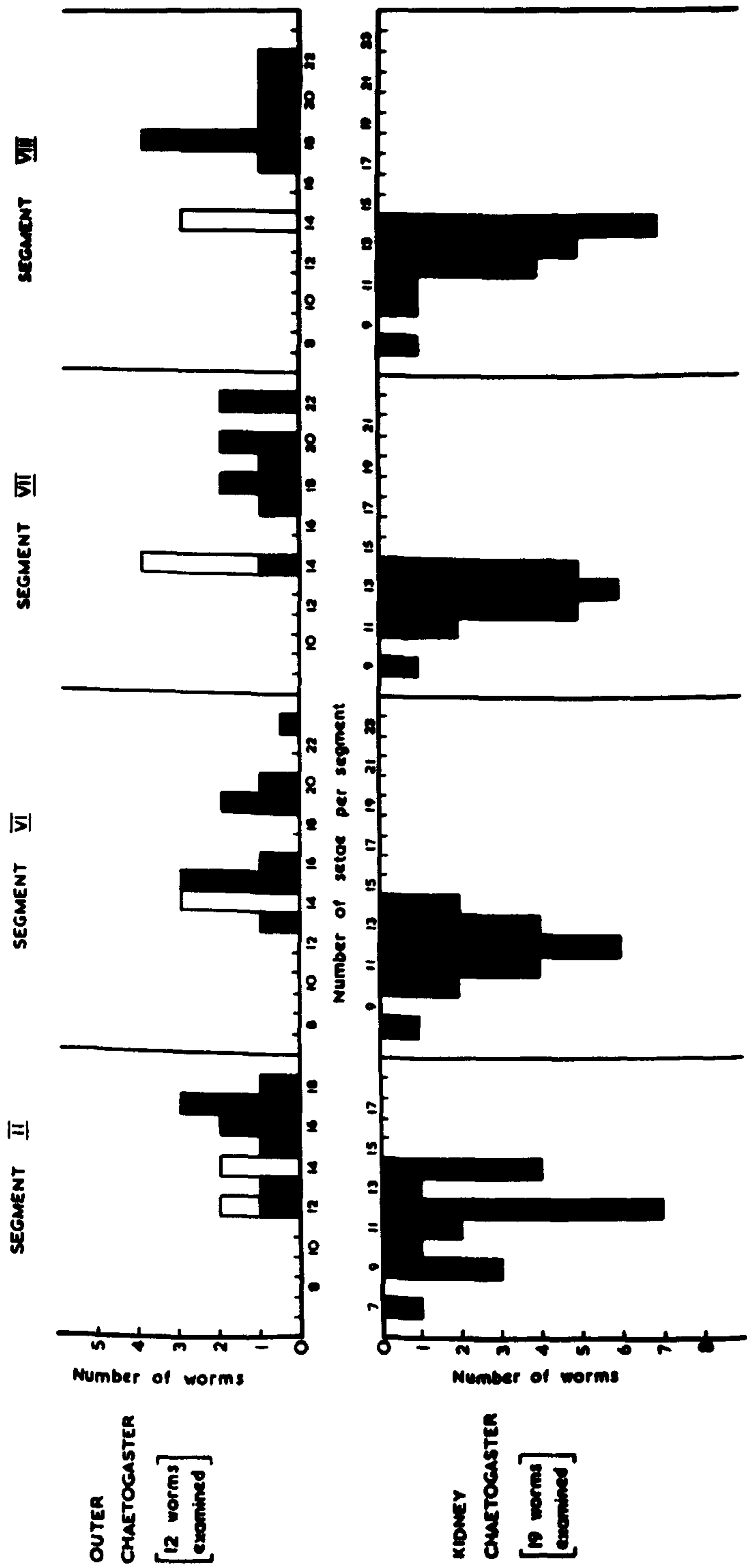


Fig. 6. The frequency distribution of setal numbers in outer and kidney forms of Ch. limnaei obtained from Frodsham.

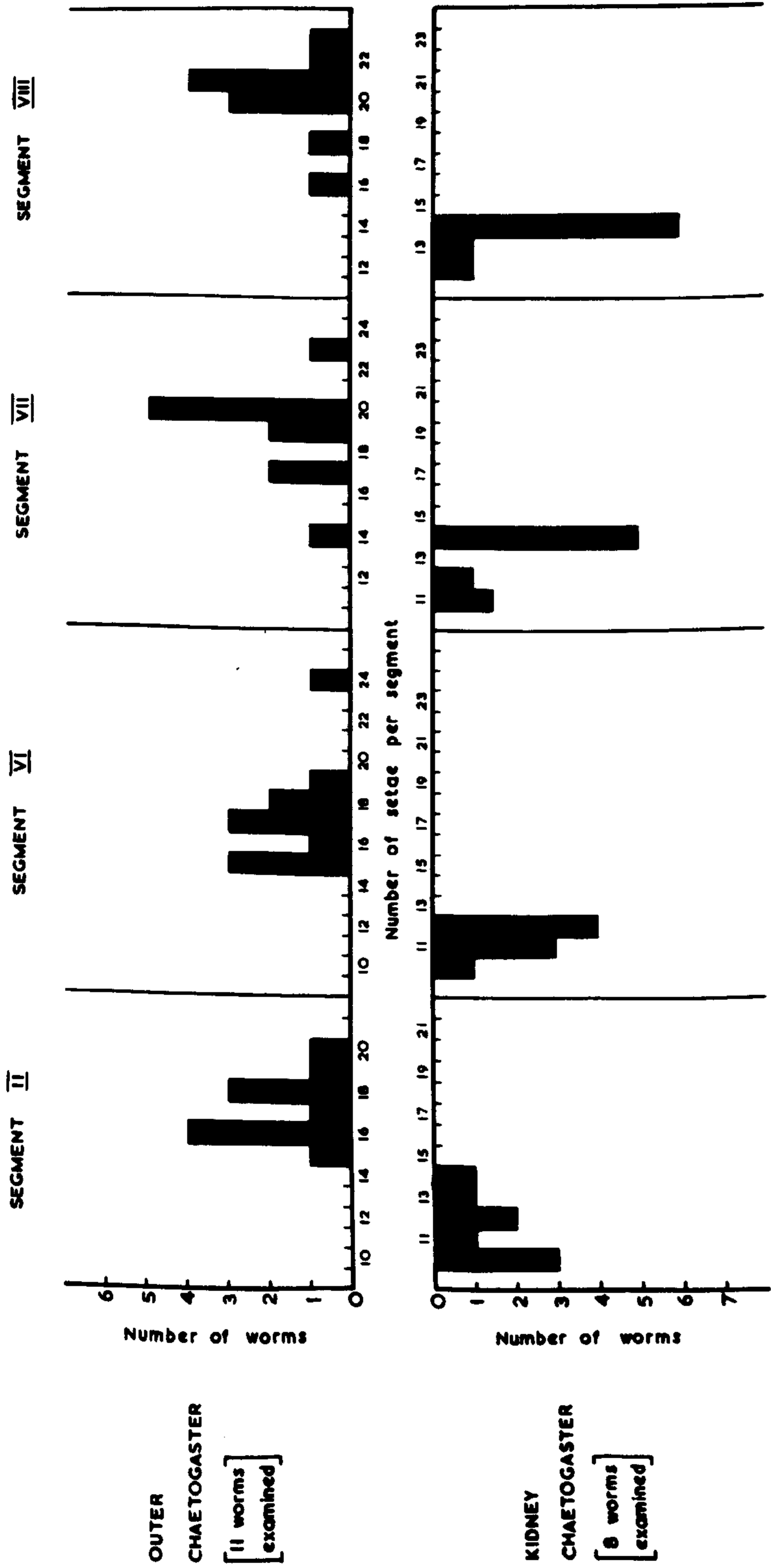


Fig. 7. The frequency distribution of setal numbers in outer and kidney forms of Ch. linnaei obtained from Walton.

After finding these differences in the setal numbers of the outer and kidney forms of the reservoir, it was decided to investigate the possibility of this difference being general and not confined to these particular populations. Samples of L. pereger obtained from various parts of Britain were examined.

(ii) Sample from a drainage ditch near Frodsham.

Chaetoxaster linnaei were obtained by dissecting a sample of L. pereger from the above location. Figure 6 shows the results of setal counts carried out on these animals. Again, the number of setae per segment never exceeded 14 in the kidney forms. Low numbers of setae per segment were more common in the outer forms here than in the reservoir animals. However, since these low numbers (i.e., 14 and below) were never found in more than one segment of any worm, the outer forms were still easily distinguishable. Three worms found on the outer surface of the host had 14 setae or less in all segments. Only kidney cells were found in their gut and it can therefore be said with certainty that these were kidney forms living temporarily on the outer surface of the snail. These are represented in the diagram by the unshaded parts of the histograms.

(iii) Sample from a Thames backwater at Walton.

The Ch. linnaei obtained by dissecting L. pereger from this habitat were again treated as above, and the results are presented in Fig. 7. All the kidney forms had 14 setae or less per segment. Here, with the exception of one segment of one individual there was no overlap between the number of setae possessed by the kidney forms and outer forms, and so the distinction between the two forms was exceptionally clear.



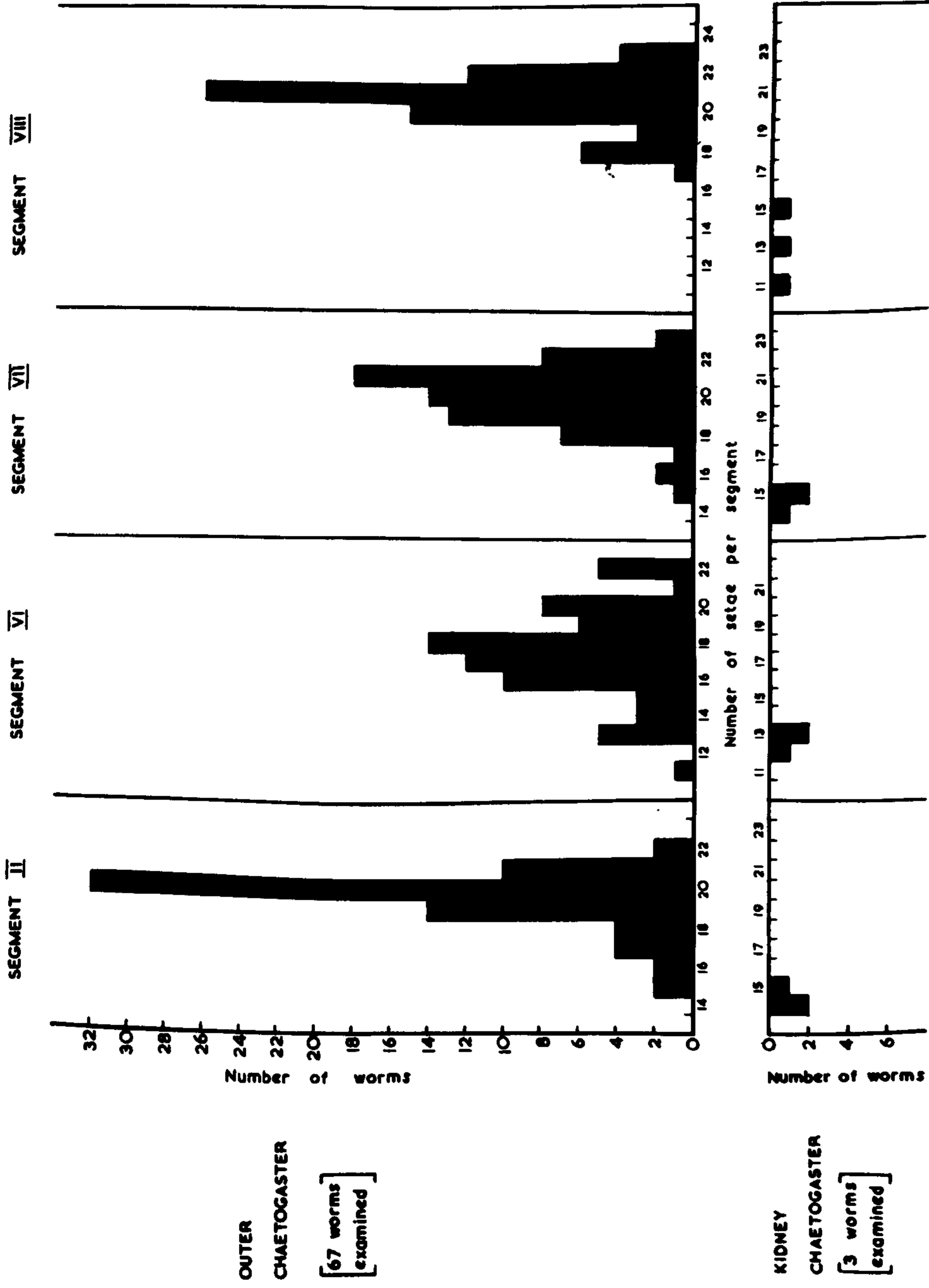


Fig. 8. The frequency distribution of setal numbers in outer and kidney forms of Ch. limnaei obtained from Foxbar.

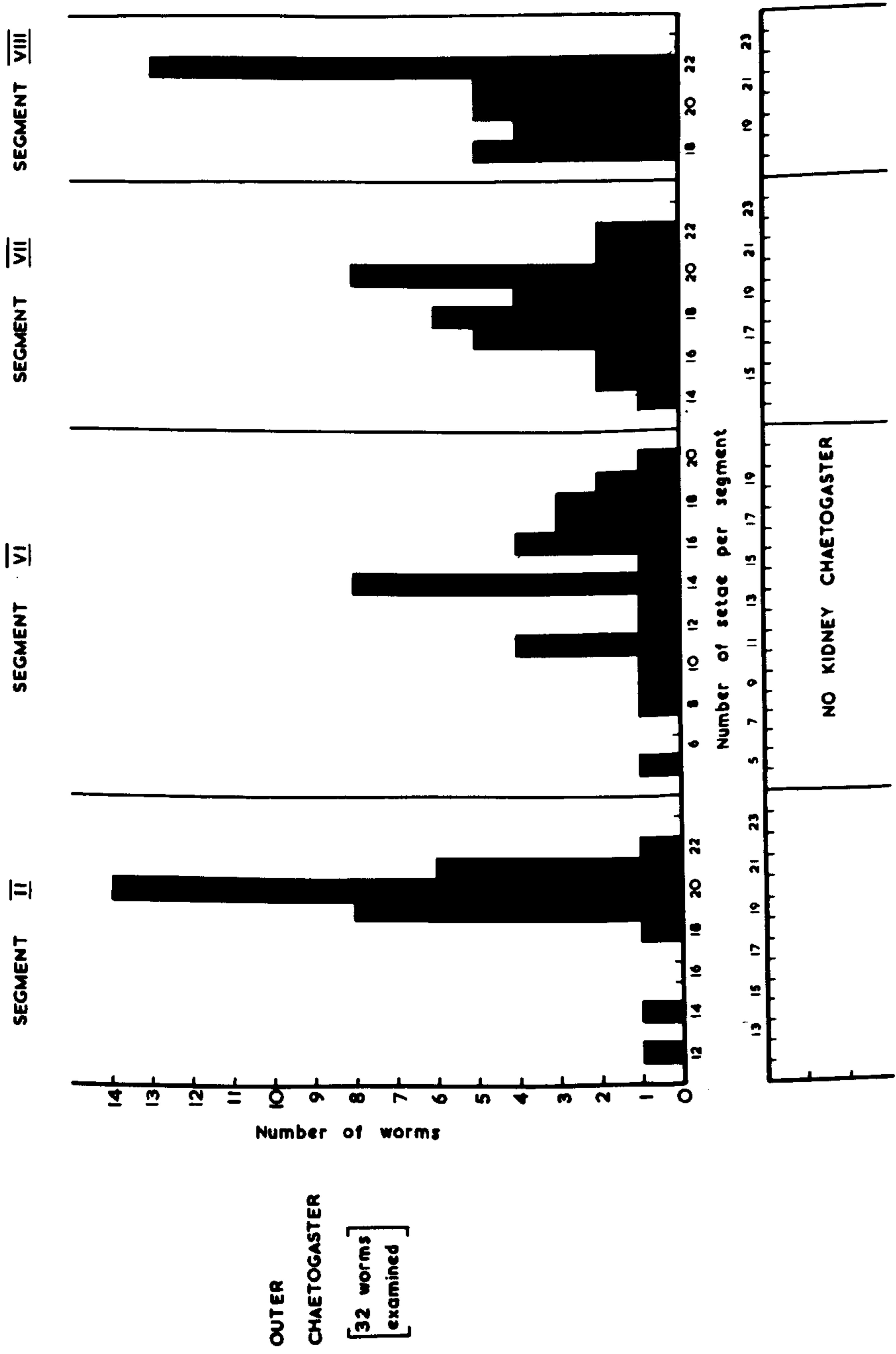
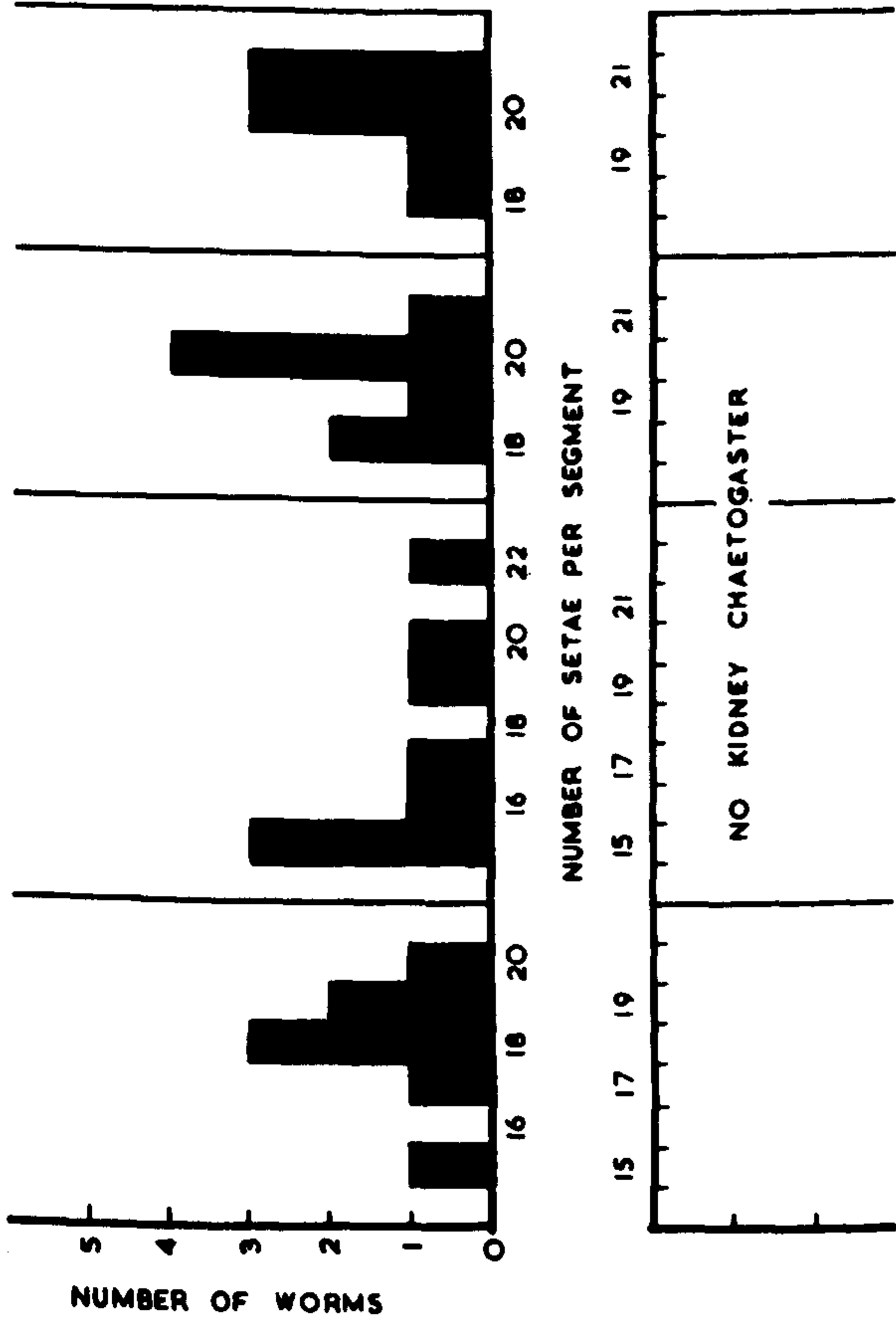


Fig. 9. The frequency distribution of setal numbers in outer and kidney forms of Ch. limnaei obtained from Helston.

Segment II      Segment VI      Segment VII      Segment VIII



Outer  
 Chaetogaster  
 [ 8 WORMS ]  
 [ EXAMINED ]

Fig. 10. The frequency distribution of setal numbers in outer and kidney forms of Ch. limnaei obtained from Reading.

(iv) Sample from a dam at Foxbar, Renfrewshire.

The Lymnaea pereger obtained from this habitat yielded many outer Chaetogaster but only three kidney forms. The three kidney forms were mature and all had three genital setae in each bundle on segment 6. These genital setae are not included in the results, which are presented in Fig. 8. It is seen that the mature kidney form often has more than 14 ordinary setae per segment. Some bundles were made up of 8 setae which is one more than the maximum seen in immature kidney forms. Only kidney cells were found in the gut of these mature animals and this indicates that they were kidney forms and that the number of setae per bundle tends to increase at maturity. Most of the outer forms had more than 14 setae in every segment, but a few had less than 14 in segment 6.

(v) Sample from Helston, Cornwall.

No kidney forms were found in the L. pereger obtained from this habitat. Most of the outer forms had more than 14 setae in all segments, but some had less in one segment, and this was usually segment 6. The results are presented in Fig. 9.

(vi) Sample from Reading.

No Ch. limnai were found in the kidney of the L. pereger in this sample either. All the outer Chaetogaster without exception had more than 14 setae in all segments. The results from this sample are presented in Fig. 10.

In conclusion it can be said that the results obtained from these widely dispersed habitats agree closely with those obtained locally at the reservoir. All the kidney forms examined, with the exception of

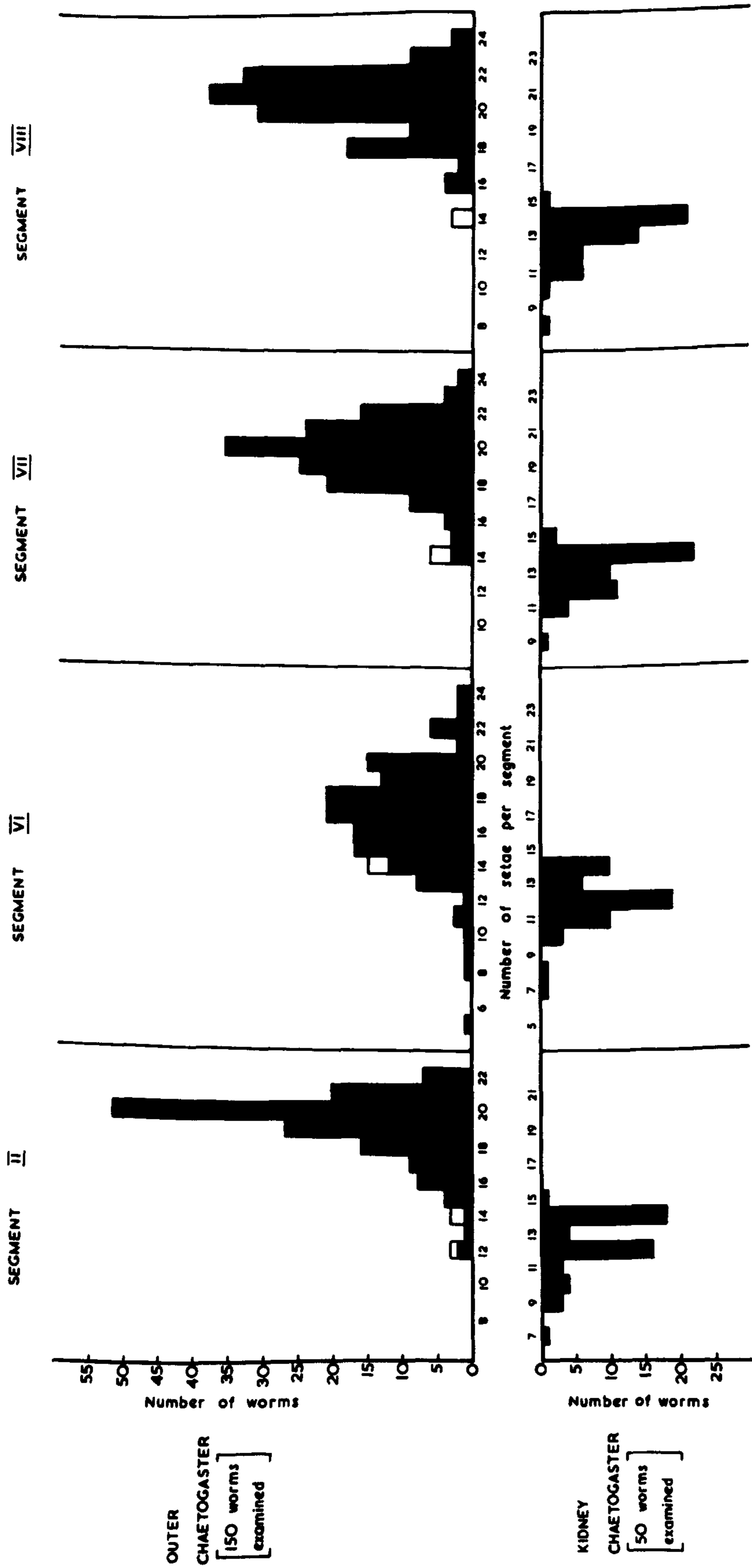


Fig. 11. The frequency distribution of setal numbers in outer and kidney forms of *Ch. limnaii*. (summation of Figs. 5, 6, 7, 8, 9 & 10)

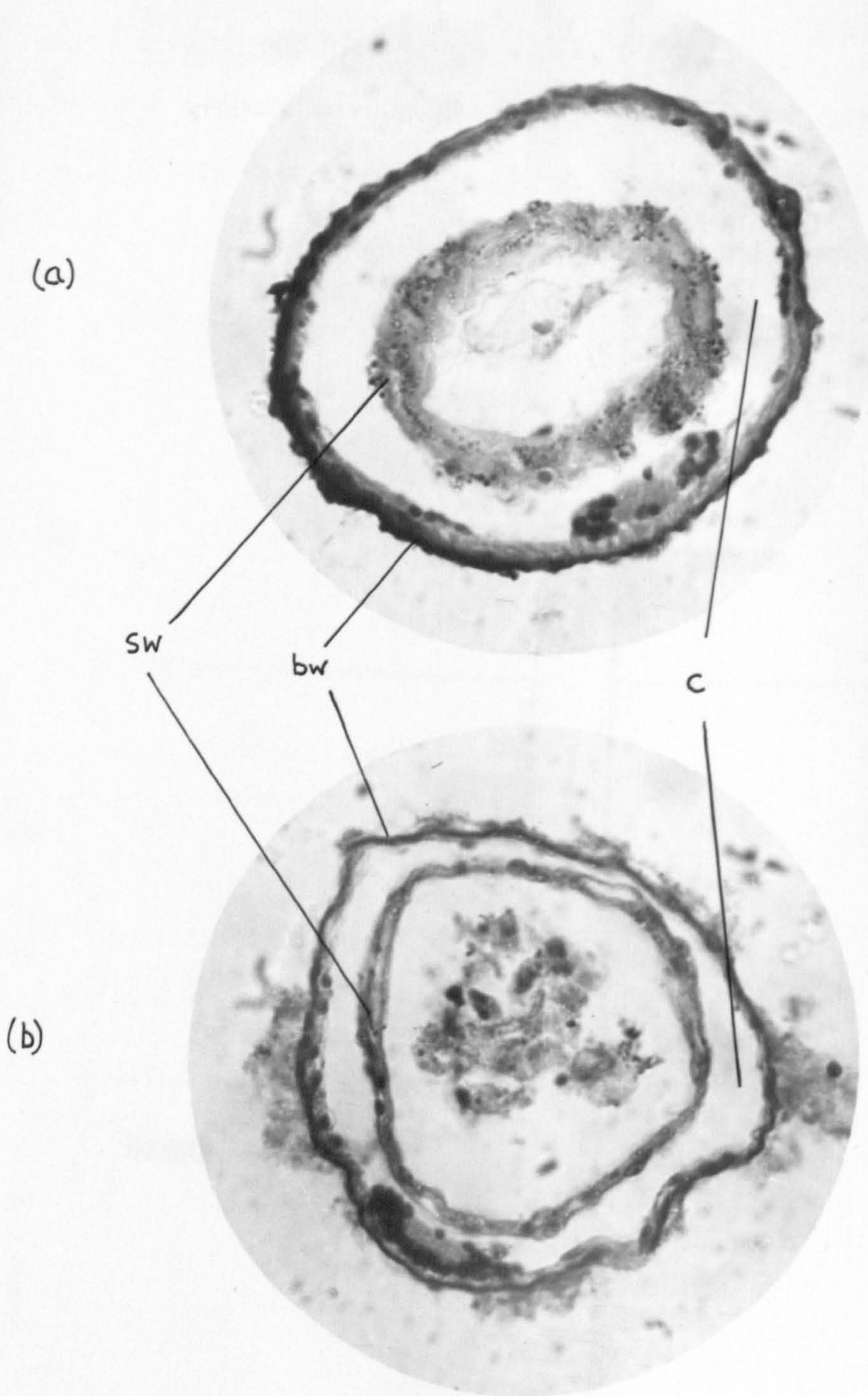


Plate 1. (a) Transverse section of the stomach region of an outer *Chaetogaster*. (x 560)  
(b) Transverse section of the stomach region of a kidney *Chaetogaster*. (x 560)  
sw, stomach wall. bw, body wall. c, coelom.

mature animals, had 14 setae or less per segment in all the four segments that were observed; this means a maximum of 7 setae per bundle. The outer forms usually had more than 14 setae per segment with a maximum of 24 per segment or, in other words, 12 per bundle. Some had less than 14 setae per segment, but in no case was this true for more than one segment of any animal. Figure 11 was constructed by pooling all the data used in Figs. 5 to 10 inclusive. The unshaded parts of the histograms represent kidney forms that were found on the outer surface of snails in the sample from Frodsham. From the pooled data it can be calculated that 4.7% of the outer forms had less than 14 setae in segment 2, 20% had less than 14 in segment 6, 4% had less than 14 in segment 7 and only 2% had less than 14 in segment 8. It is seen that the overlap between the setal numbers of the kidney and the outer forms is greatest in segment 6. This overlap is presumably caused by a loss of setae from the bundles of segment 6. If this assumption is correct, the only explanation that can be suggested is that in some way these bundles, serving as attachment organs, are subjected to greater strains than the bundles of other segments.

c. A comparison of the internal anatomy of the two forms.

Sections of both types of Ch. linnaei were cut in order to compare the anatomy of the two forms. Ten outer forms and ten kidney forms were examined in this way but no detailed histological observations were made. Differences were found in the thickness of the gut wall in the two forms. Plate 1a shows a transverse section through the stomach region of the gut of an outer Chaetogaster, Plate 1b a section of the same region

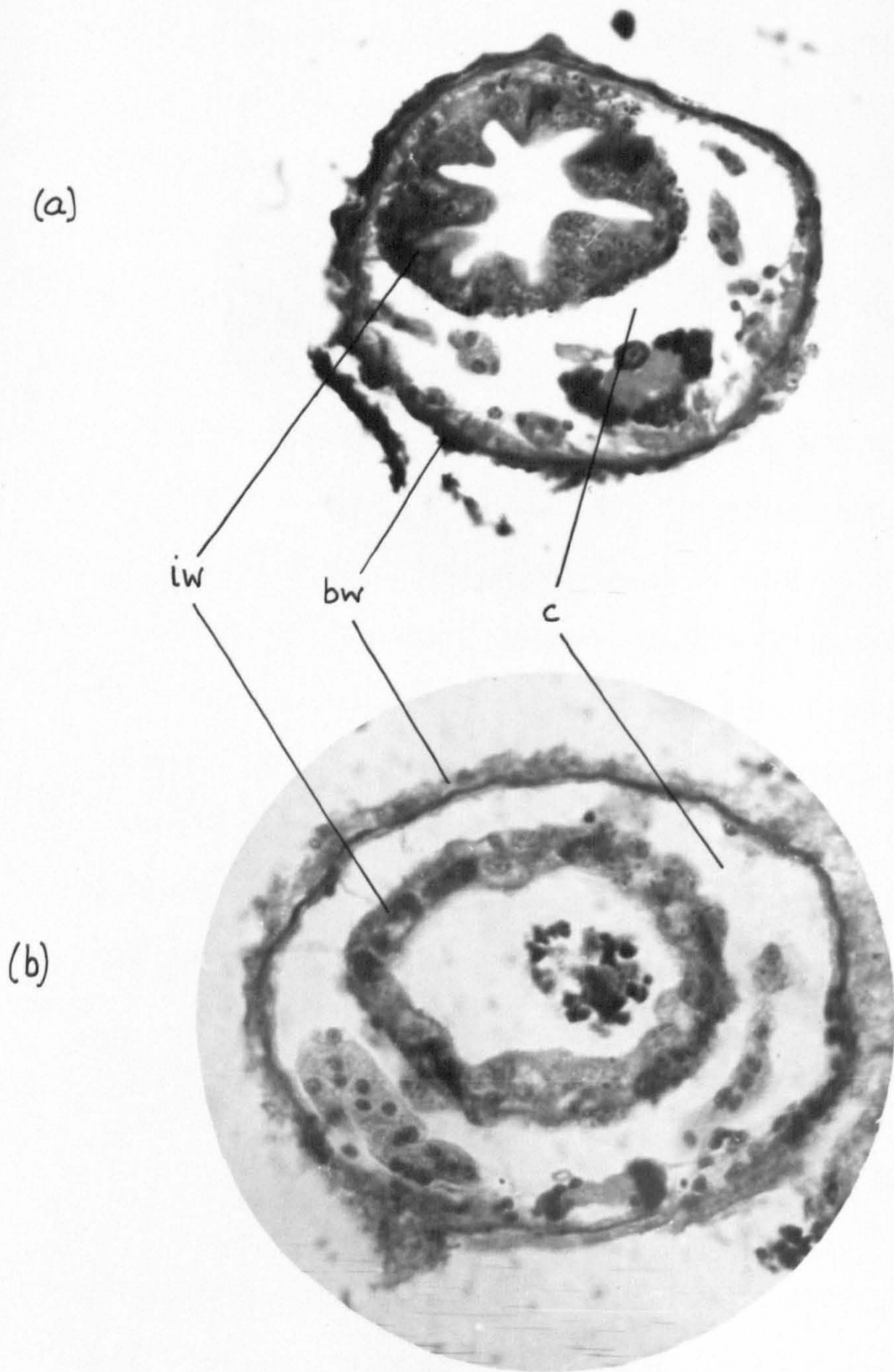


Plate 2. (a) Transverse section of the intestinal region of an outer Chaetogaster. (x 560)  
(b) Transverse section of the intestinal region of a kidney Chaetogaster. (x 560)  
iw, intestinal wall. bw, body wall. c, coelom.



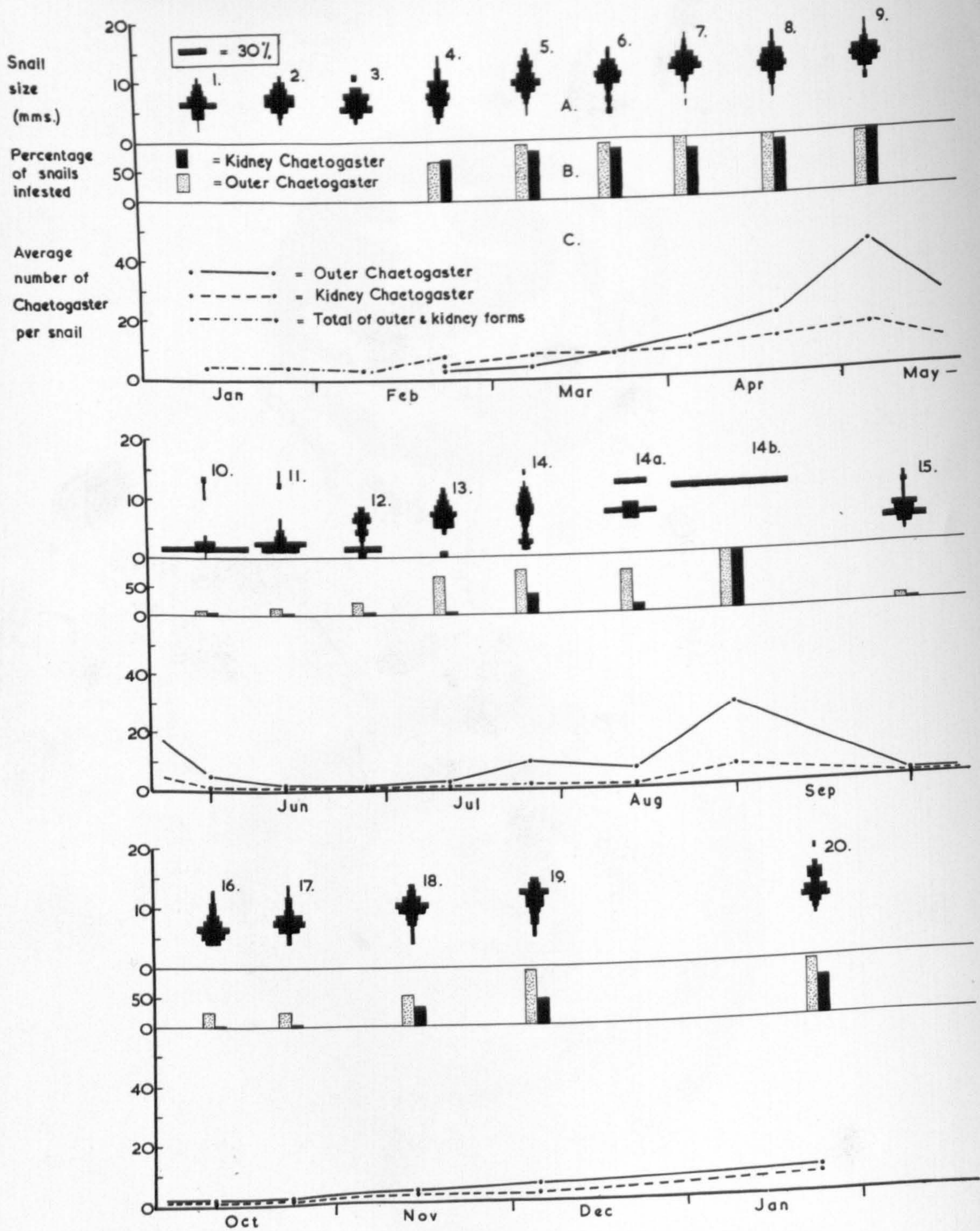


Fig. 12. (A) Frequency distribution histograms of snail size, (B) the degree of infestation of *L. pereger* by *Ch. limnaei*, and (C) the average number of *Chaetogaster* per snail in each sample taken at the reservoir during 1960.

of a kidney Chaetogaster. The endothelium of the outer form is seen to be thicker than that of the kidney form. Plates 2a and 2b show transverse sections of the intestine of an outer and a kidney form respectively. The difference between the intestinal endothelium of the two forms is more marked than the difference between the stomach endothelia. Whereas the kidney form has only a thin, regular endothelium, the outer form has a much deeper and more irregular layer of cells constituting the endothelium. It is possible that this contrast is related to the difference in the diet of the two forms, the kidney forms feeding exclusively on kidney cells (p. 52) and the outer forms feeding on a variety of planktonic plants and animals and probably having a more complex digestive mechanism.

No other major anatomical differences were found.

#### Section 5.

#### LIFE CYCLE OF LYMNAEA PERGER (Müll.)

Sampling of the LYMNAEA PERGER population started in January 1960. Figure 12A. shows the gradual increase in the size of the snails up to the beginning of May. Spawning began towards the end of March, each spawn capsule containing up to 60 eggs. Under laboratory conditions and at a temperature varying from 10 to 15°C spawning occurred when the snails reached a size of about 8 mm. The majority of snails in the winter samples were thus above the minimum breeding size, but breeding did not take place until the water temperature rose to about 8°C. Young snails appeared in the reservoir population at the end of May to the extent of 96% of the total sample. This suggests that the young snails emerged 4 to 6 weeks

after oviposition at this time of year. In the laboratory, with water temperatures at around  $15^{\circ}\text{C}$  this period shortened to 2 to 3 weeks. Young snails continued to appear in the population up to the beginning of July.

By the 27th. of June all snails of the parent generation had disappeared. No direct evidence is offered by these results as to how soon the adults die after spawning. However, at  $16$  to  $18^{\circ}\text{C}$  in the laboratory, death usually occurred 2 to 3 weeks after spawning.

The rate of increase in the size of the snails of the first generation in summer was greater than that of the parent generation in spring (Table 4), when temperatures were considerably lower (Fig.15 ), p.32. This increase continued throughout the summer up to the end of August. The samples taken on August 13th. and 31st. (samples 14a & 14b) were very small, the snails being very difficult to find. On September 4th., a small quantity of L. pereger spawn was found, and the next sample taken on September 30th., suggested that a second generation of young snails had been added to the population. The histogram produced from this sample is characteristic of a population containing a high percentage of young forms in that it has a wide base. But since the two previous samples viz. 14a and 14b, were very small, this suggestion must for the present be treated with reserve. However, the theory is supported by the fact that in contrast to the clean shells of the snails of this probable second generation, shells belonging to a few individuals of the first generation still remaining in the population were thickly coated with algae. It was this second generation together with possibly a few individuals from the first, that overwintered in the reservoir. The growth rate of the second generation in the autumn was even higher than that of the first generation

Table 4.

The average growth rate of L. pereger in mms. per week.

	Reservoir 1960	Reservoir 1961	Stream 1961
<b>Spring</b>	Feb - May 0.459	Feb - May - 0.101	Feb - Apr - 0.030
<b>Summer</b>	May - Jul 0.609	Jun - Aug 0.314	Jun - Aug 0.154
<b>Autumn</b>	Sep - Nov 0.689	Sep - Nov 0.401	Sep - Nov 0.201
<b>Winter</b>	Nov - Jan 0.144	Nov - Feb 0.206	Nov - Jan 0.050

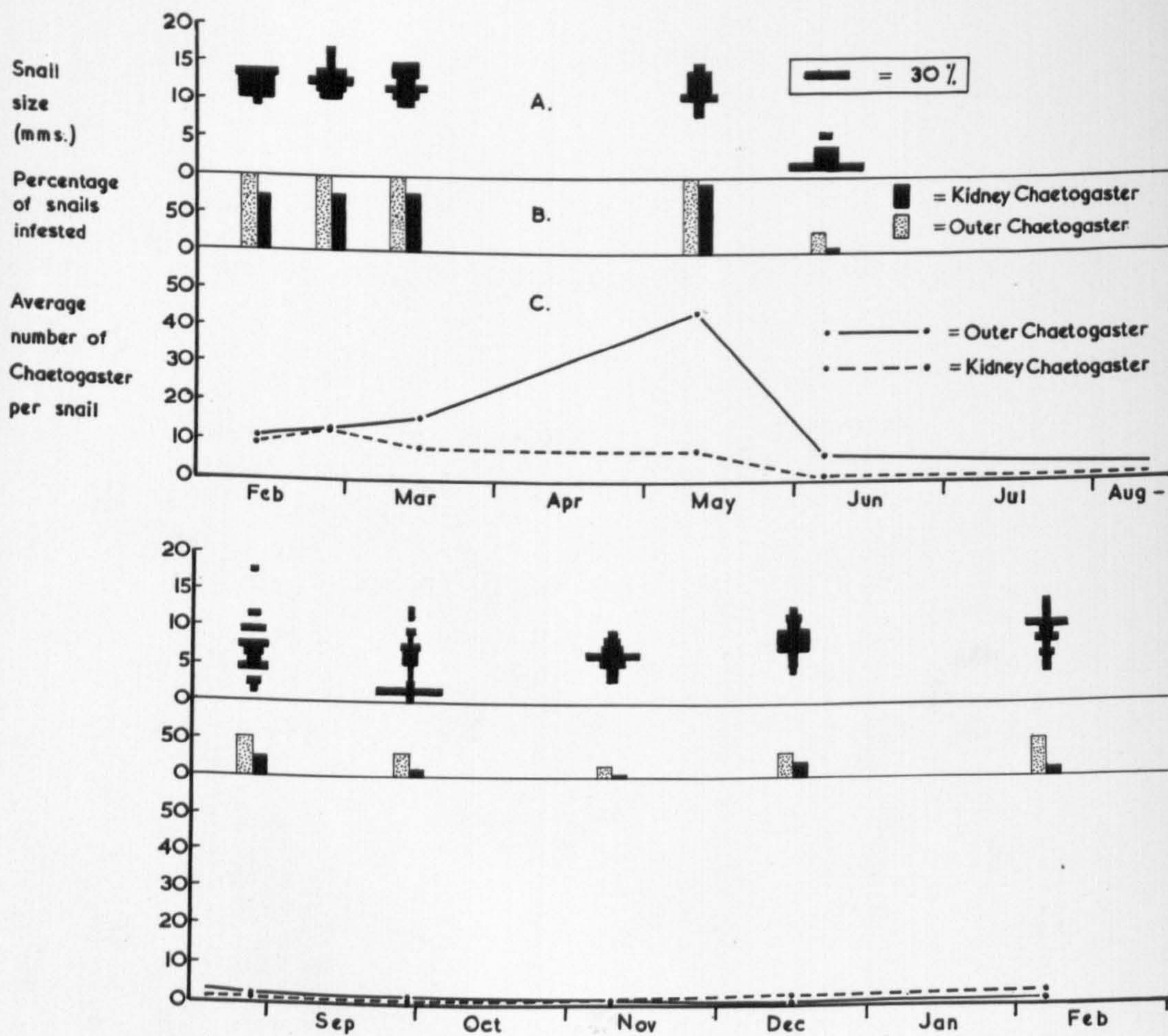


Fig. 13. (A) Frequency distribution histograms of snail size, (B) the degree of infestation of *L. pereger* by *Ch. limnaei*, and (C) the average number of *Chaetogaster* per snail in each sample taken at the reservoir during 1961.

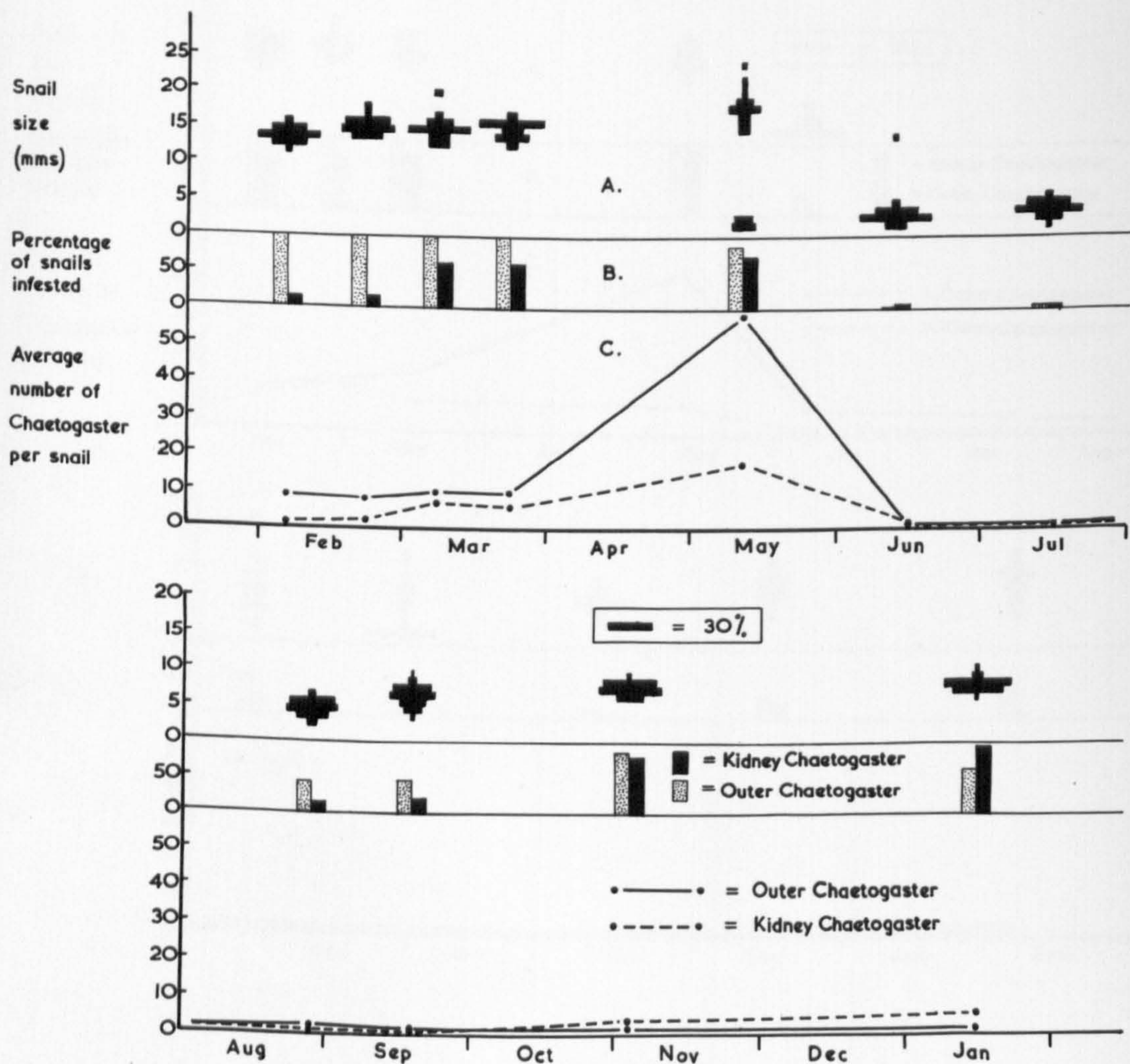


Fig. 14. (A) Frequency distribution histograms of snail size, (B) the degree of infestation of *L. pereger* by *Ch. limnaei*, and (C) the average number of *Chaetogaster* per snail in each sample taken at the Coed Mawr stream during 1961.

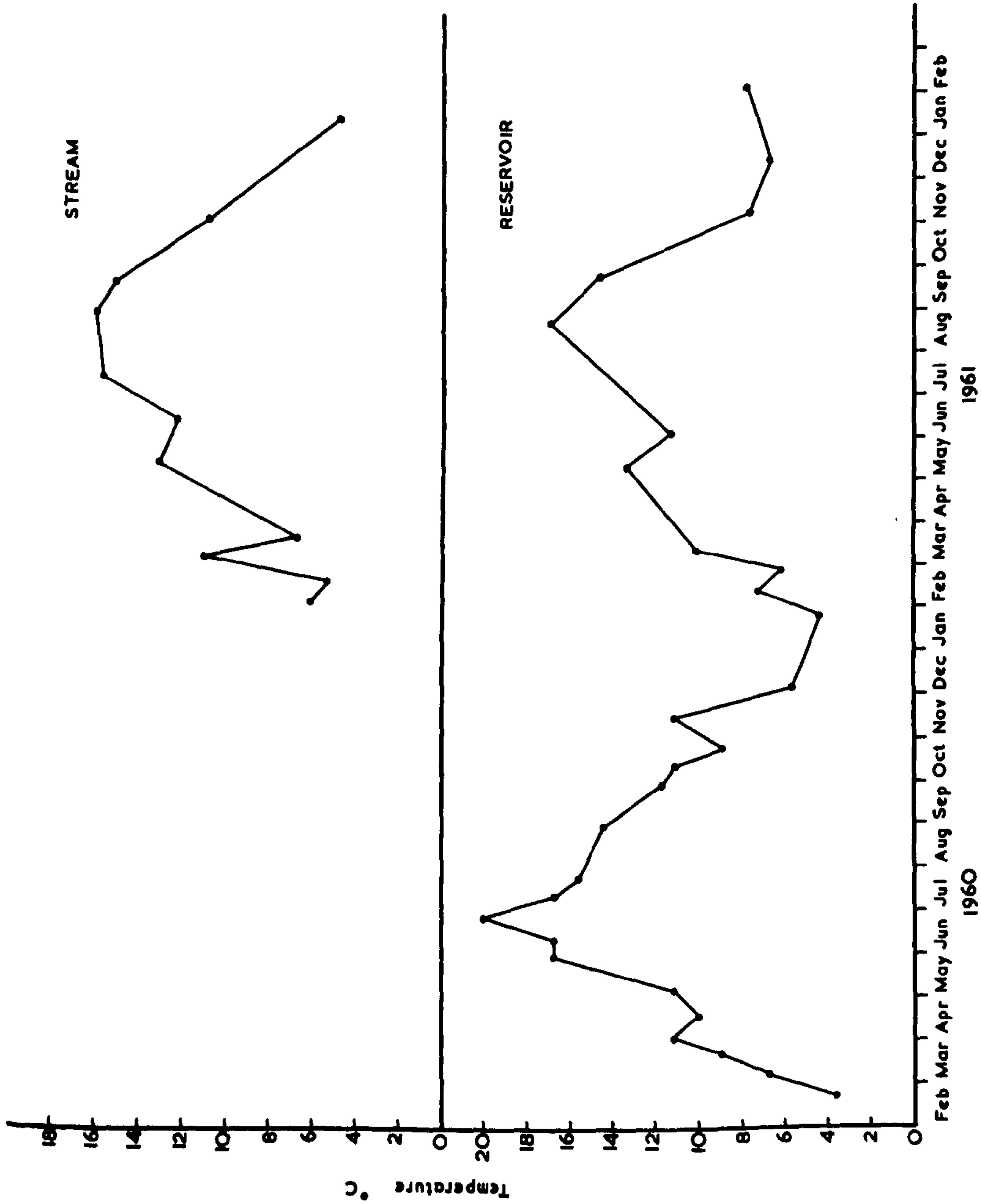


Fig. 15. Water temperatures.

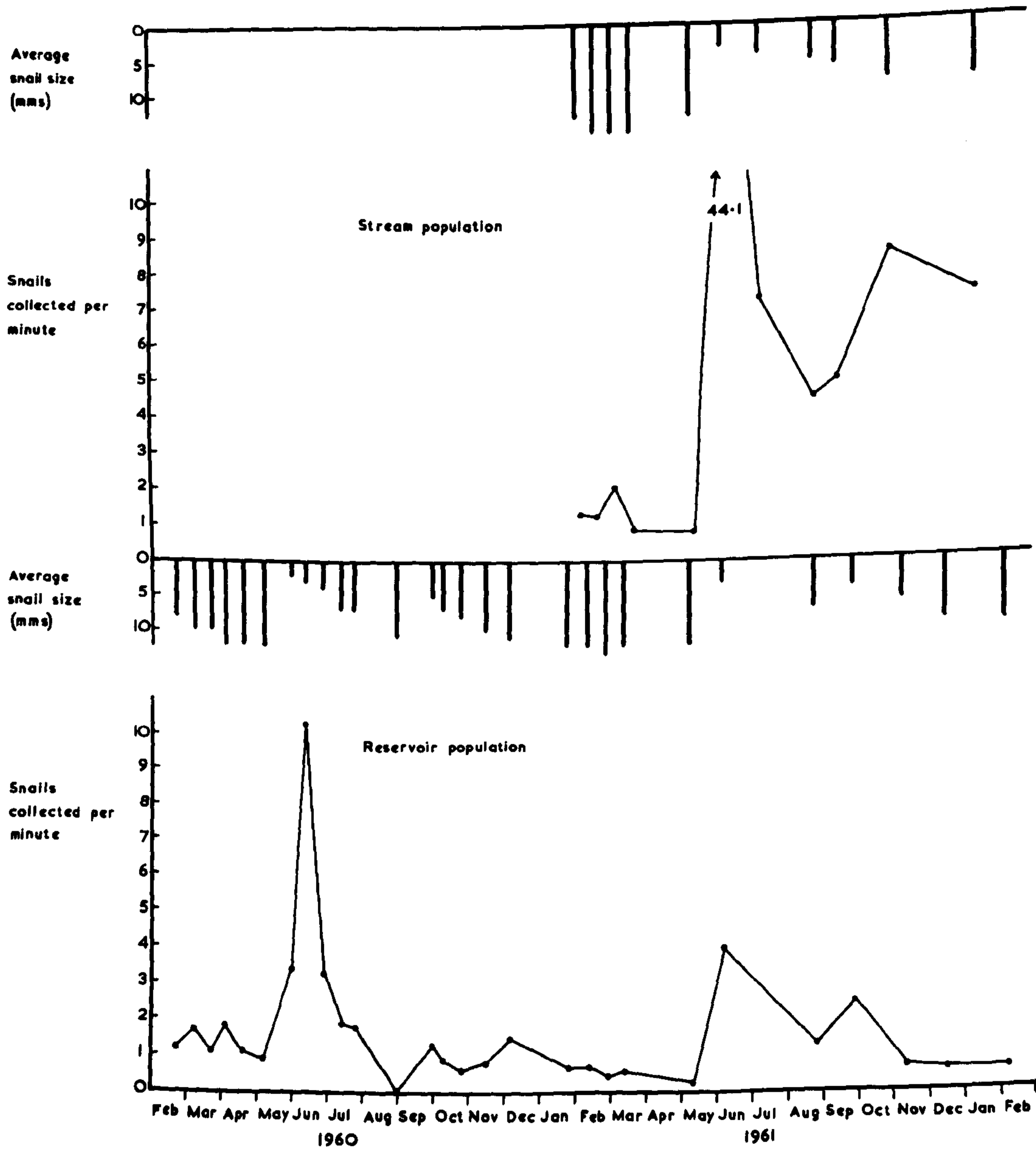


Fig. 16. An estimation of changes in the absolute size of the L. pereger populations of the reservoir and stream.



in summer (Table 4, p. 31). Growth during the winter months was retarded.

Figure 13 shows the changes in this same population from January 1961 to February 1962. This shows clearly that two generations of L. peregrina were produced in 1961 thus providing evidence confirming the conclusions drawn from the previous year's results. The growth rate here followed a similar pattern to that of 1960.

Figure 14 was drawn using results obtained throughout 1961 from a population of L. peregrina living in the stream at Coed Mawr. Although the first generation here appeared at approximately the same time as the one in the reservoir, no second generation was produced. The growth rate of the population here was generally slower than that of the population in the reservoir, although water temperatures were not widely different (Fig. 15). A slightly higher growth rate was found here again in the autumn. It is seen that the autumn growth rates in both populations were higher than the summer rates. Temperatures were falling in the autumn and so this phenomenon cannot be related to temperature. It is possible that food was more plentiful at this time, and it is likely that since the snail population was slightly smaller in the autumn than in the summer in both habitats (see below), there was less competition for food.

The index, snails collected per minute, was used to indicate fluctuation in numbers of the L. peregrina population (Fig. 16). This declined slightly from January 1960 to the beginning of May 1960 in the reservoir (a feature repeated here and in the stream population in 1961), and then rose steeply as young snails hatched. Following this rise was an equally steep fall indicating a high mortality rate in the young forms. This

infantile mortality is found in the majority of molluscs, and L. pereger requires a survival of one in five hundred for the maintenance of its numbers (Boycott 1936, Comfort 1957). Boycott (1936) states that the factors that limit the size of a mollusc population act on the infants, the adults and the eggs being fairly safe from predators. These predators that feed on young snails are listed in his paper, the most important ones being freshwater fish, leeches, especially Glossiphonia, and various carnivorous larvae and beetles such as Hydrophilus, Dytiscus, caddis larvae and glass-worm larvae. Such animals are plentiful in both the reservoir and the stream and could easily account for the mortality in the young forms in both habitats. In the second year in the reservoir a second, smaller peak in the abundance of snails occurred at the time of hatching of the second generation. This was missed in the first year because no sample was taken in mid - September when this phenomenon probably occurred.

It was noticed that during the winter months L. pereger inhabited deeper water, whilst in summer it lived in shallow water because at higher water temperatures more frequent excursions to the surface to breathe are necessary (Ghastum 1934, Hunter 1953).

Two generations per annum are not at all uncommon in populations of pulmonates. Hunter (1961) records this phenomenon in Flyza fontinalis. Here a proportion of the first generation overwintered with the second generation. De Wit (1955) on the other hand found that there was almost complete replacement of the first generation by the second, overwintering generation. Duncan (1959) and Hunter (1961) also record populations of Flyza fontinalis having only one breeding season per annum. Walton and Jones (1926) suggest that there was even a third generation

produced in one particular population of Lymnaea truncatula.

The type of life history described here involving two breeding activities by different generations, where the second generation almost completely replaced the first, has also been described in a Lymnaea perreger population by Hunter (1961). In the same paper he presents data obtained from a population of this species having only one breeding season a year. In this type of population he found that spawning occurred much later than the first spawning period in a two cycle population, although snail size and water temperatures in spring were equally favourable for early breeding. He suggests that in such cases genetic factors are involved in determining mid - summer breeding rather than spring and late - summer breeding. As was shown in Fig 14 (p. 32) the stream population did not produce a second generation. However, early breeding occurred as in the two cycle population in the reservoir. This and the fact that the growth rate in the stream was generally lower than that in the reservoir (Table 4, p. 31) suggests that the factors limiting the number of breeding seasons in the stream population are environmental rather than genetical.

#### Section 6.

#### POPULATION DYNAMICS OF CHAETOGASTER LINNAEI

Detailed quantitative observations were made on the Chaetogaster linnaei population living on the Lymnaea perreger in the reservoir at fortnightly intervals between February 1960 and January 1961. Samples of this population were also taken at monthly intervals from February 1961 to February 1962. During this latter period, monthly samples of L. PERREGER

were taken from the stream at Coed Mawr so that the Chaetogaster population could also be examined in a rather different habitat. The results obtained from the samples taken in the second year in the reservoir, and those obtained from the stream population were used chiefly for comparison with the more detailed results of the first year's sampling at the reservoir, and to check on events during periods of great change.

a. Reservoir population, 1960.

Figure 12, p. 29 shows the changes in the populations of Chaetogaster living on the outer surface of the snail and in the kidney of the snail. Figure 12 also shows the changes in the snail population which were discussed in the previous section. The graphs drawn represent the mean number of Chaetogaster per snail in each sample. Unfortunately, the only record kept of Chaetogaster numbers from samples 1, 2 and 3 was the sum totals of the kidney and outer forms found on each snail. From sample 4 on, separate records were kept for the kidney and the outer Chaetogaster. However, it is seen from samples 1, 2 and 3 that the average number of Chaetogaster per snail was very low in January and early February. In sample 4 (22nd. Feb.) the mean number of the kidney form per snail was greater than that of the outer form. By the beginning of March, both these values had increased slightly. In subsequent samples the mean value for the outer forms increased rapidly, and by the beginning of April it was greater than that of the kidney form. In early May it reached a peak at about 43 Chaetogaster per snail. The mean value for the kidney form increased rather more slowly to reach a peak at about 15 Chaetogaster per

snail also in early May.

The percentages of snails infested by the outer and kidney Chaetogaster (Fig 12) also increased during this period, the outer value reaching 100 per cent in early April and the kidney value doing so about a month later.

The next sample taken on the 30th. of May showed that the average Chaetogaster population had been reduced drastically. Young Lymnaea pereger were hatching in large numbers at this time, and the vast majority of these were not as yet infested with Chaetogaster limnaei. Ninety six per cent of this sample consisted of these young snails, and consequently the mean values for Chaetogaster per snail were very low, the kidney value being the lower of the two.

A similar pattern of events to that described above occurred during the summer between June and the end of August. The mean values increased slowly from June onwards, the outer mean again having the higher rate of increase. The peak, which was somewhat lower than that occurring in May, was detected in late August. By this time there was 100% infestation of the snails by both forms. Again the outer form reached this value sooner than did the kidney form. It is unfortunate that only 7 snails were taken on August 13th (sample 14a) and only 2 on August 30th (sample 14b). No more could be found although the whole of the sampling area was searched thoroughly. Obviously, these two samples do not give a very true picture of the state of the Chaetogaster population, but the sample taken on August 30th seems to indicate that a peak mean number of Chaetogaster per snail was probably reached at that time. This is probably true since samples taken in early summer showed a steady increase in this mean and

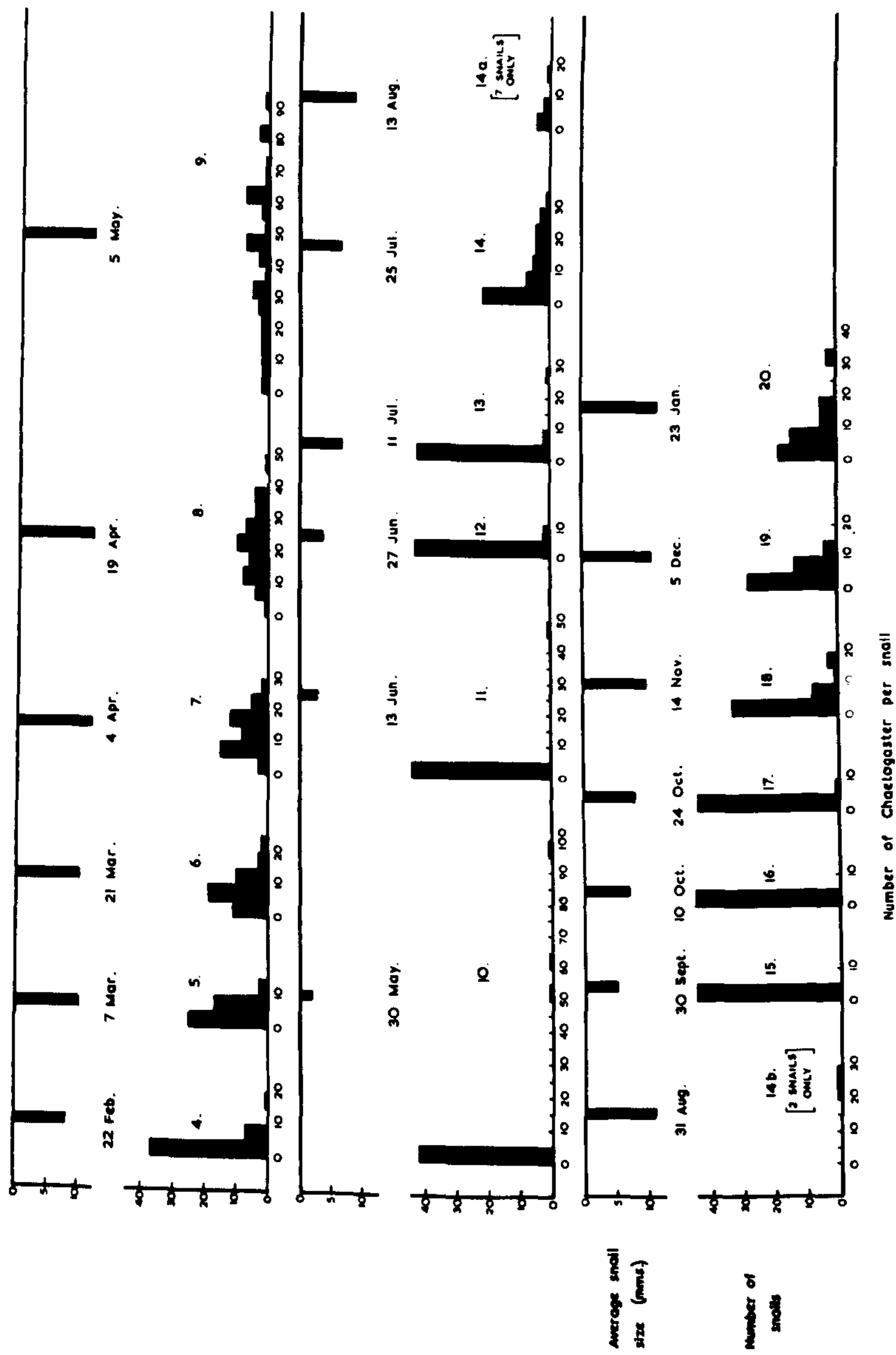


Fig. 17. The frequency distribution of the number of outer Chaetogaster per snail in each of the samples taken at the reservoir during 1960.

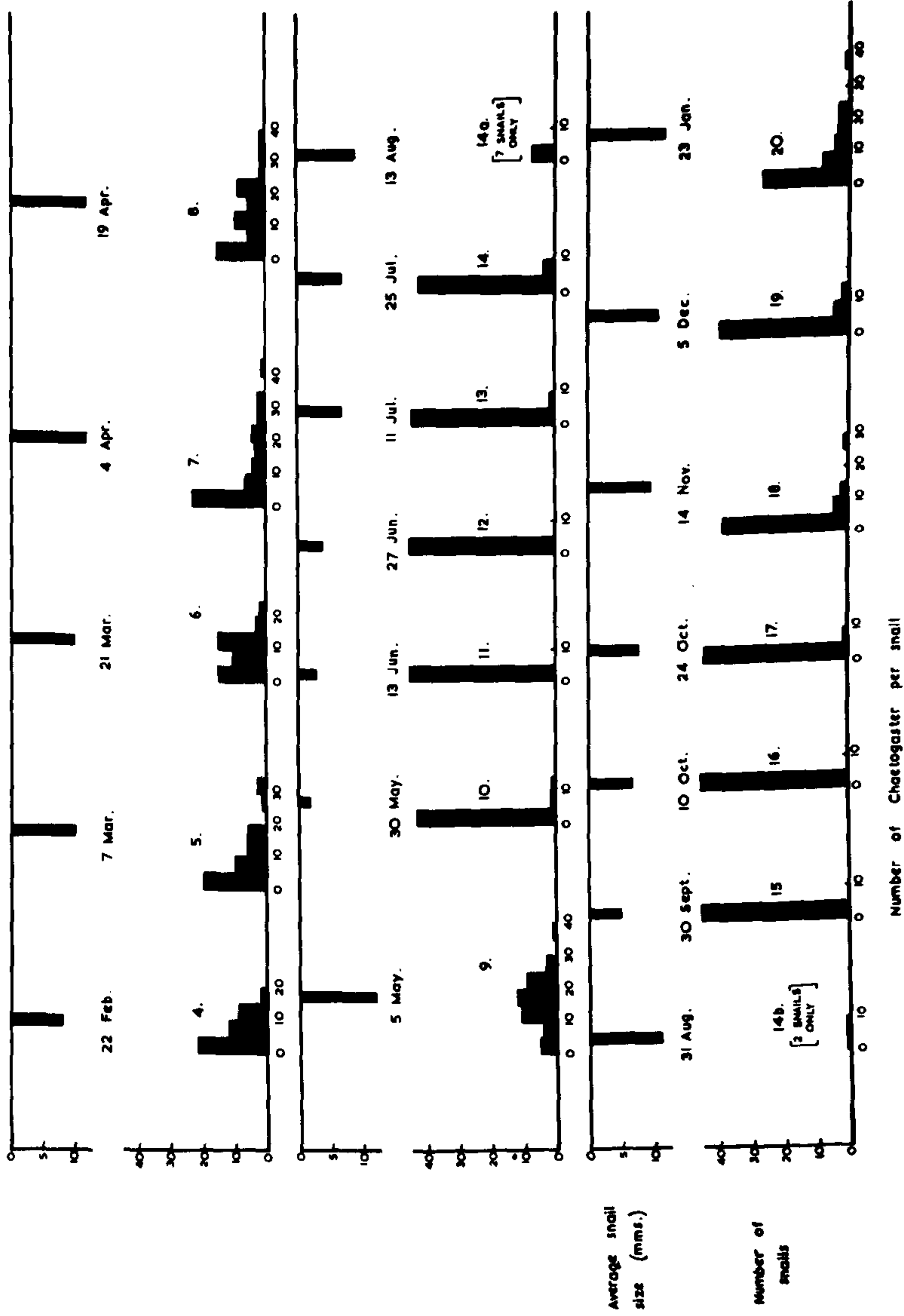


Fig. 18. The frequency distribution of the number of kidney *Chaetogaster* per snail in each of the samples taken at the reservoir during 1960.

it is reasonable to assume that this increase would continue throughout the summer until the death of the first generation of snails.

In September a second brood of young snails was produced and again the Chaetogaster sample (30th. Sept.) was diluted by young non-infested snails. As a result the mean values for Chaetogaster per snail dropped almost to zero. These low values persisted throughout October, but by November, both had increased slightly. This slow increase in both the outer and kidney populations continued throughout the winter, but here, unlike the previous winter, the mean value for the kidney form did not at any time exceed that of the outer form.

Figures 17 and 18 show histograms of each sample for the outer and kidney Chaetogaster populations respectively in relation to the host. The vertical axes represent the number of snails harbouring  $x$  Chaetogaster (horizontal axes), the total number of snails examined in each sample being 45 except where otherwise stated. At the times when the Chaetogaster population was low i.e., following the appearance of young snails, a large proportion of snails had very few Chaetogaster on them, and a picture resembling that of a Poisson distribution was obtained. A few snails had a large number of Chaetogaster on them, these being the few old snails of the previous generation still remaining in the population. As the mean number of Chaetogaster per snail increased, the distribution became more normal, the highest degree of normality occurring at the time of the Chaetogaster population peak preceding the appearance of the first generation of snails. This of course was the time at which the Chaetogaster population was at its highest.

During the two months leading up to the time of this first



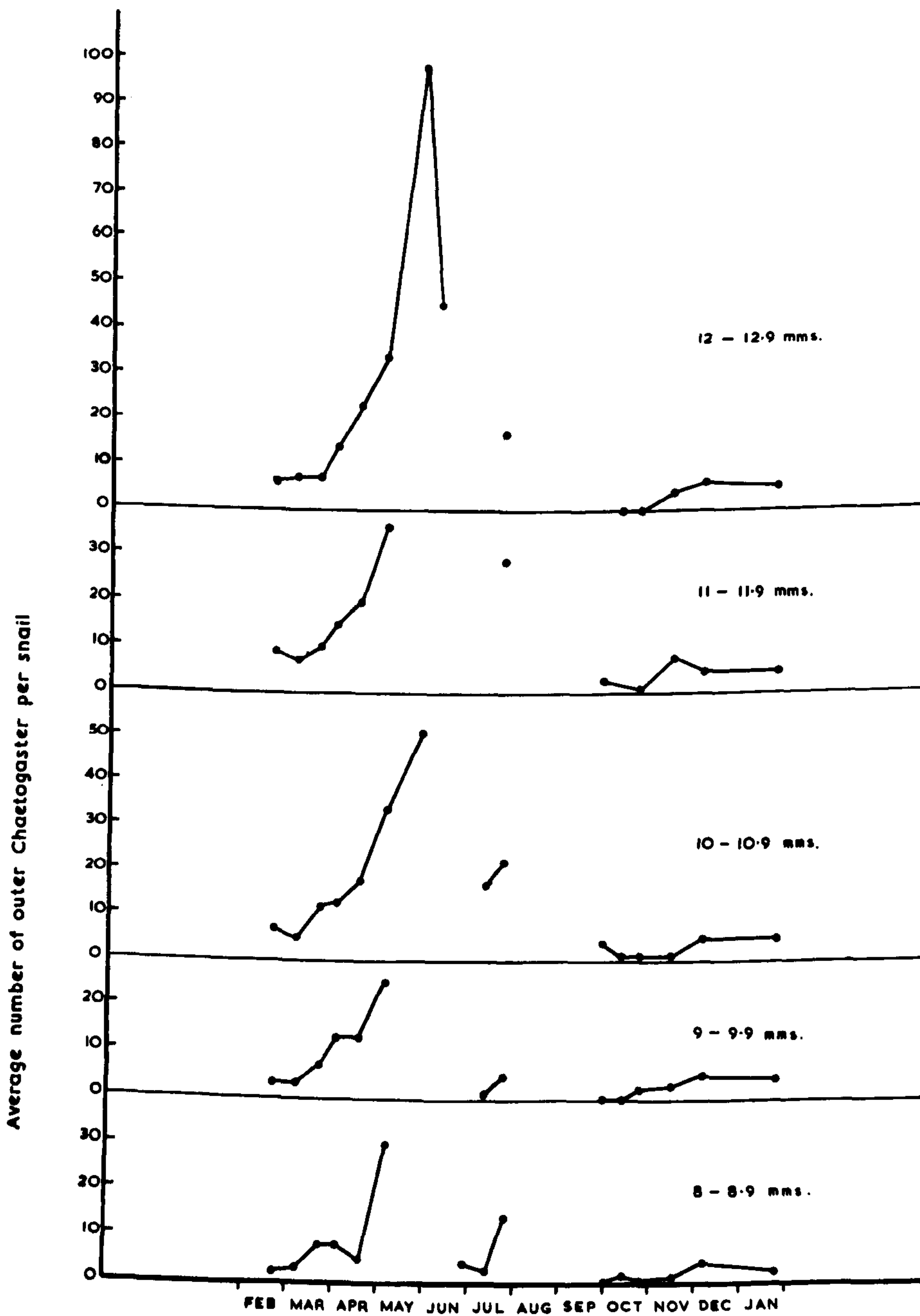


Fig. 19. The average number of outer Chaetogaster per snail throughout 1960 on five different snail size groups.

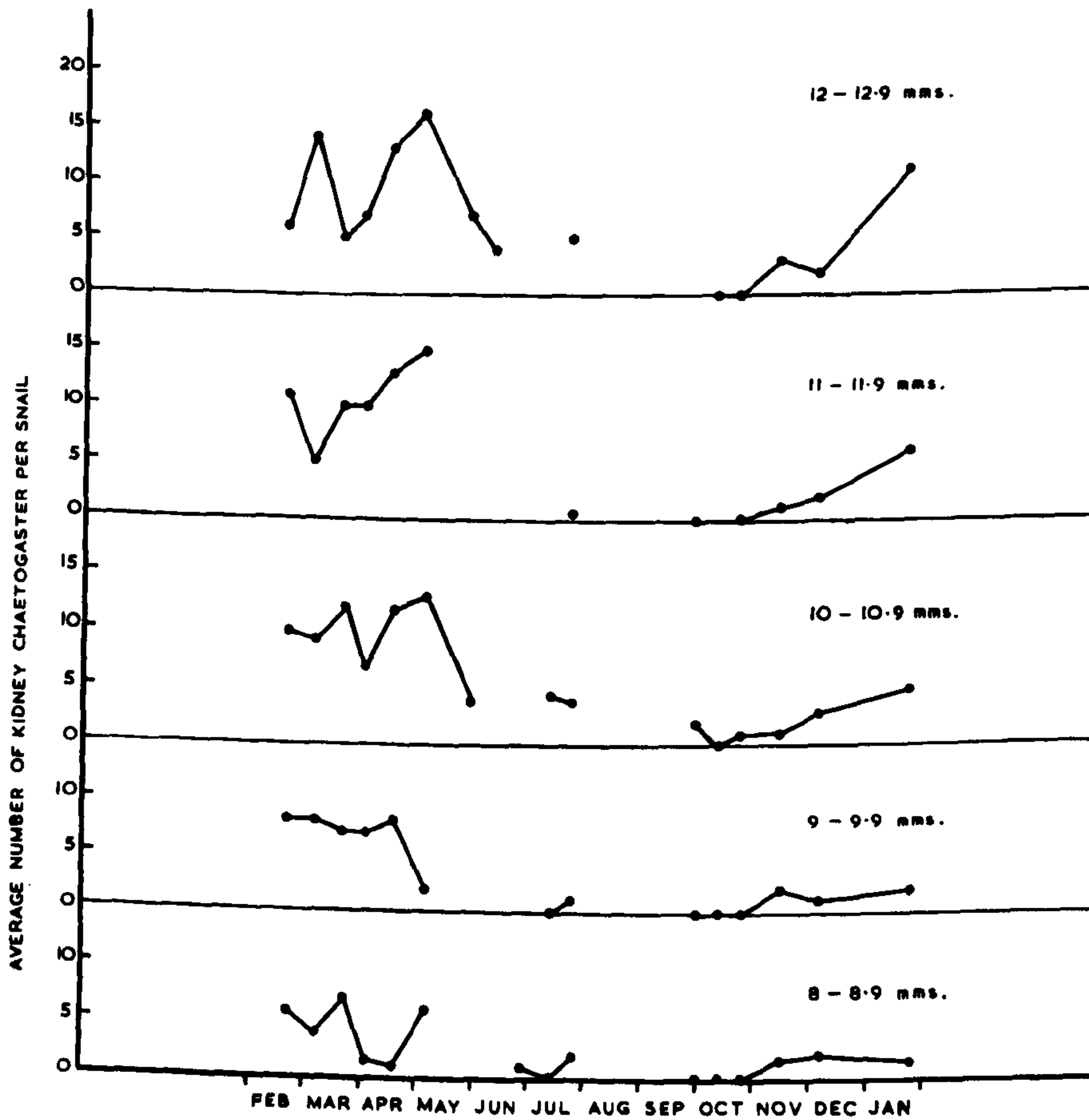


Fig. 20. The average number of kidney Chaetogaster per snail throughout 1960 on five different snail size groups.

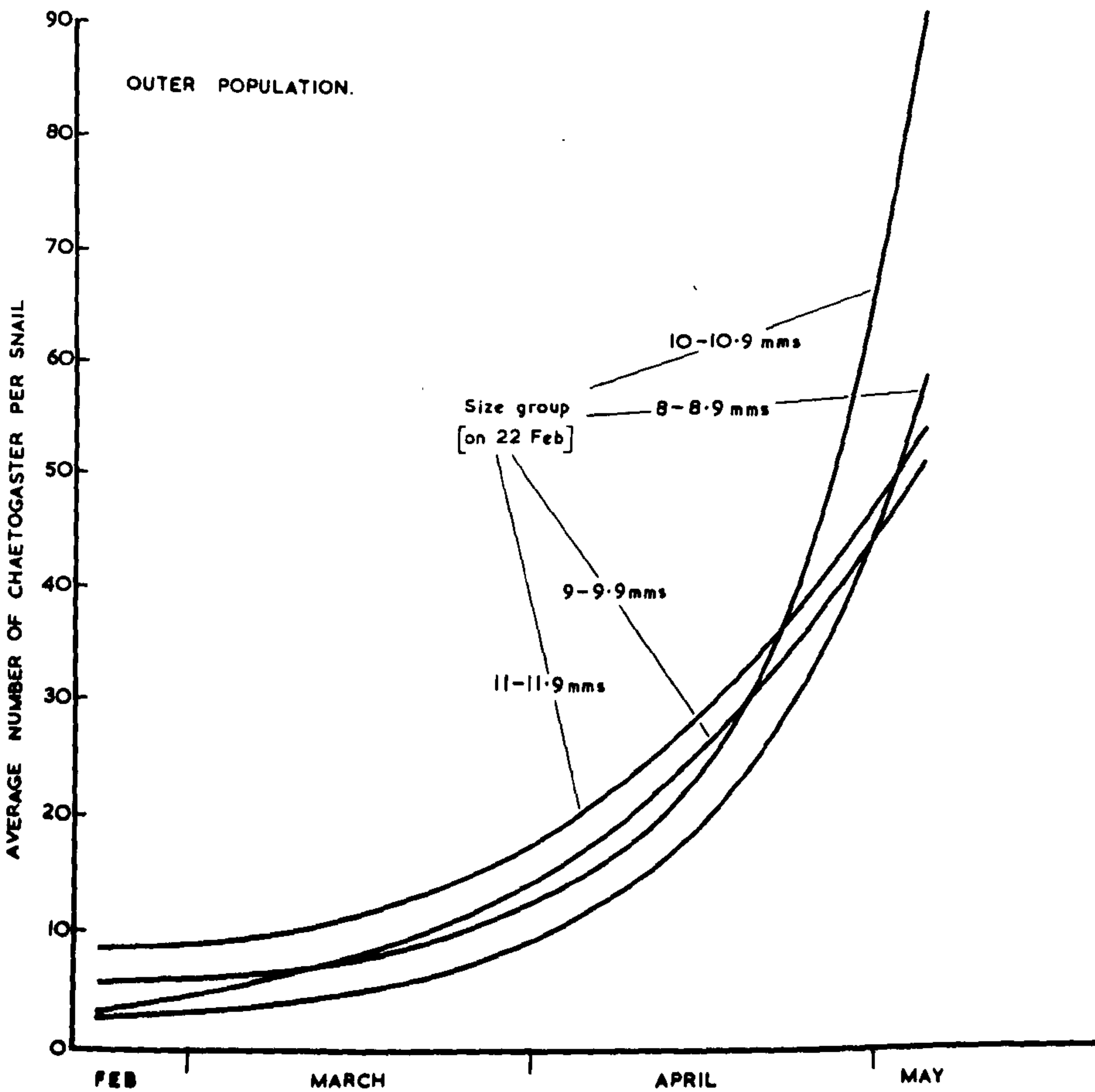
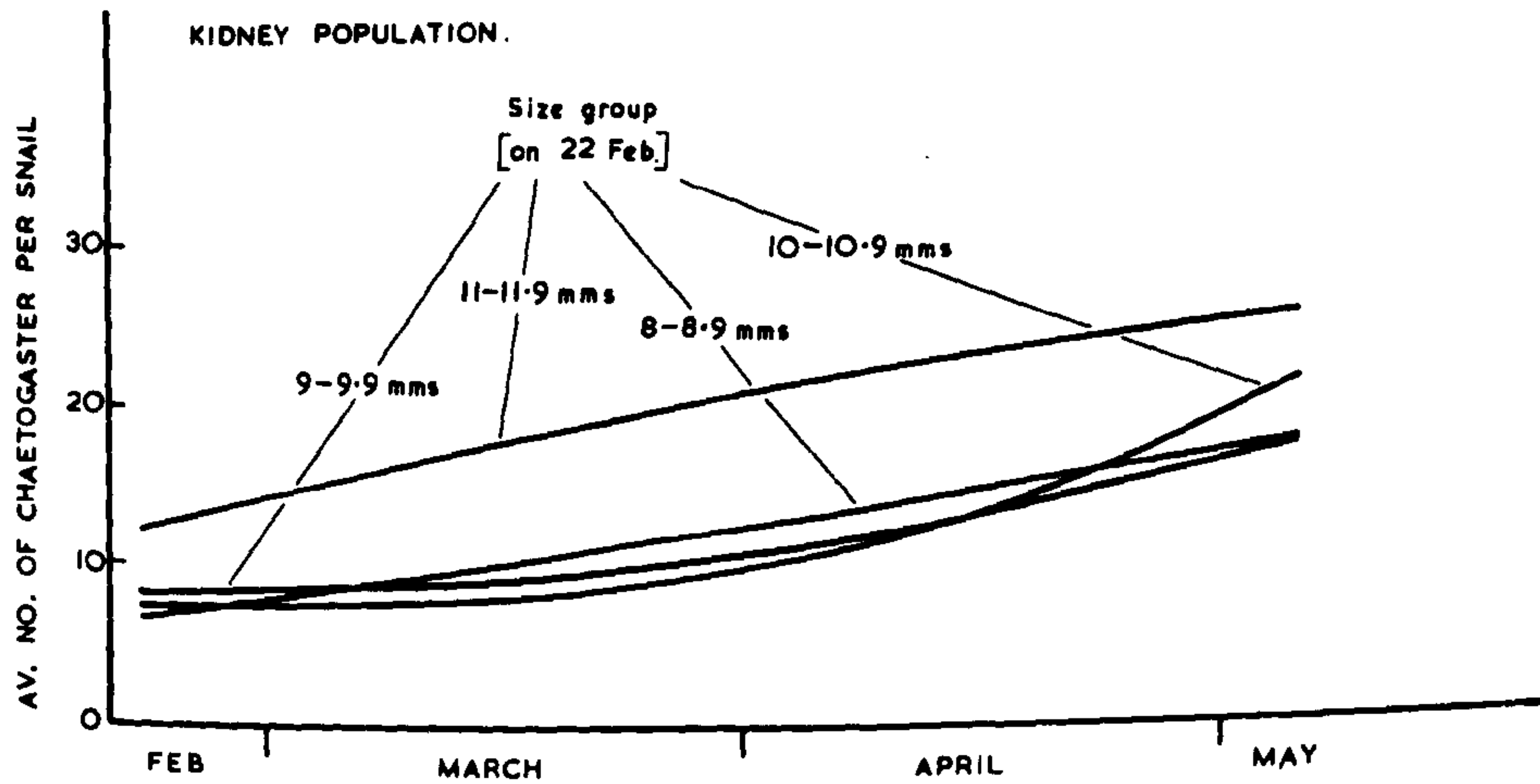


Fig. 21. The growth of the Chaetogaster populations on various snail size groups.

peak in early May 1960, the rate of increase of the mean value for the outer population became steadily greater (Fig. 12). The rate of increase of the kidney population mean however remained constantly low. This difference is demonstrated further in Figs. 19 and 20 for the outer and kidney populations respectively. Here the mean values have been calculated for five individual size groups of the snail falling between 8 and 13 mm. The outer population in all these size groups has a characteristic curve indicating an accelerating population growth rate during these two months. On the other hand, the kidney population produces a fairly constant growth rate curve; if anything, it tends to decelerate, the mean tending to reach a ceiling. These curves also show that in general the larger and presumably older snails support a bigger population of both outer and kidney Ghaetogaster than the smaller, younger snails.

During these two months in the spring of 1960, the average growth rate of the mean snail size was 0.459 mm. per week (Table 4, p. 31) and this meant that a snail would increase in size by about 1 mm. between one sampling date and the next. It was therefore quite easy to select a particular size group of snails and follow its growth and the growth of its Ghaetogaster population throughout the spring. For example, the 8 - 8.9 mm. group in sample 4 (Feb. 22nd.) would become the 9 - 9.9 mm. group in sample 5 (March 7th.), and so on. The 8, 9, 10 and 11 mm. groups of late February were followed through to early May in this way for both outer (Fig. 21) and kidney Ghaetogaster populations. The graphs represent the mean Ghaetogaster numbers for each of these groups followed through from late February to early May. Each curve in fact follows the growth of a particular pocket of the Ghaetogaster population and the four curves are

really complements of the graph of mean values of Chaetogaster per snail shown in Fig. 12 (p. 29). The shape of these curves again is such that they show the accelerating growth rate of the outer form and the slow or even decelerating growth rate of the kidney population.

b. Reservoir population, 1961.

These results are presented in Fig. 13 (p. 32.). Up to the appearance of the first generation of snails in June, the pattern is similar to that of the previous year. From June to August however, no increase was detected in either the outer or the kidney population, and no peak was reached before the appearance of the second generation of snails in September. The only effect this second dilution of the snail population had, was to lower the mean values of Chaetogaster per snail very slightly, and to reduce the percentage of snails infested. It is seen however, that these low mean values are approximately the same as the corresponding ones in the previous year where they were preceded by peak values. The percentage infestation on the other hand is slightly greater here than in the first year.

Up until February, the outer population mean remained constant at this low value reached in September. The kidney population mean however showed a slight increase from month to month. Indeed, between November and February it was found to be higher than that of the outer population as was the case early in 1960.

c. Stream population, 1961.

When sampling began in February 1961, the outer population mean was considerably higher than that of the kidney population (Fig. 14 p. 32), and moreover, the former showed 100% infestation whilst the latter showed only 14%. The 100% infestation by the outer form persisted until young snails appeared in early May, whilst the proportion of snails infested by the kidney form rose steadily and reached a peak value of 73% also in early May. As in the reservoir, both kidney and outer populations increased in numbers throughout the spring, the outer having the higher rate of increase. The peak was reached in early May, and the outer population, as in the reservoir, was considerably larger than the kidney population. With the appearance of young snails in large numbers in June, the mean values for Chaetogaster per snail dropped to near zero. After this it was not until November that any increase in these values was seen, and then only in the kidney population. The percentage of snails infested however had been increasing slowly up to this point. No second generation of young snails was produced in this habitat. By January the kidney population mean had increased further and both the mean and the percentage infestation were well above those of the outer population. Thus, apart from in the winter of 1960 - 61, the winter kidney population was always larger than the corresponding outer population in both stream and reservoir.

Taking into account the fact that only one new generation of the host snail was produced in the stream, it was seen that whilst there were minor differences between these annual cycles, the general pattern was similar in all.

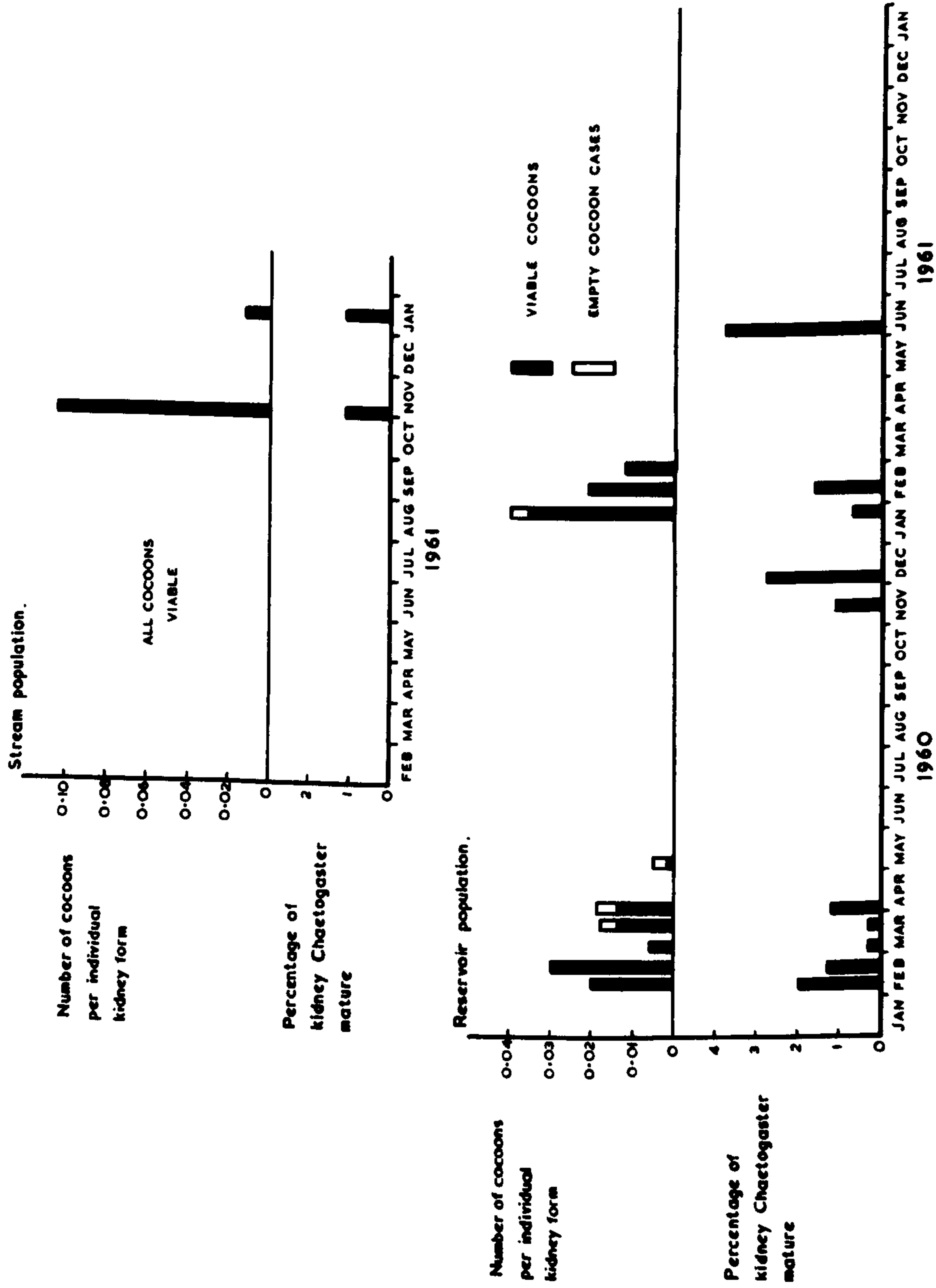


Fig. 22. The occurrence of cocoons and mature forms of Ch. Linnaei.

## Section 7.

THE LIFE CYCLE OF CHAETOGASTER LIMNAEI.a. The occurrence of mature forms.

No mature Chaetogaster limnaei of the outer type was found in either the reservoir or the stream population. Only a very small proportion of the kidney forms became sexually mature. Figure 22 shows the percentage of the total number of kidney Chaetogaster in each sample that were mature. The breeding season commenced in November or December and continued throughout the winter and spring. Except for the isolated occurrence of four mature worms in early June 1961 in the reservoir no mature individuals were found in the summer in either of the habitats.

b. The anatomy of the mature form.

Asexual reproduction by budding ceases in the mature animals. The examination of 9 mature individuals showed them to possess between 13 and 18 segments with an absence of budding zones. Genital setae are present in addition to the normal complement of setae, and an occasional bundle in these mature forms has one more seta than the maximum found in the bundles of immature forms (see p. 27). These genital setae are three in number and are situated alongside each of the setal bundles of segment 6. They are similar in shape (Fig. 1. p. 2) to the ordinary setae apart from the fact that they have only a simple hook instead of a double hook



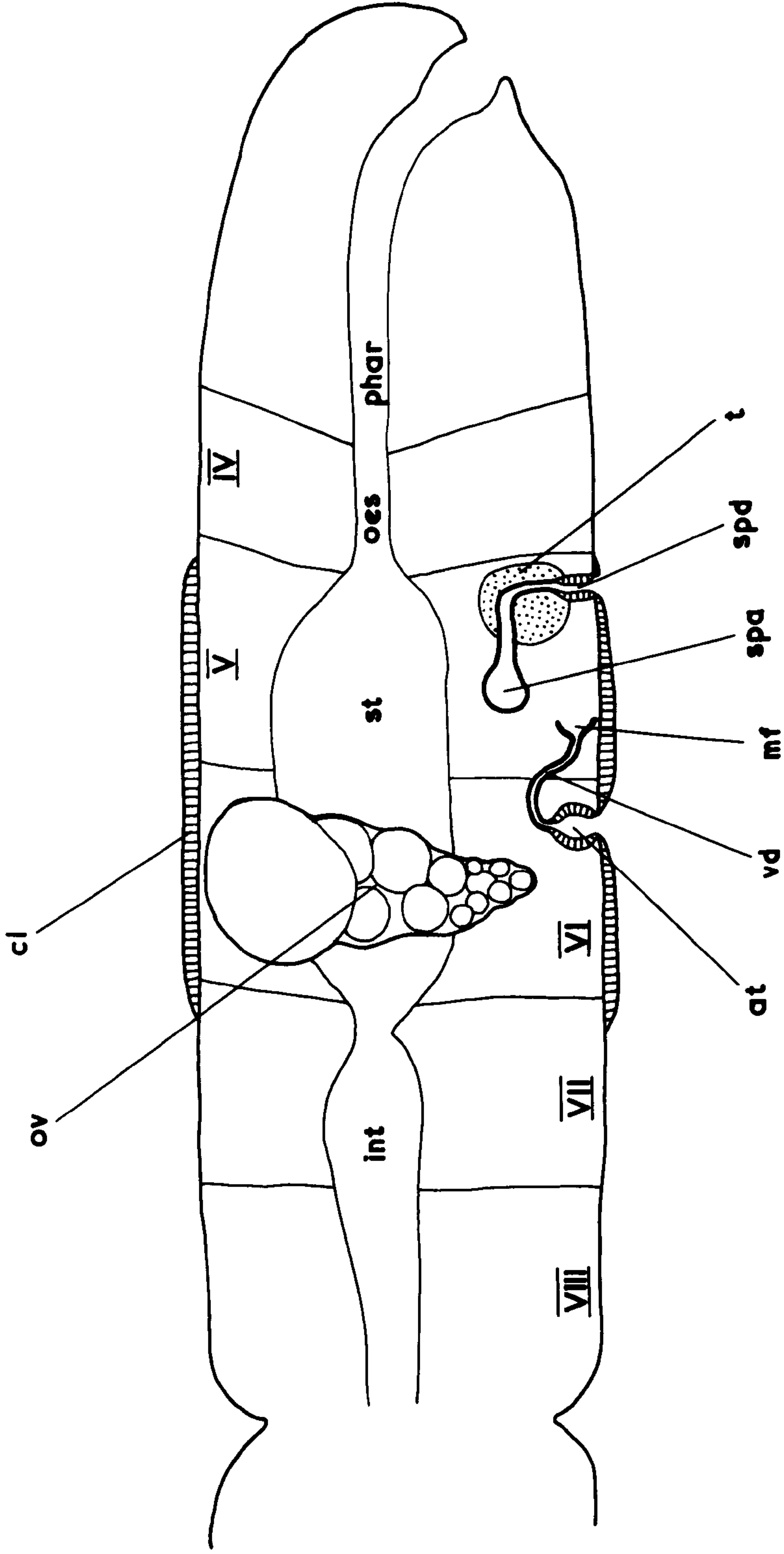


Fig. 23. The position of the reproductive organs in a mature kidney form. at, atrial ampulla. cl, clitellum. int, intestine. mf, male funnel. oes, oesophagus. ov, ovary. phar, pharynx. spa, spermathecal ampulla. spd, spermathecal duct. st, stomach. t, testis. vd, vas deferens.

as in the ordinary setae. They are however slightly longer than the average size of the ordinary setae of segment 6. They also seem to be thicker than the ordinary setae. The following measurements were made on one animal only because of the scarcity of material.

Mean length of ordinary setae on segment 6 =  $85\mu$

Mean length of genital setae on segment 6 =  $110\mu$

A diagrammatic representation of the reproductive organs of the mature kidney form is given in Fig. 23. This was drawn after examining 11 mature worms.

What seemed to be a single ovary was found in segment 6. This contained ova at various stages of development, the ripe ova being situated dorsally. A structure resembling a testis was seen in one individual. This was definite in outline and was situated on the nerve cord near to septum  $4/5$  in segment 5. The spermathecae open in segment 5 almost immediately behind septum  $4/5$ . The spermathecal duct is short and is lined by columnar epithelial cells. The spermathecal ampulla usually extends the whole length of the segment and is lined by a much more flattened epithelium than the duct. The male funnel opens into the coelom just in front of septum  $5/6$  and the vas deferens leads back from it through this septum to the atrium which in turn opens to the exterior in the anterior half of segment 6. The clitellum is seen occupying  $\frac{3}{4}$  of segment 5, segment 6 and about  $\frac{1}{4}$  or less of segment 7.

c. The occurrence of *Chaetogaster limnaii* cocoons.

All the cocoons of *Chaetogaster* that were found were taken

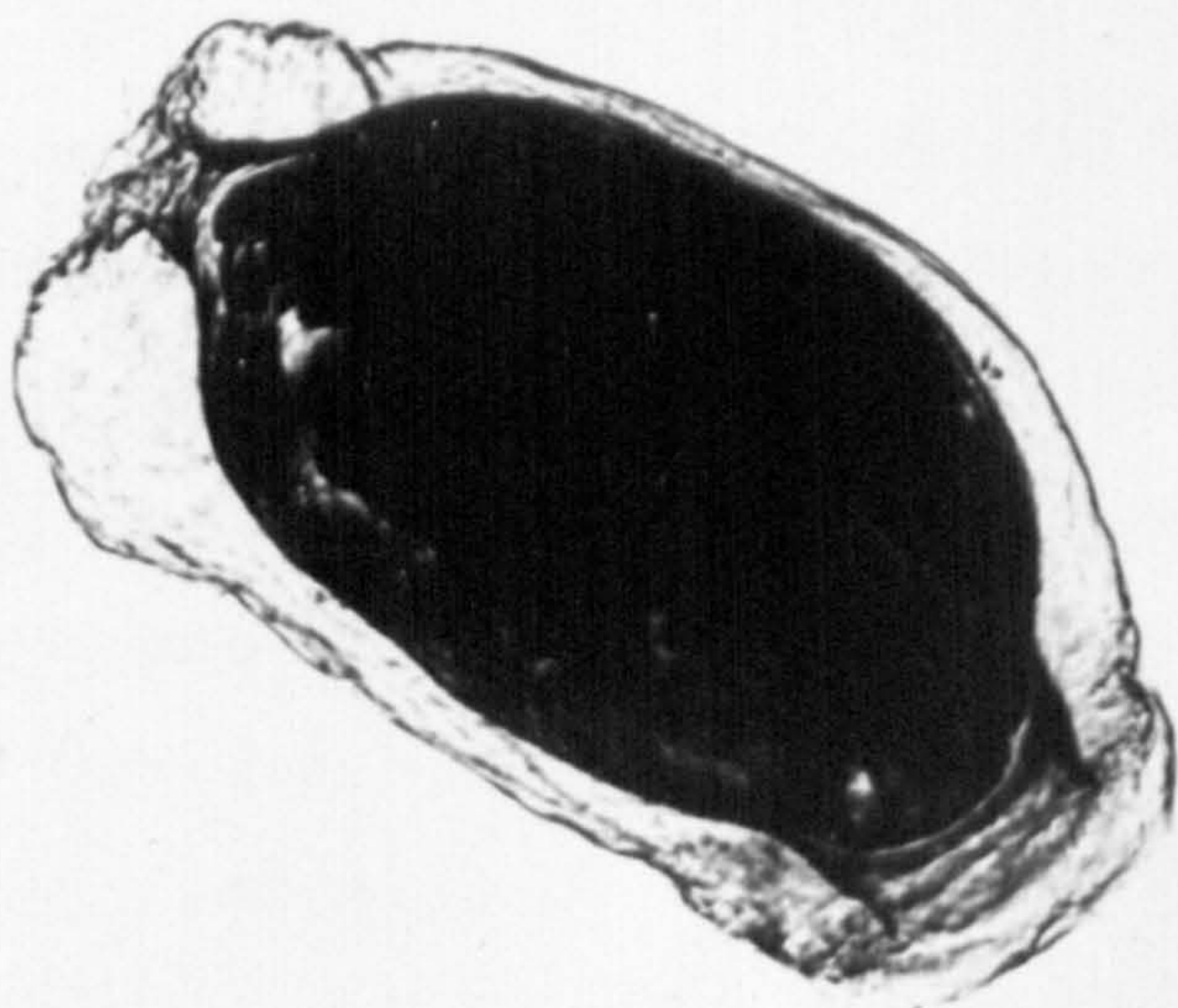


Plate 3. A Ch. limnaei cocoon found in the kidney of a  
L. pereger. (x 250)

from the snail kidney; Plate 3 shows such a cocoon. This is a typically oligochaete cocoon which is barrel shaped, each end being slightly elongated. The embryo can be seen through the transparent cocoon case. The cocoons are usually found in the portion of the kidney near the origin of the ureter.

Figure 22 (p. 41) shows the number of cocoons in relation to the number of Chaetogaster that were found in the kidney of the snails during the laboratory investigation of the samples taken at the reservoir and at the stream in 1960 and 1961. It is seen that the season in which cocoons were found corresponds to that in which mature Chaetogaster occurred. Indeed, in most cases, the cocoons were found together with mature individuals in the kidney. Many empty cocoon cases were also found in the kidney but these were not as numerous as the viable cocoons. In the spring of 1960 these empty cases did not appear until well into the breeding season and were presumably the remains of cocoons that had been deposited earlier in the season. Their presence in the kidney at this time indicates that the cocoons hatched in the kidney. However, since the empty cases were not as numerous as the viable cocoons, it is possible that some cocoons passed from the kidney through the ureter into the surrounding water.

All the cocoons found during 1960 were measured. The width varied from  $190\mu$  to  $330\mu$  with an average of  $230\mu$ . The length varied between  $230\mu$  and  $490\mu$  with an average of  $350\mu$ .

#### d. Incubation of cocoons.

Sixteen cocoons were incubated in water in small tubes at

7°C. Only three hatched as shown below.

Date found.	Date hatched.	Incubation period.	No. of buds per worm.
24:2:60	26:3:60	31 days.	None.
26:1:61	22:3:61	55 days.	One.
1:2:61	7:2:61	6 days.	None.

Six cocoons were also kept in 0.3% saline at 7°C, but not one of these hatched. A further eleven were kept in 0.3% saline at 15°C but none of these hatched either.

Out of eleven cocoons that were kept in water at 15°C two hatched as shown below.

Date found.	Date hatched.	Incubation period.
12:12:61	8:1:62	27 days.
12:12:61	8:1:62	27 days.

One of these young worms was squashed under a coverslip and the number of setae it possessed in each bundle was counted under a microscope. It was a typical kidney form in this respect. The setal bundles were fully developed since they included no exceptionally short growing setae which is typical of a developing bundle. The worm possessed one budding zone.

The second young worm was added to a dish containing a non-infested snail in pond water to see whether or not it would enter the kidney. After ten days the snail was dissected. The worm was not found on the outer surface of the snail nor in its kidney. A search of the dish revealed the worm still living on the bottom of the dish. It was then introduced to another non-infested snail in the same way. Again the worm could not be found on the snail and a search of the dish was not

successful. The worm had probably died and disintegrated.

Thirteen cocoons were incubated in kidney extract (see p. 22) at 15°C. None of the cocoons hatched.

All the cocoons incubated were kept as described for about five months and examined every three or four days.

All the cocoons that hatched were incubated in water and although the number is too small for any firm conclusions to be drawn on requirements during incubation it does appear that water is a suitable medium.

e. Factors influencing cocoon production.

It is possible that the sexual phase of the kidney form of Chaetogaster limasi is synchronised with the life cycle of the host snail. This type of synchronism has been demonstrated by Mofy and Smyth (1959) in Opalina ranarum which is parasitic in the rectum of Rana temporaria, and by Miretski (1951) in Polystoma integerrimum which is also a parasite of the frog. The former multiplies asexually during most of the year but during the breeding season of the frog it changes its reproductive pattern to a sexual one. The latter releases eggs when the frog goes into water to breed and by the time the eggs have hatched abundant tadpoles are available for infection. The sexual phase in kidney Chaetogaster occurs just prior to and during the time that spawning occurs in the L. pereger population, and such a relationship as is established between Opalina and Rana could be present here. Against this is the fact that no mature worms and cocoons were found during the second breeding season of the snail in the summer.

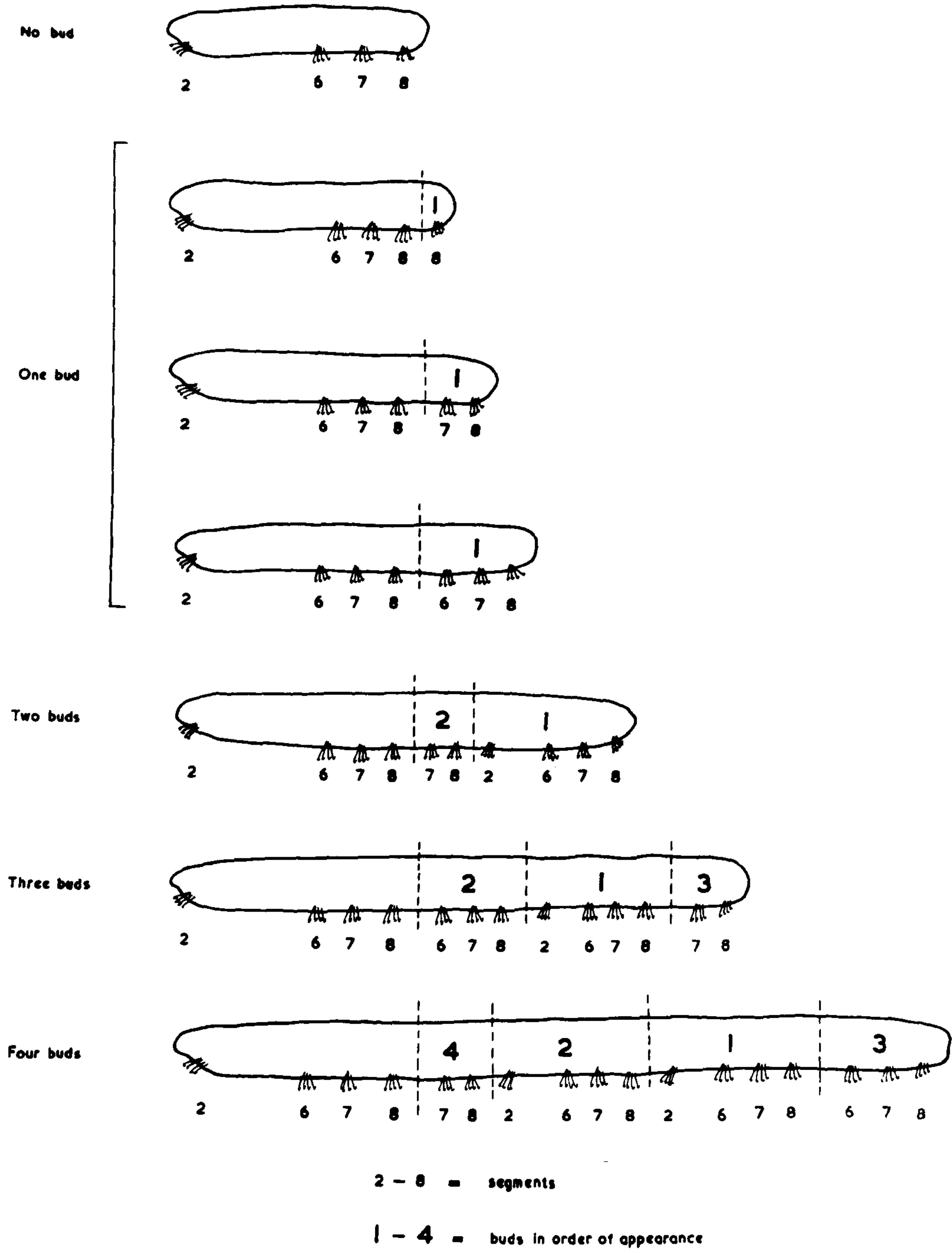


Fig. 24. The development of buds in Ch. limnaei.

The possibility that such a relationship exists however needs investigating.

Another possible stimulus for the production of cocoons is the onset of low winter temperatures. Mature individuals and cocoons appeared when the water temperature dropped to about 10 - 8°C. An attempt was made in the laboratory to induce cocoon production at low temperatures. Twenty snails were taken in May from a population of L. pereger heavily infested with kidney forms as well as with outer forms. They were then kept for 7 days in pond water at 7°C and then at 2°C for six weeks. At the end of this period the snails were dissected and the kidney Chaetogaster were removed and examined. No cocoons or mature individuals were found in any of the snails as shown in Table 5, p. 47.

Judging from the field results the percentage of mature individuals here should be at least 1 or 2 per cent assuming that low temperatures do have the effect of stimulating the Chaetogaster to become sexually mature. One can only conclude that under the conditions of this experiment low temperatures do not seem to do this. It is necessary to repeat this experiment at different times of the year so that snails at different stages in their life history can be used. This should show whether or not low temperatures can induce maturity during certain stages of the snail's life history and not at other times.

f. Budding.

Both kidney and outer Chaetogaster reproduce asexually by budding all the year round. Budding only ceases at maturity.

Figure 24 shows the growth of a Chaetogaster from a form with



Table 5.

Snail.	Outer Chaetogaster.	Kidney Chaetogaster.	Mature kidney forms.	Cocoons
1	10	3	-	-
2	9	7	-	-
3	13	7	-	-
4	25	4	-	-
5	18	8	-	-
6	14	8	-	-
7	14	3	-	-
8	21	9	-	-
9	13	5	-	-
10	13	6	-	-
11	12	10	-	-
12	19	7	-	-
13	18	8	-	-
14	15	12	-	-
15	7	10	-	-
16	5	4	-	-
17	17	9	-	-
18	11	6	-	-
19	Snail dead.			
20	Snail dead.			
<b>Total:</b>	<b>254</b>	<b>126</b>	<b>-</b>	<b>-</b>

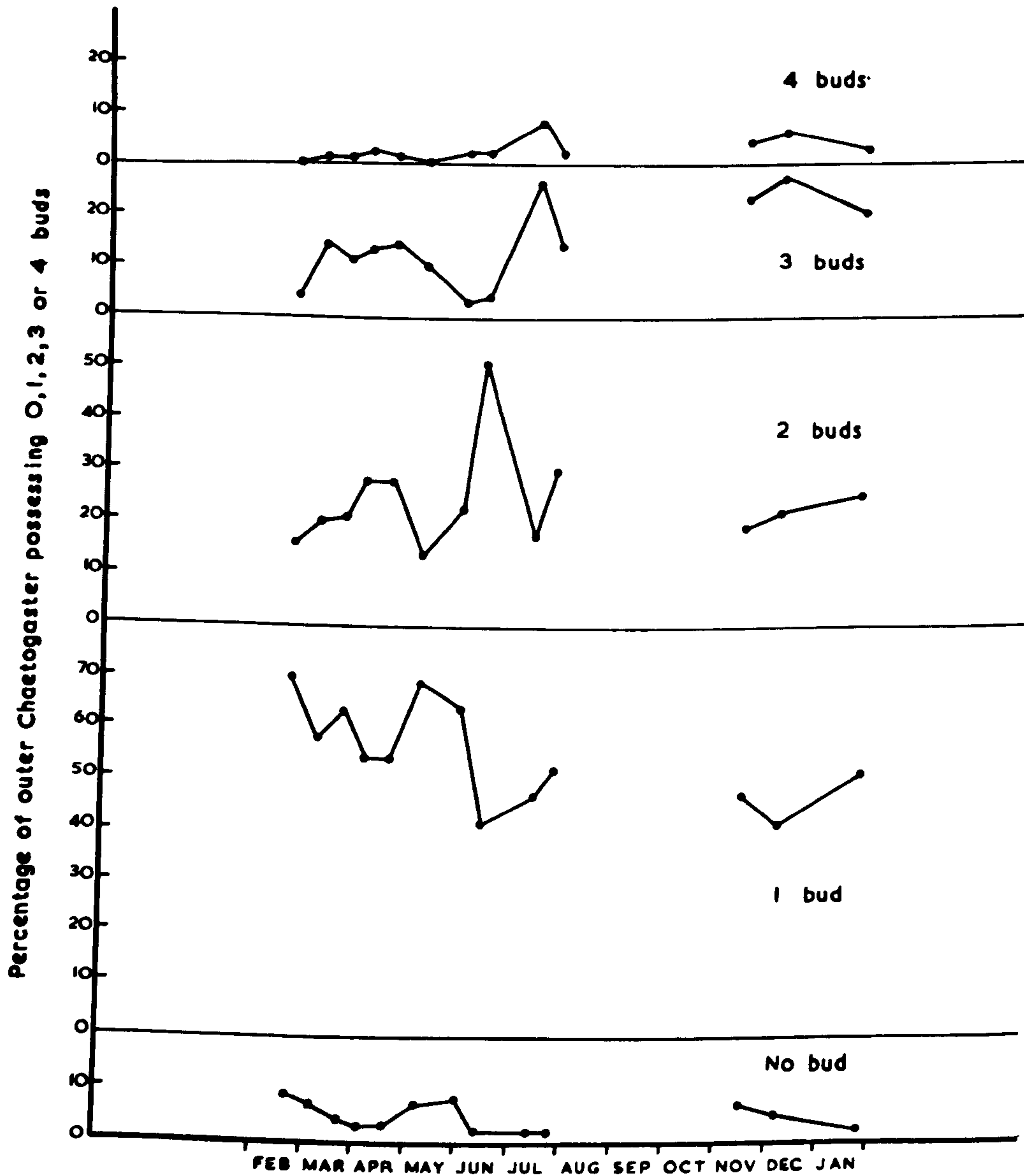


Fig. 25. The outer Chaetogaster population of the reservoir throughout 1960 split into five groups according to the number of buds per worm.

no bud to a form having four buds. This diagram applies to both outer and kidney forms.

The first budding zone appears behind segment 8. The first new segment formed, behind this budding zone, is what will eventually be segment 8 of the new individual. The second is segment 7, the third segment 6 and so on, the last formed being segment 1. This sequence is followed in the formation of all subsequent buds. The next bud appears between the parent worm and the first bud. The third bud is formed very soon after this at the posterior end of bud 1. The fourth bud is produced between the parent and the second bud. Very occasionally individuals having 5 buds are seen but unfortunately no data are available relating to the position of the fifth bud. The newly formed chain of buds seems to break away from the parent animal before the five bud stage is reached in most cases. When the animal divides it usually breaks into two parts and the number of buds remaining on the parent animal and the number of buds present on the new part depend on how many buds were originally present. It is assumed that the break occurs at the position of the first budding zone, because rough treatment of a worm having 2, 3 or 4 buds often causes a separation at this point.

g. Changes in budding activity during 1960.

(1) Outer population.

Figure 25 shows the percentages of outer Chaetogaster in each sub - sample (see p. 20) having 0, 1, 2, 3 or 4 buds. The sub - samples which contained less than 20 Chaetogaster have not been included because

the numbers were considered to be too small to be of any value.

Early in the year, when sampling began, it is seen that there was a predominance of individuals having 0, 1 or 2 buds. Thereafter until April the percentage of individuals with 0 and 1 bud dropped steadily. The percentage of the 2 and 3 bud animals however increased due to the drop in the numbers of the 0 and 1 bud animals, which had produced more buds. Very few animals grew into the four bud stage at this time and the percentage of these remained constantly low for some time. In May the percentage of 0 and 1 bud animals rose to a peak at the expense of the 2 and 3 bud animals. Obviously, the population had reached a stage where the individuals possessing the higher number of buds were dividing producing individuals with few buds. These then grew, and about a month later, in June, this peak was seen in the two bud part of the population accompanied by a fall in the percentage of 0 and 1 bud forms. The 2 bud forms in turn produced another bud and consequently a peak appeared in the percentage of 3 bud forms in July, and naturally the percentage of 2 bud forms fell. At the same time many animals grew into 4 bud forms thus producing a slight peak in the percentage of these present in the population. As before, following the peak in the percentage of forms having the higher numbers of buds came a sharp drop in these and a rise in the percentage of animals with the lower numbers of buds.

The number of worms in each sub - sample during the latter part of the summer and in the autumn was so small that they could not be used. However, it seems as though successive peaks were reached in the percentage values for the 0, 1 and 2 bud animals during this time because a peak is seen in the percentage number of the three and four

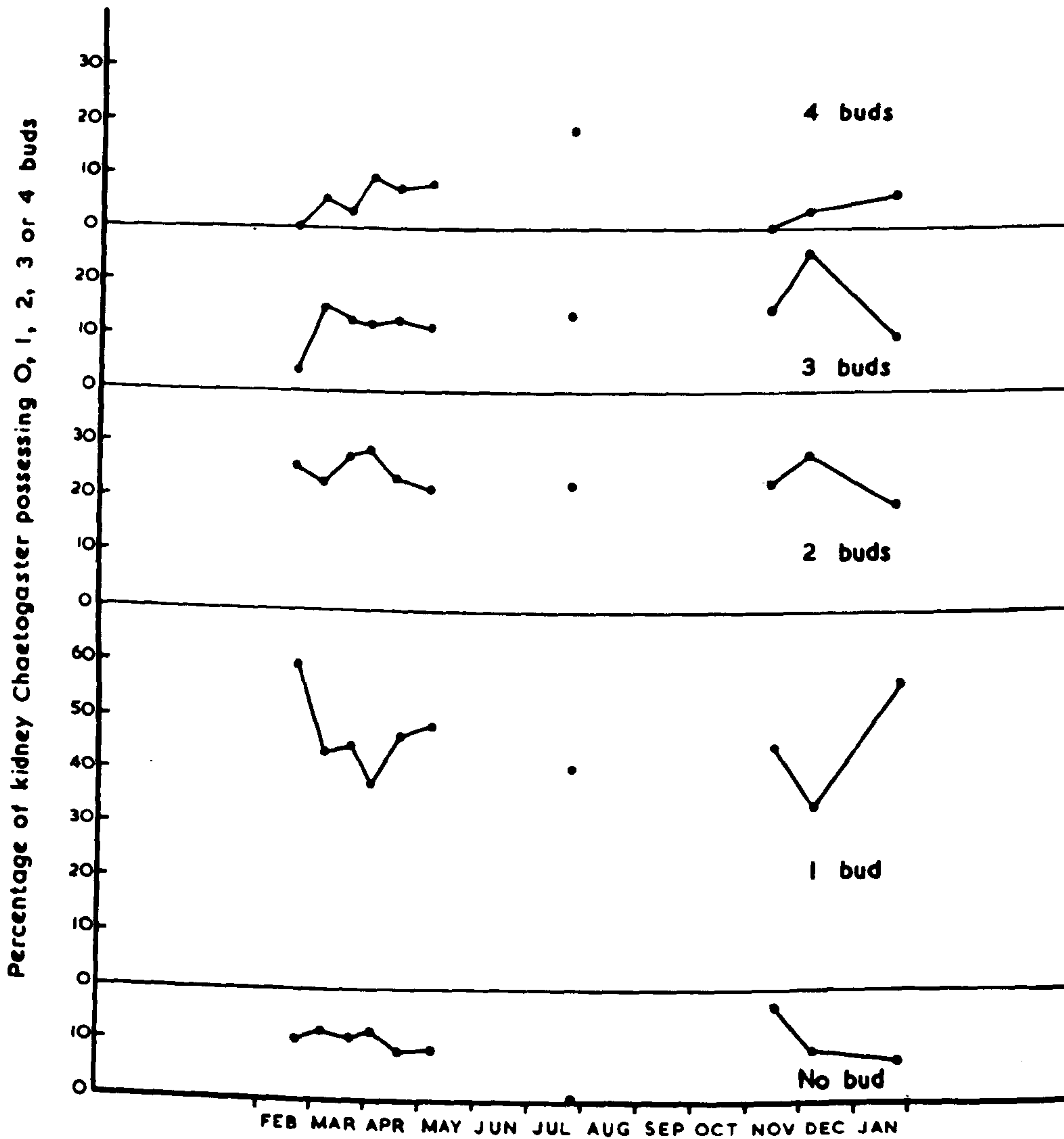


Fig. 26. The kidney Chaetogaster population of the reservoir throughout 1960 split into five groups according to the number of buds per worm.

bud animals in early winter. At this time also the percentage of 0, 1 and 2 bud animals in the population was low.

It is seen that the population oscillates frequently between two extremes, i.e., between a population of worms having 3 or more buds and a population consisting mainly of 0 and 1 bud individuals. Three such oscillations probably occurred during 1960. The amplitude of these oscillations was highest in May, June and July which suggests a greater activity in asexual reproduction at this time. It seems that this activity is little affected by changes in the life cycle of the host.

(11) Kidney population.

Figure 26 shows the percentages of kidney Chaetogaster in each sub - sample having 0, 1, 2, 3 and 4 buds. Again the sub - samples which contained less than 20 Chaetogaster have not been included.

The violent oscillations that were seen in the outer population are not seen here, or at least they are not apparent in the results. A definite trend is seen however in that between February and May, the percentage of 0 bud forms in the population steadily decreased whilst that of the 4 bud form increased. Also, after an initial drop, the proportion of 1 bud forms remained fairly steady. This drop corresponds to an initial steep increase in the percentage of 3 bud forms which thereafter remained steady. The value for the 2 bud animals varied very little during this time. It is gathered that during this period there was a steady shift from the forms with the lower number of buds towards the forms with the higher number of buds. Between May and July, and August and November, the number of Chaetogaster in each sub - sample was considered to be too low

to be of any value and they were omitted. It seems that the condition of the population in winter is the reverse to what it was in July. This is best seen by looking at the parts of the population with 0 buds and 4 buds. Between July and November the former had increased considerably whilst the latter had decreased.

It is difficult to draw conclusions from these results but it is seen that the kidney population is more stable than the outer population in that it does not display the oscillations that were seen in the latter. This suggests that conditions for the outer form are more variable than those in the kidney. It is impossible to say with certainty that asexual reproduction in the kidney population is more active at certain times of the year than at others. Again there is no evidence here to suggest that the pattern of asexual breeding activity in the kidney is affected by changes in the life cycle of the host.

## Section 8.

### FEEDING IN CHAETOGASTER LINNAEI.

The gut contents of samples of Chaetogaster linnaei taken from the reservoir were investigated at six week intervals throughout 1961. Twenty outer and twenty kidney Chaetogaster from each sample were squashed under a coverslip and the organisms observed in the gut were recorded.

#### a. The gut contents of kidney Chaetogaster.

All the kidney Chaetogaster examined throughout the year

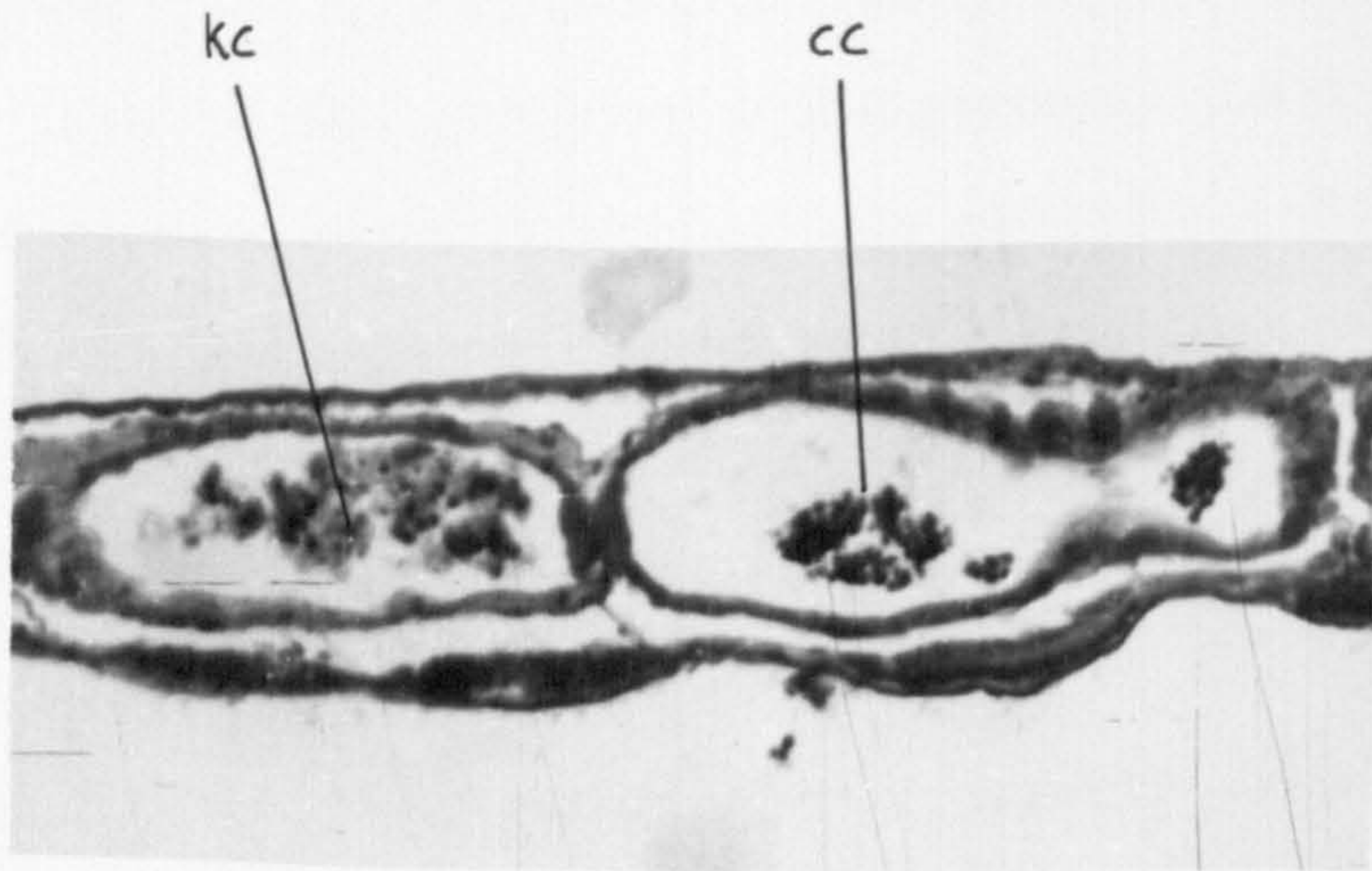


Plate 4. Longitudinal section of the stomach and intestine of a kidney form to show the crystalline concretions remaining undigested in the intestine. kc, mollusc kidney cells containing crystalline concretions present in the stomach. cc, undigested crystalline concretions in the intestine. (x 250)



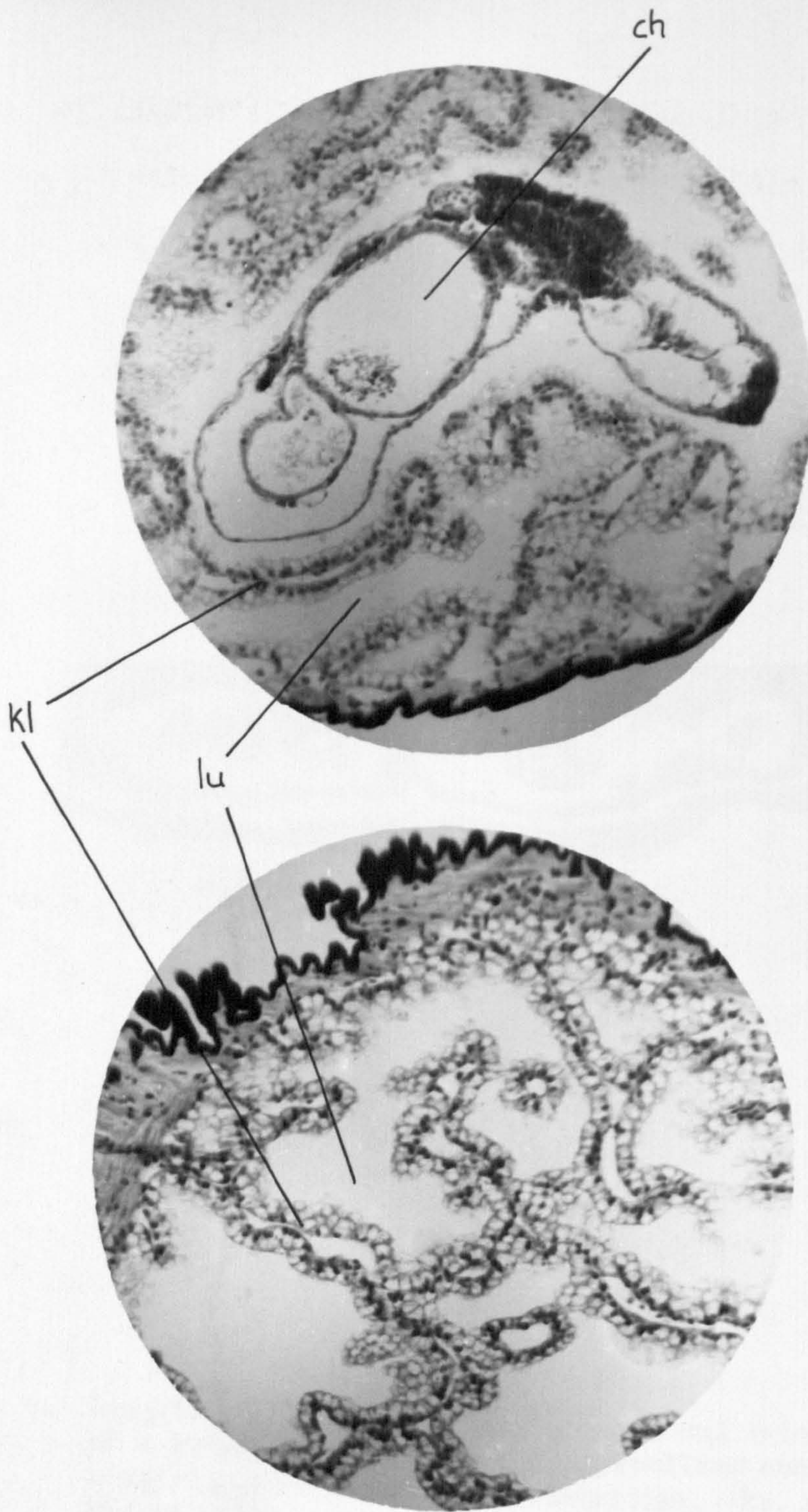


Plate 5. (a) Section through an infested kidney of a L. pereger, and (b) a section through a non-infested kidney. ch, kidney Chaetogaster. kl, kidney lamellae. lu, kidney lumen. (x 160)

contained only kidney cells and crystalline concretions derived from the kidney (Picken 1937, Pan 1958, Andrew 1959). Some individuals had only the crystals in their gut whilst others also had groups of cells containing these crystals. These cells were usually found in the portion of the gut described by Sperber (1948) as the stomach, and by the time they had passed into the intestine only the crystals were to be seen remaining undigested (Plate 4). These observations suggest that the kidney forms feed exclusively on the loose cells containing crystalline concretions which are discharged by the kidney. They may also engulf considerable quantities of the snail urine whilst feeding on these cells.

A histological examination of the kidneys of L. poroceph infested with Chaetogaster, and a comparison of these with non-infested kidneys show that the worms do not appear to damage the kidney in any way. Plate 5a. shows a section of an infested kidney and Plate 5b. a section of an uninfested kidney. The lamellae of the infested kidney do not seem to have been broken or damaged by the worms which are seen to inhabit the lumen in between the lamellae. The lamellae of the infested kidney however seem to have been forced closer together due to the presence of Chaetogaster in this organ. It is concluded that the worm does not feed on live kidney cells forming part of the intact kidney but rather on those cells which have been discharged into the kidney lumen and would normally be voided. It is pointed out in the next section (p.118) that kidney forms will not eat organisms normally consumed by other forms.

b. The gut contents of other Chaetogaster.

**(1) Introduction.**

The outer form feeds on small plants and animals present in the water in which it lives. Whilst feeding it anchors itself onto the body of the snail with all its setae except the anterior bundle on segment 2. Wagin (1931) was of the opinion that it uses its setae to anchor itself to the mucus present on the surface of the snail. It has been observed during the course of this study that whereas Chaetogaster cannot crawl on smooth, clean glass, it finds no difficulty in attaching itself to, and crawling along a mollusc mucus trail left on the same glass. Wagin's opinion was therefore probably correct since it is seen that Chaetogaster is quite capable of moving efficiently on a thin film of mucus such as would occur on the surface of the snail. The part of the worm's body anterior to segment 6 is held away from the snail and the worm can often be seen in this posture under the edge of the shell, on the tentacles and on the snail's foot. The free anterior end of the worm moves slowly from side to side in the water and when a food organism swims or floats near to its head the worm quickly engulfs its prey. During this process the pharynx is seen to dilate but the action of catching the prey is too quick to be observed with the naked eye. Wagin (1931) observed a similar behaviour in Chaetogaster lignaei feeding on cercaria.

**(11) Field Results.**

Records of the gut contents of outer Chaetogaster obtained from the samples taken during 1961 (see p. 51) are presented in Table 6 (p. 54). The values presented are the mean numbers of each organism per individual worm. The principal food was of diatoms but ciliate Protozoa and Rotifera were also taken in large numbers. Filamentous algae,

Table 6.

The average number of food organisms in the gut of each worm of the outer type in the samples taken.

Organism.	Date.							
	28:3:61	26:4:61	5:6:61	25:8:61	28:9:61	9:11:61	15:12:61	6:2:62
Diatoms	8.6	4.85	11.1	15.5	37.2	18.1	11.25	19.9
Ciliates	-	3.8	1.8	1.5	0.5	0.8	0.1	4.2
Flagellates	-	-	0.75	0.25	-	0.1	-	-
Foramni- fera	0.05	-	0.15	0.35	0.25	0.2	0.1	-
Rotifers	0.4	0.7	0.45	1.0	1.0	0.15	0.1	0.1
Filamentous algae	0.05	-	-	0.3	0.1	-	0.15	0.15
Cercaria	-	-	-	-	0.3	-	-	0.05

Foraminiferan and flagellate Protozoa were also found in the gut of Chaetogaster. Outer Chaetogaster that lived on snails infected by cercariae were often found to have eaten these organisms.

The diatoms eaten by the Chaetogaster were usually members of the genera listed below;

Genus.	Habit
Navicula	Planktonic
Gymbella	Planktonic or encrusting
Eunotia	Planktonic
Pinnularia	Planktonic
Gomphonema	Encrusting
Tabellaria	Planktonic
Synedra	Planktonic

Most of these genera are planktonic. The encrusting Gomphonema grows attached to the substratum by a short stalk.

The highest concentrations of diatoms and rotifers found in the gut were recorded in the late summer and autumn. Ciliates on the other hand seemed to be least numerous in the gut at these times.

Nauplius larvae were also found on two occasions in the gut of the worms examined.

It is seen that most of the organisms eaten by the outer Chaetogaster are planktonic.

c. Cercaria as a source of food for Chaetogaster lignesi.

Because only a small proportion of the snails from the reservoir were infected with cercariae, the number of Chaetogaster found during the routine examination of the gut contents to have eaten these was

very small. Consequently it was impossible to estimate the extent to which Chaetogaster fed on cercaria. It was therefore decided to collect a large number of snails from the reservoir and to investigate the gut contents of outer Chaetogaster from those snails that were infected with cercariae. About 300 snails were taken from the reservoir, and 20 of these were found to be liberating cercariae. The 20 cercaria - infected snails were dissected and the percentage of outer Chaetogaster on each snail having recognisable cercariae in their gut is shown in Table 7 (p.56). On average, about 16% of the Chaetogaster had cercariae in their gut at this particular time. There is a great deal of variation in the percentage value for each snail and this is partly due to the fact that the number of Chaetogaster per snail was often very low. Another possible reason is that some snails were more heavily infested with cercariae than others and that these larvae were leaving their hosts in variable numbers. It is impossible to say what proportion of the number of cercariae leaving the snail is eaten by the outer Chaetogaster. It is therefore not possible to discover from these data to what extent Chaetogaster controls the number of cercariae liberated into the water.

d. The availability of food to outer Chaetogaster.

It is possible that feeding conditions for the outer form are better on the snail than on the substratum of the pond. This hypothesis has been tested experimentally by comparing the gut contents of free-living outer Chaetogaster with those of the outer forms living on the snail.

The snails infested with outer Chaetogaster used in this

Table 7.

Snail	Percentage of <u>Chaetogaster</u> containing cercariae	Number of worms examined
1	11.27	71
2	0	1
3	75.0	4
4	14.29	14
5	0	1
6	0	3
7	0	1
8	0	1
9	100.0	1
10	15.79	38
11	36.36	11
12	4.55	22
13	7.89	76
14	4.92	62
15	8.62	58
16	9.91	111
17	8.33	36
18	8.70	69
19	5.19	77
20	6.76	74
Average:	15.88%	

experiment were kept in distilled water for 7 days. The distilled water was replaced every two days to make sure that no food organisms were available for the Chaetogaster. At the end of the 7 day period in distilled water it was seen by examining 10 of the outer Chaetogaster that their gut was completely empty. This starved Chaetogaster population was then used in the following experiment.

One snail infested with the starved outer Chaetogaster was placed in a small dish containing about 100 ml. of pond water. Another snail infested with the starved outer forms was dissected and the latter were removed and placed in another dish containing 100 ml. of pond water. These were then left for 24 hours after which time ten Chaetogaster removed from the snail and ten of the free-living ones from the second dish were examined for gut contents. The experiment was repeated a further three times and the results are presented in Table 8 (p. 58).

The results were pooled to give the total number of organisms found in 40 worms living on the host and in the 40 free-living worms. A  $\chi^2$  goodness-of-fit test performed on the totals shows that there is a significant difference between the low number of organisms found in the free-living Chaetogaster and the higher number in those living on the snail. This suggests that the availability of food is greater for the Chaetogaster living on the snail than for the free-living forms. This is especially true of non-motile food, i.e., diatoms. No diatoms had been eaten by the free-living Chaetogaster but a fair number had been taken by the worms living on the snail. Cercariae, of course, were only taken by the worms living on the snail.

There are two possible reasons why the availability of food



Table 8.

A comparison of the food taken by oyster Chaetogaster when on its host and when free-living.

	<u>Chaetogaster on the snail.</u>					<u>Free-living Chaetogaster.</u>				
	Expt 1	Expt 2	Expt 3	Expt 4	Tot	Expt 1	Expt 2	Expt 3	Expt 4	Tot
Diatoms	4	4	8	1	17	0	0	0	0	0
Rotifera	20	8	7	9	44	2	2	0	6	10
Ciliata	25	17	59	41	142	52	23	5	27	107
Geroariae	0	2	0	0	2	0	0	0	0	0

The results of  $\chi^2$  tests performed on the totals:-

Organism.	$\chi^2$	P less than
Diatoms	infinity	0.001
Rotifera	115.6	0.001
Ciliata	11.45	0.001

is greater on the snail. Firstly, the fact that the snail moves through the water means that in effect the Chaetogaster is able to search a greater volume of water for food than it would if it were crawling on the substratum, and consequently the chances of finding food are greater. Secondly, it has been observed that the snail's ciliated epithelium produces a current of water which flows in an anterior - posterior direction along the body of the snail. Again, this means that the Chaetogaster, living in this current which moves along the snail, can search a greater volume of water for food with less expenditure of energy than it could otherwise.

Since Chaetogaster feeds largely on planktonic plants and animals, its feeding behaviour is greatly assisted by the fact that it lives in a current of water. The few encrusting algae and the foraminifera found in the gut of this outer Chaetogaster (see Table 6. p. 54) were probably obtained either because these organisms were disturbed by the snail so becoming planktonic or by Chaetogaster clinging to the foot of the snail and feeding on organisms present on or near the substratum.

### Section 9.

#### LABORATORY AND FIELD EXPERIMENTS.

So that the species may be continued, Chaetogaster linnaei must in some way be able to establish itself on every new generation of the host. To do this it must leave its present host at the latest when the snail dies and presumably before it decomposes. Observations made on decomposing snails show that any outer or kidney Chaetogaster remaining on

the snail at this time are usually dead. The ease and frequency with which Chaetogaster establishes itself on a new host was investigated. First of all, an experiment was performed to see whether Chaetogaster in fact does leave the host snail. Once this was established, the transfer of Chaetogaster from one snail to another was investigated in the laboratory. In the field, experiments were carried out in an attempt to discover the method of transfer. The next group of experiments were designed to show whether or not the outer and kidney forms were habitat specific. Following on from these, it was decided to subject outer forms to adverse conditions to see whether this would cause them to enter the kidney. Since, during the transfer from one snail to another the Chaetogaster would probably adopt a free-living mode of life, several experiments were performed to see how long the outer and kidney forms could survive in the free-living state. Experiments were also performed to study the response of the worm to the mucous trails of its host.

Shortage of material prevented the repetition of many of the experiments described in this section. When the experimental work was planned, it was necessary to take into consideration the number of non-infested snails that would be available for use in such work. It was found that this factor limited the number of experiments that could be done if these were to be made in duplicate or triplicate. Therefore, it was decided that in order to extend the range and type of experiments to be performed, many of them could not be duplicated.

a. Dispersal of Chaetogaster limnasi.

The purpose of the experiment described below was to find out if and in what numbers Ghaetogaster leaves a dying snail which was infested with both outer and kidney forms. The infested snail was killed by making an incision through the brain. Abelocs (1942) and Feliksiak (1947) state that pulmonates have the power to regenerate the head, but not the head ganglia. Thus, damaging the brain was the obvious way of killing the snail in a gradual manner; the snails usually started decomposing six to ten days after damage.

After being 'killed' the snail was placed in a small dish which was searched once a day for any Ghaetogaster that had dispersed from the snail. This was continued until no more Ghaetogaster were found. The experiment was repeated a further three times and the results are presented in Table 9 (p. 62).

A control experiment in the form of four living snails in individual dishes ran concurrently with the experiment described. No Ghaetogaster were observed to leave these snails. At the end of the experiment these were dissected and the numbers of Ghaetogaster found in or on them were as follows:-

Snail 1.	Outer forms	11.	Kidney forms	5.
" 2.	" "	12.	" "	10.
" 3.	" "	14.	" "	8.
" 4.	" "	16.	" "	10.

The number of worms leaving the killed snails seems to be less than would be expected if it is assumed that the experimental and control snails had similar populations of Ghaetogaster. This is especially true of the outer forms. It must be concluded that although a considerable number are able to leave the host, under these experimental conditions

Table 9.

The dispersal of outer and kidney Chaetogaster from dying snails.

	Outer forms				Kidney forms			
	Snail	Snail	Snail	Snail	Snail	Snail	Snail	Snail
	1	2	3	4	1	2	3	4
Day 1	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0
Day 3	0	0	3	0	0	0	0	0
Day 4	0	1	0	0	0	0	0	1
Day 5	No observations made							
Day 6	0	1	0	0	0	0	0	0
Day 7	0	0	1	0	1	0	0	1
Day 8	0	1	0	0	2	1	1	0
Day 9	0	4	0	0	0	1	0	1
Day 10	3	0	0	0	0	2	0	0
Day 11	0	0	0	0	0	0	0	0
Day 12	0	0	0	0	3	0	0	0
Day 13	1	0	0	0	5	0	0	0
Day 14	0	0	0	0	0	0	0	0
Day 15	0	0	0	0	0	0	0	0
<b>Total</b>	<b>4</b>	<b>7</b>	<b>4</b>	<b>0</b>	<b>11</b>	<b>4</b>	<b>1</b>	<b>3</b>

many fail to do so.

b. Transfer of Chaetogaster from one host to another.

(1) Transfer from old to young snails.

Experiments were carried out to discover the degree of infestation of young snails which could be achieved by Chaetogaster. The worms were introduced to the young snails in three ways, (A) by adding infested snails to a dish containing small non-infested snails, (B) by adding killed infested snails and (C) by adding Chaetogaster to the dish. Non-infested snails, bred in the laboratory, were infested with outer or kidney Chaetogaster as required, for use in these experiments.

Three dishes, A, B and C, each containing 15 young snails were set up. To dish A were added 5 live adult snails infested with outer Chaetogaster only. To dish B were added 5 killed adult snails again having only outer Chaetogaster, and to dish C all the outer Chaetogaster obtained from five of the infested snails. These were left for ten days and then the young snails were examined. Table 10 (p. 64) shows the degree of infestation in each of these three dishes. It is seen that the highest degree of infestation was obtained in Dish C into which 'free' Chaetogaster had been introduced.

This experiment was repeated using kidney Chaetogaster and snails infested with kidney forms only. Dish A contained 10 young snails and 10 live adult infested snails; dish B contained 10 young snails and 10 killed infested adult snails and dish C contained 10 young snails plus all the Chaetogaster obtained on dissection from 10 infested adult snails.

Table 10.

		Number of outer <i>Chaetogaster</i> recovered		
		Dish A	Dish B	Dish C
Snail	1	0	dead	2
"	2	0	dead	1
"	3	0	1	0
"	4	0	0	0
"	5	0	1	1
"	6	0	0	1
"	7	0	0	1
"	8	0	dead	1
"	9	dead	0	1
"	10	0	4	0
"	11	1	0	1
"	12	0	1	2
"	13	1	0	1
"	14	0	0	2
"	15	dead	0	3
<b>Total</b>		<b>2</b>	<b>7</b>	<b>17</b>

dead = young snail had died before completion of the experiment.

These were left for ten days and then the young snails were dissected. The results are presented in Table 11 (p. 66).

It is difficult here to estimate in which dish the higher degree of infestation occurred, since in dish A four of the live adult infested snails died before the end of the ten day period. The degree of infestation was as high here as in dish C, and this suggests that the Chaetogaster may escape more easily from a snail dying naturally than from a snail that had been killed (cp. dish B).

The results obtained from dish A are useful however in that the situation is comparable to that in nature where the old generation is dying and the new generation is slowly becoming infested with Chaetogaster. They show that dispersal of kidney forms does occur from a dying senescent population to a young population.

(ii) Transfer from infested adult snails to non-infested adult snails.

One non-infested adult snail and one adult snail infested with Chaetogaster were placed in each of ten dishes and left for 10 days. At the end of this period one or other or both of the snails had died in five of the dishes. The death of adult snails was a difficulty experienced in all experiments in which they were used. The original non-infested snail was taken from each of the remaining five dishes and dissected and the number of Chaetogaster found on them is recorded below.

Snail.	Outer forms recovered.
1	2
2	0
3	1
4	4
5	4
Total	11



Table 11.

		Number of kidney <u>Gnastogaster</u> recovered		
		Dish A	Dish B	Dish C
Snail	1	2	0	2
"	2	1	0	1
"	3	2	0	1
"	4	0	0	3
"	5	0	0	2
"	6	1	0	1
"	7	2	0	1
"	8	2	0	0
"	9	1	1	0
"	10	0	0	dead
<b>Total</b>		<b>11</b>	<b>1</b>	<b>11</b>

dead = young snail had died before completion of the experiment.

All the five snails assumed to be infested at the start of the experiment were also dissected to check that they were in fact infested and were found to be so.

The results show that transfer did occur but it is not possible to say how this took place. It may have occurred during contact or copulation between the two snails, or it may have happened by the migration of Chaetogaster from one host to another without the snails having been in contact.

The experiment was repeated using kidney Chaetogaster instead of the outer form. At the end of this experiment both members of the pairs of snails remained alive in only four dishes. All the supposedly infested snails were dissected and were found to be infested with kidney forms. The original non-infested snails were found to have become infested as shown below.

Snail.	Kidney forms recovered.
1	0
2	1
3	0
4	3
Total	4

It is evident that transfer took place but again it is impossible to deduce from the results how this was brought about.

e. An investigation of transfer in the field.

Two frames measuring 12" x 12" x 2" were constructed of perspex and these were covered with a nylon mesh of 22 strands to the inch.

One cage was covered by a single layer of this material and the other by a double layer, these layers having a half-inch space between them. Ten large non-infested snails were enclosed in each of these cages and these were then placed in the reservoir and left for ten days. It was hoped that the snails in the two cages would show different degrees of infestation by Gn. lignasi. When the cages were recovered it was found that the water level had dropped leaving the cages out of the water and that all the snails were dead. The cages were set up a second time but with the same result. To put the cages deeper in the water so that there would be no danger of their being exposed was out of the question since part of each cage had to be out of the water to enable the snails to come to the surface for breathing (Chaetum 1934, Hunter 1953). The third time the experiment was attempted the cages were found to have been disturbed by children and the snails contained in them had escaped. It was then decided to do this experiment in the College Pond, where the water level remains constant and where the cages were safe from any interference. This was done once in December and again in July. The cages were placed in the pond with about three inches projecting above the surface of the water so that the snails could breathe. In the single layered cage contact was possible between the enclosed snails and the pond snails, but in the double layered cage this was not possible. The disadvantage of using the College Pond for this experiment was the fact that the snails here were not infested with kidney Chaetogaster and the results related to outer Chaetogaster only. After ten days the snails in the cages were dissected and the results obtained are presented in Table 12 (p. 69).

No transfer occurred in either of the cages in December, but

Table 12.

The outer Chaetogaster recovered from snails placed in cages in College Pond.

Snail	December.		July.	
	Single layered cage	Double layered cage	Single layered cage	Double layered cage
1	0	0	4	2
2	0	0	4	2
3	0	0	2	0
4	0	0	0	0
5	0	0	8	0
6	0	0	dead	0
7	0	0	dead	1
8	0	0	dead	dead
9	dead	0	dead	dead
10	dead	dead	dead	dead
<b>Total</b>	0	0	18	5

dead \* snail dead before completion of experiment.

in July the snails in both cages had become infested. If, in July, transfer in the single layered cage occurred as a result of contact between the enclosed snails and the pond snails, then it is reasonable to assume that the same result should have been obtained in December. Since this was not the case it is very likely that the snails in July became infested by Chaetogaster that were temporarily free-living. The lesser infestation in the double layered cage presumably resulted from the greater obstacle it provided to the Chaetogaster in reaching the snails. In conclusion it can be said that it seems likely that infestation occurred in July and not in December because free-living Chaetogaster were present in the pond in July and not in December. This is presumably due to the fact that at this time Chaetogaster were leaving the old dying generation and becoming temporarily free-living. This experiment also suggests that in nature young snails become infested to a greater extent by Chaetogaster that have assumed a temporary free-living mode of life and to a lesser extent through contact with infested snails, as also suggested by the experiment on transfer to young snails (p. 63).

#### d. Attraction of young snails to dead snails.

On two occasions during field sampling a cluster of adult Lymnaea peregrina were found clinging to a dead snail. It was decided to investigate the possibility that snails for some reason were attracted to a dying or dead individual. If young snails proved to be so attracted then this would provide an opportunity for the transfer of Chaetogaster from the old dying generation to the young generation of snails. The following

observations were carried out in the College Pond at a time when the adult snails were dying and the young snails were plentiful. Ten dead adult snails attached to a length of thread were placed in the pond. As controls, ten empty L. pereger shells and ten pieces of stone similar in size to the dead snails and the empty shells used, were also placed in the pond in the same way. All three sets were left in the pond for ten hours, and at hourly intervals during this time the number of young L. pereger below about 5 mm. in size found attached to each was recorded. All the results were pooled to give the total number of young snails that had been observed on each set of objects over the ten hour period.

	Empty shells	Stones	Dead snails
Total number of young snails.	44	147	18

If the young snails were not attracted differentially to any of these, the values obtained should not be significantly different from each other. A  $\chi^2$  goodness-of-fit test performed on these data shows that there is a significant difference in distribution between them and so it can be said that the young snails are attracted most by the stones and least of all by the dead L. pereger. This test also shows, when applied individually to each pair, that the snails are more attracted to the empty shells than to the dead snails and that they are less attracted to the empty shells than to the stones. It appears therefore that young snails are not attracted to dead snails and the suggestion made earlier, that transfer of Chaetogaster from the old to the young snail may be facilitated in this way, is not supported.

e. The specificity of outer and kidney Chaetogaster.

Vaghin (1946) suggested that these two forms of Chaetogaster limnaei were in fact 'biological' species of Ch. limnaei. He did not put forward any experimental evidence to support his theory, and therefore it was decided that it was necessary to investigate this possibility further by experimental means. Experiments were performed to see if kidney forms removed from the host would infest the kidney of a non-infested snail, and also whether the outer form on re-establishing itself on the snail would continue to live only on the outer surface of the snail.

The non-infested snails used in these experiments ranged from 5 to 15 mm. in size. Four series of experiments were performed and each experiment ran for ten days. The only reason for dividing these experiments into four series was that each series was carried out at a different time.

**SERIES 1.**

**Experiment A.**

Twenty-five non-infested snails were placed in each of two glass dishes. About 600 outer Chaetogaster were introduced into one dish and about 300 kidney Chaetogaster were put in the other. At the end of the ten day period the snails were dissected and the results are presented in Tables 13 and 14 (pp. 73 & 74).

The majority of the Chaetogaster returned to the type of habitat from which they were originally removed. The kidney forms that remained on the outer surface all had kidney cells only in their gut. This suggests that the worms had either fed in the kidney of their present host

Table 13.

The outer Chaetogaster recovered in Expt. A, Series 1.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	67	3
2	37	0
3	110	16
4	18	14
5	36	3
6	45	0
7	56	17
8	48	13
9	54	11
10	30	3
11	6	0
12	11	4
13	9	0
14	13	1
15	3	2
16	7	1
17	11	0
18	1	0
19	0	0
20	0	0
21	1	0
22	3	0
23		
24		
25		
<b>Total</b>	<b>566</b>	<b>88</b>

Dead = snail dead before completion of experiment.



Table 14.

The kidney Chaetogaster recovered in Expt. A, Series 1.

Snail	The Chaetogaster recovered	
	on the outer surface	in the kidney
1	0	2
2	0	5
3	1	20
4	0	7
5	0	15
6	0	11
7	0	3
8	0	9
9	0	7
10	2	7
11	0	8
12	2	3
13	2	6
14	0	2
15	0	4
16	0	3
17	0	0
18	0	0
19	0	2
20	0	2
21		dead
22		dead
23		dead
24		dead
25		dead
<b>Total</b>	<b>6</b>	<b>117</b>

dead = snail dead before completion of experiment.

or still retained food in their gut that had been obtained in the kidney of the previous host.

#### Experiment B.

This experiment was similar to the previous experiment but here four dishes were prepared with five snails only in each. To one dish, 30 kidney Chaetogaster were added and to another 30 outer Chaetogaster. This meant that if all the Chaetogaster became established on the snails each snail on average would harbour six worms. It was seen from the field results that snails of the size used in these experiments could support a much larger population of kidney and outer forms than this, and so it was considered that this number of Chaetogaster would not cause overcrowding on the snail. In the third and fourth dishes, the number of Chaetogaster introduced was designed to cause overcrowding of the worms on the snails, the numbers being 250 outer forms in one dish and 250 kidney forms in the other. This would give an average of 50 worms per snail if they all established themselves on a new host, which was greater than any average value for either form obtained from field results. It was hoped that this experiment would show whether or not outer Chaetogaster would enter the kidney in overcrowded conditions, and whether kidney forms under such conditions would emerge onto the outer surface of the snail. The results are shown in Tables 15 a & b and 16 a & b (pp. 76 & 77).

It seems that many outer Chaetogaster in the dish that should have contained excess outer Chaetogaster had presumably died and disintegrated. The results for the kidney form suggest that overcrowding in the kidney causes some of the worms to remain on the outer surface of the snail. Most of these had kidney cells in their gut.

Table 15 a.

The outer Chaetogaster recovered from Expt. B, Series 1.  
(excess Chaetogaster introduced)

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	9	0
2	12	0
3	16	0
4	dead	
5	dead	
<b>Total</b>	<b>37</b>	<b>0</b>

Table 15 b.

The kidney Chaetogaster recovered from Expt. B, Series 1.  
(excess Chaetogaster introduced)

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	4	4
2	7	24
3	11	14
4	25	8
5	2	3
<b>Total</b>	<b>49</b>	<b>53</b>

dead = snail dead before completion of experiment.

Table 16 a.

The outer Chaetogaster recovered from Expt. B, Series 1.  
(few Chaetogaster introduced)

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	4	0
2	4	0
3	4	0
4	6	0
5	6	0
<b>Total</b>	<b>24</b>	<b>0</b>

Table 16 b.

The kidney Chaetogaster recovered from Expt. B, Series 1.  
(few Chaetogaster introduced)

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	0	3
2	0	4
3	1	7
4	0	3
5	0	2
<b>Total</b>	<b>1</b>	<b>19</b>

It was possible that in obtaining Chaetogaster for use in these experiments a few outer forms were taken with the kidney forms and vice versa. In an effort to eliminate this possibility, from hereon, the Chaetogaster used for setting up an experiment with the exception of the first in every series, were usually those that had been retrieved from their 'true' habitat in the previous experiment. All the worms found in the 'wrong' habitat (i.e., kidney forms on the outer surface and outer forms in the kidney) were discarded. In this way it was hoped to filter out any outer forms contaminating the kidney forms, and any kidney forms contaminating the outer forms.

#### Experiment C.

Twenty non-infested snails were placed in each of two dishes. Fifty of the kidney Chaetogaster that had been recovered from the kidney of the snails in Expt. B were added to one dish and 50 of the outer Chaetogaster recovered from Expt. B were added to the other dish. After the usual ten days the snails were dissected and the results are shown in Tables 17 and 18 (pp. 79 & 80). Unfortunately a high percentage of the kidney forms did not succeed in establishing themselves on the snails. However, no kidney forms were found on the outer surface and no outer forms were found in the kidney.

#### Experiment D.

The 23 outer Chaetogaster recovered from Expt. C were added to a dish containing 5 non-infested snails. The two kidney forms recovered from Expt. C were also added to another dish with five non-infested snails. The results in Tables 19 a and b (p. 81) were obtained on dissection after the usual ten day period. Here again no outer forms were found in the

Table 17.

The outer Chaetogaster recovered from Expt. C, Series 1.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	0	0
2	3	0
3	1	0
4	1	0
5	0	0
6	5	0
7	1	0
8	1	0
9	3	0
10	2	0
11	0	0
12	0	0
13	2	0
14	3	0
15	0	0
16	1	0
17	0	0
18	0	0
19		
20		
<b>Total</b>	<b>25</b>	<b>0</b>

dead = snail dead before completion of experiment.

Table 18.

The kidney Chaetogaster recovered from Expt. C Series 1.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	1
13	0	0
14	0	0
15	0	1
16		dead
17		dead
18		dead
19		dead
20		dead
Total	0	2

dead = snail dead before completion of experiment.

Table 19 a.

The outer Chaetogaster recovered from Expt. D, Series 1.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	5	0
2	3	0
3	1	0
4	0	0
5	2	0
Total	11	0

Table 19 b.

The kidney Chaetogaster recovered from Expt. D, Series 1.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	0	0
2	0	0
3	0	1
4	0	0
5	0	0
Total	0	1



kidney and no kidney forms were found on the outer surface.

#### Experiment E.

The 11 outer Chaetogaster recovered in Expt. D were again added to a dish containing 5 non-infested snails and the one kidney form recovered was added to 5 non-infested snails in another dish. Tables 20 a and b (p. 83) show that all 11 outer forms were recovered from the outer surface of the snails and that the kidney Chaetogaster was not recovered.

### SERIES 2.

#### Experiment A.

This first experiment of the second series involved the use of few Chaetogaster to avoid possible overcrowding. Two dishes were set up, the first containing 15 non-infested snails and 60 outer Chaetogaster and the second 15 non-infested snails and 60 kidney Chaetogaster. All the Chaetogaster used were obtained from infested snails collected from the reservoir. All care was taken as usual to avoid contaminating the kidney Chaetogaster with outer forms and vice versa. Tables 21 and 22 (pp. 84 & 85) show that of the outer forms recovered not one was found in the kidney and only a very small proportion of the kidney forms recovered had remained on the outer surface of the snail.

#### Experiment B.

The outer forms recovered from the outer surface of the snail in Expt. A and the kidney forms recovered from the kidney were placed in separate dishes each containing 10 non-infested snails. All outer forms stayed on the surface of the snail and only one of the kidney forms that were recovered had not gone into the kidney (Tables 23 & 24, pp. 86 & 87).

Table 20 a.

The outer Chaetogaster recovered from Expt. E, Series 1.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	dead	
2	3	0
3	4	0
4	1	0
5	3	0
Total	11	0

Table 20 b.

The kidney Chaetogaster recovered from Expt. E, Series 1.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	dead	
2	dead	
3	0	0
4	0	0
5	0	0
Total	0	0

dead = snail dead before completion of experiment.

Table 21.

The outer Chaetogaster recovered from Expt. A, Series 2.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1		dead
2	1	0
3	1	0
4	0	0
5	1	0
6	4	0
7	5	0
8	7	0
9	1	0
10	1	0
11	4	0
12	1	0
13		dead
14	4	0
15	2	0
Total	32	0

dead = snail dead before completion of experiment.

Table 22.

The kidney Chaetogaster recovered from Expt. A, Series 2.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	0	2
2	0	3
3	0	2
4	0	0
5	0	1
6	0	2
7	1	1
8	0	1
9	0	6
10	1	0
11	0	4
12	0	3
13	0	5
14	0	2
15		
	dead	
<b>Total</b>	2	32

dead = snail dead before completion of experiment.

Table 23.

The outer Chaetogaster recovered from Expt. B, Series 2.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	3	0
2	2	0
3	2	0
4	4	0
5	2	0
6	1	0
7	5	0
8	4	0
9		
10		
		dead dead
<b>Total</b>	<b>23</b>	<b>0</b>

Table 24.

The kidney Chaetogaster recovered from Expt. B, Series 2.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1		dead
2	0	4
3		dead
4	0	1
5	0	4
6	1	4
7	0	2
8	0	3
9	0	4
10	0	2
<b>Total</b>	<b>1</b>	<b>24</b>

dead = snail dead before completion of experiment.

**SERIES 3.****Experiment A.**

Chaetogaster were again taken from field material for use in the first experiment of this series. An excess number of outer and kidney forms was used in an attempt to produce overcrowding on the snails. To 10 non-infested snails 250 outer forms were added, and to another ten snails were added 250 kidney forms. Tables 25 and 26 (pp. 89 & 90) show that more outer forms were recovered than were added presumably as a result of asexual reproduction. None of these were found in the kidney. On the other hand, a large proportion of the kidney forms recovered were found on the outer surface of the snails. It seems therefore that the larger the number of kidney forms that is introduced, the higher is the proportion of kidney forms found outside the kidney.

The kidney forms that were found on the outer surface of the snails in this experiment were again introduced to non-infested snails. They were added to 5 such snails. Ten worms were recovered and of these, 9 were found in the kidney as shown in Table 27 (p. 90). This result suggests that it was overcrowding that prevented these from entering the kidney in the first place.

**SERIES 4.**

By the time this series of experiments was performed it had been established that the outer and kidney forms could be recognized by the number of setae they possessed in each bundle (see p. 24). All the kidney forms used were examined to make sure that they had the 'kidney' number of setae. It was fortunate that such an examination was not necessary for the outer form since the population of snails in the College Pond were

Table 25.

The outer Chaetogaster recovered from Expt. A, Series 3.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	7	0
2	13	0
3	33	0
4	23	0
5	24	0
6	28	0
7	51	0
8	40	0
9	43	0
10	22	0
<b>Total</b>	<b>284</b>	<b>0</b>



Table 26.

The kidney Chaetogaster recovered from Expt. A, Series 3.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	5	12
2	3	4
3	9	7
4	1	18
5	8	5
6	1	4
7	2	5
8	7	6
9	4	5
10	3	6
<b>Total</b>	<b>43</b>	<b>72</b>

Table 27.

The kidney Chaetogaster recovered in the second part of Expt. A, Series 3.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	0	2
2	1	0
3	0	4
4	0	3
5	0	0
<b>Total</b>	<b>1</b>	<b>9</b>

infested with outer forms only. The latter were used in preference to outer forms obtained from snails infested with both types. In this way it was certain that neither the outer nor the kidney forms used were contaminated with the other form.

#### Experiment A.

Five non-infested snails were placed in each of two dishes. To one dish what was considered to be an excess of 150 kidney forms was added, and to the other only 25 kidney forms, a number that could easily be accommodated by 5 snails. The results are presented in Tables 28 a and b (p. 92). In the dish supplied with excess Chaetogaster a large number of worms was unaccounted for and had presumably died. In both dishes, a small proportion remained on the outer surface of the snails, but the number of setae per bundle they possessed and the fact that only kidney cells were found in their gut showed that they were typical kidney forms.

#### Experiment B.

Again, two dishes each containing 5 non-infested snails were used. Since no evidence of overcrowding of the outer form was seen in previous experiments, i.e., no outer forms were found in the kidney, it was decided that more outer forms than was used before should be used to attempt to approach overcrowded conditions. It is true that in Expt. A of Series 1 (p. 72 & Table 13, p. 73) many supposedly outer Chaetogaster did enter the kidney, but since this phenomenon did not take place again it is highly probable that the outer Chaetogaster used in that experiment were heavily contaminated with kidney forms. This, and not overcrowding, was probably the reason why many Chaetogaster were found in the kidney. Therefore, 500 outer Chaetogaster were added to one dish to try to create

Table 28 a.

The kidney Chaetogaster recovered from Expt. A, Series 4.  
(excess Chaetogaster introduced)

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	1	8
2	1	1
3	0	9
4	0	4
5	dead	
<b>Total</b>	<b>2</b>	<b>22</b>

Table 28 b.

The kidney Chaetogaster recovered from Expt. A, Series 4.  
(few Chaetogaster introduced)

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	1	0
2	0	4
3	0	2
4	0	0
5	0	0
<b>Total</b>	<b>1</b>	<b>6</b>

dead = snail dead before completion of experiment.

conditions of excess Chaetogaster, and 100 outer forms, a figure considered from the evidence of field results not to be an excess, were added to the second dish as a control. Tables 29 a and b (p. 94) show the number of worms recovered. In neither of the dishes were any Chaetogaster found in the kidney. The number of Chaetogaster recovered was greater in both cases than the number introduced and this again was presumably due to asexual reproduction.

In conclusion it can be said that outer Chaetogaster will not enter the kidney even if overcrowding occurs on the outer surface of the snail. The only exception was found in Expt. A, Series 1 (p. 72 & Table 13 p. 73) and the explanation that has been suggested is that the supposedly outer Chaetogaster used on that occasion in fact also contained kidney forms. It is probable that these kidney forms were present on the outer surface of the snails when the outer forms were collected for use in this experiment.

It appears that the space available in the kidney limits the number of Chaetogaster that either enter or can remain there. Those that either do not enter the kidney at all, or having entered leave again, apparently remain on the outer surface of the snail at least for some time. But it is not known for how long they can survive outside the kidney in this manner. It is possible that there is continuous migration in and out of the kidney in a situation of this sort, in which case their chances of survival would be increased due to the fact that they would be able to obtain food in the kidney.

Table 29 a.

The outer Chaetogaster recovered from Expt. B, Series 4.  
(excess Chaetogaster introduced)

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	268	0
2	106	0
3	125	0
4	202	0
5	62	0
Total	763	0

Table 29 b.

The outer Chaetogaster recovered from Expt. B, Series 4.  
(few Chaetogaster introduced)

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	51	0
2	28	0
3	49	0
4	44	0
5	37	0
Total	209	0

f. The effect of selected environmental conditions on the outer form.

It has been shown that under normal conditions the outer forms will not enter the kidney. The reaction of outer forms to certain abnormal conditions was investigated in the following experiments.

(i) Lack of food.

Lymnaea pereger infested with outer forms only were used so that at the end of the experiment it could be said that any worms found in the kidney were originally outer forms. Ten of these infested snails were placed in a dish containing distilled water and left for 14 days. The distilled water was replaced every other day to make sure that no food organisms were available for the Chaetogaster. After 14 days the snails were examined to see if any Chaetogaster had entered the kidney. The experiment was repeated and the results are presented in Tables 30 and 31 (pp. 96 & 97).

One Chaetogaster from each snail was examined to see if any food was present in the gut. No recognisable food organisms were found in any of them.

It is concluded that outer Chaetogaster do not move into the kidney when they are deprived of food.

(ii) Drought conditions.

Ten snails infested with outer Chaetogaster only were placed in a dish containing moist sphagnum moss and left for 14 days. The moss was kept moist throughout this period. The experiment was repeated and the results (Tables 32 & 33, pp. 98 & 99) show that none of the worms had been driven into the kidney by the dry conditions. It is judged that the

Table 30.

The results of the experiment designed to test the reaction of outer Chaetogaster when deprived of food

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	2	0
2	0	0
3	9	0
4	4	0
5	1	0
6	2	0
7	4	0
8	3	0
9	11	0
10	dead	
Total	36	0

dead = snail dead before completion of experiment.

Table II.

The results of a second experiment designed to test the reaction of outer Chaetogaster when deprived of food.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	5	0
2	6	0
3	6	0
4	7	0
5	0	0
6	15	0
7	7	0
8	9	0
9		dead
10		dead
Total	55	0

dead = snail dead before completion of experiment



**Table 32.**

The results of an experiment designed to test the reaction of outer Gastropod in drought conditions.

Snail	The <u>Gastropod</u> recovered	
	on the outer surface	in the kidney
1	1	0
2	5	0
3	2	0
4	8	0
5	7	0
6		
7		dead
8		dead
9		dead
10		dead
<b>Total</b>	<b>23</b>	<b>0</b>

dead = snail dead before completion of experiment.

**Table 33.**

The results of a second experiment designed to test the reaction of outer Chaetogaster to drought conditions.

Snail	The <u>Chaetogaster</u> recovered	
	on the outer surface	in the kidney
1	0	0
2	3	0
3	0	0
4	1	0
5	7	0
6		
7		dead
8		dead
9		dead
10		dead
<b>Total</b>	<b>11</b>	<b>0</b>

dead = snail dead before completion of experiment.

conditions of the experiment were fairly severe since half the snails in both dishes died through desiccation.

6. The reaction of Chaetogaster limaxi to the mucus of its host.

Previous experiments have shown that Chaetogaster, when introduced into a dish containing Lymnaea pereger, will establish itself on the snail. It has been demonstrated further that kidney forms will eventually establish themselves in the kidney of the molluscs whilst the outer forms will only inhabit the outer surface of the snail. The method by which the worm finds the snail could simply be the result of a chance meeting of snail and worm. On the other hand it was thought possible that both forms of Chaetogaster may actively seek a snail on which to establish themselves. It was with this possibility in mind that the following experiments were performed. No attempt was made to discover how the kidney form seeks to establish itself finally in the kidney.

Experiment 1.

It was necessary for this and subsequent experiments in this section to use containers that were coated inside with a film of algae, bacteria etc. Such a surface provided the Chaetogaster with a suitable substratum for locomotion. The film was produced by leaving pond water in the glass dishes to be used, for about a week.

Eight circles of about  $\frac{1}{2}$ " in diameter were drawn on the under surface of one of these glass dishes. These were numbered and four were selected at random. Pond water was poured into the dish and a Lymnaea

Perceger was made to crawl on the algal film inside the dish for 5 minutes within the limits of each of these four randomly selected circles. Thus when the snail was removed, four of the circles were covered with mucus secreted by the foot of the snail, and four were not. Twenty outer Chaetogaster were introduced into the dish and it was hoped that if the worms were attracted by the mucus, they would accumulate on the spots of mucus, or at least show a tendency to remain on these spots longer than they would on the circles not covered by mucus. The experiment was left for 24 hours, and at intervals during this time the number of worms present on each of the eight circles was recorded. The experiment was performed three times and the results are presented in Tables 34, 35 and 36 (pp. 102, 103 & 104). It is seen that the number of Chaetogaster on each spot of mucus tended to increase, whilst the number on each of the untreated spots tended to decrease. A  $\chi^2$  goodness-of-fit test was performed on the total numbers of Chaetogaster seen on the spots of mucus and on the untreated spots throughout the 24 hours. This showed that the number of worms found on the treated areas was significantly greater than that found on the untreated areas in all three experiments (see pp. 102, 103 & 104). This is presumably either due to Chaetogaster remaining on the spots of mucus for a much longer period than on the control spots, or to the worms remaining throughout on the spot of mucus once the mucus had been detected. The former explanation is probably nearer the truth since it is seen that on more than one occasion the number of Chaetogaster on a spot of mucus decreased, indicating that Chaetogaster did in fact leave the treated area. By observing the behaviour of the Chaetogaster in the dish it was concluded that their movements were completely random and that they did not detect

Table 34.

(1) The occurrence of Chaetogaster on spots treated and spots not treated with snail mucus.

Time	Treated spots				Untreated spots			
	1	2	3	4	5	6	7	8
0 hours	0	0	1	1	1	1	0	0
1 hour	1	1	1	1	1	1	1	0
3 hours	1	1	1	1	1	0	0	0
6 hours	1	1	1	1	1	1	1	0
18 hours	3	1	3	1	1	1	1	0
20 hours	3	1	4	1	1	1	0	0
22 hours	4	1	3	1	1	0	0	1
24 hours	3	1	2	1	1	1	0	0
<b>Total</b>	16	7	16	8	8	6	3	1

$$\chi^2 = 6.469$$

P less than 0.05

Table 35.

(ii) The occurrence of Chaetogaster on spots treated and on spots not treated with snail mucus.

Time	Treated spots				Untreated spots			
	1	2	3	4	5	6	7	8
0 hours	1	1	1	3	2	0	1	1
1 hour	1	1	1	3	2	0	1	0
2 hours	0	0	1	1	2	0	1	2
3 hours	0	1	1	1	2	0	0	0
6 hours	0	1	1	2	1	0	0	0
8 hours	1	2	1	1	1	0	0	0
21 hours	1	5	1	1	0	0	0	0
24 hours	3	3	0	1	0	0	0	0
<b>Total</b>	<b>7</b>	<b>14</b>	<b>7</b>	<b>13</b>	<b>10</b>	<b>0</b>	<b>3</b>	<b>3</b>

$$\chi^2 = 5.482$$

P less than 0.05

Table 36.

(111) The occurrence of Ghaetogaster on spots treated and on spots not treated with snail mucus.

Time	Treated spots.				Untreated spots.			
	1	2	3	4	5	6	7	8
0 hours	2	2	1	0	1	1	0	1
3 hours	8	1	0	4	0	1	0	0
6 hours	9	2	0	5	0	0	0	0
18 hours	12	2	2	4	0	0	0	0
21 hours	8	1	2	4	1	0	0	0
24 hours	9	1	2	3	1	0	0	0
<b>Total</b>	<b>48</b>	<b>9</b>	<b>7</b>	<b>20</b>	<b>3</b>	<b>2</b>	<b>0</b>	<b>1</b>

$$\chi^2 = 33.8$$

P less than 0.001

the mucus until they came upon it during the course of this random movement. This experiment therefore shows that outer Chaetogaster is stimulated to remain on the snail mucus for a much longer period of time than it would on a substratum where mucus was not present.

The experiment was repeated using kidney Chaetogaster and the results are presented in Tables 37, 38 and 39 (pp. 106, 107 & 108). It is seen that the behaviour of the kidney forms is similar to that of the outer forms.  $\chi^2$  tests were carried out on the totals as before and in two cases out of three (see pp. 106, 107 & 108) the numbers of Chaetogaster observed on the mucus throughout the 24 hours was significantly greater than that of Chaetogaster observed on the untreated areas. Although there is no significant difference between these values in the third case (p. 108), it is seen that the trend is for the Chaetogaster to aggregate on the treated areas rather than on the untreated ones. Kidney Chaetogaster however did not seem to be able to crawl along the algal film in the experimental dishes as efficiently as the outer form. Therefore, the fact that the mucus might provide a better anchoring surface than the algal film, in this case, cannot be ignored. This might also be true to some extent for the outer form, and it is a possible explanation as to why both forms are attracted by the mucus. On the other hand the mucus may be acting as a chemical stimulus to the Chaetogaster.

## Experiment 2.

This experiment was performed to investigate the behaviour of Chaetogaster when placed on a mucous trail produced by a Lyngbya peruviana. Dishes coated inside with an algal film were again used in this experiment.



Table 37.

(1) The occurrence of kidney Chaetogaster on spots treated and on spots not treated with snail mucus.

Time	Treated spots				Untreated spots			
	1	2	3	4	5	6	7	8
0 hours	1	5	1	0	1	0	1	0
1 hour	1	6	2	0	0	0	2	0
2 hours	1	6	3	0	0	0	1	0
8 hours	1	7	3	0	0	1	0	0
20 hours	2	7	4	0	0	0	0	0
24 hours	2	7	4	0	0	0	0	0
<b>Total</b>	8	38	17	0	1	1	4	0

$$\chi^2 = 23.54 \quad P \text{ less than } 0.001$$

Table 38.

(11) The occurrence of kidney Chaetogaster on spots treated and on spots not treated with snail mucus.

Time	Treated spots				Untreated spots			
	1	2	3	4	5	6	7	8
0 hours	0	1	1	1	1	0	0	1
1 hour	0	2	1	1	1	0	0	1
2 hours	0	2	1	1	1	0	0	0
3 hours	0	2	1	1	1	0	0	0
6 hours	1	2	1	1	0	0	0	1
10 hours	1	2	1	1	0	2	0	0
22 hours	1	2	1	1	0	2	0	0
24 hours	1	2	2	1	0	1	0	0
<b>Total</b>	4	15	9	8	4	5	0	3

$$\chi^2 = 6.000 \quad P \text{ less than } 0.05$$

Table 39.

(iii) The occurrence of kidney Chaetogaster on spots treated and on spots not treated with snail mucus.

Time	Treated spots				Untreated spots			
	1	2	3	4	5	6	7	8
0 hours	0	1	0	1	0	0	1	2
1 hour	0	2	1	1	0	0	2	2
4 hours	0	3	1	1	0	0	2	2
6 hours	0	3	1	1	0	0	2	1
19 hours	0	4	1	1	0	0	2	0
21 hours	0	4	1	1	0	0	1	0
24 hours	0	4	1	1	0	0	1	0
<b>Total</b>	0	21	6	7	0	0	11	7

$$\chi^2 = 2.461$$

P greater than 0.05

**(2) Outer Chaetogaster**

A Lymnaea pereger was allowed to crawl along the bottom of the dish. The path it had taken was easily discernable because of marks left by its radula on the algal film. Outer Chaetogaster were then placed on the mucous trail about 3" behind the moving snail and their movements were observed. The experiment was performed twice and 10 Chaetogaster were used each time. The movements of the worms are recorded in Table 40 a and b (p. 110). All the worms followed the snail's mucus trail. The first time the experiment was performed (Table 40 a) four of the ten worms turned in the direction of the snail and eventually caught up with it and attached themselves to it. The remaining six initially moved along the mucus trail in the opposite direction to that taken by the snail, but one of these reversed its direction and finally found the snail. The other five eventually left the trail altogether after crawling back and forth along it for at least half an hour. The second time the experiment was performed five of the worms moved along the trail towards the snail and finally attached themselves to it. The remaining five moved in the opposite direction and eventually left the mucus trail without having found the snail. These worms also remained on the mucus trail for at least half an hour but in neither experiment did the worms remain on the mucus for more than one hour.

A control experiment was set up where ten outer Chaetogaster were placed in the dish along a straight line drawn on the under surface of the dish. No mucus trail was present. All the worms moved away from the line in times varying from half a minute to four minutes as shown in Table 40 c (p. 110).

Table 40.

The behaviour of outer Cheetogaster on the mucous trail of its host.

(a)

<u>Cheetogaster</u>	Behaviour	
1	Moved towards snail.	Found snail.
2	Moved in opposite direction.	Eventually left trail.
3	Moved in opposite direction.	Eventually left trail.
4	Moved in opposite direction.	Turned back and found snail.
5	Moved in opposite direction.	Eventually left trail.
6	Moved towards snail.	Found snail.
7	Moved towards snail.	Found snail.
8	Moved in opposite direction.	Eventually left trail.
9	Moved towards snail.	Found snail.
10	Moved in opposite direction.	Eventually left trail.

(b)

<u>Cheetogaster</u>	Behaviour	
1	Moved towards snail.	Found snail.
2	Moved in opposite direction.	Eventually left trail.
3	Moved in opposite direction.	Eventually left trail.
4	Moved towards snail.	Found snail.
5	Moved in opposite direction.	Eventually left trail.
6	Moved in opposite direction.	Eventually left trail.
7	Moved towards snail.	Found snail.
8	Moved towards snail.	Found snail.
9	Moved towards snail.	Found snail.
10	Moved in opposite direction.	Eventually left trail.

(c) Control.

<u>Cheetogaster</u>	Behaviour
1	Moved away from line after $\frac{1}{2}$ minute.
2	Moved away from line after 2 minutes.
3	Moved away from line after 3 minutes.
4	Moved away from line after 4 minutes.
5	Moved away from line after $3\frac{1}{2}$ minutes.
6	Moved away from line after $\frac{1}{2}$ minute.
7	Moved away from line after 1 minute.
8	Moved away from line after $\frac{1}{2}$ minute.
9	Moved away from line after 2 minutes.
10	Moved away from line after $\frac{1}{2}$ minute.

This experiment shows conclusively that outer Chaetogaster will follow the mucous trail of a snail and if the right direction is taken initially they will overhaul the snail and attach themselves to it.

It is probable that as the trail gets older, and the mucus slowly disappears, the stimulus, whether chemical or otherwise, weakens and becomes more difficult to detect. Thus the intensity of the stimulus, which is probably greatest immediately behind the snail, decreases gradually in the direction away from the snail. It follows that if the worm, when placed at any one point on the trail, was able to detect a higher concentration of mucus in one direction and then move in that direction, it would always move towards the snail. It would appear however that the worm is incapable of detecting small differences in the concentration of the mucus and that it is a matter of chance whether or not it will move initially in the direction of the snail. However, it was decided to investigate the possibility that the worm had such a directional ability if placed only one inch away from the snail, i.e., in the region where the stimulus is likely to be strongest.

Twenty outer forms were placed individually on the mucous trail of L. pereger about one inch behind the snail. Table 41 (p. 112) shows that 11 worms turned in the direction of the snail and established themselves on it. One went in the opposite direction initially but eventually reversed its direction and found the snail. Another started moving towards the snail but turned back before reaching the snail. The remaining 7 turned in the opposite direction to that taken by the snail and did not find it. The worms that did not find the snail left the mucous trail after crawling back and forth along it for periods ranging from

Table 41.

The behaviour of outer Chaetogaster when placed on the mucous trail of its host one inch behind the snail.

<u>Chaetogaster</u>	Behaviour.	
1	Moved towards snail.	Found snail.
2	Moved towards snail.	Found snail.
3	Moved in opposite direction.	Turned back and found snail.
4	Moved in opposite direction.	Eventually left trail.
5	Moved towards snail.	Found snail.
6	Moved in opposite direction.	Eventually left trail.
7	Moved towards snail.	Turned back and eventually left trail.
8	Moved in opposite direction.	Eventually left trail.
9	Moved towards snail.	Found snail.
10	Moved towards snail.	Found snail.
11	Moved in opposite direction.	Eventually left trail.
12	Moved in opposite direction.	Eventually left trail.
13	Moved towards snail.	Found snail.
14	Moved towards snail.	Found snail.
15	Moved towards snail.	Found snail.
16	Moved in opposite direction.	Eventually left trail.
17	Moved in opposite direction.	Eventually left trail.
18	Moved towards snail.	Found snail.
19	Moved towards snail.	Found snail.
20	Moved towards snail.	Found snail.

35 to 75 minutes. This experiment confirms the conclusion drawn earlier that it is only by chance that the worm moves initially in the direction of the snail.

(11) Kidney Chaetogaster.

The experiment described above was repeated with some modifications using kidney forms. The speed at which kidney forms can crawl seems to be much slower than that of the outer forms, and to make it easier for them to overhaul the snail, they were placed on the mucous trail half an inch behind the snail instead of three inches and one inch as was done in the case of the outer forms.

Ten kidney Chaetogaster were placed on the mucous trail and observations were continued for 30 minutes. The results are presented in Table 42 a (p. 114). Not one of the Chaetogaster placed on the trail had moved from the position where they were first placed even after 30 minutes had elapsed. It was decided against continuing observations beyond 30 minutes because by that time the snail had moved a considerable distance (at least 12 inches), and the chances of a kidney Chaetogaster overhauling it at that stage were very small.

The experiment was repeated and this time the kidney forms were placed on the mucous trail immediately behind the snail. Table 42 b (p. 114) shows that again the Chaetogaster remained immobile on the mucous trail for 30 minutes with the exception of one which left the trail after 15 minutes.

A control experiment was performed by placing ten kidney Chaetogaster in the dish along a straight line drawn on the under surface of the dish. No mucous trail was present on the bottom of the dish. The



Table 42.

The behaviour of kidney Chaetogaster on the mucous trail of its host.

(a) Worms placed  $\frac{1}{2}$ " behind snail.

<u>Chaetogaster.</u>	Behaviour.
1	Remained in original position after 30 minutes.
2	Remained in original position after 30 minutes.
3	Remained in original position after 30 minutes.
4	Remained in original position after 30 minutes.
5	Remained in original position after 30 minutes.
6	Remained in original position after 30 minutes.
7	Remained in original position after 30 minutes.
8	Remained in original position after 30 minutes.
9	Remained in original position after 30 minutes.
10	Remained in original position after 30 minutes.

(b) Worms placed immediately behind snail.

<u>Chaetogaster.</u>	Behaviour.
1	Remained in original position after 30 minutes.
2	Remained in original position after 30 minutes.
3	Left trail after 15 minutes.
4	Remained in original position after 30 minutes.
5	Remained in original position after 30 minutes.
6	Remained in original position after 30 minutes.
7	Remained in original position after 30 minutes.
8	Remained in original position after 30 minutes.
9	Remained in original position after 30 minutes.
10	Remained in original position after 30 minutes.

(c) Control.

<u>Chaetogaster</u>	Behaviour.
1	Moved away from line after 23 minutes.
2	Remained near line after 30 minutes.
3	Moved away from line after 10 minutes.
4	Moved away from line after 20 minutes.
5	Remained near line after 30 minutes.
6	Moved away from line after 27 minutes.
7	Moved away from line after 5 minutes.
8	Remained near line after 30 minutes.
9	Remained near line after 30 minutes.
10	Moved away from line after 7 minutes.

worms were observed for 30 minutes and during this time six were seen to move away from the line; the remaining four still remained in their original position on the line at the end of the 30 minute period ( Table 42 c. p. 114).

It seems therefore that the mucous trail has some attraction for these kidney forms. This is shown by the fact that they would remain immobile on such a trail, more so than they would if no trail was present.

In conclusion, it can be said that the outer form shows a definite response to a mucous trail left by L. peregrin and that some stimulus causes the worm to follow this trail. It is obvious that this provides a means by which the outer Ghaetogaster can actively search for its host. The results suggest that the worm can still detect the mucus a long time after it has been deposited by the snail but it is very likely that for Ghaetogaster to be able to find a host by this method the trail would have to be a fairly recent one.

The response shown by the kidney form is not so pronounced however. It was not observed to follow the trail as did the outer form, although it did show a certain amount of attraction to the mucus. It is possible that other stimuli are required before the kidney form can be induced to search for its host.

b. The survival of outer and kidney Ghaetogaster away from the host.

During the course of the experiments described here, the Ghaetogaster were fed on Protozoa and algae and were kept at 7°U. The

algae and Protozoa were obtained from cultures prepared by boiling water - lily leaves in water and leaving them for about a week. After this time the water was found to contain ciliate Protozoa, Rotifera and various colonial and unicellular algae in large numbers. About 2 or 3 mls. of this culture were added to the dishes containing the Chaetogaster every three or four days.

#### Experiment A.

Six outer Chaetogaster were placed in each of two dishes containing pond water and fed as described above. After 40 days three Chaetogaster were still alive in each. These were examined and were found to have fed on ciliate Protozoa and a few Rotifera.

#### Experiment B.

Six outer Chaetogaster were placed in pond water in each of four dishes and six kidney Chaetogaster were put in each of another four dishes. These were fed as before and examined at intervals as shown in Table 43 (p. 117). After six days all the kidney forms had died. One outer form remained alive after 49 days. This was examined and was found to have fed largely on ciliate Protozoa.

#### Experiment C.

Dishes containing outer and kidney Chaetogaster were set up as in Expt. B. Here however, it was decided to terminate the experiment before all the Chaetogaster had died in order that the number of setae per bundle in the surviving individuals could be observed. The results are shown in Table 44 (p. 117).

After 14 days the only surviving kidney form was examined

Table 43.

The survival of free-living Chaetogaster. Expt. B.

	Dish	Days					
		0	3	6	14	29	49
Outer forms surviving.	1	6	4	0	0	-	-
	2	6	1	0	0	-	-
	3	6	1	0	0	-	-
	4	6	5	5	6	5	1
Kidney forms surviving.	1	6	2	0	-	-	-
	2	6	1	0	-	-	-
	3	6	0	0	-	-	-
	4	6	0	0	-	-	-

Table 44.

The survival of free-living Chaetogaster. Expt. C.

	Dish	Days			
		0	6	14	34
outer forms surviving	1	6	6	7	9
	2	6	6	3	5
	3	6	6	3	8
	4	6	6	6	9
kidney forms surviving	1	6	6	0	0
	2	6	6	0	0
	3	6	6	1	0
	4	6	6	0	0

found to have the number of setae per bundle that is usual in kidney forms. The outer forms were examined after 34 days and the number of setae per bundle was also normal here. This suggests that the free-living mode of life has no effect on the number of setae per bundle. More important is the fact that the outer forms survived for a much longer period than the kidney forms. The outer Chaetogaster had fed on ciliates and rotifers whilst the kidney Chaetogaster had not fed at all. So it seems that the kidney forms will not feed on organisms normally taken by the outer forms. Although this conclusion is based on the examination of one worm, it is supported by the fact that on investigating the gut content of the kidney forms found on the outer surface of snails in Expt. A, Series 4 (part e. of this section. p. 91), only kidney cells were found. This was also the case in a kidney Chaetogaster that happened to be found on the outer surface of a snail during sampling. It was also noted during the periodical examination of the dishes, using a binocular microscope, that whereas food could be seen in the gut of the outer forms, the gut of the kidney forms was empty.

#### Experiment D.

Three dishes each containing six outer Chaetogaster and another three dishes having six kidney Chaetogaster in each were set up. These were then left until all the Chaetogaster had died, the worms being fed as already described. Table 45 (p. 119) shows the number of Chaetogaster surviving at different stages in all six dishes. Again it is seen that the outer forms survive for a longer period in the free-living state than do the kidney Chaetogaster.

Table 45.

The survival of free-living Chaetogaster. Expt. D.

	Dish	Days							
		0	3	11	16	22	26	38	49
Outer forms surviving	1	6	6	6	6	4	4	1	0
	2	6	7	10	9	8	8	3	0
	3	6	6	3	3	0	0	0	0
Kidney forms surviving	1	6	6	7	7	1	0	0	0
	2	6	5	4	3	0	0	0	0
	3	6	5	5	4	3	0	0	0

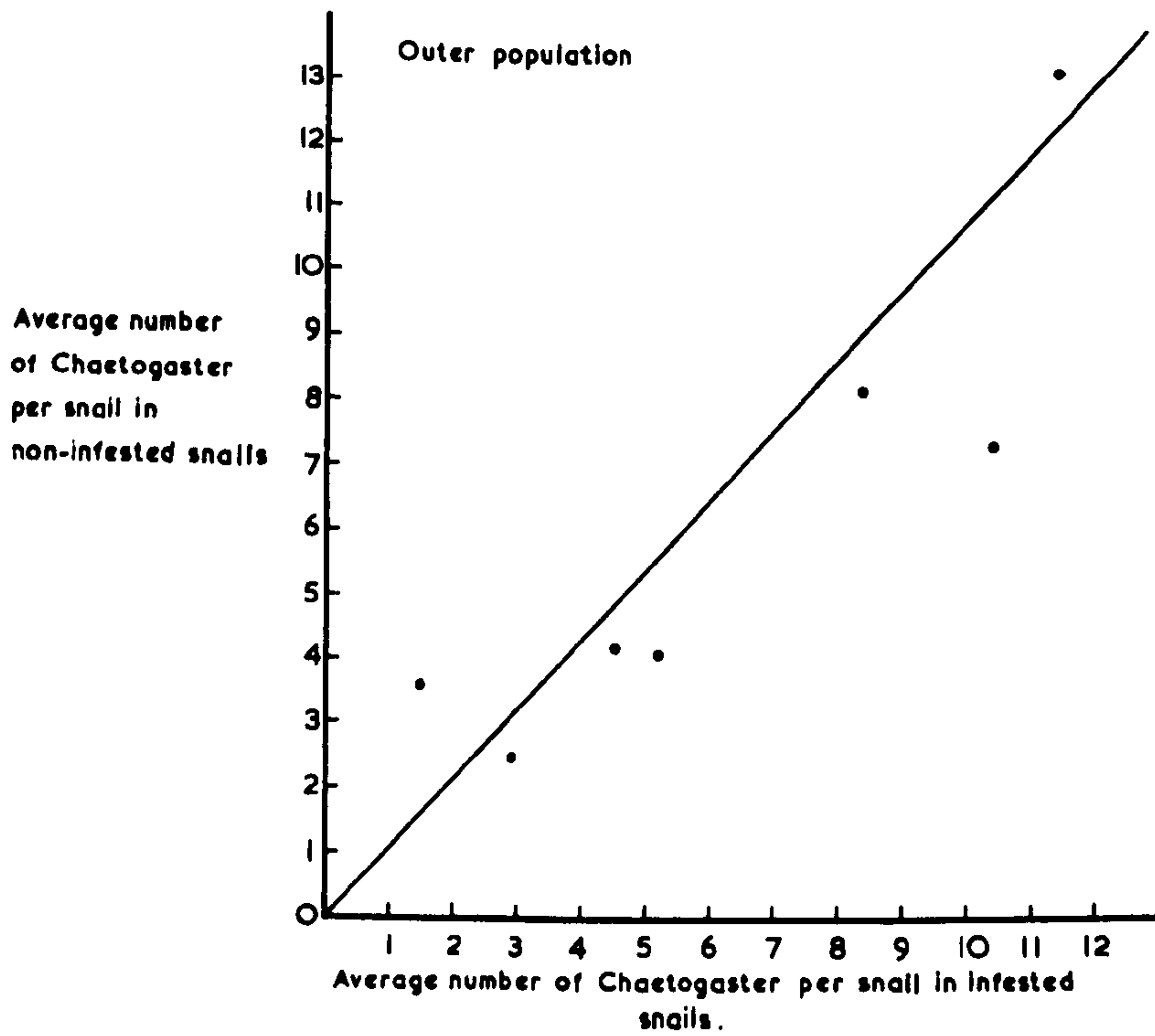
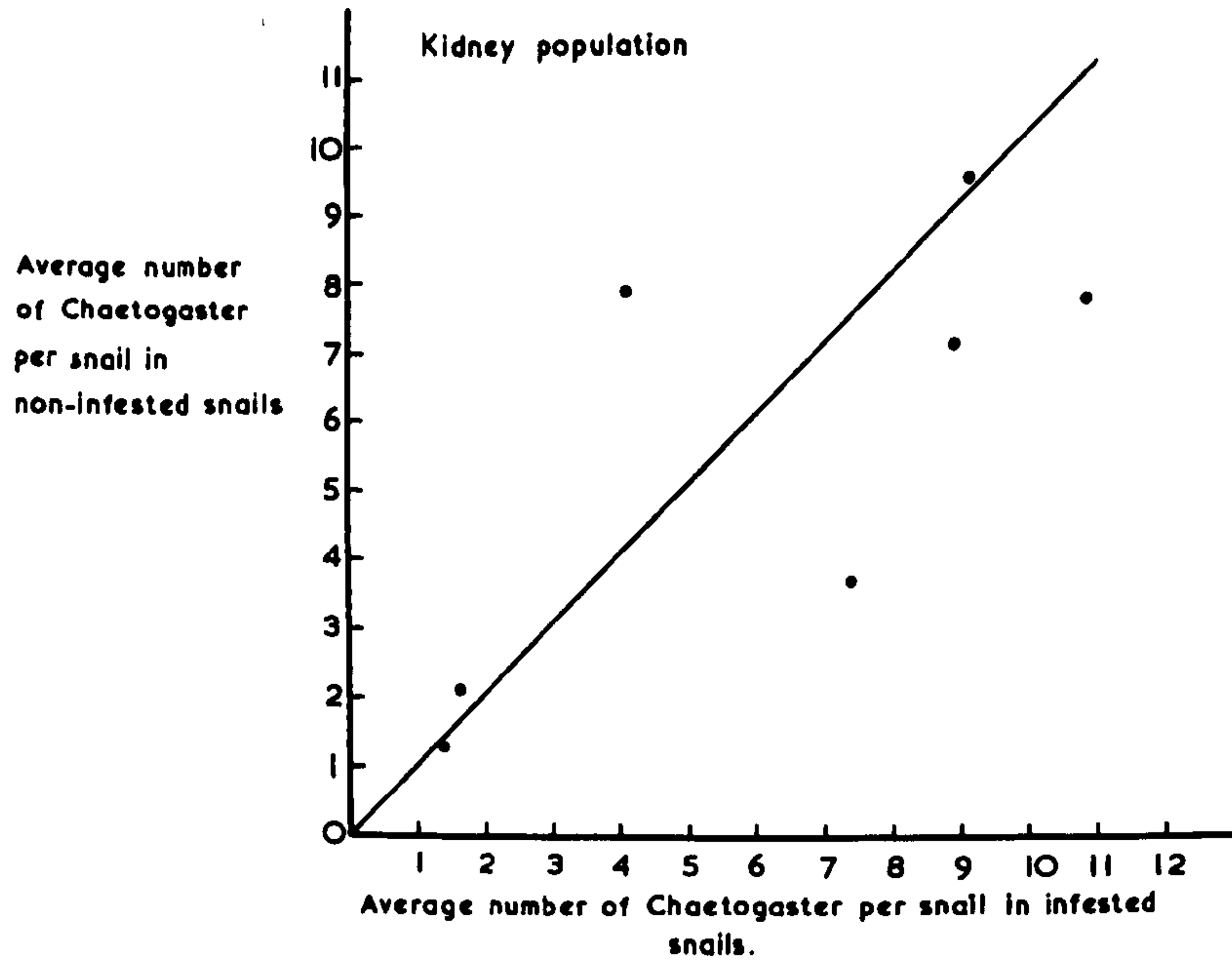


Fig. 27. A comparison of the Chaetogaster population on snails infested and on snails not infested with Glossiphonia heteroclita.

These experiments have shown that Chaetogaster limnaki can survive for a considerable period away from the host snail. In some cases evidence of asexual reproduction was seen when the number of Chaetogaster in a dish increased beyond the number that was originally introduced. They have also shown that outer forms can survive for a longer period in this free-living state than can the kidney forms. The maximum time for which an outer Chaetogaster was kept alive was 49 days, whilst that for a kidney Chaetogaster was 22 days. In Expt. D, which was allowed to continue until all the worms had died, the average survival period for the outer form was 22.05 days and that for the kidney form was 14.37 days. It was also noticed that whilst the outer Chaetogaster will feed on organisms swimming or floating in the water, the kidney form will not.

#### Section 10.

#### THE INFLUENCE OF GLOSSIPHONIA HETEROCALITA ON CHAETOGASTER LIMNAKI.

At certain times of the year Glossiphonia heterocalita<sup>(L.)</sup> was found in the mantle cavity of Lymnaea pereger in the reservoir ( see Appendix A, p. 153 ). Seven samples taken in 1960, in which leeches were plentiful, were examined to see if the presence of the leeches had any effect on the numbers of outer or kidney Chaetogaster found on the snails. The average number of Chaetogaster per snail was calculated for both outer and kidney populations, firstly on snails having leeches and secondly on the ones having no leeches in their mantle cavity. The results are presented in Fig. 27. The average number of Chaetogaster per snail in the leech-infested snails was plotted for each of the samples, against the average in



the leech - free snails. The data for the outer and the kidney populations are presented separately. Since a straight line drawn through these points almost bisects the angle between the x and the y axes in both cases, it is obvious that there is no interaction between the leech and the Chaetogaster populations.

## Section 11.

### DISCUSSION.

It has been established that Chaetogaster limnaei lives on the outer surface of the snail's body and also in the kidney of the snail. No worms were found in the mantle cavity of the snail. Von Baer (1827) and Lankester (1869a) both found the worm living in the mollusc's kidney as well as in the mantle or respiratory cavity, and on the outer surface of the snail. Between 1869 and 1946, when Vaghin published his paper on the biological species of Ch. limnaei, no reference at all was made to the kidney dwelling form. All papers appearing during this period referred to Ch. limnaei as an oligochaete worm living in the mantle cavity and on the outer surface of freshwater snails. Sperber (1950) in her key to the Naididae does not mention the kidney form at all. It is significant that the present study did not reveal any Chaetogaster living in the mantle cavity of L. pereger, and it is concluded that the worm does not inhabit this region of the snail. The reports stating that Ch. limnaei inhabits the mantle cavity of L. pereger and other freshwater pulmonates probably stem from the fact that on splitting open this cavity it is extremely easy to damage the kidney which lies in its roof. Chaetogaster would then crawl

out through the damaged part into the mantle cavity. This happens within a few seconds and it almost certainly explains the presence of any Ch. limnaei in the mantle cavity.

a. The morphology and anatomy of Chaetogaster limnaei.

Chaetogaster species are distinguished from each other by means of their setae. The shape of the distal prongs of the setae and the number present in each bundle are the most important criteria, but their length also varies from one species to another. The shape of the setae of the outer and kidney forms of Ch. limnaei is very similar. They differ however in number and length. The setae of the outer form are longer and more numerous than those of the kidney form. Pignet (1906) who described two forms of Ch. limnaei differing in respect of length and number of setae was probably describing the outer and kidney forms.

Apart from the differences in the thickness of the gut wall of the two forms, their internal anatomy is similar. The thickness of the stomach and intestinal wall is greater in the outer form than in the kidney form. Since no detailed histological examinations were carried out it is not possible to say with certainty whether these differences are due to an increase in the number or to a difference in the nature of the cells secreting digestive enzymes. It is possible that the outer form has a more complex digestive system to enable it to deal with a more varied diet. Alternatively the outer form may possess more mucous secreting cells to protect the gut wall from damage by solid structures such as the chitinous jaws of rotifers and the silicated frustules of diatoms, which

seen to pass through the gut undigested.

Lankester (1869b,c) stated that the mature form of Ch. linnaei had four genital setae on each side in segment 6. All mature individuals examined here had only three on each side. These were of course kidney forms and it is quite likely that Lankester's description refers to mature outer forms. This may also account for the fact that he found double the usual number of ordinary setae in the mature form. The mature kidney forms examined occasionally had one extra seta in each bundle. In both the immature outer and kidney forms the number of setae per segment is more or less the same on all segments, but Lankester found that the first bundle (segment 2) was made up of 12 setae whilst the remainder had only 8 per bundle. One must assume, in the light of these observations, that Lankester's observations were incorrect. The setae of the mature kidney form were not found to be longer than those of the immature form as had been reported by Lankester (1869b). Again he may have been referring to the mature outer form. Vejdovsky (1884) on the other hand found no differences between either the number or the length of the setae of mature and immature forms. It is not known whether he was referring to the kidney or the outer form.

Two spherical masses presumed to be testes were seen in segment 5 lying on the nerve cord near septum 4/5. There seems to be a great deal of controversy regarding the position and form of the testes in the genus Chaetogaster. Lankester (1869b) described the testes of Ch. linnaei as two pyriform masses in segment 5, but Sperber (1948) believes that what he saw were spermathecae. Vejdovsky (1884) and Dehorne (1916) both state that the testes of Ch. diaphanus are loose masses of spermatogonia

lying on the nerve cord in segment 5. According to Vejdovsky the spermatogonia break loose from the testes and fix themselves to various organs in the body cavity where they complete their development. Stephenson (1922) found no testes at all in Ch. orientalis. He only saw male cells attached to strands of connective tissue in the body cavity. Sperber however found unmistakable testes in Ch. diaphanus and their position agrees with the position of the spherical masses described here. It seems possible therefore that the structures seen in Ch. limnai were in fact testes.

What seemed to be a single ovary was seen in segment 6. Vejdovsky (1884) and Lankester (1869b) found paired ovaries in segment 6 of Ch. limnai. Dehorne (1916), Sperber (1948) and Vejdovsky found definite paired ovaries in segment 6 of Ch. diaphanus. Stephenson (1922) found ova in segment 6 of Ch. diaphanus but was not sure whether definite ovaries were formed. Dehorne (1923) said that the ova in Ch. diaphanus are diffusely formed everywhere in segment 6. None of the Ch. limnai ovaries examined seemed to be paired and were definite in outline. This shows a condition between the two extremes mentioned above in that the ovary is neither diffuse nor definitely paired, but is definite and single. Clearly it is necessary to examine many more individuals before this controversy over the form of the testes and ovary is finally settled.

The description of the spermathecae and the male efferent apparatus agrees with the pattern usually found in the genus Chaetogaster.

Sperber (1948, 1950) states that the genital setae of Ch. limnai have a double hook at their distal end, but a careful examination has shown that in the kidney form at least these are simple hooked (Fig. 1, p. 2).

b. The life cycle and asexual reproduction of *Chaetogaster limnaei*

In common with most Naididae the sexual form of *Ch. limnaei* is very rare. No mature individuals were found in the outer population and the few that occurred in the kidney population were found mainly during the winter months. Thus a majority of individuals of both the outer and kidney populations reproduced asexually by budding all the year round. Von Baer (1827), Lankester (1869b) and Wagin (1931) all observed mature forms of *Ch. limnaei* in very small numbers in the autumn. Vaghin (1946) stated however that the kidney population was 100% mature in late autumn. The outer forms on the other hand became mature in late summer and only to the extent of 12 - 15% of the total population. It is unfortunate that it cannot be deduced with certainty whether Baer, Lankester and Wagin were discussing mature kidney forms or not, and therefore it must be concluded from Vaghin's results that conditions are most favourable for sexual reproduction for the outer forms in late summer, i.e., two or three months earlier than the onset of maturity in the kidney forms. The results of this present work indicate that the breeding season of the kidney form was a little later than the one described by Vaghin. This is almost certainly due to the difference in temperatures between the Russian habitats on which Vaghin worked and habitats in North Wales. Although it has been suggested that the life cycle of *Ch. limnaei* is synchronised with that of its host, it is highly probable that food and temperature conditions play a major part in controlling it. Judging from the growth rate of *Lymnaea pereger*, production of food seemed to be at a maximum during late summer and autumn. It is reasonable to assume, since the snails feed on plant

material consisting mostly of encrusting diatoms, and since outer Ghaetogaster feed largely on planktonic diatoms, that food conditions for both organisms are similarly affected by the seasons. Taking this and Vaghin's evidence into consideration one would have expected to find mature worms in the outer population at about this time. Indeed, mature worms may have been present in the outer population at this time but were not detected because the total number of Ghaetogaster taken in each sample during this period was always very small. On the other hand, in winter, when mature kidney forms were found, the number of worms taken in each sample was always fairly high thus increasing the possibility of detecting mature worms. Since feeding conditions in the kidney are presumably fairly constant throughout the year it is likely that the most important factor controlling the breeding season of the kidney forms is temperature, although it could perhaps be influenced by the host's reproductive cycle. This latter suggestion has been mentioned more than once in this work but there is no evidence to support it. The fact that so few Ghaetogaster become sexually mature is perhaps evidence to the contrary, and clearly further investigation is necessary to clarify this point.

Asexual propagation was more active in the outer population in summer than in winter. This was also true to some extent in the kidney population but here the difference was not so marked. This seems to indicate that conditions in the kidney are more constant than those on the outer surface of the snail. Again, the reason is probably that the availability of food varies less in the kidney than on the outer surface of the snail. Since outer Ghaetogaster feed largely on plant material, the food available to them varies with light intensity, temperature and the

amount of nutrients present in the water. Vaghin (1946) also observed a greater activity in asexual reproduction in summer, but his maximum of 11 buds per individual in the outer population in summer seems rather high. In the populations studied here the number of buds never exceeded 5 in the outer or kidney populations. This was because the chain of buds broke in two at the first formed budding some usually before the 5 bud stage was reached, sometimes at this number but never later. The figures below represent an individual having four buds, 0 being the parent worm and the numbers 1 to 4 represent the buds in order of appearance.

0      4      2      1      3

This agrees with the sequence given by Claus (1860) for Ch. limnaei, and with that given by Herlant-Macwis (1958) for Ch. diaphanus.

#### e. Dispersal and reinfestation.

Vaghin (1946) stated that Ch. limnaei invaded another host during the process of host copulation. Although it is probably true that Chaetogaster may cross from one snail to another at this time, such behaviour does not explain how the young snails become infested. Copulation does not usually occur until the snails are about 8 mm. long, and since adult snails of the parent generation all die within about four weeks of the appearance of young snails in the population, copulation between members of different generations would seem to be impossible. It is therefore necessary to suggest another method by which a new generation of limnaea pereger is infested. It has been shown that both kidney and outer forms disperse on the death of the host and will infest another snail when

contacted. It has also been shown that transfer of Chaetogaster from one snail to another does occur when both snails are alive whether the recipient snail be young or adult. This suggests three methods of dispersal, (a) by becoming free-living for a period after the death of the host until a new host is found, (b) by transfer from one snail to another when two snails are in contact and (c) by some Chaetogaster leaving a healthy host and finding another. The relative importance of these is difficult to assess but in support of (a) it has been shown that Ch. limnaci is to be found free-living at the time when the adult snails are dying. With regard to (a) and (c) experiments have also shown that both forms of Ch. limnaci can survive free-living for at least one week, but that the outer form can survive for longer periods than the kidney form. It is not surprising therefore that the percentage of a young snail population infested by the kidney form is usually lower than that infested by the outer form. Further reasons for this are that the number of outer forms leaving the dying host generation is presumably several times greater than the number of kidney forms leaving, and the fact that kidney Chaetogaster do not seem to react to the mucous trails of the host as vigorously as the outer form thereby reducing the probability of their finding a host. It is also possible that some kidney Chaetogaster fail to leave the kidney when the snail dies.

The mucous trail of the host must be a considerable aid to the outer form in its search for a host in that the worm does not have to rely entirely on a chance meeting. It seems that the kidney form does have to rely to a greater extent on such a circumstance. However, in studying the response of the kidney form to mucus, the worms were removed from the kidney by dissection and it is possible that this would have been different



had they left the kidney of their own accord. There is also the possibility that the kidney forms react to a different stimulus in their search for a host. When one considers that the kidney form must find the external opening of the ureter, which is situated just inside the mantle cavity and above the anterior end of the pneumostome (Taylor 1894 - 1900), in order to enter the kidney, it seems likely that it does so in response to a stimulus. This could possibly be chemical and derived from the excretion of the kidney, but this problem has not been studied. If correct, it is likely that the kidney form's sense organs may respond to different stimuli from those of the outer form. The opening of the ureter is slit-like with no sphincter and has been shown to allow the entry of Chaetogaster even in very small snails. No other means of entry into the kidney is likely since in all the infested snails examined this organ was intact.

It has been shown that Chaetogaster removed from the kidney of one snail always infest the kidney if introduced to another snail. The outer forms also establish themselves only on the outer surface of another snail. All efforts to induce the outer forms to enter the kidney failed. There is some evidence however that kidney forms occasionally migrate onto the outer surface of the snail. It will be necessary to establish the extent of this migration in different seasons and under varying intensities of infestation to determine its cause.

The statement made by Amundale (1905) that Chaetogaster left the host to become free-living when the water became warm or foul needs investigating. This may be related to the fact that Chaetogaster leaves a dead snail which is decomposing and creating foul conditions.

The possibility has been mentioned that some cocoons deposited

in the kidney may pass out into the surrounding water through the ureter. If the embryos completed their development in the pond the young worms could serve to infest the next generation. Any cocoons produced by the outer population would inevitably develop and hatch on the substratum of the pond in the same way. However, the number of cocoons produced by a population of Chaetogaster is so small that their value from the point of view of dispersal is negligible. Cocoon production does of course provide for new genetical patterns essential for the evolution of the species.

d. Feeding in Chaetogaster limnaei.

The outer form of Ch. limnaei feeds on diatoms and small planktonic animals. If the host is infested with cercariae the worm will also engulf these as they emerge from the snail. The kidney form feeds exclusively on cells derived from the kidney and will not eat any of the organisms normally taken by the outer form under any of the conditions investigated.

Several authors have suggested that Ch. limnaei plays an important part in controlling cercaria numbers. This work also shows that many cercariae are destroyed by Chaetogaster but it is impossible to say whether this has any significant diminutive effect on the cercaria population because it is not known what proportion of the cercaria population is eaten.

The rate at which food is caught by the outer form when on the snail is greater than when the worm is free-living, and less energy is expended in doing so. The reason for this is that when on the snail a

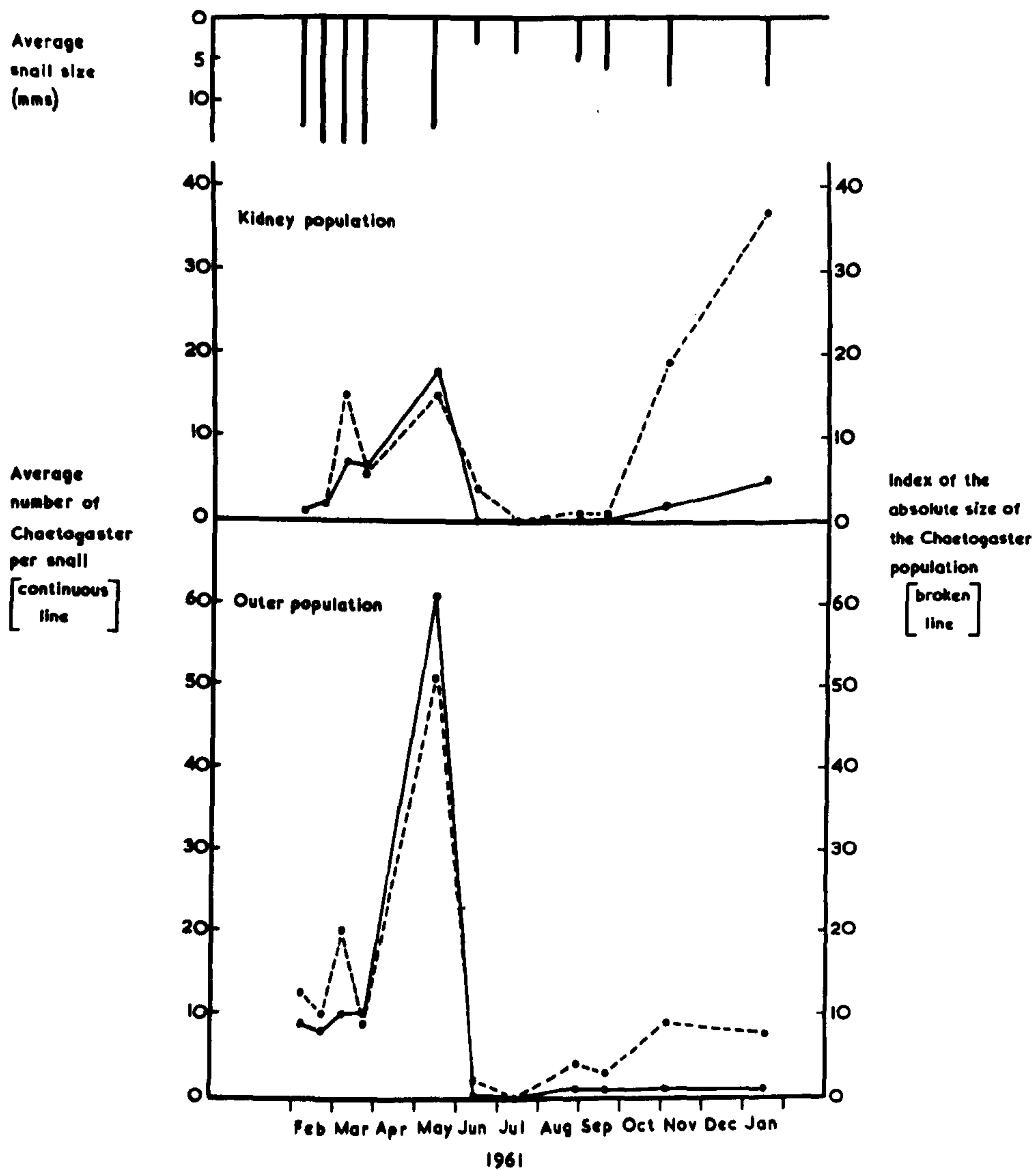


Fig. 29. A comparison of changes in the absolute size of the Chaetogaster population and in the average number of Chaetogaster per snail in the stream.

greater volume of water can be searched for food due to the ciliary currents on the snail and the movements of the snail itself. This is one reason why Chaetogaster benefits from its association with the snail.

e. Population Dynamics.

Figures 12, 13 and 14 (pp. 29, 32 & 32) were constructed using data for the average number of Chaetogaster per snail. This does not give a true indication of changes in the absolute size of the Chaetogaster population. In order to do this it is necessary to relate the average value of the density of the Chaetogaster population to that of the host population. This has been done by using the product of the average number of Chaetogaster per snail and the number of snails collected per minute (see Fig. 16, p. 32). It does not however take into account the number of Chaetogaster that are free-living at any particular time. These calculated values for both the outer and kidney populations have been plotted together with the original graphs of average values and presented in Figs. 28 and 29. It is seen that the corrected graphs, indicating the changes in the absolute size of the Chaetogaster population, are very similar in their general appearance to the original graphs based on average values per snail, but there are some minor differences.

Since the general pattern of changes in absolute numbers is very similar for the outer population and the kidney population, they will not be discussed separately. Thus, all conclusions drawn from Figs. 28 and 29 in this section will apply to both outer and kidney populations.

In general, both Chaetogaster populations increased in absolute

size continuously from the time the worms first became established on the young snails in the reservoir and in the stream until these died. A steep drop in the numbers of Chaetogaster occurred when the young snails appeared. Such an increase in the average numbers of Chaetogaster per snail could be caused by (a) multiplication of Chaetogaster, (b) snail mortality, resulting in release of Chaetogaster which could establish themselves on the remaining snails, and (c) selective mortality of snails harbouring less than the average number of Chaetogaster. Undoubtedly, asexual reproduction by Chaetogaster causes an increase in its numbers, and it is probable that Chaetogaster leaving dead snails serve to increase the average number of Chaetogaster per snail on the surviving individuals. It is unlikely however that there is selective mortality of snails harbouring only few Chaetogaster. Assuming that Chaetogaster has a harmful effect on the snail it is more likely that there would be selective mortality of heavily infested snails. A decrease in the average number of Chaetogaster per snail could be due to (a) mortality of Chaetogaster, especially during dispersal, (b) dilution of the snail population by large numbers of young non-infested snails, and (c) the selective mortality of snails having more than the average number of Chaetogaster. As mentioned above, the possibility that selective mortality of heavily infested snails occurs cannot be ignored although there is no evidence to suggest that it does so.

Since the graphs indicating changes in absolute number of Chaetogaster follow those of average number per snail very closely it seems reasonable to conclude that multiplication of Chaetogaster by asexual reproduction is the main cause of increase in average numbers per snail. This increase continues until the death of the snail generation. It is

obvious that the cause of any decrease in the absolute population size was either Chaetogaster leaving their host and becoming free-living or mortality. Such decline in population size coincided with mortality of adult snails after breeding. Thus, at first sight, it seems that the decline was due to Chaetogaster assuming a free-living mode of life. But since the absolute size of the Chaetogaster population on the new generation of snails was considerably smaller than that on the previous generation, heavy mortality of Chaetogaster must have occurred during dispersal. Although dilution of the host population by young snails contributes towards the decline in average numbers, the close correlation between the graphs of average numbers and those indicating changes in absolute population size shows that a drop in average numbers of Chaetogaster per snail is largely due to Chaetogaster mortality.

So far, in this section, only general trends common to both outer and kidney populations in the stream and in the reservoir have been discussed. There are however minor discrepancies within both the reservoir and the stream populations in the two graphs. The populations of the two habitats will be discussed separately, but since these discrepancies are common to both kidney and outer populations in each habitat, the discussions will naturally apply to both kidney and outer populations.

The peaks of May 1960 and August 1960 in the reservoir population (Fig. 28, p. 131) shown by the graph of average values are also shown by that indicating absolute population size, and so is the peak of May 1961. Neither graph shows a peak in August 1961. The August and September samples of 1961 were one month apart and it is quite possible that a small peak did occur in September and was not detected because of

the long interval between the sampling dates. This possibility is emphasised when one considers that had the samples of 1960 been taken monthly instead of fortnightly, the August peak of that year could also have been missed. Although no peak was detected, a drop in the percentage of snails infested with Chaetogaster occurred following the appearance of young snails at this time which brought the percentage down to a value comparable to those following other peak values.

The two graphs differed in the timing of peaks in August 1960 and May 1961 and detailed analysis showed that these were due to contrasts in the overlap of the host generations. In 1960 the graph of average numbers indicates a peak at the end of August whilst that for absolute numbers shows a peak at the end of July. The discrepancy is due to the fact that at the end of August the snail population was extremely small, and although the average number per snail on the surviving snails was high the absolute size of the Chaetogaster population on the snails was small. In May 1961 the snail population was extremely small at the time of the peak value in the graph of average numbers (May 11th), and as a result the absolute size of the Chaetogaster population was relatively small although the average number per snail was high. Conversely, in the next sample (June 5th), the average value was low but the presence of large numbers of young snails in the population, many of which were infested, together with a few heavily infested adults meant that the corrected value was high. Despite this, it is difficult to believe that the Chaetogaster population could have multiplied to such an extent during this short period of time. One can only assume that by May 11th. the greater part of the Chaetogaster population was free-living following the death of the majority of the snails

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of the parent generation. In the previous year most of the parent snails died immediately after the appearance of the young snails and consequently the phenomenon described above was not encountered. The subsequent drop in the absolute size of the Chaetogaster population between June and August 1961 was due to the final disappearance of heavily infested adult snails and also to the post-hatching high mortality rate amongst the young snails.

The graph indicating changes in the absolute size of the stream population (Fig. 29, p. 131) differs from that for average numbers in showing a steep increase in the Chaetogaster population size in the autumn. The apparent scarcity of the worm as indicated by the graph of average values is because the host snail became exceptionally abundant in the stream at this time. As a result, the Chaetogaster population became diluted and the increase in its size could not be detected by the average number of Chaetogaster per snail.

The accuracy of these corrected graphs which indicate changes in the absolute size of the Chaetogaster population depends of course on the accuracy of the estimates of the relative size of the snail populations; these estimates introduce another parameter which leaves room for further error.

Immediately following the appearance of young snails, the percentage of snails infested by either form of Chaetogaster was always low. This value then increased and eventually reached between 90 and 100%. The initial rate of infestation (see % infestation, Figs. 12, 13 & 14. p. 29, 32 & 32) by the outer form was usually greater than that by the kidney form (e.g., May 1960, September 1960, June 1961 and September 1961 in the

reservoir). After this initial period, infestation by both forms increased at a comparable rate, but with the outer population always retaining its initial advantage. It seems therefore that the two forms achieve maximum infestation of the host in slightly different ways. The outer form establishes itself initially on more snails than does the kidney form, probably, as explained earlier (p. 128), because the former has more efficient dispersal mechanisms. The overlap of the two generations of snails, and the possibility of members of different generations coming into contact with each other also provides an opportunity for the Chaetogaster, the outer form in particular, to transfer from one generation to another. After the initial period of infestation, further infestation of the new generation by both forms is probably brought about by contact and copulation between the snails. Because of the nature of the microhabitat in which the kidney forms dwell, it would be reasonable to assume that their migration from one host to another is very limited. The results show however that after an initial slow period, the rate of infestation by kidney forms compares with that by outer forms. This suggests that kidney forms are able to migrate onto the outer surface of the snail and reach others quite freely.

It is seen that the average number of Chaetogaster per snail in the kidney in winter (Reservoir 1959-60, 1961-62. Stream 1961-62. see Figs. 12, 13 and 14. p. 29, 32 & 32) is often greater than that of outer Chaetogaster. This situation is reversed in the spring, summer and autumn. The probable explanation is that at low winter temperatures feeding conditions in the kidney are better than in the surrounding water. At the higher temperatures of other seasons this may not be true, but here

there are other factors to be considered. Whereas the outer population has plenty of space in which to expand, this resource can be and probably is limiting in the kidney. As explained (p. 38) the average number of Chaetogaster in the kidney tends to reach a ceiling, and this is certainly partly due to the limitations of space in the kidney. When this condition is reached, many of the worms probably leave the kidney and it is at this time that one would expect to find this form on the outer surface of the snail. These worms would serve to infest other snails because saturation in the kidney often occurs at approximately the time when the snails are copulating. This is reflected in the fact that maximum infestation of the host population by kidney forms coincides with the breeding season of this snail in March - April 1960 and February 1961 in the reservoir (Figs. 12 and 13, p. 29 & 32).

f. The nature of the association between Chaetogaster limnai and Lymnaea pereger.

The outer form of Ch. limnai benefits from its association with L. pereger in that it obtains shelter and a more plentiful supply of food with less expenditure of energy than it would if it were free-living. It is sheltered from small predators, but of course it is not protected from destruction by predators of the snail, e.g., frogs and toads, fish, large insect larvae and even <sup>the triclad</sup> Dugesia lugubris (Reynoldson and Young. in press). The snail does not seem to be adversely affected in any serious way by the presence of the Chaetogaster, and so the term 'commensalism' can be safely applied to the association. In other species of freshwater pulmonates it

has been shown that Chaetogaster can be a symbiont or even a parasite (Hunter 1960. pers. comm.).

Smyth (1962) states that commensalism may be considered a type of loose association in which two animals of different species live together without metabolic dependence, although one or both organisms may receive benefit from the association. He stresses the importance of the absence of metabolic dependence in this type of association since in his opinion it is this feature which separates commensals on the one hand from parasites and symbionts on the other. He further states that parasitism is a relative phenomenon, the degree of parasitism depending on the degree to which the parasite is metabolically dependent on its host. Thus, he states that symbiosis can be regarded as a special case of parasitism in which some metabolic by-products of the parasite are of value to the host and presumably vice versa.

Other relationships of the commensal type are to be seen amongst the Oligochaeta and they all have certain characteristics in common. The most significant of these is that the worms still feed on free-living organisms and hence are not entirely dependant on the host at any stage in their life history. For example, Stephenson (1910) found Pristina longiseta, Nais communis and Nais pectinata living as commensals in Spongilla carteri. These worms are vegetable feeders and they only live in the sponge, according to Stephenson, because this habitat offers them a copious food supply. It is likely that they also gain protection from the sponge. Annandale (1906) also found a species of Chaetogaster living in association with this sponge, a closer association perhaps than that of the three worms described by Stephenson since it fed on decaying sponge

material. He also found a species of Chaetogaster living on Plumatella and feeding on Protozoa.

Parasites amongst the Oligochaeta are however less common. There are a number of records of Oligochaeta as internal parasites, but Stephenson (1930a) seems to be rather suspicious of most of them. Two have been convincingly described however. One is Allodero bauchiensis (= Nais bauchiensis, Stephenson 1930b) which is parasitic in the Harderian gland of frogs of the genus Phrynosaurus, and the other is Allodero lutsi (= Schmaradella lutsi, Michaelsen 1926) found in the ureters of the frog Hyla venulosa. Little is known of these worms but it seems that even they can survive free-living. Stephenson suggests that the former is not naturally parasitic and suspects that it is found free-living, and the latter has in fact been found free-living. Dorsal setae are present in free-living A. lutsi but not in the parasitic form. The gut of the parasitic form is also degenerate. Stephenson states that the gut of the parasitic A. bauchiensis is also degenerate. Thus, here is a situation remarkably similar to that seen in Chaetogaster limnaii and the possibility that a parasitic and free-living form of both these Naid worms exist, needs investigating.

Records of external parasites are almost entirely confined to the family Branchiobdellidae which contains only leech-like parasites of the gills and external surface of freshwater crayfishes. There seems to be some controversy as to whether they are true parasites or not. Hall (1914) states that they are not parasitic when young, the gut at this time containing vegetable material, but the adult uses its jaws to break the skin of the host in order to suck the blood. However, Smallwood (1906)

and Goodnight (1940) are rather more cautious, and the latter believed that if some Branchiobdellidae are parasitic they are only facultative parasites. Dahm (1959) suggests that the young forms live on the micro fauna and flora on the exoskeleton of the host. The adult Branchiobdellid worm he describes as having an eversible pharynx which can be forced into a wound made by the teeth in order to feed on the blood and lymphatic fluid of the host.

The metabolic dependence of the kidney form of Gn. limnaci upon its host at first sight suggests unequivocally that its 'modus vivendi' is more akin to the parasitic oligochaetes than to the commensal types. For example, because Ghaetogaster takes only kidney cells it is more dependent on its host than the Branchiobdellidae which consume a great deal of vegetable material. However, sections of infested kidney (Pl. 5a, p. 52) have shown that the tissues of this organ are not damaged by the worm and apparently only discarded cells containing metabolic wastes are eaten, and not living, functional cells. However, it cannot be denied that the kidney form is highly dependent on the metabolism of its host and cannot be regarded as a commensal; it is according to Smyth's definition a parasite. The ability of this form to live apart from its host to a limited extent does not alter this conclusion since such an attribute is a common feature of many undoubted parasites.

The kidney form also obtains shelter as a result of its relationship with the snail. It also benefits by having a constant supply of food and it is probably protected against desiccation during dry periods when the snails are driven to aestivate. In this respect it is probably better protected than the outer form.

It is surprising that since the kidney form has evolved such an obligatory relationship with the host, the worm's dispersal mechanisms do not seem to be as efficient as those of the outer form which is less dependent on the host. The problem of how the kidney form finds its host has by no means been solved, and until this has been done, no conclusions relating to the efficiency of dispersal mechanisms can be drawn.

The relationships between Ch. limnaei and one host species only were investigated. Consequently it is not known whether Ch. limnaei is host specific or not. Judging however from the work of other authors it probably is not and will colonise any suitable freshwater gastropod. If this is so, it would be extremely difficult in a mixed population of molluscs to analyse data from certain aspects, since interspecific migration of Ch. limnaei would be taking place. In the reservoir and in the stream this difficulty was not encountered since other molluscs besides L. pernix were only present in very small numbers.

g. The evolution of the outer and kidney forms of Chaetocaster limnaei.

Vaghin (1946) based his deductions, that the two forms of Ch. limnaei showed signs of divergence leading to the creation of two 'biological species', on the fact that he found differences in the habits and life cycle of the two forms. Further evidence has been accumulated here which supports his theory. The two forms display (a) morphological differences, (b) physiological differences in that they differ in their feeding habits, their ability to survive away from the host and their response to the mucous trails of the host, and (c) behavioural differences

in that they occupy different sites on the host and always return to their respective microhabitats. On this evidence it is suggested that the divergence of these two forms has reached the level of subspeciation at least, and that the kidney form should therefore be considered as a subspecies of Chaetogaster limnaei. It is further suggested that this subspecies be called Chaetogaster limnaei vaghini. Further evidence showing that the two forms do not interbreed is necessary before they can be treated as separate species. As the two forms are not completely isolated from each other and can come into contact during the migration of the kidney form onto the outer surface of the snail, crossbreeding is possible. Vaghin's evidence that the breeding seasons of the two forms do not coincide suggests that interbreeding may not occur. A solution of this problem necessitates attempting to crossbreed these two forms experimentally.

The obvious argument against recognising the kidney form as a subspecies of Ch. limnaei is to regard the former as an outer form that has in some way become conditioned to living in the kidney. This view can be discarded when the following facts are considered. On morphological grounds, no intermediate stages linking the two forms have been found, and since conditioning would presumably be a gradual change these changes should have been encountered. The kidney form, when exposed to conditions away from the host, cannot be induced to behave similarly to the outer form as would be expected if it were simply a matter of conditioning. A young worm which hatched from a cocoon that had been deposited in the kidney and reared outside the host proved to be a typical kidney form on morphological grounds. Finally, two populations of L. peregrin found in North Wales were only infested by the outer form and this suggests that the



kidney form was entirely absent and therefore is not periodically derived from the outer population. Kidney forms introduced to these snails always established themselves in the kidney. This same approach can also be used to show that an individual of the outer form has not been derived from the kidney population.

Which of the two varieties of Ch. limnaei represents the ancestral form is open to discussion. Stephenson (1950a) believed that the whole of the genus was derived from parasitic ancestors. His theory is based on the 'absence of ascending ciliary action and antiperistalsis in the intestine, the reduction of the vascular system, the absence of complete dissepiments and consequently of sperm sacs and ovisacs, the generally carnivorous habit, the thinness of the body wall and consequent transparency, and the sometimes commensal and episootic mode of life'. He also thought that the absence of dorsal setae indicated parasitic ancestors. Sperber (1948) rejected his arguments and pointed out that none of his criteria suggested parasitism. She also argued that since Chaetogaster and Amphichaeta are so obviously closely related and probably have a common ancestor and because Amphichaeta does not display all the characters mentioned by Stephenson, the mode of life cannot have been the cause of the peculiarities of the two genera. Compared with the outer form, the kidney form of Ch. limnaei shows marked reduction in the number of setae and to a lesser extent in the thickness of the gut wall. On the other hand, the outer form compared to free-living members of the genus shows adaptation to its mode of life in that it has more setae per bundle. Thus it may be said, following Stephenson's arguments, that the free-living species have been derived from parasitic forms which perhaps passed through a stage

comparable with that of the outer form of Ch. linnaei. The initial change from parasitism to commensalism would presumably have involved an increase in the number of setae brought about by selection for more efficient methods of attachment to the surface mucous film of the snail. This is of course assuming that the original parasitic forms were in a comparable situation to the present kidney form of Ch. linnaei where efficient attachment organs were not necessary. The change from commensalism to a free-living mode of life would have brought about a decrease in the number of setae because these would have been no longer necessary for anchoring the worm to another animal. Conversely, the free-living forms may have been the ancestors of the genus. The kidney form of Ch. linnaei would then clearly be the newest creation of this line of evolution, having been evolved from an intermediate commensal form, similar perhaps to the outer form of this species. It is seen however that these two arguments are based on the assumption that during the evolution of the worm the number of setae per bundle first of all increased and then decreased. Clearly, in the later stages this would have involved the reversal of its earlier evolution. As this is contradictory to the generally accepted theory of the irreversibility of evolution another explanation must be sought. A possible theory is that the kidney and outer forms each diverged independently from an ancestral free-living form. The evolution of the kidney form would have involved a reduction in the number of setae, and the evolution of the outer form an increase in the number of setae. In view of Sperber's criticism of Stephenson's other arguments this theory is probably the easier to accept, particularly since parasitic forms usually evolve from free-living forms, and thus on general grounds, the reverse process is a

very unlikely event. Consideration of these arguments and the evidence available shows that the most acceptable explanation seems to be that the kidney and outer forms of Ch. limnaei are products of divergent lines of evolution originating from an ancestral free-living form.

## Section 12.

### SUMMARY.

The ecology and population dynamics of Gaetogaster limnaei living in association with Lymnaea pereger were studied in two habitats. The habitats and sampling methods used have been described.

It has been shown that there are two distinct forms of Ch. limnaei, one living as a facultative commensal on the outer surface of the snail and the other as a parasite in the kidney of the snail.

It was established that the outer form possesses more setae per bundle and a thicker gut wall than the kidney form. The kidney form never has more than 7 setae per bundle but the outer form usually has between 8 and 12 setae per bundle. Samples obtained from various parts of Britain confirmed this observation.

Experiments performed in the laboratory have shown that both the kidney and the outer form, when removed from their host and introduced to a non-infested snail, always returned to their respective habitats.

The outer forms feed on small planktonic animals and plants, but the kidney forms have become adapted to feeding on cells derived from the kidney of the snail.

Both forms are attracted by mucous trails left by the host

snail, but the reaction shown by the outer form is much more definite than that shown by the kidney form.

Because of these morphological, behavioural and physiological differences between the two forms it has been suggested that the kidney form be considered as a subspecies of Chaetogaster limnaei.

In the reservoir and in the stream where the observations were made, the overwintering snail population was replaced in the spring by young snails. In the stream these snails showed an annual cycle. In the reservoir however, the spring generation produced a second generation of young snails in late summer and it was this second generation that overwintered. The reason for this difference in the two populations is probably environmental.

The population of both outer and kidney forms was seen to be small on the very young snails. These Chaetogaster populations increased in numbers as the snails grew. The rate of increase of the kidney population in winter was often greater than that of the outer population but the position was reversed in summer. It is thought that this was due to the greater availability of food in the kidney as compared to the outer surface of the snail in winter. The reverse is probably true in summer. The rate of infestation of a new generation of snails was greater by the outer form than by the kidney form probably because the outer forms have a better survival rate when free-living, are more active in seeking a new host and can take better advantage of contact between two snails. On the death of one generation of snails and the appearance of a new generation, both forms of Chaetogaster leave the former, become temporarily free-living and finally establish themselves on the latter. It is probable that

contact and copulation between two snails aids the dispersal of both forms. During the process of transfer from one generation of snails to the next a large proportion of the Chaetogaster population died.

Both populations of Chaetogaster increased in size mainly by asexual reproduction. Buds are produced in a chain at the posterior end of the worm and these eventually break away to form new individuals. Budding was most active during the summer and autumn. The maximum size of the kidney population seems to be restricted by the amount of space available in the kidney. Space does not limit the size of the outer population.

The sexual form of Ch. limnaei was very rare and was only found in the kidney population during the winter months. Cocoons produced by these individuals were deposited in the kidney. All cocoons found were incubated but only five out of 57 hatched. One was examined and proved to be a typical kidney form. The mature kidney form was examined and the structure and position of the reproductive organs were described briefly.

The survival of the two forms away from the host was investigated experimentally and it was found that when fed on Protozoa, Rotifera etc., the outer form outlived the kidney form by a considerable time.

It is suggested that both forms benefit by the association in that they obtain shelter from small predators and a better supply of food. The kidney forms are probably also protected to a certain degree from desiccation when the snail is driven to aestivate.

The evolution of Ch. limnaei was discussed briefly.

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## APPENDIX A.

Notes on a population of *Glossiphonia heteroclita* infesting *Lymnaea pereger*.

## Introduction.

Whilst examining samples of *Lymnaea pereger* taken from the reservoir it was noticed that many of the snails harboured the leech *Glossiphonia heteroclita* (L.) in their mantle cavity; no leeches were found in snails from the stream at Coed Mawr. The number of leeches found on each snail was recorded for a period of two years between January 1960 and February 1962. The leeches found were preserved in 70% alcohol, and at the end of the sampling period the weight of leeches taken from each sample was measured. The leeches were dried on filter paper prior to weighing.

Little is known of the life history of *Glossiphonia heteroclita*. It is reported (Mann 1962) that the Glossiphoniidae deposit their eggs in thin walled cocoons and the leech places its body over this, assuming a protective role. Eventually the embryos break free of the cocoon and attach themselves by means of an embryonic attachment organ to the ventral surface of the parent. It is thought unlikely that any nutrients are transferred from parent to young during this period. However, the parent probably provides shelter, and the movements of the parent supply water for respiratory purposes. Later, the embryos break free from their egg membranes and attach themselves to the parent by means of their posterior sucker. In *Glossiphonia complanata* the embryo usually remains in the cocoon for 5 to 6 days (Mann 1957), becomes attached by the embryonic attachment organ for 4 to 5 days and clings to the parent by means of its posterior

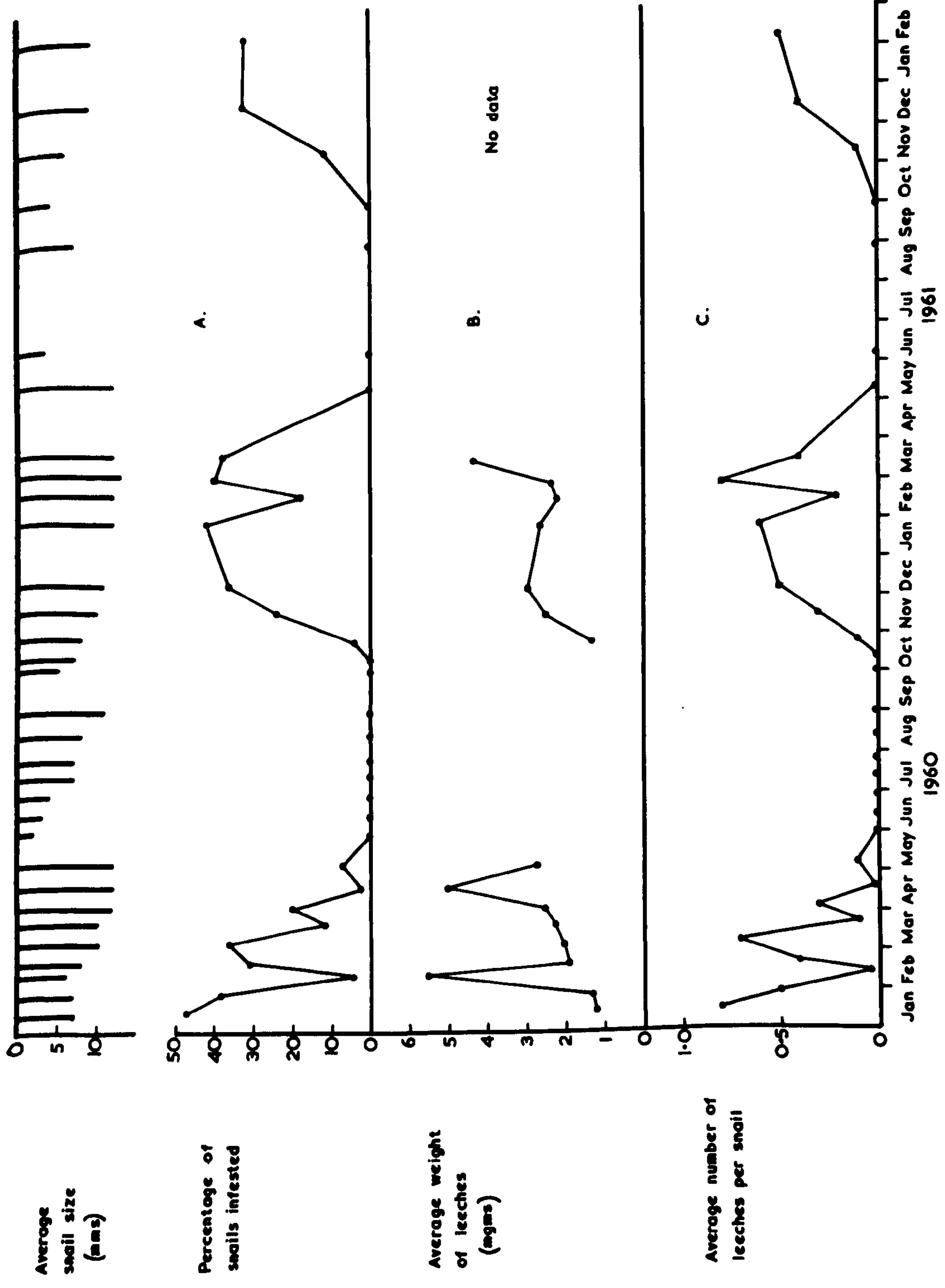


Fig. 30. (A) The percentage of snails infested with Gl. heteroclita, (B) the average weight of leeches found in each sample of L. pereger, and (C) the average number of leeches per snail in the reservoir.

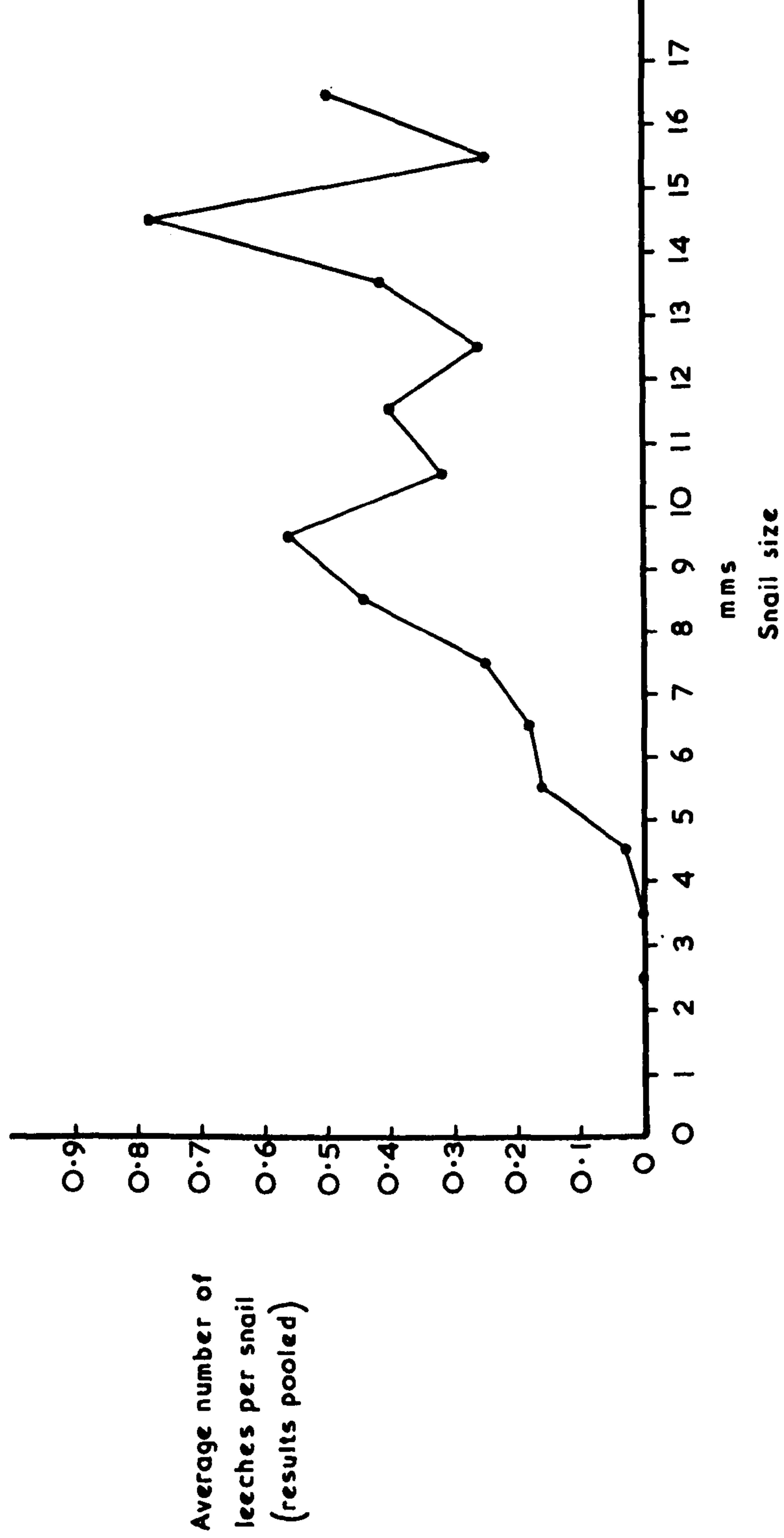


Fig. 31. The average number of Gl. heteroclita per snail obtained by pooling data from all samples in which the leeches were found.

sucker for a further 14 days or so. This leech lives for two years and breeds in both years.

### Results.

Figures 30 and 31 were drawn using the data obtained from the samples taken during 1960 and 1961. Figure 30 A shows the percentage of snails in each sample that were infested with Gl. heterocolita. During the summer months of June, July, August and September of 1960 and 1961 the snail population was not infested. Infestation began in October and maximum infestation was reached in midwinter. The percentage infestation then declined gradually, reaching zero in May. It is seen in Fig. 30 B that the average size of the leeches (indicated by average weight) increased steadily throughout the winter. The mean number of leeches per snail (Fig. 30 C) increased from zero during the first half of the winter and then decreased steadily to reach zero again in May. As would be expected, Figs. 30 A and 30 C follow a very similar pattern.

All the data obtained from leech-infested samples were pooled together and the average number of leeches found on each snail size group of 1 mm. was calculated (Fig. 31). It is seen that there is a definite tendency for the average number of leeches per snail to increase with snail size.

### Conclusions.

The results of 1960 and 1961 are so similar that they will not be discussed separately. The graphs produced from these results all show trends in a particular direction, but oscillations within these trends are rather violent. This is clearly due to the leech samples being too

small and to eliminate this, larger samples would have to be taken. Nevertheless, useful conclusions can be drawn from the graphs.

1. Glossiphonia heterocolita inhabits the mantle cavity of Lymnaea pereger between October and May only. They are not found on the snail during the summer months. The population on the snails was gradually built up between October and January, then beginning in February, the leeches gradually left their host and presumably became free-living.

2. The average weight of the leeches increased throughout the winter to reach a peak between March and May. When re-infestation occurred in the following October, the leeches were, on average, small and presumably mostly young individuals. It follows that breeding had occurred during the summer months.

3. The leech was found only to infest snails larger than 3 mm. in size. The reason for this is that the pneumostome and mantle cavity of the larger snails probably offer less resistance to the entry of the leech and more space to accommodate it. Since the leech feeds on the mollusc (Mann 1955), a large host offers a better food supply. The leeches finally disappear from the snail population at the time when the old snails are being replaced by young snails. Thus the disappearance of the large old snails may be a factor contributing towards the final disappearance of the leeches from the snail population.

It is significant that the average weight of the leech drops between May and October. As stated above, young leeches are almost certainly introduced into the population at this time. The unusual way in which the Glossiphoniidae incubate their young probably accounts for the adult leeches adopting a free-living habit during their breeding season in

early summer. This is probably the most important and perhaps the only factor causing the leech to leave the host snail and become free-living during the summer months.

The results have dealt with one aspect of the behaviour of the Gl. heterocolita population. They do not show what proportion of the leech population lives on the mollusc in winter and they do not provide any direct evidence showing what happens to this population in the summer. Indeed, until more is known about the timing of breeding and the growth rate of the young, it is not possible to account for the absence of leeches from the snails between June and September. But it is interesting to note that the breeding of Gl. heterocolita and its host are synchronised so that the period of scarcity of larger-sized snails coincides with the developmental period of leech egg and embryo. The length of time the young leech spends free-living after parental care has ceased is not known. To answer these problems it would obviously be necessary to sample the free-living part of the leech population as well as the parasitic part.

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## APPENDIX B.

The results of sampling in tabular form.

Sample	Date	No. of snails per sample	Mean Snail size	Mean No. Outer forms per snail	Mean No. Kidney forms per snail	Mean No. Leeches per snail
<u>Reservoir population (fortnightly samples).</u>						
1	11:1:60	96	6.79		3.93*	0.8
2	25:1:60	110	7.11		3.58*	0.5
3	8:2:60	124	6.33		3.22*	0.04
4	22:2:60	107	7.66	2.6	5.2	0.4
5	7:3:60	102	10.10	4.29	8.07	0.7
6	21:3:60	99	9.90	8.07	8.16	0.1
7	4:4:60	179	11.93	12.76	9.42	0.3
8	19:4:60	110	11.71	20.93	11.56	0.02
9	5:5:60	84	12.37	42.64	15.27	0.1
10	30:5:60	254	2.13	4.64	0.51	-
11	13:6:60	205	2.90	1.16	0.11	-
12	27:6:60	98	4.06	0.53	0.09	-
13	11:7:60	58	7.04	1.93	0.24	-
14	25:7:60	109	7.00	8.80	1.13	-
14a	13:8:60	7	8.58	5.57	0.1	-
14b	31:8:60	2	11.35	28.00	5.5	-
15	30:9:60	53	5.45	0.24	0.09	-
16	10:10:60	51	7.00	0.38	0.04	-
17	24:10:60	49	8.09	0.58	0.07	0.1
18	14:11:60	61	10.02	2.98	1.96	0.3
19	5:12:60	58	10.91	4.47	1.60	0.5
20	23:1:61	58	11.70	8.24	6.24	0.6
<u>Reservoir population (monthly samples).</u>						
1	13:2:61	22	11.87	11.4	8.68	0.2
2	27:2:61	20	12.65	12.85	12.55	0.8
3	13:3:61	18	12.00	15.72	7.61	0.4
4	11:5:61	12	11.60	43.5	8.25	-
5	5:6:61	60	3.35	6.71	1.33	-
6	25:8:61	25	7.03	1.76	1.28	-
7	28:9:61	62	4.05	0.97	0.40	-
8	9:11:61	28	6.45	0.21	0.25	0.1
9	15:12:61	38	8.72	0.74	2.05	0.4
10	6:2:62	25	9.08	0.72	1.80	0.5

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Sample	Date	No. of snails per sample	Mean Snail size	Mean No. Outer forms per snail	Mean No. Kidney forms per snail
<b>Stream population (monthly samples).</b>					
1	6:2:61	21	13.39	8.85	0.9
2	22:2:61	19	14.79	8.1	1.84
3	7:3:61	21	14.51	9.71	6.9
4	20:3:61	13	14.68	10.15	6.23
5	11:5:61	33	12.90	61.00	17.6
6	12:6:61	45	2.75	0.04	0.09
7	13:7:61	73	3.58	0.03	0.03
8	28:8:61	45	4.54	0.8	0.18
9	19:9:61	50	6.42	0.6	0.28
10	2:11:61	43	7.68	1.09	2.19
11	16:1:62	74	8.27	1.0	5.04

\* Average number of the sum of the outer and kidney forms per snail.