STUDIES ON THE BIOLOGY AND TREMATODE INFECTIONS OF *BITHYNIA TENTACULATA* (LINN.).

PROSOBRANCHIA: GASTROPODA.

By

Faisal Sawi Ali

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SUMMARY

The present investigation on the biology of Bithynia tentaculata has two aims. Firstly to report on the life cycle, growth, reproduction and other aspects of the biology of this snail, and secondly to identify the larval trematode infection of the snail and to report on the incidence, seasonal variation and the development of these infections.

The study area was a small artificial pond in Sefton Park, Liverpool. The biology of the snail was investigated by regular sampling of the habitat for 22 months, the snails were measured and the breeding activity was assessed. Laboratory experiments were performed on the growth, reproduction and natural diet of the snail. The larval trematode infection of the snail was investigated and experimental infections were made. A monthly survey of the incidence of these infections was made for 13 months.

The following results were obtained on the biology of the snail;

1) Growth of the snails practically ceases in the winter and the wintering population resumes growth in the spring. The new generation of snails appears in June and growth is rapid during the summer.

2) The snails begin to breed in mid-April and active breeding is limited to April-June, but sporadic egg-laying occurs in July and August.

3) There is a minimum breeding size of the snails which varied from one year to the next depending on the growth rate. In addition to size, there is a minimum period of five to six months for maturity (oviposition) of the snails.

4) The onset of egg-laying is controlled by temperature, and the snails did not oviposit at 10-12°C.

5) The snails show optimal growth when fed on detritus, and algae, growth is retarded on a diet of detritus alone.

6) Winter and unfavourable conditions cause aggregation of the snails, and organic pollution is detrimental to their distribution within the habitat.

7) The life span of Bithynia tentaculata is about 14 to 23 months, and possibly few survive to breed for a second season.

Results obtained on trematode infections of Bithynia tentaculata show the following:

1) Six species of cercariae infect the snail, a Monostome cercaria, three Gymnocephalous cercariae, a Xiphidiocercaria and a pharyngeal longifurcate monostome Furcocercaria.

2) One of these cercariae (i.e, Cercaria helvetica XIX) is a first record in British freshwater. Existing descriptions of some of the cercariae are expanded, and new descriptions of some developmental stages are added.

3) The life cycle of Notocotylus imbricatus is traced and metacercarial infectivity is demonstrated soon afer encystment. The development of Notocotylus imbricatus in the snail host is followed and descriptions of the developmental stages are presented.

4) Multiple cercariae infections are generally rare, yet certain combinations of double infections aremore frequent than expected.

5) The total cercariae infection of the snail, as well as four individual species of cercariae, show biannual peaks of incidence. No biannual peaks of metacercariae infections were detected.

6) Many cercariae infections enter the new generation of snails in their first summer. Some of these infections may mature in the same season, but most of the infections are carried through the winter in an immature state. New infections enter the snails in the spring, but few infections enter the snails in their second summer of life.

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I. GENERAL INTRODUCTION

The present investigation on the biology of Bithynia tentaculata is concerned with the life cycle of this species as well as with the larval digenetic trematode infection of the snail in the habitat studied. Introductory notes and literature reviews precede the relevant sections of the work, and the following is a brief general introduction.

Bithynia tentaculata is one of the most widely distributed freshwater gastropods in the British Isles (Boycott, 1936; Fretter and Graham, 1962; and Macan, 1969). The snail is also found throughout Europe (Zhadin, 1965) and in North America (Berry, 1943). Boycott (1936) writing on the ecology of the freshwater molluscs of the British Isles, reported on the habitat, distribution and life span of Bithynia tentaculata. Lilly (1953) was the first to report on the growth and reproduction of a natural population of the snail in Wickford, Essex. Lilly's (1953) samples were collected at three-monthly intervals, and no information was given on the sample size.

In view of the scarcity and incomplete nature of the information available on the growth, reproduction and life cycle of Bithynia tentaculata in the British Isles, the present investigation was undertaken to increase our knowledge of these as well as other aspects of the biology of this snail. In Europe reports containing information on the life cycle of this snail were contributed by Hubendick (1948), Schäfer (1953) and Frömming (1956).

In North America, Pinel-Alloul and Magnin (1971) studied the life cycle of four populations of Bithynia tentaculata in lake St. Louis, Montreal, Canada.

Bithynia tentaculata has been shown to act as the intermediate host for a number of digenetic trematodes, and numerous species of cercariae were reported infecting the snail (Dubois, 1929; Wesenberg-Lund, 1934; Wikgren, 1956; Nasir and Erasmus, 1964; Probert, 1965[a,b]; 1966a; and Pike, 1967, 1968[a], etc). In the present investigation cercariae of six species of trematodes were recognised infecting the snail. One of these is a first record in Britain, and additional information is contributed to the existing species in some cases. Moreover the life cycle of Notocotylus imbricatus was experimentally traced, and questions raised by previous workers (Pike, 1969) were clarified. In addition, the development of Notocotylus imbricatus in the snail host was followed for the first time.

The seasonal incidence of larval trematode infections in Bithynia tentaculata was reported by Pike (1968b) in a survey which covered a wide variety of invertebrates. Small and unrepresentative samples of the snail were examined, and consequently the author was unable to confirm the biannual peaks of incidence of cercariae infection in this snail. In the present work a survey was carried out for thirteen months during which 1941 snails were dissected, and results obtained confirmed the

biannual peaks of incidence of cercariae infections. The survey also revealed information on the seasonal incidence of individual cercariae species, the development of infections, and the relationship between the life cycle of the snail host and its larval digenetic trematode infection. In addition, the survey showed a greater extent of double infections with cercariae than has previously been reported in this snail.

2. THE HABITAT

The habitat studied was a small artificial pond in Sefton Park, Liverpool (Ordnance Survey Grid Reference SJ 376879). The pond is the largest of a series of four ponds which are connected and lead to the lake in Sefton Park (Fig. 1). There is a small island in the wider part of the pond, and the boundaries of this island and of the pond are made of cemented stones. The length of the pond is 160 metres and the width varies from one part to another. The greatest width of the pond is 32 metres, which narrows on either side to about four metres (Fig. 1). The depth of the pond varies from 0.6 to 1.5 metres from the bank to the centre. The area of the pond is 2310 square metres.

The pond is fenced, and there are many trees in the surrounding area (Fig. 1). The pond is supplied by rain water and the drainage area is about forty acres. The drainage water enters the pond near the broad end, and the excess water leaves through a tube 0.75 metres in diameter. The tube is situated about two metres from the extreme narrow end of the pond and is about 0.75 metres high, so most of the incoming rain water is retained and only the excess water is discharged to the adjoining pond. The water level in the pond varied seasonally, the pond being usually full in the winter and the spring. In the summer, the water level is relatively

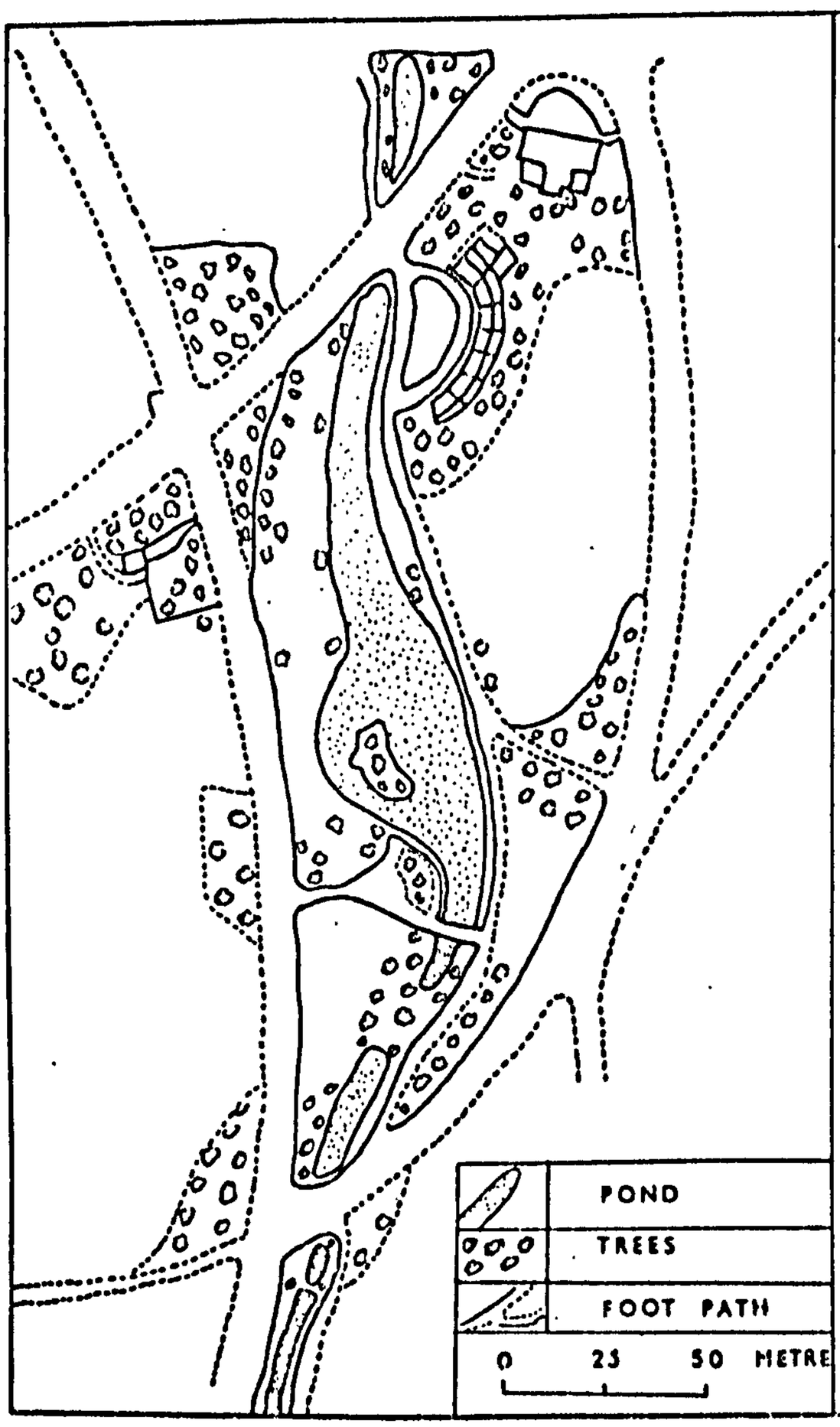


Fig. 1

The habitat, redrawn from a map of Sefton Park, supplied with the courtesy of the Liverpool Recreation and Open Space department.

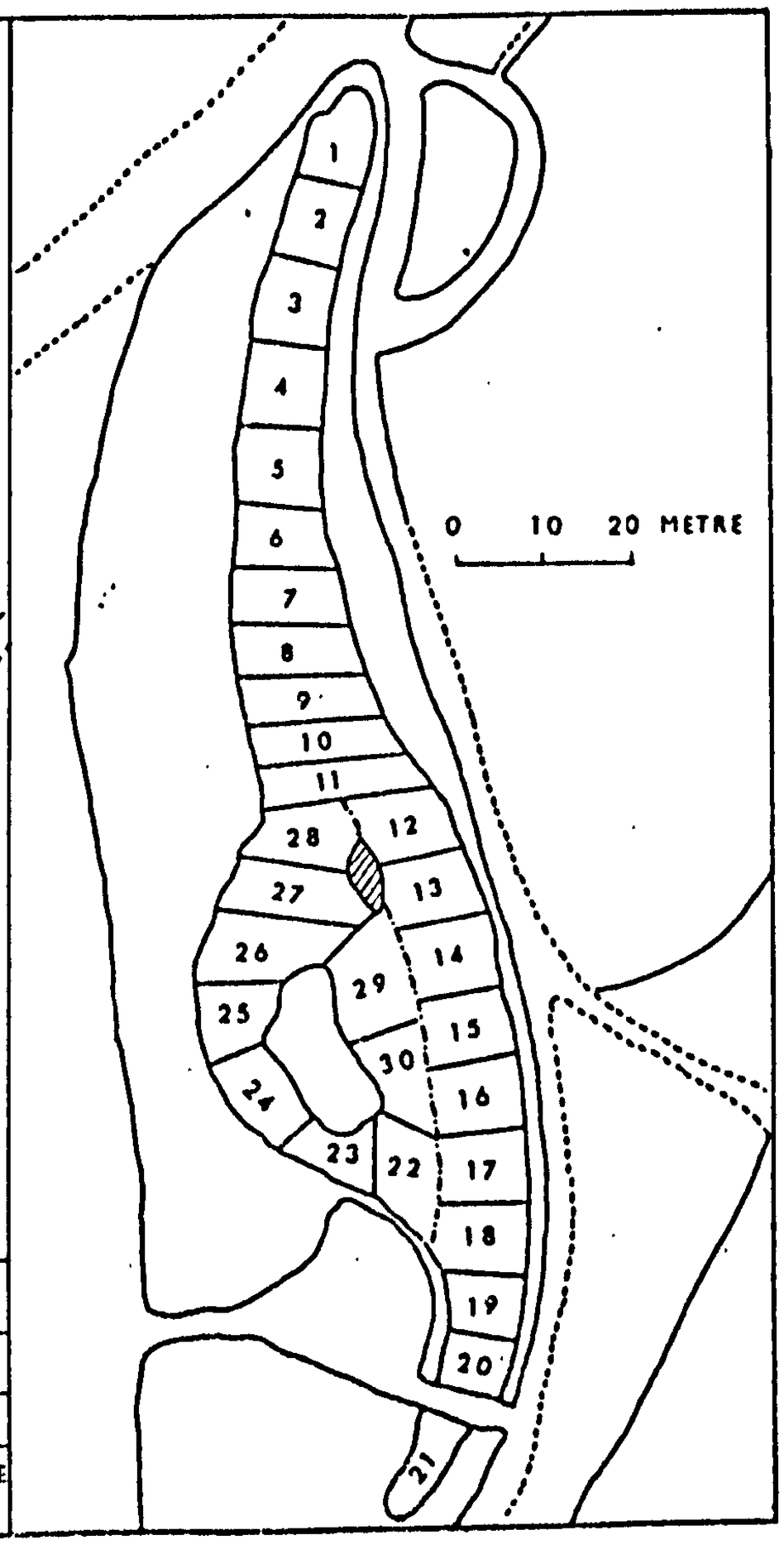


Fig. 2

The habitat divided into sampling units.

low, and the minimum depth recorded was 0.4 metres in July and August 1973.

The bottom of the pond consists of mud and decaying vegetation, with scattered stones, bricks and litter. The vegetation is mainly Elodea canadensis, which was thick in the spring and summer of 1973 and sparse in the spring and early summer of 1974. The pond fauna was identified from Macan (1959, 1969) and Young (personal communication, 1974). The molluscs were represented by Bithynia tentaculata, Lymnaea peregra, Lymnaea stagnalis, Planorbis corneus, Planorbis vortex, Sphaerium sp. and Anodonta cygnea. Of the arthropods, Asellus aquaticus, nymphs of Chloeon sp., the larvae of Chironomus sp. and Triaenodes sp. were encountered. The platyhelminths and annelids were represented by Dendrocoelum lacteum, Lumbriculus variegatus and Erpobdella octoculata. Of the vertebrates, Gasterosteus aculeatus was found.

The main seasonal change in the habitat was the growth of the Elodea canadensis in the spring and summer and its death in winter. In October and November the pond surface is covered with the shed plant leaves, which either were cleared or settled to the bottom and decayed. Another noticable change in the habitat followed the application of a chemical herbicide on 3rd September, 1973, and the clearance of the dead plants at the beginning of October 1973.

3.0 The Biology of Bithynia tentaculata

3.1 Introduction and literature review

Boycott (1936), in his major review of the ecology of the freshwater molluscs in the British Isles, stated that a good molluscan habitat is a large volume of moderately warm calcareous water, with a slow current and a fair but not excessive growth of plants. Fretter and Graham (1962) reported that inland hard waters with more plant growth tend to have a rich prosobranch fauna. Of the 36 freshwater gastropods species which Boycott (1936) reported in the British Isles, there are 10 prosobranchs.

Boycott (1936) noted that the freshwater operculate snails live in running water. He attributed this to the fact that, as gill breathers, they need water which is fairly well oxygenated and free from particles which might choke their gills. Boycott's (1936) attempts to colonise small ponds with Bithynia tentaculata were unsuccessful. McMillan (1946) found this species in a small closed pond in Cheshire, and noted that such occurrence is rare. Young (1974, personal communication) also found the snails in a small closed pond near Meols in Wirral, and he noted that it was less common than in a large pond which had a small stream leading in and out of it.

Boycott (1936), while stressing that ecological factors act together rather than individually, noted that a number of freshwater molluscs do not live in soft water with a calcium

concentration of less than 20 mg/litre. Macan (1950) considered a calcium concentration of about 20 mg/litre as a critical figure below which a number of species seldom or never occur. The operculate species which need a fair amount of lime, according to Macan (1969), are Bithynia tentaculata, B. leachii, Viviparus viviparus, V. fasciatus and Theodoxus fluviatilis. The prosobranchs which can live in soft water are Potamopyrgus jenkinsi, Valvata piscinalis and V. cristata.

Field studies on the growth, reproduction and life cycles of the freshwater gastropods are largely concerned with the pulmonates. Boycott (1936) stated that nearly all the fresh water pulmonates are annuals, with a natural duration of life of nine to fifteen months or less. Hunter (1961a) reported that a simple annual life cycle with a single breeding season is usual in the freshwater pulmonates. The annual cycle may vary in different populations of the same species in growth rate and reproduction, and Hunter (1961a) explained these inter-population variations by genetic and environmental factors.

The variations which occur in the simple annual cycle may involve slight breeding activity of the spring-born generation in late summer. Thus two generations are produced during the year, but without the replacement of one generation by the other; such as observed in Lymnaea peregra (Hunter, 1961a), Physa fontinalis (Duncan, 1959; and Hunter, 1961a) and Ancylus fluviatilis (Hunter, 1953; Goldiay, 1956). Another pattern of life cycle

involves two generations in each year, with a complete replacement of one generation by the other, as shown by Physa fontinalis (De Wit, 1955) and Lymnaea peregra (Hunter, 1961a). That three generations could occur in a year, with complete replacement of one generation by the other, was found by Walton and Jones (1926) for Lymnaea truncatula.

Hunter (1961a)noted that little information is available on the growth, reproduction and life cycles of the natural populations of the freshwater prosobranchs. Fretter and Graham (1962) reported that the freshwater prosobranchs exhibit remarkable differences in their reproductive behaviour, growth and life cycles. The sexes are separate in these snails, except in members of the genus Valvata. Potamopyrgus jenkinsi and Viviparus retain their eggs in the uterus to the hatching stages (i.e. they are viviporous) whereas the other operculates lay eggs (Fretter and Graham, 1962).

Boycott (1936) and Lilly (1953) reported that Bithynia tentaculata lives for two to three years. Pinel-Alloul and Magnin (1971) found that in some populations the life span of this snail was one year and the adults died after spawning, but that in other populations the adults died at an age of six months, after spawning. Lilly (1953), reporting on the growth of a natural population of the snail in Wickford, Essex, maintained that the rate of growth is fairly regular throughout

the year. She suggested that snails measuring 6 mm or less in January were derived from the previous year's spawn, while larger snails had survived for a second or a third year. Schäfer's (1953) report on the age distribution of the snail was based on a collection made in the summer of 1951, from which he suggested that snails measuring 3 - 4 mm in length were derived from the spring spawn and that larger individuals were one or two years old. Pinel-Alloul and Magnin (1971) found the growth rate of the snail to be fast in summer and spring and slow in winter. Frömming (1956) studied the growth of Bithynia tentaculata in the laboratory.

Hunter (1961a) reported that Valvata, Bithynia, Viviparus and Potamopyrgus can feed, and presumably grow, during the winter months. Cleland (1954) and Hunter (1961a) found that Valvata piscinalis grows slowly throughout the year, breeds when a year old, and dies off after spawning. They noted that death is not immediate as in the freshwater pulmonates. Other prosobranchs which have received considerable attention because of their medical importance are the amphibious snails Oncomelania (McMullen et al., 1951; Pesigan et al., 1958) and Pomatiopsis (Dundee, 1957; Van der Schalie and Dundee, 1955).

In the present investigation the life cycle of Bithynia tentaculata was followed for 22 months in the field. Measurement of 13,074 snails yielded information on the size composition of

the population at the different seasons of the year, and the growth rate. The collections also provided information on the changes in population density and distribution of the snails within the habitat. Moreover, the time, duration and intensity of oviposition was studied in the field, and observations were made on dispersal, behaviour and life span of the snail. The field work was supplemented by laboratory investigations on the growth and reproduction of the snail.

3.2 MATERIALS AND METHODS

To obtain information on the growth, reproduction and life cycle of Bithynia tentaculata various techniques were employed, both in the field and in the laboratory.

3.2.1. TECHNIQUES FOR FIELD STUDIES

The field study of the population of B. tentaculata in Sefton Park was carried out with a regular sampling programme for a period of 22 months, which began on 6th December 1972 and ended on 16th September 1974.

Different methods have been suggested for sampling aquatic snail populations, and these were reviewed by the World Health Organisation reports (1957, 1965). Hairston et al. (1958) discussed the advantages of the various methods and reported that there is no single method of sampling which is satisfactory for all habitats. They emphasised that the choice of method should be determined by the objective of the study, the nature of the habitat and the available facilities. Hairston (1961) pointed out that the first requirement is a technique that always obtains all, or very nearly all, the snails actually present in the area covered by the sample.

In the present investigation, a 15 x 15 cm grab (Hydrobiologie, Germany) which removed bottom material from an area of 0.0225 square metres of the habitat proved to be a suitable sampling device. The substratum of the habitat was muddy, except for occasional stones, broken bricks, glass bottles and plastic and metal cans littered around. Each sample consisted not only of emergent vegetation, but also of a quantity of bottom material. From a practical point of view, the grab was easy to transport and to operate. Furthermore, the removal of a specified area of the habitat by the grab makes it easier to estimate the population density.

Although the depth of the water in the pond varied from 0.4 to 1.4 metres from the banks to the centre, samples were taken at depths of 0.4 to 0.9 metres, thus covering 80% or more of the habitat depending on season. The deeper parts of the habitat were excluded from sampling because sampling was carried out using body waders about a metre long. Attempts to use a boat were unsuccessful.

Aquatic snail populations are usually aggregated, and hence the chance of finding or missing a single clump makes a great difference to the estimation of the mean density of the population. Hairston (1961) stated that the only method of gaining greater confidence in the estimate of the mean is to increase the number of samples. He added, "In most circum-

stances, no less than 30 samples in a habitat are required for the data to be repeatable with confidence, and 50 to 100 samples would be preferable." In the present study, 30 samples were taken on each occasion the habitat was visited. As the process of cleaning and sorting out the samples was long and laborious, it was not possible to take more.

Once the sampling device was chosen and the number of samples was determined, the next important step was to decide on the pattern of sampling. Hairston et al. (1958) stated that the sampling pattern will have a great effect upon the estimation of the population density, and recommended a planned programme so that the same procedure can be used over and over again. Hairston (1961) considered the most important factors are the randomisation of the samples and the proper representation of the different parts of the habitat in the sample. Southwood (1971) recommended the method of stratified random sampling, in which the area is divided into a number of equal sized divisions and strata, and one sample is randomly selected from each stratum.

In the present investigation, the habitat was first divided into thirty nearly equal portions (Fig. 2), with an area ranging from 69 to 84 square metres and a mean area of 77 square metres. Each portion was then further divided into 30 nearly equal areas, numbered from 0 to 29. To exclude as far as possible

any biased collection of samples, random number tables (Fisher and Yates, 1963) were used. A random selection of the first thirty numbers which fall between 0 and 29 was made, each of these numbers indicating the position where the sample should be taken in the appropriate division. Then the positions of the samples were recorded on a map which was taken to the field and followed as far as possible when taking the samples.

The samples were collected at regular intervals of two weeks from April to September, and monthly from October to March, except for the last two samples in August and September 1974 which were collected at monthly intervals. When the samples were collected, the grab was placed upon and pushed into the substratum, then all the materials which it enclosed were carefully transferred to a wide rectangular plastic dish (30 x 22.5 cms). The sample was first drained of excess water or mud through a 1mm mesh sieve (except for the sample collected on 6th December 1972), and then placed in a plastic bag for transport to the laboratory.

In the laboratory, each sample was first sieved through a 5.6 mm mesh sieve to separate the larger and the smaller components of the sample. The latter portion, which contained the smaller particles, was passed through a 1 mm mesh sieve and each of the two parts was placed in a white plastic dish

which was filled with enough water to submerge the material entirely. The snails were removed from the samples by careful visual examination. The dishes containing the sieved material were allowed to stand overnight, after the removal of the larger snails. The young snails crawled to the edge of the dish or floated in the surface film of the water and so could be easily removed. As a final precaution, the vegetation was then thoroughly checked.

After the snails were removed from the samples, each snail was measured to the nearest 0.1 mm, either with a micrometer (Moore and Wright Ltd., Sheffield, England) for large snails, or with a microscope fitted with a calibrated eyepiece micrometer for small snails. The erosion of the spire of the shell in a number of snails, where one or more whorls might be lost, offered a problem in measurement. Such snails were measured as though all the whorls were present. The youngest snails that were retained in the sample were 1.3 mm in shell length. As the newly-hatched Bithynia tentaculata measured 1 to 1.3 mm, the age distribution of the population may be considered to be properly represented. When the population was breeding, each piece of vegetation and debris in the sample was carefully examined for egg masses. The number of egg masses and egg capsules contained was determined.

The criticism of the use of exhaustive sampling methods is that repeated sampling tends to destroy the habitat (Hairston et al., 1958). This is true for small habitats where the area

covered by the sample represents an appreciable part of the total area. In the habitat under investigation, the total area covered by the sample was only 0.03% of the total area of the habitat, which was calculated as follows: Area of the grab 0.0225 square metres - hence the total area of sample is 0.0225 x 30 = 0.6750 square metres, and the percentage of area sampled to total area is: $\frac{0.6750 \times 100}{2310} = 0.0292\%$. Hence no serious damage to the habitat was expected. Macan (personal communication, 1972) pointed out that the damage done in the process of sampling is far greater than that due to the sampling itself.

In addition to the grab, a pond net (Freshwater Biological Association, Ambleside, England) was used for obtaining the last two samples and the snails required for field or laboratory experiments. Field experiments were performed to assess the dispersal rate of these snails and to gain some information on their survival in the field. Snails were marked with a quick drying paint and then released into the habitat. Field experiments were carried out to find the rate of growth of young snails in nature.

3.2.2 TECHNIQUES FOR LABORATORY STUDIES

Laboratory experiments were carried out to gain information on the reproduction and growth rate of Bithynia tentaculata. Snails collected from the field or bred in the laboratory were cultured in aquaria of 10 litres and two litres capacity, at various temperatures. The snails were fed on green lettuce, detritus and algae, the áquaria were aerated, and continuous uniform light was maintained. Various experiments were performed in the laboratory, and information was collected on the size at which the females begin to lay eggs and on the duration of the egg laying period. Observations were also made on the time required for the eggs to hatch, the size of the hatchlings, and the growth rate of young snails in the laboratory. Furthermore, a sample of field-collected snails was dissected to find the sex ratio of the natural population of B. tentaculata. The methods used in the analysis of the gut content of the snail and the growth of juveniles under different diets are described in the appropriate section.

3.3 THE LIFE CYCLE OF BITHYNIA TENTACULATA

3.3.1 Seasonal changes in the structure and growth rates of the natural population

The data collected from sampling of the natural habitat covered two breeding seasons of B. tentaculata and is summarised in Tables 1 and 2. These tables show the number and the range of shell length of the snails found in any one sample, together with its mean and standard deviation. Although the snails were measured to the nearest 0.1 mm, the lengths were grouped in classes of 0.5 mm. The percentage of individuals in each group was calculated and the results presented in histograms (Figs. 3 and 4). The sequence of events of the life cycle in the two years are described below according to the climatic seasonal changes. Accounts are given of the size composition of the population, the growth rates and the breeding activity.

3.3.1.1. The winter populations

The winter population of 1972-73 is represented by the collections made from 6th December 1972 to 20th March 1973 and that of 1973-4 comprised the collections made from 18th December 1973 to 21st March 1974. In general, both periods were characterised by low environmental temperatures (Table 3).

The winter population of 1972-73

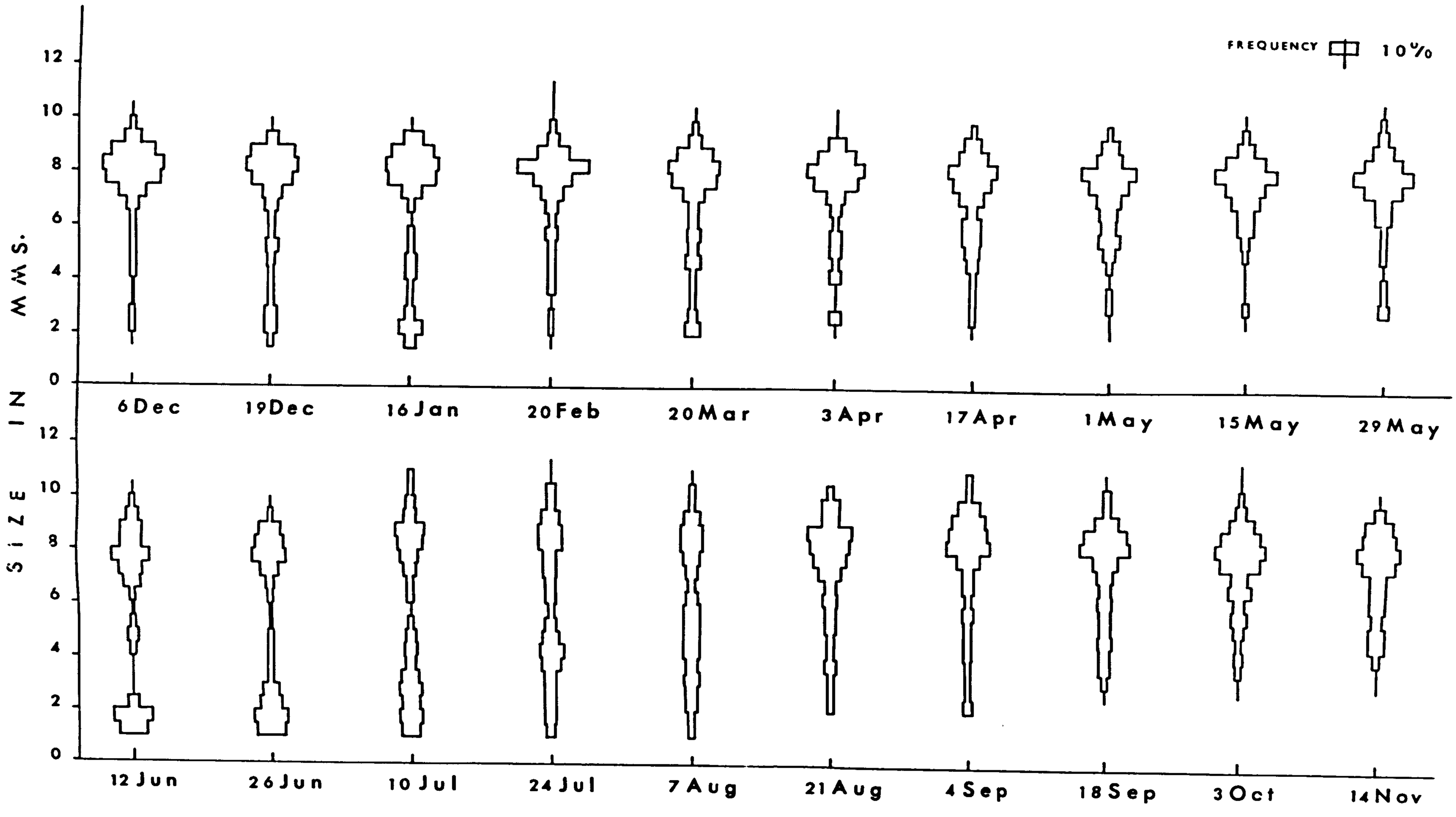
The samples collected from 6th December 1972 to 20th March 1973 were composed of snails measuring from 1.6 to 11.2 mm.

TABLE 1

A summary of the size data for the successive samples of Bithynia tentaculata collected from 6th December 1972 to 14th November 1973.

Date of Collection	Sample No.	No. of Snails Collected	Shell Length in mm.			Standard Deviation
			Minimum	Maximum	Mean	
1972						
6 December	1	383	1.8	10.8	7.6	1.7
19 December	2	583	1.6	10.8	6.8	2.2
1973						
16 January	3	505	1.9	10.9	6.7	2.4
20 February	4	403	1.9	11.2	7.4	1.9
20 March	5	602	2.1	10.7	6.9	2.1
3 April	6	494	2.1	11.9	7.2	1.9
17 April	7	532	2.3	10.9	7.1	1.7
1 May	8	417	2.3	11.6	7.3	1.7
15 May	9	386	2.6	12.4	7.7	1.5
29 May	10	333	2.8	11.6	7.7	1.8
12 June	11	328	1.3	11.0	6.0	3.1
26 June	12	483	1.3	11.5	5.5	3.1
10 July	13	480	1.3	11.9	5.5	3.0
24 July	14	450	1.3	12.9	5.8	2.7
7 August	15	478	1.5	12.1	6.1	2.6
21 August	16	314	2.1	11.3	7.4	2.0
4 September	17	483	2.1	11.7	7.6	2.1
18 Sept.	18	686	2.6	11.7	7.5	1.9
3 October	19	440	2.5	12.1	7.6	1.7
14 Nov.	20	343	3.5	11.8	7.5	1.6

Fig. 3 Histograms showing the percent size distribution of *Bithynia tentaculata* collected from 6th December 1972 to 14th November 1973.


FREQUENCY 10%
SIZE IN M M S.
0
2
4
6
8
10
12
6Dec
19Dec
16Jan
20Feb
20Mar
3Apr
17Apr
1May
15May
29May
12Jun
26Jun
10Jul
24Jul
7Aug
21Aug
4Sep
18Sep
3Oct
14Nov

in size (Table 1). Fig. 3 shows that snails measuring 1.6 to 3.0 mm. in size formed 5 to 17 percent (mean 9.8 percent) of the winter population. As individuals of the natural population in March 1973 laid eggs at a minimum size of 7 mm, then the mature snails formed 62.1 to 79.6 percent (mean 68 percent) of the winter population (Fig. 3).

Growth of the winter population, as measured by the mean size (Table 1), indicated that from 19th December 1972 (mean size 6.8 mm.) to 20th March 1973 (mean size 6.9 mm), the mean size of the population increased by 0.1 mm. The mean size of 7.6 mm. obtained on 6th December 1972 was probably due to under-representation of young snails, since a 1 mm. sieve was not used in the field for this collection. However, the high mean shell length of 7.4 mm. in the February collection was probably due to under-collection of young snails. It was noted that the pond was flooded at the time of this collection, and this might possibly be the reason. Fig. 5 shows the small change in the size composition of the population from 19th December 1972 (583 snails) to 20th March 1973 (602 snails), except for the young snails which had grown by 0.5 mm. during this period.

The winter population of 1973-74

The wintering population of 1973-74 was represented by the samples collected from 18th December 1973 to 21st March 1974. The population was composed of snails measuring from 3.5 to 11.9 mm

TABLE 2

A summary of the size data for the successive samples of Bithynia tentaculata collected from 18th December 1973 to 16th September 1974.

Date of collection	Sample No.	No.of snails collected	Shell length in mm.			Standard deviation
			Minimum	Maximum	Mean	
1973						
18 December	21	198	3.6	10.7	7.8	1.3
1974						
17 January	22	320	3.6	11.9	7.8	1.5
21 February	23	562	3.5	11.0	7.9	3.5
21 March	24	454	3.5	10.3	7.3	1.7
3 April	25	257	3.5	10.8	7.4	1.7
17 April	26	165	4.1	12.0	7.9	1.5
1 May	27	218	4.3	11.7	8.1	1.3
15 May	28	199	4.7	11.9	8.9	1.7
3 June	29	167	5.1	13.0	9.5	1.6
18 June	30	290	1.3	15.1	7.2	3.9 *
9 July	31	368	1.3	13.4	4.1	3.4 *
13 August	32	434	1.4	12.4	5.1	2.8 *
16 September	33	319	1.8	11.9	5.4	2.4 *

* Mean size of old and young populations from June to September 1974.

Date of collection	Old Population			Young Population		
	No.of snails	Mean size mm.	Std. deviation	No.of snails	Mean size mm.	Std. deviation
18 June	194	9.9	1.1	96	1.8	0.3
9 July	90	9.9	1.2	278	2.2	0.7
13 August	81	9.7	0.2	353	4.1	1.8
16 September	47	9.5	0.8	272	4.7	1.8

Fig. 4 Histograms showing the percent size distribution of Bithynia tentaculata collected from 18th December 1973 to 16th September 1974.

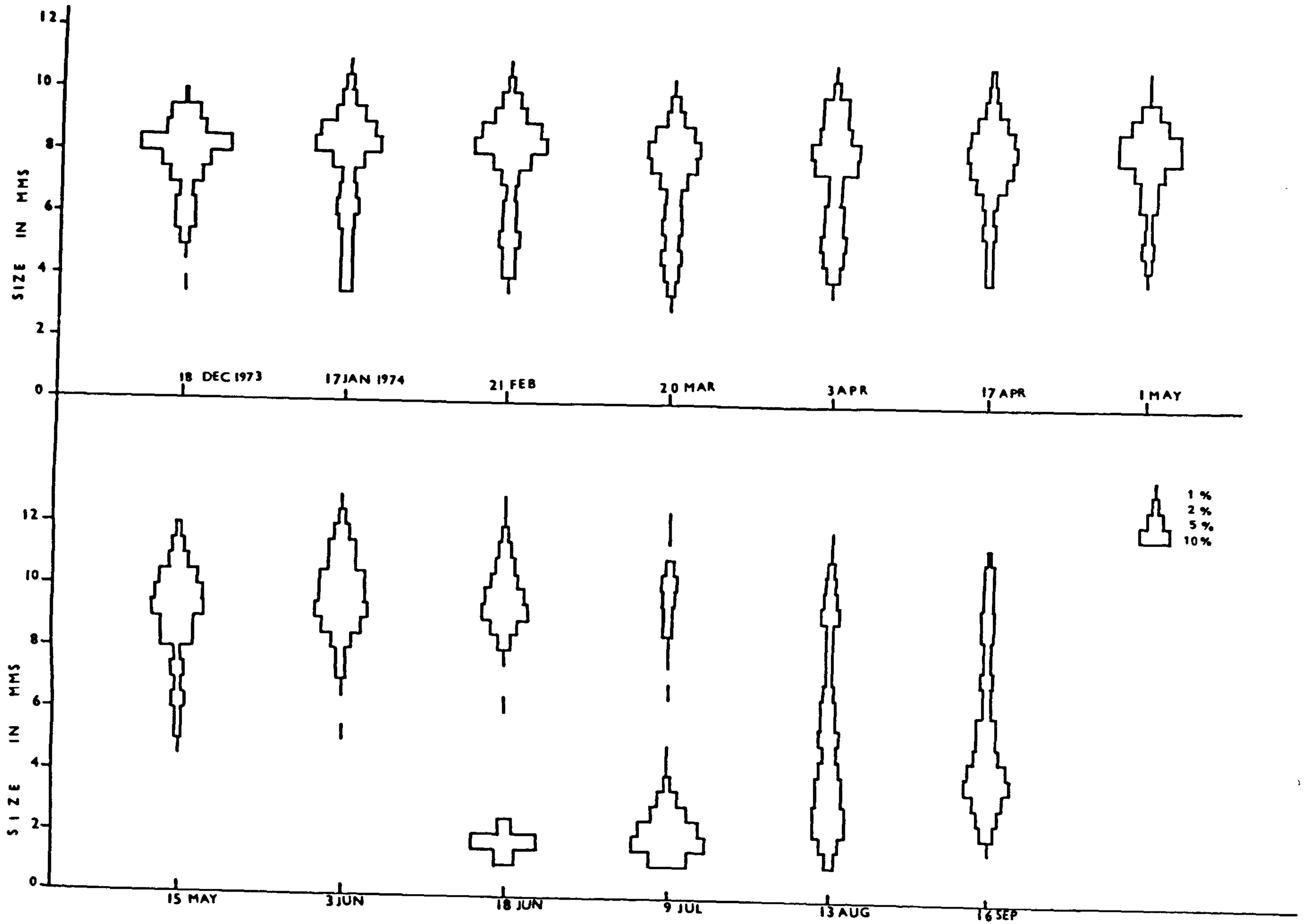

SIZE IN MMS
0
2
4
6
8
10
12
18 DEC 1973
17 JAN 1974
21 FEB
20 MAR
3 APR
17 APR
1 MAY
15 MAY
3 JUN
18 JUN
9 JUL
13 AUG
16 SEP
1 %
2 %
5 %
10 %

TABLE 3

Monthly temperature (°C) at Bidston Observatory (Cheshire).

Supplied by the courtesy of the meteorological office.

YEAR	1972			1973			1974		
MONTH	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.
January				5.0	10.8	-3.4	6.2	12.6	-1.4
February				5.2	10.1	-3.5	6.0	11.7	0.1
March				6.7	16.5	0.5	5.8	14.5	-0.6
April				6.8	13.4	-1.3	8.6	19.0	1.5
May				11.4	22.4	3.8	11.0	20.0	4.9
June				14.5	24.6	6.8	13.5	24.6	7.1
July				14.9	24.5	9.7	14.6	21.8	10.5
August				16.0	27.6	8.6	15.0	22.3	7.5
September	12.0	19.3	5.5	13.8	22.9	7.0	11.8	19.4	4.3
October	11.0	18.4	4.1	9.5	18.2	2.6			
November	7.1	16.5	1.2	6.8	15.6	-0.7			
December	5.8	13.8	-0.7	5.5	11.2	-0.8			

in size (Table 2). During the winter, 63 to 77% (mean 72%) of the snails measured more than 7 mm. in size (Fig. 4). However, in March 1974, the snails did not oviposit at a minimum size of 7 mm. as in March 1973, the minimum breeding size being 8 mm; thus 39.5% of the snails were mature in March 1974.

Table 2 shows that the mean size of the population from 18th December (7.8 mm) to 21st February (7.9mm) increased by 0.1 mm. However, the mean size of the population on 21st March (7.3 mm) and 3rd April (7.4 mm) was lower than on 21st February. Fig. 6 illustrated the small change in the size composition of the population between January (320 snails)and March (454 snails).

3.3.1.2. The Spring populations

The spring populations were obtained from the samples collected from 3rd April to 29th May 1973 and 3rd April to 3rd June 1974.

The Spring population of 1973

The proportion of young snails from 3rd April to 1st May varied from 32.6 to 38.2 percent (mean 35.4%), Fig. 3. By the 29th May, 24.6 percent of the population were young. Slow growth occurred in the spring, and the mean size of the population (Table 1) increased from 6.9 mm on 20th March to 7.7 mm on 29th May,

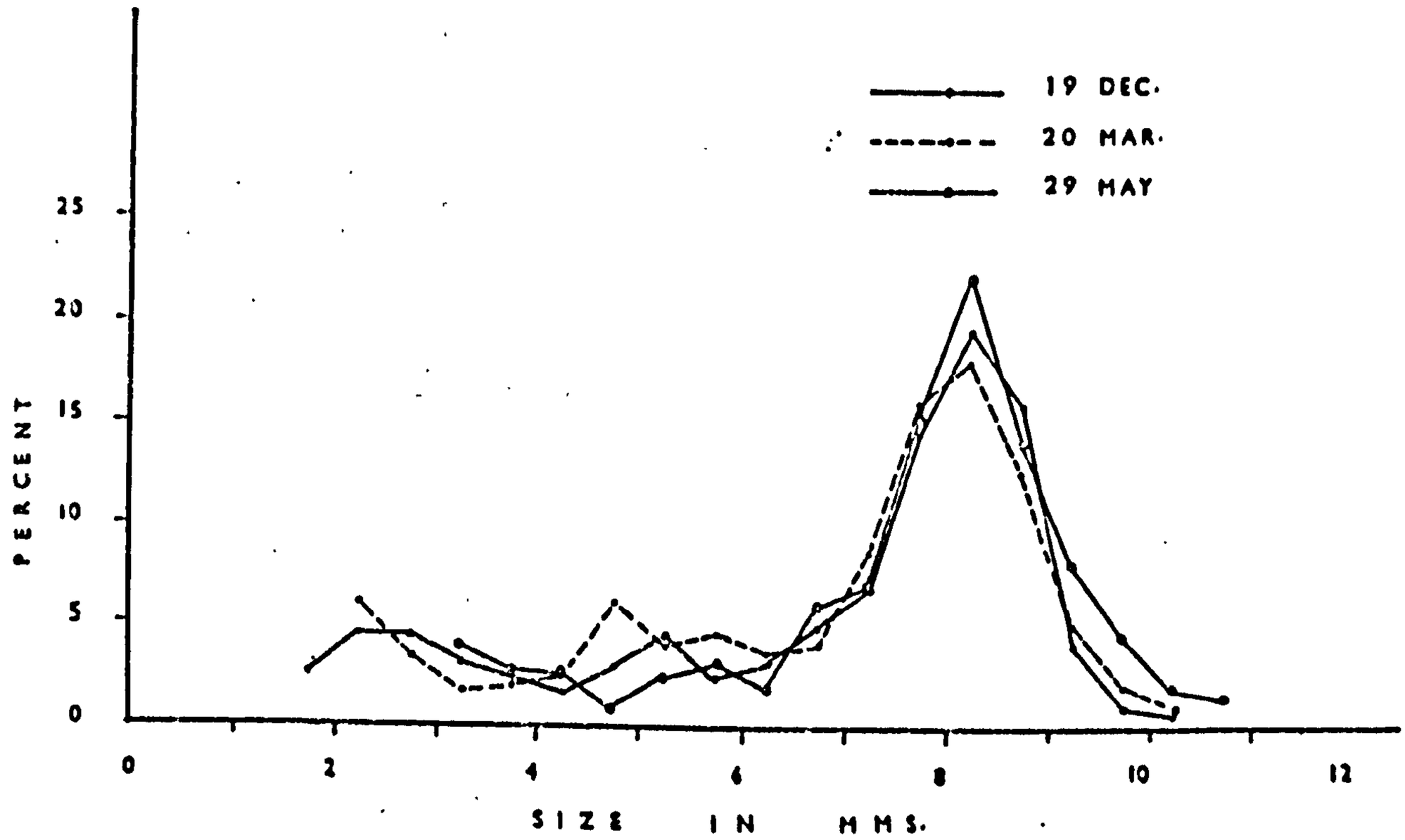


Fig. 5 A comparison of the percent size composition of the populations of Bithynia tentaculata on 19th December 1972, 20th March and 29th May 1973.

i.e, at a rate of growth of 0.08 mm per week. Fig. 5 illustrates the change in the size composition of the population.

In addition to the resumption of growth, the snails began to breed in the spring. The first egg masses were found on 17th April when the water temperature was 13°C. Many egg masses of Lymnaea peregra and Planorbis corneus were first observed on 20th March when the water temperature was 10°C. Bithynia tentaculata continued to lay eggs thereafter at an increasing rate, and although numerous eggs were present in the habitat from 17th April, no hatchlings were observed in the collection made on 29th May 1973.

The Spring population of 1974

Fig. 4 showed that 62 percent of the population on 3rd April measured more than 7 mm. in size. The population resumed active growth after the winter quiescent period, and by 3rd June only 1.8 percent of the population were less than 7 mm in size. As the minimum breeding size of the snails in March 1974 was 8 mm, 8% of the population were immature on 3rd June (Fig. 4).

The population increased in mean size from 21st March to 3rd June by 2.2 mm, i.e, at a rate of growth of 0.2 mm per week (Table 2). The active growth of the population in the spring is

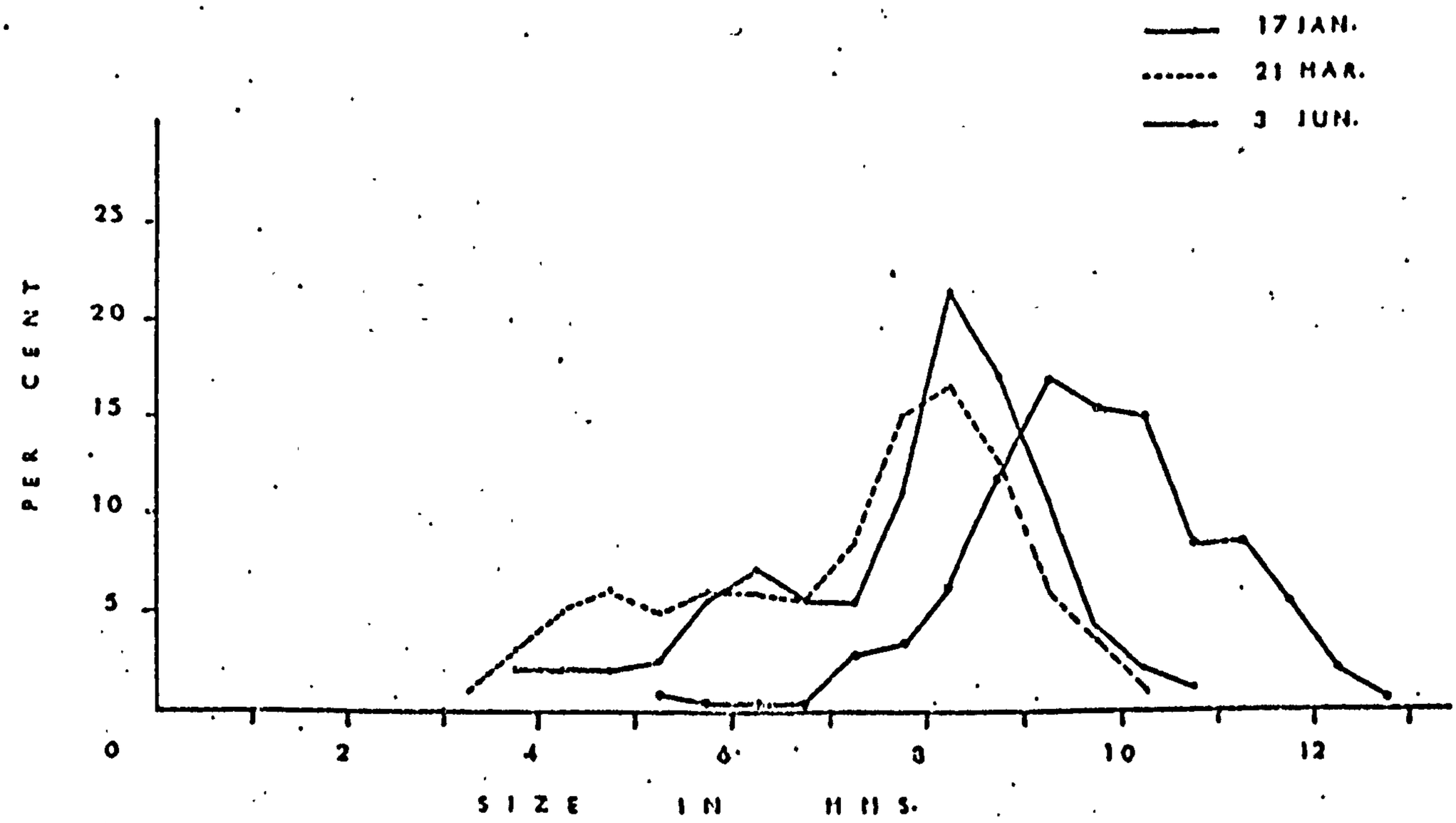


Fig. 6 A comparison of the percent size composition of the population of Bithynia tentaculata on 17th January, 21st March and 3rd June 1974.

illustrated by Fig. 6, which shows the change in the size composition of the population from 21st March to 3rd June. In addition to active growth in the spring there was a marked breeding activity. No eggs were found on 3rd April (water temperature 10°C) and an intensive search for egg masses on 9th April resulted in the finding of three egg masses (water temperature 12°C). On the 17th April, many egg masses were found, and the water temperature was 15°C. Egg masses of Lymnaea peregra and Planorbis corneus were found in the habitat from 21st March (water temperature 10°C).

3.3.1.3 The Summer populations

The summer populations collected in 1973 consisted of the samples taken from 12th June to 18th September, and those collected in 1974 included the samples taken from 18th June to 16th September.

The summer population of 1973

It was noted that the population began to oviposit actively from 17th April, and the breeding activity continued in June. In July and August, oviposition was less frequent and few egg masses were found. No more eggs were collected after 21st August 1973.

The new hatchlings were found on 12th June 1973, when numerous young snails, measuring from 1.3 to 2.5 mm, were present

in the habitat (Fig. 3). As these snails hatched between 29th May and 12th June, and since the first egg masses were found on 17th April, it presumably took the first egg masses 6 to 7 weeks to hatch. The eggs continued to hatch in large numbers, as indicated by the presence of numerous young snails and empty egg masses in the collections made in late June and through July. With the rise in temperature of the environment during this period (Table 3), the incubation period was presumably much shorter than six weeks. This was also suggested by laboratory experiments (at 20 ± 2°C eggs hatched in 19-22 days). The last hatchlings were collected on 7th August 1973, suggesting that the last egg masses laid in July had hatched.

In the laboratory the newly hatched snails measured 1.0 to 1.3 mm. Some of the newly hatched snails reached a size of 2.5 mm in two weeks, suggesting that some of these snails increased by 1.2 mm or more within two weeks of hatching (Fig. 3). The population continued to grow very quickly, and the fast-growing snails attained a size of 5 mm or more by 10th July, at an age of 6 weeks. By August, many of the young snails had reached a size of 5 to 6.5 mm, and probably the most rapid growers had merged with the adult population. By September more young snails had reached adult size, as indicated by the increase in the proportion of snails measuring more than 7 mm, and the population continued to grow in October. By November, 65% of the population measured more than 7 mm in size (Fig. 3).

As the young population was continuously emerging and quickly growing during the summer of 1973, the old population was also experiencing some changes. It could be deduced from Fig. 3 that nearly all the old population has reached the adult size of 7 mm by 10th July, except for a small number of the slow growers. The population had possibly reached its maximum size between 10th& 24th July, as the longest snail collected during the season measured 12.9 mm in length and was found in the collection made on 24th July. The old population was probably dying off gradually in late summer. At this time the new population has merged into the old, and the dying population has been replaced in size by the new generation. No complete replacement was observed, such as occurs in most of the freshwater pulmonates.

The summer population of 1974

The snails continued to lay eggs throughout the spring and early summer, and by 9th July few eggs were collected. No more eggs were found on 13th August. The new hatchlings appeared on 18th June 1974, and thus the incubation period was similar to that found in the summer of 1973. By 13th August few recently-hatched snails were collected, which suggested that most of the eggs have hatched by then; no new hatchlings were found on 16th September. The proportion of young snails in the total population continued to increase from 18th June due to the emergence of the

new hatchlings and by 16th September the population was predominantly composed of young snails (85%, Fig. 4).

The growth rate of the summer population of 1974 was followed, and separate records were kept of the old and new generations (Table 2). The distinction between the two generations was based on shell morphology. The most important characters were found to be the colour of the shell, which was light grey or horn-like in young snails and dark green or brown in old snails. Moreover the shell of young snails was glossy, thin, fragile, transparent and with a pointed spire. The shell of the old snails was dull, thick, strong, opaque and commonly eroded. As the new generation appeared after 3rd June, and the fast growers have reached a size of 2.5 mm on 18th June, the growth rate was calculated as 0.6 mm per week. By 9th July the fast growing snails had reached a size of 5 mm, a growth rate of 0.7 mm per week for the first five weeks after hatching. Fig. 7 shows the growth and percentage size composition of the new population on 9th July, 13th August, and 16th September. This indicates that by 13th August a small part (i.e, 8%) of the population has reached a shell size of more than 7 mm, and the fastest growing snails measured 10.5 mm (at an age of 9-10 weeks). However, the greater part of the population measured from 2 to 5 mm with a mean size of 4.1 mm. The new generation continued to grow actively, and 13% of the snails measured 7 mm or more by 16th September. By this date the fastest growing snail was found to measure 11.5 mm, and the bulk of the population measured 3 to 6 mm with a mean size of 4.7 mm. (Table 2).

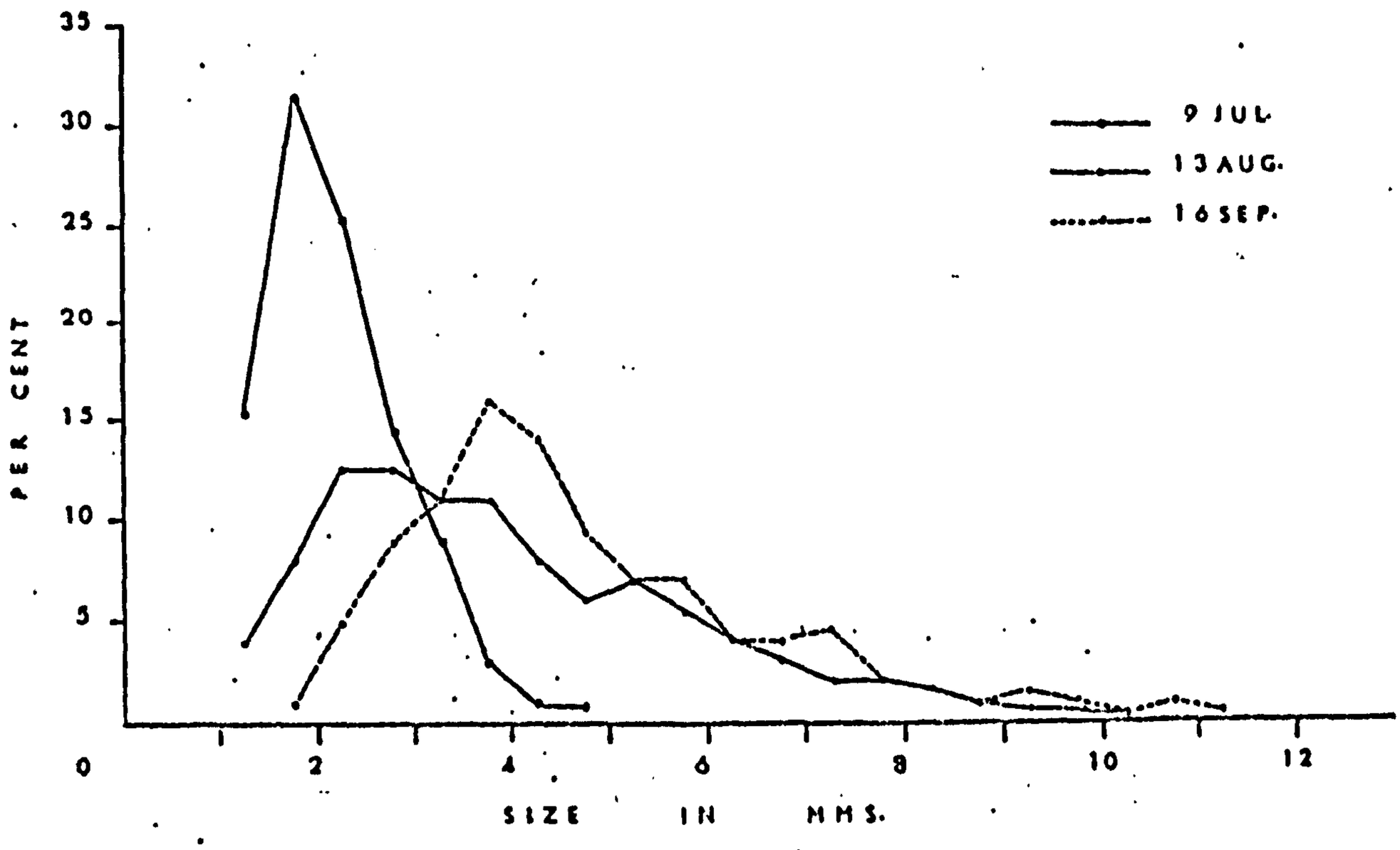


Fig. 7 Growth and percent size composition of the juveniles of Bithynia tentaculata in the summer of 1974.

The old population in the summer of 1974 continued to grow, and the maximum mean population size of 9.9 mm. was reached on 18th June (Table 2). The longest snail collected during the breeding season of 1974 was found in the collection made on 18th June, and it measured 15.1 mm. (Table 2). No further growth occurred in July, and by 13th August and 16th September there was a gradual drop in the mean size of the population to 9.7 and 9.5 mm. respectively. It was noted that the larger snails (measuring more than 12.5 mm.) had disappeared.

With the enormous increase of the young population however, the relative proportion of old snails progressively decreased. In the collection made on 16th September, the old population formed 15% of the total population.

3.3.2. Growth of Bithynia tentaculata in the laboratory

In the laboratory information was collected on the time required for hatching of the eggs, the size of hatchlings, and the growth rate of juvenile snails. Such information is complementary to the data collected from the field, and could help in the understanding of the life cycle of this species.

3.3.2.1. Hatching of eggs and size of hatchlings

Temperature is the most important factor for hatching the eggs of aquatic snails, and within the limits of tolerance of the species the higher the temperature the shorter is the period required for hatching. The eggs of Bithynia tentaculata were found to hatch in the following manner:-

Temperature	Time required for hatching
26 ± 1°C	10 - 14 days
20 ± 2°C	19 - 22 days
15 ± 2°C	28 - 32 days

The hatchlings are minatures of the adults, transparent and delicate. Measurement of 65 newly-hatched snails gave the following results:-

Size in mm.	No. of snails	Percentage
1.0	3	4.6
1.1	10	15.4
1.2	33	50.8
1.3	19	29.2

The mean size of the newly hatched snail was calculated as 1.2 mm.

3.3.2.2. Growth

The growth of one hundred recently-hatched juveniles measuring from 1.2 to 2.0 mm, with a mean size of 1.5 mm, was followed in the laboratory from 11th June 1973 to 3rd July 1974. The results obtained from the measurement of these snails at two-weekly intervals for the first 16 weeks, and then monthly till the end of the experiment, are shown in Fig. 8, together with changes in the room temperature.

In the first four weeks, the mean size of the snails increased from 1.5 mm. to 3.1 mm, i.e, at a rate of growth of 0.4 mm. per week. However, the fast growers had increased from 2 mm. to 4.9 mm. (i.e, growth rate 0.7 mm. per week). The snails continued to increase in size by a mean rate of 0.45 mm. per week from the fifth to the twelfth week, when the population rached a mean size of 6.7 mm., with a range in size of 4.2 to 8.8 mm. (Fig. 8). From the thirteenth to the sixteenth week, the growth rate was 0.35 mm, and the mean size of the population was 8.1 mm on the sixteenth week. The growth

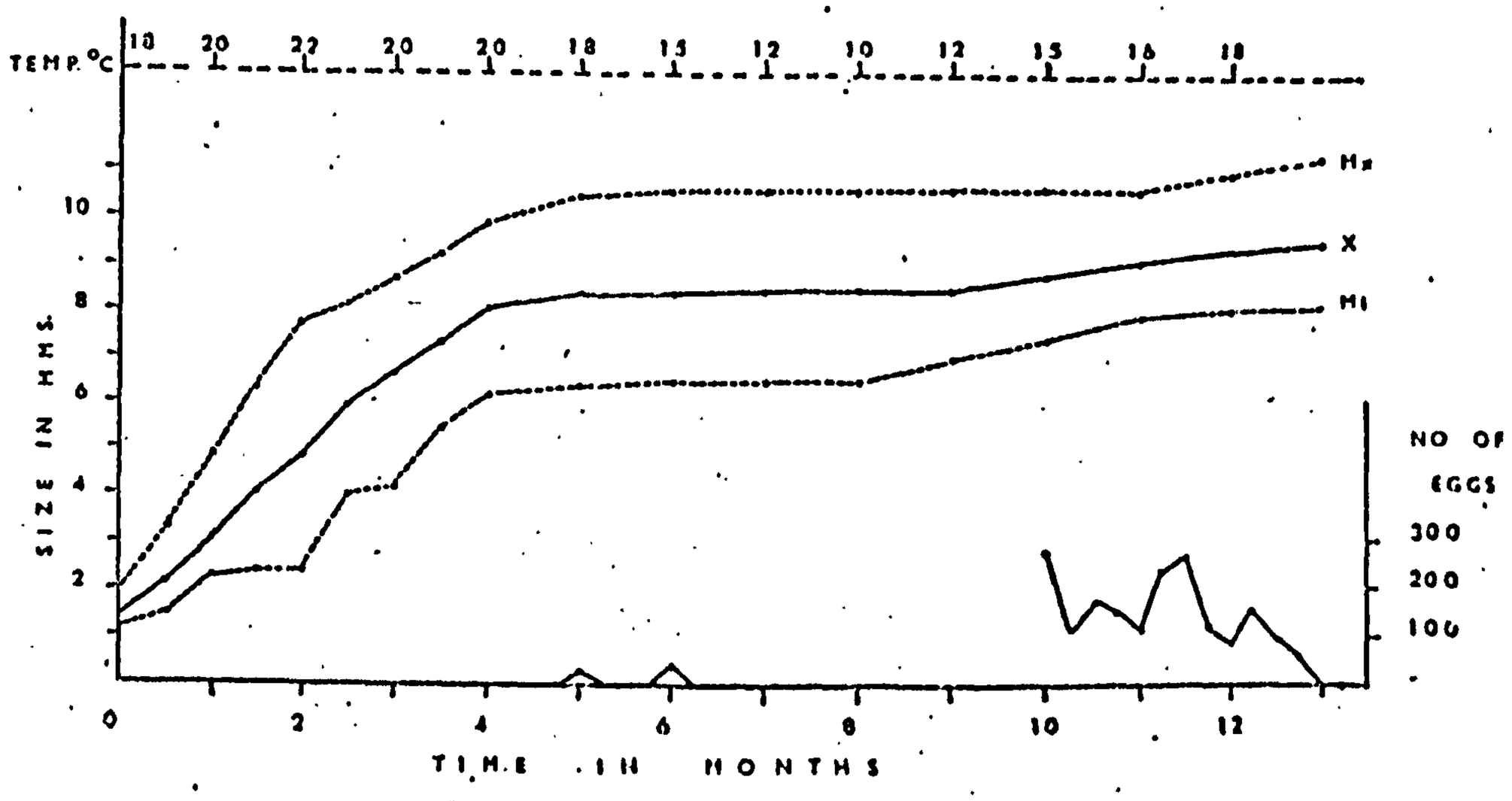


Fig. 8 The growth of Bithynia tentaculata in the laboratory in 13 months (4 weeks a month). The mean (x), maximum (Mx), and minimum (Mi) size, together with the temperature and number of eggs produced.

rate thereafter decreased, and in the next four weeks (17th to 20th week) the snails increased by 0.08 mm per week. In the following four months (i.e, from the 5th to the 9th month), the snails showed very little growth, and the mean size of the population increased by 0.1 mm. in the four months. In the last four months the mean size of the population increased by 0.23 mm per month.

Fig. 8 shows that the first few eggs were laid by the snails on 30th October and 30th November 1973, at an age of five to six months (temperature 15°C or more). No egg masses were laid in the following four months (temperature 10-12°C), but the snails laid many eggs from the tenth month onwards (Fig. 8).

3.3.3. Factors influencing the life cycle

That ecological factors act together in a complex interplay in freshwater habitats was pointed out by Boycott (1936) and Hubendick (1958). The importance of temperature as one of the main factors influencing the life cycle of aquatic snails is generally accepted. Boycott (1936) stated that in general the British freshwater molluscs are active, grow and breed only in the summer. The present findings on the activity, growth and reproduction in Bithynia tentaculata are in agreement with this statement. The growth rate of Bithynia tentaculata was rapid in the warm spring and summer months, and practically ceases in the winter months when the air temperatures were low (Table 2).

The direct influence of temperature on the initiation of oviposition is well established (Hunter, 1961a). Field and laboratory observations showed that B. tentaculata did not oviposit when the temperature is 10 - 12°C. It was observed that the pulmonate snails Lymnaea peregra and Planorbis corneus in the same habitat oviposited at a temperature of 10 - 12°C in mid-March. Duncan (1959) stated that temperatures between 7°C and 11°C were critical in the initiation of oviposition in Physa fontinalis. De Witt (1955) reported that Physa gyrina in the United States did not copulate or oviposit until the temperature reached 10 - 12°C. Hunter (1961a) reported that egg masses of Lymnaea peregra are produced shortly after the water temperature rises above 9°C in the spring.

Factors other than temperature also affect the growth rate of B. tentaculata, as illustrated by the growth rates of the spring populations of 1973 and 1974. The spring population of 1973 increased in mean size by a weekly rate of growth of 0.08 mm. from 20th March to 29th May (i.e, the mean size of the population was 6.9 and 7.7 mm. on 20th March and 29th May 1973 respectively, Table 1). The spring population of 1974 increased in mean size at a rate of 0.2 mm. per week from 21st March to 3rd June 1974 (i.e, mean size of the population was 7.3 and 9.5 mm on 21st March and 3rd June 1974 respectively, Table 2).

The greater growth rate of the spring population of 1974 is related to the conditions prevailing in the natural habitat during both seasons. The most obvious change in the habitat was the clearance of the thick growth of Elodea canndensis in October 1973, following herbicide spraying on 3rd September 1973. The clearance had resulted in the removal or death of some of the population of B. tentaculata as well as other aquatic organisms. This was suggested by the change in the relative density of the population between the spring before clearance and the spring that followed clearance (see population density, Table 9). The average mean density of the population (i.e, 14.4 snails per grab) in the spring of 1973 (3rd April to 29th May), was double the density of the population (i.e, 6.7 snails per grab) in the spring of 1974 (3rd April to 3rd June).

The effect of density on the growth rate of natural populations was demonstrated by Eisenberg (1966, 1970), who found the mean size of the population of Lymnaea cloden to show an inverse relationship to density. The author explained this relationship on the basis of food limitation. Thus the increase in the growth rate of the reduced (less dense) population of B. tentaculata in the spring of 1974 could possibly be explained by the supposition that the denser population in the previous spring suffered from food shortage which prevented the snails from attaining their maximum growth. With more food available per snail in the spring of 1974, the population not only showed a greater growth rate, but also more reproductive capacity than the population in the previous spring (Section 3.4). Hubendick (1958) reported that Kendall (personal communication) found that the absolute number of Fasciola hepatica cercariae produced by Lymnaea truncatula increased after treatment of the habitat with copper sulphate, although there was a considerable reduction in population density of the snail. Hubendick (1958) interpreted this phenomena by suggesting that the undernourished state of the population before treatment adversely affected the development of cercariae, but after the population density was reduced by treatment more food was available per snail, thus favouring the development of cercariae.

In addition to variation in the growth rate from one year to the next, the population of B. tentaculata in April 1973 laid eggs at a minimum size of 7 mm, whereas the population in April

1974 bred at a minimum size of 8 mm. Hunter (1953) found the breeding size of the adult Ancylus fluviatilis to vary from one locality to another and from one year to the next in the same locality. The author explained this variation on the basis of food availability during the growth of the young snails. Duncan (1959) reported a similar variation in the breeding size of Physa fontinalis.

3.3.4. Discussion of the life cycle

An examination of the literature showed that our present knowledge of the life cycle of B. tentaculata is derived from reports by Boycott (1936), Hubendick (1948), Lilly (1953), Schäfer (1953) and Pinel-Alloul and Magnin (1971) on natural population and by Frömming (1956) on laboratory populations. The winter population of 1972-1973 was found in the present study to be primarily composed of mature snails (more than 7 mm in size), with an appreciable number of young (Fig. 3). The adult part of the population was derived from eggs laid in the previous spring, together with a few snails surviving for a second winter. The young portion of the population consisted of the slow growers of the spring generation together with the late hatch in the summer. Although the winter population of 1973-1974 was mainly composed of snails more than 7 mm. in size, 40% of the population were mature as the minimum breeding size was above 8 mm. (Fig. 4.)

Pinel-Alloul and Magnin (1971) reported similar observations for two of the four populations of B. tentaculata studied in Montreal, Canada. They found that the population spent the winter primarily as adults, together with some young snails which hatched in late summer. The other two populations were reported by the above workers to hibernate as young snails (measuring less than 6 mm. in size). In one of these populations (Station 3) the spring generation of 1968 hatched in July, and some of the snails matured in autumn, laid eggs and died off. The eggs hatched before

the winter, and the population hibernated as young snails. The variation in the life cycle between Stations 7 and 3, which were two kilometres apart, was explained by Pinel-Alloul and Magnin on the basis of differences in the growth rates of the two populations attributed to temperature and nutrition.

Lilly (1953) found a population of B. tentaculata in Essex to be primarily composed of young snails (less than 7 mm.) in January. However, her interpretation, that these young snails were derived from the spring generation and that the larger snails present were part of the population surviving for a second winter, seems to be inaccurate. Although some of the old snails survive for a second winter, a large number of the snails derived from the spring spawn reach a size of more than 7 mm. in January, and consequently Lilly's (1953) suggestion that the large snails were adults which have survived for a second winter was partly inaccurate. Schäfer's (1953) report on the life cycle of B. tentaculata was derived from a ten day investigation in the summer of 1951, when he collected 525 snails, measured them, and plotted the number of animals against size (Abb.1, page 69). He suggested that snails 3 to 4 mm. in size were derived from eggs laid in the spring, and that larger snails were one to two years old.

The present investigation indicates that throughout the winter, growth of B. tentaculata practically ceases and the minimum, maximum and mean size of the population remain fairly constant (Table 2). However, the fall in mean size of the population in

March and early April, probably suggest the death of the large old snails (Table 2). Pinel-Alloul and Magnin (1971) reported that during the winter the adult B. tentaculata cease to grow, but the young snails grow slowly. Lilly (1953) maintained that the growth rate of B. tentaculata is fairly regular throughout the year. Hubendick, 1948 (according to Fretter and Graham, 1962) found a slow growth rate of the same species throughout the year. Cleland (1954) and Hunter (1961a) reported that Valvata piscinalis grows slowly throughout the year. In the freshwater pulmonates, Hunter (1961a) found that during the winter, the snails either grew at a reduced rate or stopped growing, with some exceptions. DeWitt (1955) reported that during the winter Physa gyrina stops growing and similarly Duncan (1959) found Physa fontinalis stops growing during the winter.

In the spring, the wintering population of B. tentaculata resume active growth, at a rate of 0.2 mm. per week (Table 2). Similarly the new generation which appeared in June 1974, grew by a mean rate of 0.3 mm. per week from 18th June to 13th August 1974 (Table 2). However, the advanced growers showed a greater rate of growth (Fig. 7). Pinel-Alloul and Magnin (1971) reported that the wintering population in Station 7 grew at a rate of 0.2 mm. per week in the spring, while the growth rate of the young snails in the summer was 0.42 mm. per week. In another population (Station 3) the above investigators found growth rates of 0.5 mm and 0.8 mm per week in Summer and spring respectively. Thus the present findings are in agreement with Pinel-Alloul and Magnin's observations in that growth of B. tentaculata is rapid in the spring and summer.

Furthermore, the growth rate of the young snails in the summer is greater than that of the overwintering population in the spring.

The presence of young snails (1.6 - 3.0 mm.) from December 1972 to March 1973 (Fig. 3), suggested that during the breeding season of 1972, the snails laid eggs in late summer which hatched into the juveniles of the late summer generation mentioned above. Similarly, Lilly (1953) found 6.7% of the population of B.tentaculata examined to measure 3 mm. in January. Pinel-Alloul and Magnin (1971) noted the late summer hatchlings of B. tentaculata. They suggested that some of the spring-born generation grow rapidly, attain maturity by late August and early September and possibly lay eggs in October to November which hatch into the late summer generation.

In the habitat examined, the breeding activity of B.tentaculata was mainly limited to the period between April and June, with few eggs laid in July (Tables 4 and 5). However, the population in 1973 laid few eggs in August and with the change in the habitat (herbicide spraying) egg laying was inhibited in September. No eggs were found in October and November 1973 and probably the low environmental temperature inhibited oviposition. The breeding season of 1974 was shorter and no eggs were found in August or September although many large snails were available. Probably the presence of a small portion of immature snails on 12th June 1973 (Fig. 3) suggest that these snails matured in July and laid eggs in August 1973. On the other hand, practically all the snails

were mature on 18th June 1974 (Fig. 4), thus suggesting that all the eggs were laid in July. Laboratory observations showed that some of the juveniles hatching in early June laid eggs in late October (Fig. 8). Hence it seems likely that in nature and under better trophic conditions, a small part of the new generation matures in late September to October, and, depending on the temperature prevailing, could possibly lay eggs which hatch into the late summer generation. Thus the presence of the young snails in the winter of 1972-1973 could possibly be due to the delayed maturity of some of last year's spawn and/or the rapid maturity of some of the same year's brood.

The life cycle and growth rate of B. tentaculata in nature, as revealed by the present work, seems to agree in general with that reported by Pinel-Alloul and Magnin (1971) for the same species in Montreal. However, as the latter work was carried out for one year and the present investigation covered nearly two years, more information was revealed by the longer term of the study. Pinel-Alloul and Magnin's suggestion that the life span of this species was one year in the four populations studied, and that (in Station 3) some of the snails died after spawning at an age of six months, does not agree with the present findings.

In the present investigation, it was found that 52.3% of the wintering population of 1972-1973 died off between June and early August 1973, with a few (10%) of the snails disappearing in

April-May. In 1974, although 53.8% of the wintering population of 1973-1974 disappeared between April and July 1974, the greatest mortality rate seemed to occur in April-May when 44.4% of the wintering population died off (see Section 3.5.1).

The surviving members of the old population die off gradually, and probably the disappearance of the larger snails in August and September 1974, together with the fall in mean size of the old population (Table 2), suggest the selective death of the larger old snails. However, many of the old snails were found on 25th November 1974 and probably the fall in mean size of the population in March and early April 1974 (Table 2) suggests the death of the old large snails. Evidence derived from marking snails showed the recovery of three marked snails 21 and 23 months old. On dissection of these marked snails, the digestive gland was found to be destroyed, the snails being heavily infested with Chaetogaster and probably in a dying condition. In the laboratory, six out of sixty snails survived for 24 months. As it is generally accepted that survival in nature is much shorter than in the laboratory, probably few B. tentaculata survive for more than 24 months. Thus the life span of B. tentaculata seem to extend from 14 to 23 months, and probably few survive to breed for a second season.

The suggestion of Pinel-Alloul and Magnin (1971) that the life span of this species is one year, and in some populations is six months, was derived from the decrease in the relative proportion

of adult snails in the total population. In their collection of 18th July 1968 they reported 93 percent of the population as measuring 1 to 4 mm, and 7 percent measuring 6.5 to 10 mm; and from this they concluded that most of the adult snails disappear in July. Hunter (1953) pointed out that the decrease in the relative proportion of adult snails in early summer was due to the emergence of numerous young rather than to the quick dying off of the adults. The histograms in Fig. 4 for the collections made on 3rd June and 9th July 1974, showed the proportion of adult snails as 100 percent and 24 percent respectively. Although the relative proportion of the old snails has greatly decreased, observations on the habitat showed many surviving adults throughout the summer.

Boycott (1936) and Lilly (1953) reported that B. tentaculata lives for 2 to 3 years. Comfort (1957) grouped some of the fresh-water snails into annuals and biennials which breed once in life. Hunter (1961a) reported that the life span of Lymnaea stagnalis is intermediate between annuals and true biennials, with a life span in nature of about 23 months, and probably few snails surviving to breed for a second season. The life span of B. tentaculata is probably similar to that of Lymnaea stagnalis.

Frömming's (1956) report on the growth of B. tentaculata in the laboratory showed that the snails attained a maximum length of 6 mm, and a minimum of 4 mm, in four months. In the present investigation the snails reached a maximum size of 9.9 mm. and a minimum size of 6.2 mm, with a mean size of 8.1 mm, in four months (Fig. 8). Results obtained from the growth of young snails fed on detritus only (see Fig. 11) showed that the snails reached a maximum size of 6.0 mm. and a minimum of 4.2 mm., with a mean size of 5 mm, in 14 weeks. Boycott (1936) stated that the growth rate of laboratory population is mainly influenced by food supply.

Other factors such as density and the frequency of changing the culture medium also affect the growth rate of the snails in the laboratory (Wright, 1960). Probably the culture conditions of Frömming's (1956) snails were not favourable, as suggested by the lower growth rates.

In summary, the life cycle of B. tentaculata in the habitat studied is as follows. The wintering population begins to breed in mid-April, and although the breeding period may extend to August, intense breeding is limited to the first two months (April to June). The adult population dies off gradually at an age of about fourteen to twenty-three months and probably few survive to breed for a second season. The young hatch in June-July, growth is fast in summer, practically stops in winter, and the wintering population resume growth in the spring at a rate slower than that of the young summer populations.

3.4 Reproduction in Bithynia tentaculata

3.4.1. Natural populations

3.4.1.1. Sex ratio

The sexes are separate in B. tentaculata and the main morphological character for sex identification is the presence of a penis in the males. The penis lies on the dorsal side of the neck, slightly to the right and near the margin of the mantle; it is curved, fleshy and bifid at its tip. Examination of the overwintering snails in March 1974 showed that the penis was very small and sometimes hard to detect in snails measuring less than 5 mm. in shell length, but it was thought that a reliable picture of the sex ratio of the population could be obtained from the examination of snails measuring more than 5 mm. in shell length. A total of 357 snails, ranging in size from 5 to 11 mm, was examined. The results, shown in Fig. 9, revealed 184 (51.54%) females and 173 (48.45%) males. Furthermore, no sexual dimorphism was observed.

3.4.1.2. Size at maturity (oviposition)

Twenty snails representing each of four size groups were collected from the field on 10th April 1973. The size groups represented were as follows: 6.0 to 6.9 mm. (mean 6.5); 7.1 to 7.9 mm. (mean 7.5); 8.0 to 8.8 mm. (mean 8.4); 9.0 to 9.9 mm. (mean 9.2). The snails were reared in the laboratory at a

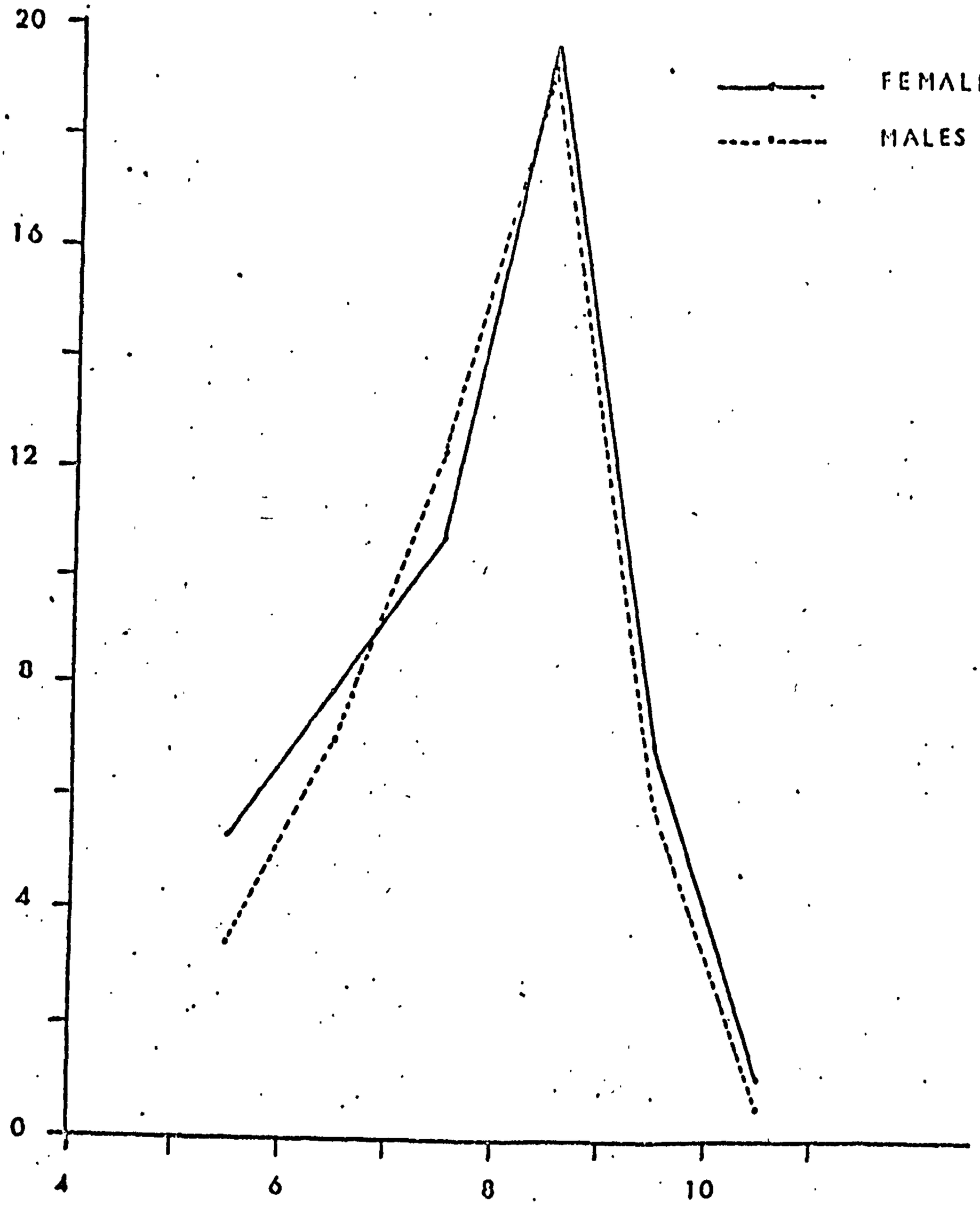


Fig. 9 The relationship between size and sex in a population of Bithynia tentaculata (357 snails) examined in March 1974.

temperature of 16 to 18°C. The snails measuring more than 7.1 mm. laid eggs within a week and continued to lay eggs for the four weeks of the experiment. This suggests that the minimum size at which B. tentaculata laid eggs in 1973 was 7.1 mm.

On 19th April 1974, twenty-five snails measuring 7.0 to 7.8 mm. (mean 7.4) were collected from the field and reared in the laboratory at 16 to 18°C. The first egg masses laid by the snails were found on the fifth week, when the snails had reached a size range of 8.4 to 10.2 mm. (mean 9 mm.). However, snails measuring 8 to 9 mm. maintained under the same conditions laid eggs within one week. This suggests that the minimum breeding size in April 1974 was 8 to 9 mm.

3.4.1.3 Egg laying

Egg masses of B. tentaculata were deposited in nature on the water plant Elodea canadensis, fallen plant leaves, floating tree branches and plastic cans and bags. No egg masses were found on stones or broken bricks. The egg masses were usually laid in two rows, but occasionally three rows were found. The eggs were laid in such a manner that those in one row alternate and interlock with those of the other row. The number of eggs in the two rows may be equal, or one row may be an egg shorter than the other.

It was pointed out in the discussion on the life cycle that the first egg masses were found in the habitat on the 17th of April 1973. A total of 4101 eggs were collected during the breeding season of 1973, and 83 % of these eggs were found in the collections made from 17th April to 29th May (Table 4); the peak was on the 15th May (38.5%). In June 11.9% of the eggs were collected, whereas 2.6% were found in July and August. No egg masses were found in September. Egg masses collected from 17th April to 15th May showed a mean number of 25.5 to 29.2 eggs per egg mass, and the maximum number of eggs per mass in these collections varied from 42 to 92 (Table 4). In the subsequent collections the egg masses contained fewer eggs per mass, except for the collection made on 26th June.

TABLE 4

Eggs of Bithynia tentaculata collected from the field during the breeding season of 1973

Date of Collection	No. of Egg Masses	Range of number of eggs per egg mass	Mean No. of eggs per mass	Total number of eggs	% of Total eggs (4101)
17.4.73	17	8 to 92	28.4	482	11.8
1.5.73	31	17 to 42	29.2	906	22.1
15.5.73	62	5 to 80	25.5	1579	38.5
29.5.73	20	7 to 41	21.8	435	10.6
12.6.73	13	3 to 17	8.1	105	2.6
26.6.73	15	4 to 70	25.3	380	9.3
10.7.73	3	16 to 28		62	1.5
24.7.73	4	7 to 18		46	1.1
1.8.73	1	48		48	1.2
21.8.73	5	8 to 22		58	1.4

The breeding activity of the snails in 1974 showed the same pattern as observed in 1973 (Table 5). The greatest proportion of eggs collected from the field (76.2%) were found between 17th April and 3rd June, with a peak on 3rd June (30.6%). On the 18th June and 9th July 18.7 and 5% respectively of the eggs were collected. No more eggs were found in August and September. Egg masses collected from 17th April to 15th May contained a mean number of 31.2 to 35.5 eggs per mass, with a maximum of 64 to 93 eggs per egg mass (Table 5). In June the mean number of eggs per mass was 26 and 25 and in July the mean number of eggs per mass was 15.7 (Table 5).

TABLE 5

Eggs of Bithynia tentaculata collected from the field during the breeding season of 1974

Date of Collection	No. of Egg masses	Range of number of eggs per egg mass	Mean No. of eggs per mass	Total number of eggs	% of Total number (8421)
17.4.74	34	12 to 93	35.50	1207	14.3
1.5.74	38	6 to 64	31.23	1187	14.1
15.5.74	41	1 to 74	35.36	1450	17.2
3.6.74	99	3 to 90	26.01	2575	30.6
18.6.74	63	9 to 60	25.01	1578	18.7
9.7.74	27	8 to 38	15.70	424	5
13.8.74	-	-	-	-	-

3.4.2. Laboratory populations

3.4.2.1. Maturity (oviposition)

The growth of B. tentaculata was followed from hatching on 11th June 1973 until 3rd July 1974.(Fig. 8). By 30th October 1973, at an age of 20 weeks from hatching, 36 snails were surviving and the first egg mass containing 24 eggs was laid. In the following month two more egg masses were laid, containing 16 and 12 eggs (Fig. 8). From December to 22nd March 1974, the room temperature dropped to 12 - 10°C, and no more eggs were laid. Attempts to induce oviposition in January, at a temperature of 26°C for a period of two weeks, were unsuccessful and four of the snails died during the experiment. In the week ending on 29th March 1974 the room temperature rose to 15°C, and the snails laid many eggs at an age of ten months from hatching.

3.4.2.2. Egg laying

B. tentaculata reared in the laboratory laid all the egg masses either on the glass walls of the aquaria or on the leaves of the water plant Ludwigia. It was noted that no eggs were deposited on the free plant leaves present in the aquaria. The eggs were laid in two rows except for a few (2%) which were deposited in three rows.

a) Laboratory-bred snails

The 31 snails which survived till 22nd March 1974, laid 15 egg masses, containing 277 eggs, in the week ending 29th March (temperature 15°C). The number of eggs laid by these snails for 14 weeks from 22nd March to 28th June 1974 is shown in Fig. 8. A total of 1892 eggs, contained in 139 egg masses, was laid, and 71.6% of these eggs were deposited during the first seven weeks of the experiment. No eggs were laid in the last two weeks. The maximum number of eggs per egg mass was 42, and the mean number of eggs per mass in the successive weeks varied from 9.6 to 22.6. The number of eggs laid per female per week was calculated, on the basis of the sex ratio (51.54% females), as 7 to 17 eggs for the first seven weeks, with a mean of 12. In the next five weeks, 10 to 3.5 eggs per female per week were laid (mean 7).

b) Snails collected from the field

Sixty snails were collected from the field on 23rd March 1973. The snails were reared in two-litre capacity beakers, each containing twenty snails, and kept at a constant temperature of 26°C. The snails were cultured in a fresh medium at weekly intervals. All the egg masses deposited were removed at weekly intervals, and the eggs were counted, throughout the experimental period of 36 weeks from 23rd March to 30th November 1973.

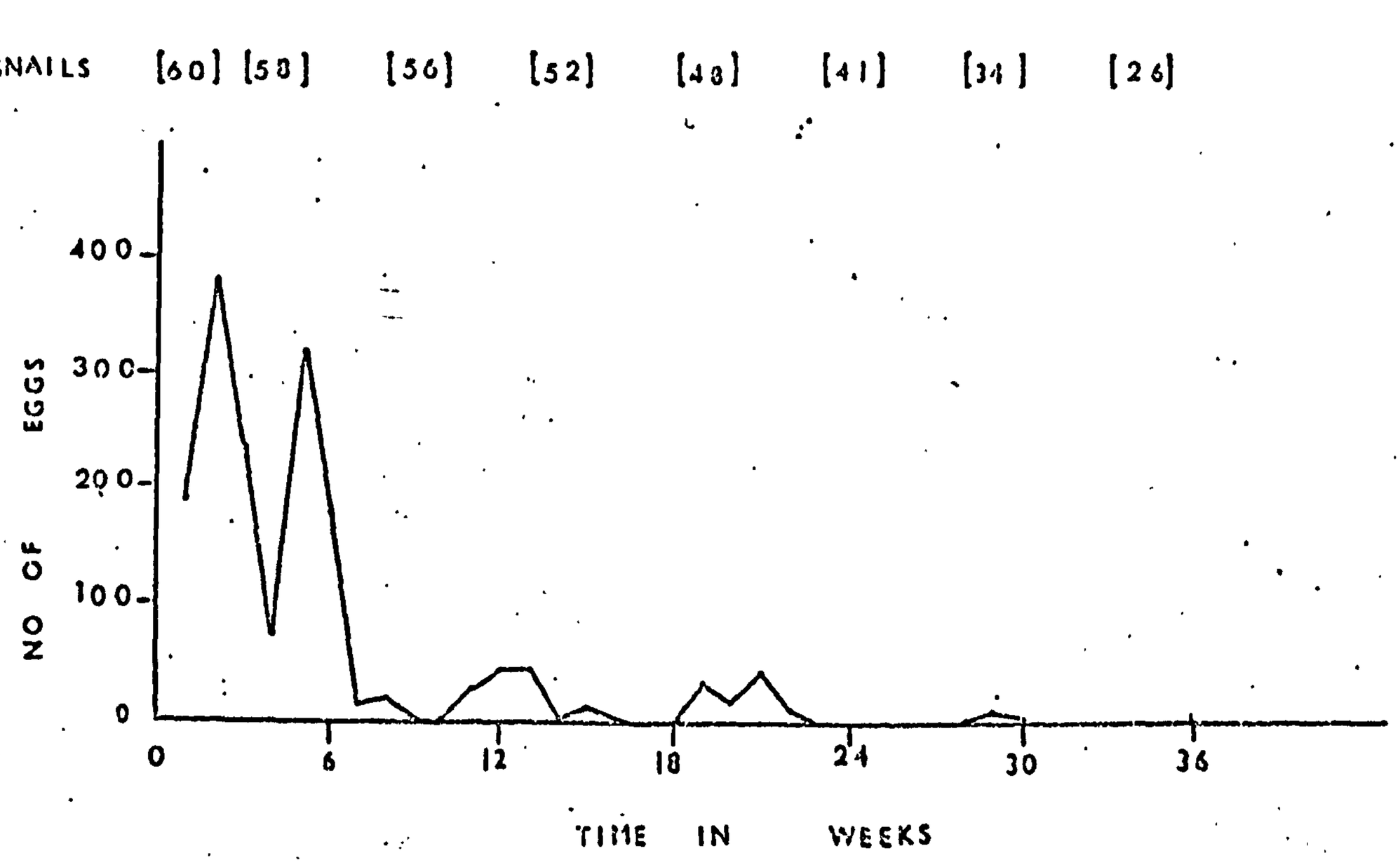


Fig. 10 Egg production by a laboratory population of Bithynia tentaculata over a period of 36 weeks at a constant temperature of 26°C.

Fig. 10, shows the number of eggs deposited at weekly intervals. Most of the eggs were laid in the first six weeks of the experimental period. A total of 1687 eggs contained in 123 egg masses was deposited by the snails during the 36 weeks of the experiment. Table 6, shows the number of eggs produced by the snails at six-weekly intervals. During the first six weeks, the snails laid 81.38% (1373 eggs) of the total egg production, and the number of eggs laid per female snail per week was calculated as 7.52. The snails showed a general decline in egg production at successive six-weekly intervals, so that from the 25th to the 30th week, 1.30% (22 eggs) of the total egg production was laid, and the female B. tentaculata was calculated to lay 0.18 eggs per week. No eggs were laid in the last six weeks of the experiment. Furthermore, it was found that the snails also showed a general decline in the number of eggs per egg mass laid (Table 6). The mean number of eggs per egg mass at the six-weekly intervals was found to be 15.96, 9.58, 7.62, 8.92, 5.50 and zero, respectively.

c) Breeding activity of snails two years old

Of the sixty snails which were collected from the field as mature snails on 23rd March 1973, and whose reproductive activity was observed for 36 weeks in the laboratory as indicated above, 26 survived until 30th November 1973. These snails were reared in a 10-litre capacity tank containing 6 litres of water and kept at room temperature which varied from 10 to 18°C. By 22nd March

TABLE 6

Egg production of Bithynia tentaculata in the laboratory over a period of 36 weeks (from 23rd March to 30th November 1973).

Time at 6 weeks interval	Mean No. of Snails	No. of females (51.54%)	No. of egg masses laid	Mean No. of eggs per egg mass	No. of eggs laid	% of Total egg production	No. of eggs laid per female/ week
First 6 weeks	59	30.4	86	15.96	1373	81.38	7.52
Second 6 weeks	57	29.37	12	9.58	115	6.81	0.65
Third 6 weeks	54	27.83	8	7.62	61	6.61	0.36
Fourth 6 weeks	44.50	22.93	13	8.92	116	6.87	0.84
Fifth 6 weeks	37.50	19.32	4	5.50	22	1.30	0.18
Last 6 weeks	26	13.40	0	0	0	0	0

1974, 18 snails were surviving, and observations were made on their breeding behaviour. When the temperature rose to 15°C in the week ending 29th March 1974, no egg masses were laid. The first egg masses (5 egg masses containing 50 eggs) laid by these snails were found on 17th May 1974, at room temperature of 18°C, when 12 snails were still alive. No more egg masses were laid until 28th June 1974, when six snails were alive.

The two year old snails were maintained under the same conditions as the 31 laboratory bred snails, which laid 1892 eggs during the same period. Moreover, two groups of 60 and 49 snails collected from the field in March 1974 and treated in the same way laid 1062 and 888 eggs respectively during the same period.

3.4.3 The relative number of eggs per egg mass laid by field and laboratory populations

A total of 173 and 303 egg masses were collected from the field during the breeding seasons of 1973 and 1974 respectively. In the laboratory the snails produced a total of 109 egg masses from 23rd March to 4th August 1973, and 296 egg masses from 22nd March to 28th June 1974. The latter eggs were produced by both laboratory raised snails (139) and mature snails collected from the field and reared in the laboratory (157).

Table 7 shows the number and percentage frequency of egg masses at an interval of 10 eggs per mass, for both field and laboratory populations in 1973 and 1974. It can be seen that 77.1 and 89.2 percent of the egg masses laid in the laboratory contained less than 20 eggs per mass, while 21.1 and 8.8% of the egg masses contained 21 to 40 eggs per mass, in 1973 and 1974 respectively. Egg masses containing 41 to 66 eggs per mass represent 1.8 and 2% of the total egg masses produced in 1973 and 1974 respectively.

The field collections showed that egg masses containing less than 20 eggs per egg mass were 46.3 and 40.3% in 1973 and 1974 respectively (Table 7). 44 and 40.3% of the egg masses contained 21 to 40 eggs, while 8.8 and 17.8% of the egg masses contained 41 to 70 eggs per mass in 1973 and 1974 respectively.

TABLE 7

The relative number of eggs per egg mass laid by field and laboratory populations of Bithynia tentaculata

No. of eggs per mass (less than)	Laboratory populations				Field populations			
	1973		1974		1973		1974	
	No. of egg masses	%	No. of egg masses	%	No. of egg masses	%	No. of egg masses	%
10	46	42.2	145	49	39	22.6	34	11.2
20	38	34.9	119	40.2	41	23.7	88	29.1
30	17	15.6	16	5.4	42	24.3	72	23.8
40	6	5.5	10	3.4	34	19.7	50	16.5
50	2	1.8	5	1.7	10	5.8	31	10.2
60					2	1.2	12	3.9
70			1	0.3	3	1.8	11	3.7
80					1	0.6	3	1
90					0		1	0.3
100					1	0.6	1	0.3

Egg masses containing 71 to 93 eggs per mass were 1.2 and 1.6% in 1973 and 1974 respectively.

3.4.4. Discussion

The structure of the reproductive system of Bithynia tentaculata was studied by Krull (1935), Lilly (1953) and Pinel-Alloul (1969). Lilly noted that there is no difference between the size of male and female B. tentaculata. A similar result was obtained in the present investigation (Fig. 9), but it was found that the females are slightly more abundant than the males (51.54: 48.46). McMullen et al. (1951) reported that the sex ratio of Oncomelania nosophora showed the females to be slightly predominant (53.07 to 46.93). Dundee (1957) found the female Pomatiopsis lapidaria to outnumber the males by 3 to 1.

Bithynia tentaculata collected from the field in April 1973 bred at a minimum size of 7 mm. The population in April 1974 as well as laboratory-raised snails, bred at a minimum size of 8 to 9 mm. Lilly (1953) reported that male B. tentaculata are mature at a size of 6 to 7 mm. whereas the females are immature at this size. Pinel-Alloul (1969) found the males of this snail mature at 7 mm. and the females at 8 to 9 mm. De Witt (1955) stated that size is the most reliable index of sexual maturity in Physa gyrina, and he found that neither field-collected nor laboratory-raised snails oviposited before reaching 7 mm. in size. Ritchie et al. (1963) found in Australorbis glabratus that size is a better criterion of maturity than age. Similarly, Van der Schalie and Davies (1965) reported the same for Oncomelania formosana. The present findings also indicate that shell lengths in B. tentaculata is a better index of maturity than age.

The failure of laboratory-raised snails to lay eggs at an age of four months after hatching, when 55% of the snails measured from 8 to 9.9 mm, suggest that the snails were sexually immature (Fig. 8). A few of these snails matured (oviposited) at an age of 5 to 6 months, as indicated by the presence of three egg masses. The intense breeding activity at the age of ten months indicates that most of the snails were mature then. These observations show that in addition to size, age is also important in the maturity of B. tentaculata. Probably there is a minimum period for the maturation of the reproductive system which is related to the growth of the snails. De Witt (1952) reared Oncomelania in the laboratory from hatching to maturity (oviposition). He found that O. nosophora and O. formosana matured in six to seven months, whereas O. quadrasi matured in about three months. Pasigan et al. (1958) reported that Oncomelania quadrasi matures in the field in about three months. DeWitt (1952) reported that the North American amphibious prosobranch Pomatiopsis lapidaria probably matures in about 12 months.

Fig. 8 shows that the mean size of the laboratory-raised B. tentaculata increased by 0.1 mm. from the fifth to the ninth month. From the 9th to the 13th month, the mean size of these snails increased by 0.9 mm. Probably the reason for the cessation of growth from the 5th to the 9th month is that, after reaching a certain size, morphological growth of the snails practically stops and physiological development of the reproductive system takes place. When sexual maturity is reached, as indicated by

active egg laying on the tenth month, the snails resume morphological growth again.

Hunter (1961a)reported that in the freshwater pulmonates studied and Valvata piscinalis, egg-laying takes place in the summer months. He added that the time for the onset of breeding is dependent on environmental (temperature) and other factors endogenous to the snail. In B. tentaculata, 62 and 40% of the population in March 1973 and 1974 respectively were mature (Figs. 3 and 4). These snails when brought into the laboratory laid many eggs within a week. In nature, no eggs were found in March or 3rd April in 1973 and 1974, when the temperature was 10 - 12°C. Thus the onset of egg-laying in the mature part of the population in early spring is controlled by temperature. However, a considerable proportion of the population in March 1973 and 1974 respectively was immature. These snails undergo a period of growth in the spring before they begin to breed.

Bithynia tentaculata laid most of the eggs collected during the breeding season within the first two months (17th April to early June, Tables 4 and 5). It is during this period that the egg masses laid contained the greatest number of eggs per egg mass. In the rest of the summer (mid-June to August) few eggs were laid, and in general, the egg masses contained fewer eggs per mass. Lilly (1953) found the eggs of B. tentaculata from April to July, and Pinel-Alloul and Magnin (1971) reported that most of the eggs of this snail are laid in June-July and that few eggs are

laid in the late summer. Cleland (1954) reported that egg masses of Valvata piscinalis are found from late April to July, with a peak in June, and Hunter (1961a) found the egg masses of the same species from late May to early September and in particular in early July. Bondesen (1950) stated that the breeding season does not last long in the freshwater pulmonates, and that by the end of July and the beginning of August, egg masses become less frequent and at the same time the number of eggs per egg mass decreases.

The oviposition behaviour of B. tentaculata in the laboratory was similar to that observed in the field. Snails reared for 36 weeks laid 81% of the total egg production in the first six weeks (Table 6). In the subsequent long period few eggs were laid, and at long-time intervals (Fig. 10). Similarly, laboratory-raised snails laid 72% of the total egg production in the first half of the fourteen weeks of the experimental period, whereas in the latter half there was a gradual decline in egg production and no eggs were laid in the last two weeks (Fig. 8). Laboratory observations on snails collected from the field and reared for two to three weeks throughout the year showed that the snails breed actively from March to May. Snails collected during the natural breeding period (April-May) readily laid eggs in less than four hours from the time they were brought into the laboratory. However, snails collected from July to January either laid few eggs or failed to lay eggs in the laboratory.

Thus field and laboratory observations indicate that intense breeding activity of B. tentaculata is limited to the natural breeding period (April-May), and could be induced in the laboratory only a few weeks earlier. However, the snails may lay few eggs for a considerable part of the year. Duncan (1959) reported that Physa fontinalis, when brought into the laboratory, oviposited during the natural breeding period only, whereas Physa actua under suitable conditions breeds throughout the year. Davies and Iwamato (1969) reported that Oncomelania hupensis nosophora collected from the field and maintained in the laboratory for one year produced the greatest number of young in July and relatively few from September to the spring months. The authors noted that laboratory-raised snails of the same species did not show seasonal periodicity.

Lilly (1953) suggested that B. tentaculata reaches maturity in the spring following hatching, breeds that year, and lives on to breed for at least a second season. The present findings suggest that few of the snails survive to breed for a second season. Laboratory observations showed that snails in their second breeding season laid 5.6 eggs per female in fourteen weeks. Laboratory-raised snails laid 118 eggs per female in fourteen weeks in their first breeding season, whereas snails collected from the field laid 34 eggs per female in fourteen weeks. Thus it is suggested that individuals of B. tentaculata which might survive for a second breeding season contribute little to the

reproductive activity of the population. Laboratory-raised snails produced more than double the eggs laid by the snails collected from the field. Probably freedom from larval trematode infection may be one of the reasons for high productivity. Pesigan et al. (1958) showed that Oncomelania quadrasi infected with Schistosoma japonicum laid few eggs compared with uninfected snails. They also demonstrated the poor hatchability of the eggs laid by the infected snails. The authors explained this by the lower vitality of infected snails rather than the direct destruction of the gonads.

Field and laboratory observations on the relative number of eggs per mass (Table 7) indicate that field populations are more productive, in the sense of laying larger egg masses, than laboratory-reared snails. 77 and 89% of the egg masses laid by laboratory populations contained less than twenty eggs per mass, whereas egg masses containing 21 to 40 eggs were frequently laid. Larger egg masses were rare, and the largest egg mass contained 66 eggs. In the field, egg masses containing less than 20 as well as 21 to 40 eggs per mass were equally abundant (i.e, each about 40%). Egg masses with more than 40 eggs were frequent and the largest egg mass contained 93 eggs. The greater productivity of the field populations is probably due to the favourable trophic conditions in the natural habitat. Lilly (1953) found the egg masses of B. tentaculata to contain 4 to 24 eggs, Nekrassow (1929) reported 98 eggs per mass, and Frömming (1956) found 10 to 70 eggs.

The productivity of field populations may vary from one year to another, as suggested by the total number of eggs collected in the spring of 1973 and 1974. From 17th April to 29th May 1973, 3402 eggs were collected (Table 4), with a mean of 850.5 eggs per collection. In 1974 and from 17th April to 3rd June, 6419 eggs were collected (Table 5), with a mean of 1604.8 eggs per collection. Thus although the spring population of 1974 was about half the density of the spring population of 1973, it produced nearly double the number of eggs. Eisenberg (1970) showed the inverse relationship between density and total number of eggs in the natural population of Lymnaea elodes. He found this inverse relationship between density and total number of eggs disappeared when more food was available. Thus the greater fecundity as well as the greater growth rate of the spring population of 1974 is probably due to better trophic conditions.

Bondesen (1950) stated that in the freshwater pulmonates a level substratum is preferred for oviposition, this being important for the attachment of the smooth capsule. A similar behaviour is observed in B. tentaculata when all the eggs in the field were laid on Elodea canadensis, free plant leaves and plastic bags and cans. No eggs were laid on broken bricks or stones. In the laboratory, eggs were laid on the glass walls of the aquaria and water plants. Furthermore, a clean substratum is necessary for the deposition of the egg masses, and in the laboratory the snails did not oviposit on free plant leaves covered with detritus or soil.

Reproduction in Bithynia tentaculata can be summarised thus. The females are slightly more abundant than the males and no sexual dimorphism was observed. The snails bred at a minimum size which varied from one year to another, depending on the growth rate. Laboratory observations showed that in addition to size there is probably a minimum age for maturity (oviposition) which is 5 to 6 months. Temperature is important for the initiation of oviposition, and the snails did not oviposit at 10-12°C. Egg-laying commences in mid-April, and by early June most of the eggs of the season are laid; few eggs were laid in the summer. Active breeding could only be induced in the laboratory a few weeks earlier than the natural breeding period.

3.5. OBSERVATIONS ON DENSITY, DISTRIBUTION, BEHAVIOUR AND DISPERSAL OF *BITHYNIA TENTACULATA*

3.5.1. Population density

From 6th December 1972 to 1st May 1974, twenty-seven samples were collected, each sample consisting of thirty grab collections. Table 8 shows the frequency of the number of snails per grab collection. The largest number of snails collected per grab was 136, found in January 1973. However, 70% or more of the grab collections contained 1 to 20 snails per grab. Collections containing 21 to 40 snails per grab were frequent (19.5 and 8.2%), whereas collections with 41 to 60 snails were rare (Table 8). Grab collections with more than sixty snails per grab were not only very rare, but were encountered from October to March. Thirteen of the eighteen grab collections with more than 60 snails were found from only October to March. All the remaining five large grab collections were found on 18th September, after herbicide spraying of the habitat on 3rd September. These observations suggest that while the snails are aggregated in their distribution, this aggregation becomes more marked during the winter and under unfavourable conditions.

The mean number of snails collected per grab in the successive samples varied greatly from one collection to the next (Table 9). The reason for such variation may lie in the aggregated distribution of the snails, and hence the finding or missing of a clump of snails will make a great difference in the estimate of the mean (Hairston, 1961). To reduce the effect of aggregation, the data in Table 9 was grouped for each of three successive

TABLE 8

Frequency distribution of the number of Bithynia tentaculata per grab collection, for the 27 samples (i.e, 810 grab collections) taken from December 1972 to 1st May 1974.

Number of snails per grab collection (less than)	Collections made from 6th December 1972 to 18th Sept, 1973. (i.e, before clearance)		Collections made from October 1973 to 1st May 1974. (i.e, after clearance)	
	No. of grabs	%	No. of grabs	%
0	25	4.6	31	11.5
10	229	42.4	155	57.4
20	149	27.6	46	17
30	77	14.3	14	5.2
40	28	5.2.	8	3
50	16	3	6	2.2
60	6	1.1	2	0.7
80	5	0.9	6	2.2
136	5	0.9	2	0.7
TOTAL	540		270	

samples. Thus the collections made from 6th December 1972 to 16th January 1973 were considered as one sample, (.i.e, 90 grab collections) and the average mean density was calculated as 16.34 snails per grab. Similarly, the average mean density from 20th February to 3rd April and from 17th April to 15th May was calculated as 16.66 and 14.83 snails per grab respectively. From 29th May to 26th June the population was made of old and young snails (Fig. 3), the total density was 12.71 and the old population density was 9.52 snails per grab. From 10th July to 7th August the total population density was 15.64, but the old population density was 7.87. The total population density from 21st August to 18th September was 16.48 snails per grab.

These observations suggest that there was no change in the population density of Bithynia tentaculata from 6th December to 3rd April. In the spring (17th April to 15th May), few (10%) of the snails die off, whereas between June and early August, 52.3% of the wintering population disappear. The new generation which appeared in June increased the population density, and in September the level population density (16.48) was the same as that which existed during the previous winter (16.5) between December 1972 and early April 1973.

The results for October and November 1973 showed a population density of 13.05 snails per grab. From 18th December to 21st March 1974, the population density was 12.78 snails per grab.

TABLE 9

The number of Bithynia tentaculata collected in the successive samples, together with the mean number of snails per grab and the standard deviation

Date of Collection	Sample Number	Number of Snails Collected	Mean Number of snails per grab	Standard deviation
1972				
6 December	1	383	12.77	24
19 December	2	583	19.43	18.5
1973				
16 January	3	505	16.83	25.2
20 February	4	403	13.43	13
20 March	5	602	20.07	11.1
3 April	6	494	16.47	12.8
17 April	7	532	17.73	11.7
1 May	8	417	13.90	10
15 May	9	386	12.87	8.8
29 May	10	333	11.10	9.9
12 June	11	328	10.93	8.8
26 June	12	483	16.10	7.5
10 July	13	480	16.00	7.5
24 July	14	450	15.00	14.3
7 August	15	478	15.93	16
21 August	16	314	10.47	11.9
4 September	17	483	16.10	18.6
18 September	18	686	22.87	33.6
3 October	19	440	14.67	18.1
14 November	20	343	11.43	21.5
18 December	21	198	6.60	8.6
1974				
17 January	22	320	10.67	15.2
21 February	23	562	18.73	26.3
21 March	24	454	15.13	17.7
3 April	25	257	8.57	9.4

cont'd...

TABLE 9

Continued....

Date of Collection	Sample Number	Number of Snails collected	Mean Number of snails per grab	Standard Deviation
17 April	26	165	5.50	5.8
1 May	27	218	7.27	7.2
15 May	28	199	6.63	*
3 June	29	167	5.57	
18 June	30	290	12.08	**
9 July	31	368	15.33	**

* Collections made on 15th May and thereafter were pooled together, and therefore no standard deviation was calculated.

** These samples consisted of 24 grab collections each.

The population density from 3rd April to 1st May and 15th May to 3rd June was 7.11, and 6.10 snails per grab respectively. The old population density from 18th June to 9th July was 5.9, whereas the total population density was 13.71 snails per grab. These results suggest that herbicide spraying of the habitat on 3rd September and the clearance of the dead plants in early October had killed or removed 20.8% of the population of B. tentaculata. Little change occurred in the population density (i.e, 2%) from October - November 1973 to the period between December 1973 and March 1974. However between 3rd April and 15th May, 44.4% of the population died off, and by June-July 1974, 53.8% of the wintering population had disappeared.

Thus it could be concluded that the population density of B. tentaculata showed little change from December to March. In the spring of 1973 few (10%) of the snails died off, whereas in the spring of 1974, a considerable proportion (44%) of the population died off. In the summer of 1973 (June to August) most of the wintering population (52.3%) disappeared, and similarly by June-July 1974, 53.8% of the population disappeared. Thus while the proportion of the wintering population which died off in the summer of 1973 and 1974 was the same, the greater part of the former population died between June-August, whereas the latter population died in April-May.

3.5.2. Distribution within the habitat

The distribution of Bithynia tentaculata throughout its habitat, like that of all other species of aquatic animals, is neither even nor random. Table 10 shows the mean number of snails collected per grab per habitat area (Fig. 2), arranged in an increasing order of density. It can be seen that in areas 14 to 22 the snails were more abundant than in other parts of the habitat. On the other hand, areas 1 and 2 contained the least number of snails. The habitat (Fig. 2) is primarily a drainage pond which is designed in such a way as to retain a maximum level of water 80-150 cms. high and to discharge the excess into another pond which leads to a small lake. The incoming water enters the pond in area 21, passes through area 20 to end in area 1. The excess water leaves through a tube about one metre above the bottom of the pond and about 2 metres from the extreme end of portion 1.

Observations in areas 1 and 2 showed that the water is usually foul in this area, possibly due to the accumulation of waste material in this end of the pond. The polluting substances were found to be the droppings of aquatic birds and other animals (such as dogs) which visit the pond, together with food material and litter thrown into the pond. Boycott (1936) suggested that one of the chief requirements of aquatic molluscs is clean transparent water, not overcharged with the products of animal

TABLE 10

Distribution of Bithynia tentaculata within the habitat. The mean number of snails per grab per habitat area in the 27 samples collected from 6th December 1972 to 1st May 1974, arranged in an increasing order.

Habitat portion	Mean number of Snails Collected	Habitat portion	Mean number of Snails Collected
1	3.4	8	12.0
2	3.7	5	12.2
30	7.5	9	12.3
28	7.6	11	13.1
12	7.6	23	13.5
29	7.6	25	14.4
27	8.0	21	17.1
24	8.0	22	18.0
26	8.3	14	19.3
3	8.6	18	20.4
7	8.9	15	21.2
6	9.3	16	25.0
4	10.1	17	29.9
13	10.1	19	32.2
10	10.9	20	37.9

or vegetable decay. Thus pollution might be one of the reasons why few snails were present in this part of the habitat. Boycott (1936) stated that domestic and domesticated ducks are the worst enemies of the freshwater molluscs. They can completely destroy the molluscs in a pond by fouling the water rather than predation.

In contrast, the water in the rest of the pond is clear in the spring and summer months, and this is probably why the population in these parts is two to ten fold that present in areas 1 and 2. Other factors are important as well, and it is the interaction of such factors as food supply, oxygen concentration, temperature etc., that might possibly make areas 14-22 more favourable than the others.

3.5.3 Behaviour

Observations on the natural habitat showed that the vertical distribution of the snails varied from one season to another. From November to March most of the water plants have died and the temperature is generally low. In these months it was difficult to find snails on the water plants or floating tree branches. Few snails could be seen on the banks of the pond, and most of the snails seemed to settle to the bottom. Here they were possibly more aggregated than in any other season, as suggested by the presence of 136 and 124 snails in one grab collection in January 1973 and February 1974 respectively.

In the laboratory, similar behaviour was observed in November 1973, as illustrated by the following experiment. On 14th November 1973, when the atmospheric temperature was 5°C, 60 snails were introduced into a large tank (34 x 23x 23 cms), containing bottom materials about 5 cms thick which consisted of soil, detritus and plant leaves from the natural habitat. About 15 litres of tap water and a few branches of the water plant Ludwigia sp. were added, and the tank was kept out of doors to simulate the natural environment. A similar tank with the same content was maintained in the laboratory at 15°C. Observations on three successive days and on the 15th day showed that none of the snails in the tank kept outside (temperature 5 - 1°C) were found on the water plants nor the walls of the container, and not more than three snails could be seen on the top of the bottom layer. When some of the plant leaves were removed from the bottom layer some of the snails were found on the under side of these leaves, but the remainder of the snails were buried in the loose detritus layer above the bottom mud. In the laboratory, at a temperature of 15°C, snails were found to be distributed over the water plant, glass walls of the tank, and the top of the bottom layer, and most of the snails were found on the glass walls. These observations are in agreement with the situation in nature, and confirm that with the onset of the cold weather the snails retreat to the bottom of the habitat and become aggregated in the most favourable parts of the habitat.

In nature the snails become more active from late March onwards, and by mid-April snails were observed in large numbers on the growing water plants, algal masses and floating tree branches, and gliding on the surface film of the water. Numerous snails were found actively crawling on the stone walls of the banks and over the plant leaves on the top of the bottom layer of the pond. This activity continued to the end of the summer, and again the snails retreat to the bottom with the onset of the cold weather in November.

3.5.4. Dispersal

In an attempt to find the rate of dispersal of B. tentaculata in its habitat, the mark-release-recapture technique was used. The snails were marked with a quick drying paint (Humbrol, nitrate cellulose dope) on the ventral surface of the body whorl. It was found in the laboratory that gentle rubbing of the shell with cotton wool or a camel-hair brush containing acetone increased the durability of the mark, and there were no loss of marks for a three-month period. Snails marked with the same paint, but whose shell was dried by filter paper or cotton wool without acetone, showed a 50% loss of mark in one month. The greater durability of the mark after acetone cleaning was probably due to the removal of the slime mould on the shell surface. After marking

the snails were kept in the laboratory for 24 to 72 hours before release, and only active snails were selected for release.

Six hundred snails were marked (blue paint) and released at the end of area 3 (Fig. 2) of the habitat on 30th July 1973. The width of the release area was about six metres and the snails were released in a straight line between the two banks. Two hundred snails were released at a distance of 1½ metres from the bank on each side, and the last two hundred snails were released in the mid-point of the line. A search on three successive days, starting twenty metres on both sides of the release area and working towards the release point, showed that the most advanced snails (6, 8 and 7 respectively) were recovered at a distance of 1 to 1½ metres on both sides of the release line. Observations on the release area showed some of the marked snails on the top branches of the water plants together with unmarked snails. A similar search on the seventh day recovered three snails within the same distance, 1 to 1½ metres, from the release line. Two, 7 and 9½ months later, marked snails (1, 2 and 1 snails respectively) were found in the samples collected near the release area, and in no other sample was a marked snail recovered.

A second group of 750 snails was marked (white paint) and released on 9th April 1974 in the middle of area 20, which is the first part of the pond to receive drainage water (see Fig. 2).

Successive search for marked snails after 24 and 48 hours resulted in the recovery of marked snails near the release area. Three more marked snails were recovered in the sample collected on 18th Une and two on 13th August 1974, all from the same area.

These observations probably suggest that active movement of Bithynia tentaculata within the habitat is limited. Passive dispersal by floods, aquatic birds or other animals no doubt moved some of the snails from one position to another, but probably the greater part of the population reacts under such conditions by settling down to the bottom where they find shelter, and remaining there until conditions are more favourable again. B. tentaculata, as a gill-breather, is able to remain on the bottom and in one position, with the operculum partly closed, for many hours, at least in the laboratory. The alternative explanation is that both active and passive dispersal are operating in the population within the habitat to a greater extent than suggested above. The failure to establish its occurrence might be due to the fact that in such a large habitat (2310 square metres) containing a population of probably over a million snails, it is most likely that the few marked snails dispersed were not detected. Moreover, the marking of the snails could have made them more prone to predators, so that they were probably greatly reduced in number. Although it is difficult to draw any conclusion from these results, the finding of three marked snails 7 and 9½ months after release in the release area suggests that dispersal in Bithynia is probably more passive than active.

3.6 OBSERVATIONS ON THE NATURAL DIET

3.6.1 Introduction

Boycott (1936) stated that freshwater snails feed on decaying plants and algae. Bovbjerg (1968) reported that although the lymnaeid species of Stagnicola and Lymnaea stagnalis are predominantly herbivorous and detritus feeders, they will ingest animal food. The gut content of some freshwater gastropods were examined by Shäfer (1952), Calow (1970), Dazo and Moreno, (1962) and Clampitt (1970), and in general detritus and algae were the main constituents.

In the present work the gut content of specimens of Bithynia tentaculata was examined. In addition, laboratory studies were carried out to find the growth response of juvenile snails reared on some of the main diet items ingested in nature.

3.6.2 The morphology of the alimentary system of Bithynia tentaculata

As the gut form and function are interrelated, a brief description of the alimentary system of B. tentaculata would be useful. The alimentary system of this species was described by Graham (1939) and is characterised by the presence of a style sac which contains a crystalline style. According to Graham (1939), this apparatus is confined to certain molluscs which are microphagous herbivores that feed by ciliary currents, or on vegetation finely comminuted by the radula, and pass a continuous stream of minute food particles into the stomach.

The buccal mass of B. tentaculata is large, and the structure of the radula has been worked out by Berry (1943) and Lilly (1953). The radula has a central, an intermediate and two lateral plates. The oesophagus runs from the posterior end of the buccal mass to open on the left side of the stomach in a rather posterior position. The stomach is elongated and complicated in structure (Graham, 1939). The posterior end of the stomach is broad and has the openings of the oesophagous and midgut duct on the left side. On its right side lies a thick cuticularised gastric shield, and between the two sides there are ciliated ridges and grooves which act as sorting areas. The intestine and style sac leave from the narrow anterior end of the gut and communicate with one another by a slit between typhlosoles. The style sac contains the crystalline style, a cylindrical rod of

concentric structure, whose lower end projects into the stomach and bears against the gastric shield. Food particles brought to the stomach by the oesophagus are carried to the head of the crystalline style. The constant rotation of the latter helps to draw the food string into the stomach and to churn the stomach content. The thoroughly mixed food enters the sorting grooves, where the smaller particles are directed towards the duct of the digestive diverticula and the larger ones are carried towards the intestine for elimination together with the particles which leave the duct of the digestive diverticula after digestion.

3.6.3 Materials and Methods

3.6.3.1 Analysis of gut content

For the analysis of the gut content, snails were collected from the field on 11th April 1973 and 2nd August 1973 to represent the summer collection, and in all 36 snails were dissected. The winter collection was made on 25th January and 6th February 1974, when 92 snails were dissected. Freshly collected snails were placed instantly in 20 percent formalin solution to halt the process of digestion and preserve the gut content. Each snail was dissected, and the viscera were removed to 50 percent glycerine on a microscope slide. The stomach was isolated under a binocular microscope and teased to remove all its contents, which were later

examined under the X40 objective of the microscope and identified. The algae was identified from West and Fritsch (1927). The different food materials present were recorded by two methods. In the first place the occurrence of each food item was noted, and then its relative abundance was approximately estimated by the points method (Hynes, 1950). Here points were allotted to the stomach according to its degree of fullness. A full stomach was given 16 points, half full 8 points, quarter full 4 points, and an empty stomach 0 points. Then the number of points allotted was subdivided to the various food items present, depending upon size and abundance.

3.6.3.2 Growth Experiments

Experiments on the growth of juvenile snails in the laboratory on different diets were carried out using the following diets:

a) Detritus

b) Algae

c) Detritus + Algae

d) Detritus + Algae + Lettuce in the presence of Ludwigia (water plant).

These diets were prepared as follows:

i) Detritus

Samples collected from the field were passed through a 1mm. sieve. The sieved mixture was then allowed to settle in a two-litre measuring cylinder and the sand in the bottom of the cylinder

was removed. This process was repeated several times before the detrital suspension was allowed to settle in the cylinder overnight. The excess water was poured off, and the detritus was boiled for 15 minutes and stored in a refrigerator.

ii) Algae

Algae brought from the field was washed several times in sterile pond water. The clean algae were then picked out and cultured in the following medium, 3 to 5 days before use. (Dr. Eaton, 1973, personal communication).

Sterile water	1 litre
$NaNo_3$	0.2 gms.
$Na_2HPO_4 12H_2O$	0.02 gms.
soil extract	50 ml.

iii) The Ludwigia water plant was taken from the tank when needed.

Snails used in these experiments were obtained from eggs collected from the field on 15th May 1973. The hatchlings were measured on 11th June 1973, and ranged in size from 1.2 - 2.0 mm. with a mean length of 1.5 mm. One hundred snails were used for each of the four diets. These snails were divided into five groups of twenty snails each. Each group of twenty snails was transferred to a two-litre fask containing one litre of filtered tank water and a spatula of sand (approximately 2 grams). Thus five replicates of each diet were used; or, in all, 20 flasks were used. Then

20 cc of detritus were added to each of the five replicates to be fed on detritus. Similarly, 20 cc of algal suspension were added to the group to be fed on algae. Each of the third and fourth groups received 10 cc of detritus + 10 cc of algae. In addition, the fourth group received a few pieces of chopped lettuce and a branch of Ludwigia.

Snails reared in the field were enclosed in Perspex tubes 15 cm. in diameter and 20 cm. long. Five tubes were used and each one enclosed twenty snails (mean size 1.5 mm). Both ends of the tube were covered with nylon cloth of about 300μ mesh, and the tube was tied with a nylon cord (3 metres) to a tree branch in the island and set free in the pond.

3.6.4 Results

3.6.4.1 Analysis of the gut content

The gut content of Bithynia tentaculata consist of a wide variety of food materials (Table 11) which fall into the following groups: unicellular and filamentous green algae, diatoms, detritus, macrophytes, animal remains, blue green algae, desmids and fungi. However, detritus, diatoms, unicellular and filamentous green algae occurred in 77.8 to 100% and 29 to 100% of the snails examined in summer and winter respectively (Table 12). These food items together formed 92.7 and 83.6 of the gut content in summer and winter respectively (Table 13). The other food items of macrophytes, animal remains, blue green algae and fungi occurred in the gut of 11.1 to 19.4% of the snails in both seasons (Table 12);

TABLE 11

Different types of food items found in the stomach of Bithynia tentaculata

(1) Unicellular algae

a) Characium
b) Euglena
c) Phacus
d) Chlamydomonas
e) Sphaerocystis
f) Chlorella

(2) Filamentous algae

a) Ulothrix
b) Cladophora
c) Oedogonium

(3) Diatoms

a) Navicula
b) Synedera
c) Cymbella
d) Gomphonema
e) Amphora
f) Epithemia

(4) Desmids

a) Penium
b) Closterium

(5) Blue green algae

a) Oscillatoria

(6) Detritus

(7) Fungi

(8) Protozoa (ciliates)

(9) Oligochaeta (small worms)

(10) Insect tracheae

(11) Macrophytes

TABLE 12

Frequency of occurrence of the items of diet in the gut of Bithynia tentaculata

	SUMMER COLLECTION 11th April, 6 Aug, 1973		WINTER COLLECTION 25th Jan, 6th Feb, 1974	
	Frequency	% Occurrence	Frequency	% Occurrence excluding empty guts
(1) No. of snails examined	36		96	
(2) No. of snails with guts containing food	36		62	
Food items				
Filamentous algae	28	77.8	18	29
Unicellular algae	33	91.7	32	51,6
Diatoms	28	77.8	34	54.8
Detritus	36	100	62	100
Sand grains	23	63.9	43	69.4
Macrophytes	7	19.4	12	19.4
Animal remains	4	11.1	10	16.1
Blue green algae	6	16.8	7	11.3
Fungi	6	16.8	10	16.1

TABLE 13

Percentage composition of the diet by volume in the gut of Bithynia tentaculata in summer and winter.

	WINTER COLLECTION		SUMMER COLLECTION	
	No. of points	%	No. of points	%
Total No. of points	388		344	
Filamentous algae	19	4.8	46	13.4
Unicellular algae	18	4.6	43	12.5
Diatoms	50	12.9	70	20.3
Detritus	238	61.3	160	46.5
Sand grains	37	9.5	13	3.8
Animal remains	6	1.6	4	1.2
Macrophytes	14	3.6	4	1.2
Blue green algae	3	0.8	2	0.6
Fungi	3	0.8	2	0.6
No. of guts examined	39		30	

and formed 3.6 and 6.8% of the gut content in summer and winter respectively (Table 13).

Of the main food items (Tables 12 and 13) detritus was always present in the gut of the snails, and it occupied 61.3 and 46.5% of the gut content in winter and summer respectively. Diatoms, unicellular and filamentous green algae occurred in 77.8 to 91.7 and 29 to 54.8% of the guts of the snails examined in summer and winter respectively. On relative abundance in the gut content diatoms were second to detritus, occupying 20.3 and 12.9% of the gut content in summer and winter respectively. Filamentous and unicellular algae formed 13.4 and 12.5% respectively of the gut content in the summer, whereas the respective values in winter were 4.8 and 4.6%.

3.6.4.2 Growth on different diets

Juvenile snails (mean size 1.5 mm) reared on detritus + algae + lettuce and in the presence of the water plant Ludwigia showed the highest growth rate throughout the fourteen weeks of the experiment (Fig. 11) and the snails attained a mean size of 7.4 mm on the 14th week. Snails reared on detritus and algae showed a lower growth rate throughout the experiment, and a mean size of 5.3 mm was reached on the fourteenth week. Snails fed on detritus alone showed a growth rate which was about

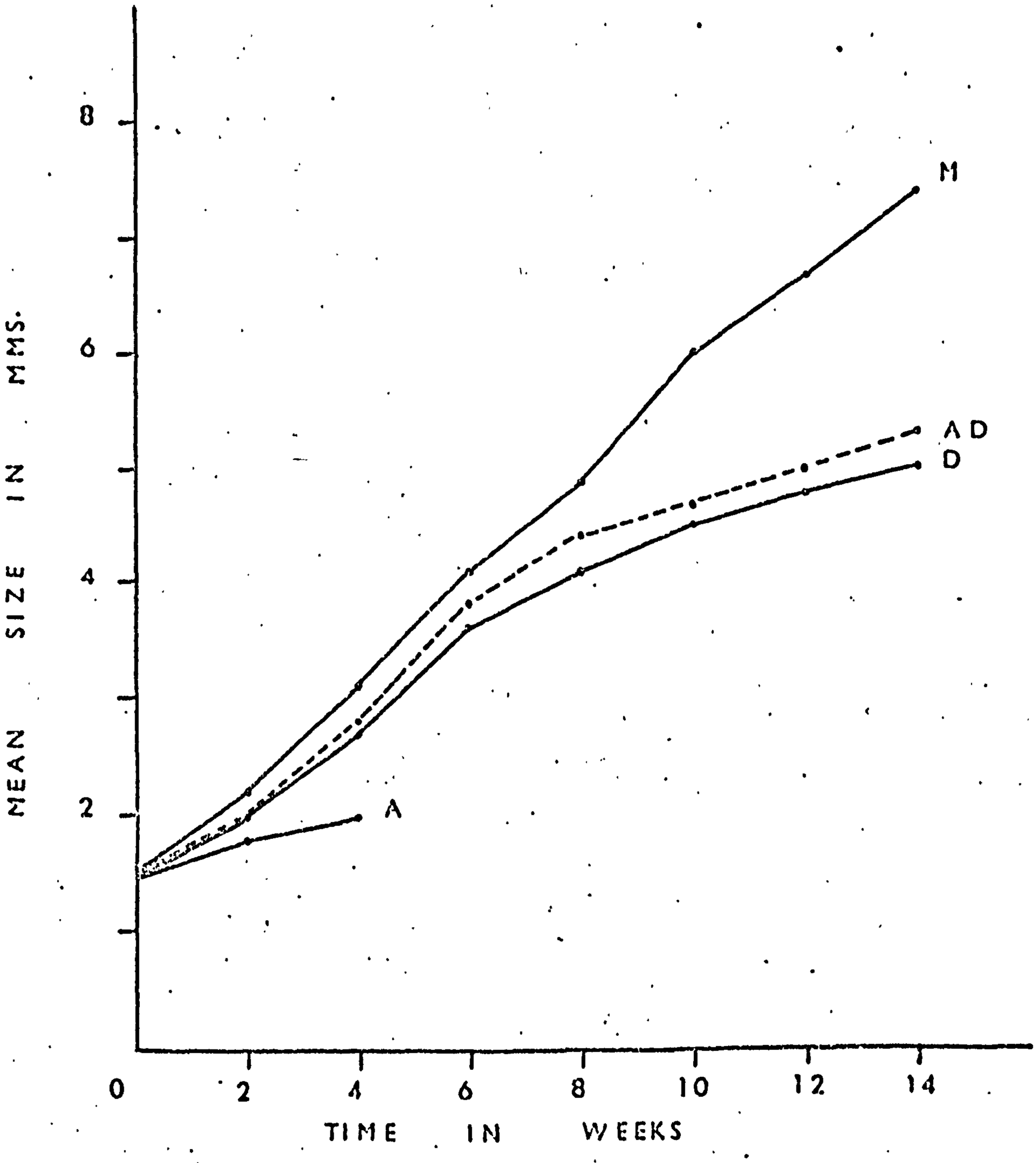


Fig. 11 Growth of Bithynia tentaculata reared on different diets. (A = Algae; D = Detritus; AD = Algae + Detritus; and M = Algae + Detritus + Lettuce + Ludwigia).

similar to that shown by the snails fed on detritus and algae. These snails reached a mean size of 5.0 mm on the 14th week (Fig. 11). The group fed on algae alone survived for four weeks only and showed the lowest growth rate during this period.

In nature, none of the snails survived for two weeks and examination of the tubes did not reveal any damage to the nylon cloth which could possibly suggest that the snails had escaped. Probably the snails died and the fragile shells disintegrated and were swept away during this period. The experiment was discontinued.

TABLE 14

Survival rate of young Bithynia tentaculata (7-10 days after hatching) fed on different diets.

Age in weeks	Algae	Detritus	Algae + Detritus	Algae + Detritus + Lettuce in presence of water plants
1	100	100	100	100
3	32	57	43	72
5	1	12	18	49
7		9	16	42
9		9	15	40
11		8	15	38
13		8	15	38
15		8	15	36
17		8	15	36
20		8	15	36

3.6.5 Discussion

The analysis of the gut content of Bithynia tentaculata showed that the snails feed on a wide variety of food materials (Table 11). However, detritus, diatoms, unicellular and filamentous algae are the main diet components. This observation is in agreement with Boycott's (1936) statement that freshwater gastropods feed on decaying plants and algae. Schäfer (1952) found detritus and unicellular algae in the gut content of B. tentaculata. Dazo and Moreno (1962) reported the gut content of Oncomelania quadrasi as green algae, detritus, diatoms, desmids, euglenoids and blue green algae as the main food constituents. Clampitt (1970) found detritus and algae in the gut content of Physa gyrina. Bovbjerg (1968) found the gut content of the Lymnaeid snails examined to be mainly made of detritus and green algae. Calow (1970) found epiphytic algae in the gut of Lymnaea peregra. Other food materials such as macrophytes and animal remains are occasionally ingested by B. tentaculata (Tables 12 and 13). Dazo and Moreno (1962) also noted the occasional ingestion of protozoa, rotifers and nematodes by Oncomelania quadrasi. Bovbjerg (1968) also reported the occasional finding of fragments and even whole bodies of rotifers and insects in the guts of the Lymnaeid snails examined.

The ingestion of a wide vareity of food materials in nature by the freshwater gastropods suggests that such a diet favours the optimal growth of these snails. Bovbjerg (1968) found

Lymnaea stagnalis to show an optimal growth rate in nature over any laboratory diet. Similarly, Clampitt (1970) found the growth rate of Physa gyrina and Physa integra in nature considerably higher than in the laboratory on a diet of green lettuce and dried maple leaves. Field data in the present investigation (Fig. 7) showed the fast growth rate of young B. tentaculata in nature.

Turner (1926) found that food supply is the main, if not the only, factor that determines the rate of growth of Lymnaea peregra in the laboratory. The optimal growth (Fig. 11) and survival (Table 14) of juvenile Bithynia tentaculata in the laboratory on a diet of detritus, algae and lettuce illustrate the importance of a varied diet. Although animal food was not introduced, the dead snails could possibly provide such part of the diet. Van der Schalie and Davies (1965) reported that optimal conditions for rapid growth and development of Oncomelania are based on the interaction and proper balance of such factors as light, soil, volume of the environment and food. Other factors being constant, the authors found that soil which is capable of supporting a rich growth of green algae and diatoms, as well as containing organic matter with its accompanying bacterial decomposers, are most suitable. Bovbjerg (1968) reported that Lymnaea stagnalis reared in the laboratory on a mixed plant and animal food showed greater growth and survival rates than snails reared on either diet alone.

That lack of certain components of the diet adversely affect the growth and survival rates of the snails is illustrated by the low growth rate (Fig. 11) and survival rate (Table 14) of juvenile Bithynia tentaculata fed on detritus alone. Van der Schalie and Davies (1965) found that Oncomelania formosana grew at a slower rate when reared on organic material with its decomposing microflora. The failure of young B. tentaculata to survive or grow for more than four weeks on an algal diet could possibly be explained by the poor growth of the algal culture rather than the unimportant role of the algae itself in the growth of these snails. Boycott (1936) stated that freshwater gastropods consume cultivated plants such as lettuce, though more readily when the plants are partly decomposed. Calow (1970) pointed out that the chloroplasts of higher plants are similar to those of unicellular algae in size and shape. He suggested that the cell wall protects such plants as Elodea from being consumed by the snails. When the cell wall is soft or partly decomposed, the chloroplasts of the higher plants are readily eaten by the snails.

Eisenberg (1966, 1970) found that natural populations of Lymnaea elodes are limited in their growth and reproduction potential by food quality rather than quantity. Probably the fast growth of B. tentaculata in the summer, when there is a rich algal growth, could possibly be caused by, among other factors, the presence of a more balanced diet (Table 13).

In winter, when the algae is reduced, the snails consume a greater proportion of detritus (Table 13); and probably the shortage of suitable food is partly responsible for the slow growth during this period.

4.0 TREMATODE INFECTIONS OF BITHYNIA TENTACULATA

4.1 CLASSIFICATION, DESCRIPTION AND IDENTIFICATION OF CERCARIAE INFECTIONS OF BITHYNIA TENTACULATA

4.1.1. Introduction and literature reviews

The study of the larval trematodes of freshwater molluscs has received considerable attention, and the literature is both deep and wide, as pointed out by Dawes (1956). In Great Britain, Thomas (1883) reported the life cycle of Fasciola hepatica and noted the cercariae infections in Lymnaea peregra. Hesse (1923) described two cercariae infections in Lymnaea peregra, and Brown (1926) reported nine species of cercariae infecting Lymnaea peregra and L. stagnalis. Subsequnt workers such as Harper (1929), Rees (1932), Iles (1959), and Khan (1960, 1961 and 1962), described many species of cercariae. Nasir and Erasmus (1964) gathered the scattered literature on the cercariae from the British freshwater molluscs into a useful key. Many species of cercariae were added by later workers and in particular from South Wales (Probert, 1965a, b, and 1966a; Pike, 1967 and 1968a).

Lühe (1909) published a monograph on cercariue in which he brought together all the forms previously described and put forward the first scheme of classification of the cercariae. Hisscheme was based to a certain extent on larval characters such as suckers, stylets, collar and collar spines and the type of tail. With the advance in knowledge some of the groups which Lühe (1909) established

were modified or subdivided and new groups were erected (Sewell, 1922; Dubois, 1929; Wesenberg-Lund, 1934; Porter, 1938). Dawes (1956), LaRue (1957) and Erasmus (1972) pointed out that Lühe's scheme had little systematic value, because many of the groups represent a collection of cercariae from different adult taxonomic groups which exhibit evolutionary convergence at the cercarial level.

As numerous cercariae were described whose adult stages are unknown, some authors have denoted their cercariae by numbers (Harper, 1929; Petersen, 1931), alphabet letters (Rees, 1932) and specific names (Wesenberg-Lund, 1934; Porter, 1938). Dawes (1956) stated that cercaria is a group name and not a generic name, yet it may take priority over later names given to the adults of the same species.

LaRue (1938) stated that the development of a natural taxonomic system for the digenea must be based upon the comparative anatomy of all the stages of the life cycle. But lack of information on post-larval development of most of the cercariae and on the larval forms of the adult worms described was the main difficulty. Next to life history as a basis of classification, it was considered (La Rue, 1938 and 1957; Cable, 1965) that the study of those systems of structure which are least modified in the course of development

are of great taxonomic value. Cort (1917) pointed out that the excretory system of cercariae of the digenetic trematodes may serve such a need, and Faust (1919) contributed a mathematical formula for expressing the arrangement and number of flame cells which came to be known as the flame cell formula. Kuntz (1950, 1951, 1952) described the two methods of formation of the excretory bladder in cercariae, and LaRue (1957) divided the digenetic trematodes into two super orders, Anepitheliocystidia and Epitheliocystidia on the basis of the structure of the excretory bladder in cercariae. He classified the digenetic trematodes into higher taxa according to its excretory system and other larval morphological data.

Richard (1971) in France, revealed the taxonomic importance of chaetotaxy in cercariae and stated, "However, these sensory receptors which are directly connected with the nervous system, must be as constant as the excretory system (LaRue, 1957), and less subject to convergences and adaptive phenomena than general morphology". The ciliary system could possibly be of more reliable taxonomic value for two reasons. Firstly, the ease with which the system can be practically elucidated by silver impregnation, at least in some groups of cercariae, e.g. Furcocercariae. Secondly, its taxonomic value at the specific level, where it allows the separation of very closely related species such as cercariae of Schistosoma mansoni and S. haematobium which are difficult to separate by other methods (Kuntz, 1950).

The difficulties of specific recognition of cercariae were expressed by Sewell (1922), in that specific diagnosis is possible only when the structure of the cercaria and of its parthenita is complete to the minutest detail, and even so there are some cases in which it is impossible to arrive at a final decision except by the recovery of the adult worms. The diagnostic value of the excretory system is recognised, and Komyia (1961) reported that the flame cell formula in the cercaria is constant in individuals of the same species with certain exceptions. However, accurate tracing of the excretory system is a difficult task. It can only be studied in the living organism, and in some cases the presence of numerous cystogenous cells and or pigment granules (e.g. in the Monostome and some of the Gymnocephalous cercariae), make it even more difficult to assess the arrangement and number of flame cells. As a result different authors may find a different number of flame cells in the same species, as illustrated by Kuntz (1950) who reported that many workers found three pairs of flame cells in the body of the cercaria of Schistosoma mansoni and S. haematobium instead of four pairs. The importance of the floating behaviour in distinguishing species of Furcocercariae was illustrated by Nasir and Erasmus (1964). Cable and Wheeler (1939) also stressed the importance of behaviour in distinguishing Pleurolophocercous cercariae.

In addition to the practical difficulties encountered, and the lack of specific diagnostic characters in cercariae, the

records and descriptions of a large number of cercariae published by various workers are inadequate to enable one with certainty to recognise and identify them, which further complicates the matter. However, in the present work all the cercariae infecting Bithynia tentaculata in the habitat studied were first fully described, and then compared with related species. In comparison with other species, the similarities as well as the differences in morphology, anatomy including the excretory system, behaviour and metacercarial nature (where possible), were assessed and their taxonomic value was considered before deciding the specific identity of the cercaria.

Finally, the general scheme of classification of the cercariae adopted in this investigation is that proposed by Lühe (1909), with modifications and subdivisions of the groupshe erected by Sewell (1922) and others.

4.1.2 Materials and Methods

Snails collected from the field were maintained in glass aquaria in the laboratory for examination. The snails were isolated individually in about 10 ml of water in small tubes (capacity 30 ml.). The isolated snails were kept either for one to four hours under direct illumination or overnight in a well-lit position in the room (temperature 25°C). When trying to find out whether shedding occurs in the dark, the snails were kept under the same conditions but in a cupboard which was completely dark. The cercariae were studied alive under coverslip pressure, and under X40 or oil immersion objectives. The intra-vitam dye neutral red was found to be useful in differentiating the alimentary system and penetration glands. Observations on swimming behaviour and floating position were made under the dissecting microscope. Measurements were made with an ocular micrometer on mature naturally-emerged cercariae, either living or fixed in 10% hot (70-80°C) formol saline or both.

The excretory system was best revealed by the technique described by Komyia (1961) in which the cercariae were examined in 0.6% saline, which is almost similar to the osmotic pressure of the freshwater molluscs. Moreover the mounting of the worms with the ventral surface upwards was recommended, because the excretory canals and the majority of the flame cells are usually

distributed near the ventral surface of the worm. One worm from a stock kept in 0.6% saline was picked with a micropipette and transferred to a coverslip with a small amount of fluid, the coverslip was turned over when the worm stuck to it and then placed on the slide glass, and thus the worm was mounted with the ventral surface upwards. The amount of fluid between coverslip and glass slide was regulated with filter paper to keep the worm in position without too much pressure, and the preparation was examined under oil immersion.

Infected snails were also crushed and dissected to study the parthenita (sporocysts or redia), and immature cercariae recovered from dissected snails provided useful information on various aspects of the morphology and anatomy of the species described. All drawings were free-hand and to scale, and represent a composite reconstruction based on numerous observations on a wide variety of specimens.

4.1.3 The Monostome cercariae

Lühe (1909) defined the Monostome cercariae as those forms which lack an acetabulum, but possess eye spots and a simple long slender tail without setae, and whose development takes place in rediae. Faust (1917) divided the Monostome cercariae into two subgroups, the Trioculate cercariae in which a median eye spot is present in addition to the paired lateral eye spots, and the Binoculate cercariae in which only the lateral eye spots are present. Sewell (1922) used the term Monostome to include all those cercariae in which an acetabulum was absent, and he divided simple and forked-tailed Monostomes into six groups, the Pleurolophocerca, Urbanensis, Ephemera, Lophocerca, Lophoides and Ubiquita. The Urbanensis and the Ephemera groups are roughly the Binoculate and Trioculate groups of Faust (1917). The cercariae of these two groups proved to be cercariae of Monostome tramatodes of the super-family Notocotyloidea. Rothschild (1938a) divided the cercariae of the super-family Notocotyloidea into two sections:

1) The Notocotyloidae,- comprising cercariae without aural lappets or collar.
2) The Pronocephalidae - comprising cercariae with aural lappets or a collar.

Rothschild (1938a) further divided the Notocotyloidae into three subgroups, the Monostomi, the Imbricata and the Yenchingensis, on the basis of differences in the structure

of the anterior transverse portion of the excretory tubules and its relation to the median eye spot. In the Monostomi group the anterior transverse portion of the excretory tubes is situated posteriorly to the median eye spot. In the Imbricata group the transverse excretory loop passes anteriorly to the median eye spot and in the Yenchingensis group, there is an unpaired finger-like projection extending from the transverse portion of the excretory tubules. Dubois (1951) divided the species of Notocotylus parasitising anserines into two biological groups on the basis of development.

1) The "Triseralis" group, whose development takes place in pulmonate gastropods and is based on Notocotylus triseralis Diesing, 1839.

2) The "Imbricata" group whose development takes place in prosobranchiate gastropods and is based on Notocotylus imbricatus (Looss, 1893) Szidat (1935).

4.1.3.1. *Cercaria imbricata* Looss. 1893

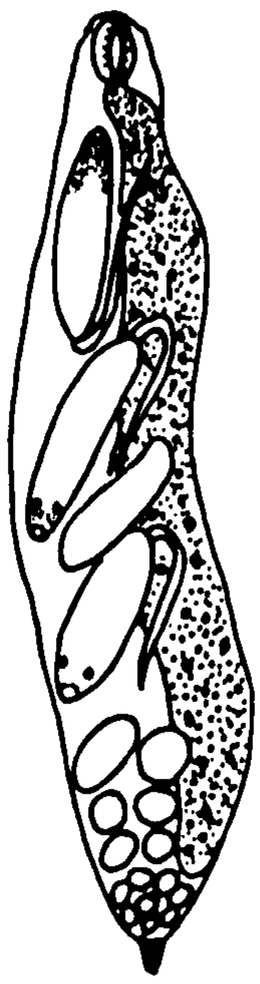
This cercaria was reported by Looss (1893) from *Bithynia tentaculata* in Germany. Szidat (1935) demonstrated that it is the larval form of *Notocotylus imbricatus*. The cercaria was fully described by subsequent workers (Dönges, 1962; Odening, 1963, 1968; Pike, 1969). Dubois (1951) reported that *Cercaria helvetica I* Dubois, 1928 is synonymous with *Cercaria imbricata*.Odening (1963, 1968) suggested that *Cercaria fennica I* Wikgren, 1956 is synonymous with *Cercaria imbricata*.

In the present investigation *Bithynia tentaculata* was found to be infected with *Cercaria imbricata*. The infection rate was 12.8%, and the following description is in agreement with previous reports.

Fig. 12 General morphology of the redia (A), cercaria (B), and metacercaria (C) of Cercaria imbricata.

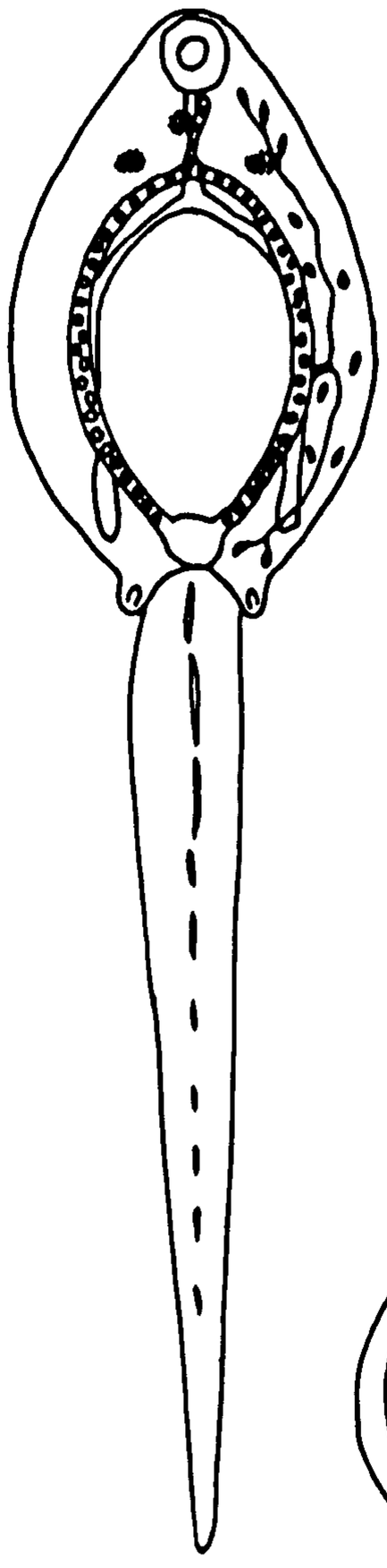
[Scale in mm.]

A



0·5

B



0·15

C

0·1

4.1.3.1.1 Description

Redia (Fig. 12A)

The rediae are found in the digestive gland of the snail host Bithynia tentaculata. Infection of the snail is usually very heavy, and the rediae are elongated in shape. Measurement of the living and fixed rediae are shown in Table 15. The length of the mature living redia varies from 500 to 1600μ and the breadth varies from 160 to 310μ. The rediae lack both a collar and posterior lappets, but have a posterior projection of the body which is muscular, contractile and probably is the locomotory organ. Mature rediae contain few germ balls and 2 to 5 cercariae in an advanced state of development. The cercariae leave the rediae to complete their development in the host tissues, which are usually packed with developing cercariae. The rediae possess a distinct pharynx which leads into a relatively wide intestine reaching almost to the posterior end of the body. The gut contents are dark brown in colour, and the birth pore is posterio-lateral to the pharynx.

Emergence and behaviour of the cercariae

The cercariae are shed in relatively large numbers in the presence of light and suitable temperature. They swim very actively, and when at rest they move in a worm-like manner. The free life of the cercariae is very short (30-

TABLE 15

Measurement in microns (μ) of 10 specimens of rediae, cercariae and metacercariae of Cercaria imbricata

	LIVING		FIXED (in 10% hot formol saline)	
	Range	Mean	Range	Mean
Rediae				
1. Body Length	500 - 1600	1078	420 - 980	693
2. Body Breadth	160 - 310	244	120 - 280	208
3. Pharynx diameter	50x50 - 100x100	82x78	50x50 - 90x80	76x70
4. Gut Length	370 - 1260	850	340 - 850	577
5. No. of developing cercariae	2 - 5	4	2 - 5	4
Cercariae				
Body Length	200 - 400	285	180 - 280	233
Body Breadth	120 - 190	155	110 - 150	126
Tail length	250 - 500	367	285 - 400	338
Tail breadth	30 - 50	42	25 - 44	35
Oral Sucker diameter	25x25 - 35x35	30x31	28x28 - 35x35	30x32
Right eye diameter	14x14 - 17x18	16x16	14x14 - 16x16	15x15
Left eye diameter	14x14 - 18x20	16x16	15x16 - 17x17	16x16
Median eye diameter	10x8 - 12x10	11x10		
Metacercariae				
1. Cyst diameter	155 - 180	168		
2. Cyst wall	15 - 20	17		
3. Outer Cyst wall	11 - 15	12.5		
4. Inner Cyst wall	4 - 6	4.5		

60 minutes), and they encyst readily in the open on glass containers, the shell of the snail host, and water plants.

The cercaria (Fig. 12B)

The cercariae are dark brown in colour and almost opaque due to the presence of cystogenous gland cells, which become more intense in mature naturally-emerged cercariae. Living cercariae have a size range of 200 to 400μ and a tail which is longer than the body (Table 15). The body wall of the cercaria is thin and devoid of finfolds, spines and hairs. The posterior end of the body is supplied with two dorso-lateral, well-developed locomotary adhesive pockets. The anterior end of the body has three pigmented eye spots arranged in the form of a triangle. The lateral eye spots have black pigment with a clear central area, and are formed at an early stage of the intra-redial phase of development. The third median eye is formed in the extra-redial phase of development from irregularly-distributed brown pigment in the anterior end of the body. The median eye is composed of diffused brown pigment compared with the compact black pigmentation of the lateral eye spots. The cystogenous gland cells of the body are numerous and packed with rounded granules.

The oral sucker is small and subterminal in position. The mouth leads into a short oesophagus, which bifurcates at the level of the transverse excretory vessel into relatively narrow intestinal caeca extending almost to the posterior end of the body, internal to but closely applied to the main excretory canals. The excretory bladder is composed of a single small spherical chamber which receives two conspicuous lateral canals filled with numerous excretory granules. These canals extend anteriorly to just behind the lateral eye spots, where they fuse to form a short median tube which extends to the posterior end of the oral sucker. In the mid-body region the primary excretory tubes give off short branches which in turn bifurcate into anterior and posterior secondary collecting tubules, the latter draining the capillaries of the flame cells of the body. A total of 24 flame cells were observed in the body, but their arrangement was difficult to work out because of the intensity of pigmentation and cystogenous gland cells, and hence no flame cell formula could be presented. The excretory pore is situated in a transverse groove between the body and tail. The caudal excretory duct is represented by remnants in mature naturally-emerged cercariae, but in immature cercariae the caudal duct is functional, extends to the middle of the tail, and opens laterally. The tail is stout, muscular, longer than the body and capable of great extension and contraction. The genital primordium is presented by a mass of cells anterior to the bladder.

Metacercaria (Fig. 12C)

The cercariae encyst in the open on glass containers, water plants and the shell of the snail host. In the process of encystment, the ventral surface is stuck hard to the substratum by the oral sucker and the adhesive pockets. Cystogenous fluid is secreted from the entire body surface enclosing the cercaria. The tail, which is lashing vigorously, is detached; the cyst wall is sub-spherical, flattened on the attached side and convex on the opposite side. Measurement of ten living metacercariae (Table 15) showed that they have a range of diameter of 155 to 180μ. The outer cyst wall measures 11 to 15μ and the inner cyst wall measures 4 to 6μ. Examination of the meta-cercariae one week after encystment showed that the median eye spot was lost and the pigmentation of the anterior end of the body had become more diffused. Three weeks after encystment all the eye spots were lost, and the body pigment-ation was greatly reduced.

4.1.3.1.2 Identification and comparison with related species

Following Rothschild's (1938a) classification the species under investigation falls into the Notocotyloidae group of cercariae, on the basis of the lack of aural lappets or collar, and furthermore, it belongs to the Yenchingensis subgroup. Cercariae belonging to the Yenchingensis subgroup and described from freshwater prosobranchiate gastropods are:

1) Cercaria imbricata Looss, 1893 (= Cercaria of Notocotylus imbricatus (Looss, 1893) Szidat, 1935) as described by Dönges, 1962; Odening, 1963, 1968; and Pike, 1969. Wesenberg-Lund (1934) described Cercaria imbricata from Bithynia tentaculata. He found the excretory system similar to that of Cercaria monostomi and the median eye inconspicuous. Rothschild (1938a) placed Cercaria imbricata Looss, 1893 as described by Wesenberg-Lund in the Monostomi subgroup. Dubois (1951) reported that Cercaria helvetica I Dubois, 1928 is synonymous to Cercaria imbricata and Odening (1963, 1968) considered Cercaria fennica I Wikgren, 1956 as synonymous to Cercaria imbricata.

2) Cercaria triophthalmia Faust, 1930.

3) Cercaria marilli Ameel, 1939.

4) Cercaria yenchingensis Faust, 1930.

5) Cercaria wesenberg-lundi Etges, 1956.

Cercaria wesenberg-lundi Etges, 1956 has minute lateral locomotory organs which are conspicuous in immature specimens, possesses a poorly differentiated pharynx and gut caeca extending to the level of the genital primordium, and is therefore clearly different from the present species. Cercaria triophthalmia Faust, 1930 has four or five concentric rows of spines around the anterior end of the body which differentiate it from the present cercaria. Cercaria marilli Ameel, 1939 is distinguished by the fact that the cystogenous cells contain rod-like granules, while in the present species the contents of the cystogenous gland cells are rounded. Cercaria yenchingensis Faust, 1930 can be differentiated by its very small size.

Cercaria imbricata Looss, 1893 as described by the above workers and the present species are similar in the general morphology of the cercaria and the size of the metacercaria. However, different observations were recorded in the excretory system and in particular the number of flame cells. Odening (1963, 1968) reported the flame cell formula as 2[(3 + 3 + 3) + (3 + 3 + 3)] = 36. In view of the difficulty of assessing the arrangement and number of flame cells, due to the presence

of pigment and cystogenous cells, the present species is referred to Cercaria imbricata Looss, 1893. The recovery of the adult worm Notocotylus imbricatus (Looss, 1893) Szidat, 1935 confirmed that the species under investigation is Cercaria imbricata.

4.1.4. Leptocercous Distome cercariae

Lühe (1909) defined the Distome cercariae as those forms which possess a ventral sucker distant from the posterior end of the body. He divided the Distome cercariae into eight groups, two of which were encountered in the present investigation, namely the Leptocercariae and the Furcocercariae.

The Leptocercariae were defined by Lühe (1909) as distomes in which the tail is simple, slender and narrower than the body at the point of its insertion. This group included the Gymnocephalous cercariae which are unarmed, the Echinostome cercariae with a head collar of spines and the Xiphidiocercariae with a stylet in the oral sucker.

In the present investigation, Bithynia tentaculata was infected with cercariae belonging to the Gymnocephalous and Xiphidiocercariae.

4.1.4.1. Gymnocephalous cercariae

Sewell (1922) considered the Gymnocephalous cercariae of Lühe (1909) as unnatural group and discarded the name "Gymnocephalous" in favour of four groups which he created, the Parapleurolophocerca, the Isospori, the Agilis and the Reflexae. Wesenberg-Lund (1934) indicated that Sewell's groups are not of any great value in the separation of cercariae which occur in Europe. He retained the group name "Gymnocephalous" and included the Pleurolophocercous cercariae (which Sewell placed in the Monostome cercariae) in the Gymnocephalous cercariae. Dawes (1956) used the group name Gymnocephalous cercariae to include Sewell's groups together with the Pleurolophocercous cercariae.

Three species of Gymnocephalous cercariae were found infecting Bithynia tentaculata in the present investigation. Two are referred to the Agilis group and the third species belongs to the Pleurolophocerca group.

4.1.4.1.1. Cercaria helvetica XIX Dubois, 1929.

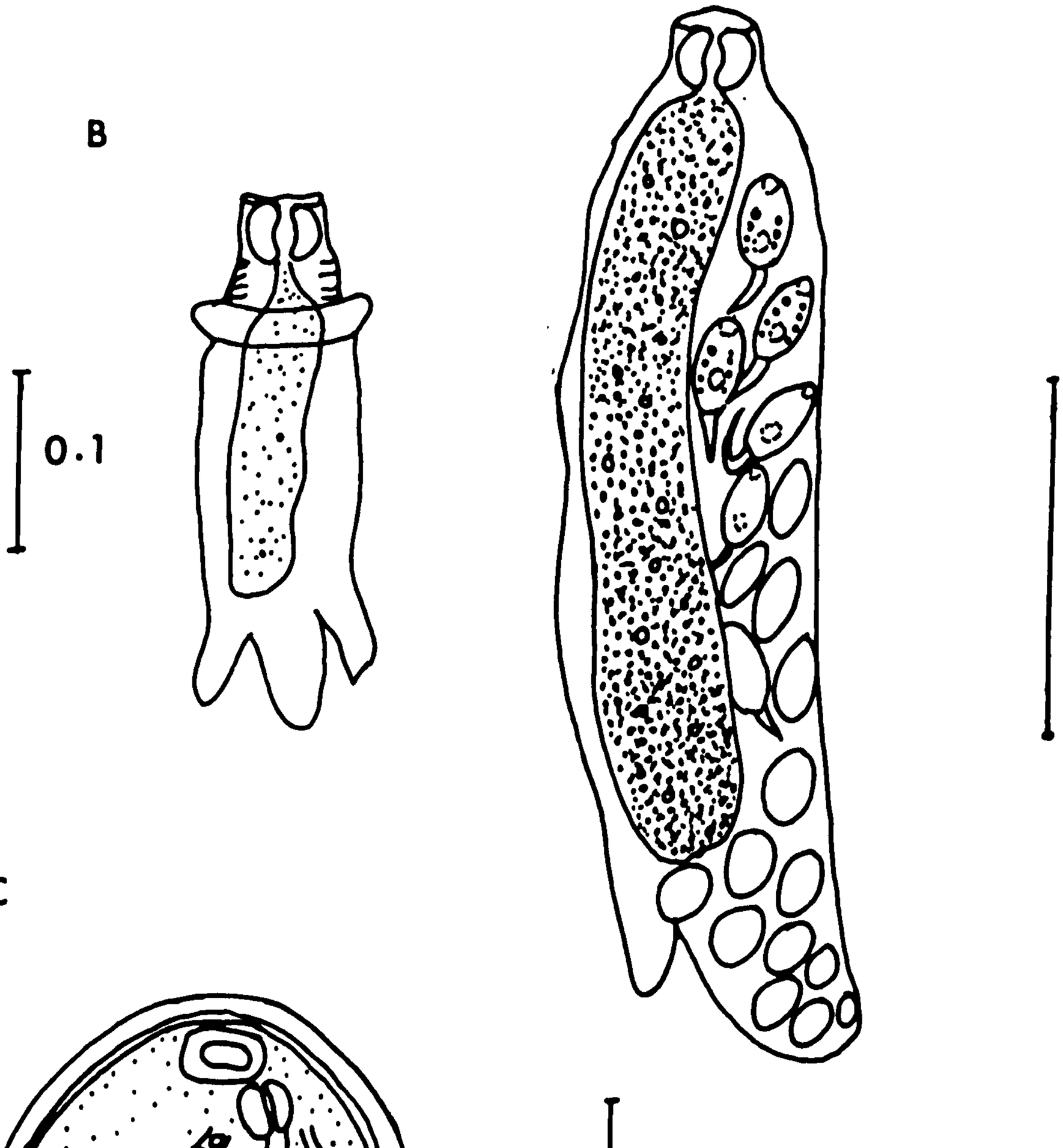
This cercaria was described by Dubois (1929) and Wesenberg-Lund (1934) from Bithynia tentaculata. The species was found infecting B. tentaculata in the present investigation and this is the first record of the species in British freshwater. The description of the cercaria is completed and the metacercaria described. The infection rate of this cercaria in B. tentaculata was 10.8%.

Fig. 13 The structure of the mature redia (A), immature redia (B) and the metacercaria of Cercaria helvetica XIX.

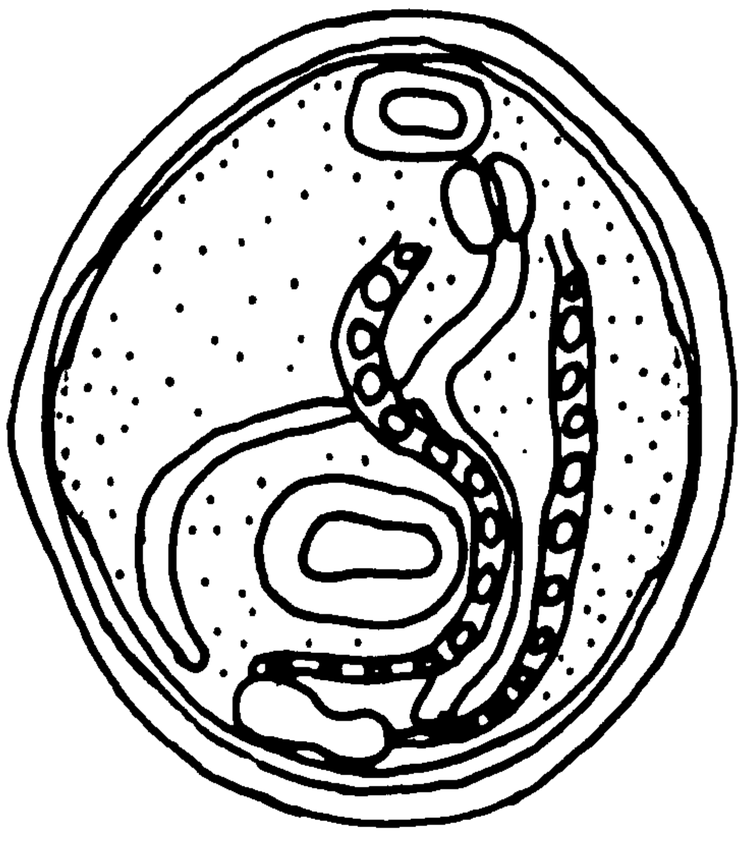
[Scale in mm.]

A

B



C



4.1.4.1.1.1. Description

Redia (Fig. 13)

The rediae occur within the digestive gland of the host and are readily separable from the host tissues. They vary in shape from sausage-like to sac-like, and their length is variable according to their degree of maturity (0.45mm to 2.0 mm) (Table 16). Mature rediae (Fig. 13A) contain 15 to 30 developing cercariae, which leave the rediae in an immature state and complete their development in the host tissues, where a large number of immature cercariae is always found. The immature redia (Fig. 13B) is rather active, and possesses a well-developed collar and ambulatory processes; the mature redia is feebly active, the collar is poorly developed, but the posterior lappets are well developed. The pharynx is large (0.078 x 0.091 mm in diameter), oval and muscular, and the anterior tip of the body is supplied with protrusible lips. The pharynx opens into a conspicuous sac-like gut that extends to more than half the body length, and in some cases as far as the posterior lappets. The gut contents are reddish-brown in colour. The birth pore is inconspicuous.

TABLE 16

Measurement in mm. of 10 rediae, cercariae and metacercariae of Cercaria helvetica XIX

	LIVING		Fixed in 10% hot formal saline	
	Range	Average	Range	Average
Rediae				
Body length	0.450 - 2.000	0.957	-	-
Body breadth	0.250 - 0.375	0.304	-	-
Pharynx diameter	0.06x0.08-0.1x0.13	0.078x0.091	-	-
Cercaria				
Body length	0.280 - 0.450	0.367	0.194 - 0.250	0.218
Body breadth	0.19 - 0.30	0.232	0.120 - 0.140	0.127
Tail length	0.32 - 0.50	0.390	0.280 - 0.350	0.312
Tail breadth at base	0.05 - 0.06	0.054	0.04 - 0.05	0.046
Oral sucker diameter	0.05x0.05-0.06x0.06	0.054 x 0.054	0.035x0.035-0.048x0.05	0.045 x 0.043
Ventral sucker diameter	0.065x0.065-0.080x0.080	0.073x0.071	0.056x0.056-0.07x0.07	0.062x0.058
Prepharynx length	0.01 - 0.012	0.011	0.006 - 0.012	0.01
Pharynx diameter	0.03x0.03 - 0.035x0.035	0.031x0.032	0.025x0.025-0.03x0.03	0.028x0.027
Metacercaria				
Cyst diameter	0.120 - 0.138	0.133		
Cyst wall	0.006 - 0.008	0.007		

Emergence and behaviour

The cercariae are shed in large numbers in the presence of light and at a suitable temperature. They are not shed in the dark. The cercariae are very active, and under laboratory conditions they swim continuously in a globular mass for one to two hours. As they become weaker they become concentrated at the bottom of the container, and move slowly in a characteristic manner in which the tail lashes and the body moves sideways. The cercariae show no special behaviour in response to light stimulation.

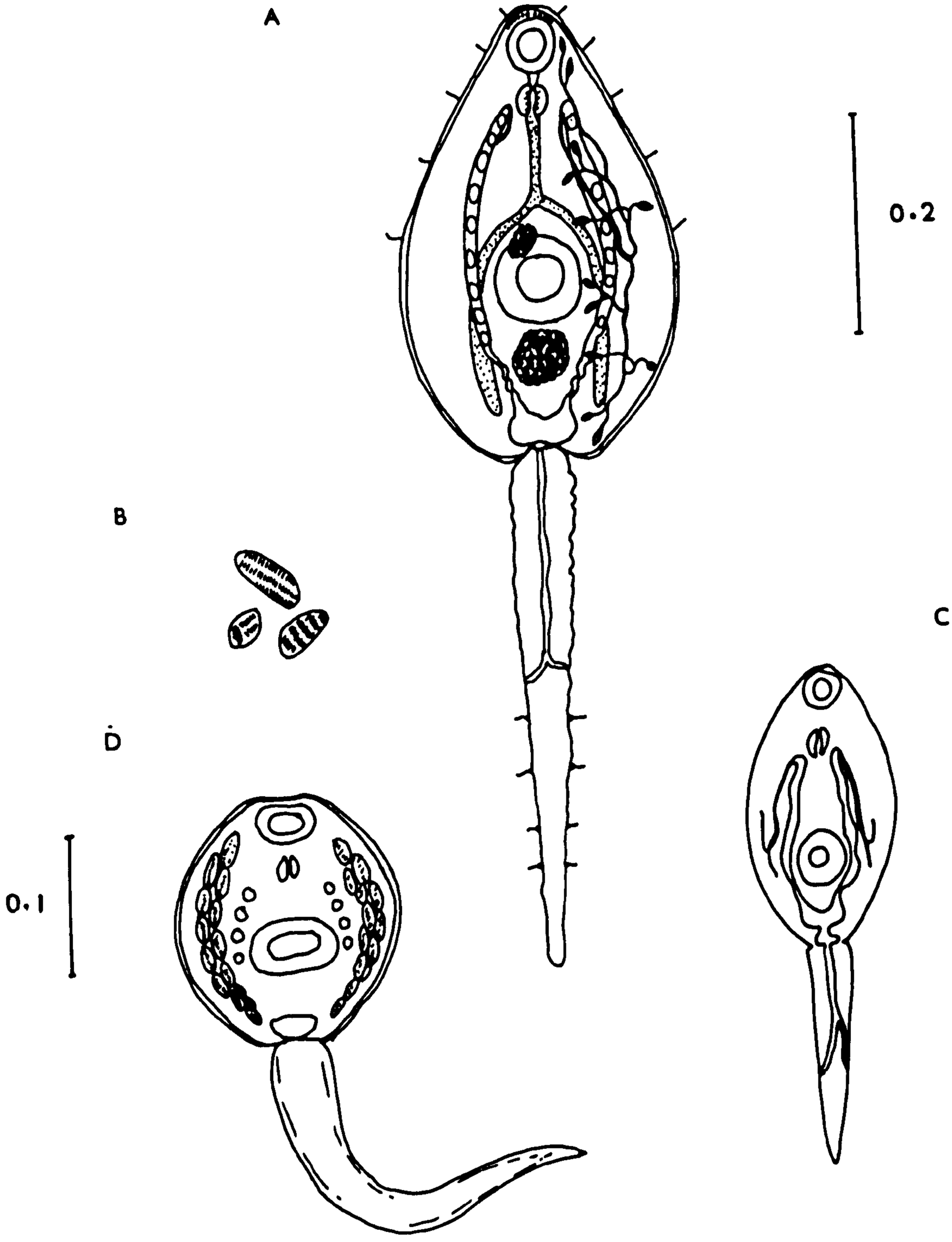
Cercaria (Fig. 14)

The shape of the body of the cercaria varies greatly according to the degree of contraction, being oval when extended and nearly spherical when contracted. Measurement of living and fixed specimens are shown in Table 16. The anterior end of the body is broad, and there seems to be a depression in the mid-body when the cercaria is moving slowly. The cercariae appear brownish in colour under the lower power of the microscope, due to the presence of cystogenous gland cells which are mainly concentrated into two lateral bands running near the edge of the body (Fig. 14D),

Fig. 14 The structure of Cercaria helvetica XIX.

Mature cercaria (A), cystogenous cells (B), immature cercaria (C) and the natural shape of the cercaria (D).

[Scale in mm.]


A
0.2
B
C
0.1
D
0.1

together with scattered cells in other parts of the body. The cystogenous gland cells are large, pear-shaped or elongated, and filled with rod-like granules arranged in bundles, each cell containing 4 to6 bundles (Fig. 14B).

The body is covered with a thick cuticle which is devoid of spines and papillae except for five pairs of hairs scattered in the anterior half of the body (Fig. 14A). The oral sucker is subterminal and nearly spherical in shape. The ventral sucker is always larger than the oral sucker, and is circular in outline, freely protrusible and placed just below the middle of the body. The mouth leads into a short prepharynx, followed by a well developed muscular pharynx and a long oesophagus that divides just anterior to the acetabulum into two long gut caeca which extend to the posterior end of the body. The gut contents are granular. The penetration gland cells are obscured by the cystogenous matter, but there are twelve openings at the anterior end of the oral sucker which are presumably the openings of the ducts of the penetration glands.

The excretory bladder is thin walled and consists of a single large rectangular chamber which is cleft anteriorly. The primary excretory ducts enter the bladder laterally, and each of these ducts is narrow and wavy posterior to the acetabulum, and wider and straight anteriorly(Fig. 14A). However,

the primary excretory ducts posterior to the acetabulum may become enlarged, depending on the contraction and expansion of the bladder. Probably there are sphincter muscles regulating the entrance of fluid into the bladder and preventing its back surge when the bladder contracts. Each of the primary excretory ducts contains 10 to 16 large refractile excretory granules which are irregular in shape and variable in size. The primary excretory vessels extend anteriorly to the level of the pharynx, where each loops on itself and passes posteriorly to bifurcate laterally to the acetabulum into anterior and posterior secondary collecting ducts. The anterior secondary collecting tubule on each side receives three ducts, each connected with two flame cells. The posterior collecting tubule receives a similar number of ducts and flame cells. The flame cell formula may be expressed as:

$$2\,[\,(2+2+2)\;+\;(2+2+2)\,]=24$$

The excretory bladder opens to the exterior through the excretory pore, which is situated in a small slit at the body-tail junction. The caudal excretory duct persists in the mature naturally-emerged cercariae, and bifurcates in the anterior half of the tail into two branches opening laterally. However, in the mature naturally-emerged cercariae, the caudal duct loses its function of expelling excretory wastes

to the outside, a role now taken over by the excretory pore at the body-tail junction. In immature cercariae (Fig. 14C) the excreta are discharged to the outside through the excretory pore located in the tail.

The tail is simple, without finfolds, and aspinose except for four pairs of hairs on papillae in the distal half of the tail. There are no caudal pockets, but the tail is a little withdrawn into the body. The tail is longer than the body, and capable of extension and contraction due to the presence of an outer layer of circular muscles and an inner layer of longitudinal muscles extending to the tip of the tail. The genital system consists of a large post-acetabular mass and a small pre-acetabular one.

Metacercariae (Fig. 13C)

Encystment never takes place in the open. The cercariae encyst in the body of Bithynia tentaculata. The cysts are not found in the tissues of the snail, but are always located between the mantle and the shell. The cysts are usually spherical in shape, but in heavy infections they may assume different shapes or become attached together in a mass. The cyst diameter varies from 0.120 to 0.138 mm. with an average of 0.133 mm (Table 16). The cyst wall is thin and measures 0.006 to 0.008 mm.

Experimental infections to obtain the adult worm were unsuccessful and hence further development is unknown.

4.1.4.1.1.2 Identification and comparison with related species

This cercaria is a member of the Agilis group of Gymnocephalous cercariae, erected by Sewell (1922), together with the Reflexae group, to include those cercariae which showed an affinity with the Echinostome cercariae yet lacked a collar of spines. Its large size, together with the absence of tail finfolds, confirms its inclusion in the Agilis group. A review of the literature revealed that about fifty-one cercariae of this group have been described. The following cercariae differ from the present species in the possession of spines either on the entire body surface or limited to certain parts of the body.

Cercaria circumstricta Faust, 1922; C. semi-robusta Faust, 1924; Cercaria of Psilotrema oligoon (Linstow, 1887) as described by Pike (1968a), (= cercaria of Psilotrema spiculigerum (Muhling, 1898) Mathias (1925), (= Cercaria tuberculata Filippi, 1854 as described by Wesenberg-Lund (1934)); Cercaria chitinostoma Faust, 1930; C. catenadena Faust, 1930; C. durbanensis Porter, 1938; cercaria of Fasciola gigantica Porter, 1938 (= C. pigmentosa Cawston, 1919); C. ornatosoma Cable, 1935 as described by Cable (1938); C. limosae Hedrick, 1943; C. ameeli Hedrick, 1943; C. palegae Goodman, 1951; C. ituriensis Fain, 1953; C. llangorsensis

Probert, 1965; C. frondicola Pike, 1968 and C. granocutis Pike, 1968.

The presence of papillae on the body characterises cercaria of Fasciola hepatica Thomas, 1883 and Cercaria papillosa Filippi, 1854 as described by Wesenberg-Lund (1934). While Cercaria cystogenata Probert, 1965 differs from the present species in the possession of numerous hairs on the body surface in having two-chambered excretory bladder and in the smaller size of the cysts. Other cercariae like Cercaria agilis Filippi, 1858; C. indicae XLI Sewell, 1922; C. planorbidis Porter, 1938; C. klarbosiae Porter, 1938; C. morijae Porter, 1938; and C. pomacea Nasir and Diaz, 1968 differ from the present species in the lack of a pharynx or oesophagus and intestinal caeca.

Cercaria gracilis O'Roke, 1917; C. redicystica Tubangui, 1928; C. congellae Porter, 1938; and C. derusti Porter, 1938 are different in the possession of gut caeca which do not extend to the posterior end of the body. Cercaria complicata Faust, 1930; C. densacutis Khan, 1960; Cercaria grandis Wesenberg-Lund, 1934; Cercaria incognita Szidat, 1937; Cercaria amnicolensis Etges ,1956; and Cercaria broederstroomiae Porter, 1938 encyst in the open and are therefore different from the present species which encysts within the snail host.

Gymnocephalous cercaria A Wikgren, 1956 has four pairs of lateral oesophageal glands. Gymnocephalous cercaria B Wikgren, 1956 is poorly described, but the presence of numerous excretory granules in the main excretory canals differentiate it from the present species. Similarly, Gymnocephalous cercaria C Wikgren, 1956 is inadequately described, and probably the bilobed excretory bladder distinguishes it from the present species.

Cercaria of Psilostomum ondatrae Price, 1931 as described by Beaver (1939), (= C. thomasi McMullen, 1938 as described by Beaver (1939) and Kuntz (1951), has a pair of lateral oesophageal diverticula, lacks cystogenous gland cells, possesses a two-chambered excretory bladder and encysts in freshwater fish. Cercaria helvetica XVII Dubois, 1929 (= C. Sphaeridiotrema globulus Szidat, 1937); and C. gigantocerca Szidat, 1937 have characteristic broad tail stems. Cercaria fusiformis O'Roke, 1917 differs from the present species in having isodiametric suckers and a marked collar. Cercaria helvetica XVIII Dubois, 1929 has a black body and a ventral sucker smaller than the oral sucker. Echinostome cercaria No.2 Petersen, 1931 is inadequately described, and the conspicuous excretory ducts are probably misinterpreted as intestinal caeca since he describes the pharynx as being posterior to the intestinal bifurcation; moreover, this cercaria has a distinct collar without collar spines. Cercaria lileta Fain, 1953 and

C. symphoriani Fain, 1953 differ from the present species in the possession of numerous flame cells in the body (68 or more flame cells).

The present cercaria closely resembles Cercaria helvetica XIX Dubois, 1929 as described by Dubois (1929) and Wesenberg-Lund (1934) and Cercaria albinea Khan, 1960. Cercaria albinea Khan, 1960 has a two-chambered excretory bladder and primary excretory ducts which join to form a median duct before opening into the bladder. Furthermore, although C. albinea has twenty-four flame cells, their pattern of arrangement is different from that encountered in the present species. In addition, Khan (1960) reported that cysts of Cercaria albinea are found in the digestive gland of the host. In the present species the cysts are always found between the shell and the mantle. Finally, in C. albinea, the cystogenous gland cells occupy almost the whole of the body surface, while in the present species two broad lateral bands of cystogenous gland cells are quite distinct. Hence it is clear that C. albinea is different from the present species.

Cercaria helvetica XIX Dubois, 1929 as described by Dubois (1929) and Wesenberg-Lund (1934) is similar to the present species in the lack of spines or papillae on the body surface and the presence of two broad bands of cystogenous cells filled with rod-like

granules. Moreover, in both species the primary excretory ducts contain few large excretory granules and the ventral sucker is larger than the oral sucker. However, Dubois (1929) and Wesenberg-Lund (1934) were not certain about the ends of the intestinal caeca, nor did they give much information on the structure of the excretory system; and no reference was made to the encystment behaviour of the cercaria.

Thus a detailed comparison of the two species is difficult, but in view of the similarity of the two species the present cercaria is referred to Cercaria helvetica XIX Dubois, 1929. A complete description of the cercaria is presented, and this is the first record of the species from British freshwater.

4.1.4.1.2 Cercaria granocutis Pike, 1968

This cercaria was described by Pike (1968a) from Bithynia tentaculata. In the habitat examined in the present work, six percent of B. tentaculata were infected with this species. The following description is in general agreement with that reported by Pike (1968a). Although some differences in observation and interpretation were noted, they were considered as insufficient for the separation of the two species. These differences were pointed out in the part dealing with identification and comparison with related species.

4.1.4.1.2.1 Description

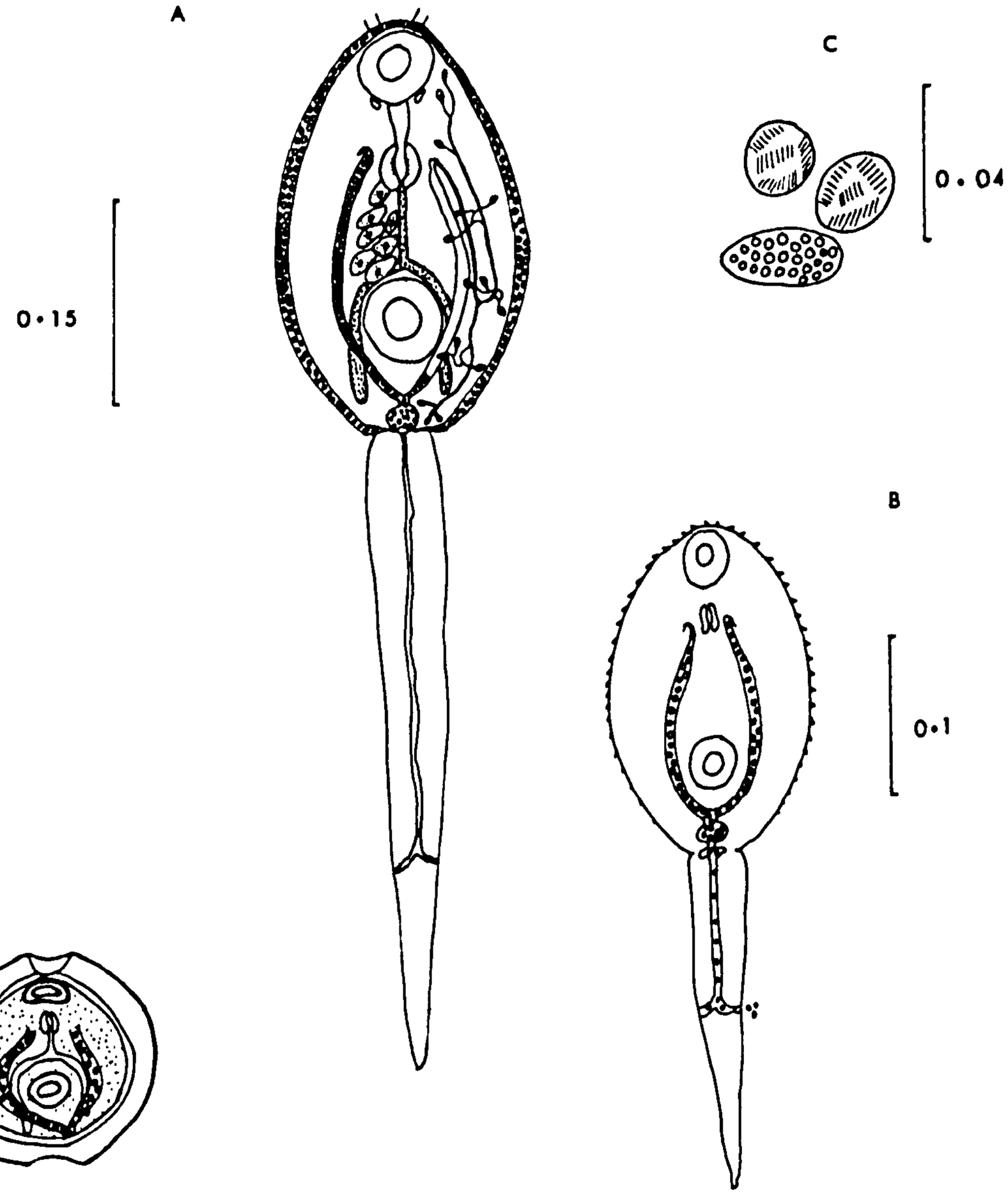
Redia

The rediae are similar to those of Cercaria helvetica XIX (Fig. 14); they are sausage-shaped, and easily separable from the host tissues. They vary in size according to the degree of maturity (0.3 to 1.5 mm.). Immature rediae are motile and possess a well-developed collar and ambulatory processes, while mature rediae show slight motility, have a poorly developed collar, and the posterior lappets are well developed. Mature rediae contain about 10 to 25 developing cercariae, which complete their development in the host tissue. The anterior end of the redia is supplied with a protrusible

Fig. 15 The structure of Cercaria granocutis.

Mature cercaria (A), immature cercaria (B), Cystogenous cells (C) and the metacercaria (D).

[Scale in mm.]


A
0·15
C
0·04
B
0·1
D
0·1

lip, the pharynx is well developed and measures 0.10 x 0.08 mm in diameter. The pharynx leads into a saccular gut that extends as far as the posterior locomotory organs. The gut contents are dark brown in colour. The birth pore is posterio-lateral to the collar.

Emergence and behaviour

The cercariae are shed in large numbers in the presence of light and at a suitable temperature. They are not shed in the dark. The cercariae swim continuously and tend to aggregate towards the illuminated part of the container. Their free life is very short, and they encyst readily in the open or die. Before death the cercariae creep on the bottom with a worm-like motion, using the suckers as adhesive organs.

Cercaria (Fig. 15)

The body of the cercaria is dark in colour due to the presence of numerous cystogenous gland cells. The shape of the body varies according to the degree of contraction, being oval when stretched and nearly spherical when contracted. The measurements of living and fixed specimens were shown in Table 17. The anterior half of the body is covered with large spines arranged in regular rows, but these are inconspicuous because they are overlaid by a thick granular layer into which

TABLE 17

Measurement in microns of 10 specimens of

Cercaria granocutis

	LIVING		FIXED	
	Range	Average	Range	Average
Body length	160 x 400	284	140 - 310	237
Body breadth	140 x 230	179	125 - 210	158
Tail length	300 - 480	383	290 - 380	327
Tail breadth	42 - 50	47	38 - 50	48
Oral sucker diameter	56x56 - 75x75	62 x 62	40x40 - 50 x50	46 x 46
Ventral sucker diameter	56x56 - 75x75	63 x 63	40x40 - 50x50	46 x 46
Pharynx diameter	32x25 - 40x30	37 x 29		
Prepharynx length	25 - 35	30		

they project (Fig. 15A). In immature unemerged cercariae (Fig. 15B), in which this layer is not yet formed, the spines are conspicuous in the anterior half of the body length, and then they become smaller in size and gradually disappear leaving the posterior third of the body devoid of spines. There are also two pairs of hair-like processes on each side of the oral sucker.

The thick cuticular granular layer of the body stains intensely with neutral red. The body is filled with numerous cystogenous gland cells of different types (Fig. 15C). Circular cells containing rod-like granules are distributed over the whole surface of the body beneath the cuticle. Below these circular cells are found large nearly oval cells with granular contents occupying the internal body surface, except for the region between the pharynx and the acetabulum. This region has twelve circular to oval nucelated cells with fine granular contents. These cells are probably gland cells of some kind, but neither their ducts nor their openings in the oral region were observed.

The oral sucker is subterminal and spherical in shape. The acetabulum is situated in the posterior half of the body, it is spherical, very protrusible, and equal in size to the oral sucker. The mouth leads into a short prepharynx which is followed by a large muscular pharynx. The oesophagus is long, and divides anterior to the acetabulum into the gut

caeca which extend to the posterior end of the body.

The excretory bladder is thin-walled and composed of two chambers, a small anterior chamber leading into a larger posterior one. The anterior chamber receives the two primary excretory ducts, and the posterior chamber opens to the outside through the excretory pore which is situated at the posterior end of the body. The bladder and the primary excretory ducts are filled with numerous refractile excretory granules, which at times are expelled, via a dilation which appears between the body and tail, to the outside through the excretory pore. The primary excretory vessels extend anteriorly to the pharynx, where each loops upon itself and runs posteriorly to bifurcate, lateral to the acetabulum, into anterior and posterior secondary collecting vessels. The anterior duct runs forwards and drains three sets of three flame cells each, while the posterior branch accommodates two sets only. There is a total of thirty flame cells, whose arrangement may be expressed by the formula:

$$2\ [\ (3 + 3 + 3)\ +\ (3 + 3)\ ]\ =\ 30$$

In immature cercariae the caudal excretory duct which bifurcates in the distal half of the tail conveys the excretory waste to the outside (Fig. 15B). In mature naturally-emerged cercariae the excretory pore is formed at the posterior end of the body, and as a result the caudal duct loses its function of conveying the excretory waste of the body to the outside.

The tail is inserted in a depression in the posterior end of the body. It is longer than the body, muscular and devoid of finfolds, spines and hairs. The genital primordium is represented by a mass of cells in the postacetabular region. It is of interest to note the presence of two oval refractile structures at the posterior border of the oral sucker. Similar structures have been reported by many workers (e.g., Probert, 1965b; Pike, 1968a) but their function is unknown.

Metacercaria (Fig. 15D)

The cercariae encyst in the open, and in the laboratory encystment was observed on the walls of the glass container and on microscope slides. Under the microscope an increase in the intensity of light stimulates the cercaria to encyst. When a piece of the water plant Ludwigia is introduced into the container, the cercariae showed no preference for it as encystment substrate. The onset of encystment is marked by the production of a sticky secretion over the entire body surface, forming a wall within which the organism is enclosed. The whole process is very rapid and is completed within a few minutes. Encystment varies with the individual organisms; some encyst shortly after their emergence from the snail host, others encyst after a short free existence. The cyst wall is formed of two layers, an outer wide layer and an inner narrow

layer. The cercaria remains active for some time within the cyst. The cyst diameter varies from 0.19 x 0.19 mm to 0.14 x 0.12 mm. A similar pattern of events has been reported by Pike and Erasmus (1967) for the cyst of Psilotrema oligoon (Linstow, 1887) Odhner, 1913.

Further development is unknown.

4.1.4.1.2.2 Identification and comparison with related species

The present species belongs to the Agilis group of Gymnocephalous cercariae and is closely similar to the cercaria of Psilotrema oligoon as described by Pike, 1968a; Cercaria complicata Faust, 1930; C. densacutis Khan, 1960; C.llangorsensis Probert, 1965; C. frondicola Pike, 1968 and C. granocutis Pike, 1968.

The cercaria of Psilotrema oligoon differs from the present species in having a single chambered excretory bladder and in the manner in which the primary excretory ducts enter the bladder. Cercaria frondicola Pike, 1968 differs in having distinct spines in naturally-emerged cercariae, in its preference for vegetation as a substratum for encystment, and in the arrangement and number of flame cells. Cercaria complicata Faust, 1930 and Cercaria densacutis Khan, 1960 possess aspinose bodies, and are therefore different from the present species. Cercaria llangorsensis Probert, 1965 has

conspicuous spines in the anterior half of the body and a larger body and cyst size, and therefore is different from the present species.

Cercaria granocutis Pike, 1968 and the present species are similar in the body spination, general structure of the excretory system and encystment behaviour, and therefore the present species is referred to Cercaria granocutis Pike, 1968. However, the following discrepancies were noted between my observations and those of Pike (1968a). Pike stated that the ventral sucker is slightly larger than the oral sucker, yet his data probably suggest that the oral and ventral suckers are isodiametric as found in the present investigation. Secondly, my observations indicate that the excretory bladder is formed of two chambers, and although Pike's figure showed the second chamber, he considered it as a common opening of the primary excretory canals. Thirdly, in mature naturally-emerged cercariae, the excretory pore is formed at the posterior end of the body. The excreta are discharged through this pore and not through the caudal duct. In immature cercariae (Fig. 15B) the excretory pore is not formed and the excreta are discharged through the caudal duct. Thus Pike's description applies to the immature cercariae, but not the mature naturally-emerged cercariae. Finally, the primary excretory ducts divide lateral to the acetabulum into the anterior and posterior secondary collecting tubules. The former drains three sets of three flame cells each, whereas the latter drains two sets of three flame cells each. Thus the flame cell formula is:

$$2\,[\,(3+3+3)\;+\;(3+3)\,]\;=\;30$$

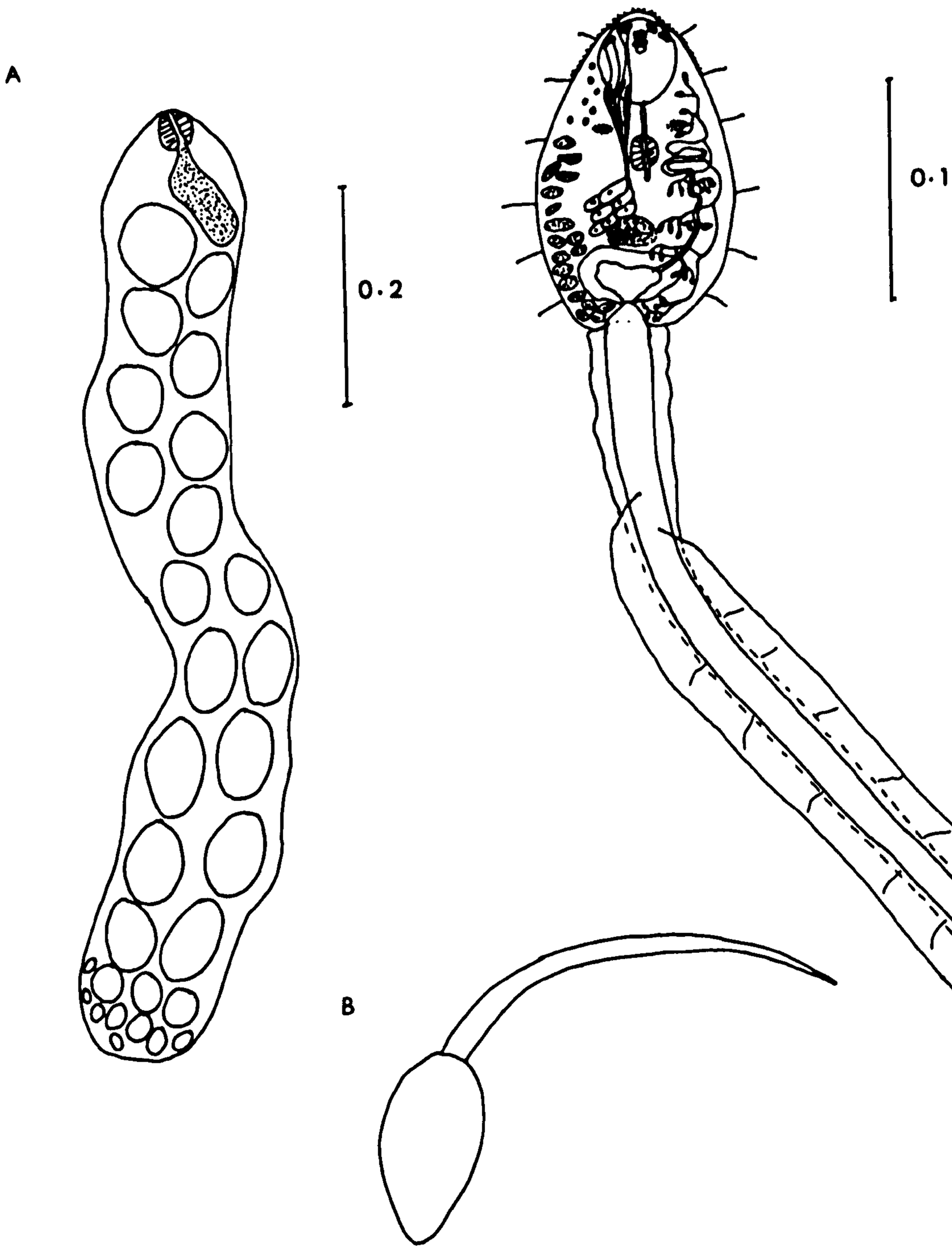
4.1.4.1.3 Cercaria lophocerca Filippi, 1859

This cercaria was reported by Filippi (1859) and later redescribed by Dubois (1929) and Wesenberg-Lund (1934) from Bithynia tentaculata. In British freshwater the species was described by Llewelyn (1957), Khan (1960) and Probert (1965b) from B. tentaculata. The following description is in agreement with earlier accounts, except that more details of the excretory system were observed. The infection rate of B. tentaculata with this species was very low (0.5%).

Fig. 16 The structure of Cercaria lophocerca.

Redia (A), resting position of the cercaria (B), the cercaria (C).

[Scale in mm.]


A
0·2
C
0·1
B

4.1.4.1.3.1 Description

Redia (Fig. 16A)

The redia are present in the digestive gland of the host. They are inactive, long, slender and lacking both collar and ambulatory processes. The pharynx is well developed, and the gut extends only a short distance behind the pharynx. The birth pore is inconspicuous. Each redia contains 15 to25 germ balls and developing cercariae. The latter leave the rediae in an early stage of development and mature in the host tissues. The development of the eye spots, tail and finfolds occurs in the tissues of the host. The largest redia measured 2.0 mm in length and 0.25 mm in width, the diameter of the pharynx is 0.04 x 0.03 mm.

Emergence and behaviour

In the laboratory, the cercariae are discharged in large numbers at a suitable temperature both under artificial illumination or in complete darkness. The cercariae swim in jerks, and when at rest the body is kept downwards and the tail is curved slightly (Fig. 16B). The cercariae react positively to light, and tend to congregate towards the illuminated side

TABLE 18

Measurement in mm of twenty naturally emerged

Cercaria lophocerca fixed in 10% hot formal saline.

	RANGE	AVERAGE
Body length	0.100 - 0.140	0.120
Body breadth	0.046 - 0.080	0.062
Tail length	0.370 - 0.400	0.378
Tail breadth	0.020 - 0.024	0.020
Oral sucker diameter	0.028x0.028 - 0.04x0.035	0.032x0.026
Right eye	0.008x0.006 - 0.01x0.008	0.01x 0.006
Left eye	0.008x0.006 - 0.01x0.008	0.01 x 0.006
Pharynx	0.009x0.01 - 0.011x0.015	0.01 x 0.013

of the container; their free life is short, and few can survive for 24 hours. Decaudation is frequent and usually occurs before the death of the cercaria.

Cercaria (Fig. 16C)

The body of the cercaria is small, oval in shape and measures 0.1 to 0.14 mm in length (Table 18). The anterior end of the body is encircled by rows of backwardly directed spines which cover the penetration organ. The rest of the body is spineless, but six pairs of long hair-like structures are present. The cercariae are golden brown in colour when viewed under the low power of the microscope, due to the presence of numerous cystogenous gland cells with coarse granular contents and pigment masses. The eye spots are elliptical in shape and are composed of small spherical granules.

The oral sucker is subterminal and globular. There are three rows of transverse spines dorsal to the mouth, each row containing five to eight anteriorly-directed spines. These spines are used as scraping devices in penetrating the second intermediate host (usually a cold blooded vertebrate). The mouth is connected to the pharynx by a poorly differentiated prepharynx. The pharynx is also poorly differentiated, it measures 0.010 x 0.013 mm in diameter, and it is situated just

posterior to the eye spots. Behind the pharynx a short narrow oesophagus is seen, but the intestinal caeca are lacking. The ventral sucker in naturally-emerged cercariae is rudimentary, and represented only by a more or less deep depression anterior to the excretory bladder. However, this organ is well developed in young immature cercariae. There are seven pair of penetration glands which open at the anterior end dorsal to the mouth. The ducts of these glands are arranged in four bundles, two median of four ducts each and two lateral of three each. The ducts follow the gut closely, and lead down into seven pairs of glands situated in the middle of the body above the genital primordium.

The excretory bladder is thick walled, large, and oval or transversely oval. The excretory pore is situated at the body-tail junction and is easily seen when the tail is lost. Two main lateral excretory ducts lead anteriorly from the bladder to the anterior third of the body. Each duct undergoes several convolutions, and divides at the level of the pharynx into anterior and posterior secondary collecting tubules (Fig.16C). Each of the anterior tubules receives two tertiary collecting tubules, each draining a set of five flame cells from the anterior end of the body. The posterior excretory tubules proceed backwards, and each receives three tertiary collecting tubules. Each of the latter drain five flame cells from the posterior end of the body. Hence the flame cell formula may be expressed as:

$$2\ [\ (5 + 5)\ +\ (5 + 5 + 5)\ ]\ =\ 50$$

The caudal excretory duct was not observed in the mature cercaria. The genital primordium is represented by a mass of deeply staining cells lying in front of the excretory bladder.

The tail is very long and set in a deep socket provided with thick folded lateral walls which were considered by Khan (1960) to be spines. The lateral finfolds begin at the anterior end of the tail, where they are broad, gradually narrow down to the anterior third, and then continue as a narrow strip around the tip of the tail. The dorsal finfold is broad and extends from the anterior third of the tail and then continues around the tip of the tail to become the ventral finfold. The latter terminates posterior to the point where the dorsal finfold begins.

4.1.4.1.3.2 Identification and comparison with related species

The present species belongs to the Pleurolophocerca group of Gymnocephalous cercariae. Rothschild (1938b) according to Dawes (1956) considered the following characters as important in the separation of the Pleurolophocercous cercariae:

1) The shape and size of the body under a coverslip at death.

2) The precise extent, position and shape of the caudal finfolds.

3) The pigmentation of the body.

4) The behaviour and length of life of the cercaria.

Cable and Wheeler (1939) reported "The precise nature of the tail, shape of the excretory vesicle and behaviour of cercariae are of great importance in distinguishing pleurolophocercous cercariae." They added that the excretory pattern may be of specific value, but is difficult to trace due to the presence of glandular structures and pigment masses in the majority of these cercariae. Martin (1950) illustrated

four types of Pleurolophocercous cercariae, separated on the basis of the shape and development of the tail finfolds.

The species under investigation can be distinguished by the presence of both lateral and dorso-ventral finfolds from other Pleurolophocercous cercariae described from freshwater molluscs, except Cercaria indicae VII Sewell, 1922; C. indicae VIII Sewell, 1922; C. lophocerca Filippi, 1859, as described by Dubois (1929), Wesenberg-Lund (1934) and Khan (1960); C. pleurolophocerca Sonsino, 1892 as described by Langeron (1924); C. parvomelianiae Tubangui, 1928; C.plotiopsis Johnston and Simpson, 1939; and C. sp. Kuntz, 1952.

Cercaria indicae VIII, C. parvomelaniae and C. sp. Kuntz, 1952, can be distinguished by the bicornuate form of the excretory bladder. C. pleurolophocerca as described by Langeron (1924) differs from the present species in the absence of a pharynx, the possession of gut caeca and a ventral sucker, while C. plotiopsis is distinguished by its spinose body and smaller size. C. indicae VII is differentiated by the lack of finfolds in the middle of the tail and the number and position of the penetration gland cells.

The present species is closely similar to Cercaria lophocerca Filippi, 1859, as reported by Dubois (1929), Wesenberg-Lund (1934), and Khan (1960) in its morphological

characters, behaviour and incidence. However, observations on the arrangement and the number of flame cells were different. Dubois (1929) saw five pairs of flame cells in the body, and Khan (1960) observed ten pairs. The present investigator found twenty-five pairs of flame cells in the body, arranged in groups of five. Such differences could be explained by the difficulty of observing the excretory system in these opaque cercariae, and by the lack of adequate material. The present worker is of the opinion that the species under investigation is Cercaria lophocerca Filippi, 1859.

4.1.4.2 Xiphidio-cercariae

The Xiphidiocercariae were defined by Lühe (1909) as Distome cercariae with a stylet at the anterior end and which possess a slender tail, lack eye spots, develop in sporocysts and encyst within an intermediate host. Lühe (1909) recognised four groups of Xiphidio-cercariae - the Microcotylae, Virgulae, Ornatae and Armatae.

The cercaria encountered in the present investigation belongs to the Microcotylae subgroup, which Lühe (1909) defined as very small cercariae whose body length is less than 0.2 mm. The acetabulum is smaller than the oral sucker and lies behind the middle of the body. The cercariae have two to four pairs of penetration glands and a simple bicornuate excretory bladder. Sewell (1922) divided the Microcotylae cercariae into the Cellulosa, Pusilla, Parapusilla and Vesiculosa groups.

4.1.4.2. 1 Cercaria parvus Khan, 1961.

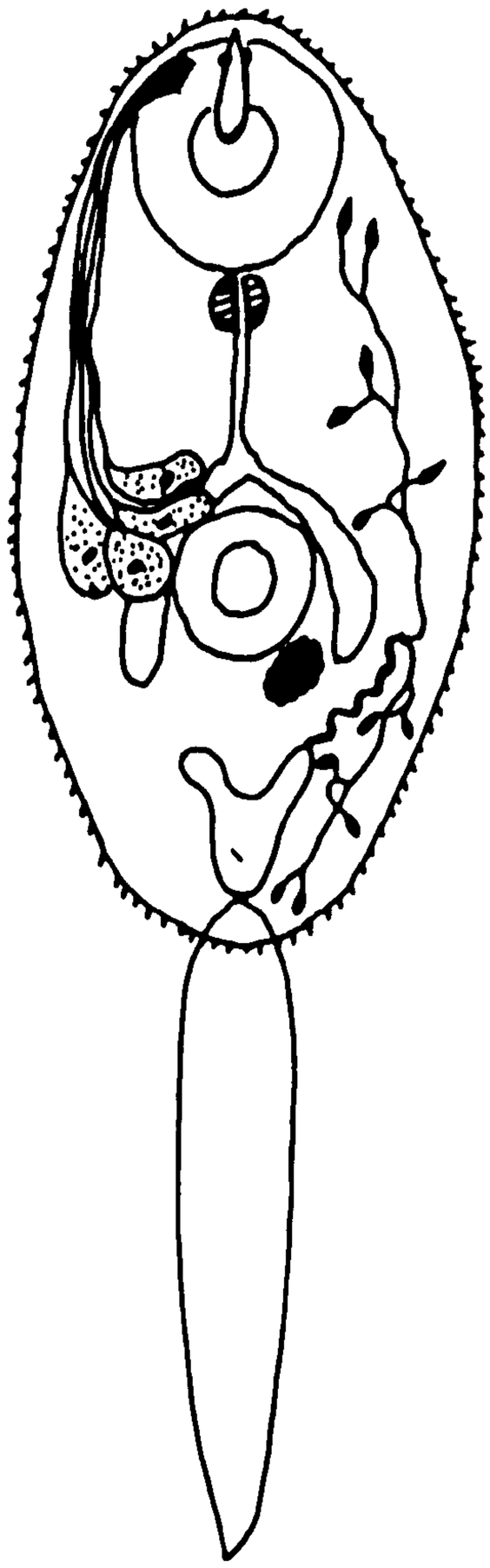
This cercaria was adequately described by Khan (1961) and Pike (1967) from Bithynia tentaculata. Pike (1967) gave an account of the metacercaria of this species. The following description is in agreement with the accounts reported by the above authors for the cercaria and metacercaria. The infection rate of B. tentaculata was 5.9%.

Fig. 17 The structure of Cercaria parvus.

The sporocyst (A), cercaria (B), stylet (C) and the metacercaria (D).

[Scale in mm.]

B



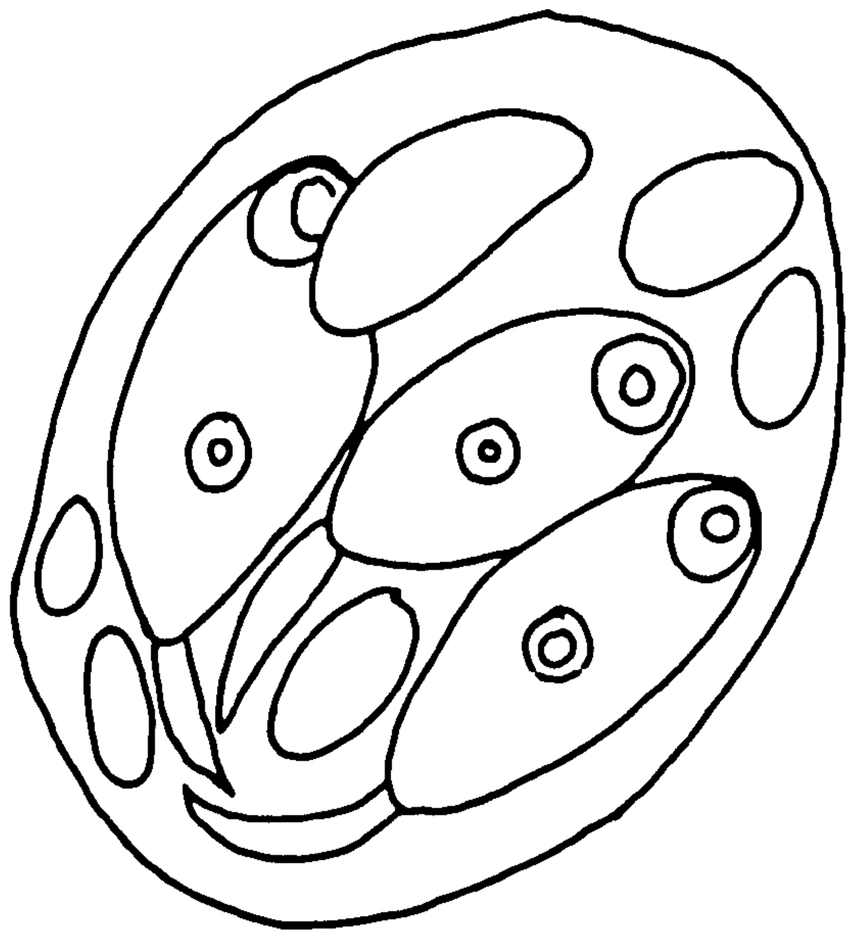
C

0.02

0.05

0.1

A



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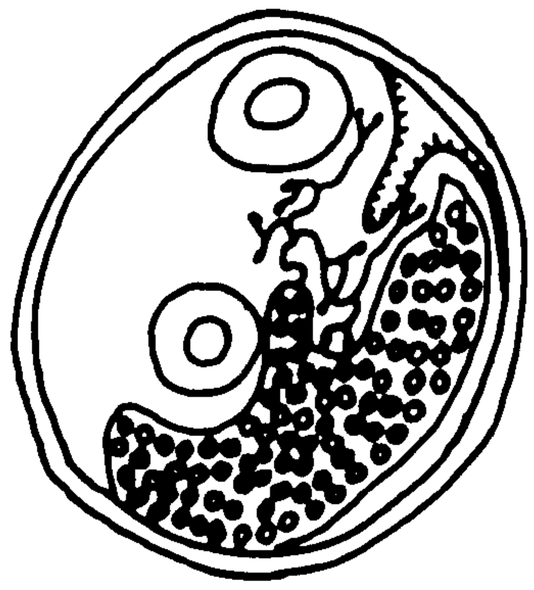

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4.1.4.2.1.1 Description

Sporocysts (Fig. 17A)

Numerous vesicular to slightly elongated sporocysts are found in the digestive gland of the snail host. The sporocysts are colourless, inactive, and contain three to five developing cercariae and a few germ balls. The birth pore is terminal, and the size of the living sporocyst varies from 0.15 to 0.30 mm in length and 0.12 to 0.18 mm in breadth.

Emergence and behaviour

The cercariae are shed in large numbers both under artificial illumination and in the dark, at a suitable temperature. The free life of the cercaria is short, and few survive for 24 hours.

The cercaria (Fig. 17B)

The cercaria is small in size (Table 19) and the whole body surface is covered with small backwardly-directed spines. The stylet lies at the anterior end of the body, it has a fine tip and thickened shoulders. The walls of the stylet are also thickened for more than half of its length (Fig. 17C).

TABLE 19

Measurement in microns of 10 living specimens of Cercaria parvus

	LIVING	FIXED
Body length	100 - 170	129
Body breadth	25 - 80	49
Tail length	30 - 120	78
Tail breadth	15 - 20	18
Oral sucker diameter	25 x 25 - 40 x 40	35 x 33
Ventral sucker diameter	15 x 15 - 25 x 25	21 x 21
Pharynx diameter	8 x 8 - 10 x 10	9 x 9
Stylet length	16 - 20	18
Stylet width at base	5	
Stylet width at shoulder	4	

The body contains numerous cystogenous gland cells with a granular content. There are four pairs of penetration glands lying anterior to the ventral sucker, and their ducts open at the anterior end near the stylet. The oral sucker is subterminal and larger in size than the ventral sucker; the latter is situated behind the middle of the body. The pharynx is small and the oesophagus divides in front of the acetabulum into wide intestinal caeca. The latter extend to just behind the level of the acetabulum.

The excretory system comprises of a bicornuate excretory vesicle at the posterior end of the body, with a long basal part in extended animals. The cornua of the bladder terminate between the ventral sucker and the posterior end of the body. The primary excretory ducts which open into the cornua are convoluted, and each divides just behind the ventral sucker into an anterior and a posterior secondary collecting tubule. Each of the latter tubules receives a set of three tertiary collecting vessels draining a pair of flame cells each. The flame cell formula could be expressed as :

$$2 \left[(2 + 2 + 2) + (2 + 2 + 2) \right] = 24$$

The genital primordium is represented by a mass of cells lying posterior to the ventral sucker. The tail is simple, aspinose and highly contractile.

The metacercaria (Fig. 17D)

In the laboratory the cercariae were found to penetrate the arthropod Asellus aquaticus. Dissection of specimens of Asellus aquaticus recently penetrated by the cercariae showed that the tail was lost, while the stylet was not lost, in penetration. However, examination of fully-developed metacercariae from natural infection of Asellus aquaticus showed no sign of the stylet, so probably this structure is lost before the formation of the cyst wall. The metacercaria are large in size, and measure from 190 x 190μ to 220 x 220μ in diameter. The cyst wall is 16μ thick and refractile. The excretory system of the metacercariae is identical with that of the cercaria, except that the cornua have become enlarged and filled with excretory granules, and occupy nearly half the body. The ventral sucker has attained a size equal to that of the oral sucker.

Further development is unknown.

4.1.4.2.1.2 Identification and comparison with related species

The present species belongs to the Microcotylae subgroup of Xiphidiocercariae, and numerous cercariae have been described in this group. However, the cercariae of the Microcotylae sub-

group with four pairs of penetration glands which are closely related to this species are Cercaria helvetica XI Dubois, 1929 as described by Probert (1965a); Cercaria vesiculosa Dies as described by Wesenberg-Lund (1934); Cercaria parvus Khan, 1961; Cercaria wentloogensis Pike, 1967 and Cercaria runmiensis Pike, 1967.

Cercaria wentloogensis and C. runmiensis have aspinose bodies, while the present species has the body covered with spines. C. helvetica XI, as described by Probert (1965a), has a cellular excretory bladder and an arrangement and number of flame cells different from the present species. Moreover Cercaria helvetica XI lacks an oesophagus and intestinal caeca, and therefore is clearly different from the present species. C. vesiculosa differs from the present species in the arrangement of the penetration glands and the opening of their ducts in the anterior end of the body. C. parvus and the present species have the same general morphology, including the excretory system. However, Khan (1961) reported that the primary excretory ducts enter the cornua of the bladder aterminally in C. parvus. Pike (1967) reported that the primary excretory ducts enter the cornua terminally in C. parvus. In the present species the main excretory ducts were observed to enter the cornua terminally. In view of the similarity in general structure of the present species to that of C. parvus, as described by Khan (1961) and Pike (1967), the present species is referred to C. parvus Khan, 1961.

4.1.5 The Furcocercariae

Lühe (1909) defined this group as Distome cercariae with a long forked tail within which the body cannot be retracted, and whose development occurs in very long sporocysts. Cort (1917) divided the Furcocercariae into three groups, on the basis of the presence or absence of a pharynx, the length of the furcal rami relative to the main tail stem and the presence or absence of eye spots. Sewell (1922) reclassified the Furcocercariae into three groups, following in the main the scheme devised by Cort (1917) but also using the arrangement of the excretory system. He combined two of Cort's groups together, and erected a third group (the Vivax group) which has a characteristic excretory system. Miller (1926) classified the Furcocercariae into the pharyngeal and apharyngeal cercariae. Each category was further divided into brevifurcate and longifurcate cercariae. The four groups created were subdivided into distome and monostome cercariae. Miller (1926) used the excretory system to establish further subdivisions.

The present cercaria belongs to the Vivax group, Sewell 1922 (i.e, pharyngeal longifurcate monostome cercariae (Miller, 1926)). Sewell (1922) divided this group into the Vivax and the Tetis subgroups on the basis of the presence or absence of a rudimentary ventral sucker and furcal finfolds,

together with the number of flame cells in the body and tail. Faust (1924) created the Leptoderma subgroup and Szidat (1933) according to Cable (1938) added the Tauiana and Vivipara subgroups. Cable (1938) recognised the following three subgroups:

a) The Vivax, Sewell, 1922

Characterised by the presence of a small rudimentary acetabulum and furcal finfolds, together with an excretory system of nine to fifteen pairs of flame cells in the body and three pairs in the tail stem.

b) The Tetis, Sewell, 1922.

Which lacks an acetabulum and furcal finfolds, but possesses an excretory system with two pairs of flame cells in the tail stem.

c) The Tauiana, Szidat, 1933.

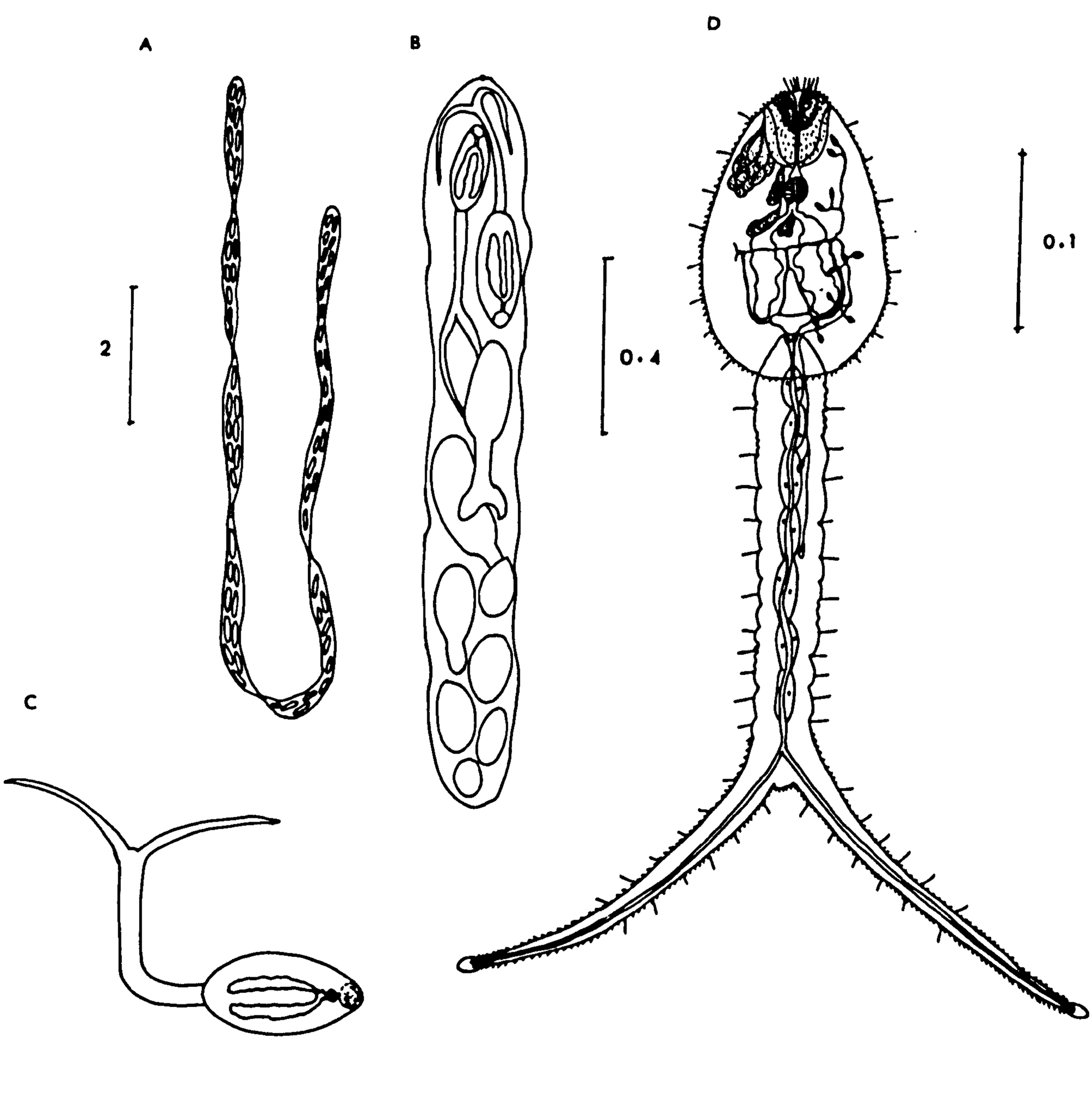
Which lacks an acetabulum, furcal finfolds, and flame cells in the tail system.

Anderson (1944) suggested the division of these cercariae into two subgroups. The Vivax subgroup which include cercariae with flame cells in the tail stem and the Tauiana subgroup to include cercariae without flame cells in the tail stem.

Fig. 18 The structure of Cercaria spatulata.

Parent sporocyst (A), daughter sporocyst (B), floating behaviour of the cercaria (C), and the cercaria (D).

[Scale in mm.]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B
C
D
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He considered that even such separation is unnatural. Khan (1962) and Nasir, Hamana and Diaz (1969) agreed that the subdivisions as they stand now of cyathocotylid cercariae fail to embrace all the larval forms of this type, and they suggested the discarding of such subdivisions in favour of only one group, the Vivax.

4.1.5.1 Cercaria spatulata Probert, 1966

This cercaria was adequately described by Probert (1966a) from Bithynia tentaculata. The following description is in agreement with the account presented by the above author, except for the observation of more penetration glands. Moreover, a description of the parent sporocyst of this species is added and attempts were made to find out the second intermediate host. The infection rate was 1.6%.

4.1.5.1.1 Description

The parent sporocyst (Fig. 18A)

Out of more than two thousand specimens of B. tentaculata dissected for larval trematode infection, only a single snail was found (on 17th April 1974) harbouring a parent sporocyst infection. This snail was infected with a single parent sporocyst, situated nearly on the outer wall of the digestive gland. The parent sporocyst was very large, and measured 18mm

in length and 0.325 mm in breadth. The body wall was thick and marked into transverse muscular ridges, and in some parts of the body there were well marked transverse constrictions. In addition to the extraordinary length of the parent sporocyst, it was found to contain about 118 daughter sporocysts packed together. The daughter sporocysts measured from 0.750 mm to 0.180 mm, each contained many germ balls, and in some cases the developing cercariae had reached the stage where the constriction between the body and tail of the cercariae was well defined.

The finding of a parent sporocyst of the Vivax group of Furcocercariae was of interest because the parent sporocysts of this group were reported to produce miracidia by Sewell (1922) and Premvati (1955) for Cercaria indicae XV and C. multiplicata respectively. Cable (1965) stated, "that germinal sacs can function in the reproductive capacity of adults was demonstrated over 40 years ago when Sewell (1922) reported a cyathocotylid sporocyst which produced miracidia. Much later, Premvati (1955) found another such species, also a cyathocotylid." Both the miracidia-producing sporocysts were reported from India and the molluscan host was Melanoides tuberculatus. The other parent sporocyst reported in the Vivax group was by Looss (1896), who found a single large parent sporocyst of Cercaria vivax Sinsino (= Cercaria of Prohemistomum vivax Odhner, 1913 (Azim, 1933)) in infected

individuals of the molluscan host Cleopatra bulimoides in Egypt.

The parent sporocysts reported from India had a maximum size of 9.6 and 5.55 mm, and the same snail host was found to be infected with several sporocysts. The parent sporocysts reported by Looss (1896) from Cleopatra in Egypt, and the present one from Bithynia tentaculata, are large in size. The infected snail harboured a single parent sporocyst which produced numerous daughter sporocysts. The parent sporocysts reported from India were capable of producing miracidia, but no miracidia were reported by Looss (1896) nor were any observed in the present investigation. The finding of this developmental stage is probably the first record from B. tentaculata.

The daughter sporocyst (Fig. 18B)

The digestive gland of the molluscan host contains numerous long coiled sporocysts which are relatively inactive and colourless. The anterior end of the sporocyst is protrusible, while the posterior extremity is rounded. The birth pore is terminal, and the sporocyst contains up to 25 cercariae at various stages of development, together with few germ balls. The cercariae leave the sporocyst through the terminal birth pore when they are fully mature. Living sporocysts measure 0.6 to 3.0 mm in length and 0.15 to 0.25 mm in breadth.

Emergence and behaviour of cercariae

The cercariae are emitted in relatively large numbers, and shedding takes place in the laboratory both under artificial illumination and in the dark at a suitable temperature. The cercariae swim actively tail foremost, and when at rest they settle with the furcae wide open and the tail shaft bent to form a right angle with the body (Fig. 18C). The cercariae react positively to light and mostly remain suspended in the top half of the container, although a few are found in the bottom half. The free life of the cercariae is short, 12 - 24 hours, but a few can survive for 36 hours. Before death the cercariae creep on the bottom of the container in a worm-like motion, and when they die the furcae are rolled over.

The cercaria (Fig. 18D).

The body of the cercaria is oval when stretched and nearly spherical when contracted. The body measures from 0.13 to 0.225 mm in living cercariae (Table 20). Under the low power of the microscope the cercariae are slightly yellow in appearance. The body is filled with cystogenous matter of small rounded bodies which are either clear or filled with fine spherical granules. The body of the cercaria is entirely covered by small backwardly-directed spines. The large sub-terminal anterior organ is armed with about 15 - 20 rows of prominant spines, which surround the oral aperture and extend

TABLE 20

Measurement in microns of 10 specimens of

Cercaria spatulata

	LIVING		FIXED	
	Range	Average	Range	Average
Body length	130 - 225	173	84 - 136	109
Body breadth	70 - 140	94	60 - 92	74
Tail length	200 - 290	252	190 - 240	215
Tail breadth	34 - 50	40	30 - 40	34
Furca length	120 - 220	182	154 - 180	165
Furca breadth	10 - 30	17	10 - 20	15
Anterior organ	-	-	38x34-50x40	41x36
Diameter				
Pharynx diameter	14x12-23x20	18x16	12x10-16x12	13x11
Prepharynx length			5 - 8	6

about two-thirds of the way down the penetration organ. The anterior end of the body is supplied with four pairs of anteriorly projecting hairs, and there are eight pairs of similar structures on the lateral margins of the body. The ventral sucker is lacking altogether. The mouth opening is funnel-shaped and leads into a short prepharynx, which opens into a well-developed nearly spherical pharynx. The oesophagus is short, and soon after its origin divides to form two wide, thin-walled intestinal caeca, with wavy margins, which extend to the level of the excretory bladder and may contain some debris.

The penetration gland cells are numerous. About 10-12 pairs are located at the posterio-lateral margin of the anterior organ, four pairs lateral to the pharynx, four pairs lateral to the gut caeca and four pairs between the intestinal caeca, just behind the oesophageal bifurcation. In addition, there are two groups of glands with clear cells within the oral organ. These glands stain intensely with neutral red, and are homologous with the slender head glands which Johnston and Beckwith (1945) described as present in Cercaria notopalae. They may possibly be ducts of some of the other glands, though no connections with other cells could be traced. The ducts of the posterio-lateral penetration glands open at the dorsal end of the penetration organ, while the ducts of all the other glands open dorsal to the mouth.

The excretory bladder is thin-walled, small and tripartite. It lies in front of the caudal insertion and opens on the dorsal surface through a small excretory pore at the posterior end of the body. Two pairs of main excretory ducts leave the bladder; the outer pair proceed forwards, closely applied to the intestinal caeca, up to near the anterior third of the intestinal caeca where they are united by a transverse excretory tube (Fig. 18D). After this the main lateral excretory vessel on each side exhibits some convolutions, and divides into anterior and posterior secondary collecting vessels. The anterior tubule receives two tertiary vessels, each draining a pair of flame cells. The posterior tubule receives four tertiary vessels draining a pair of flame cells each. The fourth pair of flame cells lies in the anterior half of the tail shaft. The two median main excretory ducts continue forwards from the excretory bladder along the inner side of the intestinal caeca up to the middle of the body, where they unite to form a single tube which opens into the transverse excretory tube.

The flame cell formula may be expressed as:

$$2\ [\ (\ 2+2\ )\ +\ (\ 2+2+2+[2]\ )\ ] = 24$$

The caudal excretory duct is surrounded by seven pairs of caudal bodies, and its branches open at the tip of the furcae. The genital rudiment is represented by a mass of cells lying in front of the excretory bladder. The tail is inserted dorsally

immediately after the bladder, and is about 0.03 mm. from the posterior extremity of the body. The tail is longer than either the body or the furcae, it has crenulated margins, and is aspinose except for eleven pairs of long hairs. The furcae are slightly longer than the body, covered with short spines, and each furca has six pairs of long hairs. The furcae have finely pointed and striated tips, which may possibly represent very much reduced finfolds as suggested by Probert (1966a) for C. spatulata.

Metacercariae

Attempts to find out whether this cyathocotylid cercaria encysts within the snail host, as reported for cercaria of Cyathocotyle bushiensis, Khan (1962), showed that these cercariae do not do so. Several attempts were made, using laboratory bred snails, but no metacercariae were recovered. The other possibility is that these cercariae encyst in freshwater fish, as found by Azim (1933) for cercaria of Prohemistomum vivax and Anderson and Cable (1950) for cercaria of Linstowiella szidati. Dissection of some of the fish present in the habitat (three-spined sticklebacks) revealed the presence of many strigeid metacercariae in the body muscles, especially in the caudal region. As the cyst wall was very thick, the internal structure of the meta-cercariae found could not be worked out. Although the cercariae failed to encyst in Bithynia tentaculata, their encystment

in fish could not be established and further development is unknown.

4.1.5.1.2 Identification and comparison with related species

The present cercaria is a member of Sewell's group 3 (Vivax group) of Furcocercariae, in view of the characteristic excretory system. As far as can be determined, thirty-eight cercariae have been described belonging to this group. The following cercariae differ from the present species in the possession of furcal finfolds, either on the entire length of the furcae or limited to its distal half. Cercaria of Prohemistomum vivax (Sonsino), Azim, 1933 (= C. vivax Sonsino, as described by Wesenberg-Lund (1934)); C. dorsocauda Tubangui, 1928; C. tatei Johnston and Angel, 1940; C.indicae XV Sewell, 1922; C. indicae LVIII Sewell, 1922; C. kasenyi Fain, 1953; C. Prohemistomum expeditum Balozet, 1953; C. kentuckiensis Cable, 1935 as described by Cable (1938); C. vivacis Iles, 1959; C. artiformis Khan, 1962; C. hirsuticauda Probert, 1966.

In having three pairs of flame cells in the tail stem and differences in morphology and resting behaviour, the following cercariae are clearly different from the species under investigation. Cercaria notopalae Johnston and Beckwith, 1945; C. Paracoenogonimus ovatus Katsunada, 1914, as described by Komyia (1938); C. leptoderma Faust, 1922; C. Linstowiella viviparae Szidat, 1933; C. Linstowiella szidati Anderson, 1944;

C. papillosoma Khan, 1962; and C. monagasica Nasir, Hamana and Diaz, 1969.

Cercaria tauiana Faust, 1930 and Furcocercaria No.4 Petersen, as described by Wesenberg-Lund (1934) differ from the present species in the lack of flame cells in the tail stem. In addition, a number of cercariae described differ from the present species in the pattern of spination. Cercaria sp. Wesenberg-Lund, 1934; and C. yankapiensis Goodman, 1951 have their body spines limited to the anterior organ only. Cercaria theodoxa Porter, 1938 and C. schoutendeni Fain, 1953 have their tail shafts spined, while the present species lacks spines on the tail shaft. In having ten pairs of flame cells in the body and other morphological characters, the present species is distinct from Cercaria indicae XXXIII Sewell, 1922; C. balthica Szidat, 1933; C. Cyathocotylis gravieri Mathias, 1935 and C. Cyathocotyle bushiensis Khan, 1962.

The present species is closely related to Cercaria spatulata Probert, 1966. Both species lack a ventral sucker, possess the same structure of the excretory system, and have similar resting behaviour. The distribution of spines and hairs on the body, tail and furcae is identical. However, the two species differ in size, the present cercaria being smaller than C. spatulata, and differences were also observed in the number

and arrangement of the penetration glands. The penetration glands lateral to the anterior organ are more numerous in the present species, and two more groups of glands were observed in the present species. One group lies within the anterior organ and the other between the intestinal cacca. On the penetration glands, Probert (1966) stated, "It is very difficult to ascertain the exact number of these cells and more are probably present." Although there is a large difference in size, the use of size as a taxonomic criterion in these elastic organisms has been underated by most workers (Wesenberg-Lund, 1934).

In view of the similarities in what could possibly be the more reliable taxonomic characters of morphology, including the excretory system and behaviour, the present species is referred to Cercaria spatulata Probert, 1966. My observations confirmed Probert's (1966) suggestion about the arrangement and number of flame cells. In addition, seven pairs of caudal bodies were observed in the tail stem.

4.2 THE LIFE CYCLE OF NOTOCOTYLUS IMBRICATUS (LOOSS, 1893) SZIDAT, 1935.

4.2.1 Introduction

Szidat (1935) demonstrated that Cercaria imbricata Looss, 1893 from Bithynia tentaculata is the larval form of Notocotylus imbricatus. The worms were obtained from laboratory investigations and described by Dönges, 1962; Odening, 1968; and Pike, 1969. Beverly-Burton (1961) described Notocotylus imbricatus from British freshwater birds.

In the present work the morphology and anatomy of the adult worm was similar to that reported by the above workers. A contribution is made to the development of Notocotylus imbricatus in the snail host.

4.2.2 Materials and methods

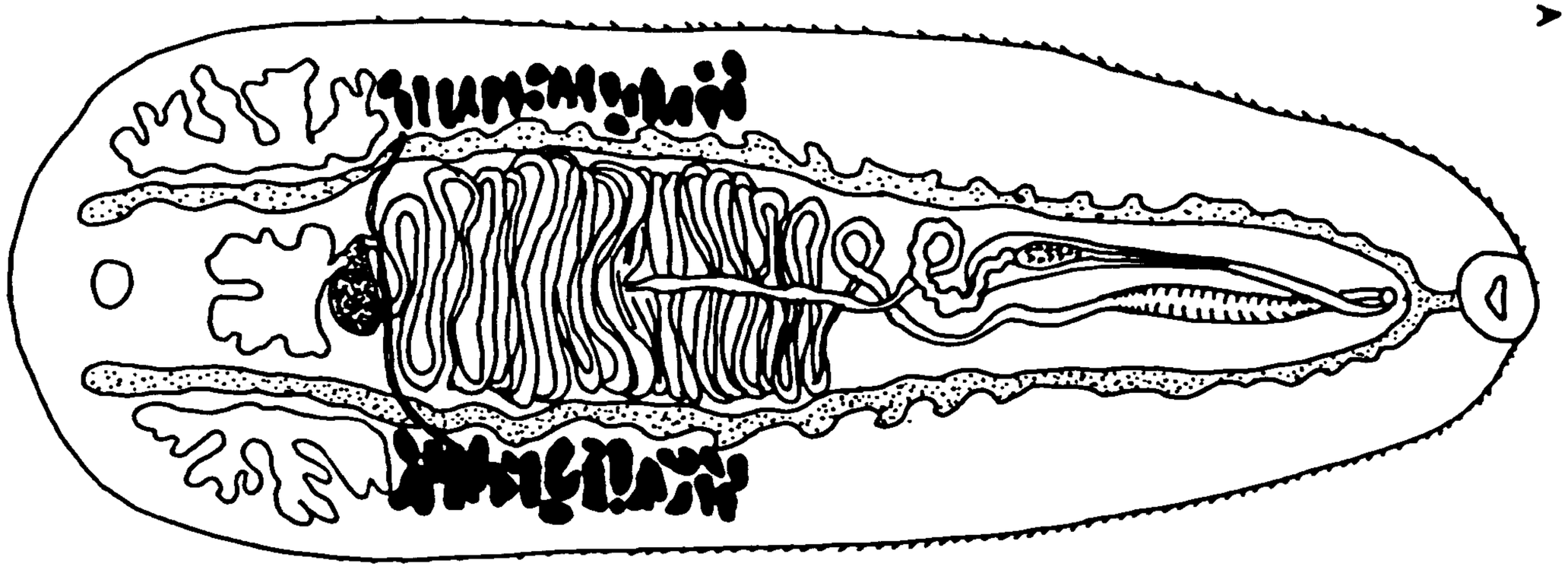
Snails known to be infected with Cercaria imbricata were isolated in small containers. The cercariae that emerged encysted on the walls of the container. The metacercariae were scraped gently from the walls, and force-fed by a syringe to six ducklings. The ducklings used in the experiment were Khaki Campbell ducklings obtained as one-day-old animals. The ducklings were killed 21 to 33 days after infection. The worms recovered were washed in 1% saline solution and examined either alive in saline solution or as fixed and stained preparations. The worms were fixed in 10% formol saline solution under slight cover-slip pressure and stained with Delafield's haematoxylin. Measurements were made on living and fixed specimens, and the figures presented were free-hand drawings to scale.

4.2.3 Metacercariae infectivity

Two four-day-old ducklings were fed metacercariae which were 2 to 24 hours old. A similar number of ducklings were fed metacercariae three weeks old. The ducks were killed 28 to 33 days after infection. The alimentary canal was dissected and each part of the system was examined. All the worms were found in the gut caeca. The ducks fed on metacercariae 2 to 24 hours old contained thirteen and one worms respectively. The ducks fed on metacercariae three weeks old contained twelve and three worms respectively.

This experiment suggests that the metacercariae of Notocotylus imbricatus are infective to the final host in less than twenty-four hours after encystment.

Fig. 19 The structure of the adult Notocotylus imbricatus (A) and the arrangement of the ventral glands (B).



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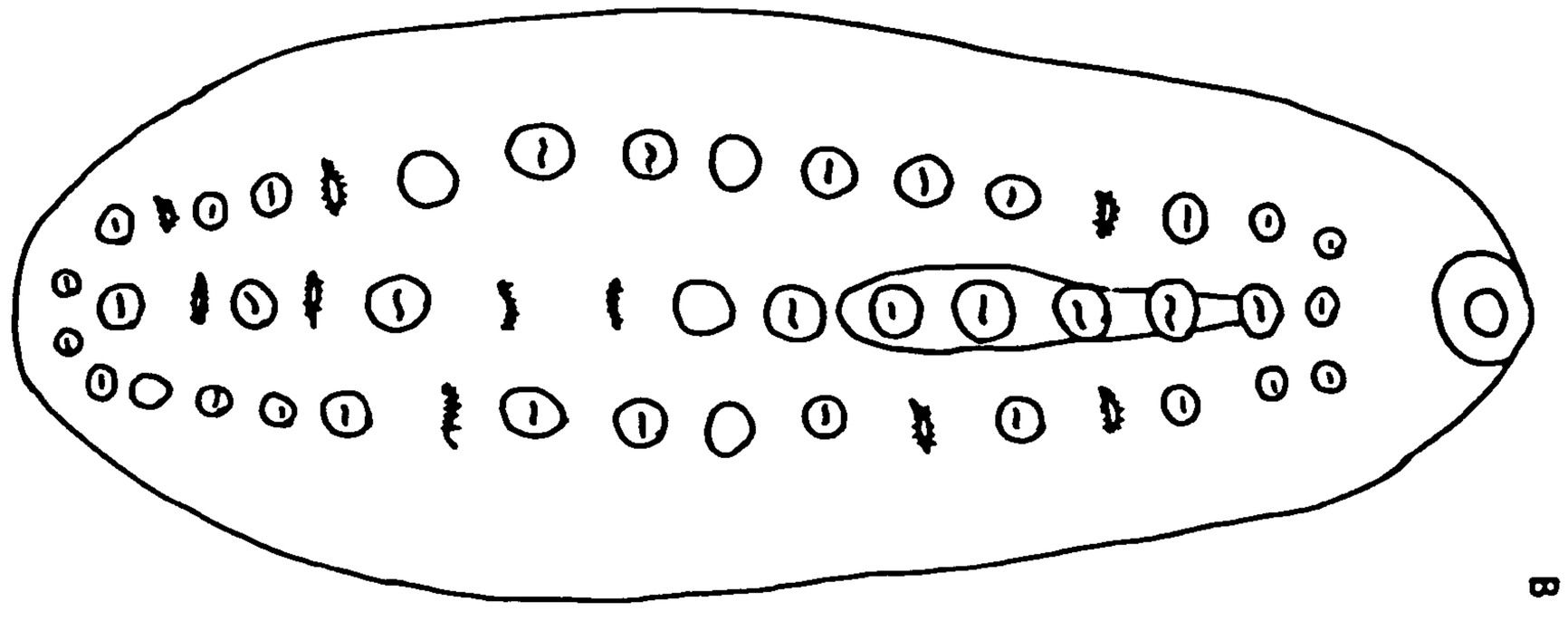


B

TABLE 21

Measurement in mm. of Notocotylus imbricatus obtained from ducks 28 to 33 days after infection

	Fixed and stained worms (8 to 13 specimens)		Living worms (21 specimens)	
	Range	Mean	Range	Mean
Body length	1.500 - 3.075	2.048	2.400 - 4.900	3.621
Body breadth	0.450 - 0.800	0.694	0.725 - 1.650	1.260
Oral sucker diameter	0.09 x 0.11 - 0.16 x 0.2	0.118 x 0.146	0.12 x 0.15 - 0.22 x 0.25	0.184 x 0.217
Oesophagus length	0.03 - 0.09	0.052	0.01 - 0.12	0.053
Testes length	0.250 - 0.450	0.325	0.375 - 0.900	0.603
Testes breadth	0.100 - 0.170	0.129		
Testes number of lobes	4 - 6	5.2	4 - 6	4.6
Distance of testes from posterior end of body	0.100 - 0.150	0.120	0.125 - 0.300	0.206
Ovary length	0.090 - 0.200	0.153		
Ovary breadth	0.090 - 0.210	0.160		
Ovary number of lobes				
Number of vitellaria	13 - 22	17	15 - 21	19.4
Anterior extent of vitellaria	0.800 - 1.400	0.983	1.300 - 2.750	1.836

continued....

TABLE 21

Continued .....

	Fixed and stained worms (8 to 13 specimens)		Living worms (21 specimens)	
	Range	Mean	Range	Mean
Length of cirrus	0.410 - 0.780	0.590		
Breadth of cirrus at base	0.070 - 0.120	0.081		
Length of metraterm	0.140 - 0.400	274		
Greatest width of metraterm	0.030 - .050	0.040		
No. of uterine loops between Vitellaria and Cirrus sac	4 - 9	6.4	4 - 8	7
Situation in relation to body length				
a) first median ventral gland	11 - 16/100	14.5/100		
b) first lateral ventral gland	10 - 14/100	12.6/100		
c) base of cirrus sac	29 - 40/100	36.5/100		
d) anterior extent of Vitellaria	44 - 52/100			
Ratio of Measurement of metraterm to cirrus sac	0.34 - 0.55	0.46		
Position of first ventral gland in relation to first lateral gland	$-\frac{1}{4}$ to $-\frac{1}{2}$ interval			

4.2.4 Morphology and anatomy of the adult worm (Fig. 19A)

The body of Notocotylus imbricatus is elongate, concave ventrally, and measures 2.4 to 4.9 mm in living specimens and 1.5 to 3.1 mm in fixed specimens (Table 21). The body is covered with backwardly directed spines which disappear in the posterior end of the body. The spines are larger in the anterior half of the ventral surface. The oral sucker is ventral and small in size.

There are three rows of eversible ventral glands characteristic of the genus Notocotylus (Fig. 19B). The median row of ventral glands is made of 14 or 15 glands, with one exception (Table 22). The lateral rows are usually equal in number except for one worm and each row contained 15 to 17 glands (Table 22). The most common arrangement of the ventral glands is 17:15:17. The first anterior median gland is situated 11 to 16/100 of the body length, while the first anterior lateral gland is situated 10 to 14/100, from the anterior end of the body length (Table 21). The first anterior median ventral gland is situated below the first lateral gland by one quarter to one half of the interval distance.

TABLE 22

Number and arrangement of the ventral glands of

Notocotylus imbricatus

	No. of glands	Living worms (44)		Fixed & Stained worms (10)	
		No.of worms	%	No.of worms	
a) median ventral gland row	14	16	36.4	5	
	15	27	61.4	5	
	16	1	2.3	-	
b) lateral row of ventral glands	15	4	-9.1	-	
	16	7	15.9	2	
	17	32	72.7	8	
c) Type of arrangement of ventral glands	15:14:15	4	9.1	-	
	16:14:16	6	13.6	2	
	16:15:16	1	2.3	-	
	17:14:16	*1	2.3	-	
	17:14:17	5	11.4	3	
	17:15:17	26	59.1	5	
	17:16:17	1	2.3	-	

* One worm was found to possess 17 and 16 glands in the right or left lateral rows respectively

The mouth is subterminal, and opens into a short narrow oesophagus which bifurcates in front of the genital pore into long gut caeca with wavy margins. The intestinal caeca are wider than the oesophagus, run posteriorly between the vitellaria and the uterus, and extend to the posterior end of the body where they terminate lateral to the excretory bladder. The excretory bladder is a small vesicle at the posterior end of the body which opens through a dorsal excretory pore. The main excretory canals form a closed circuit, as in the cercaria, and drain a network of smaller excretory tubules from all parts of the body. Few flame cells were observed.

The male reproductive system consists of two elongated testes at the posterior end of the body. The outer margins of the testes are incised to form 4 to 6 primary lobes, each of which may show further subdivision into 2 to 3 lobules. The inner margins of the testes are less markedly lobed. The vasa efferentia were not observed, and the vas deferens runs dorsal to the uterus and widens in front of the uterine loops to form the vesicula seminalis externa, which undergoes a few convolutions on the right side of the body before entering the cirrus pouch to become the vesicula seminalis interna. The latter opens into a large pyriform cavity at the base of the cirrus. The cirrus is long and covered with small spines. The base of the cirus pouch is situated between

29 and 40/100 from the anterior end of the body length. The opening of the male duct into the genital pore lies in front of the female opening, and the genital pore is always behind the oesophageal bifurcation.

The female reproductive system consists of a more compact ovary which lies between the testes in the posterior end of the body. The ovary consists of 4 to 6 lobes, the oviduct arises from the anterior end of the ovary and enters Mehlis' gland posteriorly. The latter is elongated, oval in shape, and lies in front of the ovary. The vitelline ducts leave the vitellaria at the posterior end and each duct runs across the body to unite with the other in front of the shell gland. The median vitelline duct opens into the oviduct. Laurer's canal was not observed. The uterus arises from the anterior end of Mehlis' gland, is folded into a series of transverse loops which extend to the posterior end of the cirrus pouch, and occupies more than a third of the total body length. At the anterior end the uterus runs forward to the left of the cirrus pouch and becomes the metraterm, which is thick, muscular and papillated. The metraterm measures 0.34 to 0.55 of the cirrus length. Between the anterior limit of the vitellaria and the cirrus pouch there are 4 to 9 uterine loops. The vitellaria consists of 13 to 22 vitelline cells on each side of the body. The

vitelline cells are irregular, arranged in two narrow rows, and extend from above the testes to the middle of the body. The anterior extent of the vitellaria is 44 to 52/100 from the posterior end of the body length.

The egg (Fig. 20A)

Worms recovered from the intestinal caeca of ducks 28 to 33 days after infection, when placed in saline solution, extruded masses of filamentous eggs from the genital pore within a short period. The egg is small and contains an embryo and a few yolk cells. The egg measures 18 to 22μ in length and 9 to 10μ in breadth, with an opercular diameter of 5 - 7μ. The polar filaments of the egg are very long and thin, and measure 120 - 200μ in length.

4.2.5. Experimental infection of the snails

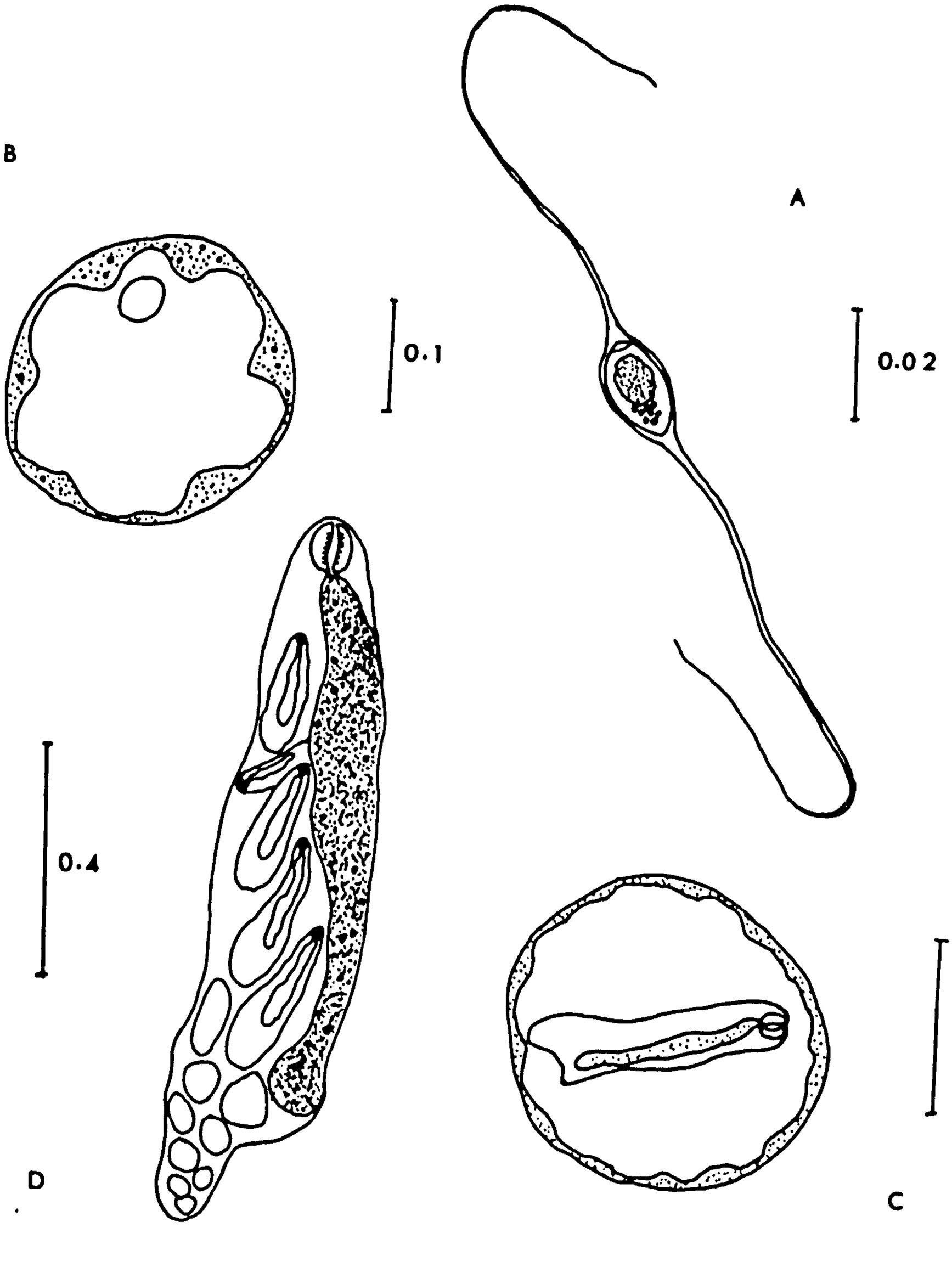
Specimens of Bithynia tentaculata raised in the laboratory and hence free of larval trematode infection, were maintained in a two-litre capacity beaker at a room temperature of 18 to 21°C with eggs produced by Notocotylus imbricatus. The experiment was started on 29th July 1974 and thirty snails were used; seven of these snails were derived from a laboratory-raised stock fifteen months old and the rest were less than three months old. Two weeks later the contents of the beaker were transferred into a large tank containing about seven litres of water, and the snails were maintained here for 12 weeks until the end of the experiment on 21st October 1974. The original water (with the eggs of the worm) was discarded four weeks after infection and the snails were subcultured in a clean fresh water.

The parent sporocysts (Fig. 20B & C)

Dissection of some of the snails four weeks after infection showed that four out of the five young snails examined were infected, while the two old snails examined were free of infection. The snails were infected with numerous parent sporocysts which were recovered from the mantle and the digestive gland, and as many as eighteen sporocysts were found in one shail. The parent sporocysts appear as small transparent vesicles when viewed under the dissecting microscope. They are slightly motile due to

Fig. 20 The morphology of Notocotylus imbricatus. The egg (A), parent sporocyst (B and C), and the mother redia (D).

[Scale in mm.]


A
0.02
B
0.1
C
0.2
D
0.4

muscular contraction and range in size from 0.150 x 0.160 to 0.200 x 0.250 mm (Table 23). No mother rediae was observed inside the sporocysts (Fig. 20B).

Six weeks after infection all of four young snails dissected were found to be infected with many parent sporocysts. Some of these sporocysts had become larger in size, and each contained a single redia (Fig. 20C). The large parent sporocysts measured from 0.25 x 0.25 to 0.40 x 0.32 mm. in size, and the enclosed redia ranged in length from 0.21 to 0.32mm. and the pharynx diameter was about 0.03 x 0.03 mm. In one snail a free mother redia was found, which measured 0.34 mm in length and 0.16 mm in breadth; the pharynx diameter was 0.04 x 0.036 mm, and the gut extended to the posterior end of the body. The mother redia contained a developing daughter redia and many germ balls.

The mother redia (Fig. 20D)

Eight weeks after infection, all of the five young snails dissected were infected, while two old snails examined were free of infection. The infection at this stage consisted of parent sporocysts and mother rediae. Some of the parent sporocysts were still young and did not contain a redia, but most of the sporocysts either contained a single redia each or were degenerating sporocysts from which the rediae had emerged. The mother rediae were numerous and varied greatly

TABLE 23

The development of the larval forms of Notocotylus imbricatus in the snail host Bithynia tentaculata (10 specimens)

Time in weeks after infection	Dominant larval forms	Range of size in mm.	Contained developing form	Range of No. of developing form	Average No.
4	Parent sporocyst	0.15x0.16 - 0.20x0.25	-		
6	Parent sporocyst	0.14x0.14 - 0.40x0.32	Mother redia	1	1
8	a) Parent sporocyst	0.15x0.15 - 0.38x0.36	Mother redia	1	1
	b) Mother redia	0.180 - 0.790	Daughter redia	1 - 3	2
10	a) Mother redia	0.710 - 1.500	Daughter redia	3 - 8	5
	b) Daughter redia	0.300 - 0.600	-		
12	a) Mother redia	0.700 - 1.500	Daughter redia	3 - 8	5
	b) Daughter redia	0.540 - 1.100	Cercaria	1 - 5	3

in size; the young rediae (body length 0.18 - 0.33 mm) were either mother rediae which have recently emerged from the parent sporocysts, or (more probably) daughter rediae produced by the old mother rediae. These young rediae contained germ balls, but no developing rediae or cercariae were observed inside these organisms. The older mother rediae were large in size, contained numerous germ balls and one to three daughter rediae, each with a well-differentiated pharynx and gut.

Ten weeks after infection four young and one old snail were dissected. All the young snails were heavily infected, but the old snail was free of infection. The infection consisted primarily of mother and daughter rediae, and few parent sporocysts containing rediae were observed. The mother rediae were large in size and contained three to eight fully-differentiated daughter rediae (Table 23). The daughter rediae were small in size, and ranged from 0.30 to 0.60 mm. in length and contained many germ balls. However, no cercariae were observed in which the division between body and tail was defined.

Daughter rediae and cercariae (Fig. 12A)

On the twelfth week after infection the remaining five snails were dissected, and of the three young snails examined two were heavily infected and the third was free of infection.

Of the two old snails, one was heavily infected and the other was free of infection. The infection was predominantly with mother and daughter rediae, except for occasional observations of a few parent sporocysts each containing a single redia. The mother rediae were large in size, measuring up to 1.50 mm in length, and contained three to eight well developed daughter rediae and many germ balls. Some of the daughter rediae were large, measuring up to 1.1 mm. in size, and contained one to five developing cercariae in which the division between body and tail was well defined. Of these developing cercariae only one had the lateral eye spots of an early stage of development; the oral sucker was not yet fully developed and body pigmentation was lacking. None of the cercariae had emerged from the rediae to complete its development in the host tissues.

4.2.6 Discussion

The general morphology and anatomy of Notocotylus imbricatus as described in the present investigation is similar to that reported by Szidat, 1935; Dubois, 1951; Beverly-Burton, 1961; Dönges, 1962; Odening, 1968; and Pike, 1969. The above authors reported variable number of ventral glands. Dubois (1951) stated that each of the lateral rows of ventral glands may contain 14 to 16 glands and the median row may contain 14-15 (16) glands. Beverly-Burton (1961) found 16 to 17 glands in each of the lateral rows and 14 to 15 in the median row. She reported a maximum of 49 ventral glands in N. imbricatus. The present investigation indicates that the median row of ventral glands may contain 14 to 16 glands and that each of the lateral rows may contain 15 to 17 glands (Table 22). The maximum number of ventral glands recorded was fifty, arranged as 17:16:17, but the most common number was 49, arranged as 17:15:17 (Table 22 and Fig. 19B). The variation in the number of ventral glands observed by the above workers could possibly be explained by that the most anterior ventral glands are rather small and sometimes difficult to recognise.

The metacercariae of Notocotylus imbricatus were found to be infective to the final host soon after encystment (i.e, in less than 24 hours). Similarly, Herber (1955) reported that the metacercariae of N. urbanensis are infective to the

final host soon after encystment. Thus Pike's (1969) suggestion that the metacercariae of N. imbricatus may undergo some development in the cyst before becoming infective is not supported by the present findings. Probably the variations in the development of the adult worms recovered by Pike (1969) was due to physiological factors, rather than metacercarial infectivity. After all, only a small proportion of the infective metacercariae develop into adult worms.

Observations on the development of N. imbricatus in the snail host Bithynia tentaculata showed that the cercariae first appeared within the rediae twelve weeks after infection at 18 to 21°C (Table 23). Probably it takes about 16 weeks for the shedding of mature cercariae, as suggested by observations on naturally infected snails. Wright and Bennett (1964) reported that the development of the larvae of N. attenuatus in Lymnaea peregra took a minimum of 59 days and an average of 69 days. Herber (1955) observed many daughter rediae of N. urbanensis containing cercariae six weeks after infection at an average temperature of 21°C. Probably the reason for the longer time required for the development of N. imbricatus is that development takes place in prosobranch gastropods, whereas the development of N. attenuatus and N. urbanensis takes place in pulmonate gastropods. Van der Schalie and Davies (1965) reported that the development of Schistosoma

mansoni in Biomphalaria glabrata takes about a month whereas the development of S. japonicum in Oncomelania takes three to four months.

The parent sporocyst of Notocotylus imbricatus produces a single redia six weeks after infection. Herber (1955) reported that the parent sporocyst of N. urbanensis in Physa gyrina contained four to eight rediae. Furthermore, the infection of N. imbricatus is established in Bithynia tentaculata by rapid redial multiplication before the appearance of cercariae inside the daughter rediae (Table 23). No mother rediae of Notocotylus imbricatus were observed to contain daughter rediae as well as cercariae. Herber (1955) found one mother redia of N. urbanensis containing rediae as well as cercariae 30 days after infection. In addition observations on snails naturally infected with N. imbricatus showed that with the onset of the extra-redial phase of development of cercariae, the mother rediae have disappeared.

The infection of one of seven old snails with N. imbricatus, compared with 19 of 21 young snails, could possibly suggest some degree of age resistance to infection in this species. Wright (1966) stated that the evidence for age resistance is inconclusive, yet he reported that miracidial infectivity may not be uniform for all stages of the normal molluscan host.

In conclusion, the metacercariae of Notocotylus imbricatus are infective to the final host soon after encystment. The morphology of the adult is similar to that reported. The development in the molluscan host differs from that reported for N. urbanensis both in the time required and details of development.

4.3 SOME ECOLOGICAL ASPECTS OF THE TREMATODE INFECTIONS OF BITHYNIA TENTACULATA

4.3.1 Introduction

The literature on the various aspects of the ecology of larval digenetic trematodes in freshwater molluscs is extensive (Sewell, 1922; Dubois, 1929; Wesenberg-Lund, 1934 and Erasmus, 1972). Kemp and Gravely (1919) suggested that trematode larvae are seasonal in their appearance, and Manson-Bahr and Fairley (1920) reported that infection of the snails with Schistosome cercariae in Egypt occur throughout the year with a peak of infection in December. Sewell (1922) found two peaks of cercarial infection during the year in Melanoides tuberculatus in India, and this phenomenon was confirmed by subsequent workers (Dubois, 1929; Rees, 1932; Rankin, 1939; Singh, 1959; Probert, 1966b and Pike, 1968b).

Sewell (1922) noted the rarity of multiple cercariae infections in the same snail host, and Cort et al. (1937) made an extensive study of the occurrence of multiple infections in Stagnicola emarginata angulata. Later workers (Ewers, 1960; Bourns, 1963) extended our knowledge of multiple infections, and Lie et al. (1968) pointed out its practical implications.

The relation of the larval trematode infection to the life cycle of the snail host was examined by Cort et al. (1940) and Cort (1941). Olleronshaw (1959, 1971) made notable contributions to the understanding of the relationship of Fasciola hepatica to the snail host and the environment.

Although numerous cercariae were described infecting Bithynia tentaculata in some parts of Europe (Dubois, 1929; Wesenberg-Lund, 1934; Wikgren, 1956; Wisienwski, 1958; Nasir and Erasmus, 1964; Probert, 1965a, b, and 1966a; and Pike, 1967, 1968a), little information is available on some aspects of the ecology of these parasites. The importance of the snail as an intermediate host of digenetic trematodes was stressed by the above workers, and Pike (1968b) reported on the incidence and seasonal changes of larval trematode infections in the snail, together with the other invertebrate fauna, in the Wentloog level in Wales.

In the present investigation, a monthly survey of the larval trematode infection was carried out for thirteen months and information was obtained on :

(i) multiple infections in the snail

(ii) seasonal variation in the incidence of individual as well as total cercariae and metacercaria infections.

(iii) the seasonal cycle of the cercariae infection in relation to the life cycle of the snail host.

4.3.2 Materials and methods

Specimens of Bithynia tentaculata were examined for larval trematode infections at approximately monthly intervals for thirteen months, from August 1973 to August 1974, and a total of 1941 snails was examined. These snails were drawn from the regular grab samples obtained during the investigation of the life cycle of the snail, except for the last two samples when a pond net was used. After the field collection was sorted out in the laboratory, the snails collected from August 1973 to May 1974 were divided into two groups according to size. One group contained snails measuring from 2 to 6.9 mm, while the other consisted of snails more than 7 mm. in size. A random selection was made from each group so that the proportion of small and large snails represented as far as possible their proportion in the natural population as found in life cycle studies.

In the collections made from June to August 1974, instead of size distinction into small and large snails, the two groups were separated on age basis as young and old snails. The former included snails which hatched from June 1974 and onwards, while the latter group represent the previous years brood. However, the proportion of each group examined was determined as above. A further collection of one hundred snails, comprising fifty young and fifty old

snails was examined on 25th November 1974.

In the examination of the snails for larval trematode infections, each snail was crushed, the shell was removed, and the digestive gland of the snail was teased with fine neddles under a dissecting microscope. The cercariae and metacercariae were identified and the stage of development of the cercariae was recorded.

4.3.3 Multiple infections of Bithynia tentaculata

4.3.3.1 Results

Of the 1941 snails dissected for larval trematode infections in the thirteen months, 660 snails contained single infections and 25 snails contained double infections (Table 24). Hosts harbouring more than two species of cercariae were not observed. The highest rate of incidence of double infections was 2.1% in May and July 1974, and the lowest rates encountered were zero and 0.3% in November 1973 and March 1974 respectively (Table 25).

The incidence in single infections of the six species of cercariae infecting the snail and the combinations of double infections observed are shown in Table 24. It was found that one of the partners was always the Xiphidiocercaria Cercaria parvus Khan, 1961, which existed in double infections with all the other cercariae infecting the snail except Cercaria lophocerca, which was of rare occurrence.

Although C. parvus was involved in all the double infections, it was the fourth prevelant cercariae infecting the snail (incidence 4.59%). Of the 25 double infections observed, 18 involved the cercaria of Notocotylus imbricatus

TABLE 24

Number and percentage of single infections and the observed and expected double infections of cercariae in 1941 Bithynia tentaculata examined.

SPECIES	Single infection No.	% incidence of single infections	No. of double infection (Observed)	Expected No. of double infection with Cercaria parvus
1. Cercaria of Notocotylus imbricatus	219	11.28	18	10
2. Cercaria helvetica XIX	197	10.15	3	9
3. Cercaria granocutis	121	6.23	2	5.6
4. Cercaris parvus	89	4.59	25	-
5. Cercaria spatulata	28	1.44	2	1.3
6. Cercaria lophocerca	6	0.31	0	0.3

(incidence 11.28%), 3 involved C. helvetica XIX (incidence 10.15%) and 2 involved each of C.granocutis and C. spatulata (incidence 6.23 and 1.44% respectively), (Table 24).

4.3.3.2 Discussion

It has been shown for more than a century that a snail may be infected with more than one species of larval trematode and, according to Cort et al. (1937), de Filippi (1857) was the first to report the presence of two species of cercariae within the same snail host. Sewell (1922) reported 18 cases of double infections in about 4,000 snails examined, and Dubois (1929) found 29 double and three triple infections in about 2,400 molluscs. Cort et al. (1937) reported 511 double, 17 triple and one quadruple infection in 7,259 specimens of Stagnicola emarginata angulata. The occurrence of triple and quadruple infections is very rare, whereas double infections are relatively more frequent; and according to Erasmus (1972) most combinations of double infections seem to be possible, although double infections with Echinostome cercariae have not been reported.

The incidence of double infections in Bithynia tentaculata in the habitat studied is low, and probably the highest incidence shown in May and July (Table 25) agree with

Rankin's (1939) remark that when double infections are present they are primarily noticed in the summer months. The involvement of Xiphidio-cercariae in double infections with other species of cercariae was pointed out by Wright (1966), and in the present work all the double infections observed involved the Xiphidio-cercaria, Cercaria parvus. In Bithynia tentaculata, Dubois (1929) reported four double infections, Wikgren (1956) found seven and Pike (1968b) reported four; and all involved a Xiphidio-cercaria. The presence of relatively more double infections in this snail in the habitat examined compared with those reported by Pike (1968b) could possibly be explained by the high incidence of the individual species of cercariae infecting the snail.

Cort et al. (1937) calculated the number of double infections that would be expected to occur by chance (on the assumption that there is no immunity or antagonism between the species of larval trematodes present) as the product of the infection rates for each of the separate species multiplied by the number of snails examined. This method has been used to calculate the number of double infections that would be expected to occur by chance between Cercaria parvus and each of the other species (Table 24).

Although 18 double infections involving C. parvus with cercariae of Notocotylus imbricatus were observed, these two species were expected to combine in double infection ten times by chance distribution. The actual occurrence of nearly

twice as many double infections as expected to occur by chance suggested that double infections in those two species were not randomly distributed. On the other hand, each of Cercaria helvetica XIX and C. granocutis were observed to combine in double infections, with C. parvus three and two times respectively, while their respective expected values of double infections were calculated as nine and 5.6 times. Combinations in double infections among species other than C. parvus were expected, and according to random distribution the three most prevelant species infecting the snail (i.e, cercariae of Notocotylus imbricatus, C. helvetica XIX, and C. granocutis) were expected to combine in double infections among themselves at values ranging from 12.3 to 22.2 times, yet no such combinations were actually observed in double infections.

According to Sewell (1922), the rarity of multiple infections is a result of the penetration of the first miracidium rendering the host physiologically unfit for further penetration, either by destroying the chemotactic stimulus normally present or by damaging the host tissue and so preventing parasitic development Cort et al. (1937) compared the number of double infections actually observed with that expected by chance in the various combinations of double infections in Stagnicola emarginata angulata. They considered that the number of double infections actually observed is expected to fall considerably below the chance

expectation even in cercariae between which there is no immunity or antagonism. This is because the probability is based on the assumptions that all double infections could be detected, and that all the snails collected were equally exposed to infection with the miracidia of the different species of larval trematodes present; conditions which are unlikely to occur in practice.

In an analysis of the observed and expected values of double infections for the strigeid cercariae of Diplostomum flexicaudum and Cotylurus communis in combination in double infections with the most common plagiorchiid cercariae of Plagiorchis muris and P. proximus, Cort et al. (1937) observed either few double infections relative to the expected value or lack of such infections altogether. The authors concluded that there must be an immunity produced by the penetration of one species of trematode which prevents penetration of the same snail by a second species; or that there is an antagonism present which prevents the development of one when the other is present. For other combinations of double infections, Cort et al. (1937) found the values observed to be roughly equal to those expected by chance, and concluded that there was no evidence of immunity or antagonism to the occurrence of double infections in these species.

The low values of double infections observed relative to the expected values in the present work between C. parvus

and each of the two Gymnocephalous cercariae, C. helvetica XIX and C. granocutis, and the lack of double infections among the three prevelant species infecting Bithynia tentaculata, could possibly be explained by the presence of an immunity or antagonism to the existence of double infections between these species. Lie et al. (1965) demonstrated experimentally that Echinostome rediae are antagonistic to the development of another species of larval trematode within the same host. In some cases the Echinostome larvae can completely eliminate a competing species, while with other species the Echinostome remains dominant, but does not completely eliminate the other species. The antagonism (Lie et al. 1968) takes the form of inhibition or destruction of certain trematode larvae by rediae, and that in addition to direct redial predation, there may be a degree of indirect inhibition exerted by rediae or sporocysts. Lie et al. (1968) found the Echinostome Paryphostomum segregatum to be antagonistic to Schistosoma mansoni in Biomphalaria glabrata. They suggested the implications of competition between the species within the snail host as a potential method of biological control of economically important trematode infections.

Ewers (1960) found the frequency of double infections of the heterophyid Strictodora sp. and the Schistosome Austrobilharzia sp. in the estuarine prosobranch Velacumantis australis to be unexpectedly high. He suggested that

infection with one of these species predisposes the snail to infection by the second species. Further evidence for the predisposition hypothesis was reported by Bourns (1963), who found the actual occurrence of double infections of some trematode species in Lymnaea stagnalis appressa to exceed the expected number by from two to 27 times. In the present investigation, the finding of 18 double infections of C. parvus with cercariae of Notocotylus imbricatus in Bithynia tentaculata, which is nearly double the expected value (Table 24), might also suggest that infection of the snail with one of the two species renders it more prone to infection by the other. Wright (1971) stated that how prior infection predisposes a snail to a second infection is not known. He suggested that chemical attractiveness of the snail might be enhanced, or some behavioural or other unknown changes might also have an influence.

In conclusion, a higher number of double infections with cercariae was found in B. tentaculata than has been reported. Some of the combinations of double infections were few compared with chance expectation or absent altogether, although they were expected to occur. This probably suggests an immunity or antagonism to the occurrence of these species of cercariae in double infections. In other instances, the number of observed double infections exceeded chance expectation, and this possibly suggests that infection of the snail with one of the two species renders it more prone to infection by the other.

4.3.4 SEASONAL VARIATION IN THE INCIDENCE OF LARVAL TREMATODE INFECTIONS IN BITHYNIA TENTACULATA

TABLE 25

A summary of the data on the incidence of larval trematode infections in Bithynia tentaculata

Date of collection	No. of snails examined	Double infections		Cercariae infections		Metacercariae infections	
		No.	%	No.	%	No.	%
1973							
7 August	90	1	1.1	25	27.8	80	88.9
4 September	90	1	1.1	31	34.4	85	94.4
3 October	106	1	0.9	41	38.7	101	95.3
14 November	60	0	0	29	48.3	57	95
18 December	60	1	1.7	27	45	55	91.7
1974							
17 January	138	2	1.5	57	41.3	133	96.4
21 February	183	3	1.6	61	33.3	171	93.4
21 March	364	1	0.3	121	33.2	350	96.2
17 April	184	3	1.6	82	44.6	178	96.7
15 May	190	4	2.1	75	39.5	184	96.8
18 June	184	3	1.6	63	34.2	150	81.5
15 July	142	3	2.1	34	23.9	124	87.3
13 August	150	2	1.3	39	26	142	94.7
TOTAL	1941	25		685		1810	
Average incidence (%)			1.3		36.2		93

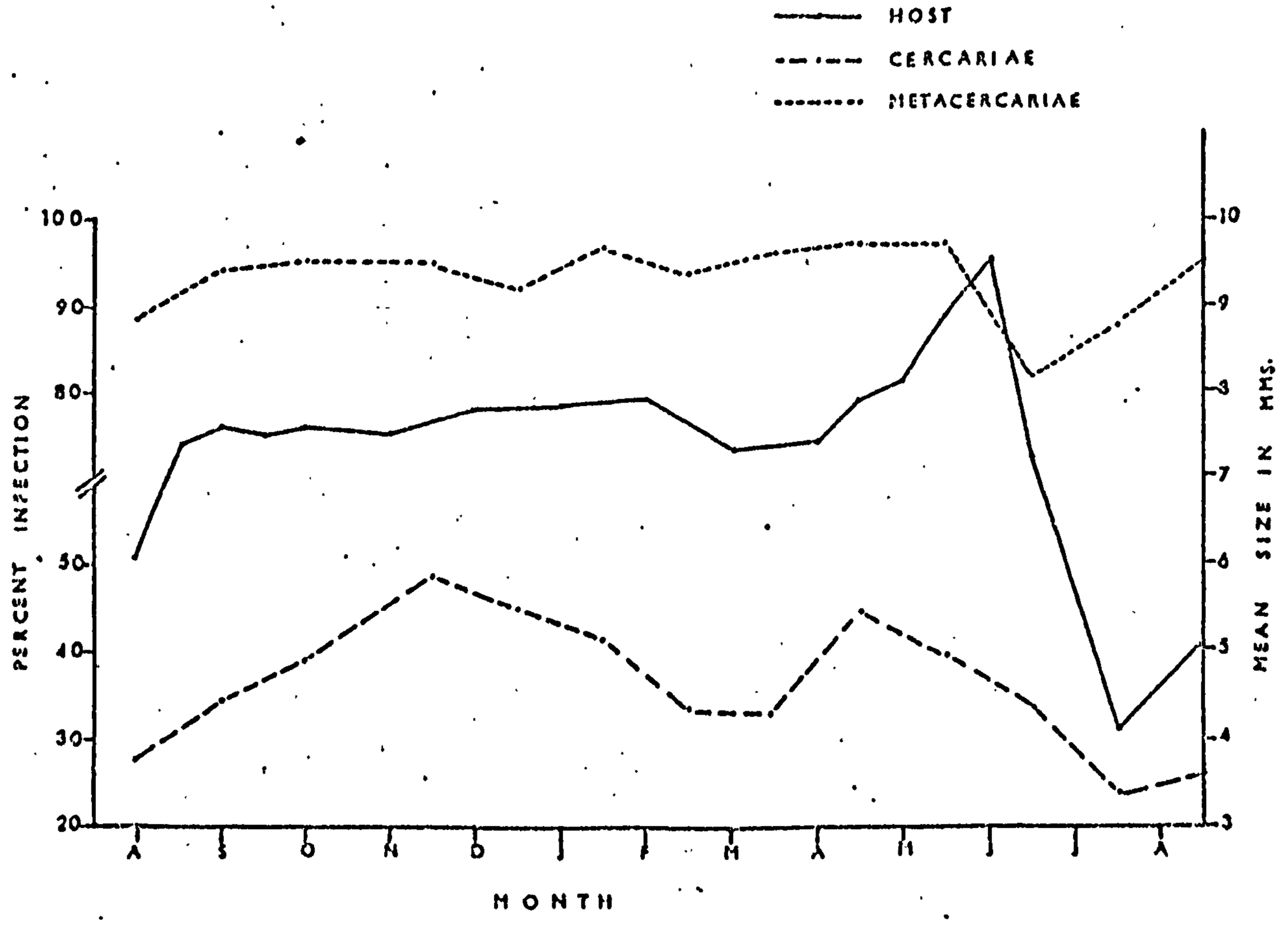


Fig. 21 Seasonal variation in the incidence of cercariae and metacercariae infections in Bithynia tentaculata, together with changes in the mean size of the snail population.

4.3.4.1 Results

Incidence and seasonal variation in cercariae infections

The average monthly incidence of the total cercariae infections in the snail was 36.2%, and the maximum and minimum infection rates were 48.3 and 23.9% respectively (Table 25). Fig. 21 showed that the rate of infection rose from August 1973 (27.8%) to reach its peak in November (48.3%) and then it gradually declined to a low level in February and March 1974 (33.2%). The number of infected snails rose again in April (44.6%) when a second peak was reached. This was followed by a subsequent decline in the infection rate and in July the population reached its lowest level of infection (23.9%) during the year.

Table 26 showed that the individual species of cercariae infecting Bithynia tentaculata were found throughout the year, except Cercaria lophocerca. The average monthly incidence of the cercariae was in the following order (Table 26); cercaria of Notocotylus imbricatus (12.8%); C. helvetica XIX (10.8%); C.granocutis (6%); C. parvus (5.9%); C. spatuluta (1.6%) and C. lophocerca (0.5%).

TABLE 26

Incidence of the six species of cercariae infecting Bithynia tentaculata

Date of collection	No.of snails examined	No.of snails infected	Cercariae of Notocotylus imbricatus		* Cercaria helvetica XIX		* Cercaria granocutis		Cercaria lophocerca		Cercaria parvus		Cercaria spatulata	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1973														
7 August	90	25	12	13.3	5	5.6	0	0	2	2.2	5	5.6	2	2.2
4 September	90	31	15	16.7	9	10	4	4.4	0	0	2	2.2	2	2.2
3 October	106	41	13	12.3	16	15.1	8	7.6	0	0	4	3.8	1	0.9
14 November	60	29	13	21.7	8	13.3	3	5	1	1.7	3	5	1	1.7
18 December	60	27	7	11.7	11	18.3	4	6.7	0	0	5	8.3	1	1.7
1974														
17 January	138	57	15	10.9	20	14.5	9	6.5	0	0	13	9.4	2	1.5
21 February	183	61	20	10.9	23	12.6	9	4.9	0	0	8	4.4	4	2.2
21 March	364	121	47	12.9	35	9.6	21	5.8	0	0	16	4.4	3	0.8
17 April	184	82	20	10.9	27	14.7	16	8.7	0	0	16	8.7	6	3.3
15 May	190	75	26	13.7	18	9.5	12	6.3	0	0	20	10.5	3	1.6
18 June	184	63	20	10.9	13	7.1	23	12.5	1	0.5	8	4.4	1	0.5
15 July	142	34	14	9.9	6	4.2	7	4.9	1	0.7	7	4.9	2	1.4
13 August	150	39	15	10	9	6	7	4.7	1	0.7	7	4.7	2	1.3
Total	1941	685	237		200		123		6		114		30	
Average incidence (%)				12.8		10.8		6		0.5		5.9		1.6

* Sometimes it was difficult to distinguish rediae of these species before differentiation of cercariae, in such cases the rediae were randomly asigned to one species.

Seasonal variation in relative abundance was shown in the four species of cercariae which had an average incidence of 5.9% or more (Table 26). Each of those cercariae showed two periods of high incidence during the year (Fig. 22), cercariae of Notocotylus imbricatus was abundant in November (21.7%) and May (13.7%), Cercaria helvetica XIX showed its peaks in December (18.3%) and April (14.7%) and Cercaria parvus was prevelant in January (9.4%) and May (10.5%). Table 26 showed that C. granocutis was abundant in October (7.6%) and June (12.5%). Cercaria spatulata, although encountered throughout the year, occurred in small numbers, and C. lophocerca was rare and mainly found from June to August.

Incidence and seasonal variation in metacercariae infections

Four metacercariae of digenetic trematodes infect the snail. Two of these (metacercariae of C. helvetica XIX and of Notocotylus imbricatus) undergo their first development in the same snail host. The Echinostome metacercariae uses the snail as a second intermediate host, while the identity of the Strigeid metacercariae could not be established. Table 25 showed the average rate of metacercariae infection as 93%, with a range of 81 to 96.8%. Fig. 21 shows the high

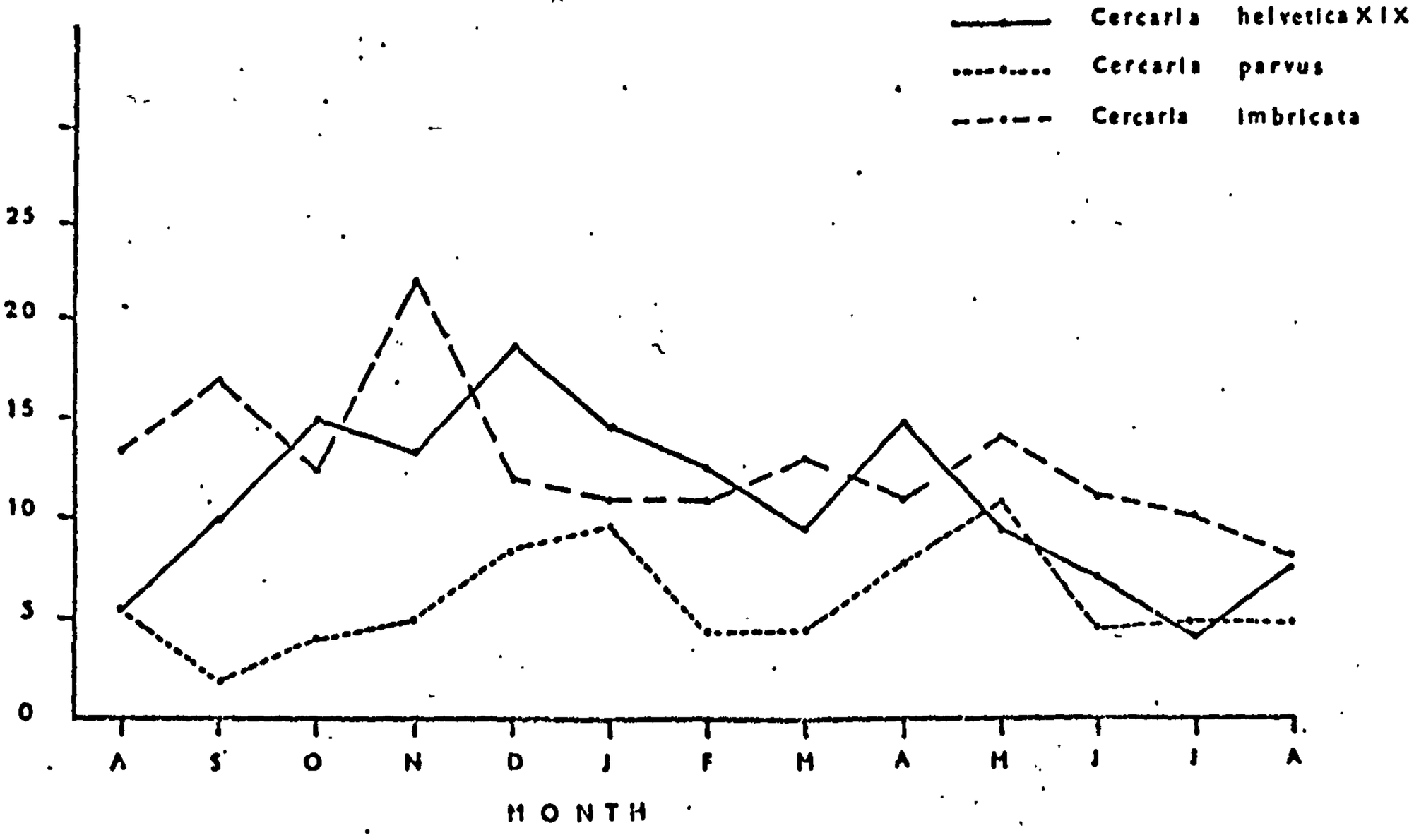


Fig. 22 Seasonal variation in the incidence of Cercaria helvetica XIX, Cercaria parvus and Cercaria imbricata infections in Bithynia tentaculata.

TABLE 27

Incidence of the different metacercariae infections in Bithynia tentaculata

Date of collection	No.of snails examined	Metacercariae of Cercaria helvetica XIX		Metacercariae of Notocotylus imbricatus		Metacercariae of Strigeid		Metacercariae of Echinostome	
		No.	%	No.	%	No.	%	No.	%
1973									
7 August	90	80	88.9	13	14.4	4	4.4	3	3.3
4 September	90	84	93.3	16	17.8	6	6.7	5	5.6
3 October	106	101	95.3	11	10.4	6	5.7	9	8.5
14 November	60	56	93.3	6	10	2	3.3	4	6.7
18 December	60	55	91.7	5	8.3	5	8.3	3	5
1974									
17 January	138	132	95.7	11	8	13	9.4	3	2.2
21 February	183	167	91.3	15	8.2	11	6	6	3.3
21 March	364	347	95.3	41	11.3	17	4.7	18	5
17 April	184	172	93.5	21	11.4	5	2.7	8	4.4
15 May	190	184	96.8	30	15.8	4	2.1	12	6.3
18 June	184	149	81	27	14.7	5	2.7	7	3.8
15 July	142	124	87.3	19	13.4	4	2.8	9	6.3
13 August	150	142	94.7	12	8	3	2	11	7.3
Total	1941	1793		227		85		98	
Average incidence (%)			92.2		11.9		4.7		5.2

level of infection in the snail population throughout the year, and indicates that although a partial drop in the infection rate occurred in June (81.5%), it was restored to its high level in August (94.7%).

The metacercaria of Cercaria helvetica XIX was the most prevelant, and few snails were free of its infection (Table 27). Even young snails less than a month old in June showed an infection rate of about 50%. The metacercaria of Notocotylus imbricatus occurred in small numbers, 8 to 17.8%, with an average rate of 11.9%. The Strigeid metacercaria was rare, with an average incidence of 4.7%; the infected snails harboured few cysts (maximum of five) and young snails were free of this infection. The Echinostome metacercariae was also found in low numbers per snail, and the infection rate ranged from 2.2 to 8.5%, with an average of 5.2% (Table 27). When naturally infected Bithynia tentaculata were fed to ducklings, two echinostome worms were recovered from the rectum. The worms were identified as Echinostoma sp. due to the presence of 37 collar spines of equal size (Dawes, 1956).

4.3.4.2 Discussion

Seasonal changes in the relative abundance of cercariae infections in freshwater molluscs were reported by Sewell, 1922; Dubois, 1929; Rees, 1932; Rankin, 1939; Singh, 1959; Probert, 1966b; and Pike, 1968b, and in general two peaks of cercariae infections occur during the year. In the present investigation a similar result was obtained, the incidence of cercariae infections in Bithynia tentaculata reaching peaks in November and April (Fig. 21).

In Britain, Rees (1932) found the two peaks of cercariae infection in Lymnaea truncatula in June and October, while the peaks of cercariae infections in Lymnaea peregra and L. palustris occurred in May and September. Probert (1966b) reported a high incidence of cercariae infections in the snails in Llangorse Lake in April-May and August - September. Pike (1968b) found the highest incidence of cercariae infections in Physa fontinalis and Lymnaea peregra to be in June and August. He suggested that the incidence of cercariae infections in Bithynia tentaculata will show similar biannual peaks.

In addition to the biannual peaks of incidence of the total cercariae infections in Bithynia tentaculata, 4 of the 6 species of cercariae infecting the snail showed similar phenomena (Table 26). The peaks of incidence of Cercaria helvetica XIX were December and April, C. parvus was more abundant in January and May, and cercariae of Notocotylus imbricatus was prevelant in November and May (Fig. 22). These findings agree with Sewell's (1922) suggestion that many, if not all, of the various forms of cercariae infecting the snail will be found to show the same double rise and fall in their numbers during the course of the year. Ollerenshaw (1971) reported the summer peak of infection of Fasciola hepatica in Lymnaea truncatula in September, and a smaller spring peak of infection in April or May.

The seasonal variation in the incidence of cercariae infections is attributed by Sewell, 1922; Dubois, 1929; and Rees, 1932 to changes in the life cycle of the snail host, the degree of infection of the final host, and environmental conditions. The effect of changes in the life cycle of the snail host on the incidence of infection was illustrated by Sewell (1922) for the cercariae infection in Belancides tuberculatus. Erasmus (1972) stated that a number of causes may be responsible for the changes in the level of cercariae infections, yet he noted that in Europe, the peaks of cercariae

infections coincide with changes within the molluscan fauna. He pointed out that the spring peak of cercariae infections in some of the British freshwater molluscs is the result of the infection acquired in the previous summer or autumn. The degree by which the infection rate drops in early summer depends on the life span of the particular mollusc. In the annual species of molluscs such as Lymnaea peregra, where the old population is completely replaced by a new generation of snails (Hunter, 1961); a sharp fall occurs in the infection rate. This is because the new generation of molluscs is either uninfected at this time, or may contain very young developing stages of the infection such as sporocysts or rediae. Erasmus added that in molluscs with a longer life span, such as B. tentaculata, the reduction in infection rate in early summer is less marked than in the annual species, due to the survival of many old infected snails.

The present findings also indicated the importance of the changes in the life cycle of B. tentaculata on the seasonal incidence of cercariae infections. The partial fall in the infection rate between June and August (Fig. 21) was due to two factors. Firstly, the appearance of the new generation of snails from mid-June through July; no infections were detected in these young snails in June and July, whereas a few very young infections were found in August.

Secondly, the survival of about half of the last year's brood at this time of the year. Many of these old snails were infected, and thus prevented a considerable reduction in the level of infection.

The second peak of cercariae infection in Bithynia tentaculata which occurred in November (Fig. 21), was due to the development of the infections acquired by the new generation of snails in the summer. The subsequent fall in the incidence of the infection from December to March was due to the following: Firstly, the fall in the environmental temperature (Table 3) inhibited the hatching of miracidia and thus the entry of new infections into the snails. Moreover, the low temperature inhibited the development of infections acquired late last summer. Erasmus (1972) stated that in spite of the presence of the appropriate hosts of the digenetic trematodes, the progress of the life cycle may be interrupted by a fall in the external temperature. Kendall (1964) stated that the development of the miracida inside the egg of Fasciola hepatica, as well as the development of the larval stages in Lymnaea truncatula, are inhibited below 10°C. Secondly, the fall in the incidence is due to the death of the old infected snails during this period. The life span of B. tentaculata as already indicated, is 14 to 23 months, and thus in their second winter most of the snails have reached the end of their life span. Ollerenshaw (1971)

reported that the decline of Fasciola hepatica infections in Lymnaea truncatula after the summer peak in September is due to the death of infected snails.

The presence of a final host infected with the adult worms is essential for subsequent infection. In the habitat examined the final hosts of the cercaria of Notocotylus imbricatus and the Gymnocephalous cercariae infecting Bithynia tentaculata are aquatic birds (Wright, 1971). These birds are fairly abundant throughout the year, and as a result some trematode eggs are likely to be deposited in the water or washed into the habitat throughout the year. Thus it is probably the temperature and the life cycle of the snail host which are the most important factors in the seasonal variation in the incidence of cercariae infections.

Seasonal variations in the relative abundance of metacercariae infections in B. tentaculata were less marked (Fig. 21). The infection rate drops in June due to the appearance of the new generation of snails. These young snails are soon infected, particularly with the metacercariae of C. helvetica XIX, and by August about 95% of the snails are infected. The level of infection remains fairly constant from September to May. Although many old infected snails disappear in their second winter and numerous cercariae are produced in the early spring, a slight change occurred in the level of infection (Fig. 21). Pike (1968b) found biannual

peaks of metacercariae infections in Physa fontinalis.

Bithynia tentaculata in the habitat studied acts as the first intermediate host for six species of cercariae and as the second intermediate host for four metacercariae. In British freshwater this snail was found (Probert, 1966b and Pike, 1968b) as the most important host of cercariae infections, as well as an important host of metacercariae infections. Wesenberg-Lund (1934) stated that B. tentaculata is the most important intermediate host of digenetic trematodes in Danish freshwater. Wiśniewski (1958) found the greatest number of cercariae species in this snail, and Dubois (1929) noted its importance as an intermediate host of digenetic trematodes. Erasmus (1972) pointed out the remarkable susceptibility of this snail to trematode infection. Probert (1966b) suggested that the wide distribution, abundance and adaptability of B. tentaculata might be the reasons for its importance in the life cycle of digenetic trematodes. Furthermore, B. tentaculata is usually associated with well-oxygenated bodies of hard water which are rich in plant growth (Boycott, 1936). Such habitats possess a more varied fauna, and are likely to attract aquatic birds as feeding places and this might be partly responsible for the importance of this snail in the life cycles of the Digenea.

Although Bithynia tentaculata is heavily infected in various parts of Europe, the extent to which the snail is infected varies with the nature of the habitat in which it

is found. In the habitat examined, the average infection rate with cercariae and metacercariae was 36.2 and 93% respectively (Table 25). Similar infection rates of this snail with cercariae were reported by Wikgren, 1956; Probert, 1966b; and Pike, 1968b, from relatively small habitats. Wiśniewski (1958), in a study of a large lake, reported an infection rate of 14.1% of B. tentaculata with 17 cercariae species and five metacercariae. Wesenberg-Lund (1934) found that, in general, infection rates are high in ponds and low in lakes, and Rankin (1939) suggested that small habitats tend to provide a high incidence of infection.

The infection rate of Bithynia with cercariae in the habitat examined was low, and Pike (1968b) also noted the low infection rate of this snail in spite of the abundance of large individuals. Pike (1968b) suggested that the operculum may at times protect the snail from miracidial penetration and thus reduce its chance of infection compared with the unprotected pulmonates. If such behaviour has an effect on miracidial penetration, it is likely to affect the entrance of cercariae into the snail host as well. Yet the present investigation, and Pike's data from Ty Mawr farm, showed more than 90% of the snails infected with metacercariae. Probably the low infection rate of B. tentaculata is related to the availability of miracidia or worm eggs and the susceptibility of the snail. Wright (1971) stated that a particular strain of fluke may show different levels of

infectivity for certain populations of its snail host. Laboratory observations (see 4.2.5) showed that 6 of 7 old B. tentaculata (15 months old) were resistant to infection with Notocotylus imbricatus, whereas two of twenty-one young snails (less than 3 months old) were resistant to the same infection. Field observations also indicated that few B. tentaculata become infected in their second summer of life.

The incidence of individual species of cercariae infecting B. tentaculata in the habitat examined (Table 26), was relatively high compared with that reported by Pike (1968b) from Ty Mawr farm. Four of the six species of cercariae had an incidence ranging from 5.9 to 12.8%. Pike (1968b) showed that one of the ten cercariae encountered had an incidence of 5.05%, and most of these cercariae occurred in less than one percent of the sample examined. The higher incidence of most of the cercariae species found in the present study is probably due to the presence of a permanent fauna of final hosts (e.g,aquatic birds).

In addition to being commonly infected, B. tentaculata has its characteristic cercarial fauna. The Furcocercariae of the Vivax group reported by Wesenberg-Lund, 1934; Wikgren, 1956; Nasir and Erasmus, 1964; and Probert, 1966a, are exclusively found in this snail.

Moreover, none of the Echinostome cercariae or the Furco-cercariae infecting Lymnaea peregra and Lymnaea stagnalis observed in the habitat studied occurred in Bithynia tentaculata. Wesenberg-Lund (1934) reported that none of the eight Echinostome observed by him, nor of the 13 Echinostomes reported by Dubois (1929), occurred in B. tentaculata. Wikgren (1956) found the cercarial fauna of this snail to be specific, and so did Probert (1966b) and Pike (1968b). Wiśniewski (1958) reported that cercarial specificity is not so narrow as commonly accepted, and he found some cercariae infecting both prosobranch and pulmonate snails at the same time (e.g. Cercaria cristata which occurred in B. tentaculata, Valvata piscinalis and Lymnaea stagnalis).

Metacercarial specificity is less marked, as illustrated by the presence of Echinostome metacercariae in B. tentaculata in the present investigation. Wesenberg-Lund (1934) stated that metacercarial specificity usually operates for one animal phylum. Pike (1968b) found metacercariae specificity to be even less marked, and he recorded the metacercariae of Sphaerostoma bramae and Cotylurus brevis as occurring in nine and eleven hosts belonging to two and three animal phyla respectively.

While Bithynia tentaculata is an important host of cercariae infections, other freshwater prosobranchs harboured few or no cercariae. Wesenberg-Lund (1934) found B. leachii

free of cercariae infections, and Wiśniewski (1958) found two species of cercariae infecting this snail. No record of the cercariae infections of Bithynia leachii is available from British freshwaters, and it would be interesting to know about the larval trematode infection of this snail in this country. The hydrobiid snail Potamopyrgus jenkinsi (Hydrobia jenkinsi) was found by Rees (1932) to be free of infection. Valvata piscinalis, Viviparus viviparus and Theodoxus fluviatilis harbour few cercariae species (Wesenberg-Lund, 1934 and Nasir and Erasmus, 1964).

To sum up, the seasonal variation in the incidence of the total as well as the individual cercariae infections in B. tentaculata showed two peaks of infection during the year, one in the spring and the other in the autumn. These variations were attributed to changes in the life cycle of the snail host and environmental temperature. Other factors such as the presence of infected final hosts and rainfall are also important. Metacercariae infections were very high throughout the year, and no biannual peaks of infection could be detected. The cercariae infections of B. tentaculata are specific, but metacercariae specificity was less marked.

4.3.5 THE SEASONAL CYCLE OF CERCARIAE INFECTIONS IN RELATION TO THE LIFE CYCLE OF BITHYNIA TENTACULATA

4.3.5.1 Results

i) Relative abundance of the parthenita and cercaria stages of development of the infection.

The data on the incidence of cercariae infections in Bithynia tentaculata (Table 25) was used to find out the relative abundance of the parthenita and cercaria stages of development of the infection. The results are shown in Fig. 23. The parthenita stage included infections which were wholly made of either rediae or sporocysts, and no free cercariae were observed in the tissues of the host. The group designated as cercaria stage refer to infections in which cercariae were observed in the tissues of the host, but this would not necessarily mean maturity. This is because the Monostome and Gymnocephalous cercariae infecting the snail undergo some development outside the rediae and in the tissues of the host before maturity is reached.

The rate of parthenita infections in the snail (Fig. 23) increased from 8.9% in August 1973 to 23.3% in November. The infection rate was high in December. It was found that 12 and 27% of the young snails (less than 7 mm. in size) were infected in August and September respectively. The infection rates of the old population were 3 and 10% in August and September respectively. In January and February the infection rate fell to 9.4 and 8.7% respectively. An increase was noted in March and in April, 14.1% of the snails were infected. The infection

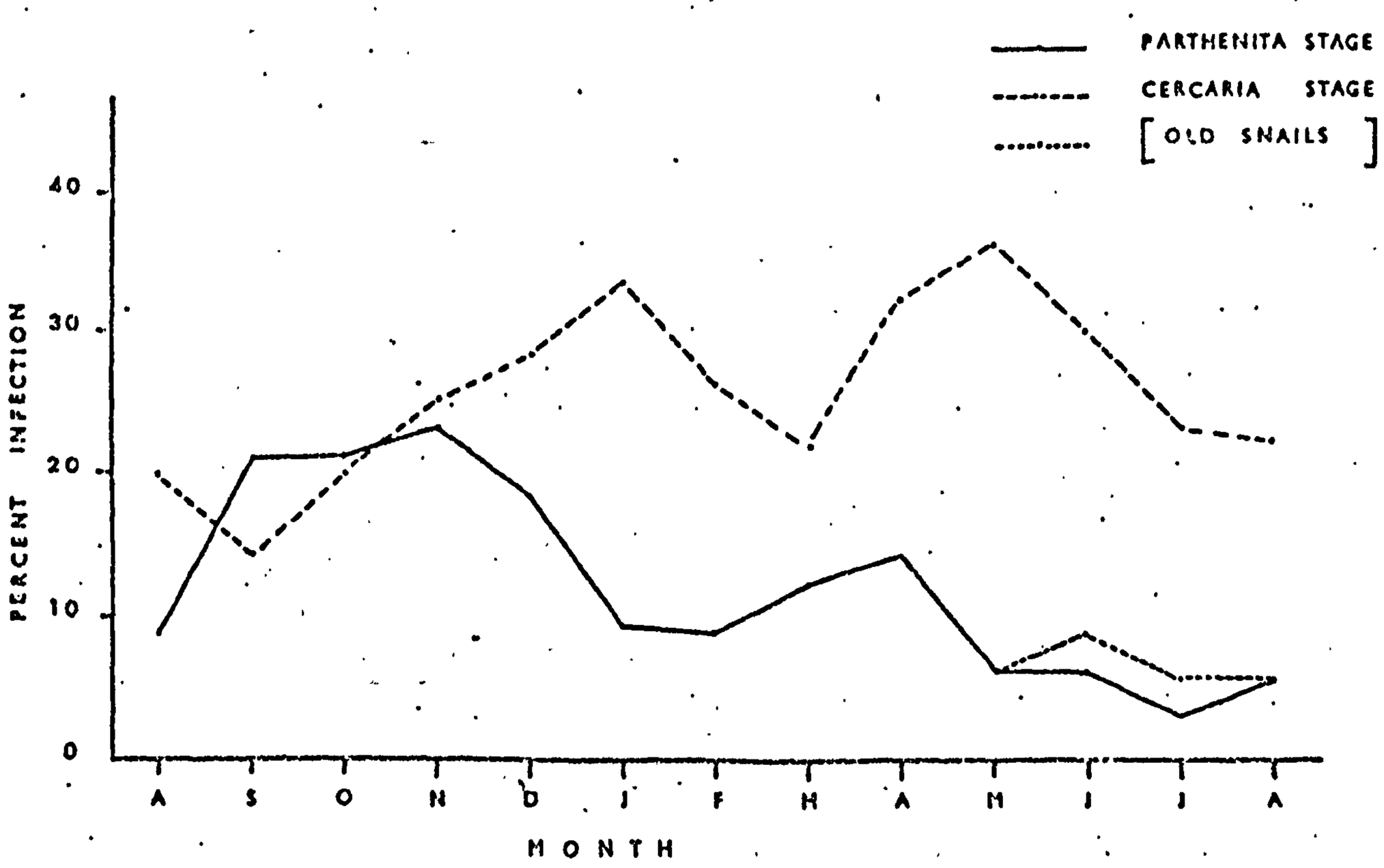


Fig. 23 The seasonal cycle of cercariae infections in Bithynia tentaculata.

rate declined in May (5.8%) and a low value was reached in July (2.8%). In August the infection rate rose to 5.3%.

As the new generation of snails appeared in June 1974 (the results presented above are for the total population), the incidence of parthenita infections in the old population from June to August were 9, 5.6 and 6% respectively. Examination of 50 old snails on 25th November 1974 revealed the presence of one parthenita infection (2%). Parthenita infections in the new generation of snails were first observed in August 1974, when five of 100 snails examined were infected, and in November 14 of 50 snails were infected (i.e, 28%).

Fig. 23 shows that 20 and 14.4% of the snails in August and September 1973 respectively, harboured the cercaria stage of the infection. The percentage of infected snails increased from October (18.9%) to reach 33.3% in January 1974. A decline in infection occurred in February (26.2%) and in March a lower level of infection was reached (21.7%). The infection rate rose in April, and in May 35.8% of the snails were infected, then the infection rate fell to 22% in August 1974. In the old population the infection rate increased to 51.6, 47.2 and 68% from June to August 1974 respectively. In the collection made on 25th November 1974, 58% of the old snails were infected while none of the infections in the young snails had produced cercariae.

TABLE 28

Incidence of the development stages of the different species of cercariae infecting Bithynia tentaculata

Date of collection	No.of snails examined	Cercariae of Notocotylus imbricatus				Gymnocephalous cercariae (except Cercaria lochocerca)				Cercaria parvus				Cercaria spatulata			
		Redia		Cercaria		* Redia		Cercaria		Sporocyst		Cercaria		Sporocyst		Cercaria	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1973																	
7 August	90	5	5.6	7	7.8	3	3.3	2	2.2	0	0	5	5.6	0	0	2	2.2
4 September	90	10	11.1	5	5.6	9	10	4	4.4	0	0	2	2.2	0	0	2	2.2
3 October	106	8	7.6	5	4.7	13	12.3	11	10.4	0	0	4	3.8	1	0.9	0	0
14 November	60	6	10	7	11.7	4	6.7	7	11.7	2	3.3	1	1.7	1	1.7	0	0
18 December	60	3	5	4	6.7	4	6.7	11	18.3	3	5	2	3.3	1	1.7	0	0
1974																	
17 January	138	2	1.5	13	9.4	4	2.9	25	18.1	7	5.1	6	4.4	0	0	2	1.5
21 February	183	3	1.6	17	9.3	4	2.2	28	15.3	5	2.7	3	1.6	4	2.2	0	0
21 March	364	10	2.8	37	10.2	28	7.7	28	7.7	2	0.6	14	3.9	3	0.8	0	0
17 April	184	6	3.3	14	7.6	14	7.6	29	15.8	2	1.1	14	7.6	4	2.2	2	1.1
15 May	190	3	1.6	23	12.1	3	1.6	27	14.2	4	2.1	16	8.4	1	0.5	2	1.1
18 June	184	1	0.5	19	10.3	9	4.9	27	14.7	1	0.5	7	3.8	0	0	1	0.5
15 July	142	1	0.7	13	9.2	2	1.4	11	7.8	1	0.7	6	4.2	0	0	2	1.4
13 August	150	7	4.9	8	5.3	1	0.7	15	10	0	0	7	4.7	0	0	2	1.3
Total	1941	65		172		98		225		27		87		15		15	

* In some cases it was difficult to distinguish the rediae of Cercaria helvetica XIX from that of Cercaria granocutis, and this is why they were combined.

Results obtained on the seasonal cycle of the infection of the different species or groups of cercariae showed some variation (Table 28). The rediae of the Gymnocephalous cercariae showed a third smaller peak of parthenita infection in June of 4.9% of the total population and 7% of the old population. The sporocysts of Cercaria parvus were found from November to May, with a peak in December - January (Table 28). Cercaria spatulata occurred in small numbers, and in general it showed the same cycle of infection as C. parvus.

ii) Observations on maturity of the infection.

Although many of the snails harboured the cercaria stage of the infection throughout the year (Table 28), it was observed that most of these infections were immature during the winter months, when few snails shed cercariae and in low numbers. Table 29 shows the relative abundance of immature and mature cercariae. The former include infections from the parthenita stage to those where some cercariae were almost mature. Mature infections refer to infections with fully developed cercariae which show the normal swimming behaviour of naturally-emerged cercariae. In February 1974, the infection was predominantly immature, 26.8% compared with 8.2% mature infections. By April, although the infection was still largely immature (27.7%), the proportion of mature infections increased (18.5%), and from May to August the infection was primarily mature (Table 29).

TABLE 29

Incidence of immature and mature cercariae infections in Bithynia tentaculata

Date of collection	No.of snails examined	Immature infections						Mature infections	
		Parthenita only		Immature cercariae		Total			
		No.	%	No.	%	No.	%	No.	%
1974									
21 February	183	16	8.7	33	18	49	26.8	15	8.2
17 April	184	26	14.1	25	13.6	51	27.7	34	18.5
15 May	190	11	5.8	12	6.3	23	12.1	56	29.5
18 June	184	11	6	0	0	11	6	55	30
15 July	142	4	2.8	0	0	4	2.8	33	23.2
13 August	150	8	5.3	0	0	8	5.3	33	22

4.3.5.2 Discussion

In the digenetic trematode life cycle, infection of the molluscan intermediate host results from the penetration of free-swimming miracidia, or from the ingestion of fluke eggs and the subsequent hatching of the contained miracidia inside the gut of the snail. In both cases the miracidia metamorphose into parent sporocysts and the latter, depending on the species, may give rise to rediae or daughter sporocysts which produce cercariae.

As no laboratory information is available on the development of the different species of trematodes within Bithynia tentaculata, except for Notocotylus imbricatus, the results obtained (Fig. 23) are discussed with reference to other published works. Laboratory observations showed that, at 18 to 21°C, the development of Notocotylus imbricatus within B. tentaculata takes about four months. Ollerenshaw (1959) stated that under conditions of average summer temperatures in Britain full development of Fasciola hepatica within the snail host will not occur in less than eight weeks. Thus it could be argued that the presence of the parthenita stage of the infection within B. tentaculata in the habitat examined between May and October could indicate that the infection was recently acquired. This is because the environmental temperature is suitable for the rapid development of the infection in the snail host (Table 3).

The abundance of parthenita infections within Bithynia tentaculata from August to November 1973 (Fig. 23) and in August and November 1974, indicate the importance of the summer infection. Although many parthenita infections were found in March and April 1974, these infections were likely to have entered the snails late the previous summer. Presumably numerous miracidia hatch between April and June, and many eggs of Notocotylus imbricatus will be ingested by the snails during this period. This is because of the rise in environmental temperature, the abundance of metacercariae, and the presence of the final hosts (aquatic birds). Thus new infections were expected to appear in the snails between June and August, yet few parthenita infections were found in the old snails at this period (Fig. 23) and these were mostly Gymnocephalous rediae (Table 28).

Brackett (1940) pointed out the importance of recognising the difference between juvenile and adult snails in the study of the seasonal incidence of trematode infections. On the basis of size differences, 12 and 27% of the young Bithynia tentaculata compared with 3 and 10% of the larger and possibly older snails in August and September 1973 respectively were infected with the parthenita stage. In August and November 1974, when the young and old snails were separated on morphological characters due to overlap in size, 5 and 28% of the young snails compared with 6 and 2% of the old snails in August and November respectively were infected. This clearly suggests that most of the new

infections in the summer were acquired by the young B. tentaculata, whereas few infections entered the adult snails. Cort (1941) found many juvenile Stagnicola emarginata angulata infected with larval trematodes in the summer, whereas few infections entered the adult snails in the latter half of their second summer; and the author suggested the possible development of immunity.

Fig. 23 suggests that some of the infections acquired by B. tentaculata in the summer of 1973 reached the cercaria stage of development in the same season (October - November). However, with the fall in the environmental temperature in November, few of these infections shed cercariae and the infection overwintered in an immature state, as observed in February 1974 (Table 29). These infections matured the following spring and numerous cercariae were produced in the spring and summer. However, in 1974 none of the summer infections in the young snails had reached the cercaria stage by November. This is probably due to temperature variations. Ollerenshaw (1971) stated that at the environmental temperatures existing at Weybridge from 1959-61, infections of Fasciola hepatica in the snail host matured in the same season when acquired before early August, whereas infections entering the snails after mid-August overwintered in a partially-developed state and maturity was reached late in the following spring or early summer.

Observations showed that the digestive gland of many of the old infected Bithynia tentaculata was damaged in November and through the winter. This is probably due to the active shedding of cercariae in the spring and second summer of the snails. Kendall (1964) stated that it is the final stages of the infection, particularly the emergence of cercariae, which cause most of the damage in the snail host. Cort (1941) remarked that in view of the damaged condition of the digestive gland of the snails, recovery from an old infection seemed to be unlikely. Thus probably the fall in the incidence of the cercaria stage of the infection in Bithynia tentaculata (Fig. 23) suggests the death of the old infected snails.

That variations exist in the development of the different species of digenean trematodes was pointed out by Kendall (1964). In the present investigation, the redia stage of both Notocotylus imbricatus and the Gymnocephalous cercariae infecting Bithynia tentaculata were abundant between August and December. The sporocysts of Cercaria parvus were mainly found from November to April, with a peak in December-January. Rankin (1939) reported that in general the sporocysts of Xiphidio-cercariae are filled with germ balls during the winter, whereas in the summer practically every sporocyst is bulging with active mature cercariae.

In summary, the course of cercariae infections in Bithynia tentaculata showed that the new generation of snails hatching in June - July is soon infected. The infections continued to enter the snails throughout the summer. Some of these infections could possibly mature in the same season, depending on temperature, but most of the infections overwinter in an immature state. In the spring, infections acquired last summer resume their development, most of these infections mature in May, and numerous cercariae are shed in the spring and summer. In addition, new infections enter the snails in the spring, but these infections are few compared with the summer infections. Fewer infections enter the snails in their second summer, and the old infected snails disappear in their second winter of life.

5. GENERAL DISCUSSION

The study of the life cycle, growth and reproduction in Bithynia tentaculata shows the importance of environmental factors, in particular temperature. The growth of the snails almost ceases in the winter, and active growth occurs in the spring and summer (Fig. 4, Table 2). Early growth of the new generation of snails is so rapid (Fig. 7) that age differences may be prominent. In addition the effect of differential growth rates, together with localised environmental inhibitors and stimuli, contribute to this heterogeneity (De Witt, 1955).

Variations occur in the growth rate of Bithynia tentaculata from one year to the next (Figs. 5 and 6), and from locality to locality as found by Pinell-Alloul and Magnin (1971). Similar variations in growth rates were reported in the freshwater pulmonate snails by Hunter (1953, 1961a and b), Duncan (1959) and Geldiay (1956). Hunter (1953, 1961b) suggested that the variations in the growth rates of Ancylus fluviatilis can be correlated with the amount of spring sunshine, because of the food and feeding behaviour of this species. Duncan (1959) and Hunter (1961b) noted that Physa fontinalis and Lymnaea peregra feed on a variety of detritus and algae, and thus light will probably be of lesser importance in influencing their growth rates.

Eisenberg (1970) found an inverse relationship between density and growth rate in Lymnaea elodes, but with the addition of food this relationship disappeared. The author suggested that the quality of food rather than quantity is the limiting factor. The present investigation suggests an inverse relationship between the density and growth rate of Bithynia tentaculata (Table 9 and Figs. 5 and 6). Hunter (1961b) found no inverse relationship between density and size in Ancylus fluviatilis, Lymnaea peregra and Physa fontinalis.

The reproductive behaviour of B. tentaculata is controlled by environmental temperature and other factors affecting the growth of the snails from hatching to maturity. The initiation of oviposition in the spring is controlled by temperature, and the snails did not lay eggs at 10 to 12°C. The freshwater pulmonates Lymnaea peregra and Planorbis corneus in the same habitat laid eggs at 10 to 12°C in early spring. Similarly, De Witt (1955), Duncan (1959) and Hunter (1961a) reported that certain freshwater pulmonates lay eggs when the temperature rises to 10 to 12°C. The sexual maturity of B. tentaculata is related to the growth rate and age of the snail, and there is a minimum breeding size as found in some of the freshwater pulmonates (Hunter, 1961a).

The population of Bithynia tentaculata in the spring of 1973 bred at a smaller size, laid fewer eggs and had a longer breeding season than in 1974 (Table 4 and 5). Such correlation between density, breeding size, number of eggs laid and length of the breeding season indicate the plasticity of the reproductive behaviour of this snail. Thus the variations in the growth rates and reproductive capacity of B. tentaculata reported by Pinel-Alloul and Magnin (1971), Lilly (1953) and Hubendick (1948) could possibly be explained by the environmental limitations. Eisenberg (1970) suggested that the population of Lymnaea elodes exists at two levels with regard to nutrition. Under poor trophic conditions the snails do not grow well and are barely capable of reproducing, but when the nutritional requirements are partly met, the snails are capable of realising some of their potential for growth and reproduction.

A late summer breeding activity of the spring-born generation is fairly common in the freshwater pulmonates (Hunter, 1961a; Duncan, 1959; De Witt, 1955), and this is due to the rapid maturity of these snails as shown by field and laboratory observations of the above authors.

B. tentaculata differs from the freshwater pulmonates in two respects. Firstly, laboratory observations showed that few of the snails mature in five to six months after hatching and

most of the snails mature in about ten months (Fig. 8). De Witt (1952) found similar long periods for the maturation of species of Oncomelania (except O. quadrasi) and Pomatiopsis lapidaria. Secondly, some of the freshwater pulmonates like Physa fontinalis and Lymnaea peregra die off soon after spawning (Hunter, 1961a). Many Bithynia tentaculata survive throughout the second summer of their life, and may produce a few eggs (Table 4), depending on the growth rate and maturity of the population (Fig. 3). Thus the presence of young snails in the winter of 1972-73 (Fig. 3) could possibly be due to the delayed maturity of some of the previous year's spawn rather than to the rapid maturity of the same year's brood as suggested by Pinel-Alloul and Magnin (1971).

Boycott (1936) noted the heavy mortality of the young snails, and similarly Hunter (1961a and b) reported that a relatively high mortality appears to be common to all species of freshwater pulmonates. Laboratory and field observations also indicate a heavy mortality of young Bithynia tentaculata (Table 14). Adult mortality is more variable, and many of the freshwater pulmonates have a life span of about one year (Boycott, 1936; Hunter, 1961a and b). The life span of B. tentaculata has been estimated from about one year or less (Pinel-Alloul and Magnin, 1971) to two or three years (Lilly, 1953; Schäfer, 1953). The present investigation suggests

that the life span of this snail is about 14 to 23 months, and that probably few of the snails survive to breed for a second season. In the laboratory a few of the snails survived for more than two years, but as laboratory populations escape the environmental limitations to which field populations are subjected (De Wit, 1955) it seems unlikely that Bithynia tentaculata would survive for more than two years in the field.

Observations on the natural diet indicate that B. tentaculata, like other freshwater snails (Boycott, 1936), feed on detritus and algae. Macrophytes and animal remains constitute a minor portion of their diet (Tables 12 and 13). The importance of food supply in the growth of laboratory populations was shown by Turner (1926), Wright (1960) and Van der Schalie and Davies (1965). A similar result was obtained in the present work, and optimum growth occurred when B. tentaculata was fed on detritus and algae supplemented with lettuce (Fig. 11). The distribution of B. tentaculata within the habitat is affected by the local conditions. Few of the snails are found where the water is stagnant and foul (Table 10). Pesigan et al. (1958) noted that Oncomelania quadrasi is absent in stagnant and foul waters, and Boycott (1936) stated that organic pollution is detrimental to the abundance of freshwater molluscs.

Nasir and Erasmus (1964) reported the revival of interest in the study of the larval trematode infections of the British freshwater molluscs. The cercariae infections of the freshwater molluscs of the Liverpool area have not been investigated before, and the present work is limited to the infections of Bithynia tentaculata. Six species of cercariae infect the snail, a Monostome, three Gymnocephalous, a Xiphidiocercaria and a pharyngeal longifurcate monostome furcocercaria. One of the Gymnocephalous cercariae, namely Cercaria helvetica XIX Dubois, 1929 is reported for the first time in British freshwater. The existing description of this cercaria was incomplete and has been expanded in the present work, and the metacercaria is described. The remaining five cercariae have been described from British freshwater, but additional information on the structure of some of these cercariae and a new description of the parent sporocyst of Cercaria spatulata Probert, 1966, are added.

The observations made on the cercariae infections of B. tentaculata indicate, as other investigators (Cable and Wheeler, 1939; Wikgren, 1956; Lewellyn, 1957) have shown, the difficulties encountered in the differentiation of closely related species. When the morphology, anatomy and behaviour of the cercariae are studied in detail, slight but consistent differences may be observed in related species, as suggested by Cable and Wheeler (1939) for Cercaria opacocorpa and Cercaria semicarinatae. However, the validity of the identification can

be established when the life cycle is completed.

Attempts to trace the life cycles of the cercariae infections of B. tentaculata were successful in one case. The metacercariae of Cercaria imbricata were infective to the final host soon after encystment, although Pike (1969) doubted this. The structure of the adult Notocotylus imbricatus is similar to previous reports (Fig. 19), and the present findings confirm Beverly-Burton (1961) and Odening (1968) suggestion that N. imbricatus may contain as many as 49 ventral glands. However, one worm was found to contain 50 ventral glands arranged as 17:16:17 (Table 22). The development of Notocotylus imbricatus in Bithynia tentaculata showed that the parent sporocyst contains a single redia. The cercariae appear inside the rediae twelve weeks after infection (Temperature 18 to 21°C), and possibly mature in about 16 weeks (Table 23). Herber (1955) found the parent sporocyst of Notocotylus urbanensis in Physa gyrina to contain four to eight rediae. Shorter periods of development were reported for Notocotylus attenuatus in Lymnaea peregra (Wright and Bennett, 1964) and N. urbanensis in Physa gyrina (Herber, 1955).

Multiple cercariae infections in Bithynia tentaculata are rare, yet a greater number of double cercariae infections is recorded than previously found in this snail (Table 24). This is probably due to the relatively higher incidence of the individual species of cercariae. All the double infections involved

Cercaria parvus, and similarly, Dubois (1929), Probert (1966b), Pike (1968b) and Wikgren (1956) found all double infections in Bithynia tentaculata to involve a Xiphidiocercaria. The combinations of double infections observed suggested that in some cases there may be an immunity or antagonism to the existance of double infections (Cort et al. 1937). However, combinations of Cercaria parvus with Cercaria imbricata in double infections were greater than expected, possibly suggesting that infection of a snail with one of these species enhances its infection with the other species (Ewers, 1960; Bourns, 1963). Wesenberg-Lund (1934) noted that double infections of B. tentaculata with Cercaria imbricata and Xiphidiocercariae were often observed.

The seasonal incidence of the cercariae infections in B. tentaculata show two peaks of infection in the course of the year, one in April and the second in November (Fig. 21). Similar biannual peaks of infection were shown by four of the cercariae species infecting the snail. These seasonal variations were attributed by Sewell (1922), Dubois (1929) and Rees (1932) to changes in the life cycle of the snail host, environmental conditions, and the degree of infection of the final hosts.

The effect of temperature on the development of the eggs of Fasciola hepatica to the hatching stage was shown by Rowcliffe and Ollerenshaw (1960), who found the development to be inhibited below 10°C. Kendall (1964) stated that at temperatures below 10°C no appreciable development of Fasciola hepatica in Lymnaea truncatula occurs. Similarly, the fall in the incidence of cercariae infections in B. tentaculata between December and

March (Fig. 21) indicate the role of temperature. Furthermore, individuals of B. tentaculata in their second winter have reached the limit of their life span, and hence the death of these snails contributes to the fall in incidence.

However, the fall in the incidence of cercariae infections in June to August (Fig. 21), when the environmental temperature is suitable for the development of the infection (Rowcliffe and Ollerenshaw, 1960; Kendall, 1964), is due to changes in the life cycle of the snail host. The fall in incidence is caused by the appearance of the new generation of snails, which are either uninfected at this time or may harbour young developmental stages of the infection (Erasmus, 1972), together with a reduction in old snails due to death. Although many old snails are present in the summer few new infections entered these snails (Fig. 23), and laboratory observations suggested age resistance to infection with Notocotylus imbricatus in Bithynia tentaculata. Cort (1941) noted that few infections enter Stagnicola emarginata angulata in the second summer of their life, and the author suggested the possible development of immunity.

Seasonal variations in the incidence of metacercariae infections were less marked and no biannual peaks were observed (Fig. 21). Observations on the habitat studied show that the cercariae infections of B. tentaculata are specific, as suggested by Wesenberg-Lund (1934), Dubois (1929) and others. The metacercarial specificity was less rigid, as found by Wesenberg-Lund (1934) and Pike (1968b).

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