STUDIES ON FRESHWATER PULMONATE SNAILS AND CERTAIN ASSOCIATED STRIGEID TREMATODES

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SECTION I

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GENERAL INTRODUCTION

Discussion of the investigations described is included in each section, but it is appropriate to consider certain general aspects more fully. To avoid repetition, individual references on points of detail are not mentioned if they have been cited in the same context in one or more of the subsequent sections.

In a recent book Elton (1958) has referred to "the Neolithic days of animal ecology, that is to say about 25 years ago." The phrase is singularly apt in relation to the ecology of freshwater snails since the observations published prior to the 1930's were of an essentially primitive nature, without a suitable quantitative basis and lacking in detail. It was only with the appearance of studies such as those of Van Cleave and Lederer (1932), Van Cleave and Altringer (1937) and van der Schalie (1938) in the United States of America, and Boycott (1936) in Britain, that the ecology of freshwater snails began to emerge on a stable basis. Until a number of other detailed studies became available in the 1950's it was not possible to reach general conclusions based on a knowledge of several species. Still there are remarkable gaps in our knowledge.

Almost certainly the largest bibliography which could be compiled for any freshwater snail would be that concerning Lymnaea stagnalis (L.). Due to its relatively large size

and wide distribution it has attracted attention over a long period. Most aspects of its anatomy, development and reproduction are known in detail and it has been the subject of many physiological investigations. Yet, prior to the observations recorded in Section II, no detailed study had been made of a natural population of this species. Opinions had varied as to how long it lived (see Section II) but it now appears that throughout most of Britain it is probably an annual although in unfavourable environments, such as the pond studied at Bellshill, its life cycle may be extended to include a second year. This agrees well with the general pattern of life cycles of freshwater snails which has emerged in Britain (Duncan, 1959; Hunter, 1957 and 1961b) where the annual variation of temperature constitutes the dominant environmental influence. Most existing studies have shown a simple annual life cycle with a fairly constant reproductive period usually occurring in the early summer. Species which are sufficiently small and fast growing may complete one or more additional generations during the warm summer conditions. L. stagnalis, on the other hand, may extend the annual pattern when conditions are not adequate to permit full development within a single year, although in most years, many individuals will probably still die when just over one-year-old.

A striking gap which remains in our knowledge of natural populations of freshwater snails in Britain concerns Planorbarius corneus (L.). Like L. stagnalis this species has been the subject of numerous laboratory investigations but information on its life cycle remains a matter of speculation. There has been general agreement that P. corneus did not have an annual life cycle and that it did not reach maturity during its first year, but the brief series of observations reported in Section IV suggests that both these assumptions have been false. Further, more detailed studies of this species will be necessary before any general picture of its life cycle can be reached with certainty.

The laboratory investigations carried out on L. stagnalis have included detailed accounts of the anatomy and histology of the reproductive system, the processes of insemination and oviposition and the development and maturation of spermatozoa and oocytes in the gonad. Most of the developmental changes involved occur in a smooth succession paralleling the steady growth rates found under the stable environmental conditions of the laboratory and these studies have excluded the influence of natural environmental conditions under which the snails show a restricted breeding season. In Section III some of the developmental changes

taking place in the reproductive system of L. stagnalis have been examined seasonally in conjunction with the field investigation described in Section II. As shown in Section II the pattern of growth of individuals in a natural population of L. stagnalis is itself seasonal and it must be expected that other developmental changes will show a corresponding seasonal pattern. This was the case with the vergic sheath and the preputium, but a more restricted period in which extensive changes took place was shown among the germinal and secretory portions of the reproductive system. This activity was largely confined to the period immediately preceding the onset of oviposition and appears to be related more to season than to size. It has already been pointed out that it is not easy to dissociate the effects of size and season when considering an animal with a regular pattern of growth and reproduction, as populations will fall into roughly the same size groups at the same time in any year, with only some variation depending on the prevailing growth conditions. Nevertheless, it is clear from Section II that the start of the breeding season was constant in the Bellshill population of L. stagnalis in both years although the respective growth conditions were somewhat different. A similar constant onset of breeding has been shown in three other freshwater pulmonate species (Hunter, 1961a).

Several species of freshwater pulmonate snails were recognized at the time of Linnaeus and most of the species found in Britain are of relatively long standing which tends to create an impression that the systematics of this group are well defined. Progress in the field of taxonomy has in recent years tended to require more and more precise definition of each species and it has become increasingly clear that it is often very hard to achieve further clarification of the characters of freshwater pulmonates, as the species tend to be far more variable than is generally the case among other groups of molluscs.

Similar views on the evolutionary significance of this have been expressed independently by Hubendick (1952 and 1954) and Hunter (1952, 1957 and 1961b) who consider that the problem arises from the transient nature of the freshwater environment. Of the existing bodies of fresh water only Lake Baikal, Lake Ochrid, Lake Tanganyika and 3 lakes on Celebes are known to be geologically old, and these, particularly Lake Tanganyika, contain a number of clearly defined endemic species of freshwater snails. On the other hand Lake Victoria, the second largest area of fresh water in the world, is not nearly so old and is known to have had a chequered history of fluctuating levels (Wayland, 1931) and this has been considered an important

feature in the evolution of some of the fish in that lake (Greenwood, 1951). Most bodies of fresh water persist for only a relatively short time geologically as a result of which their molluscan fauna has time to develop considerable infra-specific variation but insufficient time for evolution to proceed to higher levels.

Recently Hubendick (1962) has extended this view to show that the same is true of the whole part of the fauna which is exclusively limnic (for which he has proposed the term hololimnic) with the exception of fish. The difference between fish and the invertebrate hololimnic fauna is explained on the basis that fish have a high capacity for active dispersal and a low capacity for passive dispersal whereas the opposite is true of the hololimnic invertebrates. Thus if faced with a catastrophic change in their environment, fish may be able to actively move themselves to neighbouring areas which remain suitable and where the process of evolution can continue. Freshwater snails, lacking this capacity, will tend to be overtaken by the catastrophy and any evolutionary divergence developed in that locality will be lost.

While evolutionary diversification tends to be abruptly terminated by the temporary nature of the freshwater habitat, it is also encouraged by the discontinuous nature of the

habitat. Populations of hololimnic invertebrates are usually isolated from each other in small local populations thus creating a situation which is extremely suitable for the development of divergent forms. The interaction of these two aspects of the environment is thought to have led to the observed variability of freshwater snails at the infra-specific level.

In an earlier study Hubendick (1951) included a detailed study of variation in the shell of Lymnaea peregra (Müller) throughout the Scandinavian region. He concluded that the species showed a high degree of both genotypic and phenotypic variation and that this occurred in an irregular pattern over the area studied. It was felt that a study of variation in the radula of this species throughout the same area would form a useful supplement to Hubendick's observations, as it seemed likely that the radula would be fairly well protected from direct influence by the environment and would, therefore, show little ecophenotypic variation but would rather serve as a measure of the genotypic variation present in this species in the Scandinavian region. For this reason the investigation described in Section V was undertaken. The results are discussed in that section and show no inconsistency with the conclusions drawn by Hubendick from his own study. There

is no suggestion that variation in the shell is in any way paralleled by variation in the radula. Both features seem to be extremely variable in their own right and the results of the radula study are in agreement with the general theories concerning freshwater snails which have been considered above.

Hunter has also extended his views on the evolutionary significance of the infra-specific variation found among freshwater snails. He has emphasised that this gives rise to an adaptive plasticity of both form and function and has illustrated the value of this characteristic from his studies of the respiratory behaviour of freshwater pulmonate snails (Hunter, 1953 and 1957). From his own and other studies Hunter (1961a and b) has also shown that a similar plasticity exists in the reproductive behaviour and life cycles of many freshwater snails. The observations recorded in Section II provide considerable support for this hypothesis since the life cycle of the Bellshill population of L. stagnalis is very considerably modified to allow the population to survive in a relatively unfavourable environment. Hunter (1961b) considers the possession of this high degree of adaptive plasticity to be of great survival value in the freshwater environment and consequently concludes that there will have been selection pressure for

this characteristic in the course of the evolution of the freshwater pulmonates. Such an evolutionary trend would be associated with extensive genotypic variability such as was shown in L. peregra by Hubendick (1951) and by the study described in Section V.

The strigeid trematodes are very closely associated with freshwater pulmonate snails which serve as their first intermediate host and for this reason the results of two investigations on these parasites have been included here as Sections VI and VII. As a group they have not attracted much attention since they have a complicated life cycle involving two intermediate hosts and were thought to be of little economic importance. Recently they have been shown to be very suitable material for physiological studies in vitro (Bell and Hopkins, 1956; Bell and Smyth, 1958; Wyllie, Williams and Hopkins, 1960; Williams, Hopkins and Wyllie, 1961) and to be a potentially serious infection among fish being cultured in ponds causing a drastic decrease in productivity (De Bont, 1956). The details of their life cycles are known in only a few cases and the present observations have confirmed the sequence of events in the case of Diplostomum phoxini (Faust) and have also shown that two different species with very similar life cycles are found in the eyes of sticklebacks. One of these,

D. spathaceum (Rudolphi), is perhaps the best known of all the strigeid trematodes but the existence of the other has not been recorded previously. The certainty with which the metacercariae of these two species select the retina and the lens respectively as the sites for their development at this stage is clearly shown in Section VII and is a striking example of the effective habitat selection which can be exercised by larval trematodes. Section VI shows how the final development of adult flukes may be modified in different final hosts. In itself this is not surprising but the morphological differences among the worms examined from the three alternative hosts are extensive and it is obvious from the data on the relative development of the posterior lobe and on the numbers of eggs observed in the uterus that the reproductive potential is also considerably modified. The parasites apparently achieved full maturity in the three hosts but in doing so they presented three quite distinct morphological forms. Attention is directed in Section VI to the implications of these findings in relation to existing theories on the host specificity of the Strigeida. Many species have been described on the basis of a few specimens often recovered from a single host animal and in such cases great care will be necessary to prevent the same species being accorded several different names as a

result of the modifications of its development which may result from its ability to mature in several final host species.

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SECTION II

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ON THE LIFE CYCLE OF LYMNAEA STAGNALIS (L.) IN THE WEST OF SCOTLAND

INTRODUCTION.

The large pond snail, Lymnaea stagnalis (L.), has a geographical range extending over most of Europe and much of Asia and North America. Within this area it is generally absent from waters with a low calcium content (less than about 20 p.p.m; Boycott, 1936), and its minimum calcium requirements may be expected to rise with increase in latitude or in altitude (Boycott and Oldham, 1936; Hunter and Hunter, 1956). This factor certainly limits its occurrence in Scotland and the population discussed here is living at the northern edge of its range in Britain (Ellis, 1951). Forbes and Hanley (1853) and Ritchie (1920) state that L. stagnalis has been introduced to Scotland; and an apparently unsuccessful attempt to establish the species near Brora is recorded by Baillie (1887 and 1889). Such population studies as have been made on freshwater pulmonate snails in Britain are mostly recent and have, as yet, been confined to species having an annual life cycle or more than one cycle per year. L. stagnalis is among the few species thought to be able to live for a longer period but information on the biology of natural populations is lacking.

The present observations were made on a small population of L. stagnalis between October 1956 and July 1958, and two subsequent visits in 1959 have enabled wider conclusions to

be drawn. The accumulated information is presented to show the life cycle, growth and reproduction of this species in natural conditions, and these conclusions are discussed in relation to existing knowledge of other freshwater pulmonate snails.

HABITAT AND METHODS.

The population of L. stagnalis discussed lives in a small closed pond covering an area of about 200 sq. m. about a mile to the north-west of Bellshill, Lanarkshire. The bottom consists mainly of soft mud containing a high proportion of coal dust, but is partly covered at one end by large slabs of stone. There is no inflow to the pond other than by natural drainage and there is a small outflow channel which carries water only when the level is high. The principal vegetation consists of branched bur-reed, Sparganium erectum; spiked millfoil, Myriophyllum spicatum; and ivy-leaved duckweed, Lemna trisulca. Other molluscs found in the pond are Physa fontinalis (L.), Planorbis crista (L.) and Pisidium spp. The pH of the water was found to be about 7 and the calcium content was 26 p.p.m.

A collection of snails was made every four weeks with the aim of obtaining 40-50 specimens. These were gathered by hand from the part of the pond where the bottom was covered

with large stones. This method may favour the collection of large snails which are more readily seen, but there is no reason to believe that the results have been unduly biased in this way. Each slab was examined thoroughly in an attempt to collect every snail from it before any were taken from the next slab. Since the collections were always taken from the same place, so long as sufficient snails could be found, there is a danger that the repopulation of this area may be inadequate or not representative of the whole population. However, the stone surfaces formed a very suitable feeding area for the snails and, even in extremely cold weather when the snails were rather inactive, the area was satisfactorily recolonised between collections. In the breeding season of 1957 adequate repopulation occurred within a week.

The collections were removed to the laboratory for examination and not returned to the pond. The total height and maximum width of each shell were recorded as were the maximum height and width of the aperture. Considerable erosion of the spire and other parts of the shell occurred in these snails. Shell height is used throughout this paper as the most convenient criterion of size. Some anatomical studies were also made on the snails but these will form the subject of a separate communication.

RESULTS.

At the beginning of 1957 the overwintering population in the pond had a clear bimodal size distribution, with the two means at 23.15 mm and 12.46 mm (Tables 1 and 2). This was also shown by collections taken during November and December 1956 (Fig. 1) but these data have not been included in the tables. The snails in the smaller-size group had thinner shells and had obviously hatched during 1956. The larger individuals had much heavier and darker shells and were sexually mature. They had apparently bred during 1956 and were at least one year old. During February the young snails showed an increase in size and by March both groups were growing. The young snails grew more rapidly and the gap between the two groups closed. Their size ranges began to overlap in April, but their mean sizes remained quite distinct until July. By this time the shells of the larger individuals among the younger snails had also thickened and darkened, so that from July onwards it proved impossible to separate the two groups.

During July also, very young snails first began to appear in the collections. These had hatched during the previous month and grew steadily until September (Table 3). They overwintered at an average size of about 17 mm; 4 mm larger than the comparable age group the previous winter.

Meantime the snails which had been breeding grew to a mean size of 30 mm at which they overwintered. During the winter of 1957-58 the population was thus again bimodal in size distribution but in ten collections taken from September to May the larger-size group only constituted just over 7% of the snails taken. This must be attributed, at least in part, to the fact that some 300 members of this generation were removed in the collections up to July 1957, which represents a very significant proportion of the total population in a pond of this size.

From September 1957 until April 1958 there was no noticeable growth of snails. Early in 1958 a considerable proportion of smaller-sized young snails suddenly appeared in the collections and continued to appear as a distinct group until observations ended in July (Table 4). A few snails of this size group had also been taken in the collection of October 1957 and presumably represented a late hatch during the previous summer whose growth was quickly stopped by the onset of winter. When the prolonged winter conditions came to an end during April, growth was resumed by both groups of young snails and, by the end of May, the larger group had reached the same size as the year-old snails at the same time the previous year. The smaller-size group did not catch up with the larger one and still

existed as a distinct group in the July collection, by which time it was only beginning to reach breeding size. Young snails again appeared in the July collection but were slightly smaller than the previous year. Since the July collection then was taken some ten days earlier this may represent a real delay in the start of breeding resulting from the severe and prolonged winter. During spring 1958, there was no indication of growth among the survivors which had bred during 1957, which seem to have attained the maximum size for this habitat during the previous summer.

During 1959 visits were made to the pond in April and September. No great stress can be laid on these isolated observations but the data correspond well with the results from the period of continuous study. The April collection should represent approximately the state in which the population passed the winter months although a little growth may have started. Its size distribution may represent three groups. A small group of large snails could be the surviving adults from the 1958 breeding season and would seem to be from the late-hatched group since they are still too small to be from the main hatch. The bulk of the collection could be the snails hatched during the main part of the 1958 breeding season and their overwintering size of about 18-19 mm would be about the same as that of the main

hatch the previous year. There is probably a small group of late-hatched snails which may well be the offspring of the late hatch from the previous year. The September collection returns to a clear bimodal size distribution similar to that seen in January 1957 but with the average size of the snails being somewhat larger. It would appear that there was no late hatch of young during 1959, but the late hatch of 1957 did not show in the collections until January 1958.

The growth rates of the snails in the population are shown in Tables 1 - 4 and also in Fig. 2. Growth is concentrated mainly into two periods of the life cycle. One occurs during the first three months of life before the onset of colder conditions. Table 3 shows that during this period the snails which hatched in 1957 were increasing in average length by about 1 mm/wk which represents more than 30% per month. The second period of rapid growth occurs during the animals' first spring. In 1957 this growth reached its peak in April (Table 2) when the mean shell length was again increased by over 30% during the month. In 1958 the peak was again reached in April and represented an increase of just over 20% for the four-week period in the larger-size group (Table 3) but about 30% in the smaller-size group (Table 4). The latter continued to

grow rapidly until observations ended in July whereas the growth rate of the larger-size group dropped off as the largest members of the group approached their probable maximum size and breeding began.

An attempt was made to assess the relative density of the snail population at different times of year by using snails collected per hour as a unit. This was found to be impracticable since varying physical conditions, such as the presence or absence of floating duck-weed in the summer or of ice in the winter, exerted a considerable influence on the time taken. The approximate area searched to collect the samples, however, showed an interesting variation during the year. Normally the collection was made within an area of about 3 sq. yds., but during the months of June, July and August it was necessary to collect over a much wider area to obtain a collection which was still smaller than that obtained at other times of the year.

Egg masses of L. stagnalis were laid mainly on the outer leaves of bur-reed plants which were usually in some stage of decay. Some were also found on fresh green leaves of bur-reed, on stones, on submerged pieces of wood, on Lemna leaves and on the shells of other snails. The egg masses of P. fontinalis occurred mainly on green bur-reed leaves and were more numerous than the Lymnaea masses

although the total population of this species did not appear to be as great as that of L. stagnalis.

During most of the 1957 breeding season, weekly visits were made to the pond. The first egg masses were found on 21 May, and were all newly laid or in a very early stage of development. On 4 June one of the masses found was almost ready to hatch and, since the period of embryonic development lasts for about three weeks, this confirms that egg-laying started about the middle of May. About mid-June the number of egg masses had reached its maximum and all stages of development were found. By mid-July the number of egg masses had declined and most of those seen were almost ready to hatch. In mid-August only a few egg masses were observed, while the embryos in the three masses found during September and October were all dead. During the breeding season 51 egg masses were brought to the laboratory for closer examination. The average number of eggs per mass was 34.6 with a maximum of 55 and a minimum of 9. Three cases of twin embryos were observed; an incidence of 0.16%. In those egg masses where some development had occurred the proportion of eggs which had failed to develop was 8.5%. This figure is probably an underestimate since a completely infertile mass would be difficult to distinguish from a newly laid mass. During the 1958 breeding season the first

egg mass was seen on 20 May although none was found during a visit one week later. This suggests that the start of the breeding season is fairly constant in timing, but with a delay resulting from the severe spring conditions in 1958.

INFLUENCE OF WEATHER CONDITIONS.

A useful summary of the overwinter weather conditions which seem to exert most influence on freshwater snail populations has been devised by Hunter (1961a). Professor W. Russell Hunter has kindly supplied a similar analysis of the overwinter conditions for 1958-59 and Table 5 has been prepared from his data for the years 1956-59. Hunter (1961a) considered the annual variations in size, density and productivity of populations of Ancylus fluviatilis Müller, Physa fontinalis and Lymnaea peregra (Müller). He has shown that while the two latter species fluctuate from year to year in approximately the same manner, A. fluviatilis is more directly dependent on the amount of spring sunshine. This has been attributed to differences in their food requirements and feeding behaviour and on this basis L. stagnalis would be expected to react in the same way as L. peregra and P. fontinalis. Hunter showed that 1956 was a poor year and 1957 a good one, and his weather data suggest that 1958 was also a poor year and 1959 somewhat

better. The observations on L. stagnalis confirm that 1956 was a bad year for the snails since the overwintering population during 1956-57 had smaller mean sizes than in any of the following years. 1957 was certainly a good year as growth during that spring was rapid and the one-year-old snails had attained their maximum size by June and showed almost no further growth thereafter. In the late summer of 1957 growth conditions were also good and the young snails overwintered at a size about 4 mm larger than the corresponding group the previous winter. In 1958 growth began later than in the previous year and by May even the larger-size group of young snails had not reached the same mean size as the corresponding group had by that time in 1957. Little can be said about 1959 but, if the September collection is regarded as representing the overwintering state of the population, then both the old and the young snails had reached a size intermediate between those overwintering during 1956-57 and those of 1957-58. Thus the observations on annual variation of growth presented here are in complete agreement with those of Hunter (1961a) in relation to the prevailing weather conditions.

Fig. 3 shows the fluctuations in pond temperature as recorded when collections were being made. While these give a general picture of the conditions, they were mostly

taken about 11 a.m. and no information was obtained of the diurnal changes. With this reservation, it seems that the snails were affected by trends of temperature rather than by absolute temperature. Thus growth in spring began at a temperature considerably lower than that at which it ceased in the autumn; but in the first case there was an upward trend of temperature and in the second a downward trend. However, the relative abundance of available food at these two times of year may have been a critical factor. Viable egg masses appeared in 1957 at a water temperature of 12.5°C but those found in September and October when the temperature was scarcely lower contained dead embryos.

DISCUSSION.

In earlier literature various statements have been made about the length of life of L. stagnalis but little supporting evidence has been produced. It seems certain that the population at Bellshill had a biennial life cycle even although it is impossible to distinguish the adult snails after they are about one year old. During 1957 the two age groups which had overwintered became indistinguishable from July onwards but by that time the older group had already declined in numbers. Also, the large-size group overwintering from 1956-57 had not yet reached maximum size

and it is reasonable to assume that any snails breeding for a second time during 1956 would also have attained maximum size by that summer. If they had survived for another year this would have given rise to a trimodal size distribution in the population during the winter of 1956-57. It is not clear if snails which attain their maximum size at the end of their first year then die. This might be indicated by the small number of large snails which overwintered after the very favourable spring and summer of 1957 and by the lack of very large specimens in the collection taken in April 1959; but the effect of removal of snails mentioned above should be borne in mind. It is also possible that the older group overwintering during 1956-57 were members of a late hatch from 1955 and that the main hatch had already attained full size and died off. The evidence for the existence of a late hatch in 1957 is fairly clear, but the histograms for February, May and June 1957, in Fig. 1 show that there is also the possibility that a late hatch on a smaller scale occurred during 1956. These indications should not be over-emphasized because a peak of egg-laying fairly early in the breeding season must give rise to some skewing of the size group in the histograms. Such skewing can be noted also in the collection of April 1959, which could represent a late hatch again during 1958. However,

the existing evidence does not support a hypothesis that there may be two functional groups present in the population, one breeding early and the other later.

The difficulty experienced in finding snails during summer months is significant and three causes may be suggested. First, it seems that the last of the two-year-old snails die at this time, as do some of the one-year-old ones. Secondly, most of the snails' eggs are laid on weeds, whereas the usual collecting area has a bottom of large stones and very little weed. It is about August before the young snails colonise this stony area. Thirdly, during these three months the water temperature is highest (Fig. 3). Under these conditions the snails' respiratory requirements will be increased (Krogh, 1916; Berg, Lumbye and Ockelmann, 1958; Berg and Ockelmann, 1959; Jones, 1961) and the oxygen content of the water may decrease (Mortimer, 1956) so that the large snails will require to surface more frequently to renew the air in their mantle cavities. The scarcity of emergent vegetation in the collecting area could limit re-colonisation during this period. Hunter (1953b) has shown that the respiratory requirements of adult L. peregra force them to stay close to the edge of the water in Loch Lomond under summer conditions even when this removes them from their food supply causing partial starvation. At

that time the temperature of the loch did not fall below 16.8°C and the scarcity of L. stagnalis in the collecting area of the pond at Bellshill corresponds with a period during which day temperatures were probably never much less than 15°C (see Fig. 3). Since L. stagnalis is larger than L. peregra it will have proportionately less surface area available for cutaneous respiration.

The egg capsules produced by the Bellshill population are small. Bondesen (1950) gives the average number of eggs per capsule as about 100 with a maximum of about 150, and the figures which he quotes from previous literature are in general agreement with only one exception; however, some of the authors note that the first capsules laid by individual snails are smaller than those produced later. Schodduyn (1925) gives an average of 35 eggs per capsule which agrees with the Bellshill figure but his figure is based on only 5 capsules laid by a two-year-old aquarium specimen of 20 mm shell height. It thus seems likely that the size of the egg capsules from Bellshill is relatively small because the snails themselves are small and that both these features reflect the marginal nature of this habitat.

The incidence of polyvitelliny noted at Bellshill is lower than that reported by Crabb and Crabb (1927) who found 1.37% polyvitelline eggs in 284 capsules laid by

20 snails in the laboratory. It is interesting that 9 of these specimens were lab-bred from the others and they showed a higher incidence of polyvitelliny. If the original 11 snails are considered separately, the incidence drops to 0.73% while the average size only falls from 69.9 eggs per capsule to 66.9. These authors also state that polyvitelline eggs occur most frequently in larger capsules which contain 50 - 150 eggs and which tend to be deposited by larger snails. Since the average size of capsule found at Bellshill was much smaller than this, the low incidence of polyvitelliny here agrees with these earlier data.

Early authorities (Cooke, 1895; Pelseneer, 1906; Baker, 1911) assumed that a biennial life cycle was general among freshwater pulmonate snails but, until now, the few detailed studies made in Britain (Hunter, 1953a, 1957 and 1961a,b; Geldiay, 1956; Duncan, 1959) have all shown a simple annual life cycle or more than one cycle per year; and Hunter (1957) and Duncan (1959) cite further data which suggest that several more species have similar life cycles.

As a result of his observations in Scotland, Hunter (1957) considered that only Lymnaea stagnalis, L. auricularia (L.) and Planorbarius corneus (L.) could live longer and grow to a relatively great size, while Boycott (1936), from observations made mainly in the south of England, concluded

that all freshwater pulmonates were annuals except P. corneus. Berrie (1963) suggested that one population of P. corneus in the south of England had a simple annual life cycle but it is not certain that this is typical of the species. No definite information is available about L. auricularia but Boycott (1936) was certain that it was an annual.

The present study definitely shows a life cycle extending over two years but this is not a true biennial life cycle since the snails can and do breed in their first year. This suggests that under more favourable conditions the life cycle could be condensed into an annual pattern. In a brief reference to Malham Tarn, a calcareous habitat in the north of England, Campion (1956) states that Lymnaea stagnalis has a simple annual life cycle there, while unpublished observations by the present writer suggest that this is also the case at two localities in the south of England. It would seem likely that Boycott (1936) was fully justified in regarding L. stagnalis as an annual but that under unfavourable conditions, such as are normally associated with marginal habitats, its life cycle becomes extended to include a second year. The biennial life cycle, the relatively small size attained by the snails, the small size of the egg capsules and the geographical position of the habitat all tend to confirm that the pool at Bellshill

is probably unfavourable for L. stagnalis.

The largest specimen found at Bellshill had a shell length of 35 mm which is much less than the maximum sizes reported elsewhere. Crabb (1929) has shown that shortage of food and foul media are the commonest factors inhibiting growth of L. stagnalis in aquaria. Hunter (1953a and 1961a,b) has discussed the question of an environmental limitation of size in natural populations, particularly in relation to his observations on Ancylus fluviatilis where this seems to depend on the rate of water flow and the growth of algae attached to the shell. Neither of these factors was applicable to the present study nor can any other factor be clearly identified. It has already been noted that the survivors of the snails which had bred during 1957 showed no indication of growth during the spring of 1958 when the smaller snails were growing rapidly. This contrasts with the usual pattern of growth in molluscs, which is continuous throughout life if conditions are suitable, and could be interpreted as showing an environmental limitation of size as mentioned above or alternatively as "endogenous senescence" (Comfort, 1956 and 1957). In the present case it would seem very unlikely that shortage of food or of calcium was a critical factor. Fouling of the medium in natural habitats and the possible production of growth-

inhibiting or controlling substances (Berrie and Visser, 1963) is a largely unknown field but again the selective effect on large snails would be puzzling. As the snails in the population grew larger they tended to suffer more from erosion of the spire. This phenomenon has been reported previously (Ellis, 1927; Stratton, 1946) but seemed to be particularly severe in this population where, in extreme cases, several whorls were completely removed, but no evidence of a direct correlation between shell erosion and limitation of size has been found.

SUMMARY.

Observations on a population of Lymnaea stagnalis living in a small pond showed that it had a biennial life cycle and that most of the snails could breed in both years. This appears to be the first time that such a pattern has been clearly demonstrated among British freshwater pulmonates. Reproduction was seasonal and egg-laying started towards the end of May, reached a maximum about mid-June and seemed to end during August. The egg capsules were found to be much smaller than those reported elsewhere. Young snails grew rapidly until September and again from about March to June. A further period of growth could occur during their second spring but this seemed to depend

on the weather conditions which were the main factors influencing growth. It is suggested that the snails may be sensitive to trends of temperature rather than to any absolute temperature. Adult snails were relatively small and it is considered that some environmental limitation of size was responsible. The biennial life cycle, the relatively small size of the adult snails and of their egg capsules and the geographical location are all interpreted as indicating the marginal nature of the habitat.

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Sample date	No. in sample	Range of lengths mm	Mean length mm	Standard deviation mm	Increase in size since previous sample %	Growth rate mm/wk
29/1/57	17	20.5 -30.0	23.15	2.96	3.12	0.18
26/2/57	14	19.0 -27.0	22.05	2.16	-4.75	-0.28
27/3/57	2	23.0 -25.0	24.00	1.41	8.84	0.21
23/4/57	9	22.75-29.5	26.64	2.43	11.0	0.66
21/5/57	5	28.75-31.0	29.70	0.95	11.49	0.77
18/6/57	5	28.0 -33.5	30.75	2.71	3.54	0.26

Table 1. Summary of size and growth data for snails hatched in 1955.

Sample date	No. in sample	Range of lengths mm	Mean length mm	Standard deviation mm	Increase in size since previous sample %	Growth rate mm/wk
29/1/57	27	9.5 -17.5	12.46	2.09	6.77	0.20
26/2/57	26	7.25-16.5	13.54	2.31	8.67	0.27
27/3/57	41	12.0 -20.5	16.63	2.01	22.82	0.77
23/4/57	35	16.0 -27.5	21.92	2.71	31.81	1.32
21/5/57	44	11.0 -31.0	25.41	4.45	15.92	0.87
18/6/57	27	13.75-34.0	28.03	5.64	10.31	0.65
16/7/57	26	20.0 -33.25	29.24	3.89	4.32	0.30
13/8/57	7	24.5 -31.5	30.04	2.58	2.74	0.20
11/9/57	4	26.0 -31.0	29.13	1.94	-	-
9/10/57	3	28.0 -35.0	32.17	3.69	-	-
6/11/57	2	31.0 -34.0	32.50	2.24	-	-
4/12/57	3	21.75-32.0	28.42	5.80	-	-
7/1/58	2	23.75-29.5	26.63	4.10	-	-
5/2/58	3	30.25-33.25	31.92	1.47	-	-
4/3/58	4	30.0 -32.5	31.38	1.08	-	-
2/4/58	2	30.0 -34.0	32.00	2.83	-	-
30/4/58	8	28.25-32.5	31.06	1.05	-	-
27/5/58	3	26.0 -34.5	30.83	4.37	-	-

Table 2. Summary of size and growth data for snails hatched in 1956. In the lower part of the table the numbers collected are too small to provide useful figures in the last two columns.

Sample date	No. in sample	Range of lengths mm	Mean length mm	Standard deviation mm	Increase in size since previous sample %	Growth rate mm/wk
16/7/57	11	6.25-12.5	9.86	2.15	-	ca2.0
13/8/57	28	8.75-20.0	13.36	2.58	35.50	0.88
11/9/57	44	11.5 -22.25	17.13	2.31	28.97	0.99
9/10/57	42	4.5 -23.0	16.95	3.79	-1.05	-0.05
6/11/57	37	9.5 -21.0	17.52	1.94	3.36	0.14
4/12/57	45	11.0 -22.25	17.44	2.84	-0.46	-0.02
7/1/58	35	11.75-24.0	17.89	2.63	2.58	0.11
5/2/58	49	7.0 -23.5	17.11	3.25	7.95	-0.20
4/3/58	32	11.0 -22.5	17.66	2.80	-1.29	0.14
2/4/58	32	15.0 -23.0	18.99	1.96	7.53	0.33
30/4/58	20	20.0 -29.0	22.94	2.09	20.80	0.99
27/5/58	31	22.0 -30.0	25.59	1.98	11.55	0.66
29/7/58	16	26.0 -34.0	28.97	2.50	13.21	0.42

Table 3. Summary of size and growth data for snails hatched in 1957. Members of the small size group which appeared early in 1958 are excluded from this table and shown separately in Table 4.

Sample date	No. in sample	Range of lengths mm	Mean length mm	Standard deviation mm	Increase in size since previous sample %	Growth rate mm/wk
7/1/58	7	3.5 - 8.75	5.57	1.68	-	-
4/3/58	17	4.5 -10.0	6.22	1.35	11.67	0.08
2/4/58	14	5.0 - 9.5	7.48	1.38	20.26	0.32
30/4/58	17	5.0 -13.25	9.68	1.91	29.41	0.55
27/5/58	12	8.5 -15.5	13.19	1.95	36.26	0.88
29/7/58	5	16.0 -22.0	19.1	2.06	44.81	0.74

Table 4. Summary of size and growth data for the small size group of the 1957 hatch which appeared in the collections early in 1958.

Season	1955-56	1956-57	1957-58	1958-59
Mean max. temp. ^{o}C Dec. Jan. & Feb.	4.9	7.1	5.9	4.8
Mean min. temp. ^{o}C Dec. Jan. & Feb.	-0.4	1.7	0.4	-0.02
Sunshine hours Jan. Feb. & March	190.4	142.1	170.7	157.8
Number of days with air frost	51	23	53	46

Table 5. Summary of significant weather records for the years 1955 to 1959. Extracted by Professor W. Russell Hunter from records made at Springburn Park, Glasgow; except for the number of days with air frost which is the number of days on which $1^{o}F$ or more of air frost was recorded at Queen's Park, Glasgow.

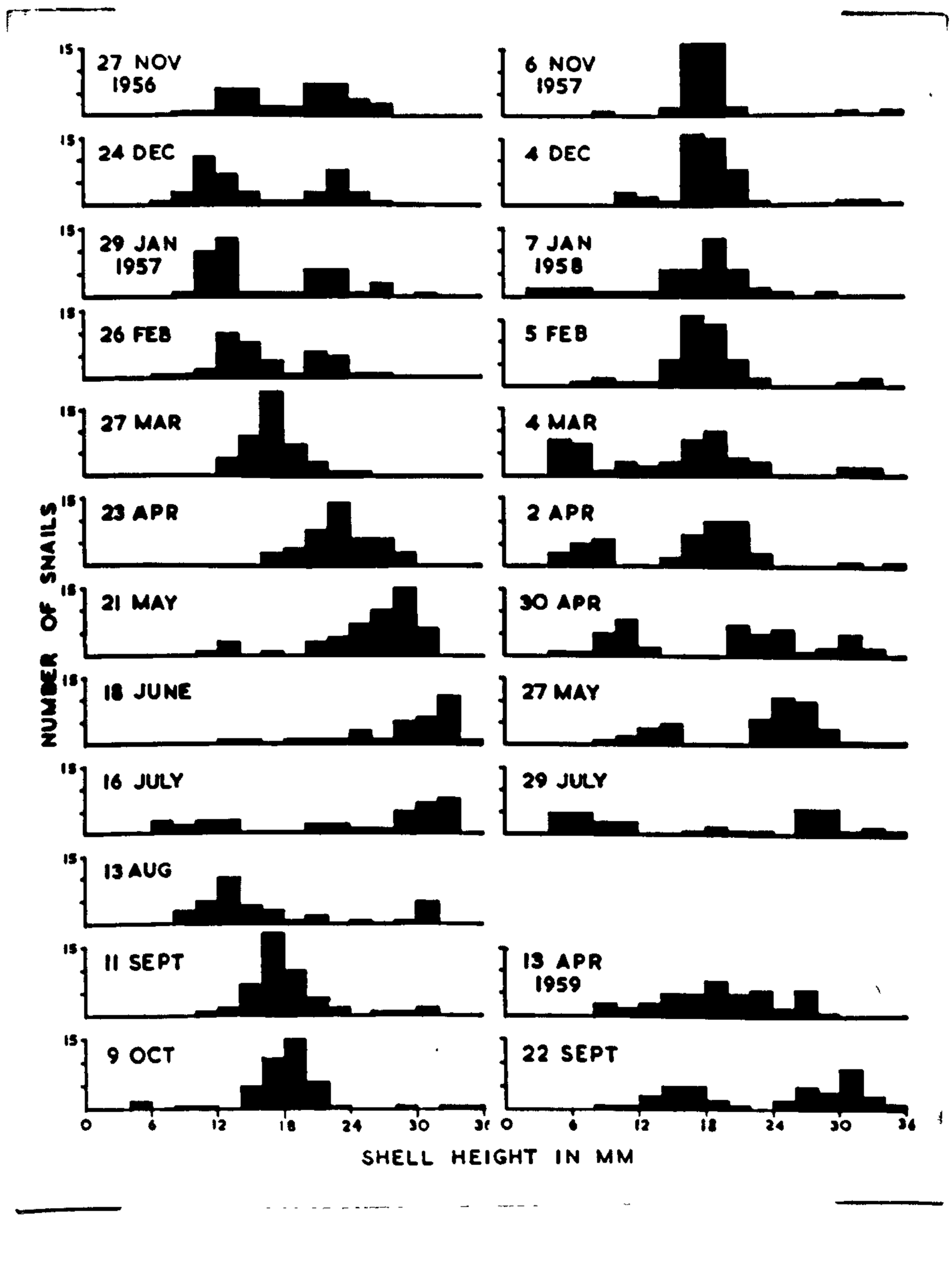

Fig. 1. Size distribution of snails in successive collections from the pond at Bellshill.

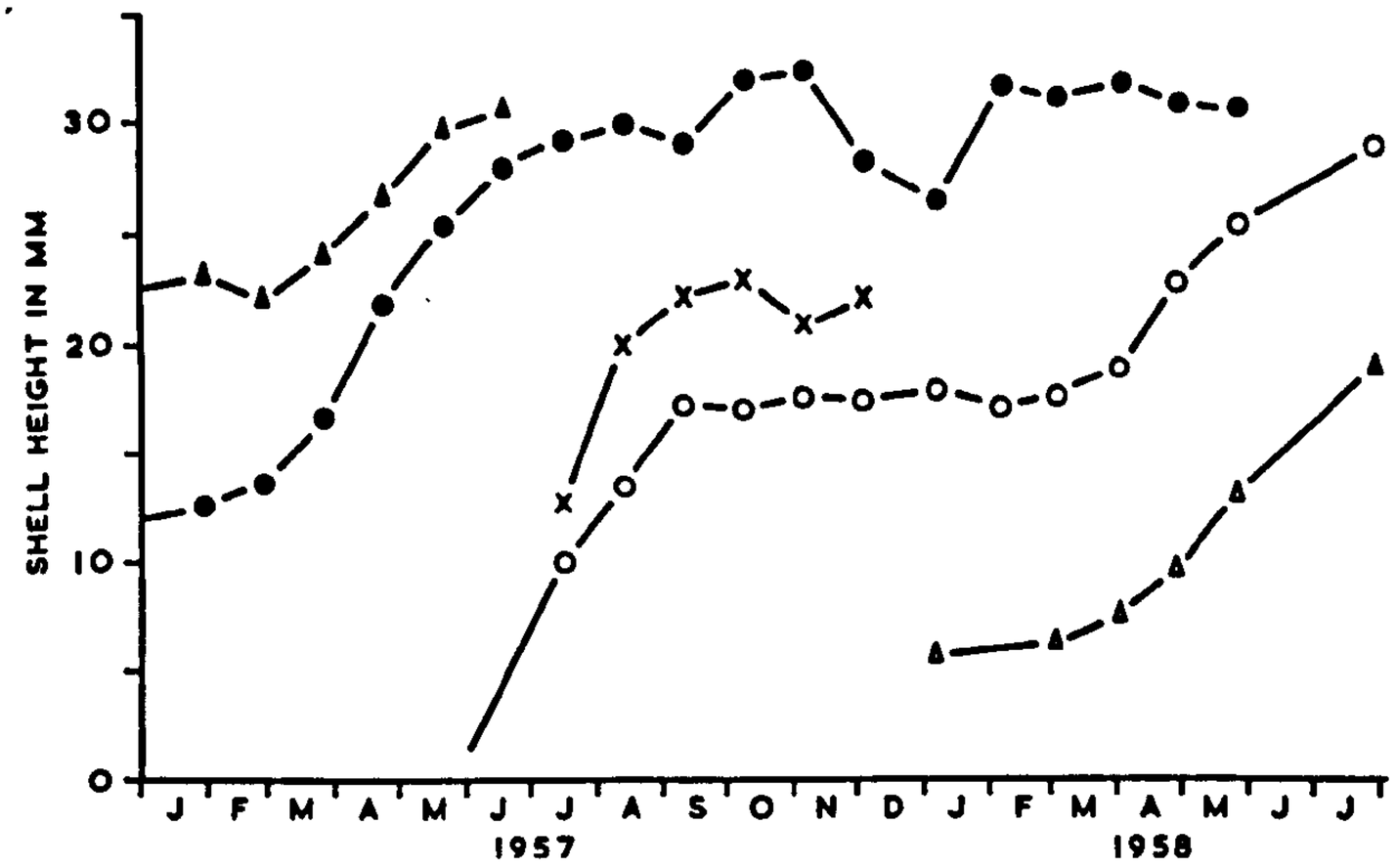

Fig. 2. Mean sizes of the various age groups present in the pond at Bellshill during the observations. The maximum size attained by young snails hatched in 1957 is also shown to indicate their maximum growth rate. ▲ snails hatched in 1955; ● snails hatched in 1956; O snails from main hatch in 1957; Δ snails from late hatch in 1957; X maximum size of young snails in 1957.

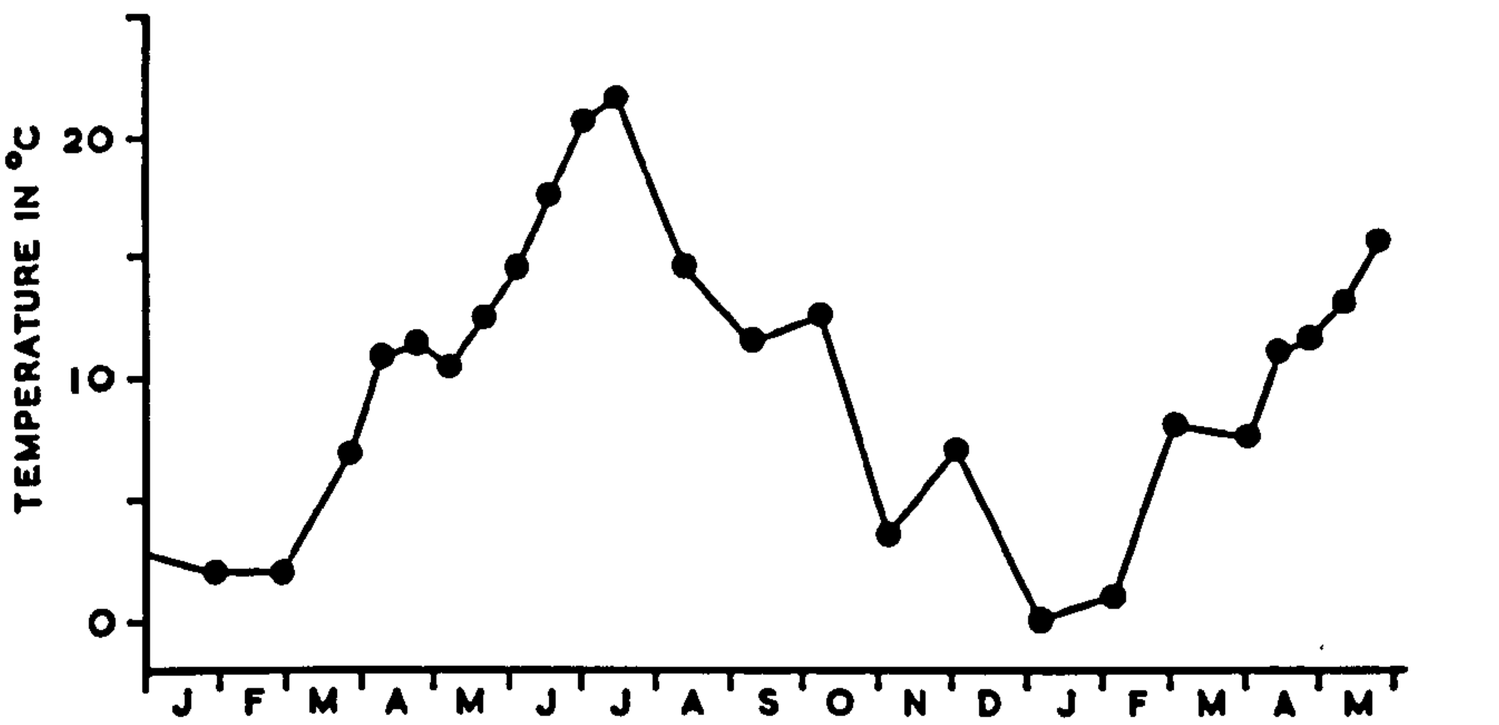

Fig. 3. Water temperatures recorded in the pond at Bellshill.

SECTION III

SECTION III

GROWTH AND SEASONAL CHANGES IN THE REPRODUCTIVE ORGANS OF LYMNAEA STAGNALIS (L.)

Although certain aspects of the biology of Lymnaea stagnalis (L.) have received extensive study, the seasonal gonadial cycle in a natural population has not yet been reported. Early developmental studies were carried out by Hogg (1854) and Lankester (1874) and, more recently, detailed descriptions have been made of the reproductive system of the American subspecies (Crabb, 1927; Holm, 1946) and of the European subspecies, including extensive consideration of the processes of insemination and ovulation (Bretschneider, 1948a and b; Bretschneider and Raven, 1951; Raven, 1958). Aubry (1954a,b,c and d, 1955 and 1956) gave a detailed account of the histology of the gonad and of the sequence of events involved in spermatogenesis and oogenesis. These studies provide adequate anatomical and histological details but none of them has been concerned with the possible effect of natural environmental conditions on the changes which they describe.

Details have been given in Section II of a series of observations carried out on the life cycle of a population of L. stagnalis in a small pond at Bellshill, Lanarkshire. This population had a biennial life cycle with most snails breeding in both years. The reproductive period extended from May to August with a peak in the middle of June. Bellshill is near the edge of the range of this species and

both the adult snails and the egg masses were smaller than those reported elsewhere, indicating that the habitat was probably relatively unfavourable for L. stagnalis. To obtain a more detailed picture of the biology of this population, an investigation was made of changes taking place in the genital system of the snails during the period of the observations. A large number of the snails collected from the pond were dissected and the gonads of several were sectioned so that such changes could be measured and related to the seasonal and growth changes observed in the field. The results of these anatomical studies are presented below.

METHODS

After measurement, snails brought from the field were placed individually in 3" x 1" glass tubes of water and were left in a refrigerator for several hours to obtain partial relaxation. The water was then poured off and replaced by Helly's solution and the snails' shells were cracked carefully in several places to ensure adequate fixation. After about twenty hours the shells were removed completely and the bodies were washed in repeated changes of water. The specimens were then dissected.

The length and width of the vergic sheath and of the preputium were measured. The size of the spermatheca was

taken as the length from its junction with its duct to its distal end (i.e. approximately its maximum diameter). The size of the albumen gland was recorded by measuring its gross dimensions and multiplying these to give a figure representative of its volume. This organ is very irregular in outline and the figure obtained is consequently very inaccurate as an exact measure of its volume; however, for the purpose of showing large changes of size it proved quite adequate. The above measurements were all carried out with a micrometer eyepiece on a dissecting microscope.

The gonads of individual snails, selected so as to be representative of the age and size groups present in the population, were cut into serial sections and stained with Heidenheim's Iron Haematoxylin. A simple system of examination was devised to obtain a picture of gross changes taking place in the gonad in relation to the size of the snail and the time of year. The number of sections in each series was counted and three sections were selected at equal intervals between the ends of the series. These were then studied in detail on a microscope fitted with a projection unit which produced an image on the bench beside the microscope. A low power magnification of 53x and a high power magnification of 570x were obtained by this method. A low power drawing of the whole section was prepared on

squared paper to show the total area of the section, the area occupied by gonad tissue and the number of eggs in the section. A table of random numbers and a grid placed over the low power drawing were used to select a point in the gonad tissue part of the section which then formed the centre of a field of which a high power drawing was prepared. On this drawing the areas occupied by different stages of spermatogenesis were marked but for the purpose of this study it was then found convenient to consider these under only two categories - mature spermatozoa and developmental stages. Thus six drawings were prepared from each gonad and, by counting the number of squares covered by each component, figures were obtained for the total size of each gonad and its internal composition.

RESULTS

PREPUTIUM AND VERGIC SHEATH

The measurements made on these organs did not produce much useful information. The length of each showed a fairly good linear relationship to shell height. Over all the specimens examined the ratio of the length of the vergic sheath to the length of the preputium had an average value of 0.235 with a standard deviation of 0.031. When the figures are grouped according to shell size or season the

various mean values obtained fall well within this overall range and no significant variation has been found. The ratio appears to be quite variable at all sizes and at all times of year.

SPERMATHECA

Details of the size of the spermatheca in various size groups of snails are shown in Table 1. It can be seen that the mean size increases fairly steadily in proportion to increase in shell size although there is considerable overlap of the range of size in successive groups, and the percentage increase is lower in large mature snails. It has not been possible to show a clear seasonal relationship to spermatheca size but the organ does show a seasonal production of an orange pigment which Bretschneider (1948) has noted to be associated with the storage of foreign sperm after copulation. The pigment is presumably first secreted when the animal is first impregnated or when it becomes receptive to impregnation and it is thus a useful indicator of the onset of reproductive activity in individual snails. The condition of the spermatheca in the snails of different year groups which were examined is shown in Fig. 1. It is clear that the change of colour occurs in nearly all snails during May and June when they are about one-year-old and that the orange colour does not disappear at the end of the breeding

season, but is retained throughout life. Only three snails in their second year were observed in which the spermatheca was colourless. These were all in the 1955 year group which had experienced the poor growth conditions of 1956 (see Section II) as a result of which a few may have failed to breed in their first year.

ALBUMEN GLAND

The mean sizes of the albumen glands in the different size groups during the period of observations are shown in Fig. 2. As stated above, the method of measurement was very inexact; also the range of sizes represented by some of the means is very great and some of the samples were very small (see tables in Section II). For these reasons no statistical analysis of the data is presented but measurements were made on almost 500 snails and it is felt that the changes in the mean sizes are sufficiently large and consistent to be regarded as significant.

In both years the albumen glands of old and young snails increased rapidly in size during March, April and May at the same time that the snails were growing rapidly. In 1957 the size prior to this increase was noticeably less in both age groups than it was in 1958. This corresponds with the relative sizes of the snails at the beginning of these two years and with the earlier conclusion that 1956 was a poor

year for snail growth and 1957 a good one. Albumen glands reached their greatest size in May 1957 at the end of the good spring growth season and by mid-June showed a considerable reduction in size; after which they remained rather constant until September. Field observations showed that a peak of egg-laying was reached just before mid-June and this seems to be confirmed by the loss of albumen at that time.

A further reduction in the size of the albumen gland occurred among the large snails between September and December. This could be attributed either to the largest snails with the largest albumen glands dying off at this time or to a genuine decrease in volume of the gland. However, during this period there was a slight increase in the mean size of the snails and the minimum size increased steadily which suggests that a genuine decrease in volume took place. This is supported by the figures for the albumen glands of individual snails during the period but the number involved is small.

THE GONAD

Details of the snails examined and the condition of the gonads are set out in Tables 2 and 3 while changes in size and internal composition of the gonad are shown in Fig. 3. A total of 45 gonads was sectioned for detailed study

and the snails were selected so as to be representative of the size range present in the population at the various times of year (Section II). Tables 2 and 3 show that the mean size of the selected snails from each collection did not differ greatly from the mean size of the whole collection.

By July 16 the gonads of the young snails, which would then be 6-8 weeks old, were not developed. Through the autumn the gonads developed to a rather small size and contained both developing spermatozoa and oocytes. From December until the beginning of April no significant changes were observed and this corresponds roughly with the period during which the water temperature probably seldom rose above $10^{o}C$ (see Fig. 3 in Section II). During April and May the gonads grew rapidly and the number and density of oocytes present also increased. Until April a large proportion contained developmental stages of spermatozoa but only a few mature spermatozoa were present. During May large numbers of spermatozoa matured but there was no decrease in the proportion of developmental stages so that a considerable potential for further production of spermatozoa still remained.

The data for the second year snails show that there is little change in the size of the gonad until the beginning of the second breeding season when there is again a rapid

expansion. In the year of study this was not accompanied by growth of the shell since the snails had already reached their maximum size for the habitat, and it may be that the expansion of the gonad is achieved at the expense of the body tissue and that this process later leads to the death of the snails. Degenerative changes in the internal organs not directly concerned with reproduction have previously been noted in senescent L. stagnalis under laboratory conditions (Noland and Carriker, 1946). "Parental death" following reproduction is a well known phenomenon in various groups of animals but the causal mechanisms are not always clear although it is easy to suggest that an evolutionary advantage results when the young do not require to compete with their parents. Existing information on these aspects of molluscan life has been reviewed by Comfort (1957). In the present case it seems likely that the life cycle of the snails is extended into a second year due to environmental conditions and that this is not typical of the species (Section II).

From December to the end of April the male elements were similar to those in young snails. A fairly constant level of developing stages was maintained with few, if any, mature spermatozoa present. Again, during May, a large number of sperm matured and the amount of space occupied by

developing stages increased. Oocytes in various stages of development were present throughout the winter although some appeared to be degenerating and their number again seemed to remain constant up to May in spite of the rapid increase in gonad size. These observations provide no evidence of any functional protandry in the life cycle of this population, such as occurs in several other hermaphrodite molluscs.

As well as the seasonal aspect of spermatogenesis it should also be noted that there is a close correlation with the size of the snails. Mature spermatozoa were never found in a snail of less than 20 mm shell height and only 5 specimens (4 taken in December) over this size did not have mature spermatozoa present. Similarly no snail of less than 20 mm shell height showed pigmentation of the spermatheca (Table 1) but in this case many larger specimens also lacked pigmentation.

DISCUSSION

Some studies of other freshwater pulmonate snails include passing reference to the condition of the reproductive system at certain times of year. In a study of Bulinus tropicus Krauss (Stiglingh, van Eeden and Ryke, 1962) it was found that the ratio of the length of the vergic sheath to the length of the preputium was very variable and

that it did not seem to change with age. Duncan (1958) noted that the spermatheca in Physa fontinalis (L.) secreted a red pigment especially during periods of reproductive activity but that this was often found before copulation had occurred. He also found that the spermatheca was distended after copulation (whereas no substantial change of size due to copulation was noted in the present observations) and that the size of the albumen gland was dependent on the sexual state of the individual, being small and difficult to detect in young snails. Abdel-Malek (1954a) found seasonal changes in the activity of the albumen gland and the gonad in Helisoma trivolvis (Say) but did not relate these to the age of the specimens. Abdel-Malek (1954b) found mature spermatozoa in the acini and seminal vesicles of Biomphalaria boissyi (Potiez and Michaud) [= B. alexandrina alexandrina (Ehrenberg)] at all times of year but this is a sub-tropical species. McCraw (1961) concluded that, in Lymnaea humilis Say, size was a better index of sexual maturity than was age. However, L. humilis is a relatively small species which appears to be able to complete three generations within a year in the northern U.S.A. where the climate produces very different growth conditions for summer and winter generations.

It appears that the development of the genital system in natural populations of L. stagnalis is related both to

the size of the snails and to the season of the year. However, these two factors are themselves closely related since the snails have a regular cycle of growth and reproduction extending, in the present case, over two years (Section II). Thus at a given time in any year the snails present in the population will fall into approximately the same size groups although the actual size will vary somewhat depending on the growth conditions which have prevailed during the life of each generation. During late spring and early summer rapid growth will nearly always occur among young snails and developmental changes in the animals will consequently be more rapid, whether they are basically related to size or to season. In particular, even in a poor growth year, most young snails can be expected to reach a size at which they can become sexually mature during this period. The only exceptions are perhaps individuals from late hatches of the previous year. Elsewhere, under conditions more favourable than those at Bellshill, young snails can reach this size during the growth period of their first summer (Berrie, unpublished observations in the Thames valley) and it would be interesting to know if they then mature and produce an autumn generation or whether maturation is delayed to a larger size in such favourable conditions.

The present observations show the size of the preputium,

of the vergic sheath and of the spermatheca to be directly related to the size of the snails. The development of the gonad also shows some relation to size but the rapid changes leading to full maturation seem to be related to season. The timing of these rapid changes in the gonads of first year snails corresponds well with expansion of the albumen gland, the start of secretion in the spermatheca and with the field observation that egg-laying started about the middle of May and reached a peak about a month later. Thus there seems to be a seasonal burst of germinal and secretory activity associated with the start of the breeding season as distinct from the more continuous growth of parts of the reproductive system not directly involved in these events.

Abeloos (1943) showed that castration of mature specimens of Limax maximus L. led to a marked regression of the albumen gland and the accessory glands of the oviduct. Further studies on terrestrial gastropods by Laviolette (1954a,b and 1956) involving grafting, removal and implantation of gonads have shown that this organ exerts a considerable influence on the development of other parts of the reproductive tract but not on the penial complex. He concluded that this mediation must be of a chemical nature since it operated from implants which were anatomically isolated from the accessory organs of the host. The

existence of such a relationship would be entirely compatible with the results of the present observations. Lūsis (1961), however, considered that the stimulus for the development of the accessory reproductive organs might not originate from the gonad since the gonad of Arion ater rufus L. sometimes showed retarded development while the accessory organs were developing at their normal rate.

SUMMARY

Changes in certain parts of the genital system of Lymnaea stagnalis were recorded at various stages in the life cycle. The sizes of the preputium, the vergic sheath and the spermatheca were related to the size of the snails. This was also partly the case with the gonad but rapid changes of size and composition were associated with the start of the breeding season. Similar seasonal changes took place in the albumen gland and in the pigmentation of the spermatheca. It is emphasised that, since the snail population which was studied undergoes a regular biennial life cycle, individuals will tend to be approximately the same size at a given time in any year. This causes some difficulty in clearly distinguishing developmental changes which are associated with the time of year from those which are related to the size of the snails.

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Size group of snails. Shell height mm	No. of snails examined	Mean size of spermatheca mm	Standard deviation mm	Incidence of pigmentation %
10.0 - 12.25	2	0.19	0.007	0.0
12.5 - 14.75	13	0.24	0.010	0.0
15.0 - 17.25	23	0.47	0.151	0.0
17.5 - 19.75	46	0.53	0.161	0.0
20.0 - 22.25	56	0.81	0.280	25.5
22.5 - 24.75	41	1.23	0.364	39.0
25.0 - 27.75	32	1.52	0.346	53.1
27.5 - 29.25	36	1.80	0.339	87.5
30.0 - 32.25	44	2.10	0.280	95.5
32.5 - 34.75	17	2.33	0.401	100.0

Table 1. The size of the spermatheca and the incidence of pigmentation in specimens of L. stagnalis at various sizes.

Collection date	16/7/57	11/9/57	4/12/57	4/3/58	2/4/58	30/4/58	27/5/58
No. of snails examined	3	3	8	7	4	4	5
Size range of snails examined mm	6.25 to 12.0	14.0 to 17.0	11.25 to 22.25	10.0 to 22.5	18.0 to 23.0	12.5 to 28.25	15.5 to 30.0
Mean size of snails examined mm	9.3	15.7	19.1	17.4	20.8	21.2	23.7
Mean size of whole collection mm	9.86	17.13	17.44	17.66	18.99	22.94	25.59
Mean area of gonad per snail (3 sections) sq mm	0.0	0.285	0.783	0.579	0.757	1.584	3.364
Internal state of gonad %							
1. Developing spermatozoa	0.0	49.1	25.6	30.5	31.2	39.6	41.7
2. Mature spermatozoa	0.0	0.0	2.4	1.2	1.0	1.9	23.0
3. Empty	0.0	50.9	72.0	68.3	67.8	58.5	35.3
Mean no. of oocytes per snail (3 sections)	0.0	3.3	1.8	5.7	6.0	42.0	48.4
No. of oocytes per sq mm	0.0	11.6	2.30	9.84	7.93	26.52	14.39

Table 2. Details of specimens of L. stagnalis from which the gonads were sectioned during the first year of life. Snail size is measured as the height of the shell.

Collection date	4/12/57	4/3/58	2/4/58	30/4/58	27/5/58
No. of snails examined	2	3	2	2	2
Size range of snails examined mm	31.5 to 32.0	30.0 to 32.5	30.0 to 34.0	29.0 to 32.5	26.0 to 34.5
Mean size of snails examined mm	31.8	31.2	32.0	30.8	30.3
Mean size of whole collection mm	28.42	31.38	32.0	31.06	30.83
Mean area of gonad per snail (3 sections) sq mm	5.358	3.836	4.245	3.008	8.010
Internal state of gonad %					
1. Developing spermatozoa	13.4	16.1	13.6	15.3	31.6
2. Mature spermatozoa	0.0	1.2	2.4	0.6	19.3
3. Empty	86.6	82.7	84.0	84.1	49.1
Mean no. of oocytes per snail (3 sections)	78.5	123.0	163.5	177.0	155.5
No. of oocytes per sq mm	14.65	32.06	38.52	58.84	19.41

Table 3. Details of specimens of L. stagnalis from which the gonads were sectioned during the second year of life. Snail size is measured as the height of the shell.

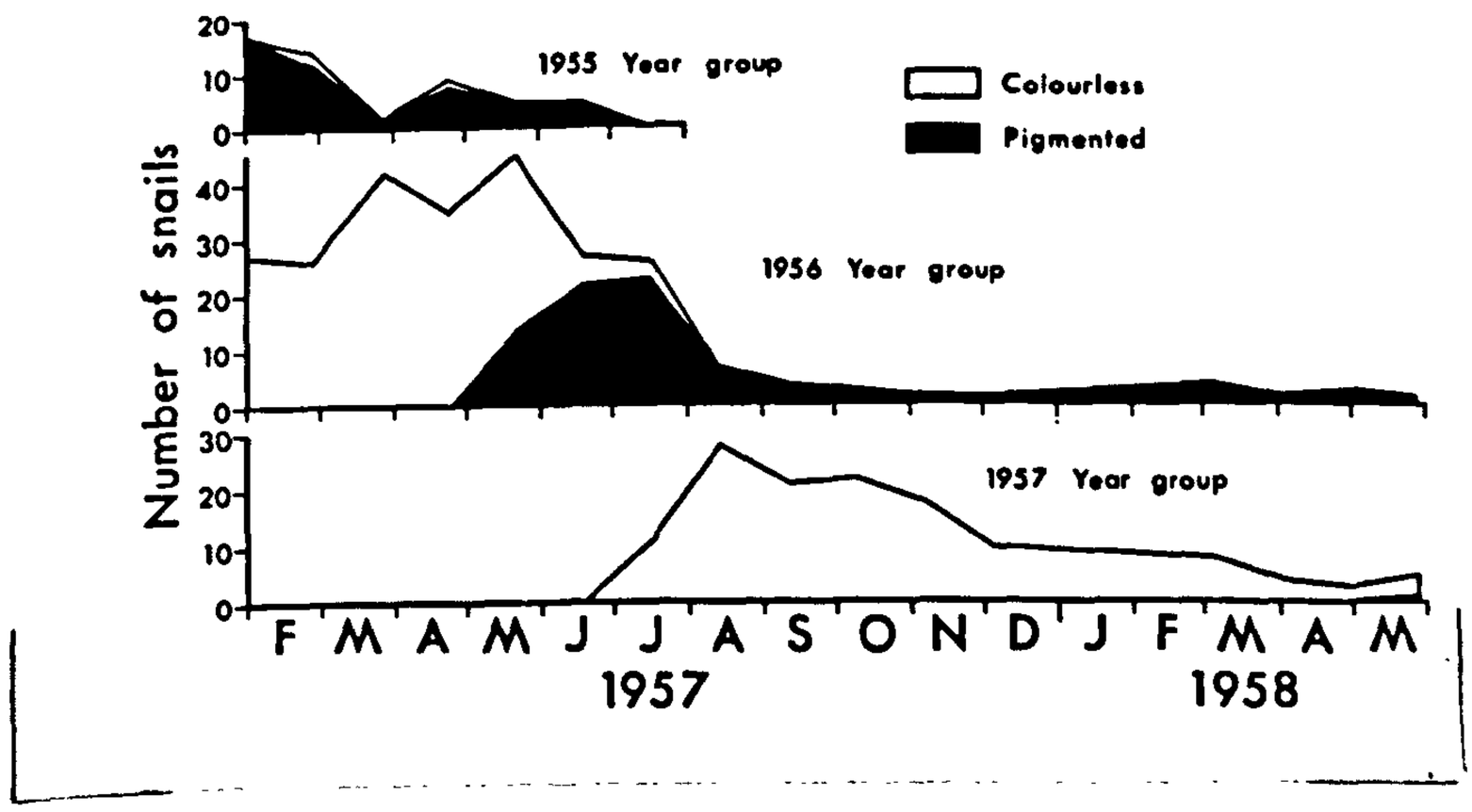

Fig. 1. Seasonal changes in the condition of the spermatheca in three successive year groups of L. stagnalis.

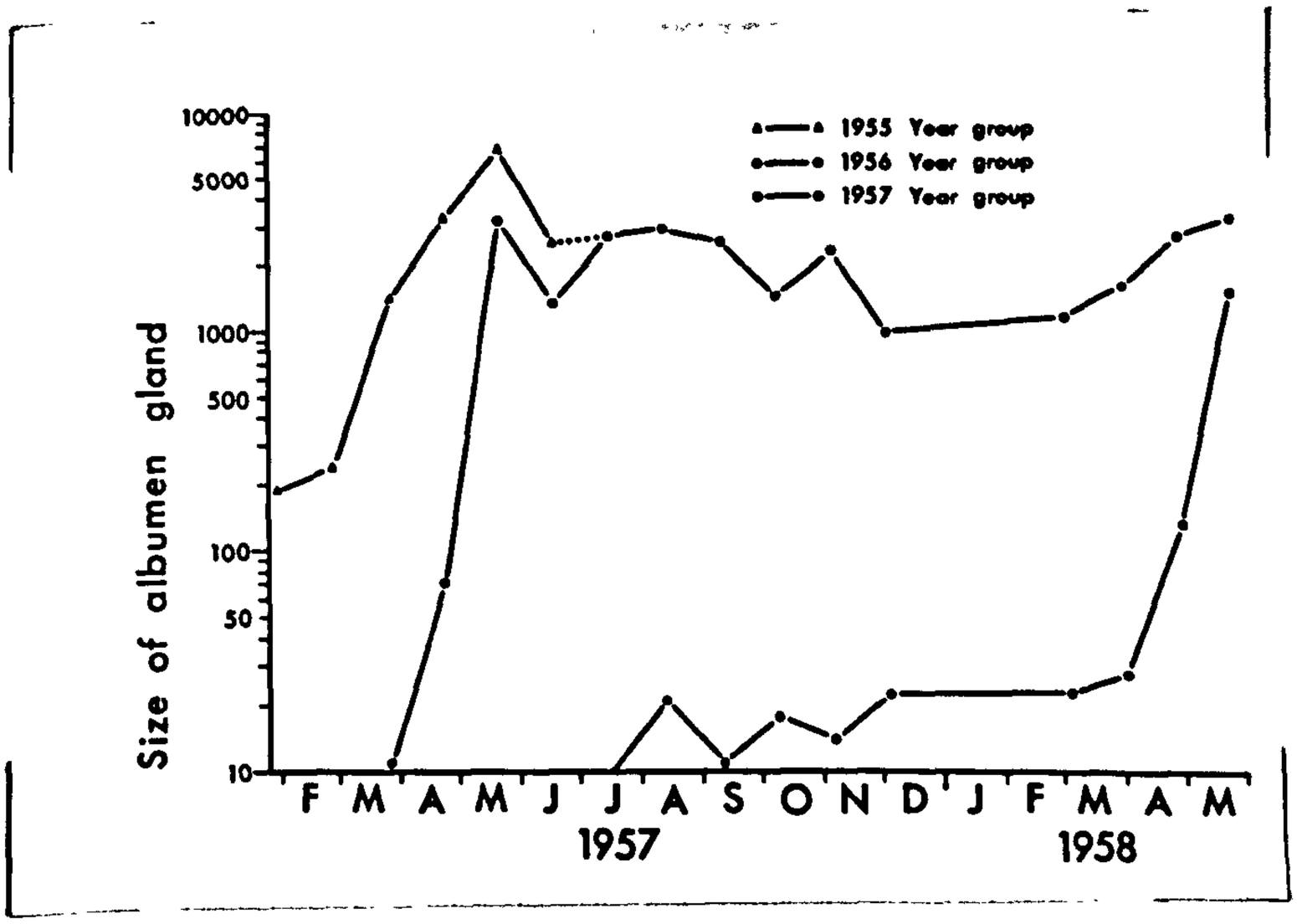

Fig. 2. Seasonal changes in the size of the albumen gland in three successive year groups of L. stagnalis.

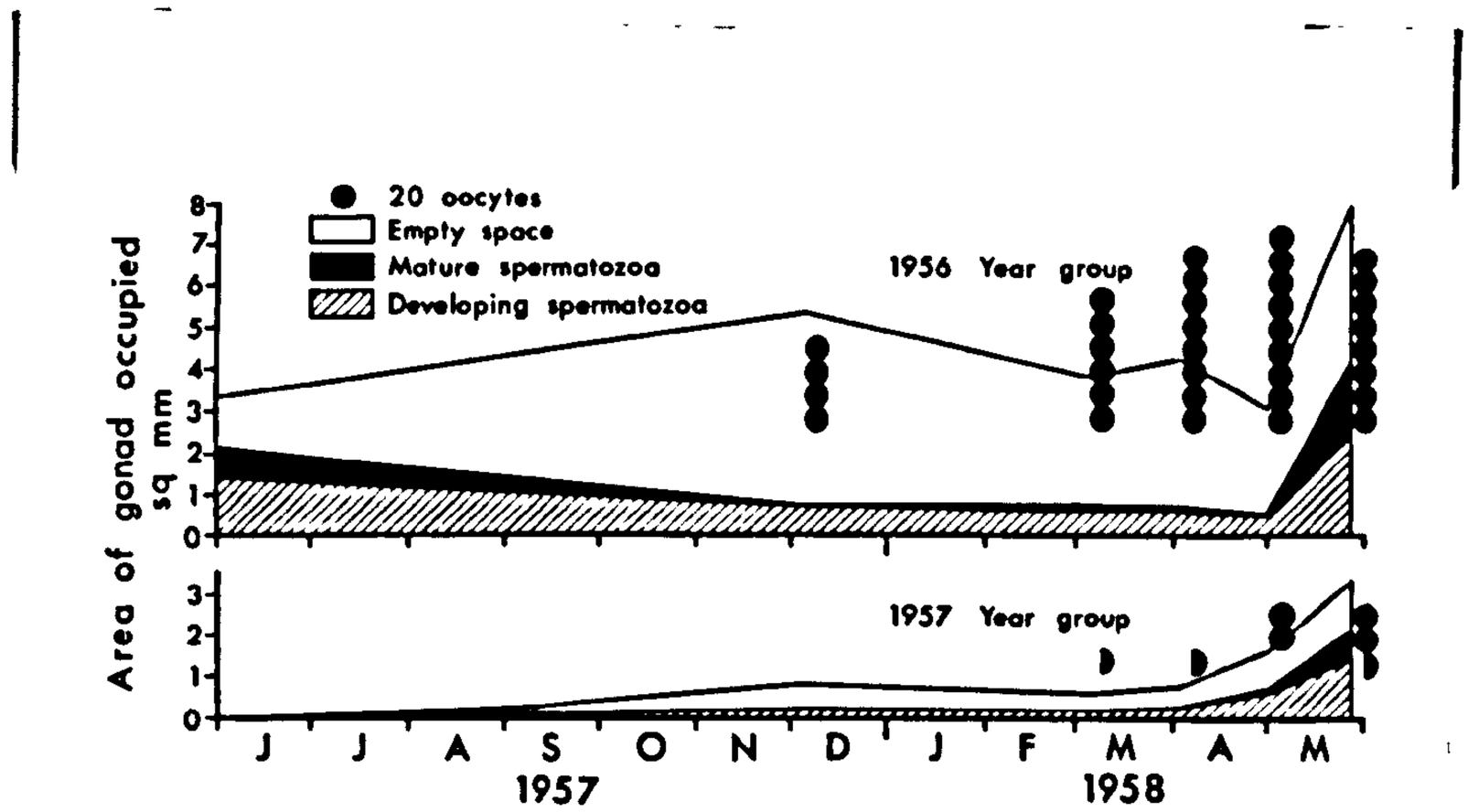

Fig. 3. Seasonal changes in the size and the internal composition of the gonad in L. stagnalis. The values for each animal were taken as the totals for the three sections examined and the figure shows the mean values of these totals throughout the year. The values shown for the 1956 year group in June 1957 are those found in the 1957 year group in May 1958.

SECTION IV

ARKIV FÖR ZOOLOGI

UTGIVET AV

KUNGL. SVENSKA VETENSKAPSAKADEMIEN

Serie 2 • Band 12 nr 27

A. D. BERRIE

Variation in the radula of the freshwater snail *Lymnaea peregra* (Müller) from northwestern Europe

ALMQVIST & WIKSELL / STOCKHOLM

1959

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By A. D. BERRIE

With 6 figures in the text

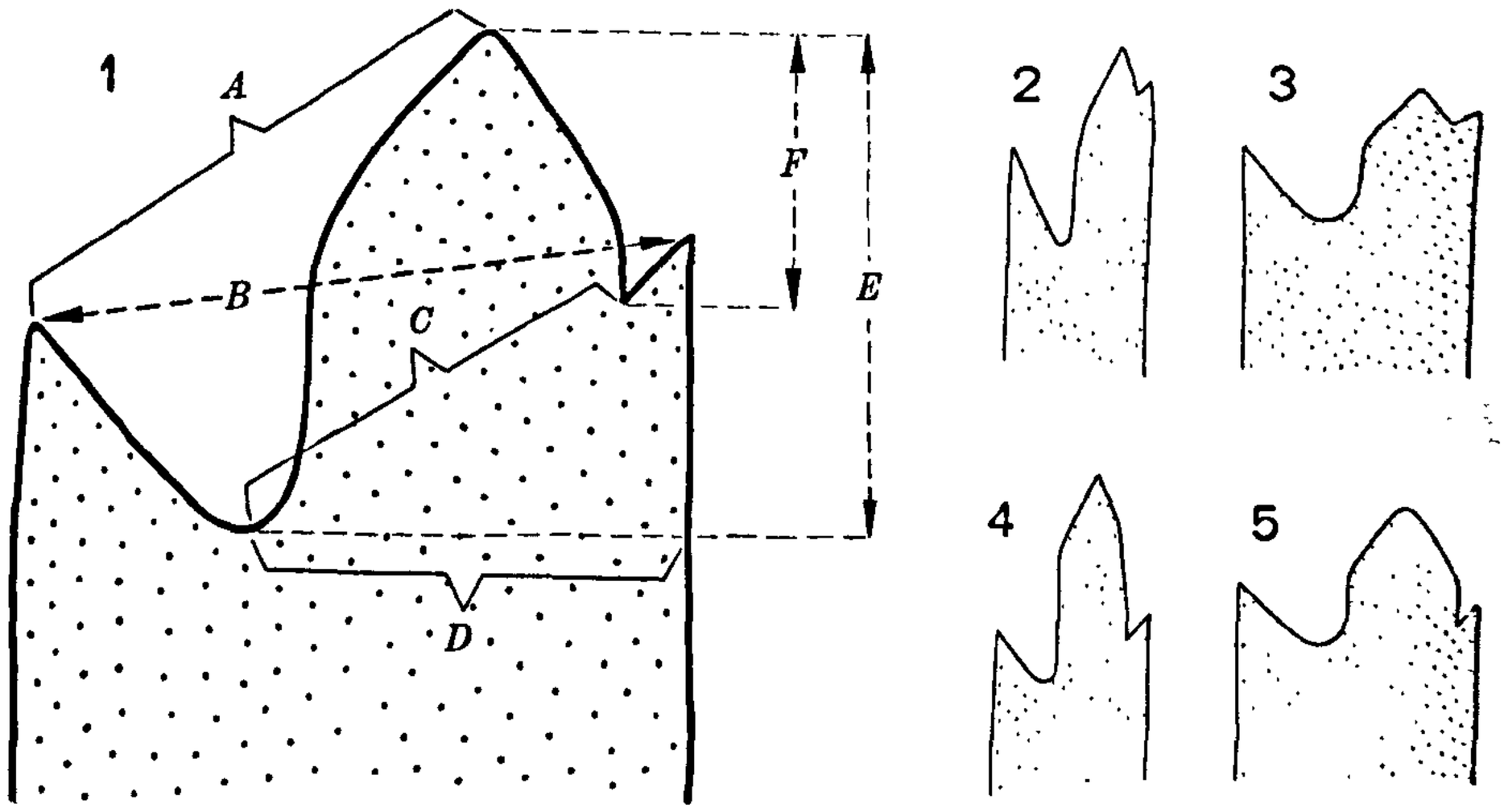
Introduction

A factor of great importance in the study of freshwater mollusca is the amount of variation which can occur within a species. This has been attributed mainly to two very distinct effects of the environment. Ecophenotypic variation is caused by varying physical and chemical conditions affecting the appearance of the snails during the lifetime of each individual. The geographic discontinuity of the environment means that populations are isolated from one another, and so genetical differences can easily arise, especially as populations may be derived from a single individual colonising a new habitat. It is thought that this genetic variation is kept at a subspecific level because of the relative impermanence of most freshwater habitats (Hubendick, 1952 and 1954, and Hunter, 1952 and 1957). This belief is strengthened by the unusual diversity of the molluscan fauna found in those habitats which are known to be of relatively great age, such as Lakes Baikal, Ochrid, and Tanganyika. Thus on theoretical grounds it may be expected that differences will exist between different populations of a freshwater molluscan species, and that these differences will not tend to conform to any pattern except where they are ecophenotypic or have had a definite selective value.

It may be possible that certain extreme environmental conditions can produce effects on the radulae of snails, but, in general, it seems unlikely that the fine structure of the teeth will show ecophenotypic variation. Small changes in the shape of the teeth appear unlikely to have a significant selective value, and, if such inheritable variations exist, their occurrence can hardly have been determined by any selection pressure specific to each habitat. Hence such changes may reasonably be presumed to express purely genotypical variation within the species and may be expected to arise in a random manner. For these reasons it was decided to conduct a comparative study of the teeth of different populations of the snail *Lymnaea peregra* (Müller).

Material and methods

The snails used in this investigation had all been preserved in alcohol. Each radula was dissected out from the buccal mass together with some of its musculature, and was heated in a dilute solution of sodium hydroxide to remove all the softer tissues.



Figs. 1–5. Fig. 1 shows the six measurements which were made on each tooth examined during the investigation. A full description is given in the text. Figs. 2–5 show the appearance of teeth having different values of the two ratios studied: Fig. 2, B/E low and F/E low: Fig. 3, B/E high and F/E low: Fig. 4, B/E low and F/E high: Fig. 5, B/E high and F/E high.

It was then rinsed in water and in 70% alcohol, and was mounted unstained in polyvinyl lactophenol. The radulae were examined under a microscope with an oil immersion lens giving a total magnification of about ×900, and measurements were made with an eyepiece micrometer.

In all parts of the study the teeth measured were from the central region of the radula, where they are fully formed but not yet subject to wear. Care was taken to avoid using any radula on which any of the teeth which had to be measured was obviously malformed compared with the other teeth. In all cases the six measurements shown in Fig. 1 were made. The first two measurements were made from the tip of the exocone. From this point A represents the distance to the tip of the mesocone, while B is the distance to the tip of the endocone. The next two measurements were made from the bottom of the gap between the mesocone and the exocone. C is the distance from this point to the bottom of the gap between the mesocone and the endocone, and D is the distance across the base of the mesocone and the endocone to the inside of the tooth, taken at right angles to this edge of the tooth. The last two measurements were made vertically down the tooth from the tip of the mesocone. E is the distance down to the level of the bottom of the gap between the mesocone and the exocone, while F is the distance down to the corresponding point between the mesocone and the endocone. It was found, however, that the limits of C and D were difficult to focus exactly and hence probably subject to considerable error, and so it was considered undesirable to make use of these figures. Some similar difficulty also occurred with E, but this could be overcome by using a standardised method of focusing the bottom of the gap between the mesocone and the exocone. For the present study it was decided to calculate the ratio B/E, which expresses the main proportions of the tooth, or, more precisely, the relation between the breadth of the tooth and the maximum height of the mesocone, and the ratio F/E, which expresses the relative positions at which the exocone on the one side and

the endocone on the other join the mesocone. Figs. 2–5 are slightly exaggerated sketches which demonstrate the appearance of teeth having different values for these ratios. The tooth in Fig. 2 has a low value of both the B/E and the F/E ratios. The one in Fig. 3 has a low F/E ratio combined with a high B/E ratio. Fig. 4 shows a tooth with a high F/E ratio and a low B/E ratio, and Fig. 5 shows the effect produced when both the ratios are high. In view of the fact that these ratios might alter at different stages of growth of the snails, as Hubendick (1945) has shown the linear dimensions to do, efforts were made to compare snails of similar sizes, as far as was possible with the available material. In no case was a young snail of less than 10 mm shell height used.

In the main part of the study a comparison was made of the teeth of specimens taken from thirteen populations in five northern European countries. Six individuals were chosen to represent each population, and measurements were made of six first lateral teeth and of six sixth lateral teeth from each individual. These teeth were studied on both sides of one transverse row of teeth. This was repeated five rows further along the radula and then repeated again five rows further along, thus giving the required number of teeth. Hence, seventy-two teeth from six individuals were measured for each population.

Apart from the main study, several other points were investigated. First, an examination was made to determine whether variation was as great between teeth on the same radula as between teeth on different radulae from the same population. At the same time the figures obtained from corresponding teeth on opposite sides of the radula were compared. Two populations were selected for this. In each case one radula was taken and the first and the sixth lateral teeth on one side of it were measured for twenty-five consecutive rows. Then twenty-five radulae were taken and the first and the sixth lateral teeth were measured on both sides of one row on each radula.

Secondly, two samples were examined, the second of which had been taken from the same population as the first after an interval of nine years. This is a relatively short interval but it enabled some check to be made on the change, if any, within a population over a period of several generations.

Thirdly, two samples were examined which had been collected from different parts of the shore of the same lake, to determine whether any difference could be detected in these circumstances.

The localities from which the population samples were obtained are shown on the map on Fig. 6. The following is a list of the habitats corresponding to the numbers on the map.

1. Small mountain lake south of Valla, near Korgen, Nordland, Norway.
2. Lake near Skalstugan, Jämtland, Sweden.
3. Freshwater rock pool on rocky island off Tvärminne, S.W. Finland.
4. West shore of Ullnasjön, Täby, about 15 km north of Stockholm, Sweden.
5. Värtan, an inlet of the Baltic Sea about 7 km north of Stockholm, Sweden.
6. The shore of Lindholmen, an island in Värtan, Sweden.
7. Orlången, Huddinge, about 10 km south of Stockholm, Sweden.
8. Skillötsjön, Vårdinge, about 50 km south-west of Stockholm, Sweden.
9. Pølaa, stream at Holmene near Teglgaardslund 0.5 km south of Hillerød, Zealand, Denmark.
10. Vaserne, 1 km north-west of Holte, Zealand, Denmark.
11. Lake Øjesø, 32 km south-west of Grenaa, Jutland, Denmark.

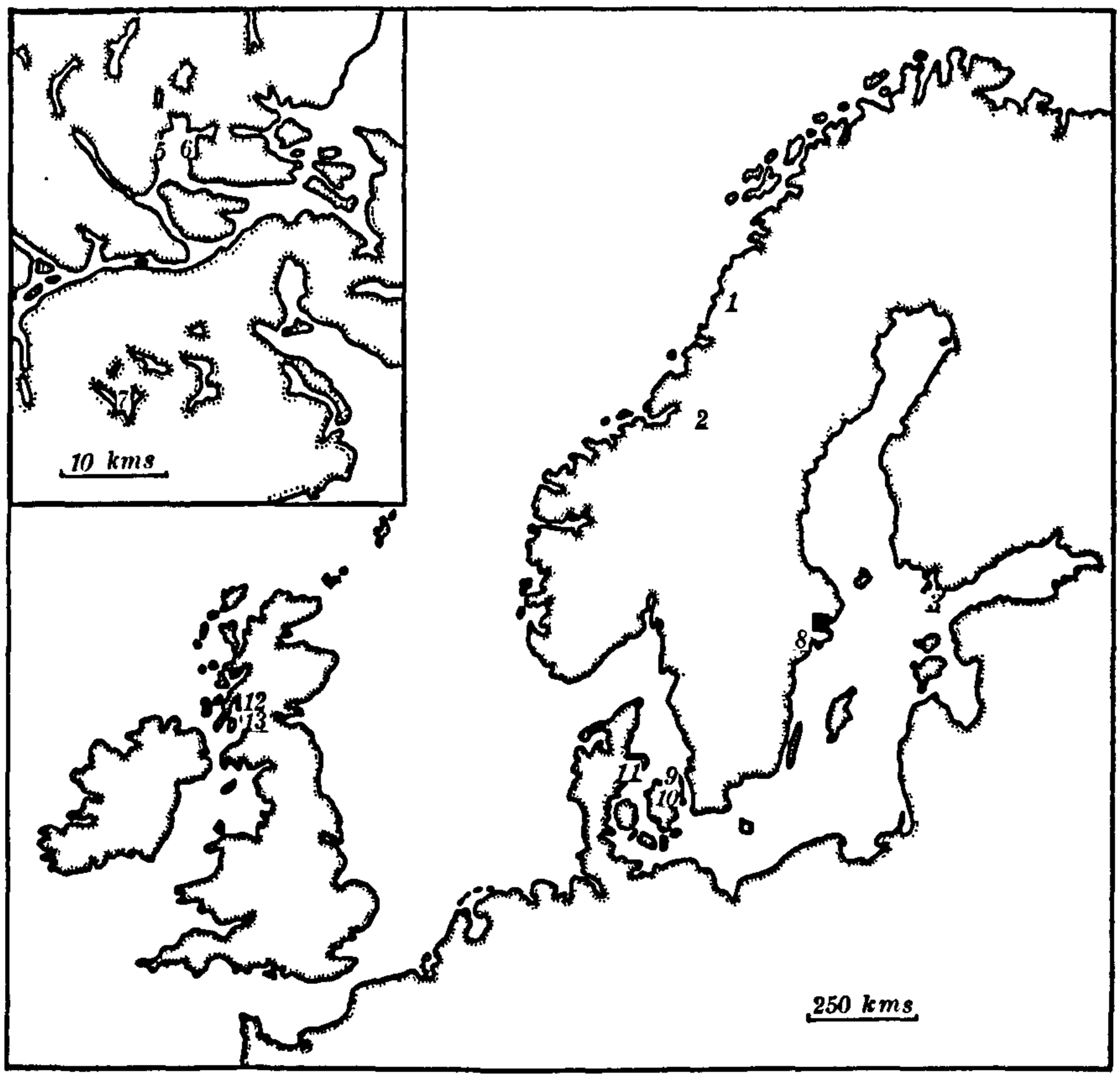


Fig. 6. The map shows the geographical distribution of the populations studied, each of which is indicated by the number under which it is listed in the text. The inset is an enlargement of the area round Stockholm designated by a black square on the main map.

12. Upper Craigton Burn, Dunbartonshire, Scotland.
13. Outfall stream from Dougalston Loch, Milngavie, Dunbartonshire, Scotland.

The localities are listed in the same order but more briefly designated in Tables 1–6. The populations numbered 1, 3, 4, 7, 8, 9 and 11 above, correspond to the populations numbered 56, 67–69, 23, 20–22, 19, 5 and 2 respectively in Table 1 of Hubendick (1951).

Results

An analysis of the figures obtained in the main part of the study is presented in Tables 1–5. In Table 1 the means and the standard errors are given for the ratios calculated from the measurements made on the thirteen populations. The standard deviations are also given below the mean in each case.

Tables 2–5 give an analysis of the differences existing between the population means for the two ratios on the two types of teeth studied. Reading along each horizontal row the comparison is given between the population named at the left

Table 1. Mean values with standard errors for the tooth ratios calculated for each population. The standard deviation is also shown below the mean in each case.

Population	B/E		F/E	
	1st lateral	6th lateral	1st lateral	6th lateral
1. Valla	1.145 ± 0.020 0.1192	0.965 ± 0.013 0.0752	0.588 ± 0.016 0.0947	0.497 ± 0.009 0.0514
2. Skalstugan	1.213 ± 0.021 0.1262	1.009 ± 0.016 0.0937	0.594 ± 0.010 0.0629	0.497 ± 0.011 0.0644
3. Tvärminne	1.156 ± 0.022 0.1323	1.022 ± 0.017 0.1047	0.545 ± 0.015 0.0902	0.436 ± 0.009 0.0561
4. Ullnasjön	1.479 ± 0.022 0.1311	1.203 ± 0.014 0.0852	0.706 ± 0.012 0.0733	0.513 ± 0.008 0.0495
5. Värtan	1.328 ± 0.040 0.2381	1.047 ± 0.034 0.2065	0.650 ± 0.014 0.0870	0.490 ± 0.018 0.1077
6 a. Lindholmen (1948)	1.185 ± 0.018 0.1054	1.003 ± 0.018 0.1109	0.647 ± 0.010 0.0590	0.479 ± 0.008 0.0492
6 b. Lindholmen (1957)	1.157 ± 0.015 0.0893	1.016 ± 0.011 0.0644	0.632 ± 0.014 0.0812	0.481 ± 0.009 0.0540
7 a. Orlången 1	1.238 ± 0.035 0.2081	1.045 ± 0.018 0.1064	0.636 ± 0.017 0.0991	0.461 ± 0.011 0.0670
7 b. Orlången 2	1.227 ± 0.014 0.0840	1.075 ± 0.013 0.0784	0.606 ± 0.013 0.0788	0.476 ± 0.008 0.0506
8. Skillötsjön	1.399 ± 0.032 0.1918	1.165 ± 0.025 0.1487	0.603 ± 0.015 0.0895	0.475 ± 0.011 0.0672
9. Hillerød	1.256 ± 0.017 0.1030	1.105 ± 0.014 0.0835	0.483 ± 0.011 0.0676	0.372 ± 0.012 0.0692
10. Holte	1.228 ± 0.017 0.1007	1.062 ± 0.012 0.0701	0.533 ± 0.008 0.0488	0.425 ± 0.010 0.0606
11. Øjesø	1.264 ± 0.024 0.1463	1.029 ± 0.016 0.0959	0.625 ± 0.012 0.0703	0.484 ± 0.010 0.0607
12. Craigton Burn	1.548 ± 0.027 0.1642	1.133 ± 0.020 0.1213	0.510 ± 0.009 0.0564	0.471 ± 0.009 0.0536
13. Dougalston Stream	1.347 ± 0.024 0.1454	1.096 ± 0.013 0.0808	0.422 ± 0.011 0.06436	0.386 ± 0.008 0.04752

hand side of the row and each other population in turn, as named at the top of the table. The differences have been estimated by applying the simple rule given by Mayr, Linsley and Usinger (1953) which uses the ratio of the difference between the means of two samples to the sum of their standard errors.

$$\frac{M_1 - M_2}{S.E._{M_1} + S.E._{M_2}}$$

If the value of this ratio is more than two then the actual population means are probably different, and this is indicated by the figure 2 in the tables. If the value is

Table 2. Differences between the means of the B/E ratio for the 1st lateral teeth. For full explanation see the text.

	Valla	Skalstugan	Tvärminne	Ullnasjön	Värtan	Lindholmen 1948	Lindholmen 1957	Orlångеn 1	Orlångеn 2	Skillötsjön	Hillerød	Holte	Øjesø	Craigton Burn	Dougalston Stream
Valla		—	—	3	2	—	—	—	2	3	2	2	2	3	3
Skalstugan	—		—	3	—	—	—	—	—	3	—	—	—	3	2
Tvärminne	—	—		3	2	—	—	—	—	3	2	—	2	3	3
Ullnasjön	3	3	3		2	3	3	3	3	—	3	3	3	—	2
Värtan	2	—	2	2		2	3	—	—	—	—	—	—	3	—
Lindholmen 1948	—	—	—	3	2		—	—	—	3	2	—	—	3	3
Lindholmen 1957	—	—	—	3	3	—		—	2	3	3	2	2	3	3
Orlångеn 1	—	—	—	3	—	—	—		—	2	—	—	—	3	—
Orlångеn 2	2	—	—	3	—	—	2	—		3	—	—	—	3	3
Skillötsjön	3	3	3	—	—	3	3	2	3		2	3	2	2	—
Hillerød	2	—	2	3	—	2	3	—	—	2		—	—	3	2
Holte	2	—	—	3	—	—	2	—	—	3	—		—	3	2
Øjesø	2	—	2	3	—	—	2	—	—	2	—	—		3	—
Craigton Burn	3	3	3	—	3	3	3	3	3	2	3	3	3		3
Dougalston Stream	3	2	3	2	—	3	3	—	3	—	2	2	—	3	

Table 3. Differences between the means of the B/E ratio for the 6th lateral teeth. For full explanation see the text.

	Valla	Skalstugan	Tvärminne	Ullnasjön	Värtan	Lindholmen 1948	Lindholmen 1957	Orlångеn 1	Orlångеn 2	Skillötsjön	Hillerød	Holte	Øjesø	Craigton Burn	Dougalston Stream
Valla		—	—	3	—	—	2	2	3	3	3	3	2	3	3
Skalstugan	—		—	3	—	—	—	—	2	3	3	—	—	3	3
Tvärminne	—	—		3	—	—	—	—	—	3	2	—	—	3	3
Ullnasjön	3	3	3		3	3	3	3	3	2	3	3	3	2	3
Värtan	—	—	—	3		—	—	—	—	2	—	—	—	—	—
Lindholmen 1948	—	—	—	3	—		—	—	2	3	3	—	—	3	3
Lindholmen 1957	2	—	—	3	—	—		—	2	3	3	2	—	3	3
Orlångеn 1	2	—	—	3	—	—	—		—	2	—	—	—	2	—
Orlångеn 2	3	2	—	3	—	2	2	—		2	—	—	—	—	—
Skillötsjön	3	3	3	—	2	3	3	2	2		—	2	3	—	—
Hillerød	3	3	2	3	—	3	3	—	—	—		—	2	—	—
Holte	3	—	—	3	—	—	2	—	—	2	—		—	2	—
Øjesø	2	—	—	3	—	—	—	—	—	3	2	—		2	2
Craigton Burn	3	3	3	2	—	3	3	2	—	—	—	2	2		—
Dougalston Stream	3	3	2	3	—	3	3	—	—	—	—	—	2	—	

Table 4. Differences between the means of the F/E ratio for the 1st lateral teeth. For full explanation see the text.

	Valla	Skalstugan	Tvärminne	Ullnasjön	Värtan	Lindholmen 1948	Lindholmen 1957	Orlången 1	Orlången 2	Skillötsjön	Hillerød	Holte	Ojeso	Craigton Burn	Dougalston Stream
Valla		—	—	3	2	2	—	—	—	—	3	2	—	3	3
Skalstugan	—		—	3	2	2	—	—	—	—	3	3	—	3	3
Tvärminne	—	—		3	3	3	2	2	2	—	2	—	2	—	3
Ullnasjön	3	3	3		2	2	2	2	3	3	3	3	3	3	3
Värtan	2	2	3	2		—	—	—	—	—	3	3	—	3	3
Lindholmen 1948	2	2	3	2	—		—	—	—	—	3	3	—	3	3
Lindholmen 1957	—	—	2	2	—	—		—	—	—	3	3	—	3	3
Orlången 1	—	—	2	2	—	—	—		—	—	3	3	—	3	3
Orlången 2	—	—	2	3	—	—	—	—		—	3	3	—	3	3
Skillötsjön	—	—	—	3	—	—	—	—	—		3	3	—	3	3
Hillerød	3	3	2	3	3	3	3	3	3	3		2	3	—	2
Holte	2	3	—	3	3	3	3	3	3	3	2		3	—	3
Ojeso	—	—	2	3	—	—	—	—	—	—	3	3		3	3
Craigton Burn	3	3	—	3	3	3	3	3	3	3	—	—	3		3
Dougalston Stream	3	3	3	3	3	3	3	3	3	3	2	3	3	3	

Table 5. Differences between the means of the F/E ratio for the 6th lateral teeth. For full explanation see the text.

	Valla	Skalstugan	Tvärminne	Ullnasjön	Värtan	Lindholmen 1948	Lindholmen 1957	Orlången 1	Orlången 2	Skillötsjön	Hillerød	Holte	Ojeso	Craigton Burn	Dougalston Stream
Valla		—	3	—	—	—	—	—	—	—	3	3	—	—	3
Skalstugan	—		3	—	—	—	—	—	—	—	3	3	—	—	3
Tvärminne	3	3		3	2	2	2	2	2	2	3	3	2	—	3
Ullnasjön	—	—	3		—	2	—	2	2	2	3	3	—	2	3
Värtan	—	—	2	—		—	—	—	—	—	3	2	—	—	3
Lindholmen 1948	—	—	2	2	—		—	—	—	—	3	2	—	—	3
Lindholmen 1957	—	—	2	—	—	—		—	—	—	3	2	—	—	3
Orlången 1	—	—	2	2	—	—	—		—	—	3	—	—	—	3
Orlången 2	—	—	2	2	—	—	—	—		—	3	2	—	—	3
Skillötsjön	—	—	—	2	—	—	—	—	—		3	2	—	—	3
Hillerød	3	3	3	3	3	3	3	3	3	3		2	3	3	3
Holte	3	3	—	3	2	3	2	—	2	2	2		2	2	2
Ojeso	—	—	2	—	—	—	—	—	—	—	3	2		—	3
Craigton Burn	—	—	—	2	—	—	—	—	—	—	3	2	—		3
Dougalston Stream	3	3	2	3	3	3	3	3	3	3	3	2	3	3	

Table 6. For each population is given the number of other populations from which it differs significantly in respect of each ratio studied. The total number of differences recorded for each population is also given.

Population	B/E		F/E		Total
	1st lateral	6th lateral	1st lateral	6th lateral	
Valla	9	10	7	4	30
Skalstugan	4	6	7	4	21
Tvärminne	7	5	9	10	31
Ullnasjön	12	13	14	9	48
Värtan	6	2	8	4	20
Lindholmen 1948	6	6	8	5	25
Lindholmen 1957	9	8	6	4	27
Orlången 1	3	4	6	3	16
Orlången 2	6	6	6	5	23
Skillötsjön	11	10	5	4	30
Hillerød	8	7	13	14	42
Holte	6	5	12	12	35
Øjesø	6	6	6	4	22
Craigton Burn	13	9	11	4	37
Dougalston Stream	10	7	14	14	45

three or more then the actual population means are almost certainly different, and the figure 3 is inserted in the tables. For the purpose of this paper both these cases will be regarded as showing significant differences, and all others, indicated by a dash, will be considered as not being significantly different. Tables 2–5 show that many of the population means are significantly different from each other.

Table 6 gives a partial summary of Tables 2–5. For each population it records the number of other populations from which that population differs significantly.

It is important to determine whether there is any correlation between the ratios calculated in this study and the shell size of the snails used, because although it was desirable to use specimens of almost the same size throughout, this was not always possible. In particular, the samples which were available with the *ampla* type of shell, from Orlången and Skillötsjön, were nearly all larger than the largest specimens available from the other populations. In view of this a check was carried out to find whether or not there was any evidence of such a correlation. The most convenient cases for testing this might have been the two populations from which twenty-five individuals had been examined, but in these cases the specimens were all similar in size, with a range of only about 4 mm, which was not very suitable for testing the correlation. In view of this it was decided to use the snails forming the main part of the study, treating each snail individually and lumping all the population samples together. The seventy-eight individuals which were considered gave a large size range extending from 10.3 mm to 25.5 mm. Correlation coefficients were calculated for each of the four ratios compared with the shell height. For the first lateral teeth the correlation coefficient for the B/E ratio was -0.054, and that for the F/E ratio was 0.153. In the case of the sixth lateral teeth the value for the B/E ratio was 0.113, and that for the F/E ratio was 0.172. When the significance of these coefficients is tested using Student's t Tables at the 5 % level of significance it is found that in no case has a significant correlation been established.

In a study of the radula of *Goniobasis livescens*, Howe (1930) quotes three tooth dimensions and the shell heights for forty-two individuals. As a further check for the present study the three possible ratios of these tooth dimensions have been calculated from his figures for the twenty-five snails of more than 10 mm shell height. When the correlation coefficients were calculated comparing shell height with these three ratios the following values were obtained:

width of central cusp/length of central cusp:	0.292
width of central cusp/width of median tooth:	0.326
length of central cusp/width of median tooth:	0.007

Again, in no case was a significant correlation established. Hubendick (1945), in a study of the radula of *Lymnaea limosa* (=*peregra*), presents, with graphs, data showing the increase in the width and in the length of the first lateral teeth with increase in shell height. The slopes of the two graphs do not suggest that there would be any significant change in the ratio of these two tooth dimensions above 5 mm shell height. Hence, in the present study, it seems that such variations as do occur in shell size between the various samples can be reasonably ignored.

Another possible correlation which could influence the results of this study is between the two ratios calculated for all the teeth. If a scatter diagram is constructed in which the B/E values for the first lateral teeth are plotted against the B/E values for the sixth lateral teeth, the result shows that there is a definite correlation between these. The same is true for the F/E values for these teeth. However, if the B/E values for the first lateral teeth are plotted against the F/E values for the same teeth, there is no evidence of correlation. This is the case also when the B/E and the F/E values for the sixth lateral teeth are plotted together. This shows that, while the same ratio tends to vary similarly for the two teeth studied, the B/E ratios and the F/E ratios of the teeth vary independently of each other. This means that these two ratios, used throughout this study, give indepedent records of the variation between the populations involved.

Before proceeding to the inter-population variation it is better to consider the information derived on intra-population variation. The comparison of the variation between the teeth on one radula and the teeth on different radulae from the same population yielded the results given in Tables 7 and 8. The results from both of the populations studied in this respect are very similar. In comparing the figures from twenty-five specimens with those from single specimens the mean is of little value, and the important figure is the standard deviation. In all cases it is much lower for the ratios of the teeth from the single specimens, which shows that there is less variation between the teeth on one radula than between those on different radulae from the same population. This is as might be expected, but, as the standard deviations also show, there is, nevertheless, an appreciable amount of variation between teeth from the same radula. This last statement is supported by the comparison, in the tables, of the figures obtained from the teeth on the left and those on the right side of the radulae. Again there is slight variation, although this never approaches the level of statistical significance.

A comparison of the same population at different times was made by studying two samples from Lindholmen, which were collected on the shore of this island in 1948 and 1957. These samples are recorded in Table 1 as numbers 6a and 6b respectively. It can be seen that the mean values in each case are similar, and Tables 2–5 show

Table 7. Comparison of the variation between the teeth on 25 different radulae and those on a single radula, all from the Valla population. Means and standard errors for each ratio of each tooth studied are given, with the standard deviation below the mean in each case. In the case of the 25 specimens, the figures obtained from teeth on the left and the right sides of the radulae are given separately and then combined.

Ratio	25 specimens				1 specimen	
	1st lateral		6th lateral		1st lateral	6th lateral
B/E	left side 1.195 ± 0.027 0.1328	right side 1.187 ± 0.018 0.08903	left side 0.960 ± 0.019 0.09251	right side 0.995 ± 0.019 0.09643		
	both sides 1.191 ± 0.016 0.1119		both sides 0.978 ± 0.013 0.09517		1.191 ± 0.012 0.06303	0.977 ± 0.009 0.04519
F/E	left side 0.573 ± 0.016 0.08125	right side 0.584 ± 0.015 0.07729	left side 0.473 ± 0.012 0.05933	right side 0.482 ± 0.011 0.0561		
	both sides 0.578 ± 0.011 0.07639		both sides 0.478 ± 0.008 0.05743		0.633 ± 0.008 0.04231	0.514 ± 0.009 0.04438

that no significant difference was found between them. It would have been more valuable if material collected at a much wider interval had been available. However, this does show that the population does not appear to have changed during a period of nine years in respect of the ratios studied.

In order to see whether any difference could be detected between two samples from different parts of the same lake, two samples from Orlången were examined. These had been collected on the same day and, although they were not very far apart, they were taken in different ecological areas. The figures obtained are given in Table 1 as numbers 7a and 7b, and in both cases are similar. Tables 2–5 show that no significant differences were found between the means, which indicates that a certain homogeneity exists among the snails in the lake.

It should also be noted here that Lindholmen is an island in Värtan. It seems very unlikely that there is any active contact between the populations studied from the island shore and from the mainland shore, but they would seem to be much more favourably situated for the occasional transfer of individuals to occur than are any of the other populations. It is interesting to find that the sixth lateral teeth in the Värtan and the Lindholmen populations are very similar, but that differences exist between the first lateral teeth which are significant in the case of the B/E ratio.

The extent and nature of the variation between the different populations, which formed the main purpose of this investigation, may now be considered. It should be remembered that the differences being considered at this stage are between the actual means of the populations concerned, as determined by the method outlined at the beginning of this section. The most distinctive population is that from Ullna-

Table 8. Comparison of the variation between the teeth on 25 different radulae and those on a single radula, all from the Lindholmen 1948 population. Means and standard errors for each ratio of each tooth studied are given, with the standard deviation below the mean in each case. In the case of the 25 specimens, the figures obtained from teeth on the left and the right sides of the radulae are given separately and then combined.

Ratio	25 specimens				1 specimen	
	1st lateral		6th lateral		1st lateral	6th lateral
B E	left side 1.192 ± 0.031 0.1539	right side 1.203 ± 0.032 0.1576	left side 1.030 ± 0.029 0.1432	right side 1.043 ± 0.026 0.1303		
	both sides 1.198 ± 0.022 0.1542		both sides 1.037 ± 0.019 0.1356		1.219 ± 0.013 0.064	0.917 ± 0.008 0.041
F/E	left side 0.583 ± 0.014 0.06752	right side 0.612 ± 0.012 0.0607	left side 0.487 ± 0.008 0.0417	right side 0.493 ± 0.010 0.04939		
	both sides 0.598 ± 0.009 0.06516		both sides 0.490 ± 0.006 0.04531		0.638 ± 0.006 0.030	0.454 ± 0.005 0.026

sjön, showing a high value in all its ratios and being significantly different from forty-eight out of fifty-six ratios of other populations. The two Scottish populations, Dougalston Stream and Craigton Burn, have high values for both B/E ratios and low values for both F/E ratios. However, although they show this similar trend their values are very significantly different from each other in three out of the four cases. The two Zealand populations, Hillerød and Holte, are similar in having intermediate values for both B/E ratios and low ones for both F/E ratios. In both these cases, however, the F/E ratios are significantly different from each other. Tvärminne shows low values for all four ratios while Valla has low B/E values and intermediate ones for F/E. Skillötsjön has high values of B/E and low ones of F/E. No general trend related to the geographical distribution of the populations seems to be present, except perhaps in the similar conditions found within the pairs from Scotland and Zealand. As stated above, however, they also differed considerably from each other, although in both cases the populations are only a few kilometers apart.

Mayr, Linsley and Usinger (1953) discuss a method of deciding whether or not two populations should be separated subspecifically. This involves calculating the coefficient of difference, which is obtained by dividing the difference between the two means by the sum of their standard deviations.

$$\text{C.D.} = \frac{M_b - M_a}{S.D._a + S.D._b}$$

If the coefficient is equal to 1.28 this signifies that 75 % of the one population differs

from 97 % of the second, which is the conventional level of subspecific difference. A higher coefficient means a greater degree of difference, and they suggest that if the value is above 1.5 two different subspecies are usually involved. It should be noted that the differences now being discussed are between the actual populations involving their entire ranges of values and not merely their mean values as above.

It is of interest to note the cases in which a subspecific level of difference exists between the populations now under consideration, as determined by the coefficient of difference. Cases in which the coefficient has a value greater than 1.5 occur only in respect of the first lateral teeth. Considering the B/E ratio, this level is reached in comparing Craigton Burn, which has the highest value of this ratio, with Lindholmen 1957, which has one of the lowest values. More cases exist in respect of the F/E ratio. Ullnasjön, which has the highest value, differs at this level from the three populations with the lowest values, namely Dougalston Stream, Hillerød and Craigton Burn. Dougalston Stream, which has the lowest value, differs at this level from Värtan, Lindholmen 1948 and Øjesø, in addition to Ullnasjön. In comparing the F/E ratio of the first lateral teeth from snails at Ullnasjön and Dougalston Stream the coefficient of difference is 2.06 which signifies only a very small degree of overlap between the populations. If the various cases in which the coefficient of difference is greater than 1.28 are taken into consideration fourteen other cases are added to those mentioned above. None of the populations considered here differs to this extent from each of the others, and there is nothing to suggest that any distinct subspecies are, in fact, present.

As mentioned previously, the populations from Orlången and Skillötsjön possessed the *ampla* type of shell, which is perhaps the most extreme shape assumed by *L. peregra*. In the present study Skillötsjön snails proved to be fairly distinctive in their B/E ratio, as is apparent from Tables 2 and 3, although they were well below the maximum value recorded. Otherwise these two populations are unobtrusive in the tables, and it would seem that their radulae do not show any extreme modification in parallel with their shells.

Discussion

The variation in the shell of two species of *Lymnaea* has been studied in Canada by Mozley (1935). He states that "in *L. palustris* the range of variation in any one locality tends to approximate to that which occurs over the whole territory occupied by this animal," and that "in *L. emarginata* the range of variation in any one locality forms only a part of the geographical variation". Thus it would seem that the variation in *L. peregra* in the present study corresponds to that found in *L. emarginata*. However, Mozley attributes the difference in the type of variation shown by the two species in his investigation to the fact that *L. palustris* is of very common occurrence in the territory studied, and thus has frequent opportunities for mixture of stock to take place. *L. emarginata*, on the other hand, is of relatively rare occurrence which would make the transfer of individuals less likely and so allow genetic differences to arise between the various colonies. In this respect it would seem that, since *L. peregra* is of common occurrence in the area of the present study, its variation might be expected to resemble that of *L. palustris* in Mozley's study rather than that of *L. emarginata*. Thus the conclusions reached by Mozley are not in accord with those of the present investigation. It has been pointed out by Diver (1939) that Mozley's results could also be explained if it was shown that *L. emarginata*

possessed a greater ecological plasticity than did *L. palustris*. In this connection it is interesting and perhaps significant that Hubendick (1951) opens his review of *L. emarginata* with the following sentence: "It is difficult, perhaps impossible, to give a concise diagnosis of the shell of this species because of its very great variability."

The number of populations studied here is not large enough to enable such broad conclusions to be reached as was possible in Hubendick's (1951) study of the shell variation in *L. peregra*. However, there is no discernable inconsistency between the results obtained in the two investigations. There is a certain amount of variation within the individual populations, but this is always very much less than the range of variation of the species. Indeed, individual populations may differ from one another to such an extent in respect of some character that, in the absence of further information on the variation of this character within the species, they could legitimately be established as separate subspecies. What is most striking about this is that the populations concerned may be found relatively close to each other. Although no cases in close proximity were mentioned among the instances listed above, it may now be added that in comparing Ullnasjön with Lindholmen 1957 the coefficient of difference was found to be 1.46. This is only very slightly below the 1.5 level, and the populations concerned are only a few kilometers apart. It is equally true that populations which are far removed from one another may be quite similar in their characters. Good examples of this are provided by the populations at Skalstugan and Øjesø, and by those at Tvärminne and Holte, between which no significant differences are recorded. Further cases can be found in the tables. As far as can be ascertained from a careful study of the results, the geographical distribution of the populations does not appear to bear any relation to the nature of their variation. This seems to occur in a random manner and there is no evidence to suggest the existence of any cline.

SUMMARY

The radulae of specimens of *L. peregra* from thirteen different populations in north-western Europe were studied. Measurements were made on the first and the sixth lateral teeth and these were used to derive two ratios expressing the proportions of the teeth. These ratios were shown to vary independently of the shell size of the snails studied and also of each other. No difference could be detected between two samples collected from different parts of the same lake shore on the same day, nor between two samples collected from the same locality with an interval of nine years. Teeth on the same radula show a certain amount of varaition, but this is much less than the variation between radulae from the same population. The variation within each population is, in turn, very much less than the total variation of the species, and populations can differ very significantly from each other. The variation observed does not appear to bear any relation to the geographical distribution of the populations.

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SECTION VI

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The Influence of Various Definitive Hosts on the Development of *Diplostomum phoxini* (Strigeida, Trematoda).

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Several papers have recently appeared dealing with various aspects of the biology of *Diplostomum phoxini* (Faust, 1918). The life cycle was first reported from France by Arvy and Buttner (1954 and 1955), and then from Wales by Rees (1955 and 1957). A study of the development of the adult stage was carried out by Bell and Hopkins (1956), and in association with this, the present writer started a study of the life history, which was curtailed when the work of Dr. Rees became known. Several points of interest arose, and these are recorded here in supplement to the above mentioned works.

The metacercaria, *Diplostomulum phoxini*, is common in the Glasgow area of Scotland. It occurs in the brain of the minnow (*Phoxinus phoxinus* (L.)), and has been found in Loch Lomond, Loch Eck, River Clyde, River Endrick and the Craigton Burn at Milngavie. It has previously been recorded from Loch Lubnaig and from a stream near Edinburgh by Ashworth and Bannerman (1927). Although the incidence of infection is almost 100% the degree of infection seems to be less than that recorded by some other workers.

Molluscan Host

Arvy and Buttner (1954 and 1955) found that the first intermediate host in the Paris area was *Lymnaea auricularia* (L.). It seemed impossible that this could be the intermediate host in Scotland as this mollusc is calciphile in this part of its range, whereas the minnow infections recorded are from soft waters. Snails were

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collected from the River Endrick at a point where infected minnows had been caught, and were examined for infection with larval trematodes. Several specimens of *Lymnaea peregra* (Müller) were found from which furcocercous cercariae were emerging. No source of uninfected minnows was known, so infected fish were kept in the laboratory for over four weeks, by which time those metacercariae already present should have completed their development (Arvy and Buttner, 1954 and 1955). Two Breffit jars were set up each containing six of these minnows. A dense suspension of cercariae which had emerged from the *L. peregra* was added to Breffit A while Breffit B was kept as a control. All the minnows in Breffit A died within a few hours and showed signs of severe subcutaneous haemorrhage presumably due to cercarial penetration, though no cercariae were found to have reached the brains. A further six fish were placed in Breffit A with a smaller number of cercariae. One of these also died within a few hours, but the other five survived and were killed after ten days. Examination of their brains showed that each contained a massive infestation of small metacercariae in addition to the older, larger ones. The controls were also killed on the same day and their brains were found to contain only large metacercariae. Thus, in the Glasgow area, *L. peregra* acts as the first intermediate host of *D. phoxini*, as it also does in Wales (Rees, 1957). The ubiquity of the infection in minnows in the Glasgow area is not surprising in view of the widespread occurrence of this snail in the same area.

Laboratory Hosts for the Adult Stage

Adult flukes were first obtained by feeding the brains of infected minnows to young ducklings a few days old, which were autopsied after three days. This is the same definitive host that has been used by other workers (Arvy and Buttner, 1954 and 1955; Rees, 1955; Bell and Hopkins, 1956). Attempts were made to infect various other laboratory animals. The number of infected brains administered to each individual varied from 5 to 20 according to the size of the host animal.

Six young chickens were fed and one, killed after 24 hours, yielded 10 immature flukes. The other five, killed at intervals up to 96 hours, were all negative. Two pigeons were fed, but both were negative after 24 hours. Six rats were fed by stomach tube. Two were killed after 24 hours and one of these contained numerous

immature flukes. The other was negative, as were two killed after 48 hours and two killed after 72 hours. It was found possible to recover a small number of adult flukes from mice infected by stomach tube. Finally, herring-gull chicks were tried and these proved to be very suitable definitive hosts, giving a good yield of adult flukes. Thus three laboratory hosts were available for experimental purposes.

ADULT FLUKES

All the flukes used in this study were fixed in 70% alcohol using a modified version of the technique described by Johri and Smith (1956). Two small blobs of vaseline were placed on a slide so that they would support two edges of a cover slip. The live flukes were placed in 70% alcohol between the blobs, a cover slip was placed over them and pressed gently down so that the flukes were dorso-ventrally compressed as far as possible, without damaging them. The flukes were left thus until fixed in that position. Later they were stained in Gower's Carmine and mounted. This method shows the internal structure very clearly. Although this technique affects the dimensions of the flukes, it was more satisfactory than any other which was tried. It is considered that the comparative measurements used in this study are quite satisfactory since all the specimens were treated in the same manner and by the same person.

All the flukes from ducks and gulls which were included in this investigation had been recovered after exactly four days in the definitive host. In order to obtain a large enough number of specimens from mice, however, it was necessary to include 15 specimens which had been recovered after five days, and 12 which had been recovered at periods between three and four days. The measurements of these different age groups from mice have been studied separately, and there is no evidence to suggest that any significant effect is produced in the conclusions presented here as a result of this slight age difference.

When flukes recovered from the three experimental hosts were compared, it was obvious that they differed from one another. To illustrate the differences some measurements were made and the results are presented in Table 1. The table shows the mean values obtained from the measurements, and the standard error of each mean is given in brackets. When the standard error of the difference is calculated for each of the pairs of means from mice and ducks it

is found that the respective figures are all significantly different, with those from ducks being the larger. By the same method the pairs of figures from ducks and gulls are also found to be significantly different, except in the case of the B/A ratio, and those from gulls are always the larger. It follows that the corresponding figures from mice and gulls must be very significantly different indeed.

	Mice	Ducks	Gulls
Number of flukes examined	34	57	44
Length in mm.: A	0·593 (0·017)	0·896 (0·017)	1·264 (0·029)
Breadth in mm.	0·307 (0·012)	0·363 (0·008)	0·500 (0·015)
Length of posterior lobe in mm.: B ...	0·243 (0·010)	0·447 0·010)	0·663 (0·019)
Ratio B/A	0·403 (0·008)	0·498 (0·006)	0·521 (0·007)
Number of eggs in uterus	0·588 (0·135)	3·526 (0·210)	7·182 (0·711)

Table 1. Mean values of the measurements of adult flukes recovered from three different hosts, with standard errors in brackets.

Perhaps the most important conclusion from these figures is given in the lower two rows. The ratio of the length of the posterior lobe to the body length shows that the posterior lobe is relatively best developed in gulls and most poorly developed in mice. Since this lobe contains the genitalia and develops almost entirely in the final host from a small lobe of the metacercaria, its degree of development may reasonably be used as an index of the sexual development

of the flukes. Hence the flukes achieve their best development as well as their largest size in gulls. The figures for the average number of eggs contained by each fluke also indicate that the greatest sexual productivity is reached in gulls. It may be concluded that gulls are the most satisfactory of the various experimental hosts used in this study, and mice the least so. It seems quite possible that gulls may be the natural definitive host of this parasite in the west of Scotland.

Finally, it should be noted that adult flukes containing eggs and mature sperm could be obtained from both avian and mammalian hosts. Dubois (1944) claims that the Strigeida show a strong specificity to their host, and he uses the specificity of members of this group to either birds or mammals as a major distinction in his system of classification (Dubois, 1938 and 1953). The present study shows that *D. phoxini* is not highly specific as regards its definitive host, but that it will develop more satisfactorily in one host than in another. According to Dubois the genus *Diplostomum* is found in birds. It may be argued that the infection of mice in this study is only a laboratory infection which could probably never occur in nature, and that the flukes which were obtained from this host were abnormal. Nevertheless, they underwent considerable development and produced eggs and mature sperm. New species of parasites are often described on the basis of a very few specimens recovered from a single host individual, and it is considered that in these circumstances too great a reliance on the host specificity of the worms may only give rise to confusion.

SUMMARY

1. Infection with *Diplostomulum phoxini* is very common among minnows in the Glasgow area of Scotland.

2. The first intermediate host of the parasite in this area is shown to be the freshwater snail *Lymnaea peregra*.

3. Adult flukes were obtained experimentally in mice, ducklings and herring-gull chicks.

4. Significant differences are shown to occur between adult flukes recovered from different host species.

5. The implications of these findings in relation to the host specificity and the classification of the Strigeida are briefly discussed.

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SECTION VII

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Two *Diplostomulum* Larvae (Strigeida, Trematoda) in the Eyes of Sticklebacks (*Gasterosteus aculeatus* L.)

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The life cycle of *Diplostomum spathaceum* (Rud.) is one of the few from this genus which is known, having been constructed from the work of Braun (1894) and Szidat (1924). It is known that the furcocercous cercariae can develop in the pulmonate snails *Lymnaea stagnalis* (L.) and *Lymnaea peregra* (Müller), and that on leaving this host they may enter any one of a considerable number of species of freshwater fish. There they migrate to the eyes, where they enter the lens and complete their larval development as metacercariae. This involves a considerable increase in size and probably takes about one month, as was demonstrated for *Diplostomulum phoxini* (Faust) by Arvy and Buttner (1954). Thus new infections can always be distinguished at once from older ones by the smaller size of the metacercariae.

During some studies on the parasites of freshwater fish, sticklebacks were collected by Gray (unpublished) from a pond situated in ground south of Mossend Railway Station, Lanarkshire. Gray found that in the eyes of these fish there were apparently two types of metacercariae which could be distinguished by slight morphological differences and also by the fact that one type was found in the lens whereas the other occurred in the pigment of the retina (Fig. 1). Several explanations were possible, but two seemed to be the most

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probable. The two types might be specifically identical, with the metacercariae showing some ecophenotypic variation dependent on the part of the eye in which they developed. Alternatively two species might be present. An investigation was therefore carried out, the results of which are presented below.

Natural Infection of Intermediate Hosts

Twenty specimens of *L. peregra* were collected from the pond at Mossend, placed in tubes of water in the laboratory, and observed for cercarial infections. From six of the snails there emerged moderate numbers of strigeid cercariae which, when floating in the water, showed a flexure of the body which is characteristic of *Cercaria C* Szidat, the known larval stage of *D. spathaceum*. One snail showed a heavy infection with another strigeid cercaria which did not flex its body when at rest, while another had also a heavy infection of this same cercaria but a few *Cercaria C* Szidat were also present.

Sticklebacks collected from Loch Lomond were found to have both types of metacercariae in the eyes, but the degree of infection was extremely low compared with that of the Mossend fish (Table 1). This is probably due to the different ecological conditions influencing the fish and the snails in a large lake as compared with a small closed pond. Because of the low incidence of natural infection, it was decided to use Loch Lomond fish for experimental infection with the cercariae from the Mossend snails.

These observations were made during August and September. Snails and fish were also collected from Mossend during the month of January. None of the latter snails shed cercariae during the two weeks that they were kept in the laboratory, although several proved to have infections when dissected at the end of this period. The fish were found to have only fully developed metacercariae in their eyes. Apparently under cold conditions no transmission occurs from snails to fish.

Experimental Procedure

Two tanks were set up each containing about twenty sticklebacks. The fish were kept in the laboratory for eighteen days so that any immature metacercariae already present would be at least almost

mature and readily distinguishable from a new infection. The six snails which were shedding *Cercaria C* Szidat were then placed in one tank, and the two which were shedding the other strigeid cercaria were placed in the other tank. This meant that a very few *Cercaria C* Szidat were present in the second tank; however, as will be seen, this did not confuse the results. The snails were removed from both tanks after twenty-four hours. By this time almost half of the fish in the first tank had died, probably due to internal haemorrhage caused by the penetration of large numbers of cercariae. Fish from both tanks were killed at intervals starting six days after exposure and the eyes were dissected.

Table 1. Natural infection rates in sticklebacks.

Source of fish	No. of fish examined	Total No. of metacercariae in retina	Total No. of metacercariae in lens
Loch Lomond ...	40	17	4
Mossend	22	161	280

Table 2. Numbers and locations of metacercariae recovered from Loch Lomond sticklebacks after exposure to *Cercaria C* Szidat. Each line represents one fish.

No. of days since exposure	Left eye				Right eye			
	in retina		in lens		in retina		in lens	
	large	small	large	small	large	small	large	small
6	0	0	0	47	0	0	0	59
7	0	0	0	71	1	0	0	53
7	0	0	0	34	0	0	0	71
8	2	0	0	56	0	0	0	79
9	0	0	0	41	0	0	0	81
9	0	0	0	67	0	0	0	92
9	1	0	0	3	0	0	0	3
9	0	0	0	28	0	0	0	49
12	0	0	1	66	0	0	0	5
15	0	0	0	8	0	0	0	11
17	0	0	0	97	0	0	0	90

Results

The results are presented in Tables 2 and 3. Table 2 shows that the fish exposed to *Cercaria C* Szidat all had small metacercariae in the lens of each eye, indicating a recent infection. In nearly all cases the number found was very large, but in no case was a small metacercaria found anywhere other than in the lens. Thus even under the crowded conditions of a heavy infection all the metacercariae of *D. spathaceum* entered the lens. Table 3 shows that the fish exposed

Table 3. Numbers and locations of metacercariae recovered from Loch Lomond sticklebacks after exposure to unidentified strigeid cercaria. Each line represents one fish.

No. of days since exposure	Left eye				Right eye			
	in retina		in lens		in retina		in lens	
	large	small	large	small	large	small	large	small
6	0	19	0	0	0	13	0	0
7	0	16	0	0	0	23	0	1
7	1	19	0	0	1	11	0	0
7	0	9	0	0	0	21	0	0
7	0	9	0	0	0	19	0	1
8	0	13	0	0	0	15	0	0
8	2	24	0	0	1	19	0	0
12	0	14	1	0	0	15	0	0
12	0	13	0	0	0	11	0	0
12	0	12	0	0	0	19	0	0
12	1	24	0	0	2	13	0	0
12	0	29	0	0	0	32	0	0
12	0	18	0	0	0	19	0	0
12	0	19	0	0	0	30	0	0
13	0	25	0	0	0	11	0	0
15	0	30	0	0	0	23	0	0
16	0	17	1	0	1	22	0	0
16	0	22	0	0	0	16	0	0
17	0	18	0	0	0	15	0	0
73	38	0	0	0	15	0	0	0
73	40	0	0	0	41	0	0	0

to the other furcocercous cercaria all had small metacercariae in the retina of each eye. In two cases a single small metacercaria was found in the lens, almost certainly due to a few *Cercaria C* Szidat having emerged into this tank from the snail with the double infection. The last two records in Table 3 are from fish which were killed more than two months after exposure. Again there is ample evidence of the heavy infection, although by this time all the metacercariae had completed their development and are recorded as large.

A similar experiment was conducted using fish from Mossend, and the results in this case were equally clear. It is evident from these experiments that two species of strigeid metacercariae are present in the eyes of the Mossend sticklebacks, and that they each exhibit a strong habitat selection within the eye, irrespective of the presence or absence of the other.

Finally the two types of metacercariae were fed separately to young ducklings. These were killed four days later and adult flukes were recovered in both cases. Thus, as well as having the same intermediate hosts, the two species of parasites can complete their life cycles in the same definitive host.

SUMMARY

Two distinct *Diplostomulum* metacercariae were shown to inhabit the eyes of sticklebacks. Both these species could complete their life cycles in the same three host species. Observations at different seasons indicated that under cold winter conditions no transmission occurred from snails to fish in the field.

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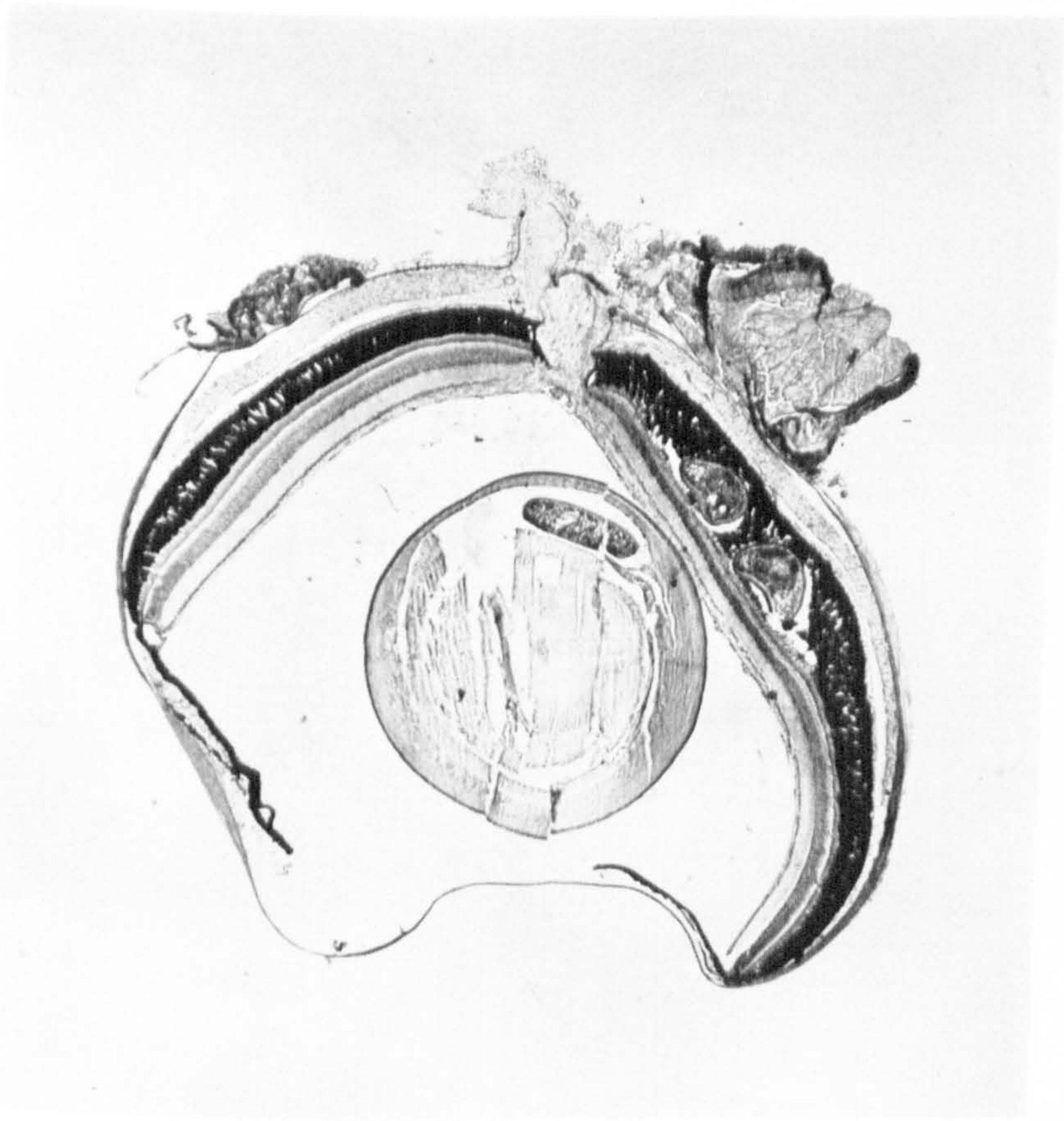

Fig. 1.—Section through an eye of an infected stickleback showing two metacercariae in the pigment layer and one in the lens.